Single Molecule Imaging of FRET with fluorescence Anisotropy

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To unravel the complexity of biological systems, information at a single molecule level is essential to accurately probe molecular properties. In fact, single molecule imaging provides real time access to conformational dynamics [1], which can elucidate heterogeneity in molecular distribution in terms of dipole orientation, spectra or intramolecular distance, in both stable and unstable systems. In addition to these structural data, real-time functional information can also be accessed and reaction kinetics determined.
Fluorescence anisotropy is a method of contrast, which by quantifying the fluorescence depolarization, can access a fluorophore's rotational correlation time or quantify energy transfer (e.g. FRET). Fluorescence anisotropy techniques may also probe the fluorophore's environment in terms of viscosity, interactions between molecules and ligand-substrate binding.
In this presentation, we probe the conformational changes of single FRET biosensors by acceptor fluorescence anisotropy [2, 3]. We present an adapted and optimized Total Internal Reflection Fluorescence (TIRF) microscope combined with steady state fluorescence anisotropy detection for single molecule imaging. Our set-up is used for FRET imaging of purified Immunoglobulin E (IgEFc) FRET biosensor by measuring the depolarisation of the acceptor (mRFP) upon donor excitation (eGFP). Single molecule imaging with a fluorescence anisotropy read-out, are presented and future prospects discussed.

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