# Analysis of Kinesin 1 and Kinesin 5

Ku Kim
Isabelle Le Blanc
MCB170L

# Background

ScCIN8 Kinesin 5 - antiparallel sliding of Microtubules

Specifically required for maintenance of bipolar spindle

Commercial Kinesin 1 possesses role in vesicular transport

# Questions

How does ScCIN8P compare to commercial Kinesin 1 in terms of Specific and Enzymatic activities?

# Cloning and Transformation

(Experiment 1.3 & 1.7)

-Sc-CIN8 fragment was ligated into Bacterial Vector pET24b-HIS

-Plasmids were transformed into a bacterial strain E. coli DH5***α***

-Plated on $AMP^{100}$

-BLR transformation

# Results from Gel Analysis


Digestion with BamHI and HindIII

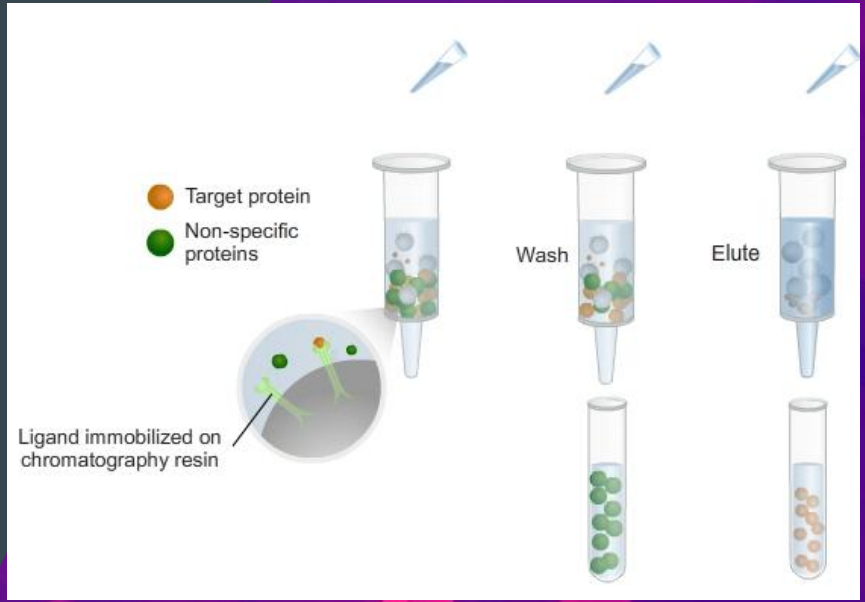
-Lane 5
Why did we choose it?
Why are there three bands?

Did we correctly insert Sc-CIN8 vector into pET24b plasmid?

# Expression & purification (Exp. 3.4-3.5)

-Protein purification of Cin8 using columns

-Assay of flow through, washes, and elution fractions



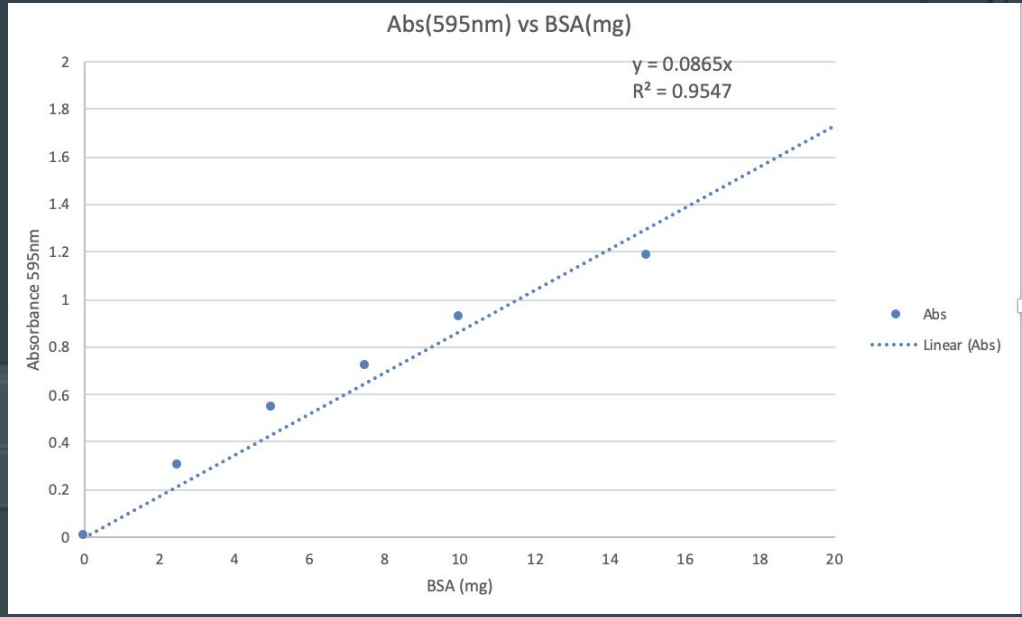
# Bradford Assay (Exp 3.5)

-Finding protein concentration using standard curve

-Pooled(E2+E3+E4)

y=0.0865x

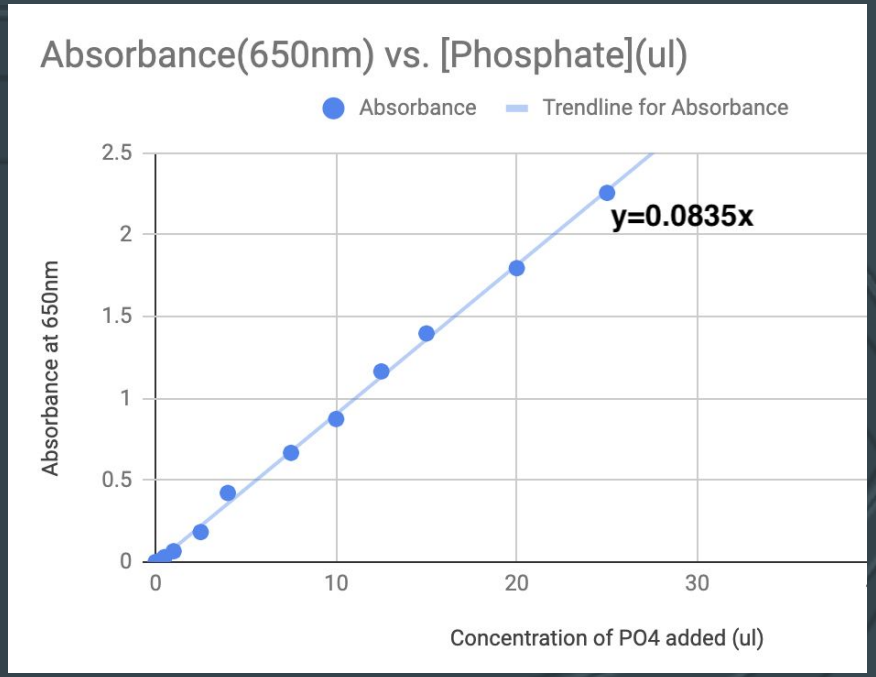
| Fraction | Absorbance | ug protein | Protein concentration ug/ul |
|---|---|---|---|
| FT | 1.27 | 14.7 | 14.7 |
| E1 | .165 | 1.9 | 0.38 |
| E2 | .448 | 5.2 | 1.04 |
| E3 | .246 | 2.8 | 0.57 |
| E4 | .222 | 2.6 | 0.51 |
| E5 | .182 | 2.1 | 0.42 |
| Pool | .215 | 2.5 | 0.50 |



# Malachite Green Assay

-Establishing ε (Experiment 3.2)
Measured ε=83.5mM
Class ε=84.2mM

-Calculating Pi Concentration using A=εCl
Enzymatic Activity umol/min
Specific Activity umol/min/mg



# Comparisons

| Fraction | A650 | [Pi] | pi (umol) | EA (umol/min) | Protein (mg) | SA (umol/min/mg |
|---|---|---|---|---|---|---|
| comm+ATP+MT | 1.289 | 0.0149017341 | 0.000014901734 | 0.000000745086 | 0.000835 | 0.000892319407 |
| E1+ATP+MT | 0.106 | 0.001225433526 | 0.000001225433 | 0.000000061271 | 0.0079154 | 0.000007740818 |
| E2+ATP+MT | 0.32 | 0.003699421965 | 0.000003699421 | 0.000000184971 | 0.0079154 | 0.000023368509 |
| E3+ATP+MT | 0.238 | 0.002751445087 | 0.000002751445 | 0.000000137572 | 0.0079154 | 0.000017380328 |
| E4+ATP+MT | 0.132 | 0.001526011561 | 0.000001526011 | 0.000000076300 | 0.0079154 | 0.000009639510 |
| E5+ATP+MT | 0.089 | 0.001028901734 | 0.000001028901 | 0.000000051445 | 0.0079154 | 0.000006499366 |
| Pool | 0.145 | 0.001676300578 | 0.000001676300 | 0.000000083815 | 0.010415 | 0.000008047530 |
| Pool+ATP | 0.25 | 0.00289017341 | 0.000002890173 | 0.000000144508 | 0.010415 | 0.000013875052 |
| Pool+ATP+MT | 0.2 | 0.002312138728 | 0.000002312138 | 0.000000115606 | 0.010415 | 0.000011100041 |

Enzymatic Activity and Specific Activity of Commercial Kinesin 1

Enzymatic Activity and Specific Activity Pooled of Sc-CIN8 Kinesin 5

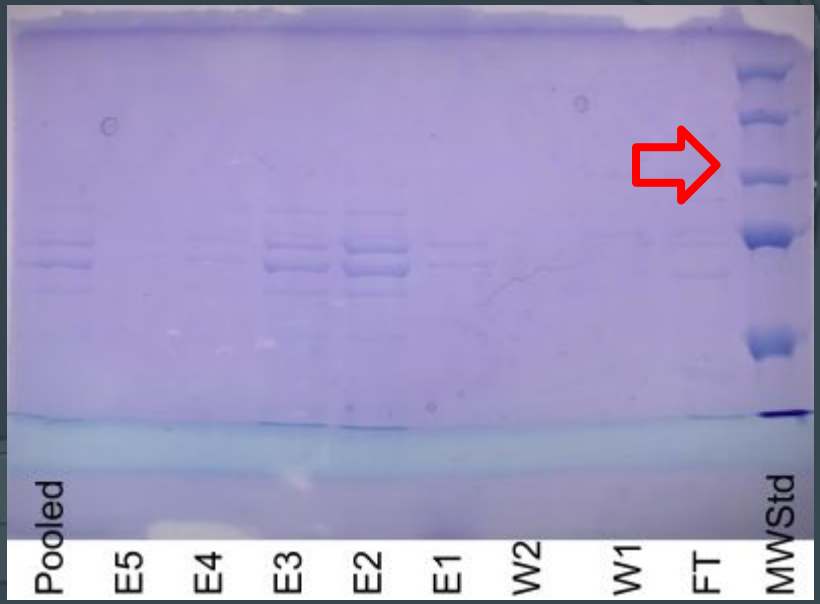
# COOMASSIE Staining

- How successful were we with purification?

-Why are there multiple bands?

-Size of ScCIN8 is expected to be 119kD

-Multiple bands denote other proteins



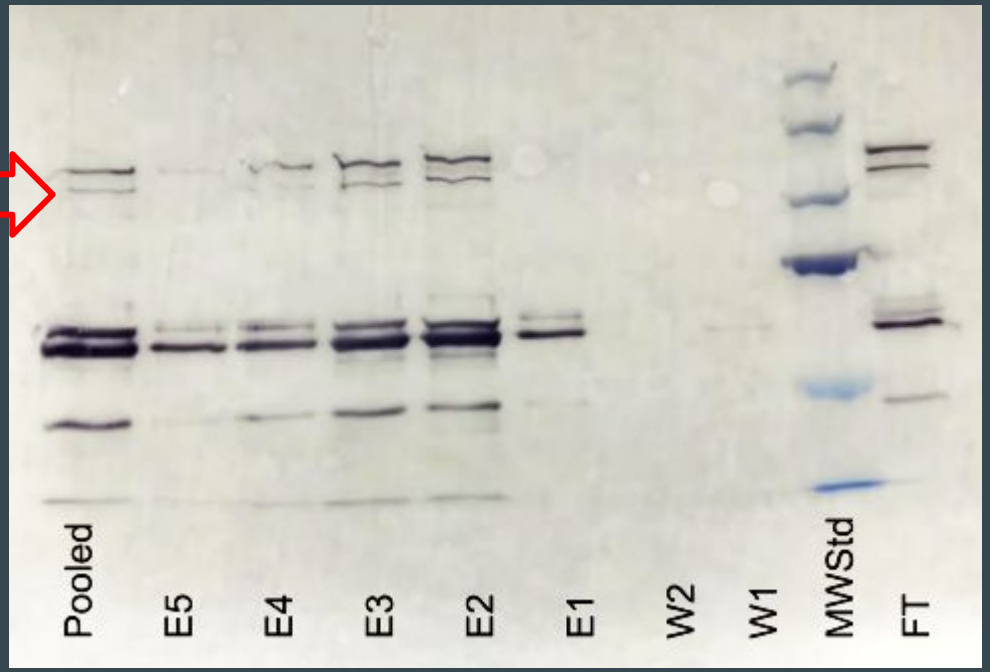
# Western Blot

-Bringing back HIS tag

-Signal Amplification with Antibodies

-Western Blot vs. COOMASSIE

-His Binding of Sheared and Unsheared proteins

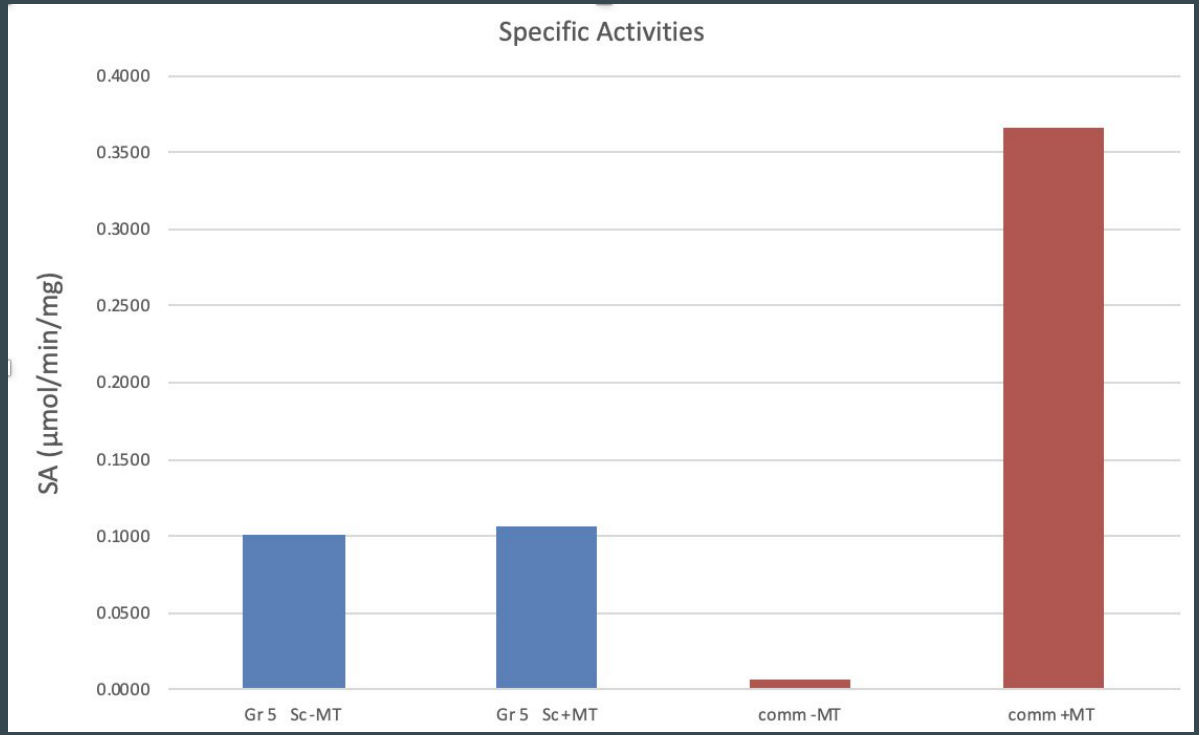


# Comparisons using NADH ATPase Assay

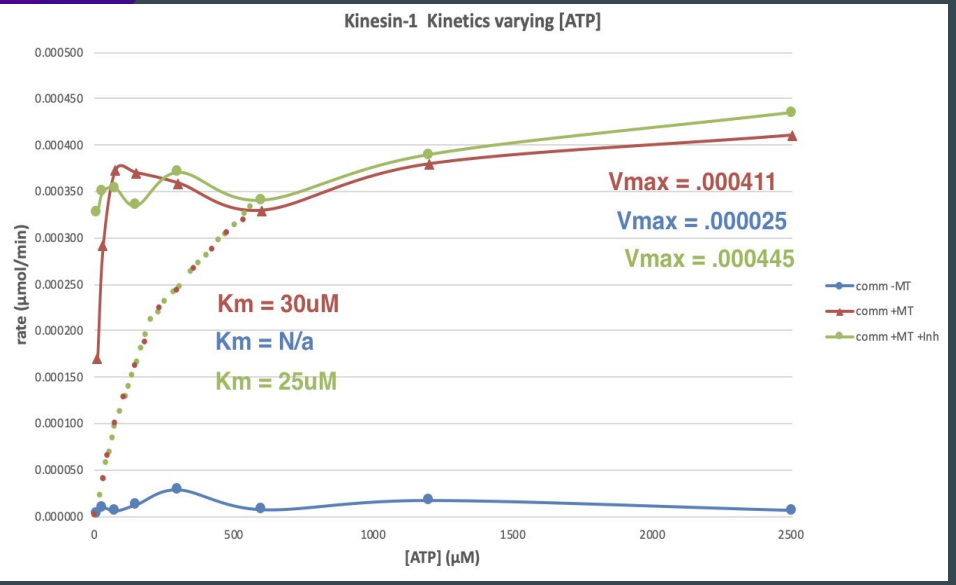
-Kinesin 1 has a much higher specific activity in the presence of Microtubules (.36umol/min/mg) than Kinesin 5 (.11umol/min/mg).

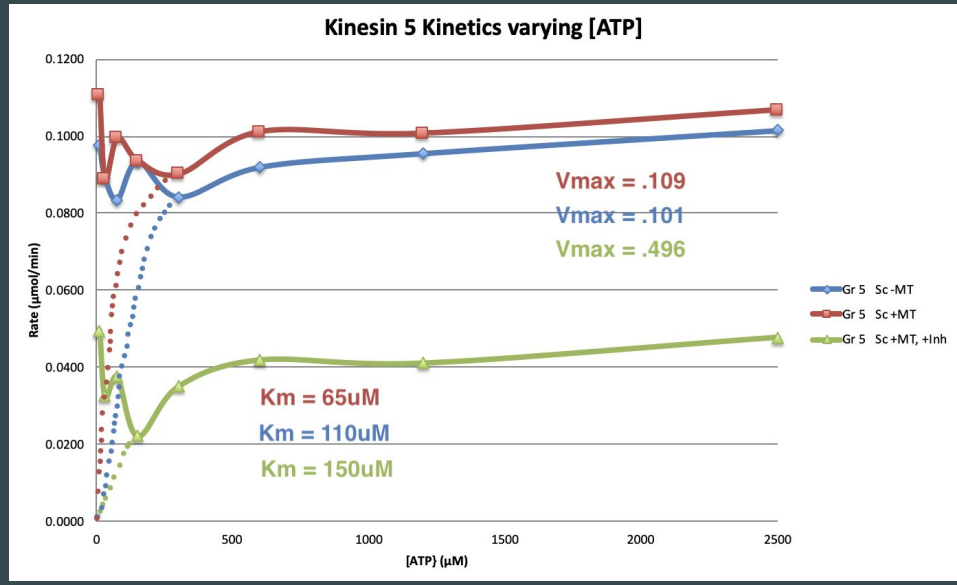
-In the absence of MT, Kinesin 1 has much lower specific activity(.01umol/min/mg) than Kinesin 5(.10umol/min/mg)

-What might this mean?



# Km and Vmax


Kinesin-1 Kinetics varying [ATP]
rate (µmol/min)
0.000500
0.000450
0.000400
0.000350
0.000300
0.000250
0.000200
0.000150
0.000100
0.000050
0.000000
0
500
1000
1500
2000
2500
[ATP] (µM)
Vmax = .000411
Vmax = .000025
Vmax = .000445
Km = 30uM
Km = N/a
Km = 25uM
comm -MT
comm +MT
comm +MT +Inh


Kinesin 5 Kinetics varying [ATP]
Rate (µmol/min)
0.1200
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
0
500
1000
1500
2000
2500
[ATP} (µM)
Vmax = .109
Vmax = .101
Vmax = .496
Km = 65uM
Km = 110uM
Km = 150uM
Gr 5 Sc -MT
Gr 5 Sc +MT
Gr 5 Sc +MT, +Inh

# Conclusion

Specific activity and Enzymatic activity

Areas of Improvement

Future Directions?
-use a different inhibitor and see how Kinesin 5 and Kinesin 1 compares in
    -ADP not an effective inhibitor, we can possibly use “BI8” by Kozielski group which binds a novel pocket of Eg5.
    -Eg5 inhibitors target Loop 5 of Kinesin 5

# References

Waitzman, J. S., & Rice, S. E. (2014). Mechanism and regulation of kinesin-5, an essential motor for the mitotic spindle. *Biology of the cell*, *106*(1), 1–12. doi:10.1111/boc.201300054

Overview of Affinity Purification. (n.d.). Retrieved from https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-affinity-purification.html