Surface Sampling with Electrospray Mass Spectrometry

Gary J. Van Berkel[1], Stephen J. Kennel[1], Mitchel J. Doktycz[1], Michael J. Ford[1], Amaury D. Sanchez[2], and J. Martin E. Quirke[2]

[1]Oak Ridge National Laboratory
[2]Florida International University, Miami FL

Overview

- Surface sampling electrospray ionization probe developed to read-out TLC plates, surface deposited proteins and affinity interaction captured material
- Experimental set-up optimized to accommodate 10 x 10 cm glass backed TLC plates and 2.5 x 7.5 cm glass slides
- Sampling operations enhanced through introduction of remote control sample platform
- Complete testing of the current probe design with respect to applications and operating conditions

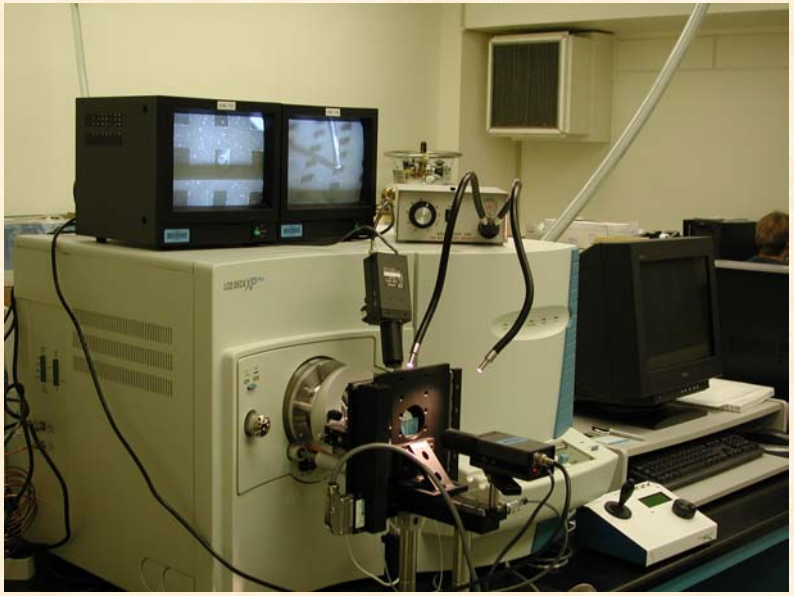
Introduction I

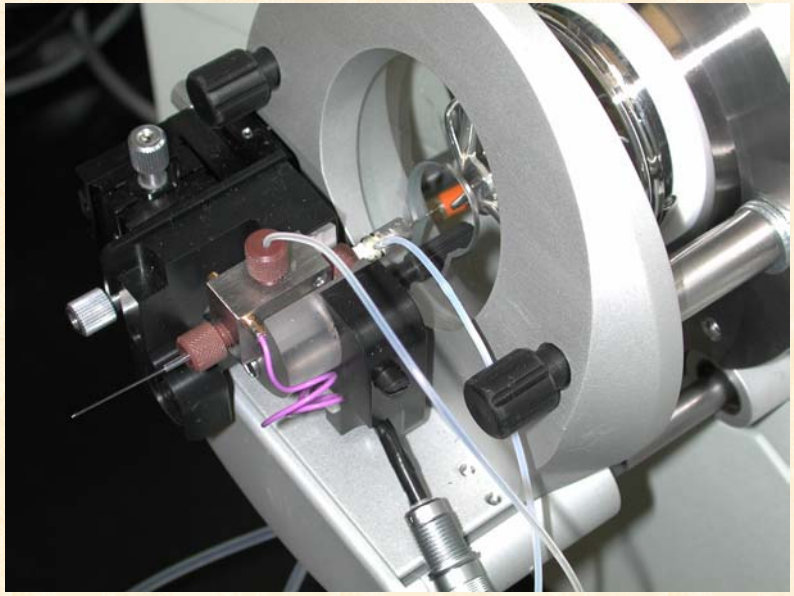
- Mass spectrometer interfaces available for surface analysis: *FAB, SIMS, MALDI*
  - Problems: *sample compatibility, sample preparation*, *system stability and cost*
- Electrospray ionization based surface sampler
  - Advantages: *sample preparation not a cornerstone of ionization mechanism, compatible with a broad range of organic compounds, robust ionization interface, atmospheric pressure ionization technique, no sample size limitation*

Introduction II

- Electrospray surface sampling demonstrated technology; Wachs and Henion, Anal. Chem. (2001), Van Berkel *et al.,* Anal. chem. (2002)
- Optimization of the probe operating conditions leads to a plethora of un-exploited applications
  - TLC-MS: direct read-out of TLC lanes
  - Affinity surfaces: Identification of captured moiety
  - HPLC fractions: increase MS events per unit volume
  - SPE disks: decrease routine analysis time

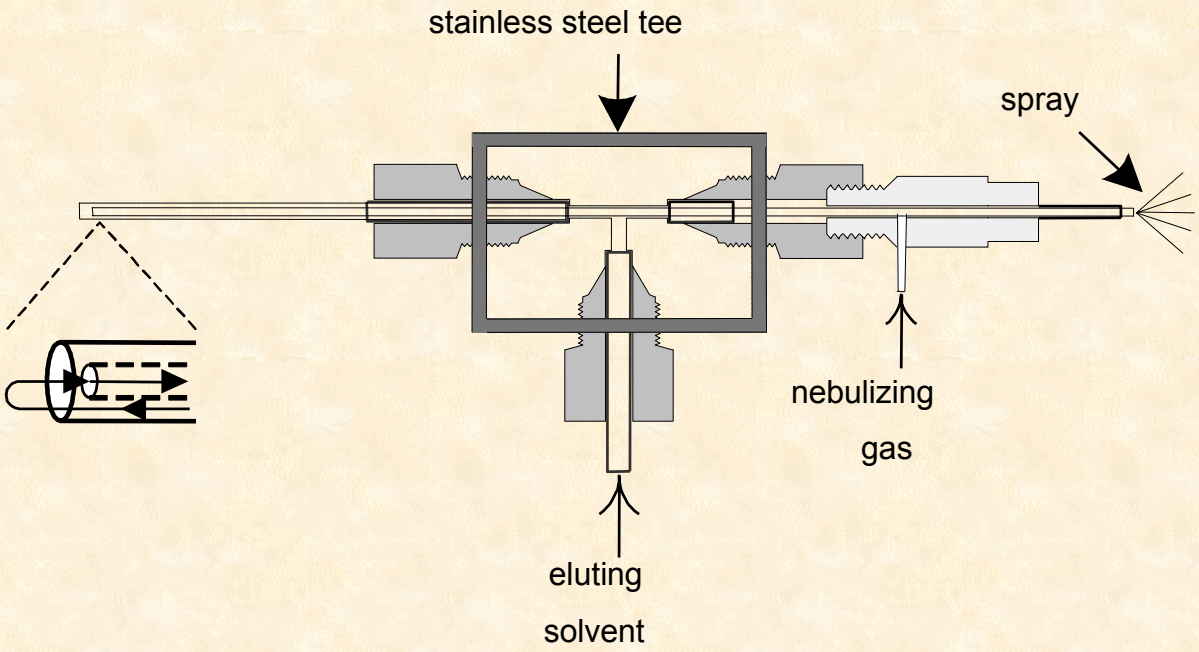
Experimental Set-up



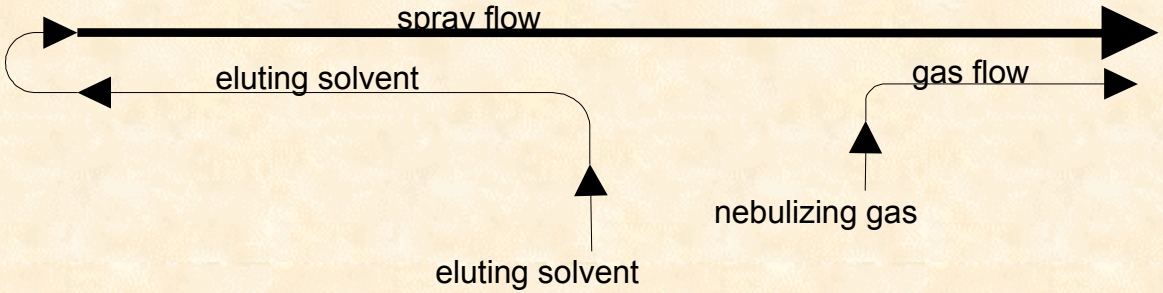


- Probe mounted on a Finnigan LCQ Deca XP Plus mass spectrometer
- Platform illumination improves liquid junction observation
- Modified Finnigan nanospray source
- Orientation of probe relative to ion inlet

Probe Design

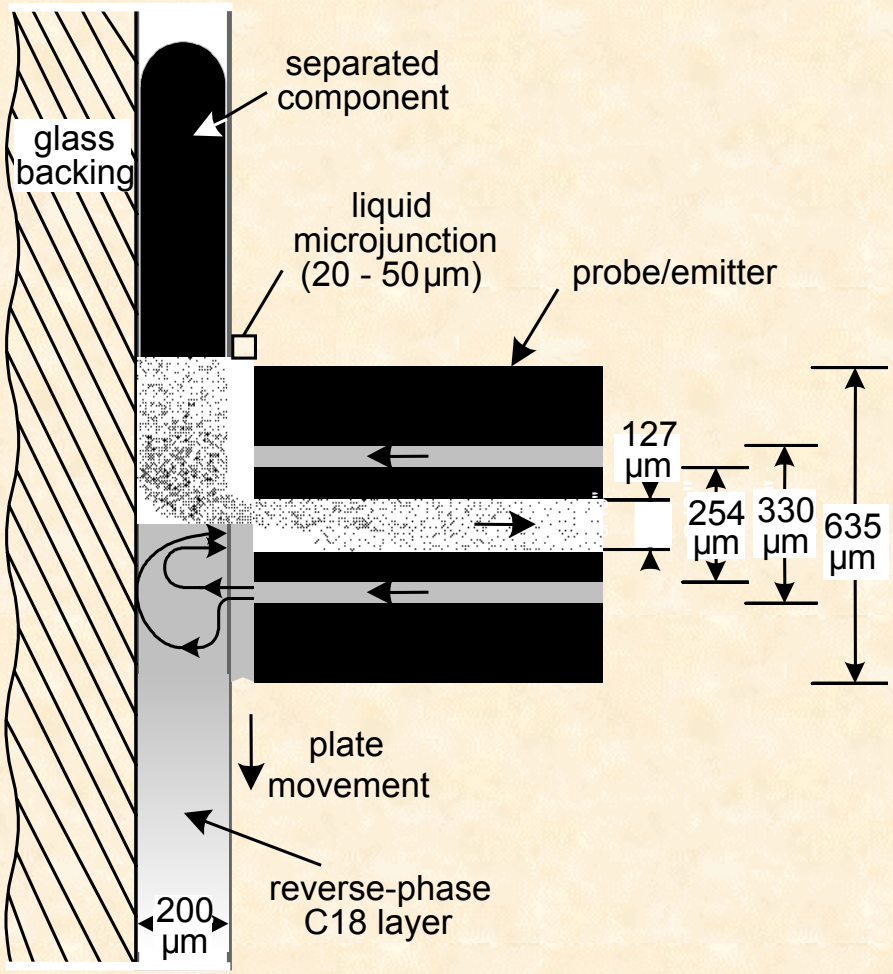


- Sampling probe design details
- Inset detail shows the recess between the outer and inner capillaries



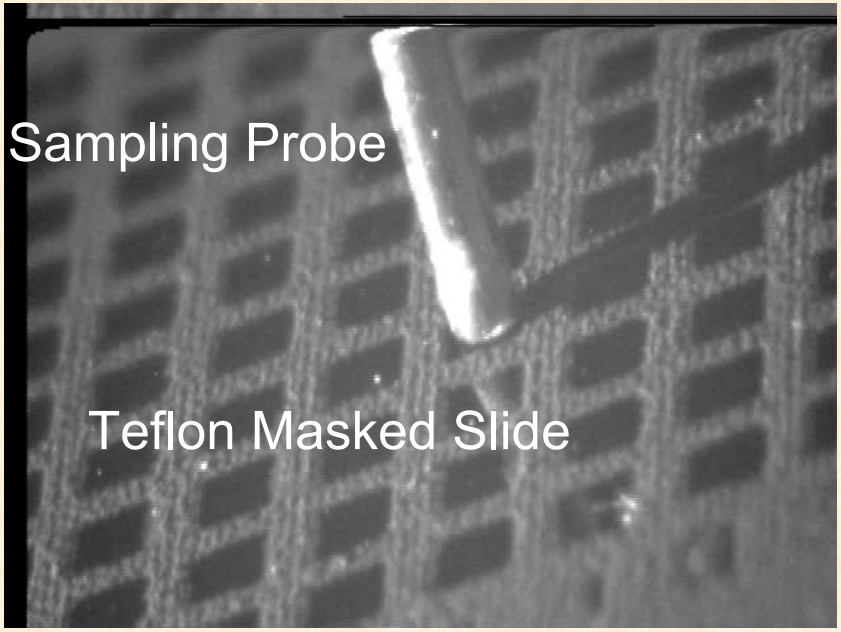
- Path of the eluting solvent and nebulizing gas through the probe assembly

Sampling Detail I

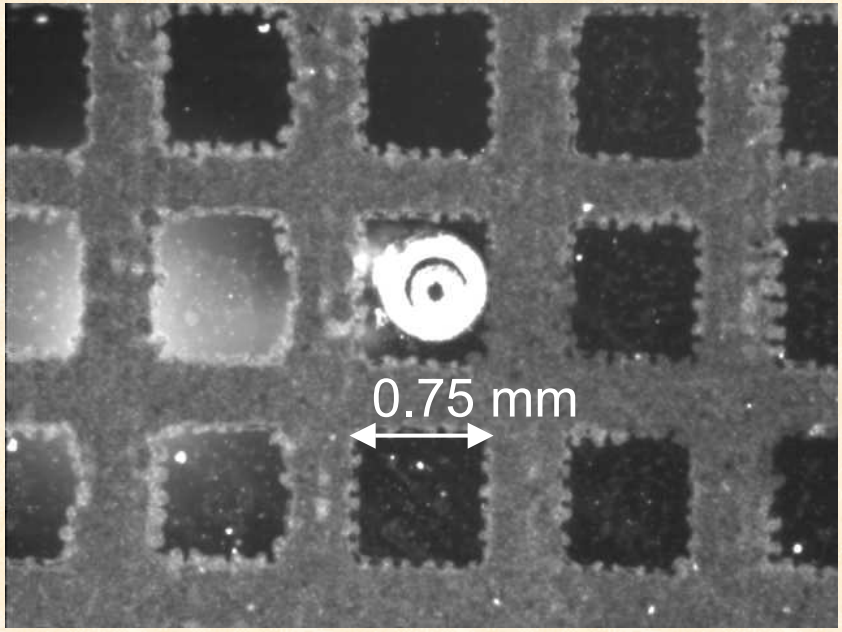


- Sampling action of the probe
- Formation of liquid micro-junction
- Solvent dissolves the surface target
- Solvent/solute aspirated by the probe

Sampling Detail II



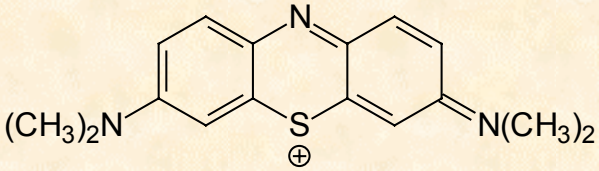
- Sampling probe lowered towards capture site for sampling event
- Formation of 20 µm liquid junction



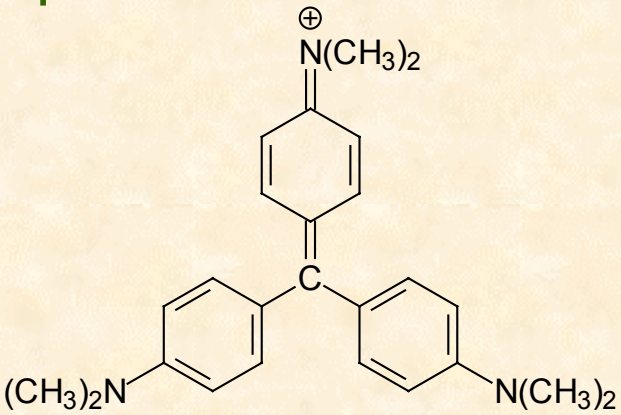
- Sampling event pictured from the reverse side of the sample slide

TLC-ESI/MS I

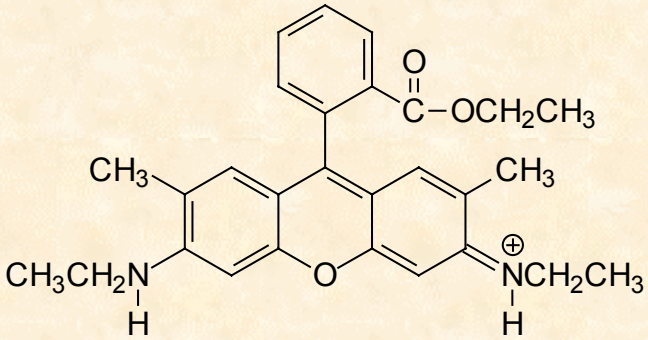
- Easy to visualize test mix separated on RP-C18 plate
- Plate analyzed with novel electrospray sampling probe



methylene blue
$(M)^+$ = *m/z* 284

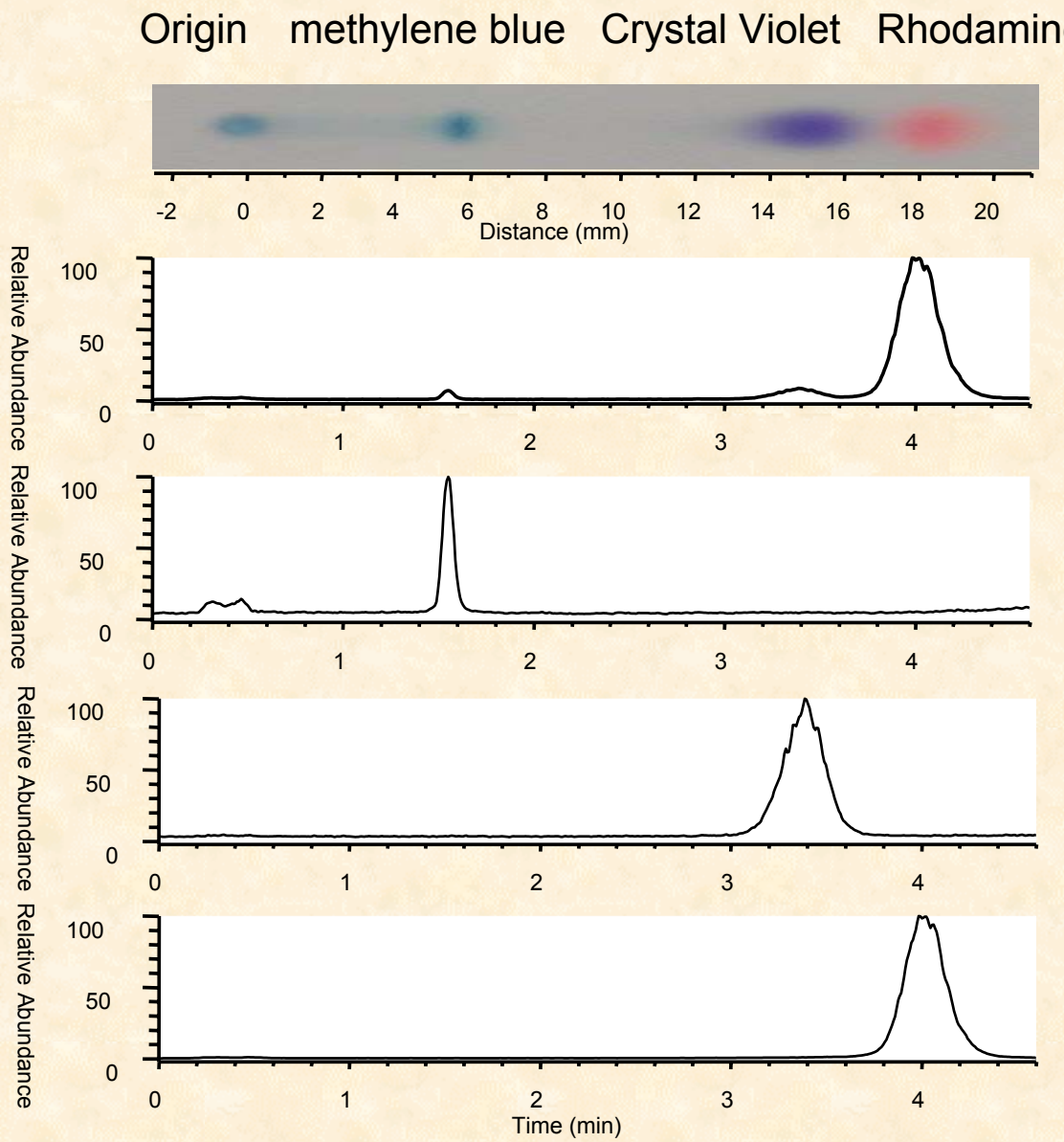


crystal violet
$(M)^+$ = *m/z* 372



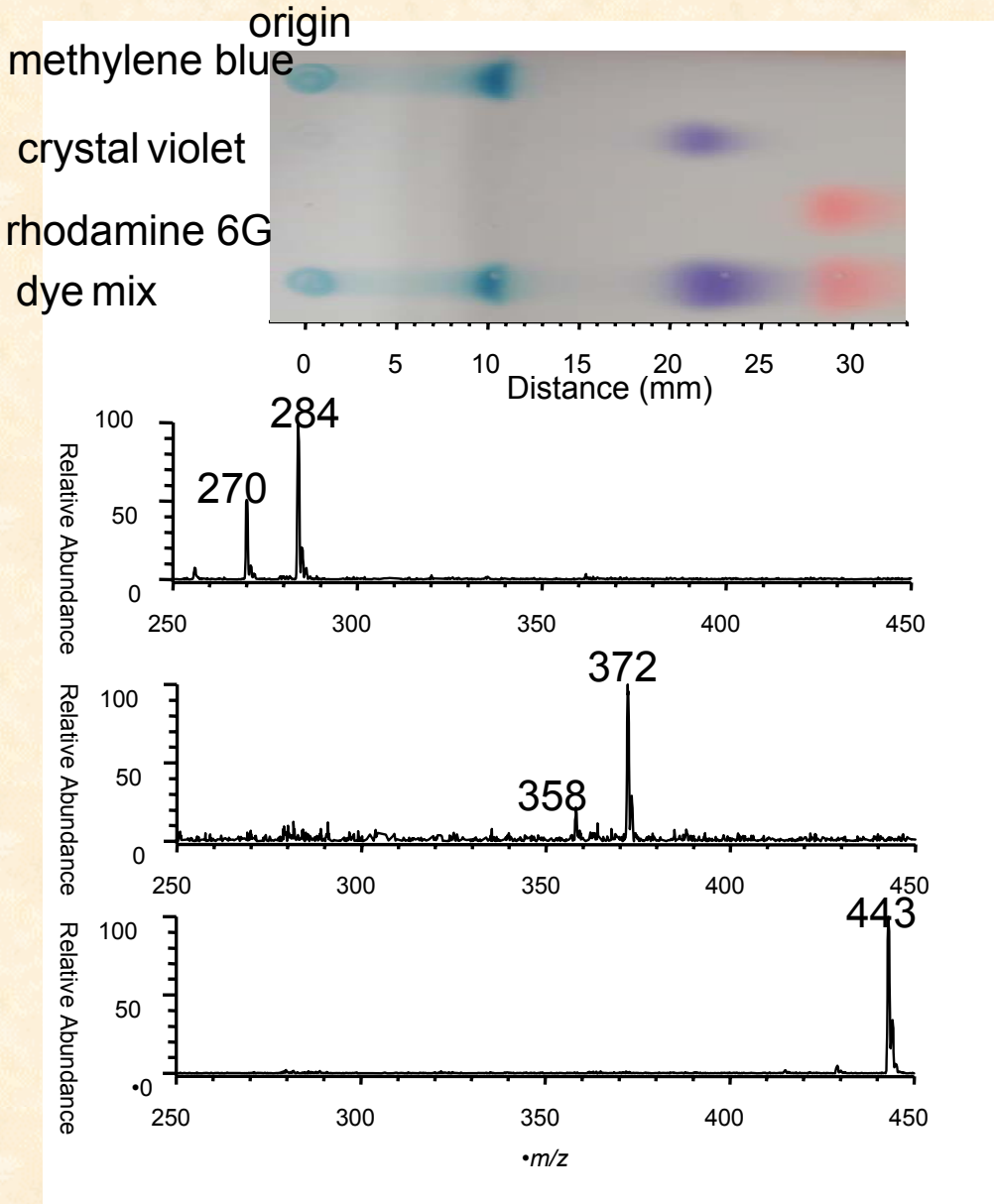
rhodamine 6G
$(M)^+$ = *m/z* 443

TLC-ESI/MS II



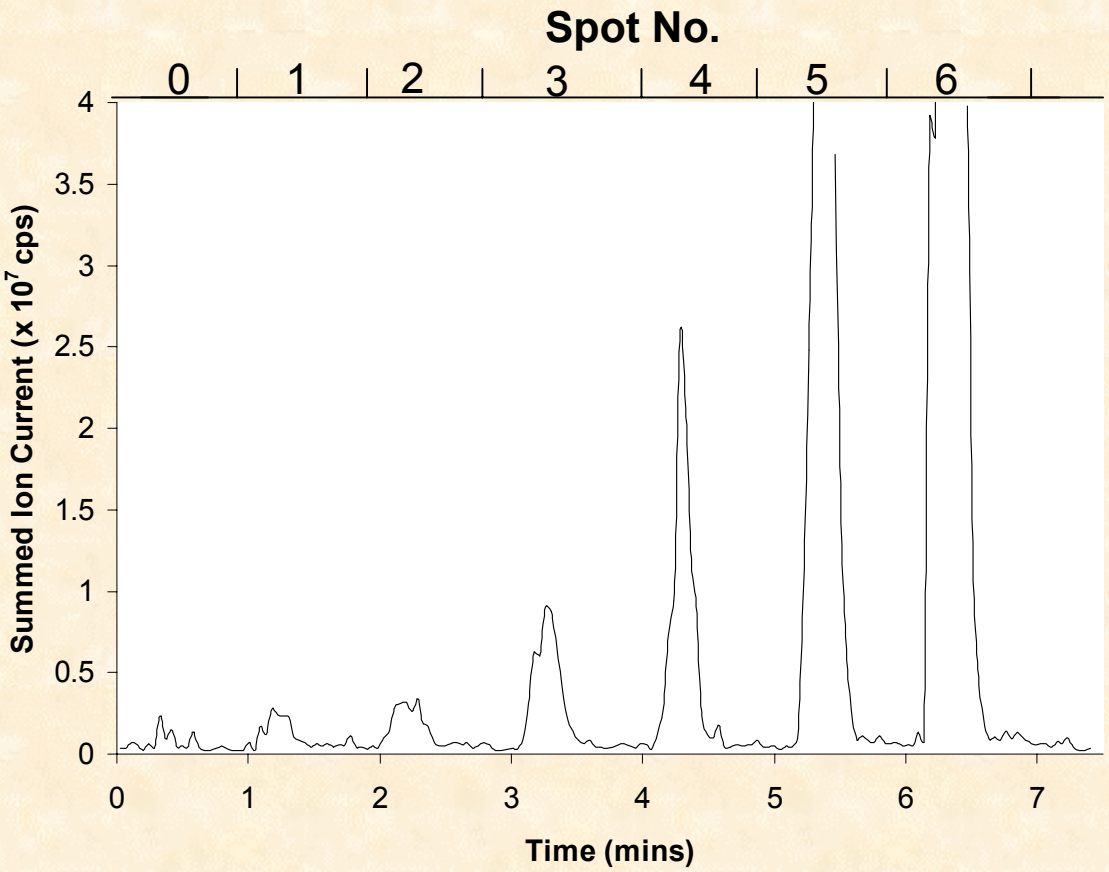
- TLC Separation
- TIC
- 284 *m/z*
- 372 *m/z*
- 443 *m/z*

TLC-ESI/MS III



- TLC separation of 3 component dye mix
- Methylene blue $[M]^+ = 284$ *m/z*
- Crystal violet $[M]^+ = 372$ *m/z*
- Rhodamine 6G $[M]^+ = 443$ *m/z*

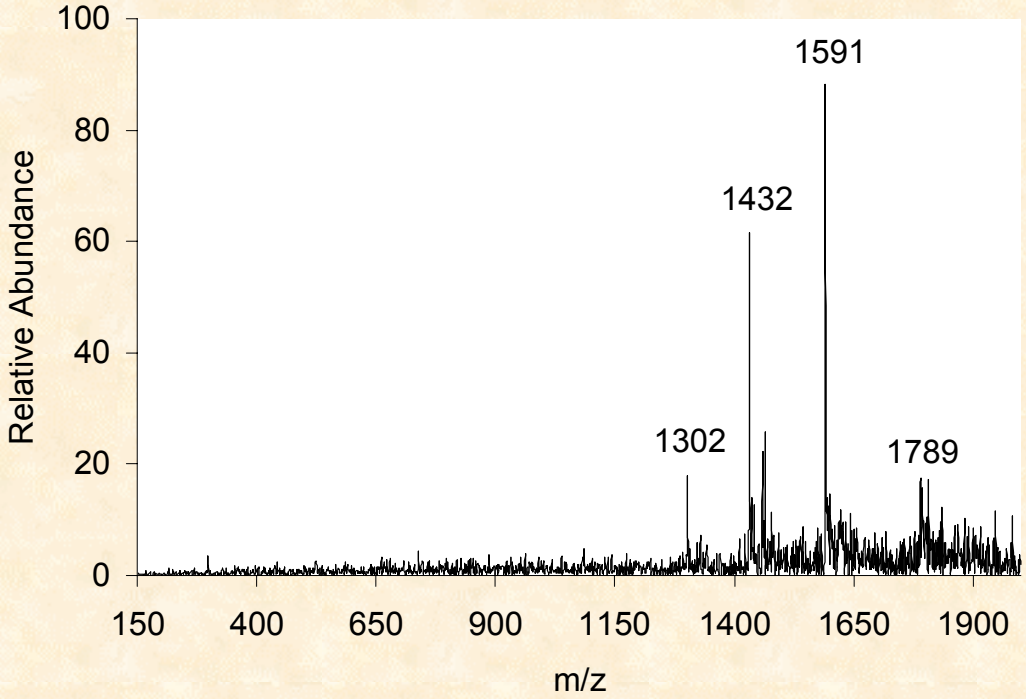
Sampling of Surface Spotted Protein I



- Piezo-electric spotted Lysozyme
- Teflon masked well sampled for 30 secs
- Chromatogram shows summed ion current for +8 to +14 charge states of Lysozyme

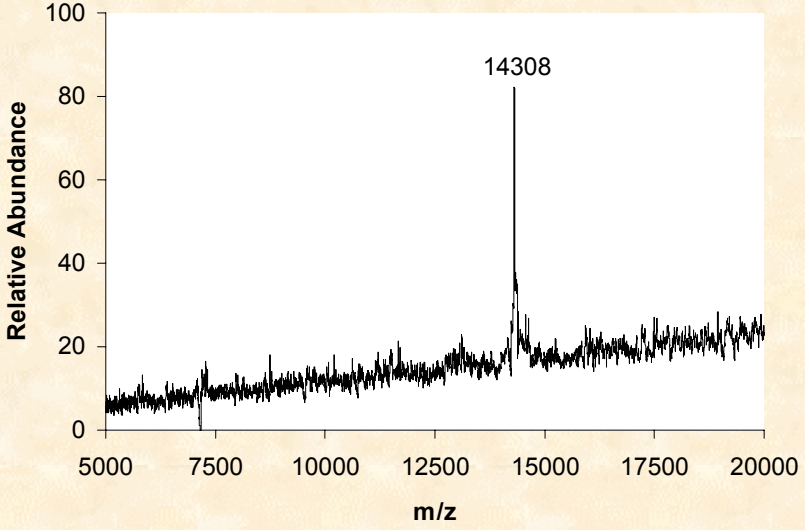
Spot	[Lysozyme] ng	[Lysozyme] fmol
0	0	0
1	0.01	1.0
2	0.07	5.0
3	0.14	10.0
4	0.28	20.0
5	0.70	50
6	1.40	100

Sampling of Surface Spotted Protein II

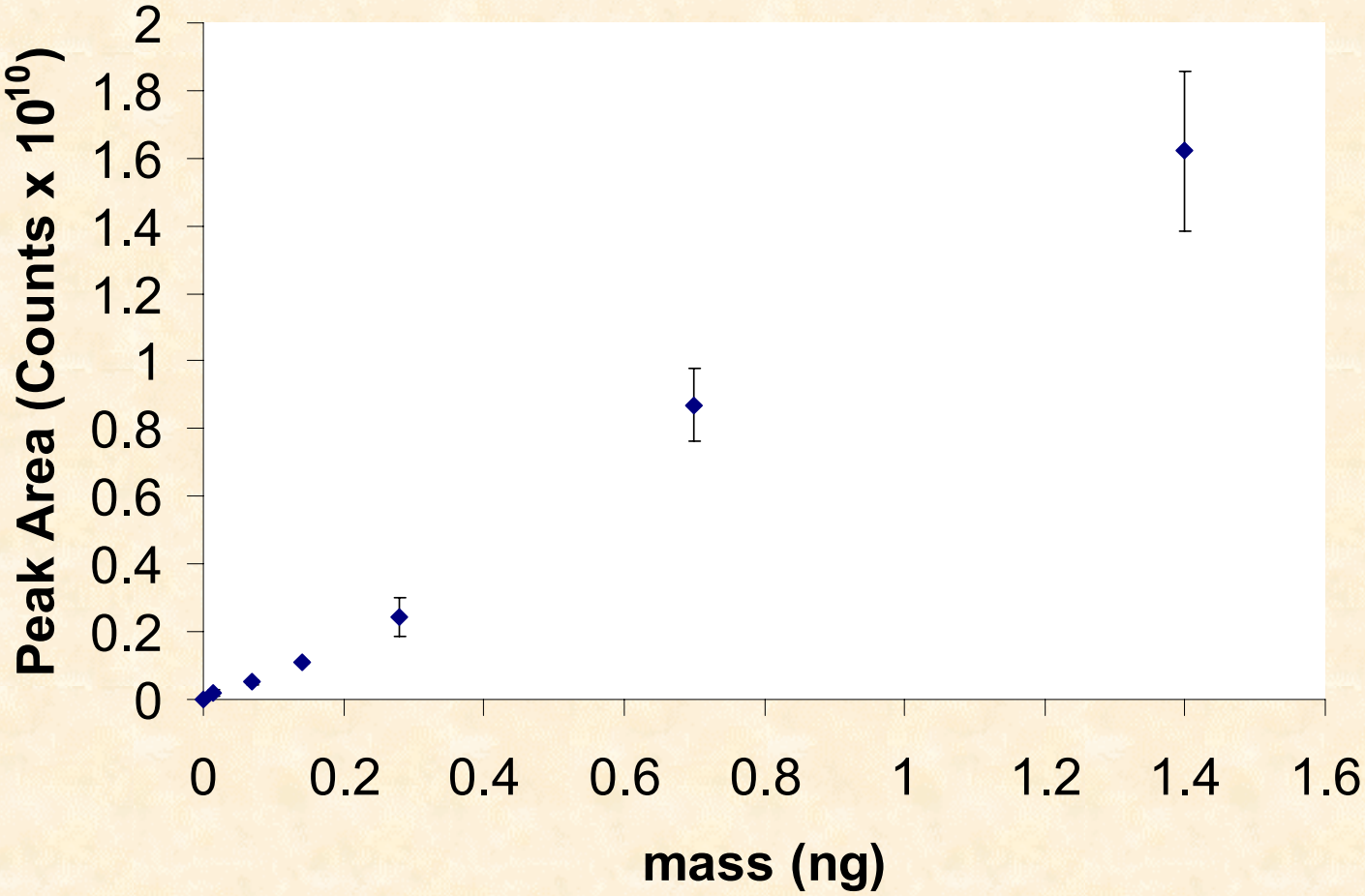


- Mass spectrum taken from a 5 fmol surface spotted lysozyme
- +8 to + 11 charge states of lysozyme are labeled

- Deconvoluted Lysozyme spectrum



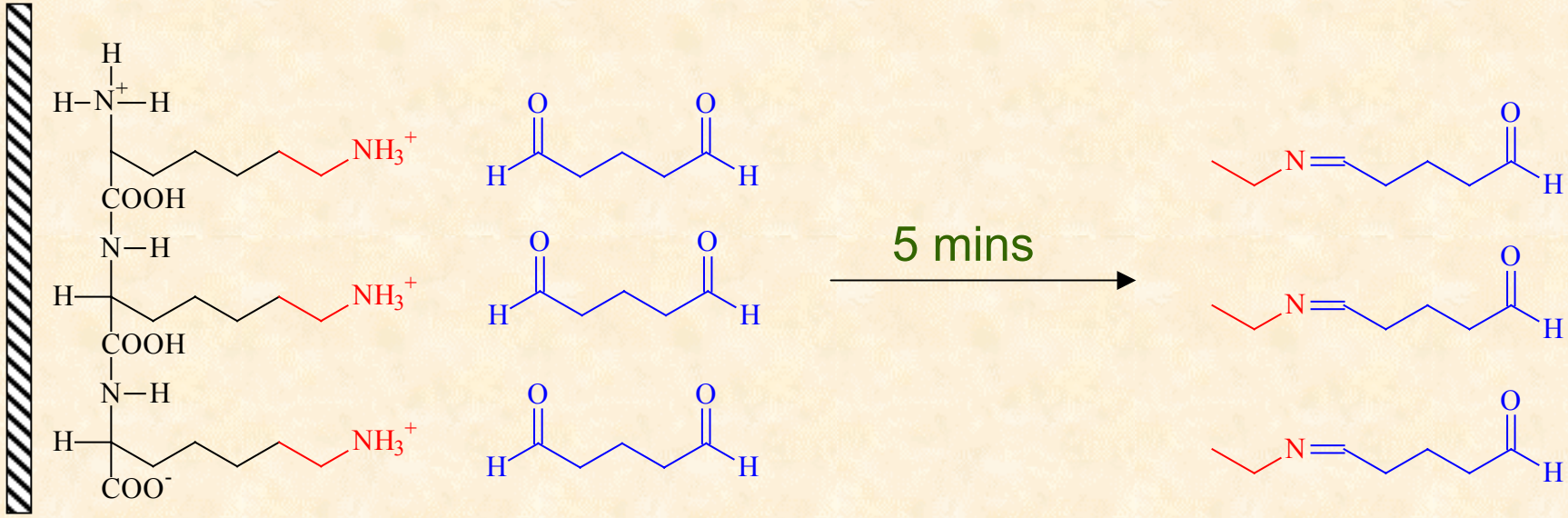
Sampling of Surface Spotted Protein III



- Peak area data collected from triplicate analyses of surface spotted lysozyme

Sampling of Surface Captured Protein I

- Surface Capture Chemistry I

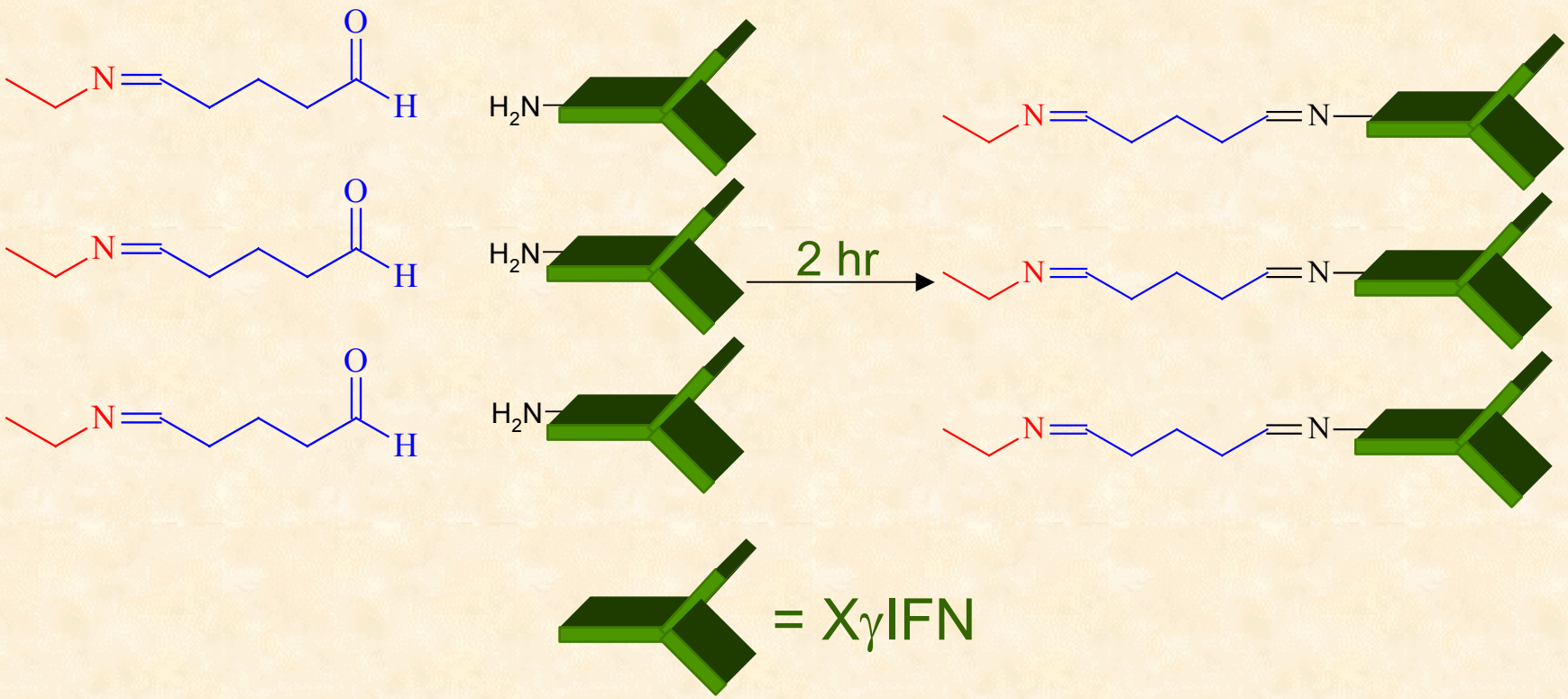


glass

- Glass slides were treated with poly-L-lysine (PLL) and incubated with glutaraldehyde to produce a Schiff's base

Sampling of Surface Captured Protein II

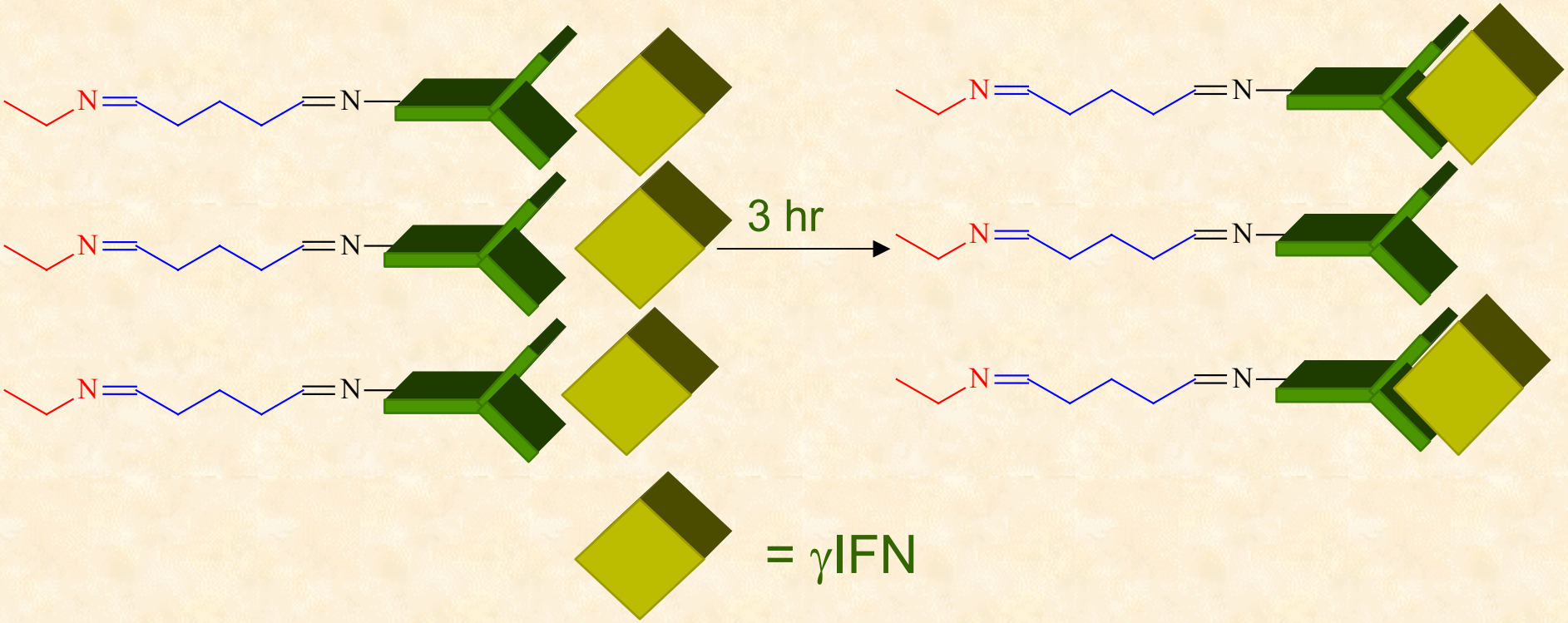
- Surface Capture Chemistry II



- Schiff's base loaded chip soaked in anti Gamma interferron (XγIFN), formation of a further Schiff's base immobilizes the antibody on the glass substrate

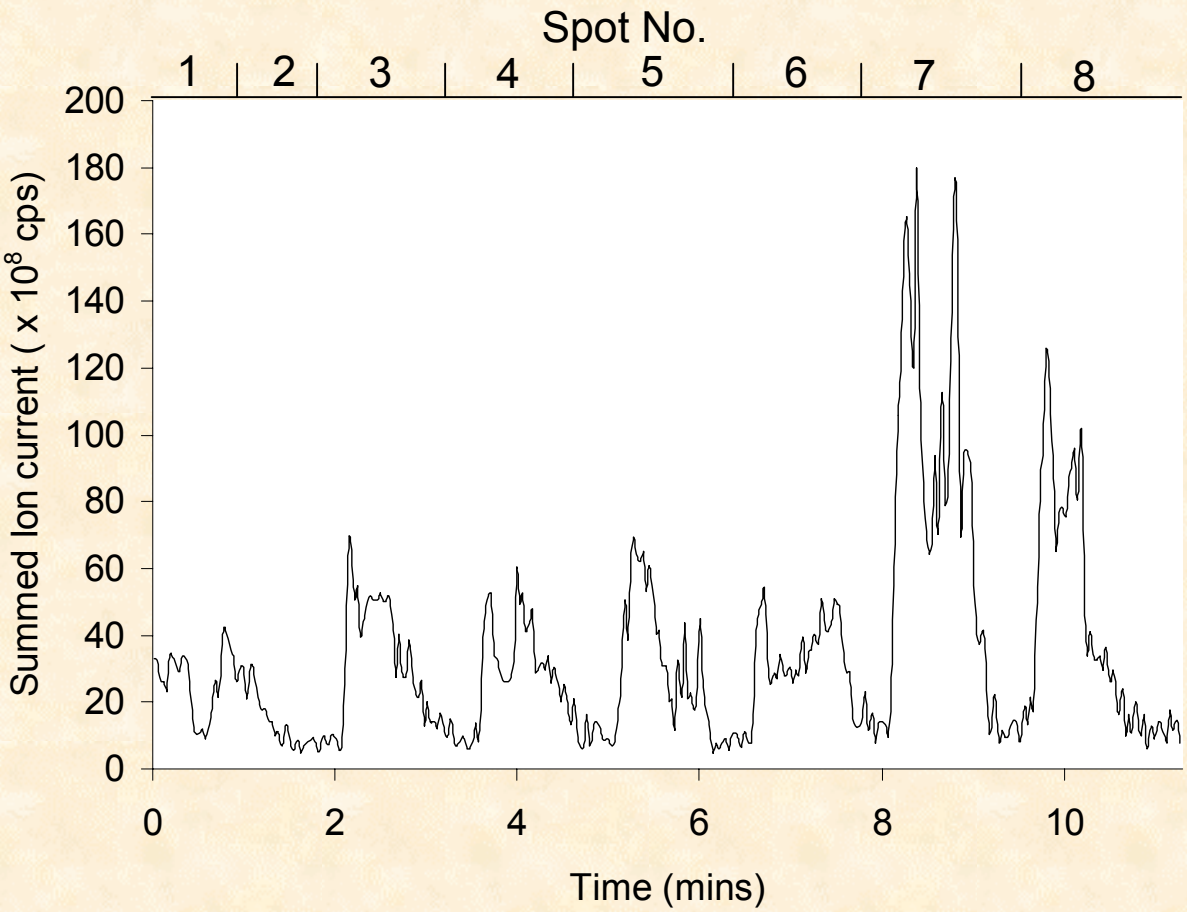
Sampling of Surface Captured Protein III

- Surface Capture Chemistry III



- Aqueous antigen pipetted onto capture array slide surface
- After 3 hr period capture array washed with water and analyzed

Sampling of Surface Captured Protein IV



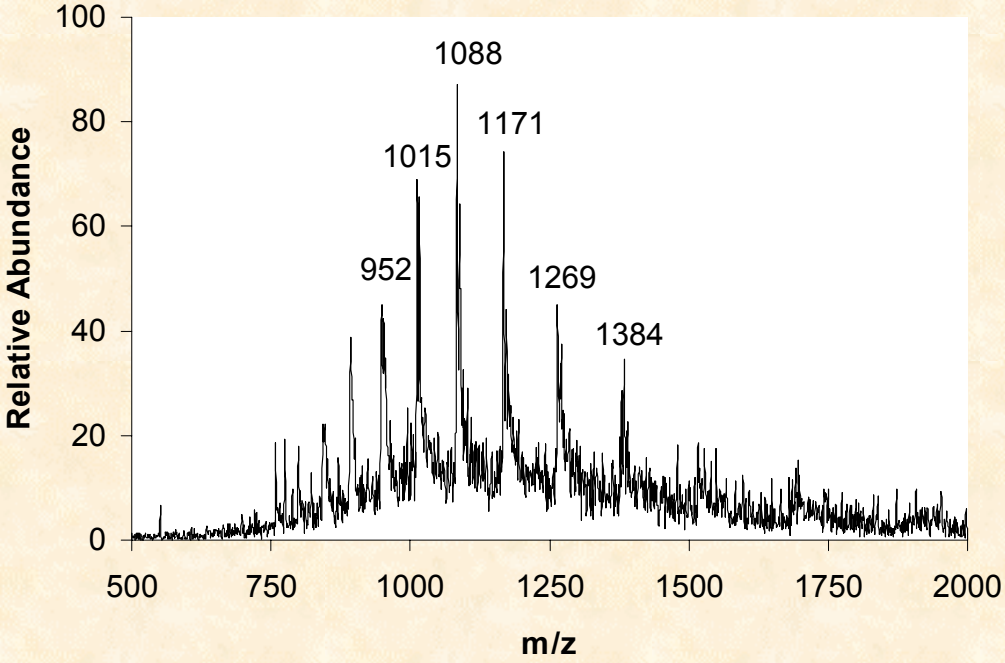
- Mass chromatogram from the sampling of 4 different concentrations of surface captured γIFN
- Chromatogram shows summed ion current for the +12 to +18 charge states of γIFN

Spot	Mass γIFN (ng)		Amount γIFN capt. fmol
	Inc.	capt.	
1	3.1	0.31	20
2	3.1	0.31	20
3	12.5	1.25	82
4	12.5	1.25	82
5	50	5	329
6	50	5	329
7	200	20	1319
8	200	20	1319

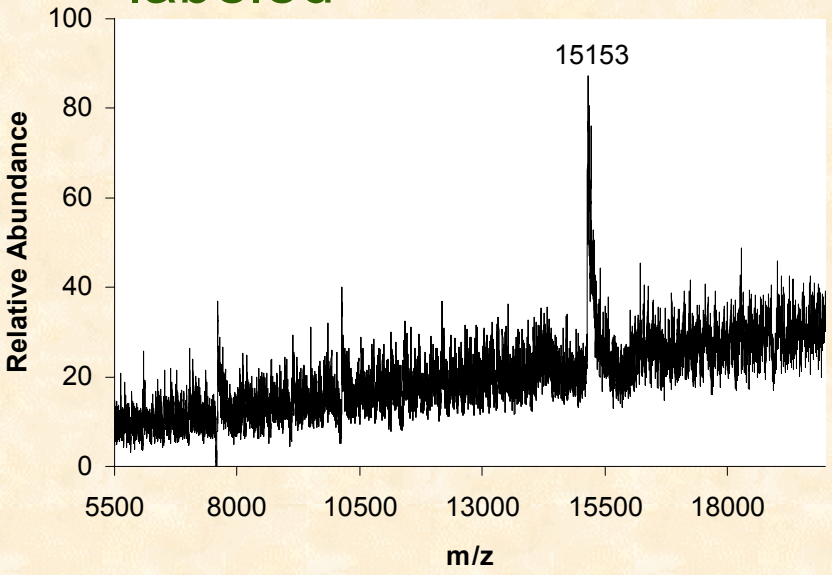
Inc. = incubated
capt. = captured

- Capture experiments were carried out in duplicate
- Capture efficiency determination via parallel radio-labeled capture assay

Sampling of Surface Captured Protein V

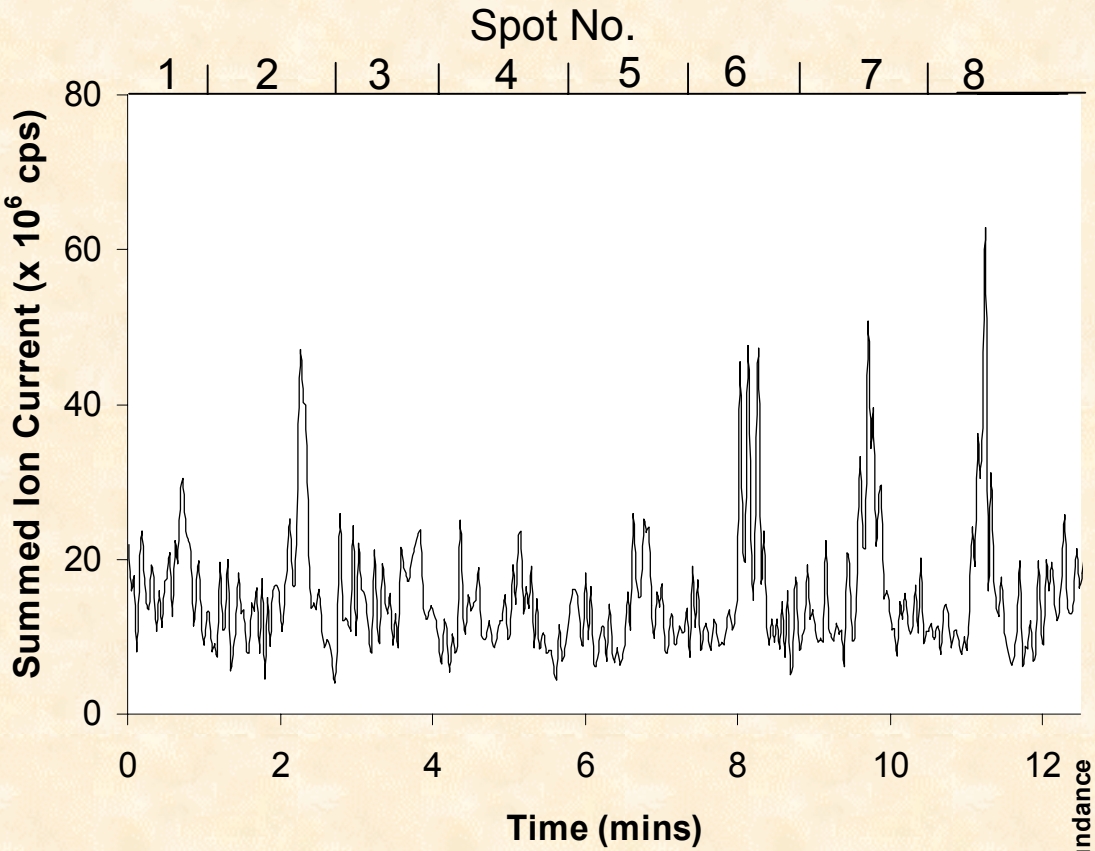


- γIFN electrospray mass spectrum acquired from spot 8 (329 fmol Captured)
- +11 to + 16 charge states of γIFN are labeled



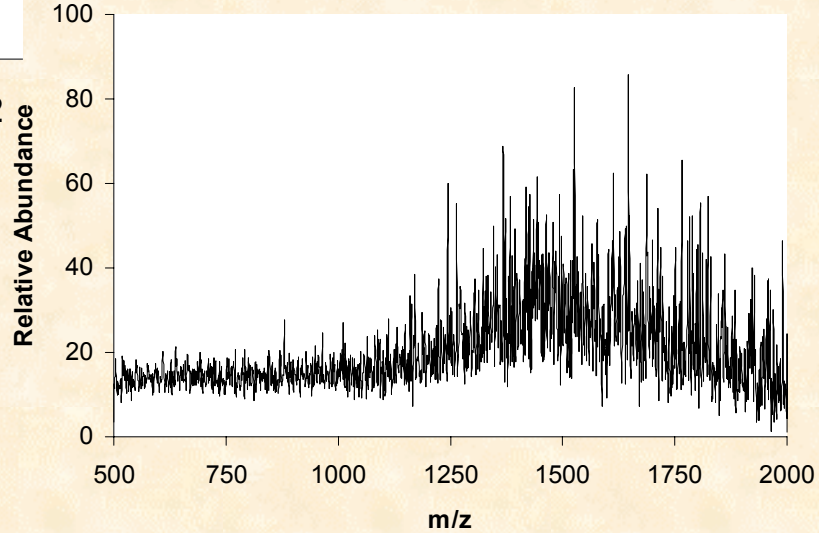
- Deconvoluted γIFN mass spectrum

Sampling of Surface Captured Protein VI



- Mass chromatogram from the analysis of a γIFN array treated control array
- The array is treated in the same way as the previous experiment but with a normal rat IgG capture agent
- Chromatogram shows summed ion current for the +12 to +18 charge states of γIFN

- Mass spectrum extracted from spot 8



Conclusions

- Surface sampling probe provides:
  - TLC-ESI/MS
    - Mass chromatogram
    - Full scan mass spectrum from chromatographic peaks
  - Surface Spotted Protein
    - Demonstrable biological surface sampling
    - Low limit of detection
  - Affinity Captured Protein
    - Proof of principle
    - Sampling of surface captured protein

Future Work

- Reduction in probe volume to increase system sensitivity
- Development of fluorescence based capture assay allowing measurement of sampling efficiency
- Coupling of other spray ionization techniques such as APPI and APCI

Acknowledgements

- This research was sponsored by the ORNL Laboratory Director's Research and Development Program, U.S. Department of Energy, under contract No. DE-AC05-00OR22725 with Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC.
- Dr. Timothy Wachs, Cornell University
- Dr. Heinz E. Hauck, Merck, Darmstadt, Germany
- Dr. Fred Rabel, EM Science, Gibbstown, New Jersey
- Dr. Peixen He, CH Instruments, Inc., Austin, Texas
- John Zemek, Spencer Doidge and co-workers, ASI, Inc., Eugene, Oregon