

Single-molecule studies of the assembly of a quaternary receptor (interleukin-2/-15R) in model membranes

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Cellular signalling - the transmission and processing of information by cells - is an essential feature of life and critical to coordinating the immune response. Cytokines – a family of small signalling proteins – play a pivotal role in orchestrating both innate and adaptive immune responses. The cytokines interleukin-2 (IL-2) and interleukin-15 (IL-15), for example, set T- and NK-cells on course to respond to pathogens and tumours. IL-2 and IL-15 both transduce signals by binding to membrane receptors. IL-2 and IL-15 bind to a shared heterodimeric receptor consisting of IL-2/15R β and IL-2/15R γ in complex with a private subunit (IL-2R α and IL15-R α , respectively). Signalling is transduced solely through the shared receptors, with no apparent structural differences between the IL-2 and IL-15 quaternary complexes. However, distinct and competing functional differences in IL-2 and IL-15 signalling indicate the existence of a ligand discrimination mechanism. To elucidate potential ligand discrimination mechanisms based on the kinetics of receptor assembly, we apply multi-colour single-molecule imaging on a reconstituted membrane-receptor system to follow individual receptor subunits and their assembly upon ligand binding. Kinetic rates of the ligand-receptor complex are then extracted from colocalization events. To this end, we built a bespoke four-colour single-molecule total internal reflection fluorescence (TIRF) microscope based on the open hardware framework miCube. It features a flat-top illuminated field of view of 222 x 222 μm^2 , a focus stabilisation system and simultaneous three-colour detection.

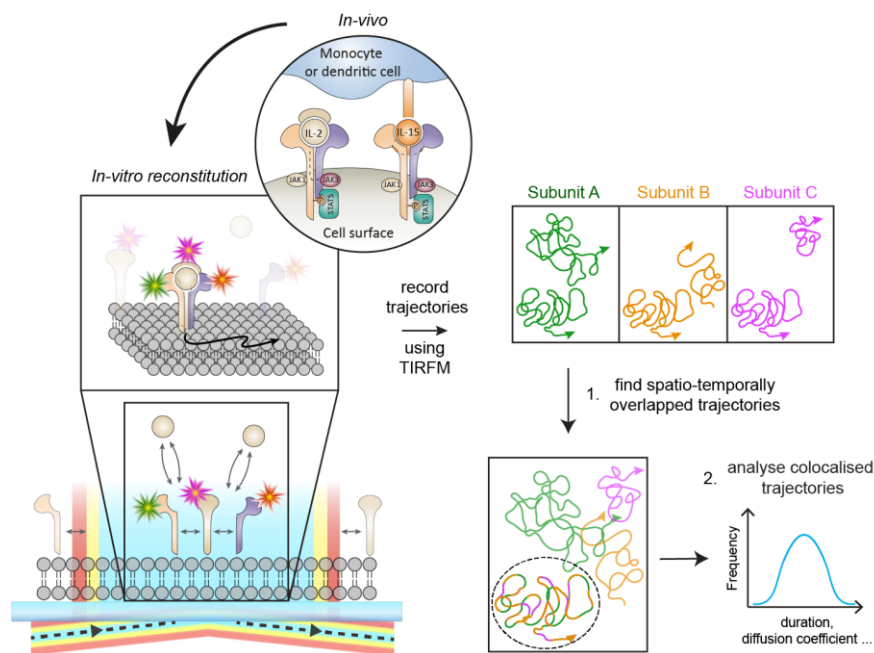


Figure: Single-molecule fluorescence microscopy studies of the assembly of a quaternary receptor (interleukin-2/-15R) in model membranes