Epitope mapping of an interleukin receptor for three therapeutic antibodies by HDX/MS

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INTRODUCTION

Hydrogen-Deuterium Exchange Mass Spectrometry (HDX/MS) is an innovative tool for the characterisation of therapeutic monoclonal antibodies. Besides the study of higher-order structures, HDX/MS can also be used for epitope mapping studies in order to determine to which region of the target a monoclonal antibody binds. In this poster, we present a case study in which we performed epitope mapping for three monoclonal antibodies binding to an interleukin receptor.

PRINCIPLE OF THE METHOD

Proteins in solution are diluted in an excess of D₂O so that H atoms are exchanged for D atoms on the amide nitrogen of the peptide bond (Figure 1). Side chain H-atoms also exchange but at too fast a rate, and are consequently entirely back-exchanged during the LC/MS analysis.

After quenching of the deuteration by lowering both the temperature and the pH, the protein is digested online by a protease. The resulting peptides are quickly desalted and separated at low temperature.

Mass spectrometry measures the uptake of deuterium using the difference in peptide mass, due to the mass difference between exchanged D (2.0141 Da) and H (1.0078 Da). This exchange of D for H occurs at rates varying by a factor of as much as 10⁵ depending on hydrogen bonding and solvent accessibility.

Binding of a given protein region to another protein typically decreases its deuterium uptake rate because the involved amino acids become more buried and/or establish new H-bonds. The epitope can be mapped by monitoring regions that display reduced deuterium uptake upon binding.

EXPERIMENTAL CONDITIONS

Sample preparation:
- 25 µM each protein in 10 mM phosphate buffer, pH 6.8
- 10x dilution in 10 mM phosphate buffer, 100 mM NaCl in D₂O, pHapparent 6.4
- Incubation 30 min at 20°C
- Quench at 1°C by 2x dilution in 100 mM phosphate buffer, 400 mM TCEP, 4M guanidine, pH 2.3 for 2 min

HPLC column:
- Enzyme BEH Pepsin, 2.1 x 30 mm, 5 µm (Waters)
- Acquity BEH C18 Vanguard 2.1 mm x 5 mm, 1.7 µm (Waters)
- Acquity BEH C18 1.0 mm x 100 mm, 1.7 µm (Waters)

Mobile phases:
- A: 0.19% FA in water, pH 2.5
- B: 0.19% FA in ACN

Digestion/desalting:
- 100% A at 200 µL/min for 3 min at 20 °C

Analytical gradient:
- 5% – 40% B in 10 min at 0°C

Detection:
- ESI/MS (Xevo G2-XS QTOF)

SEQUENCE COVERAGE

- Sequence coverage, peptide length and average peptide redundancy per amino acids are important parameters regarding the quality of the results.
- Shorter peptides give higher resolution results, especially when combined with high redundancy where partially overlapped peptides allow narrowing the sequence of the binding site.
- A high redundancy also increase the confidence in the results as it provides a means to evaluate the consistency of the results.
- An excellent sequence coverage together with a very good average redundancy were obtained (99.6%, 6.44 peptides per aa; Figure 5) from 137 peptides identified using both PLGS and UNIFI.

EPITOPE DETERMINATION

Using HDX/MS, the epitope of three mAbs were straightforwardly inferred, in solution, for a same interleukin receptor target. Qualitative and quantitative analysis allowed pinpointing the peptide regions of the antigen that are protected from deuteration upon antibody binding. The resulting epitope location is analogous for all three candidates, although one mAb seems to also bind a secondary site.