



# Identification and Possible Implications of a Human Plasma Purified Anodal Variant of Alpha-1-Antitrypsin

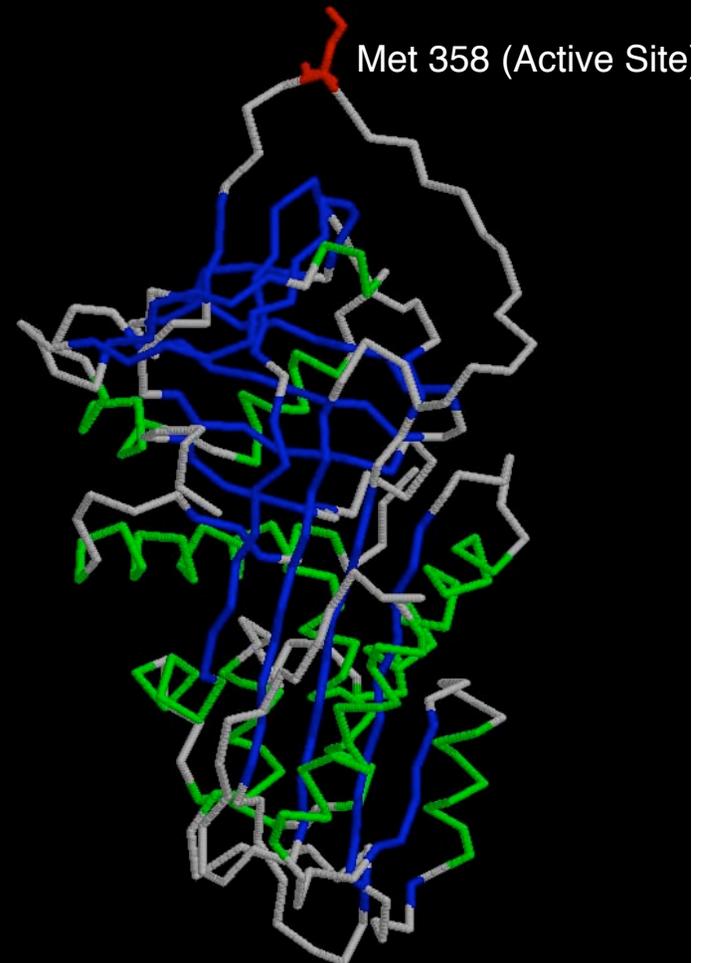
BPAC Meeting Nov 4, 2005

---

Mark Brantly, MD  
University of Florida

# Alpha-1-Antitrypsin (AAT)

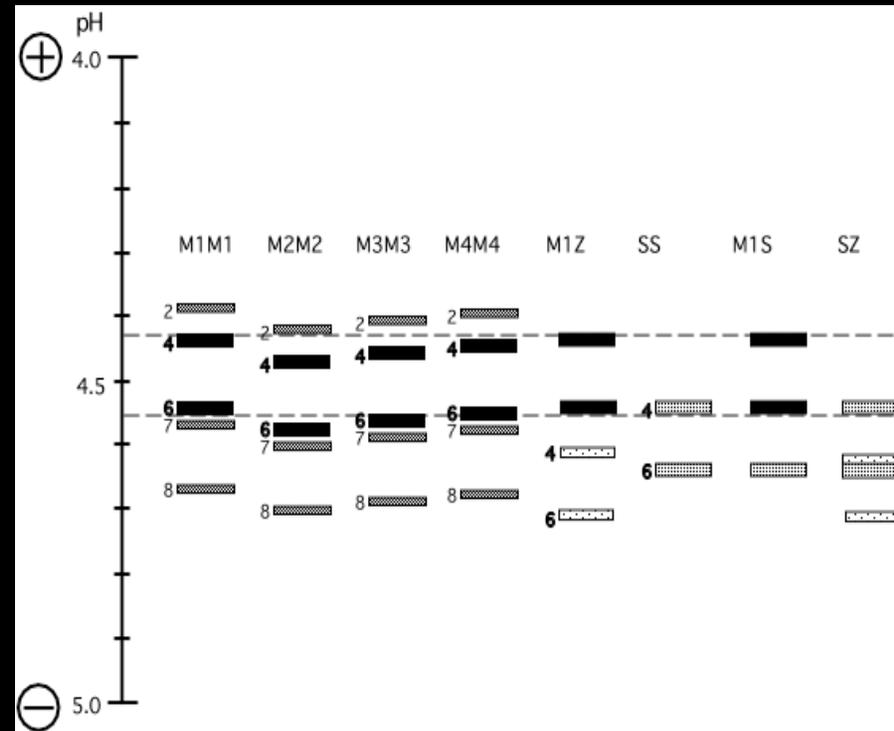
- 394 amino acids
- Glycoprotein
- 3 N-linked carbohydrate attachment sites
- Molecular weight ~52,000 Da
- Single cysteine
- Isoelectric point ~4.2-4.8
- Functions:
  - Inhibition of serine proteinases
  - Inhibition of neutrophil defensins
  - Broad anti-inflammatory properties
- Also called Alpha-1-Proteinase Inhibitor (A1PI)



AAT Structure

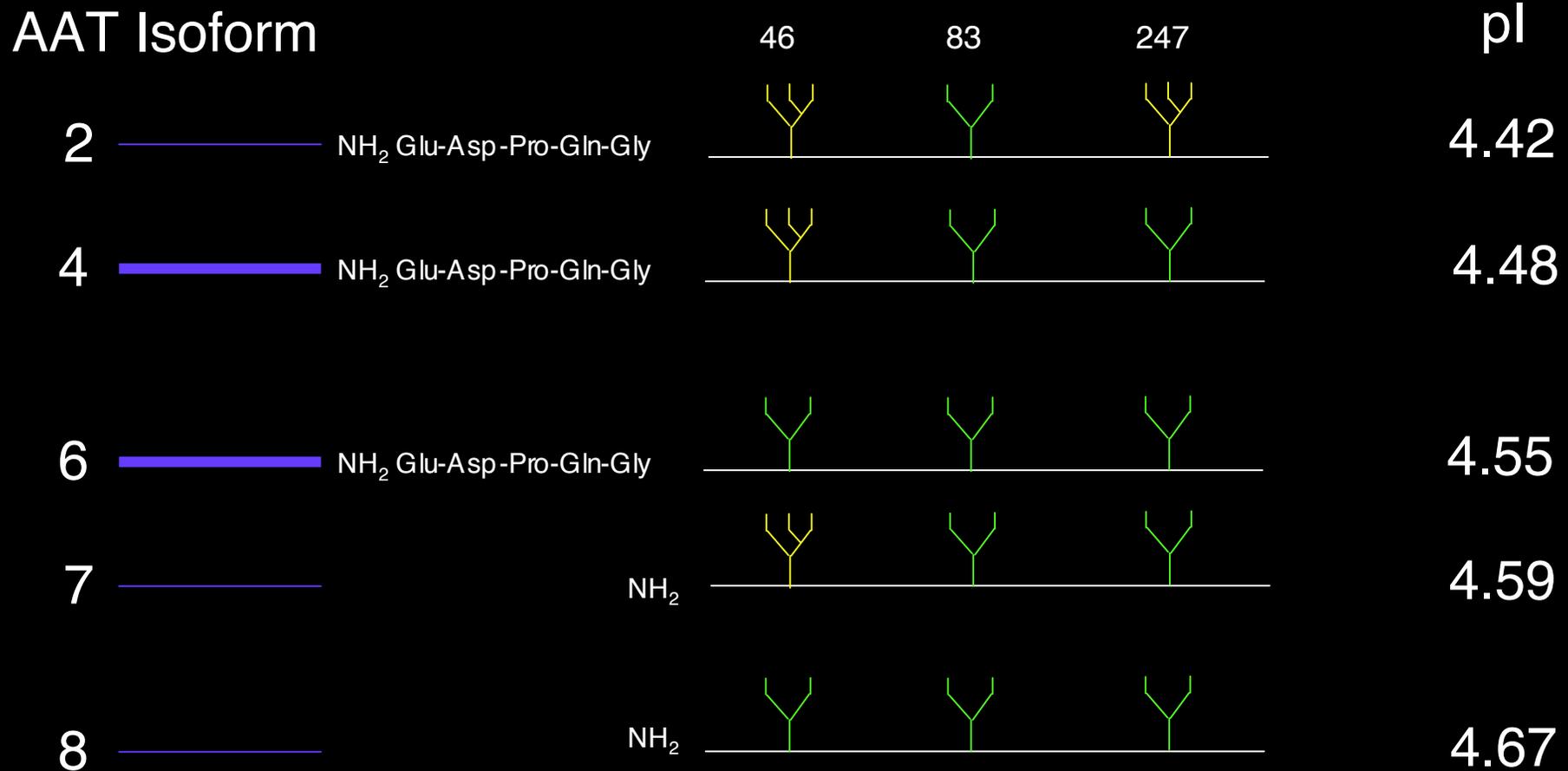
# Characterization of AAT by Isoelectric Focusing (IEF)

- IEF-Method of separating protein isoforms by charge
- Isoelectric point is the place on the pH scale where a protein has no net charge
- Traditionally used in the laboratory diagnosis of AAT Deficiency



Schematic AAT IEF Gel of AAT Variants

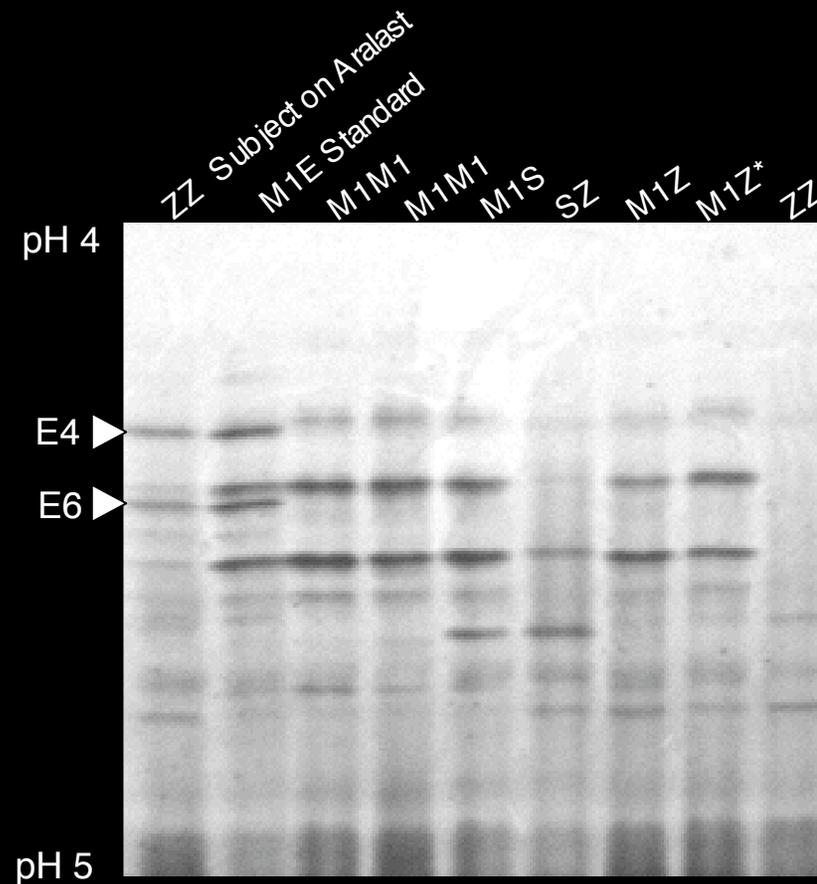
# AAT Isoform Microheterogeneity



Adapted from Jeppsson, JO. J Chromat 1985; 326: 173-177

# Identification of the Anodal Variant in Aralast™

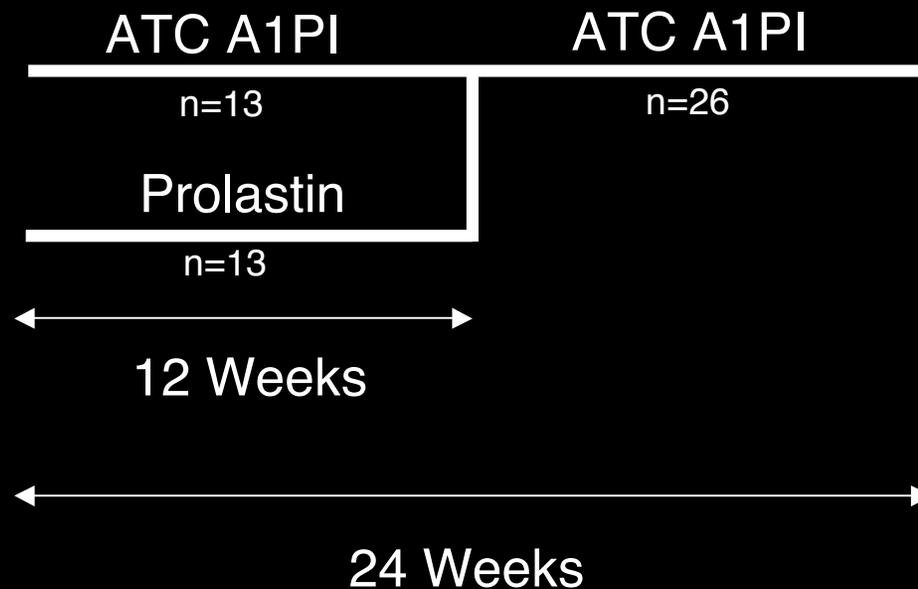
- Individuals with AAT deficiency most commonly are ZZ
- Augmentation therapy IEF pattern- MZ
- “E” region variant was identified by 2 labs
- Genotypically the subjects were ZZ
- All subjects were receiving Aralast™ augmentation therapy



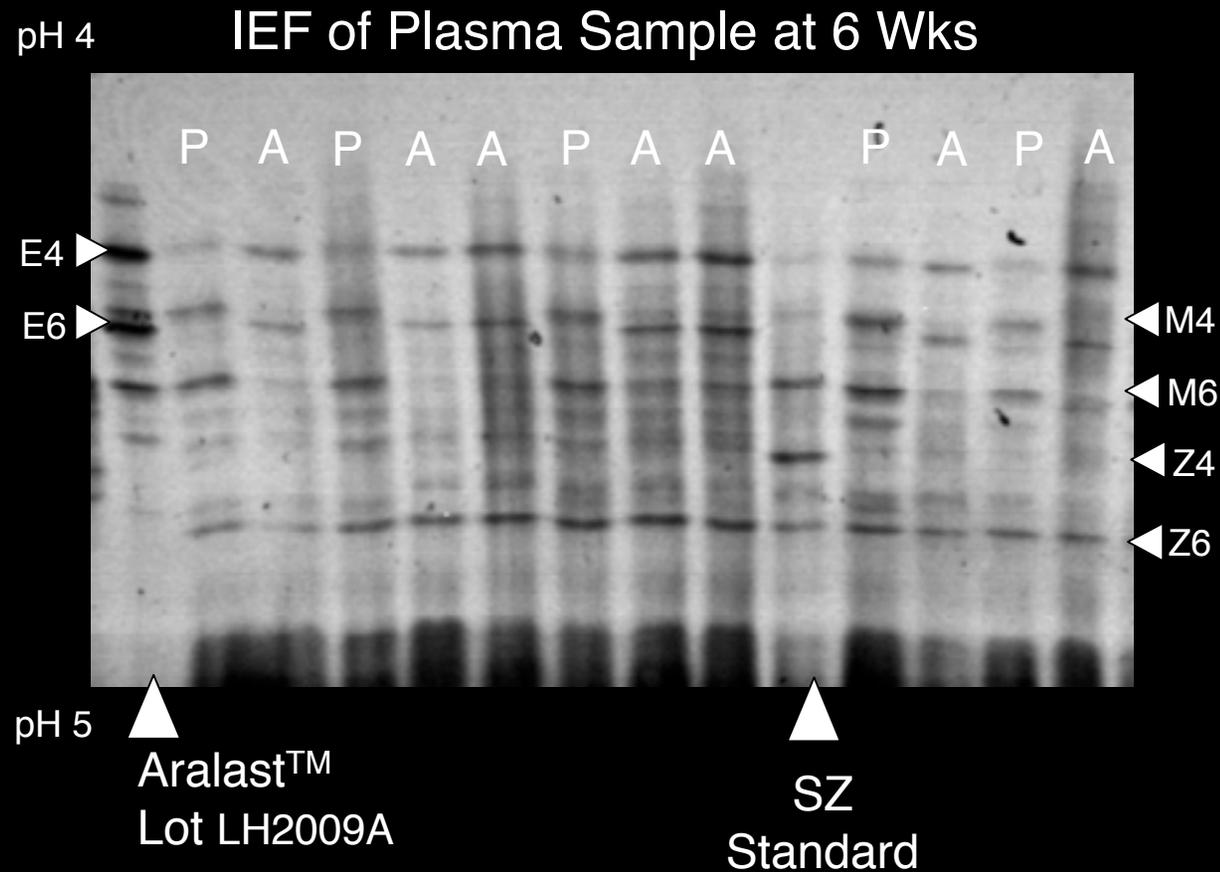
\*Subject on Prolastin™

# Alpha Therapeutics Corporation (ATC) Pivotal Study

- Aralast™ developed by ATC
- Clinical trial from 1996-1999
- 28 deficient subjects enrolled for 6 months-2 early drop outs
- Comparator: Prolastin™
- Primary Outcome Variable-Not Inferior to Comparator (Total & Functional AAT)
- Central Lab-Brantly-NIH/UF

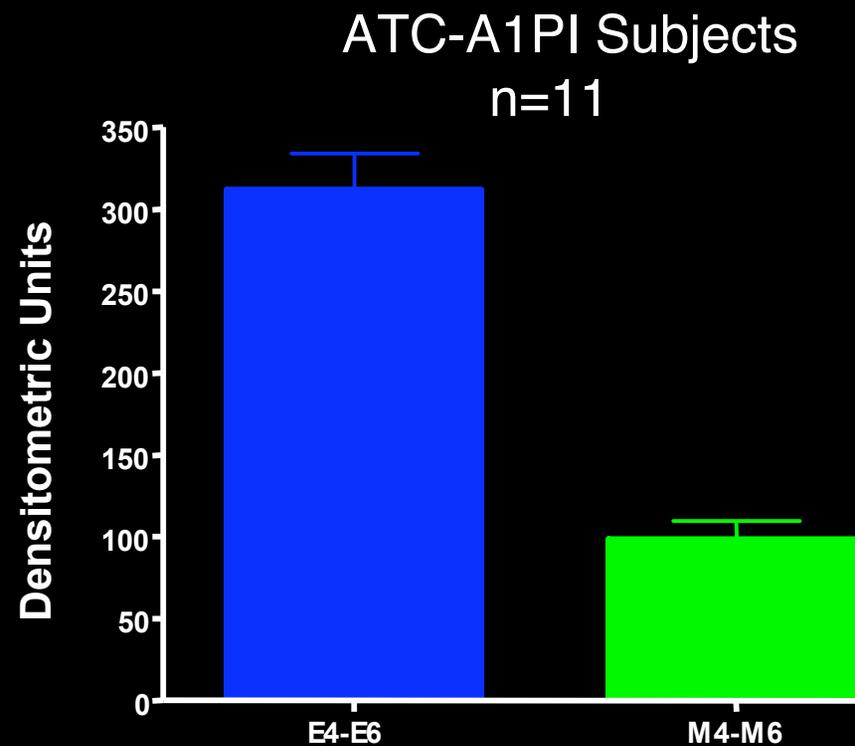


# Anodal Variant Present in Pivotal Study



# Proportion of E & M Isoforms in Study Samples

- Determine the proportion of “E” (modified variant) compared to M
- Densitometry of IEF isoforms following 6 weeks of study drug
- ~76% of total nadir AAT was modified form



# ATC Pivotal Study Results

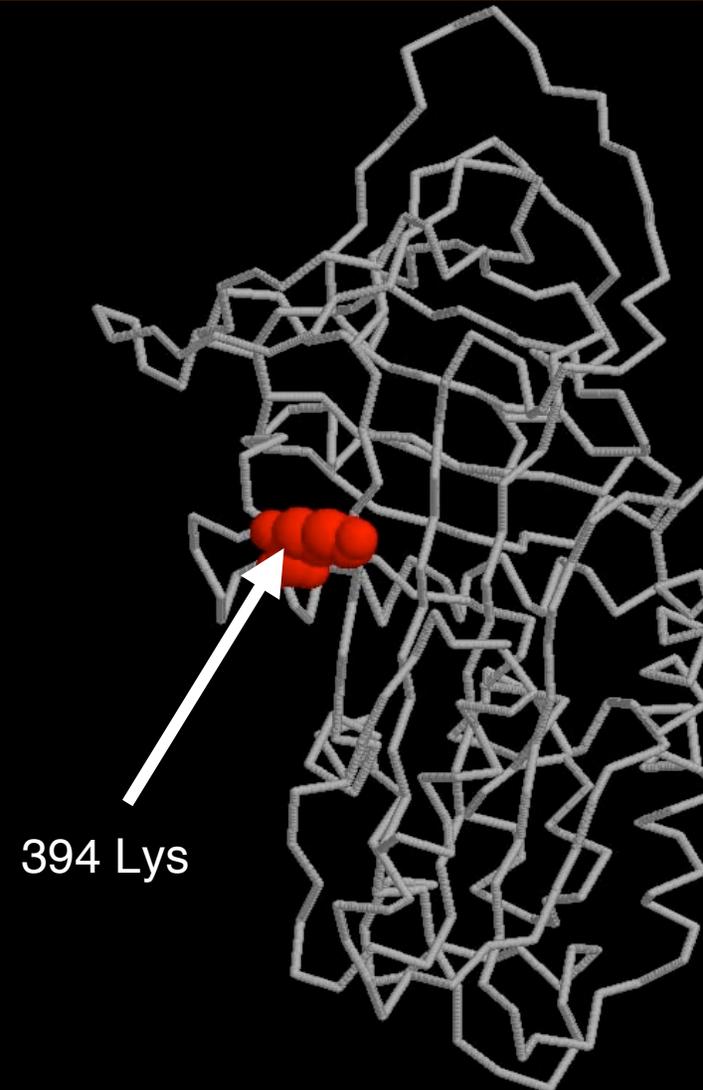
---

- Antigenic and functional amount (anti-protease activity) of ATC-A1PI similar to comparator (Prolastin)
- No serious safety signal in small short study
- Following 6 weeks of ATC-A1PI “E” (modified AAT variant) is ~76% of total nadir AAT
- Half-life similar for ATC-A1PI & Prolastin

# Explanation for Anodal Variant

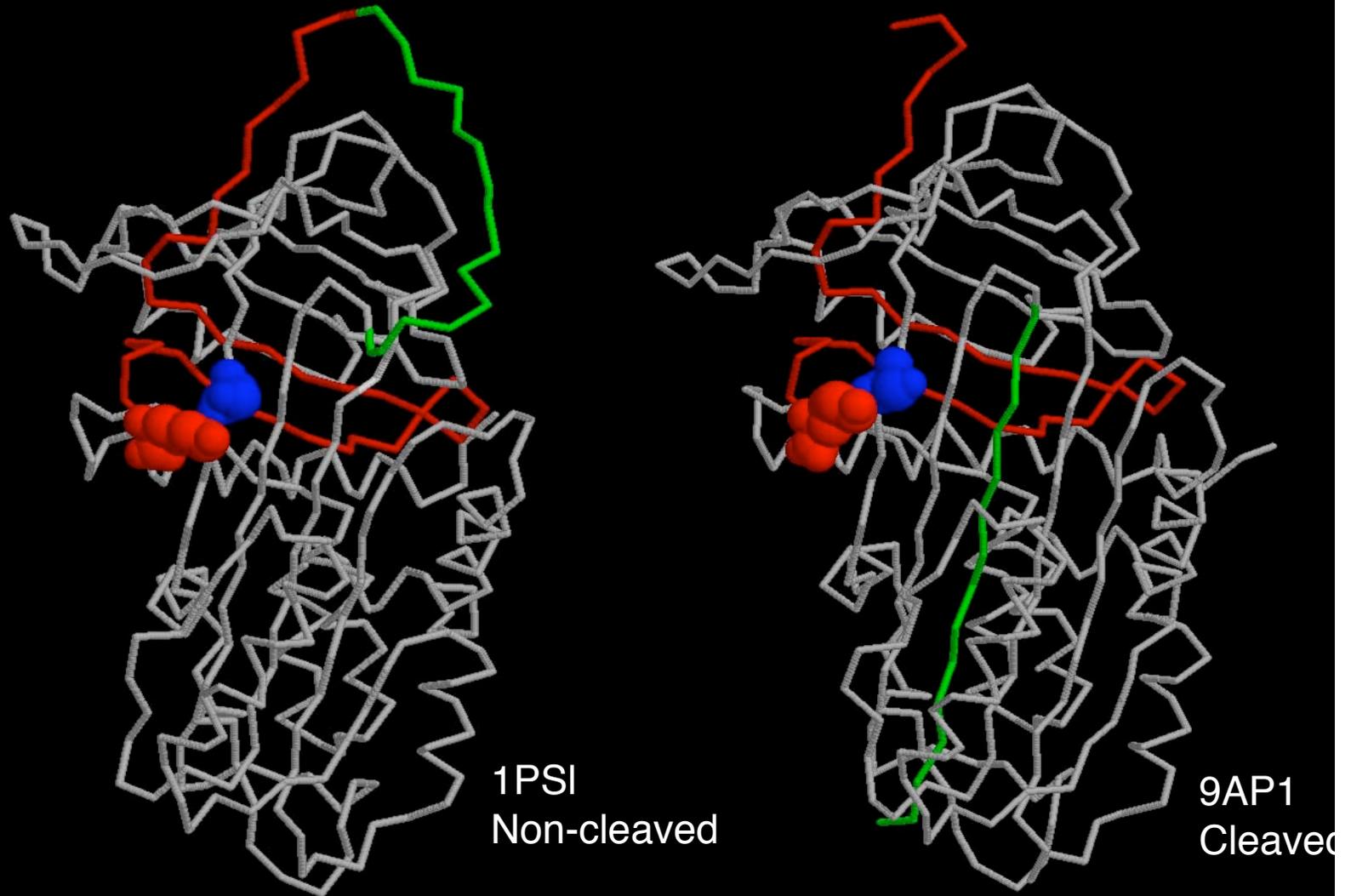
---

- Anodal variant secondary to loss of C-Terminal positive charged Lys
- Loss of C-Terminal residue likely secondary to carboxypeptidase activity



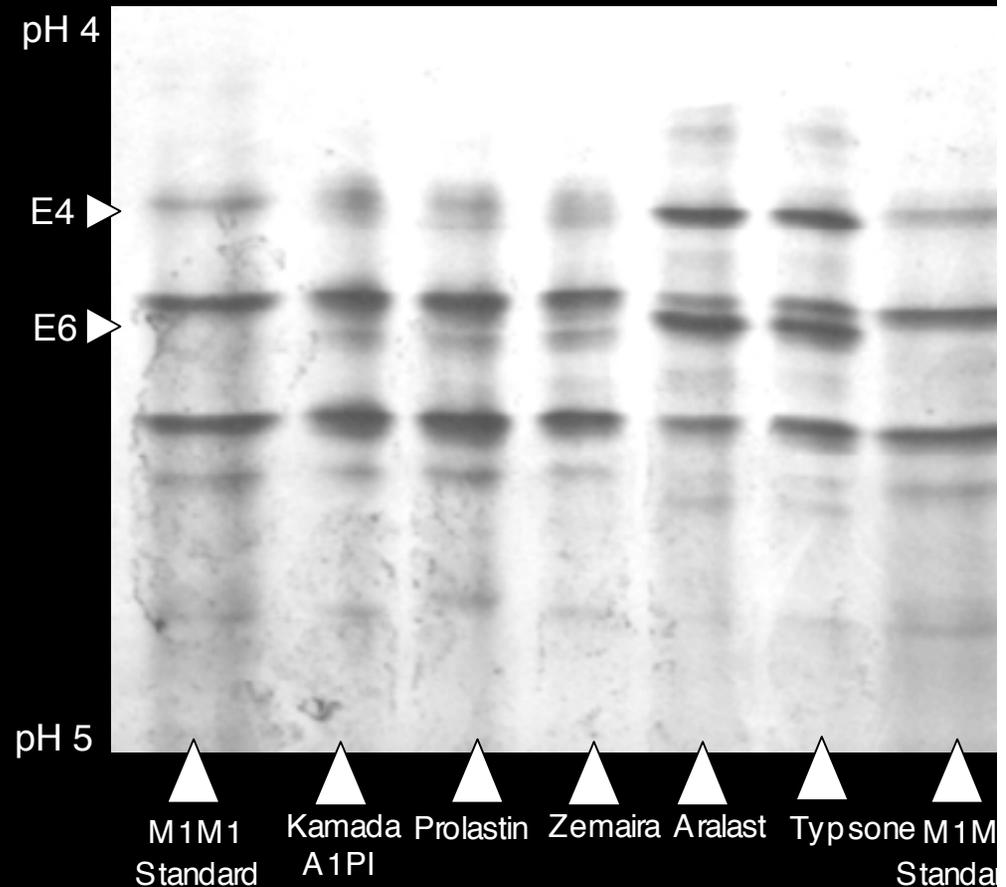
# Lys 394 Before and After Protease Cleavage of Reactive Site Loop

---



# IEF Comparison of Several Manufactures of Plasma Purified AAT

- No product is identical to native AAT
- Greater than 65% of Trypsone & Aralast is modified AAT variant
- Prolastin and Zemaira have between 2-6% modified AAT Variant
- Other modifications are known to occur in these products



# Is There Reason for Concern in Using a Modified Form AAT for Augmentation Therapy?

---

- Small clinical study established safety profile
- Modified Form of AAT
  - ~35-Fold more in Aralast compared to Prolastin
  - Potential of antigenicity because of the loss of surface amino acid
  - Charge difference may result in a different tissue distribution and/or clearance
  - Active site cleavage of the modified form creates a modified C-terminal fragment
- There is reason to believe that ATC did their animal studies using a different form of A1PI

# Summary

---

- Anodal variant is a modified form of AAT (truncated C-terminus) which occurs during purification
- At least 65% of AAT in Aralast is modified form
- Truncation of AAT may alter
  - antigenicity
  - tissue distribution/clearance
  - stability and/activity of the reactive site loop
- This modified form of AAT may or may not have all functional properties of native AAT
- Careful monitoring of patients taking this group of products is warranted