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Exploring The Low Affinity Limit of Dual-color Fccs Analysis of Protein Binding to a Membrane-bound Receptor

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The binding of ligand molecules to receptors in biological membranes is a vital step in cellular signaling and recognition, but quantitative measurements of their mutual affinity are very challenging and often not possible in situ. Especially for lower affinities the background of free ligands must be accounted for in the analysis. We use dual-color fluorescence cross-correlation spectroscopy (FCCS) to study the binding of a soluble receptor-binding domain (RBD) construct of the spike-protein from SARS-CoV-2 to different variants of membrane-bound Angiotensin-converting enzyme 2 (ACE2) receptor expressed in a human cell line. As the affinity is in the nanomolar to lower micromolar range, free RBD contributes significantly to the fluorescence signal. We develop a theoretical model describing the resulting intensities, FCS-derived particle numbers and cross-correlation amplitudes and compare different methods using these quantities to derive binding curves. The resulting affinities for all variants are compared to micro-scale thermophoresis (MST) measurements that we have performed on soluble constructs and found to be in good agreement with the FCCS results.