TOWARDS THE INVESTIGATION OF TRANSIENT PEPTIDE-MEMBRANE INTERACTIONS WITH TIR-FCS

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The accurate determination of binding rates to membranes or membrane-bound proteins is of key relevance for quantitative biology. In particular, the reversible binding of amphipathic helices is essential for a multitude of processes, such as membrane remodeling, signaling and spatiotemporal organization. Despite the existence of multiple methods to characterize interactions with membranes, there are still many experimental challenges regarding simplicity of use and general applicability. We developed a simple and versatile single-molecule fluorescence approach for the accurate determination of binding rates to surfaces or surface-bound receptors. Our approach combines Fluorescence Correlation Spectroscopy (FCS) with Total Internal Reflection (TIR) Fluorescence microscopy and a camera-based detection. This combination not only yields a high surface selectivity but also resolves association and dissociation rates over a wide time range. Previously, we quantified the transient hybridization of single-stranded DNA to the complementary handles of immobilized DNA origamis. Here, we present our progress on the transfer of this assay to the otherwise challenging quantification of transient interactions between peptides and lipid bilayers.

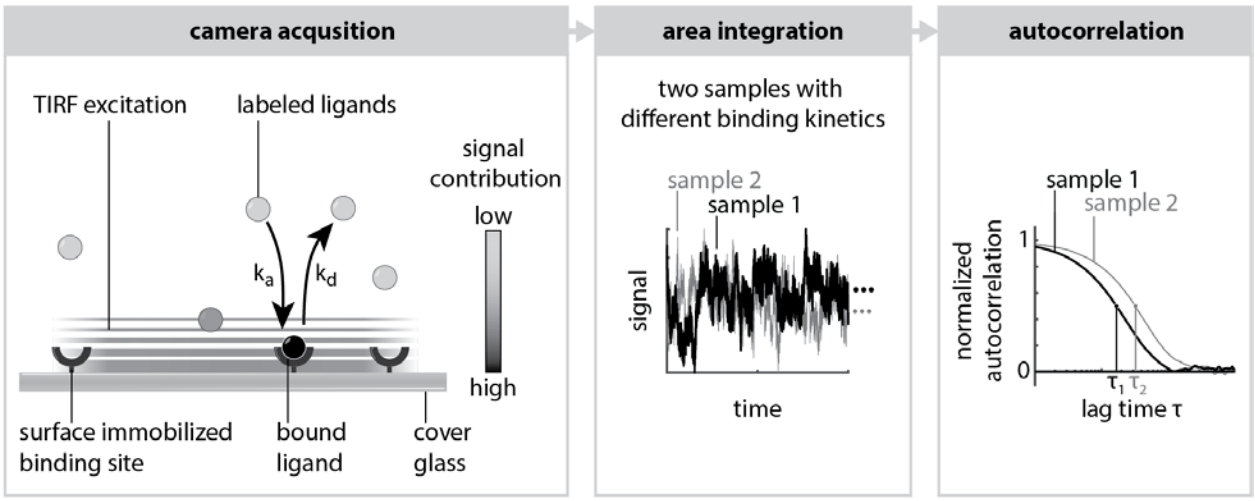

Figure 1: Workflow for determining surface binding kinetics with TIR-FCS. Binding and unbinding create a fluctuating detector signal. The autocorrelation extracts the binding kinetics from the intensity fluctuations.