

BOOSTING FLUORESCENCE RESONANCE ENERGY TRANSFER EFFICIENCY ON NANO-COATED MICROSCOPY COVERSLEIPS IN LIVE CELL EXPERIMENTS

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For probing biomolecular interactions in a live-cell setting the distance depending Fluorescence resonance energy transfer (FRET) is often the method of choice. The design of cellular FRET probes is crucial with the goal to keep the original functionality and binding kinetics of the molecular complex despite the dual-fluorophore tagging. Thus, FRET probe design usually requires labeling compromises often resulting in limited FRET efficiencies.

Here, we present an approach to optimize the energy transfer without changing the design of the FRET probe [1]. We show that gold coated glass cover slips allow reinforcing the otherwise forbidden donor-acceptor energy transfer by virtual optimization of the dipole orientation. We show resulting enhanced FRET on our nano-coatings for G protein-coupled receptors, the largest known class of molecular targets with proven therapeutic value, which mediate cellular responses and communication across cellular membranes. We monitor the ligand driven activation of M1 muscarinic acetylcholine receptors labeled with a CFP-FlAsH pair, and demonstrate that both brightness and FRET efficiency of the GPCR FRET probe is significantly enhanced. As our approach amplifies signal responses without interfering with the well-characterized molecular assay we believe that it has particular potential for pharmaceutical drug screening to boost non-ideal FRET probes.

[1] B. Schreiber; M. Kauk, H.S. Heil, M. Emmerling, I. Tessmer, M. Kamp, S. Höfling, U. Holzgrabe, C. Hoffmann, K.G. Heinze, “Enhanced fluorescence energy transfer in G protein-coupled receptor probes by nanocoated microscopy coverslips”, *ACS Photonics* **5**, 6, 2225-2233 (2018).