A Tethered Ligand Assay to Probe SARS-CoV-2:ACE2 Interactions

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SARS-CoV-2 attaches to the ACE2 receptor on human hosts cells via its receptor-binding domain (RBD) on the Spike protein. This critical first step occurs in dynamic environments, where external forces act on the binding partners, creating an urgent need for assays that can quantitate SARS-CoV-2 interactions with ACE2 under mechanical load. We present a tethered ligand assay that comprises the RBD and the ACE2 ectodomain joined by a flexible peptide linker. Using magnetic tweezers [1,2] and atomic force spectroscopy, we investigate the RBD:ACE2 interaction over the whole physiologically relevant force range [3]. Combined with steered molecular dynamics simulations, we observe and assign fully consistent unbinding and unfolding events across the three techniques and establish ACE2 unfolding as a molecular fingerprint. We quantify the force dependence and kinetics of the RBD:ACE2 bond in equilibrium and find significant differences between SARS-CoV-1 and 2, which helps to rationalize the different infection patterns of the two viruses [3]. Finally, we probe how different RBD mutations affect force stability and speculate how mechanical coupling promotes increased transmissibility in variants of concern.

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