Structural studies of nucleotide binding and oligomerisation in "stand-alone" ATP-cone domains

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The small, ~100-residue ATP-cone domain, which was first characterized in ribonucleotide reductase (RNR) subunits, is crucial for the activity regulation of this enzyme[1]. Through allosteric regulation, the presence of ATP leads to activation of RNR, whereas dATP leads to oligomerization and inactivation[2]. ATP-cones are also found in RNR transcriptional regulator NrdR and in a few 2-phosphoglycerate kinases[1].

Interestingly, in various bacterial species, ATP-cones exist as stand-alone proteins, not connected to any other modules. We denoted these proteins AcoP (ATP-Cone Proteins). While ATP-cones have been extensively studied in RNRs and their transcriptional regulator NrdR[3], AcoPs remain an enigma. Our aim is to understand the biological function of AcoP, identify its interaction partner/s and reveal its molecular mechanism of action.

Here, we present structural studies of AcoP from *Streptococcus pneumoniae* (SpAcoP), *Streptococcus thermophilus* (StAcoP), and *Lactococcus lactis* (LlAcoP). Biophysical studies demonstrated that AcoP can bind two molecules of ATP or dATP, but not both simultaneously. The nature of the bound nucleotide affects the oligomeric state of the AcoP. We focused on solving the protein structures using X-ray crystallography, investigating the mechanism of nucleotide binding and the oligomeric states using small-angle X-ray scattering (SAXS). Our results show the variety of intermolecular interactions that AcoPs exhibit. Despite their structural similarities, they show organism-dependent behaviors in oligomerization and differ in their nucleotide binding from what is seen when they are integrated into a ribonucleotide reductase polypeptide, which makes them an exciting target for our biochemical, biological, and structural studies.

[1] Aravind, L., Y.I. Wolf, and E.V. Koonin, *The ATP-cone: an evolutionarily mobile, ATP-binding regulatory domain.* J Mol Microbiol Biotechnol, 2000. **2**(2): p. 191-4.

[2] Rozman Grinberg, I., et al., *Novel ATP-cone-driven allosteric regulation of ribonucleotide reductase via the radical-generating subunit.* Elife, 2018. **7**

[3] Rozman Grinberg, I., et al., *A nucleotide-sensing oligomerization mechanism that controls NrdR-dependent transcription of ribonucleotide reductases.* Nature Communications, 2022. **13**(1): p. 2700.