Aims of the Project

Myosin VI walking mechanism

According to the widely established lever arm model, the step sizes of myosin molecular motors are expected to be proportional to their lever arm length. The lever arm length is defined by the number of the light chain binding domains that form the neck region of myosin molecules. Myosin VI was shown to walk processively on the actin filaments with a step size of 36 nm. However, according to the lever arm model, with only two light chains myosin VI step size was predicted to be ~10 nm. If myosin VI does not utilize a fundamentally different stepping mechanism than other characterized myosins in the myosin super family then it must make use of some mechanically extensible elements to span the 36 nm distance on actin. This putative element can be in principle N-terminal or C-terminal to the calmodulin light chain binding domain. One major objective of this work was to elucidate the myosin VI stepping mechanism, via single molecule analysis. If myosin VI walks hand-over-hand, the displacement of one head dependent on the position of the putative flexible element makes different and testable predictions in a single molecule TIRF experiment. The measured step size can distinguish between a hand-over-hand walking mechanism utilizing a putative C-terminal flexible element and a possible inchworm model[67].

Single molecule high resolution colocalization

Single molecule fluorescence techniques have provided access to valuable information about the molecular mechanism of action of numerous enzymes that is otherwise not obtainable from bulk assays. Specifically, the spatial tracking of single molecules attached to enzymes has yielded a wealth of new insights. Recently it has been possible to localize a fluorophore with a 1.5 nm spatial resolution which allowed circumvention of diffraction limited resolution for visible light of about 250 nm. These methods allow precise tracking of one fluorophore at a time. However, information about the relative distances in macromolecules or complexes is for the most part inaccessible with these methods. Single molecule FRET is capable of measuring distances up to 10 nm.

Thus, there is a ‘spectroscopic gap’ between the upper resolution limit of single molecule FRET technique and the lower resolution limit of the far field fluorescence microscopy. To allow distance measurements in a range that is relevant to a wide range of macromolecules between 10 nm and 200 nm, this work aimed to help developing a new single molecule method that allowed tracking of two chromatically different dyes simultaneously through time with high precision[68].

Regulation of *Dictyostelium* Myosin II ATPase

Biochemical data show that the actin activated ATPase activity of the *Dictyostelium* full-length myosin II is increased 6-fold upon phosphorylation of the regulatory light chain (RLC), whereas the single headed myosin II S1 appears not to be regulated[17,69]. The molecular mechanism of this regulation is poorly understood, partially because the RLC in the crystal structure is approximately ~100 Å away from the head domain and thus does not directly contact the catalytic domain[7,9,11,12]. Unexpectedly, during the course of the studies on the single headed myosin II S1 it was discovered that the RLC can crosslink to the catalytic domain in the myosin S1 suggesting a physical interaction between them in solution. The dependence of the RLC phosphorylation on this crosslinking was further characterized to gain insights into the mechanism of the RLC phosphorylation on the actin activated ATPase of *Dictyostelium* myosin II.