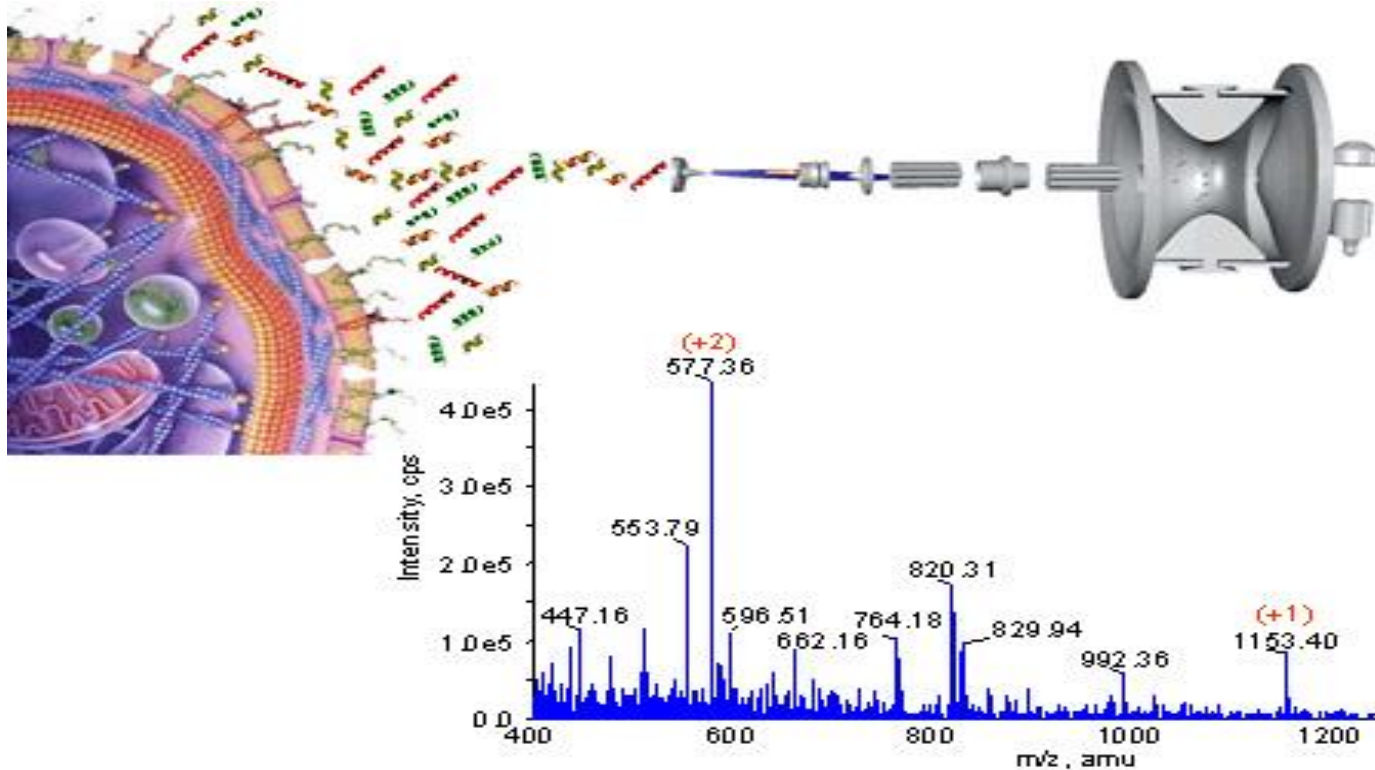
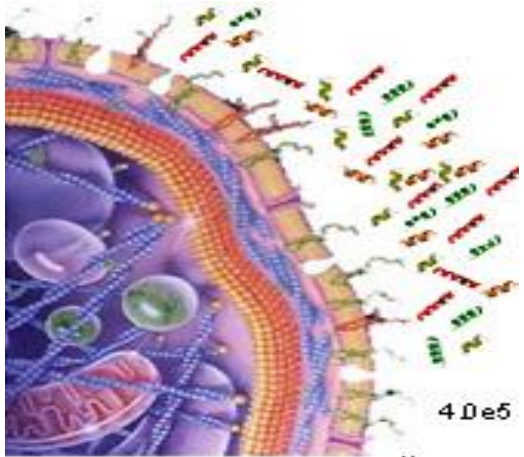
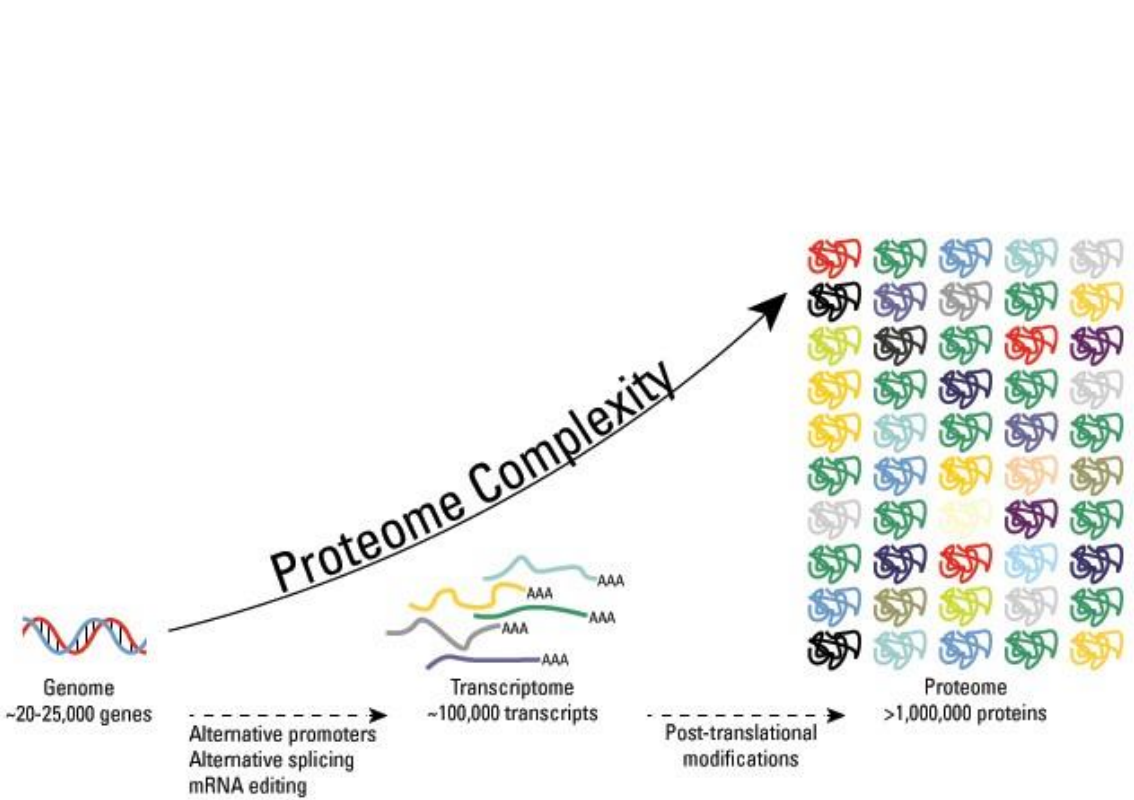


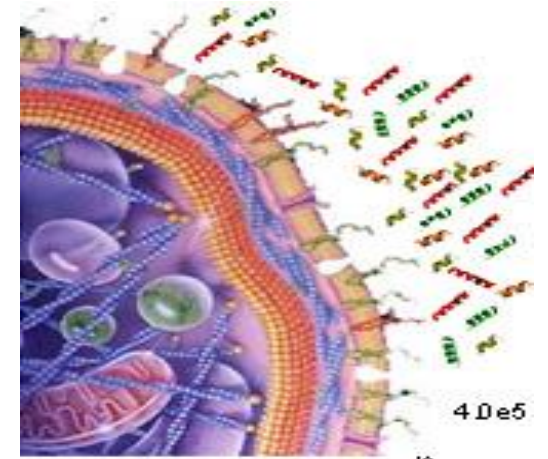
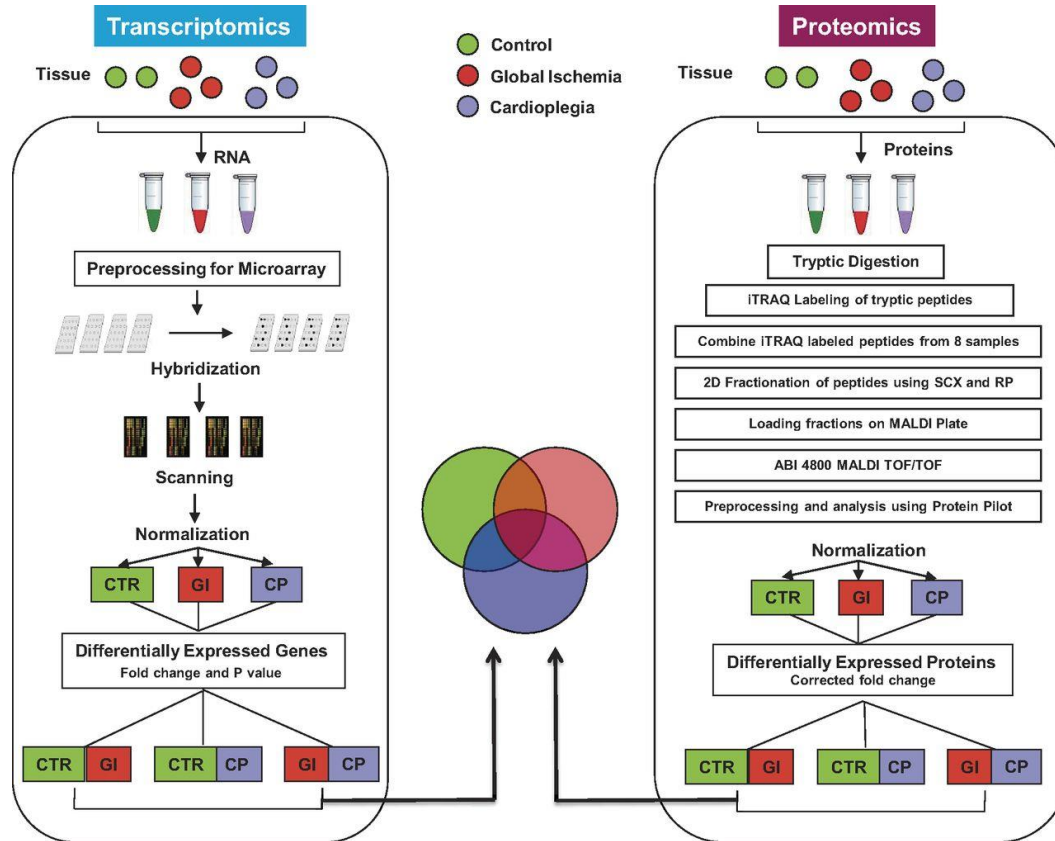
Analytical Mass Spectrometry and Proteomics



Analytical Mass Spectrometry and Proteomics



Analytical Mass Spectrometry and Proteomics



Analytical Mass Spectrometry and Proteomics

Analytical Realities

MS & Proteomics is not the PCR reaction.

- Not template driven
- No primers
- No amplification

**Orders of Magnitude matter
i.e. concentration**

Which of these is more abundant?

- Ribosomes
- Heat shock proteins
- Tubulin
- Albumin
- Sox-2 pluripotent stem cell transcription factor

MS analysis is not very biased

What will ionize?

- Proteins
- Lipids
- Carbohydrates
- Small molecules
- Salts

**MS analysis is very sensitive,
accurate, and fast.**

What do these overcome?

- complexity
- isomers
- isobars
- Bad experimental design
- Keratin and other contaminants

Targeted proteomics

Analysis of a preselected group of proteins delivers more precise, quantitative, sensitive data to more biologists. Vivien Marx reports.

Although the number and identity of protein-coding genes in humans and many other organisms are known to a certain level of approximation, the numbers of proteins produced by each of these genes remains a mystery. Further complicating matters, given the many possible splice forms and post-translational modifications, the potential number of proteins is “staggering,” says Arizona State University researcher Josh LaBaer, who is also president-elect of the US Human Proteome Organization. A protein is also dynamic. “It’s phosphorylated this minute; it’s not phosphorylated the next minute,” he says. This is fascinating science, but it makes proteins in a complex, dynamic sample hard to precisely measure.

Understanding disease-related changes, for example, calls for reliable, quantitative ways of assessing protein levels, and mass spectrometers are instruments able to nail that task. But the data from so-called discovery proteomics experiments in which mass spectrometry is used to identify a

“I personally can’t wait until we stop hearing about someone describing how big of a list of proteins, peptides or phosphopeptides they detected,” says one researcher critical of discovery proteomics who did not wish to be identified. Proteomics has been doing “my list is bigger than your list” for far too long. “It is way more important to measure the one right protein than 10,000 wrong ones.”

Scientists wanting to follow well-founded hunches about dozens or hundreds of proteins seek a focused, reproducible, quantitative view of a small subset of the whole proteome in their lab vials. High-throughput biology experiments, which include DNA sequencing, genome analysis and gene expression analysis, are generating massive



Ruedi Aebersold hopes many laboratories

are not inherently the same,” says Ruedi Aebersold, from the Institute of Molecular Systems Biology at the Swiss Federal Institute of Technology in Zurich. Neither person is necessarily wrong: the contra-



Kate Vicari

Targeted proteomics detects proteins of interest with high sensitivity, quantitative accuracy and reproducibility.

**aka – USE your Brain
& Your skills as a cellular biologist, molecular biologist, protein biochemist, etc...even as a clinical physician.**

Untargeted MS by blind faith is time consuming.

- **Generates a lot of data**
- **Produces lots of possibilities**
- **Produces even better pictures**
- **Leaves you overwhelmed**
- **Without true direction to move in**
- **Unless you have an army**

Analytical Mass Spectrometry and Proteomics

Analytical Realities

Direct measurement of proteins and other molecules

- Often closest to reality
- Controls Still Matter!

MS analysis is not very biased

- Mass accuracy and chemical formulas do not lie
- You can separate based on
 - Class of molecules
 - Location
 - Separation (chromatography)

Orders of Magnitude matter *i.e.* concentration

Enrichment Strategies

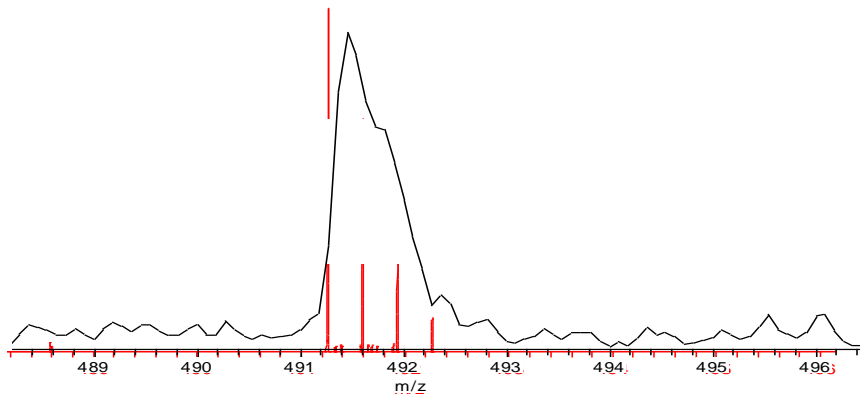
- Again Separation
(tissue, cellular, molecular, protein class levels)
- Antibody enrichment (assuming)
- Affinity tags

MS analysis is very sensitive, accurate, and fast.

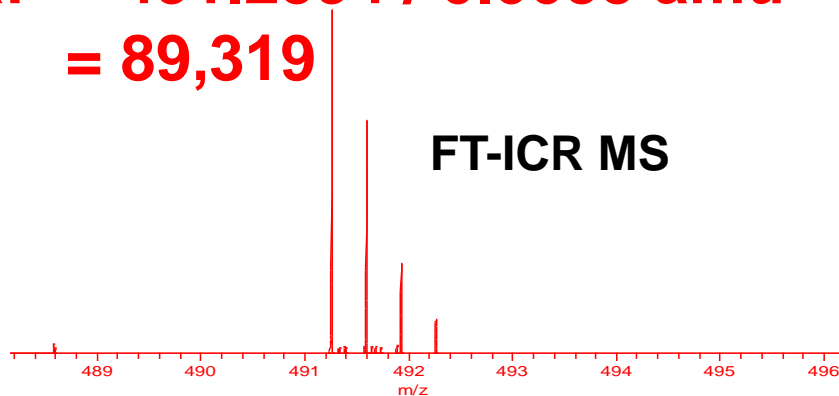
Targeting Strategies

- Put the blinders on (focus)
- Create unique tags between samples
- Deplete abundant things
- Apply what is already known
- Ask specific questions

Mass Resolution = $m / \Delta m$ 50%

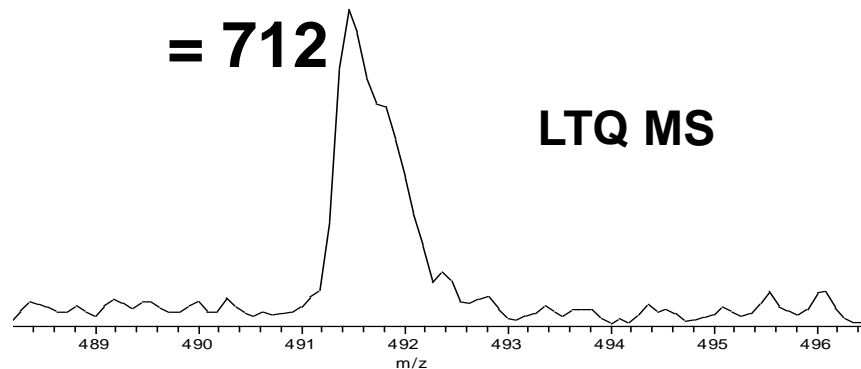


$$\text{RP} = 491.2594 / 0.0055 \text{ amu} \\ = 89,319$$



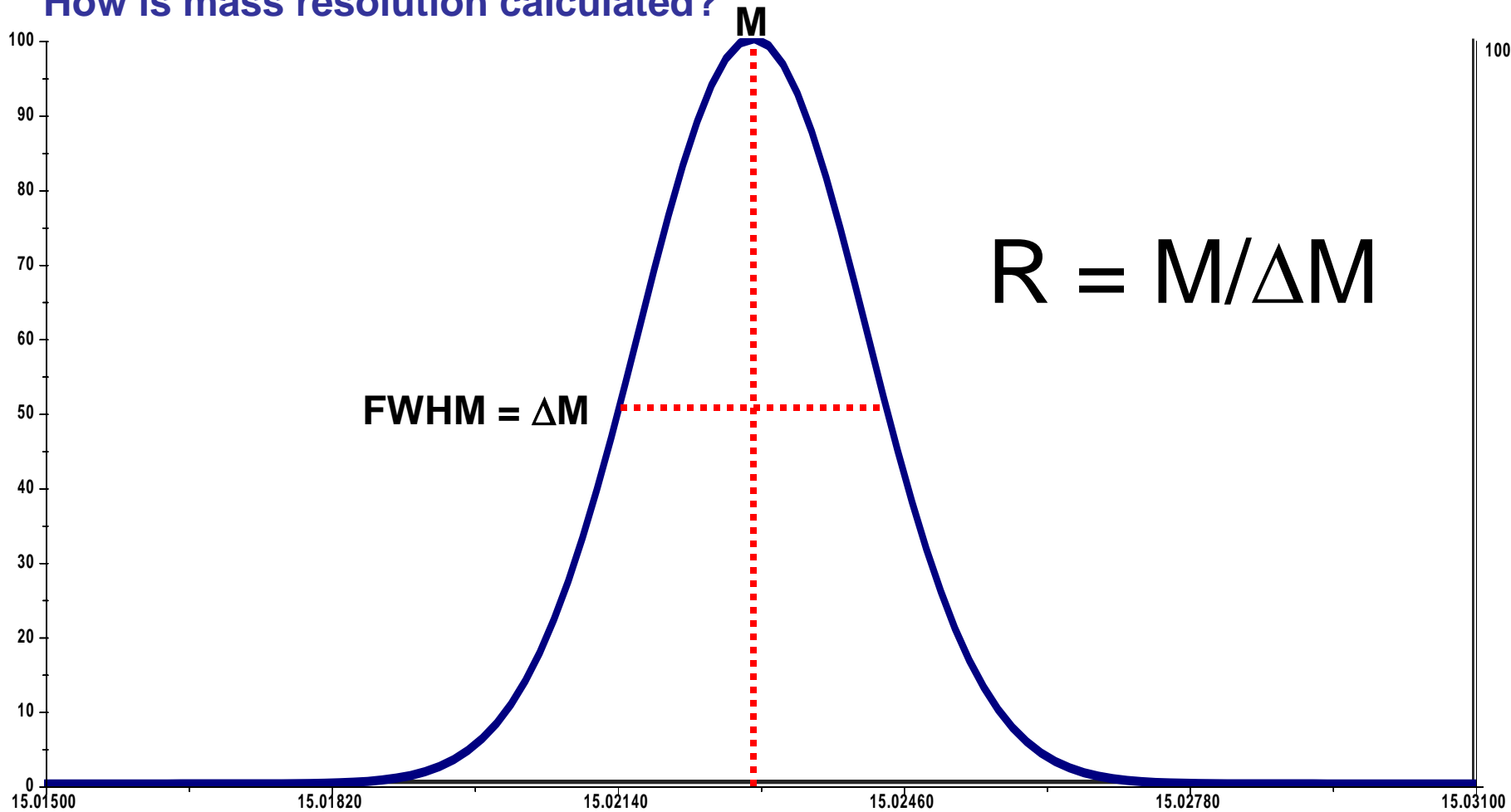
FT-ICR MS

$$\text{RP} = 491.45 / 0.69 \text{ amu} \\ = 712$$



LTQ MS

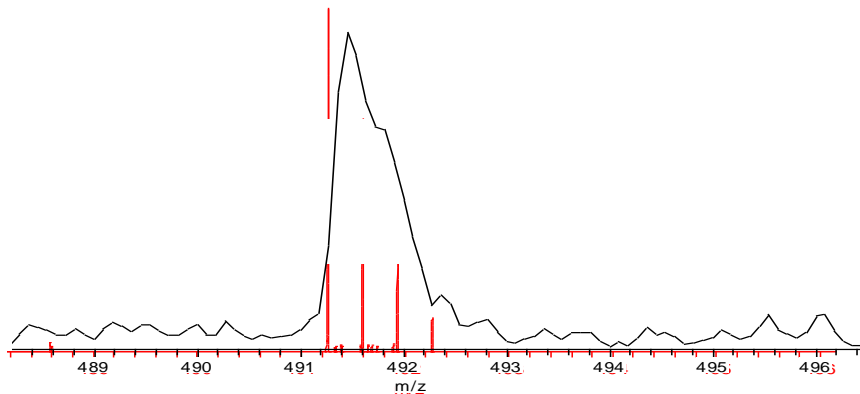
How is mass resolution calculated?



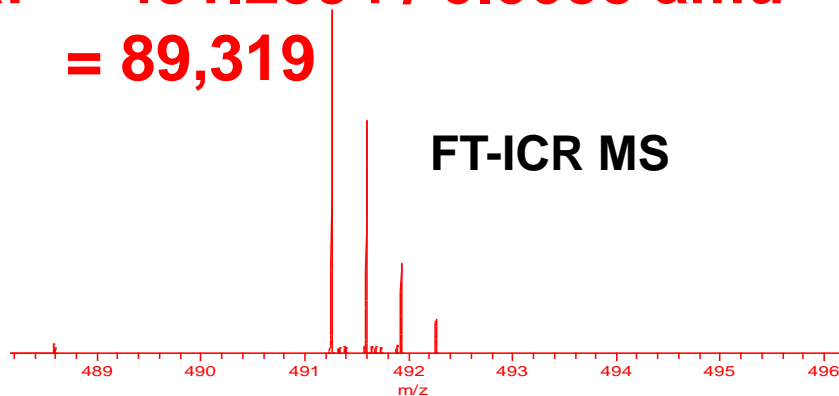
Stable isotopes of most abundant elements of peptides

Element	Mass	Abundance
H	1.0078	99.985%
	2.0141	0.015
C	12.0000	98.89
	13.0034	1.11
N	14.0031	99.64
	15.0001	0.36
O	15.9949	99.76
	16.9991	0.04
	17.9992	0.20

Mass Resolution = $m / \Delta m$ 50%

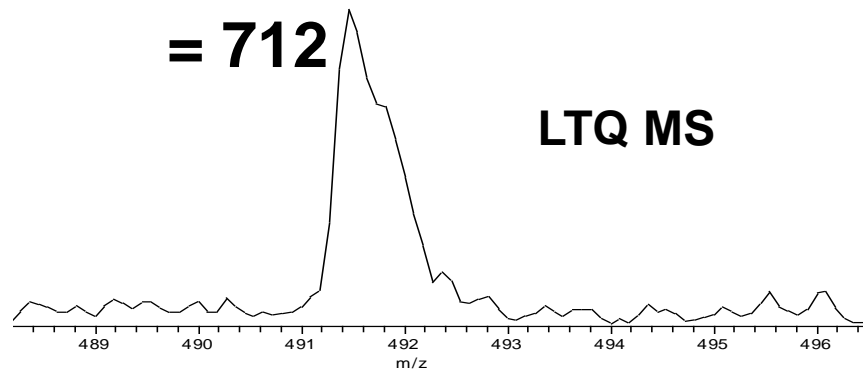


$$\text{RP} = 491.2594 / 0.0055 \text{ amu} \\ = 89,319$$



FT-ICR MS

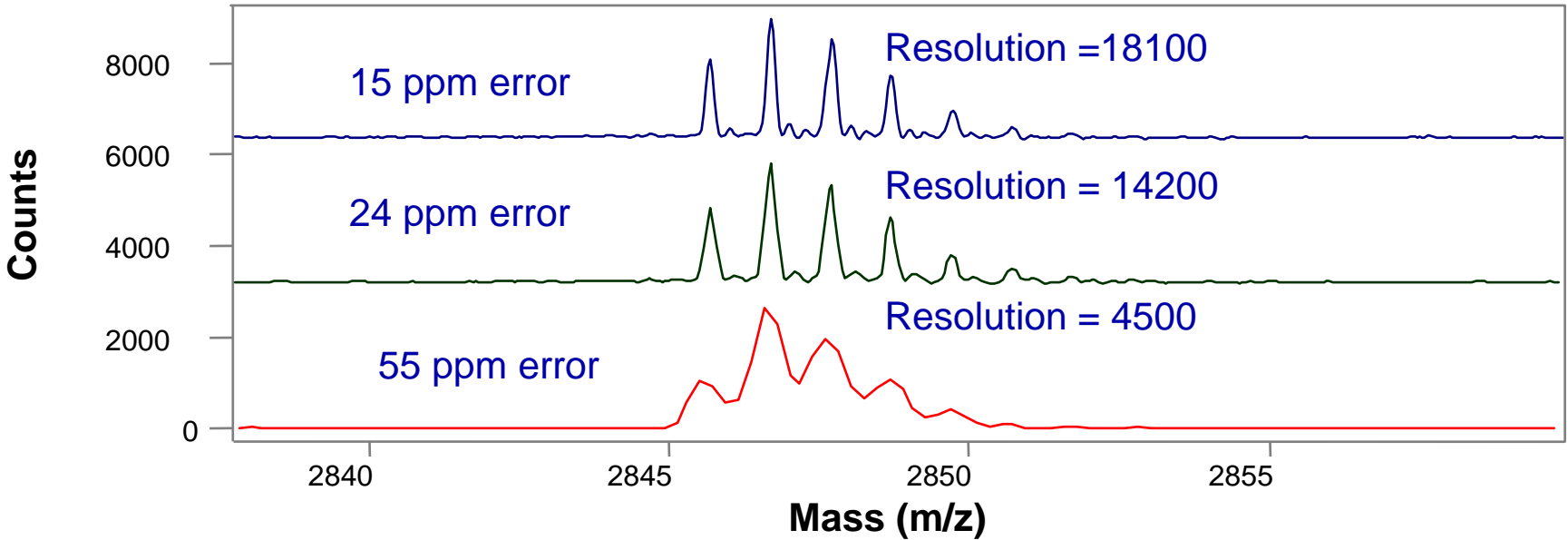
$$\text{RP} = 491.45 / 0.69 \text{ amu} \\ = 712$$



LTQ MS

Mass measurement accuracy depends on resolution

High resolution means better mass accuracy

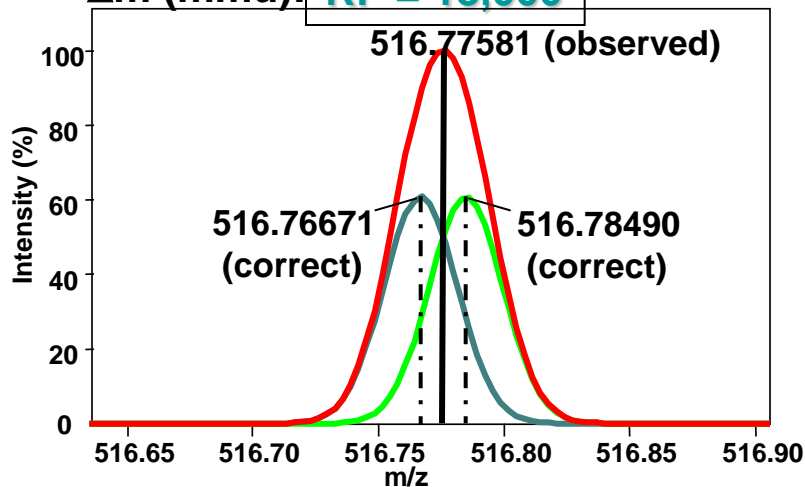


Two peptides - same nominal mass - simulation

Peptide mixture: [Val⁵]-Angiotensin II
Sequence: DRVYVHPF
Formula: C₄₉H₆₉N₁₃O₁₂
Exact mass: [M+2H]²⁺ = 516.76671

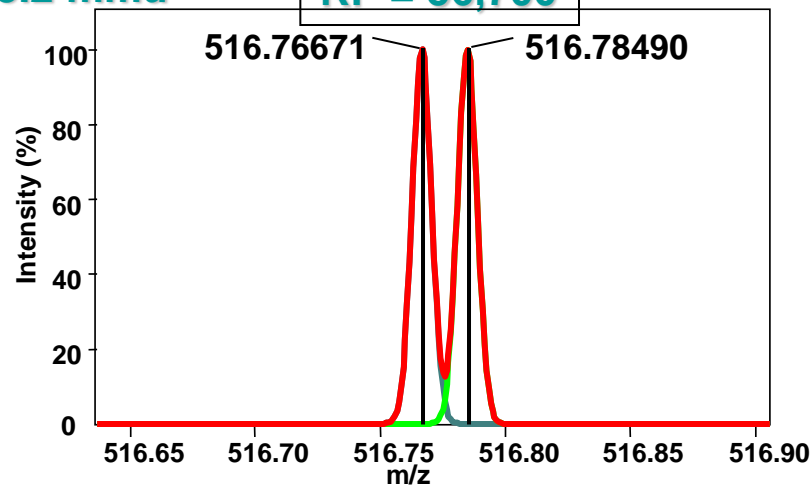
Lys-des-Arg⁹-Bradykinin
KRPPGFSPF
C₅₀H₇₃N₁₃O₁₁
[M+2H]²⁺ = 516.78490

Δm (mmu): **RP = 18,000**



18.2 mmu

RP = 56,700

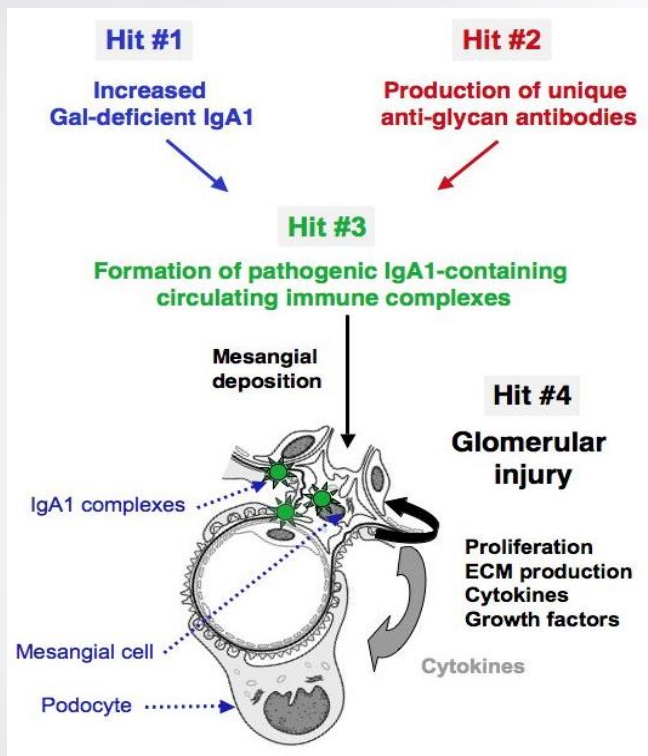


Is Mass Accuracy Important ?

Results for error limit up to 5 ppm

	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	516.76671	0.0	0.0	21.0	C ₄₉ H ₇₁ O ₁₂ N ₁₃
	516.76647	0.5	0.2	15.0	C ₄₉ H ₇₉ O ₁₁ N ₉ S ₂
2 ppm (10)	516.76638	0.6	0.3	12.0	C ₄₁ H ₇₅ O ₁₄ N ₁₅ S ₁
	516.76705	-0.7	-0.3	11.5	C ₄₃ H ₇₇ O ₁₅ N ₁₂ S ₁
	516.76604	1.3	0.7	16.0	C ₄₈ H ₇₅ O ₁₆ N ₉
	516.76738	-1.3	-0.7	20.5	C ₅₁ H ₇₃ O ₁₃ N ₁₀
	516.76604	1.3	0.7	21.5	C ₄₇ H ₆₉ O ₁₁ N ₁₆
5 ppm (23)	516.76580	1.8	0.9	15.5	C ₄₇ H ₇₇ O ₁₀ N ₁₂ S ₂
	516.76772	-2.0	-1.0	16.5	C ₄₄ H ₇₃ O ₁₁ N ₁₆ S ₁
	516.76773	-2.0	-1.0	11.0	C ₄₅ H ₇₉ O ₁₆ N ₉ S ₁
	516.76805	-2.6	-1.3	25.5	C ₅₂ H ₆₉ O ₉ N ₁₄
	516.76537	2.6	1.3	16.5	C ₄₆ H ₇₃ O ₁₅ N ₁₂
	516.76807	-2.6	-1.4	7.0	C ₃₈ H ₇₉ O ₁₄ N ₁₅ S ₂
	516.76513	3.0	1.6	10.5	C ₄₆ H ₈₁ O ₁₄ N ₈ S ₂
	516.76513	3.1	1.6	16.0	C ₄₅ H ₇₅ O ₉ N ₁₅ S ₂
	516.76839	-3.3	-1.7	16.0	C ₄₆ H ₇₅ O ₁₂ N ₁₃ S ₁
	516.76479	3.7	1.9	20.0	C ₅₂ H ₇₅ O ₁₁ N ₉ S ₁
	516.76872	-3.9	-2.0	25.0	C ₅₄ H ₇₁ O ₁₀ N ₁₁

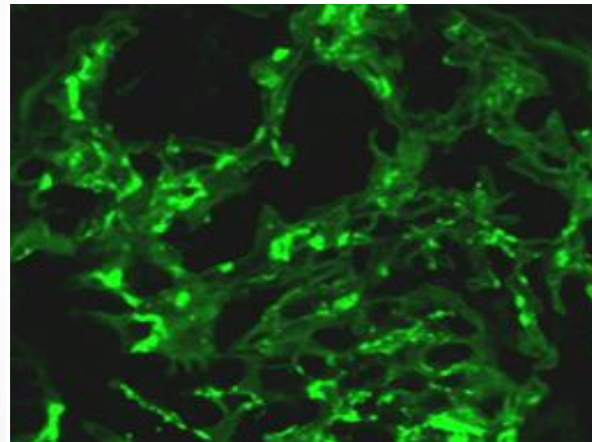
IgAN Pathogenesis: Multi-hit Hypothesis



Suzuki *JCI* 2008, 2009, *JASN* 2011
Lai *AJP* 2008,
Novak *Semin Immunopathol* 2012

Autoimmune features

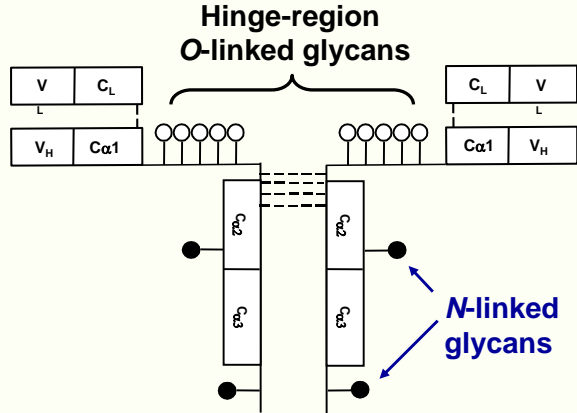
- Autoantigen (Gal-deficient IgA1)
- Autoantibody (binds the autoantigen)



Bertinetto *NDT* 2012; Gharavi *JASN* 2008, Feehally *JASN* 2010, Gharavi *Nat Genet* 2011, Yu *Nat Genet* 2011, Kiryluk *KI* 2011; Kiryluk *PLoS Genet* 2012, Wyatt & Julian *NEJM* 2013

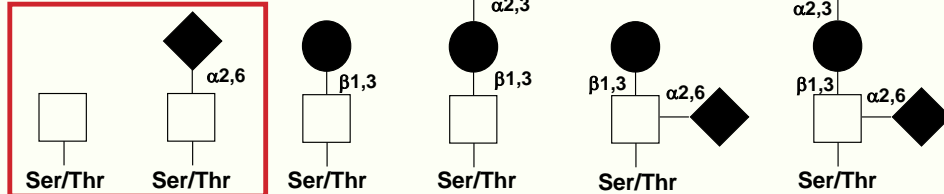
IgA1 O-glycosylation

IgA1



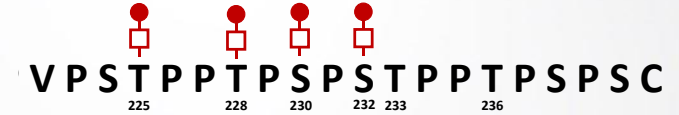
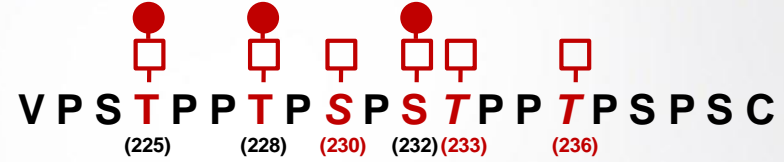
N-linked glycans

Gal-deficient glycans



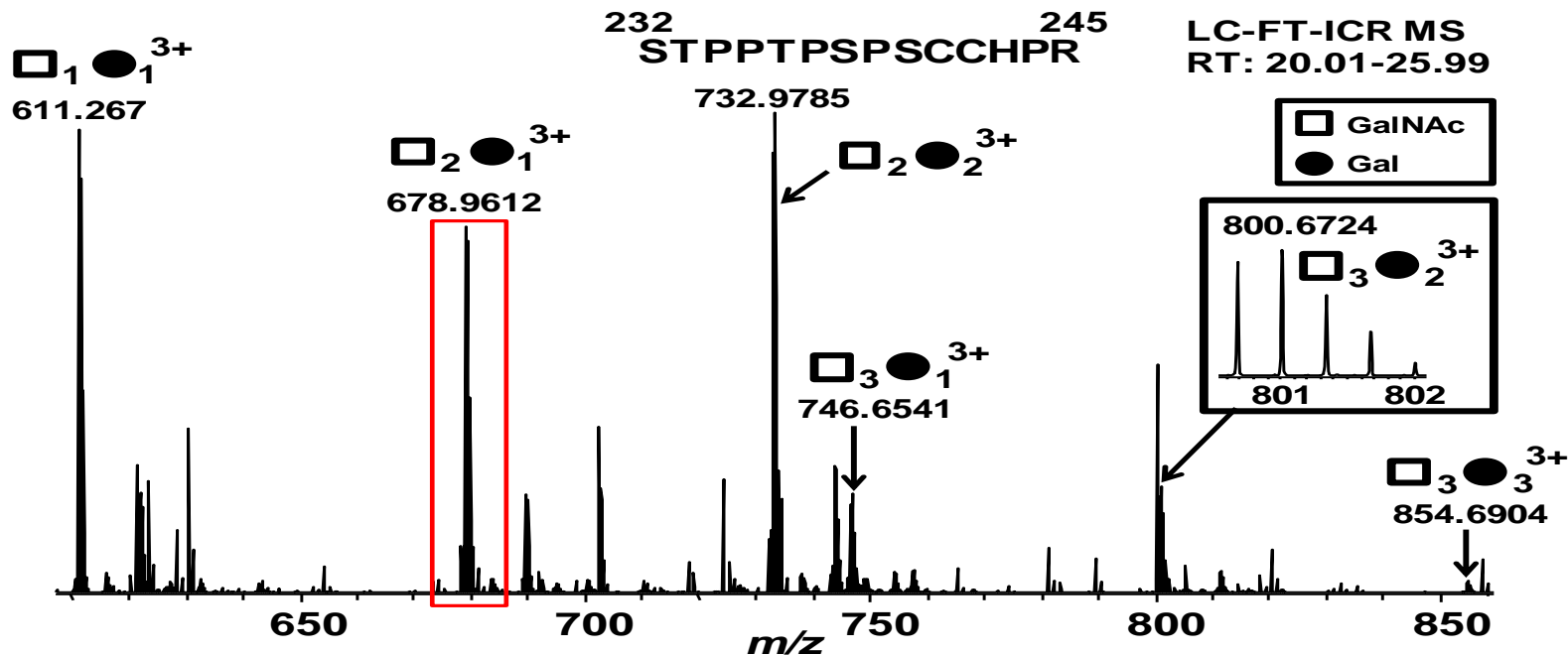
O-glycans

IgA1 O-glycoforms



LC-FT-ICR MS

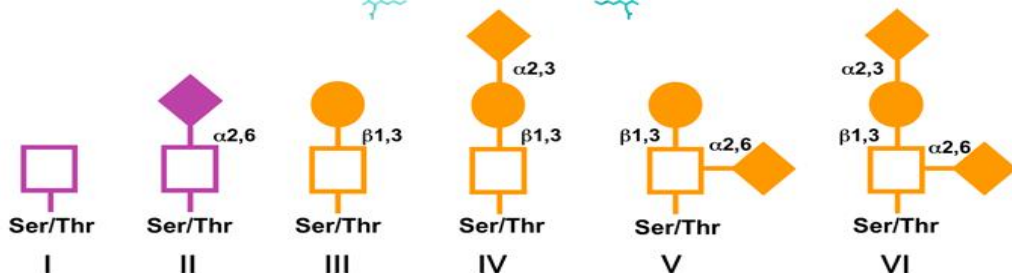
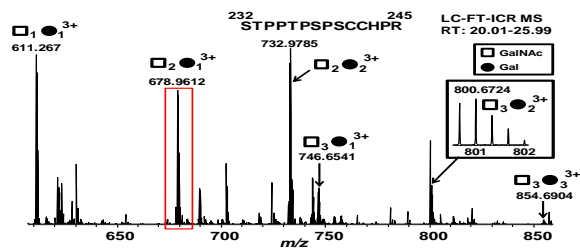
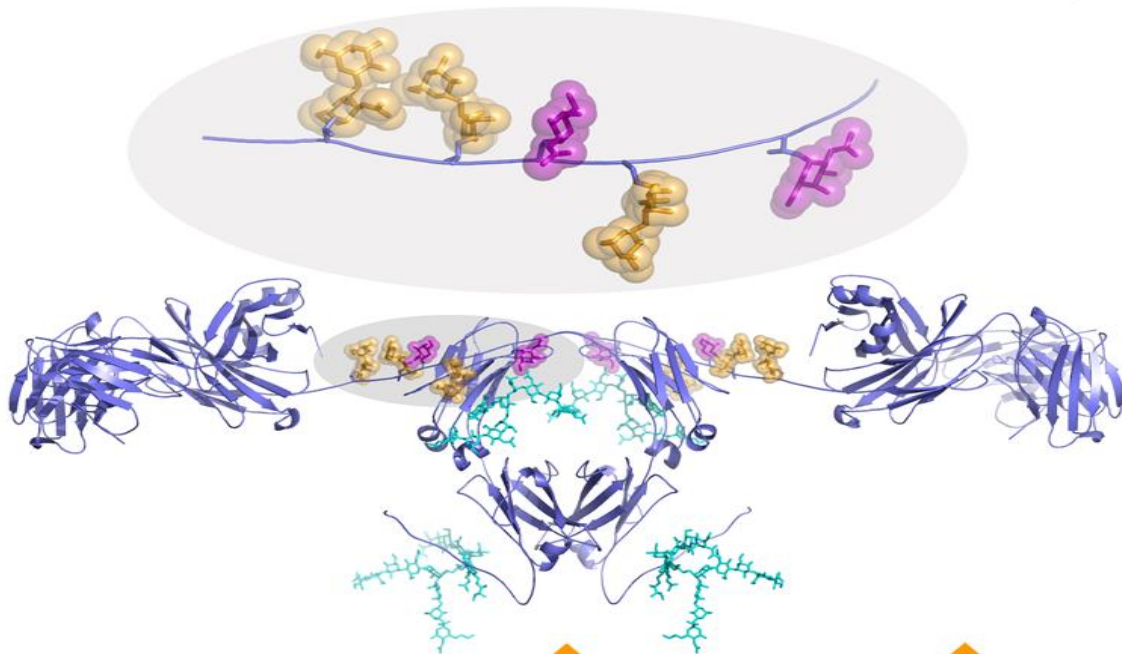
Analysis of IgA1 O-glycosylation



Measuring molecular mass

PeptideAA's
+
Glycan residues
+
H⁺ (protons)

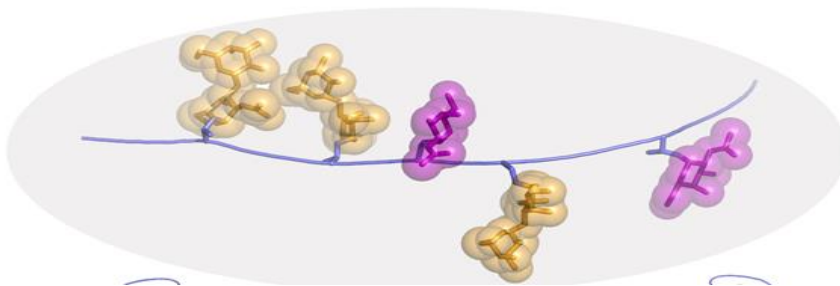
-Pro-Val-Pro-Ser-²²⁵Thr-Pro-Pro-²²⁸Thr-Pro-²³⁰Ser-Pro-²³²Ser-Thr-Pro-Pro-²³⁶Thr-Pro-Ser-Pro-Ser-Cys-



Measuring Formula Mass + protons

Peptide AA's
+
Glycan residues
+
H⁺ (protons)
1.00727
(not H 1.00782)

-Pro-Val-Pro-Ser-²²⁵Thr-Pro-Pro-²²⁸Thr-Pro-²³⁰Ser-Pro-²³²Ser-Thr-Pro-Pro-²³⁶Thr-Pro-Ser-Pro-Ser-Cys-



Elemental Composition: C60 H96 N19 O20 S2

STPPTPSPSCCHPR

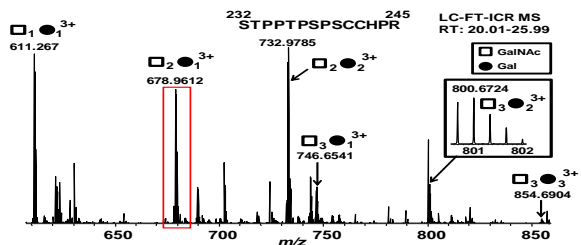
MH ⁺¹ (av)	MH ⁺¹ (mono)	MH ⁺³ (av)	MH ⁺³ (mono)
1467.6756	1466.6515	489.8968	489.5553

Elemental Composition: C82 H132 N21 O35 S2

STPPTPSPSCCHPR

+ 2 N-acetylgalactosamine + 1 galactose

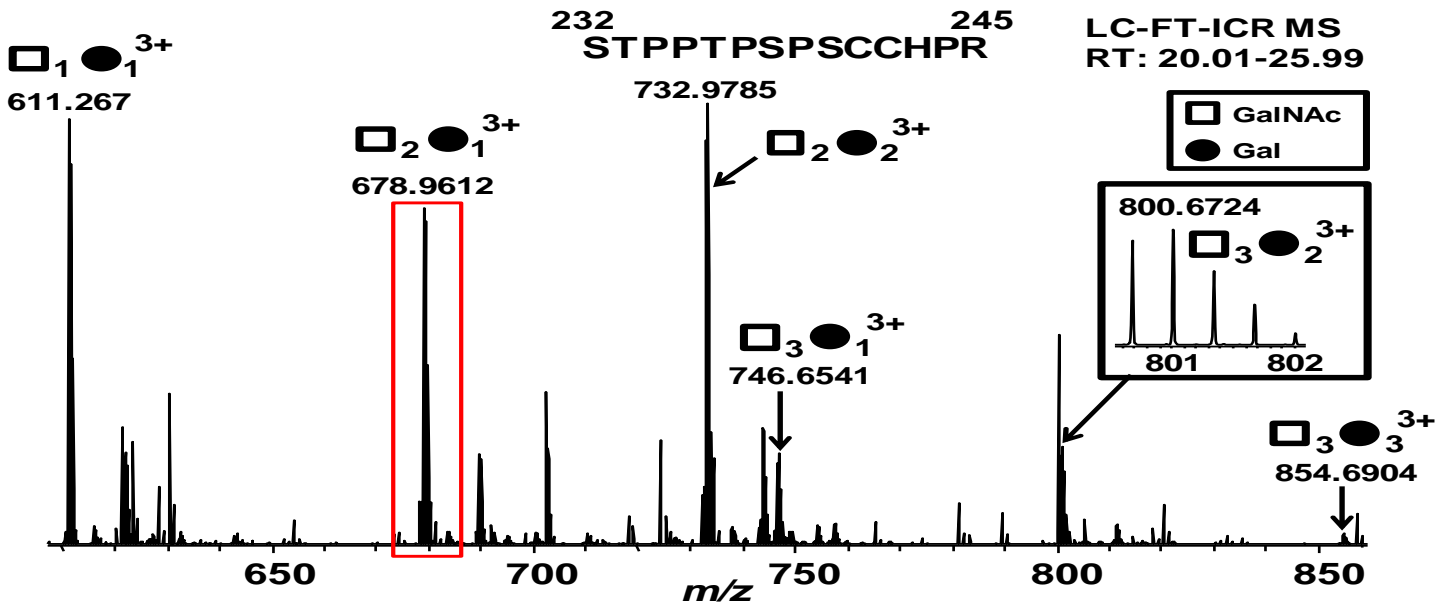
MH ⁺¹ (av)	MH ⁺¹ (mono)	MH ⁺³ (av)	MH ⁺³ (mono)
2035.7710	2034.8631	679.2620	678.9592





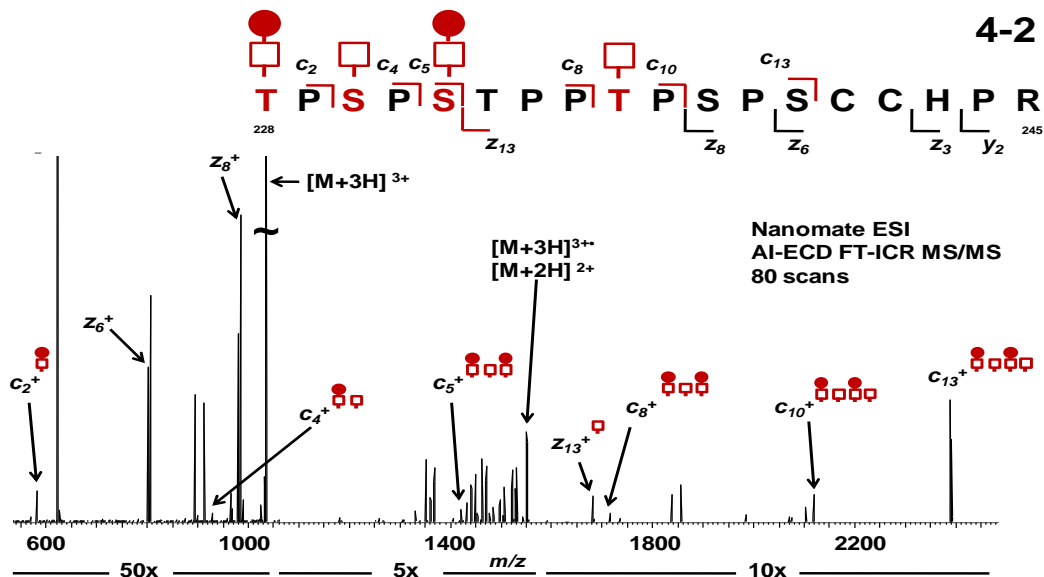
[STPTPSPSCCHPR + 3H]³⁺

678.9592 – 678.9612 = 0.0020 2.96 ppm mass error

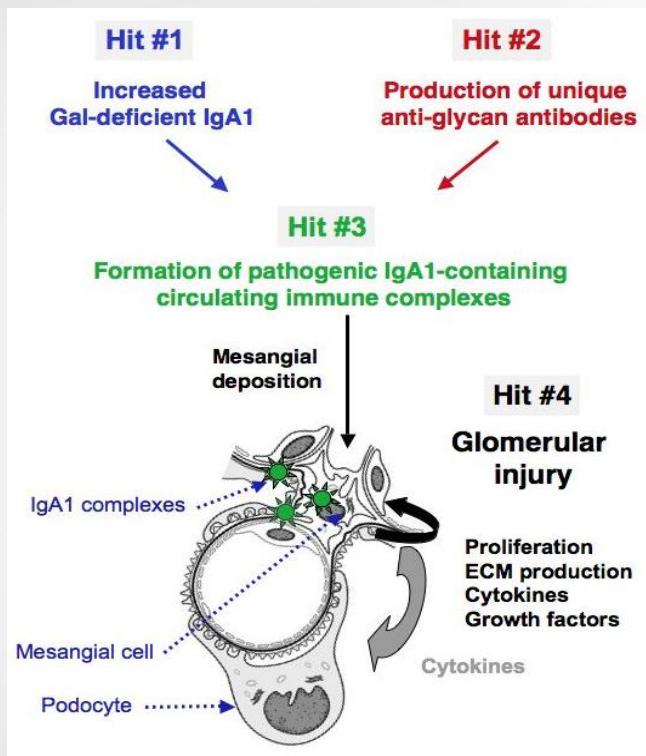


Tandem Mass Spectrometry

Terminal GalNAc at Ser²³⁰ Thr²³⁶



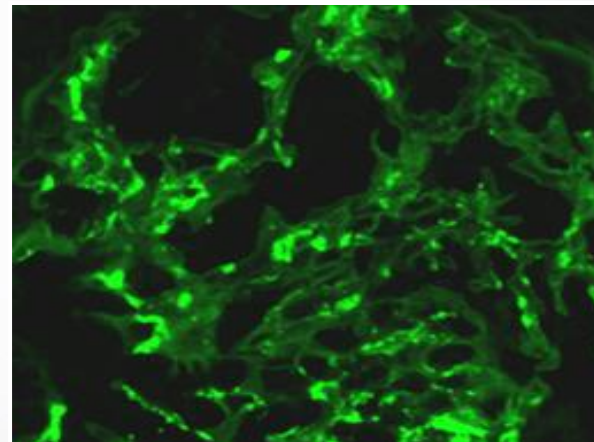
IgAN Pathogenesis: Multi-hit Hypothesis



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Autoimmune features

- Autoantigen (Gal-deficient IgA1)
- Autoantibody (binds the autoantigen)

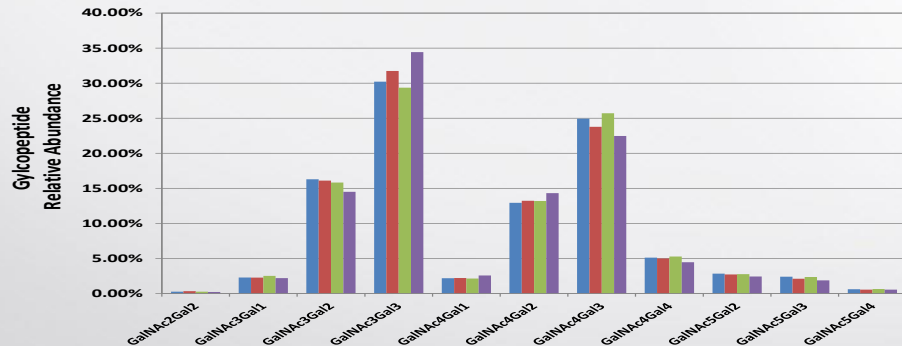
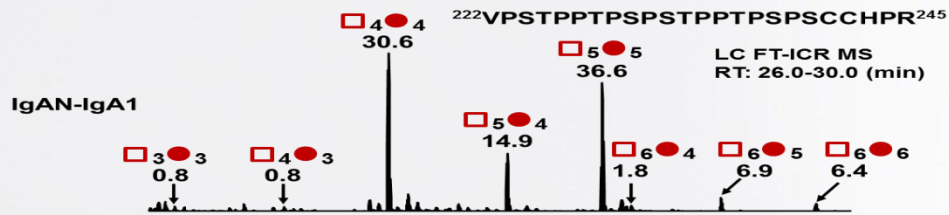


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Relative abundance of individual site microheterogeneity (%) ^a						
	225T	228T	230S	232S	233T	236T
Absent	0.4	N.D.	10.4	N.D.	64.0	65.4
Gal-deficient	0.3	N.D.	27.0	0.3	11.8	24.2
Disaccharides	99.3	100.0	62.6	99.7	24.2	10.3

Defining IgA1 O-glycan Heterogeneity by High Resolution Mass Spectrometry

- Range of O-glycoforms
- Sites of attachment
- Sites of Gal-deficiency
- Isomers
- Quantitative value for each O-glycosylated form
- Highly reproducible

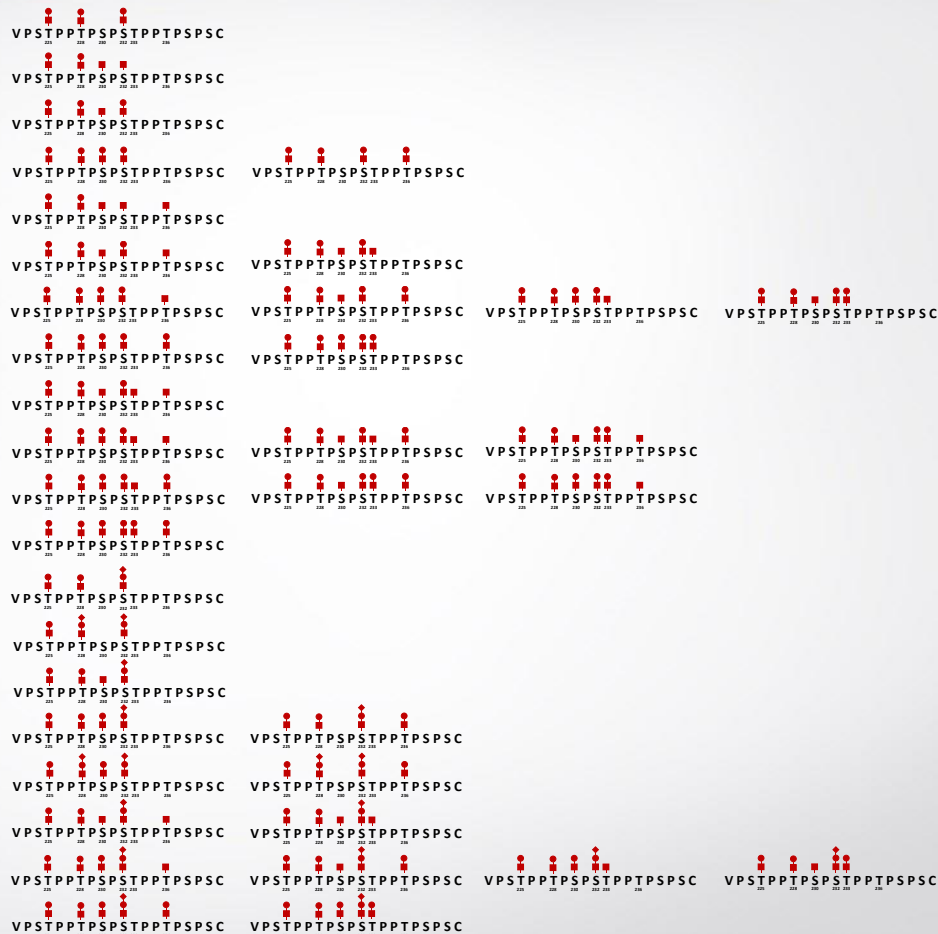
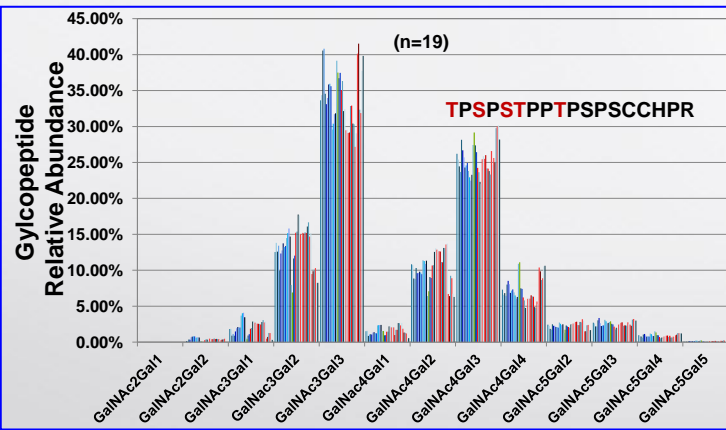
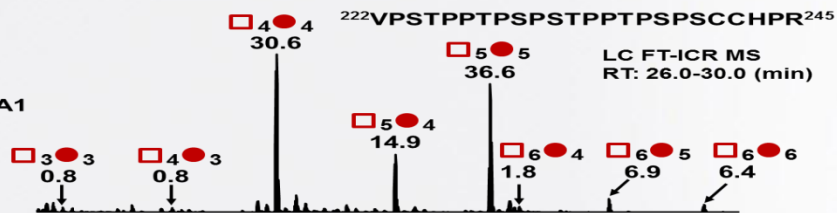


Relative abundance of individual site microheterogeneity (%)^a

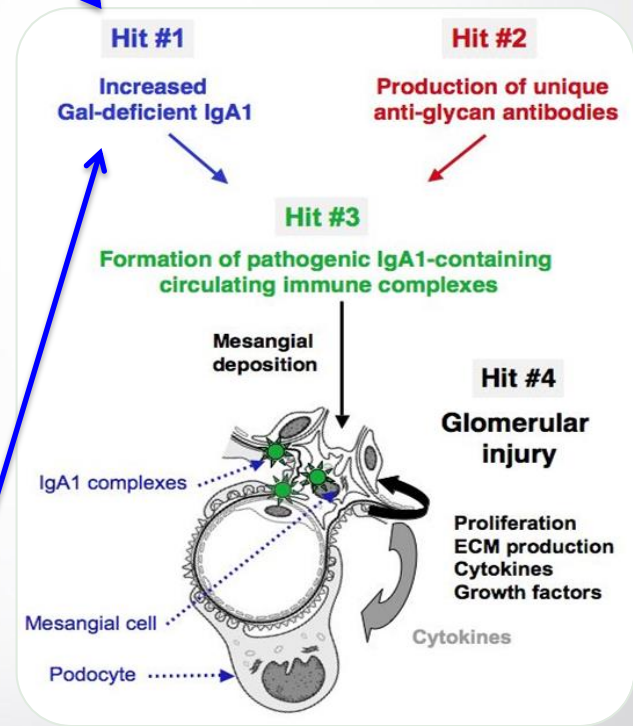
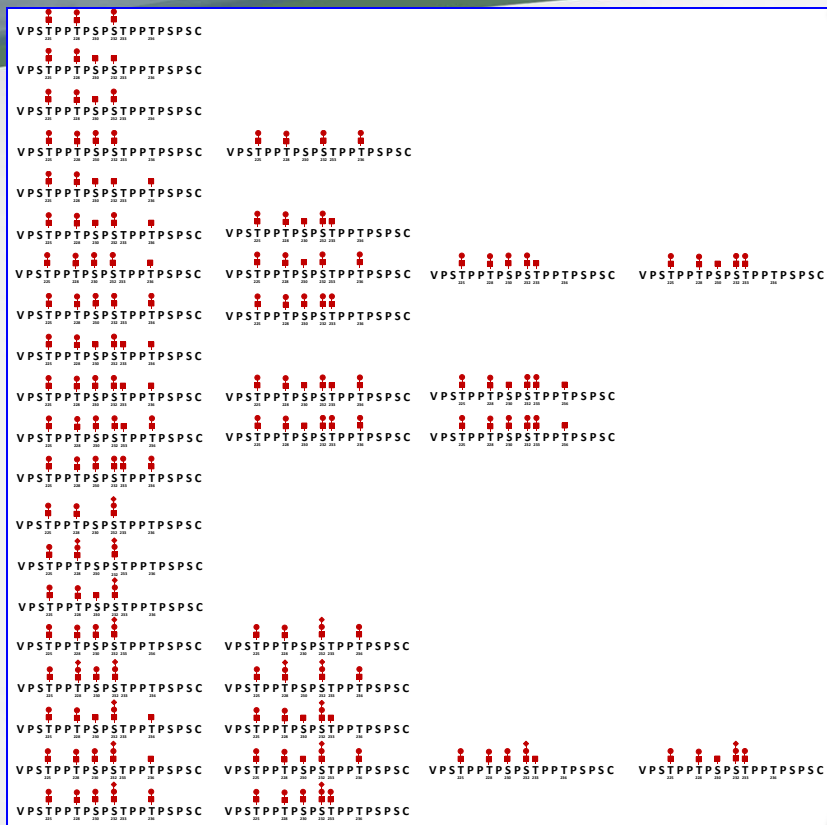
	225T	228T	230S	232S	233T	236T
Absent	0.4	N.D.	10.4	N.D.	64.0	65.4
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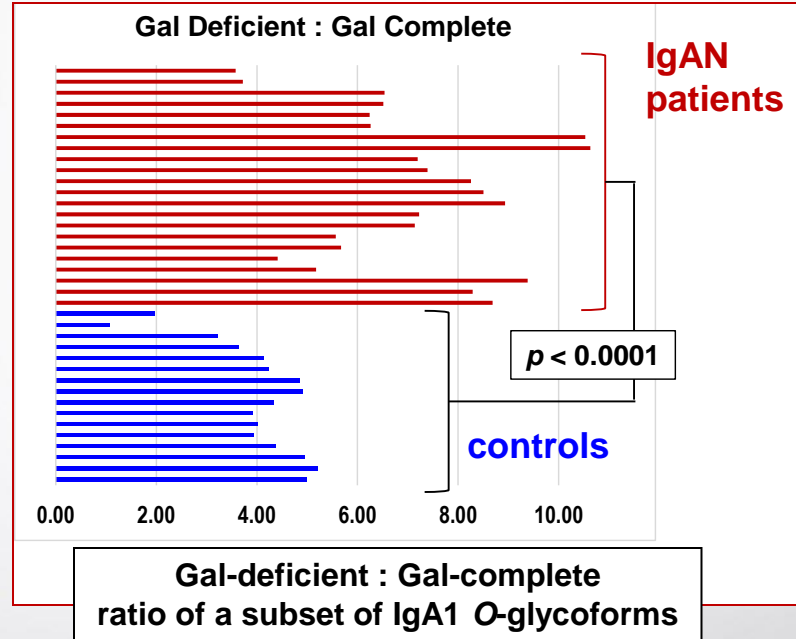
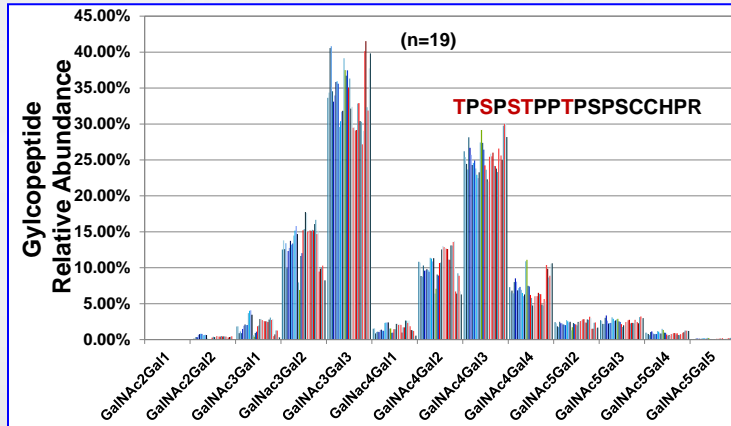
IgAN-IgA1



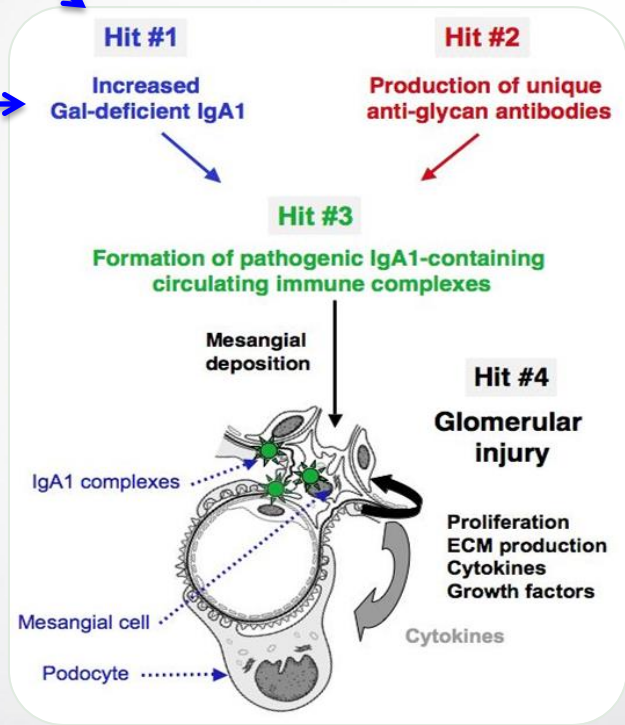
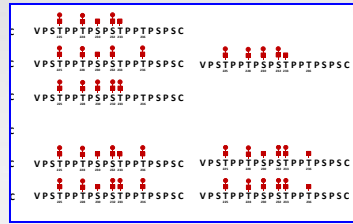
← Profiled IgAN patient sera



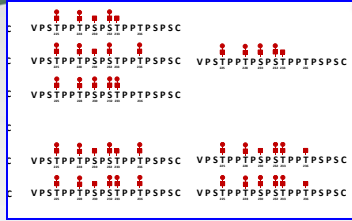
Gal-deficient IgA1 : Gal-complete IgA1 (IgAN patients vs. age / race-matched Healthy Controls)



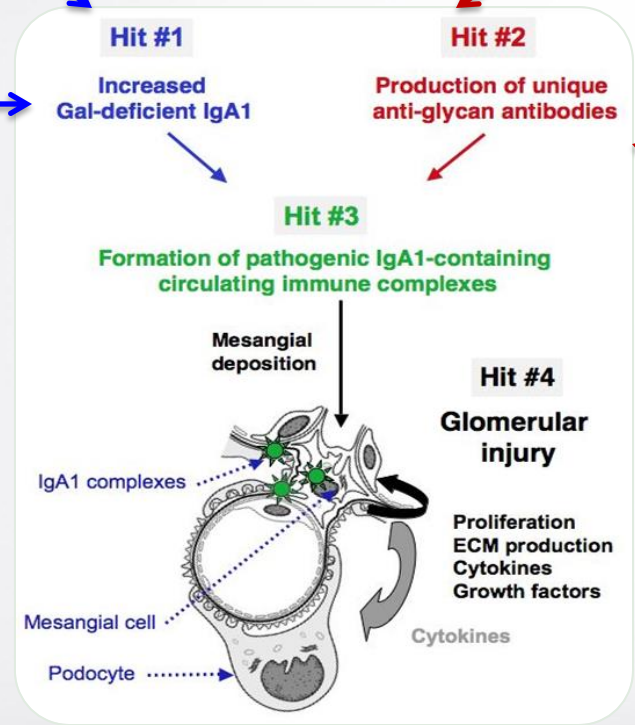
IgAN Pathogenesis: Multi-hit Hypothesis



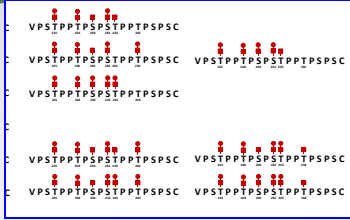
IgAN Pathogenesis: Multi-hit Hypothesis



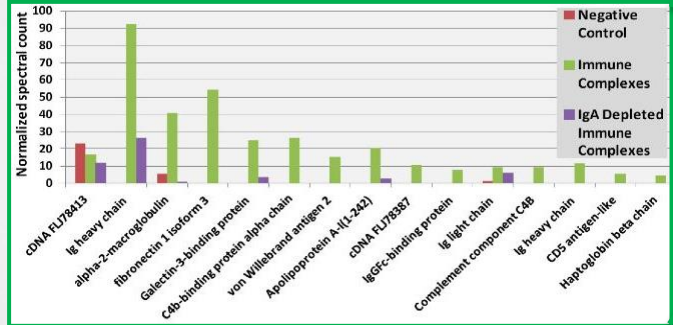
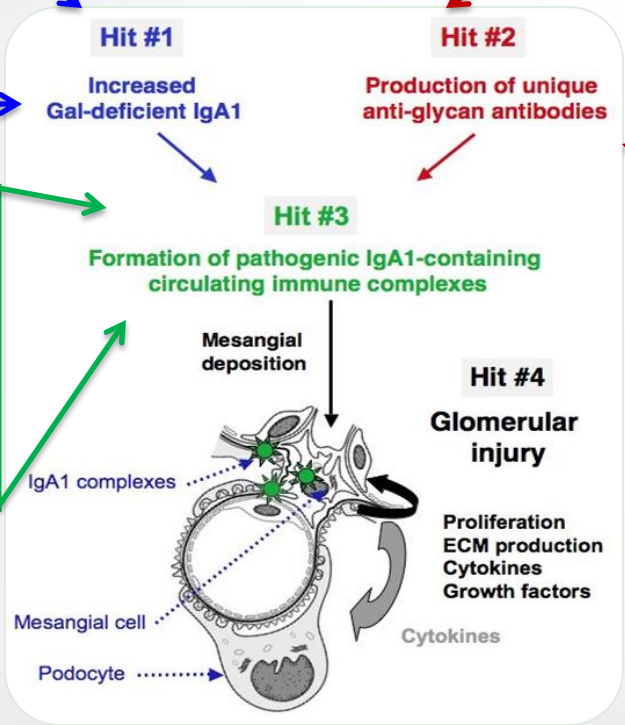
- AEDTAVYYCAK
- AEDTAVYYCAR**
- AEDTAVYYCSR**
- SEDTAVYYCAR
- AEDTAIYYCAR
- AEDTAIYYCSR
- ADDTAVYYCAR
- ADDTAVYYCSR
- LSSVTAADTAVYYCAR



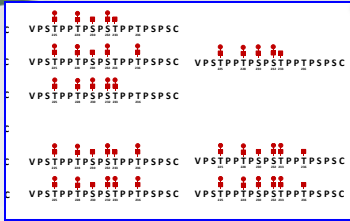
IgAN Pathogenesis: Multi-hit Hypothesis



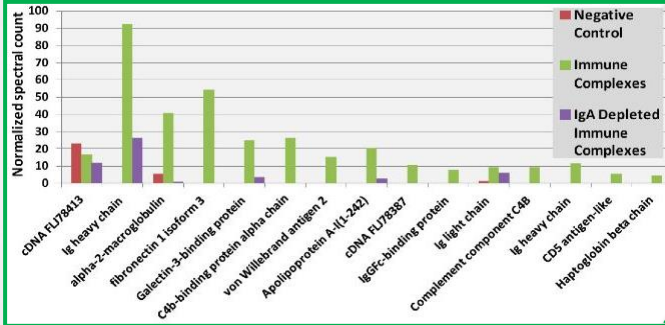
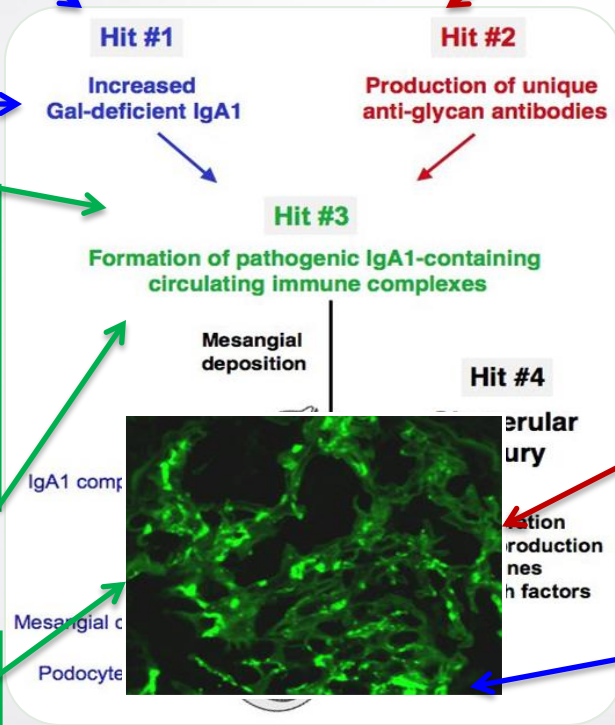
- AEDTAVYYCAK
- AEDTAVYYCAR**
- AEDTAVYYCSR**
- SEDTAVYYCAR
- AEDTAIYYCAR
- AEDTAIYYCSR
- ADDTAVYYCAR
- ADDTAVYYCSR
- LSSVTAADTAVYYCAR



IgAN Pathogenesis: Multi-hit Hypothesis



- AEDTAVYYCAK
- AEDTAVYYCAR**
- AEDTAVYYCSR**
- SEDTAVYYCAR
- AEDTAIYYCAR
- AEDTAIYYCSR
- ADDTAVYYCAR
- ADDTAVYYCSR
- LSSVTAADTAVYYCAR



- AEDTAVYYCAK
- AEDTAVYYCAR**
- AEDTAVYYCSR**
- SEDTAVYYCAR
- AEDTAIYYCAR
- AEDTAIYYCSR
- ADDTAVYYCAR
- ADDTAVYYCSR
- LSSVTAADTAVYYCAR

