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Title : Fusion of mitochondrial membranes – biophysical, computational and biochemical characterization of mitofusins

Abstract

Fusion of mitochondrial outer membranes (MOM) involves mitofusins, large GTPases of the Dynamin-Related Proteins (DRPs) superfamily, one of which is Fzo1. Recent data in M. Cohen's team indicate that the GTPase domain of Fzo1 would induce a conformational switch from a closed to an open state concomitant with mitochondrial tethering. Such a conformational switch resembles the bacterial dynamin-like protein (BDLP), a close relative of Fzo1 (20% identity and 41% similarity), whose structure is known in two conformational states: binding of GTP analogs by BDLP results in a switch from a compact structure, seen with GDP binding, to an 'open' conformation that stimulates BDLP oligomerization and favors its ability to shape the morphology of liposomes. BDLP structural properties and Fzo1 behavior during MOM fusion suggest that mitofusins may genuinely promote membrane fusion by switching from closed to open conformation, although the molecular fusion mechanism is unknown.

The key idea of this thesis project is to use molecular modeling and atomistic simulations in close link to experiment to study Fzo1 based on BDLP structures to decipher the region within Fzo1 that would be required for its potential conformational switch. The roles of this region in MOM fusion and ubiquitinylation of Fzo1 will then be dissected.

We will first homology model open and closed Fzo1 conformations based on available BDLP structures. The models will be refined, for instance using available knowledge on a variety of mutants, and their stability will be assessed in extensive molecular dynamics simulations. In turn, the refined models may generate hypotheses for the conformational transition between open and closed forms that lead to testable mutations. Analysis of their effects on MOM fusion and tethering will be carried out. Low and high-resolution structural data (cryo-EM, crystallography) will be integrated with the modelling as soon as the data becomes available. Once these models are fully validated, simulation of Fzo1 conformational changes in an *in vivo*-like environment can be attempted to gain insight into the fusion mechanism. Since sequence and function of mitofusins are conserved throughout evolution, our approach may unravel novel functional domains and provide fundamental insights about molecular mechanisms by which mitofusins and more generally DRPs catalyze membrane fusion.

This thesis project is directly relevant to the LABEX DYNAMO task 2 (Membrane biogenesis and dynamics) sub-task on remodelling membranes: fission and fusion. It involves three experts in molecular modelling: Marc Baaden (molecular dynamics of membrane proteins), Jérôme Hénin (enhanced sampling and quantification of membrane processes) and Antoine Taly (homology modeling and design). Experimental fusion and tethering assays will be carried out in Mickael Cohen's team. Cryo-EM acquisitions are ongoing in collaboration with Werner Kuhlbrandt (Max-Planck, Francfort). Crystallographic screening is about to start in collaboration with Hugues Nury (IBS, Grenoble).