Experimental procedure for HDX analysis (antibody:ligand, protein:receptor)

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HDX MS - sample preparation for the analysis of SARS-CoV-2 S-protein binding to recombinant ACE-2 entry receptor ectodomain (and mAb when available)

Introduction.

Previous cryo EM studies have shown that the SARS-CoV-2 S protein binds with high affinity to the ACE-2 receptor (15nM affinity)[1]. The structure of the S protein RBD (receptor binding domain) bound to ACE2 has also been determined[2]. HDX-MS experiments can be carried out with SARS-CoV-2 S (Receptor binding domain), ACE2 and mAbs (when these become available), and this would be complementary to the work proposed by Michal Sharon's group. HDX could also be used to investigate the effects of glycosylation on binding[3].

For HDX analysis, we have used the following protocol previously.

HDX MS - Buffers

Equilibration Buffer

87.09 mg K_2HPO_4

68.05 mg KH_2PO_4

100 mL H2O pH 7.00 (adjust with 1M HCl)

Labelling Buffer

78.86 mg K_2HPO_4

61.61 mg KH_2PO_4

100 g D_2O (100 g of D_2O 90.55 mL due to density) pH 6.6 (adjust with 20% DCl; pD = 7)

Quench Buffer

217.73 mg K_2HPO_4

170.11 mg KH_2PO_4

25 mL H_2O pH 2.66 (adjust with conc. HCl)

Wash Solution

0.5 M Guanidine HCl, 4 % ACN

Reducing, Denaturing Quench Buffer (for use when analyzing antibodies)

217.73 mg K_2HPO_4

170.11 mg KH_2PO_4

625 mg TCEP· HCl 25 mL 2M Guanidine HCl

HDX MS - sample preparation (possible combinations indicated in table).

	Sample number i.d								
Viral protein/Ab	1	2	3	4	5	6	7	8	9
SARS-CoV-2 S RBD (10-20µM)	**√**	**√**				**√**			
ACE-2 receptor (10-20µM)		**√**	**√**		**√**			**√**	
mAb(s) (when available) (10-20µM)				**√**	**√**	**√**			**√**
SARS-CoV S RBD(previous SARS – if available) (10-20µM)							**√**	**√**	**√**

1. Mix the samples below at room temperature in equivalent proportions:

2. Incubate for 2 hours at room temperature (22-23 °C), after which these are added to suitable glass vials for LCMS acquisition.

HDX MS - data acquisition

HDX-MS experiments are carried out using a Waters nano-Acquity UPLC system (incorporating a HDX chamber), coupled to a separate autosampler (LEAP Technologies dual-armed robot). ESI detection is facilitated using a Waters Synapt G2S mass spectrometer operating in positive ion/resolution mode, acquiring data over the m/z range 290-2500. Protein solutions of free ligand and ligand (~20 µM) complexed are loaded directly. All samples are run as three technical replicates, and three biological replicates (9 analyses for each condition).

All samples are diluted 20-fold in 10 mM phosphate buffer in either H_2O or D_2O at pH/pD 7. and incubated at 20°C for 0 minutes, 30 seconds, 1 minute, 10 minutes, 1 hour and 4 hours (in D_2O) prior to the quench step.

HDX quenching is achieved by mixing the reaction solution 1:1 with a cooled solution of 200 mM phosphate + 0.5 M TCEP + 1.5 M Guanidine Hydrochloride (pH 2.5, 0°C). Samples are then injected into the HDX chamber (0°C), and washed over a pepsin column (Waters Enzymate BEH Pepsin, 2.1 x 30 mm) in 0.1% HCOOH in H_2O, pH 2.5, at 200 $\mu L min^{-1}$.

Resulting peptides are trapped on a VanGuard C18 trap column, and separated on a C18 analytical column (Waters Acquity UPLC BEH C18 1.7 µm, 1.0 x 10 mm). The gradient is developed at a flow rate of 40 $\mu L/min^{-1}$ over 16 mins as follows: 0 min: 5% B, 7 min: 35% B, 8 min: 85% B, 11 min: 5% B, 12 min: 95% B, 13 min: 5% B, 14 min: 95% B, 15 min: 5% B (mobile phases A: water + 0.1 % formic acid and mobile phase B: acetonitrile + 0.1 % formic acid respectively), as described by the manufacturer. The mass spectrometer is operated in ToF only (Resolution) mode and Leu Enk (m/z 556.2771) peptide is used as Lock Spray.

Data is acquired using Waters MassLynx software v4.1, with the LEAP robot controlled by HDx Director 1.0.3.9. Data processing and analysis are carried out using Waters ProteinLynx Global Server 3.0.1 and subsequently Waters DynamX 3.0 software. HDX heat map data is mapped

onto PDB structures using PyMOL software (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).

1. Wrapp, D., Wang, N., Kizzmekia, S. *et al*. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation *Science* (2020). https//doi.org/10.1126/science.abb250
2. Lan, J., Ge, J., Yu, J. *et al.* Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* (2020). https://doi.org/10.1038/s41586-020-2180-5
3. Zhang Y., Zhao W., Mao S. *et al.* Site-specific N-glycosylation Characterization of Recombinant SARS-CoV-2 Spike Proteins using High-Resolution Mass Spectrometry. bioRxiv preprint (2020) https://doi.org/10.1101/2020.03.28.013276