

Characterizing Selective Binding of Cul3 Using Chimeric Analysis



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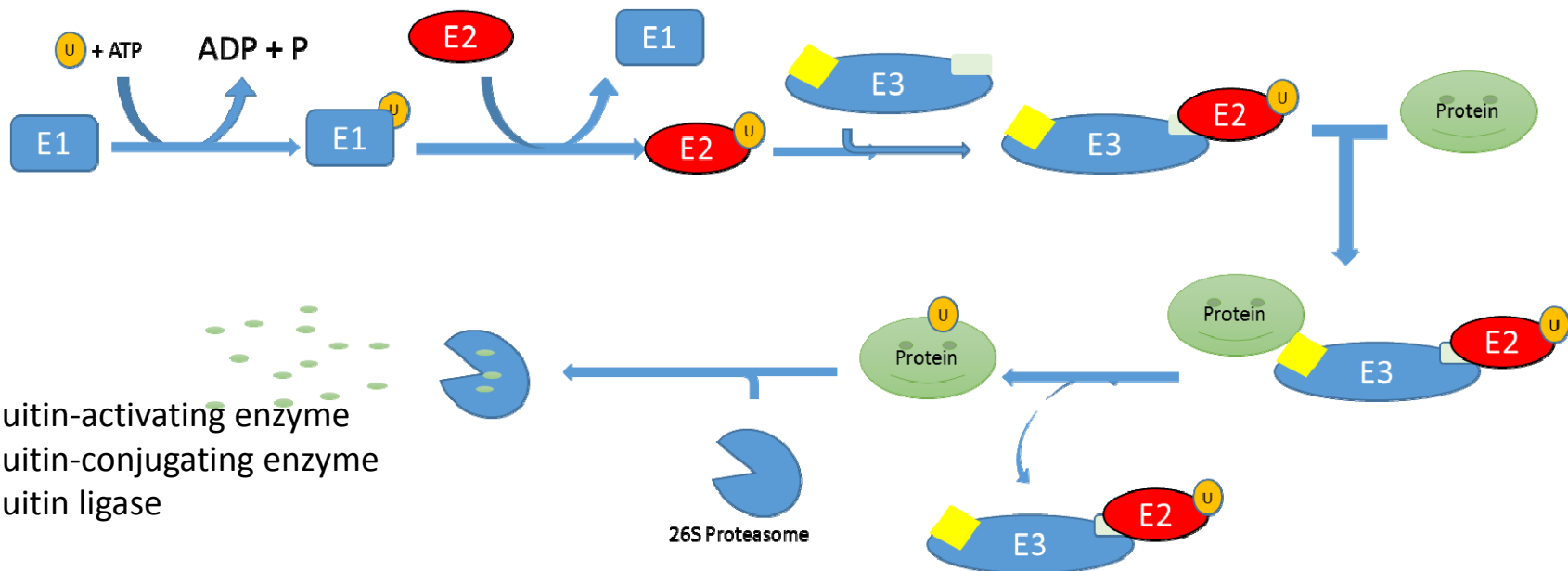


Lecture overview

- General introduction to the system
- Our question and how we plan to answer it
- Results
- Relevance

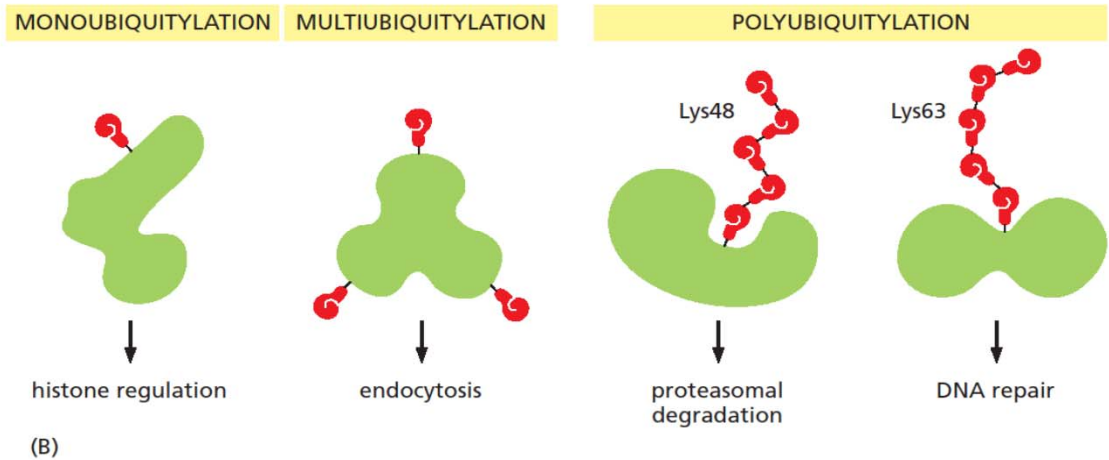
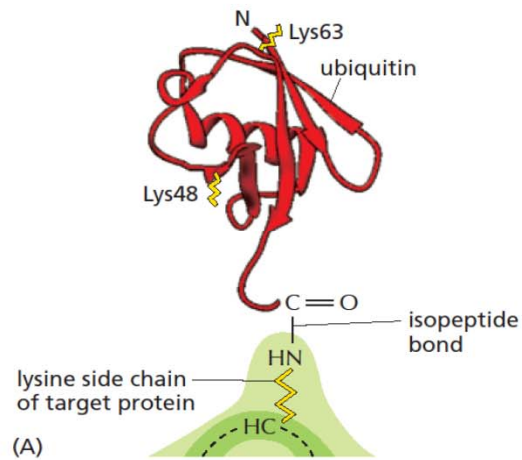
Ubiquitin system

- Ubiquitination is a process which tags proteins for proteolytic and non-proteolytic fates
 - Relies on a concert of enzymes



Fate after ubiquitin

- The E2 is responsible for specificity of ubiquitination



Cul3

- Cul3 is part of a family of RING-type E3s called Cullins
- Cullins are molecular scaffolds which form a structural backbone
- There are 7 known Cullins in the human system
 - Cul1, Cul2, Cul3, Cul4a, Cul4b, Cul5, & Cul7
 - Crystallography and sequence data has shown Cullins share a highly conserved three helix structure on their N-terminus.
 - The C-termini is also highly conserved in sequence and function

Cullin Complex

- All Cullins have 3 major sections

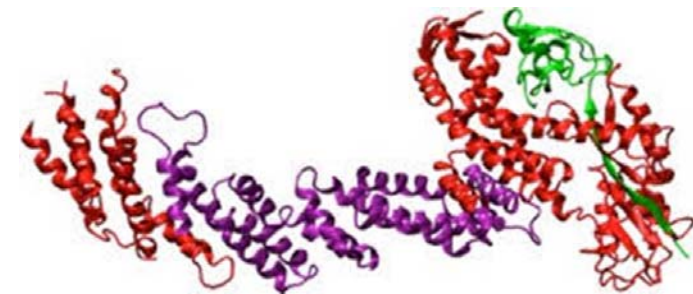
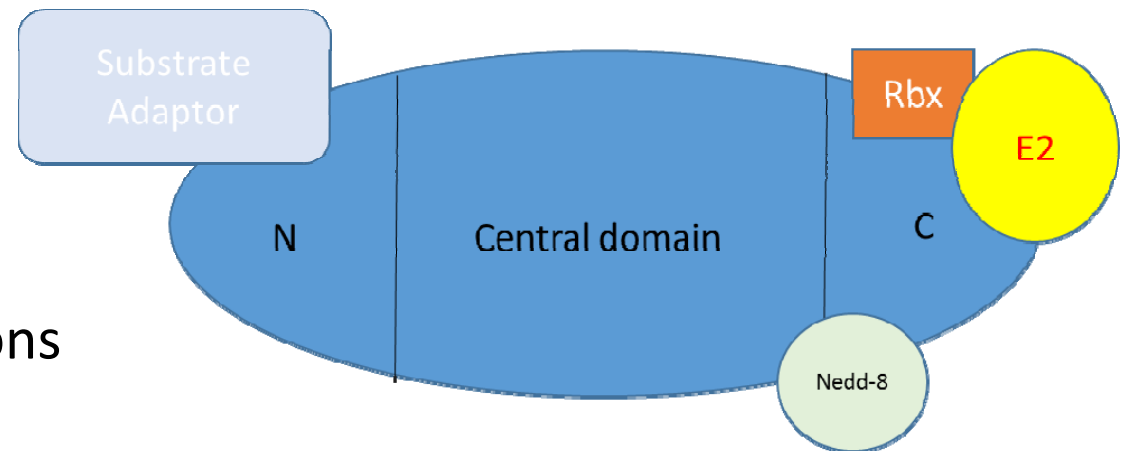
- Carboxyl terminus (CTD)

- Binds activation peptide Neural-precursor-cell-Expressed Developmentally Down-regulated 8 (NEDD8)
 - RING-box protein 1 (Rbx1)
 - Recruits E2
 - E2 (ubiquitin-conjugating enzyme)
 - Binds ubiquitin to substrates

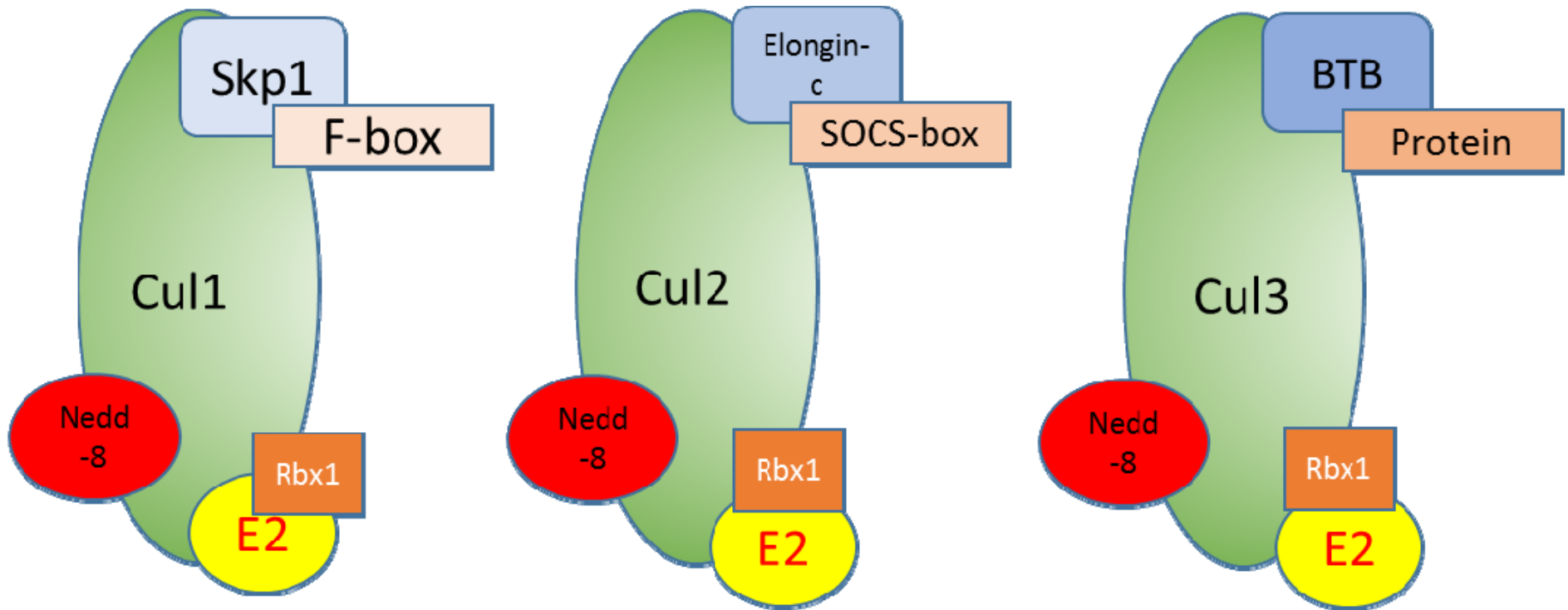
- *A stalk-like central domain*

- N-terminus (NTD)

- Binds substrate adapter which recruits target substrates



Cullin complexes



The Question

The Cullins are so similar yet bind different proteins and target different substrates for specific fates.

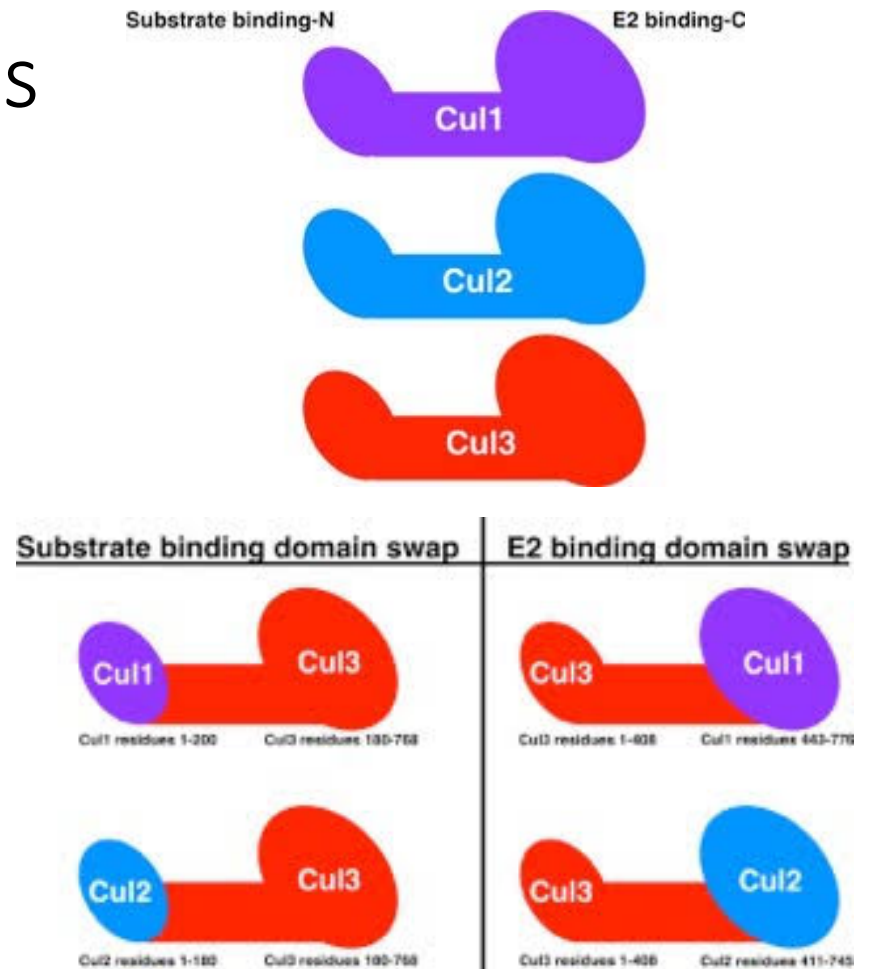
Do both the substrate binding and the E2 binding domains contribute to substrate specificity?

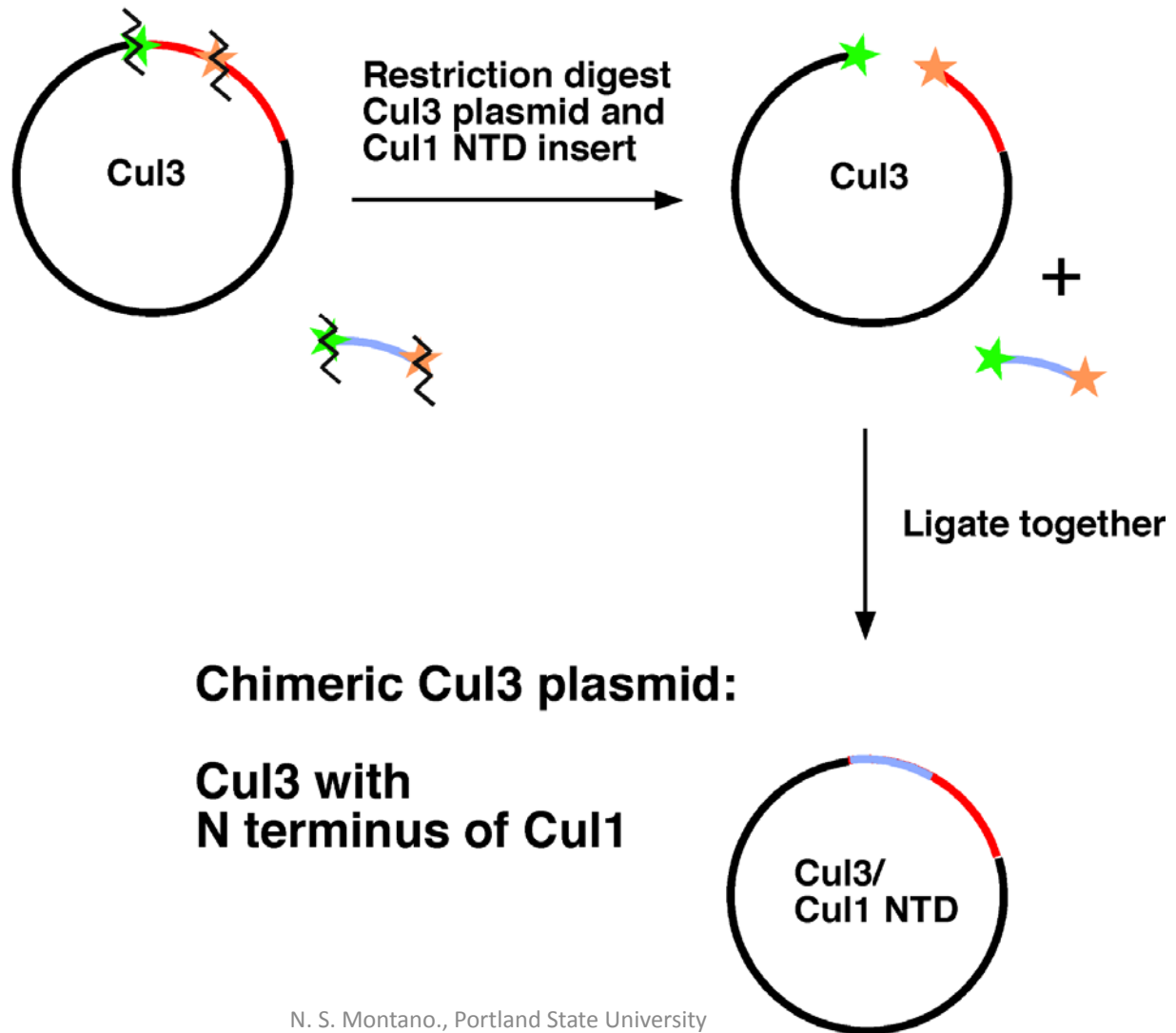
How do we test if one region has an affect on functional specificity?

By switching the C-termini and N-termini of different Cullins with its siblings

Creating Chimeric Mutants

- In Greek mythology, the **chimera** was a fire-breathing monster that combined the parts of a goat, a lion and a serpent.
- Using Cul3, Cul2, and Cul1 plasmids we swapped the NTD and CTD

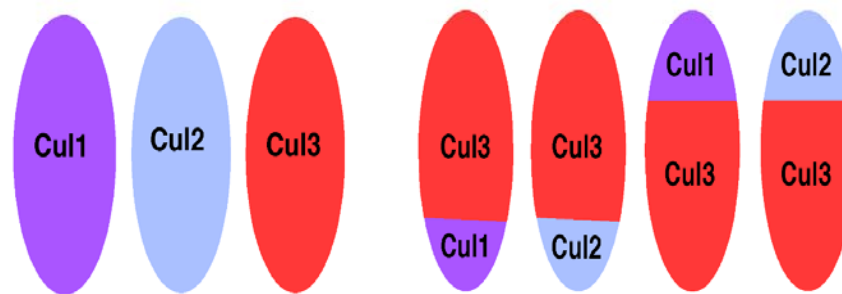
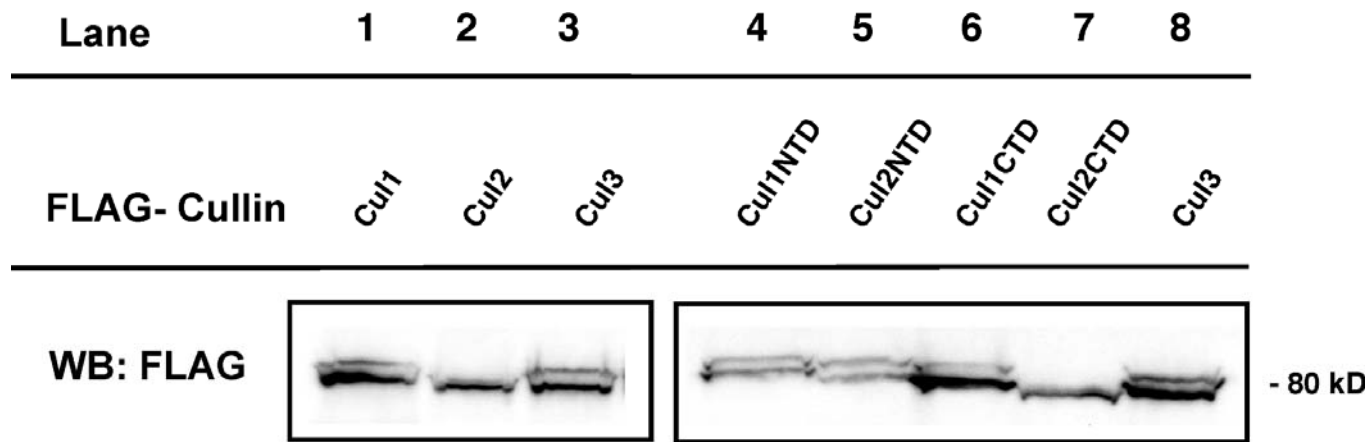




Chimeric Cul3 plasmid:

**Cul3 with
N terminus of Cul1**

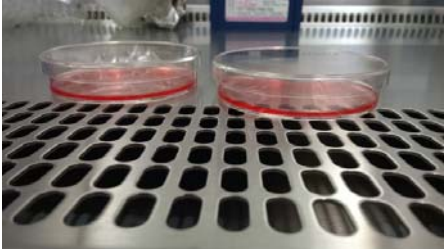
Do the chimeras express?



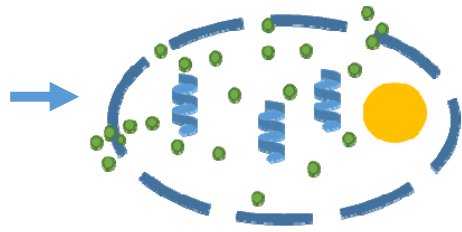
How will we qualify their binding preference?

- Immunoprecipitation and immunoblotting
 - The chimeras and the binding partners are co-transfected in cells
 - After harvesting the cells the lysate are placed with antibody beads isolating the chimera and what it is bound to
 - Next, we run the proteins on an acrylamide gel separating it out by size and transferring it to a membrane which binds proteins.
 - The membrane is soaked in the second antibody specific to the binding partner
 - Which we then attach another molecule for imaging

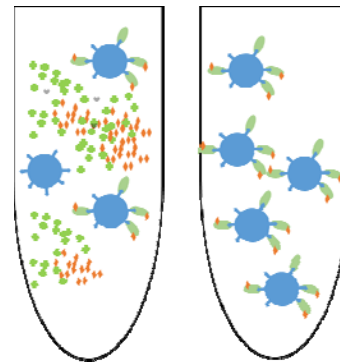
DNA + Cells



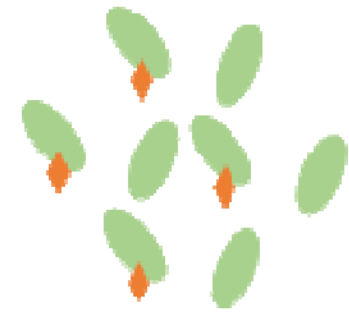
Harvest Cell



Remove Noise

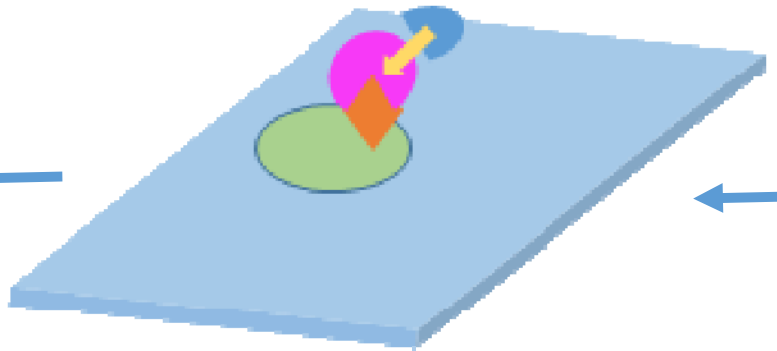


Isolate

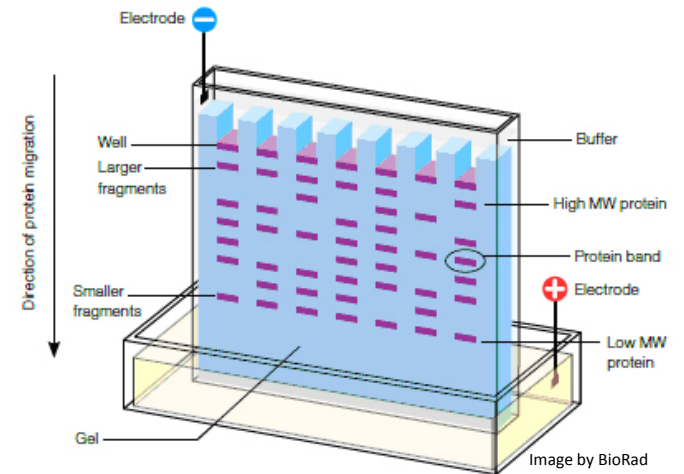
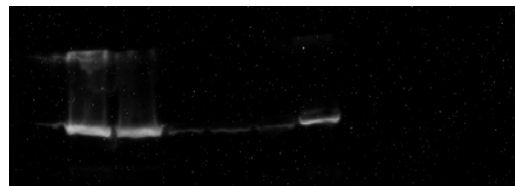


Run on Gel

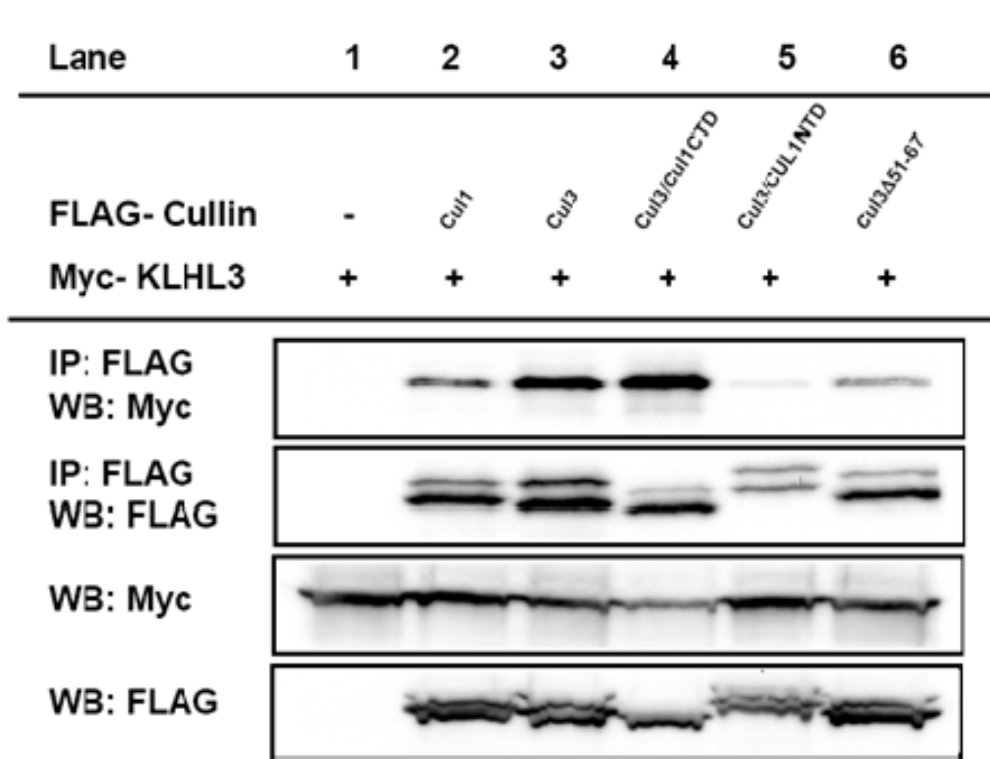
X-fer To Membrane and Add Photo Agent



Image

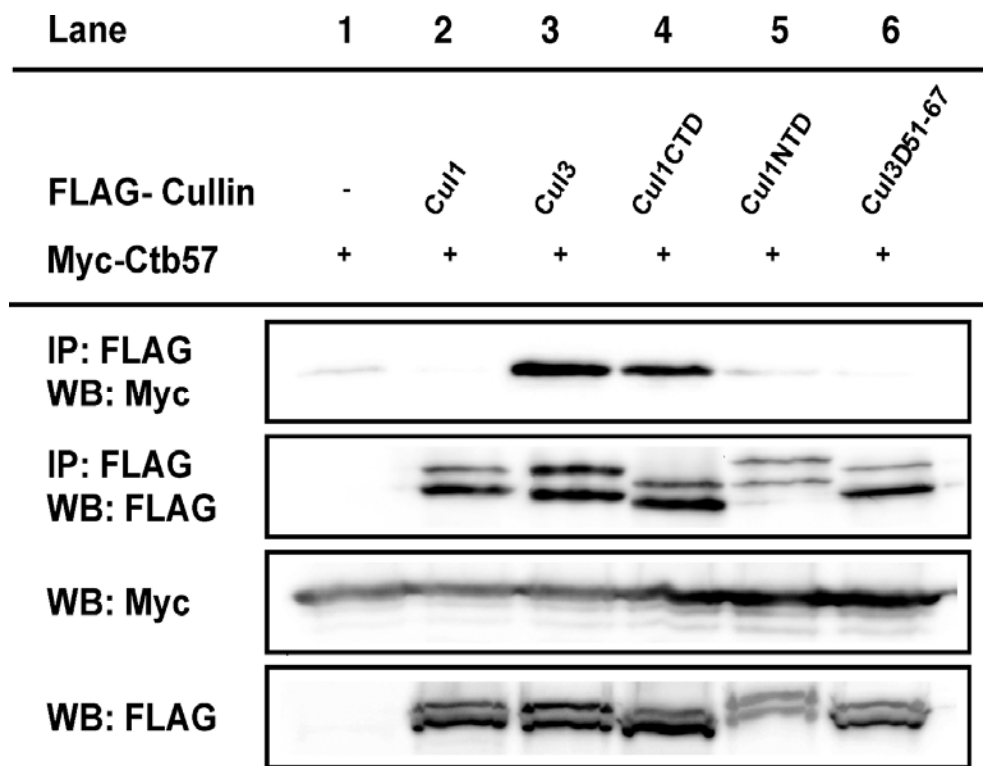


Results



- KLHL3 is a binding partner of Cul3 in normal conditions
 - BTB domain protein
- Results
 - Robust binding is only seen in WT Cul3 and Cul3/Cul1CTD

Results



- Ctb57 is a BTB containing protein
- Results
 - Binding in Cul3 WT as expected
 - Binding was also seen in the Cul3
- Cul3/Cul1 CTD binds similar to that of the Cul3 WT

Direction and continuation

- Binding of more proteins is needed to validate chimeras
- What E2 will be recruited
- Will the chimeras ubiquitinate target substrates?
- Will the chimeras function in the cell in the same manor as the wild type?

Why does this protein matter?

- Cul3 has been shown to be vital to development
 - Deletion of gene causes death in development
 - Cell cycle arrest
- Cul3 regulation has been connected to multiple diseases
 - Familial Hyperkalemic Hypertension (FHHT)
 - Hypertension caused my Cul3-Klhl3 mutant (confirmed, studied by my lab and OHSU)
 - Multiple forms of cancer

Special Thanks

A close-up photograph of a white mouse with long whiskers, sitting and holding a small brown teddy bear in its paws. The mouse is looking directly at the camera. The background is a plain, light-colored surface.

- Mentor: Dr. Singer
- Lab Mates: Brittney and Shaun
- REU Program: Dr. Jiao and Dr. Sanchez
- REU Babysitter: Ellie
- Funding: National Science Foundation
- You people

Questions?



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