Mass Photometry on SARS-CoV-2

APPLICATION NOTE

Mass photometry allows quantification of the mass distribution of biomolecules in solution. Its utility in analysing the oligomeric state and quantifying protein-protein interactions is here used to study the spike protein of the newly emergent SARS-CoV-2 virus and its interaction with the ACE2 receptor, thought to be the main entry route into human cells.

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The emergence of the current SARS-CoV-2 pandemic has sparked a lot of functional and structural studies relating to the mechanism of the coronavirus entry into host cells. The entry is mediated by the transmembrane spike glycoprotein that forms homotrimers protruding from the viral surface through a tight interaction with human angiotensin-converting enzyme 2 (ACE2), that acts as a functional receptor for the spike protein. This protein is subsequently cleaved by host proteases thereby activating the protein for membrane fusion via extensive irreversible conformational changes.

Here we present a mass photometry-based assay which can inform on some key aspects of the oligomeric state of the spike glycoprotein, its interaction with ACE2 as well as the conformational state of the receptor-binding domain (RBD) which engages directly with ACE2. These RBDs can be either in an "up" conformation or a "down" conformation, which either includes or excludes them from a possible interaction with ACE2 respectively (Fig. 1).

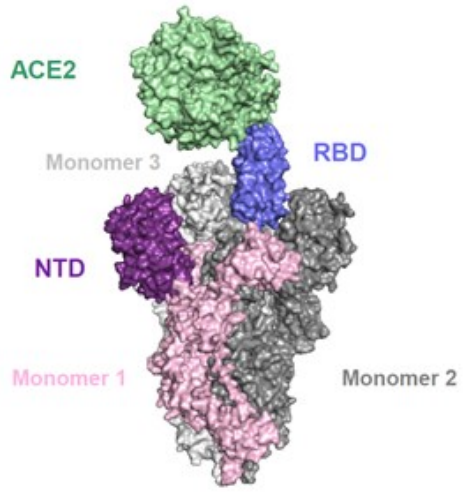

Fig 1. Structure of SARS-CoV2 spike ectodomain in complex with ACE2 with one RBD in "up" and two RBD in "down" position. The model was constructed by overlaying the cryo-EM structure of SARS-CoV2 spike (6VSB) onto the crystal structure of ACE2 in complex with SARS-CoV2 RBD (6M17). ACE2 is shown in green. Trimeric spike protein with the monomer having the RBD in the binding competent "up"-conformation coloured. The NTD is shown in purple and RBD in blue. The other monomers are shown in light-and dark grey respectively.

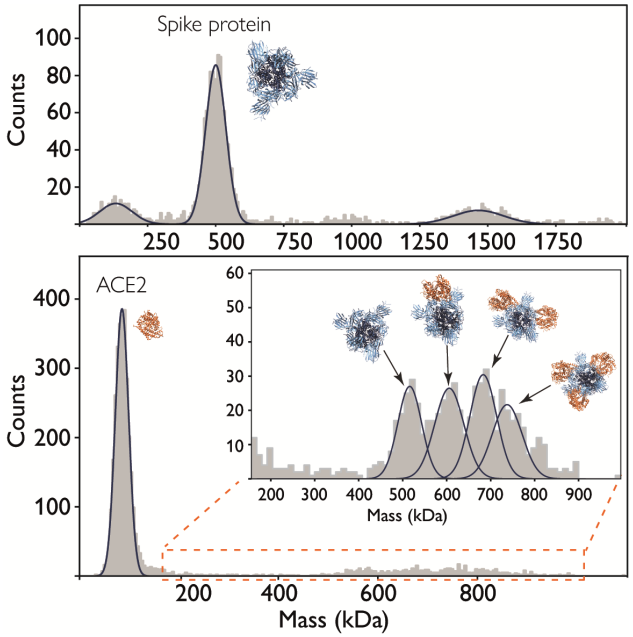

Fig 2. Mass photometry characterisation of the SARS-CoV-2 spike ectodomain and its interaction with ACE2. Mass photometry histogram of recombinant SARS-CoV-2 spike ectodomain (upper panel). The protein forms a trimer (top view of the cryo-EM structure). The SARS-CoV-2 spike ectodomain can bind multiple copies of ACE2, indication that within a trimer several RBDs can be in a binding-competent "up" position (lower panel).

We could demonstrate that the recombinantly produced SARS-CoV-2 spike ectodomain forms a well-defined trimer (Fig. 2, upper panel). Upon challenge with ACE2, one can identify distinct populations that correspond to binding of one or several copies of ACE2 per trimer (Fig. 2, lower panel) This indicates a mixed population of RBDs in either the "up" or "down" conformation as well as functional binding of ACE2 to the accessible RBDs within the spike protein.

Here we presented the use of mass photometry to study the binding mechanism of the SARS-CoV-2 spike protein to the ACE2 receptor. One could further extend this assay to monitor how antibodies engage with the trimer and eventually disrupt the interaction with ACE2.

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