

Preliminary ICAT Study on 1.5 pmol (Injected) of a 1:2 (d₀:d₈) Derivatized Sample of BSA

- **Aliquots of BSA were derivatized with either d₀ or d₈-ICAT reagent.**
- **They were then mixed in a 1:2 (d₀:d₈) ratio and digested with trypsin.**
- **The trypsin digest was step-eluted from a cation exchange cartridge.**
- **LC/MS analysis carried out on a (on-loan) Micromass Q-Tof-API at a flow rate of 300 nl/min on a 75 micron x 15 cm Vydac C-18 column equilibrated with 0.5% acetic acid, 5% acetonitrile and eluted with a 60 min acetonitrile gradient.**
- **LC/MS scans were taken at a rate of one full scale MS scan/sec. *One of the 3,600 scans* from this LC/MS analysis is shown on the next page.**
- **Automated processing of all 3,600 scans using a beta test version of the Micromass MCAT software resulted in 6 ICAT tryptic peptide pairs being identified and quantified and one incorrectly assigned pair of ions (data not shown).**
- **The mean value for the 6 ICAT pairs was 0.59 ±0.047, which corresponds to an average precision of ±7.9% and "apparent" error of +18%.**

MS Scan from LC/MS of 1.5 pmol (Injected) of Tryptic Digest of 1:2 (d₀:d₈) Derivatized BSA

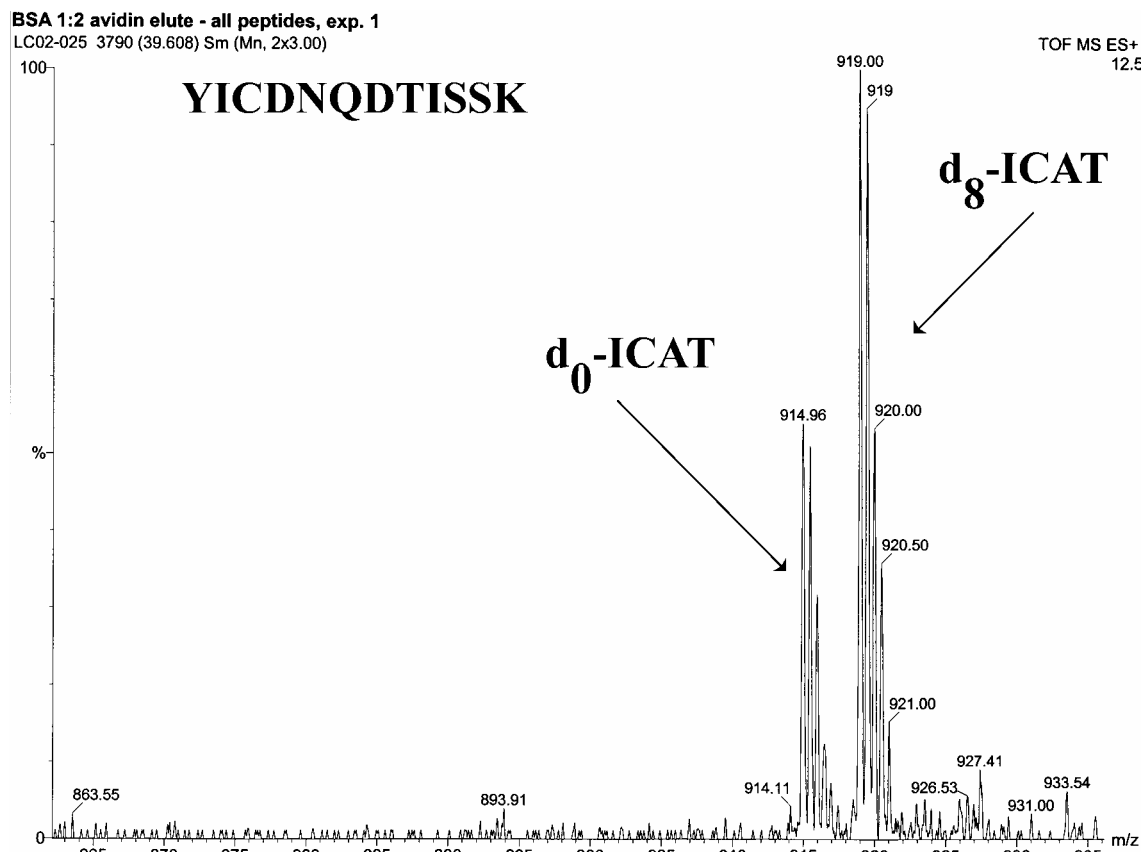


Table 1: First Keck LC/MS ICAT Study on a 1:2 (Light:Heavy) Isotope Derivatized (1.5 pmol) Sample of BSA

Light ICAT Peptide Mass	Heavy ICAT Peptide Mass	Charge	Light ICAT Isotope Area	Heavy ICAT Isotope Area	Ratio
546.244	550.264	2	57.75	93.79	0.62
610.255	612.936	3	93.39	132.7	0.70
718.788	722.813	2	43.42	82.76	0.52
572.28	576.304	2	120.35	207.2	0.58
914.921	918.94	2	19.65	37.08	0.53
572.281	576.304	2	125.16	212.41	0.59

Summary of Data from 6 Automatically Assigned ICAT Peptide Pairs

Expected Ratio	0.50
Observed Mean Ratio	0.59
Precision	±7.9%
Error	+18%