



## Polymeric gel nanoparticle pH sensors for intracellular measurements

**Almdal, Kristoffer; Andresen, Thomas Lars; Benjaminsen, Rikke Vicki; Christensen, Nynne Meyn; Henriksen, Jonas Rosager; Sun, Honghao**

*Publication date:*  
2011

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*

Almdal, K., Andresen, T. L., Benjaminsen, R. V., Christensen, N. M., Henriksen, J. R., & Sun, H. (2011). *Polymeric gel nanoparticle pH sensors for intracellular measurements*. Poster session presented at Frontiers in Polymer Science : Second International Symposium, Lyon, France, .

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

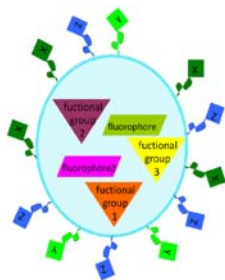
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Polymeric gel nanoparticle pH sensors for intracellular measurements

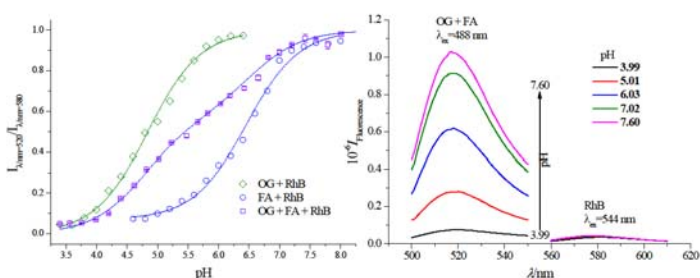
Kristoffer Almdal, Thomas L. Andresen, Rikke V. Benjamiensen, Nymne M. Christensen, Jonas R. Henriksen, and Honghao Sun

DTU Nanotech, Department of Micro- and Nanotechnology, Technical University of Denmark, Ørsted Plads, Building 423  
DK-2800 Kgs. Lyngby, Denmark

Precise measurements of pH in cells and intracellular compartments are of importance to both the fundamental understanding of metabolism and to the development of drugs that are released from the endosomal-lysosomal pathway. We have developed polymer gel nanoparticles as carriers of covalently bound fluorophores for ratiometric measurements of pH. One or more pH sensitive fluorophores serve to give the desired pH dependence of the output. With one pH sensitive fluorophore a dynamic range of ~2 pH units is achieved. The physiologically interesting pH range is approximately 4 pH units and thus a nanoparticle sensor with two pH sensitive fluorophores is appropriate. Suitable choice of fluorophores is e.g. Rhodamine B as reference and Oregon green (OG) and Fluorescein (FA) as pH sensitive fluorophores. The fluorophores are derivatized to allow for covalent bonding to the gel nanoparticle. The synthesis also allows for introduction of charged groups. Particle charge influences the cell uptake heavily.



## Sensor calibration



- Green diamonds are Oregon Green (OG)/Rhodamine B (RhB) labeled particles
- Blue circles are Fluorescein (FA)/Rhodamine B (RhB) labeled particles
- Violet squares are OG/FA/RhB labeled particles

## Sensor calibration curves

- The intensity ratio of fluorescence at  $\lambda=580$  nm and  $\lambda=520$  nm,  $I_{\lambda=580}/I_{\lambda=520}$  is generated for each pH
- For single pH sensitive fluorophore sensors the curve follows  $R = \frac{I_{\lambda=580}}{I_{\lambda=520}} = \frac{R_{max} - R_{min}}{10^{pK_a - pH} + 1} + R_{min}$
- Following a fit of  $R$  the data is rescaled as  $\frac{I_{\lambda=580} - R_{min}}{I_{\lambda=520} - R_{min}}$
- The double pH sensitive fluorophore (OG/FA/RhB) curve are fitted with

$$R = \frac{I_{\lambda=580}}{I_{\lambda=520}} = \frac{R_{max} - R_2 - R_{min}}{10^{pK_{a1} - pH} + 1} + \frac{R_2}{10^{pK_{a2} - pH} + 1} + R_{min}$$

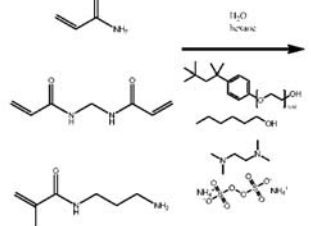
and rescaled similar to the single pH sensitive fluorophore curves

## Conclusions

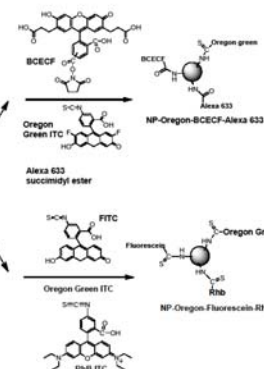
- Nanoparticle fluorescent ratiometric sensors covering the physiologically relevant pH range have been obtained.
- The relation between micro- and miniemulsion in the synthesis step is not fully understood.
- The sensors can be used to follow cellular processes.
- The availability of the broad pH range is important in characterizing the cellular processes.

This work is part of the Danish Research Council for Technology and Production (Grant 274-07-0172) sponsored project LiMES - Time-resolved metabolite quantifications in living cells using optical nanosensors.

## Microemulsion synthesis of amino functionalized particles



## Fluorophore attachment to particles



## Nanoparticle sensor synthesis

- Microemulsion synthesis allows for control of particle size.
- microemulsions are sensitive systems:
  - unclear whether a true microemulsion exist throughout the course of the synthesis. In other published work often bimodal particles size distributions arise.<sup>1</sup>
  - probably the microemulsion<sup>2</sup> changes to a miniemulsion<sup>3</sup> or emulsion during the course of the reaction. Thus the stirring rate becomes important.
- DLS and microscopy analysis indicate monomodal particle size distribution with 77 nm particles.
- Fluorophores can alternatively be covalently bonded to the particles at the polymerization step through acrylate functionalization prior to polymerization.
- The amine conjugation method allows for a higher fluorophore loading of the particles.
- The choice of non-ionic surfactants for forming the microemulsion is deliberate. Use of anionic surfactants (such as AOT) lead to particles with a negative  $\zeta$ -potential. It is impossible to completely wash out the anionic surfactant.

<sup>1</sup> Heather A. Clark, Susan L. R. Baker, Murphy Beaman, Michael T. Miller, Eric Monson, Steve Parus, Zhong-You Shi, Antonim Song, Bjorn Thorarud, Raoul Kopelman, Alex Ade, Walter Metzner, Brian Ashley, Marion Hoyer, Dwayne Hill, Rhonda Lightle, Martin A. Philbert, *Sensors and Actuators B: Chemical*, 51, 12-16 (1998); Heather A. Clark, Marion Hoyer, Martin A. Philbert, Raoul Kopelman, *Anal. Chem.*, 71, 4831-4836 (1999); Anja Graefel, Samuza E. Stanca, Sandoz Nietzsche, Lenka Kubcova, Ramer Beckert, Christoph Bischof, *Colloid Polym. Sci.*, 288, 6326-6331 (2010)

<sup>2</sup> A microemulsion is an equilibrium mixture of water, oil and surfactants.

<sup>3</sup> A miniemulsion is a mixture of water, oil and surfactants with a characteristic length scale in the nanometer range that is stabilized by osmotic and high shear.

## Kinetics of acidification of acidification in the endosomal-lysosomal system

- HepG2 cells. Nanoparticle sensor treatment for 1.5, 2.0 and 24 h
- Differential interference contrast (DIC) images, fluorescence images and overlay.
- Particle colours according to the pH as determined by a calibration curve.
- After 1.5 hours unspecific endocytosis (no receptor targeting), pH= 5.1  $\pm$  0.6
- After 2 hours shift of the pH distribution toward lower pH pH= 4.9  $\pm$  0.6
- After 24 hours most particles have reached the acidic compartments pH= 4.5  $\pm$  0.4

