Potential Clinical Assays (not FDA approved)

Outline

- Glycated β-Hemoglogin ~ (HbA1c)
- Albumin / Creatinine in Urine (microalbuminuria)
- Mass Spectrometric Immunoassay (MISA)
- Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA)
- Oligo-nucleotide applications

Why MALDI-TOF ?

- accurate across a wide mass range
  linear mode 100 – 100,000s and beyond in single spectrum

- precise, quantitative

- requires << 1µL of sample

- fast, high throughput
  - Analysis of multiple replicates can be done minutes
  - reanalysis of same target is possible if necessary
  - adaptive to numerous sample prep formats

- Potential for the provision of addition information

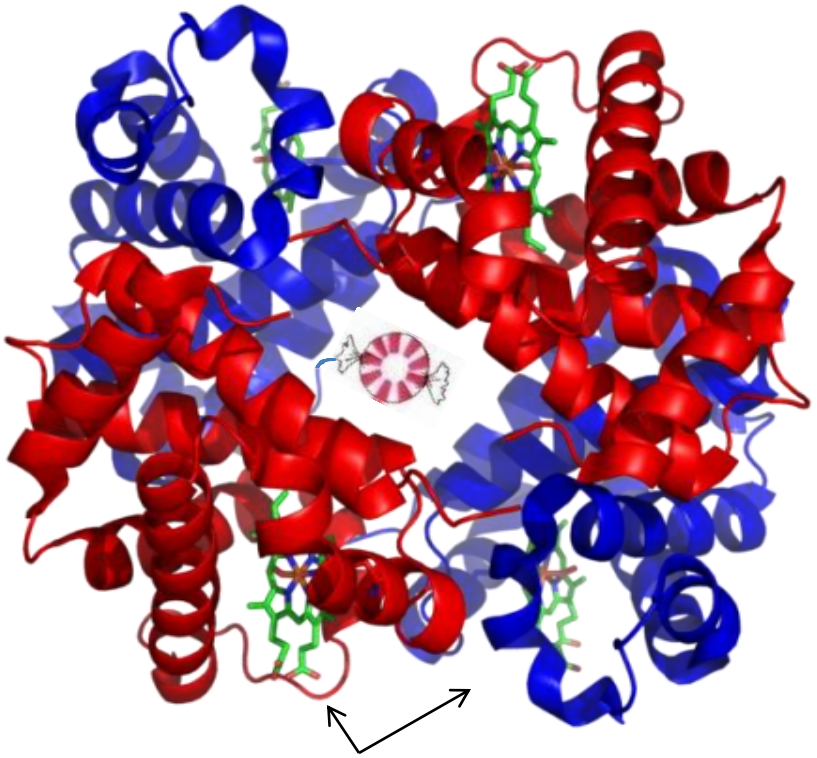
Hemoglobin (Hb) / Hb-A1c

Hemoglobin

Protein in red blood cells (erythrocytes)

- Primary function is respiratory
  - Transports oxygen

- Four globulin chains
  - Normal adult
    - 2 alpha-chains (α-Hb)
    - 2 beta-chains (β-Hb)
  - All chains contain an embedded heme-group



Heme (green)

Hemoglobin A1c

- Glycation of N-terminal valine of the Hb beta-chain
  - Non-enzymatic, [glucose]-dependent

-Serves as an average measure of blood glucose over the past 2 to 3 months

- β-Hb contains 11 lysine residues that can also undergo glycation

Diabetes Mellitus / diagnosis and monitoring

<u>Diabetes</u>

- ***Group of metabolic diseases that result in high blood sugar levels***
- 9% of the US population (30 million people) have diabetes
- 8 million are undiagnosed
- 80 million people are pre-diabetic; 90% of them are undiagnosed

<u>Diagnosis and monitoring</u>

-***Quantification of blood glucose***: (fluctuates with diet)

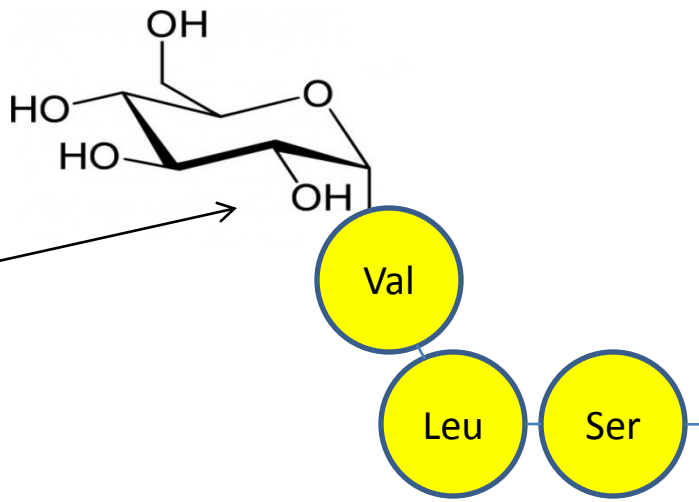
Fasting levels:	normal	70 and 99 mg/dL
	pre-dia.	100-125 mg/dL
	diabetic	126 mg/dL

-***Quantification of HbA1c*** (% N-terminal beta chain glycosylation)

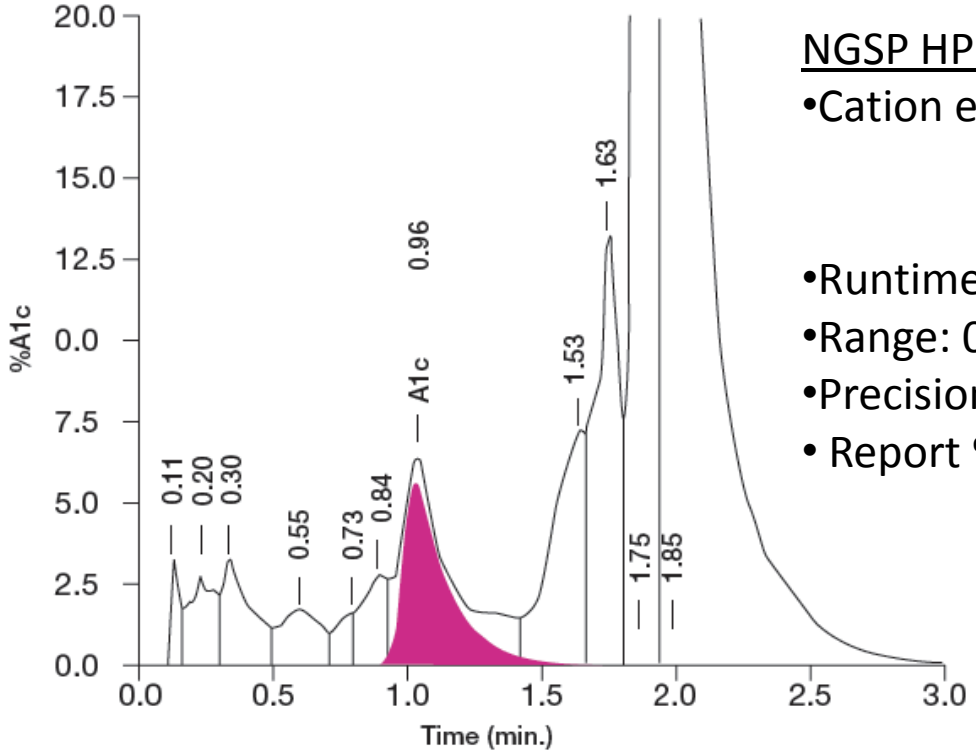
-HPLC, ELISA

normal	< 5.7%
pre-diabetic	5.7-6.4%
diabetic	> 6.5%

NGSP techniques and MALDI-TOF



- Glycation causes change in molecular charge and mass
  - Glucose addition
    - reduction in molecular charge (-1)
    - addition (+ 162 Da) in molecular mass



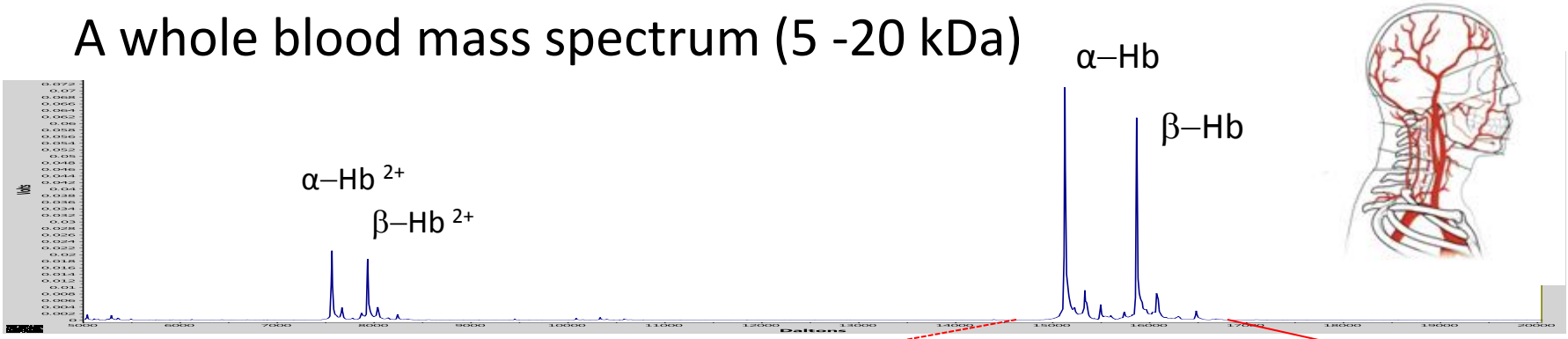
Example HPLC Chromatogram

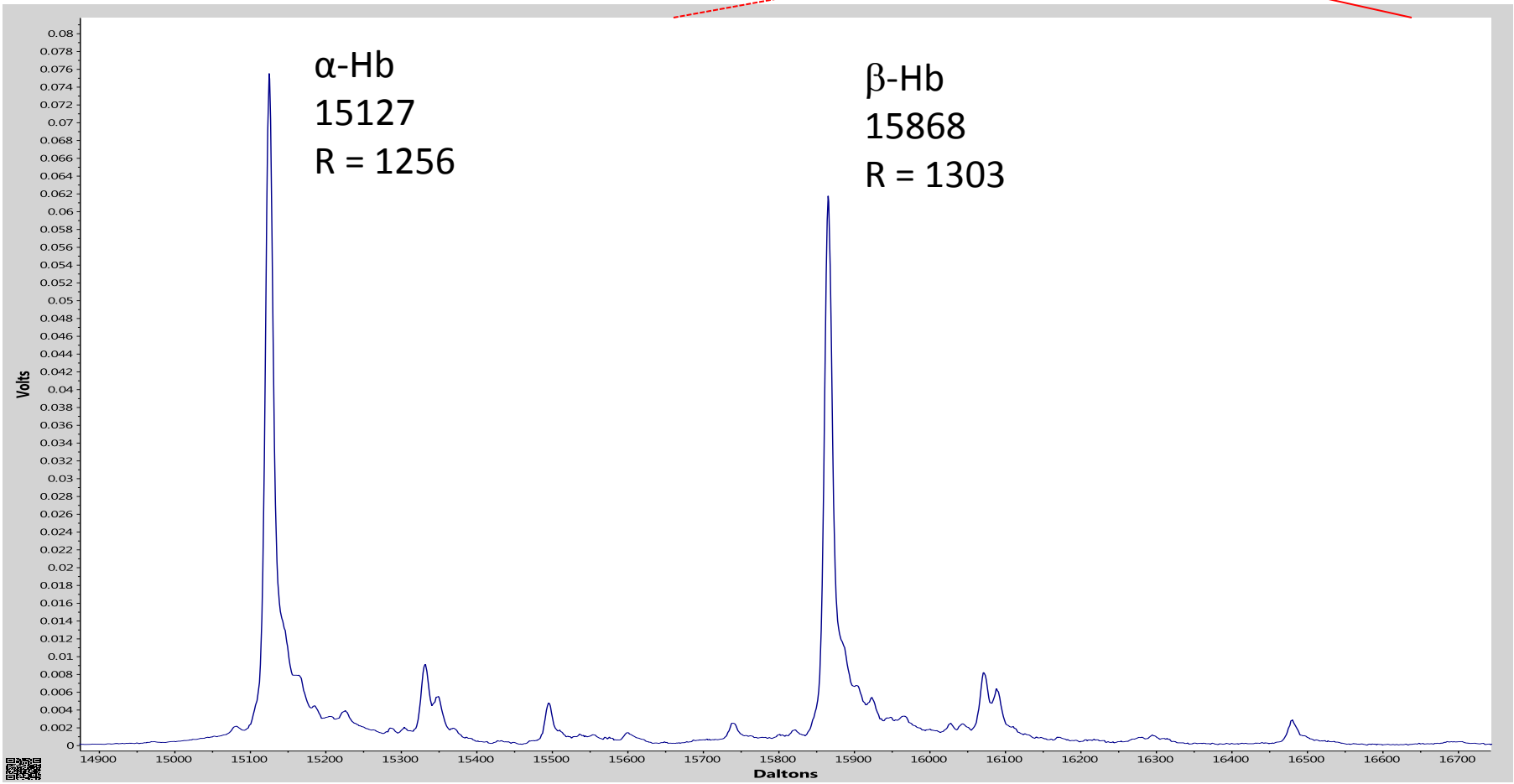
<u>NGSP HPLC Method</u>

- Cation exchange chromatography
  - difference in charge state
  - Heme abs. (415nm)
- Runtime: 3 min
- Range: 0 – 20% HbA1c
- Precision ≤ 2%
- Report % A1c as a ratio

  [A1c / (Hβ + A1c)] * 100 = % A1c

A whole blood mass spectrum (5 -20 kDa)



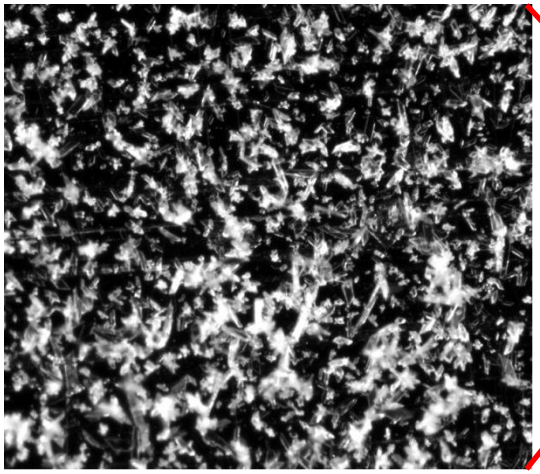


Sample preparation

- Sinapinic acid (30% CH_3CN, 0.1% TFA)
- µFocus MALDI Plate 2600 µm (Hudson Surface Technology)
- Samples spotted as 5x replicates

Other matrices examined

Alpha-cyano
DHB
Super-DHB
HABA
3-HPA
Ferulic acid
trans 3,5-Bis (trifluoromethyl) cinnamic acid
3,4,5 trimethoxycinnamic acid



Crystals ~ 10-20 µM

Sample Acquisition

Acquisition parameters

-linear mode using positive-ion polarization

-acceleration voltage 20 KV

-mass range 5000 – 20,000 Dalton

detector response (saturation, recovery)

limits file size data storage

quickens data manipulation

-focus mass 15,600

optimum resolution

-laser pulse frequency 1000 Hz

data collection rate

-laser pulse energy 12 µJ

sensitivity, signal / noise

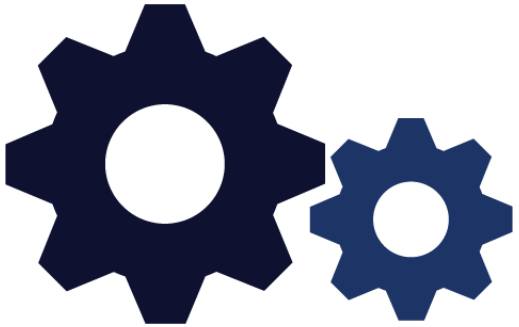
-scan rate 1 mm/min

- Sample spot size 2.6 mm

-100 µm raster to cover each sample position

Red = adjustable parameters that determine acquisition speed

Post acquisition data processing



-***Average all spectra > 20 mV*** signal intensity / spot

-***Baseline correct*** spot-averaged spectra

-***Calibrate*** spot-averaged spectra

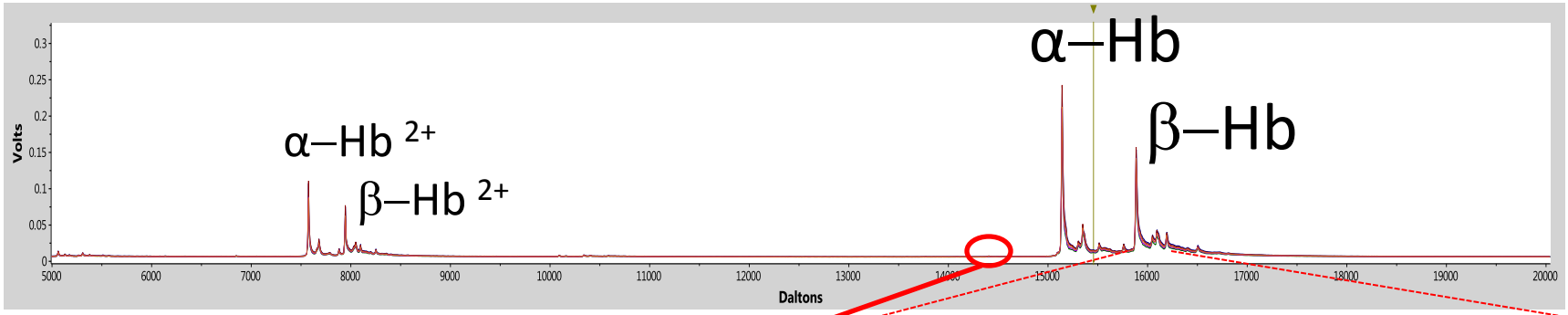
- M^{+1} and M^{+2} ions of hemoglobin α and β subunits (7564, 15127, 7934, 15868 Da)

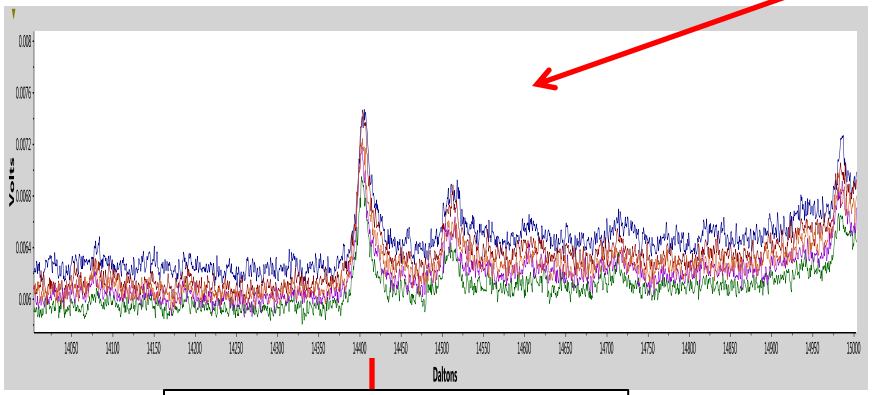
-***Quantify*** by integration of signals from β–Hb and (β–Hb + 162 (glucose))

-***Report*** as a ratio of the percentage of total glycation on the β chain

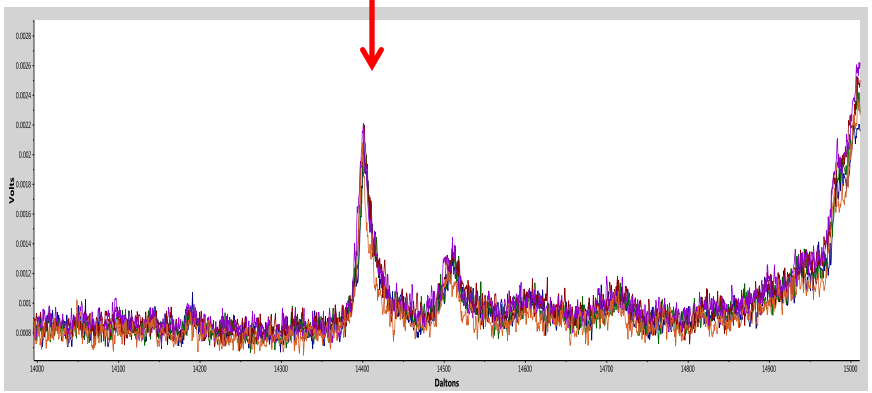
$$[\ H\beta\ /\ (H\beta\ +\ (H\beta{+}162))\ ]\ \ *\ 100\ =\ \%\ Glyco\text{-}\beta\text{–}Hb$$

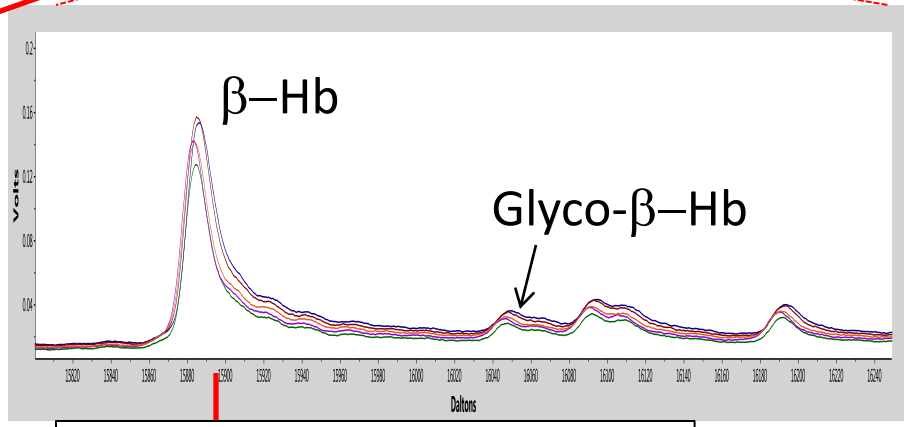
Data Processing



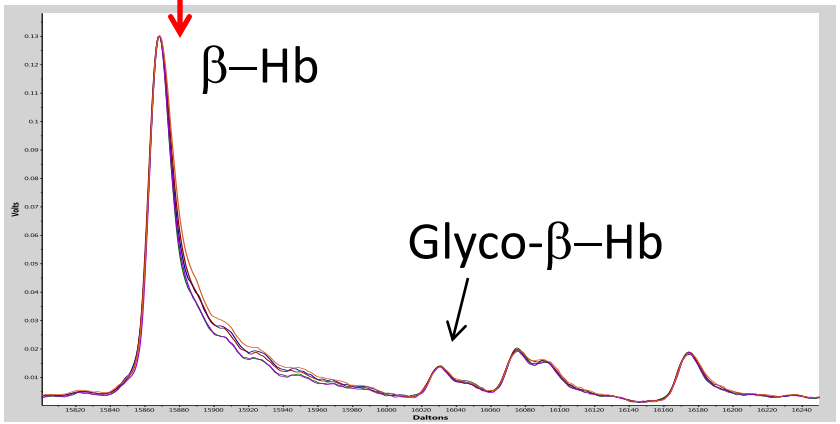


1. Baseline correction

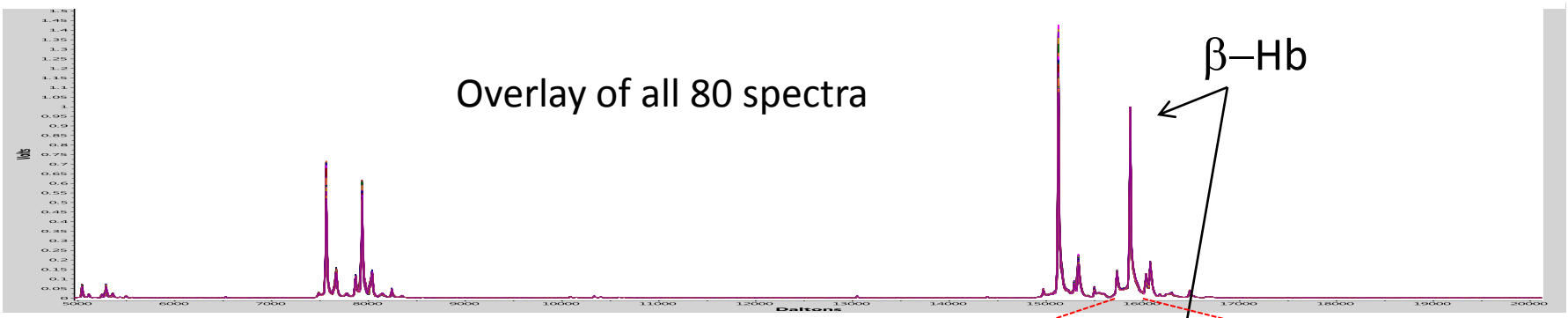




2. Calibration & normalization



Reproducibility

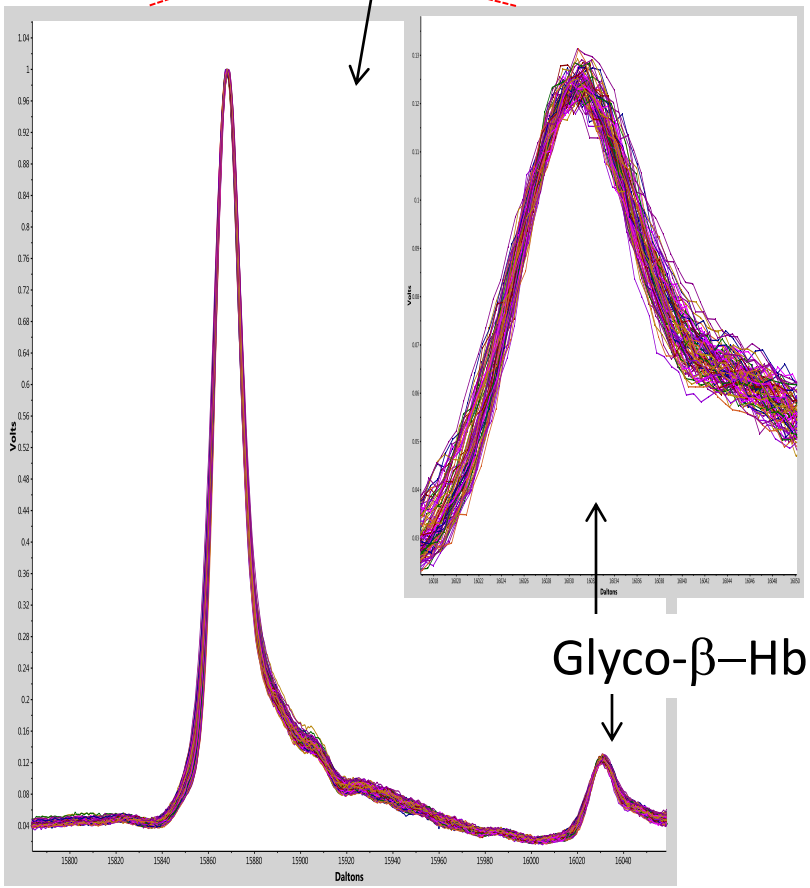


<u>Reproducibility experiment</u>

- 1 sample across plate
- processed 16 samples
  5x replicates
- Ave CV for 16 < 1.00%
- CV for 16 Glyco ratios 1.22 %

80 spots
~ 16,000 spectra

	GlyHb/Hb%	CV %
T 1	13.52	1.39
T 2	13.48	0.71
T 3	13.20	0.62
T 4	13.07	0.99
T 5	13.07	0.96
T 6	13.11	0.90
T 7	12.98	0.67
T 8	12.99	1.19
T 9	13.05	0.96
T 10	12.99	0.99
T 11	13.08	0.92
T 12	13.01	0.66
T 13	13.16	1.00
T 14	13.15	0.84
T 15	13.22	0.70
T 16	13.17	0.85
Average	13.14	0.90
Std Dev	0.16	
Rel Std Dev	1.22	



Reference Materials

Lyphochek Hemoglobin A1C Linearity Set
12000070
Human whole blood based control designed to verify linearity throughout the patient reportable range of HbA1C assays (6 x 0.5 mL, 1 of each level)

Assayed Values Typically Available for Common Analyzers

BIO-RAD ANALYZERS
- D-10™
- in2it™
- VARIANT™
- VARIANT™ II /TURBO

OTHER ANALYZERS
- Beckman Coulter® Synchron® and AU Systems
- Abbott Architect™ Series
- Roche COBAS® and Hitachi®
- Siemens ADVIA®, DCA and Dimension® Series
- TOSOH G7 and G8
- Trinity Biotech Series
- Ortho VITROS® Series
- Pointe Scientific

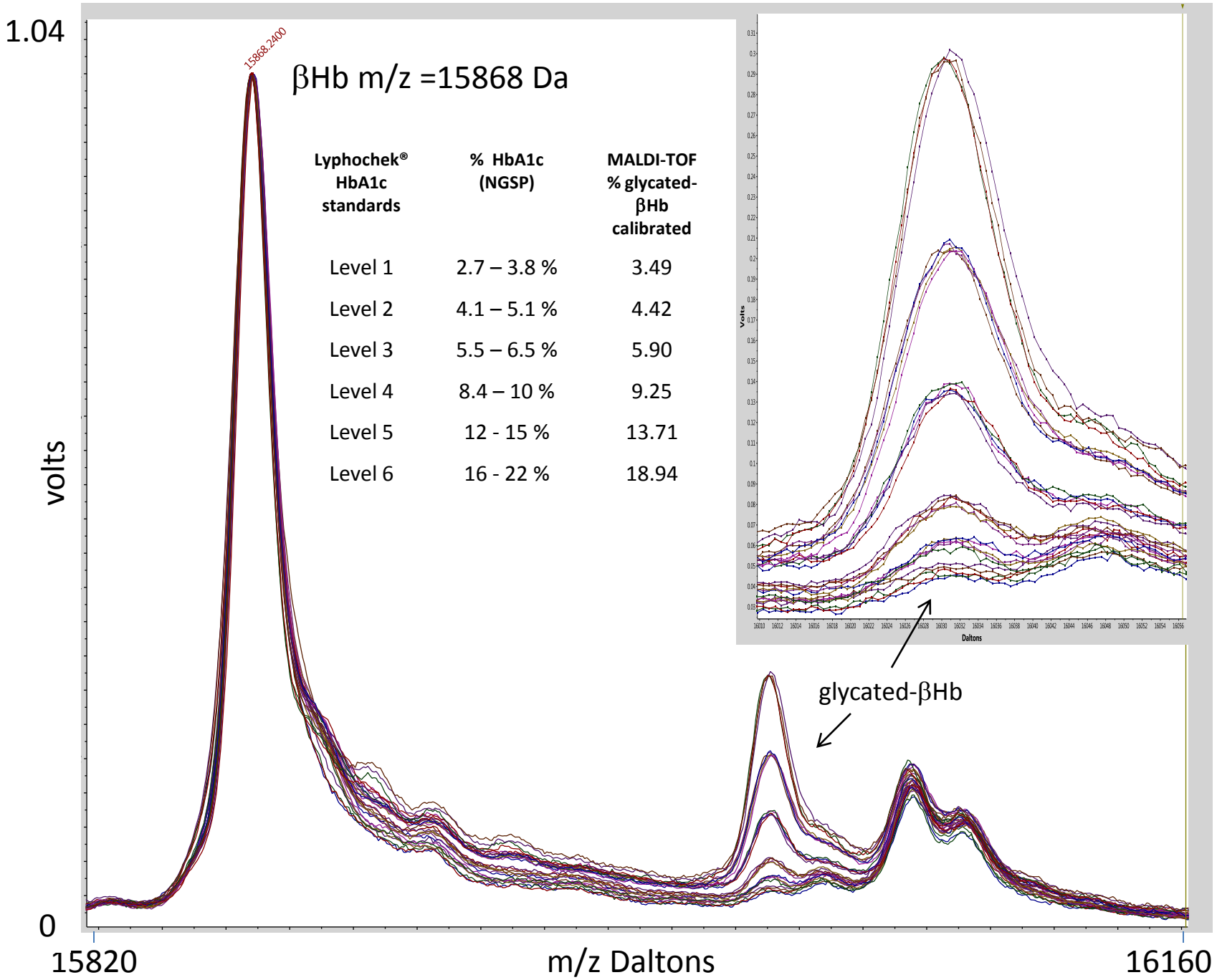
Expected Values for Hemoglobin A1C (%NGSP)*

Level 1	2.7 - 3.8%
Level 2	4.1 - 5.1%
Level 3	5.5 - 6.5%
Level 4	8.4 - 10%
Level 5	12 - 15%
Level 6	16 - 22%

* Ranges reflect typical recovery values for this product. Exact values may vary depending on test/assay methodology, instruments, reagent, system calibrators or laboratory technique.

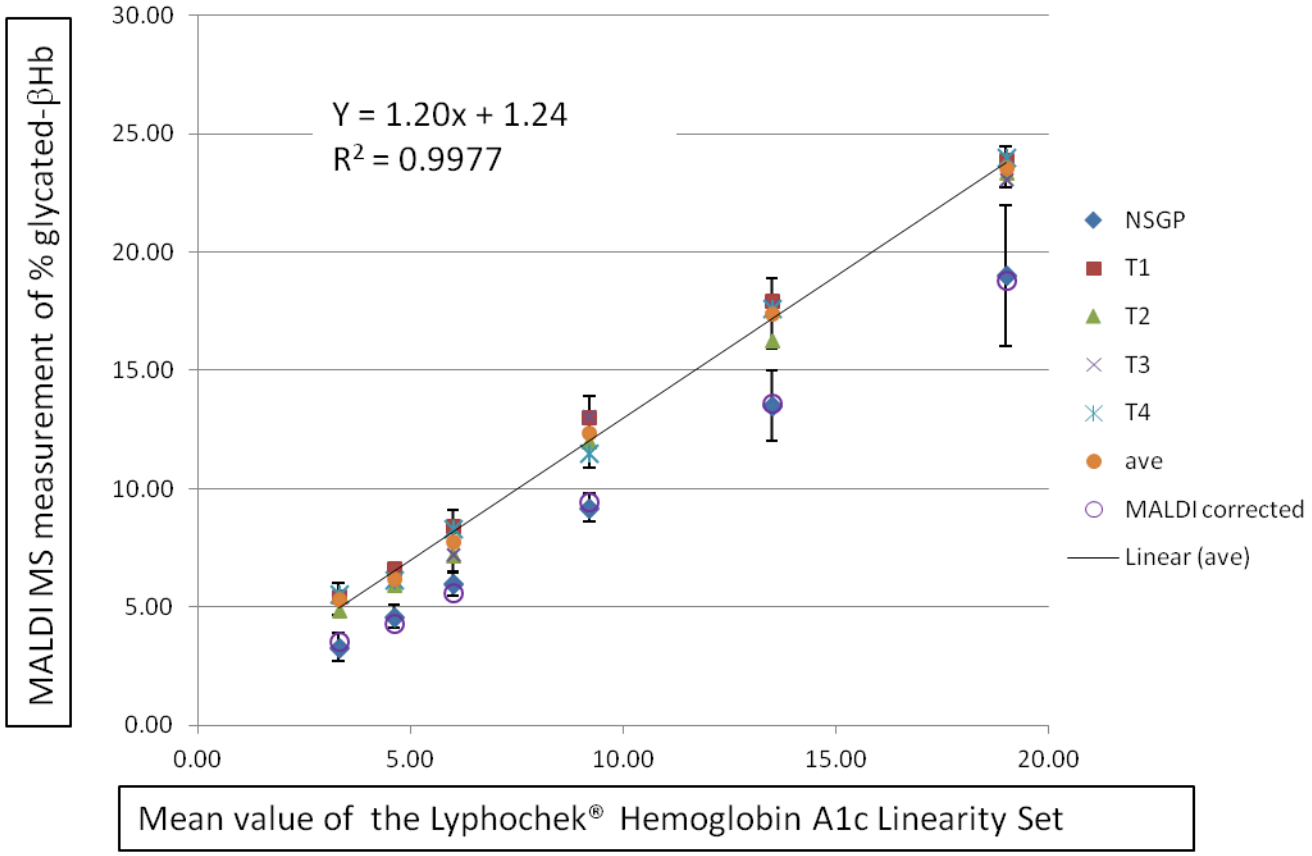
Urine Albumin reference poster here at conference

Wednesday 3:00 PM ; ***Proteomics***
"*A Reference Measurement System for Urine Albumin*"
Ashley Beasley Green, NIST


1.04
0
volts
15868.2400
βHb m/z =15868 Da
Lyphochek® HbA1c standards
% HbA1c (NGSP)
MALDI-TOF % glycated-βHb calibrated
Level 1
2.7 – 3.8 %
3.49
Level 2
4.1 – 5.1 %
4.42
Level 3
5.5 – 6.5 %
5.90
Level 4
8.4 – 10 %
9.25
Level 5
12 - 15 %
13.71
Level 6
16 - 22 %
18.94
glycated-βHb
Volts
Daltons
15820
m/z Daltons
16160

Calibration

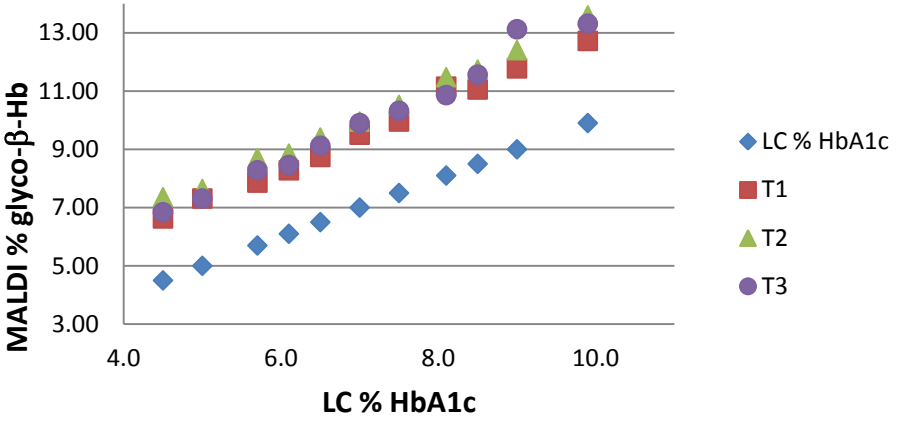
Establish the relationship between the signals

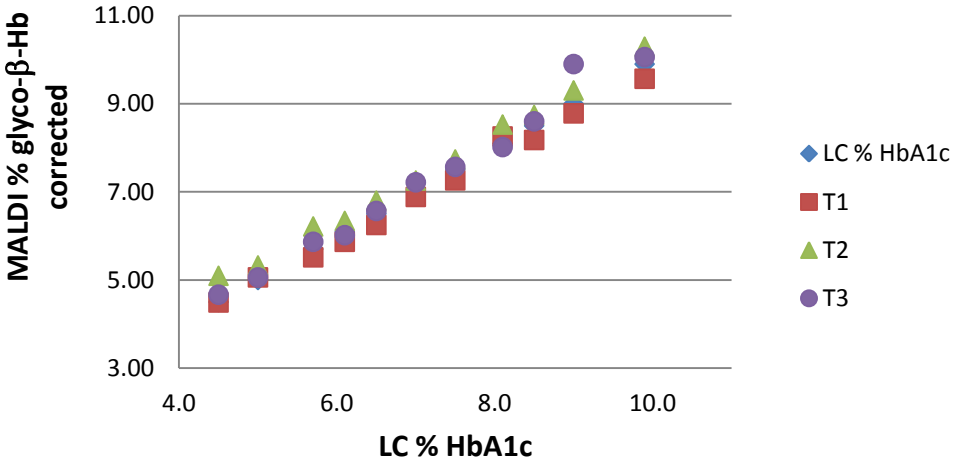


Blood Samples Jan. 2015, 3 plates, 11 samples

β - Hb

LC % A1c	T1	T2	T3	T1 Cor.
9.00	11.78	12.40	13.12	8.78
9.90	12.72	13.59	13.31	9.57
8.10	11.15	11.47	10.86	8.26
8.50	11.05	11.73	11.56	8.18
7.50	9.95	10.52	10.32	7.26
6.50	8.74	9.40	9.12	6.25
6.10	8.28	8.85	8.46	5.87
5.00	7.31	7.64	7.31	5.06
4.50	6.63	7.35	6.84	4.49
7.00	9.50	9.95	9.90	6.89
5.70	7.86	8.70	8.28	5.51
Ave % CV	2.09	2.43	2.06	





Overlay of spectra plate 3

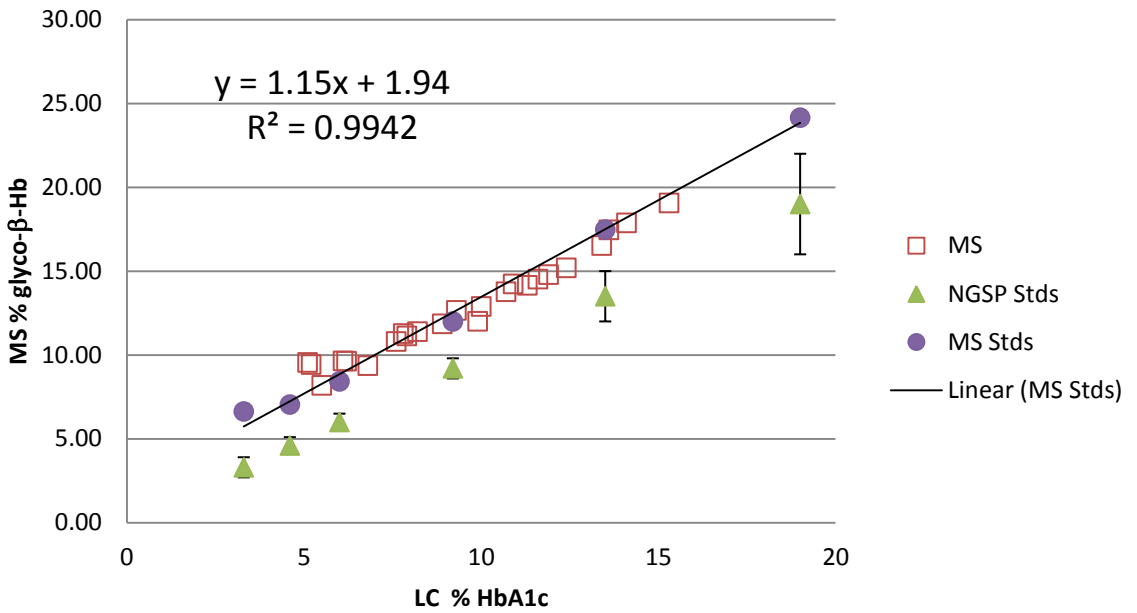
Corrected data

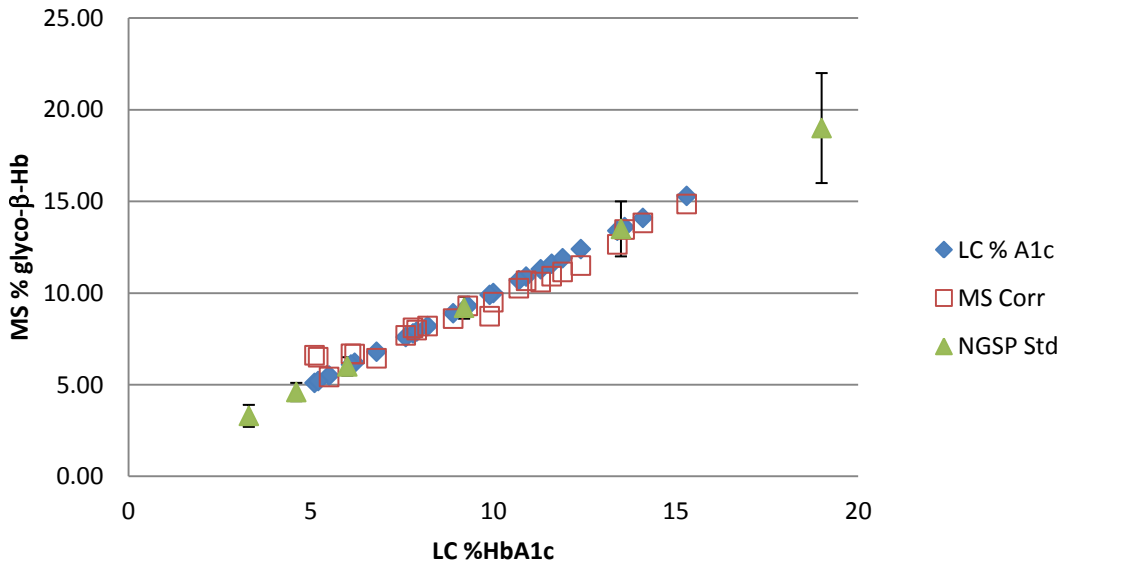
$MS_{corr} = (MS - 1.24) / 1.20$

3 plates, 11 samples, 5x replicates

Data Set 2

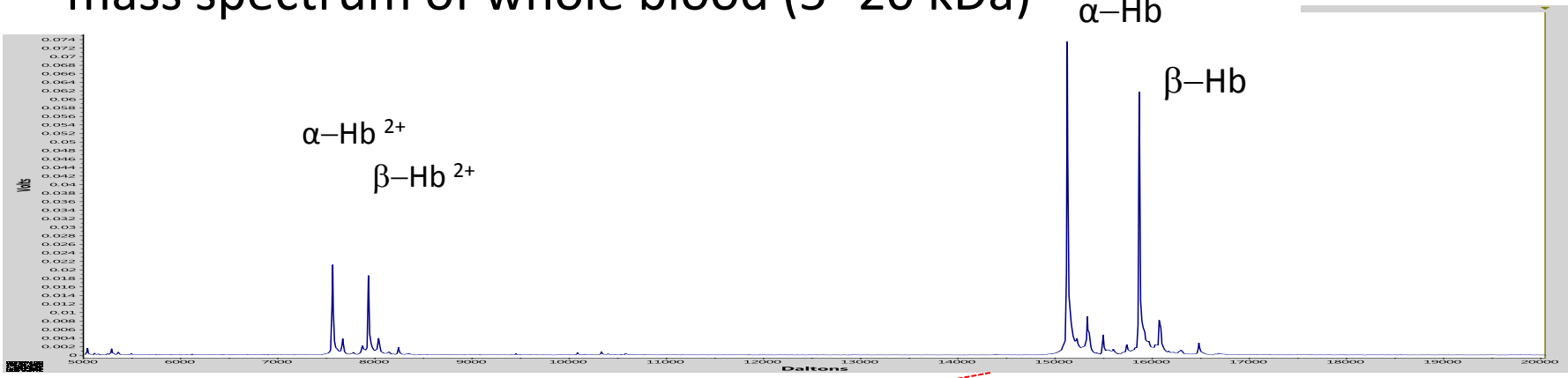
LC % A1c	MS	MS Corr
14.10	17.88	13.83
10.90	14.24	10.67
8.20	11.39	8.20
10.00	12.89	9.50
10.70	13.77	10.26
7.80	11.28	8.10
12.40	15.20	11.50
11.30	14.15	10.60
13.60	17.46	13.47
11.60	14.53	10.92
11.90	14.79	11.15
9.90	12.01	8.74
7.60	10.80	7.69
6.80	9.36	6.44
15.30	19.07	14.86
6.20	9.63	6.67
5.50	8.19	5.42
6.10	9.65	6.69
5.10	9.55	6.61
5.20	9.43	6.50
7.90	11.14	7.98
8.90	11.86	8.61
13.40	16.53	12.66
9.30	12.66	9.30

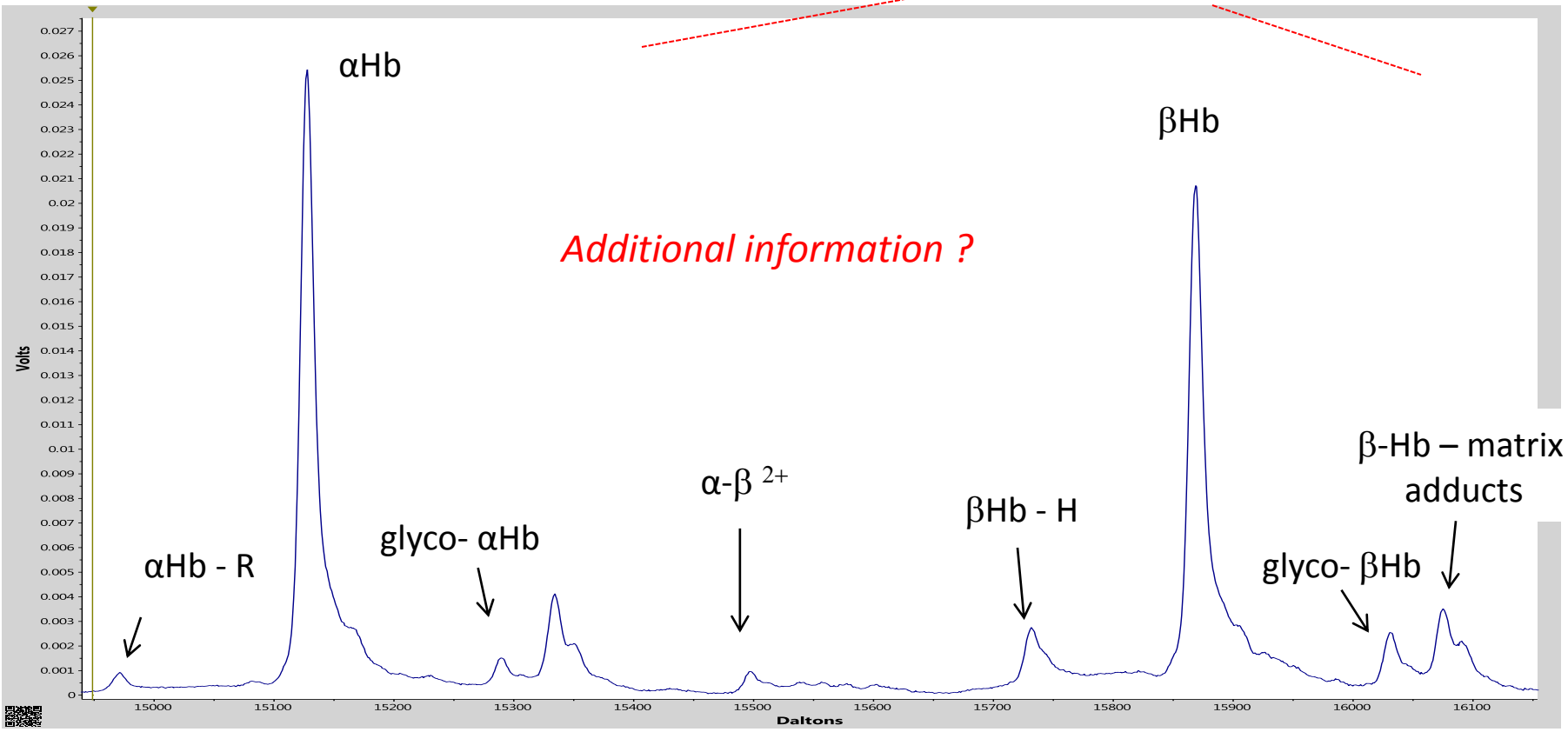




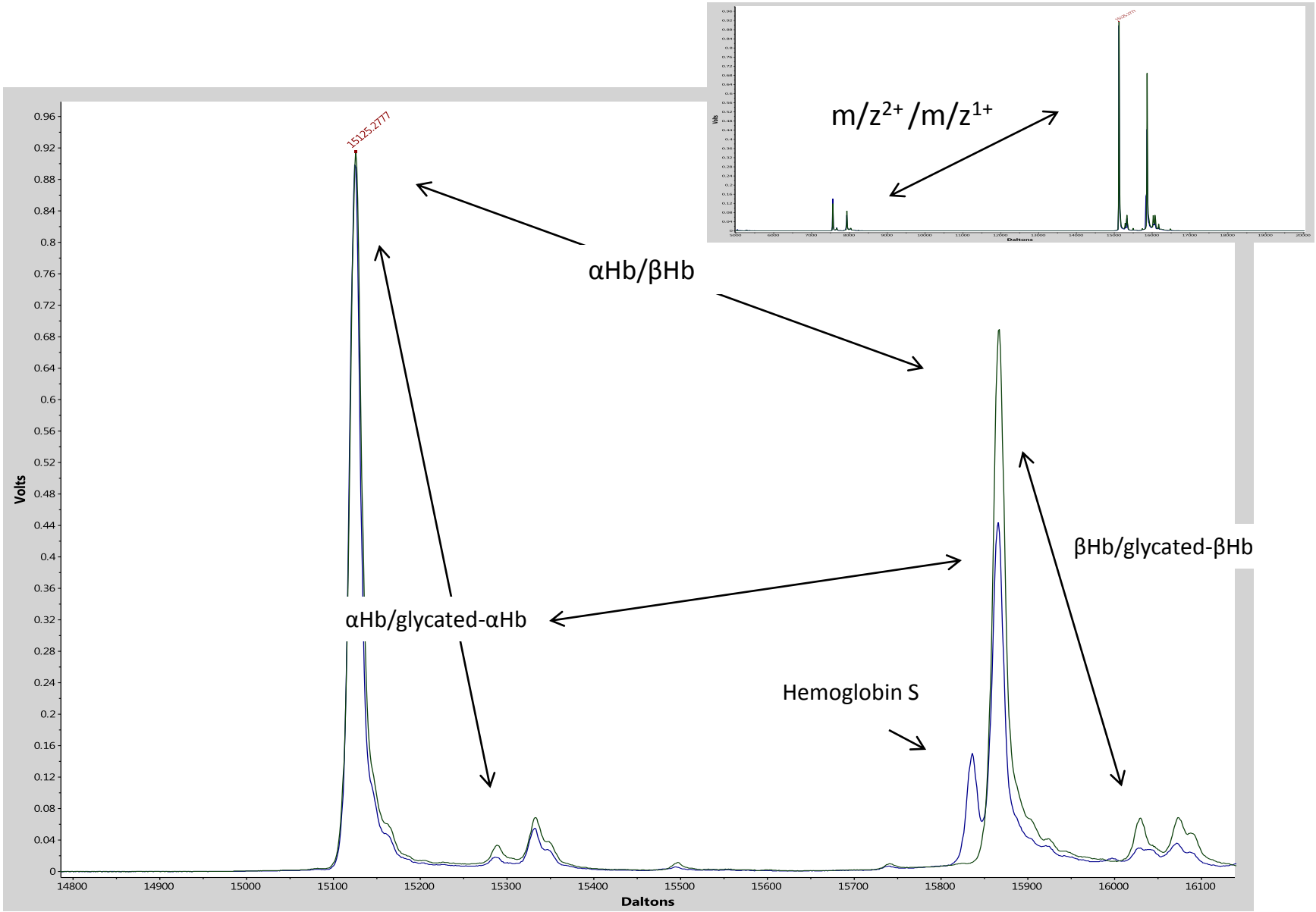
MS correction = (MS – 1.94) / 1.15

mass spectrum of whole blood (5 -20 kDa)

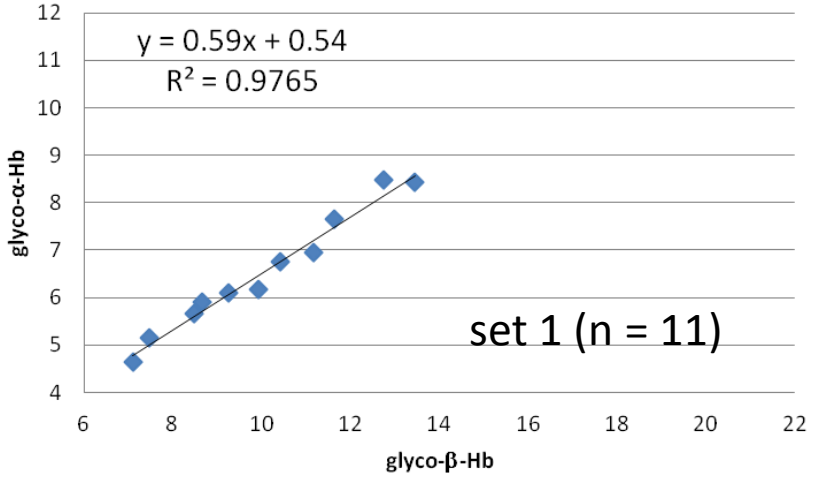


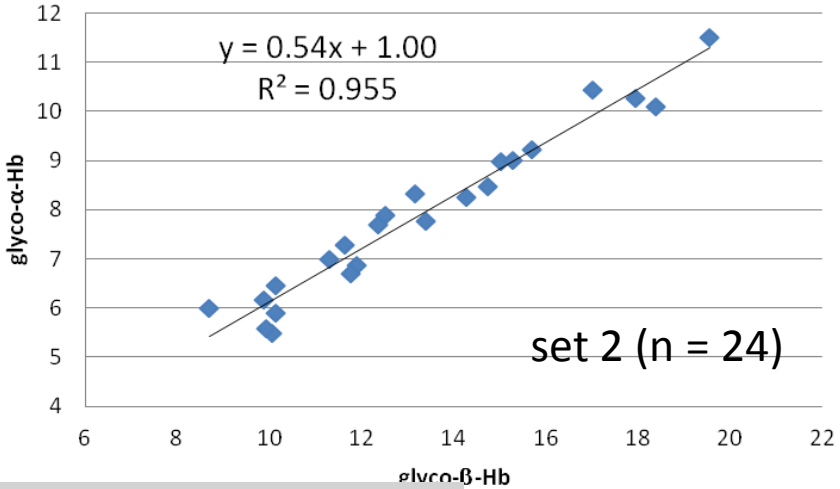


Numerous relationships that can be monitored to provide corroborative information

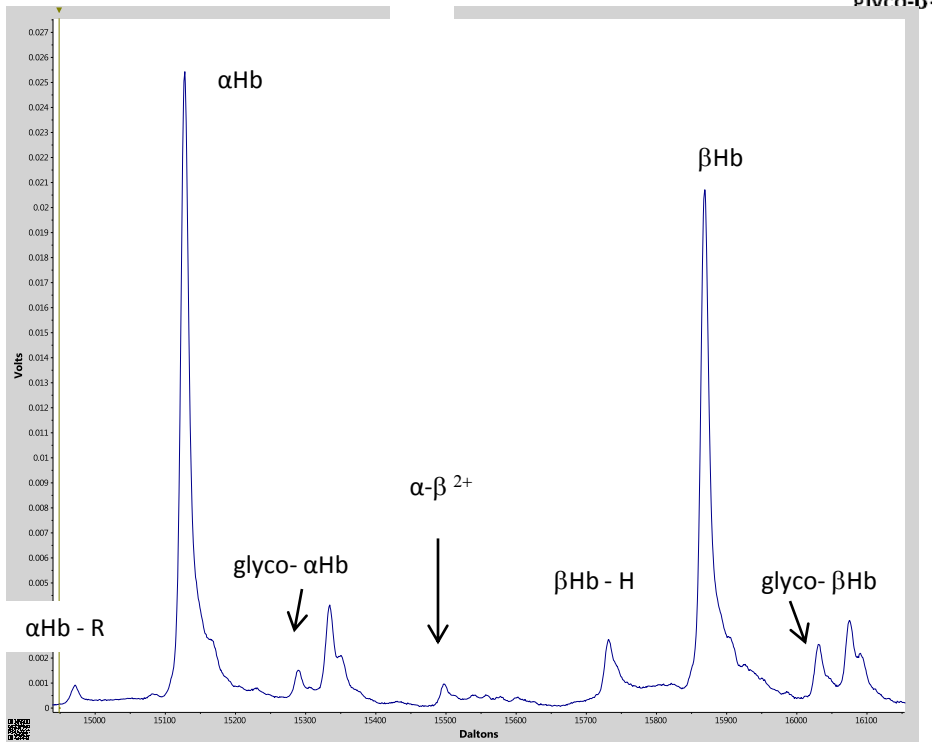


Relationship between α-chain and β-chain glycation





Glyco-β	Glyco-α	Glyco-α / Glyco-β
12.43	8.38	0.67
13.21	8.07	0.61
11.16	6.77	0.61
11.45	7.15	0.62
10.27	6.36	0.62
9.09	5.82	0.64
8.53	5.96	0.70
7.42	4.91	0.66
6.94	4.43	0.64
9.78	5.88	0.60
8.28	5.41	0.65
	Ave	0.64
	Std Dev	0.03
	CV (n=11)	4.80

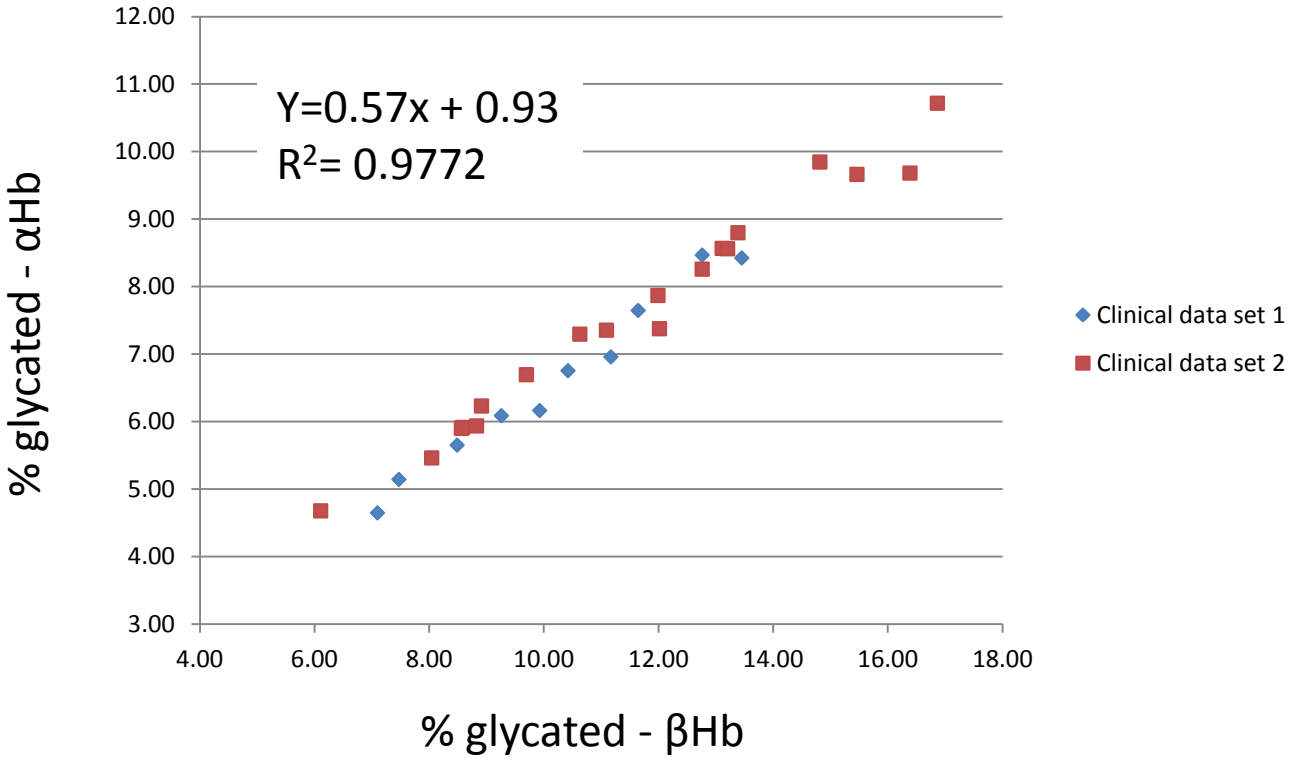


Data set 2
glyco-αHb / glyco-βHb

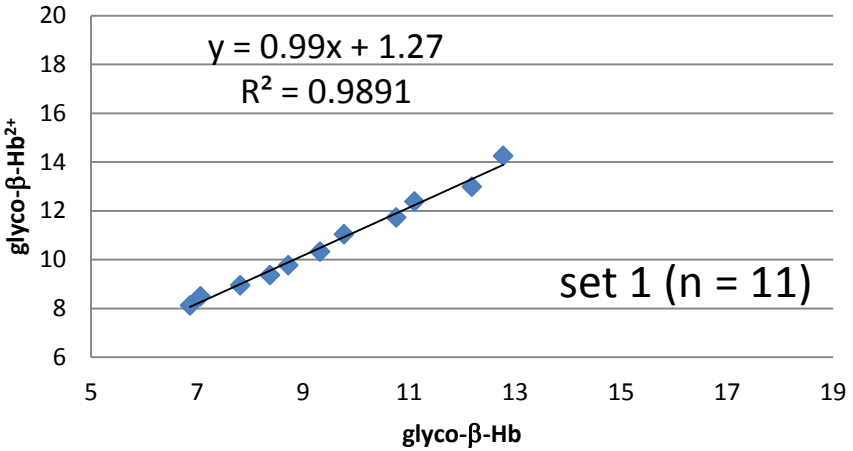
Ave	0.63
Std Dev	0.04
CV (n=24)	6.51

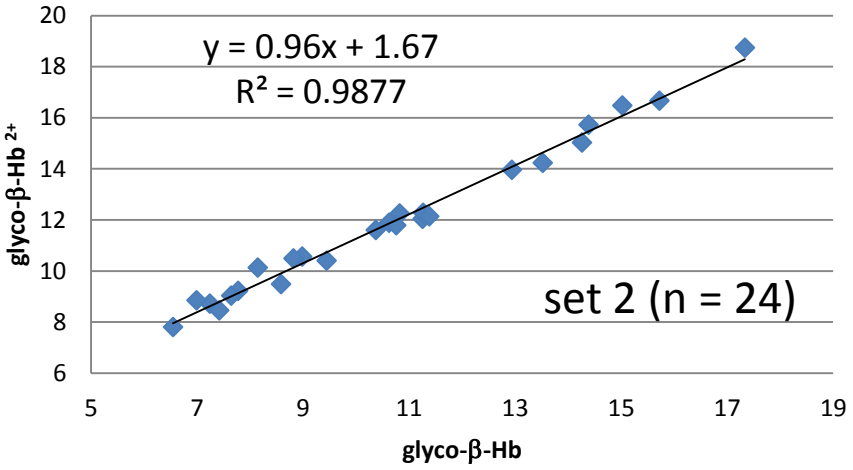
High = 0.73
Low = 0.57

Sample set overlay

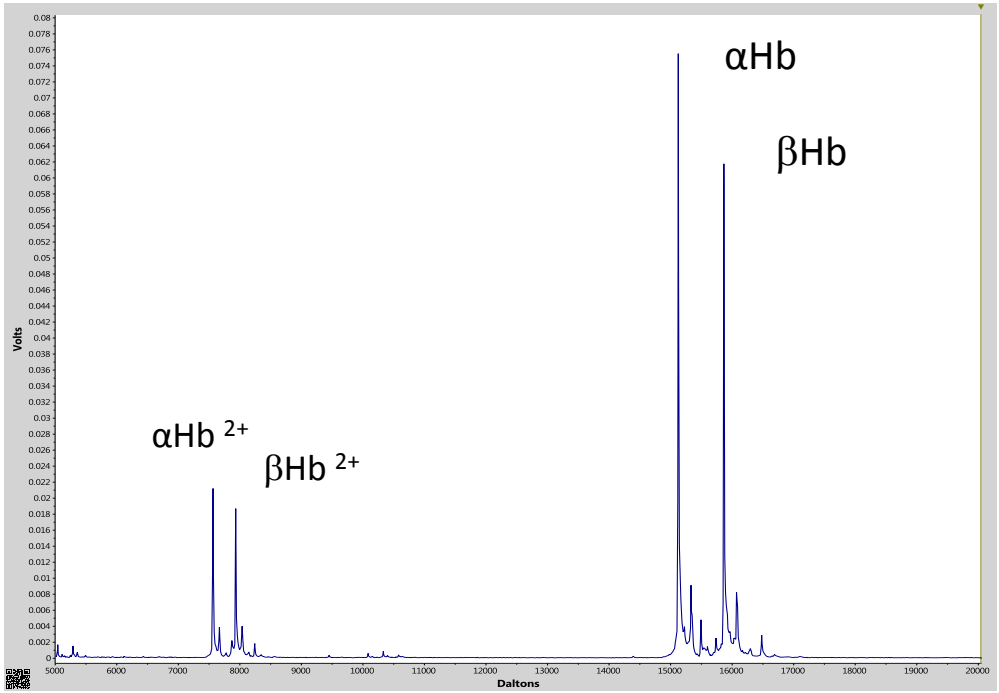


Relationship between singly and doubly charged β-chain glycation





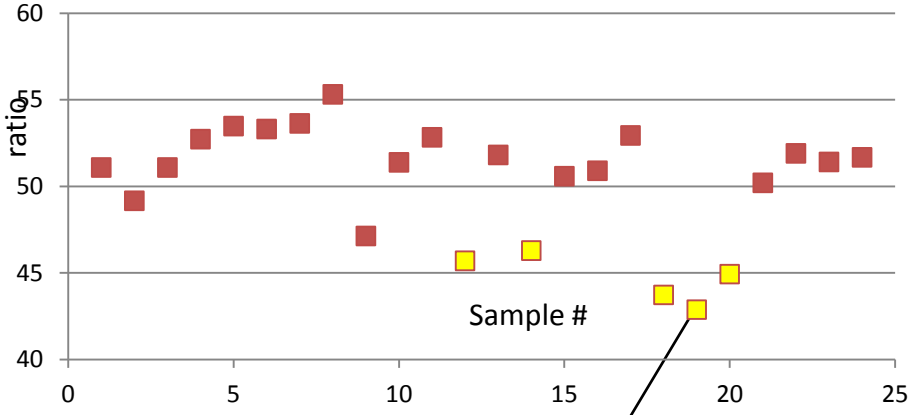
Glyco-β	Glyco-β²⁺	Glyco-β²⁺ / Glyco-β
12.43	12.99	1.04
13.21	14.25	1.08
11.16	11.73	1.05
11.45	12.38	1.08
10.27	11.04	1.08
9.09	9.77	1.08
8.53	9.37	1.10
7.42	8.50	1.15
6.94	8.12	1.17
9.78	10.33	1.06
8.28	8.95	1.08
	Ave	1.09
	Std Dev	0.04
	CV (n=11)	3.55

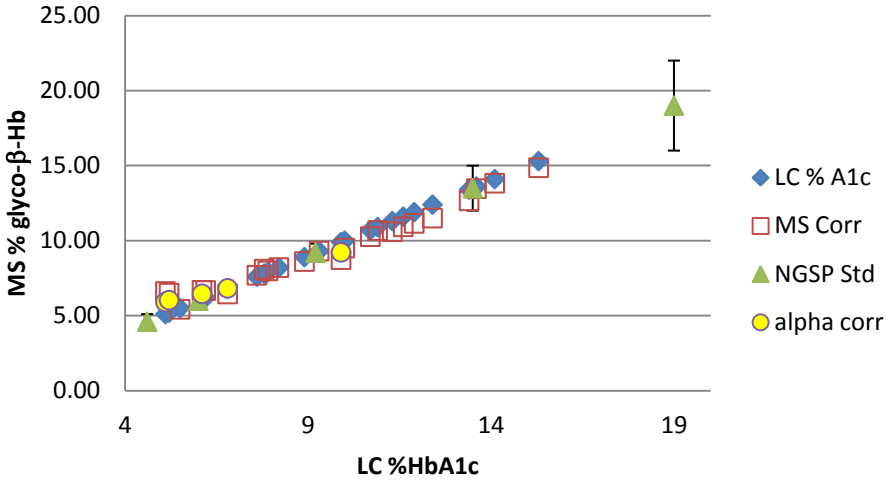


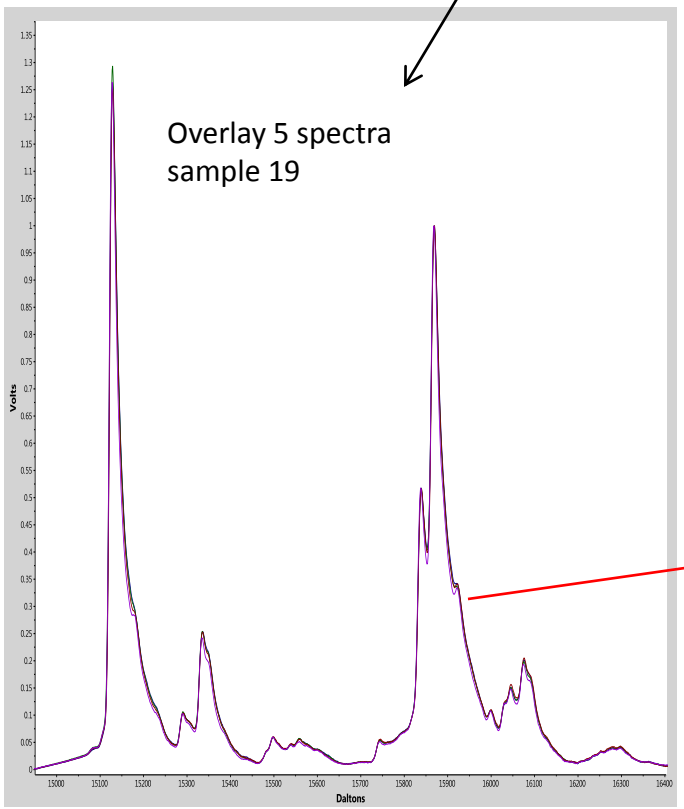
Ave	1.13
Std Dev	0.06
CV (n=24)	5.40

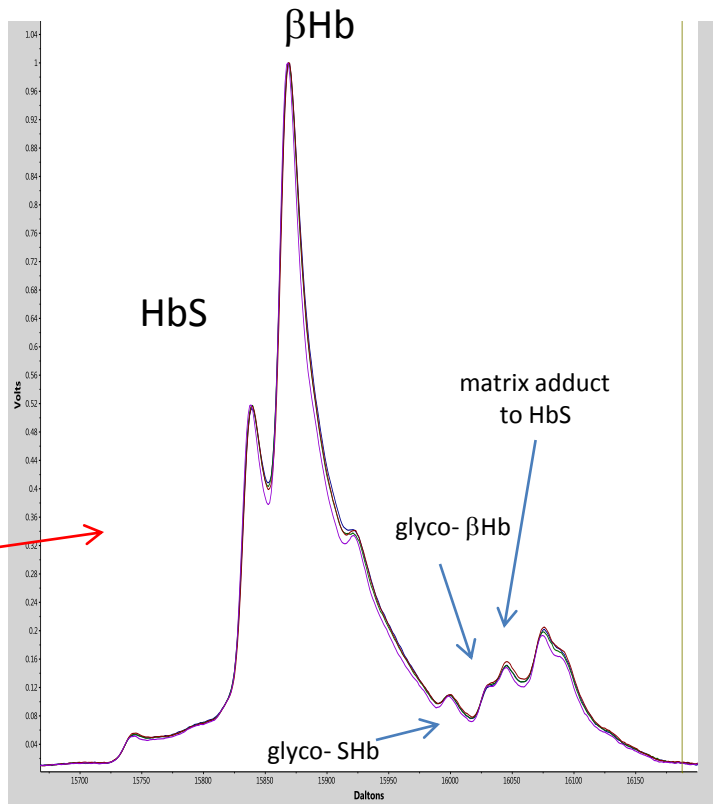
High = 1.27
Low = 1.05

Ratio between the main chain α and β peaks

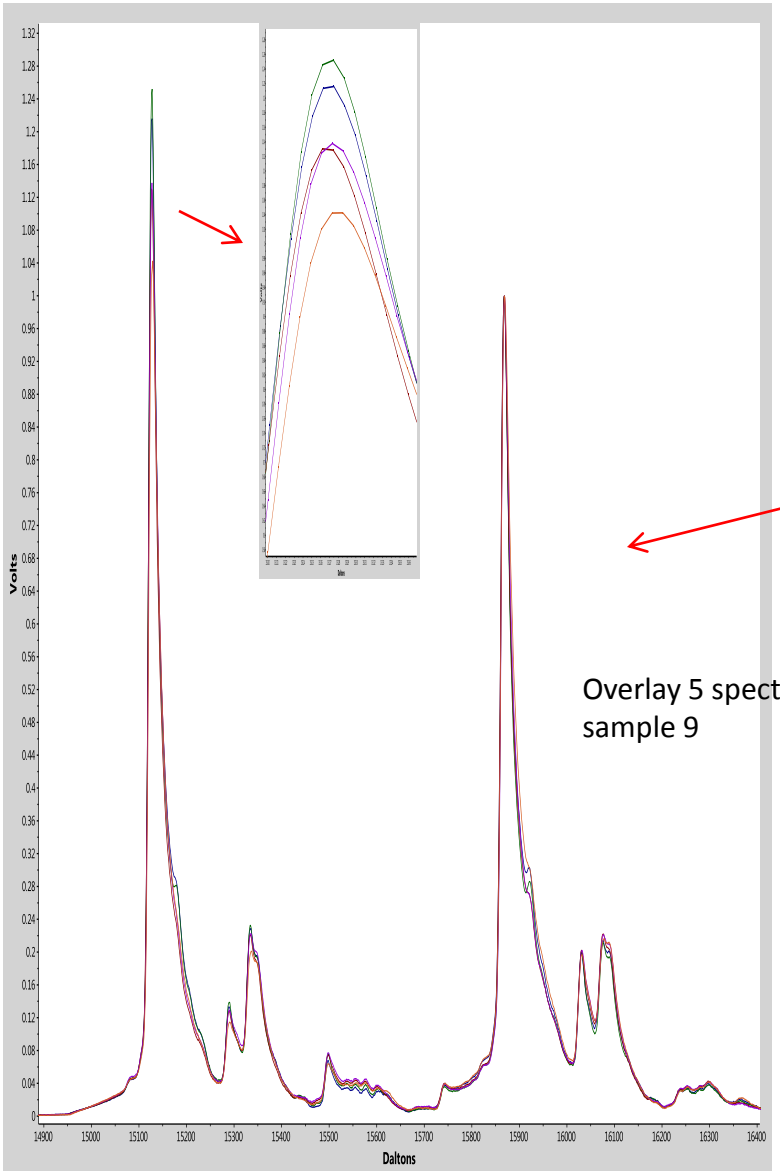




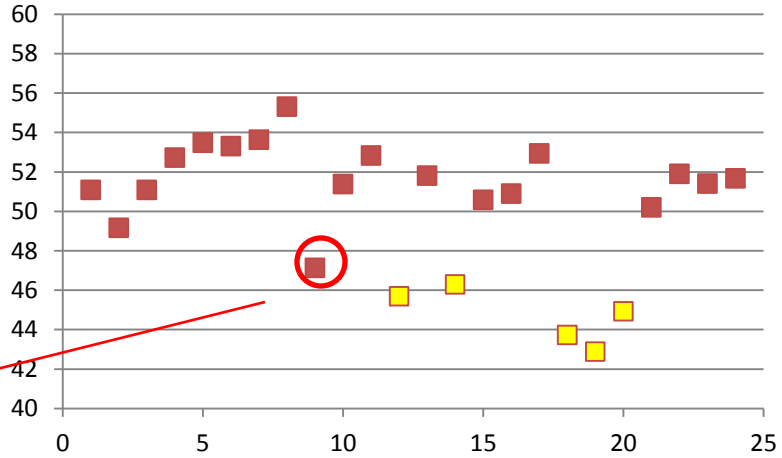




Ratio between the main chain α and β peaks



Overlay 5 spectra
sample 9



Proteinuria / Microalbuminuria / Hematuria

•Proteinuria (pro-tee-NU-ree-uh) and microalbuminuria = *Protein in urine*

-Low levels of protein in urine can be normal

-Temporarily high levels of protein in urine aren't unusual either, particularly after exercise or during an illness

- Often *monitored in cases of diabetes* and newly developing or increasing amounts of protein in urine may be an earliest sign of *diabetic kidney damage*.

•Hematuria = *blood in urine*

-It's important to determine the cause

-not necessarily painful and can occur without other signs or symptoms

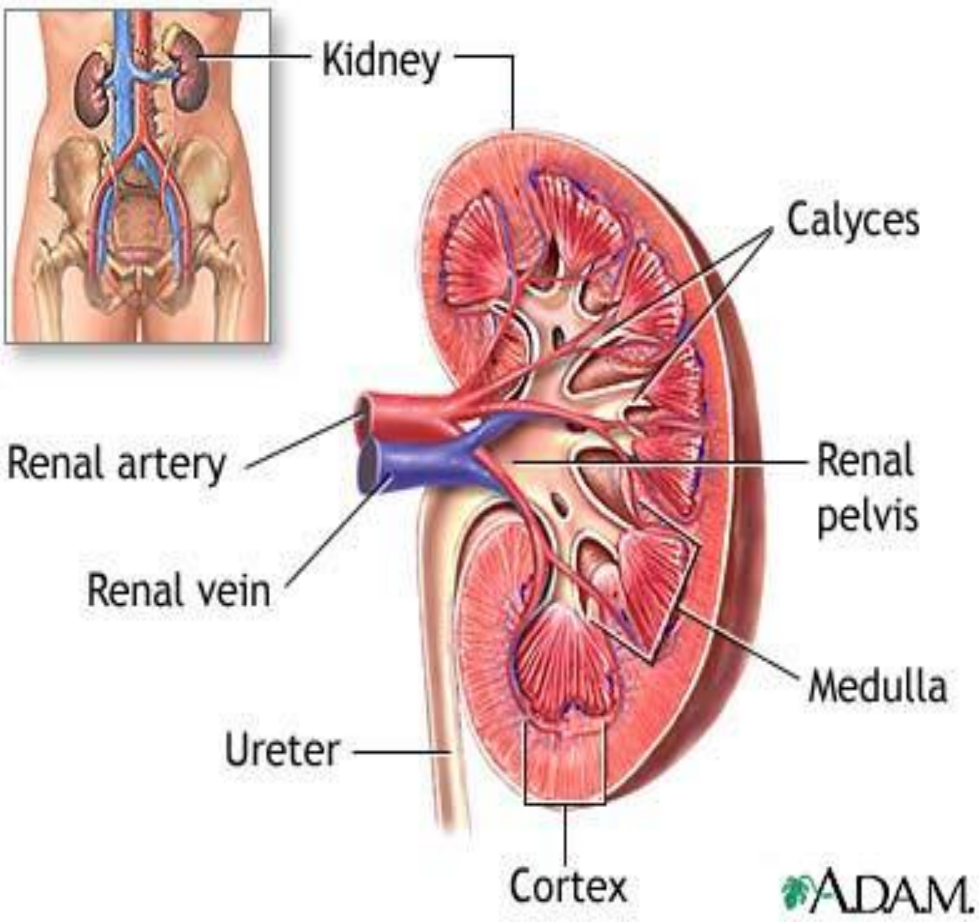
When to see a doctor ?

Make an appointment to see your doctor anytime you notice blood in your urine.

http://www.mayoclinic.org/diseases-conditions/blood-in-urine/basics/definition

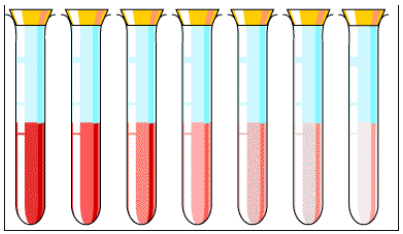
“Urias”, although not necessarily bad, are not considered healthy either

Causes
Urinary tract infection
Kidney infection
Bladder or kidney stone
Enlarged prostate
Kidney disease
Cancer
Inherited disorders
Kidney injury
Medications
Illness
Strenuous exercise



http://www.mayoclinic.org/diseases-conditions/blood-in-urine/basics/causes

Quantification of Albumin in Urine Using MALDI-TOF MS



Create Calibration Curve with spiked albumin and Internal Standard

-- Albumin standard weighed and diluted with pooled urine to 100 µM

-- 10x dilution of pooled Std with individual control sample (Ken or Steve) to create 10µM

-- Dilute 10µM by 2x six times using same urine sample
span clinically range 10.00 to 0.31 µM

--All standard further diluted 1:10 (sample:matrix) containing internal std.
0.075 µM Lysozyme in 10 mg/mL alpha-cy 75% CH_3CN, 0.1% TFA

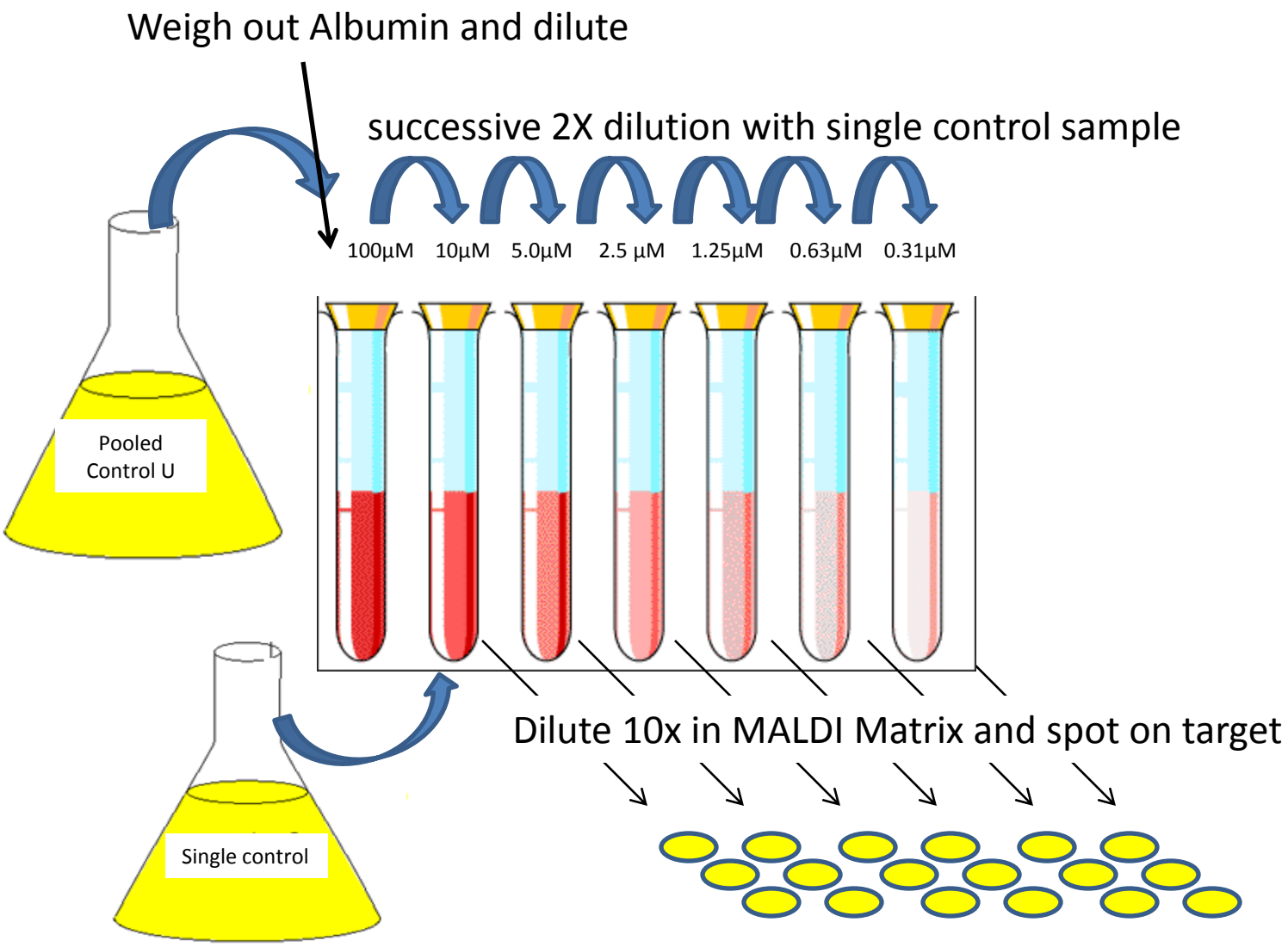
-- All concentrations spotted in 6x replication

--All spectra normalized to Lysozyme singly charged peak

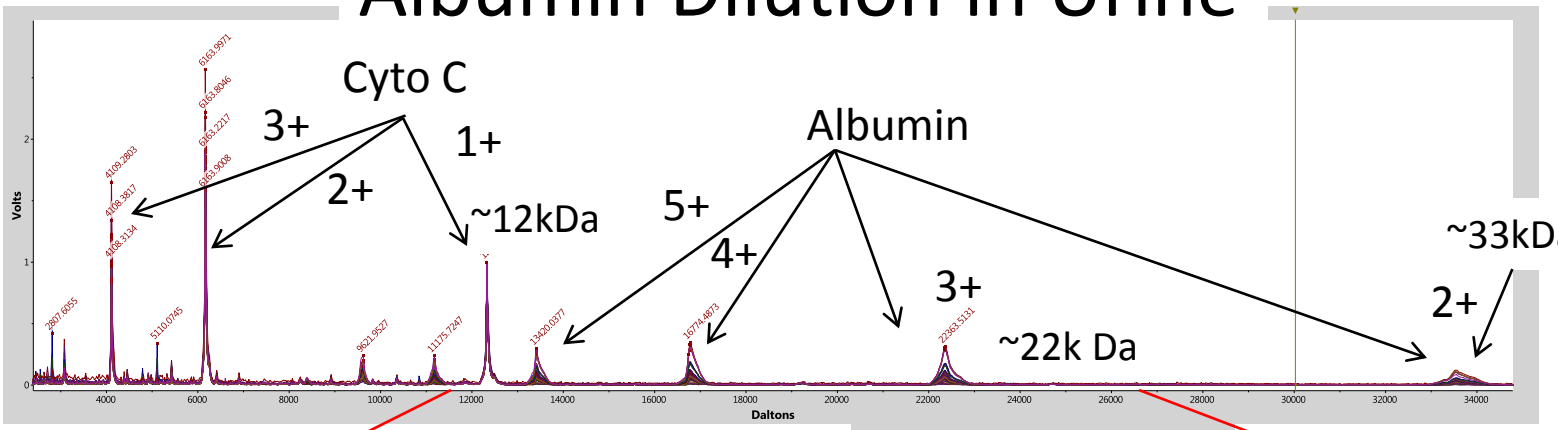
-- Integration Albumin response used to create calibration curve for quantitation of unknowns.

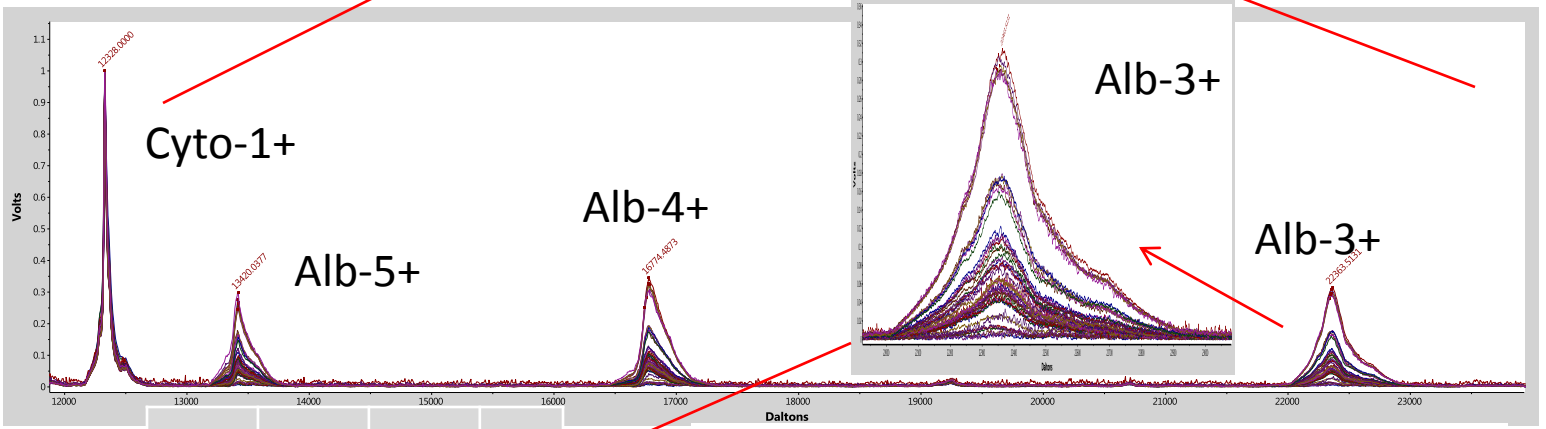
Patient sample diluted 10x with matrix and internal Std.
-run in 3 x replication
-all spectra normalized to Lysozyme signal
-signal quantitated using calibration curve constructed from Standards

Create dilution series of Albumin in Control Urine

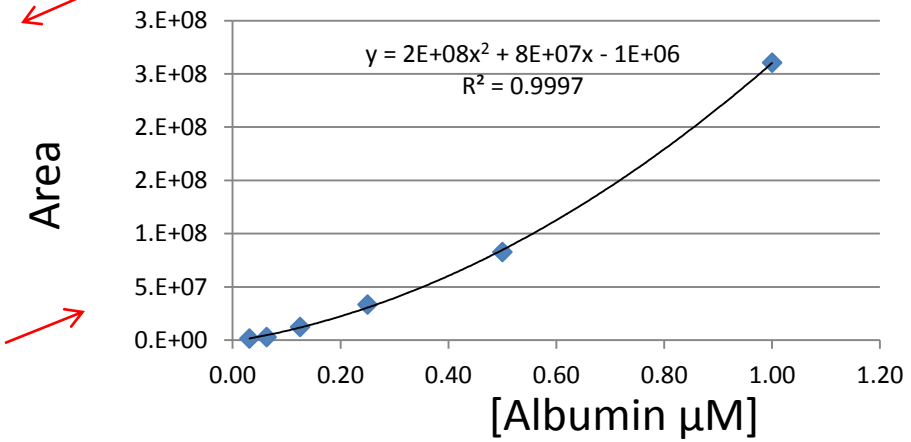


Albumin Dilution in Urine





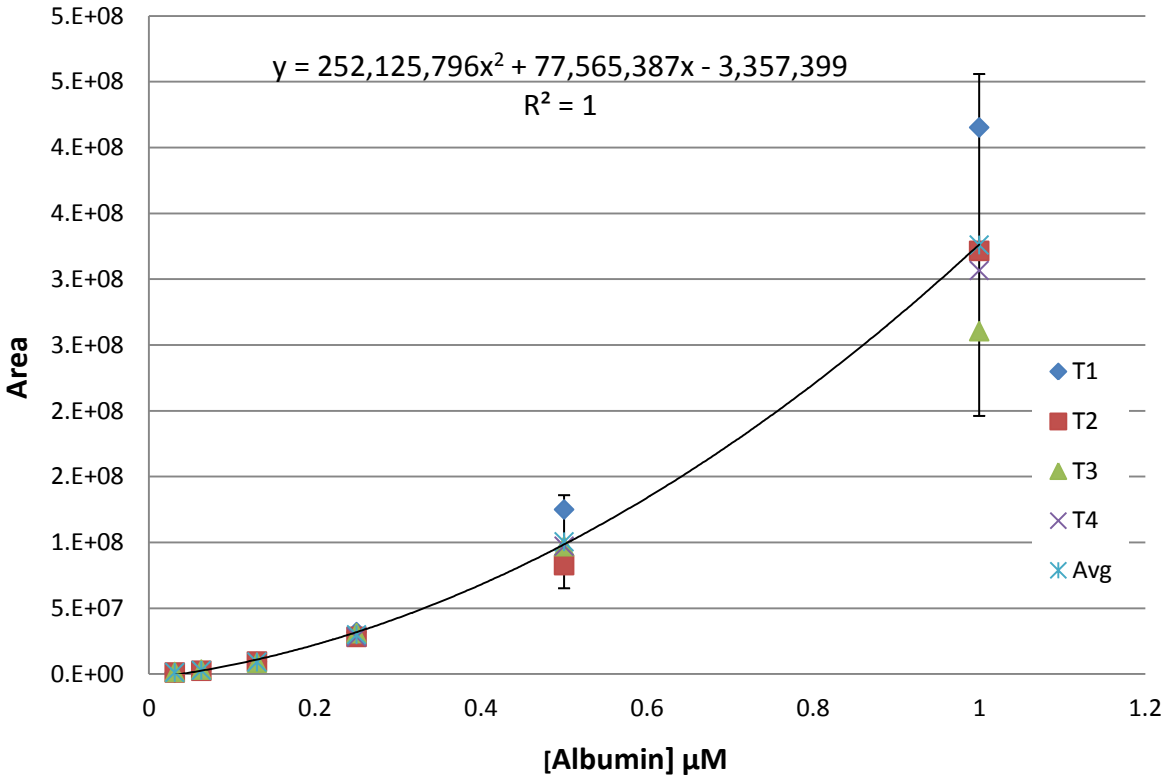
[Albumin]	Area	Std Dev	CV %
0.03	1.55E+06	1.02E+05	6.58
0.06	2.90E+06	1.58E+05	5.43
0.13	1.24E+07	1.01E+06	8.12
0.25	7.63E+07	2.27E+06	6.78
0.50	8.27E+07	5.10E+06	6.16
1.00	2.60E+08	1.53E+07	5.86



Multiple Calibration curves of Albumin spiked in Urine measured of over weeks

[Alb] µM	Area	Std Dev	CV
0.03	1.21E+06	3.14E+05	26.00
0.06	2.80E+06	4.89E+05	17.50
0.13	9.09E+06	4.35E+05	4.78
0.25	2.98E+07	1.92E+06	6.44
0.50	1.01E+08	1.77E+07	17.59
1.00	3.26E+08	6.50E+07	19.93
		Avg =	15.37

Data fits best to a 2nd order quadratic regression
Alb^{3+}



Error bars = ± (2 x Std Dev)

Quantification of Creatinine Using Isotopically Labeled Creatinine (d3)

Established quantitative response across clinically relevant range using control

-Deuterated creatinine (d3) spiked into urine at 4 different concentrations to span clinically relevant range

-Samples analyzed and normalized to native creatinine peak

-Calibration curve constructed based and spiked [creatinine] vs. peak area for d3 peak

Performed relative quantitation in unknowns using a single point spike

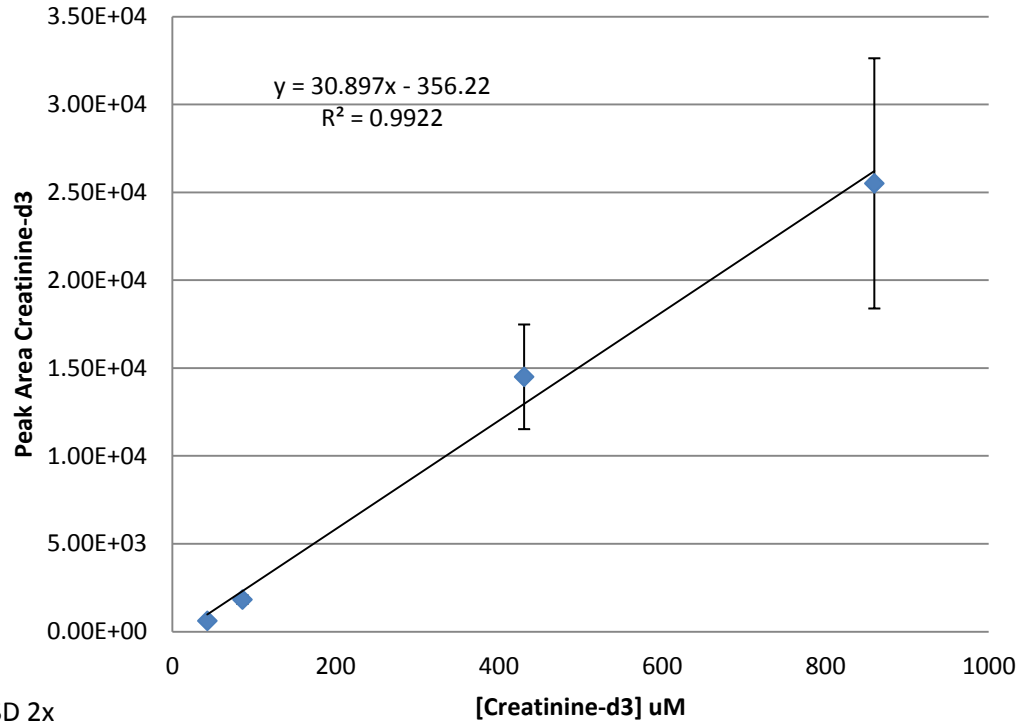
-With signal response calibrated across clinically relevant range

-[Creatinine] in unknowns were quantitated by relative comparison of native creatinine signal against that of a single (mid-range) spike of creatinine-d3 included in MALDI matrix

ken

Normal range urine (male)
3.5-26.5 mM

urine diluted 100x
35 – 265 µM

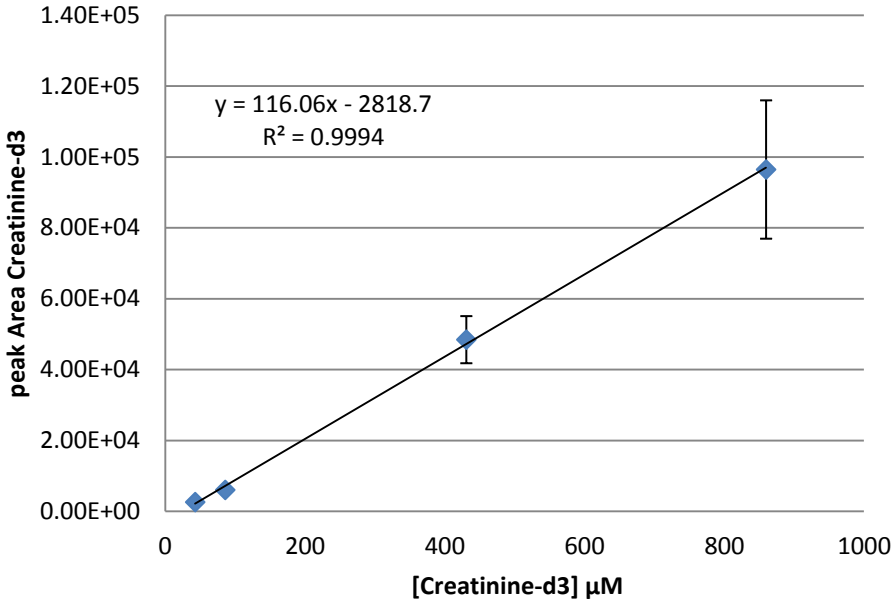


[Creatinine-d3]	Area (n=6)	SD	CV	SD 2x
43.00	615.10	70.07	11.39	140.13
86.00	1823.91	123.38	6.76	246.76
431.00	14501.29	1488.90	10.27	2977.80
860.00	25508.39	3559.71	13.96	7119.42

	Area 113.14 (n=6)	SD	CV	[Calculated]
T1	10246.81	381.05	3.71	343.14
T2	11137.06	980.24	8.80	371.95
T3	10816.40	391.24	3.61	361.57
T4	10941.94	806.46	7.37	365.64
		Avg		360.58
		SD		12.38
		CV %		3.43

Normal range urine (male)
3.5-26.5 mM

urine diluted 100x
35 – 265 µM



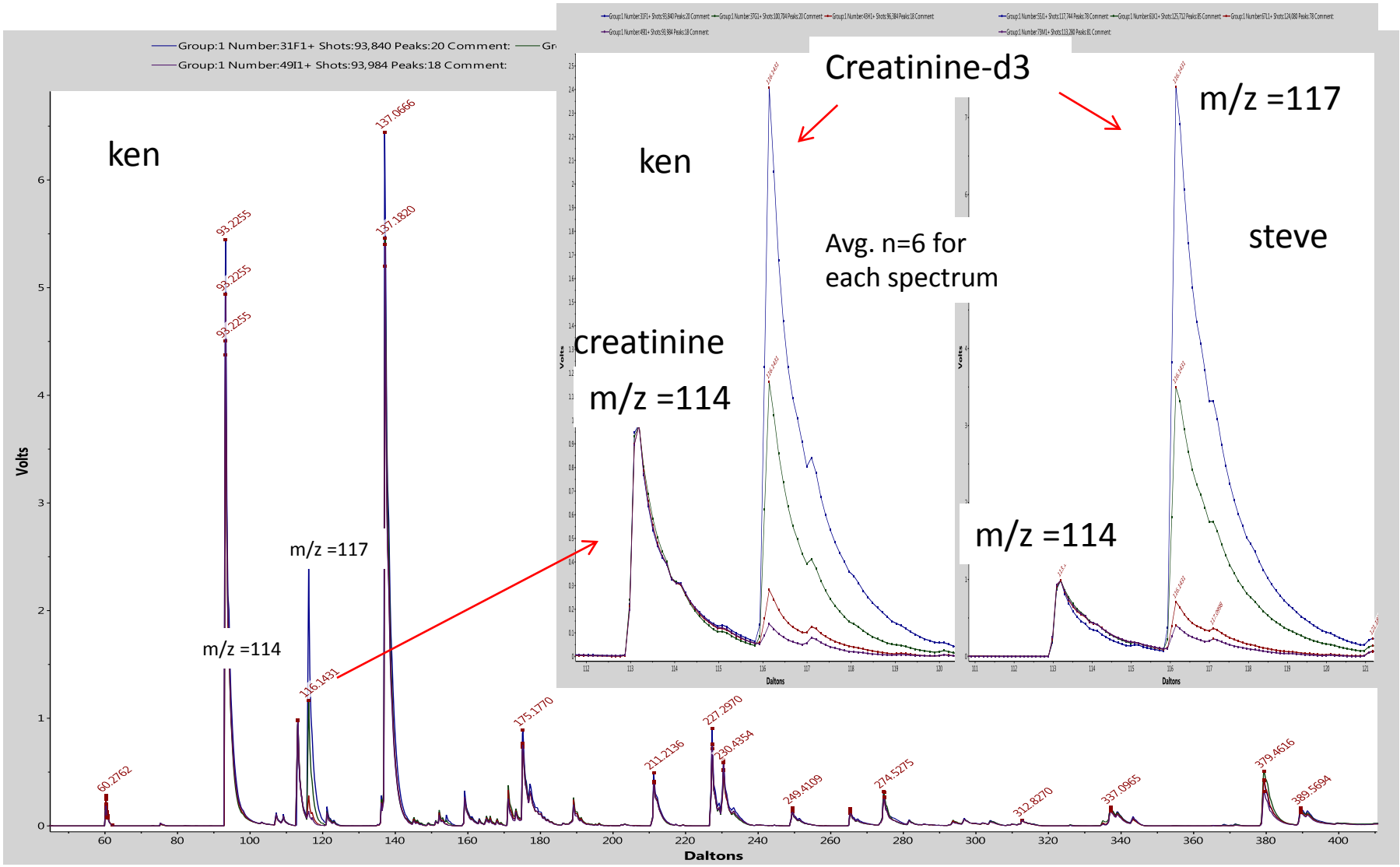
[Creatinine-d3]

µM	Area (n=6)	SD	CV	SD 2x
43.00	2597.03	367.62	14.16	735.24
86.00	6026.36	308.41	5.12	616.82
431.00	48442.19	3324.68	6.86	6649.37
860.00	96462.58	9762.23	10.12	19524.46

Native Creatinine

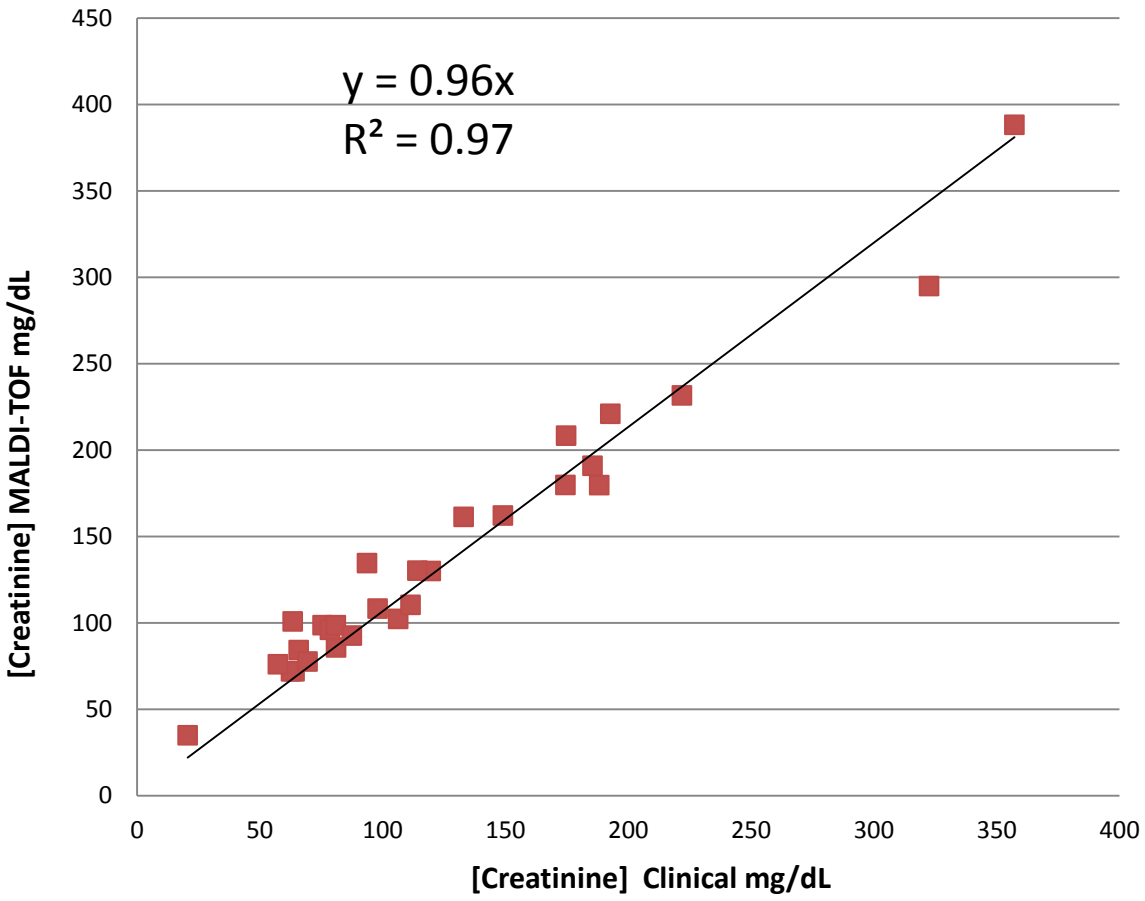
	Area 113.14 (n=6)	SD	CV	[Calculated]
T1	13167.51	1884.66	14.31	137.74
T2	14977.70	1072.87	7.16	153.34
T3	11546.79	199.43	1.73	123.78
T4	10163.53	351.93	3.46	111.86
			Avg	131.68
			SD	17.90
			CV	13.59

Creatinine overlay of avg. spectra for calibration curve



Comparison of [Creatinine] Clinical vs. MALDI-TOF relative quantitation against 9 mg/dL Creatinine d3 spike

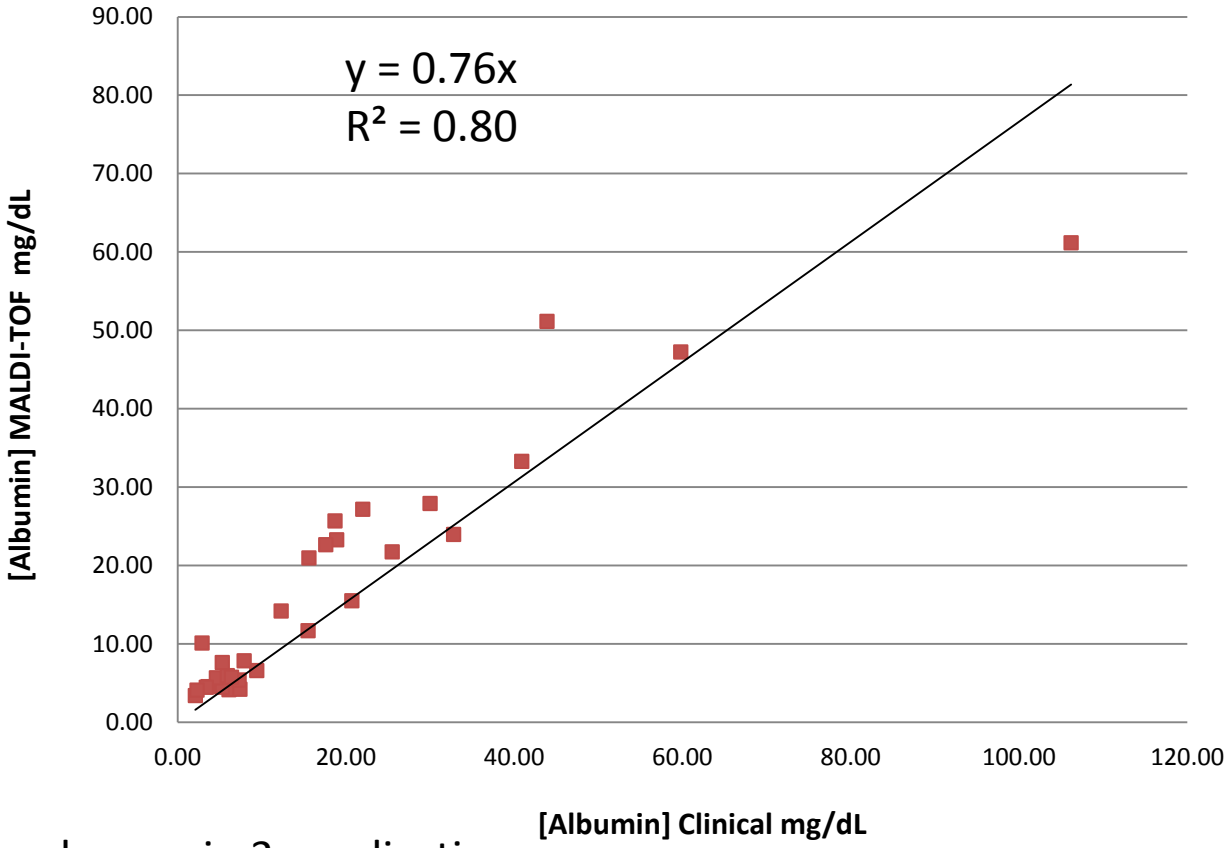
Sample	Clinical mg/dL	MALDI mg/dL	CV %
1			
2			
3	63.4	100.8	1.1
4	192.7	221.0	3.3
5	93.6	134.6	7.8
6	98.0	108.3	3.8
7	119.6	130.0	3.6
8	65.8	84.3	1.4
9	81.0	85.8	0.7
10	75.5	98.7	2.1
11	64.1	72.0	1.4
12	62.7	71.9	2.7
13	221.9	231.7	1.5
14	188.2	179.7	1.1
15	357.3	388.3	2.6
16	114.2	130.2	0.9
17	174.4	179.8	5.0
18	133.0	161.4	3.5
19	185.5	190.9	3.9
20	149.0	162.1	2.6
21	78.5	96.0	1.7
22	322.5	294.9	6.1
23	174.7	208.4	2.0
24	80.9	98.7	9.0
25	106.3	102.3	0.2
26	111.4	110.4	0.9
27	69.4	77.6	3.5
28	87.5	92.7	0.8
29	57.3	76.1	1.2
30	20.6	34.9	4.7
		Avg. =	2.81



Measurements done in 3x replication

Comparison of [Albumin] Clinical vs. MALDI-TOF

Sample	Clinical mg/dL	MALDI mg/dL	CV %
1	3.6	4.6	14.5
2	6.1	4.2	17.5
3	43.9	51.2	31.7
4	5.3	4.5	3.3
5	18.9	23.3	28.4
6	2.1	3.4	17.8
7	32.8	24.0	24.8
8	4.6	5.7	37.5
9	9.4	6.6	34.2
10	22.0	27.2	17.5
11	25.5	21.8	17.8
12	7.4	4.2	12.1
13	20.7	15.5	36.4
14	15.5	11.7	18.8
15	5.9	6.0	33.1
16	7.3	5.4	10.8
17	3.4	4.5	10.1
18	17.6	22.7	11.4
19	7.9	7.9	27.2
20	12.3	14.2	22.0
21	18.7	25.7	3.2
22	6.4	5.8	4.3
23	106.2	61.2	30.2
24	2.9	10.1	37.3
25	59.8	47.3	9.2
26	2.3	4.1	5.4
27	40.9	33.3	20.9
28	5.3	7.6	13.6
29	30.0	27.9	24.7
30	15.6	21.0	2.7
		Avg. =	19.51

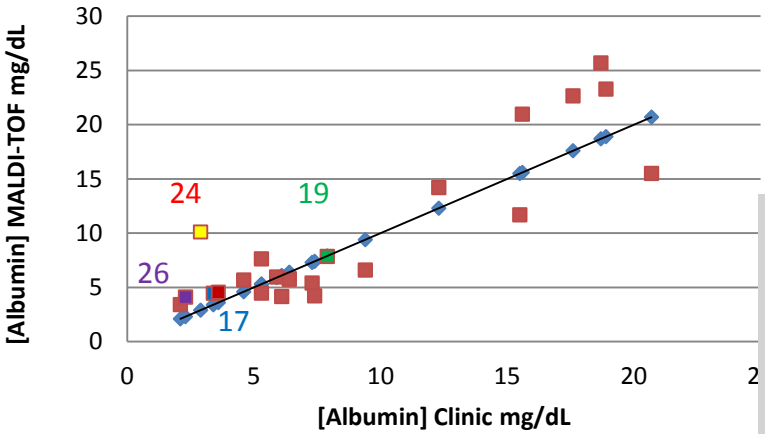


All samples run in 3x replication

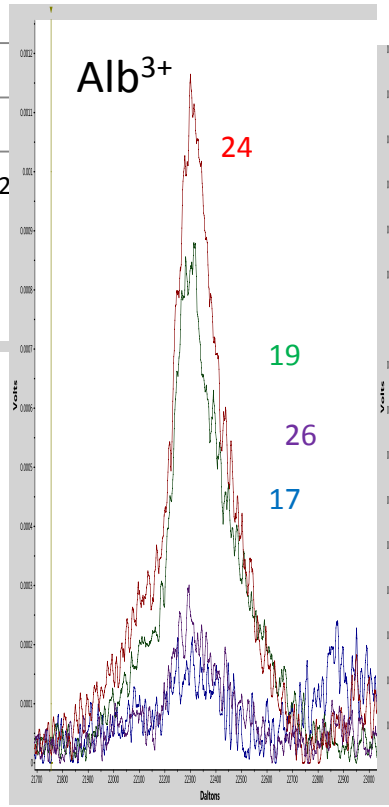
Wednesday 3:00 PM ; ***Proteomics***
"A Reference Measurement System for Urine Albumin"
Ashley Beasley Green, NIST

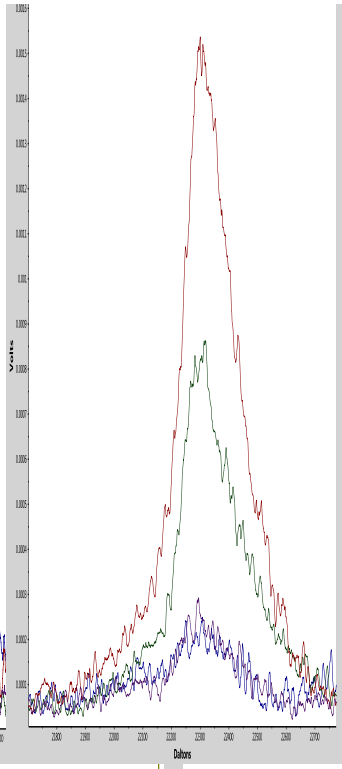
Closer look at low end measurements of [Albumin]

Sample #	[Albumin] Clinical	[Albumin] MALDI-TOF
6	2.1	3.4
26	2.3	4.1
24	2.9	10.1
17	3.4	4.5
1	3.6	4.6
8	4.6	5.7
4	5.3	4.5
28	5.3	7.6
15	5.9	6.0
2	6.1	4.2
22	6.4	5.8
16	7.3	5.4
12	7.4	4.2
19	7.9	7.9
9	9.4	6.6
20	12.3	14.2
14	15.5	11.7
30	15.6	21.0
18	17.6	22.7
21	18.7	25.7
5	18.9	23.3
13	20.7	15.5

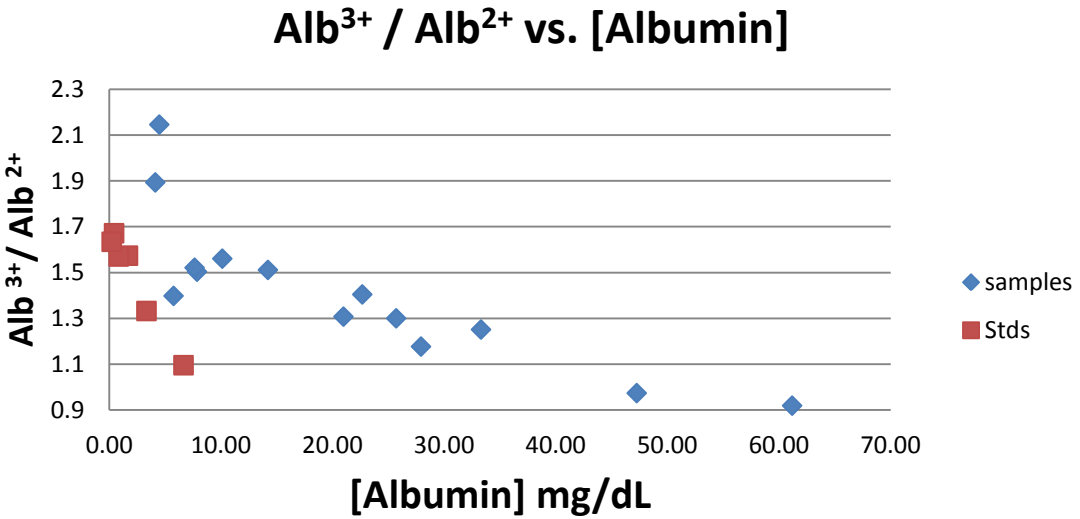


No normalization

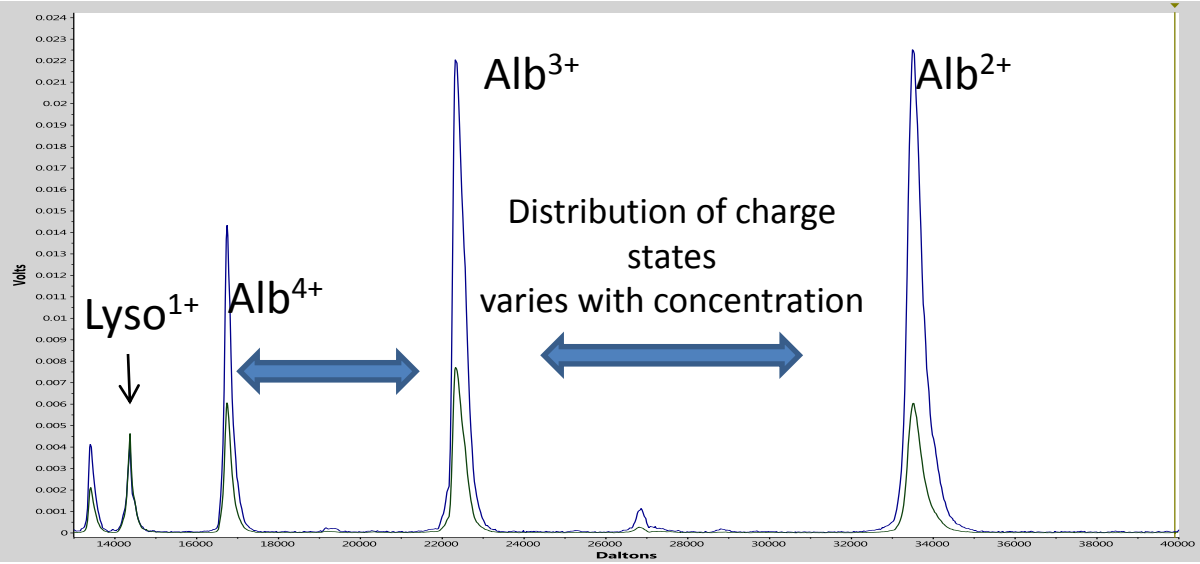




As [Albumin] increases the signal distribution between charged states changes

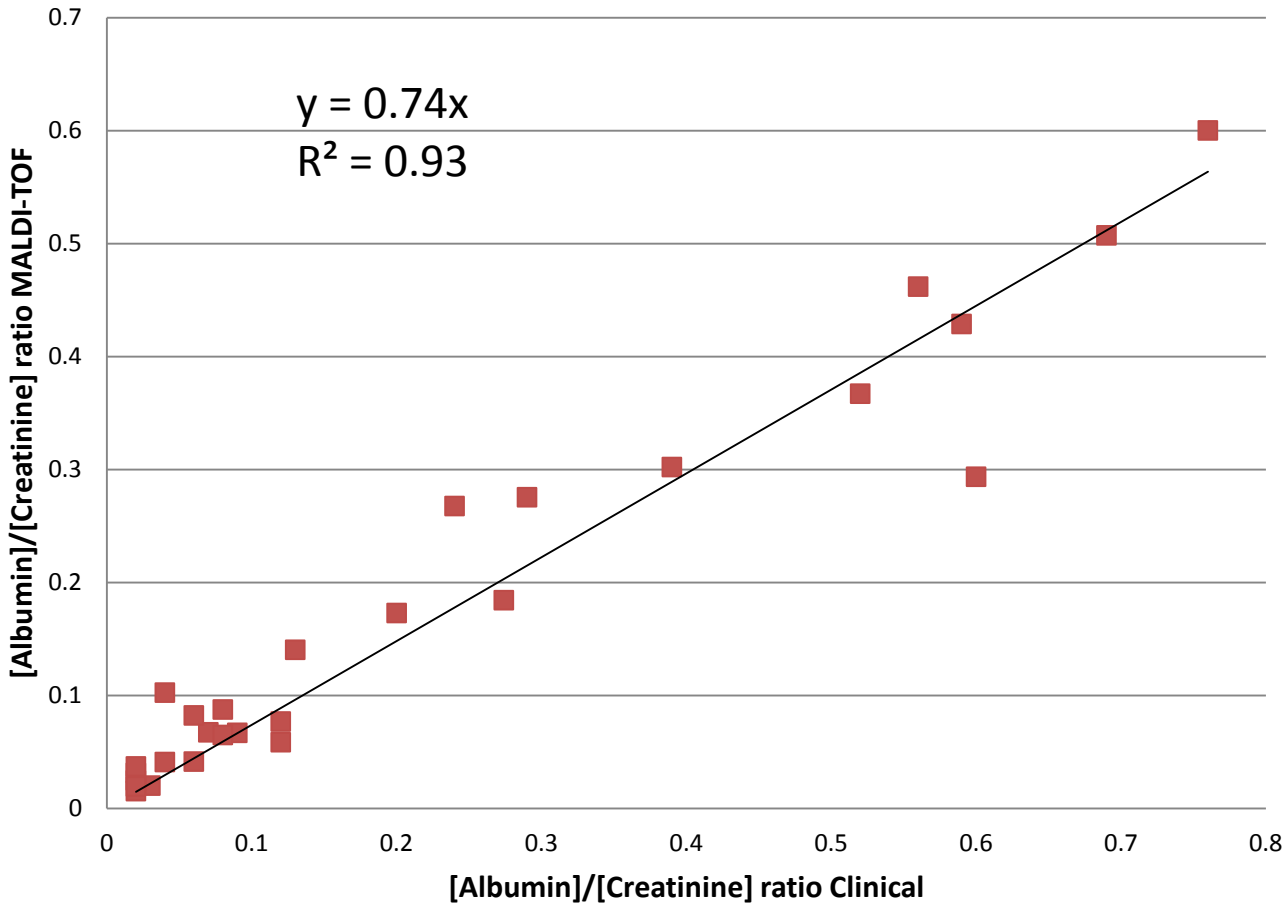


impacting [albumin] calculation ?



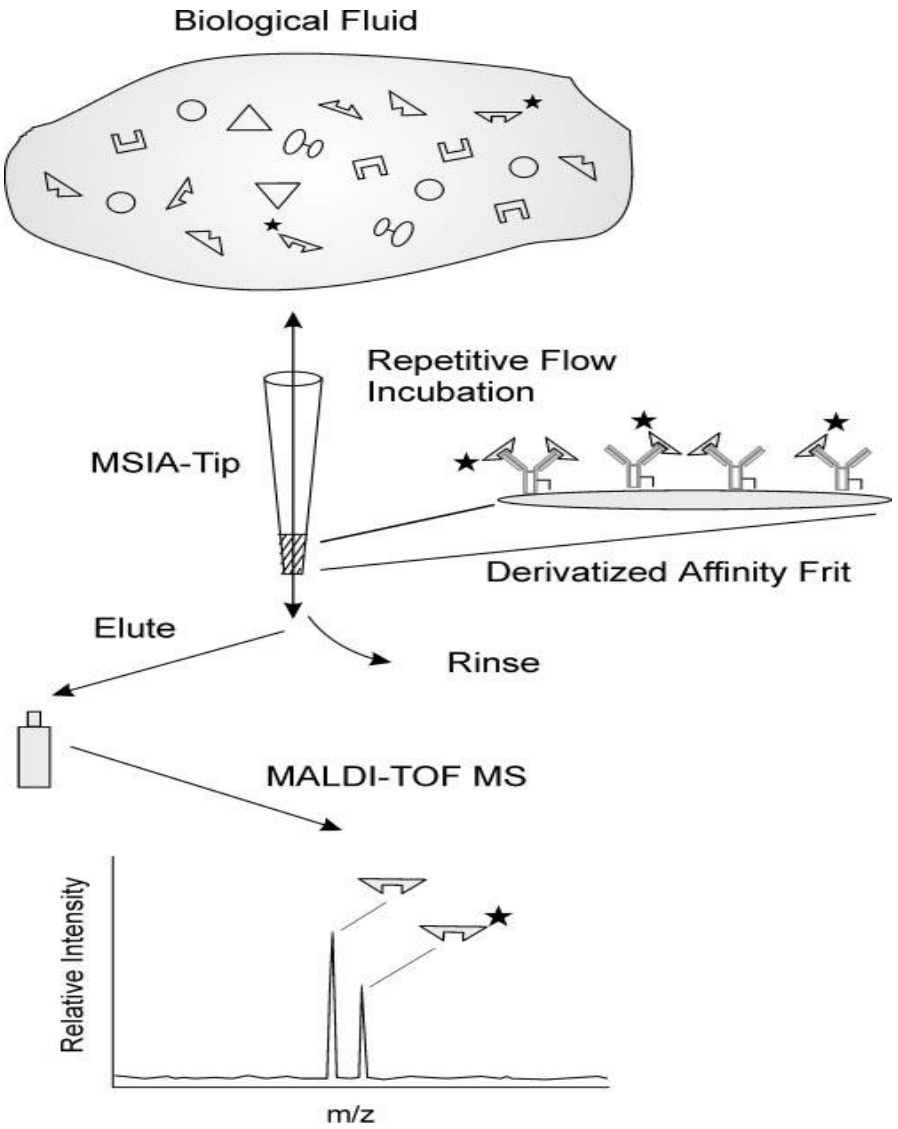
Comparison of Albumin/Creatinine ratio Clinic vs. MALDI-TOF (mg/mg)

Sample	Clinical Ratio	MALDI Ratio
1		
2		
3	0.69	0.51
4	0.03	0.02
5	0.20	0.17
6	0.02	0.03
7	0.27	0.18
8	0.07	0.07
9	0.12	0.08
10	0.29	0.28
11	0.39	0.30
12	0.12	0.06
13	0.09	0.07
14	0.08	0.07
15	0.02	0.02
16	0.06	0.04
17	0.02	0.02
18	0.13	0.14
19	0.04	0.04
20	0.08	0.09
21	0.24	0.27
22	0.02	0.02
23	0.60	0.29
24	0.04	0.10
25	0.56	0.46
26	0.02	0.04
27	0.59	0.43
28	0.06	0.08
29	0.52	0.37
30	0.76	0.60



Mass Spectrometric Immunoassay (MISA)

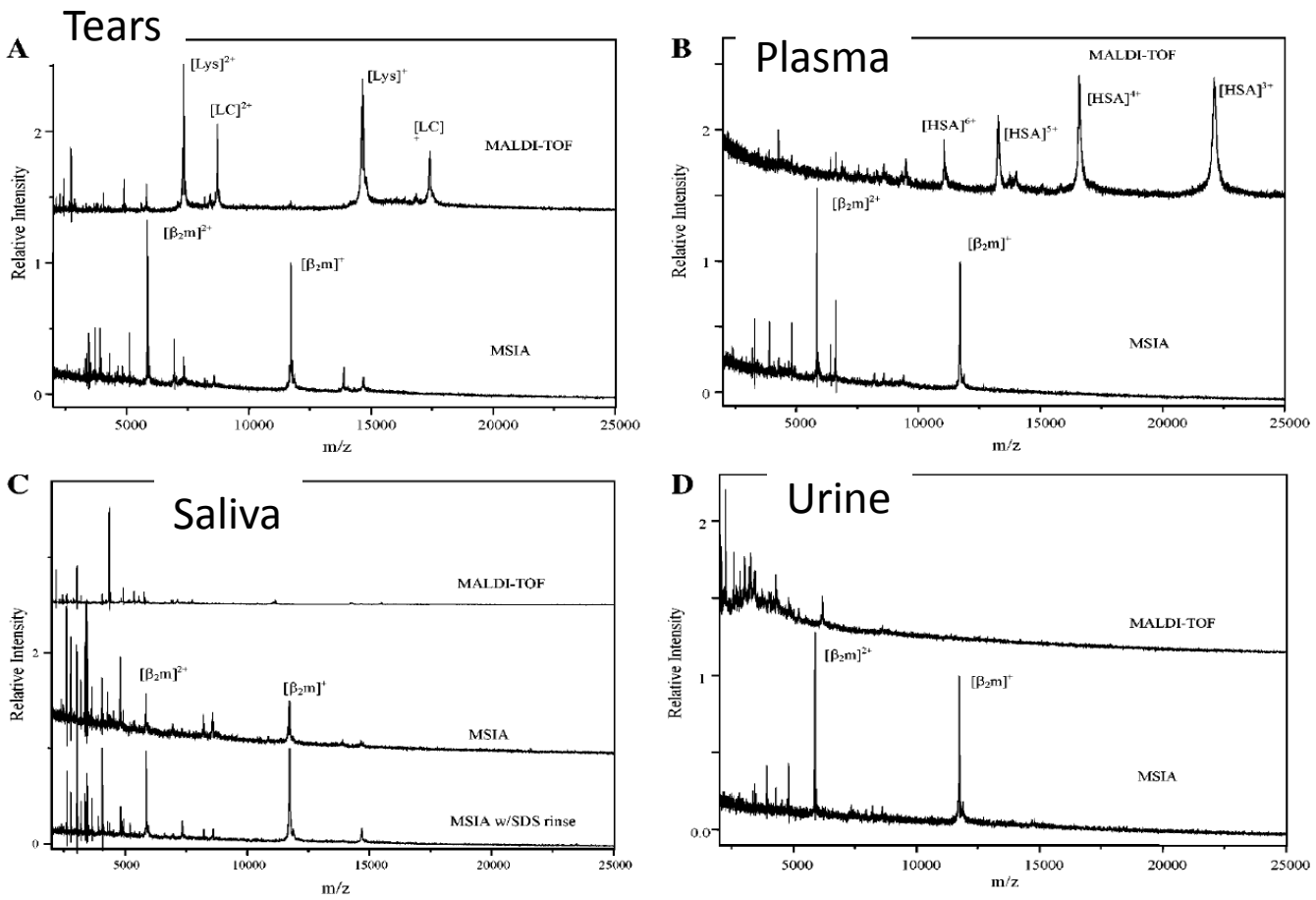
- Solid support is imbedded into a pipette tip to create a solid phase affinity capture
- Media to capture and enrich target analyte
- Repetitive flow through tip help ensure adequate time interaction
- Nonspecifically interacting compounds are washed away
- Retained species eluted directly onto a MALDI target using MALDI
- Matrix
- Inclusion of mass-shifted variants of the analyte in question can be incorporated for
- quantitation



Kemmons A. Tubbs, Dobrin Nedelkov, and Randall W. Nelson
"*Detection and Quantification of b-2-Microglobulin Using Mass Spectrometric Immunoassay*" Analytical Biochemistry **289, 26–35 (2001)**

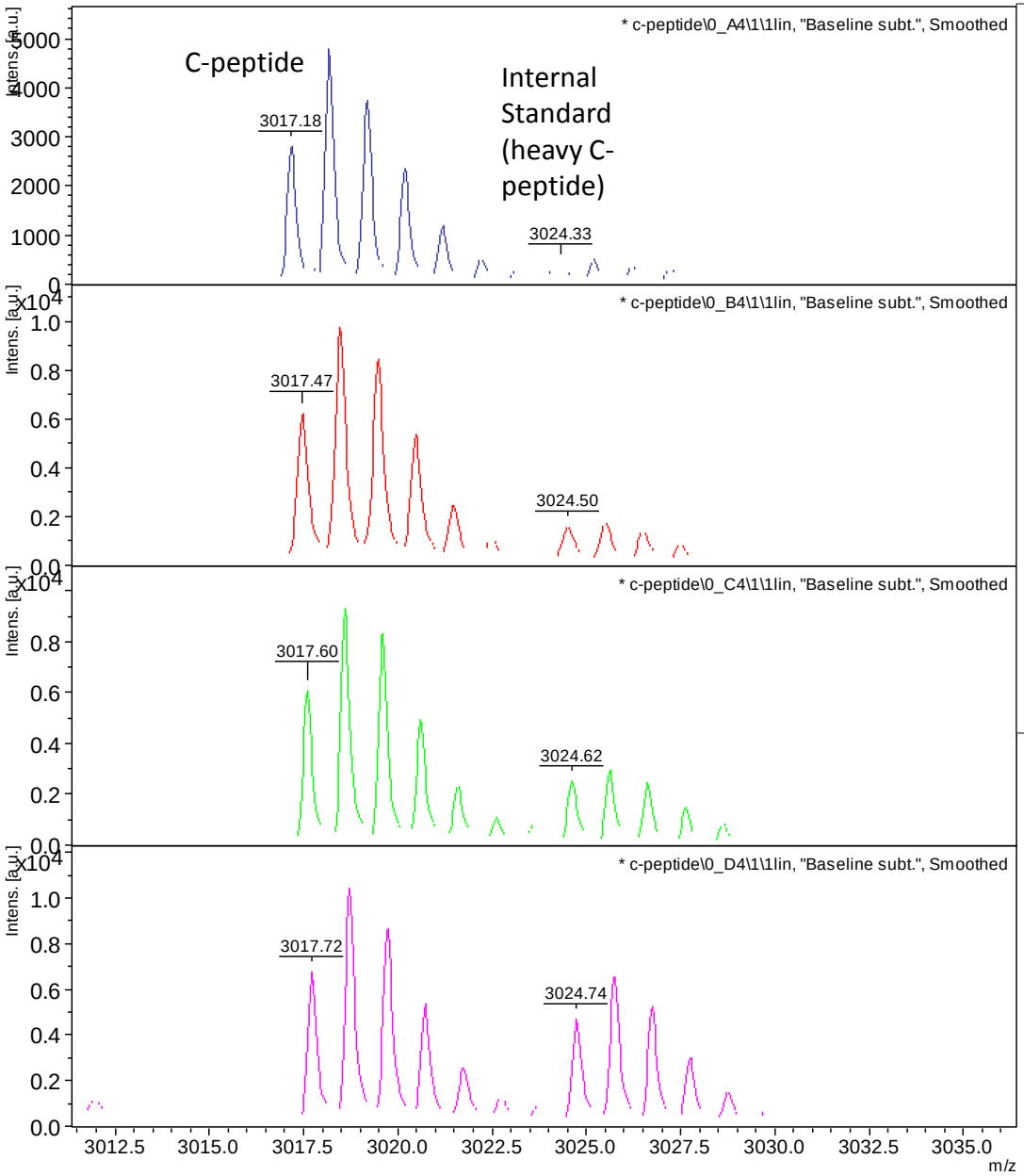
Intrinsic Bioprobes, Inc., 625 South Smith Road, Suite 22, Tempe, Arizona 85281

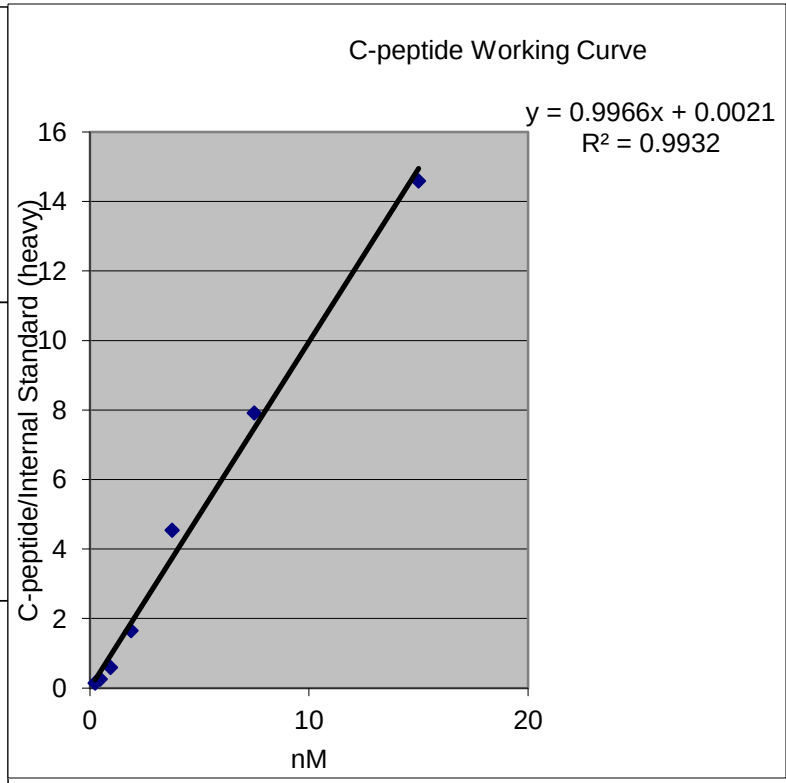
MISA for analyte enrichment from complex biological fluids



Kemmons A. Tubbs, Dobrin Nedelkov, and Randall W. Nelson
"*Detection and Quantification of b-2-Microglobulin Using Mass Spectrometric Immunoassay*"
Analytical Biochemistry **289, 26–35 (2001)**

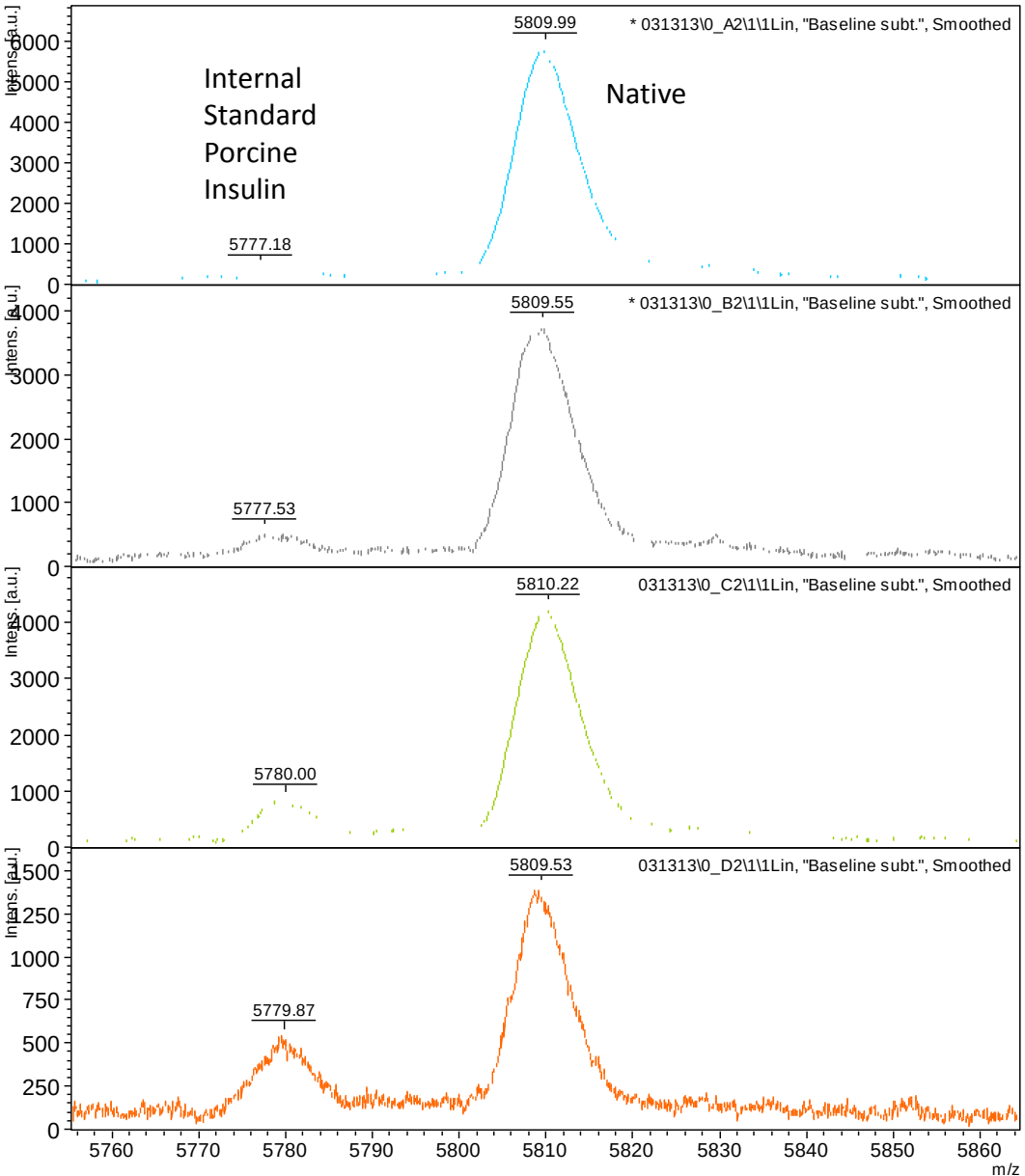
C-peptide Serial Dilution Working Curve (234pM-15pM) 3-5k shots in negative reflectron mode

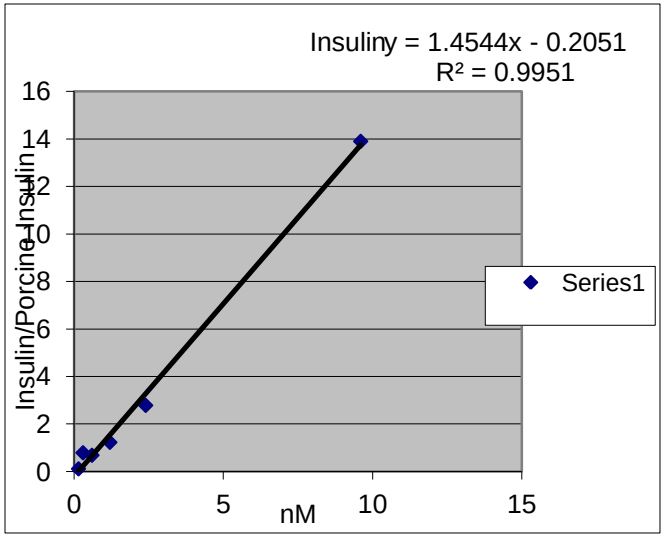




Paul E. Oran, Jason W. Jarvis, Chad R. Borges and Randall W. Nelson
"C-peptide microheterogeneity in type 2 diabetes populations"
Proteomics Clin. Appl. 2010, 4, 1–6

Insulin Serial Dilution Working Curve (110pM-19.2nM) Positive Linear Mode\
Of note these curves are 10X higher than our usual

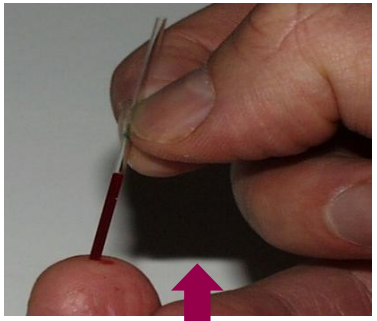




MISA for large scale protein profiling

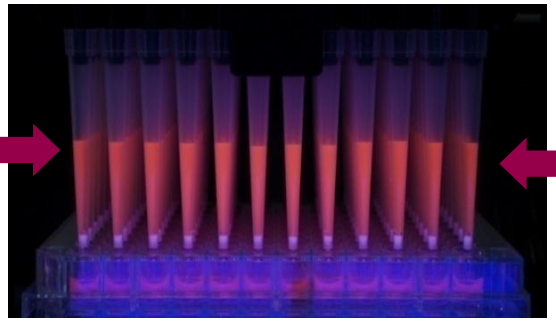
Sample Collection

Samples (plasma, serum, urine) are collected from control and disease cohorts and prepared for analysis. A single sample preparation is generally used for multiple assays.



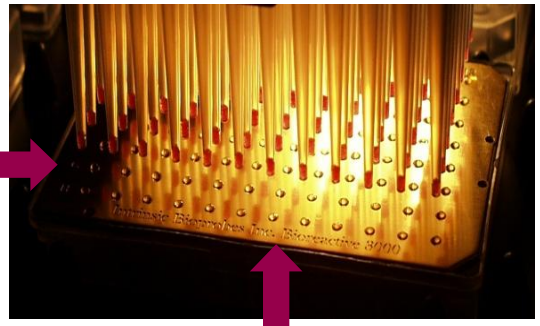
Targeted Affinity Extraction

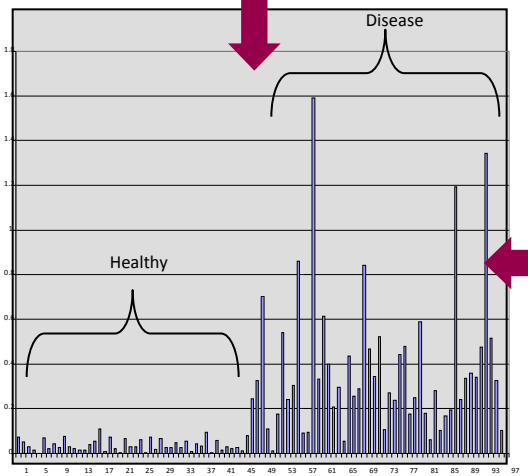
Proteins and variants are extracted from biofluid using affinity microcolumns – pipettes fitted with monolithic supports derivatized with affinity ligand (e.g., antibodies, aptamers, small molecules).

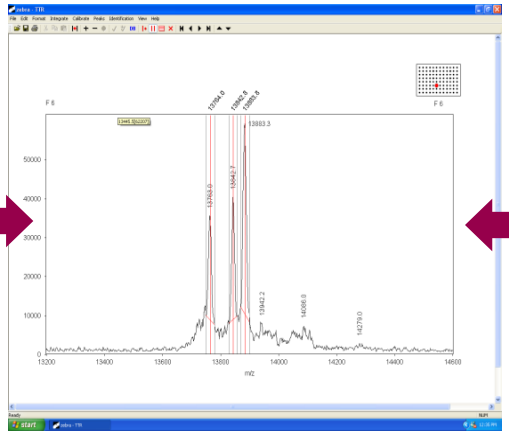


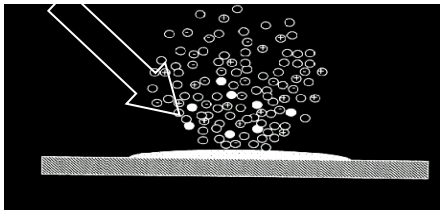
Sample Processing

Ligand immobilization, protein extraction, rinses, elution/deposition and digestion are performed using 96-well parallel robotics & tailored devices. Typically, 96-samples are prepared for analysis in less than 1-hour.









Population Studies

Unique structural changes and/or quantitative modulations are observed between healthy and disease populations. Oftentimes, these changes are small relative to the wild-type protein.

Data Processing

Data are batch processed to determine mass-shifts (structural variants) and relative abundance. These data form the basis for population-based statistical investigations and comparative studies.

Mass Spectrometry

MALDI mass spectrometry used in high information content analyses.

Nelson R.W., Mapping Protein Microheterogeneity using Mass Spectrometric Immunoassay, HUPO 2011 Geneva Switzerland, September 6, 2011

“Sick Proteins”

Apolipoprotein A1

DEPPQSPWDRVKDLATVYVDVLKDSGRDYVSQFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQEMSKDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEG
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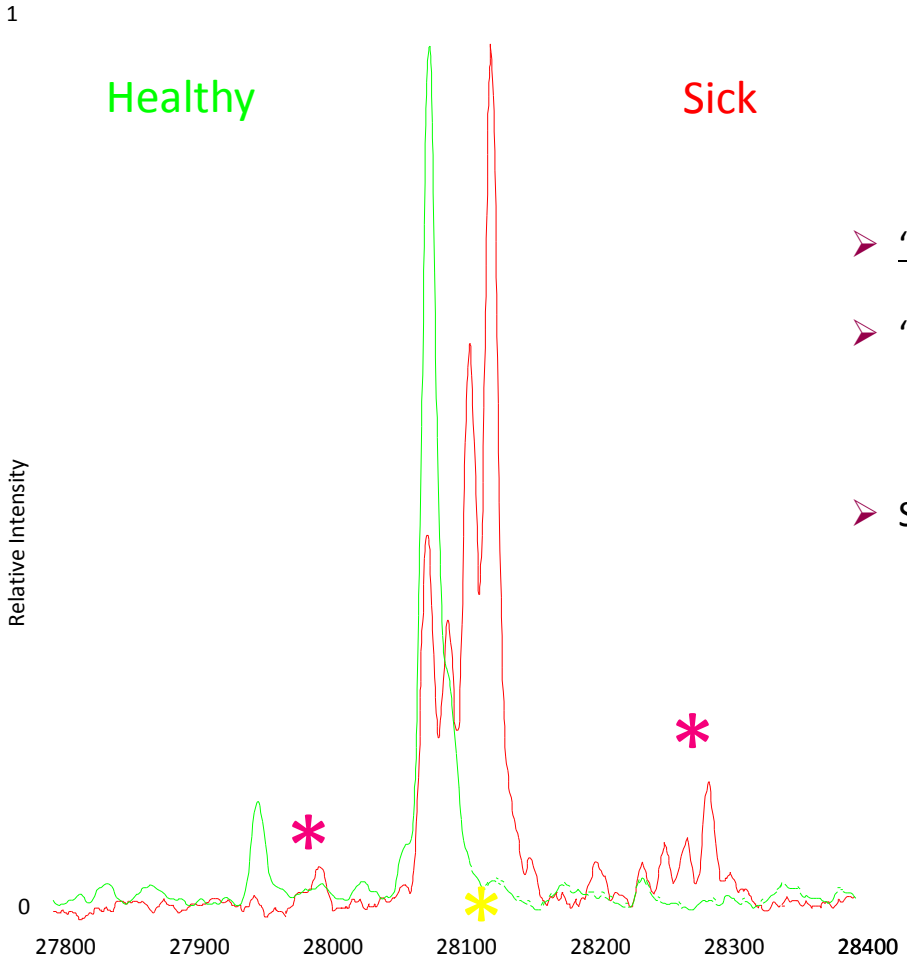
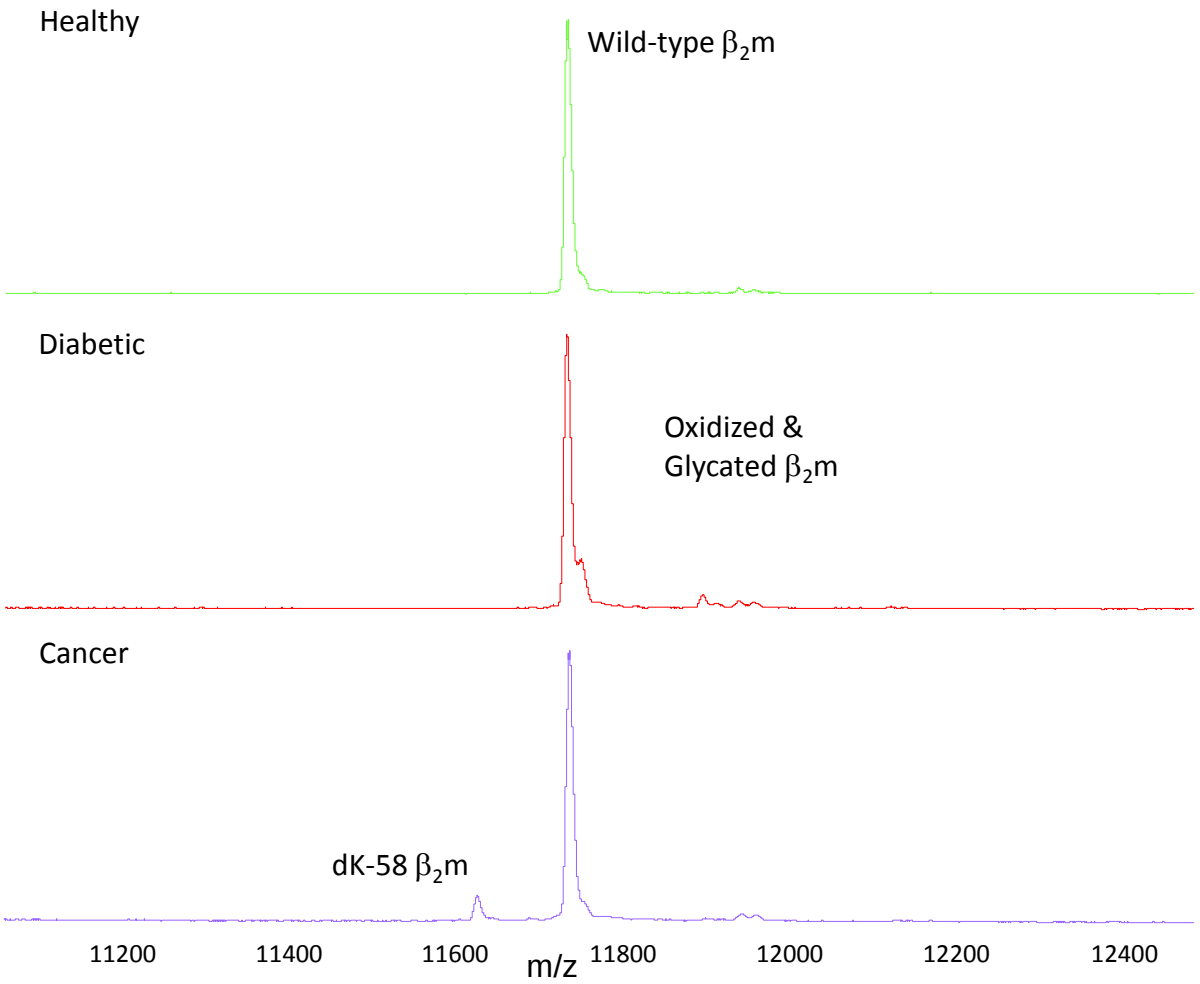


- “Sick Proteins”
- “Multiplexed” Apo A1 Assay
  - Posttranslational Modifications
  - 12 Evident & Resolvable Forms
- Stoichiometry is Important

Nelson R.W., Mapping Protein Microheterogeneity using Mass Spectrometric Immunoassay, HUPO 2011 Geneva Switzerland, September 6, 2011

The Possibility of Disease-Specific Variants

Alpha-1 Antitrypsin
Alpha-1 Glycoprotein
Apolipoprotein A1
Apolipoprotein B
Beta2 Microglobulin
Beta2 Microglobulin
Beta2 Microglobulin
C-Reactive Protein (CRP)
Ceruloplasmin
Complement C3
Complement C4
Haptoglobin
Immunoglobulin A (IgA)
Immunoglobulin E (IgE)
Immunoglobulin G (IgG)
Immunoglobulin M (IgM)
Lp(a)
Microalbumin (u)
Prealbumin
Rheumatoid Factor (RF)
Transferrin



Nelson R.W., Mapping Protein Microheterogeneity using Mass Spectrometric Immunoassay, HUPO 2011 Geneva Switzerland, September 6, 2011

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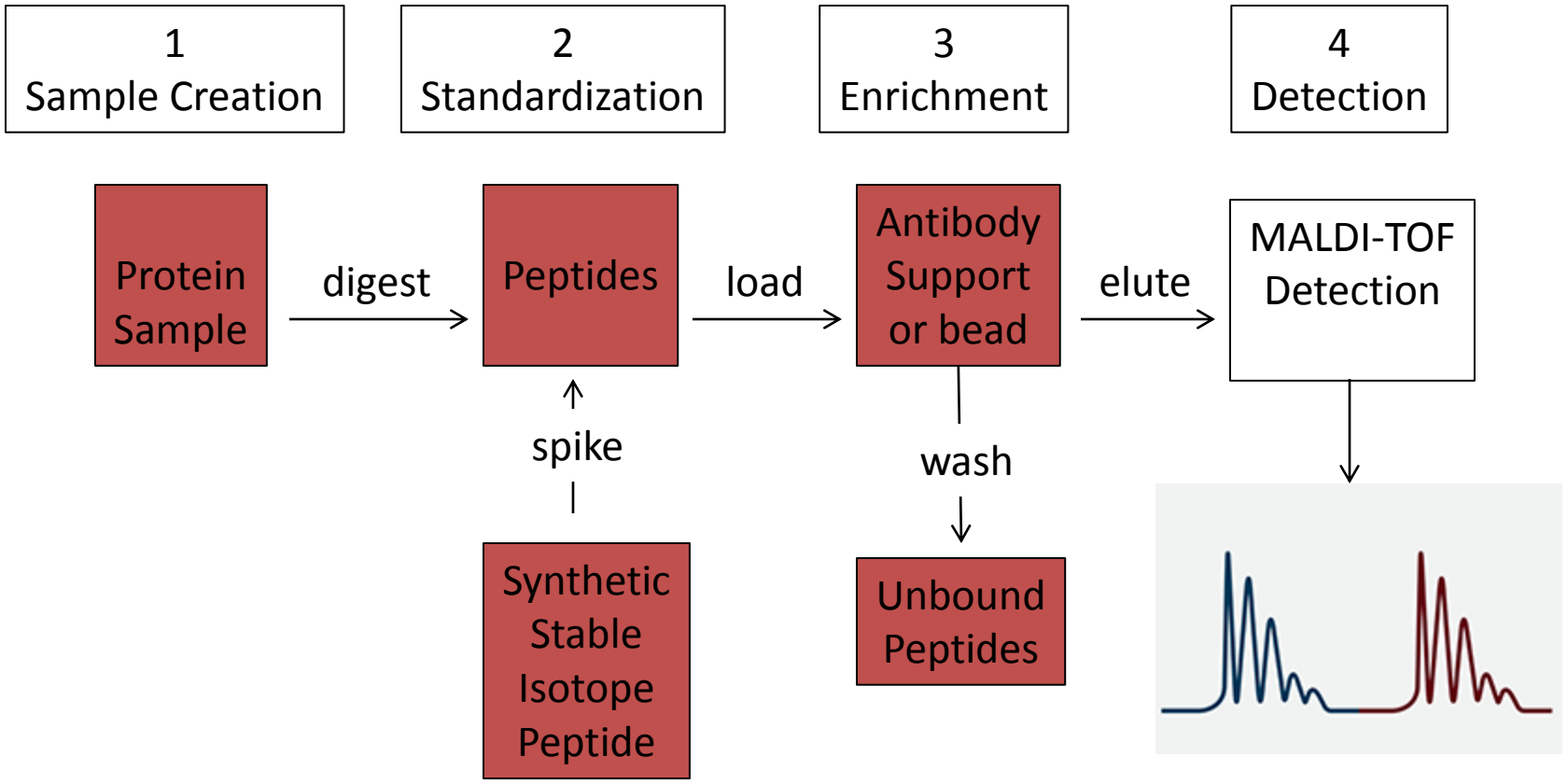
Stable **I**sotope **S**tandards and **C**apture by **A**nti-**P**eptide **A**ntibodies



http://www.siscapa.com/

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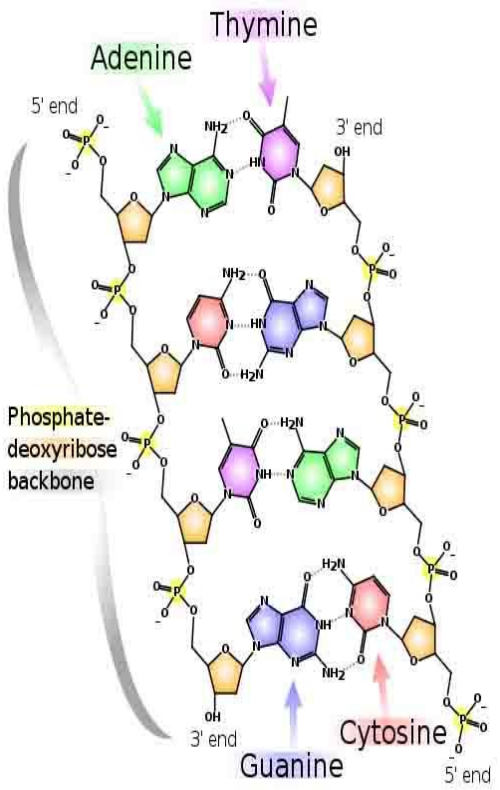
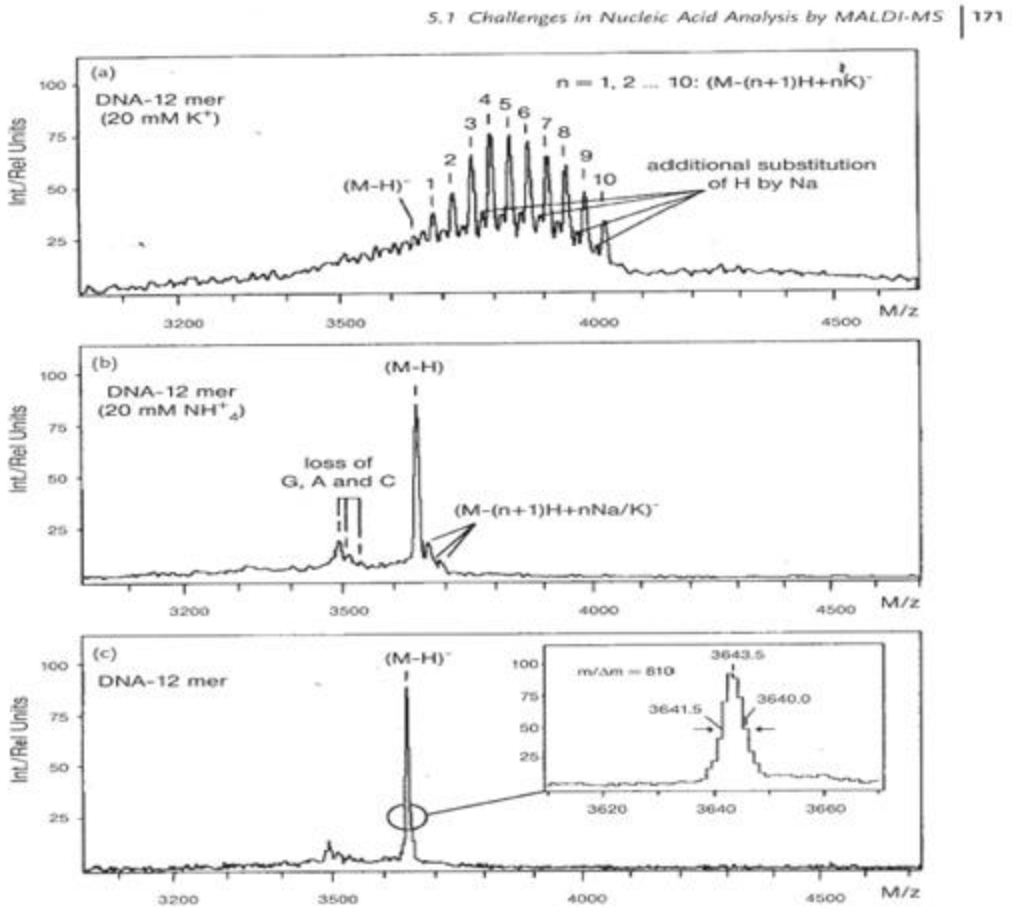
Nucleic Acid Analyses





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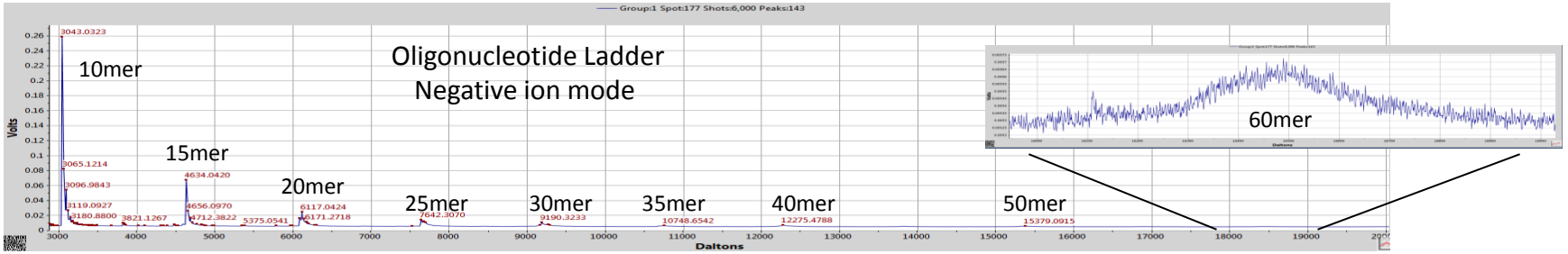
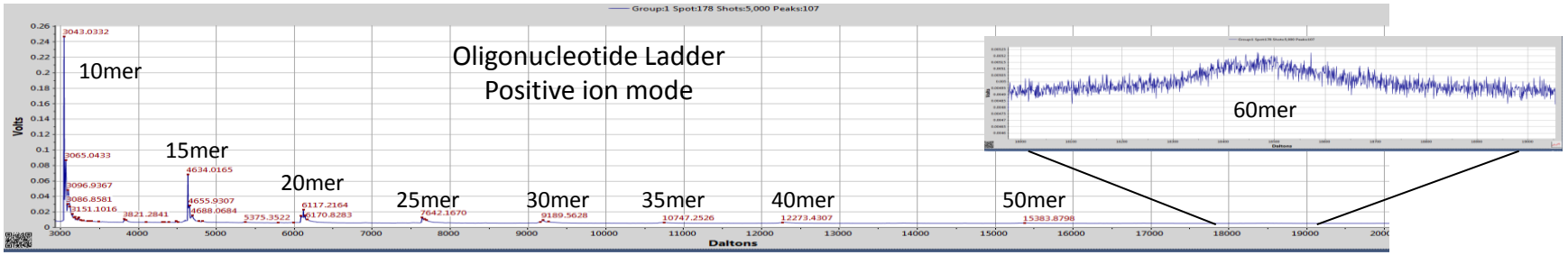
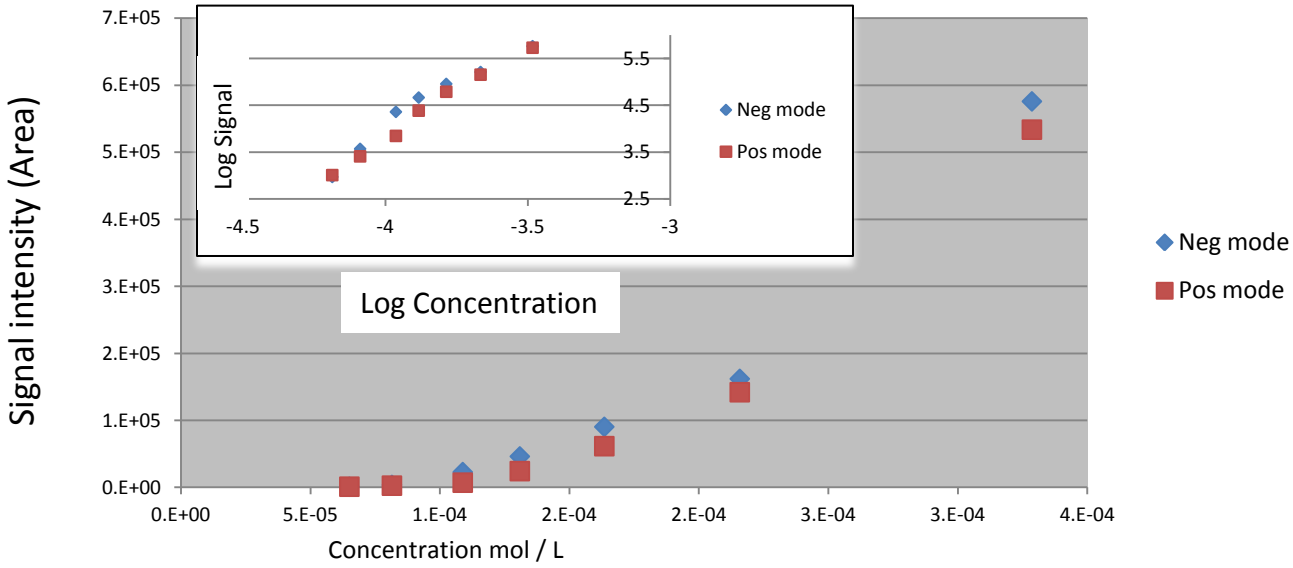
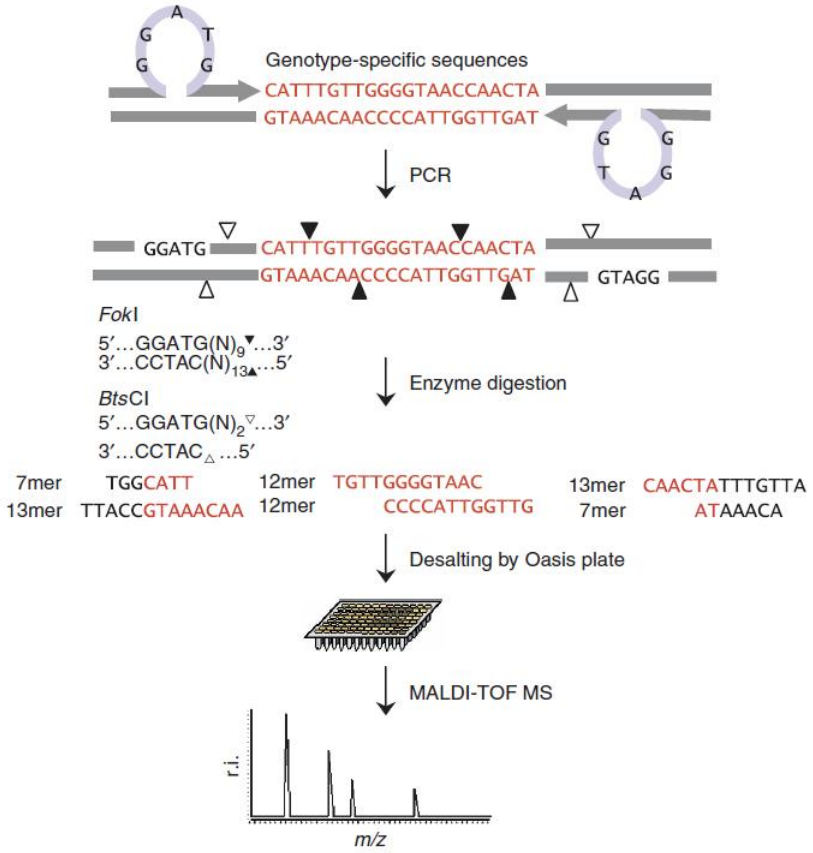
Single Charged similar response in + and - ion mode







MALDI Analysis of Oligonucleotides is used in several assays



Can perform Isotopic labeling for quantitation

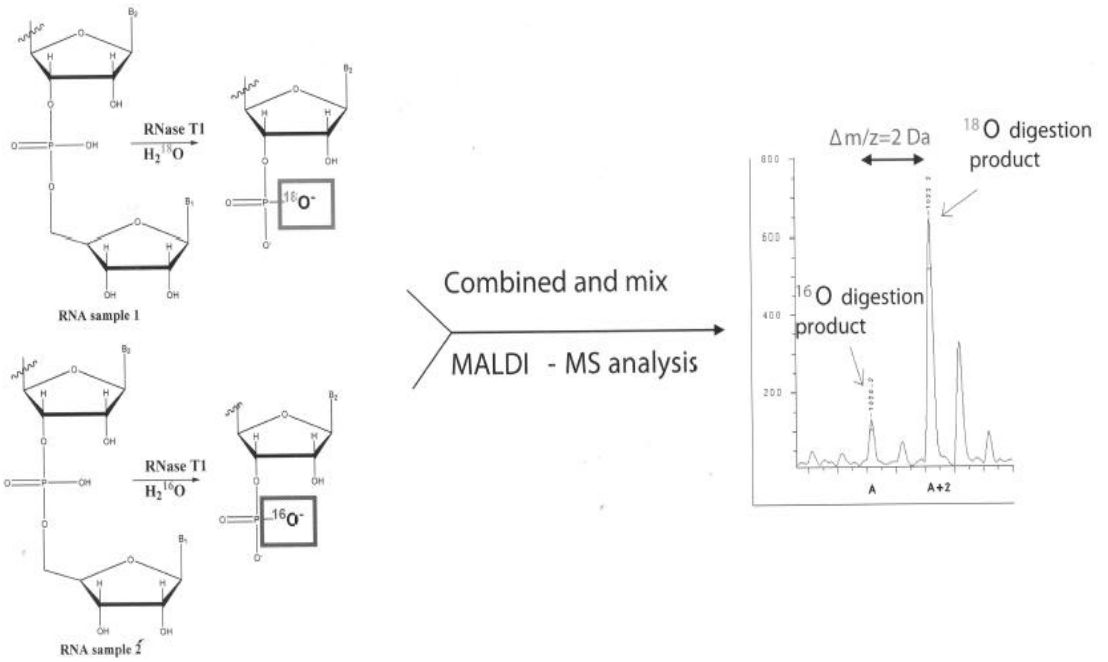


Figure 5.7 General overview of the described ^{18}O labeling and MALDI-MS approach for quantitation of RNA samples. Reproduced with permission from Ref. [94]. © 2005 American Chemical Society.

5.2 Genetic Markers | 185

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