Structural Characterization Of The Tec Family Kinase's Lipid Binding Module

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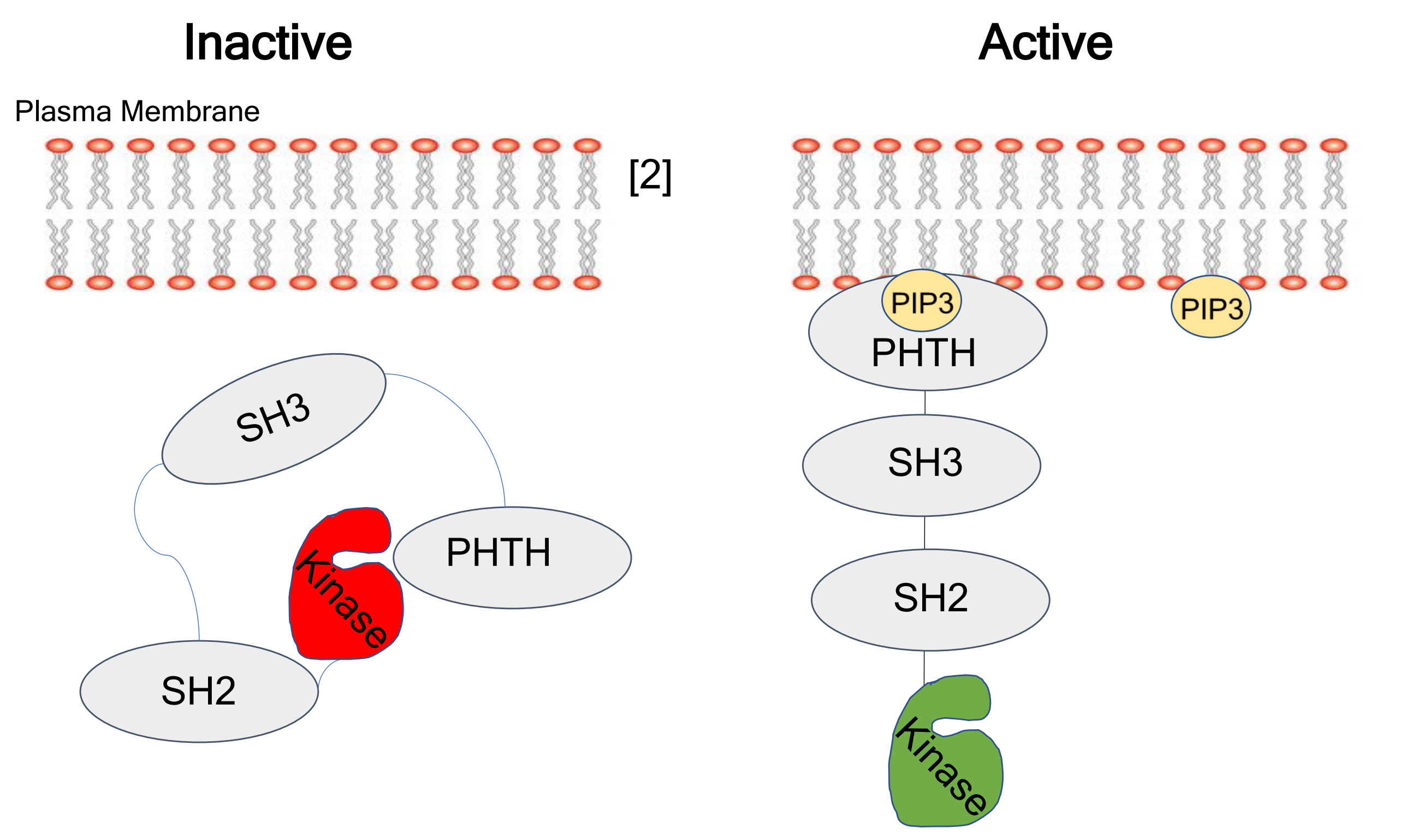
Abstract

Protein kinases play a major role in cell signal transduction. Here we consider the Tec family of non-receptor tyrosine kinases, specifically Tec and interleukin-2-inducible tyrosine kinase (Itk). The Tec kinases comprise of a Pleckstrin homology and Tec homology (PHTH) region, the kinase, Src homology 2 (SH2), and Src homology 3 (SH3) domains. The SH2 and SH3 domains are involved in substrate recognition, and the Kinase domain is the functional part of the protein that transfers a phosphate group from ATP to a substrate. In the inactive form in solution, the PHTH domain is bound to the Kinase domain, blocking access to the active site. Itk is activated when the PHTH domain recognizes and binds to PIP3 lipids in the membrane, thus freeing the Kinase domain and allowing the active site to become accessible.[1] However, there is no crystal structure of the Itk PHTH domain. A crystal structure of the Btk PHTH domain was obtained by mutating Arg 28 to Cys. We carried out a similar mutation R29C to see if we could obtain crystals of the Itk PHTH domain. We cloned the R29C Itk mutation, expressed it in bacteria, and purified the Itk PHTH protein. We then attempted to obtain crystals of the purified Itk PHTH domain.

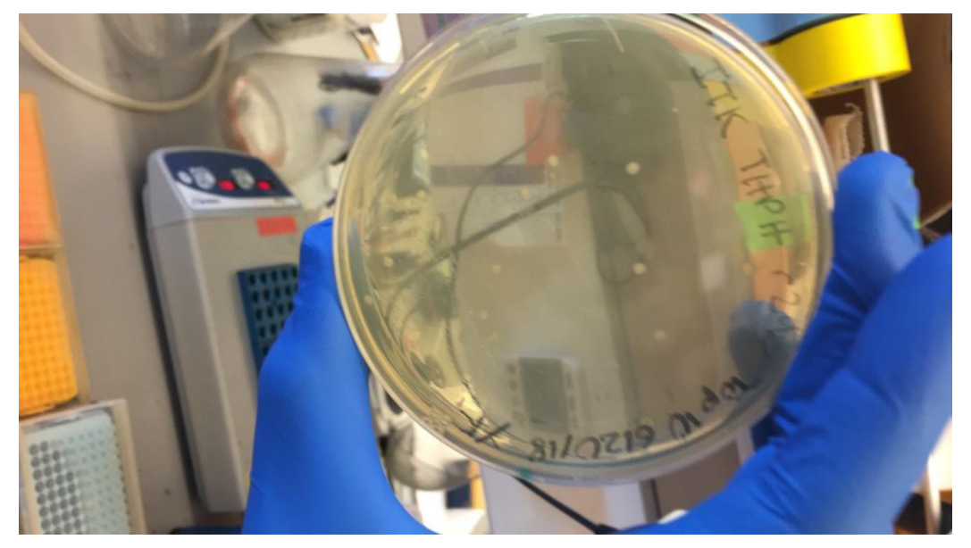
We also studied the dynamics of the PHTH domain by running molecular dynamics simulations of the Tec PHTH domain. The Tec PHTH domain is expected to be structurally similar to the Itk PHTH domain, so we expect the dynamics of this domain to also be similar to that of the Itk PHTH domain.

Activation of Itk Kinase



- ❑ These two models represent how Itk is inactive (left) and active (right)
- ❑ If PIP3 is not present, the PHTH region blocks the active site of the Kinase domain
- ❑ This inactive conformation inhibits the Kinase domain from transferring a phosphate group from ATP to a substrate and Itk cannot provide a cell signaling mechanism
- ❑ In the presence of PIP3 lipid, the PHTH region is able to bind to it and the Kinase domain's active site is free and thus Itk is activated

Methods



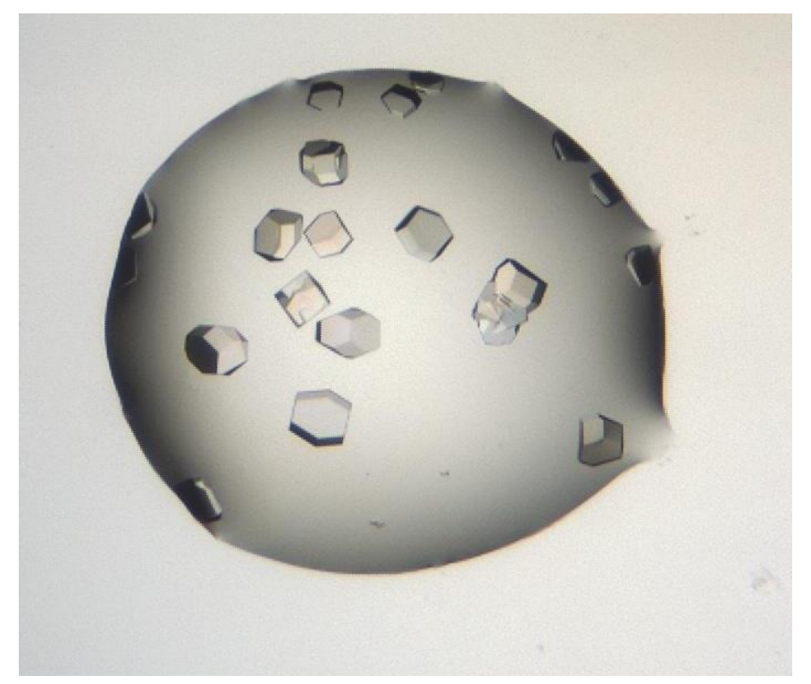
Introduce R29C Mutation

Express in Bacteria for Growth

[3]

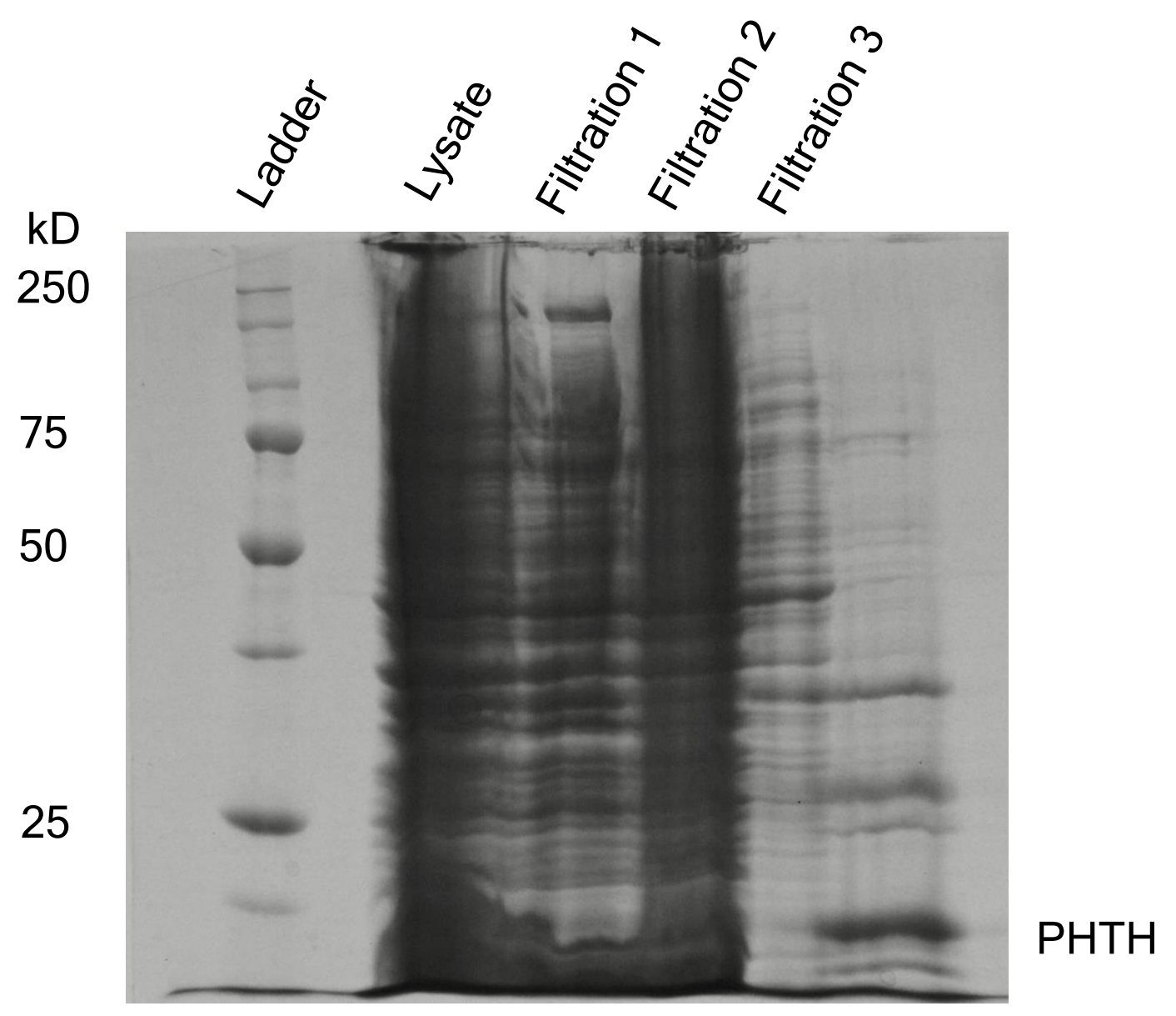
Protein Purification

[4]



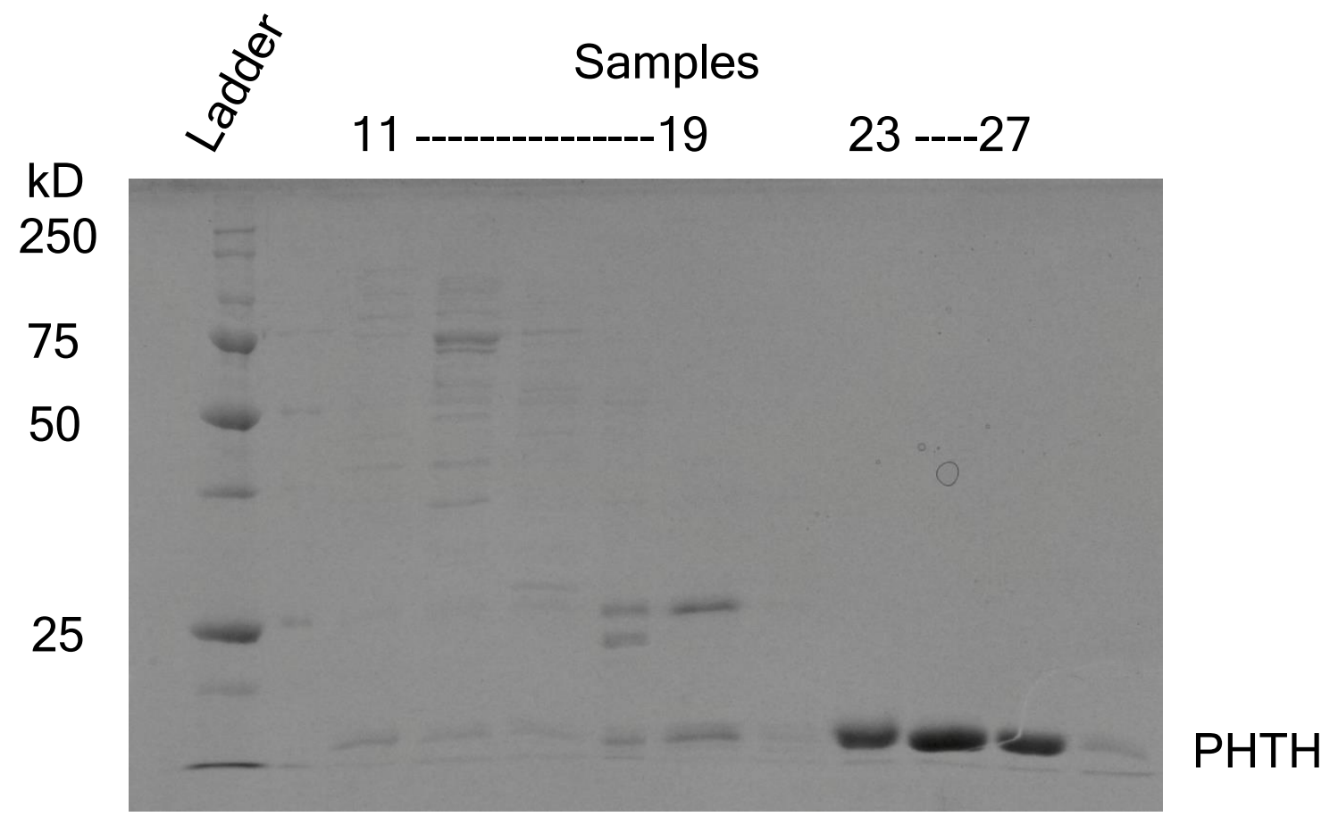
Crystallization

Purification of Itk PHTH Domain



After 1st Nickel Column

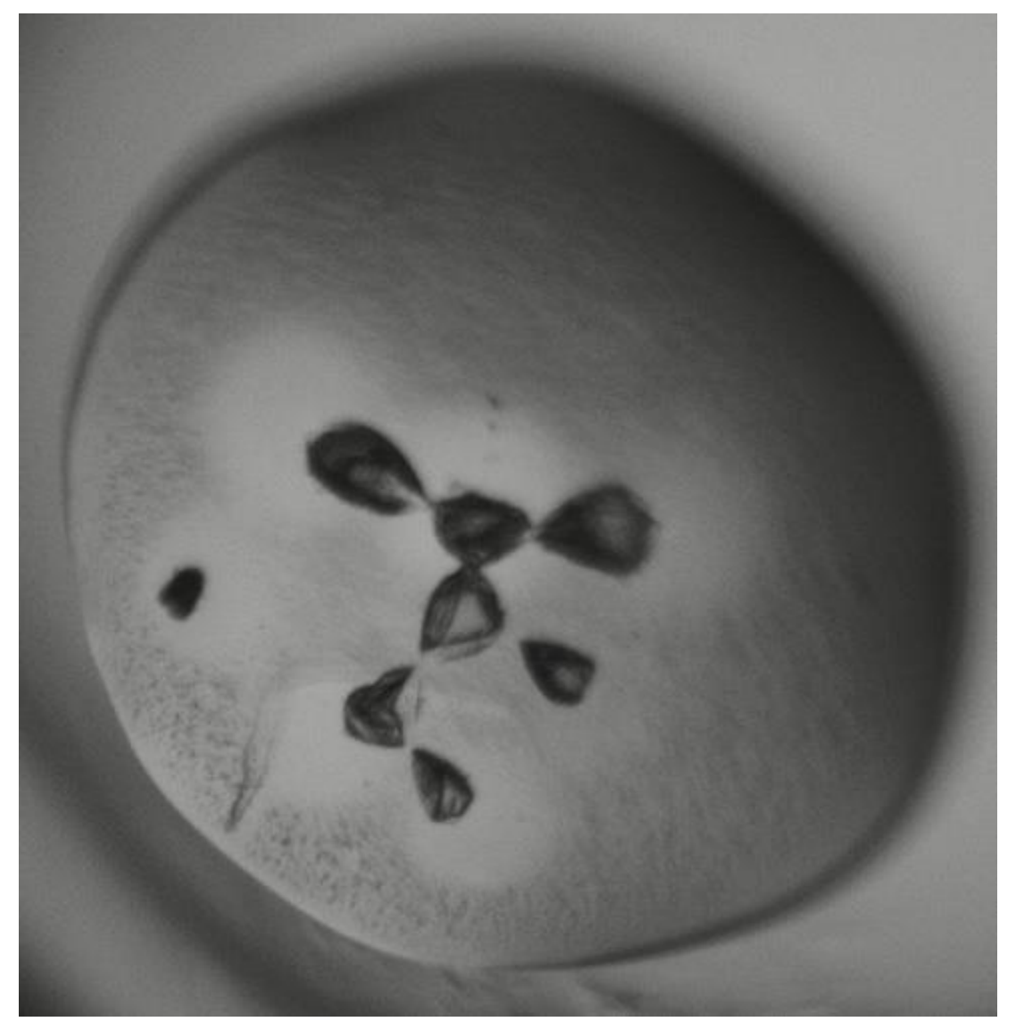
- ❑ This image shows heavy over expression of many proteins as seen by the dark bands of color through all the samples
- ❑ The lane with the PHTH has other bands above it indicating other proteins



After Superdex Column

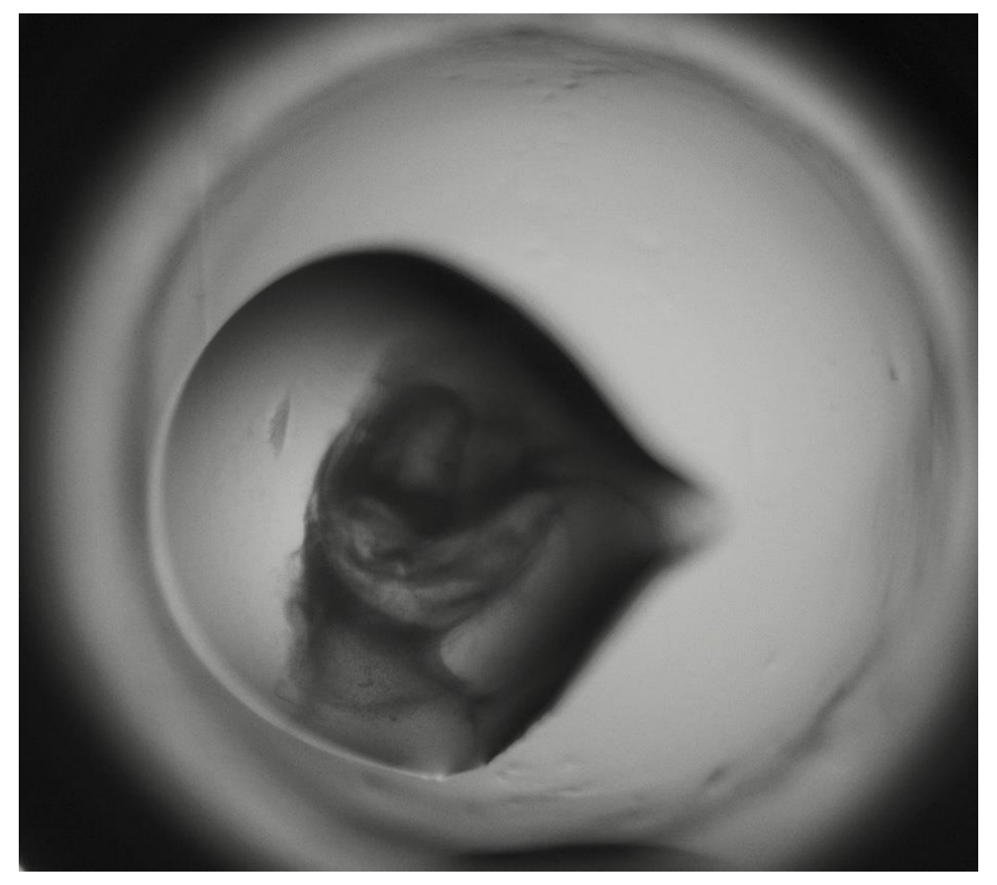
- ❑ A few insignificant bands in samples 11 - 19
- ❑ Samples 23 - 17 only have the PHTH protein expressed as there are no other bands above it

Crystallization of Itk PHTH Domain



Aggregate

- ❑ Unsuccessful in forming crystals
- ❑ Image to the left looks like a crystal with its defined lines and 3D shapes
- ❑ However, it is likely it is an aggregate of salt or an air bubble

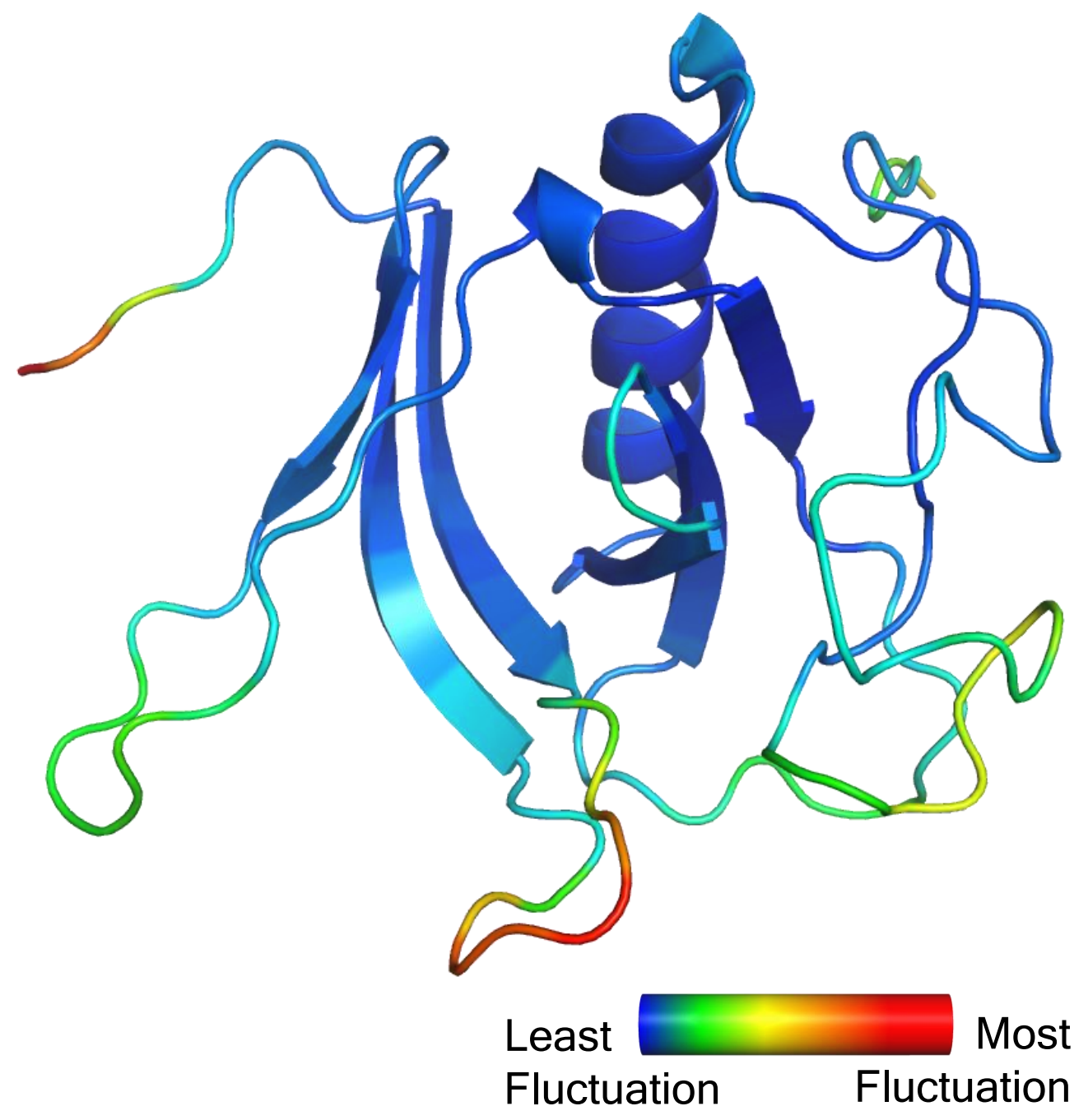


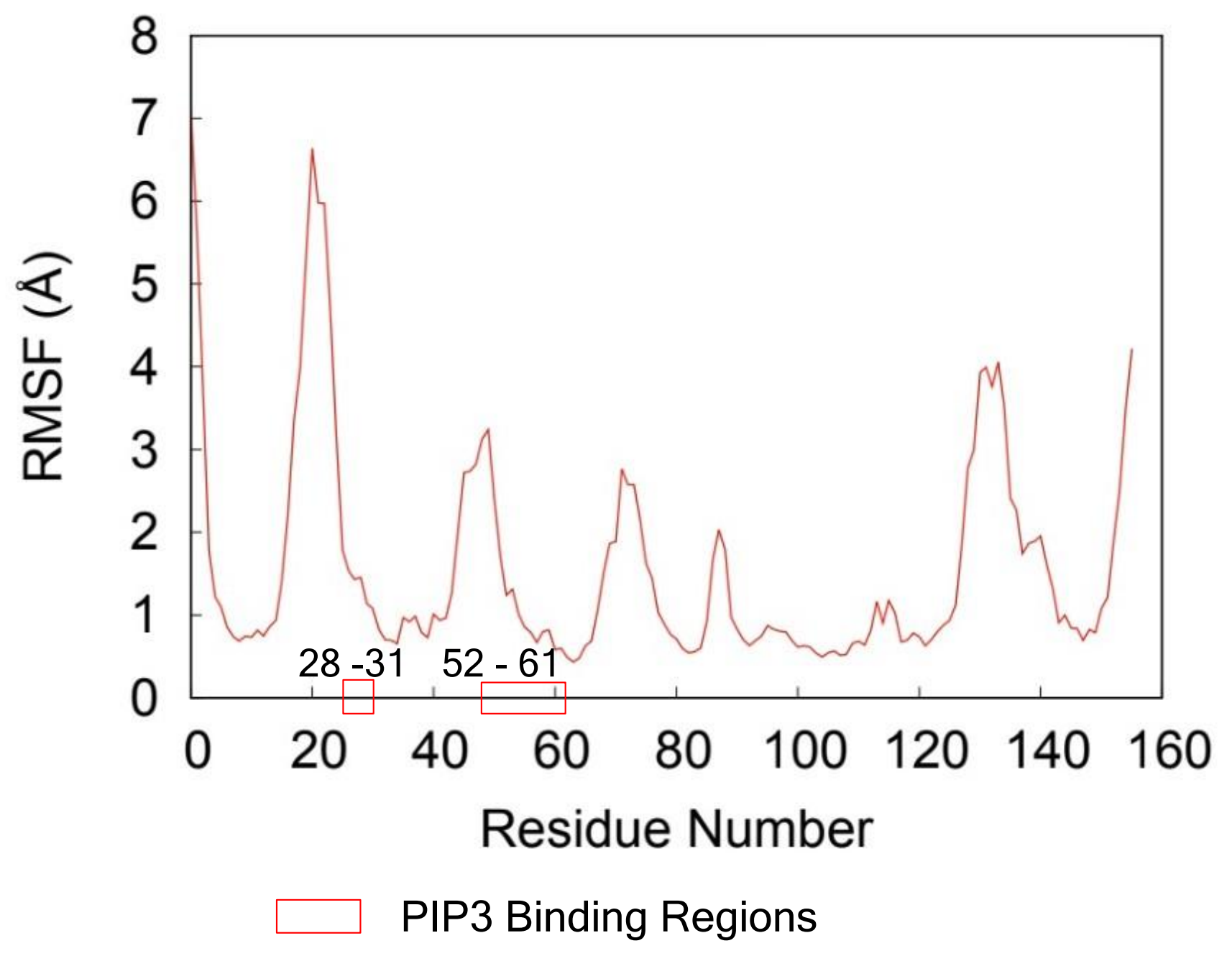
Precipitate of Itk PHTH

- ❑ The dark swirls in the image on the right contain protein
- ❑ However, it precipitated and went beyond the point of solubility

Dynamics of Tec PHTH Domain

[5]





- ❑ The Root Mean Square Fluctuation (RMSF) is a measure of the fluctuation of each residue over the period of the simulation
- ❑ The figure on the left shows the regions of the protein colored by the amount of fluctuations they undergo
- ❑ Blue indicates the least amount of fluctuation and red indicates the most
- ❑ The figure on the right shows the RMSF with respect to the residues of the Tec PHTH domain.
- ❑ PIP3 binding regions show the most amount of fluctuation.

Conclusions

- ❑ Successfully purified the PHTH domain of Itk protein
- ❑ Crystallization yielded aggregates
- ❑ Molecular dynamics simulations reveal that PIP3 binding regions had high fluctuations

Future Work

- ❑ Crystallize Itk
- ❑ From crystals create crystal structure
- ❑ Run simulations of Itk PHTH crystal structure using molecular dynamics and compare to that of other Tec Family members

References

[1] Devkota, Sujan et al. "An Autoinhibitory Role for the Pleckstrin Homology Domain of ITK and Its Interplay with Canonical Phospholipid Recognition." *Biochemistry* 56.23 (2017): 2938-2949. *PMC*. Web. 25 June 2018.
[2] https://study.com/academy/lesson/lipid-bilayer-definition-structure-function.html
[3] https://www.gelifesciences.com/en/mc/shop/chromatography/resins/affinity-tagged-protein/histrap-ff-crude-histidine-tagged-protein-purification-columns-p-00407
[4] http://www.embl-hamburg.de/services/spc/Services-and-resources/protein-crystallization/
[5] [PDB: 2lul]

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