LC-MS Confirmation of Single Amino Acid Correction by Base Editing

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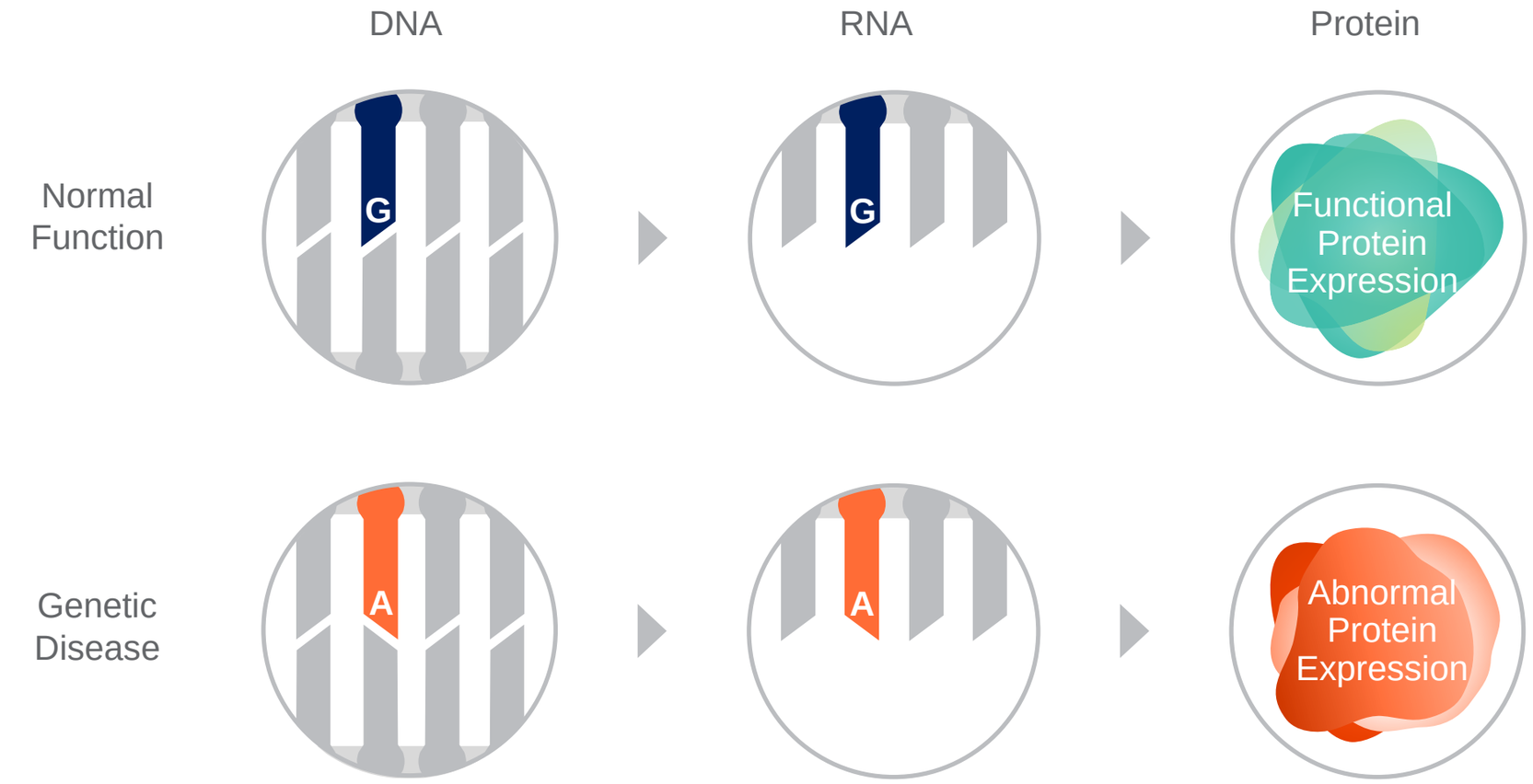
DISCLOSURE

- I am a Beam employee and shareholder

Base Editing: direct conversion of one base pair to another at a target location, without double-stranded breaks

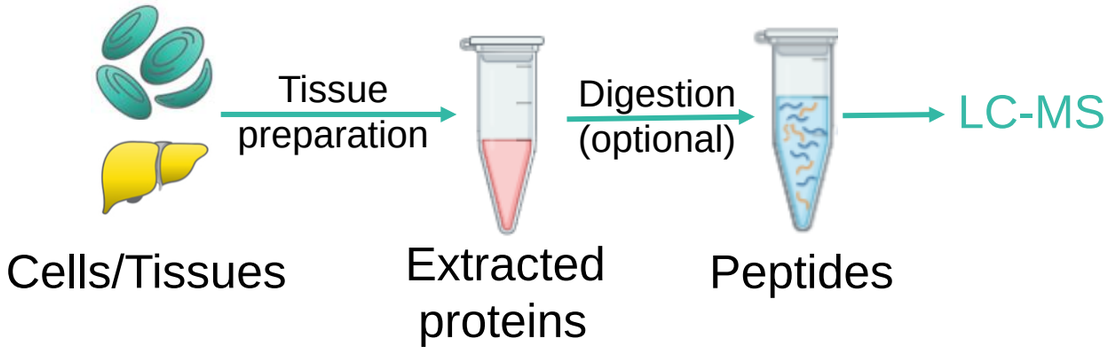
>3 Billion bases (A, G, C, T) in the human genomic code

Even **a single letter can be the difference** between health and disease



Mass spectrometry offers unique solutions for confirming single amino acid mutations

Option 1: Liquid chromatography mass spectrometry (LC-MS) - *Recommended*



PRO:
- No requirement on antibody availability
- Qualitative and quantitative measurement
- Streamlined method development strategy for different programs

CON:
More complex sample preparation workflow

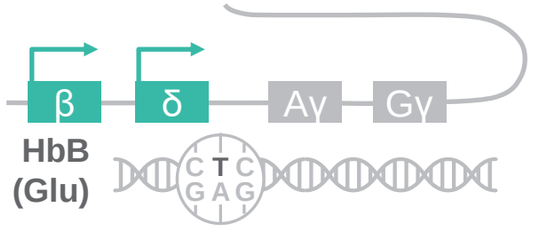
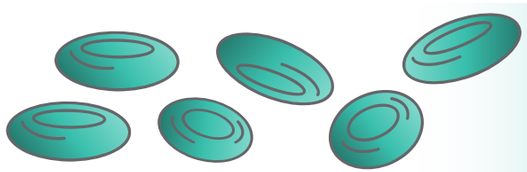
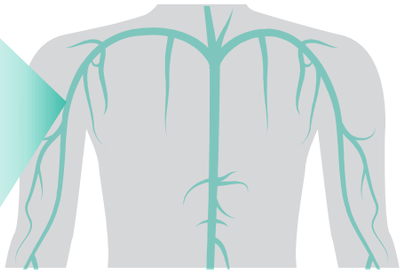
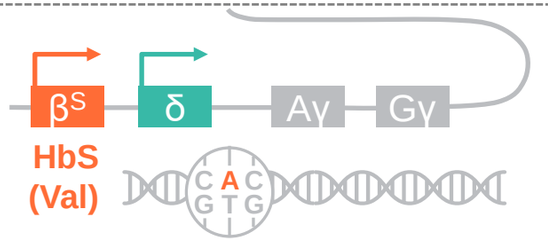
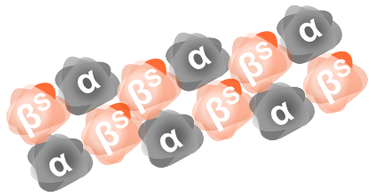
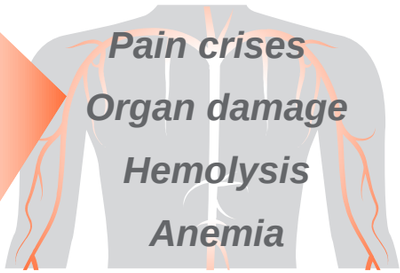
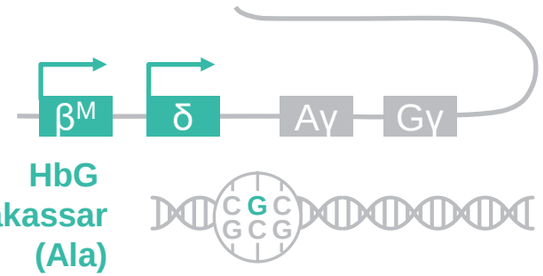
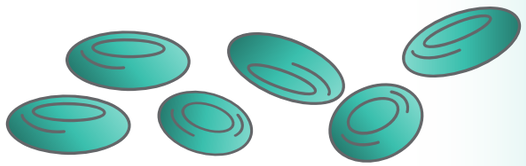
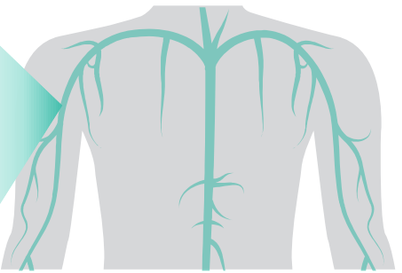
Option 2: Western Blot or ELISA

PRO:
Easier method development, transfer & qualification.

CON:
Depends on the availability of antibodies which can recognize single amino acid mutations

Additional requirement for the assay:
Accurate and precise
High sensitivity
High specificity
Broad linear range
Suitable for R&D & GxP environments

Case study – 1: precise base editing of the sickle cell disease mutation

	Globin Genes	Hemoglobin Proteins	Red blood cells	System
Normal (Adult)	 After birth, γ-globin is repressed and β-globin is expressed	 Normal β-γ hemoglobin tetramers form	 Red blood cells from and function normally	
Sickle cell disease (Adult)	 After birth, γ-globin is repressed and sickle causing β^S-globin is expressed	 β^S-globin tetramers form polymers under hypoxia	 Polymers cause red blood cells to sickle under hypoxia	 ***Pain crises*** ***Organ damage*** ***Hemolysis*** ***Anemia***
Naturally occurring Makassar variant		 HbG-Makassar functions like wild-type β-globin	 Red blood cells with HbG-Makassar have normal function	

Blackwell 1970; Pagnier 1993; Prehu 1999; Viprakasit, 2002

Mass spectrometry strategies for confirming V6A editing

1a. Bottom-up peptide mapping

- Confirm single amino acid correction
- High quantitation accuracy

1b. Intact LC-UV-MS

- Globin quantitation and molecular weight analysis
- Easy setup with fast data turn-around time

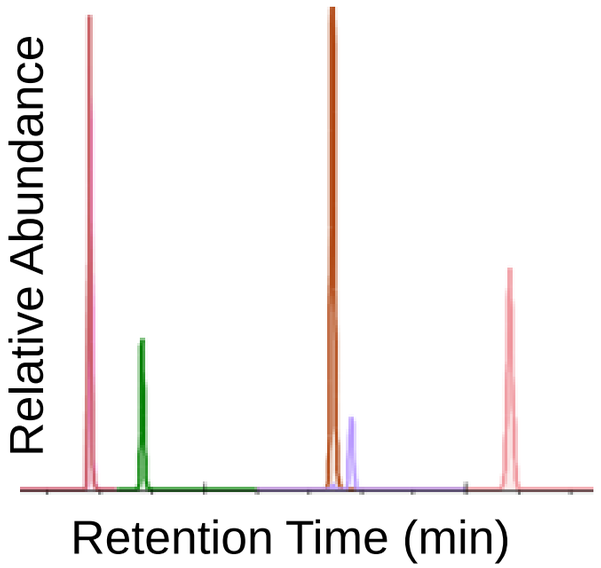
1c. Top-down MS/MS

- Preliminary structure elucidation
- Identify unknowns with limited sample consumption

1d. Tetramer formation native MS

- Identification of globin tetramers
- Quick readout with qualitative assessment

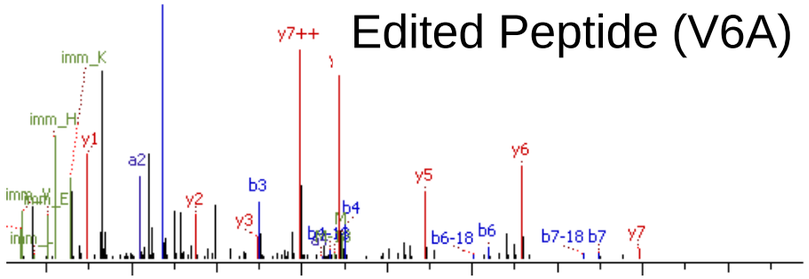
1a. Quantify multiple peptides/proteins in the same run



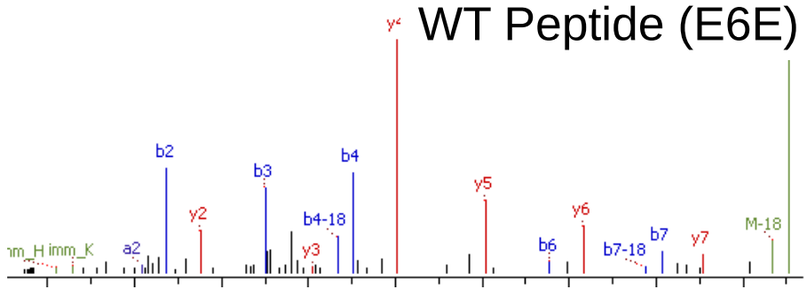
- Edited Peptide (V6A)
- WT Peptide (E6E)
- Sickle Peptide (E6V)
- Additional Peptide - 1
- Additional Peptide - 2
- Additional Peptide - 3

MS/MS confirmation of editing at the single amino acid level

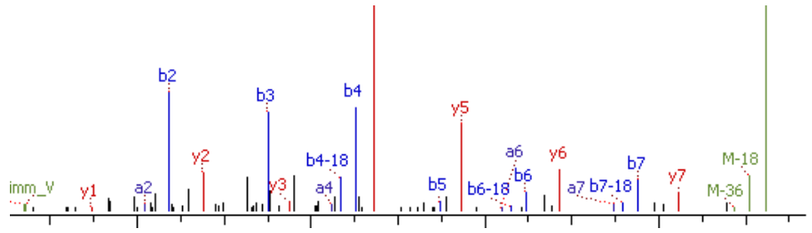
Edited Peptide (V6A)



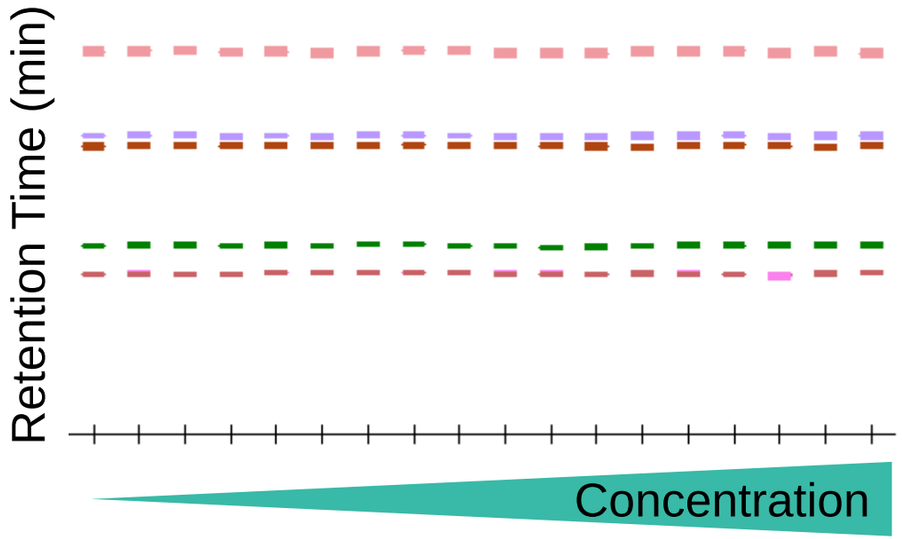
WT Peptide (E6E)



Sickle Peptide (E6V)



Reproducible retention time across ~ 3 orders of magnitudes

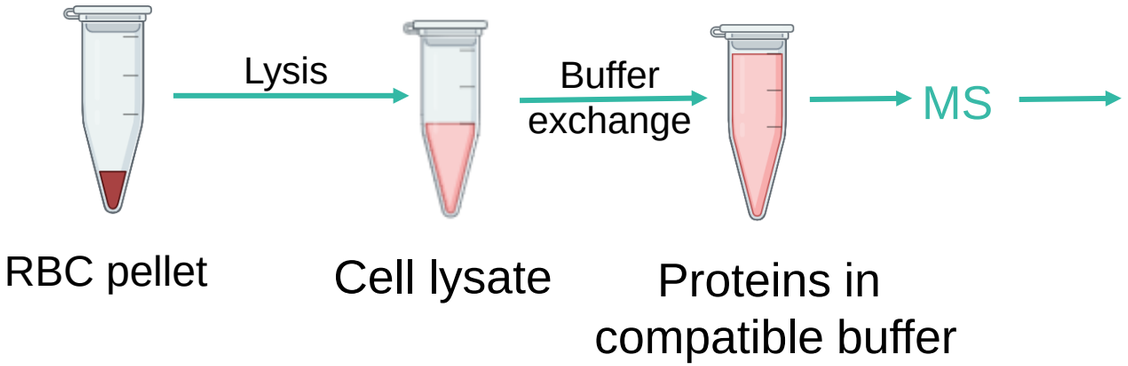


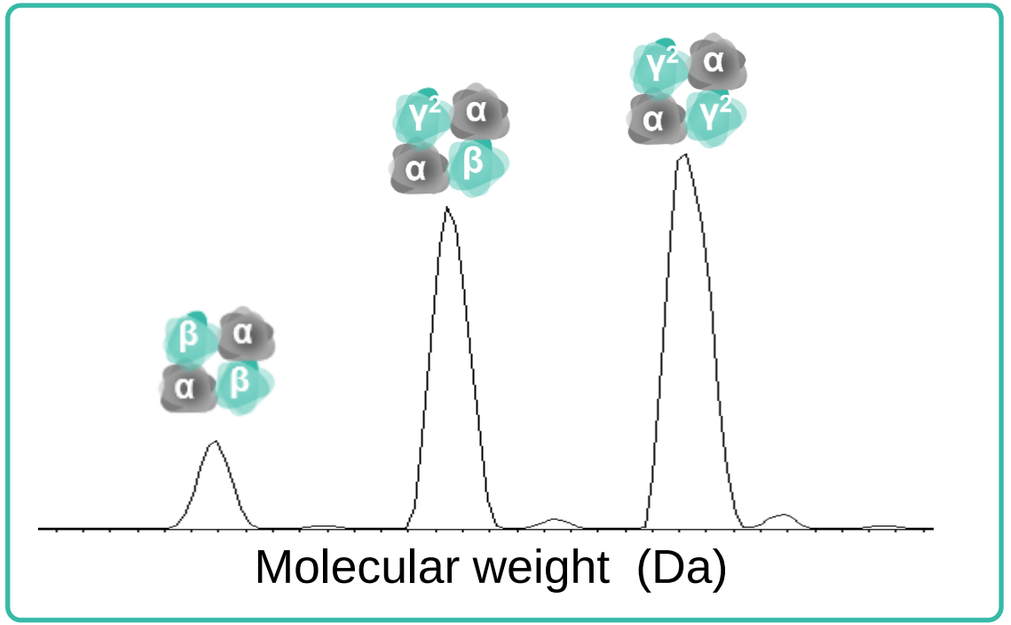
%CVs of peak area in replicate runs: ≤ 5%

Wide linear range with $R^2 > 0.99$

Low sample consumption

1d. Tetramer formation native MS





PRO:

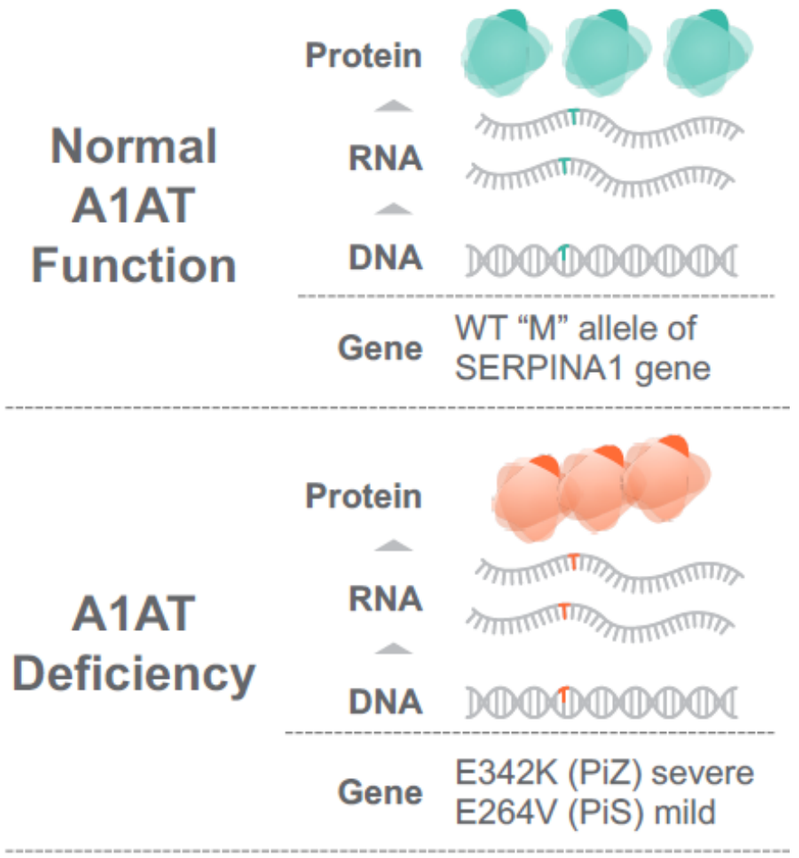
- Confirm non-covalent complex (such as protein-protein interaction)
- Confirm hybrid tetramer species
- Straight-forward sample preparation

CON:

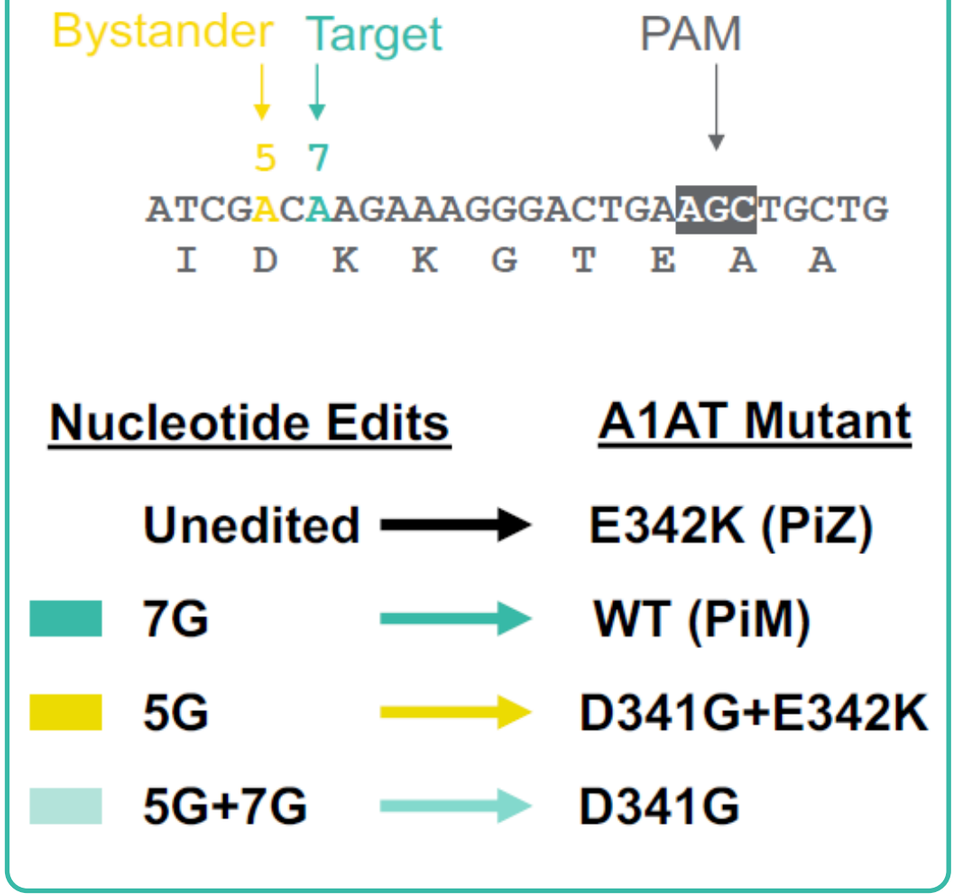
- Challenges in method development

Case Study 2. Confirmation of base editing correction of the disease-causing mutation in Alpha-1 Antitrypsin Deficiency by LC-MS/MS

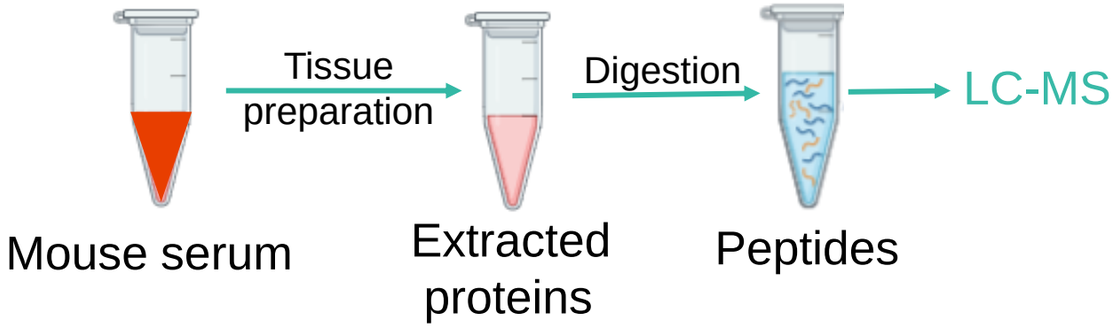
Disease mechanism



Editing Strategy



2. Targeted proteomics for A1AT proteoform quantitation in serum by LC-MS

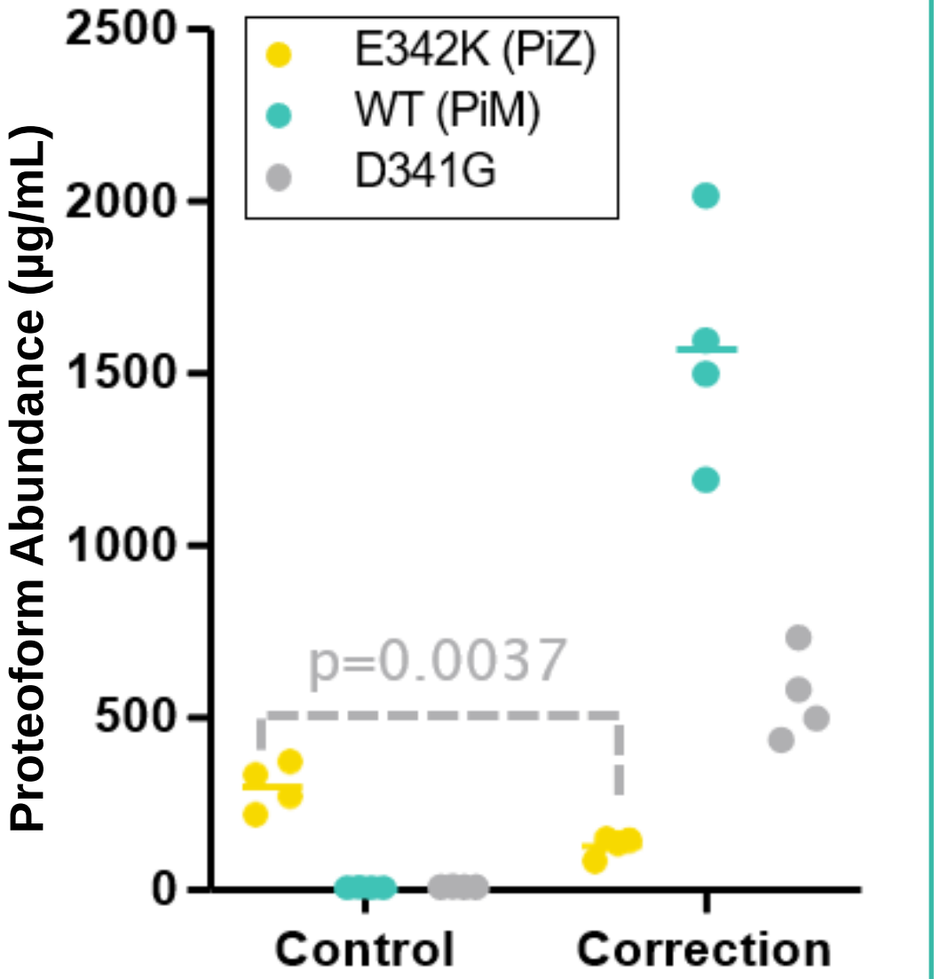


PRO:

- Quantify multiple proteins without requirement of antibodies
- Method can be easily modified for different species, such as mouse, rat, etc.
- Wide linear range (> 4 orders of magnitude, R^2=0.99)

CON:

- Instrumentation not widely available at CRO/CDMO for assay transfer/qualification



Summary

- Mass spectrometry offers novel and unique solutions for base editing research and development
- Taken together, our results highlight the depth of our understanding of base editing and confirm specificity and efficacy of our base editors in both ex vivo and in vivo programs

Thank you!

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