Resolving active ion transport at the single molecule level for the first time

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Electrochemical gradients across cellular membranes control a plethora of vital biological processes. These gradients are generated by primary active transporters and are subsequently used to drive the exchange of other solutes through secondary active transporters and to facilitate signaling via ion channels. The macroscopic biological phenomena that channels and transporters give rise to are intimately connected to how they function at the single molecule level. For decades, patch clamp recording has been used to observe the functional dynamics of single ion channels revealing discrete on and off states, subconductance states, and other mechanistically important features that macroscopic experiments cannot probe.1,2 Currently, there aren’t any techniques available to investi- rate transport function at the single molecule level, thus they are only studied using ensemble biochemical methods. For a decade we have been developing quantitative fluorescence microscopy based assays of arrayed proteoliposomes for investigating membrane proteins.3–6 Here we extended the platform to monitor the single-molecule activity and the regulation of a prototypic P-type ATPase, Arabidopsis thaliana H+–ATPase (AHAA). For the first time we have shown that individual proton pumps are not active continuously but rather transitioning between active and inactive states separated by a large activation barrier (kJMol−1).7 We found that the dynamics of these states form the basis of the regulation of the macroscopic activity either by regulatory R-domain, pH gradients, or ATP. Like for ion channels we often found that regulatory inputs do not affect the intrinsic pumping rates but rather active probabilities.

References: