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.
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