# Developing a Disulfide Replacement Picture of APOBEC3G

Mitch Biermann and Dr. Hiroshi Matuso, BMBB, University of Minnesota

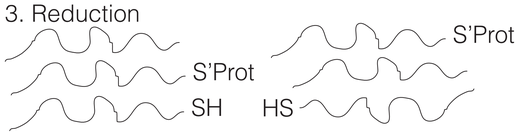
Structure
matsuo lab

## Concept

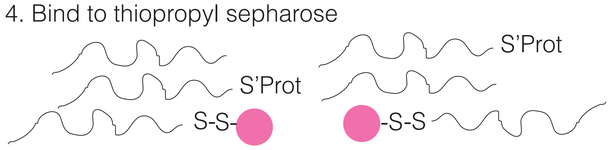
1. Mixture of Peptides


2. Protection of free thiols

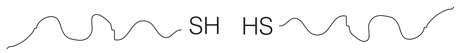

3. Reduction



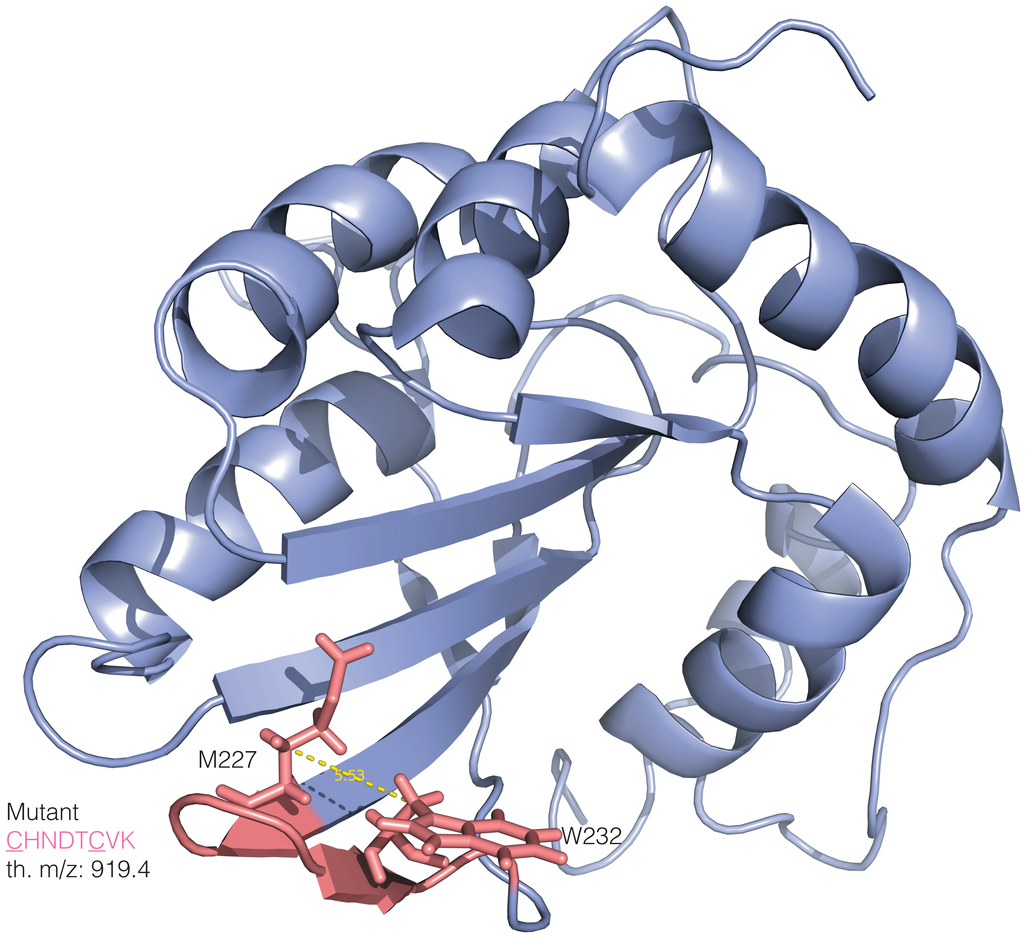

4. Bind to thiopropyl sepharose



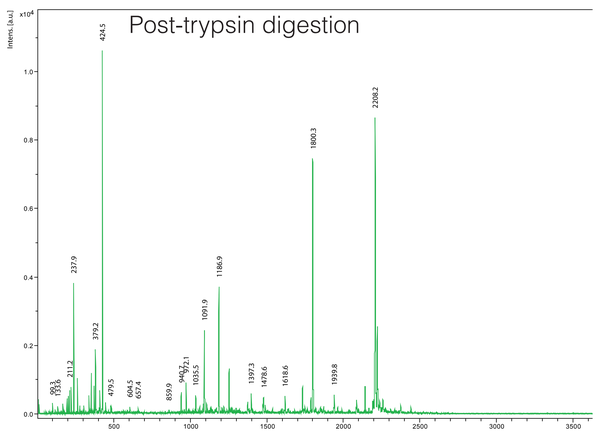

5. Wash and elute

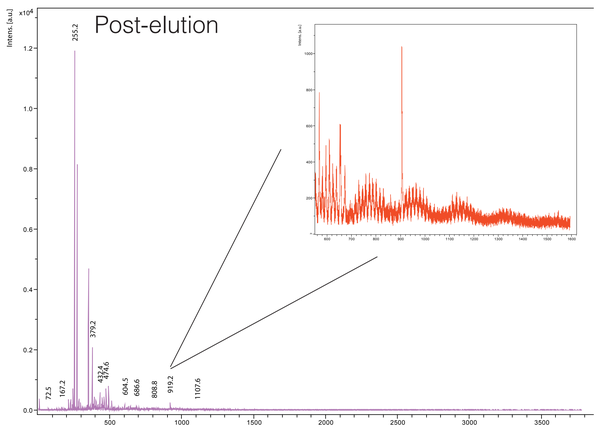



## First application




Residues 227 and 232 of the Ctd of A3G(2K3A) were mutated to cysteine. The C beta-C beta distance from 227 to 232 was 5.53 angstroms based our lab's most recent NMR structure (2KEM). The protein was digested, and a small amount eluted from the thiopropyl sepharose, suggesting C227 and C232 formed a disulfide bond.

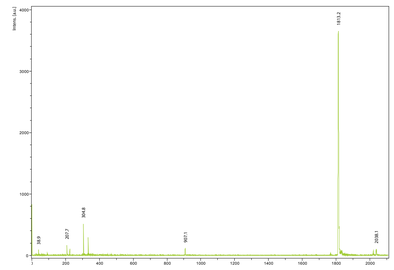


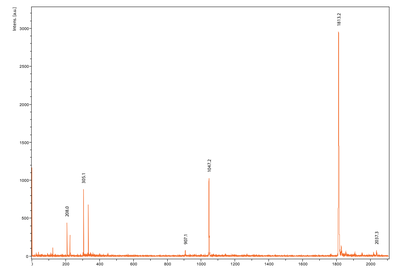

## Optimization

The oxidized (mw: 1813.2) and reduced (mw: 907.1) forms of the commercial peptide HCKFWW were separated and detected with mass spectrometry.

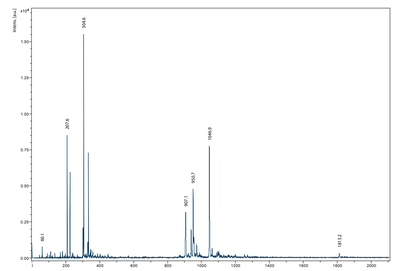
Starting peptide



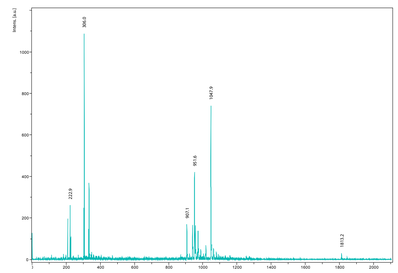

Partial reduction, followed by protection



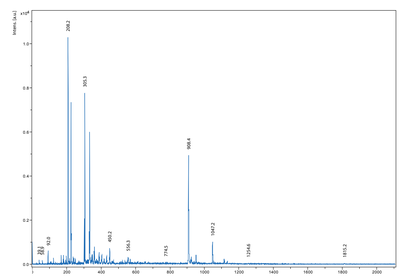

Complete reduction




Flow-through from thiopropyl sepharose column




Elution from column




## Summary

The human protein APOBEC3G (A3G) interferes with HIV infection by acting as a cytidine deaminase, an enzyme that induces numerous mutations in HIV's genetic material that ultimately destroy it. But A3G is only successful at this for a time. The HIV protein viral infectivity factor (Vif) destroys A3G. Developing a way to mask A3G from Vif is a major therapeutic goal. Uncovering the three-dimensional structure of A3G is crucial to rational drug design. The catalytic C-terminal domain of A3G has been solved, but the crucial Vif-interacting N-terminal domain remains invisible to medicinal chemists. A major obstacle toward this goal is the N-terminal domain's poor solubility. Here we explore a novel technique, disulfide replacement, in which pairs of cysteine residues are incorporated into the protein at hypothetically close positions and checked for disulfide bonding. We isolated a model peptide containing a disulfide bond from its reduced form, and we observed an engineered disulfide from the Ctd of A3G at two residues known to be spatially close. However, the sensitivity of the approach in digested peptide samples must be improved. We would like to acknowledge Yongjian Lu, Takahide Kono, the Chemistry Department Mass Spectrometry Facility, and all the other members of the Matsuo lab for their support.