Tripartite assembly of RND multidrug efflux pumps

Laetitia Daury[1,2], François Orange[1,2] Jean-Christophe Taveau[1,2], Alice Verchère[3], Laura Monlezun[3], Ravi K. R. Marreddy[4], Martin Picard[3], Isabelle Broutin[3], Klaas M. Pos[4] and Olivier Lambert[1,2]

[1]Univ. Bordeaux, CBMN UMR 5248, Bordeaux INP, IECB, F-33600 Pessac, France.
[2]CNRS, CBMN UMR5248, F-33600 Pessac, France,
[3] Laboratoire de Cristallographie et RMN Biologiques, UMR 8015, CNRS, Université Paris Descartes, Faculté de Pharmacie, 4 Avenue de l'Observatoire, 75006, Paris, France.
[4] Institute of Biochemistry, Goethe-University Frankfurt, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany

Tripartite multidrug efflux systems of Gram-negative bacteria export a large variety of antimicrobial compounds at the expense of ATP or the proton motive force, thereby conferring resistance to a wide variety of antibiotics. *Pseudomonas aeruginosa* MexAB-OprM and *Escherichia coli* AcrAB-TolC, are prototypic proton motive force-driven efflux systems from Resistance Nodulation and cell Division (RND) family. These efflux systems, composed of an inner membrane transporter, an outer membrane channel and a periplasmic adaptor protein, are assumed to form ducts inside the periplasm, facilitating drug exit across the outer membrane. We previously reported the architecture of OprM/MexA complex reconstituted into lipid membranes, using cryo-electron tomography.

We present here the reconstitution of native MexAB-OprM and AcrAB-TolC in a lipid nanodisc system. Single particle analysis by electron microscopy revealed the lipid nanodisc-embedded inner and outer membrane protein components linked together via the periplasmic adaptor protein, this forming a tripartite setup. This intrinsic *in vitro* self-assembly of the native components was emphasized by the formation of a stable interspecies AcrA-MexB-TolC complex, providing evidence for a common mechanism of tripartite assembly with cognate and non-cognate components. The projection structures of all three RND complexes presented here emphasize the role of the periplasmic adaptor protein as part of the exit duct with no physical interaction between the inner and outer membrane components.