

Mapping Discontinuous Antibody Epitopes to Reveal Protein Structure and Changes in Structure Related to Function

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Abstract

We are developing a new method for protein structure determination that overcomes limitations in traditional methods, such as x-ray crystallography or nuclear magnetic resonance, and requires about a thousand fold less protein. The method, called Antibody Imprinting, uses antibodies against target proteins and random peptide probe libraries to map the epitopes of antibody binding sites on target proteins. Virtually all known antibody epitopes are highly discontinuous and are “assembled” by folding together regions of the protein that are far apart in the primary sequence. The Antibody Imprinting method seeks to rapidly and efficiently “mine” the antibody epitope information to reveal the structure of the target proteins.

1. Introduction

We are developing a new method for protein structure determination that overcomes limitations in traditional methods, such as x-ray crystallography or nuclear magnetic resonance, and requires about a thousand fold less protein. The method, called Antibody Imprinting, uses antibodies against target proteins and random peptide probe libraries to map the epitopes of antibody binding sites on target proteins [1]. The first step in the process is to experimentally determine a subset of the probes that have a high affinity to the antibody. Once this set of probes is found, we apply an existing algorithm we have developed [3,4] to find the best alignments of each probe sequence to the target protein sequence. Binding sites of the antibodies are surfaces, not just continuous linear sequences and reveal long-range proximity information since they are “assembled” from solvent-exposed regions that may be far apart in the primary sequence. In this poster, we report on new work to synthesize the set of probe-target alignments into a single map of the epitope surface of the protein. We have developed a program called EPIMAP that creates a *surface neighbor graph* on

the epitope residues and finds a planar embedding which is most consistent with the alignments found.

2. Epitope Mapping

We use a graph-based approach to merge and visualize the collective surface proximity information provided in the entire set of top-scoring alignment sets, one set for each probe. In this approach each residue of the target protein constitutes a vertex in a weighted surface-neighbor graph. Edge weights in this graph indicate how strongly the alignment data supports the conclusion that the residues at each endpoint are neighbors on the surface of the protein. The specific procedure employed for calculating edge weights is as follows: for each probe, compute the set of top scoring alignments. Suppose there are n such alignments and that a particular pair of residues are neighbors in k of these alignments. Then n/k is added to the weight of the edge between the two residues in question. After this procedure is repeated for each probe, edges that have comparatively high weights are most likely to link residues that are true surface neighbors.

We are currently investigating algorithms for finding planar embeddings of the surface neighbor graphs and *lattice embeddings* that place residue vertices at lattice points in a way that is maximally consistent with the collection of probe-target alignments

3. Experiments and Validation

We have studied cases where the precise antibody epitopes are known from x-ray analysis of the target protein-antibody structures, cases where the protein structure is known but the antibody epitope is not, cases where the protein structure is currently unknown, cases where antibodies recognize protein-protein interaction regions, and a case where antibodies recognize active conformations of a protein where only the resting, inactive conformation is known.

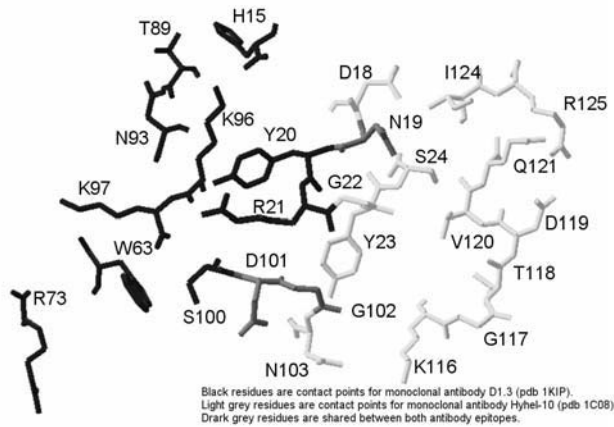


Figure 1 – Hen egg lysozyme epitope regions for antibodies D1.3 (pdb 1KIP) and Hyhel 10 (pdb 1C08).

We have used well-defined epitopes of hen egg lysozyme (Figure 1) as a validation case and present surface neighbor graphs (Figures 2 and 3) that correctly identify regions of the protein that are exposed to solvent and distant regions in the sequence that are in close proximity in the folded protein. Rhodopsin is the best understood member of the G protein-coupled receptor (GPCR) superfamily and the resting/dark-adapted conformation has been solved to 2.6Å by x-ray crystallography. The light-excited conformation, which stimulates the visual signaling network, is poorly understood but is thought to be homologous to the agonist-excited conformations of other GPCRs. We have identified monoclonal antibodies (mAbs) that stabilize light-excited conformations of rhodopsin and antibody imprinting is providing information on the conformations of the light-excited protein. As an extension of prior work [1], we have characterized the epitopes of 25 mAbs that recognize at least 5 distinct regions of human neutrophil flavocytochrome b, an integral membrane electron transferase that produces host defensive superoxide. We are using antibody imprint analysis to help predict the flavocytochrome structure in advance of attempts using X-ray crystallography.

4. Summary

Antibody imprinting is a new and promising approach to determining three dimensional structures proteins. Accurate epitope mapping has the potential to be extremely useful to improve *ab initio* protein structure predictions.

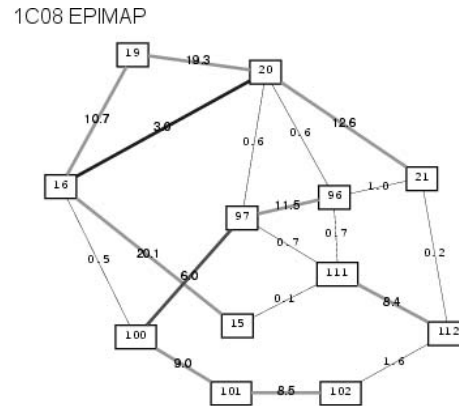


Figure 2 – Surface neighbor graph found from Hyhel 10 (pdb 1C08) alignments.

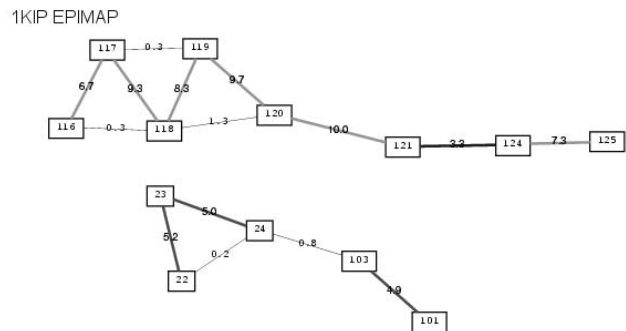


Figure 3 – Surface neighbor graph found from D1.3 (pdb 1KIP) alignments.

References

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