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 D_2O 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 10^8 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 uptakes upon binding.

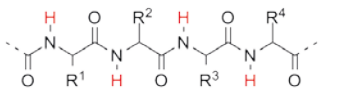

Figure 1: Exchangeable hydrogen atoms on a peptide backbone, at the HDX/MS timescale

Figure 2: Waters Acquity UPLC M-Class system with HDX technology used for HDX/MS studies

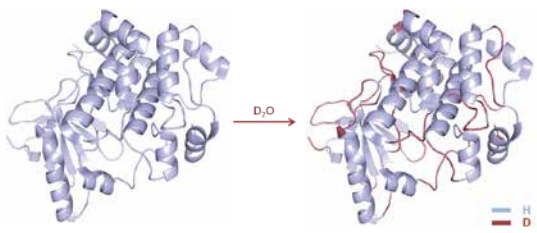

Figure 3: D-labeling of a protein in the presence of an excess of D2O

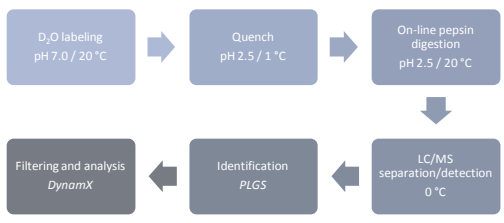

Figure 4: Analytical workflow

EXPERIMENTAL CONDITIONS

Sample preparation:	• 25 µM each protein in 10 mM phosphate buffer, 100 mM NaCl, pH 6.8 • 10x dilution in 10 mM phosphate buffer, 100 mM NaCl in D_2O, $pH_{apparent}$ 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:	• Enzymate 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 then 0°C
Analytical gradient:	5 – 40% B in 10 min at 0°C
Detection:	ESI/MS (Xevo G2-XS QTOF)

SEQUENCE COVERAGE

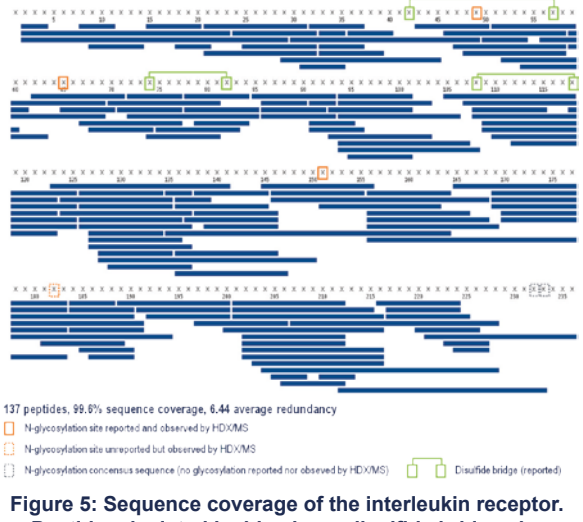

Figure 5: Sequence coverage of the interleukin receptor. Peptides depicted by blue bars, disulfide bridges by green lines, and N-glycosylation sites by orange frames.

- 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

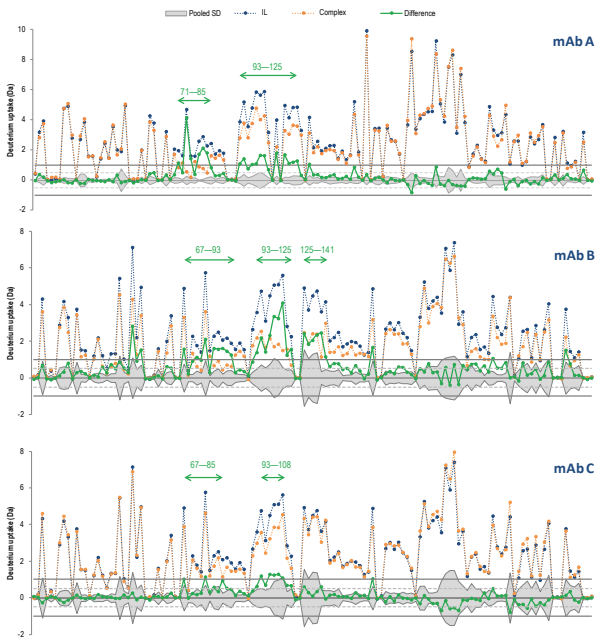

Figure 6: Deuterium uptakes of the interleukine receptor (blue) and complex (orange) peptides (from N- to C term), and corresponding difference (green). The pooled standard deviation is shown as a mirrored grey area. Arbitrary thresholds at ± 0.5 and ± 1.0 Da are illustrated by dashed and plain grey lines, respectively.

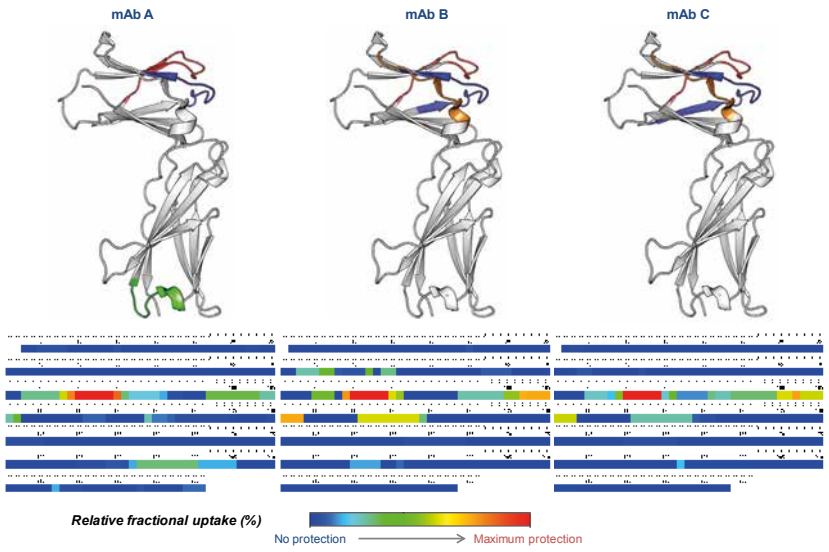

Figure 7: Heatmap of the deuteration differences between the unbound and complexed interleukin receptor. The inferred epitope site 1 (blue, red and orange) and site 2 (green) are shown on the 3D structure.

- Deuteration D was calculated as an average of the experimental replicates
- The differences between both states were calculated peptide by peptide (Figure 6) between the two states (bound *vs.* unbound).
- Regions with $\Delta D > 1.0$ Da are likely to constitute the epitope.
- These regions are clearly highlighted on the heatmap (Figure 7).

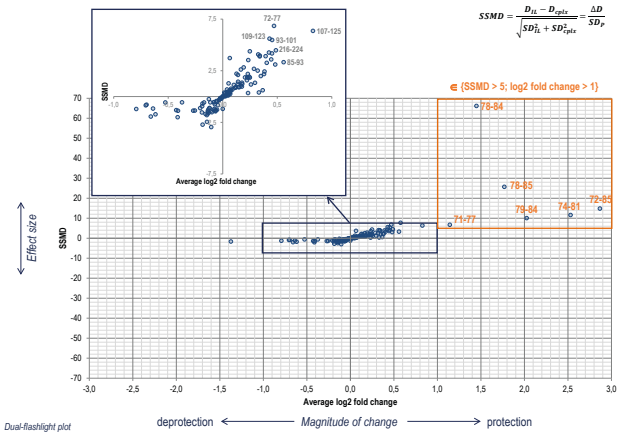

Figure 8: Dual-flashlight plot for the discovery of peptides with a statistically significant protection

- An alternative visualisation of the results was set up, taking into account both the magnitude of the change in deuteration and the pooled standard deviation. This allows to quickly identify the peptides with the most significant changes in deuteration upon binding.
- This was achieved by calculation of the SSMD (strictly standardised mean difference).
- The best peptides (located in the top right-hand corner, Figure 8) are located on the hypothesised primary epitope site, for all three mAb candidates.

CONCLUSION

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 peptidic 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.