Implementation of simultaneous single molecule FRET and force spectroscopy measurements to characterize mechano-activation of adhesion GPCRs

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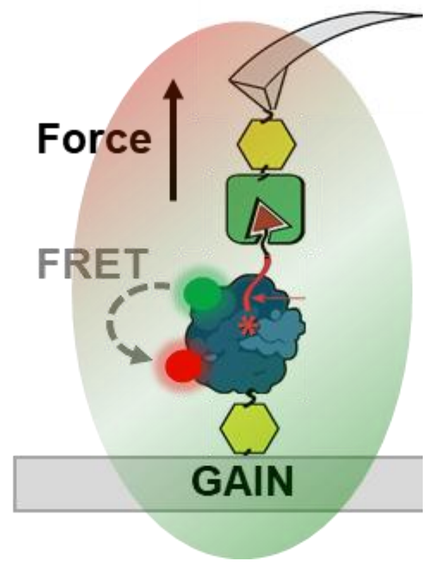
Overview

Mechanical forces are increasingly recognized as key regulators of membrane receptor function and cellular behavior. However, a direct mechanistic understanding of how defined mechanical stimuli are translated into structural changes that govern receptor activation at the molecular level remains elusive. This is due to the lack of approaches capable of simultaneously accessing mechanical inputs and conformational outputs in real time at the single-molecule level. To address this challenge, we propose to develop a **multiparametric and simultaneous approach combining single-molecule Förster resonance energy transfer (smFRET) with single-molecule force spectroscopy using atomic force microscopy (SMFS-AFM)**. This correlative methodology will enable the quantitative and simultaneous readout of inter-dye distances (nm), conformational dynamics (µs–ms), and force-extension responses (pN–nN), directly linking mechanical stimulation to structural changes within the same molecule.

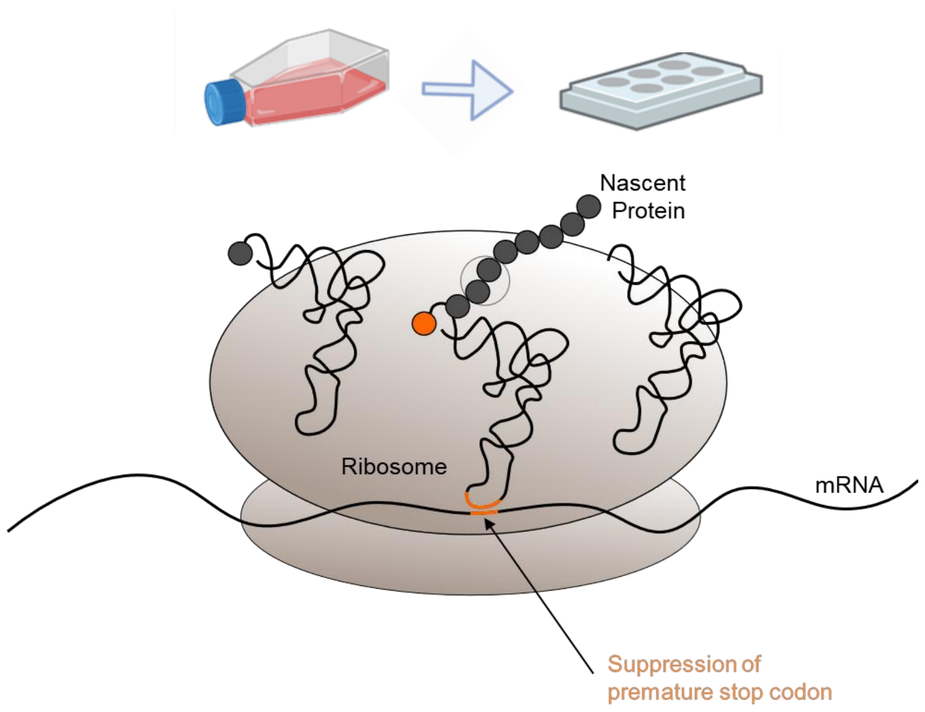
Using **adhesion G protein-coupled receptors** (aGPCRs) as a model system, which play a crucial role in various physiological processes such as tissue repair, immune response, neuronal and organ development and are associated with different diseases including for instance cardiovascular diseases, neurodevelopmental disorders, autoimmune diseases and cancer metastasis, this approach will provide access **to force-dependent conformational landscapes**, including intermediate states that are not accessible by current techniques. In addition, we will exploit the possibility of applying cantilever-induced oscillations at defined frequencies in order to allow for the selective excitation of conformational modes, the evaluation of molecular mechanical transfer functions, and the amplification of small-amplitude dynamics, thereby enabling **access to fast and weakly populated states** that are otherwise difficult to detect, such as for instance partially unfolded states.

Altogether, this project aims to **establish a novel multiparametric framework to quantitatively link mechanical forces to protein conformational changes at the single molecule level**, by developing a novel, integrative, and correlative multiparametric technique tailored to the molecular basis of mechanotransduction.

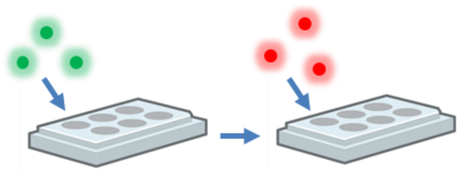


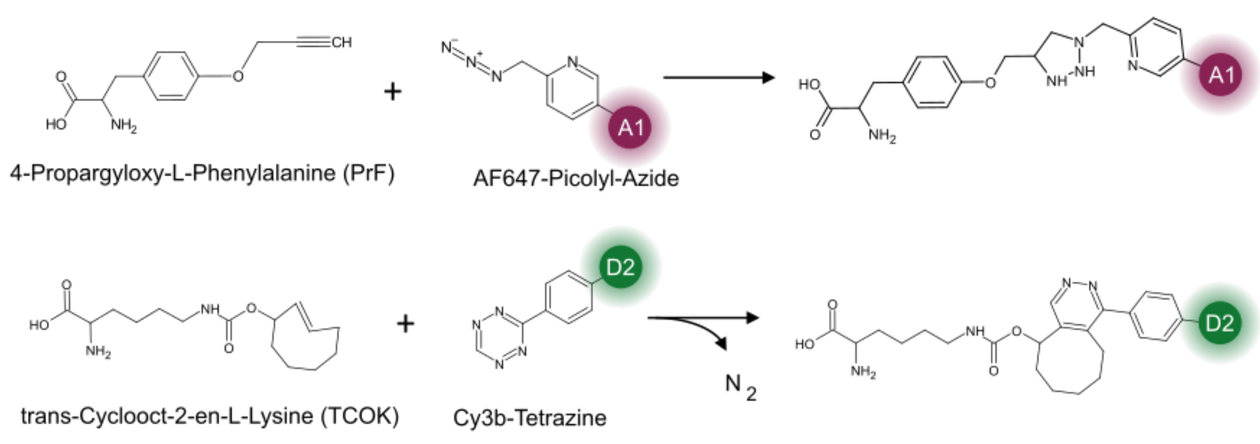
Methods

Genetic code expansion for site-specific incorporation of unnatural amino acids in human cells

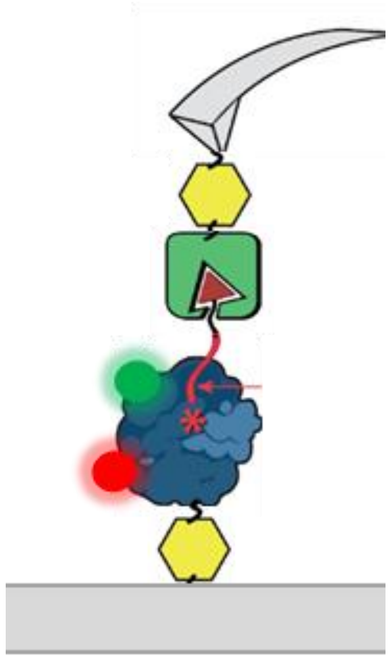


Bioorthogonal click chemistries for site-specific fluorophore labeling

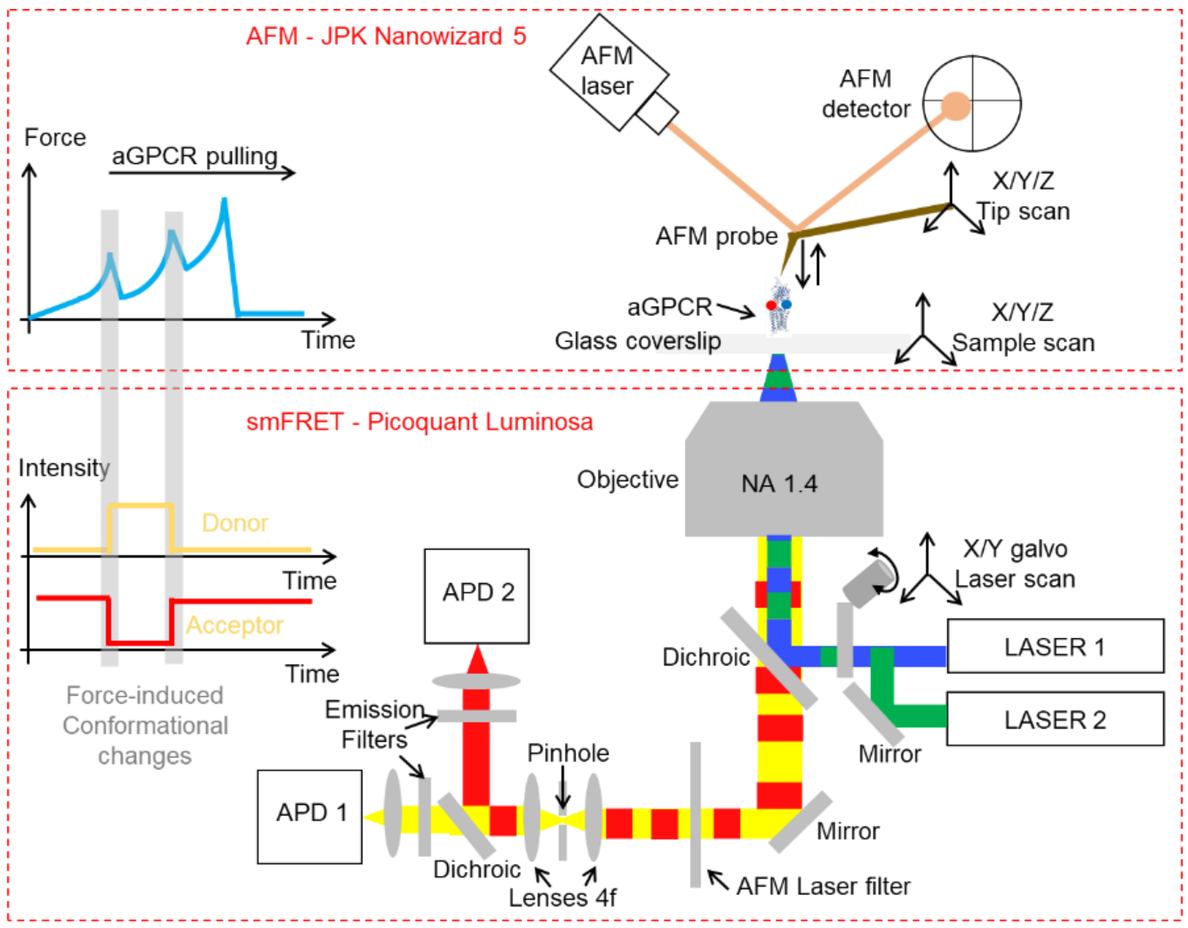




Receptor immobilization and tip functionalization



Implementation of simultaneous single molecule force spectroscopy and single molecule FRET measurements



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