UAB STRUCTURAL PROTEOMICS RESOURCE

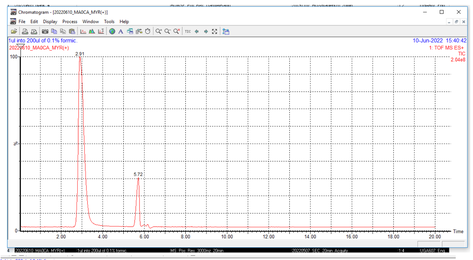
Director – Peter Prevelige Ph.D.

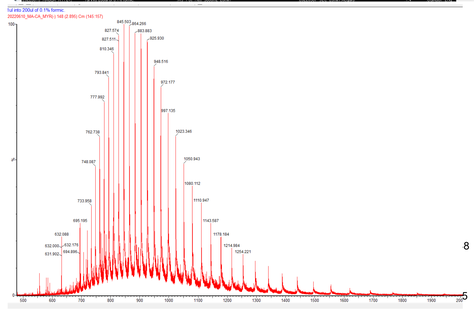
Intact Protein Mass Determination

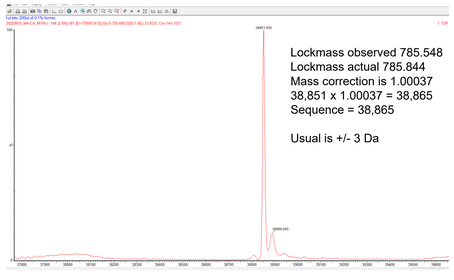
check for sequence, PTM, protolysis

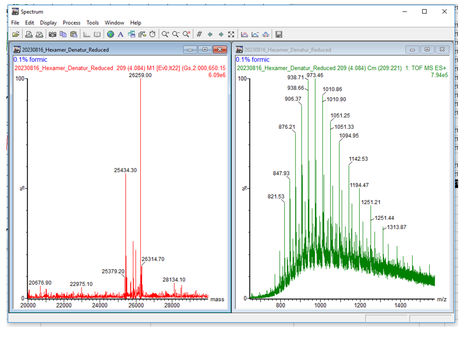
5-10 ul of protein
1-5 uM concentration

Desalt sample over small pore SEC column in 0.1% formic acid (pH 2.5)
Protein is denatured – becomes highly (stochastically) charged
non-covalent ligands are lost









Native Mass Spec

determine stoichiometry, detect ligand binding

5 ml @ 1mM concentration
Desalt into 20-200 mM ammonium acetate
low electrospray voltage
low collision energy
High m/z ToF instruments – 100,000 m/z

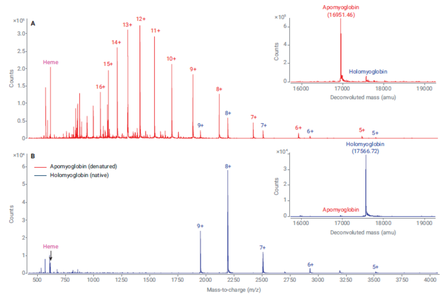
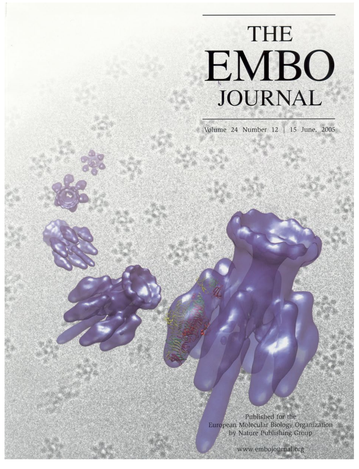
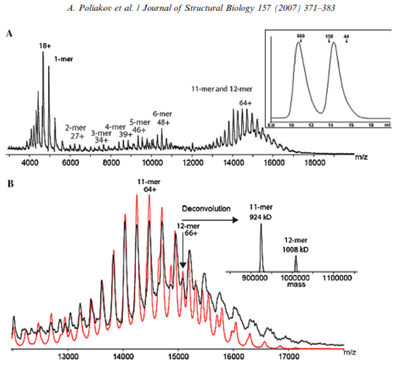


Figure 2. LC/MS analysis of intact myoglobin sample. A) Myoglobin sample was analyzed under denatured LC/MS conditions (previous studies). The heme group was dissociated from the protein complex and the majority of the protein was apomyoglobin (inset figure). B) Native MS analysis of myoglobin. The holomyoglobin (with heme) structure was preserved and only trace amount of heme was detected.



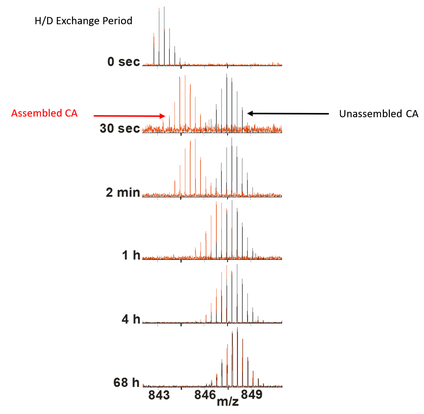


Hydrogen-Deuterium Exchange

characterize changes in protein dynamics
due to ligand binding, mutations, polymerization

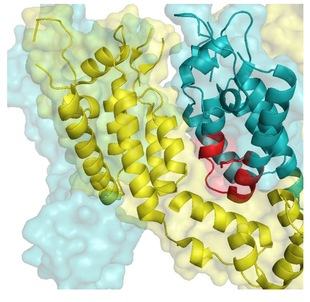
relatively pure protein (90%)
100 μl @ 10 μM

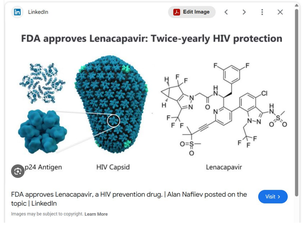
dilute into D2O buffer
sample over time
digest to 10-15 aa peptides
count deuterons
map onto structure



Lanman et al, J. Mol. Biol. 325:759 (2003)

Interface formed upon HIV Maturation





This resource is supported by the Integrative Structural Biology Center

"Mass Don't Lie"