The crystal structure of phospholipase PlaB from *Legionella pneumophila* reveals the basis of tetramerization-dependent inactivation by a central metabolite

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The Gram-negative bacterium *Legionella pneumophila* is the causative agent of Pontiac fever and Legionnaires' disease, a potentially fatal form of pneumonia. It is taken up by inhalation of contaminated freshwater and enters lung macrophages, where it establishes a protected niche known as the "*Legionella*-containing vacuole" *via* injecting a set of several hundred proteins into the host cell. A significant fraction of these proteins are phospholipases that damage host cell membranes, provide nutrients to the bacterium and modulate host cell signaling by impacting on the second messenger pool. Amongst these enzymes, PlaB is unusual in that it localizes to *Legionella's* outer membrane and that it is only active at higher dilutions, which has been linked to a dimer/tetramer equilibrium that inactivates PlaB at higher concentration [1].

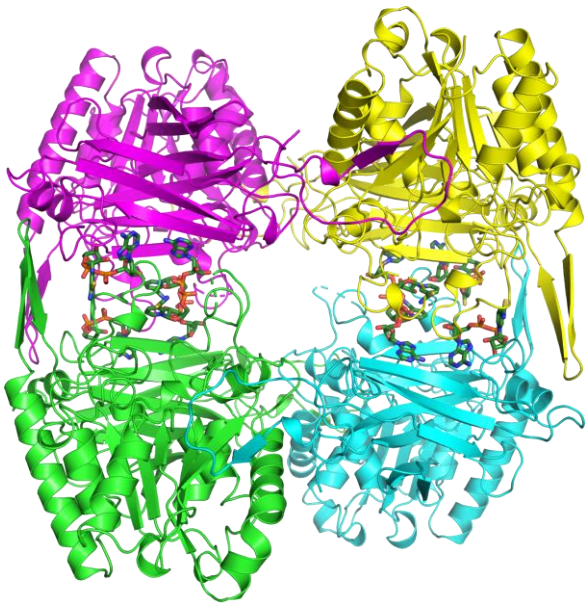

Fig.1: Structure of the PlaB tetramer bound to thio-NAD^+ shown in sticks

After some difficulty, we obtained well-diffracting crystals of PlaB and determined the crystal structure of the tetramer by experimental phasing, using seleno-*L*-methionine-labelled protein (Fig. 1). The structure provides an explanation for the inactivity of the tetramer and reveals building blocks that can be linked to dimerization and insertion into the outer membrane of *L. pneumophila*. Surprisingly, we found additional electron density in the dimer/dimer interface. This electron density was interpreted as eight NAD(H) molecules and hints at a stabilizing role of this central metabolite in the formation of the tetrameric form. Indeed, the addition of NAD(H) derivatives improved crystallization significantly and was also found to inhibit the enzymatic activity of PlaB [2].

The finding of an NAD(H)-stabilized inactive tetramer suggests a mechanism for self-protection against the phospholipase activity: while the protein resides within *L. pneumophila*, it is inactivated by high intracellular levels of NAD(H). Once PlaB is exported to the extracellular milieu, the concentration of NAD(H), leading to dissociation of the inactive tetramer into active dimers that subsequently associate with the outer membrane. To our knowledge, PlaB is the first example of an enzyme that is inhibited by ligand-mediated oligomerization.

[1] Kuhle K, Krausze J, Curth U, Rössle M, Heuner K, Lang C, Flieger A. Oligomerization inhibits Legionella pneumophila PlaB phospholipase A activity. J. Biol. Chem., 289, 18657-18666 (2014)

[2] Diwo MG, Michel W, Aurass P, Kuhle-Keindorf K, Pippel J, Krausze J, Wamp S, Lang S, Blankenfeldt W, Flieger A. NAD(H)-mediated tetramerization controls the activity of Legionella pneumophila phospholipase PlaB. Proc. Natl. Acad. Sci. USA, 118, e2017046118 (2021)

A.F. and W.B. acknowledge support by DFG under grant numbers FL359/4-2/3 and 281361126/GRK2223.