Understanding the substrate access mechanism in Phospholipase A from *Pseudomonas aeruginosa*

Sabahuddin Ahmad[1], Stephan Schott-Verdugo[1], Christoph Strunk[2], Filip Kovacic[2], Karl-Erich Jaeger[2], Holger Gohlke[1,3]

[1]Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

[2]Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany and Institute of Bio- and Geosciences IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Jülich, Germany

[3]John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), and Institute of Biological Information Processing (IBI-7: Structural Biochemistry), Forschungszentrum Jülich GmbH, Jülich, Germany

The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen and frequent cause of nosocomial infections, affecting primarily immune-compromised patients. PlaF from *P. aeruginosa* is a novel phospholipase A1 (PLA1). It is an integral inner membrane protein and has been shown to be a relevant virulence factor. A recent crystal structure of PlaF together with crosslinking and micro-scale thermophoresis experiments revealed PlaF to be in monomeric and dimeric configurations, although the protein was found to be active only in the monomeric state. Our previous free energy computations and wet-lab experiments indicated that, depending on the protein concentration, the dimer-to-monomer equilibrium is shifted to either side. The dimer-to-monomer transition is associated with a change in the orientation of the active site tunnel with respect to the membrane: A) in the dimeric form, the tunnel is *parallel* to the membrane *surface*, and the entrance resides more than 5 Å above it, which will likely hamper substrate access; B) in the monomeric form, PlaF tilts and the active site tunnel is in direct contact with the membrane, which will likely facilitate the substrate access from the membrane. We therefore hypothesize that the tilting of monomeric PlaF leads to this form being active by facilitating substrate access to the active site tunnel.

On further investigation, it was found that there are other cavities than the active site tunnel, resulting in total four access pathways (Tunnel 1-4) to/from the active site. However, which of these pathways is most favorable for substrate access remained elusive. To answer this, we performed configurational free energy computations of substrate access from the membrane to the active site. We considered different phospholipid substrates to understand this. Since a phospholipid substrate can enter by its head or either one of the two tails, we tested all three scenarios. Our computations reveal that A) Tunnel 2 is energetically most favorable for substrate access and B) the substrate entering with tail 1 first is the most favorable access mode. This access mode agrees with the fact that PlaF is a PLA1 that cleaves tail 1 of phospholipids. To validate our hypothesis about tunnel 2, we proposed tryptophan substitutions for all the four tunnels. Among proposed substitutions, the ones corresponding to tunnel 2 reduced the activity of PlaF by ~70%. Hence, it was confirmed that the tunnel 2 of Plaf is the preferred access tunnel for substrates. At present we aim to understand the egress pathway of the PlaF products. Together with our previous studies, our results can provide an explanation to the activity mechanism of PlaF at the atomistic level.