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Label-Free, All-Optical Detection, Imaging and Nanometric Tracking of Single Proteins
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Optical detection of single proteins requires fluorescent labelling due to their intrinsically small scattering cross-sections. Label-free detection approaches rely on the amplification of the single molecule signals either by cavity, nanotube or plasmonic enhancement, but come at the expense of complex experimental setups that are unable to perform imaging by design. In addition, plasmonic enhancement relies on nanoscopic detection volumes that are difficult to address in a controlled fashion at the single molecule level. All-optical alternatives exist, yet only detect molecules with strong transition dipoles — a property that most biological particles lack. Here, we used interferometric scattering microscopy (iSCAT) to detect and image the processive motion of single myosin 5a molecules on actin filaments without labelling or any nanoscopic amplification at up to 1 kHz bandwidths. The achievable high signal-to-noise ratios enabled tracking with < 5 nm accuracy at 25 Hz, directly comparable to state-of-the-art single molecule fluorescence detection. Together with the simple experimental arrangement, an intrinsic independence from strong electronic transition dipoles and a detection limit for proteins with < 50 kDa, our approach paves the way towards non-resonant, label-free sensing and imaging of nanoscopic objects down to the single protein level.

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Simultaneous Confocal based 3D Tracking and Fluorescence Imaging
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The response of the receptor, IgE-FcεRI, to binding of a multivalent antigen begins a cellular signaling cascade resulting in the release of histamine, part of the human allergic response. The receptor's modes of motion and internalization process are investigated using a confocal based 3D tracking microscope. A Nipkow spinning disk has been added to the microscope to allow simultaneous confocal 3D tracking and wide field spinning disk imaging. The receptor is tracked in live cells while simultaneously imaging cellular structures labeled with fluorescent proteins.

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Photostable Fluorophores for Single-Molecule Imaging
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Fluorophores exhibit instability (blinking and photobleaching) that limits the spatial and temporal resolution for single-molecule imaging. Although protective agents in solution can mitigate fluorophore instability, their poor solubility in water limits further improvements. Moreover, it is very challenging, if not impossible, to use protective agents in solution for live cell imaging. To overcome these limitations, we linked protective agents to Cy5 fluorophores. In doing so, we have achieved remarkable enhancements in photostability. We further demonstrated that this strategy provides a general method for improving the photostability of chemically distinct fluorophores across the visible spectrum.

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The Activation Dynamics of Class C Gpcrs Revealed by Single Molecule FRET
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G-protein-coupled receptors (GPCRs) are the largest and most diverse family of membrane signaling proteins. Binding of an extracellular signal to a GPCR activates G protein, which in turn modulates the activity of different downstream cellular effectors. Recent biochemical, pharmacological and biophysical studies suggest that GPCRs do not simply act as on-off switches but instead exist in multiple conformational and functional states where they may signal to distinct G proteins and other partners. However, despite recent advances, many aspects of signal transduction through GPCRs is still not well understood. Here we report a novel functional assay based on single molecule FRET to probe real-time conformational dynamics of isolated receptors. Using our assay, we studied metabotropic glutamate receptors (mGluRs), dimeric members of class C GPCR family, which are involved in modulation of synaptic transmission and excitability of nerve cells. Experiments on mGluR2 and mGluR3 homodimers reveal multiple stable conformational states. Strikingly, in spite of more than 70% sequence identity between mGluR2 and mGluR3, we observe substantial differences in their inherent dynamics and basal activity in absence of ligand. Our approach should make it possible to directly quantify how drugs stabilize conformational states of receptors and provide new insights into the molecular mechanisms of GPCR signaling.

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Single-Molecule Imaging of Von Willebrand Factor Activation by Flow
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Von Willebrand Factor (VWF) is a long multimeric blood protein that plays a central role in platelet adhesion at sites of elevated hydrodynamic stresses, such as in injured or stenotic vessels. The function of VWF is closely related to thrombogenesis in cardiovascular diseases and the most common hereditary bleeding disorder von Willebrand disease. The effectiveness of the current therapies is limited partly due to the poor understanding of the activation mechanism of VWF, which is regulated by the hydrodynamic stresses in the blood stream. VWF only binds platelets under high shear or elongational flow. Moreover, the length of VWF is regulated by the ADAMTS13 protease cleavage under high shear flow. Studies suggest a hypothesis that the elevated hydrodynamic stresses exert tensile forces on VWF to unfold the tertiary and secondary structures and expose the binding sites for the platelet membrane receptor GP1b and the protease ADAMTS13.
This study elucidates the mechanism of VWF function regulation at single-molecule level under tethered shear flow and elongational flow, which are more effective at activating VWF than pure shear flow according to molecular dynamics simulation. We infuse a FRET tension sensing module in each VWF monomer to report the shape and the tensile force of the multimers, and image them under flow with a microscope system that simultaneously records the length and the fluorescence spectrum of a single VWF multimer. For elongational flow, real-time feedback is applied to trap the fast moving single VWF molecules within the high hydrodynamic stress region and the field of view. By adding fluorescently labeled GP1b and ADAMTS13, the rates of binding and cleavage activities in VWF is also measured. These results provide experimental basis for quantitative modeling of the intra-molecular interactions that are responsible for VWF function regulation.

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Reconstructing Global Conformational Dynamics of a Multi-Domain Protein
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Time-dependent large-amplitude conformational changes of a single protein can be directly probed via single-molecule Förster-type resonance energy transfer (smFRET), typically one pair of dyes at a time. Monitoring complex motions within a multi-domain protein requires additional distance constraints that could be provided by separate measurements using multiple dye pairs installed at various locations. To infer the possible global conformations and to extract the associated conformational dynamics, we develop a generalizable resampling scheme that integrates high-resolution smFRET data and