

Dynamic structural biology of membrane transporters: tools and applications

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Membrane transporters are vital to any living system and involved in the translocation of a wide variety of substrates. Despite their importance, all proposed molecular models for transport are based on indirect evidence due to the inability of classical biophysical and biochemical techniques to visualize dynamic structural changes. My group has recently started to use single-molecule fluorescence microscopy to characterize conformational states and changes in active membrane transporters in vitro to directly observe how different steps in transport are coordinated.[1-3]

In the first part of my talk I focus on the homodimeric GlnPQ complex, a bacterial ABC-importer, possessing two different substrate-binding proteins (SBDs) per single translocator. To decipher how conformational changes within the different subdomains drive transport, we use a combination of single-molecule methods and classical biochemical techniques (calorimetry and uptake assays). We demonstrate by single-molecule Förster resonance energy transfer (FRET) that the two SBDs intrinsically transit from open to closed ligand-free conformation, and the proteins capture their amino acid ligands via an induced-fit mechanism. High-affinity ligands elicit transitions without changing the closed-state lifetime, whereas low-affinity ligands dramatically shorten it. We show that SBDs in the closed state compete for docking onto the translocator. We find that the rate-determining steps for translocation depend on the SBD and the amino acid transported. We conclude that the lifetime of the closed conformation controls both SBD docking to the translocator and substrate release.[1]

In the second part of the talk, I describe our latest developments of “enabling technology” for mechanistic studies: using photophysical tricks, we present a simple two-colour FRET assay that allows can either monitor multiple distances within protein complexes or simultaneously reveals one FRET-based distance and the presence of a second protein.[4] I finally summarize our contributions towards the development of "self-healing" organic fluorophores and their applications in single-molecule FRET or super-resolution microscopy.[2]

[1] G. Gouridis et al., *Nature Structural & Molecular Biology* 22 (2015) 57-64

[2] J.H.M. van der Velde et al., *Nature Communications* 7:10144 (2016)

[3] A. A. Jazi et al., *Biochemistry* 56 (2017) 2031-2041

[4] E. Ploetz and E. Lerner et al., *Scientific Reports* 6:33257 (2016)