

The Spike-ACE2 interaction underlying SARS-CoV-2 infection and inhibition is enhanced by intermolecular crosslinking

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The interaction between the SARS-CoV-2 spike trimer with the host dimeric angiotensin-converting enzyme 2 (ACE2) receptor is the first step in virus entry. Consequently, efforts aimed at therapeutic intervention have focused on blocking spike-ACE2 binding by designing ACE2-based decoys. The multivalency of ACE2 and spike is fundamental to our understanding of the molecular mechanism underlying ACE2-spike affinity, manifested by the observation of much tighter binding of dimeric ACE2 to spike compared with monomeric ACE2. While structural evidence suggests that intra-spike avidity is unlikely to explain these observations, the alternative, intermolecular avidity mechanism through ACE2-mediated crosslinking, is challenging to detect and quantify owing to the inherent heterogeneity of crosslinked oligomeric complexes. Mass photometry, single-molecule mass measurement, provides accurate quantification of binding stoichiometries and affinities by detecting biomolecular complexes in solution. Using mass photometry, we quantified the free energies for the spike-ACE2 interaction building blocks; the spike receptor binding domain and soluble monomeric or dimeric ACE2. Similarly, we quantified the interaction between monomeric ACE2 and trimeric soluble spike. In all cases, intermolecular avidity was not possible, and the interaction free energies were similar, additive, and consistent with previously reported affinities on the order of $K_D=20$ nM. For dimeric ACE2 and trimeric spike, we found that ACE2 coexists with spike trimer occupied by zero, one or two ACE2 dimers. In addition, however, the mass balance was shifted, predominantly favoring high-mass oligomers with increasing ACE2 concentration. Our results show that in solution, ACE2 and spike can form crosslinking interactions, which coupled with the enhanced inhibitory effect of dimeric ACE2 compared to monomeric ACE2 on viral infection, suggests that intermolecular ACE2-spike avidity may enhance spike-ACE2 interaction. These are relevant not only for our understanding of the cell-virus interaction, but also for the search and design of therapeutics.