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Publications:

An assessment of the importance of exposure routes to the uptake and internal localisation of fluorescent nanoparticles in zebrafish (Danio rerio), using light sheet microscopy

A major challenge in nanoecotoxicology is finding suitable methods to determine the uptake and localisation of nanoparticles on a whole-organism level. Some uptake methods have been associated with artefacts induced by sample preparation, including staining for electron microscopy. This study used light sheet microscopy (LSM) to define the uptake and localisation of fluorescently labelled nanoparticles in living organisms with minimal sample preparation. Zebrafish (Danio rerio) were exposed to fluorescent gold nanoparticles (Au NPs) and fluorescent polystyrene NPs via aqueous or dietary exposure. The in vivo uptake and localisation of NPs was investigated using LSM at different time points (1, 3 and 7 days). A time-dependent increase in fluorescence was observed in the gut after dietary exposure to both Au NPs and polystyrene NPs. No fluorescence was observed within gut epithelia regardless of the NP exposure route indicating no or limited uptake via intestinal villi. Fish exposed to polystyrene NPs through the aqueous phase emitted fluorescence signals from the gills and intestine. Fluorescence was also detected in the head region of the fish after aqueous exposure to polystyrene NPs. This was not observed for Au NPs. Aqueous exposure to Au NPs resulted in increased relative swimming distance, while no effect was observed for other exposures. This study supports that the route of exposure is essential for the uptake and subsequent localisation of nanoparticles in zebrafish. Furthermore, it demonstrates that the localisation of NPs in whole living organisms can be visualised in real-time, using LSM.

General information

State: Accepted/In press
Organisations: Department of Environmental Engineering, Environmental Chemistry, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Gothenburg, Roskilde Universitet
Authors: Skjolding, L. M. (Intern), Ašmonaitė, G. (Ekstern), Jølck, R. I. (Intern), Andresen, T. L. (Intern), Selck, H. (Ekstern), Baun, A. (Intern), Sturve, J. (Ekstern)
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Applying fluorescence correlation spectroscopy to investigate peptide-induced membrane disruption

There is considerable interest in understanding the interactions of antimicrobial peptides with phospholipid membranes. Fluorescence correlation spectroscopy (FCS) is a powerful experimental technique that can be used to gain insight into these interactions. Specifically, FCS can be used to quantify leakage of fluorescent molecules of different sizes from large unilamellar lipid vesicles, thereby providing a tool for estimating the size of peptide-induced membrane disruptions. If fluorescently labeled lipids are incorporated into the membranes of the vesicles, FCS can also be used to obtain information about whether leakage occurs due to localized membrane perturbations or global membrane destabilization. Here, we outline a detailed step-by-step protocol on how to optimally implement an FCS-based leakage assay. To make the protocol easily accessible to other researchers, it has been supplemented with a number of practical tips and tricks.

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Department of Chemistry
Authors: Kristensen, K. (Intern), Henriksen, J. R. (Intern), Andresen, T. L. (Intern)
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**Combined Colorimetric and Gravimetric CMUT Sensor for Detection of Phenylacetone**

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Organisations: Department of Micro- and Nanotechnology, MEMS-AppliedSensors, Surface Engineering, Colloids and Biological Interfaces
Authors: Mølgaard, M. J. G. (Intern), Laustsen, M. (Intern), Thygesen, I. L. (Intern), Jakobsen, M. H. (Intern), Andresen, T. L. (Intern), Thomsen, E. V. (Intern)
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**Convenient one-step synthesis of 5-carboxy-seminaphthofluoresceins**

The one-step synthesis and characterization of a series of regioisomerically pure 5-carboxy-seminaphthofluoresceins (5-carboxy-SNAFLs) is reported. The optical properties were determined in aqueous buffer at around biological pH, and highly pH sensitive, large Stokes-shift fluorophores with emission in the deep-red to near-infrared region were identified.

**General information**
State: Published
Organisations: Department of Chemistry, Center for Nanomedicine and Theranostics, X-ray Crystallography, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Organic Chemistry, Lund University
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Delivery of TLR7 agonist to monocytes and dendritic cells by DCIR targeted liposomes induces robust production of anti-cancer cytokines

Tumor immune escape is today recognized as an important cancer hallmark and is therefore a major focus area in cancer therapy. Monocytes and dendritic cells (DCs), which are central to creating a robust anti-tumor immune response and establishing an anti-tumorogenic microenvironment, are directly targeted by the tumor escape mechanisms to develop immunosuppressive phenotypes. Providing activated monocytes and DCs to the tumor tissue is therefore an attractive way to break the tumor-derived immune suppression and reestablish cancer immune surveillance. To activate monocytes and DCs with high efficiency, we have investigated an immunotherapeutic Toll-Like Receptor (TLR) agonist delivery system comprising liposomes targeted to the dendritic cell immunoreceptor (DCIR). We formulated the immune stimulating TLR7 agonist TMX-202 in the liposomes and examined the targeting of the liposomes as well as their immune activating potential in blood-derived monocytes and DCs to the tumor tissue is therefore an attractive way to break the tumor-derived immune suppression and reestablish cancer immune surveillance. To activate monocytes and DCs with high efficiency, we have investigated an immunotherapeutic Toll-Like Receptor (TLR) agonist delivery system comprising liposomes targeted to the dendritic cell immunoreceptor (DCIR). We formulated the immune stimulating TLR7 agonist TMX-202 in the liposomes and examined the targeting of the liposomes as well as their immune activating potential in blood-derived monocytes, myeloid DCs (mDCs), and plasmacytoid DCs (pDCs). Monocytes and mDCs were targeted with high specificity over lymphocytes, and exhibited potent TLR7-specific secretion of the anti-cancer cytokines IL-12p70, IFN-α 2a, and IFN-γ. This delivery system could be a way to improve cancer treatment either in the form of a vaccine with co-formulated antigen or as an immunotherapeutic vector to boost monocyte and DC activity in combination with other treatment protocols such as chemotherapy or radiotherapy. Cancer immunotherapy is a powerful new tool in the oncologist's therapeutic arsenal, with our increased knowledge of anti-tumor immunity providing many new targets for intervention. Monocytes and dendritic cells (DCs) are attractive targets for enhancing the anti-tumor immune response, but systemic delivery of immunomodulators has proven to be associated with a high risk of fatal adverse events due to the systemic activation of the immune system. We address this important obstacle by targeting the delivery of an immunomodulator, a Toll-like receptor agonist, to DCs and monocytes in the bloodstream. We thus focus the activation,
potentially avoiding the above-mentioned adverse effects, and demonstrate greatly increased ability of the agonist to induce secretion of anti-cancer cytokines.

**General information**

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Department of Biotechnology and Biomedicine, Disease Systems Immunology


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**Enzyme sensitive liposomes in chemotherapy and potentiation of immunotherapy**

Cancer is one of the leading causes of death in the world, and improved treatment approaches are urgently needed. One of the major treatment regimes used in the clinic today is chemotherapy. However, chemotherapeutic drugs are often hampered by poor circulation and low specificity, leading to low efficacy and induction of severe adverse effects. Interestingly, the pharmacokinetics and biodistribution of drugs can be substantially altered by encapsulation in liposomal drug delivery vehicles. The first chapter of this thesis gives a brief introduction to cancer followed by a discussion of the
applicability of liposomes as drug delivery vehicles in cancer therapy. The second chapter describes the development of a liposome system with an inbuilt release mechanism triggered by secretory phospholipase A2 (sPLA2). This enzyme is expressed at elevated levels in many human cancers, and as such represents a potential cancer specific trigger mechanism. The presented study validates the concept of sPLA2-induced release. However, in vivo evaluation reveals severe toxicity, potentially related to off-target activation of the trigger mechanism.

In the third chapter, a matrix metalloproteinase (MMP) sensitive liposome system is evaluated. Here cationic liposomes are engineered with an MMP cleavable PEG construct, aimed at shedding the PEG layer upon encounter with cancer expressed MMP enzymes, leading to exposure of the cationic charge and enhanced uptake in cancer cells. It is demonstrated that although exposure of cationic charge leads to enhanced uptake, it does not necessarily lead to enhanced bioavailability of the drug, underlining the importance of a release mechanism.

The fourth chapter explores the impact of the immune system on the efficacy of liposomal oxaliplatin. The chapter starts with an introduction to the cancer-immunity cycle and to how treatment approaches can aid this interplay. Subsequently it demonstrates that the presence of a functional immune system is important in the efficacy of liposomal oxaliplatin, and that this efficacy can be substantially enhanced by combination with the immune modulatory agent R848. In the fifth and last chapter it is concluded that the potential of liposomes in cancer drug delivery is highly dependent on extensive knowledge of the interplay between the intrinsic parameters of the liposome, the phenotype of the cancer and the potential effects on off-target tissues. Furthermore, it is underlined that effective cancer therapy requires approaches that target several different aspects of the cancer, and that in the case of liposomal oxaliplatin this can be partially achieved by combination with an effective immune modulatory agent. Finally it is speculated that further improvement of the presented treatment strategy might be obtained by targeting additional aspects of the cancer-immunity interplay not already addressed.

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Relations
Projects:
Injectable iodine-125 labeled tissue marker for radioactive localization of non-palpable breast lesions
We have developed a 125I-radiolabeled injectable fiducial tissue marker with the potential to replace current methods used for surgical guidance of non-palpable breast tumors. Methods in routine clinical use today such as radioactive seed localization, radio-guided occult lesion localization and wire-guided localization suffers from limitations that this injectable fiducial tissue marker offers solutions to. The developed 125I-radiolabeled injectable fiducial tissue marker is based on highly viscous sucrose acetate isobutyrate. The marker was readily inserted in NMRI mice and proved to be spatially well-defined and stable over a seven day period with excellent CT contrast (>1500 HU), enabling fluoroscopic visualization of markers during placement. The radioactivity remains strongly associated with the marker during the implantation period, which limits exposure to healthy tissue. Biodistribution studies show that there is negligible radioactivity in all non-tumor tissues sampled, with the exception of the thyroid gland, where limited accumulation was observed (0.06% of injected dose after 7 days). Based on the excellent performance of the marker and the fact that it can be delivered through thin hypodermic needles (≥27G), the marker holds great promise for clinical application, since patient discomfort is reduced significantly compared to current methods. Statement of Significance. A new type of tissue marker for local administration to non-palpable breast tumors has been developed. The surgical guidance marker is based on derivatives of the biomaterial sucrose acetate isobutyrate and unlike currently used markers it is injectable in the tissue using thin needles, reducing the discomfort to the patients significantly. The marker confers CT contrast and has radioactive properties, meaning it also could find use in brachytherapy. The design of the iodine-125 labeled fiducial tissue marker enables control of dosimetry as well as a choice of iodine isotope used. The marker is anticipated to be clinical applicable due to its contrast performance in mice and its potential for enhanced flexibility in surgical procedures, compared to current methods.

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Organisations: Department of Chemistry, Center for Nuclear Technologies, The Hevesy Laboratory, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Organic Chemistry, University of Copenhagen
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Liposome accumulation in irradiated tumors display important tumor and dose dependent differences
Radiation therapy may affect several important parameters in the tumor microenvironment and thereby influence the accumulation of liposomes by the enhanced permeability and retention (EPR)-effect. Here we investigate the effect of single dose radiation therapy on liposome tumor accumulation by PET/CT imaging using radiolabeled liposomes. Head and neck cancer xenografts (FaDu) and syngenic colorectal (CT26) cancer models were investigated. Radiotherapy displayed opposite effects in the two models. FaDu tumors displayed increased mean accumulation of liposomes for radiation doses up to 10 Gy, whereas CT26 tumors displayed a tendency for decreased accumulation. Tumor hypoxia was found negatively correlated to microregional distribution of liposomes. However, liposome distribution in relation to hypoxia was improved at lower radiation doses. The study reveals that the heterogeneity in liposome tumor accumulation between tumors and different radiation protocols are important factors that need to be taken into consideration to achieve optimal effect of liposome based radio-sensitizer therapy.

General information
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Liquid fiducial marker applicability in proton therapy of locally advanced lung cancer

Background and purpose: We investigated the clinical applicability of a novel liquid fiducial marker (LFM) for image-guided pencil beam scanned (PBS) proton therapy (PBSPT) of locally advanced lung cancer (LALC). Materials and methods: The relative proton stopping power (RSP) of the LFM was calculated and measured. Dose perturbations of the LFM and three solid markers, in a phantom, were measured. PBSPT treatment planning on computer tomography scans of five patients with LALC with the LFM implanted was performed with 1-3 fields. Results: The RSP was experimentally determined to be 1.164 for the LFM. Phantom measurements revealed a maximum relative deviation in dose of 4.8% for the LFM in the spread-out Bragg Peak, compared to 12-67% for the solid markers. Using the experimentally determined RSP, the maximum proton range error introduced by the LFM is about 1. mm. If the marker was displaced at PBSPT, the maximum dosimetric error was limited to 2 percentage points for 3-field plans. Conclusion: The dose perturbations introduced by the LFM were considerably smaller than the solid markers investigated. The RSP of the fiducial marker should be corrected in the treatment planning system to avoid errors. The investigated LFM introduced clinically acceptable dose perturbations for image-guided PBSPT of LALC.
Multicompartment Artificial Organelles Conducting Enzymatic Cascade Reactions inside Cells

Cell organelles are subcellular structures entrapping a set of enzymes to achieve a specific functionality. The incorporation of artificial organelles into cells is a novel medical paradigm which might contribute to the treatment of various cell disorders by replacing malfunctioning organelles. In particular, artificial organelles are expected to be a powerful solution in the context of enzyme replacement therapy since enzymatic malfunction is the primary cause of organelle dysfunction. Although several attempts have been made to encapsulate enzymes within a carrier vehicle, only few intracellularly active artificial organelles have been reported to date and they all consist of single-compartment carriers. However, it is noted that biological organelles consist of multicompartment architectures where enzymatic reactions are executed within distinct subcompartments. Compartmentalization allows for multiple processes to take place in close vicinity and in a parallel manner without the risk of interference or degradation. Here, we report on a subcompartmentalized and intracellularly active carrier, a crucial step for advancing artificial organelles. In particular, we develop and characterize a novel capsosome system, which consists of multiple liposomes and fluorescent gold nanoclusters embedded within a polymer carrier capsule. We subsequently demonstrate that encapsulated enzymes preserve their activity intracellularly, allowing for controlled enzymatic cascade reaction within a host cell.
PET imaging with copper-64 as a tool for real-time in vivo investigations of the necessity for crosslinking of polymeric micelles in nanomedicine: Imaging the influence of polymeric micelle crosslinking

Polymeric micelles in nanomedicine are often crosslinked to prevent disintegration in vivo. This typically requires clinically problematic chemicals or laborious procedures. In addition, crosslinking may interfere with advanced release strategies. Despite this, it is often not investigated whether crosslinking is necessary for efficient drug delivery. We used PET imaging with $^{64}$Cu to demonstrate general methodology for real-time in vivo investigations of micelle stability. Triblock copolymers with 4-methylcoumamin cores of ABC-type (PEG-PHEMA-PCMA) were functionalized in the handle region (PHEMA) with CB-TE2A chelators. Polymeric micelles were formed by dialysis and one half was core-crosslinked by UV light (CL) and the other half was not (nonCL). Both CL and nonCL were radiolabeled with 64 Cu and compared in vivo in tumor-bearing mice, with free $^{64}$Cu as control. Accumulation in relevant organs was quantified by ROI analysis on PET images and ex vivo counting. It was observed that CL and nonCL showed limited differences in biodistribution from each other, whereas both differed markedly from control (free $^{64}$Cu). This demonstrated that 4-methylcoumarin core micelles may form micelles that are stable in circulation even without crosslinking. The methodology presented here where individual unimers are radiolabeled is applicable to a wide range of polymeric micelle types.
Recent advances in compartmentalized synthetic architectures as drug carriers, cell mimics and artificial organelles

Compartmentalization is a key feature of biological cells which conduct their metabolic activity in individual steps isolated in distinct, separated compartments. The creation of architectures containing multiple compartments with a structure that resembles that of a biological cell has generated significant research attention and these assemblies are proposed as candidate materials for a range of biomedical applications. In this Review article, the recent successes of multicompartment architectures as carriers for the delivery of therapeutic cargo or the creation of micro- and nanoreactors that mimic metabolic activities, thus acting as artificial cells or organelles, are discussed. The developed technologies to assemble such complex architectures are outlined, the multicompartment carriers' properties which contribute to their performance in diverse applications are discussed, and their successful applications are highlighted. Finally, future directions and developments in the field are suggested.

General information
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Authors: York-Durán, M. J. (Intern), Gallardo, M. G. (Intern), Labay, C. P. (Intern), Urquhart, A. (Intern), Andresen, T. L. (Intern), Hosta-Rigau, L. (Intern)
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Revisiting the use of sPLA$_2$-sensitive liposomes in cancer therapy

The first developed secretory phospholipase A$_2$ (sPLA$_2$) sensitive liposomal cisplatin formulation (LiPlaCis®) is currently undergoing clinical evaluation. In the present study we revisit and evaluate critical preclinical parameters important for the therapeutic potential and safety of platinum drugs, here oxaliplatin (L-OHP), formulated in sPLA$_2$ sensitive liposomes. We show the mole percentage of negatively charged phospholipid needed to obtain enzyme-sensitivity for saturated systems is $\geq$ 25% for 16-carbon chain lipid membranes, and $> 40\%$ for 18-chain lipid membranes, which was surprising as 25% is used clinically in LiPlaCis®. Efficient sPLA$_2$-dependent growth inhibition of colorectal cancer cells was demonstrated in vitro, where cell membrane degradation and cytolysis depends on the sensitivity of the formulation towards the enzyme.
and is governed by the amount of lysolipids generated and the presence of serum proteins. We found that serum proteins did not affect the lipase activity of the enzyme towards the membranes but instead sequester the lysolipid byproducts consequently inhibiting their detergent-like cytotoxic properties. In vivo therapeutic potential and safety of the liposomes was investigated in nude mice bearing sPLA₂-deficient FaDu squamous carcinoma and sPLA₂-expressing Colo205 colorectal adenocarcinoma. After intravenous injections, the tumor growth was suppressed for liposomal L-OHP relative to free drug, but only a weak response was observed for both slow- and fast-releasing sPLA₂-sensitive formulations compared to non-sensitive liposomes. Also, the mice did not show longer survival. In turn, for the highly sPLA₂-sensitive liposomes, multiple high doses caused petechial cutaneous hemorrhages, along with multifocal hepatonecrotic lesions, suggestive of premature activation in skin and liver irrespective of sPLA₂-status of the tumor engraft. These results indicate that although liposomal carriers can improve the antitumor efficacy of platinum drugs, sPLA₂-triggered release suffers from a narrow therapeutic index and has safety concerns.
Secretory phospholipase A2 responsive liposomes exhibit a potent anti-neoplastic effect in vitro, but induce unforeseen severe toxicity in vivo

The clinical use of liposomal drug delivery vehicles is often hindered by insufficient drug release. Here we present the rational design of liposomes optimized for secretory phospholipase A2 (sPLA2) triggered drug release, and test their utility in vitro and in vivo. We hypothesized that by adjusting the level of cholesterol in anionic, unsaturated liposomes we could tune the enzyme specificity based on membrane fluidity, thus obtaining liposomes with an improved therapeutic outcome and reduced side effects. Cholesterol is generally important as a component in the membranes of liposome drug delivery systems due to its stabilizing effects in vivo. The incorporation of cholesterol in sPLA2 sensitive liposomes has not previously been possible due to reduced sPLA2 activity. However, in the present work we solved this challenge by optimizing membrane fluidity. In vitro release studies revealed enzyme specific drug release. Treatment of two different cancer cell lines with liposomal oxaliplatin revealed efficient growth inhibition compared to that of clinically used stealth liposomes. The in vivo therapeutic effect was evaluated in nude NMRI mice using the sPLA2 secreting mammary carcinoma cell line MT-3. Three days after first treatment all mice having received the novel sPLA2 sensitive lipidome formulation were euthanized due to severe systemic toxicity. Thus the present study demonstrates that great caution should be implemented when utilizing sPLA2 sensitive liposomes and that the real utility can only be disclosed in vivo. The present studies have clinical implications, as sPLA2 sensitive formulations are currently undergoing clinical trials (LiPlaCis®).
Within the field of nanoparticle-assisted photothermal cancer therapy, focus has mostly been on developing novel heat-generating nanoparticles with the right optical and dimensional properties. Comparison and evaluation of their performance in tumor-bearing animals are commonly assessed by changes in tumor volume; however, this is usually a late-occurring event. This study implements 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose positron emission tomography imaging to perform early evaluation of the treatment outcome of photothermal therapy. Silica-gold nanoshells (NS) are administered intravenously to nude mice bearing human neuroendocrine tumor xenografts and the tumors are irradiated by a near-infrared laser. The animals are positron emission tomography scanned with 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose one day before and one day after treatment. Using this setup, a significant decrease in tumor uptake of 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose is found already one day after therapy in the group receiving NS and laser treatment compared to control animals. At this time point no change in tumor volume can be detected. Moreover, the change in tumor uptake, is used to stratify the animals into responders and non-responders, where the responding group matched improved survival. Overall, these findings support the use of 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose positron emission tomography imaging for preclinical and clinical evaluation and optimization of photothermal therapy.

**General information**

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Copenhagen

Authors: Norregaard, K. (Ekstern), Jørgensen, J. T. (Ekstern), Simón, M. (Ekstern), Melander, F. (Intern), Kristensen, L. K. (Ekstern), Bendix, P. M. (Ekstern), Andresen, T. L. (Intern), Oddershede, L. B. (Ekstern), Kjær, A. (Ekstern)

**18F-FDG PET/CT-based early treatment response evaluation of nanoparticle-assisted photothermal cancer therapy**

Within the field of nanoparticle-assisted photothermal cancer therapy, focus has mostly been on developing novel heat-generating nanoparticles with the right optical and dimensional properties. Comparison and evaluation of their performance in tumor-bearing animals are commonly assessed by changes in tumor volume; however, this is usually a late-occurring event. This study implements 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose positron emission tomography imaging to perform early evaluation of the treatment outcome of photothermal therapy. Silica-gold nanoshells (NS) are administered intravenously to nude mice bearing human neuroendocrine tumor xenografts and the tumors are irradiated by a near-infrared laser. The animals are positron emission tomography scanned with 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose one day before and one day after treatment. Using this setup, a significant decrease in tumor uptake of 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose is found already one day after therapy in the group receiving NS and laser treatment compared to control animals. At this time point no change in tumor volume can be detected. Moreover, the change in tumor uptake, is used to stratify the animals into responders and non-responders, where the responding group matched improved survival. Overall, these findings support the use of 2-deoxy-2-\([\text{F-18}]\)fluoro-D-glucose positron emission tomography imaging for preclinical and clinical evaluation and optimization of photothermal therapy.

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Cancer therapy, Drug delivery, Liposome, Oxaliplatin, Secretory phospholipase A(2), Triggered release
Targeting transferrin receptors at the blood-brain barrier improves the uptake of immunoliposomes and subsequent cargo transport into the brain parenchyma

Drug delivery to the brain is hampered by the presence of the blood-brain barrier, which excludes most molecules from freely diffusing into the brain, and tightly regulates the active transport mechanisms that ensure sufficient delivery of...
nutrients to the brain parenchyma. Harnessing the possibility of delivering neuroactive drugs by way of receptors already present on the brain endothelium has been of interest for many years. The transferrin receptor is of special interest since its expression is limited to the endothelium of the brain as opposed to peripheral endothelium. Here, we investigate the possibility of delivering immunoliposomes and their encapsulated cargo to the brain via targeting of the transferrin receptor. We find that transferrin receptor-targeting increases the association between the immunoliposomes and primary endothelial cells in vitro, but that this does not correlate with increased cargo transcytosis. Furthermore, we show that the transferrin receptor-targeted immunoliposomes accumulate along the microvessels of the brains of rats, but find no evidence for transcytosis of the immunoliposome. Conversely, the increased accumulation correlated both with increased cargo uptake in the brain endothelium and subsequent cargo transport into the brain. These findings suggest that transferrin receptor-targeting is a relevant strategy of increasing drug exposure to the brain.

The diffusion dynamics of PEGylated liposomes in the intact vitreous of the ex vivo porcine eye: A fluorescence correlation spectroscopy and biodistribution study
The diffusion dynamics of nanocarriers in the vitreous and the influence of nanocarrier physicochemical properties on these dynamics is an important aspect of the efficacy of intravitreal administered nanomedicines for the treatment of
posterior segment eye diseases. Here we use fluorescence correlation spectroscopy (FCS) to determine liposome diffusion coefficients in the intact vitreous ($D_{\text{Vit}}$) of ex vivo porcine eyes using a modified Miyake-Apple technique to minimize the disruption of the vitreous fine structure. We chose to investigate whether the zeta potential of polyethylene glycol functionalized (i.e. PEGylated) liposomes altered liposome in situ diffusion dynamics in the vitreous. Non-PEGylated cationic nanocarriers have previously shown little to no diffusion in the vitreous, whilst neutral and anionic have shown diffusion. The liposomes investigated had diameters below 150nm and zeta potentials ranging from -20 to +12mV. We observed that PEGylated cationic liposomes had significantly lower $D_{\text{Vit}}$ values ($1.14 \mu\text{m}^2\text{s}^{-1}$) than PEGylated neutral and anionic liposomes ($2.78$ and $2.87 \mu\text{m}^2\text{s}^{-1}$). However, PEGylated cationic liposomes had a similar biodistribution profile across the vitreous to the other systems. These results show that PEGylated cationic liposomes with limited cationic charge can diffuse across the vitreous and indicate that the vitreous as a barrier to nanocarriers ($\Theta< 500$ nm) is more complicated than simply an electrostatic barrier as previously suggested.

**General information**

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Scopus rating (2012): SJR 1.542 SNIP 1.655 CiteScore 4.1
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ISI indexed (2011): ISI indexed yes
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Scopus rating (2007): SJR 1.182 SNIP 1.542
Web of Science (2007): Indexed yes
Transfection of primary brain capillary endothelial cells for protein synthesis and secretion of recombinant erythropoietin: a strategy to enable protein delivery to the brain

Treatment of chronic disorders affecting the central nervous system (CNS) is complicated by the inability of drugs to cross the blood-brain barrier (BBB). Non-viral gene therapy applied to brain capillary endothelial cells (BCECs) denotes a novel approach to overcome the restraints in this passage, as turning BCECs into recombinant protein factories by transfection could result in protein secretion further into the brain. The present study aims to investigate the possibility of transfecting primary rat brain endothelial cells (RBECs) for recombinant protein synthesis and secretion of the neuroprotective protein erythropoietin (EPO). We previously showed that 4% of RBECs with BBB properties can be transfected without disrupting the BBB integrity in vitro, but it can be questioned whether this is sufficient to enable protein secretion at therapeutic levels. The present study examined various transfection vectors, with regard to increasing the transfection efficiency without disrupting the BBB integrity. Lipofectamine 3000™ was the most potent vector compared to polyethylenimine (PEI) and Turbofect. When co-cultured with astrocytes, the genetically modified RBECs secreted recombinant EPO into the cell culture medium both luminally and abluminally, and despite lower levels of EPO reaching the abluminal chamber, the amount of recombinant EPO was sufficient to evolve a biological effect on astrocytes cultured at the abluminal side in terms of upregulated gene expression of brain-derived neurotrophic factor (BDNF). In conclusion, non-viral gene therapy to RBECs leads to protein secretion and signifies a method for therapeutic proteins to target cells inside the CNS otherwise omitted due to the BBB.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Aalborg University, University of Leipzig
Authors: Burkhart, A. (Ekstern), Andresen, T. L. (Intern), Aigner, A. (Ekstern), Thomsen, L. B. (Ekstern), Moos, T. (Ekstern)
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Scopus rating (2015): SJR 0.513 SNIP 1.465 CiteScore 5.68
Acylation of Therapeutic Peptides: Interaction with Lipid Membranes and its Implications in Oral Delivery

Oral administration of therapeutic peptides could benefit millions of chronically ill people worldwide, through easier and less stigmatized therapy, and likely improve the long-term effects of currently widespread disease mismanagement. However, oral peptide delivery is a formidable task due to the harsh and selective gastrointestinal system, and development has lacked far behind injection therapy.

Peptide acylation is a powerful tool to alter the pharmacokinetics, biophysical properties and chemical stability of injectable peptide drugs, primarily used to prolong blood circulation, but it is not widely studied in an oral context. As acylation furthermore increases interactions with the lipid membranes of mammalian cells, it offers several potential benefits for oral delivery of therapeutic peptides, and we hypothesize that tailoring the acylation may be used to optimize intestinal translocation.

This work aims to characterize acylated analogues of two therapeutic peptides by systematically increasing acyl chain length in order to elucidate its influence on membrane interaction and intestinal cell translocation in vitro. The studied peptides are the 33 amino acid Glucagon-like peptide-2 (GLP-2), which promotes intestinal growth and is used to treat bowel disorders such as inflammatory bowel diseases and short bowel syndrome, and the 32 amino acid salmon calcitonin (sCT), which lowers blood calcium and is employed in the treatment of post-menopausal osteoporosis and hypercalcemia. The two peptides are similar in size and structure, but oppositely charged at physiological pH. Both peptides were acylated with linear acyl chains of systematically increasing length, where sCT was furthermore acylated at two different positions on the peptide backbone.

For GLP-2, we found that increasing acyl chain length caused increased self-association and binding to lipid and cell membranes, whereas translocation across intestinal cells displayed a nonlinear dependence on chain length. Short and medium chains improved translocation compared to the native peptide, whereas long chain acylation displayed no improvement in translocation. This indicates an initial translocation benefit for shorter chains through increased interaction
with the cell membrane, which reverts to a hindrance for long chains, i.e. the analogues get stuck in the cell membrane. Co-administration of a paracellular absorption enhancer was found to increase translocation similarly for each analogue while retaining acyl chain length dependence. A transcellular enhancer displayed increased synergy with the long chain acylation, consistent with increased membrane fluidization 'liberating' bound peptide, although medium chain acylation remained optimal overall. The results indicate that rational acylation of GLP-2 can increase its in vitro intestinal absorption, alone or in combination with permeation enhancers, and are consistent with the initial project hypothesis. For sCT, an unpredicted effect of acylation largely superseded the anticipated membrane interactions; i.e. acylated sCT acted as its own in vitro intestinal permeation enhancer. Acylated analogues permeabilized lipid membranes, causing drastically increased peptide permeability through reversible cell effect similar to transcellular permeation enhancers. The effect likely stems from a synergy between the positive peptide charge and membrane-active acyl moiety, supported by its pH-dependency, whereby the effect increased with decreasing pH and concomitant charge increase. The extent of permeation enhancing effect was highly dependent on acylation chain length and position, with highest peptide permeability for short chain N-terminal acylation or medium/long chain Lys18 acylation, whereas permeability and cell membrane binding appeared correlated only for some analogues. However, prolonged heating and/or solution storage of certain acylated sCT analogues caused aggregation in physiological buffer solutions, potentially forming fibril-like structures. Lys18 acylation appeared superior to N-terminal acylation, most clearly exemplified by the short chain analogues, however, no systematic dependence on acylation chain length was apparent. All analogues could be monomerized by addition of cyclodextrin, however, their separate permeability enhancing effects were reduced in the mixtures, and the additive was not investigated further. Thus, acylation of sCT for oral delivery purposes may not be indiscriminately applicable, and requires rational choices of acylation details and/or additives.

Further investigations of the most cell-permeable sCT analogues unveiled quite distinct permeation enhancer effects, ranging from high membrane binding / high permeability and non-specific enhancing effect on model compounds, to very low membrane binding / high permeability and very limited unspecific permeation enhancer effects, i.e. selective and efficient translocation. Peptide receptor potency was retained for GLP-2 analogues following acylation, whereas sCT analogues displayed substantially reduced potency, depending on acylation position and length. Overall, rational acylation of the studied peptides can increase in vitro intestinal permeability, modestly for GLP-2 and drastically for sCT, and might benefit oral delivery. GLP-2 results provide a well-founded predictive power for future peptide analogues, whereas sCT results hold great promise for future analogues, albeit with a larger uncertainty in predictions.

**General information**

State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Novo Nordisk AS
Authors: Trier, S. (Intern), Andresen, T. L. (Intern), Henriksen, J. R. (Intern), Jensen, S. B. (Ekstern)
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Publication: Research › Ph.D. thesis – Annual report year: 2016

**Affinity Induced Surface Functionalization of Liposomes Using Cu-Free Click Chemistry**

Functionalization of nanoparticles is a key element for improving specificity of drug delivery systems toward diseased tissue or cells. In the current study we report a highly efficient and chemoselective method for post-functionalization of liposomes with biomacromolecules, which equally well can be used for functionalization of other nanoparticles or solid surfaces. The method exploits a synergistic effect of having both affinity and covalent anchoring tags on the surface of the liposome. This was achieved by synthesizing a peptide linker system that uses Cu-free strain-promoted click chemistry in combination with histidine affinity tags. The investigation of post-functionalization of PEGylated liposomes was performed with a cyclic RGDFE peptide. By exploring both affinity and covalent tags a 98 ± 2.0% coupling efficiency was achieved, even a diluted system showed a coupling efficiency of 87 ± 0.2%. The reaction kinetics and overall yield were quantified by HPLC. The results presented here open new possibilities for constructing complex nanostructures and functionalized surfaces.

**General information**

State: Published
A microfluidic cell culture device with integrated microelectrodes for barrier studies.

We present an eight cell culture microfluidic device fabricated using thiol-ene ‘click’ chemistry with embedded microelectrodes for evaluating barrier properties of human intestinal epithelial cells. The capability of the microelectrodes for trans-epithelial electrical resistance (TEER) measurements was demonstrated by using confluent human colorectal epithelial cells (Caco-2) and rat fibroblast (CT 26) cells cultured in the microfluidic device.

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Fluidic Array Systems and Technology, University of Copenhagen
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A Mouse Positron Emission Tomography Study of the Biodistribution of Gold Nanoparticles with Different Surface Coatings Using Embedded Copper-64

By taking advantage of the ability of (64)Cu to bind non-specifically to gold surfaces, we have developed a new methodology to embed this radionuclide inside gold nanoparticles (AuNPs). (64)Cu enables the in vivo imaging of AuNPs by positron emission tomography (PET). AuNPs have a multitude of uses within health technology and are useful tools for general nanoparticle research. (64)Cu-AuNPs were prepared by incubating AuNP seeds with (64)Cu(2+), followed by the entrapment of the radionuclide by grafting a second layer of gold on the surface. This resulted in radiolabeling efficiencies of 53 ± 6%. The radiolabel showed excellent stability when challenging with EDTA for two days (>95% radioactivity retention) and showed no loss of (64)Cu when incubated with 50% mouse serum for two days. The methodology was chelator-free, and circumvents traditional concerns over chelator instability and altered AuNP properties due to surface modification. Radiolabeled (64)Cu-AuNP cores were prepared in a biomedically relevant size of 30 nm and used to investigate the in vivo stability of three different AuNP coatings by PET imaging in a murine xenograft tumor model. We found the longest plasma half-life (T½ = 9 hours) and highest tumor accumulation (3.9 %ID/g) by using polyethylene glycol (PEG) coating, while faster elimination from the bloodstream was observed with both a Tween 20-stabilized coating and a zwitterionic coating based on a mixture of sulfonic acids and quaternary amines, which has previously been reported to be superior to PEG. The new embedding method provides the utilization of PET imaging in combination with the multitude of uses that AuNPs have found in health technology, and the method can equally well be utilized for therapeutic copper radioisotopes for use in radiotherapy.

General information
An ATP Binding Cassette Transporter Mediates the Uptake of α-(1,6)-Linked Dietary Oligosaccharides in Bifidobacterium and Correlates with Competitive Growth on These Substrates

The molecular details and impact of oligosaccharide uptake by distinct human gut microbiota (HGM) are currently not well understood. Non-digestible dietary galacto- and gluco-(1,6)-oligosaccharides from legumes and starch, respectively, are preferentially fermented by mainly bifidobacteria and lactobacilli in the human gut. Here we show that the solute binding protein (BiG16BP) associated with an ATP binding cassette (ABC) transporter from the probiotic Bifidobacterium animalis subsp. lactis Bl-04 binds -(1,6)-linked glucosides and galactosides of varying size, linkage, and monosaccharide composition with preference for the trisaccharides raffinose and panose. This preference is also reflected in the -(1,6)-galactoside uptake profile of the bacterium. Structures of BiG16BP in complex with raffinose and panose revealed the basis for the remarkable ligand binding plasticity of BiG16BP, which recognizes the non-reducing -(1,6)-diglycoside in its
ligands. BiG16BP homologues occur predominantly in bifidobacteria and a few Firmicutes but lack in other HGMs. Among seven bifidobacterial taxa, only those possessing this transporter displayed growth on -(1,6)-glycosides. Competition assays revealed that the dominant HGM commensal Bacteroides ovatus was out-competed by B. animalis subsp. lactis BI-04 in mixed cultures growing on raffinose, the preferred ligand for the BiG16BP. By comparison, B. ovatus monocultures grew very efficiently on this trisaccharide. These findings suggest that the ABC-mediated uptake of raffinose provides an important competitive advantage, particularly against dominant Bacteroides that lack glycan-specific ABC-transporters. This novel insight highlights the role of glycan transport in defining the metabolic specialization of gut bacteria.

**General information**

**State:** Published

**Organisations:** Department of Systems Biology, Enzyme and Protein Chemistry, Department of Chemistry, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Lund University, University of Groningen

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Scopus rating (2009): SJR 4.158 SNIP 1.344

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Scopus rating (2008): SJR 4.289 SNIP 1.375

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Binding of human serum albumin to liposomes studied by fluorescence correlation spectroscopy

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Department of Chemistry
Authors: Kristensen, K. (Intern), Urquhart, A. (Intern), Thormann, E. (Intern), Andresen, T. L. (Intern)
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Binding of human serum albumin to liposomes: insights into binding numbers and dynamics by fluorescence correlation spectroscopy

Liposomes for medical applications are often administered by intravenous injection. Once in the bloodstream, the liposomes are covered with a "protein corona", which impacts the behavior and eventual fate of the liposomes. Currently, many aspects of the liposomal protein corona are not well understood. For example, there is generally a lack of knowledge about the liposome binding affinities and dynamics of common types of blood plasma proteins. Fluorescence correlation spectroscopy (FCS) is a powerful experimental technique that potentially can provide such knowledge. In this study, we have used FCS to investigate the binding of human serum albumin (HSA) to standard types of PEGylated fluid-phase liposomes (consisting of DOPC and DOPE-PEG2k) and PEGylated gel-phase liposomes (consisting of DSPC and DSPE-PEG2k) with various PEG chain surface densities. We detected no significant binding of HSA to the PEGylated fluid-phase liposomes. In contrast, we found that HSA bound tightly to the PEGylated gel-phase liposomes, although only a low number of HSA molecules could be accommodated per liposome. Overall, we believe that our data provides a useful benchmark for other researchers interested in studying the liposomal protein corona.
**General information**
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Department of Chemistry
Authors: Kristensen, K. (Intern), Urquhart, A. (Intern), Thormann, E. (Intern), Andresen, T. L. (Intern)
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Scopus rating (2016): CiteScore 7.46 SJR 2.769 SNIP 1.459
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.842 SNIP 1.588 CiteScore 7.97
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.651 SNIP 1.676 CiteScore 7.64
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.55 SNIP 1.469 CiteScore 6.89
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.761 SNIP 1.346 CiteScore 6.08
ISI indexed (2012): ISI indexed yes
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Scopus rating (2011): SJR 2.494 SNIP 1.448 CiteScore 5.69
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Scopus rating (2010): SJR 1.827 SNIP 0.62
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**Contructive delivery of cancer chemotherapeutics using virus inspired liposomes.**

**General information**
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Technical University of Denmark, Harvard Medical School
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Poster_Contractive_delivery_of_cancer_chemo.pdf
Delivery of TLR7 agonist to monocytes and dendritic cells by DCIR targeted liposomes induces robust production of anti-cancer cytokines.

Elucidating the role of free polycations in gene knockdown by siRNA polyplexes

Future improvements of non-viral vectors for siRNA delivery require better understanding of intracellular processing and vector interactions with target cells. Here, we have compared the siRNA delivery properties of a lipid derivative of bPEI 1.8. kDa (DOPE-PEI) with branched polyethyleneimine (bPEI) with average molecular weights of 1.8. kDa (bPEI 1.8. kDa) and 25. kDa (bPEI 25. kDa). We find mechanistic differences between the DOPE-PEI conjugate and bPEI regarding siRNA condensation and intracellular processing. bPEI 1.8. kDa and bPEI 25. kDa have similar properties with respect to condensation capability, but are very different regarding siRNA decondensation, cellular internalization and induction of reporter gene knockdown. bPEI 1.8. kDa and bPEI 25. kDa have similar properties with respect to condensation capability, but are very different regarding siRNA decondensation, cellular internalization and induction of reporter gene knockdown. Lipid conjugation of bPEI 1.8. kDa improves the siRNA delivery properties, but with markedly different formulation requirements and mechanisms of action compared to conventional PEIs. Interestingly, strong knockdown using bPEI 25. kDa is dependent on the presence of a free vector fraction which does not increase siRNA uptake. Finally, we have investigated the effect on lysosomal pH induced by these vectors to elucidate the differences in the proton sponge effect between lipid conjugated PEI and conventional PEI: Neither DOPE-PEI nor bPEI 25. kDa affected lysosomal pH as a function of time, underlining that the possible proton sponge effect is not associated with changes in lysosomal pH. Statement of Significance: Gene silencing therapy has the potential to treat diseases which are beyond the reach of current small molecule-based medicines. However, delivery of the small interfering RNAs (siRNAs) remains a bottleneck to clinical implementation, and the development of safe and efficient delivery systems would be one of the most important achievements in medicine today. A major reason for the lack of progress is insufficient understanding of cell-polyplex interaction. We investigate siRNA delivery using polyethyleneimine (PEI) based vectors and examine how crucial formulation parameters determine the challenges associated with PEI as a delivery vector. We further evaluate how lipid conjugation of PEI influences formulation, cytotoxicity and polymer interaction with cells and cargo as well as the proton sponge capabilities of the vectors.
General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Northeastern University
Authors: Klauber, T. C. B. (Intern), Søndergaard, R. V. (Intern), Sawant, R. R. (Ekstern), Torchilin, V. P. (Ekstern), Andresen, T. L. (Intern)
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Scopus rating (2016): CiteScore 6.66 SJR 1.789 SNIP 1.921
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.997 SNIP 1.99 CiteScore 6.58
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.814 SNIP 2.324 CiteScore 6.53
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 1.963 SNIP 2.269 CiteScore 6.41
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.904 SNIP 2.125 CiteScore 5.51
ISI indexed (2012): ISI indexed yes
Scopus rating (2011): SJR 1.808 SNIP 1.91 CiteScore 5.15
ISI indexed (2011): ISI indexed yes
Scopus rating (2010): SJR 1.794 SNIP 1.964
Web of Science (2010): Indexed yes
Scopus rating (2009): SJR 1.399 SNIP 1.662
Scopus rating (2008): SJR 1.404 SNIP 1.981
Scopus rating (2007): SJR 1.199 SNIP 1.493
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Evaluating liposomal nanoparticles for controlled release of chemotherapeutics in vitro and in vivo

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Copenhagen
Authors: Østrem, R. G. (Intern), Nielsen, O. L. (Ekstern), Hansen, A. E. (Intern), Andresen, T. L. (Intern)
Number of pages: 3
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Gold Nanoparticles with Stably Embedded \(^{64}\text{Cu}\) and Their Use in Nanoparticle Research

\(^{64}\text{Cu}\) is a popular radionuclide for PET imaging and when \(^{64}\text{Cu}^{2+}\) is mixed with gold nanoparticles (AuNPs) it adheres to the gold surface. Taking advantage of this, we developed methods to trap the \(^{64}\text{Cu}\) within the AuNPs by embedding under additional layers of gold. This resulted in radiolabeling efficiencies around 53 ± 6%. EDTA challenge for two days revealed the embedded \(^{64}\text{Cu}\) to possess excellent stability with 94-98% of the radioactivity remaining associated with the AuNPs. Testing for two days against serum likewise showed no loss of \(^{64}\text{Cu}\) from the \(^{64}\text{Cu}\)-AuNPs. Accordingly, the technology was shown to yield a very stable radiolabel that can accurately trace AuNPs in vivo. Such chelator-free radiolabeling removes traditional concerns over the use of chelators for \(^{64}\text{Cu}\), notably instabilities of chelators, such as DOTA, and their ability to alter the surface and thus the biodistribution of the compounds onto which they are attached. Radiolabeled \(^{64}\text{Cu}\)-AuNPs were prepared in biomedically relevant sizes of 20-30 nm and decorated with three different coatings, in order to investigate their in vivo performance by PET imaging in a murine xenograft model. We found the longest plasma half-life (T½ about 9 hours) to result from a polyethylene glycol (PEG) coating, while faster elimination from the bloodstream was observed for both a Tween-20 stabilized coating and a zwitterionic coating based on sulfonic acids and quaternary amines. Accordingly, our data concluded the PEG coating to be most beneficial for long circulation in vivo. In the in vivo model, the \(^{64}\text{Cu}\) was observed to closely follow the AuNPs for each coating, again attributing to the excellent stability of the radiolabel. Further, \(^{64}\text{Cu}\)-AuNPs were prepared in three different sizes ranging from 30 to 70 nm and injected intravenously (I.V.) or intratumorally (I.T.) in murine xenograft models, either coated with PEG or stabilized by citrate (only 30 nm). In the I.T. experiments, citrate-stabilized \(^{64}\text{Cu}\)-AuNPs were retained best in the tumors with about 100 %ID/g after 24 hours. For the PEG-coated \(^{64}\text{Cu}\)-AuNPs, a tendency for increased retention as larger particles were injected was observed (30 nm: ~ 30 %ID/g, 70 nm: ~ 60%ID/g). In the I.V. experiments, the opposite tendency was observed, with smaller particles showing higher tumor accumulation and citrate stabilized \(^{64}\text{Cu}\)-AuNPs being rapidly taken up in liver and spleen. Our group continues work with embedding of radionuclides in solid nanoparticles and further results will be presented as available.

General information
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Organisations: Center for Nuclear Technologies, The Hevesy Laboratory, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Technical University of Denmark, University of Copenhagen
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Publication date: 2016
Conference: Annual Congress of the European Association of Nuclear Medicine, Barcelona, Spain, 15/10/2016 - 15/10/2016
Main Research Area: Technical/natural sciences
Injectable silver nanosensors: in vivo dosimetry for external beam radiotherapy using positron emission tomography

Development of safe and efficient radiotherapy routines requires quantification of the delivered absorbed dose to the cancer tissue in individual patients. In vivo dosimetry can provide accurate information about the absorbed dose delivered during treatment. In the current study, a novel silver-nanosensor formulation based on poly(vinylpyrrolidinone)-coated silver nanoparticles formulated in a gelation matrix composed of sucrose acetate isobutyrate has been developed for use as an in vivo dosimeter for external beam radiotherapy. In situ photonuclear reactions trigger the formation of radioactive (106)Ag, which enables post treatment verification of the delivered dose using positron emission tomography imaging. The silver-nanosensor was investigated in a tissue equivalent thorax phantom using clinical settings and workflow for both standard fractionated radiotherapy (2 Gy) and stereotactic radiotherapy (10- and 22 Gy) in a high-energy beam setting (18 MV). The developed silver-nanosensor provided high radiopacity on the planning CT-scans sufficient for patient positioning in image-guided radiotherapy and provided dosimetric information about the absorbed dose with a 10% and 8% standard deviation for the stereotactic regimens, 10 and 22 Gy, respectively.

General information
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Organisations: Department of Applied Mathematics and Computer Science, Image Analysis & Computer Graphics, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Copenhagen, Copenhagen University Hospital, Technical University of Denmark
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In vitro toxicity of cationic micelles and liposomes in cultured human hepatocyte (HepG2) and lung epithelial (A549) cell lines

The aim of this study was to compare the effects of cationic micelle and liposome drug delivery systems on liver and lung cells in a toxicological in vitro screening model, with observations on cytotoxicity and genotoxicity. A screening battery was established for assessment of a broad range of parameters related to adverse effects. Clear concentration response effects were observed related to impairment of mitochondrial function, membrane integrity and oxidative stress markers, but no effect was observed on genotoxicity. The adverse effects were highest for the liposomes. The High Content Screening seems optimal for initial screening of adverse effects, and combined with standard cytotoxicity measurements initial screening can be performed for predictive toxicological screening.
The objective of this study was to evaluate the potential of PEGylated 64Cu-liposomes in clinical diagnostic positron emission tomography (PET) imaging and PEGylated 177Lu-liposomes in internal tumor radiotherapy through in vivo characterization and dosimetric analysis in a human xenograft mouse model. Liposomes with 5 and 10 mol% PEG were characterized with respect to size, charge, and 64Cu- and 177Lu-loading efficiency. The tumor imaging potential of 64Cu-loaded liposomes was evaluated in terms of in vivo biodistribution, tumor accumulation and tumor-to-muscle (T/M) ratios, using PET imaging. The potential of PEGylated liposomes for diagnostic and therapeutic applications was further evaluated through dosimetry analysis using OLINDA/EXM software. The 64Cu-liposomes were used as biological surrogates to estimate the organ and tumor kinetics of 177Lu-liposomes. High remote loading efficiency (>95%) was obtained for both 64Cu and 177Lu radionuclides with PEGylated liposomes, and essentially no leakage of the encapsulated radionuclides was observed upon storage and after serum incubation for 24 h at 37 °C. The 10 mol% PEG liposomes showed higher tumor accumulation (6.2±0.2 %ID/g) than the 5 mol% PEG liposomes, as evaluated by PET imaging.
The dosimetry analysis of the 64Cu-liposomes estimated an acceptable total effective dose of 3.3∗10−2 mSv/MBq for diagnostic imaging in patients. A high absorbed tumor dose (114 mGy/MBq) was estimated for the potential radiotherapeutic 177Lu-liposomes. The overall preclinical profile of PEGylated 64Cu-liposomes showed high potential as a new PET theranostic tracer for imaging in humans. Dosimetry results predicted that initial administered activity of 200 MBq of 64Cu-liposomes should be acceptable in patients. Work is in progress to validate the utility of PEGylated 64Cu-liposomes in a clinical research program. The high absorbed tumor dose (114 mGy/MBq) estimated for 177Lu-liposomes and the preliminary dosimetric studies justify further therapeutic and dosimetry investigation of 177Lu-liposomes in animals before potential testing in man.

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Department of Chemistry, Center for Nuclear Technologies, The Hevesy Laboratory, University of Copenhagen
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Scopus rating (2012): SJR 2.034 SNIP 1.749 CiteScore 3.98
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Scopus rating (2010): SJR 1.725 SNIP 1.457
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BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.357 SNIP 1.249
Scopus rating (2007): SJR 1.599 SNIP 1.414
Scopus rating (2006): SJR 1.473 SNIP 1.453
Scopus rating (2005): SJR 1.085 SNIP 1.806
Web of Science (2005): Indexed yes
Liposome based radiosensitizer cancer therapy: Potential of enzymesensitive liposomes

Liposome-encapsulated chemotherapeutics have been used in the treatment of a variety of cancers and are feasible for use as mono-therapeutics as well as for combination therapy in conjunction with other modalities. Despite widespread use of liposomal drugs in cancer patient care, insufficient drug bioavailability following accumulation in solid tumors remains one of the major obstacles limiting their clinical efficacy. Long-circulating stimuli-responsive liposomes offer apart from increased tumor accumulation, control over the level of drug exposure from an ability to trigger the release of entrapped biomolecules. By modulating the liposomal membrane, liposomes can become sensitive towards enzymatically-driven destabilization and/or functionalization, thereby allowing control of the release of encapsulated therapeutics within the diseased tissue upon intrinsic stimulation from tumor-associated enzymes. And may thereby improve therapeutic outcome. Two types of enzymes commonly overexpressed in solid cancers and exploited for liposomal drug delivery purposes, are secretory phospholipase A₂ (sPLA₂) and matrix metalloproteinases (MMPs). Furthermore, as platinum-based chemotherapeutic compounds are renowned for their radiosensitizing capacity, tumor-associated enzyme-sensitive liposomal platinum drugs can enhance the effect of radiotherapy (RT) specifically in the tumor tissue. In this thesis, we investigate the utility of enzyme-sensitive liposomal oxaliplatin (L-OHP) to improve inhibition of tumor growth and increase survival in tumor-bearing mice. The safety and efficacy of sPLA₂-sensitive liposomal L-OHP was assessed in sPLA₂-deficient FaDu hypopharyngeal squamous cell carcinoma and sPLA₂-expressing Colo205 colorectal adenocarcinoma. Also, the feasibility of multimodal cancer therapy employing L-OHP encapsulated in MMP-sensitive liposomes with fractionated RT was evaluated in MMP-proficient FaDu cancer xenografts.

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Organisations: Department of Micro- and Nanotechnology
Authors: Pourhassan, H. (Intern), Andresen, T. L. (Intern), Hansen, A. E. (Intern)
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Liquid fiducial marker performance during radiotherapy of locally advanced non small cell lung cancer
We analysed the positional and structural stability of a long-term biodegradable liquid fiducial marker (BioXmark) for radiotherapy in patients with locally advanced lung cancer. Markers were injected via endoscopic- or endobronchial ultrasound in lymph nodes and reachable primary tumours. Marker volume and Hounsfield Units (HU) changing rates were estimated using breath-hold CBCT. Inter-fraction variation in marker position relative to gross tumour volume (GTV) position was established, as well as the inter-fraction variation in mediastinal marker registration relative to a carina registration through the treatment. Fifteen patients were included and 29 markers analysed. All markers that were in situ at planning were visible through the treatment. Mean HU was 902±165HU for lymph node and 991±219HU for tumour markers. Volume degradation rates were -5% in lymph nodes and -23% in primary tumours. Three-dimensional inter-
fraction variation for marker position relative to the GTV position was -0.1±0.7mm in lymph nodes and -1.5±2.3mm in primary tumours. Inter-fraction variations in marker registration relative to carina registration were -0.4±1.2mm in left-right, 0.2±2.0mm in anterior-posterior and -0.5±2.0mm in cranio-caudal directions. The liquid fiducial markers were visible and stable in size and position throughout the treatment course.

General information
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Main Research Area: Technical/natural sciences
Manose 6-Phosphate Receptor Is Reduced In -Synuclein Overexpressing Models of Parkinson's Disease

Increasing evidence points to defects in autophagy as a common denominator in most neurodegenerative conditions. Progressive functional decline in the autophagy-lysosomal pathway (ALP) occurs with age, and the consequent impairment in protein processing capacity has been associated with a higher risk of neurodegeneration. Defects in cathepsin D (CD) processing and α-synuclein degradation causing its accumulation in lysosomes are particularly relevant for the development of Parkinson's disease (PD). However, the mechanism by which alterations in CD maturation and α-synuclein degradation leads to autophagy defects in PD neurons is still uncertain. Here we demonstrate that MPR300 shuttling between endosomes and the trans Golgi network is altered in α-synuclein overexpressing neurons. Consequently, CD is not correctly trafficked to lysosomes and cannot be processed to generate its mature active form, leading to a reduced CD-mediated α-synuclein degradation and α-synuclein accumulation in neurons. MPR300 is downregulated in brain from α-synuclein overexpressing animal models and in PD patients with early diagnosis. These data indicate MPR300 as crucial player in the autophagy-lysosomal dysfunctions reported in PD and pinpoint MRP300 as a potential biomarker for PD.

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Scopus rating (2013): SJR 1.74 SNIP 1.147 CiteScore 3.94
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Matrix metalloprotease-sensitive doxorubicin-loaded liposomes for enhanced anticancer activity.

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Copenhagen
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Multiplexed Dosing Assays by Digitally Definable Hydrogel Volumes
Stable and low-cost multiplexed drug sensitivity assays using small volumes of cells or tissue are in demand for personalized medicine, including patient-specific combination chemotherapy. Spatially defined projected light photopolymerization of hydrogels with embedded active compounds is introduced as a flexible and cost-efficient method for producing multiplexed dosing assays. The high spatial resolution of light projector technology defines multiple compound doses by the volume of individual compound-embedded hydrogel segments. Quantitative dosing of multiple proteins with a dynamic range of 1–2 orders of magnitude is demonstrated using fluorescently labeled albumins. The hydrogel matrix results from photopolymerization of low-cost poly(ethylene glycol) diacrylates (PEGDA), and tuning of the PEGDA composition enables fast complete dosing of all tested species. Dosing of hydrophilic and hydrophobic compounds is demonstrated using two first-line chemotherapy regimens combining oxaliplatin, SN-38, 5-fluorouracil, and folinic acid, with each compound being dosed from a separate light-defined hydrogel segment. Cytotoxicity studies using a colorectal cancer cell line show equivalent effects of dissolved and released compounds. Further control of the dosing process is demonstrated by liposomal encapsulation of oxaliplatin, stable embedding of the liposomes in hydrogels for more than 3 months, and heat-triggered complete release of the loaded oxaliplatin.

General information
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Organisations: Department of Micro- and Nanotechnology, Polymer Microsystems for Cell Processing, Colloids and Biological Interfaces, Amphiphilic Polymers in Biological Sensing
Nanomechanical IR spectroscopy for fast analysis of liquid-dispersed engineered nanomaterials

The proliferated use of engineered nanomaterials (ENMs), e.g. in nanomedicine, calls for novel techniques allowing for fast and sensitive analysis of minute samples. Here we present nanomechanical IR spectroscopy (NAM-IR) for chemical analysis of picograms of ENMs. ENMs are nebulized directly from dispersion and efficiently collected on nanomechanical string resonators through a non-diffusion limited sampling method. Even very small amounts of sample can convert absorbed IR light into a measurable frequency detuning of the string through photothermal heating. An IR absorption spectrum is thus readily obtained by recording this detuning of the resonator over a range of IR wavelengths. Results recorded using NAM-IR agree well with corresponding results obtained through ATR-FTIR, and remarkably, measurement including sample preparation takes only a few minutes, compared to ~2 days sample preparation for ATR-FTIR. Resonator dimensions play an important role in NAM-IR, a relationship which will be elaborated here.
Polymeric pH nanosensor with extended measurement range bearing octaarginine as cell penetrating peptide

A synthetic peptide octaarginine which mimics human immunodeficiency virus-1, Tat protein is used as cell penetrating moiety for new pH nanosensors which demonstrate enhanced cellular uptake and expanded measurement range from pH 3.9 to pH 7.3 by simultaneously incorporating two complemental pH-sensitive fluorophores in a same nanoparticle. The authors believe that this triple fluorescent pH sensor provides a new tool to pH measurements that can have application in cellular uptake mechanism study and new nanomedicine design.

Redox-Sensitive liposomes for glioblastoma treatment.

Redox-Sensitive liposomes for glioblastoma treatment.
Redox-Sensitive liposomes for glioblastoma treatment.

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Lund, M. A. (Intern), Bak, M. (Intern), Kamaly, N. (Intern), Andresen, T. L. (Intern)
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sPLA₂ sensitive fluid phase liposomes induce severe toxicity in murine cancer model

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Copenhagen
Authors: Østrem, R. G. (Intern), Nielsen, O. L. (Ekstern), Hansen, A. E. (Ekstern), Andresen, T. L. (Intern)
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Targeting HER2-positive cancer using multifunctional nanoparticles
Advanced delivery of chemotherapeutics to tumor tissue is an active field of research, as it offers several benefits over conventional cancer therapies. In the three introductory chapters of this thesis, the development of liposomes as drug carriers, including novel strategies to improve delivery efficiency, is thoroughly reviewed.
Chapter 4 encompasses a comprehensive manuscript, which describes the in vitro and in vivo evaluation of a novel liposomal delivery platform designed to target the HER2 receptor on cancer cells and be activated by enzyme activity in the tumor.
In Chapter 5, an alternative HER2-targeted liposome formulation was assessed in vitro. Rather than being enzyme-sensitive, these liposomes were responsive to reducing conditions. Such conditions are found in several cancers due to hypoxia as well as in endocytic compartments.
The progressive in vitro optimization of a complex multifunctional liposomal formulation is reviewed in Chapter 6. This formulation is similar to the one described in Chapter 4, but the lipid composition of the liposomes has been changed to make the formulation sensitive to low pH and prone to engage in advantageous interactions with other lipid membranes. The final study, described in Chapter 7, comprises an in vivo evaluation of the potential benefits of combining enzyme-sensitive liposomal oxaliplatin with the HER2-targeted antibody trastuzumab.
As concluded in the final comments in Chapter 8, the extensive in vitro and in vivo data reported in this thesis demonstrate the potential of using HER2-targeting in combination with advanced drug release mechanisms and present exciting new perspectives for the development of novel delivery platforms.

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Authors: Juul, C. A. (Intern), Andresen, T. L. (Intern), Hansen, A. E. (Intern)
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Targeting HER2-positive cancer using multifunctional nanoparticles

Targeting the DCIR Receptor with a TLR7 Agonist Specifically Activates Monocytes and DCs

The impact of transferrin receptor targeting on immunoliposomal cargo delivery across the blood-brain barrier.

Ultrasound-mediated delivery of novel bio-responsive nanoparticles
Three dimensional (3D) biomaterial microarrays hold enormous promise for regenerative medicine because of their ability to accelerate the design and fabrication of biomimetic materials. Such tissue-like biomaterials can provide an appropriate microenvironment for stimulating and controlling stem cell differentiation into tissue-specific lineages. The use of 3D biomaterial microarrays can, if optimized correctly, result in a more than 1000-fold reduction in biomaterials and cells consumption when engineering optimal materials combinations, which makes these miniaturized systems very attractive for tissue engineering and drug screening applications.
Acetylation of salmon calcitonin modulates in vitro intestinal peptide flux through membrane permeability enhancement

Acetylation of peptide drugs with fatty acid chains has proven beneficial for prolonging systemic circulation, as well as increasing enzymatic stability and interactions with lipid cell membranes. Thus, acetylation offers several potential benefits for oral delivery of therapeutic peptides, and we hypothesize that tailoring the acetylation may be used to optimize intestinal translocation. This work aims to characterize acetylated analogues of the therapeutic peptide salmon calcitonin (sCT), which lowers blood calcium, by systematically increasing acyl chain length at two positions, in order to elucidate its influence on intestinal cell translocation and membrane interaction. We find that acetylation drastically increases in vitro intestinal peptide flux and confers a transient permeability enhancing effect on the cell layer. The analogues permeabilize model lipid membranes, indicating that the effect is due to a solubilization of the cell membrane, similar to transcellular oral permeation enhancers. The effect is dependent on pH, with larger effect at lower pH, and is impacted by acylation chain length and position. Compared to the unacylated peptide backbone, N-terminal acylation with a short chain provides 6- or 9-fold increase in peptide translocation at pH 7.4 and 5.5, respectively. Prolonging the chain length appears to hamper translocation, possibly due to self-association or aggregation, although the long chain acetylated analogues remain superior to the unacylated peptide. For K(18)-acylation a short chain provides a moderate improvement, whereas medium and long chain analogues are highly efficient, with a 12-fold increase in permeability compared to the unacylated peptide backbone, on par with currently employed oral permeation enhancers. For K(19)-acylation the medium chain acylation appears to be optimal, as elongating the chain causes greater binding to the cell membrane but similar permeability, and we speculate that increasing the chain length further may decrease the permeability. In conclusion, acetylated sCT acts as its own in vitro intestinal permeation enhancer, with reversible effects on Caco-2 cells, indicating that acylation of sCT may represent a promising tool to increase intestinal permeability without adding oral permeation enhancers.
吸附于固体表面的阳离子肽

Cationic membrane-active peptides have been studied for years in the hope of developing them into novel types of therapeutics. In this article, we investigate an effect that might have significant experimental implications for investigators who wish to study these peptides, namely, that the peptides adsorb to solid surfaces of glass and plastic. Specifically, we use analytical HPLC to systematically quantify the adsorption of the three cationic membraneactive peptides mastoparan X, melittin, and magainin 2 to the walls of commonly used glass and plastic sample containers. Our results show that, at typical experimental peptide concentrations, 90% or more of the peptides might be lost from solution due to rapid adsorption to the walls of the sample containers. Thus, our results emphasize that investigators should always keep these adsorption effects in mind when designing and interpreting experiments on cationic membrane-active peptides. We conclude the article by discussing different strategies for reducing the experimental impact of these adsorption effects.

General information

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Department of Chemistry, Physical and Biophysical Chemistry
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A hydrogel based nanosensor with an unprecedented broad sensitivity range for pH measurements in cellular compartments

Optical pH nanosensors have been applied for monitoring intracellular pH in real-time for about two decades. However, the pH sensitivity range of most nanosensors is too narrow, and measurements that are on the borderline of this range may not be correct. Furthermore, ratiometric measurements of acidic intracellular pH (pH < 4) in living cells are still challenging due to the lack of suitable nanosensors. In this paper we successfully developed a multiple sensor, a fluorophore based nanosensor, with an unprecedented broad measurement range from pH 1.4 to 7.0. In this nanosensor, three pH-sensitive fluorophores (difluoro-Oregon Green, Oregon Green 488, and fluorescein) and one pH-insensitive fluorophore (Alexa 568) were covalently incorporated into a nanoparticle hydrogel matrix. With this broad range quadruple-labelled nanosensor all physiological relevant pH levels in living cells can be measured without being too close to the limits of its pH-range. The nanosensor exhibits no susceptibility to interference by other intracellular ions at physiological concentrations. Due to its positive surface charge it is spontaneously internalized by HeLa cells and localizes to the lysosomes where the mean pH was measured at 4.6. This quadruple-labelled nanosensor performs accurate measurements of fluctuations of lysosomal pH in both directions, which was shown by treatment with the V-ATPase
inhibitor bafilomycin A1 or its substrate ATP in HeLa cells. These measurements indicate that this novel quadruple-labelled nanosensor is a promising new tool for measuring the pH of acidic compartments in living cells.
Development of microfluidic cell culture devices towards an in vitro human intestinal barrier model

Existing in vitro models of the human intestine such as the established epithelial cell line, Caco-2, cultured on porous membranes have been extensively used for assessing and predicting permeability and absorption of oral drugs in the pharmaceutical industries. However, such in vitro human intestinal models fail to support any form of luminal flow conditions on the cells in order to more closely mimic in vivo conditions. Although these existing systems are easy to use, they require a large amount of cells, culture media, samples and reagents. Microfluidics is a technology that has the potential to revolutionise the way of in vitro cell culture. In particular, microfluidics provides avenues for researchers to tailor the cellular microenvironment to better mimic the cell-cell and cell-extracellular matrix interactions, while at the same time reducing the scale of the experimental studies. Moreover, microfluidics also offers the possibility of dynamic cell culture in microperfusion systems to deliver continuous nutrient supplies for long term cell culture. When combined with electronic or optical components such as sensors, actuators, and control logic, microfluidics has the potential to enable real-time detection of cell responses, adjustment of cellular stimulation etc. leading to establishment of conditional experiments. In this project, microfluidic systems engineering was leveraged to develop an eight chamber multi-layer microchip for intestinal barrier studies. Sandwiched between the layers was a modified Teflon porous membrane for cell culture. The novelty lies in modifying the surface of the porous Teflon support membrane using thiol-ene ‘click’ chemistry, thus allowing the modified Teflon membrane to be bonded between the chip layers to form an enclosed microchips.

Successful application of the multi-layer microchip was demonstrated by integrating the microchip to an existing cell culture fluidic system to culture the human intestinal epithelial cells, Caco-2, for long term studies. Under the continuous low flow conditions, the cells differentiated into columnar cells displaying folds that closely resembled the intestinal villi and formation of a tight barrier. Furthermore, the microelectrodes embedded in the microchip also allow real-time monitoring of the barrier integrity by means of measuring the trans-epithelial electrical resistance. Demonstrations of transport studies using different compounds on the in vitro human intestinal model in the microfluidic device showed comparable results with static cultures. In addition, a normal commensal intestinal bacteria, Escherichia coli (E. coli) was successfully co-cultured on the luminal surface of the cultured epithelium without compromising the epithelial cell viability and barrier function. Such a platform paves the way towards an alternative in vitro intestinal model for high throughput screening of drugs, chemicals, pathogens, intestinal diseases as well as toxicological studies.

Development of nanoparticle based delivery systems for sublingual immunotherapy

The prevalence of IgE mediated allergic diseases is increasing dramatically in industrialized countries. Sublingual immunotherapy (SLIT) has been demonstrated to be a safe and efficacious treatment for IgE mediated allergic diseases, but requires protracted treatment duration. Even though SLIT is considered to have a better safety profile than subcutaneous immunotherapy, SLIT can still cause adverse events requiring clinical supervision for the first administration. Optimization of SLIT, by reducing the administration dose and treatment duration, would improve safety profile. For this purpose, development of nanoparticle delivery systems that can carry antigen across oral mucosa and improve targeting of antigen to the oral immune system would be favourable. This thesis presents seven delivery systems, including liposomes (neutral and cationic) and acylated peptide, which were tested in an ovalbumin (OVA)-induced allergic airway inflammation model for their ability to improve immune tolerance induction of ovalbumin (protein and peptide) when delivered sublingually. In the liposome study, mice were treated sublingually during two weeks with free or liposome encapsulated OVA (OVA-liposomes) followed by intraperitoneal injections and intranasal challenge. Mice treated sublingually with OVA-liposomes showed a significant reduction of airway eosinophilia, OVA-specific IgE antibodies and splenocyte proliferation in comparison to free OVA. In addition, reduced levels of IFN-γ and IL-5 were observed in spleen cell supernatants from OVA-liposome treated mice compared to the sham-treated group. A non-significant reduction of IL-4 and IL-10 in comparison to the sham-treated group was seen. In the study with acylated peptide, mice were SLIT treated with free or acylated OVA323-339 peptide during two weeks followed by intraperitoneal injection. Mice treated with acylated OVA323-339 showed a non-significant tendency of downregulating the proliferation of spleen cells compared to free OVA323-339. The same down-regulating tendency was seen for IFN-γ, IL-4, IL-5 and IL-10.
Facile Large-Scale Synthesis of 5- and 6-Carboxyfluoresceins: Application for the Preparation of New Fluorescent Dyes

A series of fluorescein dyes have been prepared from a common precursor through a very simple synthetic procedure, giving access to important precursors for fluorescent probes. The method has proven an efficient access to regioisomerically pure 5- and 6-carboxyfluoresceins on a large scale, in good yields, and with high regioisomeric purity. Furthermore, we have applied the method to the development of a new type of mixed fluorescein derivatives of 5-carboxyfluorescein. We have demonstrated the scope of the procedure by synthesizing a new type of double chromophore within the fluoro-Jade family.
Facing the Design Challenges of Particle-Based Nanosensors for Metabolite Quantification in Living Cells

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Authors: Søndergaard, R. V. (Intern), Christensen, N. M. (Intern), Henriksen, J. R. (Intern), Ek, P. K. (Intern), Almdal, K. (Intern), Andresen, T. L. (Intern)
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Harnessing Endogenous Systems for Cancer Therapy

In the recent decade, two strategies in particular have attracted attention due to the prospect of significantly improving cancer treatment: Gene silencing therapy and immunotherapy. Both strategies work by manipulating endogenous mechanisms and theoretically promise very strong effect on the diseased cells and minimal effect on the healthy ones. This thesis regards the investigation of important mechanistic aspects of gene silencing mediated by delivery of small interfering RNA (siRNA) using synthetic vectors (Project I) as well as the development of a delivery platform for targeted immunotherapy (Project II).

Transfer into the clinic of therapies based on gene silencing by siRNA delivered by synthetic vectors has yet to happen. A major reason is the lack of efficiency in the delivery process, partly due to insufficient understanding of cellular uptake and processing of the siRNA-containing particles.

Project I aims to provide new mechanistic understanding of intracellular processing and vector interaction with target cells by investigating siRNA delivery using branched polyethylenimine (bPEI), which is a well-known synthetic vector for DNA delivery, and comparing the properties of bPEI with a lipid derivative thereof (DOPE-PEI). We demonstrate mechanistic differences between the bPEI conjugate and conventional bPEI with respect to siRNA condensation and intracellular processing and also show that lipid conjugation of bPEI results in markedly different formulation requirements compared to the conventional PEIs. However, lipid conjugation did not sufficiently reduce the
inherent toxicity associated with high molecular weight PEI, and lipid conjugation of bPEI did also not change the ability of bPEI to affect lysosomal pH as a function of time. In contrast to gene silencing therapy, cancer immunotherapy is starting to produce positive results in the clinic. A major target in cancer immunotherapy is the immunosuppressive tumor microenvironment generated directly or indirectly by the tumor. Tumor tissues have been shown to be heavily infiltrated by macrophages and DCs but due to the immunosuppressive environment they frequently adopt an inactive or tumor-promoting phenotype. Project II describes the development of a platform which enables the highly specific targeting of monocytes and DCs in the bloodstream. Using this platform to deliver a TLR7 agonist, we were able to demonstrate activation of the targeted cells and increased potency of the agonist. While the described platform targets selected immune cells in the blood and not in itself targets the tumor tissue, we believe that because tumor associated inflammation has been shown to recruit monocytes and DCs to the tumor tissues, our strategy could be an elegant and efficient way of providing activated monocytes, monocyte-derived macrophages, and DCs to the tumor site. If the duration of cytokine secretory activity extends post-extravasation, this will not only provide activated innate immune cells to the tumor site, but may also contribute to the re-polarization of the tumor microenvironment thereby promoting antitumor immunity.

Impedimetric Toxicity Assay Evaluating Free and Liposome-loaded Anticancer drugs

In this work, we have developed a microfluidic cytotoxicity assay for a cell culture and detection platform, which enables both fluid handling and electrochemical/optical detection. The cytotoxic effect of anticancer drugs doxorubicin (DOX), oxalipatin (OX) as well as OX-loaded liposomes, developed for targeted drug delivery, was evaluated using real-time impedance monitoring. The time-dependent effect of DOX on HeLa cells was monitored and found to have a delayed onset of cytotoxicity in microfluidics compared with static culture conditions based on data obtained in our previous study. The result of a fluorescent microscopic annexin V/propidium iodide assay, performed in microfluidics, confirmed the outcome of the real-time impedance assay. In addition, the response of HeLa cells to OX-induced cytotoxicity proved to be slower than toxicity induced by DOX. A difference in the time-dependent cytotoxic response of fibrosarcoma cells (HT1080) to free OX and OX-loaded liposomes was observed and attributed to incomplete degradation of the liposomes, which results in lower drug availability. The matrix metalloproteinase (MMP)-dependent release of OX from OX-loaded liposomes was also confirmed using laryngopharynx carcinoma cells (FaDu). The comparison and the observed differences between the cytotoxic effects under microfluidic and static conditions highlight the importance of comparative
studies as basis for implementation of microfluidic cytotoxic assays.

**General information**

State: Published

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ISI indexed (2013): ISI indexed yes

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BFI (2010): BFI-level 2

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Injectable Colloidal Gold for Use in Intrafractional 2D Image-Guided Radiation Therapy

In the western world, approximately 50% of all cancer patients receive radiotherapy alone or in combination with surgery or chemotherapy. Image-guided radiotherapy (IGRT) has in recent years been introduced to enhance precision of the delivery of radiation dose to tumor tissue. Fiducial markers are often inserted inside the tumor to improve IGRT precision and to enable monitoring of the tumor position during radiation therapy. In the present article, a liquid fiducial tissue marker is presented, which can be injected into tumor tissue using thin and flexible needles. The liquid fiducial has high radio-opacity, which allows for marker-based image guidance in 2D and 3D X-ray imaging during radiation therapy. This is achieved by surface-engineering gold nanoparticles to be highly compatible with a carbohydrate-based gelation matrix. The new fiducial marker is investigated in mice where they are highly biocompatible and stable after implantation. To investigate the clinical potential, a study is conducted in a canine cancer patient with spontaneous developed solid tumor in which the marker is successfully injected and used to align and image-guide radiation treatment of the canine patient. It is concluded that the new fiducial marker has highly interesting properties that warrant investigations in cancer patients.

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Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
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Interdependence of initial cell density, drug concentration and exposure time revealed by real-time impedance spectroscopic cytotoxicity assay

We investigated the combined effect of the initial cell density (12,500, 35,000, 75,000, and 100,000 cells cm⁻²) and concentration of the anti-cancer drug doxorubicin on HeLa cells by performing time-dependent cytotoxicity assays using real-time electrochemical impedance spectroscopy. A correlation between the rate of cell death and the initial cell seeding density was found at 2.5 μM doxorubicin concentration, whereas this was not observed at 5 or 100 μM. By sensing the changes in the cell-substrate interaction using impedance spectroscopy under static conditions, the onset of cytotoxicity was observed 5 h earlier than when using a standard colorimetric end-point assay (MTS) which measures changes in the mitochondrial metabolism. Furthermore, with the MTS assay no cytotoxicity was observed after 15 h of incubation with 2.5 μM doxorubicin, whereas the impedance showed at this time point cell viability that was below 25%. These results indicate that impedance detection reveals cytotoxic events undetectable when using the MTS assay, highlighting the importance of combining impedance detection with traditional drug toxicity assays towards a more in depth understanding of the effect of anti-cancer drugs on in vitro assays. Moreover, the detection of doxorubicin induced toxicity determined with impedance under static conditions proved to be 6 times faster than in perfusion culture.
Investigation of enzyme-sensitive lipid nanoparticles for delivery of siRNA to blood–brain barrier and glioma cells

Clinical applications of siRNA for treating disorders in the central nervous system require development of systemic stable, safe, and effective delivery vehicles that are able to cross the impermeable blood–brain barrier (BBB). Engineering nanocarriers with low cellular interaction during systemic circulation, but with high uptake in targeted cells, is a great challenge and is further complicated by the BBB. As a first step in obtaining such a delivery system, this study aims at designing a lipid nanoparticle (LNP) able to efficiently encapsulate siRNA by a combination of titratable cationic lipids. The targeted delivery is obtained through the design of a two-stage system where the first step is conjugation of angiopep to the surface of the LNP for targeting the low-density lipoprotein receptor-related protein-1 expressed on the BBB. Second, the positively charged LNPs are masked with a negatively charged PEGylated (poly(ethylene glycol)) cleavable lipopeptide, which contains a recognition sequence for matrix metalloproteinases (MMPs), a class of enzymes often expressed in the tumor microenvironment and inflammatory BBB conditions. Proteolytic cleavage induces PEG release, including the release of four glutamic acid residues, providing a charge switch that triggers a shift of the LNP charge from weakly negative to positive, thus favoring cellular endocytosis and release of siRNA for high silencing efficiency. This work describes the development of this two-stage nanocarrier-system and evaluates the performance in brain endothelial and glioblastoma cells with respect to uptake and gene silencing efficiency. The ability of activation by MMP-triggered dePEGylation and charge shift is demonstrated to substantially increase the uptake and the silencing efficiency of the LNPs.

General information
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BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.039 SNIP 1.589 CiteScore 3.85
In vivo toxicity of cationic micelles and liposomes

This study investigated toxicity of nanocarriers comprised of cationic polymer and lipid components often used in gene and drug delivery, formulated as cationic micelles and liposomes. Rats were injected intravenously with 10, 25 or 100 mg/kg and sacrificed after 24 or 48 h, or 24 h after the last of three intravenous injections of 100 mg/kg every other day. Histological evaluation of liver, lung and spleen, clinical chemistry parameters, and hematology indicated little effect of treatment. DNA strand breaks were increased in the lung and spleen. Further, in the dose response study we found unaltered expression levels of genes in the antioxidant response (HMOX1) and repair of oxidized nucleobases (OGG1), whereas expression levels of cytokines (IL6, CXCL2 and CCL2) were elevated in lung, spleen or liver. The results indicate that assessment of genotoxicity and gene expression add information on toxicity of nanocarriers, which is not obtained by histology and hematology.
Monocyte targeting and activation by cationic liposomes formulated with a TLR7 agonist

Objectives: Monocytes are one of the major phagocytic cells that patrol for invading pathogens, and upon activation, differentiate into macrophages or antigen-presenting dendritic cells (DCs) capable of migrating to lymph nodes eliciting an adaptive immune response. The key role in regulating adaptive immune responses has drawn attention to modulate monocyte responses therapeutically within cancer, inflammation and infectious diseases. We present a technology for targeting of monocytes and delivery of a toll-like receptor (TLR) agonist in fresh blood using liposomes with a positively charged surface chemistry.

Methods: Liposomes were extruded at 100 nm, incubated with fresh blood, followed by leukocyte analyses by FACS. Liposomes with and without the TLR7 agonist TMX-202 were incubated with fresh blood, and monocyte activation measured by cytokine secretion by ELISA and CD14 and DC-SIGN expression.

Results: The liposomes target monocytes specifically over lymphocytes and granulocytes in human whole blood, and show association with 75 - 95% of the monocytes after 1 h incubation. Formulations of TMX-202 in cationic liposomes were potent in targeting and activation of monocytes, with strong induction of IL-6 and IL-12p40, and differentiation into CD14+ and DC-SIGN+ DCs.

Conclusion: Our present liposomes selectively target monocytes in fresh blood, enabling delivery of TLR7 agonists to the intracellular TLR7 receptor, with subsequent monocyte activation and boost in secretion of proinflammatory cytokines. We envision this technology as a promising tool in future cancer immunotherapy.
On-line monitoring of 2D and 3D cell cultures: electrode configurations for impedance based sensors

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Positron Emission Tomography Based Elucidation of the Enhanced Permeability and Retention Effect in Dogs with Cancer Using Copper-64 Liposomes
Since the first report of the enhanced permeability and retention (EPR) effect, the research in nanocarrier based antitumor drugs has been intense. The field has been devoted to treatment of cancer by exploiting EPR-based accumulation of nanocarriers in solid tumors, which for many years was considered to be a ubiquitous phenomenon. However, the understanding of differences in the EPR-effect between tumor types, heterogeneities within each patient group, and
dependency on tumor development stage in humans is sparse. It is therefore important to enhance our understanding of
the EPR-effect in large animals and humans with spontaneously developed cancer. In the present paper, we describe a
novel loading method of copper-64 into PEGylated liposomes and use these liposomes to evaluate the EPR-effect in 11
canine cancer patients with spontaneous solid tumors by PET/CT imaging. We thereby provide the first high-resolution
analysis of EPR-based tumor accumulation in large animals. We find that the EPR-effect is strong in some tumor types but
cannot be considered a general feature of solid malignant tumors since we observed a high degree of accumulation
heterogeneity between tumors. Six of seven included carcinomas displayed high uptake levels of liposomes, whereas one
of four sarcomas displayed signs of liposome retention. We conclude that nanocarrier-radiotracers could be important in
identifying cancer patients that will benefit from nanocarrier-based therapeutics in clinical practice.

General information
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(Intern), Elema, D. R. (Intern), Rosenschöld, P. M. A. (Ekstern), Kristensen, A. T. (Ekstern), Kjær, A. (Ekstern), Andresen,
T. L. (Intern)
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 5.923 SNIP 2.723 CiteScore 12.49
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ISI indexed (2011): ISI indexed yes
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Scopus rating (2010): SJR 5.313 SNIP 2.065
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Quantification and comparison of visibility and image artifacts of a new liquid fiducial marker in a lung phantom for image-guided radiation therapy

**Purpose:** A new biodegradable liquid fiducial marker was devised to allow for easy insertion in lung tumors using thin needles. The purpose of this study was to evaluate the visibility of the liquid fiducial markers for image-guided radiation therapy and compare to existing solid fiducial markers and to one existing liquid fiducial marker currently commercially available.

**Methods:** Fiducial marker visibility was quantified in terms of contrast to noise ratio (CNR) on planar kilovoltage x-ray images in a thorax phantom for different concentrations of the radio-opaque component of the new liquid fiducial marker, four solid fiducial markers, and one existing liquid fiducial marker. Additionally, the image artifacts produced on computer tomography (CT) and cone-beam CT (CBCT) of all fiducial markers were quantified.

**Results:** The authors found that the new liquid fiducial marker with the highest concentration of the radio-opaque component had a CNR > 2.05 for 62/63 exposures, which compared favorably to the existing solid fiducial markers and to the existing liquid fiducial marker evaluated. On CT and CBCT, the new liquid fiducial marker with the highest concentration produced lower streaking index artifact (30 and 14, respectively) than the solid gold markers (113 and 20, respectively) and the existing liquid fiducial marker (39 and 20, respectively). The size of the image artifact was larger for all of the liquid fiducial markers compared to the solid fiducial markers because of their larger physical size. **Conclusions:** The visibility and the image artifacts produced by the new liquid fiducial markers were comparable to existing solid fiducial markers and the existing liquid fiducial marker. The authors conclude that the new liquid fiducial marker represents an alternative to the fiducial markers tested.

**General information**

State: Published

Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Rigshospitalet

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BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.523 SNIP 1.631 CiteScore 2.79
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Scopus rating (2013): SJR 1.766 SNIP 1.767 CiteScore 3.17
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Scopus rating (2012): SJR 1.42 SNIP 1.669 CiteScore 3.08
Remote Loading of $^{64}$Cu$^{2+}$ into Liposomes without the Use of Ion Transport Enhancers

Due to low ion permeability of lipid bilayers, it has been and still is common practice to use transporter molecules such as ionophores or lipophilic chelators to increase transmembrane diffusion rates and loading efficiencies of radionuclides into liposomes. Here, we report a novel and very simple method for loading the positron emitter $^{64}$Cu$^{2+}$ into liposomes, which is important for in vivo positron emission tomography (PET) imaging. By this approach, copper is added to liposomes entrapping a chelator, which causes spontaneous diffusion of copper across the lipid bilayer where it is trapped. Using this method, we achieve highly efficient $^{64}$Cu$^{2+}$ loading (>95%), high radionuclide retention (>95%), and favorable loading kinetics, excluding the use of transporter molecule additives. Therefore, clinically relevant activities of 200-400 MBq/patient can be loaded fast (60-75 min) and efficiently into preformed stealth liposomes avoiding subsequent purification steps. We investigate the molecular coordination of entrapped copper using X-ray absorption spectroscopy and demonstrate high adaptability of the loading method to pegylated, nonpegylated, gel- or fluid-like, cholesterol rich or cholesterol depleted, cationic, anionic, and zwitterionic lipid compositions. We demonstrate high in vivo stability of $^{64}$Cu-liposomes in a large canine model observing a blood circulation half-life of 24 h and show a tumor accumulation of 6% ID/g in FaDu xenograft mice using PET imaging. With this work, it is demonstrated that copper ions are capable of crossing a lipid membrane unassisted. This method is highly valuable for characterizing the in vivo performance of liposome-based nanomedicine with great potential in diagnostic imaging applications.

General information

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Organisations: Department of Chemistry, Center for Nanomedicine and Theranostics, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, X-ray Crystallography, Center for Nuclear Technologies, The Hevesy Laboratory, University of Copenhagen
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Single-vesicle detection and analysis of peptide-induced membrane permeabilization

The capability of membrane-active peptides to disrupt phospholipid membranes is often studied by investigating peptide-induced leakage of quenched fluorescent molecules from large unilamellar lipid vesicles. In this article, we explore two fluorescence microscopy-based single-vesicle detection methods as alternatives to the quenching-based assays for studying peptide-induced leakage from large unilamellar lipid vesicles. Specifically, we use fluorescence correlation spectroscopy (FCS) to study the leakage of fluorescent molecules of different sizes from large unilamellar lipid vesicles dispersed in aqueous solution, and we use confocal imaging of surface-immobilized large unilamellar lipid vesicles to investigate whether there are heterogeneities in leakage between individual vesicles. Of importance, we design an experimental protocol that allows us to quantitatively correlate the results of the two methods; accordingly, it can be assumed that the two methods provide complementary information about the same leakage process. We use the two methods to investigate the membrane-permeabilizing activities of three well-studied cationic membrane-active peptides: mastoparan X, melittin, and magainin 2. The FCS results show that leakage induced by magainin 2 is less dependent on the size of the encapsulated fluorescent molecules than leakage induced by mastoparan X and melittin. The confocal imaging results show that all three peptides induce leakage by a heterogeneous process in which one portion of the
vesicles are completely emptied of their contents but another portion of the vesicles are only partially emptied. These pieces of information regarding leakage induced by mastoparan X, melittin, and magainin 2 could not readily have been obtained by the established assays for studying peptide-induced leakage from lipid vesicles.

**General information**

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Department of Chemical and Biochemical Engineering, CAPEC-PROCESS, Department of Chemistry, Physical and Biophysical Chemistry
Authors: Kristensen, K. (Intern), Ehrlich, N. (Intern), Henriksen, J. R. (Intern), Andresen, T. L. (Intern)
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Scopus rating (2015): SJR 1.686 SNIP 1.308 CiteScore 4.33
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.816 SNIP 1.391 CiteScore 4.59
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.895 SNIP 1.356 CiteScore 4.55
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.177 SNIP 1.382 CiteScore 4.37
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.051 SNIP 1.357 CiteScore 4.42
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 2.148 SNIP 1.4
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 2.156 SNIP 1.351
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.383 SNIP 1.34
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.449 SNIP 1.434
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.375 SNIP 1.428
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Synthesis of Cross-Linked Polymeric Micelle pH Nanosensors: An Investigation of Design Flexibility

The design flexibility that polymeric micelles offer in the fabrication of optical nanosensors for ratiometric pH measurements is investigated. pH nanosensors based on polymeric micelles are synthesized either by a mixed-micellization approach or by a postmicelle modification strategy. In the mixed-micellization approach, self-assembly of functionalized unimers followed by shell cross-linking by copper-catalyzed azide-alkyne cycloaddition (CuAAC) results in stabilized cRGD-functionalized micelle pH nanosensors. In the postmicelle modification strategy, simultaneous cross-linking and fluorophore conjugation at the micelle shell using CuAAC results in a stabilized micelle pH nanosensor. Compared to the postmicelle modification strategy, the mixed-micellization approach increases the control of the overall composition of the nanosensors. Both approaches provide stable nanosensors with similar pKₐ profiles and thereby nanosensors with similar pH sensitivity.

General information
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Authors: Ek, P. K. (Intern), Jølck, R. I. (Intern), Andresen, T. L. (Intern)
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.807 SNIP 1.136 CiteScore 4.72
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Scopus rating (2013): SJR 1.762 SNIP 1.137 CiteScore 4.78
ISI indexed (2013): ISI indexed yes
Acylation of Glucagon-like peptide-2: Interaction with lipid membranes and in vitro intestinal permeability

These results show that membrane interactions play a prominent role during intestinal translocation of an acylated peptide. Acylation benefits permeation for shorter and medium chains due to increased membrane interactions, however, for longer chains insertion in the membrane becomes dominant and hinders translocation, i.e. the peptides get ‘stuck’ in the cell membrane. Applying a transcellular absorption enhancer increases the dynamics of membrane insertion and detachment by fluidizing the membrane, thus facilitating its effects primarily on membrane associated peptides.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Novo Nordisk A/S
Authors: Trier, S. (Intern), Linderoth, L. (Ekstern), Bjerregaard, S. (Ekstern), Andresen, T. L. (Intern), Rahbek, U. L. (Ekstern)
Number of pages: 10
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BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
Biodistribution of rhodamine B fluorescence-labeled cationic nanoparticles in rats

We investigated the biodistribution following the administration of nanosized (about 50 and 90 nm) cationic (ζ: +30 and +50 mV) micelles and liposomes intended for drug delivery. The particles were stable and well characterized with respect to size and ζ potential. Ten 5- to 6-week-old male rats were used. The animals were randomly allocated to five groups receiving either cationic micelles or cationic liposomes by single intravenous (IV) administration at a dose of 100 mg/kg bodyweight by single intracerebroventricular (ICV) injection at a dose of 50 μg or no treatment. ICV administration was used to study local distribution in the brain and IV administration to study the systemic distribution of the particles. For both types of particles, ICV administration showed distribution in all ventricles in the brain while IV delivery displayed distribution to the major organs liver, spleen, kidney and lung, but not to the brain. Our data suggest that cationic micelles and liposomes are widely distributed in the body, indicating that these could potentially be used as drug delivery carriers to the major organs, but they do not cross the blood–brain barrier to a significant extent, without a targeting ligand attached. However, they are able to persist in the ventricles of the brain up to 24 h after ICV administration, demonstrating a new ability.
Cross-linked self-assembled micelle based nanosensor for intracellular pH measurements

A micelle based nanosensor was synthesized and investigated as a ratiometric pH sensor for use in measurements in living cells by fluorescent microscopy. The nanosensor synthesis was based on self-assembly of an amphiphilic triblock copolymer, which was chemically cross-linked after micelle formation. The copolymer, poly(ethylene glycol)-b-poly(2-aminoethyl methacrylate)-b-poly(styrene) (PEG-b-PAEMA-b-PS), was synthesized by isolated macroinitiator atom transfer radical polymerization that forms micelles spontaneously in water. The PAEMA shell of the micelle was hereafter cross-linked by an amidation reaction using 3,6,9-trioxaundecandioic acid cross-linker. The cross-linked micelle was functionalized with two pH sensitive fluorophores and one reference fluorophore, which resulted in a highly uniform ratiometric pH nanosensor with a diameter of 29 nm. The use of two sensor fluorophores provided a sensor with a very broad measurement range that seems to be influenced by the chemical design of the sensor. Cell experiments show that the sensor is capable of monitoring the pH distributions in HeLa cells.

Design, calibration and application of broad-range optical nanosensors for determining intracellular pH.

Particle-based nanosensors offer a tool for determining the pH in the endosomal-lysosomal system of living cells. Measurements providing absolute values of pH have so far been restricted by the limited sensitivity range of nanosensors, calibration challenges and the complexity of image analysis. This protocol describes the design and application of a polyacrylamide-based nanosensor (~60 nm) that covalently incorporates two pH-sensitive fluorophores, fluorescein (FS) and Oregon Green (OG), to broaden the sensitivity range of the sensor (pH 3.1-7.0), and uses the pH-insensitive fluorophore rhodamine as a reference fluorophore. The nanosensors are spontaneously taken up via endocytosis and directed to the lysosomes where dynamic changes in pH can be measured with live-cell confocal microscopy. The most important focus areas of the protocol are the choice of pH-sensitive fluorophores, the design of calibration buffers, the
determination of the effective range and especially the description of how to critically evaluate results. The entire procedure typically takes 2-3 weeks.
Differential toxicological response to positively and negatively charged nanoparticles in the rat brain

We investigated the potential for systemic and local toxicity after administration of empty nanosized anionic and cationic PEGylated-micelles and non-PEGylated liposomes, without a ligand attached, intended for use in drug-delivery systems. The particles were administered to 5–6-week-old male rats by three intravenous (IV) administrations over a period of one week at a dose of 100 mg/kg bodyweight or after a single intracerebroventricular (ICV) injection at a dose of 50 µg. The particles were stable and well characterised with respect to size and zeta potential. ICV administration of cationic particles was associated with histological changes near the injection site (hippocampus). Here, we detected focal infiltration with phagocytic cells, loss of neurons and apoptotic cell death, which were not observed after administration of the vehicle. No significant difference was found after IV or ICV administration of the anionic micelles with regard to haematology, clinical chemistry parameters or at the pathological examinations, as compared to control animals. Our study suggests that ICV delivery of cationic particles to the brain tissue is associated with toxicity at the injection site.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, National Food Institute, H. Lundbeck A/S, DHI Denmark, University of Copenhagen
Authors: Knudsen, K. (Ekstern), Northeved, H. (Ekstern), Ek, P. K. (Intern), Permin, A. (Intern), Andresen, T. L. (Intern), Larsen, S. (Ekstern), Wegener, K. M. (Ekstern), Lam, H. R. (Intern), Lykkesfeldt, J. (Forskerdatabase)
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  Web of Science (2016): Indexed yes
  BFI (2015): BFI-level 2
  Scopus rating (2015): CiteScore 7.14
  Web of Science (2015): Indexed yes
  BFI (2014): BFI-level 2
  Scopus rating (2014): CiteScore 5.92
  Web of Science (2014): Indexed yes
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  ISI indexed (2012): ISI indexed yes
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  BFI (2011): BFI-level 1
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DOIs:
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Source: dtu
Source-ID: n::oai:DTIC-ART:bl/427641608::38149

Polyethylene glycol (PEG)-based hydrogels are widely used for biomedical applications, including matrices for controlled drug release. We present a method for defining drug dosing in screening assays by light-activated cross-linking of PEG-diacylate hydrogels with embedded drug-loaded liposome nanoparticles in freely definable areas of micro wells.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Polymer Microsystems for Cell Processing, Colloids and Biological Interfaces, Amphiphilic Polymers in Biological Sensing
Authors: Faralli, A. (Intern), Melander, F. (Intern), Larsen, E. K. U. (Intern), Andresen, T. L. (Intern), Larsen, N. B. (Intern)
Number of pages: 1
Publication date: 2014

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Main Research Area: Technical/natural sciences
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Publication: Research - peer-review › Conference abstract in proceedings – Annual report year: 2014

Effective Nanoparticle-based Gene Delivery by a Protease Triggered Charge Switch

Gene carriers made from synthetic materials are of interest in relation to gene therapy but suffer from lack of transfection efficiency upon systemic delivery. To address this problem, a novel lipo-peptide-PEG conjugate constituted by a lipid-anchor, a peptide sensitive to proteases and a poly (ethylene glycol) (PEG) chain is investigated. Utilizing ethanol-mediated nucleic acid encapsulation to prepare lipo-nanoparticles (LNPs), LNPs that are stable in serum are obtained.

The LNPs constitute a highly effective gene delivery systems in vitro and possess the right features for further investigation in vivo including a PEG layer and a net negative charge that should ensure long-circulating properties before being activated by proteases in diseased tissue. Protease activation leads to detachment of PEG and a charge switching where the LNPs become positive due to the presence of glutamates in the cleaved peptide moiety. The cationic lipid DOTAP is used mainly to complex DNA and proton titratable DODAP is used to increase endosomal escape and enhance transfection efficiency. The idea of using a mixture of permanently charged and titratable cationic lipids shielded by a protease sensitive negatively charged lipo-peptide-PEG coat appears to be a highly efficient solution for achieving effective non-viral gene delivery and the results warrant further investigations.

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics
Authors: Gjetting, T. (Intern), Jelck, R. I. (Intern), Andresen, T. L. (Intern)
Number of pages: 12
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Scopus rating (2016): CiteScore 5.26 SJR 1.906 SNIP 1.108
Injectable Colloidal Gold in a Sucrose Acetate Isobutyrate Gelating Matrix with Potential Use in Radiation Therapy

External beam radiation therapy relies on the ability to deliver high radiation doses to tumor cells with minimal exposure to surrounding healthy tissue. Advanced irradiation techniques, including image-guided radiation therapy (IGRT), rely on the ability to locate tumors to optimize the therapeutic benefit of these techniques. Today, radiopaque fiducial tissue markers are placed in or around tumors, for example, in prostate cancer patients to enhance the precision of daily and/or real-time IGRT. A liquid injectable fiducial marker (nanogel) is developed based on PEGylated gold nanoparticles and sucrose acetate isobutyrate (SAIB) with improved properties compared to current solid fiducial markers. The developed nanogel is investigated in vitro and subsequently evaluated in vivo in immunocompetent NMRI mice. The nanogel shows high CT-contrast and excellent stability in vivo over a period of 12 weeks. The nanogel is found to be biocompatible and well tolerated. No induction of the inflammatory cytokines INF-γ, IL-6, or TNF-α is observed throughout the study period. The developed nanogel seems to be a safe injectable fiducial marker ideally suited for IGRT that may further enhance the effect of radiation.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Center for Nanomedicine and Theranostics, Colloids and Biological Interfaces, University of Copenhagen
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Pages: 1680-1687
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Main Research Area: Technical/natural sciences
Nanomechanical IR Spectroscopy for the fast analysis of picogram samples of engineered nanomaterials

The proliferation of engineered nanomaterials (ENMs), e.g. in nanomedicine, demands for novel sensitive techniques allowing for the analysis of minute samples. We present nanoelectromechanical system-based IR spectroscopy (NEMS-IR) of picograms of polymeric micelles. The micelles are nebulized with electrospray directly from dispersion and then efficiently collected on the sensor, which detects the IR-wavelength-dependent photothermal sample heating. Only 10 nL of sample (~0.1 mg/mL) is required for the acquisition of an IR spectrum. Measurement, including sample preparation, takes only a few minutes, compared to 2 days for analysis by ATR-FT-IR. NEMS-IR constitutes a promising technique for the fast analysis of ENMs.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Nanoprobes, Colloids and Biological Interfaces
Authors: Andersen, A. J. (Intern), Ek, P. K. (Intern), Andresen, T. L. (Intern), Yamada, S. (Intern), Boisen, A. (Intern), Schmid, S. (Intern)
Number of pages: 2
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NEMS-IR, Engineereed nanoparticles, Nanomedicine, Infrared spectroscopy
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Positron Emission Tomography Based Analysis of Long-Circulating Cross-Linked Triblock Polymeric Micelles in a U87MG Mouse Xenograft Model and Comparison of DOTA and CB-TE2A as Chelators of Copper-64

Copolymers of ABC-type (PEG-PHEMA-PCMA) architecture were prepared by atom transfer radical polymerization and formulated as micelles with functionalizable primary alcohols in the shell-region (PHEMA-block) to which the metal-ion chelators DOTA or CB-TE2A were conjugated. Using this micelle system we compared the in vivo stabilities of DOTA and CB-TE2A as chelators of 64Cu in micelle nanoparticles. The coumarin polymer (PCMA-block) micelle core was cross-linked by UV irradiation at 2 W/cm2 for 30 min. The cross-linked micelles were labeled with 64Cu at room temperature for 2 h (DOTA) or 80 °C for 3 h (CB-TE2A), giving labeling efficiencies of 60–76% (DOTA) and 40–47% (CB-TE2A). 64Cu-micelles were injected into tumor-bearing mice (8 mg/kg) and PET/CT scans were carried out at 1, 22, and 46 h postinjection. The micelles showed good blood stability (T1/2: 20–26 h) and tumor uptake that was comparable with other nanoparticle systems. The DOTA micelles showed a biodistribution similar to the CB-TE2A micelles and the tumor uptake was comparable for both micelle types at 1 h (1.9% ID/g) and 22 h (3.9% ID/g) but diverged at 46 h with 3.6% ID/g (DOTA) and 4.9% ID/g (CB-TE2A). On the basis of our data, we conclude that cross-linked PEG-PHEMA-PCMA micelles have long circulating properties resulting in tumor accumulation and that DOTA and CB-TE2A 64Cu-chelates show similar in vivo stability for the studied micelle system.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Center for Nuclear Technologies, The Hevesy Laboratory, Center for Nanomedicine and Theranostics, Colloids and Biological Interfaces, Copenhagen University Hospital
Authors: Jensen, A. T. I. (Intern), Binderup, T. (Ekstern), Ek, P. K. (Intern), Kjær, A. (Ekstern), Rasmussen, P. (Intern), Andresen, T. L. (Intern)
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  - Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
  - Scopus rating (2015): SJR 2.134 SNIP 1.449 CiteScore 6.05
  - Web of Science (2015): Indexed yes
- BFI (2014): BFI-level 2
  - Scopus rating (2014): SJR 2.207 SNIP 1.652 CiteScore 6.38
  - Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 1
  - Scopus rating (2013): SJR 2.085 SNIP 1.617 CiteScore 6.07
  - ISI indexed (2013): ISI indexed yes
  - Web of Science (2013): Indexed yes
- BFI (2012): BFI-level 1
  - Scopus rating (2012): SJR 2.317 SNIP 1.677 CiteScore 5.72
  - ISI indexed (2012): ISI indexed yes
  - Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 1
  - Scopus rating (2011): SJR 2.213 SNIP 1.777 CiteScore 5.74
  - ISI indexed (2011): ISI indexed yes
  - Web of Science (2011): Indexed yes
- BFI (2010): BFI-level 1
  - Scopus rating (2010): SJR 2.333 SNIP 1.66
  - Web of Science (2010): Indexed yes
- BFI (2009): BFI-level 1
  - Scopus rating (2009): SJR 2.288 SNIP 1.6
  - Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 1
  - Scopus rating (2008): SJR 2.228 SNIP 1.487
  - Web of Science (2008): Indexed yes
- Scopus rating (2007): SJR 2.173 SNIP 1.528
  - Web of Science (2007): Indexed yes
- Scopus rating (2006): SJR 1.854 SNIP 1.492
  - Scopus rating (2005): SJR 1.643 SNIP 1.467
  - Web of Science (2005): Indexed yes
- Scopus rating (2004): SJR 1.454 SNIP 1.34
  - Web of Science (2004): Indexed yes
- Scopus rating (2003): SJR 1.17 SNIP 1.187
  - Scopus rating (2002): SJR 1.033 SNIP 1.148
  - Web of Science (2002): Indexed yes
- Scopus rating (2001): SJR 0.787 SNIP 0.804
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Propargylamine-isothiocyanate reaction: efficient conjugation chemistry in aqueous media.
A coupling reaction between secondary propargyl amines and isothiocyanates in aqueous media is described. The reaction is high-yielding and affords cyclized products within 2-24 h. A functionalized ether lipid was synthesized in 8 steps, formulated as liposomes with POPC and conjugated to FITC under mild conditions using this method.

General information
State: Published
Organisations: Department of Chemistry, Organic Chemistry, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Technical University of Denmark
Authors: Viart, H. M. (Intern), Larsen, T. S. (Ekstern), Tassone, C. (Intern), Andresen, T. L. (Intern), Clausen, M. H. (Intern)
Pages: 7800-7802
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Main Research Area: Technical/natural sciences

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Ratings:
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Web of Science (2017): Indexed Yes
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Scopus rating (2016): CiteScore 6.06 SJR 2.506 SNIP 1.159
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.664 SNIP 1.314 CiteScore 6.7
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.701 SNIP 1.446 CiteScore 6.83
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.755 SNIP 1.38 CiteScore 6.73
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.09 SNIP 1.347 CiteScore 6.21
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.857 SNIP 1.322 CiteScore 5.96
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 2.709 SNIP 1.232
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 2.588 SNIP 1.252
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.791 SNIP 1.236
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.851 SNIP 1.237
Quantification of leakage from large unilamellar lipid vesicles by fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (FCS) is a powerful experimental technique that in recent years has found numerous applications for studying biological phenomena. In this article, we scrutinize one of these applications, namely, FCS as a technique for studying leakage of fluorescent molecules from large unilamellar lipid vesicles. Specifically, we derive the mathematical framework required for using FCS to quantify leakage of fluorescent molecules from large unilamellar lipid vesicles, and we describe the appropriate methodology for successful completion of FCS experiments. By use of this methodology, we show that FCS can be used to accurately quantify leakage of fluorescent molecules from large unilamellar lipid vesicles, including leakage of fluorescent molecules of different sizes. To demonstrate the applicability of FCS, we have investigated the antimicrobial peptide mastoparan X. We show that mastoparan X forms transient transmembrane pores in POPC/POPG (3:1) vesicles, resulting in size-dependent leakage of molecules from the vesicles. We conclude the paper by discussing some of the advantages and limitations of FCS as compared to other existing methods to measure leakage from large unilamellar lipid vesicles.
Real-time monitoring of drug-induced cytotoxicity kinetics using a tailor-made impedance platform

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Nanoprobes, Bioanalytics, Colloids and Biological Interfaces, Molecular Windows
Authors: Caviglia, C. (Intern), Canepa, S. (Intern), Zor, K. (Intern), Heiskanen, A. (Intern), Andresen, T. L. (Intern), Emnéus, J. (Intern)
Number of pages: 1
Publication date: 2014

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Main Research Area: Technical/natural sciences
Real-time multi-parameter cell-based analysis platform: towards new tools for biomedical research

Monitoring cellular dynamics such as cell surface interactions, metabolic processes, and exocytosis, can help unravel the causes behind the evolution of diseases associated with cellular dysfunction. A better understanding of cellular behaviour opens up possibilities for the development of new biomedical diagnostic techniques, drug discovery and screening.

My project focused on the further development, improvement and exploration of the EXCELL microfluidic platform with particular interest in drug kinetic monitoring and neurotransmitter detection. The aim was to perform multi-parameter real-time cell based assays for their future application as complementary tools in biomedical research. Specifically, the research has focused on: (1) Characterization of the cell culture and detection platforms (batch system for static conditions and microfluidics for perfusion conditions) and optimization of protocols and procedures for performing different cellular assays. (2) Electrochemical impedance spectroscopy (EIS) applied for drug screening and drug delivery in cancer research and wound healing studies. (3) Amperometry for monitoring of neurotransmitter exocytosis, relevant in research on Parkinson’s disease. (4) The combination of amperometry, EIS monitoring and microscopic visualization in microfluidics assays for real-time multi-parameter analysis on the same cell population.

The research carried out in this thesis branches out from the context of the EU-funded FP7 project EXCELL (Exploring Cellular Dynamics at Nanoscale) aimed at developing innovative systems for the investigation of real time cellular dynamics. The main focus of the EXCELL project was related to the development of a multi-parameter microfluidic cell culture and detection platform, combining electrochemical and optical techniques.

Side Chain Hydrophobicity Modulates Therapeutic Activity and Membrane Selectivity of Antimicrobial Peptide Mastoparan-X

The discovery of new anti-infective compounds is stagnating and multi-resistant bacteria continue to emerge, threatening to end the “antibiotic era”. Antimicrobial peptides (AMPs) and lipo-peptides such as daptomycin offer themselves as a new potential class of antibiotics; however, further optimization is needed if AMPs are to find broad use as antibiotics. In the present work, eight analogues of mastoparan-X (MPX) were investigated, having side chain modifications in position 1, 8 and 14 to modulate peptide hydrophobicity. The self-association properties of the peptides were characterized, and the peptide-membrane interactions in model membranes were compared with the bactericidal and haemolytic properties. Alanine substitution at position 1 and 14 resulted in higher target selectivity (red blood cells versus bacteria), but also decreased bactericidal potency. For these analogues, the gain in target selectivity correlated to biophysical parameters showing an increased effective charge and reduction in the partitioning coefficient for membrane insertion. Introduction of an unnatural amino acid, with an octyl side chain by amino acid substitution, at positions 1, 8 and 14 resulted in increased bactericidal potency at the expense of radically reduced membrane target selectivity. Overall, optimized membrane selectivity or bactericidal potency was achieved by changes in side chain hydrophobicity of MPX. However, enhanced potency was achieved at the expense of selectivity and vice versa in all cases.
Sucrose acetate isobutyrate-based nanogels as liquid fiducial tissue markers with potential use in image guided radiotherapy

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Center for Nanomedicine and Theranostics, Department of Chemistry, Organic Chemistry, Colloids and Biological Interfaces, Technical University of Denmark, Copenhagen University Hospital
Authors: Bruun, L. M. (Intern), Schaarup-Jensen, H. (Intern), Jelck, R. I. (Intern), Hansen, A. E. (Intern), Christiansen, A. N. (Ekstern), Clausen, M. H. (Intern), Kjær, A. (Ekstern), Scherman, P. J. B. (Ekstern), Andresen, T. L. (Intern)
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Main Research Area: Technical/natural sciences
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Poster presentation
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2014

Sucrose acetate isobutyrate-based nanogels as liquid fiducial tissue markers with potential use in image guided radiotherapy

The poster presents the development of a liquid fiducial tissue marker based on sucrose acetate isobutyrate (SAIB) and uniform, coated gold nanoparticles (AuNPs). The PNIPAM-coated AuNP-SAIB gel provided high CT contrast and high in vivo stability and was assessed to be a suitable tissue marker for image guided radiotherapy (IGRT).

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Center for Nanomedicine and Theranostics, Department of Chemistry, Organic Chemistry, Colloids and Biological Interfaces, Technical University of Denmark, Copenhagen University Hospital
Authors: Bruun, L. M. (Intern), Schaarup-Jensen, H. (Intern), Jelck, R. I. (Intern), Hansen, A. E. (Intern), Christiansen, A. N. (Ekstern), Clausen, M. H. (Intern), Kjær, A. (Ekstern), Scherman, P. J. B. (Ekstern), Andresen, T. L. (Intern)
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Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2014

Sucrose acetate isobutyrate-based nanogels as liquid fiducial tissue markers with potential use in image guided radiotherapy

The poster presents the development of a liquid fiducial tissue marker based on sucrose acetate isobutyrate (SAIB) and uniform, coated gold nanoparticles (AuNPs). The PNIPAM-coated AuNP-SAIB gel provided high CT contrast and high in vivo stability and was assessed to be a suitable tissue marker for image guided radiotherapy (IGRT).

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Department of Chemistry, Organic Chemistry, Technical University of Denmark, Copenhagen University Hospital
Authors: Bruun, L. M. (Intern), Schaarup-Jensen, H. (Intern), Jelck, R. I. (Intern), Hansen, A. E. (Intern), Christiansen, A. N. (Ekstern), Scherman, P. J. B. (Ekstern), Clausen, M. H. (Intern), Kjær, A. (Ekstern), Andresen, T. L. (Intern)
Number of pages: 1
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Main Research Area: Technical/natural sciences
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Poster
Publication: Research › Poster – Annual report year: 2014
These systems were shown to have superior drug efficacy in vitro.

targeting moiety on the surface to guide the uptake, in addition to an enzymatically cleavable peptide sequence, whose liposomes, limiting the therapeutic efficacy of the liposomes. We developed liposomal formulations which present a in order for it to circulate in the blood stream. However, the presence of the polymer obstructs the uptake pattern of the liposomes as drug delivery agents. The presence of a shielding polymer layer on the surface of the liposome is important revealing the biodistribution of drug delivery systems. Chapter four deals with one of the large dilemmas, when using experiments using fluorescent phospholipids and in vivo experiments using radiolabeled liposomes and a PET imaging technique. The results were encouraging and proved the large potential of radiolabeled liposomes as candidates for releasing the drug from the liposome interior is often needed. Several approaches have been suggested to work as release mechanisms such a pH changes, the presence of enzymes or external applied stimulus as heat or light. Chapter two deals with the synthesis of the functionalized phospholipids, which function as the targeting moiety on the surface of the liposomes. Several examples of synthetic procedures known from the literature are presented. The chapter is completed with a study covering the conjugation efficiencies of a variety of

Chapter one gives an introduction to the strategies used in liposomal drug delivery today. The important issues as...

Advanced Drug Delivery Systems - a Synthetic and Biological Applied Evaluation

Specific delivery of drugs to diseased sites in the body is a major topic in the development of drug delivery system today. Especially, the field of cancer treatment needs improved drug delivery systems, as the strong dose-limiting side effects of chemotherapy today often present a barrier for an effective cure. Liposomes have attracted much attention since they were first proposed as potential drug carrier agents in the 1970s. Chapter one gives an introduction to the strategies used in liposomal drug delivery today. The important issues as enhanced specific uptake in diseased tissue and effective unloading of the encapsulated drug have been tried optimized in a variety of ways. Many propose the use of small molecules, such as vitamins and peptides, for active targeting of the liposomes to overexpressed receptors on the cancerous tissue. Once located close to the diseased site a trigger mechanism for releasing the drug from the liposome interior is often needed. Several approaches have been suggested to work as release mechanisms such a pH changes, the presence of enzymes or external applied stimulus as heat or light. Chapter two deals with the synthesis of the functionalized phospholipids, which function as the targeting moiety on the surface of the liposomes. Several examples of synthetic procedures known from the literature are presented. The chapter is completed with a study covering the conjugation efficiencies of a variety of chemical functionalities. Large differences are revealed between the conjugation efficiency in solution and directly on the surface of the pre-formed liposomes. In chapter three the efficiency of the targeted liposomes is investigated.

Liposomes have attracted much attention since they were first proposed as potential drug carrier agents in the 1970s. Especially, the field of cancer treatment needs improved drug delivery systems, as the strong dose-limiting side effects of chemotherapy today often present a barrier for an effective cure. Liposomes have attracted much attention since they were first proposed as potential drug carrier agents in the 1970s. Chapter one gives an introduction to the strategies used in liposomal drug delivery today. The important issues as enhanced specific uptake in diseased tissue and effective unloading of the encapsulated drug have been tried optimized in a variety of ways. Many propose the use of small molecules, such as vitamins and peptides, for active targeting of the liposomes to overexpressed receptors on the cancerous tissue. Once located close to the diseased site a trigger mechanism for releasing the drug from the liposome interior is often needed. Several approaches have been suggested to work as release mechanisms such a pH changes, the presence of enzymes or external applied stimulus as heat or light. Chapter two deals with the synthesis of the functionalized phospholipids, which function as the targeting moiety on the surface of the liposomes. Several examples of synthetic procedures known from the literature are presented. The chapter is completed with a study covering the conjugation efficiencies of a variety of chemical functionalities. Large differences are revealed between the conjugation efficiency in solution and directly on the surface of the pre-formed liposomes. In chapter three the efficiency of the targeted liposomes is investigated. In vitro experiments using fluorescent phospholipids and in vivo experiments using radiolabeled liposomes and a PET imaging technique. The results were encouraging and proved the large potential of radiolabeled liposomes as candidates for revealing the biodistribution of drug delivery systems. Chapter four deals with one of the large dilemmas, when using liposomes as drug delivery agents. The presence of a shielding polymer layer on the surface of the liposome is important in order for it to circulate in the blood stream. However, the presence of the polymer obstructs the uptake pattern of the liposomes, limiting the therapeutic efficacy of the liposomes. We developed liposomal formulations which present a targeting moiety on the surface to guide the uptake, in addition to an enzymatically cleavable peptide sequence, whose cleavage would result in removal of the polymer layer as well as uncovering cationic charges on the liposomal surface. These systems were shown to have superior drug efficacy in vitro.
An in-vivo PET study of DOTA vs. CB-TE2A and the effect of crosslinking using core-crosslinked ny triblock polymeric micelles labeled with Cu-64 in the shell-region

General information
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Organisations: Center for Nuclear Technologies, The Hevesy Laboratory, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, University of Copenhagen
Authors: Jensen, A. T. I. (Intern), Ek, P. K. (Intern), Binderup, T. (Forskerdatabase), Andresen, T. L. (Intern), Kjær, A. (Ekstern), Rasmussen, P. (Intern)
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Cationic liposomal drug delivery system for specific targeting of human cd14+ monocytes in whole blood
This invention concerns a liposome comprising lipids and at least one active ingredient, wherein at least one of the lipids is a cationic lipid; said liposome exhibiting a net positive charge at physiological conditions at which said liposome preferentially adheres to monocytes in freshly drawn blood when compared to adherence to granulocytes, T-lymphocytes, B-lymphocytes and/or NK cells in freshly drawn blood, to a lipid-based pharmaceutical composition comprising said liposomes and their use in monocytic associated prophylaxis, treatment or amelioration of a condition such as cancer, an infectious disease, an inflammatory disease, an autoimmune disease or allergy.

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Department of Chemistry, Physical and Biophysical Chemistry
Authors: Andresen, T. L. (Intern), Henriksen, J. R. (Intern), Johansen, P. T. (Intern), S. Jensen, S. (Ekstern)
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Complement activation by PEG-functionalized multi-walled carbon nanotubes is independent of PEG molecular mass and surface density
Carboxylated (4%) multi-walled carbon nanotubes were covalently functionalized with poly(ethylene glycol)1000 (PEG1000), PEG1500 and PEG4000 with a PEG loading of approximately 11% in all cases. PEG loading generated non-uniform and heterogeneous higher surface structures and increased nanotube width considerably, but all PEGylated nanotube species activated the complement system in human serum equally. Increased PEG loading, through adsorption of methoxyPEG2000(or 5000)-phospholipid conjugates, generated fewer complement activation products; however, complement activation was never completely eliminated. Our observations address the difficulty in making carbon nanotubes more compatible with innate immunity through covalent PEG functionalization as well as double PEGylation strategies. From the Clinical EditorComplement-mediated toxicity is a major limiting factor in certain nanomedicine applications. This study clarifies that PEGylation of carbon nanotubes is unlikely to address this complication.
Current Challenges and Future Directions in Nanomedicine

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Authors: Berg, R. H. (Intern), Andresen, T. L. (Intern)
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Journal: JSM Nanotechnology & Nanomedicine
**Evolution of the EPR effect in dogs with spontaneous tumors and its implications on nanotherapies**

Nanoparticles are well established as effective drug delivery systems and have potential in biomedical imaging as a diagnostic tool. We have recently developed a highly efficient method for utilizing liposomes as agents in positron emission tomography (PET) imaging giving high resolution images and allowing direct quantification of liposome tissue distribution and blood clearance. Our approach is based on remote loading of a copper-radiouclide (64Cu) into preformed liposomes and copper entrapment by an encapsulated copper-chelator. We show that the 64Cu-liposomes provide quantitative in vivo imaging in canines with spontaneous tumors using PET. Seven canines with spontaneous tumors were included in the study where the main focus was to evaluate the EPR effect in large animals with spontaneous tumors and the performance of the developed liposome imaging agent. None of the included dogs displayed any anaphylactic, toxic or adverse reactions. Liposome circulating half-life ranged from 24.2 hours to 54.2 hours, with a mean half-life of 35.0 ± 4.24 hours. The study showed that the EPR effect assures substantial tumor accumulation in some but not all spontaneous tumors in canines. The included carcinomas displayed higher mean and maximum uptake levels of liposomes relative to the included sarcomas. The 64Cu-liposomes have potential as a diagnostic tracer in cancer diagnostics. We envision that the 64Cu-liposomes will be an important tool for evaluating liposome performance in future and may become an important tool in selection of cancer patients for nanoparticle based chemotherapy.

**References:**


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**Formulation of solid nano-sized particles in a gel-forming system**

The present invention relates to novel formulations comprising a plurality of nano-sized solid particles and a gel-forming system, useful e.g. for imaging of the body of a mammal. Also described are kits comprising such formulations and imaging methods utilizing such formulations or kits.

**General information**

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Bjerg, L. N. (Intern), Jelck, R. I. (Intern), Andresen, T. L. (Intern), Albrechtsen, M. (Ekstern)
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**Publication information**

Labeling of gold nanoparticles with Cu-64 and Zr-89 for combined CT/PET-imaging

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Quantitative single-vesicle analysis of antimicrobial peptide-induced leakage

Although the research field of antimicrobial peptides has attracted considerable scientific attention in the past decades, the microbicidal mechanisms of antimicrobial peptides still remain elusive. One of the keys to a more profound comprehension of the function of these peptides is a deeper understanding of their interactions with phospholipid membranes. In this study, the membrane-permeabilizing effects of antimicrobial peptides were scrutinized by combining two biophysical techniques. Confocal fluorescence microscopy to visualize leakage from individual surface-immobilized lipid vesicles was combined with fluorescence correlation spectroscopy to quantify leakage from a bulk collection of lipid vesicles in aqueous solution. Quantitative correlation between the two techniques was achieved through a detailed experimental protocol. The potential of combining the two techniques was tested using three canonical antimicrobial peptides: melittin, magainin 2, and mastoparan X. The results demonstrate an unprecedented level of insight into the molecular processes governing antimicrobial peptide-induced permeabilization of phospholipid membranes.

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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Kristensen, K. (Intern), Ehrlich, N. (Intern), Henriksen, J. R. (Intern), Andresen, T. L. (Intern)
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Quantitative studies of antimicrobial peptide-lipid membrane interactions

The increasing occurrence of multi-drug-resistant bacteria poses a serious threat to modern society. Therefore, novel types of anti-infective therapeutics are highly warranted. Antimicrobial peptides are a class of naturally occurring host-defense molecules that potentially might be developed into such novel therapeutics. However, limited understanding of the mechanisms underlying microbicidal activity of antimicrobial peptides has slowed down this development. A central step toward understanding the microbicidal mechanisms of action of antimicrobial peptides is to understand the mechanisms by which antimicrobial peptides interact with phospholipid membranes. Motivated by that fact, the scope of this thesis is to study these antimicrobial peptide-lipid membrane interactions. In particular, we attempt to study these interactions with a quantitative approach. For that purpose, we consider the three archetypal α-helical antimicrobial peptides mastoparan X, melittin, and magainin 2 as model peptides. These three peptides are investigated by three different experimental techniques.

The first of these experimental techniques is analytical HPLC. We use this technique to document an effect that might pose a significant problem for quantitative studies of antimicrobial peptide-lipid membrane interactions; namely that antimicrobial peptides adsorb to surfaces of glass and plastic. Specifically, we demonstrate that under standard experimental conditions, this effect is significant for mastoparan X, melittin and magainin 2. Consequently, we conclude that investigators should always take this adsorptive effect into account when designing and interpreting their experiments on antimicrobial peptides.

The second experimental technique is fluorescence correlation spectroscopy (FCS). We optimize this technique so that it can be used to quantify antimicrobial peptide-induced leakage of fluorescent markers from large unilamellar lipid vesicles in solution. For that purpose, we derive the mathematical framework required to calculate leakage from the FCS data, and we identify a number of experimental pitfalls that might lead to inaccurate conclusions, or even completely wrong conclusions, when interpreting the FCS data. We show that, if all of the pitfalls are avoided, then FCS is a technique with a large potential for quantitative studies of antimicrobial peptide-induced leakage of fluorescent markers from large unilamellar lipid vesicles in solution. Particularly interesting is our finding that FCS might be used for studying peptide-induced leakage of markers of different sizes, thereby providing a novel approach for rapid sizing of transmembrane pores formed by antimicrobial peptides. We demonstrate the applicability of FCS by using the technique to study partial transient leakage induced by mastoparan X, melittin, and magainin 2. The leakage data demonstrate that magainin 2 forms larger and/or more stable transmembrane pores in POPC/POPG (3:1) lipid bilayers than do mastoparan X and melittin.

The third and final technique is confocal imaging. Specifically, we use this technique to visualize fluorescently-labeled surface-tethered large unilamellar lipid vesicles. We design an experimental protocol that allows us to directly correlate antimicrobial peptide-induced leakage of fluorescent markers from these surface-tethered vesicles to antimicrobial peptide-induced leakage of fluorescent markers from lipid vesicles in solution. Thereby, we have developed a direct and flexible approach for quantitative evaluation of antimicrobial peptide-induced leakage from large unilamellar lipid vesicles on the single-vesicle level, allowing us an unprecedented level of insight into the leakage process. For example, the surface-tethered lipid vesicles can be used to directly visualize how the single-vesicle leakage profiles depend on the marker size. We employ the surface-tethered vesicles to study partial transient leakage induced by mastoparan X, melittin and magainin 2 from POPC/POPG (3:1) large unilamellar lipid vesicles. The results show that on the single-vesicle level, all three peptides induce heterogenous leakage in the sense that they induce complete emptying of some vesicles and only partly emptying of other vesicles. This heterogenous leakage profile is observed regardless of the size of the lumen.
Quantitative Studies of Antimicrobial Peptide Pore Formation in Large Unilamellar Vesicles by Fluorescence Correlation Spectroscopy (FCS)

In spite of intensive research efforts over the past decades, the mechanisms by which membrane-active antimicrobial peptides interact with phospholipid membranes are not yet fully elucidated. New tools that can be used to characterize antimicrobial peptide-lipid membrane interactions are therefore highly warranted. Fluorescence correlation spectroscopy is a biophysical technique that can be used to quantify leakage of fluorescent probes of different sizes from large unilamellar vesicle, thereby potentially becoming such a new tool. However, the usage of fluorescence correlation spectroscopy to quantify leakage from large unilamellar vesicles is associated with a number of experimental pitfalls. Based on theoretical and experimental considerations, we discuss how to properly design experiments to avoid these pitfalls. Subsequently, we apply fluorescence correlation spectroscopy to quantify leakage of fluorescent probes of different sizes through transmembrane pores formed by each of the three representative antimicrobial peptides: melittin, magainin 2, and mastoparan X. The experimental results demonstrate that leakage assays based on fluorescence correlation spectroscopy offer new and detailed insight into the size and cooperative nature of transmembrane pores formed by antimicrobial peptides that is not available from the conventional quenching-based leakage assays.

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Real-time impedimetric monitoring of Poly(ethylenimine)s-mediated cytotoxicity during gene transfection

Poly(ethylenimine)s (PEIs) are able to condense DNA and RNA into stable toroidal and globular nanostructures (polyplexes) and are among the most efficient and promising synthetic transfectants, but they induce severe cytotoxicity. The mechanisms of PEI-mediated cytotoxicity have not been fully delineated but PEI toxicity appears to predominantly depend on membrane perturbing effects in cellular compartments in which they accumulate. Electrochemical Impedance Spectroscopy (EIS) is used as a non-invasive biophysical approach for the investigation of the electrical properties of biological materials according to their physiological and morphological changes. In this work, EIS has been used to evaluate impedance changes due to the polycation perturbations on a cell population. HeLa cells have been cultured on laminin-coated gold interdigitated electrode arrays integrated into a tailor-made microfluidic cell culture platform. Multiplexed EIS data from each sensor element were acquired using a 24-channel miniaturized potentiostat (30 points between 100 Hz and 100k-Hz). Two alternative sensing configuration approaches (the standard “vertical” configuration (a single working electrode (WE) versus a large, distant counter electrode (CE)), and the interdigitated configuration (WEa
Comb versus Web comb have been used and compared on the same cell population, providing optimal detection conditions. The experiments have been performed by initially seeding about 10^3 cells into each chamber, continuously perfused with fresh culture medium. The platform was incubated in a humidified atmosphere. After 24 hours, different concentrations of PEIs have been introduced in the culture medium and the incubation continued for other 24 hours. Cell adhesion, growth and PEI-cytotoxicity have been detected in real-time by following impedance changes. Microscopic imaging and MTS assays have been combined to the electrochemical detection. Complementary ongoing experiments aim to monitor in real-time gene transfection in order to detect the cytotoxic effects (apoptosis and necrosis) induced by different cationic polyplexes. This approach can contribute to a clearer and detailed mechanistic understanding of polycation-modulated cellular functions and cell death and could initiate rational approaches for design and engineering of safer vectors for nucleic acid transfection.

Single-Walled Carbon Nanotube Surface Control of Complement Recognition and Activation

Carbon nanotubes (CNTs) are receiving considerable attention in site-specific drug and nucleic acid delivery, photodynamic therapy, and photoacoustic molecular imaging. Despite these advances, nanotubes may activate the complement system (an integral part of innate immunity), which can induce clinically significant anaphylaxis. We demonstrate that single-walled CNTs coated with human serum albumin activate the complement system through C1q-mediated classical and the alternative pathways. Surface coating with methoxypoly(ethylene glycol)-based amphiphiles, which confers solubility and prolongs circulation profiles of CNTs, activates the complement system differently, depending on the amphiphile structure. CNTs with linear poly(ethylene glycol) amphiphiles trigger the lectin pathway of the complement through both l-ficolin and mannan-binding lectin recognition. The lectin pathway activation, however, did not trigger the amplification loop of the alternative pathway. An amphiphile with branched poly(ethylene glycol) architecture also activated the lectin pathway but only through l-ficolin recognition. Importantly, this mode of activation neither generated anaphylatoxins nor induced triggering of the effector arm of the complement system. These observations provide a major step toward nanomaterial surface modification with polymers that have the properties to significantly improve innate immunocompatibility by limiting the formation of complement C3 and C5 convertases.
Synthesis and Characterization of a Micelle-Based pH Nanosensor with an Unprecedented Broad Measurement Range

A new cross-linked micelle pH nanosensor design was investigated. The nanosensor synthesis was based on self-assembly of an amphiphilic triblock copolymer, poly(ethylene glycol)-b-poly(2-amino ethyl methacrylate)-b-poly(coumarin methacrylate) (PEG-b-PAEMA-b-PCMA), which was synthesized by isolated macroinitiator atom transfer radical polymerization. Micelles were formed by PEG-b-PAEMA-b-PCMA self-assembly in water, giving micelles with an average diameter of 45 nm. The PCMA core was employed to utilize coumarin-based photoinduced cross-linking in the core of the micelles, which was performed by UV irradiation (320 nm <λ <500 nm), and the progress of the cross-linking was monitored by UV spectroscopy. The formed cross-linked core–shell–corona micelle was converted into ratiometric pH nanosensors by binding the pH-sensitive fluorophores oregon green 488 and 2′,7′-bis-(2-carboxyethyl)-5-(and-6) carboxyfluorescein and a reference fluorophore Alexa 633 to the PAEMA shell region of the micelles. Fluorescence measurements show that these pH nanosensors are sensitive in a surprisingly broad pH range of 3.4–8.0, which is hypothesized to be due to small differences in the individual fluorophores' local environment. It was found that the utilization of self-organization principles to form the nanoparticles, followed by cross-linking to ensure sensor integrity, provides a fast and highly flexible method that can be utilized in a broad range of nanosensor designs.
Nanosensor, Cross-linked micelle, Coumarin, Core−shell–corona micelle, Ratiometric sensor, pH sensor

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Targeting monocytes using a novel liposome based delivery system

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Scopus rating (2008): SJR 0.236 SNIP 0.078
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 0.286 SNIP 0.141
Scopus rating (2006): SJR 0.421 SNIP 0.125
Scopus rating (2005): SJR 0.999 SNIP 0.642
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Theoretical and experimental comparison of microelectrode sensing configurations for impedimetric cell monitoring

A theoretical and experimental comparison between vertical and coplanar interdigitated sensing configurations for impedimetric cell growth tracking is presented. These widely-adopted approaches are quantitatively compared on the same cell populations and on the same 10 μm interdigitated microelectrodes using a versatile custom-made monitoring platform including a 24-channel miniaturized potentiostat. The characterization of bare microelectrodes in buffer and tracking experiments with HeLa cells over 16 hours demonstrate that the coplanar configuration provides a higher sensitivity to cell adhesion and spreading (Cell Index = 1.6 vs. 0.4) albeit at a higher frequency of maximum sensitivity (100 kHz vs. 24 kHz) shifting over time. © 2014 Taylor & Francis Group.
A GALA lipopeptide mediates pH- and membrane charge dependent fusion with stable giant unilamellar vesicles

Peptides capable of mediating fusion between lipid membranes are widely observed in nature, and have attracted considerable attention in the liposome drug delivery field. However, studies that are proving the benefit of small synthetic fusion peptides as components in drug delivery systems remain sporadic and there is a strong need to characterize and increase our understanding of the membrane fusion properties of these peptides. Many fusion studies have focused on
the ability of free peptides in solution that mediate fusion between liposomes. For drug delivery purposes it is a necessity to attach the peptides to the surface of the liposome drug delivery carrier, without impairing the peptide functionality. Here we present the synthesis and characterization of a new diacylated derivative of the previously described fusion peptide GALA. Two myristoyl chains were attached to the GALA peptide through the 1,2-diamino propanoic acid (Dap) moiety, yielding the lipopeptide dimyristoyl-Dap-GALA (DMDGALA). We have investigated DMDGALA as a component in large unilamellar vesicles (LUVs) and demonstrate pH-triggered fusion of peptide containing LUVs with stable target giant unilamellar vesicles (GUVs), which were used as simple mimics of cell membranes. The number of fusion events was large at pH 5.0, which is a physiologically relevant pH-range for a drug delivery system.

**General information**

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ISI indexed (2013): ISI indexed yes  
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Scopus rating (2009): SJR 2.516 SNIP 1.534  
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BFI (2008): BFI-level 1  
Scopus rating (2008): SJR 2.562 SNIP 1.392  
Web of Science (2008): Indexed yes  
Scopus rating (2007): SJR 2.482 SNIP 1.458  
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Analysis and Improvement of the Gene Delivery Properties of Polyethylenimine – An In Vitro Study

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Mattebjerg, M. A. (Intern), Andresen, T. L. (Intern)
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Publication: Research › Ph.D. thesis – Annual report year: 2012

Charge triggering of self-organized nanoparticles

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Department of Chemistry, Physical and Biophysical Chemistry
Authors: Jølck, R. I. (Intern), Henriksen, J. R. (Intern), Gjetting, T. (Intern), Andresen, T. L. (Intern)
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Source: dtu
Publication: Research › Patent – Annual report year: 2012

Comparison of microelectrode sensing configurations for impedimetric cell monitoring
A theoretical and experimental comparison between vertical and coplanar interdigitated sensing configurations for impedimetric cell growth tracking is presented. For the first time, these widely-adopted approaches are quantitatively compared on the same cell populations and on the same 10μm interdigitated microelectrodes using a versatile custom-made monitoring platform including a 24-channel miniaturized potentiostat. As expected, characterization of bare microelectrodes in buffer and tracking experiments with HeLa cells over 16 hours demonstrate that the coplanar configuration provides a higher sensitivity to cell adhesion and spreading (Cell Index = 1.6 vs. 0.4) albeit at a higher frequency of maximum sensitivity (100kHz vs. 24 kHz).

General information
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Design and application of optical nanosensors for pH imaging in cell compartments

Measurements of pH in acidic cellular compartments of mammalian cells is important for our understanding of cell metabolism, and organelle acidification is an essential event in living cells especially in the endosomal-lysosomal pathway where pH is critical for cellular sorting of internalized material. Intracellular pH can be measured by the use of fluorescence ratio imaging microscopy (FRIM), however, available methods for pH measurements in living cells are not optimal. Nanoparticle based optical sensor technology for quantification of metabolites in living cells has been developed over the last two decades. However, even though these sensor systems have proven themselves as superior to conventional methods, there are still questions about the use of these sensors that need to be addressed, especially regarding sensor design and calibration. We have developed a new triple-labelled pH nanosensor designed with two pH sensitive dyes and one reference dye covalently attached to the nanoparticle matrix. The effective pH sensitivity range of this sensor was determined to be at least 3.1 pH units, which is twice the range of conventional dual-labelled nanosensors which is 1.4 pH units. The triple-labelled nanosensor was demonstrated to be superior to a dual-labelled nanosensor when performing measurements of pH in lysosomes in response to treatment of the cells with Bafilomycin A1. The triple-labelled nanosensor could follow the resulting increase in pH from a mean value around pH 4.3 up to 5.6, whereas the duallabelled nanosensor failed to measure the pH of up to 70% of the nanosensor containing vesicles. In order to perform reliable measurements of pH, proper calibration and image analysis have to be performed. We investigated nanosensors calibration and provide a suitable equation for fitting calibration curves which can be adapted to both dual- and triple-labelled sensors as well as sensors with even more sensitive dyes. Furthermore, we describe how image analysis can be performed correcting for both background fluorescence and differences in laser power.

We further demonstrated the use of the triple-labelled pH nanosensor in answering biological questions. The triple-labelled nanosensor was shown to specifically localize in lysosomes where the pH was measured in response to the treatment of the cells with polyethylenimine (PEI), a potent transfection agent. We found no change in lysosomal pH within a timeframe of up to 24 h in response to any of the investigated PEIs. In relation to these findings we do not reject the "proton sponge" hypothesis, but suggest that the effect is not associated with changes in lysosomal pH. Finally, we investigated the pH profiles of a positively charged nanosensor in six different cell lines as well as the profile of a hyaluronic acid conjugated nanosensor tested in one cell line. After 24 h of incubations all sensors resided in compartments with low pH, recognized as lysosomes in HeLa cells, and responded with an increase in pH to the treatment with Bafilomycin A1. This indicates that all internalized material eventually ends up in the lysosomes, even though the hyaluronic acid conjugated nanosensor showed uptake directed by the CD44 receptor. The initial uptake pathway employed by this nanosensor could potentially be different from the one employed by the positively charged nanosensor. In conclusion, we have developed a triple-labelled pH nanosensor which was shown to be superior to conventional dual-labelled nanosensors with respect to the pH sensitive range. With proper calibration and image analysis we performed pH measurements of lysosomes in different mammalian cells in response to the transfection agent PEI and in relation to different functionalizations.

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**Design, synthesis and characterisation of gurmarin and melanocortin analogues of gurmarin**

In this Ph.D. project the possibility of grafting melanocortin receptor binding sequences into the inhibitor cystine knotted peptide gurmarin was investigated. The goal of designing these analogues was to produce highly stable peptide agonists with potential for obesity treatment.

Inhibitor cystine knotted peptides are a group of peptides found in a variety of plant and animal species. They all contain six cysteines forming three disulphides which determine their tertiary structure and provide them with exceptional proteolytic stability. It has been demonstrated that these peptides can be used as molecular scaffolds into which peptide sequences of pharmaceutical interest can be incorporated, thus providing highly stable drug candidates.

The melanocortin receptors are a family of five G protein-coupled receptors distributed throughout the body. The melanocortin 4 receptor is found in the brain where it regulates appetite and energy expenditure. The melanocortin receptors are activated by peptides containing a His-Phe-Arg-Trp tetrapeptide sequence. Consequently, inhibitor cystine knotted peptides in which this tetrapeptide are grafted could provide novel appetite regulating peptides for the treatment of obesity.

First, a stable method to synthesise gurmarin was developed. The peptide was oxidised by a random oxidation method using a buffer containing a glutathione/cystamine redox pair. Various attempts were made to confirm the disulphide connectivity of the randomly oxidised gurmarin. The native disulphide connectivity of the randomly oxidised gurmarin was ultimately confirmed by a synthetic approach. This approach used a combination of thermolysin cleavage of the randomly oxidised peptide and the development of an orthogonal oxidation method. In this method, the disulphides in gurmarin were oxidised sequentially using three different pairs of cysteine side chain protection groups, which allowed selective disulphide formation.

Secondly, the redox buffer oxidation was used to synthesise six melanocortin analogues of gurmarin. These peptides were designed based on a structural alignment of gurmarin and agouti-related protein, an endogenous antagonist of the melanocortin receptors. The C-terminal part of agouti-related protein contains multiple disulphides similar to the inhibitor cystine knot of gurmarin. In addition, two tetra-disulphide analogues of gurmarin was designed and synthesised based on the alignment with agouti-related protein. The melanocortin analogues were characterised for their binding to the melanocortin receptors. In general, it was proved that it is possible to synthesise gurmarin analogues which bind to the melanocortin 4 receptor. The analogue with highest affinity to the melanocortin 4 receptor had a binding affinity of 500 nM which is an order of magnitude lower affinity than the endogenous agonist α-melanocyte stimulating hormone.

Finally, it was demonstrated that it was possible to synthesise melanocortin analogues of the cyclic cystine knotted peptide kalata B1, a plant peptide which two termini are cyclised. These analogues were shown by NMR spectroscopy to adopt similar threedimensional folds as kalata B1. One analogue was found to bind to the melanocortin 4 receptor with a binding affinity of 29 nM, which is higher affinity than α-melanocortin stimulating hormone. However, the analogue was less potent than α-melanocortin stimulating hormone at activating the melanocortin 4 receptor. In addition, the analogues were shown to be highly resistant to proteolysis compared to α-melanocyte stimulating hormone.

In summary, the possibility of grafting melanocortin binding sequences into cystine knotted peptides was confirmed using two different peptides as molecular scaffolds. The ability of these melanocortin analogues to adopt native folds was confirmed. In addition, it was demonstrated that the synthesised peptides in fact are able to bind to and activate the melanocortin 4 receptor. Moreover, the peptides were resistant to proteolysis. This novel melanocortin grafting approach provides new candidates for the development of drugs treating obesity.
Obesity is an increasingly important global health problem that lacks current treatment options. The melanocortin receptor 4 (MC4R) is a target for obesity therapies because its activation triggers appetite suppression and increases energy expenditure. Cyclotides have been suggested as scaffolds for the insertion and stabilization of pharmaceutically active peptides. In this study, we explored the development of appetite-reducing peptides by synthesizing MC4R agonists based on the insertion of the His-Phe-Arg-Trp sequence into the cyclotide kalata B1. The ability of the analogues to fold similarly to kalata B1 but display MC4R activity were investigated. Four peptides were synthesized using t-butoxycarbonyl peptide chemistry with a C-terminal thioester to facilitate backbone cyclization. The structures of the peptides were found to be similar to kalata B1, evaluated by Ho NMR chemical shifts. KB1(GHFRWG;23-28) had a Ki of 29 nM at the MC4R and was 107 or 314 times more selective over this receptor than MC1R or MC5R, respectively, and had no detectable binding to MC3R. The peptide had higher affinity for the MC4R than the endogenous agonist, α-melanocyte stimulation hormone, but it was less potent at the MC4R, with an EC50 of 580 nM for activation of the MC4R. In conclusion, we synthesized melanocortin analogues of kalata B1 that preserve the structural scaffold and display receptor binding and functional activity. KB1(GHFRWG;23-28) is potent and selective for the MC4R. This compound validates the use of cyclotides as scaffolds and has the potential to be a new lead for the treatment of obesity.

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, University of Queensland, Novo Nordisk A/S
Authors: Eliasen, R. (Intern), Daly, N. L. (Ekstern), Wulff, B. S. (Ekstern), Conde-Frieboes, K. W. (Ekstern), Andresen, T. L. (Intern), Conde-Frieboes, K. W. (Ekstern), Craik, D. J. (Ekstern)
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.254 SNIP 1.222 CiteScore 4.5
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 3.369 SNIP 1.231 CiteScore 4.87
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.361 SNIP 1.244 CiteScore 4.97
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Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.495 SNIP 1.26 CiteScore 4.97
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.923 SNIP 1.342
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 4.158 SNIP 1.344
Bibliographical note
Background: Cyclotides are useful scaffolds to stabilize bioactive peptides.
Results: Four melanocortin analogues of kalata B1 were synthesized. One is a selective MC4R agonist.
Conclusion: The analogues retain the native kalata B1 scaffold and introduce a designed pharmacological activity, validating cyclotides as protein engineering scaffolds.
Significance: A novel type of melanocortin agonist has been developed, with potential as a drug lead for treating obesity.
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Source-ID: n::oai:DTIC-ART:compendex/375974211::26081
Publication: Research - peer-review › Journal article – Annual report year: 2013

Entrapment of Radionuclides in Nanoparticle Compositions
The present invention is directed to the technical field of imaging compositions useful for diagnosing cancer and other diseases in a subject. In particular, the invention relates to a class of diagnostic compounds comprising a novel liposome composition with encapsulated metal entities such as radionuclides, for example 61Cu and 64Cu copper isotopes. The invention further relates to a novel method for loading delivery systems, such as liposome compositions, with metal entities such as radionuclides, and the use of liposomes for targeted diagnosis and treatment of a target site, such as cancerous tissue and, in general, pathological conditions associated with leaky blood vessels. The present invention provides a new diagnostic tool for the utilization of positron emission tomography (PET) imaging technique.

General information
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Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nuclear Technologies, The Hevesy Laboratory, Department of Chemistry, Physical and Biophysical Chemistry
Authors: Andresen, T. L. (Intern), Petersen, A. L. (Intern), Henriksen, J. R. (Intern), Rasmussen, P. (Intern), kjær, A. (Ekstern)
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Factors Controlling Nanoparticle Pharmacokinetics: An Integrated Analysis and Perspective

Intravenously injected nanoparticulate drug carriers provide a wide range of unique opportunities for site-specific targeting of therapeutic agents to many areas within the vasculature and beyond. Pharmacokinetics and biodistribution of these carriers are controlled by a complex array of interrelated core and interfacial physicochemical and biological factors. Pertinent to realizing therapeutic goals, definitive maps that establish the interdependency of nanoparticle size, shape, and surface characteristics in relation to interfacial forces, biodistribution, controlled drug release, excretion, and adverse effects must be outlined. These concepts are critically evaluated and an integrated perspective is provided on the basis of the recent application of nanoscience approaches to nanocarrier design and engineering. The future of this exciting field is bright; some regulatory-approved products are already on the market and many are in late-phase clinical trials. With concomitant advances in extensive computational knowledge of the genomics and epigenomics of interindividual variations in drug responses, the boundaries toward development of personalized nanomedicines can be pushed further.
Handling a tricycle: Orthogonal versus random oxidation of the tricyclic inhibitor cystine knotted peptide gurmarin

Gurmarin is a 35 amino acid peptide with three disulfide bridges in an inhibitor cystine knot. It is found in the plant Gymnema sylvestre, and has been identified as a sweet taste inhibitor in rodents. In this article we provide an efficient route for the synthesis of gurmarin by a controlled random oxidation strategy. We compared two oxidation procedures to form the three disulfide bridges. In the first, based on random oxidation, reduced gurmarin was synthesized using trityl for cysteine protection, and oxidized for 48h in a Tris–HCl buffer containing cystamine and reduced glutathione to facilitate disulfide scrambling. The second was based on step-wise deprotection followed by oxidation in which the cysteine pairs are orthogonally protected with tert-Butylthio, trityl and acetamidomethyl. To verify that the native gurmarin oxidation product was obtained, thermolysin cleavage was used. Cleavage of random oxidized gurmarin showed two possible disulfide combinations; the native and a non-native gurmarin disulfide isomer. The non-native isomer was therefore synthesized using the orthogonal deprotection-oxidation strategy and the native and the non-native gurmarin isomers were analyzed using UPLC. It was found that the random oxidation procedure leads to native gurmarin in high yield. Thus, the synthetic route was simple and significantly more efficient than previously reported syntheses of gurmarin and other cysteine rich peptides. Importantly, native gurmarin was obtained by random oxidation, which was confirmed by a synthetic approach for the first time.

General information

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Authors: Eliasen, R. (Intern), Andresen, T. L. (Intern), Conde-Frieboes, K. W. (Ekstern)
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BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.11 SNIP 0.921 CiteScore 2.81
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.957 SNIP 0.936 CiteScore 2.74
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.971 SNIP 0.937 CiteScore 2.89
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.801 SNIP 0.902 CiteScore 2.72
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Hyaluronic Acid Immobilized Polyacrylamide Nanoparticle Sensors for CD44 Receptor Targeting and pH Measurement in Cells

Our ability to design receptor-targeted nanocarriers aimed at drug release after endocytosis is limited by the current knowledge of intracellular nanoparticle (NP) trafficking. It is not clear if NP size, surface chemistry, and/or targeting of cell surface receptors changes the intracellular fate of NPs; i.e., will all NPs enter acidic compartments and eventually end up in lysosomes or are there escape mechanisms or receptor-specific signaling that can be induced to change the cellular processing of an internalized NP? To give new insight into the intracellular trafficking of NPs that target the CD44 receptor, which is overexpressed on the surface of a broad variety of cancer cells, we have synthesized an NP pH sensor system that targets CD44. We used a polyacrylamide nanoparticle matrix bearing hyaluronic acid (HA) on the surface as a CD44 targeting ligand. The HA-coated NPs were prepared by radical polymerization followed by post functionalization with sensor fluorophores and physically absorbed or chemically conjugated HA. Cell uptake studies showed significant uptake of HA-coated nanosensors in HeLa cells and no uptake under the same conditions without the HA targeting ligand. The pH distribution profile in cells was measured for nanosensors with HA, cationic, and noncharged NP surface coatings giving a clear indication of the intracellular pH environment that the different NPs experience after internalization. The pH profile of cationic nanosensors in comparison to HA conjugated nanosensors indicates that the intracellular trafficking is aimed at lysosomes regardless of whether CD44 receptor-specific or unspecific uptake is induced.
Liposomal delivery of radionuclides for cancer diagnostics and radiotherapy: From material development to in vivo applications using positron emission tomography (PET) imaging

Molecular imaging is increasingly being used as an integrated discipline in designing radiotherapeutic agents and diagnostic imaging agents, and in developing drugs in general. In recent years, the use of the radionuclide and positron emitter copper-64 (64Cu) has become increasingly important, as the use of positron emission tomography (PET) scanners for molecular and diagnostic imaging has become more attractive. Furthermore, the importance of molecular and diagnostic imaging in nanotechnology has also been recognized, and significant research has been conducted on radio-labeled liposomes for scintigraphy and single photon emission computed tomography (SPECT) imaging. Preclinical
as well as clinical SPECT studies on radiolabeled liposomes have contributed with valuable information on the pharmacokinetics of liposomes during several liposomal drug developments. SPECT has lower detection sensitivity compared to PET, and developing new radio-labeling and loading methods, and designing liposomes useful in PET imaging are therefore of great importance.

The first two sections of this thesis (Introduction and Project I) review current liposomal radio-labeling and loading strategies, and describe the use of 64Cu in PET imaging. Article I and Patent I present our work focused on developing a remote (active) loading technique using a new lipophilic chelator (2-hydroxyquinoline) to load 64Cu into preformed (pre-manufactured) liposomes. We optimized the remote loading technique through several liposomal loading experiments and isothermal titration calorimetry (ITC) measurements. Various chelators, ionophores and lipophilic chelators were tested at different pH and temperature conditions.

Liposomes passively accumulate in tumors due to the enhanced permeability and retention (EPR) effect. In Article I, an in vivo study is presented, where passive tumor accumulation of 64Cu loaded liposomes (64Cu-liposomes) in tumor-bearing mice was quantified directly by PET and computed tomography (CT) imaging. Furthermore, Article I present an evaluation and quantitative measurement of the biodistribution of 64Cu-liposomes in healthy and tumor-bearing mice.

Project II summarizes considerations required in designing diagnostic liposomal radiotracers such as liposomal properties (e.g. particle size and poly(ethylene glycol) PEG coating), importance of background clearance in diagnostic imaging, and radiation exposure during PET scanning and internal radiotherapeutic applications. The circulation half-life of liposomes can be modulated by tuning the particle size and surface coating by varying the degree of PEG. Article II presents our work comparing two liposomal formulations coated with PEG (5 mol% PEG and 10 mol% PEG) in vivo in tumor-bearing mice. In addition, we perform remote loading experiments of the radionucleide, lutetium-177, (177Lu) into preformed liposomes useful in internal radiotherapy. Furthermore, Article II presents a dosimetric evaluation of PEGylated 64Cu-liposomes as clinical PET radiotracers and PEGylated 177Lu-liposomes useful in internal tumor radiotherapy.

While liposomes passively accumulate in tumor sites due to the EPR-effect, active targeting strategies using ligands directed towards over-expressed receptors on tumor cells can enhance tumor accumulation of liposomes. Project III briefly describes some active tumor-targeting strategies that have been tested with liposomes. In addition, Project III describes over-expressed somatostatin receptors (SSTRs) in neuroendocrine (NE) tumors and how SSTRs can be targeted with radiolabeled somatostatin analogs in imaging and radiotherapy of NE tumors in cancer patients. Article III presents our work, in which we investigate the capability of 64Cu-liposomes to actively target NE tumors when a somatostatin analog, octreotate (TATE), as targeting ligand is covalently attached to the distal end of DSPE-PEG2000. We also compare the biodistribution of PEGylated 64Cu-liposomes with and without TATE, and their ability to image NE tumors in tumor-bearing mice using PET. Further, we compare the 64Cu tumor accumulation and imaging capability with that of the radiolabeled somatostatin analog 64Cu-DOTA-TATE.

During the past 30 years, ionophores or lipophilic chelators have commonly been used for remotely loading radionuclides into liposomes. During the optimization of our remote loading method (Project I), we discovered a much simpler and even more efficient approach to load the radionuclide 64Cu, indium-111 (111In) and 177Lu into preformed liposomes, a so called "unassisted" loading, excluding any use of ionophores and lipophilic chelators. Project IV presents results from this invention (Patent II), where a presentation of various parameters affecting the efficiency of the unassisted loading method is given.

Section 5 summarizes the regulatory requirements governing quality assurance and considerations during development of a liposomal PET radiopharmaceutical for clinical use. In addition, the opportunity to use larger animals as clinical cancer patients for improving 64Cu-liposomes as PET imaging agents is briefly described followed by a short summary of possible clinical applications of a liposomal PET radiotracer.

Section 6 summarizes the work of this thesis and brings the work into perspective.

**General information**

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Authors: Petersen, A. L. (Intern), Andresen, T. L. (Intern), Rasmussen, P. H. (Ekstern)
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**Liposome imaging agents in personalized medicine**

In recent years the importance of molecular and diagnostic imaging has increased dramatically in the treatment planning of many diseases and in particular in cancer therapy. Within nanomedicine there are particularly interesting possibilities for combining imaging and therapy. Engineered liposomes that selectively localize in tumor tissue can transport both drugs and imaging agents, which allows for a theranostic approach with great potential in personalized medicine. Radiolabeling of liposomes have for many years been used in preclinical studies for evaluating liposome in vivo performance and has been an important tool in the development of liposomal drugs. However, advanced imaging systems now provide new possibilities for non-invasive monitoring of liposome biodistribution in humans. Thus, advances in imaging and developments in liposome radiolabeling techniques allow us to enter a new arena where we start to consider how to use
imaging for patient selection and treatment monitoring in connection to nanocarrier based medicines. Nanocarrier imaging agents could furthermore have interesting properties for disease diagnostics and staging. Here, we review the major advances in the development of radiolabeled liposomes for imaging as a tool in personalized medicine.

**General information**

State: Published

Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, Department of Electrical Engineering, Shaare Zedek Medical Center and Hebrew University

Authors: Petersen, A. L. (Intern), Hansen, A. E. (Intern), Gabizon, A. (Ekstern), Andresen, T. L. (Intern)

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- BFI (2015): BFI-level 2
- Scopus rating (2015): SJR 5.066 SNIP 4.303 CiteScore 16.01
- BFI (2014): BFI-level 2
- Scopus rating (2014): SJR 4.839 SNIP 4.389 CiteScore 15.08
- Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 2
- ISI indexed (2013): ISI indexed yes
- BFI (2012): BFI-level 2
- Scopus rating (2012): SJR 4.488 SNIP 3.419 CiteScore 12.16
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): SJR 4.846 SNIP 3.822 CiteScore 13.15
- ISI indexed (2011): ISI indexed yes
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- BFI (2010): BFI-level 2
- BFI (2009): BFI-level 2
- BFI (2008): BFI-level 2
- Scopus rating (2008): SJR 3.106 SNIP 2.897
- Scopus rating (2007): SJR 3.041 SNIP 2.977
- Scopus rating (2006): SJR 2.741 SNIP 2.938
- Scopus rating (2005): SJR 2.674 SNIP 3.285
- Scopus rating (2004): SJR 2.723 SNIP 3.044
- Scopus rating (2003): SJR 1.916 SNIP 2.302
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Nanoparticles, Nanomedicine, Theranostics, Nanotheranostics, Nuclear imaging, Diagnostics, Radionuclides, SPECT, PET, Cancer

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Micropatterning of Functional Conductive Polymers with Multiple Surface Chemistries in Register

A versatile procedure is presented for fast and efficient micropatterning of multiple types of covalently bound surface chemistry in perfect register on and between conductive polymer microcircuits. The micropatterning principle is applied to several types of native and functionalized PEDOT (poly(3,4-ethylenedioxythiophene)) thin films. The method is based on contacting PEDOT-type thin films with a micropatterned agarose stamp containing an oxidant (aqueous hypochlorite) and applying a nonionic detergent. Where contacted, PEDOT not only loses its conductance but is entirely removed, thereby locally revealing the underlying substrate. Surface analysis showed that the substrate surface chemistry was fully exposed and not affected by the treatment. Click chemistry could thus be applied to selectively modify re-exposed alkyne and azide functional groups of functionalized polystyrene substrates. The versatility of the method is illustrated by micropatterning cell-binding RGD-functionalized PEDOT on low cell-binding PMOXA (poly(2-methyl-2-oxazoline)) to produce cell-capturing microelectrodes on a cell nonadhesive background in a few simple steps. The method should be applicable to a wide range of native and chemically functionalized conjugated polymer systems.

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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
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Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.895 SNIP 1.356 CiteScore 4.55
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.177 SNIP 1.382 CiteScore 4.37
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.051 SNIP 1.357 CiteScore 4.42
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 2.148 SNIP 1.4
Nanoparticle-guided radiotherapy
The present invention relates to a method and nano-sized particles for image guided radiotherapy (IGRT) of a target tissue. More specifically, the invention relates to nano-sized particles comprising X-ray-imaging contrast agents in solid form with the ability to block x-rays, allowing for simultaneous or integrated external beam radiotherapy and imaging, e.g., using computed tomography (CT).

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Andresen, T. L. (Intern), Albrechtsen, M. (Ekstern)
Publication date: 2012

Publication information
Country: Denmark
IPC: A61B6/03
Patent number: WO2012007567
Date: 19/01/2012
Original language: English

Bibliographical note
DTU reference number: 92502-10
Main Research Area: Technical/natural sciences
Publication: Research › Patent – Annual report year: 2012

Particulate Systems for Targeting of Macrophages: Basic and Therapeutic Concepts
Particulate systems in the form of liposomes, polymeric micelles, polymeric nano- and microparticles, and many others offer a rational approach for selective delivery of therapeutic agents to the macrophage from different physiological portals of entry. Particulate targeting of macrophages and intracellular drug release processes can be optimized through modifications of the drug carrier physicochemical properties, which include hydrodynamic size, shape, composition and
PET imaging of liposomes labeled with an \(^{18}F\)-fluorocholesteryl ether probe prepared by automated radiosynthesis

A novel \(^{18}F\)-labeled cholesteryl ether lipid probe was prepared by synthesis of the corresponding mesylate, which was \(^{18}F\)-fluorinated by a \(^{18}F\)KF, Kryptofix-222, K2CO3 procedure. Fluorination was done for 10 minutes at 165 degrees C and took place with conversion between 3 and 17%, depending on conditions. Radiolabelling of the probe and subsequent in situ purification on SEP-Paks were done on a custom-built, fully automatic synthesis robot. Long-circulating liposomes were prepared by hydration (magnetic stirring) of a lipid film containing the radiolabeled probe, followed by fully automated extrusion through 100-nm filters. The \(^{18}F\)-labeled liposomes were injected into nude, tumor-bearing mice, and positron emission tomography (PET) scans were performed several times over 8 hours to investigate the in vivo biodistribution. Clear tumor accumulation, as well as hepatic and splenic uptake, was observed, corresponding to expected liposomal pharmacokinetics. The tumor accumulation 8 hours postinjection accounted for 2.25 +/- 0.23 (mean +/- standard error of the mean) percent of injected dose per gram (%ID/g), and the tumor-to-muscle ratio reached 2.20 +/- 0.24 after 8 hours, which is satisfactorily high for visualization of pathological lesions. Moreover, the blood concentration was still at a high level (13.9 +/- 1.5 %ID/g) at the end of the 8-hour time frame. The present work demonstrates the methodology for automated preparation of radiolabeled liposomes, and shows that \(^{18}F\)-labeled liposomes could be suitable as a methodology for visualization of tumors and obtaining short-term pharmacokinetics in vivo.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Center for Nuclear Technologies, The Hevesy Laboratory, Colloids and Biological Interfaces, Copenhagen University Hospital
Authors: Jensen, A. T. I. (Intern), Binderup, T. (Forskerdatabase), Andresen, T. L. (Intern), Kjær, A. (Ekstern), Rasmussen, P. (Intern)
tumor Neoplasms (MeSH) neoplastic disease, Rodentia Mammalia Vertebrata Chordata Animalia (Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates) - Muridae [86375] mouse common, 10-cholesteryloxy-1-[fluorine-18]fluoro-decanol diagnostic-drug pharmacokinetics, [fluorine-18]-fluorocholesteryl ether probe, fluorine-18-labeled cholesteryl ether diagnostic-drug pharmacokinetics, Kryptofix-222 23978-09-8, liposome, potassium carbonate 584-08-7, 10511, Biophysics - Bioengineering, 12504, Pathology - Diagnostic, 12512, Pathology - Therapy, 14004, Digestive system - Physiology and biochemistry, 15002, Blood - Blood and lymph studies, 15004, Blood - Blood cell studies, 22002, Pharmacology - General, 24003, Neoplasms - Immunology, 24004, Neoplasms - Pathology, clinical aspects and systemic effects, 34502, Immunology - General and methods, 34508, Immunology - Immunopathology, tissue immunology, Pharmacology, liver digestive system, spleen immune system, blood and lymphatics, automated radiosynthesis method laboratory techniques, custom-built fully automatic synthesis robot laboratory equipment, magnetic stirring method laboratory techniques, positron emission tomography imaging PET imaging imaging and microscopy techniques, diagnostic techniques, Biomaterials, Methods and Techniques, Pharmaceuticals, Tumor Biology
Polymeric gel nanoparticle pH sensors for Expanding the dynamic measurement range for intracellular pH-measurements by polymeric nanoparticle sensors

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Amphiphilic Polymers in Biological Sensing, Colloids and Biological Interfaces
Authors: Almdal, K. (Intern), Andresen, T. L. (Intern), Benjaminsen, R. V. (Intern), Christensen, N. M. (Intern), Henriksen, J. R. (Intern), Sun, H. (Intern)
Number of pages: 1
Publication date: 2012

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Main Research Area: Technical/natural sciences
Conference: 49th Nordic Polymer Days 2012, Copenhagen, Denmark, 29/05/2012 - 29/05/2012
Publication: Research - peer-review › Conference abstract in proceedings – Annual report year: 2012

Positron emission tomography evaluation of somatostatin receptor targeted (64)Cu-TATE-liposomes in a human neuroendocrine carcinoma mouse model.
Targeted therapeutic and diagnostic nanocarriers functionalized with antibodies, peptides or other targeting ligands that recognize over-expressed receptors or antigens on tumor cells have potential in the diagnosis and therapy of cancer. Somatostatin receptors (SSTRs) are over-expressed in a variety of cancers, particularly neuroendocrine tumors (NETs) and can be targeted with somatostatin peptide analogs such as octreotate (TATE). In the present study we investigate liposomes that target SSTR in a NET xenograft mouse model (NCI-H727) by use of TATE. TATE was covalently attached to the distal end of DSPE-PEG(2000) on PEGylated liposomes with an encapsulated positron emitter (64)Cu that can be utilized for positron emission tomography (PET) imaging. The biodistribution and pharmacokinetics of the (64)Cu-loaded PEGylated liposomes with and without TATE was investigated and their ability to image NETs was evaluated using PET. Additionally, the lipid accumulation and imaging capability was compared with free radiolabelled TATE peptide administered as (64)Cu-DOTA-TATE. The presence of TATE on the liposomes resulted in a significantly faster initial blood clearance in comparison to control-liposomes without TATE. PEGylated liposomes with or without TATE accumulated at significantly higher quantities in NETs (5.1±0.3 and 5.8±0.2 %ID/g, respectively) than the free peptide (64)Cu-DOTA-TATE (1.4±0.3 %ID/g) 24h post-injection. Importantly, (64)Cu-loaded PEGylated liposomes with TATE showed significantly higher tumor-to-muscle (T/M) ratio (12.7±1.0) than the control-liposomes without TATE (8.9±0.9) and the (64)Cu-DOTA-TATE free peptide (7.2±0.3). The higher T/M ratio of the PEGylated liposomes with TATE suggests some advantage of active targeting of NETs, although no absolute benefit in tumor accumulation over the non-targeted liposomes was observed. Collectively, these data showed that (64)Cu-loaded PEGylated liposomes with TATE conjugated to the surface could be promising new imaging agents for visualizing tumor tissue and especially NETs using PET.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics, The Hevesy Laboratory, Center for Nuclear Technologies, Physical and Biophysical Chemistry, SUND ph.d. skole, Copenhagen University Hospital
Authors: Petersen, A. L. (Intern), Binderup, T. (Forskerdatabase), Jølck, R. I. (Intern), Rasmussen, P. (Intern), Henriksen, J. R. (Intern), Pfeifer, A. K. (Forskerdatabase), Kjær, A. (Ekstern), Andresen, T. L. (Intern)
Pages: 254-263
Publication date: 2012
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Controlled Release
Volume: 160
Issue number: 2
ISSN (Print): 0168-3659
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
We present here a highly efficient and chemoselective liposome functionalization method based on oxime bond formation between a hydroxylamine and an aldehyde-modified lipid component. We have conducted a systematic and quantitative comparison of this new approach with other state-of-the-art conjugation reactions in the field. Targeted liposomes that recognize overexpressed receptors or antigens on diseased cells have great potential in therapeutic and diagnostic applications. However, chemical modifications of nanoparticle surfaces by postfunctionalization approaches are less effective than in solution and often not high-yielding. In addition, the conjugation efficiency is often challenging to characterize and therefore not addressed in many reports. We present here an investigation of PEGylated liposomes functionalized with a neuroendocrine tumor targeting peptide (TATE), synthesized with a variety of functionalities that have been used for surface conjugation of nanoparticles. The reaction kinetics and overall yield were quantified by HPLC. Reactions were conducted in solution as well as by postfunctionalization of liposomes in order to study the effects of steric
hindrance and possible affinity between the peptide and the liposome surface. These studies demonstrate the importance of hoosing the correct chemistry in order to obtain a quantitative surface functionalization of liposomes.

**General information**

State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Feldborg, L. N. (Intern), Jelck, R. I. (Intern), Andresen, T. L. (Intern)
Pages: 2444
Publication date: 2012
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Bioconjugate Chemistry
Volume: 23
Issue number: 12
ISSN (Print): 1043-1802
Ratings:
  - BFI (2018): BFI-level 1
  - BFI (2017): BFI-level 1
  - Web of Science (2017): Indexed Yes
  - BFI (2016): BFI-level 1
  - Scopus rating (2016): CiteScore 4.63 SJR 1.781 SNIP 1.071
  - Web of Science (2016): Indexed yes
  - BFI (2015): BFI-level 1
  - Scopus rating (2015): SJR 1.686 SNIP 1.073 CiteScore 4.64
  - Web of Science (2015): Indexed yes
  - BFI (2014): BFI-level 1
  - Scopus rating (2014): SJR 1.704 SNIP 1.177 CiteScore 4.85
  - BFI (2013): BFI-level 1
  - Scopus rating (2013): SJR 2.012 SNIP 1.208 CiteScore 5.12
  - ISI indexed (2013): ISI indexed yes
  - BFI (2012): BFI-level 1
  - Scopus rating (2012): SJR 2.069 SNIP 1.267 CiteScore 4.8
  - ISI indexed (2012): ISI indexed yes
  - Web of Science (2012): Indexed yes
  - BFI (2011): BFI-level 1
  - Scopus rating (2011): SJR 2.28 SNIP 1.292 CiteScore 5.26
  - ISI indexed (2011): ISI indexed yes
  - Web of Science (2011): Indexed yes
  - BFI (2010): BFI-level 1
  - Scopus rating (2010): SJR 2.252 SNIP 1.203
  - Web of Science (2010): Indexed yes
  - BFI (2009): BFI-level 1
  - Scopus rating (2009): SJR 2.03 SNIP 1.111
  - Web of Science (2009): Indexed yes
  - BFI (2008): BFI-level 1
  - Scopus rating (2008): SJR 2.154 SNIP 1.154
  - Web of Science (2008): Indexed yes
  - Scopus rating (2007): SJR 2.029 SNIP 1.284
  - Scopus rating (2006): SJR 1.693 SNIP 1.145
  - Scopus rating (2005): SJR 1.661 SNIP 1.159
  - Scopus rating (2004): SJR 1.346 SNIP 1.154
  - Scopus rating (2003): SJR 1.343 SNIP 1.251
  - Web of Science (2003): Indexed yes
  - Scopus rating (2002): SJR 1.087 SNIP 1.197
  - Web of Science (2002): Indexed yes
Quantitative Label-Free Cell Proliferation Tracking with a Versatile Electrochemical Impedance Detection Platform

Since the use of impedance measurements for label-free monitoring of cells has become widespread but still the choice of sensing configuration is not unique though crucial for a quantitative interpretation of data, we demonstrate the application of a novel custom multipotentiosstat platform to study optimal detection strategies. Electrochemical Impedance Spectroscopy (EIS) has been used to monitor and compare adhesion of different cell lines. HeLa cells and 3T3 fibroblasts have been cultured for 12 hours on interdigitated electrode arrays integrated into a tailor-made cell culture platform. Both vertical and coplanar interdigitated sensing configuration approaches have been used and compared on the same cell populations.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Bioanalytics, Colloids and Biological Interfaces, Politecnico di Milano
Authors: Caviglia, C. (Intern), Carminati, M. (Ekstern), Heiskanen, A. (Intern), Vergani, M. (Ekstern), Ferrari, G. (Ekstern), Sampietro, M. (Ekstern), Andresen, T. L. (Intern), Emnéus, J. (Intern)
Pages: 012029
Publication date: 2012
Main Research Area: Technical/natural sciences

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Journal: Journal of Physics: Conference Series (Online)
Volume: 407
Issue number: 1
ISSN (Print): 1742-6596
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.45 SJR 0.24 SNIP 0.383
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.24 SNIP 0.373 CiteScore 0.35
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.253 SNIP 0.344 CiteScore 0.32
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.231 SNIP 0.272 CiteScore 0.25
ISI indexed (2013): ISI indexed no
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.28 SNIP 0.354 CiteScore 0.33
ISI indexed (2012): ISI indexed no
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.292 SNIP 0.352 CiteScore 0.43
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.288 SNIP 0.344
Web of Science (2010): Indexed yes
very similar to the crosslinked micelles. In addition, crosslinked micelles (with 64Cu bound to CB-TE2A) were compared with non-crosslinked micelles. To our surprise, we found that the non-crosslinked micelles exhibited good stability in circulation and obtained a biodistribution as shown in the figure. It was concluded that there did not seem to be a significant difference between DOTA and CB-TE2A in-vivo.

Some of the prepared micelles were found to exhibit gross instabilities, especially with raised temperature, which allowed longer scans (up to 48 hours), which mirrors the duration of nanoparticle pharmacokinetics. It is a metal and must be attached to polymeric micelles by covalently conjugated chelators. DOTA and CB-TE2A are two such chelators, but DOTA is widely believed to be unstable in-vivo. DOTA and CB-TE2A were conjugated to triblock polymeric micelles in the shellregion. Here, they were thought to be shielded by the outer PEG-layer. The micelles were crosslinked in their coumarin-containing cores by exposure to UV light. Subsequently, the micelles were labeled with 64Cu, followed by removal of unspecifically bound 64Cu by EDTA. Good labeling efficiency was achieved with both chelators (40-70%).

Radiolabeling of liposomes and polymeric micelles with PET-isotopes

This thesis is divided into three separate chapters that can be read independently. Chapter 1 is a general introduction, touching upon liposomes and polymeric micelles and radiolabeling with 18F and 64Cu. Chapter 2 and 3 address two separate research projects, each described below. A complete reference list is compiled in the end, immediately after the three chapters. This is followed by the supplementary information, divided into appropriate sections. Finally, the two first-authored manuscripts are attached as appendices.

Chapter 1. The field of nanoparticulate drug delivery has been hailed as a revolution in modern therapeutics, especially in chemotherapy. A major reason is the ability of nanoparticles to accumulate in tumor tissue. Liposomes are the classic nanoparticle, consisting of a lipid membrane with an aqueous core. Polymeric micelles are made from amphiphilic detergent-like copolymers, that self-assemble in water. Therapy with nanoparticles is hampered by often poor tumor accumulation, combined with massive uptake by macrophages in the liver and spleen. For this reason, visualizing nanoparticle pharmacokinetics in-vivo is a valuable tool in the on-going research. Such visualization can be done by labeling with radio isotopes. Isotopes that emit positrons (PET-isotopes) can be detected by PET (positron emission tomography) technology, an accurate technique that has gained popularity in recent years. PET-isotopes of interest include 18F and 64Cu. In addition to being a research tool, radiolabeled nanoparticles hold promise as a radiopharmaceutical in themselves, as a means of imaging tumor tissue, aiding in diagnosis and surgery.

Chapter 2. A method for labeling liposomes with 18F (97% positron decay, T1/2 = 110 min) was investigated. 18F is widely available, but is hampered by a short half-life only allowing up to 8 hours scans. 18F must be covalently attached to components of the liposome. By binding to a lipid, it can be stably lodged in the membrane. A glycerolipid and a cholesteryl ether were synthesized with free primary alcohols and a series of their sulphonates (Ms, Ts, Tf) were prepared.

[18F]Radiofluorination of these substrates was performed on fully automated equipment using a classic Kryptofix222-mediated procedure in DMSO. Yields were poor, 3-17% depending on conditions. The [18F]fluorinated probes were purified in-situ on SEP-Paks. The cholesteryl ether mesylate performed best. This substrate was radiolabeled and formulated in long-circulating liposomes by drying the probe and the lipids together, followed by hydration by magnetic stirring. The liposomes were extruded through 100 nm filter on fully automated equipment. Animal studies were done in tumor-bearing mice, and PET-scans were performed over 8 hours. Clear tumor uptake, as well as hepatic and splenic uptake, was observed, corresponding to expected liposomal pharmacokinetics. Tumor uptake was quantifiable (tumor-muscle ratio at 8 h: 2.20), showing that the maximum scan duration with 18F is sufficient for visualizing tumor tissue. Because of the low [18F]radiofluorination yields obtained, we investigated ways of labeling lipophilic substrates in nonpolar solvents. This involved the transfer of [18F]HF gas from a solution of concentrated sulphuric acid into a receiving vial containing the substrate in toluene. A phosphazene base was present to bind [18F]HF and mediate fluorination. This procedure made it possible to fluorinate highly lipophilic substrates in 71% yields.

Chapter 3. Radiolabeling of polymeric micelles with 64Cu (18% positron decay, T1/2 = 12.7 h) was investigated. 64Cu allows longer scans (up to 48 hours), which mirrors the duration of nanoparticle pharmacokinetics. It is a metal and must be attached to polymeric micelles by covalently conjugated chelators. DOTA and CB-TE2A are two such chelators, but DOTA is widely believed to be unstable in-vivo. DOTA and CB-TE2A were conjugated to triblock polymeric micelles in the shell region. Here, they were thought to be shielded by the outer PEG-layer. The micelles were crosslinked in their coumarin-containing cores by exposure to UV light. Subsequently, the micelles were labeled with 64Cu, followed by removal of unspecifically bound 64Cu by EDTA. Good labeling efficiency was achieved with both chelators (40-70%). Some of the prepared micelles were found to exhibit gross instabilities, especially with raised temperature, which prevented their in-vivo use. Other micelles were stable and were investigated in xenografted mice. These micelles were 20-45 nm. They showed good tumor uptake (4-5 %ID/g, 48h) and limited uptake in liver (5-7 %ID/g, 48h) and spleen (3-6 %ID/g, 48h). It was concluded that there did not seem to be a significant difference between DOTA and CB-TE2A in-vivo. In addition, crosslinked micelles (with 64Cu bound to CB-TE2A) were compared with non-crosslinked micelles. To our surprise, we found that the non-crosslinked micelles exhibited good stability in circulation and obtained a biodistribution very similar to the crosslinked micelles.
Role of Synthetic and Dimensional Synthetic Organic Chemistry in Block Copolymer Micelle Nanosensor Engineering

This thesis investigated the role of amphiphilic triblock copolymer micelle nanomaterials in nanosensors, with emphasis on the synthesis of micelle particle sensors. The thesis is focused on the role of synthetic and dimensional synthetic organic chemistry in amphiphilic triblock core-shell-corona micelle based ratiometric fluorescence pH nanosensor fabrications. Two synthetic strategies such as post micelle modification and mixed micellisation (co-micellisation) were employed for pH nanosensor synthesis.

In the post micelle modification strategy, dimensional synthetic modifications on polymer micelles were performed. The structural potential of amphiphilic functional triblock copolymer selfassembly to provide regioselective functionalization and cross-linking was the key factor for this approach. Initially, functional amphiphilic triblock copolymers (functional unimers) were prepared by synthesis based on isolated macroinitiator ATRP of protected functional monomers. Selfassembly of these functional unimers in water resulted in functional core-shell-corona micelles. The functional micelles were stabilized by covalent cross-linking at the distinct functional shell or core domains of the micelle. The cross-linked micelles were converted into ratiometric pH nanosensors by conjugating pH sensitive and reference fluorophores at the shell region. The amphiphilic triblock copolymers, PEG-b-PAEMA-b-PS, PEG-b-PAEMA-b-PES and PEG-b-PAEMA-b-PCMA, were used for the preparation of functional micelles. Shell cross-linking on PEG-b-PAEMA-b-PS micelles was performed by amidation reactions between the amino groups of PAEMA blocks using a di-carboxylic acid cross-linker. Also a dendritic cross-linker based click chemistry was used to stabilize the PEG-b-PAEMA-b-PES micelle having click readied PES core. In another study, UV radiation was used to induce non-reversible and reversible photo core crosslinking of core-shell-corona functional micelles were also investigated. A PEG-b-PAEMA-b-PES micelle core was photo cross-linked by UV induced oxidative coupling between alkyne groups present at the micelle core. In a different system, reversible photo dimerization of coumarin was used to construct reversibly photo core cross-linked PEG-b-PAEMA-b-PCMA micelle. By conjugating pH sensitive and reference fluorophores at the shell regions of the shell and core crosslinked micelles, pH nanosensors were synthesized with sensitivity ranges that were appropriate for pH measurements in living cells. The sensitivity ranges of the nanosensors were simply altered by changing the fluorophores conjugated to the shell region. Nanosensors having targeting capabilities were synthesized by mixed micellisation or co-micellisation strategy. In this approach, the amphiphilic triblock copolymers synthesized by ATRP were further modified, and conjugated with targeting ligands and fluorophores. The co-micellisation of this functionalized amphiphilic triblock copolymers resulted in functionalized mixed micelle nanosensors. Post polymer modifications were easier to implement and quantify than post micelle modifications; hence the co-micellisation strategy provided more precise knowledge about the composition of the nanosensor.

Targeted non-cross-linked and targeted cross-linked ratiometric pH nanosensors were prepared by mixed micellisation or a co-micellisation strategy. Fluorophores and octaarginine conjugated amphiphilic triblock copolymers were synthesized by post-polymer modifications of PEG-b-PHEMA-b-PMMA and NH2-PEG-b-PHEMA-b-PMMA. Additionally, these functionalized triblocks resulted in octaarginine surface functionalized mixed micelle pH nanosensors. Similarly, a cross-linked cyclic RGD peptide targeted mixed micelle nanosensor was also prepared. The cyclic RGD peptide (cRGDfK) and fluorophores were conjugated to the amphiphilic triblock copolymers. NH2-PEG-b-PAzEMA-b-PMMA and PEG-b-PAzEMA-b-PMMA. Mixed micellisation of these functionalized unimers followed by dendritic click shell cross-linking resulted in a stable cRGDfK targeted mixed micelle pH nanosensor.

Thus, the engineerability of triblock core-shell-corona micelle was utilized for the synthesis of ratiometric pH nanosensor having desired pH sensitivity ranges.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology
Authors: Ek, P. K. (Intern), Andresen, T. L. (Intern), Almdal, K. (Intern)
Number of pages: 249
Publication date: 2012
Publication information
Publisher: Technical University of Denmark (DTU)
ISBN (Print): 978-87-995321-3-1
Original language: English
Main Research Area: Technical/natural sciences
Electronic versions:
Radiolabeling_of_liposomes.pdf
Publication: Research › Ph.D. thesis – Annual report year: 2013
Smart Surface Chemistries of Conducting Polymers: for Guiding Cell Behavior in Polymeric Microsystems

In this thesis we investigate post-polymerization covalent modifications of poly(3,4-dioxythiophene (PEDOT)-type conducting polymers. The aim of the modifications is to gain specific control of the interaction between the material and living mammalian cells. The use of “click-chemistry” to modify an azide-modified PEDOT, poly(3,4-(1-azidomethylene)-dioxythiophene) (PEDOT-N3), is studied in detail, and found to be a valuable approach. This is concluded, as we are able to obtain delicate control of cellular adhesion, by covalently attaching appropriate bio-functional molecules onto PEDOT-N3 thin film substrates.

Complementing these findings, we introduce a novel technique for fabricating surface chemical gradients on PEDOT-N3 substrates. The technique is based on applying “electro-click chemistry” to locally induce covalent modifications. Further supplementing these results, we develop a straightforward and in-expensive method for patterning conducting polymer thin films into microelectrodes, without losing control of the surface chemistry of the samples. On the contrary, the method provides direct control of the surface chemistry of both the fabricated micro-electrodes and the gaps between them. The method is based on locally removing PEDOT-type polymers to expose underlying non-conducting functional polymer substrates. Thereby, multifunctional substrates are obtained. By applying this method, we are able to fabricate allpolymer micro-systems with multiple types of localized functional (bio)-chemistries.

In the course of our studies, we find that PEDOT-N3 thin films undergo a significant yet reversible swelling when exposed to dimethyl-sulfoxide (DMSO). This swelling is found to be of practical use for controlling the reaction density and depth. This, for example, enables the fabrication of dense poly-ethylene-glycol-coatings of the conducting polymer substrates. These coatings render the substrates resistant to protein adsorption. Hence, the choice of solvent is found to be a key parameter for achieving functional post-polymerization modifications of PEDOT-N3.

The methods developed in this thesis are highly generic, and can therefore be applied for fabricating a diversity of microsystems based on conducting polymers, with multiple types of localized and highly bio-specific surfaces chemistries.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology
Authors: Lind, J. U. (Intern), Larsen, N. B. (Intern), Andresen, T. L. (Intern)
Number of pages: 256
Publication date: 2012
Synthesis and Stability Studies of α,α-Difluoro Ester Phospholipids

The synthesis of two new α,α-difluoro ester phospholipid conjugates is described and the stability of their liposomal formulations in three different aqueous buffers (pH 4.5, 7.5 and 8.5) has been investigated. The studies confirmed that α,α-difluoro esters are much more prone to hydrolysis when positioned close to the hydrophilic head group of phospholipids than when the functionality is placed in the lipophilic part of the bilayer in liposomes. This observation lends further support to the concept of protecting hydrolysable functionalities by formulation as part of the membrane of liposomes.
Synthesis of tocopheryl succinate phospholipid conjugates and monitoring of phospholipase A2 activity
Tocopheryl succinates (TOSs) are, in contrast to tocopherols, highly cytotoxic against many cancer cells. In this study the enzyme activity of secretory phospholipase A2 towards various succinate-phospholipid conjugates has been investigated. The synthesis of six novel phospholipids is described, including two TOS phospholipid conjugates. The studies revealed that the TOS conjugates are poor substrates for the enzyme whereas the phospholipids with alkyl and phenyl succinate moieties were hydrolyzed by the enzyme to a high extent.

General information
State: Published
Organisations: Department of Chemistry, Organic Chemistry, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Center for Nanomedicine and Theranostics
Authors: Pedersen, P. J. (Intern), Viart, H. M. (Intern), Melander, F. (Intern), Andresen, T. L. (Intern), Madsen, R. (Intern), Clausen, M. H. (Intern)
Pages: 3972-3978
Publication date: 2012
Main Research Area: Technical/natural sciences

Publication information
Journal: Bioorganic & Medicinal Chemistry
Volume: 20
Issue number: 13
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Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.978 SNIP 0.969 CiteScore 2.96
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.038 SNIP 1.058 CiteScore 3
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.01 SNIP 1.101 CiteScore 2.87
Targeting monocytes using a novel liposome based delivery system

General information
State: Published
Organisations: Department of Chemistry, Physical and Biophysical Chemistry, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces, Bioneer A/S
Authors: Jensen, S. S. (Ekstern), Johansen, P. T. (Ekstern), Wern, J. E. (Forskerdatabase), Gad, M. (Forskerdatabase), Henriksen, J. R. (Intern), Andresen, T. L. (Intern)
Pages: 769-770
Publication date: 2012
Conference: 3rd European Congress of Immunology, Glasgow, United Kingdom, 05/09/2012 - 05/09/2012
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Immunology
Functionalization of Self-Organized Nanoparticles for Biological Targeting and Active Drug Release

Functional nanomaterials have attracted much attention due to the unique properties of these nanoconstructs. In recognition of the huge potential within this field, much research has been devoted to develop sophisticated nanoparticles for medical diagnostics, sensors, contrast agents, vaccines and drug delivery. The objective of this PhD thesis was to expand the field of liposomal drug delivery by developing novel methods to efficiently functionalize and subsequently sensitize liposomes towards internal stimuli, such as matrix metalloproteinases. Initially, we investigated a novel method to post-functionalize and directly quantify the degree of conversion without prior purification. Based on Cu-free Click chemistry, quantitative conversion under very mild conditions was achieved using conditions which might be equally suited for other nanomaterials. Being able to rapidly determine ligand surface density after post-functionalization is highly important, to ensure batch-to-batch reproducibility and to ensure that the desired ligand surface density has been accomplished. A systematic study was furthermore conducted to elucidate the optimal post-functionalization chemistry, in addition to the importance of the relative position of the reactive functionalities. Surface conjugation reactions of octreotate by Michael addition, Click chemistry, Cu-free Click chemistry or oxime bond formation were investigated. From these studies it was evident that chemical reactions performed directly on the surface of functionalized liposomes were slower than the solution phase counterpart and often far from quantitative. The effect of active targeting with 64Cu octreotate liposomes targeting the somatostatin receptor 2 was evaluated to improve tumor bioimaging for diagnostic applications, using positron emission tomography. Targeted liposomes had a sufficient circulation profile to passively accumulate in tumor tissue of H727 xenografts in nude mice, but no statistical difference was detected in the tumor accumulation compared to control-liposomes. Despite this, the tumor-to-muscle ratio for targeted liposomes was significantly higher than for the control liposomes, which indicated that active targeting can improve tumor-to-muscle contrast, thus, improving bioimaging for diagnostic applications.

Finally, a novel drug delivery system based on charge-triggering of matrix metalloproteinase 2/9 sensitive PEGylated lipopeptides was designed. Methods to efficiently synthesize conjugates with the lipid-peptide-PEG motif were developed, and the use of these conjugates to shield positively charged liposomeand lipoplex formulations were described. The synthesized conjugates efficiently shielded cationic charges present at the surface of the nanoconstructs, resulting in anionic nanoparticles with long circulation properties in xenograft HT1080 tumor-bearing mice. Charge reversal by peptide hydrolysis was achieved in the presence of proteases, resulting in cationic particles which were readily internalized by cells in vitro. The use of matrix metalloproteinase sensitive liposomes and lipoplexes dramatically enhanced the cytotoxicity of known chemotherapeutics and facilitated effective gene transfection in vitro.

Concluding on the work of this PhD thesis, we managed to expand the field of functional nanomaterials by developing novel methods to conjugate and directly quantify the surface density of immobilized ligands. Furthermore, a unique drug delivery system based on charge shielding and subsequent charge triggering by matrix metalloproteinase 2 has been established. This system is currently being further investigated in vivo, in order to test the therapeutic capacity of the system.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces
Authors: Jølck, R. I. (Intern), Andresen, T. L. (Intern), Berg, R. H. (Intern)
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Main Research Area: Technical/natural sciences

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Syntese og karakterisering af lipidderivater til inkorporering i målrettede drug delivery systemer

General information
State: Published
Organisations: Department of Micro- and Nanotechnology
Authors: Andersen, S. (Intern), Andresen, T. L. (Intern)
Publication date: Sep 2011

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Place of publication: Kgs. Lyngby, Denmark
Publisher: Technical University of Denmark (DTU)
Biophysical Characterization of Interactions of α-helical amphipathic peptides with membranes

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Biomedical Tracers, Radiation Research Division, Risø National Laboratory for Sustainable Energy, Organic Chemistry, Department of Chemistry
Authors: Etzerodt, T. P. (Intern), Andresen, T. L. (Intern), Rasmussen, P. (Intern), Clausen, M. H. (Intern)
Publication date: Aug 2011

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Place of publication: Kgs. Lyngby, Denmark
Publisher: Technical University of Denmark (DTU)
Original language: English
Main Research Area: Technical/natural sciences
Source: orbit
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Publication: Research › Ph.D. thesis – Annual report year: 2011

64Cu loaded liposomes as positron emission tomography imaging agents
We have developed a highly efficient method for utilizing liposomes as imaging agents for positron emission tomography (PET) giving high resolution images and allowing direct quantification of tissue distribution and blood clearance. Our approach is based on remote loading of a copper-radionuclide (64Cu) using a new ionophore, 2-hydroxyquinoline, to carry 64Cu(II) across the membrane of preformed liposomes and deliver it to an encapsulated copper-chelator. Using this ionophore we achieved very efficient loading (95.5 ± 1.6%) and retention stability (>99%), which makes the 64Cu-liposomes highly applicable as PET imaging agents. We show the utility of the 64Cu-liposomes for quantitative in vivo imaging of healthy and tumor-bearing mice using PET. This remote loading method is a powerful tool for characterizing the in vivo performance of liposome based nanomedicine, and has great potential in diagnostic and therapeutic applications.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, Biomedical Tracers, Radiation Research Division, Risø National Laboratory for Sustainable Energy, University of Copenhagen
Authors: Petersen, A. L. (Intern), Binderup, T. (Ekstern), Rasmussen, P. (Intern), Henriksen, J. R. (Intern), Elema, D. R. (Intern), Kjaer, A. (Ekstern), Andresen, T. L. (Intern)
Pages: 2334-2341
Publication date: 2011
Main Research Area: Technical/natural sciences

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Scopus rating (2016): CiteScore 8.89 SJR 2.853 SNIP 1.879
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.425 SNIP 2.028 CiteScore 9.35
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.289 SNIP 2.186 CiteScore 9.31
A simple protocol for preparation of a liposomal vesicle with encapsulated plasmid DNA that mediate high accumulation and reporter gene activity in tumor tissue

The systemic delivery of gene therapeutics by non-viral methods has proven difficult. Transfection systems that are performing well in vitro have been reported to have disadvantageous properties such as rapid clearance and short circulation time often resulting in poor transfection efficiency when applied in vivo. Large unilaminary vesicles (LUV) with encapsulated nucleic acids designated stabilized plasmid- lipo-particle (SPLP) have showed promising results in terms of systemic stability and accumulation in tumor tissue due to the enhanced permeability and retention effect (EPR). We have developed a simple protocol for the research-scale preparation of SPLPs from commercially available reagents with high amounts of encapsulated plasmid DNA. The SPLPs show properties of promising accumulation in tumor tissue in comparison to other organs when intravenously injected into xenograft tumor-bearing nude mice. Although transcriptionally targeted suicide gene therapy was not achieved, the SPLPs were capable of mediating reporter gene transfection in subcutaneous flank tumors originating from human small cell lung cancer.

General information
Biodistribution, Gene delivery, Stabilized plasmid–lipid particle (SPLP), Suicide gene therapy, Liposome, Xenograft tumor model

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Source: orbit
Source-ID: 286969
Publication: Research - peer-review › Journal article – Annual report year: 2011

Biological studies of matrix metalloproteinase sensitive drug delivery systems

Cancer, which is a group of diseases characterized by cells with elevated replication rate and compromised DNA damage response, is often treated with cytotoxic drugs, chemotherapeutics, inducing DNA damage that results in cell death. The use of chemotherapeutics in the clinic, however, is limited due to severe side effects as a result of drug distribution to healthy tissues. To enhance efficacy of treatment and improve life quality of patients, tumor specific drug delivery strategies, such as liposome encapsulated drugs, which accumulate in tumor tissue, has gained increased attention. Several strategies have been developed to target tumor tissue, however, liposomal systems developed so far rely on passive drug diffusion or unspecific association between liposomes and cells after accumulation in tumor tissue, resulting in low drug efficiency. Therefore, development of rationally designed systems for delivery of drugs to specific tissues or cells utilizing biological knowledge of cancer tissue is getting increased attention.

In this thesis a novel matrix metalloproteinase-2 (MMP-2) sensitive poly-ethylene glycol (PEG) coated liposomal drug delivery system for treatment of cancer was developed. The system exploits the increased MMP-2 activity present in tumor tissue as a site-specific trigger of liposomal activation and controlled drug release after accumulation due to the enhanced permeability and retention effect. Enzymatic activity of MMP-2 results in shedding of a novel PEG coating, consisting of a negatively charged lipopeptide-PEG conjugates containing a MMP-2 cleavable peptide, which leads to cationic liposomes with enhanced ability to interact with negatively charged cell membranes.

Activation of the liposomal formulation developed here resulted in enhanced association of liposomes with cancer cells in vitro and in addition, the liposomes were not associated with phagocytosing cells in human blood without prior enzymatic activation. Encapsulation of oxaliplatin resulted in significantly increased toxicity compared with both the free drug and oxaliplatin encapsulated in PEG-coated neutral liposomes in vitro. High MMP-2 expression and activity was furthermore observed in human clinical samples of different types of cancer as well as in patient-derived colon cancer xenografts in mice, whereas only low concentrations of pro-enzymes were observed in healthy mouse tissues. These data support the use of MMP-2 as a trigger for liposomal activation in tumor tissue. Thus, this new strategy provides a promising system for specific delivery of encapsulated drugs and controlled release in tumor tissues, resulting in enhanced drug bioavailability and decreased systemic side effects.

In addition, we investigated the interaction between liposomes and cell populations in the blood, resulting in a novel liposomal system for specific targeting to CD14+ monocytes. Monocytes play an important role in inflammatory diseases, which are commonly treated with steroids, through their secretion of pro-inflammatory cytokines, such as TNF-alpha and IL-1beta. However, the use of these drugs has limitations due to side effects as a result of distribution to healthy tissues, which can be circumvented by the use of specific drug delivery systems targeting the inflammatory cells.

We found that liposome formulations with a positive charge between 7.5 - 10 % were optimal for targeting specific to monocytes and it was observed that this association rapidly occurred in freshly drawn human blood. It was furthermore revealed that the use of newly drawn blood was essential for mimicking in vivo conditions closest possible, as the monocytes ability to phagocyte liposomes relatively fast declined after the blood was drawn. In addition, we demonstrated that these formulations were not toxic to cells in vitro. The formulations described in this study enable specific targeting to
monocytes potentially resulting in enhanced inflammatory effect and decreased side effects of anti-inflammatory drugs.

**Catalyst-Free Conjugation and In Situ Quantification of Nanoparticle Ligand Surface Density Using Fluorogenic Cu-Free Click Chemistry**

A highly efficient method for functionalizing nanoparticles and directly quantifying conjugation efficiency and ligand surface density has been developed. Attachment of 3-azido-modified RGD-peptides to PEGylated liposomes was achieved by using Cu-free click conditions. Upon coupling a fluorophore is formed, which could be utilized to monitor conjugation efficiency and the obtained ligand surface density in situ, without prior purification.

**General information**

State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, Amphiphilic polymers in biological sensing Group
Authors: Jølck, R. I. (Intern), Sun, H. (Intern), Berg, R. H. (Intern), Andresen, T. L. (Intern)
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  - Scopus rating (2016): CiteScore 5.03 SJR 2.247 SNIP 1.046
  - BFI (2015): BFI-level 2
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  - BFI (2014): BFI-level 2
  - Scopus rating (2014): SJR 2.487 SNIP 1.219 CiteScore 5.51
  - BFI (2013): BFI-level 2
  - Scopus rating (2013): SJR 2.604 SNIP 1.239 CiteScore 5.68
  - ISI indexed (2013): ISI indexed yes
  - BFI (2012): BFI-level 2
  - Scopus rating (2012): SJR 2.884 SNIP 1.294 CiteScore 5.55
  - ISI indexed (2012): ISI indexed yes
  - Web of Science (2012): Indexed yes
Micrometer scale electrical circuits of PEDOT (poly(3,4-dioxythiophene)) were created by locally oxidizing PEDOT thin films with an agarose stamp containing the oxidizing agent NaOCl. The oxidized PEDOT was removed completely by applying detergents. The process was sufficiently mild that chemical groups on the underlying substrate, such as azides or
alkynes, were preserved for subsequent specific functionalization. Moreover entire PMOXA (poly(2-methyl-2-oxazoline)) films preventing cell binding could be hidden below the PEDOT and be re-exposed upon stamping, allowing for cell capturing microelectrodes on a cell non-adhesive background. Chemically functionalized PEDOT types permitted the introduction of multiple additional types of micropatterned chemistry.

**General information**

State: Published
Organisations: Department of Micro- and Nanotechnology, The Danish Polymer Centre, Department of Chemical and Biochemical Engineering, Eidgenössische Technische Hochschule
Authors: Lind, J. U. (Intern), Daugaard, A. E. (Intern), Andresen, T. L. (Intern), Acikgöz, C. (Ekstern), Textor, M. (Ekstern), Larsen, N. B. (Intern)
Publication date: 2011
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Surface modification, PEDOT, Conducting polymers, Micropatterning, Click chemistry
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Source: orbit
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Publication: Research - peer-review » Poster – Annual report year: 2011

**Elucidating the interplay between DNA-condensing and free polycations in gene transfection through a mechanistic study of linear and branched PEI**

In the present study we compare LPEI and BPEI characteristics related to DNA condensation and their role as free polycation chains in gene transfection. Using radioactive 32P labeled DNA, we investigated the effect of free PEI chains on the cellular uptake of polyplexes. Our investigations show different properties of BPEI and LPEI polyplexes in condensation and de-condensation processes as well as in cellular uptake, which was tightly correlated with transfection efficiency. In agreement with earlier reports we find all DNA to be condensed at N/P = 3. Further added PEI chains remain free in solution. We found that both the cellular uptake and gene transfection of BPEI polyplexes is much more efficient than LPEI polyplexes at a low N/P ratio of 3 (i.e., without free PEI chains). When N/P is high (10, with 7 portions of free PEI), the LPEI and BPEI polyplexes have similar transfection efficiency even though the cellular uptake of the LPEI polyplexes is significantly lower. In addition, we found that addition of free short or long PEI chains (2.5 and 25 kDa) leads to a comparable gene transfection efficiency.

**General information**

State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, Chinese University of Hong Kong
Authors: Dai, Z. (Intern), Gjetting, T. (Intern), Mattebjerg, M. A. (Intern), Wu, C. (Ekstern), Andresen, T. L. (Intern)
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Scopus rating (2016): CiteScore 8.89 SJR 2.853 SNIP 1.879
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.425 SNIP 2.028 CiteScore 9.35
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.289 SNIP 2.186 CiteScore 9.31
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.395 SNIP 2.185 CiteScore 9.02
ISI indexed (2013): ISI indexed yes
Our ability to engineer nanomaterials for biological and medical applications is continuously increasing, and nanomaterial designs are becoming more and more complex. One very good example of this is the drug delivery field where nanoparticle systems can be used to deliver drugs specifically to diseased tissue. In the early days, the design of the nanoparticles was relatively simple, but today we can surface functionalize and manipulate material properties to target diseased tissue and build highly complex drug release mechanisms into our designs. One of the most promising strategies in drug delivery is to use ligands that target overexpressed or selectively expressed receptors on the surface of diseased cells. To utilize this approach, it is necessary to control the chemistry involved in surface functionalization of nanoparticles and construct highly specific functionalities that can be used as attachment points for a diverse range of targeting ligands such as antibodies, peptides, carbohydrates and vitamins. In this review we provide an overview and a critical evaluation of the many strategies that have been developed for surface functionalization of nanoparticles and furthermore provide an overview of how these methods have been used in drug delivery systems.

Engineering Liposomes and Nanoparticles for Biological Targeting

Our ability to engineer nanomaterials for biological and medical applications is continuously increasing, and nanomaterial designs are becoming more and more complex. One very good example of this is the drug delivery field where nanoparticle systems can be used to deliver drugs specifically to diseased tissue. In the early days, the design of the nanoparticles was relatively simple, but today we can surface functionalize and manipulate material properties to target diseased tissue and build highly complex drug release mechanisms into our designs. One of the most promising strategies in drug delivery is to use ligands that target overexpressed or selectively expressed receptors on the surface of diseased cells. To utilize this approach, it is necessary to control the chemistry involved in surface functionalization of nanoparticles and construct highly specific functionalities that can be used as attachment points for a diverse range of targeting ligands such as antibodies, peptides, carbohydrates and vitamins. In this review we provide an overview and a critical evaluation of the many strategies that have been developed for surface functionalization of nanoparticles and furthermore provide an overview of how these methods have been used in drug delivery systems.
Evaluating Nanoparticle Sensor Design for Intracellular pH Measurements

Particle-based nanosensors have over the last decade been designed for optical fluorescent-based ratiometric measurements of pH in living cells. However, quantitative and time-resolved intracellular measurements of pH in endosomes and lysosomes using particle nanosensors is challenging and there is a need to improve measurement methodology. In the present paper, we have successfully carried out time resolved pH measurements in endosomes and lysosomes in living cells using nanoparticle sensors and show the importance of sensor choice for successful quantification.
We have studied two nanoparticle-based sensor systems that are internalized by endocytosis, and elucidated important factors in nanosensor design that should be considered in future development of new sensors. From our experiments it is clear that it is highly important to use sensors that have a broad measurement range, as erroneous quantification of pH is an unfortunate result when measuring pH too close to the limit of the sensitive range of the sensors. Triple-labeled nanosensors with a pH measurement range of 3.2-7.0, which was synthesized by adding two pH-sensitive fluorophores with different pKa to each sensor, seem to be a solution to some of the earlier problems found when measuring pH in the endosome-lysosome pathway.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Benjaminsen, R. V. (Intern), Sun, H. (Intern), Henriksen, J. R. (Intern), Christensen, N. M. (Intern), Almdal, K. (Intern), Andresen, T. L. (Intern)
Pages: 5864-5873
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Main Research Area: Technical/natural sciences

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Journal: A C S Nano
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 13.65 SJR 6.916 SNIP 2.65
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 5.923 SNIP 2.723 CiteScore 12.49
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 6.646 SNIP 2.735 CiteScore 13.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 7.131 SNIP 2.689 CiteScore 11.92
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Scopus rating (2011): SJR 6.204 SNIP 2.447 CiteScore 11.05
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
Scopus rating (2010): SJR 5.313 SNIP 2.065
Web of Science (2010): Indexed yes
Scopus rating (2009): SJR 4.098 SNIP 1.739
Web of Science (2009): Indexed yes
Scopus rating (2008): SJR 2.384 SNIP 1.012
Web of Science (2008): Indexed yes

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Expanding the dynamic measurement range for polymeric nanoparticle pH sensors

Conventional optical nanoparticle pH sensors that are designed for ratiometric measurements in cells have been based on utilizing one sensor fluorophore and one reference fluorophore in each nanoparticle, which results in a relatively narrow dynamic measurement range. This results in substantial challenges when conducting live cell measurements, which often leads to misleading results. In the present work we provide a simple solution to this problem.
Loading technique for preparing radionuclide containing nanoparticles

Source: US2012213698A

The present invention relates to a novel composition and method for loading delivery systems such as liposome compositions with radionuclides useful in targeted diagnostic and/or therapy of target site, such as cancerous tissue and, in general, pathological conditions associated with leaky blood vessels. The composition and methods of the invention find particular use in diagnosing and imaging cancerous tissue and, in general, pathological conditions associated with leaky blood vessels in a subject. The present invention provides a new diagnostic tool for the utilization of positron emission tomography (PET) imaging technique. One specific aspect of the invention is directed to a method of producing nanoparticles with desired targeting properties for diagnostic and/or radio-therapeutic applications.

Material properties in complement activation

Uncontrolled complement activation can induce many inflammatory and life threatening conditions. Accordingly, the role of complement in initiation of adverse reactions to polymers and nanoparticulate drug carriers is receiving increasing attention and has prompted extensive ‘structure-immune performance’ relationship studies in nanomedicine research at many fronts. The interaction between nanomaterials and the complement system is complex and regulated by inter-related factors that include nanoscale size, morphology and surface characteristics. Each of these parameters may affect complement activation differently and through different sensing molecules and initiation pathways. The importance of material properties in triggering complement is considered and mechanistic aspects discussed. Mechanistic understanding of complement events could provide rational approaches for improved material design and nanoengineering strategies for clinical medicine.
Membrane fusion of pH-sensitive liposomes – a quantitative study using giant unilamellar vesicles
This article presents a methodology for developing small-signal behavioral electromagnetic (EM) models of p-i-n photodiodes (PDs) for high-speed applications. The EM model includes RC bandwidth limitation effect and transit-time effect. The model is capable of accurately modeling arbitrary complex parasitics of PD chips. It can be used to predict the optical-to-electrical (O/E) response of PDs with various p-i-n junction structures in the frequency domain at the behavioral level. Compared to equivalent circuit models, EM models avoid developing complicated circuit network to represent complex chip parasitics as well as extracting parasitic values and provide straightforward access to EM characteristics of devices.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology
Authors: Trier, S. (Intern), Henriksen, J. R. (Intern), Andresen, T. L. (Intern)
Pages: 9027-9034
Publication date: 2011
Main Research Area: Technical/natural sciences

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Journal: Soft Matter
Volume: 7
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Scopus rating (2016): SJR 1.573 SNIP 1.219 CiteScore 3.7
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.67 SNIP 1.33 CiteScore 3.97
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.751 SNIP 1.267 CiteScore 4.11
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.745 SNIP 1.208 CiteScore 4.2
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.898 SNIP 1.155 CiteScore 3.96
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.006 SNIP 1.314 CiteScore 4.56
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.165 SNIP 1.376
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.516 SNIP 1.534
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.562 SNIP 1.392
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.482 SNIP 1.458
Web of Science (2007): Indexed yes
Polymeric gel nanoparticle pH sensors for intracellular measurements

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Ecosystems, Biosystems Division, Risø National Laboratory for Sustainable Energy
Authors: Almdal, K. (Intern), Andresen, T. L. (Intern), Benjaminsen, R. V. (Intern), Christensen, N. M. (Intern), Henriksen, J. R. (Intern), Sun, H. (Intern)
Publication date: 2011
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Main Research Area: Technical/natural sciences
Electronic versions:
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Publication: Research - peer-review › Journal article – Annual report year: 2011

Revisit complexation between DNA and polyethylenimine — Effect of length of free polycationic chains on gene transfection

Our revisit of the complexation between DNA and polyethylenimine (PEI) by using a combination of laser light scattering and gel electrophoresis confirms that nearly all the DNA chains are complexed with PEI to form polyplexes when the molar ratio of nitrogen from PEI to phosphate from DNA (N:P) reaches ~3, irrespective of the PEI chain length and solvent. Each solution mixture with N:P>3 contains two kinds of PEI chains: bound to DNA and free in the solution. It has been shown that it is those free PEI chains that play a vital role in promoting the gene transfection. The effects of the length of the bound and free chains on the gene transfection were respectively studied. Both short and long PEI chains are capable of condensing DNA completely at N:P~3 but long ones are ~102-fold more effective in the gene transfection, apparently due to their fast endocytosis and intracellular trafficking. The cellular uptake kinetics studied by flow cytometry reveals that long free chains increase the uptake rate constant of the DNA/PEI complexes. In the intracellular pathway, they are able to prevent the development of the later endolysosomes, and facilitate the subsequent release of the polyplexes from the endosomes. Our result shows that the “proton sponge” effect is not dominant because the shut-down of the proton pump only partially attenuates the transfection efficiency. A possible mechanism is speculated and presented.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Chinese University of Hong Kong
Authors: Yue, Y. (Ekstern), Jin, F. (Ekstern), Deng, R. (Ekstern), Cai, J. (Ekstern), Dai, Z. (Ekstern), Lin, M. C. (Ekstern), Kung, H. (Ekstern), Mattebjerg, M. A. (Intern), Andresen, T. L. (Intern), Wu, C. (Ekstern)
Pages: 143-151
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Conference: Symposium on Innovative Polymers for Controlled Delivery, 01/01/2011
Main Research Area: Technical/natural sciences

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Secretory Phospholipase A(2) Activity toward Diverse Substrates

We have studied secretory phospholipase A(2)-IIA (sPLA(2)) activity toward different phospholipid analogues by performing biophysical characterizations and molecular dynamics simulations. The phospholipids were natural substrates, triple alkyl phospholipids, a prodrug anticancer etherlipid, and an inverted ester. The latter were included to study head group-enzyme interactions. Our simulation results show that the lipids are optimally placed into the binding cleft and that water molecules can freely reach the active site through a well-defined pathway; both are indicative that these substrates are efficiently hydrolyzed, which is in good agreement with our experimental data. The phospholipid analogue with three alkyl side chains forms aggregates of different shapes with no well-defined sizes due to its cone-
shape structure. Phosphatidylglycerol and phosphatidylcholine head groups interact with specific charged residues, but relatively large fluctuations are observed, suggesting that these interactions are not necessarily important for stabilizing substrate binding to the enzyme.

**General information**

State: Published
Organisations: Physical Chemistry, Department of Chemistry, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Madsen, J. J. (Intern), Linderoth, L. (Intern), Subramanian, A. K. (Intern), Andresen, T. L. (Intern), Peters, G. H. (Intern)
Pages: 6853-6861
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Scopus rating (2016): CiteScore 3.03 SJR 1.348 SNIP 1.02
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.367 SNIP 1.096 CiteScore 3.25
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.44 SNIP 1.14 CiteScore 3.28
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.494 SNIP 1.2 CiteScore 3.53
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.92 SNIP 1.251 CiteScore 3.66
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.78 SNIP 1.226 CiteScore 3.62
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.849 SNIP 1.214
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.232 SNIP 1.349
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.543 SNIP 1.381
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.346 SNIP 1.282
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.369 SNIP 1.415
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.275 SNIP 1.474
Selective Acylation Enhances Membrane Charge Sensitivity of the Antimicrobial Peptide Mastoparan-X

The partitioning of the wasp venom peptide mastoparan-X (MPX) into neutral and negatively charged lipid membranes has been compared with two new synthetic analogs of MPX where the Nα-terminal of MPX was acylated with propanoic acid (PA) and octanoic acid (OA). The acylation caused a considerable change in the membrane partitioning properties of MPX and it was found that the shorter acylation with PA gave improved affinity and selectivity toward negatively charged membranes, whereas OA decreased the selectivity. Based on these findings, we hypothesize that minor differences in the embedding and positioning of the peptide in the membrane caused by either PA or OA acylation play a critical role in the fine-tuning of the effective charge of the peptide and thereby the fine-tuning of the peptide's selectivity between neutral and negatively charged lipid membranes. This finding is unique compared to previous reports where peptide acylation enhanced membrane affinity but also resulted in impaired selectivity. Our result may provide a method of enhancing selectivity of antimicrobial peptides toward bacterial membranes due to their high negative charge—a finding that should be investigated for other, more potent antimicrobial peptides in future studies.
Solvent Composition Directing Click-Functionalization at the Surface or in the Bulk of Azide-Modified PEDOT

Thin films of the conducting polymer poly(3,4-(1-azidomethylethylene)dioxythiophene) tosylate (PEDOT−N3) can be functionalized by reaction with alkynated reagents in aqueous solutions. Reaction in pure water resulted in surface specific modification of PEDOT−N3 films, whereas both surface and bulk reaction was achieved in solvent mixtures of water and DMSO. These reaction patterns were confirmed by a combination of AFM and XPS measurements on the front- and backside of the film. The phenomenon is attributed to a strong dependence of the swelling of PEDOT−N3 on the solvent mixture used. Liquid AFM studies showed increasing film thickness with increasing DMSO content, with the measured thickness in pure DMSO being >250% of the thickness in pure water. A similar, but less pronounced, behavior was observed for unmodified poly(3,4-ethylenedioxythiophene) tosylate (PEDOT). High-density grafting of a number of alkynated compounds onto PEDOT−N3 was achieved via controlled swelling of the polymer. In particular, grafting of alkynated poly(ethylene glycol) (PEG) was optimized to minimize protein adsorption to the conductive polymer surface.
Intermediate swelling of PEDOT−N3 during the reaction, using ~50% DMSO, resulted in the formation of a dense PEG surface layer with low protein adhesiveness without adversely affecting the conductive properties of the film.

**General information**

State: Published

Organisations: Polymer Microsystems for Cell Processing Group, Polymer Micro and Nano Engineering Section, Department of Micro- and Nanotechnology, The Danish Polymer Centre, Department of Chemical and Biochemical Engineering, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section

Authors: Lind, J. U. (Intern), Hansen, T. S. (Intern), Daugaard, A. E. (Intern), Hvilsted, S. (Intern), Andresen, T. L. (Intern), Larsen, N. B. (Intern)

Pages: 495-501

Publication date: 2011

Main Research Area: Technical/natural sciences

**Publication information**

Journal: Macromolecules

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Ratings:

BFI (2017): BFI-level 2

Web of Science (2017): Indexed yes

BFI (2016): BFI-level 2

Scopus rating (2016): CiteScore 5.76 SJR 2.557 SNIP 1.507

Web of Science (2016): Indexed yes

BFI (2015): BFI-level 2

Scopus rating (2015): SJR 2.407 SNIP 1.638 CiteScore 5.82

Web of Science (2015): Indexed yes

BFI (2014): BFI-level 2

Scopus rating (2014): SJR 2.534 SNIP 1.721 CiteScore 5.83

Web of Science (2014): Indexed yes

BFI (2013): BFI-level 2

Scopus rating (2013): SJR 2.576 SNIP 1.754 CiteScore 6.09

ISI indexed (2013): ISI indexed yes

Web of Science (2013): Indexed yes

BFI (2012): BFI-level 2

Scopus rating (2012): SJR 2.779 SNIP 1.58 CiteScore 5.35

ISI indexed (2012): ISI indexed yes

Web of Science (2012): Indexed yes

BFI (2011): BFI-level 2

Scopus rating (2011): SJR 2.556 SNIP 1.593 CiteScore 5.15

ISI indexed (2011): ISI indexed yes

Web of Science (2011): Indexed yes

BFI (2010): BFI-level 2

Scopus rating (2010): SJR 2.51 SNIP 1.51

Web of Science (2010): Indexed yes

BFI (2009): BFI-level 2

Scopus rating (2009): SJR 2.962 SNIP 1.533

Web of Science (2009): Indexed yes

BFI (2008): BFI-level 2

Scopus rating (2008): SJR 2.819 SNIP 1.54

Web of Science (2008): Indexed yes

Scopus rating (2007): SJR 3.102 SNIP 1.613

Web of Science (2007): Indexed yes

Scopus rating (2006): SJR 2.987 SNIP 1.714

Web of Science (2006): Indexed yes

Scopus rating (2005): SJR 2.579 SNIP 1.654
Thermodynamic and biological evaluation of a thrombin binding aptamer modified with several unlocked nucleic acid (UNA) monomers and a 2'‐C‐piperazino‐UNA monomer

Thrombin binding aptamer is a DNA 15-mer which forms a G-quadruplex structure and possess promising anticoagulant properties due to specific interactions with thrombin. Herein we present the influence of a single 2'‐C‐piperazino‐UNA residue and UNA residues incorporated in several positions on thermodynamics, kinetics and biological properties of the aptamer. 2′-C-Piperazino-UNA is characterized by more efficient stabilization of quadruplex structure in comparison to regular UNA and increases thermodynamic stability of TBA by 0.28–0.44 kcal/mol in a position depending manner with retained quadruplex topology and molecularity. The presence of UNA-U in positions U3, U7, and U12 results in the highest stabilization of G-quadruplex structure (ΔΔG37°=−1.03 kcal/mol). On the contrary, the largest destabilization mounting to 1.79 kcal/mol was observed when UNA residues were placed in positions U7, G8, and U9. Kinetic studies indicate no strict correlation between thermodynamic stability of modified variants and their binding affinity to thrombin. Most of the studied variants bind thrombin, albeit with decreased affinity in reference to unmodified TBA. Thrombin time assay studies indicate three variants as being as potent as TBA in fibrin clotting inhibition.
Thermodynamic profiling of Peptide membrane interactions by isothermal titration calorimetry: a search for pores and micelles.

Antimicrobial peptides are known to interact strongly with negatively charged lipid membranes, initially by peripheral insertion of the peptide into the bilayer, which for some antimicrobial peptides will be followed by pore formation, and successive solubilization of the membranes resulting in mixed peptide-lipid micelles. We have investigated the mode of action of the antimicrobial peptide mastoparan-X using isothermal titration calorimetry (ITC) and cryo-transmission electron microscopy (cryo-TEM). The results show that mastoparan-X induces a range of structural transitions of POPC/POPG (3:1) lipid membranes at different peptide/lipid ratios. It has been established that ITC can be used as a fast method for localizing membrane transitions and when combined with DLS and cryo-TEM can elucidate structural changes, including the threshold for pore formation and micellization. Cryo-TEM was employed to confirm the structural changes associated with the thermodynamic transitions found by ITC. The pore-formation process has furthermore been investigated in detail and the thermodynamic parameters of pore formation have been characterized using a system-specific temperature where the enthalpy of peptide partitioning becomes zero (T(zero)). This allows for an exclusive study of the pore-formation process. The use of ITC to find T(zero) allows for characterization of the thermodynamic parameters of secondary processes on lipid membranes.

General information
Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: The challenges ahead

Since their introduction, poly(ethylene glycol)-phospholipid (PEG-PL) conjugates have found many applications in design and engineering of nanosized delivery systems for controlled delivery of pharmaceuticals especially to non-macrophage targets. However, there are reports of idiosyncratic reactions to certain PEG-PL engineered nanomedicines in both experimental animals and man. These reactions are classified as pseudoallergy and may be associated with cardiopulmonary disturbance and other related symptoms of anaphylaxis. Recent studies suggest that complement activation may be a contributing, but not a rate limiting factor, in eliciting hypersensitivity reactions to such nanomedicines in sensitive individuals. This is rather surprising since PEGylated structures are generally assumed to suppress protein adsorption and blood opsonization events including complement. Here, we examine the molecular basis of complement activation by PEG-PL engineered nanomedicines and carbon nanotubes and discuss the challenges ahead.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, University of Brighton, Applied Sciences University, University of Copenhagen, Semmelweis University
Authors: Moghimi, S. (Ekstern), Andersen, A. J. (Intern), Hashemi, S. (Ekstern), Lettiero, B. (Ekstern), Ahmadvand, D. (Ekstern), Hunter, A. (Ekstern), Andresen, T. L. (Intern), Hamad, I. (Ekstern), Szebeni, J. (Ekstern)
Pages: 175-181
Publication date: 2010
Conference: Nanomedicine and Drug Delivery, Indianapolis, United States, 05/10/2009 - 05/10/2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Controlled Release
Volume: 146
Issue number: 2
ISSN (Print): 0168-3659
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 7.56 SJR 2.393 SNIP 1.84
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.725 SNIP 2.08 CiteScore 8.11
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.416 SNIP 2.092 CiteScore 6.86
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.416 SNIP 2.044 CiteScore 6.31
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.417 SNIP 2.061 CiteScore 5.84
Complex Surface Concentration Gradients by Stenciled "Electro Click Chemistry"

Complex one- or two-dimensional concentration gradients of alkynated molecules are produced on azidized conducting polymer substrates by stenciled "electro click chemistry". The latter describes the local electrochemical generation of catalytically active Cu(I) required to complete a "click reaction" between alkynes and azides at room temperature. A stencil on the counter electrode defines the shape and multiplicity of the gradient(s) on the conducting polymer substrate, while the specific reaction conditions control gradient steepness and the maximum concentration deposited. Biologically active ligands including cell binding peptides are patterned in gradients by this method without losing their biological function or the conductivity of the polymer.

General information

State: Published
Organisations: Department of Micro- and Nanotechnology, Polymer Microsystems for Cell Processing Group, Polymer Micro and Nano Engineering Section, The Danish Polymer Centre, Department of Chemical and Biochemical Engineering, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section
Authors: Hansen, T. S. (Intern), Lind, J. U. (Intern), Daugaard, A. E. (Intern), Hvilsted, S. (Intern), Andresen, T. L. (Intern), Larsen, N. B. (Intern)
Pages: 16171-16177
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information

Journal: Langmuir
Volume: 26
Issue number: 20
ISSN (Print): 0743-7463
Ratings:
BFI (2017): BFI-level 2
Distinct Polymer Architecture Mediates Switching of Complement Activation Pathways at the Nanosphere-Serum Interface: Implications for Stealth Nanoparticle Engineering

Nanoparticles with surface projected polyethyleneoxide (PEO) chains in 'mushroom-brush' and "brush" configurations display stealth properties in systemic circulation and have numerous applications in site specific targeting for controlled drug delivery and release as well as diagnostic Imaging. We report on the "structure-activity" relationship pertaining to surface immobilized PEO of various configurations on model nanoparticles, and the initiation of complement cascade, which is the most ancient component of innate human immunity, and its activation may induce clinically significant adverse reactions in some individuals. Conformational states of surface chains, arising from the block copolymer poloxamine 908 adsorption, on polystyrene nanoparticles trigger complement activation differently. Alteration of copolymer architecture on nanospheres from mushroom to brush configuration not only switches complement activation from C1q dependent classical to lectin pathway but also reduces the level of generated complement activation, products C4d, Bb, C5a, and SC5b-9. Also changes in adsorbed polymer configuration trigger alternative pathway activation differently and through different initiators. Notably, the role properdin mediated activation of alternative pathway was only restricted to particles displaying PEO chains in a transition mushroom-brush configuration. Since nanoparticle-mediated complement activation is of clinical concern our findings provide a rational basis for improved surface engineering and design of immunologically safer stealth and targetable nanosystems with polymers for use in clinical medicine.

General information

State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Hamad, I. (Ekstern), Al-Hanbali, O. (Ekstern), Hunter, A. (Ekstern), Rutt, K. (Ekstern), Andresen, T. L. (Intern), Moghimi, S. (Ekstern)
Pages: 6629-6638
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information

Journal: A C S Nano
Volume: 4
Issue number: 11
ISSN (Print): 1936-0851
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 13.65 SJR 6.916 SNIP 2.65
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 5.923 SNIP 2.723 CiteScore 12.49
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 6.646 SNIP 2.735 CiteScore 13.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 7.131 SNIP 2.689 CiteScore 11.92
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Scopus rating (2011): SJR 6.204 SNIP 2.447 CiteScore 11.05
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
Scopus rating (2010): SJR 5.313 SNIP 2.065
Web of Science (2010): Indexed yes
Scopus rating (2009): SJR 4.098 SNIP 1.739
Enzyme-triggered nanomedicine: Drug release strategies in cancer therapy (Invited Review)

Nanomedicine as a field has emerged from the early success of nanoparticle-based drug delivery systems, in particular for treatment of cancer, and the advances made in nano- and biotechnology over the past decade. A prerequisite for nanoparticle-based drug delivery systems to be effective is that the drug payload is released at the target site. A large number of drug release strategies have been proposed that can be classified into certain areas. The simplest and most successful strategy so far, probably due to relative simplicity, is based on utilizing certain physico-chemical characteristics of drugs to obtain a slow drug leakage from the formulations after accumulation in the cancerous site. However, this strategy is only applicable to a relatively small range of drugs and cannot be applied to biologicals. Many advanced drug release strategies have therefore been investigated. Such strategies include utilization of heat, light and ultrasound sensitive systems and in particular pH sensitive systems where the lower pH in endosomes induces drug release. Highly interesting are enzyme sensitive systems where overexpressed disease-associated enzymes are utilized to trigger drug release. The enzyme-based strategies are particularly interesting as they require no prior knowledge of the tumour localization. The basis of this review is an evaluation of the current status of drug delivery strategies focused on triggered drug release by disease-associated enzymes. We limit ourselves to reviewing the liposome field, but the concepts and conclusions are equally important for polymer-based systems.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, Purdue University, Danish Technological Institute
Authors: Andresen, T. L. (Intern), Thompson, D. H. (Ekstern), Kaasgaard, T. (Ekstern)
Pages: 353-363
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Molecular Membrane Biology
Volume: 27
Issue number: 7
ISSN (Print): 0968-7688
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.068 SNIP 0.518 CiteScore 2.1
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.061 SNIP 0.513 CiteScore 1.99
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.847 SNIP 0.456 CiteScore 1.71
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.109 SNIP 0.644 CiteScore 2.72
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.443 SNIP 0.751 CiteScore 2.95
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.747 SNIP 0.715 CiteScore 2.55
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Flourescent hydrogel particles in the Nanometer Range for Detection of Metabolites in living cells

General information
State: Published
Organisations: Amphiphilic polymers in biological sensing Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces Group
Authors: Almdal, K. (Intern), Andresen, T. L. (Intern), Sun, H. (Intern), Benjaminsen, R. V. (Intern), Arleth, L. (Ekstern)
Publication date: 2010
Event: Poster session presented at Frontiers in Polymer Science : International Symposium Celebrating the 50th anniversary of the Journal Polymer, Mainz, Germany.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 259564
Publication: Research - peer-review › Poster – Annual report year: 2010

Isomerization of all-(E)-Retinoic Acid Mediated by Carbodiimide Activation - Synthesis of ATRA Ether Lipid Conjugates

Treatment of the lysolipid 1-O-hexadecyl-sn-phosphatidylcholine with all-(E)-retinoic acid, DCC and DMAP resulted in poor acylation and caused (Z)/(E) isomerization of the alpha-beta double bond. In the presence of a proton source, the carbodiimide-activated all-(E)-retinoic acid undergoes fast isomerization to give a final mixture of (13E)/(13Z) isomers in a 3:1 ratio. Similar treatment of (13Z)-retinoic acid leads to the same isomer ratio. The isomerization was circumvented successfully by using a Mitsunobu reaction, which provided an efficient synthesis of all-(E)-retinoic acid sn-2-conjugated to phosphatidylcholine and phosphatidylglycerol etherlipids.

General information
State: Published
Organisations: Department of Chemistry, Organic Chemistry, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Christensen, M. S. (Intern), Pedersen, P. J. (Intern), Andresen, T. L. (Intern), Madsen, R. (Intern), Clausen, M. H. (Intern)
Pages: 719-724
Publication date: 2010
Main Research Area: Technical/natural sciences
Publication information
Issue number: 4
ISSN (Print): 1434-193X
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.74 SJR 1.133 SNIP 0.653
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.198 SNIP 0.758 CiteScore 2.88
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.181 SNIP 0.767 CiteScore 2.96
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.292 SNIP 0.796 CiteScore 2.96
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.471 SNIP 0.811 CiteScore 2.93
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.536 SNIP 0.857 CiteScore 3.2
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.572 SNIP 0.785
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.497 SNIP 0.778
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.652 SNIP 0.759
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.711 SNIP 0.84
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.505 SNIP 0.849
Scopus rating (2005): SJR 1.246 SNIP 0.763
Scopus rating (2004): SJR 1.2 SNIP 0.81
Scopus rating (2003): SJR 1.19 SNIP 0.802
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.382 SNIP 0.829
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.159 SNIP 0.816
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.192 SNIP 1.048
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.877 SNIP 0.976
Original language: English
Retinoic acid, Acylation, Isomerization, Mitsunobu reaction, Phospholipids
DOIs: 10.1002/ejoc.200901128
Source: orbit
Source-ID: 263379
Publication: Research - peer-review › Journal article – Annual report year: 2010
Liposomal cancer therapy: exploiting tumor characteristics

Importance of the field: More than 10 million people worldwide are diagnosed with cancer each year, and the development of effective cancer treatments is consequently of great significance. Cancer therapy is unfortunately hampered by severe dose-limiting side effects that reduce the efficacy of cancer treatments. In the search for more effective cancer treatments, nanoparticle-based drug delivery systems, such as liposomes, that are capable of delivering their drug payload selectively to cancer cells are among the most promising approaches. Areas covered in this review: This review provides an overview of current strategies for improving the different stages of liposomal cancer therapy, which involve transporting drug-loaded liposomes through the bloodstream, increasing tumor accumulation, and improving drug release and cancer cell uptake after accumulation at the tumor target site. What the reader will gain: The review focuses on strategies that exploit characteristic features of solid tumors, such as abnormal vasculature, overexpression of receptors and enzymes, as well as acidic and thiolytic characteristics of the tumor microenvironment. Take home message: It is concluded that the design of new liposomal drug delivery systems that better exploit tumor characteristic features is likely to result in more efficacious cancer treatments.

General information
State: Published
Organisations: Department of Chemistry, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Kaasgaard, T. (Intern), Andresen, T. L. (Intern)
Pages: 225-243
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Expert Opinion on Drug Delivery
Volume: 7
Issue number: 2
ISSN (Print): 1742-5247
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 1.469 SNIP 1.45 CiteScore 5.49
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.439 SNIP 1.449 CiteScore 5.06
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.439 SNIP 1.423 CiteScore 4.78
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.507 SNIP 1.363 CiteScore 4.85
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.882 SNIP 1.577 CiteScore 5.48
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.683 SNIP 1.323 CiteScore 5.03
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.542 SNIP 1.237
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.12 SNIP 1.024
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.937 SNIP 1.008
Scopus rating (2007): SJR 1.068 SNIP 0.857
Scopus rating (2006): SJR 0.583 SNIP 0.581
Liposomal Formulation of Retinoids Designed for Enzyme Triggered Release

The design of retinoid phospholipid prodrugs is described based on molecular dynamics simulations and cytotoxicity studies of synthetic retinoid esters. The prodrugs are degradable by secretory phospholipase A(2) IIA and have potential in liposomal drug delivery targeting tumors. We have synthesized four different retinoid phospholipid prodrugs and shown that they form particles in the liposome size region with average diameters of 94-118 nm. Upon subjection to phospholipase A(2), the lipid prodrugs were hydrolyzed, releasing cytotoxic retinoids and lysolipids. The formulated lipid prodrugs displayed IC50 values in the range of 3-19 μM toward HT-29 and Colo205 colon cancer cells in the presence of phospholipase A(2), while no significant cell death was observed in the absence of the enzyme.

General Information

State: Published
Organisations: Organic Chemistry, Department of Chemistry, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Number of pages: 11
Pages: 3782-3792
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication Information

Journal: Journal of Medicinal Chemistry
Volume: 53
Issue number: 9
ISSN (Print): 0022-2623
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.06
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.529 SNIP 1.631 CiteScore 5.66
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.259 SNIP 1.693 CiteScore 5.55
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.293 SNIP 1.78 CiteScore 5.65
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.33 SNIP 1.756 CiteScore 5.52
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.259 SNIP 1.706 CiteScore 5.48
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.99 SNIP 1.586
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Polycation cytotoxicity: a delicate matter for nucleic acid therapy-focus on polyethylenimine

This article provides a critical assessment of the major challenges facing nucleic acid therapeutics, focusing on the safety and efficacy of delivery strategies using synthetic polycations and particularly polyethylenimines. Deficiencies in the field and avenues for further research are identified that could help with design and selection of an expanded and improved library of safer polycationic vectors for clinical gene therapy and RNA interference delivery.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Parhamifar, L. (Ekstern), Larsen, A. K. (Ekstern), Hunter, A. C. (Ekstern), Andresen, T. L. (Intern), Moghimi, S. M. (Ekstern)
Pages: 4001-4009
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Soft Matter
Volume: 6
Issue number: 17
ISSN (Print): 1744-683X
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.573 SNIP 1.219 CiteScore 3.7
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.67 SNIP 1.33 CiteScore 3.97
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.751 SNIP 1.267 CiteScore 4.11
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.745 SNIP 1.208 CiteScore 4.2
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Prostaglandin phospholipid conjugates with unusual biophysical and cytotoxic properties
The synthesis of two secretory phospholipase A(2) IIA sensitive 15-deoxy-Delta(12,14)-prostaglandin J(2) phospholipid conjugates is described and their biophysical and biological properties are reported. The conjugates spontaneously form particles in the liposome size region upon dispersion in an aqueous buffer and both phospholipids are hydrolyzed by phospholipase A(2), but with different conversion rates and extent of hydrolysis. The cytotoxicity was evaluated in HT-29 and Colo205 cells and the conjugates induced cell death in the presence of phospholipase A(2) and surprisingly also in the absence of the enzyme.

General information
State: Published
Organisations: Organic Chemistry, Department of Chemistry, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, LiPlasome Pharma ApS
Authors: Pedersen, P. J. (Intern), Adolph, S. K. (Ekstern), Andresen, T. L. (Intern), Madsen, M. W. (Ekstern), Madsen, R. (Intern), Clausen, M. H. (Intern)
Pages: 4456-4458
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Bioorganic & Medicinal Chemistry Letters
Volume: 20
Issue number: 15
ISSN (Print): 0960-894X
Ratings:
Solid-Phase Synthesis of PEGylated Lipopeptides Using Click Chemistry

A versatile methodology for efficient synthesis of PEGylated lipopeptides via CuAAC "Click" conjugation between alkyne-bearing solid-supported lipopeptides and azido-functionalized PEGs is described. This new and very robust method offers a unique platform for synthesizing PEGylated lipopeptides with a high level of complexity. These molecules, obtained in a single purification step, are ideally suited for functionalization of solid-supported lipid bilayers and liposomal drug delivery systems and are particularly valuable in enzyme activation strategies.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Synthesis and Characterization of Self-organized Cross-linked Nanoparticle Sensors for Intracellular pH Measurements

General information
State: Published
Organisations: Department of Micro- and Nanotechnology
Authors: Ek, P. K. (Intern), Christensen, N. M. (Intern), Benjaminsen, R. V. (Intern), Andresen, T. L. (Intern)
Number of pages: 1
Publication date: 2010
Event: Poster session presented at International NanoBio Conference, Zurich, Switzerland.
Main Research Area: Technical/natural sciences

Bibliographical note
Source: dtu
Source-ID: u::3820
Publication: Research - peer-review › Poster – Annual report year: 2010

Understanding Detergent Effects on Lipid Membranes: A Model Study of Lysolipids
Lysolipids and fatty acids are the natural products formed by the hydrolysis of phospholipids. Lysolipids and fatty acids form micelles in solution and act as detergents in the presence of lipid membranes. In this study, we investigate the detergent strength of a homologous series of lyso-phosphatidylcholine lipids (LPCs) on 1-palmitoyl-2-oleyl-sn-glycerol-3-phosphatidylcholine (POPC) lipid membranes by use of isothermal titration calorimetry and vesicle fluctuation analysis. The membrane partition coefficient (K) and critical micelle concentration (cmc) are determined by isothermal titration calorimetry and found to obey an inverse proportionality relation (cmc. K similar to 0.05-0.3). The partition coefficient and critical micelle concentration are used for the analysis of the effect of LPCs on the membrane bending rigidity. The dependency of the bending rigidity on LPC membrane coverage has been analyzed in terms of a phenomenological model based on continuum elastic theory, which yields information about the curvature-inducing properties of the LPC molecule. The results reveal: 1), an increase in the partition coefficient with increasing LPC acyl-chain length; and 2), that the degree of acyl-chain mismatch between LPC and POPC determines the magnitude of the membrane mechanical perturbation per LPC molecule in the membrane. Finally, the three-stage model describing detergent membrane interaction has been extended by a parameter D-MCI, which governs the membrane curvature stability in the detergent concentration range below the cmc-value of the LPC molecule.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section,
Department of Micro- and Nanotechnology
Authors: Henriksen, J. R. (Intern), Andresen, T. L. (Intern), Feldborg, L. N. (Intern), Duelund, L. (Ekstern), Ipsen, J. (Ekstern)
Pages: 2199-2205
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Biophysical Journal
Volume: 98
Issue number: 10
ISSN (Print): 0006-3495
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.06 SJR 1.946 SNIP 1.018
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.145 SNIP 1.173 CiteScore 3.3
Web of Science (2015): Indexed yes
A Concise Synthesis of Castanospermine by the Use of a Transannular Cyclization

A nine-step synthesis of (+)-castanospermine has been accomplished in 22% overall yield from methyl alpha-D-glucopyranoside. The key transformations involve a zinc-mediated fragmentation of benzyl-protected methyl 6-iodoglucopyranoside, ring-closing olefin metathesis, and strain-release transannular cyclization to afford the indolizidine skeleton of the natural product.

General information
State: Published
Organisations: Department of Chemistry, Solar Energy Programme, Risø National Laboratory for Sustainable Energy, Organic Chemistry
Authors: Jensen, T. (Intern), Mikkelsen, M. (Intern), Lauritsen, A. (Intern), Andresen, T. L. (Intern), Gottfredsen, C. H. (Intern), Madsen, R. (Intern)
Pages: 8886-8889
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Organic Chemistry
Volume: 74
Issue number: 22
ISSN (Print): 0022-3263
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 1.976 SNIP 1.03 CiteScore 4.59
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.018 SNIP 1.174 CiteScore 4.69
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.003 SNIP 1.222 CiteScore 4.69
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.078 SNIP 1.176 CiteScore 4.51
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.272 SNIP 1.23 CiteScore 4.31
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.27 SNIP 1.261 CiteScore 4.43
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 2.112 SNIP 1.173
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 2.186 SNIP 1.254
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.309 SNIP 1.208
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.37 SNIP 1.309
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.222 SNIP 1.31
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.995 SNIP 1.3
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.945 SNIP 1.315
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.865 SNIP 1.355
Web of Science (2003): Indexed yes
Administration of liposome- and polymer-based clinical nanomedicines, as well as many other proposed multifunctional nanoparticles, often triggers hypersensitivity reactions without the involvement of IgE. These anaphylactic reactions are believed to be secondary to activation of the complement system, giving rise to the release of anaphylatoxins C3a and C5a that initiate a wide array of responses through their effect on mast cells, polymorphonuclear cells, platelets and monocytes. Additionally, the terminal complement C5b-9 complex induces platelet activation, thereby enhancing their procoagulant activity, and has the capacity to elicit non-lytic stimulatory responses from vascular endothelial cells. Here we discuss the molecular basis of complement activation by liposomes, including poly(ethylene glycol) coated vesicles, and other related lipid-based and phospholipid-poly(ethylene glycol) conjugate stabilized entities. We have further considered the role of these complement activating entities in experimental oncology since intra-tumoural complement activation is suggested to induce tumour growth and progression.
Complement-mediated tumour growth: implications for cancer nanotechnology and nanomedicines

The recent unexpected observation that complement activation helps tumour growth and progression has an important bearing on the future development of cancer nanomedicines for site-specific tumour targeting as these entities are capable of triggering complement. These issues are discussed and suggestions are provided for future design and development of safer and effective cancer nanomedicines.

General information
State: Published
Organisations: Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Moghimi, S. M. (Ekstern), Andresen, T. L. (Intern)
Pages: 1571-1572
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Molecular Immunology
Volume: 46
Issue number: 8-9
ISSN (Print): 0161-5890
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.523 SNIP 0.961 CiteScore 3.2
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.572 SNIP 0.928 CiteScore 3.16
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.474 SNIP 0.916 CiteScore 2.89
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.46 SNIP 0.94 CiteScore 2.89
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.406 SNIP 0.862 CiteScore 2.94
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.403 SNIP 0.874 CiteScore 3.01
ISI indexed (2011): ISI indexed yes
Drug Delivery by an Enzyme-Mediated Cyclization of a Lipid Prodrug with Unique Bilayer-Formation Properties

Special delivery: Liposomal drug-delivery systems in which prodrugs are activated specifically by disease-associated enzymes have great potential for the treatment of severe diseases, such as cancer. A new type of phospholipid-based prodrug has the ability to form stable small unilamellar vesicles (see picture). Activation of the prodrug vesicles by the enzyme sPLA2 initiates a cyclization reaction, which leads to the release of the drug.
Liposomes containing alkylated methotrexate analogues for phospholipase A(2) mediated tumor targeted drug delivery

Two lipophilic methotrexate analogues have been synthesized and evaluated for cytotoxicity against KATO III and HT-29 human colon cancer cells. Both analogues contained a C-16-alkyl chain attached to the gamma-carboxylic acid and one of the analogues had an additional benzyl group attached to the alpha-carboxyl group. The cytotoxicity of the gamma-alkylated compound towards KATO III (IC50 = 55 nM) and HT-29 (IC50 = 400 nM) cell lines was unaffected by the alkylation, whereas the additional benzyl group made the compound nontoxic. The gamma-derivative with promising cytotoxicity was incorporated into liposomes that were designed to be particularly susceptible to a liposome degrading enzyme, secretory phospholipase A(2) (sPLA(2)), which is found in high concentrations in tumors of several different cancer types. Liposome incorporation was investigated by differential scanning calorimetry (DSC), and sPLA(2) hydrolysis was examined by fluorescence spectroscopy and high performance liquid chromatography (HPLC). The results showed that the methotrexate (MTX)-analogue could be incorporated into liposomes that were degradable by
sPLA(2). However, the in vitro cytotoxicity of the MTX-liposomes against KATO III and HT-29 cancer cells was found to be independent of sPLA(2) hydrolysis, indicating that the alkylated MTX-analogue was available for cancer cell uptake even in the absence of liposome hydrolysis. Using a DSC based method for assessing the anchoring stability of alkylated compounds in liposomes, it was demonstrated that the MTX-analogue partitioned into the water phase and thereby became available for cell uptake. It was concluded that liposomes containing alkylated MTX-analogues show promise as a drug delivery system, although the MTX-analogue needs to be more tightly anchored to the liposomal carrier. Also, the developed DSC-assay for studying the anchoring stability of alkylated drugs will be a useful tool in the development of liposomal drug delivery systems.
Mechanistic Study of the sPLA₂ Mediated Hydrolysis of a Thio-ester Pro Anticancer Ether Lipid

Secretory phospholipase A₂ (sPLA₂) is an interesting enzyme for triggered liposomal drug delivery to tumor tissue due the overexpression of sPLA₂ in cancerous tissue. A drug delivery system based on the triggered release of therapeutics from sPLA₂-sensitive liposomes constituted of pro anticancer ether lipids, which become cytotoxic upon sPLA₂-catalyzed hydrolysis has previously been established. To optimize the hydrolysis rate of the lipids and thereby optimizing the release profile of the drugs from the liposomes, we have synthesized a thio-ester pro anticancer ether lipid. Liposomes constituted of this lipid showed an altered rate of hydrolysis by sPLA₂. We have tested the cytotoxicity of the thio-ester pro anticancer ether lipids toward cancer cells, and the results showed that the cytotoxicity is indeed maintained upon sPLA₂ exposure. To further understand the origin for the observed different hydrolysis rates for the esters, we have applied molecular dynamics simulations and density functional theory. The combination of these theoretical methods has given valuable insight into the molecular mechanism for sPLA₂ action on sulfur-containing phospholipids. It appears that the enzyme-catalyzed hydrolysis of thio-esters follow a different pathway compared to the hydrolysis pathway of the free thio-ester.
Polymeric nanosensors for measuring the full dynamic pH range of endosomes and lysosomes in mammalian cells

Polymer nanoparticle sensors have been constructed for studying pH in the endocytic pathway in mammalian cells. The pH sensors for fluorescence ratiometric measurements were prepared using inverse microemulsion polymerization with rhodamine as reference fluorophor and fluorescein and oregon green as pH sensitive dyes, which gave a dynamic pH measurement range from 4.1-7.5. Thus, the sensors cover the pH range of almost all intracellular compartments in mammalian cells. Both neutral and cationic polyacrylamide particles were synthesized where (3-acrylamidopropyl) trimethylammonium chloride was used to introduce a net positive charge in the cationic particles. It was found that the positively charged particle sensors were internalized spontaneously by HepG2 cancer cells. These new pH nanosensors are potential tools in time resolved quantification of pH in the endocytic pathway of living cells.

General information
State: Published
Organisations: Amphiphilic polymers in biological sensing Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology, Colloids and Biological Interfaces Group
Authors: Sun, H. (Intern), Andresen, T. L. (Intern), Benjaminsen, R. V. (Intern), Almdal, K. (Intern)
Pages: 676-682
Publication date: 2009

Original language: English
DOIs: 10.1021/ja901412j
Source: orbit
Source-ID: 257078
Publication: Research - peer-review › Journal article – Annual report year: 2009
Synthesis and Biophysical Characterization of Chlorambucil Anticancer Ether Lipid Prodrugs

The synthesis and biophysical characterization of four prodrug ether phospholipid conjugates are described. The lipids are prepared from the anticancer drug chlorambucil and have C16 and C18 ether chains with phosphatidylcholine or phosphatidylglycerol headgroups. All four prodrugs have the ability to form unilamellar liposomes (86-125 nm) and are hydrolyzed by phospholipase A2, resulting in chlorambucil release. Liposomal formulations of prodrug lipids displayed cytotoxicity toward HT-29, MT-3, and ES-2 cancer cell lines in the presence of phospholipase A2, with IC50 values in the 8-36 μM range.

General information
State: Published
Organisations: Department of Chemistry, Department of Micro- and Nanotechnology, University of Southern Denmark, LiPlasome Pharma ApS
Authors: Pedersen, P. J. (Intern), Christensen, M. S. (Intern), Ruysschaert, T. (Ekstern), Linderoth, L. (Intern), Andresen, T. L. (Intern), Melander, F. (Ekstern), Mouritsen, O. G. (Ekstern), Madsen, R. (Intern), Clausen, M. H. (Intern)
Pages: 3408-3415
Publication date: 2009
Main Research Area: Technical/natural sciences
Molecular basis of phospholipase A₂ activity toward phospholipids with sn-1 substitutions

We studied secretory phospholipase A₂ type IIA (sPLA₂) activity toward phospholipids that are derivatized in the sn-1 position of the glycerol backbone. We explored what type of side group (small versus bulky groups, hydrophobic versus polar groups) can be introduced at the sn-1 position of the glycerol backbone of glycerophospholipids and at the same time be hydrolyzed by sPLA₂. The biophysical characterization revealed that the modified phospholipids can form multilamellar vesicles, and several of the synthesized sn-1 functionalized phospholipids were hydrolyzed by sPLA₂.
Molecular dynamics simulations provided detailed insight on an atomic level that can explain the observed sPLA(2) activity toward the different phospholipid analogs. The simulations revealed that, depending on the nature of the side chain located at the sn-1 position, the group may interfere with an incoming water molecule that acts as the nucleophile in the enzymatic reaction. The simulation results are in agreement with the experimentally observed sPLA(2) activity toward the different phospholipid analogs.
Metabolite quantification in living cells using optical nanosensor particles

General information
State: Published
Organisations: Cell Biology, Biosystems Division, Risø National Laboratory for Sustainable Energy, Polymers for Biological and Medical Technology, Polymer Department, Polymer Department. Management
Authors: Scharff-Poulsen, A. (Intern), Sun, H. (Intern), Andresen, T. (Intern), Jakobsen, I. (Intern), Almdal, K. (Intern)
Publication date: 2007
Event: Poster session presented at SBE-DTU's 1st annual symposium for biotechnological research, Lyngby (DK).
Main Research Area: Technical/natural sciences
Links:
Source: orbit
Source-ID: 215739
Publication: Research › Poster – Annual report year: 2007

Optical nanosensor particles for detection of metabolites in living cells

General information
State: Published
Organisations: Cell Biology, Biosystems Division, Risø National Laboratory for Sustainable Energy, Polymers for Biological and Medical Technology, Polymer Department, Polymer Department. Management
Authors: Scharff-Poulsen, A. (Intern), Sun, H. (Intern), Andresen, T. (Intern), Jakobsen, I. (Intern), Almdal, K. (Intern)
Publication date: 2007
Event: Paper presented at Bioimaging Workshop Copenhagen, University of Copenhagen, Denmark.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 215740
Publication: Research › Paper – Annual report year: 2007

Optical nanosensor particles for detection of metabolites in living cells

General information
State: Published
Organisations: Cell Biology, Biosystems Division, Risø National Laboratory for Sustainable Energy, Polymers for Biological and Medical Technology, Polymer Department, Polymer Department. Management
Authors: Scharff-Poulsen, A. (Intern), Sun, H. (Intern), Andresen, T. (Intern), Jakobsen, I. (Intern), Almdal, K. (Intern)
Publication date: 2007
Oxidative stability of Liposomes composed of docosahexaenoic acid-containing phospholipids

Oxidative stability of liposomes made of (Docosahexaenoic acid) DHA-containing phosphatidylcholine (PC) was examined during preparation and storage. After preparation of the liposomes, the concentration of primary (conjugated dienes) and secondary oxidation products (Thiobarbituric acid-reactive substances, TBARS) were significantly higher compared to the initial value. During cold storage, formation of conjugated dienes and TBARS remained more or less constant in large unilamellar vesicles (LUV), whereas in multilamellar vesicles (MLV) they were seen to increase over a period of 21 days. Evaporation of solvent traces from a lipid film should preferably be done under nitrogen as vacuum evaporation was found to increase oxidation of the phospholipid.

General information
State: Published
Organisations: Department of Chemistry, Polymers for Biological and Medical Technology, Polymer Department, Risø National Laboratory for Sustainable Energy, Center for Biological Sequence Analysis, Department of Systems Biology, National Food Institute
Authors: Vikbjerg, A. F. (Intern), Andresen, T. L. (Intern), Jørgensen, K. (Intern), Mu, H. (Intern), Xu, X. (Intern)
Pages: 631-637
Publication date: 2007
Main Research Area: Technical/natural sciences
Secretory Phospholipase A₂ Hydrolysis Phospholipid Analogs is Dependent on Water Accessibility to the Active Site

A new and unnatural type of phospholipids with the head group attached to the 2-position of the glycerol backbone has been synthesized and shown to be a good substrate for secretory phospholipase A₂ (sPLA₂). To investigate the unexpected sPLA₂ activity, we have compared three different phospholipids by using fluorescence techniques and HPLC, namely: (R)-1,2-dipalmitoyl-glycero-3-phosphocholine (hereafter referred to as 1R), (R)-1-O-hexadecyl-2-palmitoyl-glycero-3-phosphocholine (2R), and (S)-1-O-hexadecyl-3-palmitoyl-glycero-2-phosphocholine (3S). Furthermore, to understand the underlying mechanisms for the observed differences, we have performed molecular dynamics simulations to clarify on a structural level the substrate specificity of sPLA₂ toward phospholipid analogues with their head groups in the 2-position of the glycerol backbone. We have studied the lipids above 1R, 2R, and 3S as well as their enantiomers 1S, 2S, and 3R. In the simulations of sPLA₂−1S and sPLA₂−3R, structural distortion in the binding cleft induced by the phospholipids showed that these are not substrates for sPLA₂. In the case of the phospholipids 1R, 2R, and 3S, our simulations revealed that the difference observed experimentally in sPLA₂ activity might be caused by reduced access of water molecules to the active site. We have monitored the number of water molecules that enter the active site region for the different sPLA₂−phospholipid complexes and found that the probability of a water molecule reaching the correct position such that hydrolysis can occur is reduced for the unnatural lipids. The relative water count follows 1R > 2R > 3S. This is in good agreement with experimental data that indicate the same trend for sPLA₂ activity: 1R > 2R > 3S.

General information
State: Published
Organisations: Department of Chemistry, Risø National Laboratory for Sustainable Energy, Technical University of Denmark, LiPlasome Pharma ApS
Authors: Peters, G. H. (Intern), Møller, M. S. (Ekstern), Jørgensen, K. (Ekstern), Rönnholm, P. (Ekstern), Mikkelsen, M. (Ekstern), Andresen, T. L. (Intern)
Number of pages: 11
Pages: 5451-5461
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: American Chemical Society. Journal
Volume: 129
ISSN (Print): 0002-7863
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 7.368 SNIP 2.584 CiteScore 13.18
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 6.826 SNIP 2.632 CiteScore 12.81
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 6.273 SNIP 2.578 CiteScore 11.92
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 5.953 SNIP 2.455 CiteScore 11.38
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 6.141 SNIP 2.379 CiteScore 10.37
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 5.447 SNIP 2.336 CiteScore 9.94
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 5.076 SNIP 2.132
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 4.883 SNIP 2.176
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 4.936 SNIP 2.116
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 5.023 SNIP 2.126
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 4.546 SNIP 2.22
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 4.284 SNIP 2.207
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 3.754 SNIP 2.178
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 3.267 SNIP 2.215
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 3.527 SNIP 2.346
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 3.449 SNIP 2.199
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 3.573 SNIP 2.224
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 3.56 SNIP 2.182

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10.1021/ja067755b
Source: orbit
Source-ID: 216330
Synthesis of sn-1 functionalized phospholipids as substrates for secretory phospholipase A2

Secretory phospholipase A2 (sPLA2) represents a family of small water-soluble enzymes that catalyze the hydrolysis of phospholipids in the sn-2 position liberating free fatty acids and lysophospholipids. Herein we report the synthesis of two new phospholipids (1 and 2) with bulky allyl-substituents attached to the sn-1 position of the glycerol backbone. The synthesis of phospholipids 1 and 2 is based upon the construction of a key aldehyde intermediate 3 which locks the stereochemistry in the sn-2 position of the final phospholipids. The aldehyde functionality serves as the site for insertion of the allyl-substituents by a zinc mediated allylation. Small unilamellar liposomes composed of phospholipids 1 and 2 were subjected to sPLA2 activity measurements. Our results show that only phospholipid 1 is hydrolyzed by the enzyme. Molecular dynamics simulations revealed that the lack of hydrolysis of phospholipid 2 is due to steric hindrance caused by the bulky side chain of the substrate allowing only limited access of water molecules to the active site.

General information
State: Published
Organisations: Department of Chemistry, Physical and Biophysical Chemistry, Sustainable and Green Chemistry, Colloids and Biological Interfaces Group, Self-organizing materials for nanotechnology Section, Department of Micro- and Nanotechnology
Authors: Linderoth, L. (Intern), Peters, G. H. (Intern), Jørgensen, K. (Ekstern), Madsen, R. (Intern), Andresen, T. L. (Intern)
Number of pages: 13
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Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
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Volume: 125
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ISSN (Print): 0009-3084
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.976 SNIP 0.862 CiteScore 2.78
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.957 SNIP 0.957 CiteScore 2.75
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.885 SNIP 1.039 CiteScore 2.62
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.82 SNIP 1.055 CiteScore 2.66
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.803 SNIP 0.974 CiteScore 2.41
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.727 SNIP 0.984 CiteScore 2.56
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.874 SNIP 0.964
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.9 SNIP 0.995
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.114 SNIP 1.057
Scopus rating (2007): SJR 1.083 SNIP 1.091
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.808 SNIP 0.881
Activation of interfacial enzymes at membrane surfaces

A host of water-soluble enzymes are active at membrane surfaces and in association with membranes. Some of these enzymes are involved in signalling and in modification and remodelling of the membranes. A special class of enzymes, the phospholipases, and in particular secretory phospholipase A2 (sPLA2), are only activated at the interface between water and membrane surfaces, where they lead to a break-down of the lipid molecules into lysolipids and free fatty acids. The activation is critically dependent on the physical properties of the lipid-membrane substrate. A topical review is given of our current understanding of the physical mechanisms responsible for activation of sPLA2 as derived from a range of different experimental and theoretical investigations.
Activation of the human complement system by cholesterol-rich and pegylated liposomes - Modulation of cholesterol-rich liposome-mediated complement activation by elevated serum LDL and HDL levels

Intravenously infused liposomes may induce cardiopulmonary distress in some human subjects, which is a manifestation of "complement activation-related pseudoallergy." We have now examined liposome-mediated complement activation in human sera with elevated lipoprotein (LDL and HDL) levels, since abnormal or racial differences in serum lipid profiles seem to modulate the extent of complement activation and associated adverse responses. In accordance with our earlier observations, cholesterol-rich (45 mol% cholesterol) liposomes activated human complement, as reflected by a significant rise in serum level of S-protein-bound form of the terminal complex (SC5b-9). However, liposome-induced rise of SC5b-9 was significantly suppressed when serum HDL cholesterol levels increased by 30%. Increase of serum LDL to levels similar to that observed in heterozygous familial hypercholesterolemia also suppressed liposome-mediated SC5b-9 generation considerably. While intravenous injection of cholesterol-rich liposomes into pigs was associated with an immediate circulatory collapse, the drop in systemic arterial pressure following injection of liposomes preincubated with human lipoproteins was slow and extended. Therefore, surface-associated lipoprotein particles (or apolipoproteins) seem to lessen liposome-induced adverse haemodynamic changes, possibly as a consequence of suppressed complement activation in vivo. PEGylated liposomes were also capable of activating the human complement system, and the presence of surface projected methoxypoly(ethylene glycol) chains did not interfere with generation of C3 opsonic fragments. We
also show that poly(ethylene glycol) is not responsible for PEGylated liposome-mediated complement activation. The net anionic charge on the phosphate moiety of the phospholipid-mPEG conjugate seemed to play a critical role in activation of both the classical and alternative pathways of the complement system.
Methylation of the phosphate oxygen moiety of phospholipid-methoxy(polyethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production

Methoxy(polyethylene glycol), mPEG, -grafted liposomes are known to exhibit prolonged circulation time in the blood, but their infusion into a substantial percentage of human subjects triggers immediate non-IgE-mediated hypersensitivity reactions. These reactions are strongly believed to arise from anaphylatoxin production through complement activation. Despite the general view that vesicle surface camouflaging with mPEG should dramatically suppress complement activation, here we show that bilayer enrichment of noncomplement activating liposomes [di-palmitoylphosphatidylcholine (DPPC) vesicles] with phospholipid-mPEG conjugate induces complement activation resulting in vesicle recognition by macrophage complement receptors. The extent of vesicle uptake, however, is dependent on surface mPEG density. We have delineated the likely structural features of phospholipid-mPEG conjugate responsible for PEGylated liposome-induced complement activation in normal as well as C1q-deficient human sera, using DPPC vesicles bearing the classical as well as newly synthesized lipid-mPEG conjugates. With PEGylated DPPC vesicles, the net anionic charge on the phosphate moiety of phospholipid-mPEG conjugate played a key role in activation of both classical and alternative pathways of complement and anaphylatoxin production (reflected in significant rises in SC5b-9, C4d, and C3a-desarg levels in normal human sera as well as SC5b-9 in EGTA-chelated/Mg2+ supplemented serum), since methylation of the phosphate oxygen of phospholipid-mPEG conjugate, and hence the removal of the negative charge, totally prevented complement activation. To further corroborate on the role of the negative charge in complement activation, vesicles bearing anionic phospholipid-mPEG conjugates, but not the methylated phospholipid-mPEG, were shown to significantly decrease serum hemolytic activity and increase plasma thromboxane B2 levels in rats. In contrast to liposomes, phospholipid-mPEG micelles had no effect on complement activation, thus suggesting a possible role for vesicular zwitterionic phospholipid head-groups as an additional factor contributing to PEGylated liposome-mediated complement activation. Our findings provide a rational conceptual basis for development of safer vesicles for site-specific drug delivery and controlled release at pathological sites.

General information
State: Published
Organisations: Department of Chemistry, University of Brighton, Semmelweis University
Authors: Moghimi, S. (Ekstern), Hamad, I. (Ekstern), Andresen, T. L. (Intern), Jørgensen, K. (Intern), Szebeni, J. (Ekstern)
Pages: 2591-2593
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Faseb Journal
Volume: 20
Issue number: 14
ISSN (Print): 0892-6638
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.68 SJR 2.57 SNIP 1.22
Web of Science (2016): Indexed yes
Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release

Tumor specific drug delivery has become increasingly interesting in cancer therapy, as the use of chemotherapeutics is often limited due to severe side effects. Conventional drug delivery systems have shown low efficiency and a continuous search for more advanced drug delivery principles is therefore of great importance. In the first part of this review, we present current strategies in the drug delivery field, focusing on site-specific triggered drug release from liposomes in cancerous tissue. Currently marketed drug delivery systems lack the ability to actively release the carried drug and rely on passive diffusion or slow non-specific degradation of the liposomal carrier. To obtain elevated tumor-to-normal tissue drug ratios, it is important to develop drug delivery strategies where the liposomal carriers are actively degraded specifically in the tumor tissue. Many promising strategies have emerged ranging from externally triggered light- and thermo-sensitive liposomes to receptor targeted, pH- and enzymatically triggered liposomes relying on an endogenous trigger mechanism in the cancerous tissue. However, even though several of these strategies were introduced three decades ago, none of them have yet led to marketed drugs and are still far from achieving this goal. The most advanced and prospective technologies are probably the prodrug strategies where nontoxic drugs are carried and activated specifically in the malignant tissue by overexpressed enzymes. In the second part of this paper, we review our own work, exploiting
secretory phospholipase A(2) as a site-specific trigger and prodrug activator in cancer therapy. We present novel prodrug lipids together with biophysical investigations of liposome systems, constituted by these new lipids and demonstrate their degradability by secretory phospholipase A2. We furthermore give examples of the biological performance of the enzymatically degradable liposomes as advanced drug delivery systems.

**General information**

State: Published
Organisations: Department of Chemistry
Authors: Andresen, T. L. (Intern), Jensen, S. S. (Intern), Jørgensen, K. (Intern)
Pages: 68-97
Publication date: 2005
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Progress in Lipid Research
Volume: 44
Issue number: 1
ISSN (Print): 0163-7827
Ratings:

- BFI (2017): BFI-level 2
- Web of Science (2017): Indexed Yes
- BFI (2016): BFI-level 2
- Scopus rating (2016): SJR 4.59 SNIP 3.359 CiteScore 11.67
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
- Scopus rating (2015): SJR 5.217 SNIP 3.229 CiteScore 11.6
- BFI (2014): BFI-level 2
- Scopus rating (2014): SJR 5.061 SNIP 3.813 CiteScore 11.6
- BFI (2013): BFI-level 2
- Scopus rating (2013): SJR 4.996 SNIP 3.645 CiteScore 12.62
- ISI indexed (2013): ISI indexed yes
- BFI (2012): BFI-level 2
- Scopus rating (2012): SJR 4.511 SNIP 3.238 CiteScore 11.71
- ISI indexed (2012): ISI indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): SJR 4.025 SNIP 3.541 CiteScore 11.13
- ISI indexed (2011): ISI indexed yes
- BFI (2010): BFI-level 2
- Scopus rating (2010): SJR 3.708 SNIP 2.949
- BFI (2009): BFI-level 2
- Scopus rating (2009): SJR 4.779 SNIP 3.268
- BFI (2008): BFI-level 1
- Scopus rating (2006): SJR 5.698 SNIP 4.402
- Scopus rating (2005): SJR 4.68 SNIP 3.641
- Web of Science (2005): Indexed yes
- Scopus rating (2004): SJR 4.508 SNIP 3.86
- Web of Science (2004): Indexed yes
- Scopus rating (2003): SJR 4.602 SNIP 3.423
- Scopus rating (2002): SJR 2.85 SNIP 2.109
- Scopus rating (2001): SJR 2.779 SNIP 2.044
- Scopus rating (2000): SJR 3.158 SNIP 3.044
- Scopus rating (1999): SJR 3.47 SNIP 2.868

Original language: English

enzymatic degradation, specific release, targeting, liposome, PEG, triggering, drug delivery, lipid vesicles, prodrug

DOIs:

10.1016/j.plipres.2004.12.001
Synthesis and Biological Activity of Anticancer Ether Lipids That Are Specifically Released by Phospholipase A2 in Tumor Tissue

The clinical use of anticancer lipids is severely limited by their ability to cause lysis of red blood cells prohibiting intravenous injection. Novel delivery systems are therefore required in order to develop anticancer ether lipids (AELs) into clinically useful anticancer drugs. In a recent article (J. Med. Chem. 2004, 47, 1694) we showed that it is possible to construct liposome systems composed of masked AELs that are activated by secretory phospholipase A2 in cancerous tissue. We present here the synthesis of six AELs and evaluate the biological activity of these bioactive lipids. The synthesized AEL 1-6 were tested against three different cancer cell lines. It was found that the stereochemistry of the glycerol headgroup in AEL-2 and 3 has a dramatic effect on the cytotoxicity of the lipids. AEL 1-4 were furthermore evaluated for their ability to prevent phosphorylation of the apoptosis regulating kinase Akt, and a correlation was found between their cytotoxic activity and their ability to inhibit Akt phosphorylation.

General information
State: Published
Organisations: Department of Chemistry
Authors: Andresen, T. L. (Intern), Jensen, S. S. (Intern), Madsen, R. (Intern), Jørgensen, K. (Intern)
Pages: 7305-7314
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: The Journal of Medicinal Chemistry
Volume: 48
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BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.06
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.529 SNIP 1.631 CiteScore 5.66
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.259 SNIP 1.693 CiteScore 5.55
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.293 SNIP 1.78 CiteScore 5.65
ISI indexed (2013): ISI indexed yes
Synthesis and membrane behavior of a new class of unnatural phospholipid analogs useful as phospholipase A2 degradable liposomal drug carriers

A new and unnatural type of lipid analogs with the phosphocholine and phosphoglycerol head groups linked to the C-2 position of the glycerol moiety have been synthesized and the thermodynamic lipid membrane behavior has been investigated using differential scanning calorimetry. From the heat capacity measurements, it was observed that the pre-transition was abolished most likely due to the central position of the head groups providing better packing properties in the low temperature ordered gel phase. Activity measurements of secretory phospholipase A2 (PLA2) on unilamellar liposomal membranes revealed that the unnatural phospholipids are excellent substrates for PLA2 catalyzed hydrolysis. This was manifested as a minimum in the PLA2 lag time in the main phase transition temperature regime and a high degree of lipid hydrolysis over a broad temperature range. The obtained results provide new information about the interplay between the molecular structure of phospholipids and the lipid membrane packing constrains that govern the pre-transition. In addition, the PLA2 activity measurements are useful for obtaining deeper insight into the molecular details of the catalytic site of PLA2. The combined results also suggest new approaches to rationally design liposomal drug carriers that can undergo a triggered activation in diseased tissue by overexpressed PLA2.

General information
State: Published
Organisations: Department of Chemistry
Authors: Andresen, T. L. (Intern), Jørgensen, K. (Intern)
Pages: 1-7
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Biochimica Et Biophysica Acta-biomembranes
Volume: 1669
Issue number: 1
Synthesis of anti-tumour phosphatidylinositol analogues from glucose by the use of ring-closing olefin metathesis

A divergent strategy is described for synthesis of the novel phosphatidylinositols 1-3. The synthetic approach commences from benzyl-protected methyl 6-iodo-6-deoxy-a-D-glucopyranoside, which undergoes zinc-mediated reductive fragmentation followed by vinyl Grignard addition and ring-closing metathesis to afford the key conduritol B intermediate 7.
This can trifurcate to form three different benzyl-protected myo-inositol headgroups 4-6, which after phosphorylation and attachment of the glycerolipid part give phosphatidylinositols 1-3. Preliminary biological testing against human colon adenocarcinoma cells reveals that analogues 1-3 are significant anti-tumour agents.
Secreted phospholipase A(2) as a new enzymatic trigger mechanism for localised liposomal drug release and absorption in diseased tissue

Polymer-coated liposomes can act as versatile drug-delivery systems due to long vascular circulation time and passive targeting by leaky blood vessels in diseased tissue. We present an experimental model system illustrating a new principle for improved and programmable drug-delivery, which takes advantage of an elevated activity of secretory phospholipase A(2) (PLA(2)) at the diseased target tissue. The secretory PLA2 hydrolyses a lipid-based proenhancer in the carrier liposome, producing lyso-phospholipids and free fatty acids, which are shown in a synergistic way to lead to enhanced liposome destabilization and drug release at the same time as the permeability of the target membrane is enhanced. Moreover, the proposed system can be made thermosensitive and offers a rational way for developing smart liposome-based drug delivery systems. This can be achieved by incorporating specific lipid-based proenhancers or prodestabilisers into the liposome carrier, which automatically becomes activated by PLA2 only at the diseased target sites, such as inflamed or cancerous tissue.
Projects:

**Statistical Modelling of TCR Repertoires for Immunotherapy and Drug Delivery Systems**

Department of Micro- and Nanotechnology  
Period: 15/10/2017 → 14/10/2020  
Number of participants: 3  
Phd Student: Vujovic, Milena (Intern)  
Supervisor: Kaplinsky, Joseph John (Intern)  
Main Supervisor: Andresen, Thomas Lars (Intern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Fonde  
Project: PhD

**Development of advanced drug delivery systems for therapeutic radionuclides in cancer treatment**

Department of Micro- and Nanotechnology  
Period: 01/10/2017 → 30/09/2020  
Number of participants: 4  
Phd Student: Magnus, Charlotte Busk (Intern)  
Supervisor: Andresen, Thomas Lars (Intern)  
Herth, Matthias (Ekstern)  
Main Supervisor: Jensen, Andreas Tue Ingemann (Intern)  

**Financing sources**  
Source: Internal funding (public)
**Reconstituted high-density lipoproteins for immuno- and chemotherapeutic drug delivery**

Department of Micro- and Nanotechnology  
Period: 01/10/2017 → 30/09/2020  
Number of participants: 3  
PhD Student:  
Pedersbæk, Dennis (Intern)  
Supervisor:  
Andresen, Thomas Lars (Intern)  
Main Supervisor:  
Simonsen, Jens Bæk (Intern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Institut stipendie (DTU)  
Project: PhD

**CodeSphere - Molecular encoding of Nanoparticles for targeted cargo delivery**

National Veterinary Institute  
Period: 01/09/2017 → 31/08/2020  
Number of participants: 4  
PhD Student:  
Moss, Keith Henry (Intern)  
Supervisor:  
Andresen, Thomas Lars (Intern)  
Jakobsen, Søren Nyboe (Intern)  
Main Supervisor:  
Hadrup, Sine Reker (Intern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Samfinansieret - Andet  
Project: PhD

**Development of Targeted Drug Delivery Systems for The Brain**

Department of Micro- and Nanotechnology  
Period: 01/06/2017 → 31/05/2020  
Number of participants: 3  
PhD Student:  
Kostrikov, Serhii (Intern)  
Supervisor:  
Hempel, Casper (Intern)  
Main Supervisor:  
Andresen, Thomas Lars (Intern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Fonde  
Project: PhD

**Development of novel drug delivery systems for cancer immunotherapy**

Department of Micro- and Nanotechnology  
Period: 15/03/2017 → 14/03/2020  
Number of participants: 3  
PhD Student:  
Stavnsbjerg, Camilla (Intern)
Supervisor: 
Hansen, Anders Elias (Intern) 
Main Supervisor: 
Andresen, Thomas Lars (Intern)

**Financing sources**
Source: Internal funding (public) 
Name of research programme: Offentlig finansiering 
Project: PhD

**Drug delivery of cancer immunotherapeutics**
Department of Micro- and Nanotechnology 
Period: 15/03/2017 → 14/03/2020 
Number of participants: 3 
Phd Student: 
Weywadt, Matilda Felicia de Val (Intern) 
Supervisor: 
Hansen, Anders Elias (Intern) 
Main Supervisor: 
Andresen, Thomas Lars (Intern)

**Financing sources**
Source: Internal funding (public) 
Name of research programme: Offentlig finansiering 
Project: PhD

**The Protein Corona of Liposomes for Drug Delivery**
Department of Micro- and Nanotechnology 
Period: 01/12/2016 → 30/11/2019 
Number of participants: 4 
Phd Student: 
Lassen, Rasmus Mikkel Münter (Intern) 
Supervisor: 
Kristensen, Kasper (Intern) 
Simonsen, Jens Bæk (Intern) 
Main Supervisor: 
Andresen, Thomas Lars (Intern)

**Financing sources**
Source: Internal funding (public) 
Name of research programme: Institut stipendie (DTU) 
Project: PhD

**Targeted adjuvant delivery to antigen presenting cells**
Department of Micro- and Nanotechnology 
Period: 15/11/2016 → 14/11/2019 
Number of participants: 3 
Phd Student: 
Christensen, Esben (Intern) 
Supervisor: 
Parhamifar, Ladan (Intern) 
Main Supervisor: 
Andresen, Thomas Lars (Intern)

**Financing sources**
Source: Internal funding (public) 
Name of research programme: Forskningsrådsfinansiering 
Project: PhD
Design, synthesis and development of biologically inspired polymeric nanomedicines for the treatment of advanced atherosclerosis

Department of Micro- and Nanotechnology
Period: 15/10/2016 → 14/10/2019
Number of participants: 4
Phd Student:
Basak, Suman (Intern)
Supervisor:
Almdal, Kristoffer (Intern)
Andresen, Thomas Lars (Intern)
Main Supervisor:
Kamaly, Nazila (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Nanomedicine Development for Combination with Ultrasound Mediated Brain Cancer Therapy

Department of Micro- and Nanotechnology
Period: 01/10/2016 → 30/09/2019
Number of participants: 3
Phd Student:
Sereti, Viktoria (Intern)
Supervisor:
Urquhart, Andrew (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Design, synthesis and development of hypoxia reactive drug delivery systems

Department of Micro- and Nanotechnology
Period: 15/09/2016 → 14/09/2019
Number of participants: 3
Phd Student:
Björk Sigurdardóttir, Sara (Intern)
Supervisor:
Kamaly, Nazila (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Fonde
Project: PhD

Liposome based vaccines in cancer immunotherapy

Department of Micro- and Nanotechnology
Period: 15/09/2016 → 14/09/2019
Number of participants: 3
Phd Student:
Jæhger, Ditte Elisabeth (Intern)
Supervisor:
Parhamifar, Ladan (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Fonde
Project: PhD

Liposome based vaccines in cancer immunotherapy
Department of Micro- and Nanotechnology
Period: 01/09/2016 → 31/08/2019
Number of participants: 3
Phd Student:
Hübbe, Mie Linder (Intern)
Supervisor:
Kaplinsky, Joseph John (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Grundforskningsfonden
Project: PhD

Formulation of Radionuclides and Organometallic Anticancer Compounds in Gels and Liposomes
Department of Micro- and Nanotechnology
Period: 15/02/2016 → 14/02/2019
Number of participants: 5
Phd Student:
Wang, Wenbo (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Elema, Dennis Ringkjøbing (Intern)
Jensen, Andreas Tue Ingemann (Intern)
Main Supervisor:
Henriksen, Jonas Rosager (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

Liposome based nanomedicines for the treatment of diabetic retinopathy
Department of Micro- and Nanotechnology
Colloids and Biological Interfaces
Period: 01/10/2015 → 30/09/2018
Number of participants: 3
Phd Student:
Arta, Anthoula (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Main Supervisor:
Urquhart, Andrew (Intern)
Project
Immune cell targeted drug delivery systems for combination with chemotherapy

Department of Micro- and Nanotechnology
Period: 01/10/2015 → 30/09/2018
Number of participants: 3
Phd Student:
Madsen, Ditte Villum (Intern)
Supervisor:
Parhamifar, Ladan (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Anden EU-finansiering
Project: PhD

Immunotherapy and combined therapies for cancer treatment

Department of Micro- and Nanotechnology
Period: 01/10/2015 → 30/09/2018
Number of participants: 3
Phd Student:
Jørgensen, Jennifer Solgaard (Intern)
Supervisor:
Parhamifar, Ladan (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Eksternt finansieret virksomhed
Project: PhD

Novel nanomedicines for the treatment of diabetic retinopathy

Department of Micro- and Nanotechnology
Period: 01/10/2015 → 30/09/2018
Number of participants: 3
Phd Student:
Arta, Anthoula (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Main Supervisor:
Urquhart, Andrew (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Eksternt finansieret virksomhed
Project: PhD

Carriers Containing Multiple Compartments for Lysosomal Storage Diseases

Department of Micro- and Nanotechnology
Period: 01/09/2015 → 31/08/2018
Number of participants: 3
Phd Student:
York-Durán, María José (Intern)
Supervisor:
Hosta-Rigau, Leticia (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Financing sources
Source: Internal funding (public)
Name of research programme: Samfinansierede - Virksomhed
Project: PhD

Nanocarrier mediated transport of macromolecules across the blood brain barrier
Department of Micro- and Nanotechnology
Period: 01/09/2015 → 31/08/2018
Number of participants: 3
Phd Student:
Lund, Mette Aagaard (Intern)
Supervisor:
Kamaly, Nazila (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Eksternt finansieret virksomhed
Project: PhD

Prodrugs and Linker Systems for Degradation in Diseased Tissue as part of Liposomal Drug Delivery Systems
Department of Micro- and Nanotechnology
Period: 01/09/2015 → 31/08/2018
Number of participants: 2
Phd Student:
Kræmer, Martin Kisha (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Eksternt finansieret virksomhed
Project: PhD

Chemical/Biological sensing using CMUTs
Department of Micro- and Nanotechnology
Period: 01/05/2015 → 21/05/2018
Number of participants: 4
Phd Student:
Mølgaard, Mathias Johannes Grøndahl (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Jakobsen, Mogens Havsteen (Intern)
Main Supervisor:
Thomsen, Erik Vilain (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Development of drug delivery systems for treatment of atherosclerosis
Department of Micro- and Nanotechnology
Period: 15/12/2014 → 14/12/2017
Number of participants: 3
Phd Student:
Petersen, Lars Ringgaard (Intern)
Remote loading strategies for incorporation of therapeutic compounds and contrast agents into gels and liposomes

Department of Chemistry
Period: 15/12/2014 → 14/12/2017
Number of participants: 5
Phd Student:
Engudar, Gokce (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Jensen, Andreas Tue Ingemann (Intern)
Thormann, Esben (Intern)
Main Supervisor:
Henriksen, Jonas Rosager (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

New nanomedicines for the treatment of diabetic retinopathy

Department of Micro- and Nanotechnology
Colloids and Biological Interfaces
Period: 01/10/2014 → 30/09/2017
Number of participants: 3
Phd Student:
Eriksen, Anne Zebitz (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Main Supervisor:
Urquhart, Andrew (Intern)

Liquid Fiducial Markers for Potentiation of Radiotherapy

Department of Micro- and Nanotechnology
Period: 01/10/2014 → 30/09/2017
Number of participants: 3
Phd Student:
Larsen, Trine Bjernbo (Intern)
Supervisor:
Hansen, Anders Elias (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Offentlig finansiering
Project: PhD
New nanomedicines for the treatment of diabetic retinopathy
Department of Micro- and Nanotechnology
Period: 01/09/2014 → 29/11/2017
Number of participants: 3
Phd Student:
Eriksen, Anne Zebitz (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Main Supervisor:
Urquhart, Andrew (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Synthesis of biomaterials for use in drug delivery to the brain
Department of Micro- and Nanotechnology
Period: 15/04/2014 → 14/07/2017
Number of participants: 2
Phd Student:
Bak, Martin (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Eksternt finansieret virksomhed
Project: PhD

Liposome based chemopainting in cancer radiotherapy
Department of Micro- and Nanotechnology
Period: 15/01/2014 → 14/06/2017
Number of participants: 5
Phd Student:
Østrem, Ragnhild Garborg (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Henriksen, Jonas Rosager (Intern)
Moos, Torben (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Samfinansieret - Andet

Relations
Publications:
Enzyme sensitive liposomes in chemotherapy and potentiation of immunotherapy

New cancer drugs based on MMP sensitive drug delivery systems
Department of Micro- and Nanotechnology
Period: 15/11/2013 → 27/09/2017
Number of participants: 6
Phd Student:
Brogaard, Rikke Yding (Intern)
Supervisor:
Melander, Fredrik (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Kamaly, Nazila (Intern)
Foged, Camilla (Ekstern)
Cruz, Edgar (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Anden EU-finansiering
Project: PhD

Development of Novel Biomaterials for Potentiating Radiotherapy
Department of Micro- and Nanotechnology
Period: 01/08/2013 → 14/06/2017
Number of participants: 5
Phd Student:
Bruun, Linda Maria (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Kamaly, Nazila (Intern)
Linderoth, Lars (Intern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut, samfinansiering

Relations
Publications:
Development of Novel Biomaterials for Potentiation of Radiotherapy
Project: PhD

Elucidating the transport behavior of HER2 targeted nanoparticles and their use in novel nanotherapies
Department of Micro- and Nanotechnology
Period: 01/06/2013 → 08/02/2017
Number of participants: 5
Phd Student:
Juul, Christian Ammitzbøll (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Berg, Rolf Henrik (Intern)
Moos, Torben (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.

Relations
Publications:
Targeting HER2-positive cancer using multifunctional nanoparticles
Project: PhD
Liposome based radiosensitizer cancer therapy

Department of Micro- and Nanotechnology
Period: 15/04/2013 → 08/02/2017
Number of participants: 6
Phd Student:
Pourhassan, Houman (Intern)
Supervisor:
Hansen, Anders E. (Ekstern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Henriksen, Jonas Rosager (Intern)
Christensen, Dennis (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Anden EU-finansiering

Relations
Publications:
Liposome based radiosensitizer cancer therapy
Project: PhD

Nano-Sensitizer Cancer Cell targeted Radiotherapy

Department of Micro- and Nanotechnology
Colloids and Biological Interfaces
Period: 01/04/2013 → 31/03/2018
Number of participants: 2
Acronym: NaSTaR
Project Manager, organisational:
Møller, Majken Lerche (Intern)
Project Manager, academic:
Andresen, Thomas Lars (Intern)

Investigations of drug transport phenomena through cell layers using lab-o-a-chip technologies

Department of Micro- and Nanotechnology
Period: 01/09/2012 → 30/09/2016
Number of participants: 7
Phd Student:
Tan, Hsih-Yin (Intern)
Supervisor:
Dufva, Martin (Intern)
Kutter, Jörg Peter (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Svendsen, Winnie Edith (Intern)
Dittrich, Petra Stephanie (Ekstern)
Nielsen, Carsten Uhd (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering

Relations
Publications:
Development of microfluidic cell culture devices towards an in vitro human intestinal barrier model
Project: PhD

Development of radioisotope labeling methods for nanoparticle contrast agents
Department of Micro- and Nanotechnology
Period: 01/08/2012 → 16/03/2016
Number of participants: 6
Phd Student:
Frellsen, Anders Floor (Intern)
Supervisor:
Jensen, Andreas Tue Ingemann (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Henriksen, Jonas Rosager (Intern)
Cai, Weibo (Ekstern)
Herth, Matthias (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: 1/3 FUU, 1/3 inst 1/3 Andet
Project: PhD

Development of stimuli triggered liposomes for controlled drug release in cancer therapy
Department of Micro- and Nanotechnology
Period: 01/06/2012 → 12/12/2013
Number of participants: 2
Phd Student:
Tassone, Chiara (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: 1/3 FUU, 1/3 inst 1/3 Andet
Project: PhD

Ruthenium-Catalyzed Tandem RCM/Isomerization Sequences
Department of Chemistry
Period: 15/03/2012 → 03/06/2015
Number of participants: 6
Phd Student:
Ishøy, Mette (Intern)
Supervisor:
Nielsen, Thomas Eiland (Intern)
Main Supervisor:
Tanner, David Ackland (Intern)
Examiner:
Andresen, Thomas Lars (Intern)
Nelson, Adam S. (Ekstern)
Poulsen, Thomas Bjørn (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD
Implications of membrane interactions of acylated peptides in oral drug delivery

Department of Micro- and Nanotechnology
Period: 15/01/2012 → 08/02/2017
Number of participants: 7
Phd Student: Trier, Sofie (Intern)
Supervisor: Henriksen, Jonas Rosager (Intern)
Jensen, Simon Bjerregaard (Ekstern)
Main Supervisor: Andresen, Thomas Lars (Intern)
Examiner: Urquhart, Andrew (Intern)
Brayden, David J. (Ekstern)
Nielsen, Hanne Mørck (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: ErhvervsPhD-ordningen VTU

Relations
Publications:
Acylation of Therapeutic Peptides
Project: PhD

Development of nanoparticles based delivery systems for sublingual Immunotherapy

Department of Micro- and Nanotechnology
Period: 01/01/2012 → 12/02/2016
Number of participants: 7
Phd Student: Alija, Hava (Intern)
Supervisor: Brimnes, Jens (Ekstern)
Rask, Carola S. (Ekstern)
Main Supervisor: Andresen, Thomas Lars (Intern)
Examiner: Urquhart, Andrew (Intern)
Christensen, Dennis (Ekstern)
Rådinger, Madeleine (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: ErhvervsPhD-ordningen VTU
Project: PhD

Imaging of nanostructures in cells

Department of Micro- and Nanotechnology
Period: 01/09/2011 → 12/12/2014
Number of participants: 6
Phd Student: Købler, Carsten (Intern)
Supervisor: Vogel, Ulla Birgitte (Intern)
Main Supervisor: Mølhave, Kristian (Intern)
Examiner: Andresen, Thomas Lars (Intern)
Alexandra, Porter (Ekstern)
Martinez, Karen (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: 1/3 FUU, 1/3 inst 1/3 Andet
Project: PhD

Development of peptide based non-viral gene transfection Systems
Department of Micro- and Nanotechnology
Period: 15/08/2011 → 18/06/2015
Number of participants: 5
Phd Student:
Klauber, Thomas Christopher Bogh (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Berg, Rolf Henrik (Intern)
Moos, Torben (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Development of safer non-viral gene transfection vectors
Department of Micro- and Nanotechnology
Period: 01/05/2011 → 12/12/2014
Number of participants: 6
Phd Student:
Caviglia, Claudia (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Main Supervisor:
Emnéus, Jenny (Intern)
Examiner:
Rozlosnik, Noemi (Intern)
Guiseppi-Elie, Anthony (Ekstern)
Ruzgas, Tautgirdas (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD

Synthesis and Characterization of non-viral transfection systems
Department of Micro- and Nanotechnology
Period: 15/03/2011 → 07/06/2011
Number of participants: 2
Phd Student:
Jensen, Christina Mernøe (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
**Delivery of Biologics across the blood brain barrier through nanoencapsulation**

Department of Micro- and Nanotechnology  
Period: 01/02/2011 → 20/08/2014  
Number of participants: 5  
Phd Student:  
Bruun, Jonas (Intern)  
Main Supervisor:  
Andresen, Thomas Lars (Intern)  
Examiner:  
Berg, Rolf Henrik (Intern)  
Moos, Torben (Ekstern)  
Nylander, Tommy (Ekstern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: ErhvervsPhD-ordningen VTU  
Project: PhD

**Quantification of biomolecular interactions with soft material**

Department of Micro- and Nanotechnology  
Period: 01/09/2010 → 19/02/2014  
Number of participants: 5  
Phd Student:  
Kristensen, Kasper (Intern)  
Main Supervisor:  
Andresen, Thomas Lars (Intern)  
Examiner:  
Marie, Rodolphe (Intern)  
Ipsen, John Hjorth (Intern)  
Wimley, William C. (Ekstern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Institut, samfinansiering  
Project: PhD

**In vivo trafficking of liposome labelled by novel [18F]-phospholipid probes**

Department of Micro- and Nanotechnology  
Period: 01/03/2010 → 18/12/2012  
Number of participants: 6  
Phd Student:  
Jensen, Andreas Tue Ingemann (Intern)  
Supervisor:  
Andresen, Thomas Lars (Intern)  
Main Supervisor:  
Rasmussen, Palle (Intern)  
Examiner:  
Lindvold, Lars René (Intern)  
Goins, Beth A. (Ekstern)  
Madsen, Jacob (Intern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Institut, samfinansiering  
Project: PhD
Syntese af Gurmarin Analogier

Department of Micro- and Nanotechnology
Period: 01/07/2009 → 22/11/2012
Number of participants: 6
Phd Student:
Eliasen, Rasmus (Intern)
Supervisor:
Berg, Rolf Henrik (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Jakobsen, Mogens Havsteen (Intern)
Bernkop Schnürch, Andreas (Ekstern)
Jensen, Knud Jørgen (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: ErhvervsPhD-ordningen VTU
Project: PhD

Analysis and Improvement of the Gene Delivery Properties of Polyethyleneimine - An in Vitro Study

Department of Micro- and Nanotechnology
Period: 15/04/2009 → 31/10/2012
Number of participants: 5
Phd Student:
Mattebjerg, Maria Ahlm (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Larsen, Niels Bent (Intern)
Gao, Jinming (Ekstern)
Moos, Torben (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD

Development of Enzymatically Triggered Polymersomes as Drug Delivering Systems for Treating Cancer

Department of Micro- and Nanotechnology
Period: 01/04/2009 → 21/06/2013
Number of participants: 5
Phd Student:
Bjerg, Lise Nørkjær (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Berg, Rolf Henrik (Intern)
Moos, Torben (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD
Smart surfaces for guiding cellular behaviour

Department of Micro- and Nanotechnology
Period: 01/02/2009 → 23/05/2012
Number of participants: 5
Phd Student: Lind, Johan Ulrik (Intern)
Supervisor: Andresen, Thomas Lars (Intern)
Main Supervisor: Larsen, Niels Bent (Intern)
Examiner: Rozlosnik, Noemi (Intern)
Berggren, Magnus (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD

Synthesis and Characterization of Nanoparticle Sensors for Metabolite Quantification in Cells

Department of Micro- and Nanotechnology
Period: 01/01/2009 → 26/06/2012
Number of participants: 6
Phd Student: Ek, Pramod Kumar (Intern)
Supervisor: Almdal, Kristoffer (Intern)
Main Supervisor: Andresen, Thomas Lars (Intern)
Examiner: Ndoni, Sokol (Intern)
Bechgaard, Klaus (Intern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

Time-resolved pH imaging in cancer cell compartments: Towards the development of new drug delivery systems for treatment of cancer

Department of Micro- and Nanotechnology
Period: 01/05/2008 → 09/02/2012
Number of participants: 5
Phd Student: Søndergaard, Rikke Vicki (Intern)
Supervisor: Almdal, Kristoffer (Intern)
Main Supervisor: Andresen, Thomas Lars (Intern)
Examiner: Mølhave, Kristian (Intern)
Bechgaard, Klaus (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD
Syntese og karakterisering af protease sensitive liposomer

Department of Micro- and Nanotechnology
Period: 15/03/2008 → 09/02/2012
Number of participants: 6
Phd Student:
Jølck, Rasmus Irmeng (Intern)
Supervisor:
Berg, Rolf Henrik (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Ndoni, Sokol (Intern)
Bechgaard, Klaus (Intern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

Celte Biologiske studier af protease aktiverede liposomale drug delivery

Department of Micro- and Nanotechnology
Period: 01/03/2008 → 19/04/2012
Number of participants: 6
Phd Student:
Johansen, Pia Thermand (Intern)
Supervisor:
Jensen, Simon S. (Ekstern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Pedersen, Susanne Brix (Intern)
Fichtner, Iduna (Ekstern)
Vogel, Ulla Birgitte (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Ansat ekstern
Project: PhD

Liposomal Drug Delivery of Radionuclides for Cancer Diagnostics and Therapy

Department of Micro- and Nanotechnology
Period: 01/02/2008 → 26/06/2012
Number of participants: 6
Phd Student:
Petersen, Anncatrine Luisa (Intern)
Supervisor:
Rasmussen, Palle H. (Ekstern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Jensen, Mikael (Intern)
Gabizon, Alberto A. (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Syntese og karakterisering af lipidderivater til inkorporering i målrettede drug delivery systemer

Department of Micro- and Nanotechnology
Period: 15/01/2008 → 21/09/2011
Number of participants: 6
Phd Student:
Andersen, Simon (Intern)
Supervisor:
Jensen, Simon Skjøde (Ekstern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Berg, Rolf Henrik (Intern)
Larsen, Kim L. (Ekstern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: ErhvervsPhD-ordningen VTU
Project: PhD

Synthesis and Characterization of Membrane Active Peptides - Towards the Development of Novel Drug Delivery Systems

Department of Micro- and Nanotechnology
Period: 01/09/2007 → 24/08/2011
Number of participants: 7
Phd Student:
Etzerodt, Thomas Povl (Intern)
Supervisor:
Claussen, Mads Hartvig (Intern)
Rasmussen, Palle (Intern)
Main Supervisor:
Andresen, Thomas Lars (Intern)
Examiner:
Berg, Rolf Henrik (Intern)
Thompson, David H. (Ekstern)
Westh, Peter (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut/centerfinansieret
Project: PhD

Liposomal prodrug systems - synthesis, biofysiske og biologiske studier

Department of Chemistry
Period: 01/03/2007 → 01/09/2010
Number of participants: 7
Phd Student:
Pedersen, Palle Jacob (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Madsen, Robert (Intern)
Main Supervisor:
Claussen, Mads Hartvig (Intern)
Examiner:
Tanner, David Ackland (Intern)
Jensen, Knud Jørgen (Intern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

Syntese og biologiske studier af lipid-baserede lægemidler
Department of Chemistry
Period: 01/10/2006 → 30/06/2007
Number of participants: 4
Phd Student:
Jørgensen, Pernille Nyvang (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Peters, Günther H.J. (Intern)
Main Supervisor:
Madsen, Robert (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: DTU-lønnet stipendie
Project: PhD

Organic Synthesis and Biophysical Investigation of Novel Targeted Drug Delivery Systems
Department of Chemistry
Period: 01/03/2005 → 29/08/2008
Number of participants: 7
Phd Student:
Linderoth, Lars (Intern)
Supervisor:
Andresen, Thomas Lars (Intern)
Jørgensen, Kent (Intern)
Peters, Günther H.J. (Intern)
Main Supervisor:
Madsen, Robert (Intern)
Examiner:
Tanner, David Ackland (Intern)
Thompson, David H. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: DTU-lønnet stipendie
Project: PhD

De Novo Organic Synthesis abd Biophysical Investigation of Novel Liposome Systems in relation to Drug Delivery
Department of Chemistry
Period: 01/04/2001 → …
Number of participants: 9
Phd Student:
Andresen, Thomas Lars (Intern)
Supervisor:
Begtrup, Mikael (Ekstern)
Jørgensen, Kent (Intern)
Lundt, Inge (Intern)
Mouritsen, Ole G. (Intern)
Main Supervisor:
Madsen, Robert (Intern)
Examiner:
Peters, Günther H.J. (Intern)
Barenholz, Yechezkel (Ekstern)
Wengel, Jesper (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Erhvervsforskerordningen
Project: PhD

Activities:

Quantifying antimicrobial peptide interactions with lipid membranes: Presented at the department of Chemistry, Purdue University
Period: 1 Mar 2009
Thomas Lars Andresen (Speaker)
Department of Micro- and Nanotechnology
Self-organizing materials for nanotechnology Section
Colloids and Biological Interfaces Group

Description
Place: West Lafayette, USA

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations

Secretary phospholipase A2 hydrolysis of lipid membranes: Presented at Biozentrum, University of Basel
Period: 12 Feb 2009
Thomas Lars Andresen (Speaker)
Department of Micro- and Nanotechnology
Self-organizing materials for nanotechnology Section
Colloids and Biological Interfaces Group

Description
Place: Basel, Switzerland

Related external organisation
Unknown external organisation
Activity: Talks and presentations › Conference presentations