Light sensitivity of Cell free expressed Archaerhodopsin-3 in microfluidics

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Many studies have highlighted the importance of transmembrane receptor proteins in the plasma membranes of biological cells. Photoreceptors such as Archaerhodopsin (Arch) constitute a class of transmembrane proteins that are highly sensitive to light. Light sensitivity of proteins has been intensely investigated during the past decades. However, there is a lack of information regarding the electrophysiological properties of the pore forming Archaerhodopsin (Arch), as studied in vitro. Here, we use a PDMS microfluidic chip with two channels that form unsupported bilayers between them. The technique enables simultaneous optical and electrical assessments of the bilayer in real time. In this work we recombinantly produced Arch-3 using a cell-free expression system. We synthesized a GFP labelled variant of Arch-3 to track its synthesis, using fluorescence microscopy. The electrophysiological properties of Arch-3 incorporated in a suspended bilayer were studied using a green laser for excitation. From the measurements we estimated the pore size of Arch-3 as 1.2 nm. Due to rapid cell-free prototyping just by changing the DNA, our setup enables us to study also photoelectrical properties of modified transmembrane protein constructs with ease. Moreover, Arch possesses the generic transmembrane structure of G-proteins. Our work represents a first step to study their cascade signaling in conjunction with coupled receptor proteins.