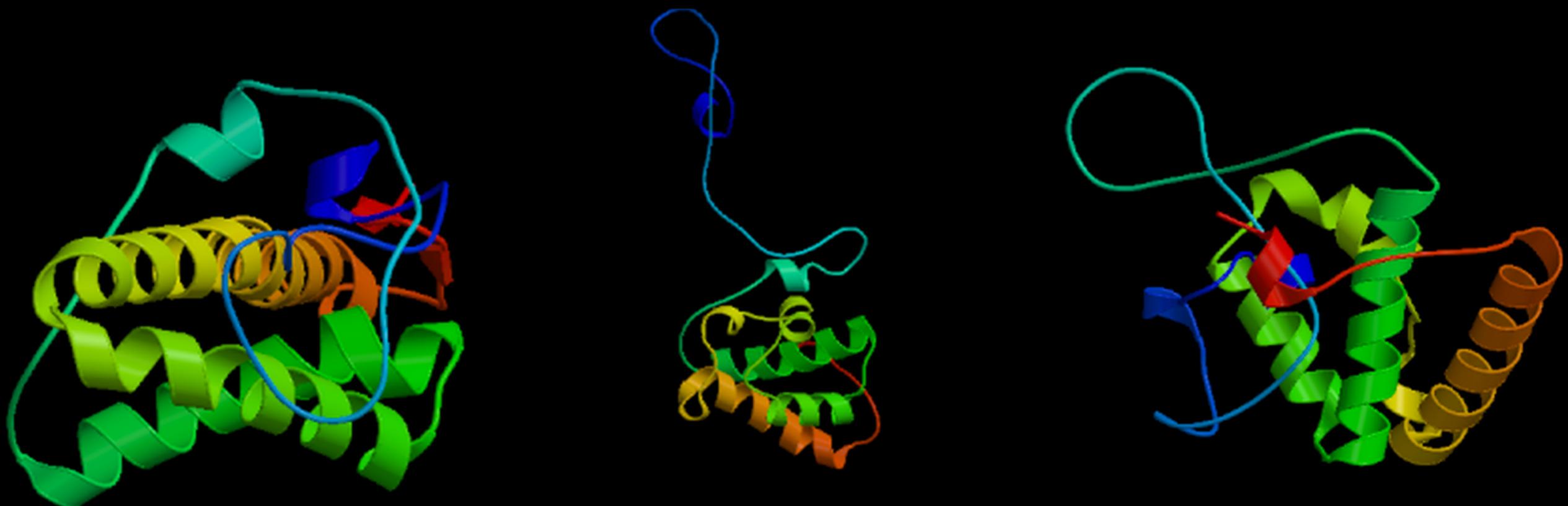


HAstV1 - Capsid Acidic Domain

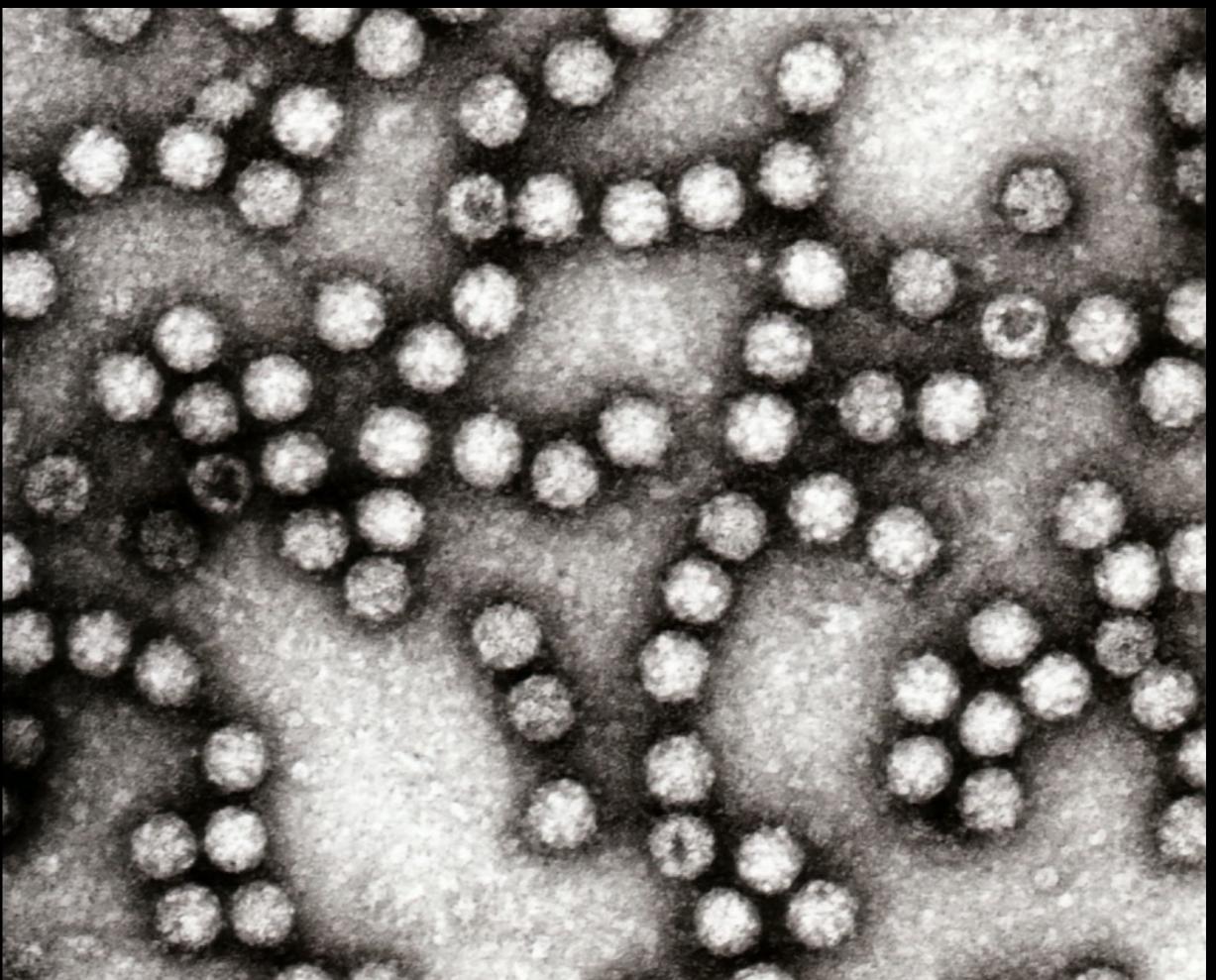


HAstV1 Epidemiology

- Causes gastrointestinal symptoms
- Seasonality
- Variants in many mammals/birds

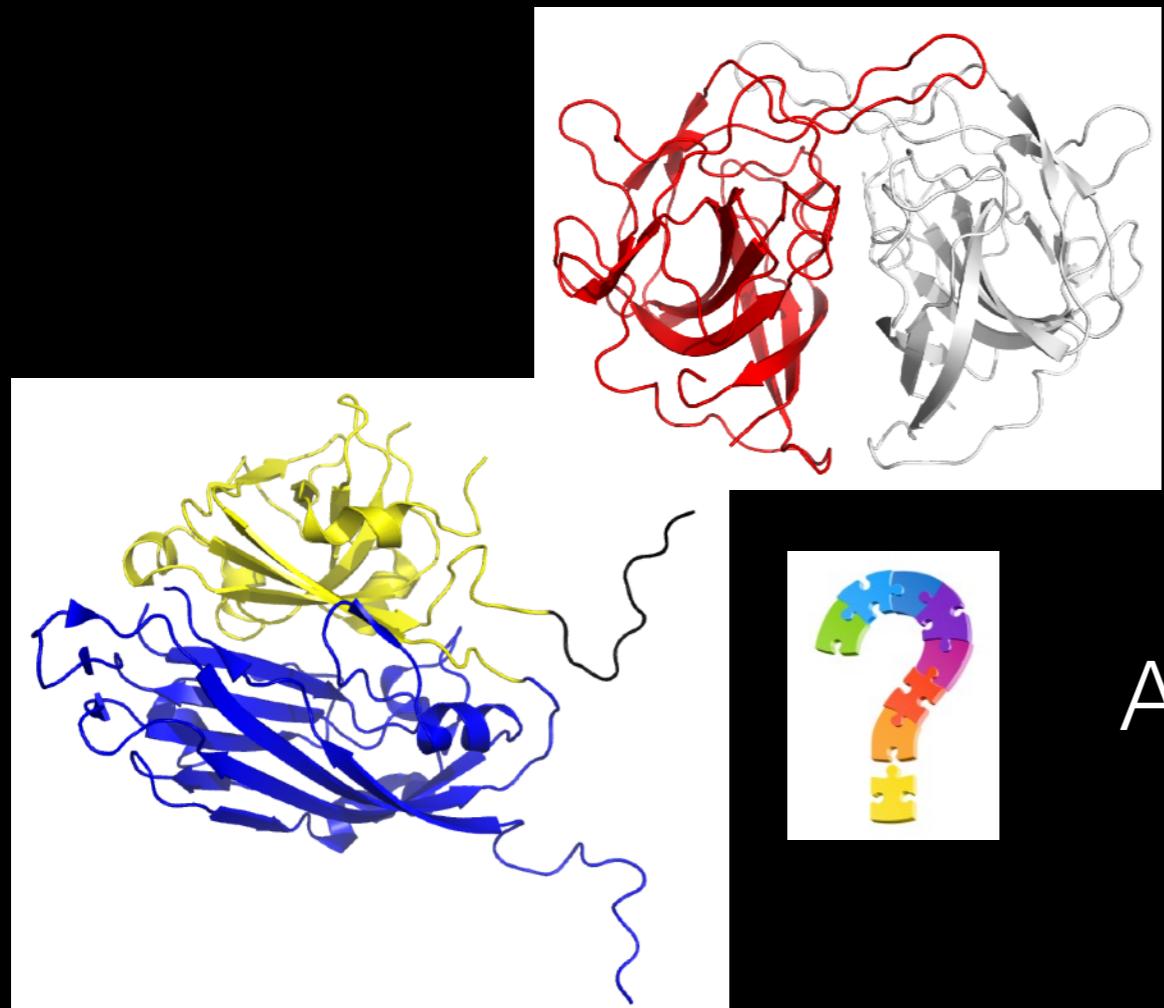
HAstV1 Biology

- SS RNA genome
- Non-enveloped



* Transmission Electron Micrograph from the 70's when Astrovirus was discovered - showing star shape

HAstV1 Capsid Structure

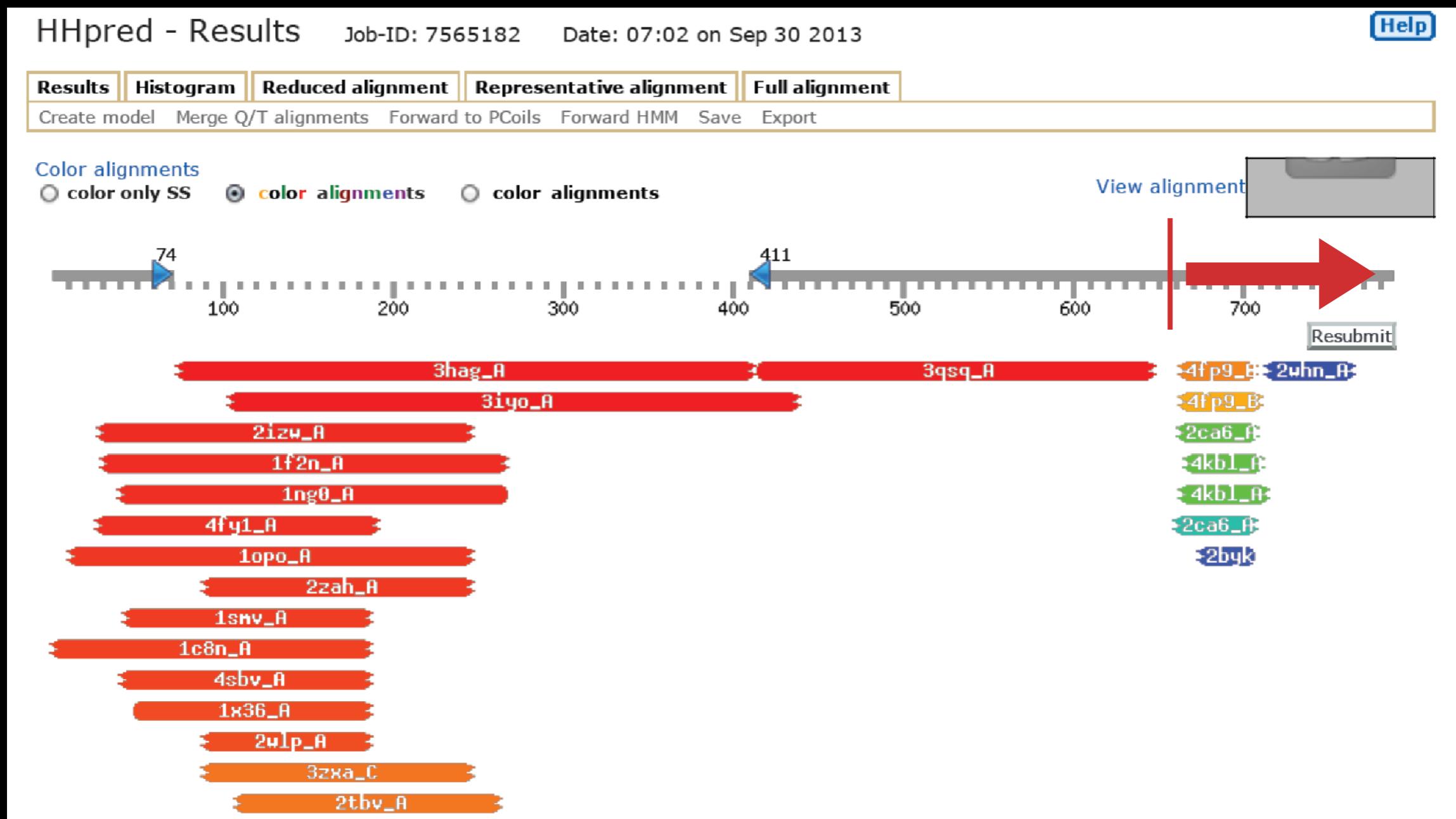


Spike Domain Solved

Acidic Domain Unknown

Capsid Domain Predicted (structure similarity)

HHPred



Previous Work...

- Primers designed/ordered
- PCR
- Get insert into plasmid and transform into E. coli
(pending)

From Last Quarter

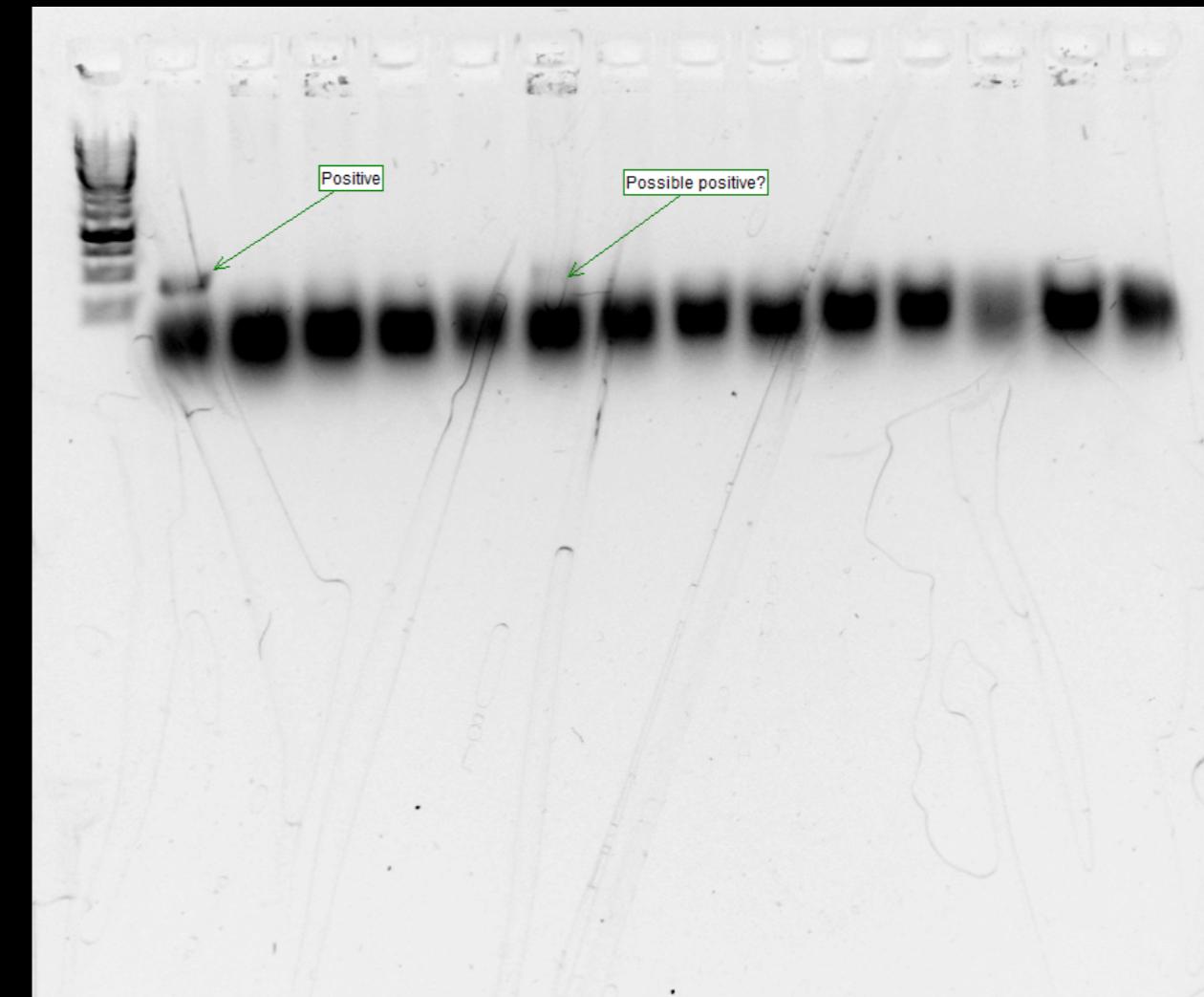
Next Steps

- Use a **large** amount of both vector and insert to perform ligation
- Try the other set of primers ordered (w/different restriction site)

Large Scale Digest and Ligation

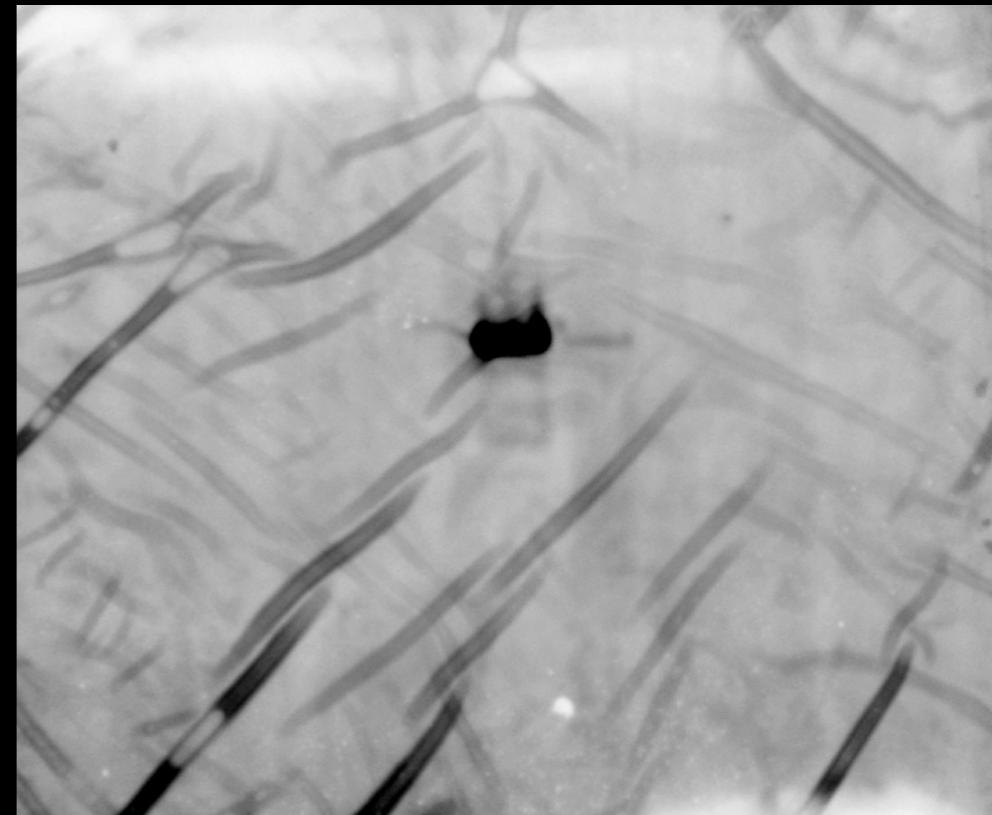
Finally worked!

- Colonies on 4 of 6 plates
- Colony PCR performed to confirm presence of insert
- Sent for sequencing



Small Scale Expression/ Purification Tests

- 50 mL cultures, induced with IPTG at O.D.= 0.8
- Overnight expression
- Expression detected in the BL21 cell line (confirmed by western blot)
- Found to be in the soluble fraction

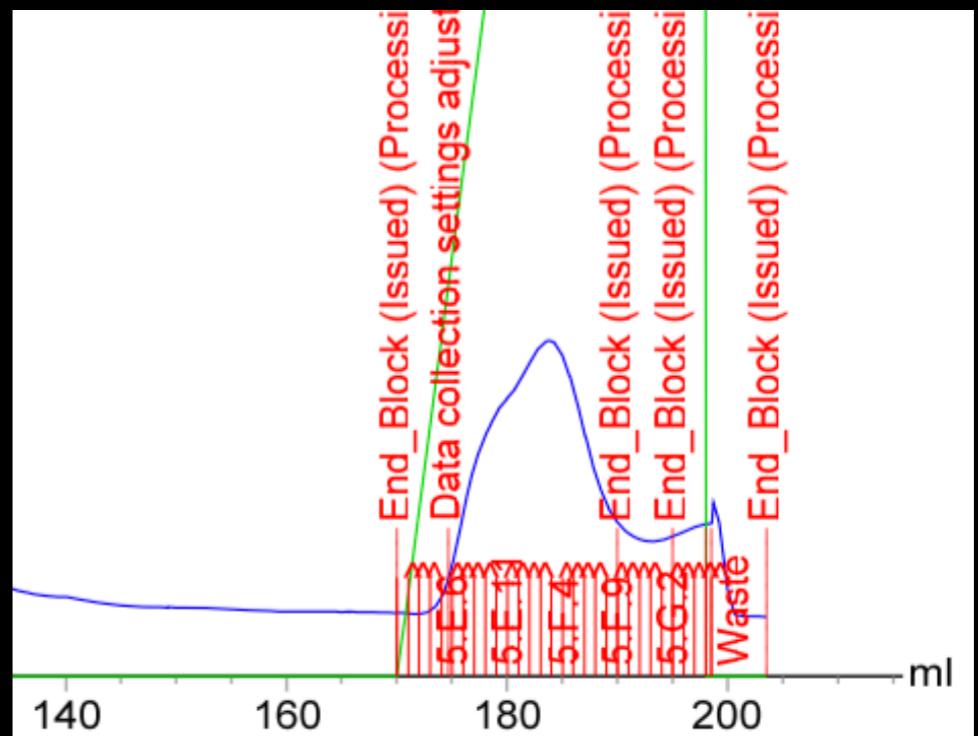


Large Scale Expression

- 7.5 liter culture, induced at O.D. ~0.8
- Overnight expression
- Sonication, centrifugation, filtered supernatant
- Prepared to run on His Trap

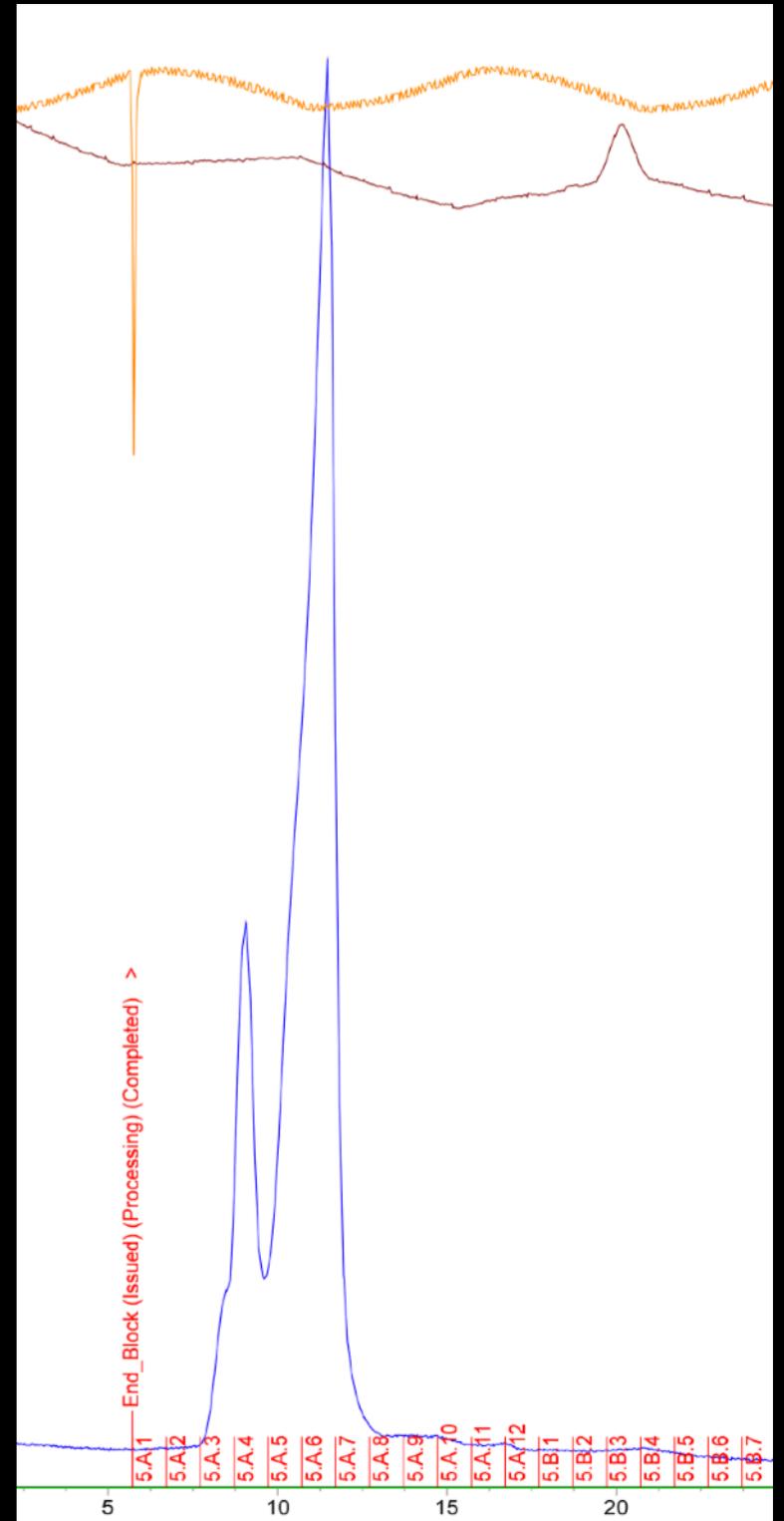
His Trap Purification

- Affinity tag on the C-terminus
- Elution with 500 mM Imidazole
- Run gel of results



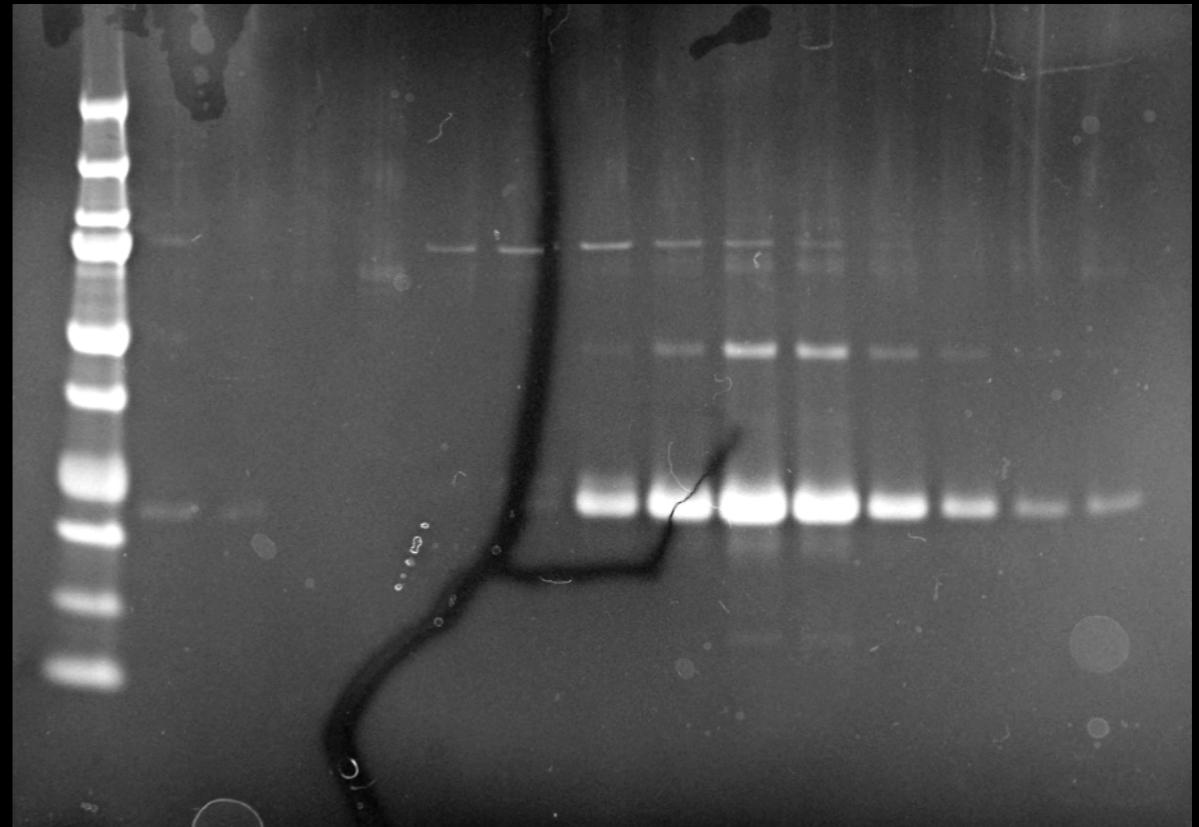
Sizing Column

- Runs like a much larger protein
-> implies lack of structure



Anion Exchange Column

- Protein is negatively charged at pH 8
- Eluted with 1M NaCl
- Still not entirely pure



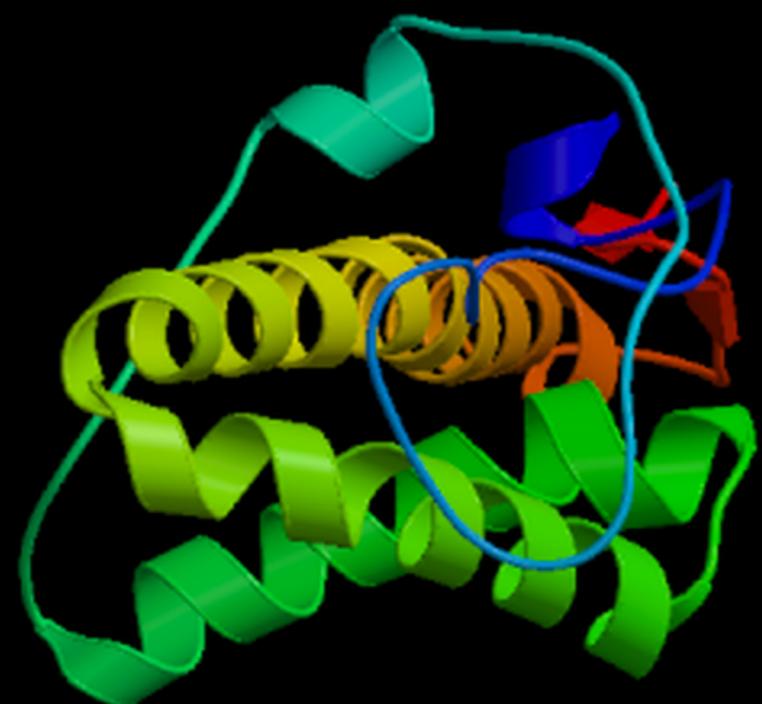
Structure Prediction

- Rosetta protein structure prediction server predicts that ~ half of the protein is disordered
- 4 helixes predicted at the C-terminus

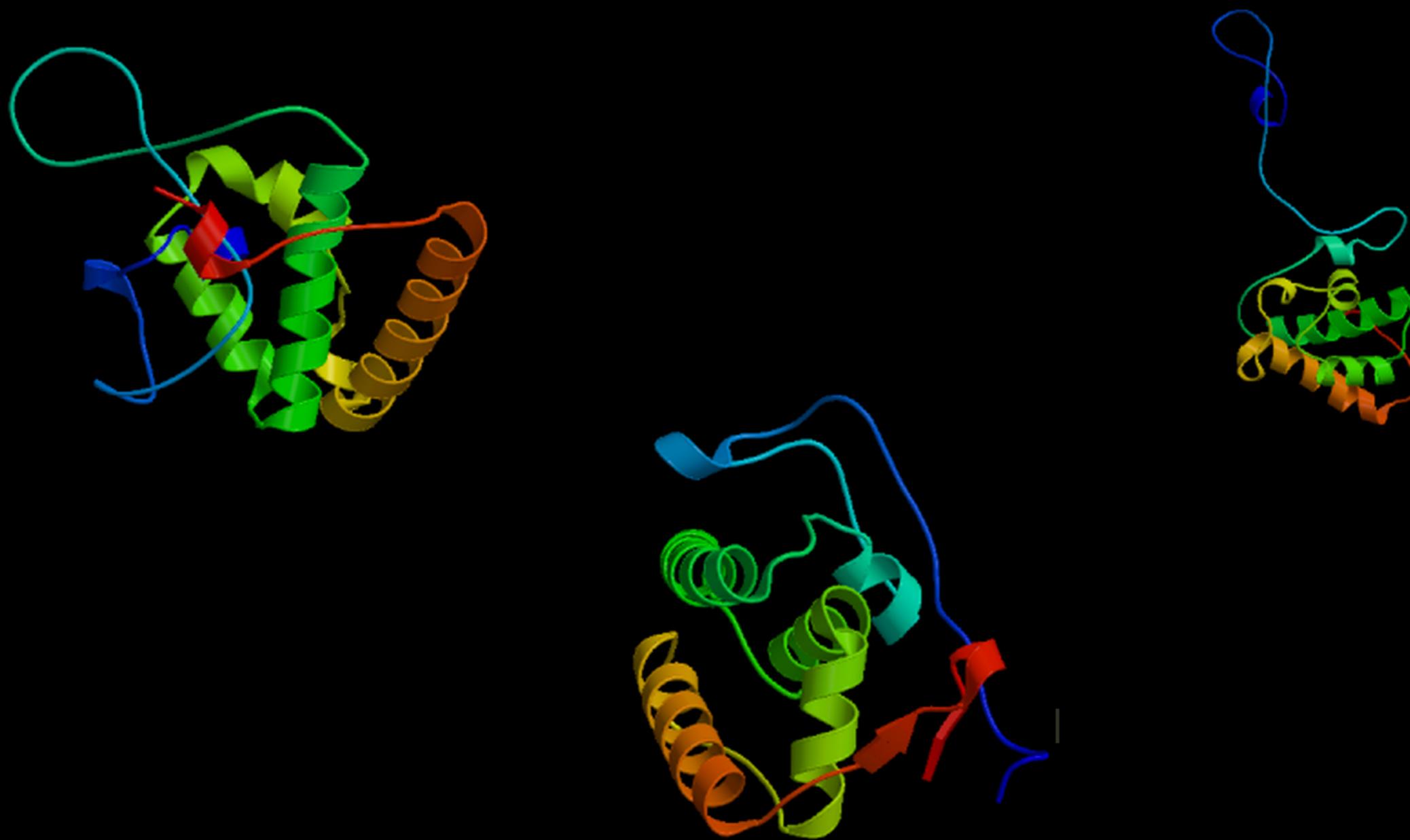
1	. 10 .	20 .	30 .	40 .	50 .	60 .	70 .	80 .	90 .	100 .	110 .	120 .	130 .	140
SRASGYGYESDNTEYLDAPDSADQFNEDIEDTDTIEDDEADRFDIIDTSDEEDESETDRVTLSTLVNQGMTITRATKIAARRAFPTLSRIKRGVYMDLLASGASPNAWSHACEEARKAAGEINPCTSGSRGHAE														
----- XXXXXXXXXXXXXXXXXX-----XXXXXXXXXXXXXXXXXX-----														
----- XXXXX--XXX-----XXXXXXXXXXXXXXXXXX-----XXXXXXXXXXXXXXXXXX-----														
----- HHHHHHHHHHHHHHHHHHHH-----HHHHHHHHHH-----HHHH-----EEEE-----HHHHHHHHHHHHHHHHHH-----														

Structure Prediction

- A number of tertiary structure predictions were also generated



Structure Prediction



Complications

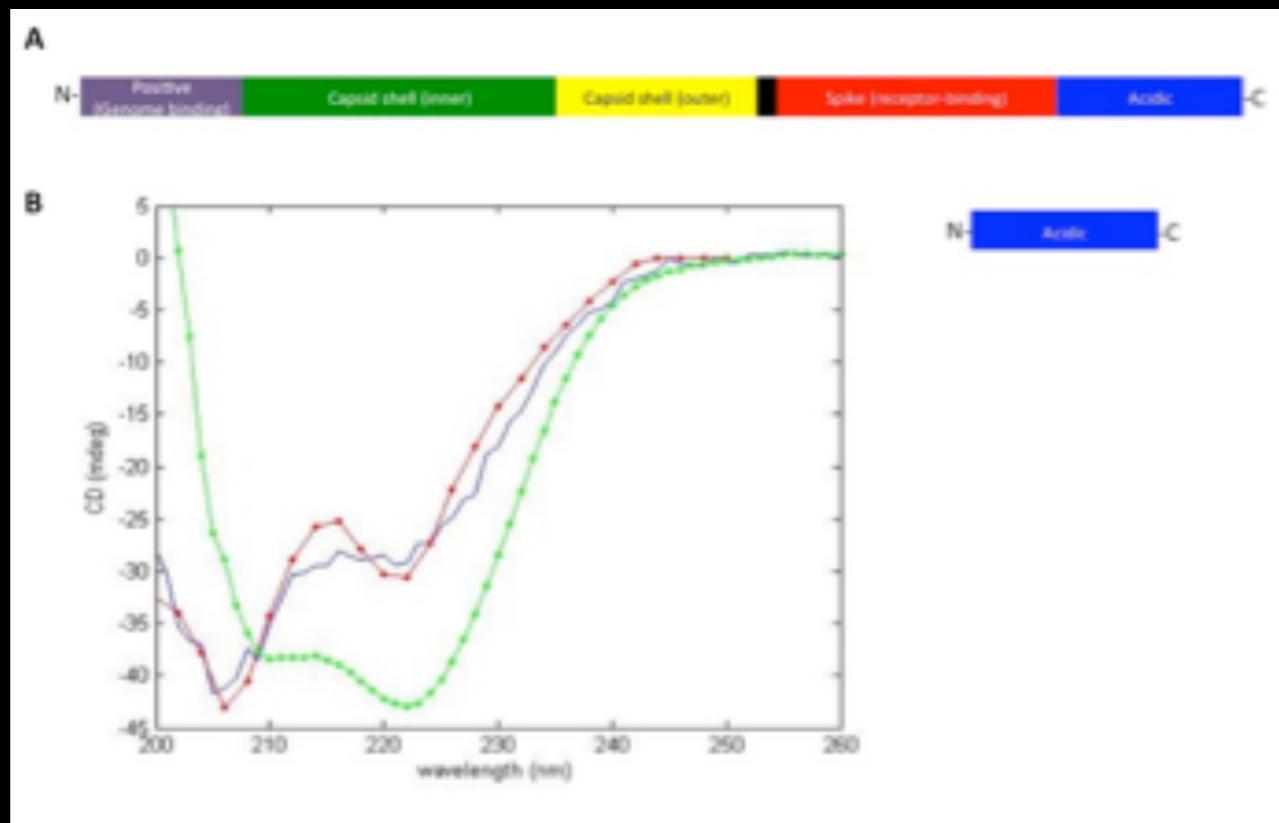
- Protein not entirely pure, even after multiple techniques
- Runs on a sizing column as a much larger protein (~150-200 kDa)
- DTT added to eliminate possible disulfide bonds
- Aggregating? Pore forming? Need to learn more about structure/function.

1D NMR

- 1D NMR can give only general structure information
- The results imply that the protein is mostly unstructured (with or without DTT)

Circular Dichroism

- CD can give more detailed structure information, such as the ratio of secondary structure types
- Suggested composition: 40% helix, 60% coil

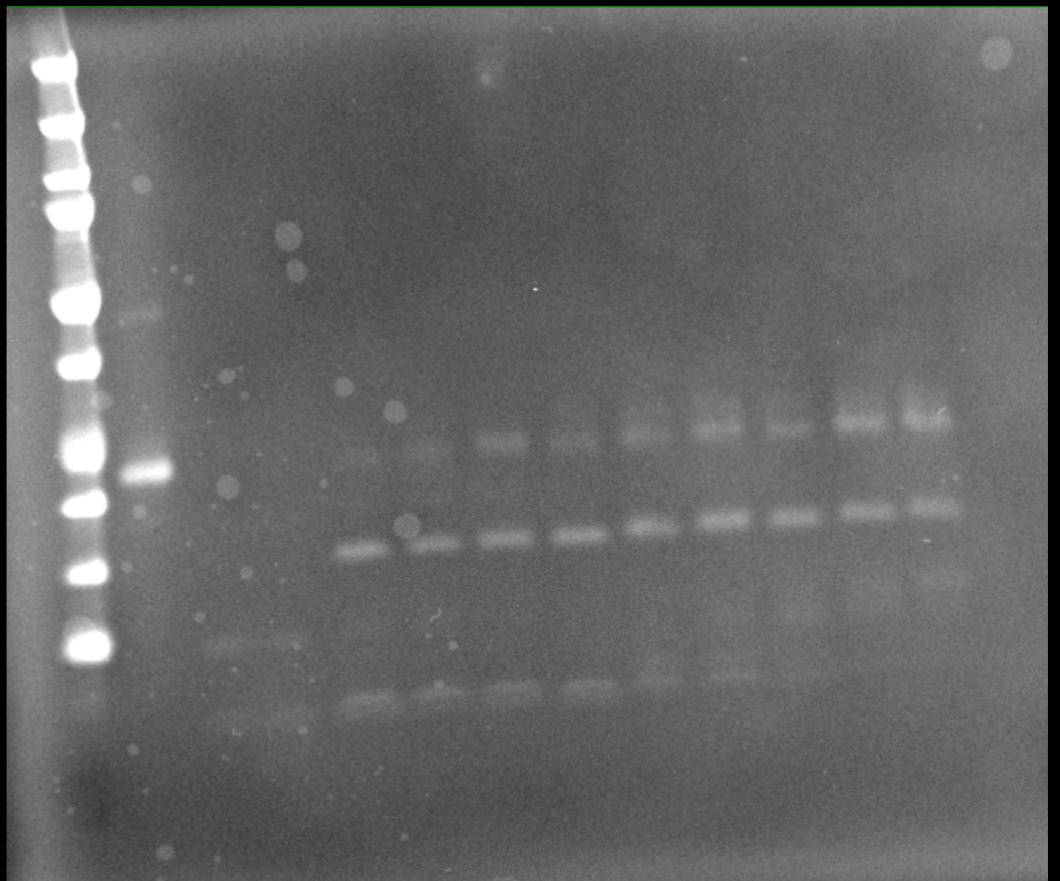


Crystallography

- If the protein is >half unstructured, crystallography may be difficult (and give limited information)
- It might also be easier to crystallize the helical portion of the protein on it's own

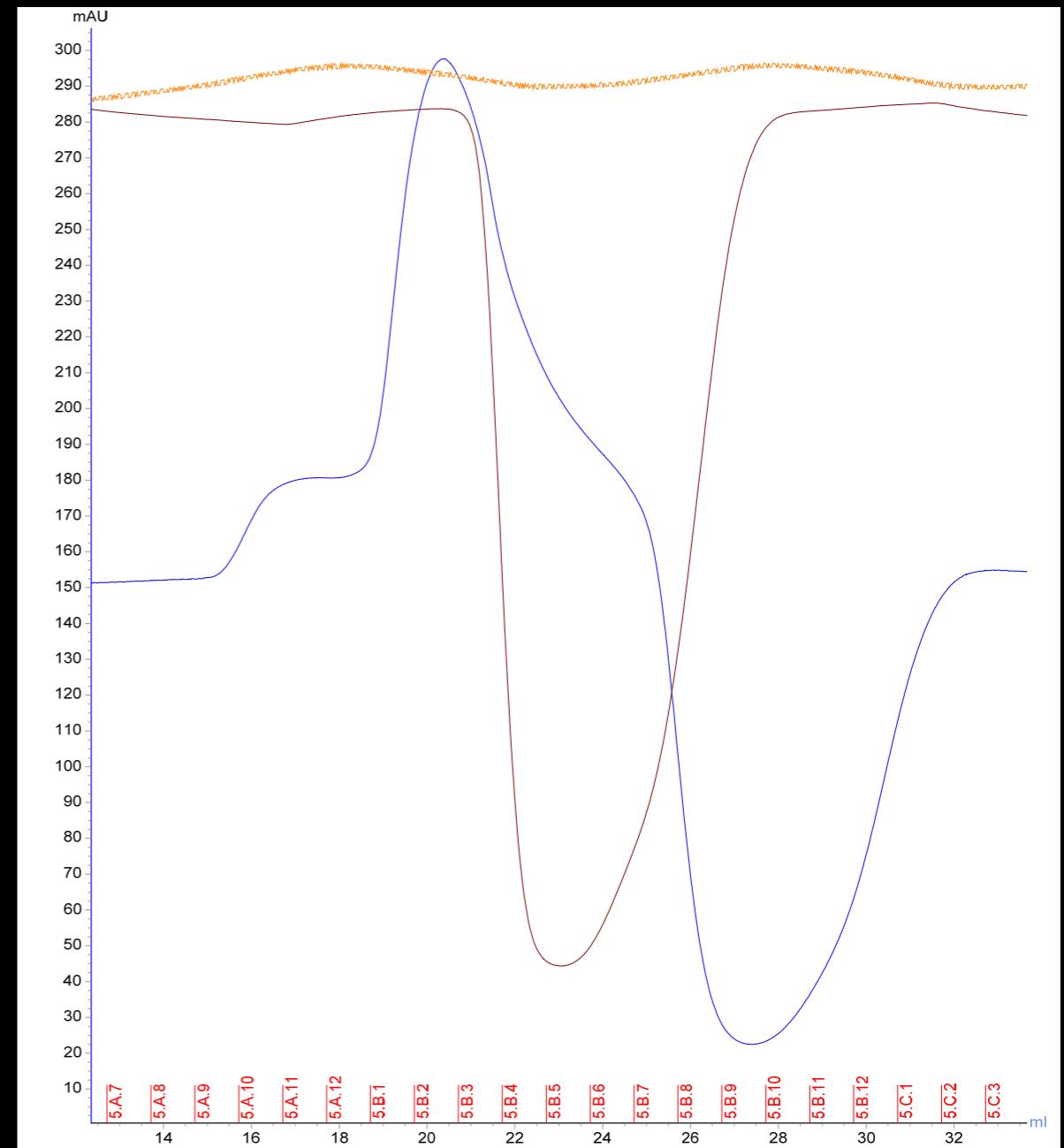
Trypsin Digest

- Results suggest that there is a stable helical bundle of about 10 kDa

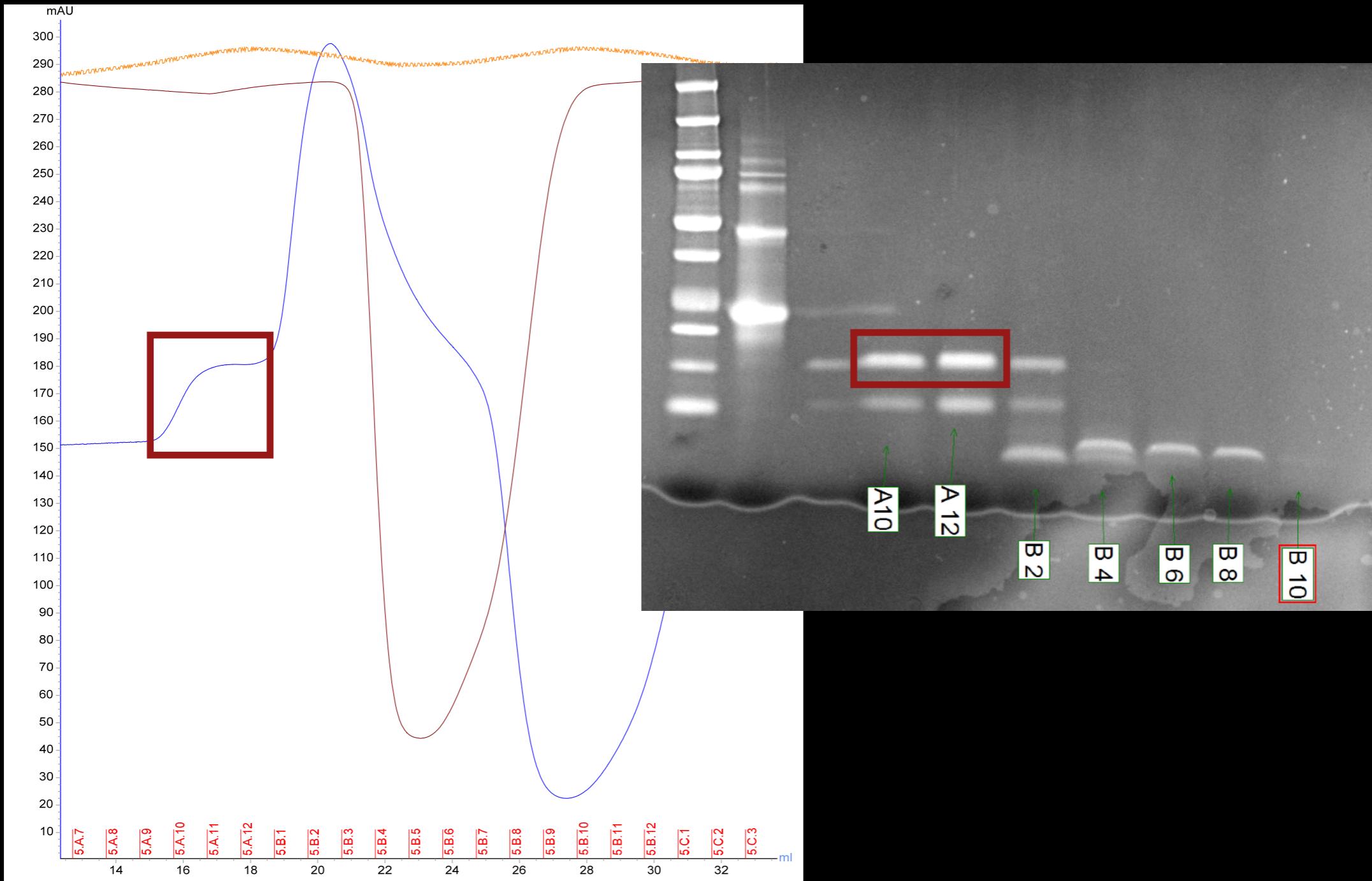


Post-Trypsin Sizing

- After digest, the protein no longer runs too large



Post-Trypsin Sizing



Next Steps

- Crystallography
 - Concentrate samples, and setup trays with full length construct AND digested version (tomorrow)
- Test for metal binding
- Think about cell-based assays for localization/ function
- Consider trying to solve the structure using NMR

Have a great Spring Break!

