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Structural and functional investigation of MexA/MexB/OprM, a multidrug efflux pump from *Pseudomonas aeruginosa*

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Among the various mechanisms developed by the bacteria to counter to the effect of antibiotics, active efflux is on the front line. In *Pseudomonas aeruginosa*, a gram-negative bacteria, efflux transporters are organized as multicomponent systems where MexB, the efflux pump located in the inner membrane, works in conjunction with MexA, a periplasmic protein, and OprM, an outer membrane protein. MexB acts as a proton motive force-dependent pump with broad substrate specificity.

We have determined the high resolution X-ray structure of OprM and combined this structural study with a normal mode analysis of the internal dynamics of the porin. In this dynamic analysis, some key residues appeared to be important for keeping the porin pore closed. In order to monitor the activity of this protein, we have used a microfluidic biomimetic chip for the monitoring of functional ion channels reconstituted within suspended artificial bilayer lipid membranes (BLM). The microfluidic chip permits long-term electrical investigation of ion-channel conductance.

As a complementary approach, we are working on the functional reconstitution of the pumps into proteoliposomes. Very recently, we have designed a functional test for MexB. This original activity assay uses bacteriorhodopsin (BR), a light-activated proton pump, to generate a tunable, robust and reversible proton gradient. In this system, upon illumination with visible light, the photo-induced proton gradient created by the BR is shown to be coupled to the active transport of substrates through the pump. We are now working on the reconstitution of the whole efflux pump.

Finally, in collaboration with the group of P. Minard, we are adapting a new set of synthetic scaffolds (dubbed α-reps for "artificial alpha repeat protein") to help us crystallize the efflux pumps. Such interactants are selected through *in vitro* screening of the membrane proteins targets. We make use of amphipathic polymers (amphipols) to stabilize and immobilize the proteins onto solid support so that the library of possible interactants can be screened.

Biography

Martin Picard received his Ph.D. in Biochemistry from the University Pierre and Marie Curie in 2004 (funded HFSP). In the group of Professor Marc Mayor, under the supervision of Dr. P. Champeil, they studied the Ca (2 +)-ATPase of sarcoplasmic reticulum (SERCA1a), a structural and functional point of view. In particular, they showed that, under certain conditions, the crystallization conditions can sometimes induce artifactual structures. In 2005, he made a post-doctoral fellowship at the University of Aarhus (FEBS long-term fellowship) in the group of Professor Poul Nissen for studying calcium from a structural point of view crystallography pump. The controversy about fixing the AMPPCP, a non-hydrolysable analogue of ATP, was resolved and the structure of the enzyme in the autophosphorylated state of high energy has been determined. Back in Paris, Martin Picard joined the group of Dr. Jean-Luc Popot to work on the use of amphiphilic polymers, amphipols, designed to stabilize membrane proteins in solution. He was hired in 2007 as a CNRS researcher in the group of Prof. Arnaud Ducruix group now led by Dr. Isabelle Broutin. He works on the efflux pumps of gram-negative bacteria *Pseudomonas aeruginosa* a structural point of and functional.

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