Structural characterization of the N-terminal domain of the MexXY-OprM efflux pump response regulator ParR

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Pseudomonas aeruginosa (PA) is one of the most important infectious agents in cystic fibrosis. Introduction of last resort antibiotics such as colistin and polymixin B against multi-resistant strains of PA shows activation of the two-component system ParRS leading to MexXY efflux pump overexpression. The presence in the environment of PA of such drugs and their interaction with lipopolysaccharide triggered uncharacterized signal that is detected by the membrane-sensor Histidine Kinase ParS. In response, ParS auto-phosphorylates its cytosolic domain on a conserved histidine residue. A phospho-transfer allows the activation of the response regulator ParR on a conserved aspartate residue. Once ParR is activated, it will regulate the expression of several genes including *mexXY* operon.

In this study, we purpose the structural characterization of the wild-type response regulator ParR together with a gain of function mutant M59I from the strain PAOW2. Our first results show that unphosphorylated ParR (u-ParR) is a monomeric protein in solution with two domains as suggested by homology modeling and confirmed by SAXS experiment. Interestingly, we show that u-ParR is not able to bind its DNA sequence target. SAXS result also shows folded protein containing flexible region. This was confirmed by limited proteolysis, which leads to the C-terminal domain (CTD) isolation. In addition, the N-terminal domains carrying (NTDw2) or not (NTDwt) the mutation M59I was cloned and purified. Structural investigation in solution showed that NTDwt is a well-folded and globular domain. Crystallization screens on NTDwt gave diffracting crystals that were optimized and X-ray data were then collected at ESRF and SOLEIL synchrotrons. Molecular replacement solution and structure refinement of this domain is now in progress. These structures will shed light on the role of the particular mutation M59I of ParR and will offer a new target for *in silico* drug-design in order to restore AG activity.