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SPADE web service for prediction of allergen IgE epitopes

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ABSTRACT

The specific interaction of allergens with IgE antibodies and the allergen mediated cross-linking of receptor-bound IgE are key events of allergic diseases. The elucidation of the IgE binding sites (the epitopes) on the allergen surface is an important goal of allergy research. Only few allergen-specific IgE epitopes have been determined experimentally to date. Epitope prediction methods represent a viable alternative to experimental methods and have worked well with linear epitopes. However, as most IgE epitopes are of conformational and/or discontinuous nature sequence based prediction methods have had limited success in these cases. Here, we present the web server of the program SPADE (https://spade.uni-graz.at), which is the server implementation of a previously published program (1). In this approach we utilize the structural homology of cross-reactive allergens combined with the immunological cross-reactivity data for the discrimination of putative IgE-binding sites from non-cross-reactive surface patches. The method, although predictive, does not rely on machine-learning algorithms and does not require training data. The SPADE server features an easy-to-use interface, an automated pipeline consisting of third-party, as well as own, newly developed routines and a comprehensive output page.

INTRODUCTION

The recognition of allergens by specific IgE antibodies and the allergen mediated cross-linking of receptor-bound IgE are hallmark events in the progression of allergic diseases (2–4). In order to understand these key events it is important to detect and characterize the specific IgE interaction sites (i.e. conformational epitopes) on the allergen surface. The most accurate method would be the structural determination of the specific allergen-antibody complexes. Although many major environmental allergens have been structurally characterized and >120 non-redundant allergen structures have been deposited in the protein data bank (PDB) (5,6), there are still only very few allergen-antibody complexes available. Disregarding Hen-egg-white lysozyme (HEL or Gal d 4), which accounts for 32 complex structures with Fab or scFv, the Fab complex structures of only eight distinct allergens are available in the PDB (5,6). These include Bet v 1 (7), Api m 2 (8), Bos d 5 (9), Bla g 2 (10,11), Der p 1 (12), Der f 1 (13), Phl p 2 (14) and Phl p 7 (15). Only two of these structures, the complexes of Bos d 5 and Phl p 2 actually contain Fab's derived from IgE antibodies. Methods for the experimental determination of conformational epitopes, e.g. co-crystallization, NMR-based or MS based hydrogen exchange methods (16–19), chemical shift mapping (20,21) and mutational analysis (22,23) are in general very time consuming and/or work only with monoclonal antibodies. Therefore epitopes can only be determined one at the time and never represent the polyclonal situation found *in vivo*.

Alternatively, in the absence of allergen-antibody complexes, conformational epitopes can be predicted with *in silico* methods. Several methods have been developed for this purpose (24–31), however these methods have two fundamental drawbacks: they are based on sequence comparison rather than structural similarities and they do not account for experimental cross-reactivity data. To overcome these disadvantages and to improve the prediction accuracy we have developed computational methods for the localization of cross-reactive IgE-epitopes by structure-based comparison of allergen surfaces including the correlation of the surface similarity scores with immunological data (1,5).

MATERIALS AND METHODS

In this section we describe the input, output and workflow of the SPADE program and briefly touch on the underlying algorithms and the used third-party programs. For more in depth information on the methodology the reader is referred to the original SPADE paper (1) and our review about allergen structures and structure based epitope map-

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ping, which also contains several examples of evaluation of the method (5).

Server input

The SPADE method relies on the structural, i.e. 3D-coordinate-based comparison of cross-reactive and/or non-cross-reactive allergens. Therefore the program needs atomic resolution structures of molecules in PDB format, which are usually experimental structures determined by X-ray crystallography or NMR methods.

On the front webpage, the user may enter a 4-letter PDB code per structure, in which case that structure is fetched from a local database or from the official PDB repository (32). The local database represents a manually curated subset of allergen structures. Alternatively, the coordinates are uploaded by the user from her/his computer. The latter option is useful for investigating unpublished structures with the SPADE program, but it may also be necessary if a published structure represents a homo- or hetero-oligomer. By default the program assumes monomeric allergens and hence takes the first chain in the PDB-file for analysis while ignoring the rest. In case the user intends to analyze a higher chain, the easiest possibility is to extract the chain from the deposited PDB-file for direct upload.

Structure files have to be provided for at least two molecules: the target allergen for which to predict epitopes and a cross-reactive second allergen, which is typically a sequence/structure homologue. Further structures, in particular non-allergenic homologues, allow for contrasting the multiple comparisons in order to increase prediction specificity. Homology models as prediction target may in principle also be used as prediction targets (via the upload method), but this option has not been tested extensively and bears the risk of contortion of the results due to model bias.

In addition to the structural data, immunologic cross-reactivity values are essential, at least for the more precise multi-comparison variant with contrasting. The cross-reactivity, given as a percentage, has either been determined experimentally, e.g. by IgE inhibition experiments, or estimated (*pro forma* values), e.g. 100% for the quantitative use in a single comparison to a cross-reactive allergen.

Workflow

All server-side computations are performed in a CGI framework, which generates a pipeline of third-party programs, as well as own software tools. In a first step, the input models are stripped from unwanted (typically crystallographic) content such as water and solvent molecules, and a single amino-acid chain/molecule out of a complex, or a single-conformation model out of an NMR ensemble, respectively, is extracted from the overall structure. Characteristics that are derivable from the amino-acid type, namely polarity and lipophilicity of the residues, are assigned to the structure by means of sequence (primary structure) annotation for later use. For the inclusion of coordinate-based, respectively volumetric, information the structures to be compared are first 3D-aligned and superimposed to the target allergen, and subsequently quantitative features such as the molecular surface itself and the superimposed electrostatic potential are calculated for every molecule. SPADE employs Multiprot (33) for 3D-alignment, MSMS (34) for calculation of solvent-excluded molecular surfaces and PDB2PQR/APBS (35,36) for the calculation of electrostatic potential grids.

The superposition and comparison algorithm, based on the initial alignment with 1:1 residue mapping, is performed by an own program where multiple local, i.e. structure-subgroup, superpositions for the allergen-homologue pair of models are interleaved with the actual surface comparison. This yields multiple preliminary surface similarity scores, which are then assembled by means of weighted averaging. The chosen procedure accounts for possible conformational differences between homologous structures, in particular at surface loops, where the more conserved helix or sheet elements of secondary structure are absent. For the same reason amino acid side chains, which are not involved in intramolecular contacts, are assimilated before superposition by means of taking the most likely conformations from a rotamer library (37).

The per-residue scores of surface similarity are based on the geometrical shape agreement of the surface, which is represented by multiple triangular face elements, and the similarity of electrostatic potential grid points at the spatial position of those faces. In addition, previously determined per-residue features, such as lipophilicity are included to the score. In case of multiple comparisons to the target, i.e. when two or more homologous structures have been provided, the separate sets of scores have to be merged. For all cross-reactive cases, this is done by means of weighted averaging where the cross-reactivity percentage values (as decimal fraction) are taken as weights. If however no-/low-cross-reactivity molecules are among the comparisons, the according scores are defined as 'negative', meaning that they will be subtracted from the positive averages, again in a weighted manner: the less cross-reactive, the higher the negative weight. This results in a reactivity-contrasted similarity map where similar surface regions among cross-reactive homologues can be discriminated from other similarity patches that are meaningless in our allergy context.

The final step is the spatial filtering of residues on the target allergen surface. That step combines a similarity threshold with the spatial clustering of filtered residues: Amino-acid residues above the threshold must, as a second requirement, be very close in space in order to form a contiguous surface patch. Due to the fact that surface similarity scores depend on various aspects, there is no absolute threshold. Our program routine iteratively reduces an initial threshold of 70% until one or more reasonably sized patches are found, which represent the predicted epitopes.

Server output

The SPADE server presents its results on two separate output pages, and designates the epitope candidates as *predicted epitope patches* (PEP). One page is for 2D and one for 3D representation of results.

The 2D page lists the PEPs as such, all their contributing amino-acid residues by number and three-letter code, and quantitative global features of the PEP: overall solvent-solvent-accessible surface area (SASA), the relative accessibility as compared to 'free' residues (38) and

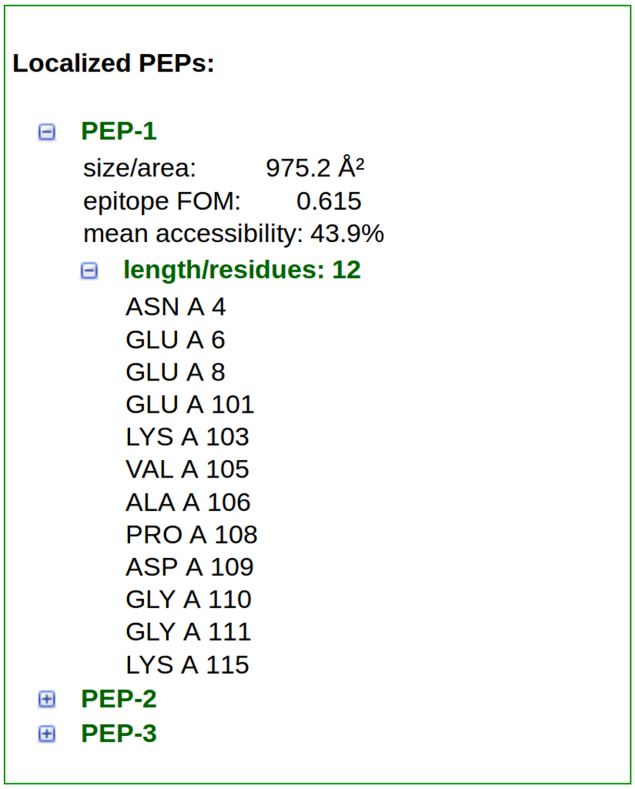

Figure 1. Prediction result for birch pollen allergen Bet v 1 (PDB: 1bv1), as obtained by comparison to the cherry allergen Pru av 1 (PDB: 1e09). This type of presentation lists of the three predicted epitope patches (PEPs), where the PEP-1 node is expanded twice to show global statistics, as well as the list of participating amino acid residues.

the effective average similarity to the cross-reactive homologues. This overview table is arranged like a folder list, with a collapsible item tree (Figure 1). The same page presents a bar-chart where individual similarly scores after the merging/contrasting step are plotted versus the residue number in sequence and PEP residues are tagged by markers with PEP color below the bars (Figure 2).

The 3D pages employs a Jmol graphics window (39) in HTML5 mode for the 3D-model display, associated to a JMOL control area where several data visualizations and structure representations can be chosen. The actual PEP visualization displays the molecular surface in light-grey with PEP-colored residues in individual colours, where e.g. blue is always used for PEP#1 (Figure 3A). Alternatively one can map similarity scores onto the surface using a color ramp that ranges from red via white to blue for minimal to maximal scores (Figure 3B). The same information, patches or similarity scores, may be combined with a cartoon representation of the structure, highlighting fold and secondary structure elements. Finally the target allergen may be inspected for physico-chemical features mapped to the surface, independent of the comparison to other molecules. This uses the same JMOL display window with volumetric mapping of the electrostatic potential, for instance.

All server results are stored in a user folder, which is protected from accidental access or guessing by means of a 24-digit alphanumerical (plus other allowed ASCII characters) name string. The output pages allow downloading of the results folder as compressed tar archive, which is a recommended action due to the fact that results are not permanently stored.

Server technicalities and policy

The SPADE service is using an Apache2 web server (2.4.7) with PHP5 (5.5.9) installed on a Linux machine (4-core Intel Xeon 2.5 MHz running Ubuntu 14.04.4. The used protocol for client communication is HTTPS. Javascript is used for input form evaluation and as interface to the JMOL on the results page. The back-end uses CGI as implemented to a Python 2.7 framework. A pipelined prediction job is designed as tool chain of subsequent computation processes using different programs. Some of the tasks are managed by Python scripts while heavier computation is done by compiled software tools. This concerns third-party programs as mentioned in the workflow section and own programs. Our core modules are 'spade_confalign' for side-chain replacement, 'spade_compare' for multiple superposition and surface comparison and 'spade_patchfind' for clustering of filtered residues, all of which are written in C. Regarding job management, there is no queuing system used at the moment.

SPADE was tested with the following browser versions:

- Firefox Quantum (65.0) on MacOS 10.14/10.9 and Windows 10
- Google Chrome (72.0) on MacOS 10.14 and Windows 10, (67.0) on MacOS 10.9
- Apple Safari (12.0.3) on MacOS 10.14 and (9.1.3) on MacOS 10.9
- Microsoft Edge (42.17134) on Windows 10.

The website is free and open to all users and there is no login requirement, nor mandatory request for an e-mail address. A job-id is presented upon submission for later reference. Users have the option to provide their e-mail address if they prefer to be notified on job completion via e-mail. This e-mail contains the results-page link, which includes the unique job-id, as also presented on the waiting page.

RESULTS

Performance

A typical prediction job with three involved structures (i. e. two comparisons followed by post-processing) takes about four minutes from submission to results display on our current hardware. This scales with the number of structures, but also depends significantly on the size of the structures in terms of atom numbers. An exact time benchmarking has not been performed.

Regarding the prediction performance we assessed our results by means of comparison to experimentally determined reference epitopes, as per crystal structures of antibody complexes:

- The epitope of cow milk allergen beta-lactoglobulin as in complex with IgE-Fab (PDB 2R56) was predicted with 33% sensitivity and 42% specificity

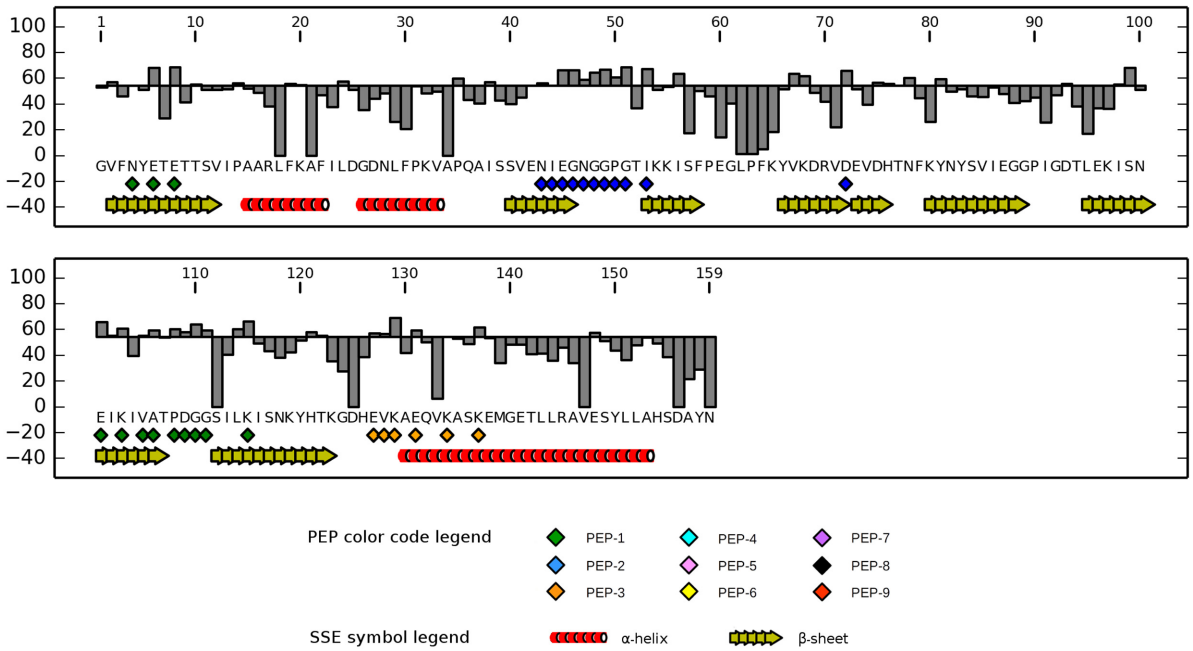

Figure 2. Sequential plot of surface similarity scores for the same prediction as in Figure 1. This bar chart is polar with respect to a baseline at the similarity threshold of 54%: residues with higher similarity (passing the epitope filter) have up-facing bars while values below the threshold correspond to down-facing bars. Actual PEP residues are tagged by diamond marks below the graph, in the color of their PEP, and secondary structure elements are annotated as well.

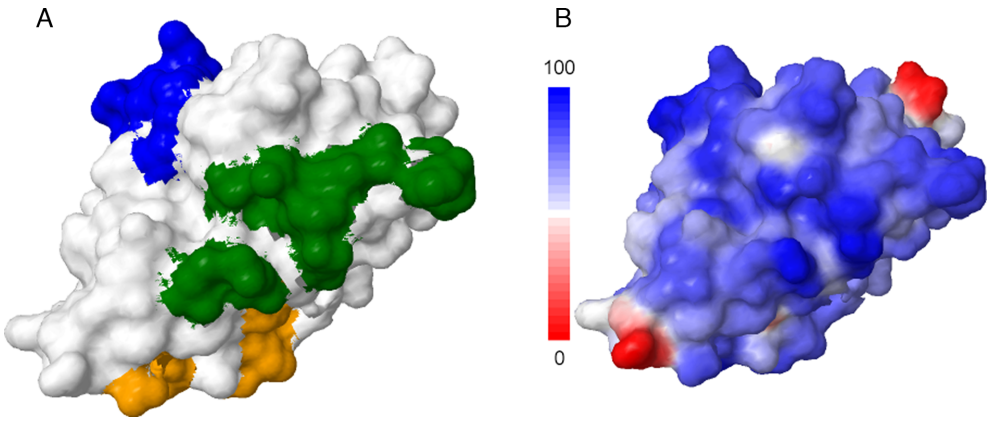

Figure 3. Two variants of 3D visualization on the SPADE results page. (**A**) The Jmol window shows a molecular surface representation of Bet v 1 with PEPs in unique colors. (**B**) Upon changing the display type, the surface is colored with a gradual scheme from red via white to blue according to the surface similarity of each residue. The surface orientation is the same in both displays so that the PEPs can clearly be aligned to most similar regions, i.e. the most intensely blue shading.

- The epitope of timothy grass pollen allergen Phl p 2 as in complex with IgE-Fab (PDB 2VXQ) was predicted with 57% sensitivity and 71% specificity.

Comparison to other web services

To the best of our knowledge, there is no other web-service, or software tool in general, that would specialize on IgE epitope prediction or would use a combination of structural and clinical data in its method. Likewise the existing web-services, as known to us, take single antigen structures and derive antigenicity scores from the intrinsic feature space of those targets, instead of comparing to other structures.

To name a few of the existing webserver-based tools, Discotope (29) and Epitopia (40) derive the likelihood of residues belonging to epitopes from statistical analyses (*t*- and *G*-tests, respectively) on physicochemical and structural properties which serve as classifiers for machine learning. ElliPro (28), on the other hand, implements a non-trained prediction method employing a residue protrusion index based on a purely structural analysis. More details and a wider coverage of other web-service implementations can be found in a review by Dall'Antonia *et al.* (5).

CONCLUSIONS AND OUTLOOK

The theory behind the epitope prediction algorithms implemented in SPADE was described in detail (1) and some test cases were also described in this paper.

SPADE can be applied in two basic modes: in the similarity mode (i.e. two or more cross-reactive allergens are available) and in the difference mode (when at least one structure of a proven non-cross-reactive protein is available for comparison). The first mode has been tested thoroughly (1,5,23), but for the second mode test cases are currently rare, as there is a lack of allergen families, which contain both the structure of an allergen as well as the structure of a 'non-allergen' (i.e. a structurally related protein, which is not IgE reactive or does not exhibit cross-reactivity to the allergen). With the event of more allergen structures being determined and IgE epitopes being determined with experimental methods, there will be the opportunity to evaluate the difference mode. Specifically, it will be interesting to see, whether the inclusion of a proven non-cross-reactive protein in the analysis can improve the specificity of the prediction.

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