Séminaire

CONFÉRENCIER INVITÉ

Vendredi 25 Mai 2018 à 11h

Salle des séminaires

Institut de biologie structurale - 71 avenue des Martyrs CS 10090 38044 Grenoble Cedex 9 - T.+33 (0)4 57 42 85 00

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DEER, X-ray and MD simulations unveil the mechanics of a heterodimeric ABC transporter

ATP binding and hydrolysis in the nucleotide-binding domains of ABC transporters are chemo-mechanically coupled to conformational changes in the transmembrane domains, leading to substrate translocation. Here we show that the combination of biochemical assays, site-directed mutagenesis, EPR and MD simulations allow to deepen the understanding of the molecular details of the large-scale movements underlying the functional working cycle of the heterodimeric ABC exporter TM287/288, whose structure had been solved only in two inward-facing states.

Unbiased multi-microsecond MD simulations in an explicit membrane/water environment show how in response to ATP binding, TM287/288 undergoes spontaneous conformational transitions from the inward-facing (IF) state via an occluded (Occ) intermediate to an outward-facing (OF) state, the latter two being similar to crystal structures from homologous transporters. The OF structural model was experimentally verified by Double Electron Electron Resonance (DEER, also known as PELDOR) performed on transporters spin-labeled with nitroxide probes in detergents as well as in liposomes. The validation of the models as well as the effects of the membrane bilayer on the structural response of the transporter will be presented.

The OF structure was recently solved by x-ray (unpublished data from M. Seeger's lab) using a synthetic nanobody, which causes strong inhibition of ATP hydrolysis. Comparison between the crystallized and MD-obtained models will be presented. To monitor the effects of this nanobody on the conformational cycle of TM287/288, we spin-labeled it with a gadolinium probe, which is spectroscopically distinguishable from the nitroxide probes in the transporter. Thereby, we could follow on the same sample, both the movements of the transporter and the binding of the sybody.

Hôte : Paul Schanda (IBS/Groupe NMR)