Cin8 as an Anti-cancer Drug Target

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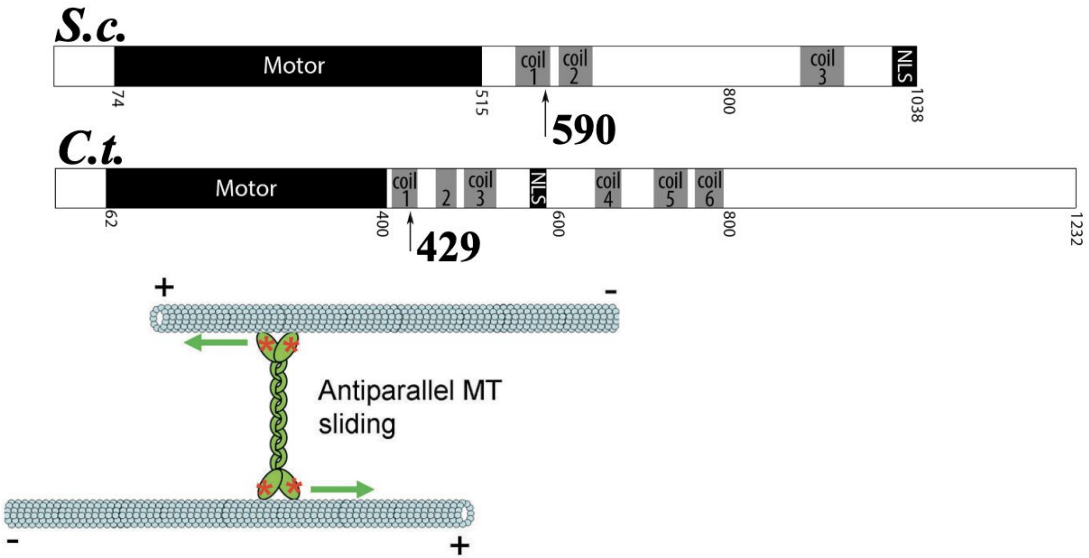
Background

Research: **substantial relationship** between **kinesin-5 protein overexpression** and **cancer development**

Approach: study a kinesin-5 protein in a simpler organism ⟶ enhance understanding of the underlying biochemical and biophysical processes ⟶ highlight desirable characteristics in potential drug

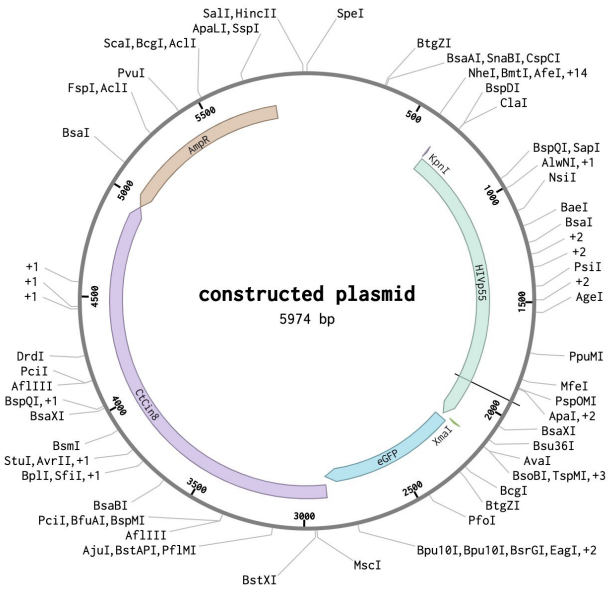


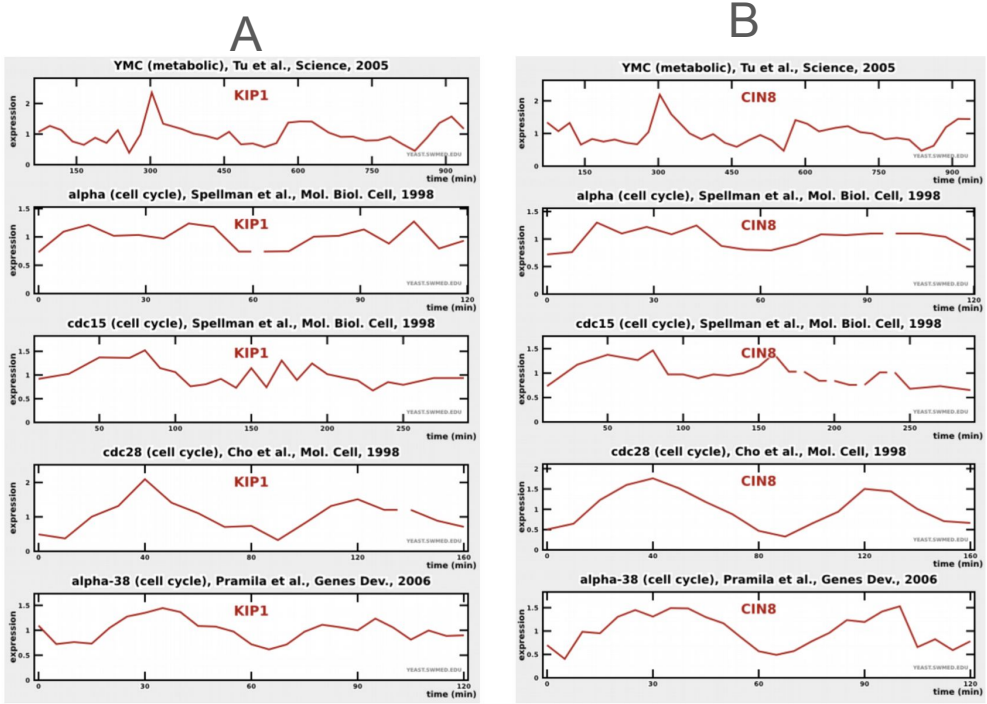
Questions to explore

- *what is Cin8p's mechanism of action?*
- *under what conditions is the protein most active?*
- *how can overall expression be reduced?*
- *what is the significance of the motor domain?*

References: Sheng, J., Li, C., Dong, M., and Jiang, K. (2020) Identification by Comprehensive Bioinformatics Analysis of KIF15 as a Candidate Risk Gene for Triple-Negative Breast Cancer. Cancer Manag. Res. Volume 12, 12337–12348, Christina Thiede, Vladimir Fridman, Adina Gerson-Gurwitz, Larisa Gheber & Christoph F. Schmidt (2012) Regulation of bi-directional movement of single kinesin-5 Cin8 molecules, BioArchitecture, 2:2, 70-74, DOI: 10.4161/bioa.20395

Experimental Setup





Expression of KIP1 and CIN8 during cell cycle.

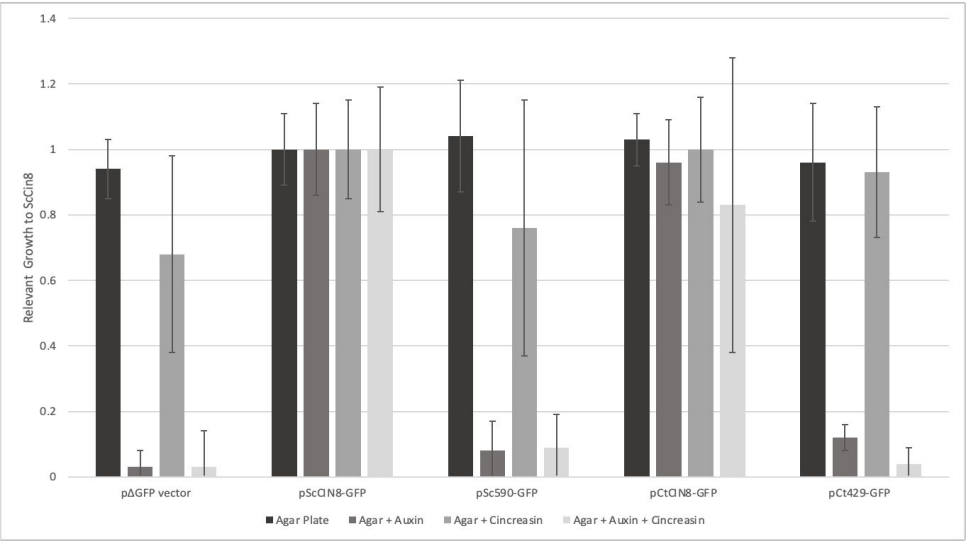
(A) Expression of KIP1 during cell cycle vs. time.

(B) Expression of Cin8 during cell cycle vs. time.

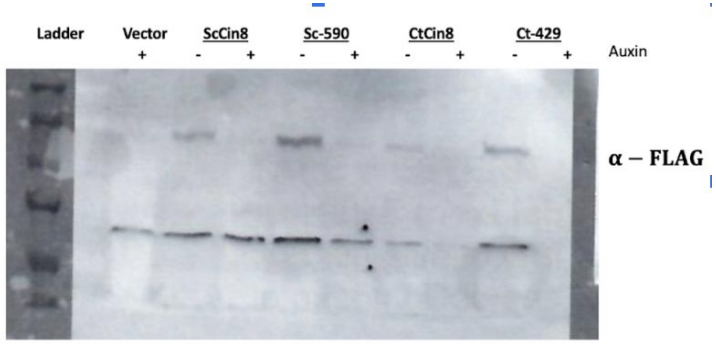
Previously completed (Module 1)

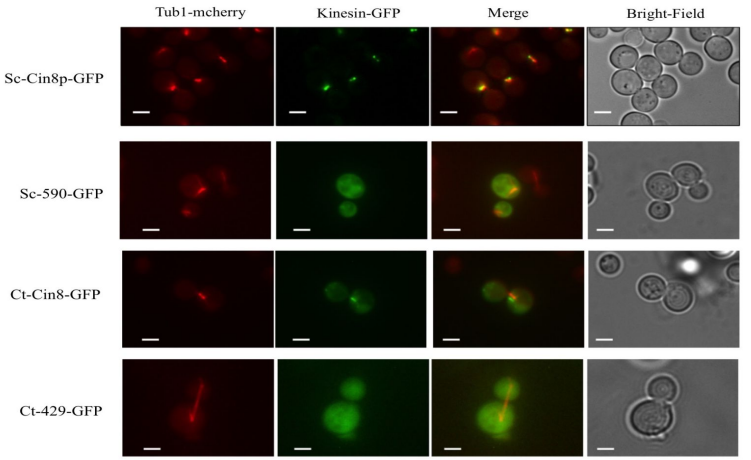
- PCR → ligation → transformation in *E.Coli*, yeast backbones

Rescue in auxin to be tested **in vivo**, localization to Tub-1



Yeast Growth Assay





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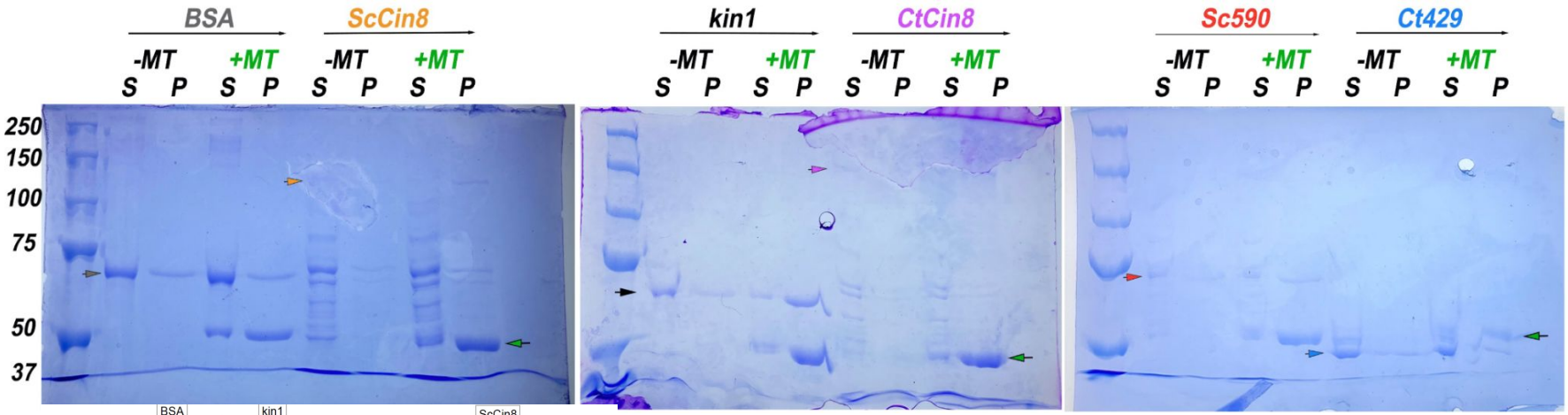
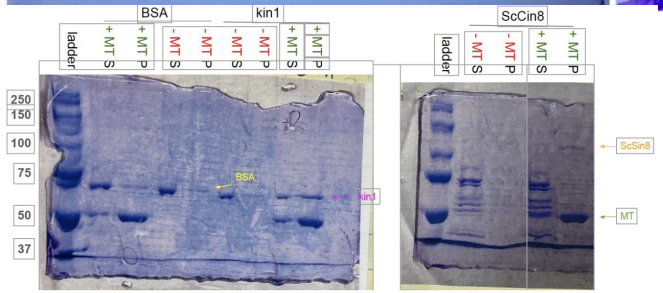
Mechanism of Action involves microtubule binding





Coomassie stains of constructs, separated by soluble and pellet, in the presence and absence of microtubules

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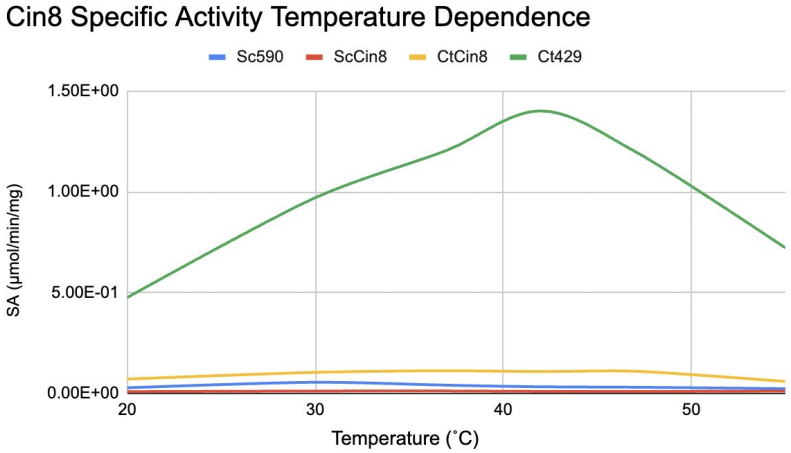
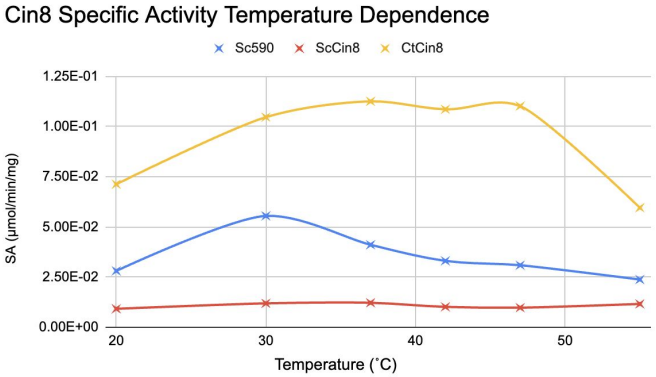
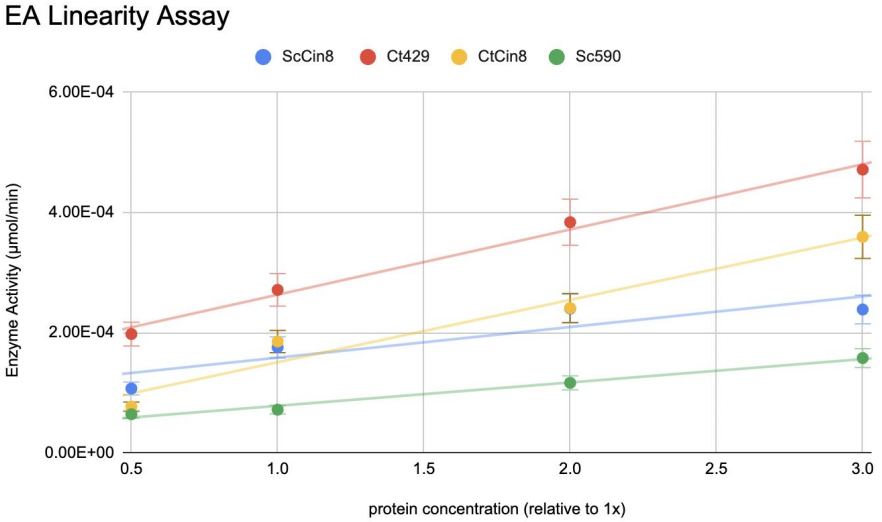
Complex Formation is **temperature-dependent**







Establish 1X [protein] in linear range

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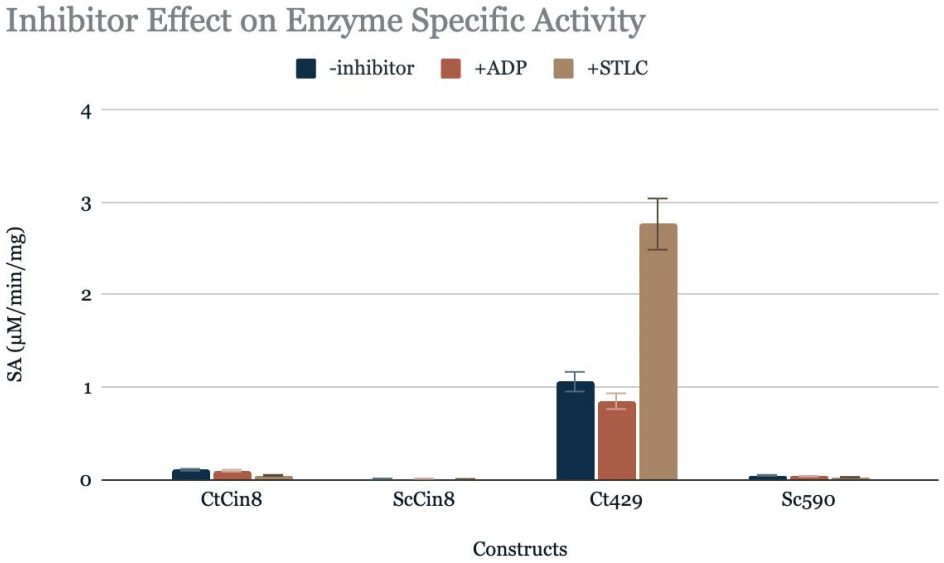
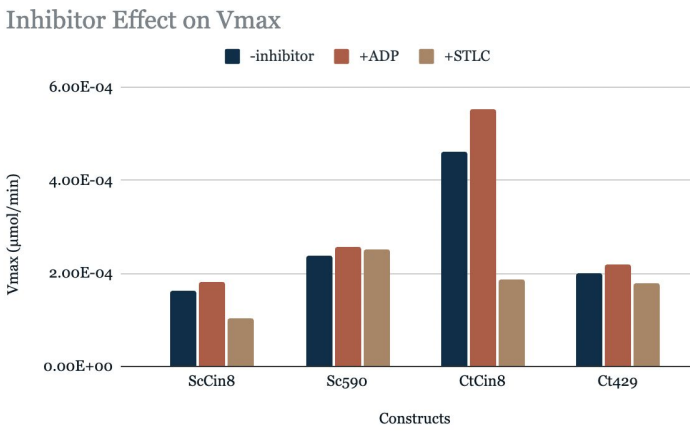
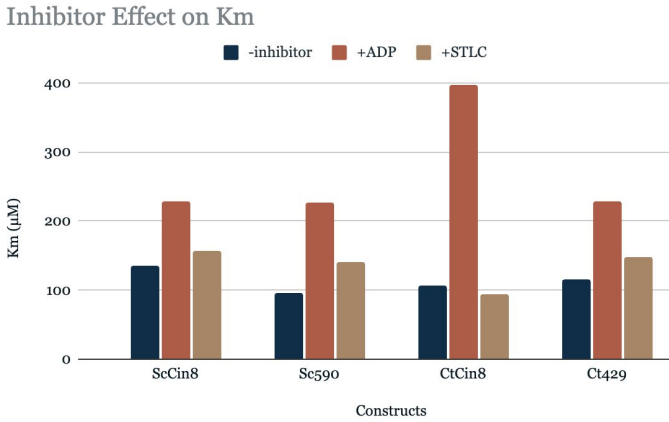
Inhibition effects on activity

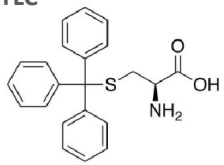






"**STLC** has been identified as an ATP-noncompetitive and reversible inhibitor of human mitotic kinesin Eg5 with potential as an antimitotic chemotherapeutic agent. **STLC** has also been reported as a potent anticancer agent" (Radwan et. al).

STLC



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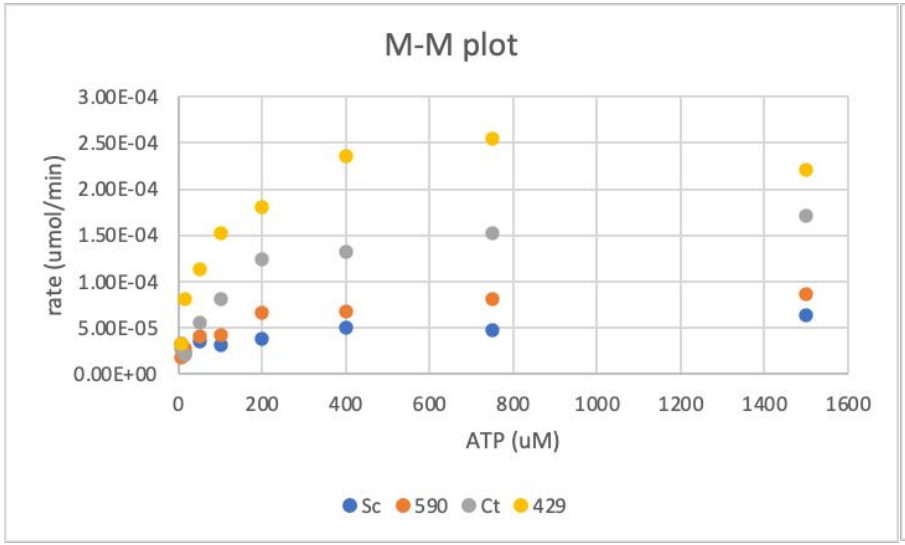
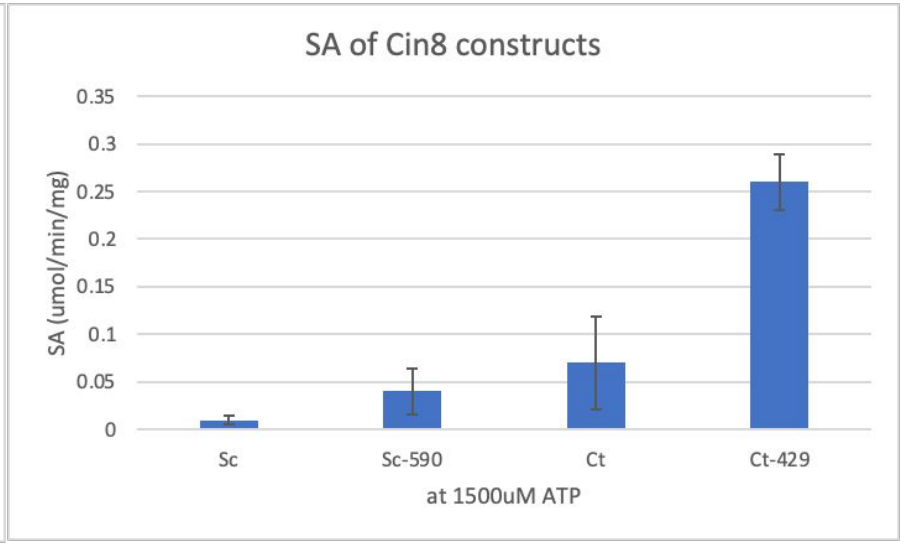
ATP concentration affects enzyme activity





NADH-Coupled Assay

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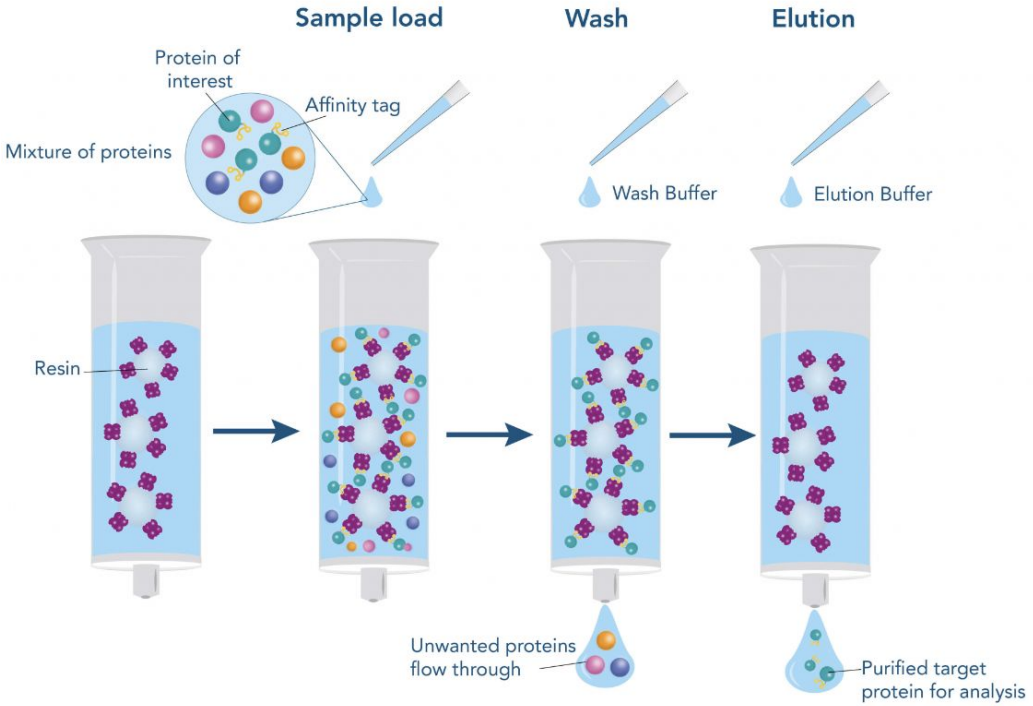
Antibody-Affinity Chromatography as an improvement

Pros

- very high specificity ⟶ high recovered purity
- no histidine tag required; FLAG tag for antigen fusion
- no denaturation conditions

Cons

- expensive, sensitive antibodies, regeneration



Credit: https://www.neuromics.com/protein-affinity-chromatography

Conclusion

Experimental

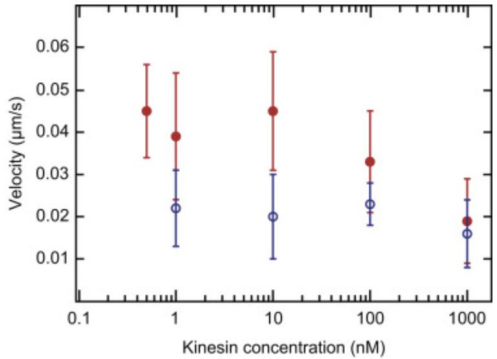
- Comparison between Ct and Sc
- Comparison between truncated and non-truncated version

Refer back to cancer treatment

- Mutations in gene that destabilize the motor

Future direction

1.Microtubule corkscrewing assay (Cy5 labeling)



2. Replicate work with a human kinesin-5 protein.

Pei, Y.-Y., Li, G.-C., Ran, J., and Wei, F.-X. (2017) Kinesin family member 11 contributes to the progression and prognosis of human breast cancer. Oncol. Lett. 14, 6618–6626