

Epitope Mapping by Combination of Amide Hydrogen/Deuterium Exchange Mass Spectrometry and Docking

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Objectives

- To develop a widely-applicable, high-resolution, and reliable method to determine the epitope of antigen-antibody interactions.
- Our strategy is to use amide hydrogen/deuterium exchange (H/D-Ex) mass spectrometry (MS) results as constraints for computational docking (H/D-DOCK).

Methods: H/D Exchange MS (Figures 1 and 2)

- An antigen (cytochrome c) was incubated in neutral deuterated buffer to initiate the exchange of amide hydrogens to deuteriums.
- The deuterated antigen was loaded onto an antibody column (E8 monoclonal antibody).
- After being washed with neutral aqueous buffer, the antibody column was incubated for the same duration as the first step. All deuteriums on the antigen amides were back to hydrogens except for the ones at epitope which is protected by the antibody.
- Acid washing of the antibody column stopped the exchange reactions and eluted out the antigen from the antibody column.
- After pepsin digestion of the antigen, the mass of each peptic fragment was analyzed by LC-MS. The peptides which gained the mass following this process are the H/D exchange identified epitope (Figures 2 and 3).

Methods: Docking (Figures 4 – 6)

- Unbound crystal structure of cytochrome c (1HRC) and either unbound crystal structure of E8 (1QBL) or homology modeled E8 variable region (by RosettaAntibody) were used as starting structures for the docking. Docking calculations were performed using ZDOCK, ZRANK, and RDOCK.

Results (Figures 4 – 6)

- When H/D exchange results were used as constraints for the docking of the antigen and antibody (H/D-DOCK), iRMSD between the top pose of docking results and co-crystal structure was 1.1 angstrom. Moreover, the top docking pose correctly identified all interacting residues in the complex.
- When unbound crystal structure of E8 was replaced with homology modeled E8 variable region, the iRMSD was still native like 1.5 angstrom.

Conclusion

- The combination method used here successfully identified the discontinuous conformational epitope of cytochrome c – E8 antibody interaction. This method is very powerful and can be applicable to most of antigen – antibody interactions, as (1) no crystallization is required unlike X-ray method and (2) the method is compatible with conformational epitopes unlike peptide library approaches.

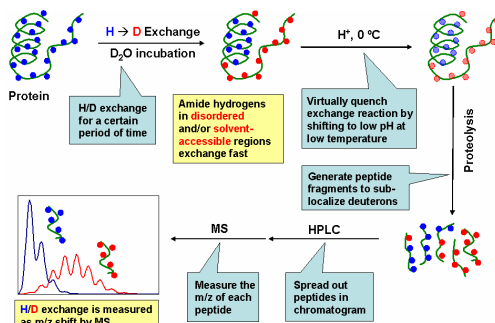


Figure 1. General Method: H/D-Ex by MS

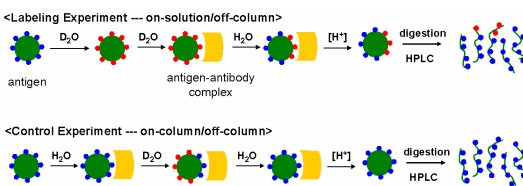


Figure 2. Epitope Mapping Method: On/Off Exchange

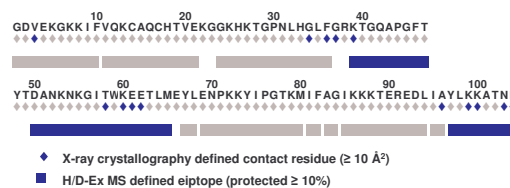


Figure 3. Epitope Mapping by H/D-Ex MS: Cytochrome C-E8

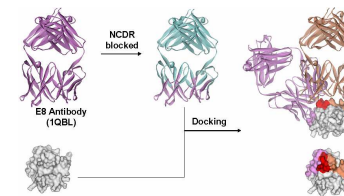


Figure 4. Docking of Cytochrome c – E8

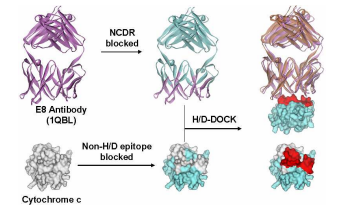


Figure 5. H/D-DOCK of Cytochrome c – E8

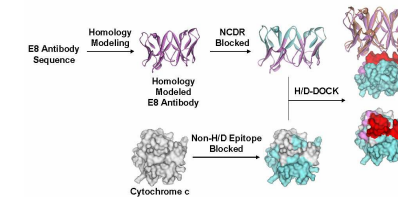


Figure 6. H/D-DOCK of Cytochrome c – E8 Starting with Homology Modeled Antibody

Concept of “docking” for an antigen-antibody interaction (left) and docked pose (right) for cytochrome c – E8 antibody interaction. Unbound crystal structures of cytochrome c (white surface accessible area, 1HRC) and E8 antibody (pink, 1QBL) were docked after the residues of NCDR in the antibody (light blue) were “blocked”. Gold ribbon is E8 antibody binding to cytochrome c in the X-ray co-crystal structure (1WEJ). Pink ribbon is the highest ranked pose of “docking”. Red on cytochrome c is the contact residues identified by both poses. Gold on cytochrome c is the contact residues unique to the co-crystal structure. Pink on cytochrome c is the contact residues unique to the “Docking” pose.

Concept of “H/D-DOCK” for an antigen-antibody interaction (left) and docked pose (right) for cytochrome c – E8 antibody interaction. Unbound crystal structures of cytochrome c (white surface accessible area, 1HRC) and E8 antibody (pink, 1QBL) were docked after the residues of NCDR in antibody and non-epitope residues identified by H/D-Ex MS in antigen (shown in light blue) were “blocked”. Gold ribbon is E8 antibody binding to cytochrome c in the X-ray co-crystal structure (1WEJ). Pink ribbon is the highest ranked pose of “docking”. Red on cytochrome c is the contact residues identified by both poses. Gold on cytochrome c is the contact residues unique to the co-crystal structure. Pink on cytochrome c is the contact residues unique to the “Docking” pose.

Unbound crystal structures of cytochrome c (1HRC) and homology modeled variable region of E8 antibody (by RosettaAntibody) were docked after the residues of NCDR in the antibody and non epitope residues in cytochrome c identified by H/D-Ex MS (light blue) were “blocked”. Gold ribbon is E8 antibody binding to cytochrome c in the X-ray co-crystal structure (1WEJ). Pink ribbon is the highest ranked pose of “docking”. Red on cytochrome c is the contact residues identified by both poses. Pink on cytochrome c is the contact residues unique to the “H/D-DOCK” pose.