ArrayPitope: Automated Analysis of Amino Acid Substitutions for Peptide Microarray-Based Antibody Epitope Mapping

Identification of epitopes targeted by antibodies (B cell epitopes) is of critical importance for the development of many diagnostic and therapeutic tools. For clinical usage, such epitopes must be extensively characterized in order to validate specificity and to document potential cross-reactivity. B cell epitopes are typically classified as either linear epitopes, i.e. short consecutive segments from the protein sequence or conformational epitopes adapted through native protein folding. Recent advances in high-density peptide microarrays enable high-throughput, high-resolution identification and characterization of linear B cell epitopes. Using exhaustive amino acid substitution analysis of peptides originating from target antigens, these microarrays can be used to address the specificity of polyclonal antibodies raised against such antigens containing hundreds of epitopes. However, the interpretation of the data provided in such large-scale screenings is far from trivial and in most cases it requires advanced computational and statistical skills. Here, we present an online application for automated identification of linear B cell epitopes, allowing the non-expert user to analyse peptide microarray data. The application takes as input quantitative peptide data of fully or partially substituted overlapping peptides from a given antigen sequence and identifies epitope residues (residues that are significantly affected by substitutions) and visualize the selectivity towards each residue by sequence logo plots. Demonstrating utility, the application was used to identify and address the antibody specificity of 18 linear epitope regions in Human Serum Albumin (HSA), using peptide microarray data consisting of fully substituted peptides spanning the entire sequence of HSA and incubated with polyclonal rabbit anti-HSA (and mouse anti-rabbit-Cy3). The application is made available at: www.cbs.dtu.dk/services/ArrayPitope.
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.

General information
State: Published
Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, Network Engineering of Eukaryotic Cell Factories, Integrative Systems Biology, Roche NimbleGen, Universidad de Costa Rica
Number of pages: 1
Publication date: 2016
Event: Poster session presented at The 12th Congress of the Pan-American Section of the International Society on Toxinology, Miami Beach, United States.
Main Research Area: Technical/natural sciences
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.
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.
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.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Fluidic Array Systems and Technology, Department of Systems Biology, Center for Biological Sequence Analysis, National Food Institute, University of Vienna, Roche NimbleGen
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Number of pages: 1
Pages: 90-90
Publication date: 2015
Conference: European Academy of Allergy and Clinical Immunology Congress 2015, Barcelona, Spain, 06/06/2015 - 06/06/2015
Main Research Area: Technical/natural sciences

Publication information
Journal: Allergy
Volume: 70
Issue number: Suppl. 101
Article number: 183
ISSN (Print): 0105-4538
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 6.23 SJR 2.724 SNIP 2.475
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 3.13 SNIP 2.127 CiteScore 5.73
Web of Science (2015): Indexed yes
A novel approach for characterisation of conformational allergen epitopes combining phage display and high-throughput sequencing

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Fluidic Array Systems and Technology, Department of Systems Biology, Center for Biological Sequence Analysis, Immunological Bioinformatics, National Food Institute, Division of Toxicology and Risk Assessment
Authors: Christiansen, A. (Intern), Hansen, C. S. (Intern), Kringelum, J. V. (Intern), Lund, O. (Intern), Bøgh, K. L. (Intern), Dufva, M. (Intern)
Pages: P27
Publication date: 2014
Characterisation of the Ara h 1-specific IgE repertoire in peanut allergic patients using phage display technology and next generation sequencing

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Fluidic Array Systems and Technology, National Food Institute, Division of Toxicology and Risk Assessment, Department of Systems Biology, Center for Biological Sequence Analysis, Immunobiometrics, Institute of Food Research, Medical University of Vienna
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Number of pages: 1
Pages: 140-140
Publication date: 2014
Conference: European Academy of Allergy and Clinical Immunology Congress 2014, Copenhagen, Denmark, 07/06/2014 - 07/06/2014
Main Research Area: Technical/natural sciences
Projects:

Prediction of antigen_BCR interactions based on high throughput peptide chip data and prediction of MHC-peptide-TCR Interactions

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