Identification of bacterial PolyP-ome – proteins associated with inorganic polyphosphate (PolyP)

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Inorganic polyphosphate (PolyP) is a linear anionic polymer of hundreds to thousands of phosphate (P_i) molecules held together by high-energy phosphoanhydride bonds. PolyP was found in all domains of life: bacteria, archaea, and eukaryotes, but its origin seems to be pre-biotic as one of the first energy-rich molecules on earth and precursor to RNA, DNA, and proteins. In microorganisms, PolyP functions as an energy and phosphate reservoir, it plays an essential role in cell survival, cation homeostasis, stress response, and contributes to sporulation, quorum-sensing, and pathogenesis. In mammals, PolyP appears to play an equally large number of distinct roles, such as stimulation of blood clotting, bone mineralization, mTOR activation, and apoptosis triggering.

The molecular mechanism by which such simple molecule exerts all these diverse functions is likely to be related to its ability to bind to specific proteins, either through ionic interactions or through recently described covalent post-translational modification called lysine polyphosphorylation. However, only a handful of reports have been published so far describing proteins associated with PolyP and relevance of such interactions. This was mainly due to technical difficulties with discriminative isolation of PolyP together with associated partners and limitations of mass spectrometry in polyphosphorylation detection.

Currently in our lab, we develop methods for isolation of intact PolyP granules together with associated proteins from bacteria grown under different conditions – both favorable and nonoptimal. Using ultracentrifugation in HistoDenz isopycnic gradient and pull-down assays with recombinant PolyP binding domain from *Escherichia coli* PolyP phosphatase (*Ec*PPX) we plan to selectively purify proteins physiologically associated with PolyP. The identification of potential targets will be done by mass spectrometry and subsequently confirmed by series of *in vitro* and *in vivo* experiments. In further steps, we want to characterize the biochemistry and molecular function of such interaction based on selected candidates. Our work will provide a novel tool to study bacterial and presumably eukaryotic PolyP-omes in the future.