Functional and structural study of two homologous ABC tripartite efflux pumps from Gram-negative bacteria

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ATP-Binding Cassette (ABC) tripartite efflux pumps are membrane protein machineries that perform vectorial export of a large variety of drugs and virulence factors from Gram-negative bacteria using ATP hydrolysis as energy source [1-3]. MacAB TolC in *Escherichia coli* and PvdRT OpmQ in *Pseudomonas aeruginosa* form homologous tripartite efflux pumps with MacB and PvdT belonging to the type VII subfamily of ABC transporters.

While the functional roles of these proteins are relatively well studied, the mechanistic aspects of transport are still not fully understood. In our laboratory, a combination of functional and structural experiments has led to the description of their likely operation.

An initial study of reconstituted MacAB TolC in lipid environments enables the measurement of both ATP hydrolysis and substrate transport activity. We observed that these two events are concomitant, suggesting a role for ATP hydrolysis in substrate efflux by MacAB TolC. These results facilitate a more in-depth discussion of the catalytic cycle of this pump and its critical events [4].

A second study on MacAB TolC focuses on allosteric effects on the ATP hydrolysis activity of MacB. We first confirmed what the literature reports: MacA enhances the ATPase activity of MacB. Furthermore, we demonstrated that TolC increases MacB activity even more in the presence of MacA. The reconstitution of the proteins in lipid environments increases MacB ATP hydrolysis activity in the presence of MacA or both MacA and TolC. These results allow us to reevaluate the available structural data in the literature [5].

Finally, we established the production of the three components of the tripartite efflux pump PvdRT OpmQ, enabling their functional and structural study. PvdT is an active protein in detergent micelles, and the presence of substrate pyoverdine or lipids has an activating effect on PvdT ATPase activity. We also obtained preliminary structural results of PvdRT using an *in silico* model constructed in collaboration and combined with negative-staining results. This opens up research prospects for its future structural study using single-particle cryo-electron microscopy.

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