# Top-down Analysis of Modifications on Cysteine 34 of Albumin in Human Serum Samples

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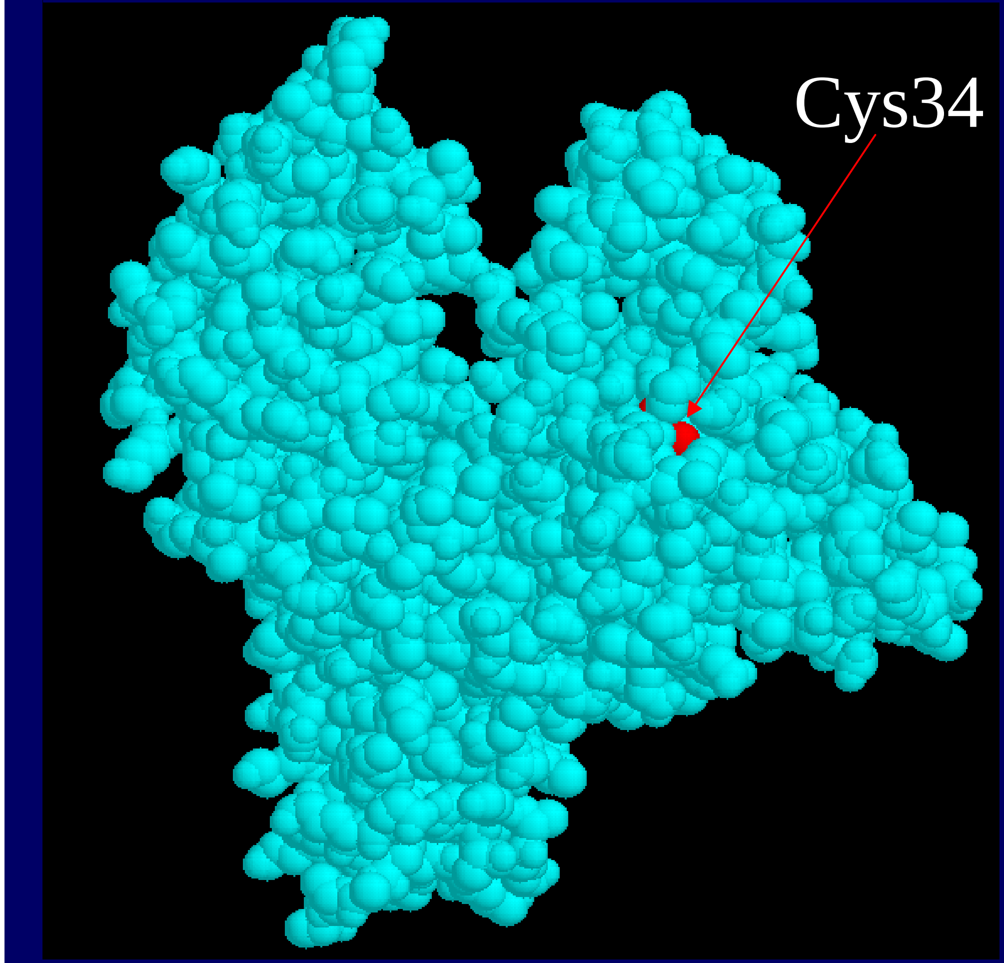
# Human Serum Albumin (HSA)



Generated with Protein Explorer

http://www.umass.edu/microbio/chime/pe_beta/pe/protexpl/frntdoo2.htm

- most abundant protein in serum
- 585 amino acid residues
- 35 cysteine residues:

17 disulfide bonds
+ 1 free cys34 with –SH group

# Modifications of –SH on Cys34 of HSA

- Thiols and disulfides in serum

  --- cysteine, homocysteine, cysteinylglycine and glutathione

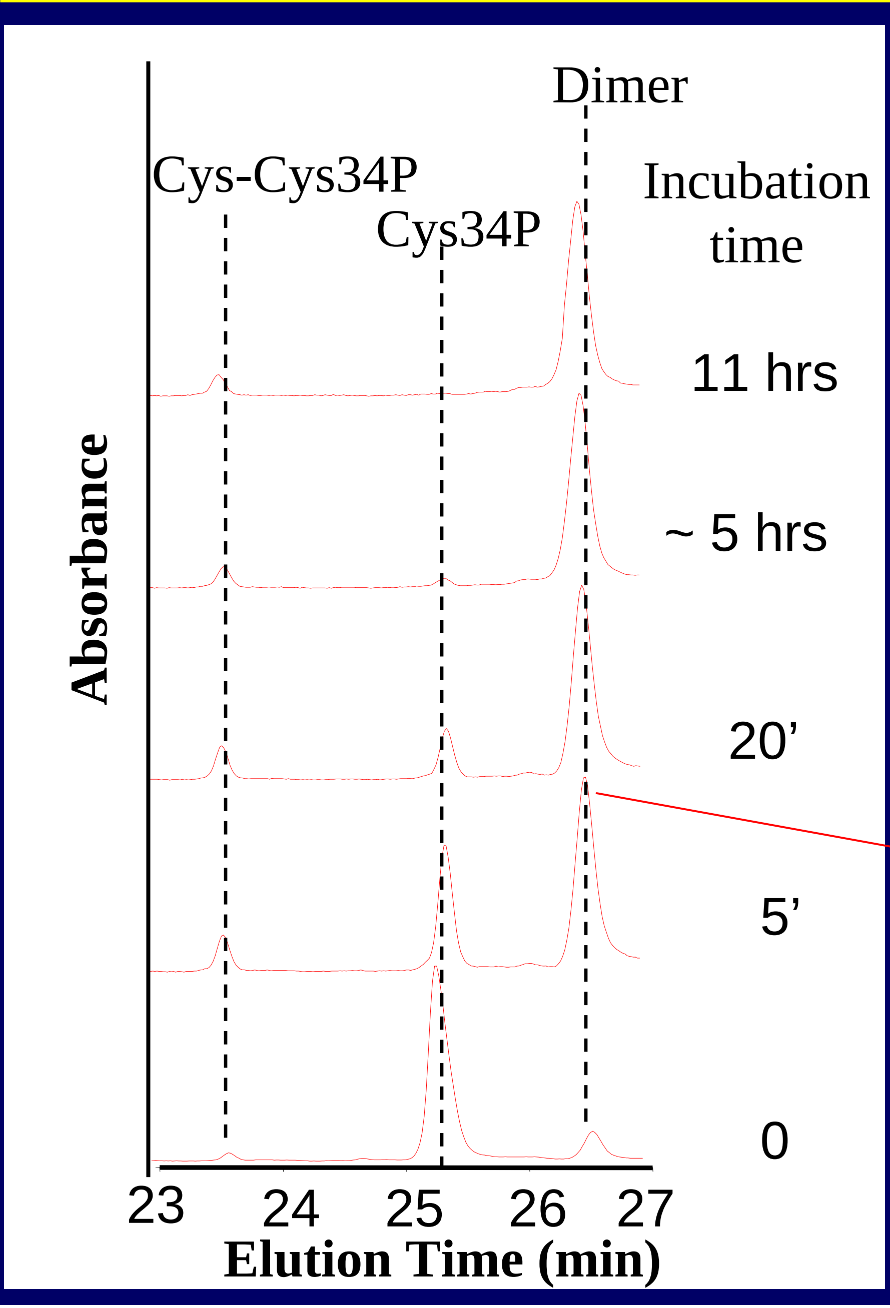
  --- disulfides formed by thiols
- Cys34 modified by thiols or disulfides

  --- maybe related to ischemia (restriction in blood supply )

  --- potential biomarker for acute coronary syndromes
- Study Method

  --- bottom-up & multiple reaction monitoring (MRM)

# Artificial Modification of Cys34 with Bottom-up Approach



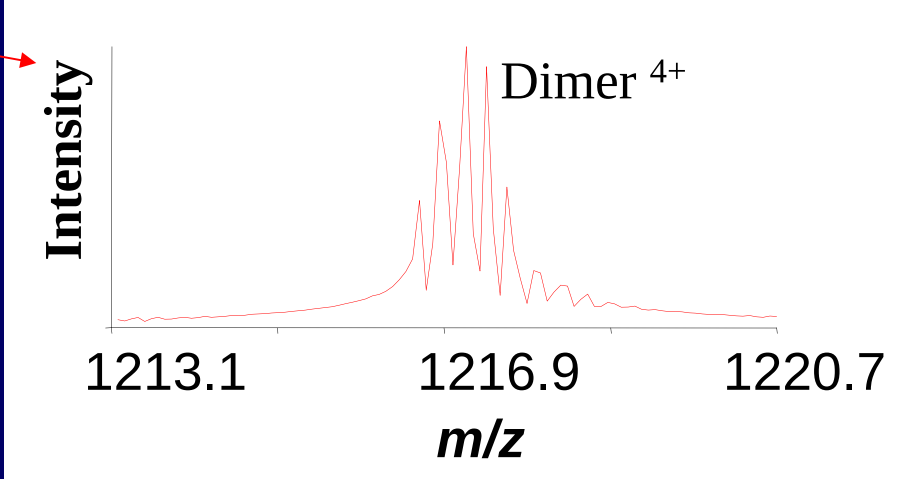
Mock serum digestion experiment:

600 µM synthetic
Cys34 tryptic peptide (Cys34P)

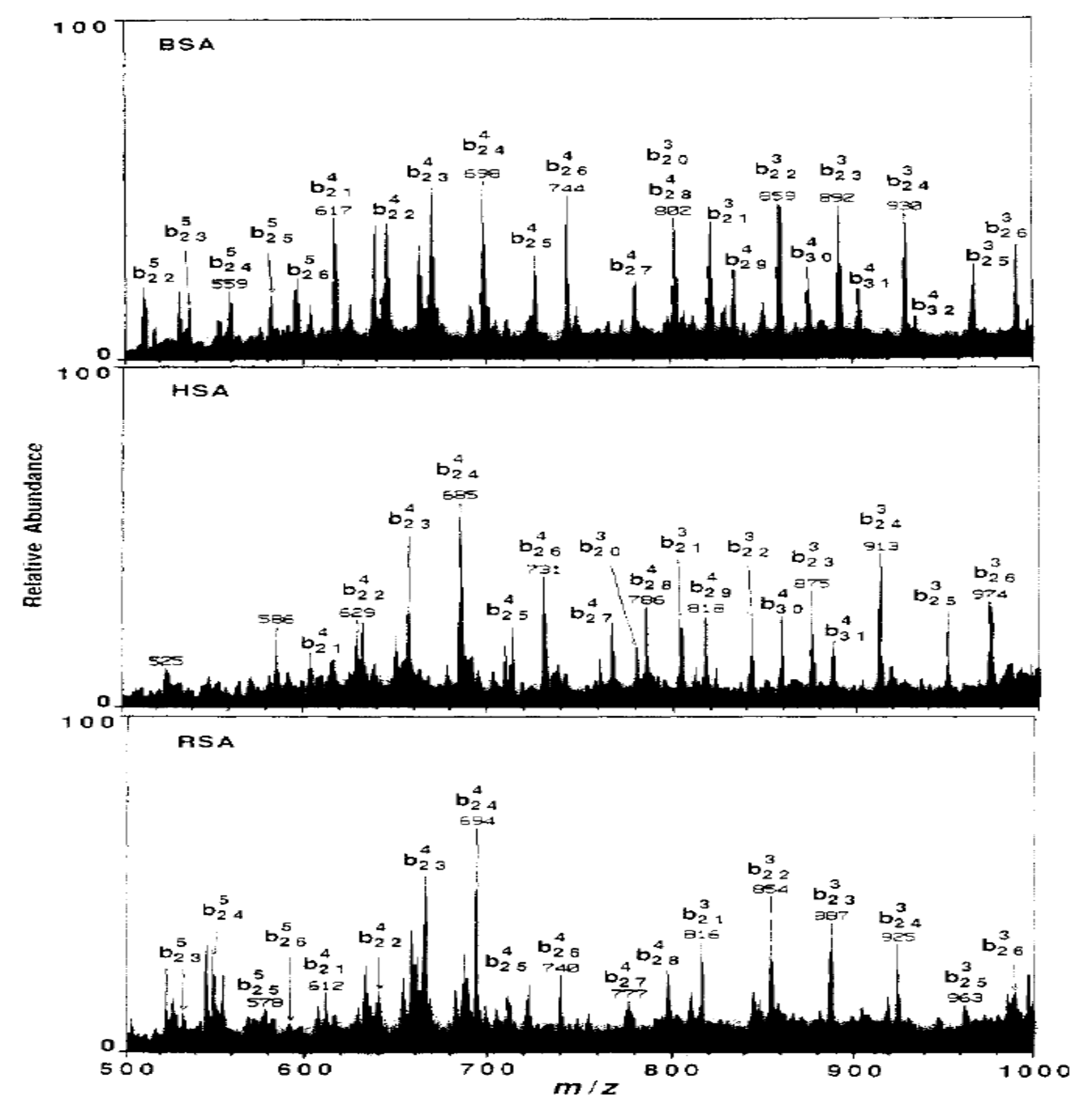
6 mM Cysteine

50 mM $NH_4HCO_3$
37°C

Cys-Cys34P + Cys34P-Cys34P (Dimer)
(Predominant)



# History of Top-down Analysis of Serum Albumin[1,2]



1. Smith RD et al, Anal Chem, 1990, 62, 882
2. Loo JA et al, Anal Chem, 1991, 63, 2488

# Top-down Approach

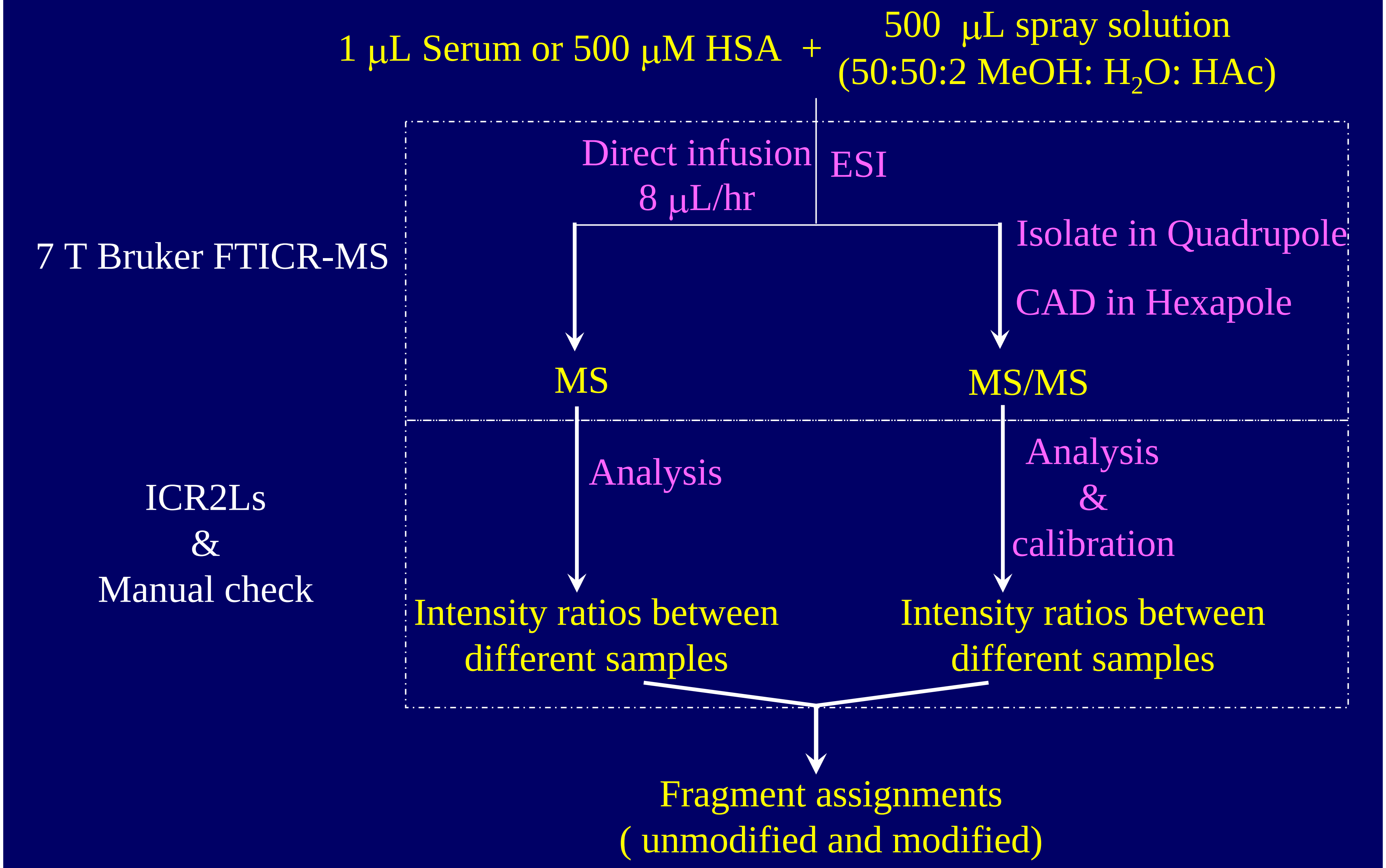
Advantages:

- Direct Analysis
  - --- fast
  - --- decreased artificial modifications
- 100% sequence coverage
- Easier to identify protein isoforms and mutations

Challenges:

- Low sensitivity
- Difficult fragment assignments
  - --- overlapping peaks
  - --- internal fragments
  - --- frequency shift due to space-charge effect

# Experimental



1 μL Serum or 500 μM HSA + 500 μL spray solution ($50:50:2$ MeOH: $H_2O$: HAc)

ESI

Direct infusion
8 μL/hr

7 T Bruker FTICR-MS

Isolate in Quadrupole

CAD in Hexapole

MS

MS/MS

ICR2Ls
&
Manual check

Analysis

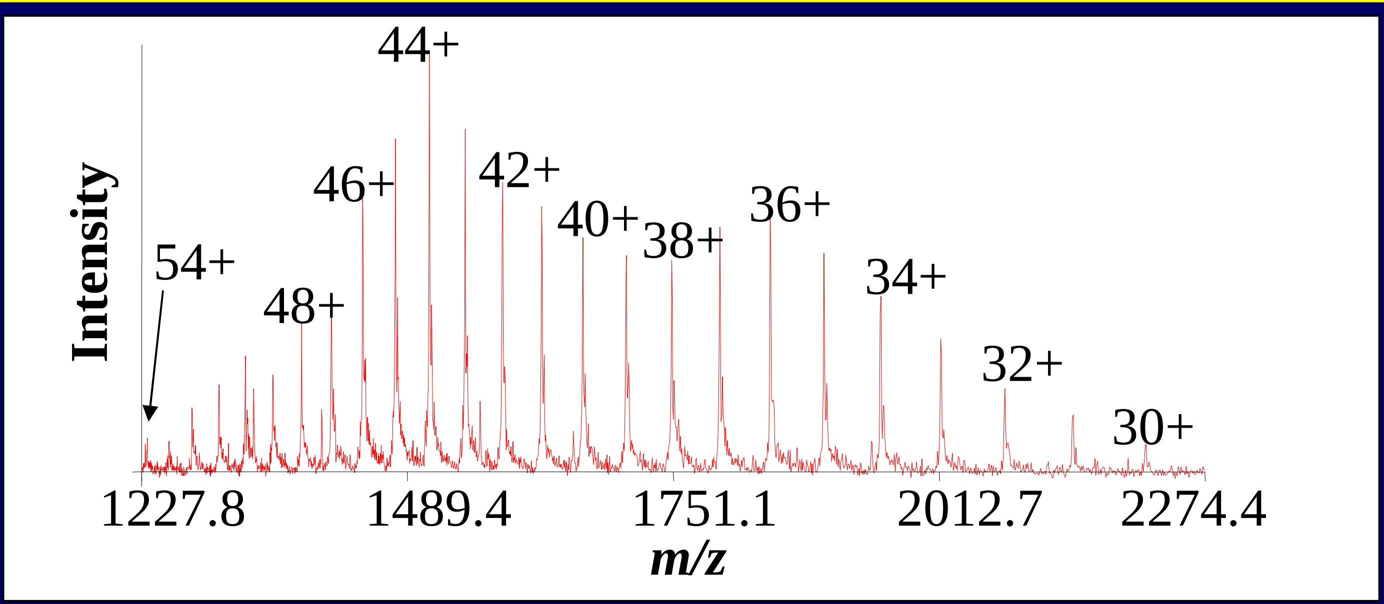
Analysis
&
calibration

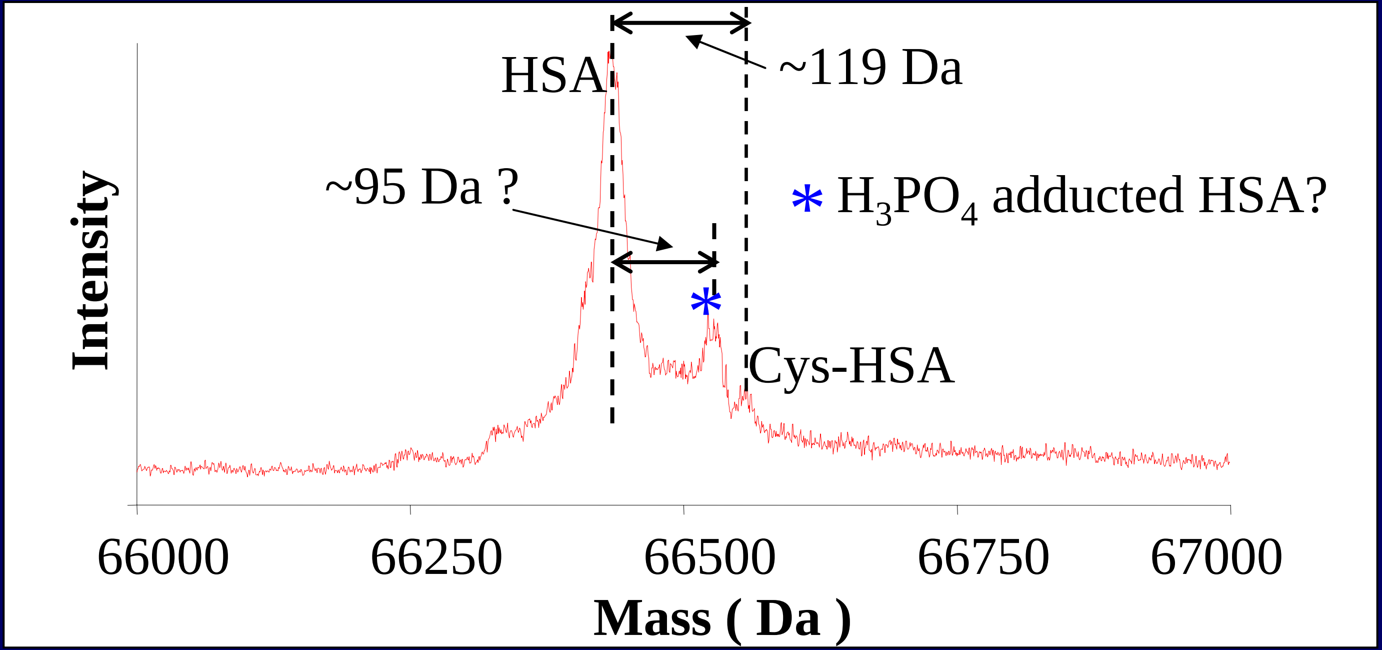
Intensity ratios between different samples

Intensity ratios between different samples

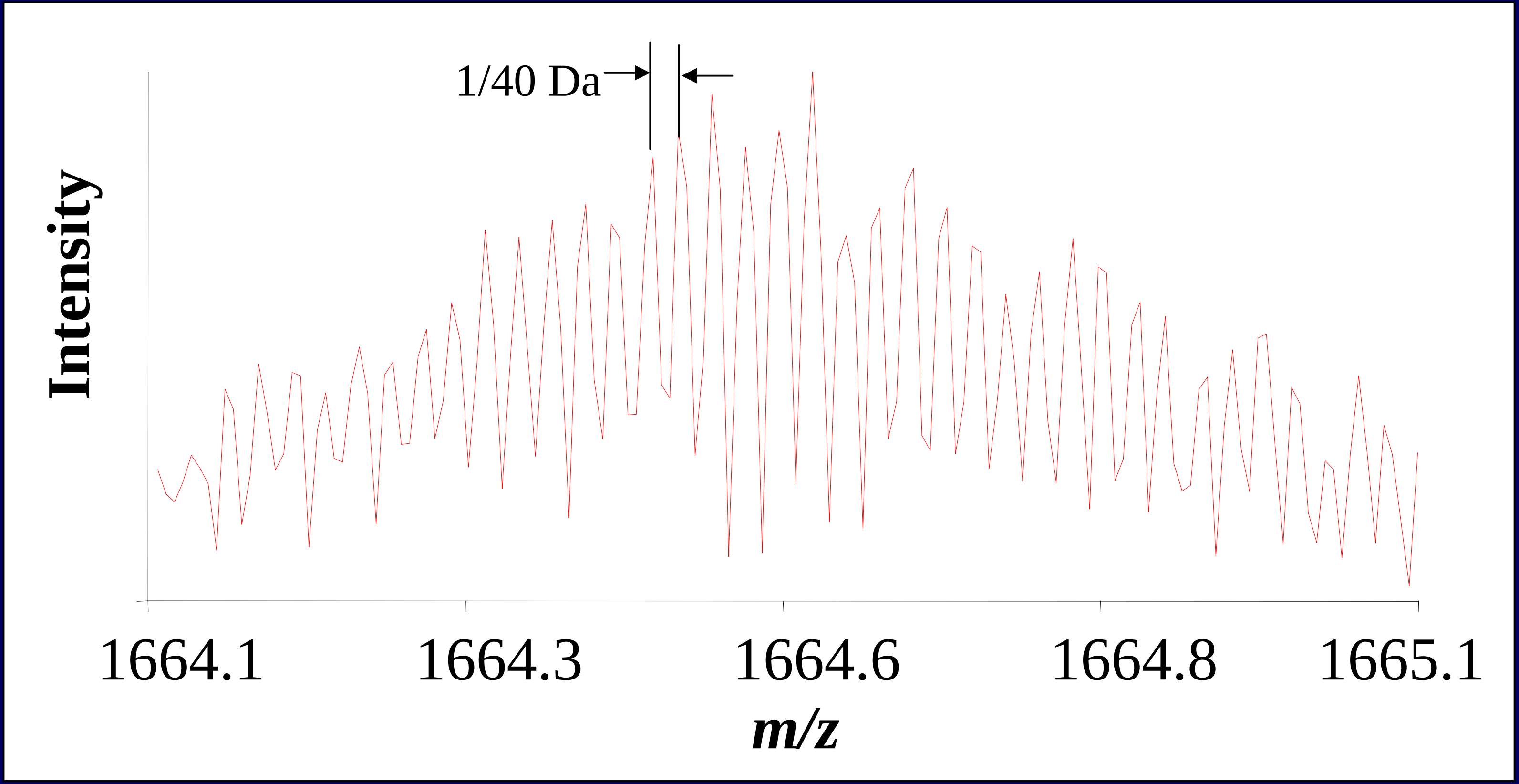
Fragment assignments
( unmodified and modified)

# MS Spectrum of HSA in Diluted Serum Sample



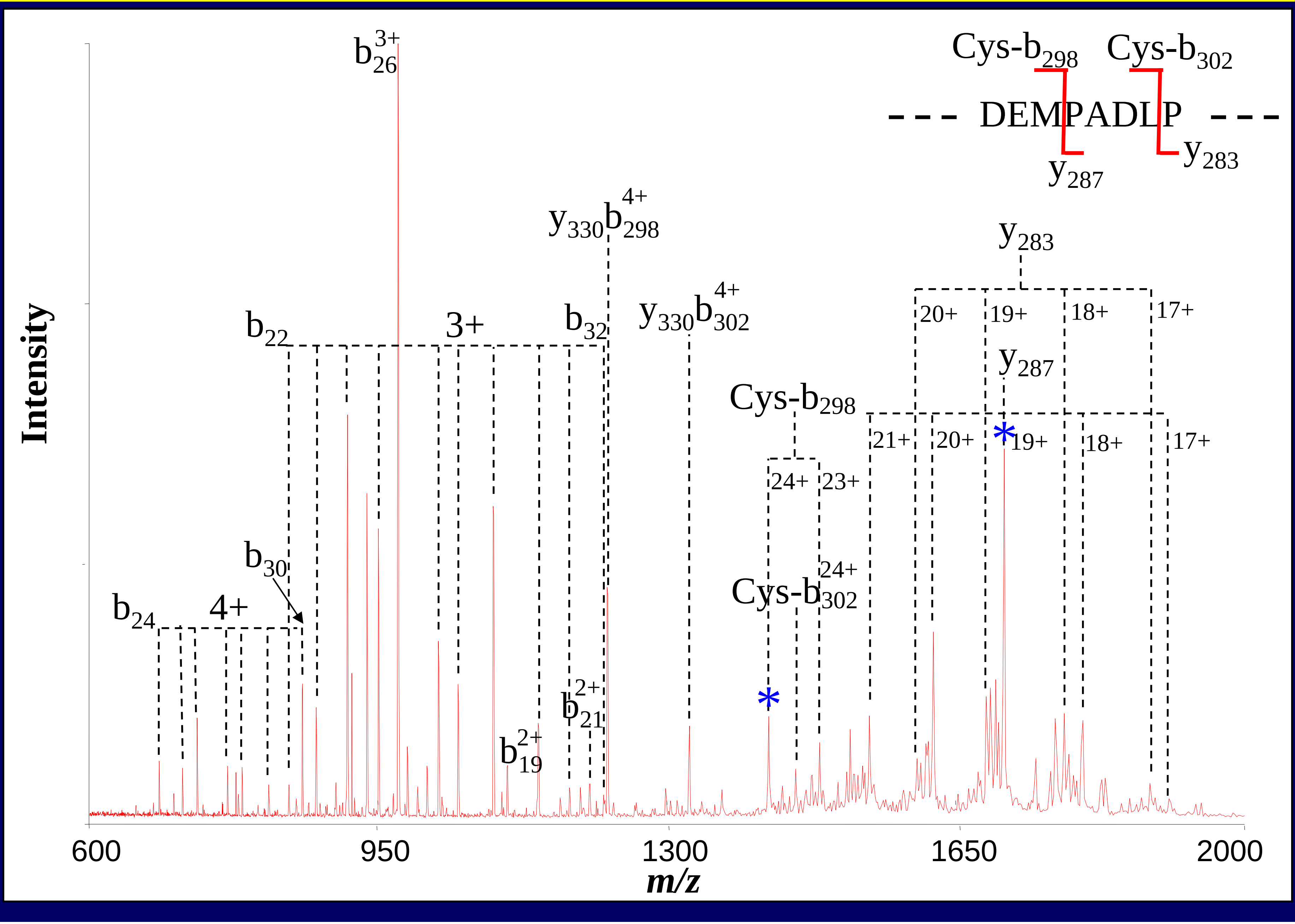


# Isotopic Resolution of Cys-HSA$^{40+}$ in Sigma HSA

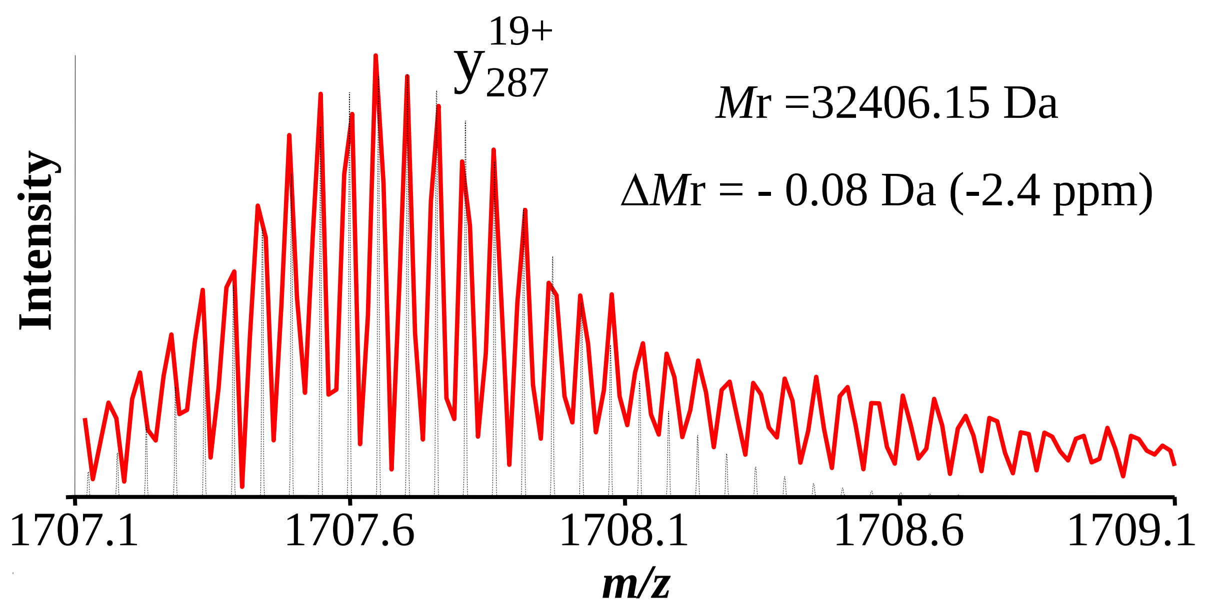


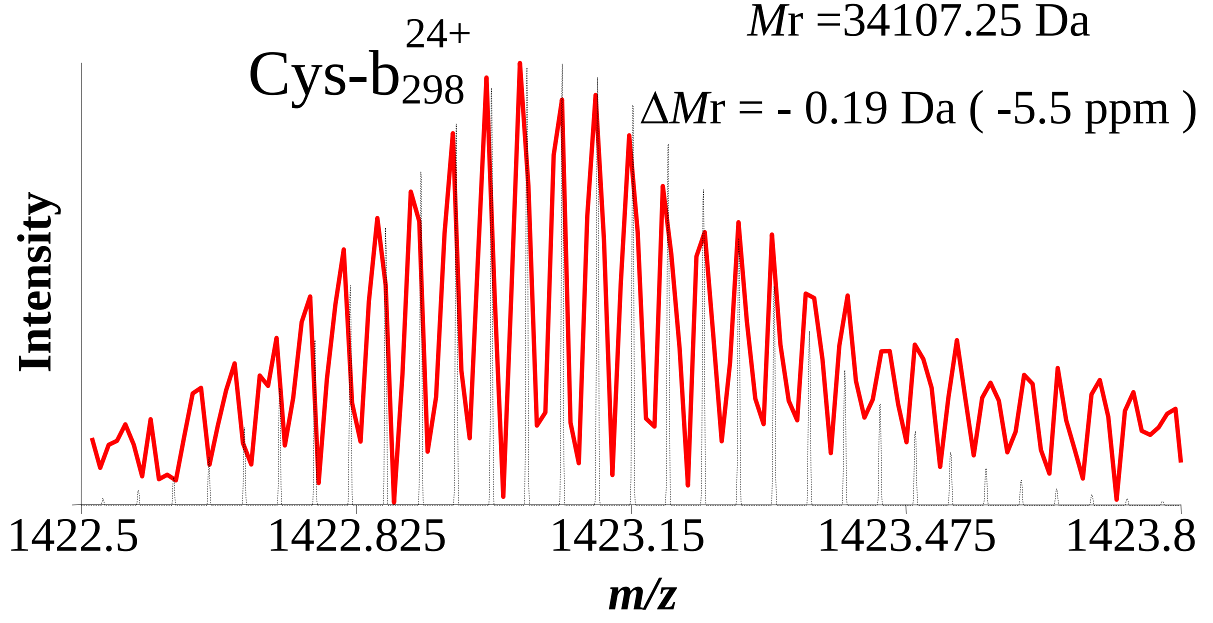
High resolution detection makes it possible to do top-down analysis.

# MS/MS Spectrum of Sigma HSA$^{44+}$



# Two Complementary Fragments of Sigma HSA $^{44+}$





Monoisotopic *M*r of Cys-HSA

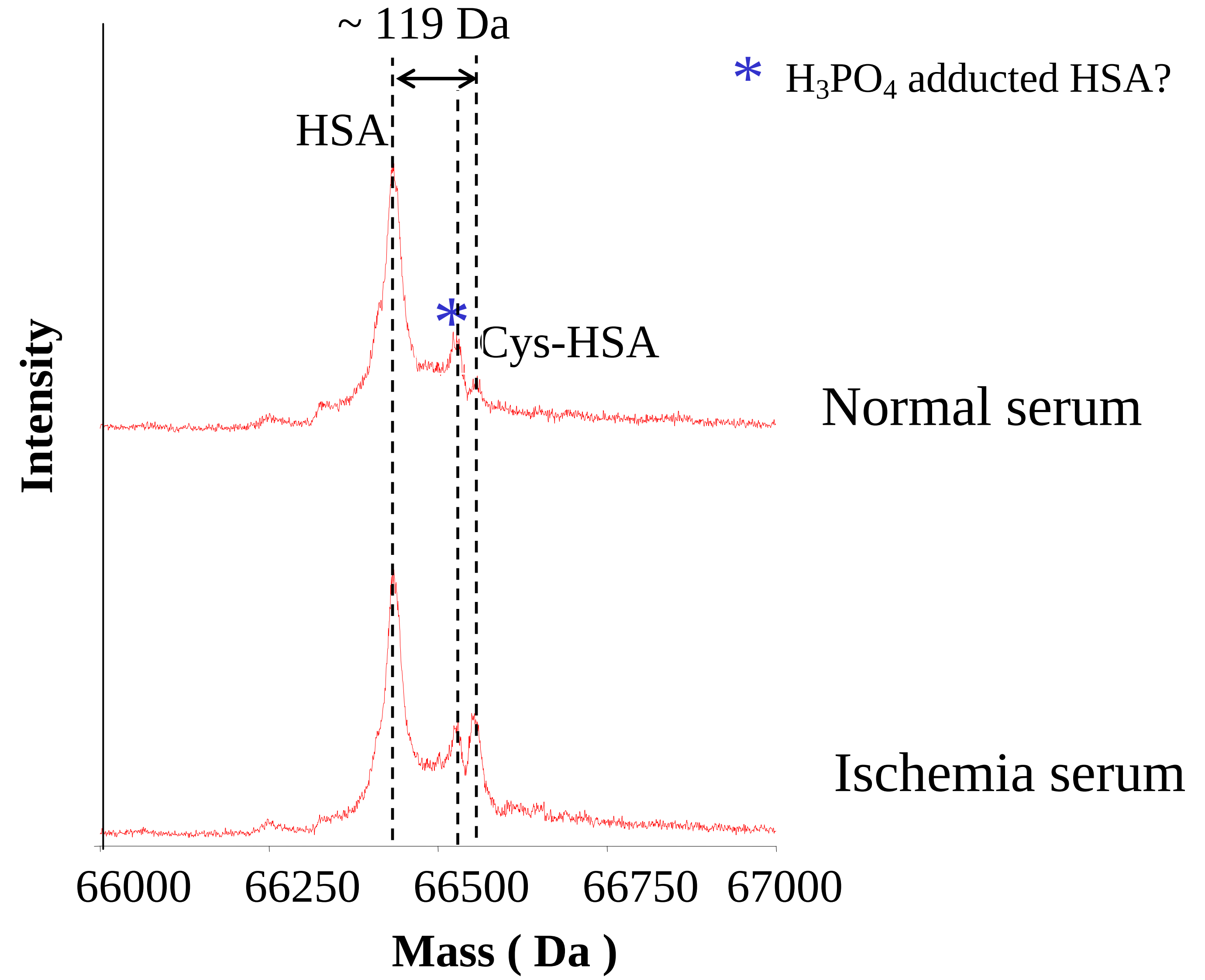
Experimental: 66513.40 Da

Theoretical: 66513.66 Da

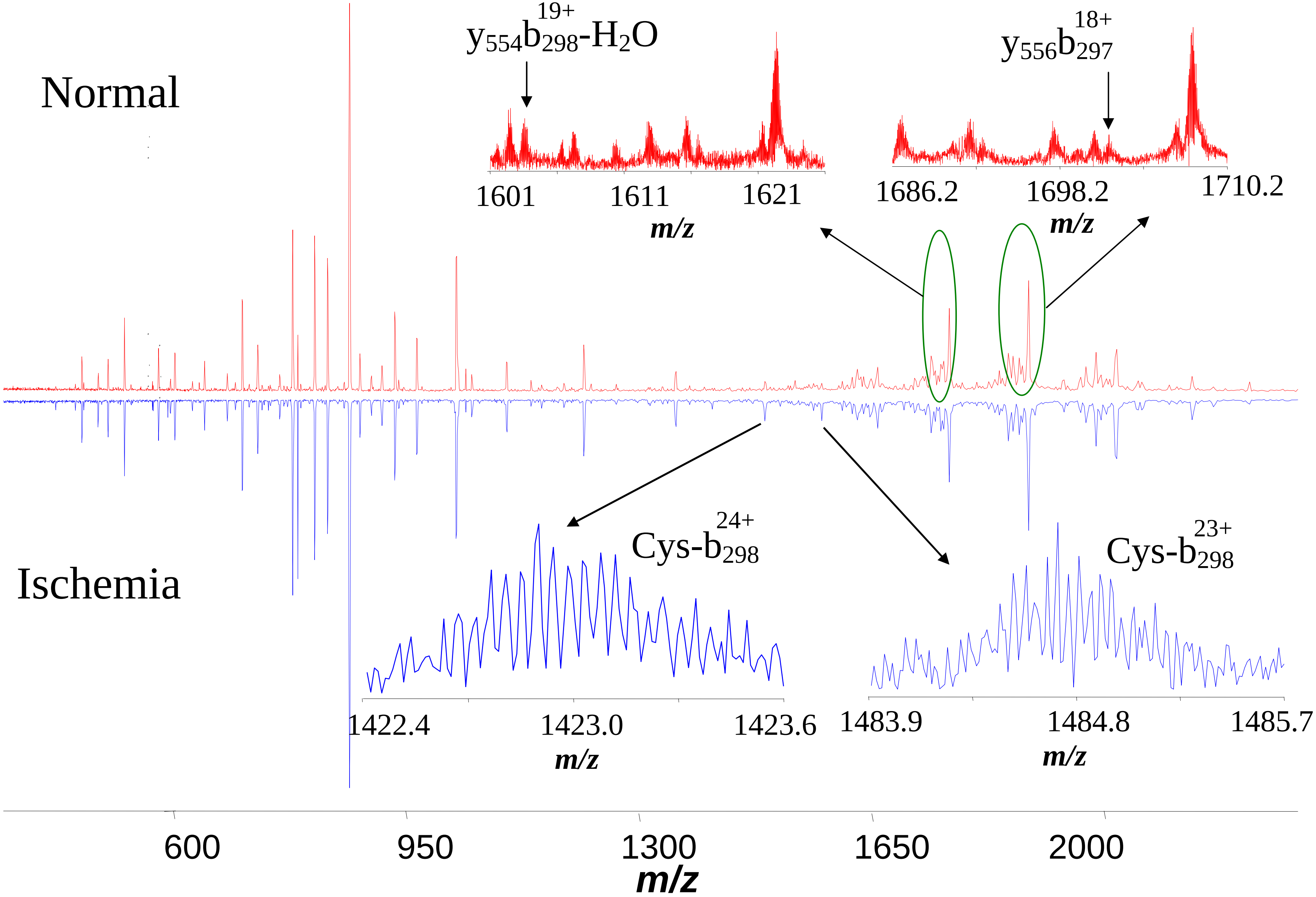
Δ*M*r = - 0.26 Da

- 4.4 ppm

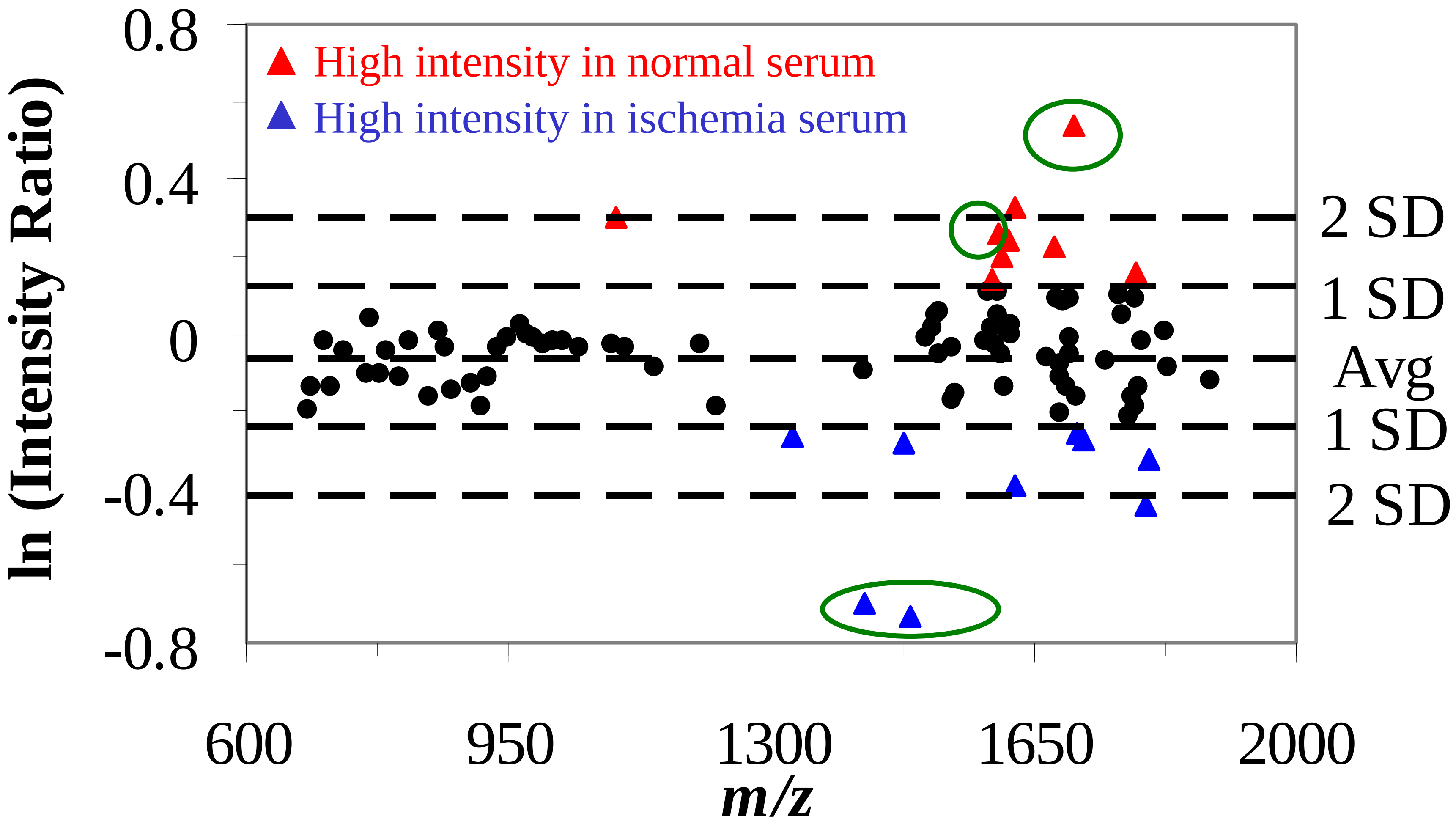
# Abundances of Different Forms of HSA in Serum Samples



# Cys34 Related HSA Fragments in Serum Samples



# Intensity Ratios of HSA Fragments in Serum Samples



# Summary

- Top-down approach is a more reliable method for the quantitation of modifications on Cys34 of HSA.
- Isotopic resolution and high mass accuracy MS/MS analysis of HSA were achieved for large fragments.
- Relative intensities of the fragments for different samples help assign the fragments or confirm the assignments.
- Modified and/or unmodified fragments may be used for relative quantitation.

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