

# Epitope Mapping by Hydrogen/Deuterium (H/D)–Exchange Mass Spectrometry (MS)

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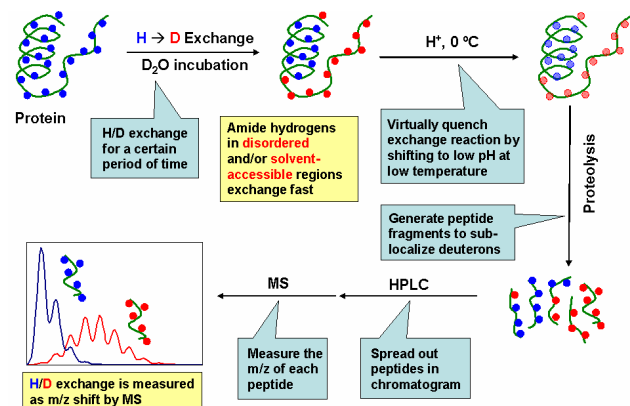
## Abstract

Amide hydrogen/deuterium (H/D) exchange coupled with proteolysis, HPLC separation, and mass spectrometry (MS) was used to map antigen-antibody interactions. Briefly, (i) an antigen was deuterated in solution by mixing with neutral deuterated buffer (on-exchange), (ii) the deuterated antigen was loaded onto an antibody column, (iii) the antibody column was washed with neutral aqueous buffer (off-exchange), (iv) the antigen was eluted from the antibody column by a cold acidic buffer, (v) the eluted antigen was digested by acid stable protease(s), and (vi) the deuteration levels of each antigen peptide were determined by LC-MS.

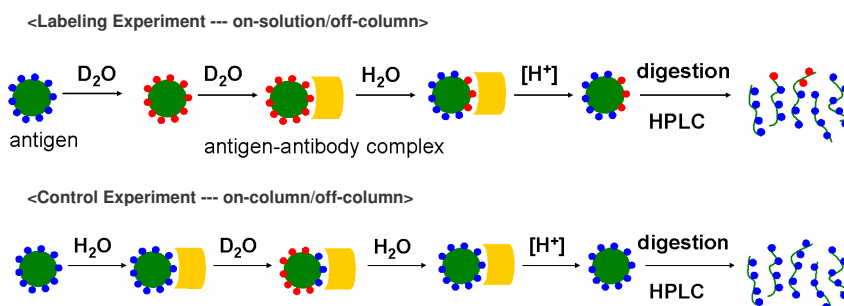
The binding of an antibody to its antigen should retard the exchange of amide hydrogens to deuterium through solvent exclusion, and/or restriction of conformational fluctuations at the antigen-antibody interface. If an amide is within the epitope, the amide should carry a significant amount of deuterium after on-exchange in solution and off-exchange in the column reactions. On the other hand, if an amide is not in the epitope, the amide should carry very little deuterium after on/off exchange reactions.

H/D-exchange technology was used to map the epitopes of three antigen-antibody interactions. All of them were validated by X-ray crystallographic data. All three epitopes identified were discontinuous conformational epitopes. The success rate of H/D-exchange epitope mapping is over 90%. H/D-exchange technology is widely applicable for the epitope mapping of various antigen-antibody interactions providing epitope resolution between 5-15 amino acids per region.

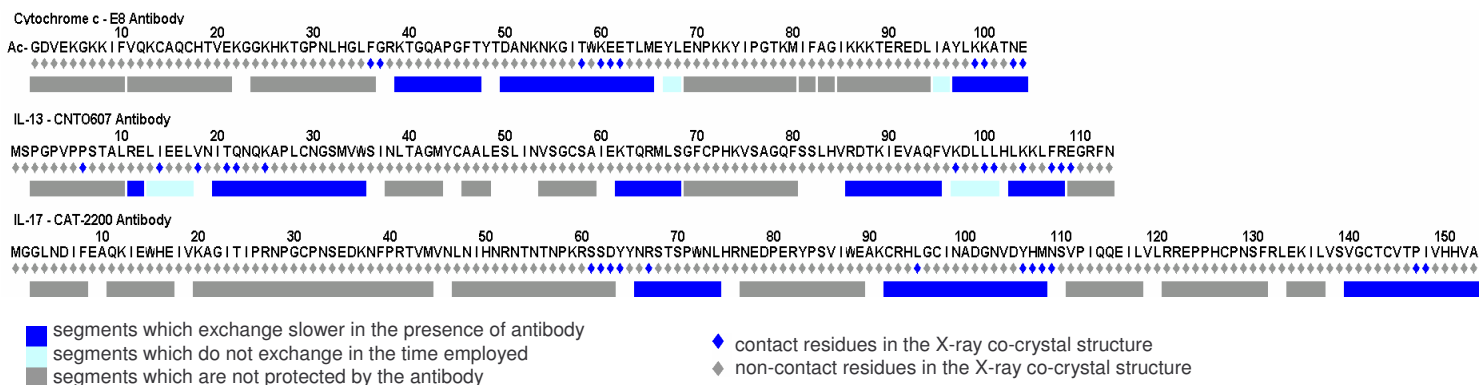
## Method: H/D-Exchange by MS



## On/Off Exchange for Epitope Mapping



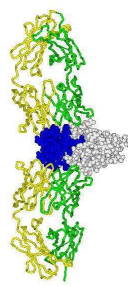
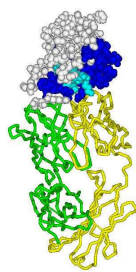
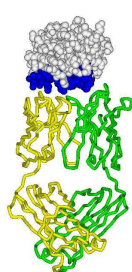
## Results: Epitope Mapping by H/D-Exchange and X-Ray Crystallization



Cytochrome C – E8 Antibody

IL-13 – CNT0607 Antibody

IL-17A – CAT2200 Antibody



CPK (space-filling model) is antigen  
Blue is epitope residues  
Green is heavy chain of mAb  
Yellow is light chain of mAb

## Conclusion

- Amide hydrogen/deuterium (H/D) exchange coupled with proteolysis, HPLC separation, and mass spectrometry (MS) was used to map three antigen-antibody interactions, all of which were found to have discontinuous conformational epitopes.
- H/D-exchange is a widely-applicable (> 90% success rate), medium-resolution (5–15 amino acids), medium-throughput technology (1–2 wks per epitope).
- Each of the three epitopes identified by H/D-exchange MS agreed well with the contact residues of the corresponding X-ray co-crystal structures.
- ExSAR performs epitope mapping by H/D-exchange MS on a fee for service basis.