Structural study of a bacterial multidrug RND efflux pump reconstituted in membrane mimetic systems

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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 [1]. MexB and OprM inserted in lipid nanodisc self-assembled in the presence of MexA [2]. The structure of tripartite system has been studied by electron microscopy and the 3D structure is currently analyzed by single particle cryo-EM. Taking advantage of the recent development of several membrane mimetic systems, we analyze the formation of tripartite systems using Amphipol 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.

References

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