CryoEM study of a bacterial multidrug efflux pump involved in antibiotic resistance

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The dangerous increase of pathogenic strains of bacteria resistant to most of available antibiotics emerges as a worldwide public health concern. It is urgent to develop original solution and focus on unconventional targets. Active efflux is one of the four main mechanisms by which bacteria exhibit resistance to antibiotic.

In Gram-negative bacteria, tripartite efflux pumps spanning the cell envelope can mediate the efflux of a wide variety of antimicrobial compounds, and participate to the antibiotic resistance. These efflux systems share a similar structural architecture composed of an inner membrane transporter and an outer membrane channel bridged by a periplasmic adaptor protein. The prototypical and clinically relevant efflux pump from *Pseudomonas aeruginosa*, MexAB-OprM is involved in the transport of drugs from the periplasm to the extracellular medium through the use of the proton gradient.

We have developed a method to reconstitute a tripartite assembly from native components using lipid nanodiscs. MexB and OprM inserted in lipid nanodisc self-assembled in the presence of MexA. The structure of tripartite system has been studied by electron microscopy [1]. The 3D structure analyzed by single particle cryoEM of the tripartite efflux pump is at 6.4 A resolution revealing the α helical loops of OprM and MexA in a tip to tip interaction shows that OprM and MexB components are linked together via MexA emphasizing its role as part of the exit duct with no physical interaction between the inner and outer membrane components. Taking advantage of the recent development of several membrane mimetic systems, we studied the formation of tripartite systems using Amphipol [2] that are negatively charged amphiphilic polymers stabilizing membrane proteins without the need of lipids. Results are discussed in the context of the key parameters governing the assembly of this tripartite system.

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[2]. Tribet, C., Audebert, R., & Popot, J. L. (1996). Amphipols: polymers that keep membrane proteins soluble in aqueous solutions. Proceedings of the National Academy of Sciences, 93(26), 15047-15050.