Mass Photometry on SARS-CoV-2

APPLICATION NOTE

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

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The emergence of the current SARS-CoV-2 pandemic has sparked numerous functional and structural studies relating to the mechanism of the coronavirus's 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, and the conformational state of the receptor-binding domain (RBD) which engages directly with ACE2. These RBDs can be either in an "up" conformation, which enables interaction with ACE2, or a "down" conformation, which excludes a possible interaction (Fig. 1).

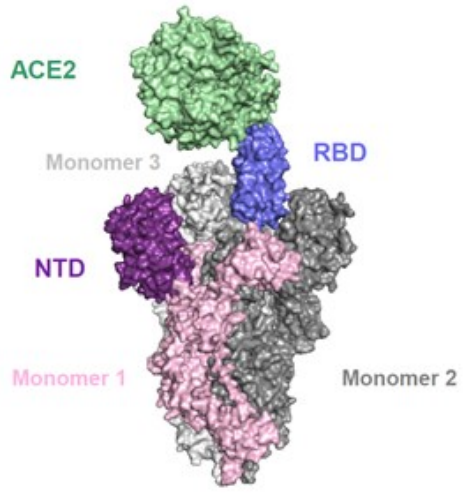

Fig. 1 Structure of SARS-CoV2 spike ectodomain in complex with ACE2, with one RBD in the "up" and two RBDs in the "down" position. The model was constructed by overlaying the cryo-EM structure of SARS-CoV2 spike (6VSB) onto the crystal structure of ACE2 in complex with SARS-CoV2 RBD (6M17). ACE2 is shown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

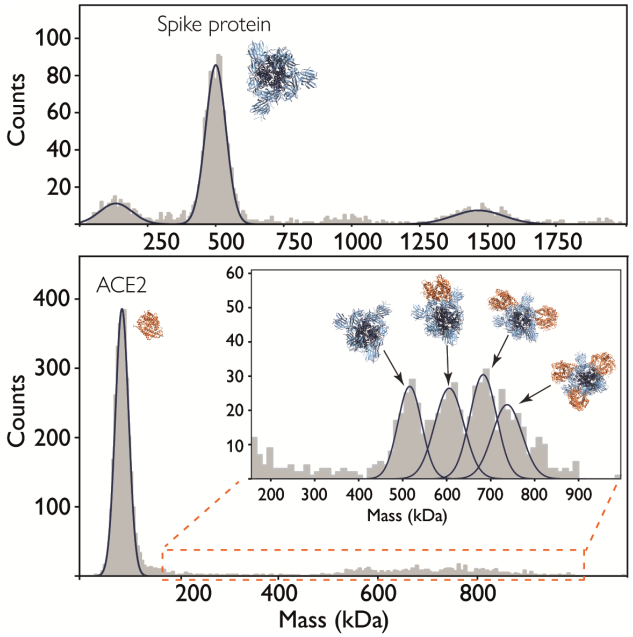

Fig. 2 Mass photometry characterisation of the SARS-CoV-2 spike ectodomain and its interaction with ACE2. Mass histogram of recombinant SARS-CoV-2 spike ectodomain (upper panel), also showing the top view of a cryo-EM structure of the protein in its trimeric form. Mass histogram of ACE2 (lower panel).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

We could demonstrate that the recombinantly produced SARS-CoV-2 spike ectodomain forms a well-defined trimer (Fig. 2). Upon challenge with ACE2, one can identify distinct populations that correspond to the binding of one or several copies of ACE2 per trimer (Fig. 2) 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 mechanism by which the SARS-CoV-2 spike protein binds to the ACE2 receptor. One could further extend this assay to monitor how antibodies engage with the spike protein trimer and eventually disrupt the interaction with ACE2.

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