

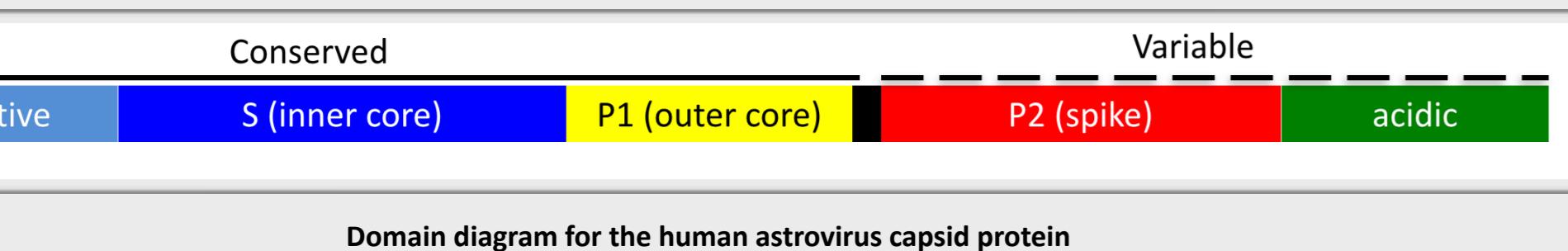
Structural Studies of the Human Astrovirus 1 Capsid Acidic Domain

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Background:

Astroviruses are a major cause of diarrhea in children and immunocompromised populations. Infections are seasonal in nature, following the rainy season in tropical areas and the winter season in temperate areas. The different serotypes have varying clinical presentations, with diarrhea, fever, and vomiting being common symptoms.

Human astroviruses (HAstV) are non-enveloped, single-stranded, positive sense RNA viruses. The HAstV capsid protein contains 5 distinct structural domains. One of these (known as the acidic domain), is thought to be involved in virus maturation and assembly.

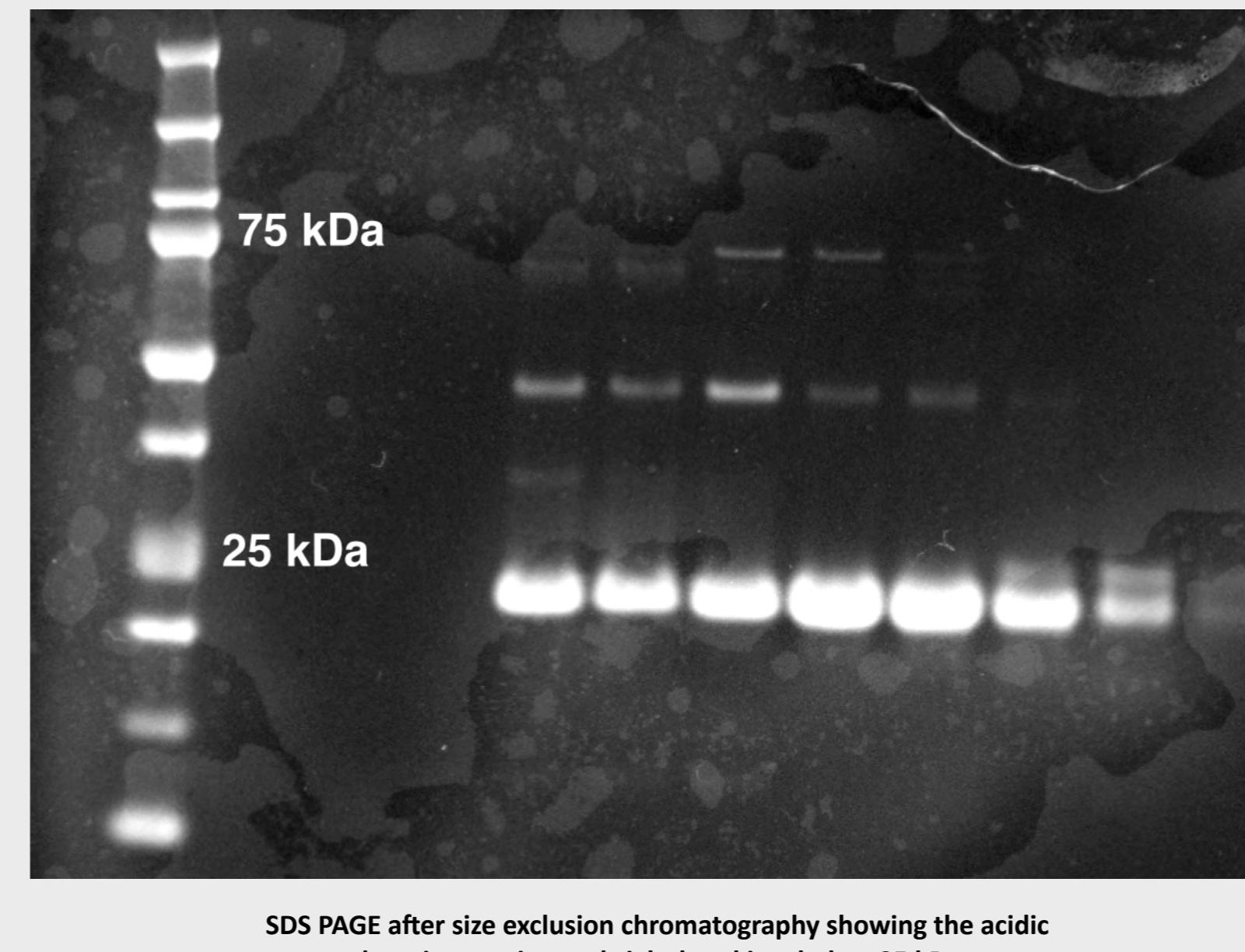


Methods:

- Cloning was performed to produce a pET52b expression plasmid containing the acidic domain nucleotide sequence
- Protein was expressed in the BL21 *E. coli* cell line
- Purification was performed by affinity and size exclusion chromatography
- Computational tools were used to predict secondary and tertiary structure
- Nuclear magnetic resonance (NMR) and circular dichroism (CD) were performed to corroborate predicted secondary structural characteristics
- A trypsin digest was performed to isolate the putative helical bundle
- Screening trays were set up to find ideal crystallization conditions

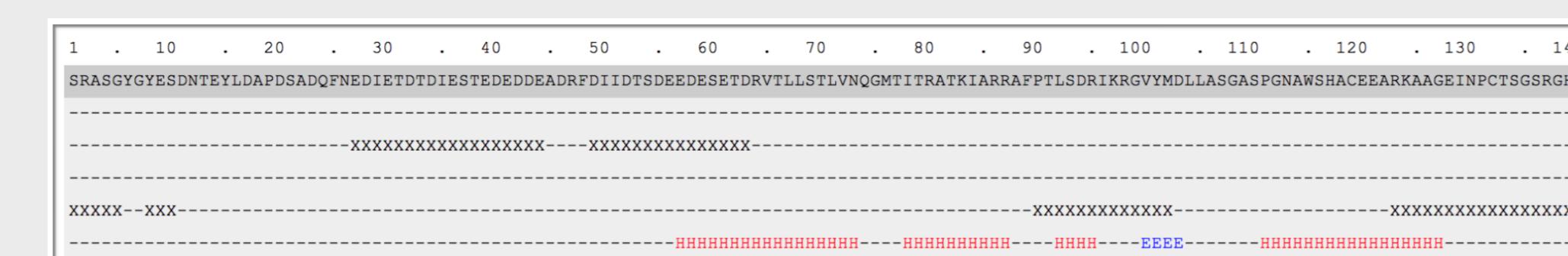
Results:

Cloning, expression, and purification produced an ample supply of stable acidic domain protein.

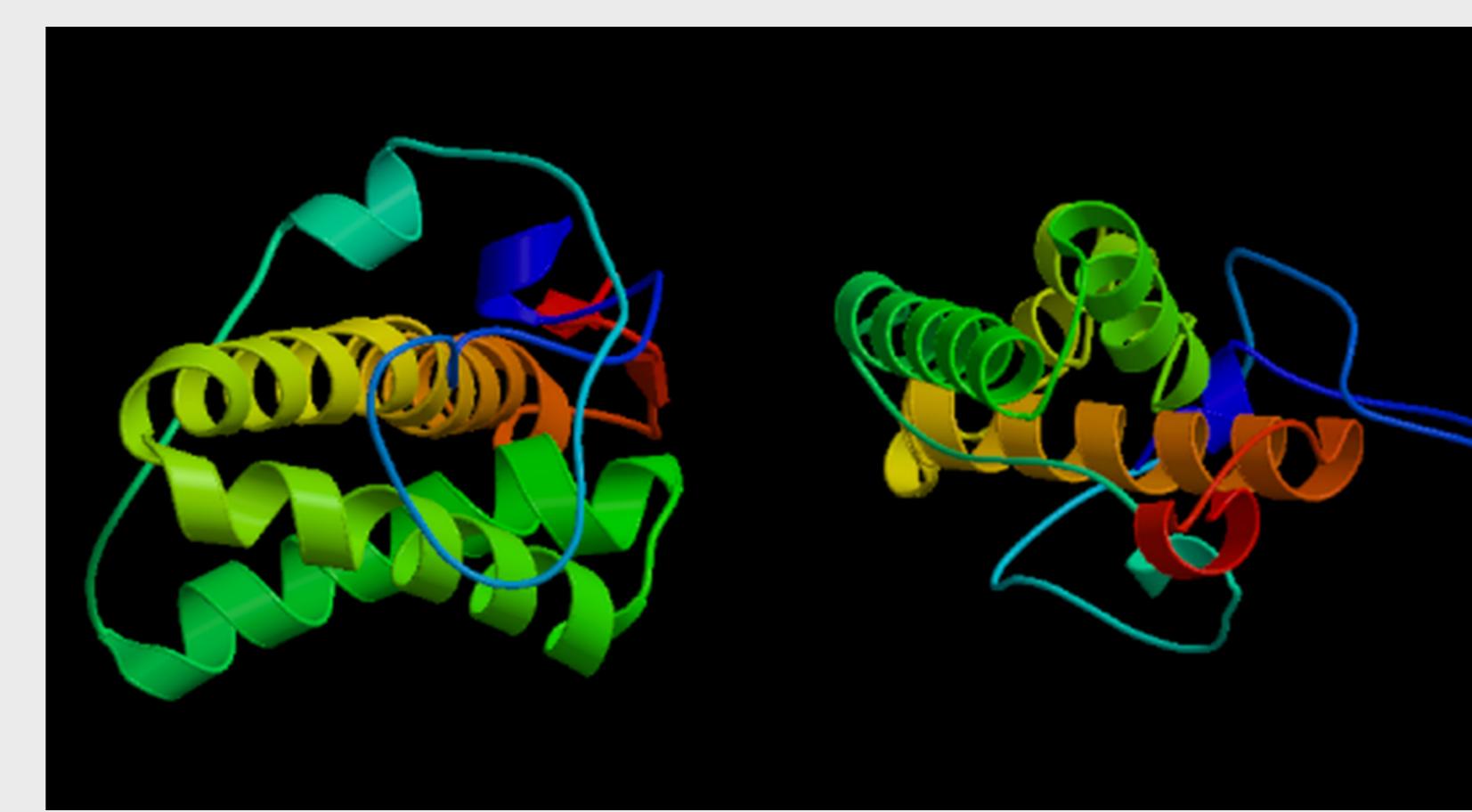


SDS PAGE after size exclusion chromatography showing the acidic domain protein as a bright band just below 25 kDa

Computational tools predicted an N-terminal disordered region and a C-terminal region consisting of four helices. Several tertiary structure predictions were also produced.

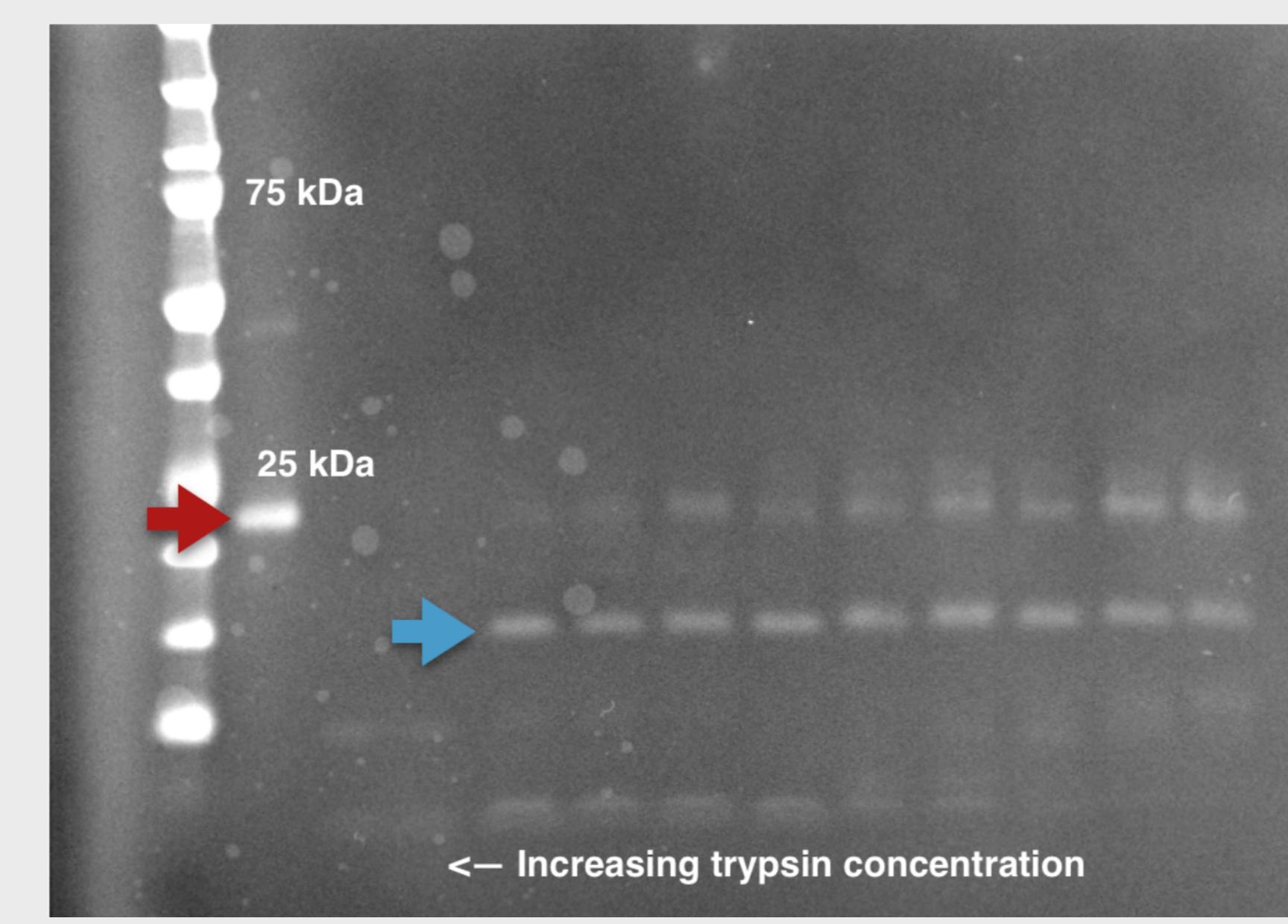


Robetta secondary structure prediction, showing predicted helices as red "H's"



NMR and CD studies were consistent with a secondary structure of approximately 40% helices and 60% unstructured regions.

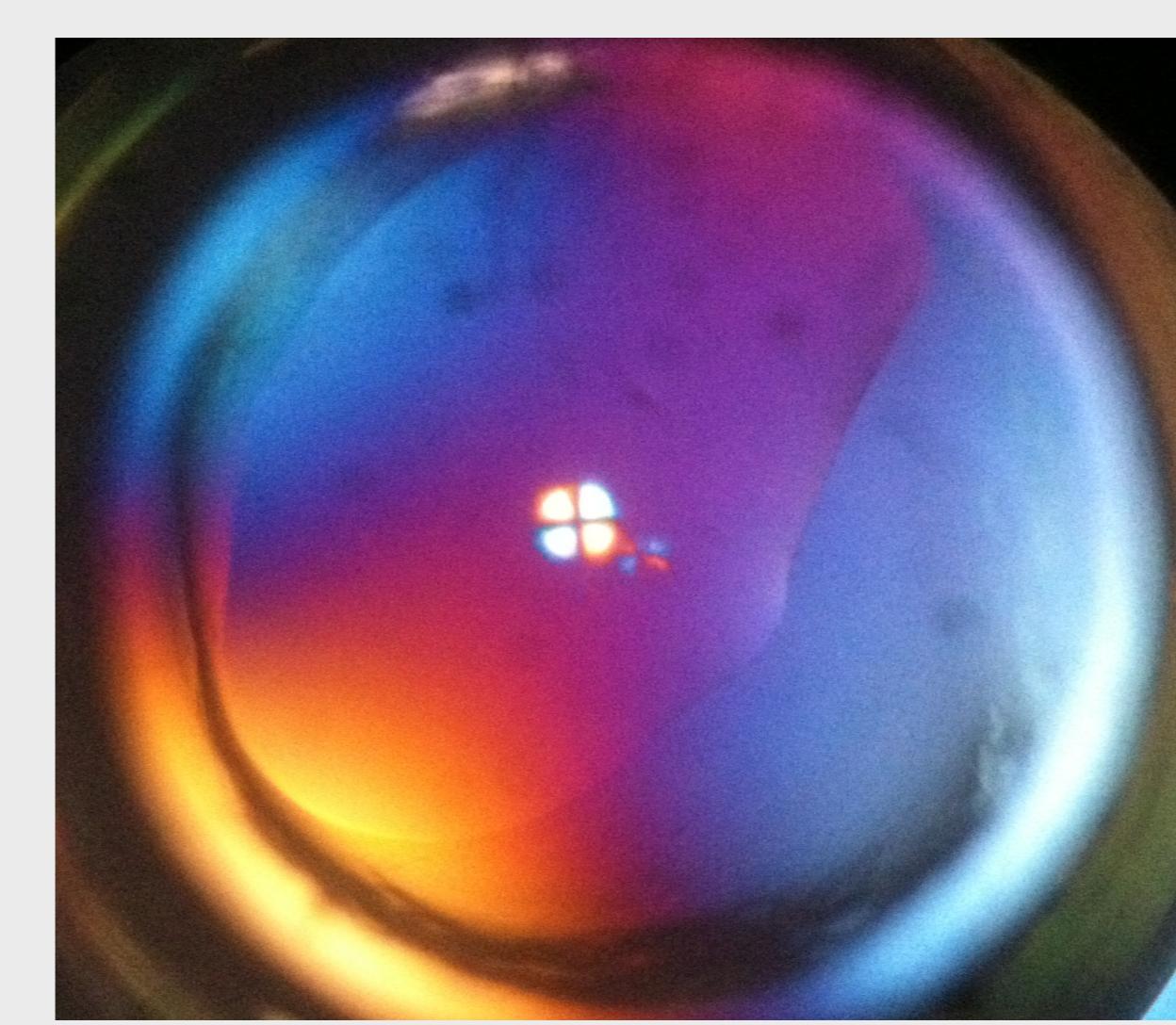
To isolate the helices, limited proteolysis was performed with trypsin. The result was a stable band present at all protease concentrations. This band was separated using a size exclusion column.



Trypsin digest concentration test ranging from 10:1 to 2500:1 acidic domain:trypsin ratio (by mass). The red arrow corresponds to the full length construct, and the blue arrow corresponds to the stable band

Full length protein and trypsinized fragment were concentrated and used to prepare crystallization condition screening trays. Unfortunately, degradation of the fragment had already occurred due to residual trypsin in the sample.

No crystals were observed in full length protein or intact fragment. A variety of conditions produced crystals in the degraded fragment, but even these were bundles of crystals, unsuitable for diffraction.



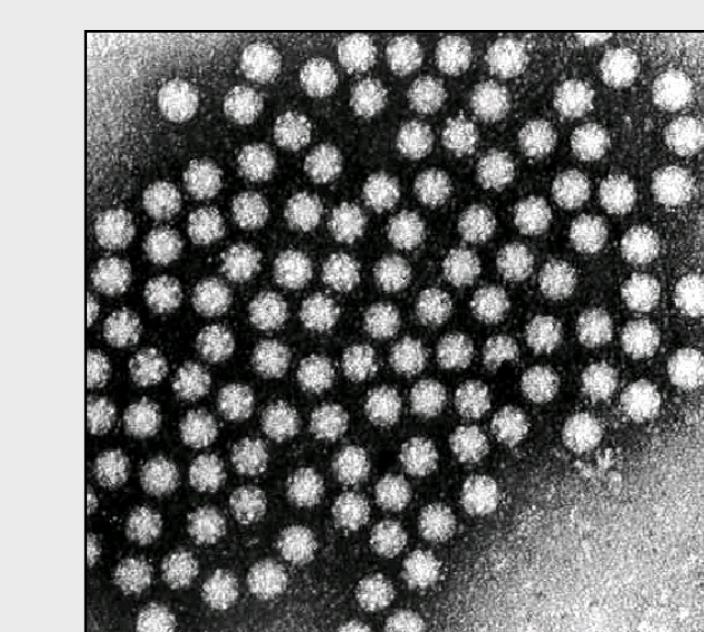
Crystal bundles formed from degraded protein sample

Conclusions:

While the tertiary structure of the acidic domain is still unknown, protocols for successfully producing and purifying it have been demonstrated here.

Several lines of evidence (including tryptic digest pattern, circular dichroism, and sizing behavior) have helped confirm the predicted secondary structure of approximately 40% helix and 60% unstructured.

Attempts to crystallize both the entire domain and the putative helices isolated by tryptic digest were ultimately unsuccessful.



Electron micrographs of a single immature astrovirus (left) and a group of mature astroviruses (right). (Dreyden et al. 2012, Creative Commons 2.0, respectively)

Future Directions:

- Cloning was performed to produce an expression plasmid with only the helical regions of the acidic domain. (avoiding trypsin digestion) Expression and purification tests will be performed using this new construct.
- Functional assays are currently in development, which aim to localize the protein within the cell and determine what effect (if any) this domain has on mammalian cells without the presence of the full virus.
- If crystallization proves difficult, a structure for one or both constructs may be pursued via NMR.

Acknowledgments:

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