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How can we identify the common molecular signatures underlying polyphosphate accumulation?

Since releasing our pub on polyphosphate-forming proteins in bacteria, we've noticed the community has similar problems studying this process in diverse organisms. We're actively seeking feedback with a focus on advancing basic discoveries and useful tools in this space!

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Purpose

We recently performed a comparative analysis of protein sequence and structural similarities to identify signatures of bacterial polyphosphate accumulation phenotypes. Since releasing this work, new questions have emerged and we're now seeking feedback from the broader community on next steps. We're particularly interested in

further computational exploration by connecting gene neighborhoods and regulatory motifs with our structural results. Additionally, we're beginning to explore wet-lab approaches and assays that we can apply to begin validating our computational hypotheses. We hope that by starting an open discussion, others will be inspired to follow up on this work on their own as well.

- This is a follow-up to work described in a **prior pub**, "Discovering shared protein structure signatures connected to polyphosphate accumulation in diverse bacteria." Visit that pub for complete background info and context.

Background on the original pub

We recently shared a pub comparing the structural similarity of phosphate-accumulating PPK1 enzymes from diverse microbes **[1]**. We were interested in understanding why certain bacteria in wastewater treatment plants are particularly good at accumulating polyphosphate and others are not. If we could better predict the molecular mechanisms contributing to microbial polyphosphate accumulation, this could enable rational engineering approaches to make wastewater treatment processes more reliable. Additionally, polyphosphate accumulation is important for virulence and biofilm formation in bacterial pathogens and plays a role in many processes in mammalian cells (albeit through unknown pathways).

We hypothesized that, despite great sequence divergence, protein structure could be a better indicator of function and explain why some bacteria accumulate large amounts of polyphosphate. We found through a comparative analysis of 28,000 PPK1 proteins that this can sometimes be the case, but doesn't fully explain things **[1]**.

New questions emerge

In an attempt to identify structural or genetic signatures of effective polyphosphate accumulation, a commenter suggested that we consider whether the presence of phosphatase enzymes would reflect an organism's ability to accumulate

polyphosphate, as an organism should possess the ability to mobilize the polyphosphate in some capacity.

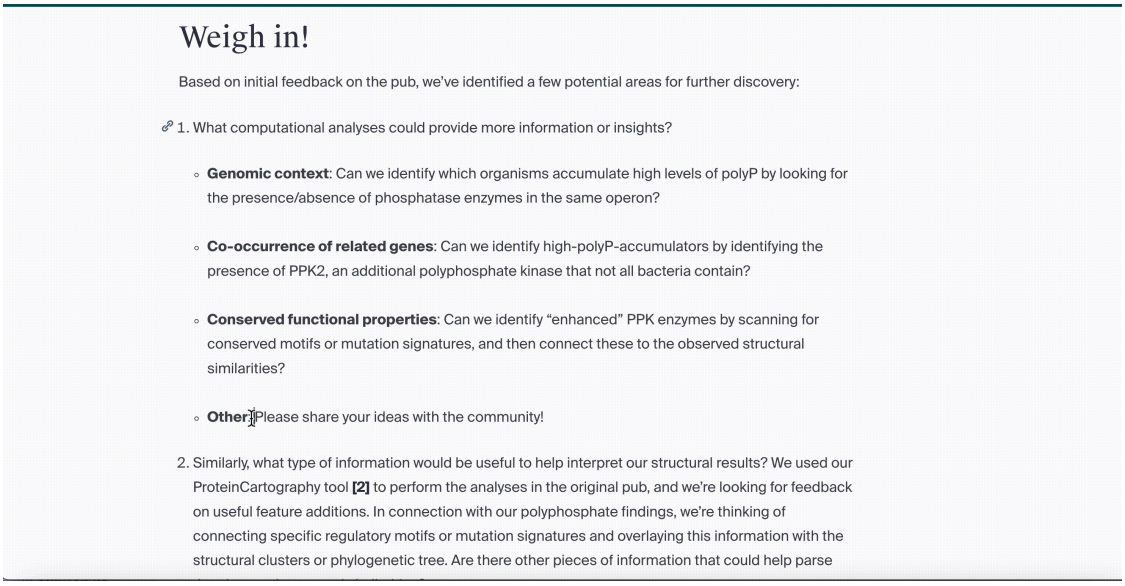
With this in mind, we're seeking feedback on potential next steps. We're specifically focusing our current efforts on two areas, but invite others to actively pursue these questions on their own as well:

- Computational analyses that give us more insights into our structural similarity results, such as looking at gene neighborhoods and mutation signatures
- Comparing *in vitro* and *in vivo* approaches to test the function and activity of PPK1 homologs from understudied organisms that are hypothesized to have enhanced polyphosphate accumulation activity, specifically those that are similar to Accumulibacter

To address these areas, we're seeking feedback from the community on some key questions. Our goal is to start a public conversation on this research topic, as we believe polyphosphate accumulation is of broad interest to many research communities. While the two areas above are of greatest interest to us, we hope that others will join in and work on these and other questions described below. Arcadia isn't pursuing climate technologies in the near term since our focus is on therapeutics, but there are clear implications in this space that we hope other groups pursue.

How can I weigh in?

We hope you'll respond publicly to our questions below by selecting/highlighting the question you'd like to answer, clicking the comment icon, and typing in your thoughts (as shown in the GIF below)! You'll need a PubPub account to do this, but it's free and quick to make one. Here's a quick tutorial on all of this.



Weigh in!

Based on initial feedback on the pub, we've identified a few potential areas for further discovery:

1. What computational analyses could provide more information or insights?
    - **Genomic context**: Can we identify which organisms accumulate high levels of polyP by looking for the presence/absence of phosphatase enzymes in the same operon?
    - **Co-occurrence of related genes**: Can we identify high-polyP-accumulators by identifying the presence of PPK2, an additional polyphosphate kinase that not all bacteria contain?
    - **Conserved functional properties**: Can we identify "enhanced" PPK enzymes by scanning for conserved motifs or mutation signatures, and then connect

these to the observed structural similarities?

- **Other**: Please share your ideas with the community!

2. Similarly, what type of information would be useful to help interpret our structural results? We used our ProteinCartography tool **[2]** to perform the analyses in the original pub, and we're looking for feedback on useful feature additions. In connection with our polyphosphate findings, we're thinking of connecting specific regulatory motifs or mutation signatures and overlaying this information with the structural clusters or phylogenetic tree. Are there other pieces of information that could help parse the observed structural similarities?
3. What experimental assays could validate these computational observations?
4. What learnings from polyphosphate accumulation in bacteria could be translated to understanding polyphosphate synthesis and related processes in mammalian cells?

We appreciate any feedback on the above questions or anything else that came up while reading the pub! We're particularly interested in how to move forward on bacterial polyphosphate discoveries with a focus on tool-building. We feel that an open conversation on this project will benefit the polyphosphate community as a whole, and we hope others will build on the ideas presented here and in future comments.

References

1 Avasthi P, Celebi FM, McDaniel EA. (2024). Discovering shared protein structure signatures connected to polyphosphate accumulation in diverse bacteria. https://doi.org/10.57844/ARCADIA-AC10-23E7

2 Avasthi P, Bigge BM, Celebi FM, Cheveralls K, Gehring J, McGeever E, Mishne G, Radkov A, Sun DA. (2024). ProteinCartography: Comparing proteins with structure-based maps for interactive exploration. https://doi.org/10.57844/ARCADIA-A5A6-1068