ChemProt-3.0: a global chemical biology diseases mapping

ChemProt is a publicly available compilation of chemical-protein-disease annotation resources that enables the study of systems pharmacology for a small molecule across multiple layers of complexity from molecular to clinical levels. In this third version, ChemProt has been updated to more than 1.7 million compounds with 7.8 million bioactivity measurements for 19 504 proteins. Here, we report the implementation of global pharmacological heatmap, supporting a user-friendly navigation of chemogenomics space. This facilitates the visualization and selection of chemicals that share similar structural properties. In addition, the user has the possibility to search by compound, target, pathway, disease and clinical effect. Genetic variations associated to target proteins were integrated, making it possible to plan pharmacogenetic studies and to suggest human response variability to drug. Finally, Quantitative Structure-Activity Relationship models for 850 proteins having sufficient data were implemented, enabling secondary pharmacological profiling predictions from molecular structure.
High-throughput epitope profiling of snake venom toxins: unveiling the complexity of antigen-antibody interactions of antivenoms

Insight into the molecular details of polyclonal antivenom antibody specificity is a prerequisite for accurate prediction of cross-reactivity and can provide a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear elements in epitopes in 82 toxins from four African mamba and three neurotoxic cobra snakes obtained from public databases.

High-throughput immuno-profiling of mamba (Dendroaspis) venom toxin epitopes using high-density peptide microarrays

Snakebite envenoming is a serious condition requiring medical attention and administration of antivenom. Current antivenoms are antibody preparations obtained from the plasma of animals immunised with whole venom(s) and contain antibodies against snake venom toxins, but also against other antigens. In order to better understand the molecular interactions between antivenom antibodies and epitopes on snake venom toxins, a high-throughput immuno-profiling study on all manually curated toxins from Dendroaspis species and selected African Naja species was performed based on custom-made high-density peptide microarrays displaying linear toxin fragments. By detection of binding for three different antivenoms and performing an alanine scan, linear elements of epitopes and the positions important for binding were identified. A strong tendency of antivenom antibodies recognizing and binding to epitopes at the functional sites of toxins was observed. With these results, high-density peptide microarray technology is for the first time introduced in the field of toxinology and molecular details of the evolution of antibody-toxin interactions based on molecular recognition of distinctive toxic motifs are elucidated.
Synthesis and biological evaluations of cytotoxic and antiangiogenic triterpenoids-jacaranone conjugates

Background: The development of antiangiogenic agents arises as a more effective and selective therapeutic approach for the treatment of cancer. In addition to reduced acute toxicity, the efficacy of chemotherapy could be improved when administered in combination specific antiangiogenic with cytotoxic agents. The conjugation or hybridization of bifunctional molecules is one of the alternative rational design strategies for co-administration of anticancer drugs. Objective and
Methods: The goal of this work is to prepare the conjugates of an antiangiogenic triterpene, 3-oxo oleanolic acid, and structurally related triterpenoids with a cytotoxic semibenzoquinone, jacaranone. The cytotoxic, antiproliferative and antiangiogenic activities of segments and conjugates were determined. The possible targets of conjugates 6a-6h were predicted using Similarity Ensemble Approach (SEA). Results: The results showed that these conjugates are more potent in both cytotoxic and antiangiogenic assays than their corresponding parent molecules, and are also selectively more active against melanoma cells B16 and metastatic B16BL6 than the two other cancer cell lines (A549 and MCF-7) tested. The predicted antiangiogenesis related targets could involve glycogen phosphorylase, neuraminidase, interferon gamma, and tubulin beta chain. Conclusion: The bifunctional conjugates could be useful as dual acting antitumor/antiangiogenic agents.

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BFI (2014): BFI-level 1
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BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 1.44 SJR 0.402 SNIP 0.624
Web of Science (2012): Impact factor 1.373
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
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Web of Science (2011): Impact factor 1.496
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Discovery of Peptidic Anti-cobratoxins by Next Generation Phage Display

Antivenoms are still being produced by animal immunization protocols and are therefore associated with high immunogenicity for human recipients. Here we report the first step towards discovery of synthetic antitoxins that could be used for development of a fully synthetic antivenom against neurotoxin from cobras (*Naja* genus).

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High-throughput sequencing enhanced phage display enables the identification of patient-specific epitope motifs in serum

Phage display is a prominent screening technique with a multitude of applications including therapeutic antibody development and mapping of antigen epitopes. In this study, phages were selected based on their interaction with patient serum and exhaustively characterised by high-throughput sequencing. A bioinformatics approach was developed in order to identify peptide motifs of interest based on clustering and contrasting to control samples. Comparison of patient and control samples confirmed a major issue in phage display, namely the selection of unspecific peptides. The potential of the bioinformatic approach was demonstrated by identifying epitopes of a prominent peanut allergen, Ara h 1, in sera from patients with severe peanut allergy. The identified epitopes were confirmed by high-density peptide micro-arrays. The present study demonstrates that high-throughput sequencing can empower phage display by (i) enabling the analysis of complex biological samples, (ii) circumventing the traditional laborious picking and functional testing of individual phage clones and (iii) reducing the number of selection rounds.

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Ultra-high density peptide arrays demonstrate unique patient-specific IgE and IgG4 epitope patterns for peanut allergens that persist over multiple years

Clinicians are seeing a growing number of cashew nut allergic patients. One of the peculiarities of this allergy is that a minimal amount of cashew nut allergen may cause severe allergic reactions, suggesting high potency of the allergen comparable to other tree nuts and peanuts. The double blind placebo controlled food challenge (DBPCFC) test is currently the gold standard to establish cashew nut allergy. The development of predictive tools in diagnosing cashew nut allergy is
needed and research should be done on cross-sensitization between cashew nut and other botanically related allergens.

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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 5.51 SJR 2.529 SNIP 2.161
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
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BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.89 SJR 2.221 SNIP 1.801
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A novel approach for characterisation of conformational allergen epitopes combining phage display and high-throughput sequencing

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Characterisation of the Ara h 1-specific IgE repertoire in peanut allergic patients using phage display technology and next generation sequencing
Pharmacology profiling of chemicals and proteins

The central tenet in drug-development of one drug selectively interacts with one target is increasingly challenged by the vast amount of data released to the public domain in the past 10-15 years, documenting multiple targets for most of the FDA approved pharmaceuticals [1]. Unintended interactions between pharmaceuticals and proteins in vivo potential leads to unwanted adverse effects, toxicity and reduced half-life, but can also lead to novel therapeutic effects of already approved pharmaceuticals. Hence identification of in vivo targets is of importance in discovery, development and repurposing of pharmaceuticals, a process referred to as pharmacology profiling. Pharmacology profiling of chemical and protein based pharmaceuticals has been proven valuable in a number studies [2],
however missing values in the drug-protein interaction matrix limits the profile for novel or less studied compounds. This limitation complicates adverse effect assessment in the early drug-development phase, thus contributing to drugattrition. Prediction models offer the possibility to close these gaps and provide more complete pharmacology profiles, however improvements in performances are required for these tools to serve as an alternative to experimentally obtained measurements.

Here I present several different tools that aid pharmacology profiling of the two main classes of pharmaceuticals; chemicals (small molecules) and proteins (biopharmaceuticals). Biopharmaceuticals have the inherent risks of eliciting an immune response due to its nonself origin, which potentially alters the pharmacology profile of the substance. The neutralization of biopharmaceuticals by antidrug antibodies (ADAs) is an important element in the immune response cascade, however studies of ADA binding site on biopharmaceuticals, referred to as B-cell epitopes, are complicated by expensive experimental procedures thus making prediction models an appealing alternative. In general, B-cell epitope prediction tools have moderate performances, which to some extent originates from an incomplete understanding of what constitute a B-cell epitope and incomplete datasets used for model building and benchmarking. In the first paper included in this thesis we analyze the B-cell epitopes obtained from co-crystalized antibody-antigen complexes from the PDB database. We were able to describe the epitope area as a flat oblong area residing on the protein surface consisting of a hydrophobic core surrounded by hydrophilic/charged residues. This finding prompted us to update the B-cell epitope prediction method DiscoTope [3] by introducing a novel scoring function for describing the spatial neighborhood surrounding each residue as described in paper II of this thesis. Using the developed method we assessed the impact on performance using a more realistic benchmark definition compared to privies studies, by including multiple epitopes for each antigen and the biological unit used for raising the antibody response, when available. On average, the Area Under the roc-Curve(AUC) performance was improved from 0.791 to 0.824 for the 13 proteins were additional information could be obtained, thereby indicating that the performance of B-cell epitope prediction tools in general are under-estimated.

Novel techniques such as Next Generation Sequencing (NGS) and peptide microarray facilitate novel strategies for experimental identification of B-cell epitopes. In chapter 4, a novel method for epitope identification is described, combining NGS with phage-display. Epitopes in peanut allergen ara h1 were successfully detected using sera from peanut allergic patients and confirmed using peptide micro-array technology, demonstrating the applicability of both methods. Adverse effect of small molecule based pharmaceutical is rarely mediated through an immune response but is predominantly the consequence of interactions with unintended proteins in vivo. To assists researchers in determine the binding profile of chemicals, thus their pharmacology profile, a database of chemical-protein interactions were developed and presented in chapter 5. The database integrates chemical-protein interaction information from 10 different databases as well as disease, functional and pathway mapping of proteins, SNP data through the Ensembl database and prediction tools for filling out gaps in the chemical-protein interaction matrix. Graphical representation of the pharmacology space is accomplished by the use of zoomable heatmaps, which enable traversing from an overview of the entire space to specific pharmacology profiles of a single chemical by zooming on specific areas of the heatmap. The compiled dataset together with the implemented visualization and prediction tools, facilitate pharmacology profiling of chemicals in all development stages, hence potentially enable identification of adverse effects in early drugdevelopmentor identification of novel treatment paradigms for approved pharmaceuticals.

Finally, the visualization of the pharmacology space is addressed by developing a 2 dimensional zoomable heatmap inspired by country and city maps. Chemicals sharing similar scaffold or features are placed together on the map, thus enable a more detailed visualization of the pharmacology landscape surrounding one or more chemicals of interest. The tool, presented in chapter 6, enables researchers to couple scaffold and feature hopping with bioactivity data for the use in drug-discovery and development, thus avoiding unwanted adverse effects. In summary, here I present several different tools that can assists researchers in determine essential properties in the pharmacology profile of both protein and small molecule pharmaceuticals and potentially detect adverse effects in drug-development.

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ChemProt-2.0: visual navigation in a disease chemical biology database
ChemProt-2.0 (http://www.cbs.dtu.dk/services/ChemProt-2.0) is a public available compilation of multiple chemical-protein annotation resources integrated with diseases and clinical outcomes information. The database has been updated to > 1.15 million compounds with 5.32 millions bioactivity measurements for 15 250 proteins. Each protein is linked to quality-scored human protein-protein interactions data based on more than half a million interactions, for studying diseases and biological outcomes (diseases, pathways and GO terms) through protein complexes. In ChemProt-2.0, therapeutic effects as well as adverse drug reactions have been integrated allowing for suggesting proteins associated to clinical outcomes.
New chemical structure fingerprints were computed based on the similarity ensemble approach. Protein sequence similarity search was also integrated to evaluate the promiscuity of proteins, which can help in the prediction of off-target effects. Finally, the database was integrated into a visual interface that enables navigation of the pharmacological space for small molecules. Filtering options were included in order to facilitate and to guide dynamic search of specific queries.

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Scopus rating (2016): CiteScore 9.28 SJR 7.883 SNIP 2.744
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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 9.48 SJR 7.358 SNIP 2.631
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 8.74 SJR 6.64 SNIP 2.552
Web of Science (2014): Impact factor 9.112
Web of Science (2014): Indexed yes
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Scopus rating (2013): CiteScore 8.46 SJR 6.801 SNIP 2.284
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ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 2
Web of Science (2012): Impact factor 8.278
ISI indexed (2012): ISI indexed yes
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BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 7.86 SJR 5.976 SNIP 2.19
Web of Science (2011): Impact factor 8.026
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Scopus rating (2010): SJR 5.381 SNIP 2.034
Structural analysis of B-cell epitopes in antibody:protein complexes

The binding of antigens to antibodies is one of the key events in an immune response against foreign molecules and is a critical element of several biomedical applications including vaccines and immunotherapeutics. For development of such applications, the identification of antibody binding sites (B-cell epitopes) is essential. However experimental epitope mapping is highly cost-intensive and computer-aided methods do in general have moderate performance. One major reason for this moderate performance is an incomplete understanding of what characterizes an epitope. To fill this gap, we here developed a novel framework for comparing and superimposing B-cell epitopes and applied it on a dataset of 107 non-similar antigen:antibody structures extracted from the PDB database. With the presented framework, we were able to describe the general B-cell epitope as a flat, oblong, oval shaped volume consisting of predominantly hydrophobic amino acids in the center flanked by charged residues. The average epitope was found to be made up of ~15 residues with one linear stretch of 5 or more residues constituting more than half of the epitope size. Furthermore, the epitope area is predominantly constrained to a plane above the antibody tip, in which the epitope is orientated in a −30° to 60° angle relative to the light to heavy chain antibody direction. Contrary to previously findings, we did not find a significant deviation between the amino acid composition in epitopes and the composition of equally exposed parts of the antigen surface. Our results, in combination with previously findings, give a detailed picture of the B-cell epitope that may be used in development of improved B-cell prediction methods.
Reliable B cell epitope predictions: Impacts of method development and improved benchmarking.

The interaction between antibodies and antigens is one of the most important immune system mechanisms for clearing infectious organisms from the host. Antibodies bind to antigens at sites referred to as B-cell epitopes. Identification of the exact location of B-cell epitopes is essential in several biomedical applications such as; rational vaccine design, development of disease diagnostics and immunotherapeutics. However, experimental mapping of epitopes is resource intensive making in silico methods an appealing complementary approach. To date, the reported performance of methods for in silico mapping of B-cell epitopes has been moderate. Several issues regarding the evaluation data sets may however have led to the performance values being underestimated: Rarely, all potential epitopes have been mapped on an antigen, and antibodies are generally raised against the antigen in a given biological context not against the antigen monomer. Improper dealing with these aspects leads to many artificial false positive predictions and hence to incorrect low performance values. To demonstrate the impact of proper benchmark definitions, we here present an updated version of the DiscoTope method incorporating a novel spatial neighborhood definition and half-sphere exposure as surface measure. Compared to other state-of-the-art prediction methods, Discotope-2.0 displayed improved performance both in cross-validation and in independent evaluations. Using Discotope-2.0, we assessed the impact on performance when using proper benchmark definitions. For 13 proteins in the training data set where sufficient biological information was available to make a proper benchmark redefinition, the average AUC performance was improved from 0.791 to 0.824. Similarly, the average AUC performance on an independent evaluation data set improved from 0.712 to 0.727. Our results thus demonstrate that given proper benchmark definitions, B-cell epitope prediction methods achieve highly significant predictive performances suggesting these tools to be a powerful asset in rational epitope discovery. The updated version of DiscoTope is available at www.cbs.dtu.dk/services/DiscoTope-2.0.

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Scopus rating (2016): CiteScore 4.41 SJR 3.243 SNIP 1.363
Web of Science (2016): Impact factor 4.542
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.69 SJR 3.476 SNIP 1.442
Pre-clinical exploration of cancer neoepitope immunotherapy
The complex nature of cancer has complicated the development of an effective treatment immensely. This has resulted in an increased interest for immuno-oncology treatment strategies that focus on the deliberate use of the patient’s immune system to fight the cancer. Particularly interesting are personalised vaccines based on tumour-specific neoepitopes, which are created by the unique set of mutations characteristic of the individual tumour. However, pre-clinical approaches require time-consuming screening steps to identify neoepitopes and current in silico (computational) tools for efficient identification of neoepitopes are scarce and of limited accuracy. The overall objective of the project is to develop an advanced, pre-clinically validated in silico pipeline for the identification of murine cancer neoepitopes. In parallel, the development of a human pipeline will enable the translation of pre-clinical findings to clinically relevant information for the construction of personalised human cancer vaccines. A range of features, which are hypothesised to be linked to
neoeptipe immunogenicity, are determined through the development of various computational tools using data mining and advanced machine learning methods, and subsequently incorporated into a complete neoeptipe identification pipeline. The aim of this is to improve the predictive performance of the pipeline enabling a link between mouse and human models to be established, which will ultimately allow for translation of project findings into human cancer vaccines.

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