BCHM 421/422 – 2022-23 – Jia Lab Project #1

Project Outline:

Inorganic polyphosphate (polyP) is a polymer composed of three to over 1,000 phosphate residues linked together via high-energy phosphoanhydride bonds. PolyP has been implicated in an astonishing array of biological functions—ranging from phosphorus storage to molecular chaperone activity to blood coagulation. In bacteria, polyP is synthesized by polyphosphate kinase (PPK) enzymes. Remarkably, *ppk* deletion has been shown to reduce the virulence of many pathogenic bacteria, thus indicating PPK as an attractive drug target. The Jia Lab recently discovered a new family of drugs that inhibit PPK enzymes in *Pseudomonas aeruginosa*, thereby attenuating the virulence of this important pathogen (1). We further validated the efficacy of our best PPK inhibitor against the superbugs *Acinetobacter baumannii* and *Klebsiella pneumoniae* (2). The main goals of this project are to improve the potency of our PPK inhibitors, and elucidate the mechanistic links between polyP, PPKs, and bacterial virulence. To improve inhibitor potency, we will use a combination of X-ray crystallography, enzyme kinetic studies, and structure-activity relationship (SAR) to guide the synthesis of new inhibitor analogues. To investigate the mechanisms by which polyP modulates virulence, we will monitor bacterial phenotypes such as biofilm formation, toxin secretion, and stress survival in wildtype, Δ*ppk*, and inhibitor-treated bacteria.

In parallel to our bacteria work, the Jia Lab recently discovered that human and yeast proteins with stretches of consecutive histidine residues exhibit a NuPAGE mobility shift in the presence of polyP, much like that observed for lysine polyphosphorylation (3, 4). We therefore seek to explore the possibility that this interaction could be a novel post-translation modification. We aim to elucidate the biochemical determinants of this interaction via mutagenesis, shift assays, and ^{31}P NMR. Potential physiological roles will be studied via activity assays and fluorescence microscopy in the presence or absence of polyP, using ΔHis proteins as negative controls.

Supervisor: Dr. Zongchao Jia

Project Title: Polyphosphate in bacterial virulence and mammalian physiology

Project Goals:

- Improve the potency of our lead PPK inhibitor compound gallein
- Decipher the mechanistic links between PPKs, polyP, and virulence in bacteria
- Investigate the biochemistry and physiological relevance of histidine-polyP interactions

Experimental Approaches:

- Cloning and site-directed mutagenesis
- Protein expression and purification
- *In vitro* enzyme inhibition and kinetics
- X-ray crystallography
- Fluorescence microscopy
- *P. aeruginosa* virulence phenotype assays (e.g. biofilm, pyoverdine, motility)

References:

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2. Roberge, N., Neville, N., Douchant, K., Noordhof, C., Boev, N., Sjaarda, C., Sheth, P. M., and Jia, Z. (2021) Broad-spectrum inhibitor of bacterial polyphosphate homeostasis attenuates virulence factors and helps reveal novel physiology of Klebsiella pneumoniae and Acinetobacter baumannii. *Frontiers in Microbiology*. **12**, 3229
3. Azevedo, C., Livermore, T., and Saiardi, A. (2015) Protein polyphosphorylation of lysine residues by inorganic polyphosphate. *Mol. Cell*. **58**, 71–82
4. Bentley-DeSousa, A., Holinier, C., Moteshareie, H., Tseng, Y.-C., Kajjo, S., Nwosu, C., Amodeo, G. F., Bondy-Chorney, E., Sai, Y., Rudner, A., Golshani, A., Davey, N. E., and Downey, M. (2018) A screen for candidate targets of lysine polyphosphorylation uncovers a conserved network implicated in ribosome biogenesis. *Cell Rep*. **22**, 3427–3439