NANOSCIENCE COLLOQUIUM

Single-molecule characterisation of an osmoregulatory transporter

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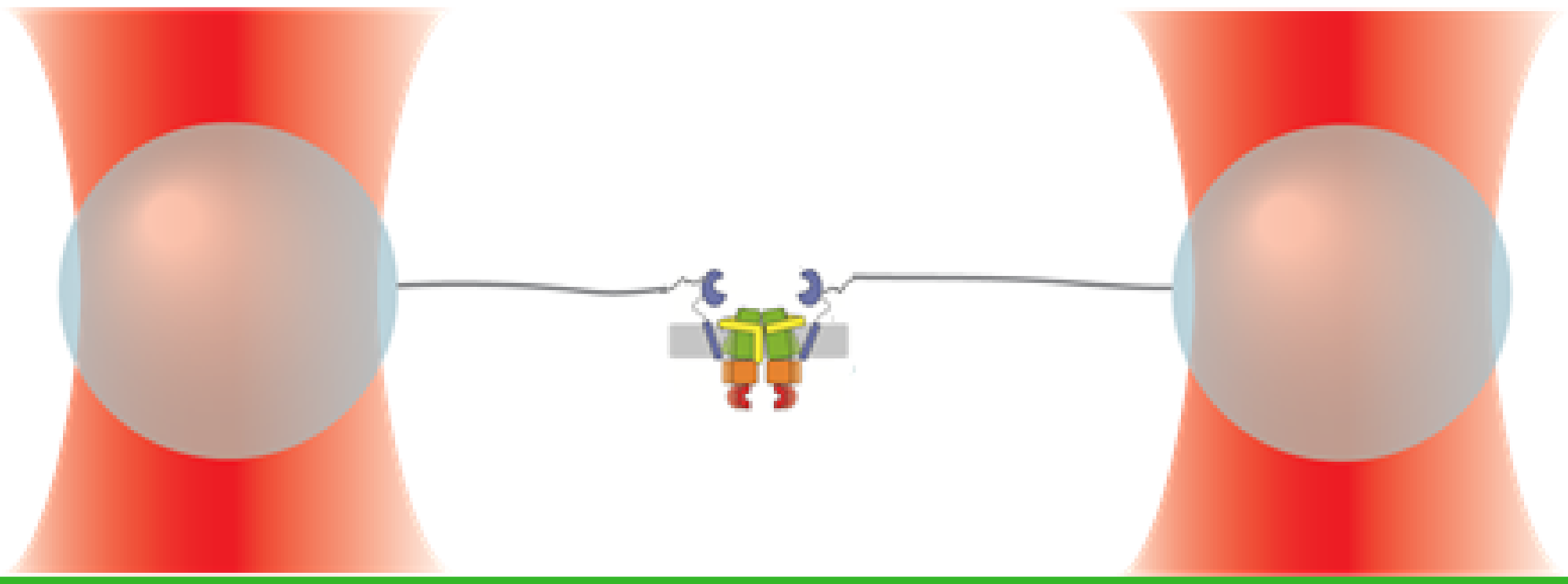
Abstract: Using the optical tweezers is, by now, a well-established technique for the study of protein (un)folding and for the observation of function-related protein motions. In my lab, we seek to expand the capabilities of this method to enable (amongst other things) simultaneous mechanical and fluorescent readouts of inter- and intra-molecular distances and dynamics, characterisation of the function-related motions of membrane proteins, and assessment of the impact of lipid membrane composition on protein function.
I will present our most recent work-in-progress: the single-molecule characterisation of the bacterial osmoregulatory ATP-binding cassette transporter OpuA.
Adaptation to stress is an essential feature of living cells, and a hypertonic environment leads to a potentially dangerous loss of turgor of the cell, due to outflow of water. Fortunately, the cell has mechanisms by which it is able to restore its volume. OpuA restores the cell volume by accumulating large amounts of compatible solute, glycine betaine. OpuA is gated by ionic strength and inhibited by the second messenger cyclic-di-AMP. Despite being extensively studied, molecular-level details of the transport mechanism, such as rates governing the interactions between the substrate binding domains and the transmembrane domain, are lacking. Using a combination of single-molecule FRET and optical tweezers measurements we are unravelling these functionally-relevant details with the goal of obtaining a quantitative overview of the functional cycle of OpuA.



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17.05.2022
1:15 pm | R. 301 / CHyN

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