Semi-Synthetic K-Ras 4B Protein : A Platform for Investigating the Biological Behavior of Ras Protein

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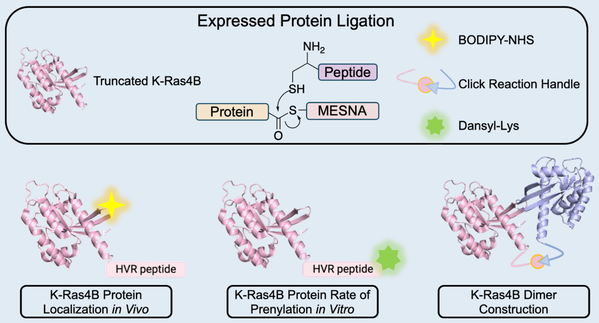
Construction of Semi-synthetic K-Ras 4B Protein and Application



Introduction & Background

As a lipid post-translational modification on proteins, prenylation is a critical process in regulating protein membrane interactions. Ras proteins, a class of small GTPases are prenylated and also involved in several complex signal transduction pathways including the MAPK and PI3K-AKT pathways, which regulated cellular function. Recent research has shown that, up to 30% of human tumors carry mutated Ras genes. Targeting Ras prenylation is a potential strategy to study Ras-related cancer. Farnesyltransferase inhibitors (FTIs), can successfully inhibit cancers driven by H-Ras mutations. However, tumors which primarily contain K-Ras mutations, such as pancreatic adenocarcinoma, have a limited reponse to FTIs. Therefore, new approaches to understand the mechanism of Ras prenylation in cancer cells in crucial.

Prenylated Ras protein have been found to form clusters on the cell membrane. Recent reports highlight some downstream signaling proteins which interact with prenylated Ras protein dimer in the cytoplasm. This indicates the possibility of Ras protein dimerization on the cell membrane and thereby enhancing cellular signal transduction. Computational studies have been used to provide a structural model for the Ras dimer. However, as a membrane protein, it has been challenging to extract the dimer from the cell membrane while maintaining the intact dimer. Performing Ras dimerization *in vitro* under relevant cellular concentrations is challenging. Therefore, developing an in vitro method to form Ras dimers to prove its structure is important.

The aim of this project is to construct a semi-synthetic K-Ras 4B protein using a well-known bio-orthogonal protein engineering method called Expressed Protein Ligation (EPL) to investigate the biological behavior of Ras protein , a critical protein involved in cancer.

References & Acknowledgements

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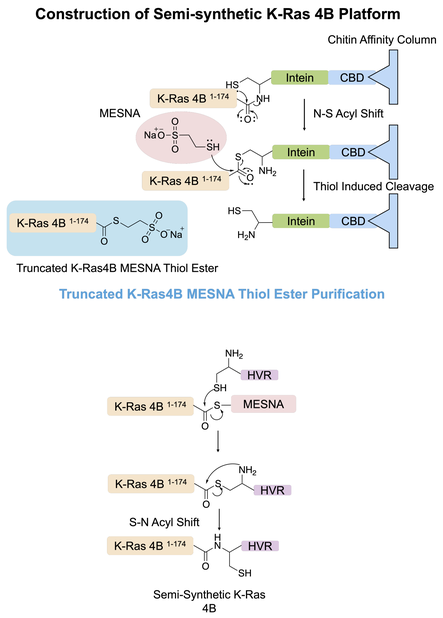
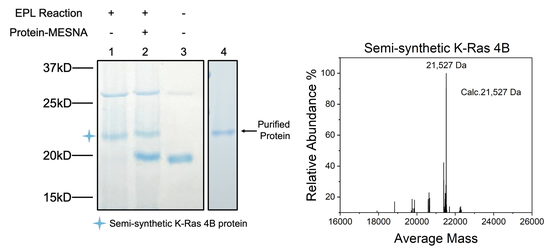
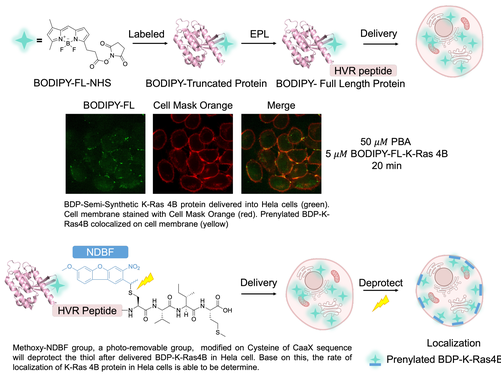
Dr. Yongxiang Chen in Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology at Tsinghua University.

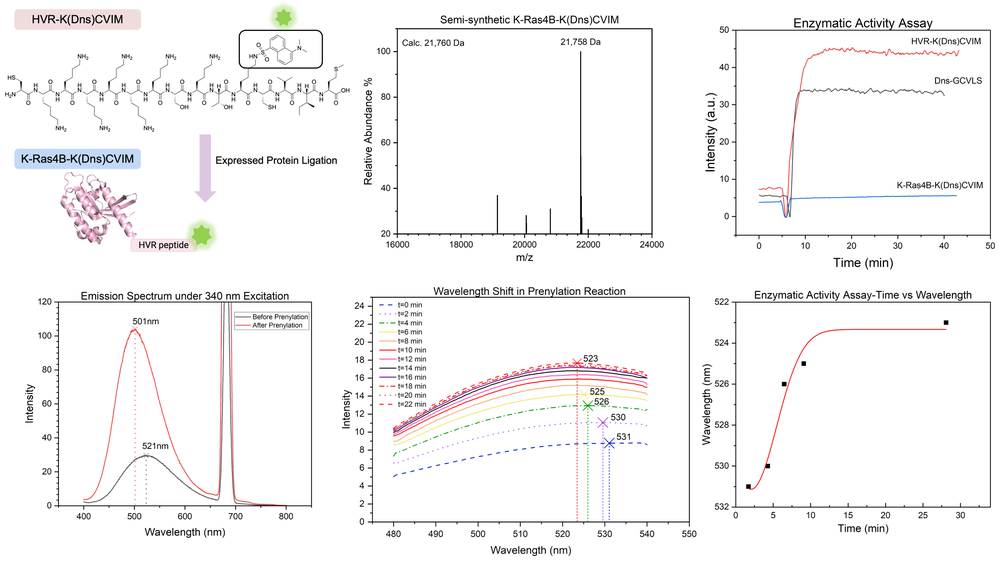
Construction of Semi-synthetic K-Ras 4B Protein and Determination of Rate of Membrane Localization



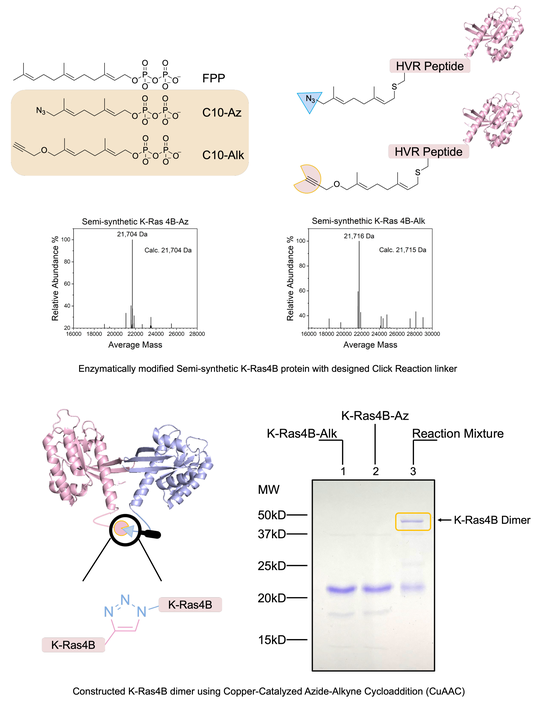




Semi-synthetic K-Ras 4B-K(Dansyl)CVIM: Estimate the Rate of Prenylation *in Vitro* and *in Vivo*



Construction of K-Ras 4B Dimer to Investigate the Impact of Ras Dimerization



Conclusions & Future Directions

- Expressed protein ligation is able to successfully produce BODIPY-Semi-synthetic K-Ras 4B protein which show same ability in localization compared to the native K-Ras 4B protein. Using same method, a photo-removable group NDBF, will be applied on semi-synthetic K-Ras4B. This should help to determine the rate of localization of Ras protein *in Cellulo*.
- A Semi-synthetic K-Ras 4B-K(Dns)CVIM is prepared. That should allow us to measure the rate of prenylation of full length K-Ras4B protein *in vitro* and even *in cellulo*.
- By comparing the rate of localization *in Cellulo* versus the rate *in Vitro*, it should be possible to determine whether other cellular components play a role in controlling Ras prenylation and ultimately localization and function.
- With the formation of Ras dimer established vis SDS-PAGE, future work will focus on structural determination and functional investigation.

Overall, these results highlight Semi-synthetic K-Ras 4B protein as a platform in investigating the biological behavior of Ras protein.

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