Marie Skłodowska-Curie Actions Individual Fellowship-2017 CALL

Candidate Look Out

Transduction of transmembrane electric field into protein motion studied by High Speed Atomic Force Microscopy

FM4B Lab, U1006 and LAI, French National Health Institute INSERM, Aix-Marseille Université, Marseille

Candidate requirement: (i) Residency our of France, or (ii) Residency in France for less than a year

Project awarded the Seal of Excellence of the European Commission in 2016

Outlline: To determine the biophysical mechanisms of the transduction of transmembrane electric field into protein motion high-resolution techniques must be used to assess the different protein conformations. In particular the voltage open and close gating-states of the channels. Nevertheless, previous works where the crystal structure of some voltage-gated channels was obtained only studied the structure of the activation state (at 0 mV), whereas the resting state (closed, 70mV) structure remains structurally unknown. The reason being that electric field dependent resting state cannot be exited during the 3-D crystal protein structure determination measurement. From electrophysiological studies (patchclamp, black lipid membranes) we know of the presence of an exponential-like dependence between the open-closed transition rates and the membrane potential. Theoretically, several competing models describing the voltage-gated channel movements are found in the literature (sliding helix, paddle model). Nevertheless to discriminate among them only visualization of the transduction of transmembrane electric field into protein motion will clarify this important molecular function that regulates the action of the nervous system, neurons and the brain.

The atomic force microscope (AFM) has become an essential tool in structural biology. Only the AFM allows structural characterization of biomolecules under native-like conditions, in physiological buffer and temperature and ambient pressure. In the case of membrane proteins, topographies are acquired at a lateral resolution of ~10Å and a vertical resolution of ~1Å. Thanks to the high signal-to-noise ratio of the AFM, individual membrane proteins can be imaged in native membranes and the protein assemblies studied, and the inter-protein interactions understood. The imaging speed of the conventional AFM of one to several minutes per image did not allow observing the dynamics of the immense majority of biomolecular processes. In response to the need for faster imaging rates for monitoring the essential molecular movements a new generation of faster High-Speed Atomic Force Microscopes (HS-AFMs) have been developed recently. This instrument features an increased imaging speed by three orders of magnitude compared to previous conventional AFMs, and it is now achieving maximum imaging rates of 20 ms per frame with single molecule resolution. Therefore HS-AFM represents the ideal candidate for characterizing the dynamics of the electromechanical coupling in cell membranes and will be used here, for the first time, to study a complex dynamic process.

For this project a new set-up for the application of an electric field during HS-AFM imaging is under development, proof of concept results are available and must be included in the 2017 MSCA call.

Contact (as soon as possible): Ignacio Casuso, ignacio.casuso@inserm.fr

Webs: https://sites.google.com/view/fm4b-lab/home // https://labadhesioninflammation.org/

Deadline for fellowship submission: 14th September

References: B.Martinac, Y. Saimi, C. Kung (2008) Physiol. Rev. 88(4):1449-1490 ; I. Casuso et Al. (2012) Nature Nanotechnology 7 (8), 525-529