Soutenance ibs

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Soutenance de thèse

Mardi 28 février 2012

A 14h - Salle des séminaires de l'IBS

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Structural characterization of membrane proteins by hydrogen/deuterium exchange mass spectrometry

Thèse de Doctorat de l'Université Joseph Fourier

TATP-Binding Cassette exporters are known to be responsible for resistance against a broad spectrum of antibiotics and chemotherapeutic drugs in bacteria and mammalian cells. Amide hydrogen deuterium exchange coupled to mass spectrometry (HDX-MS) was applied to investigate the conformational changes in two different bacterial ABC exporters, BmrA and BmrC/BmrD, in the presence and absence of nucleotide. Local H/D exchange kinetics showed highly dynamic nature of ICDs in apo form which was not anticipated from X-ray structure of the homologue proteins. In outward facing (closed form) conformation the movement of ICDs were largely reduced for both transporters. The H/D exchange kinetics of closed form were determined by applying H/D exchange on mutants unable to hydrolyze nucleotides or on wild-type inhibited by vanadate. The dynamics of NBDs particularly for those regions which interact during ATP hydrolysis were also reduced in closed form as compared to open one. The addition of different drugs which are known to be transported by ABC transporters did not affect dynamics of NBDs. We further applied H/D exchange kinetics on a prokaryotic homologue of pentameric ligandgated ion channel (pLGIC) GLIC. Local H/D exchange kinetics were in full agreement with the available structure and change in pH showed differences in deuterium level for interacting regions of the subunits.