Mass Spectrometry in Medicine

BI-793 – Lecture 12

Catherine E. Costello
Center for Biomedical Mass Spectrometry
Boston University School of Medicine
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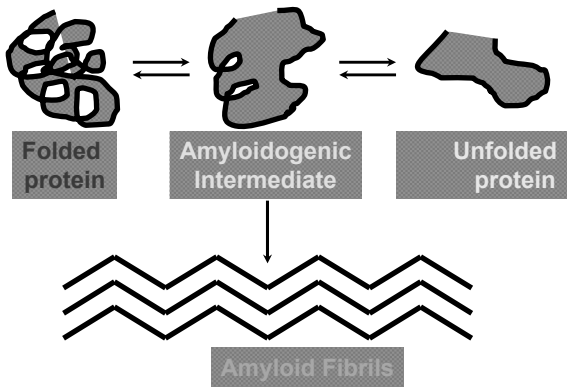
Mass Spectrometry in Medicine

- Amyloid proteins and hemoglobin mutations
- Virus capsid maturation
- CD1 presentation of antigenic lipids
- Urinary tract infections

Protein folding gone awry: amyloid proteins

- BUSM MS Resource: Zhenning Hong, Amareth Lim, Mark E McComb, Roger Théberge, Yan Jiang, Marianna Budnik
- *Collaborators:*
- BUSM Amyloid Program: Lawreen H Connors, Jon Kingsbury, Tatiana Prokaeva, Martha Skinner, Mary Walsh†
- Cleveland Clinic: Donald M Jacobsen
- Univ. Pavia: Francesca Lavatelli, Giampaolo Merlini

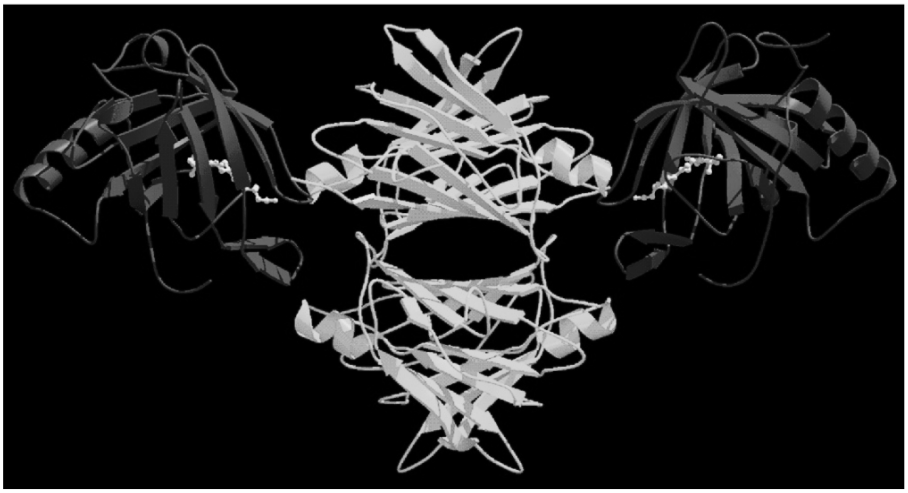
Amyloid fibril formation



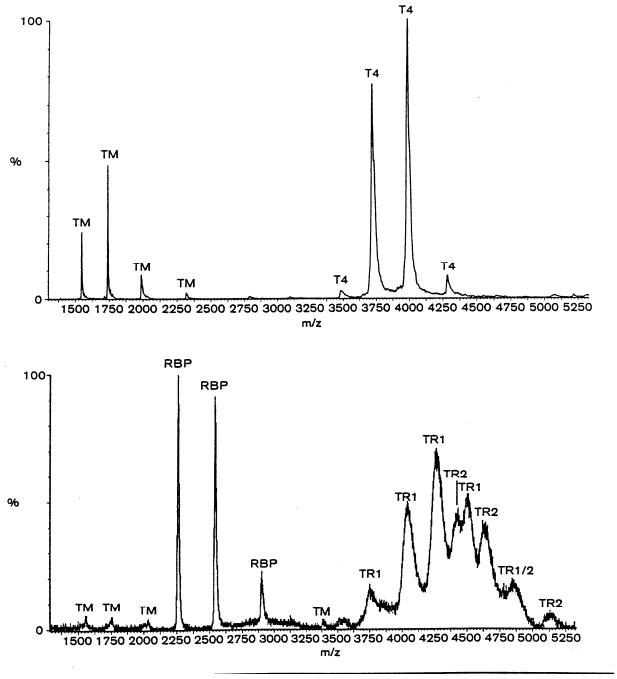
Amyloidogenic proteins

Amyloid Protein	Precursor	System/Local	Syndrome/Tissue
AL (AH)	IgG light (heavy) chain	S.L	Primary/Myeloma
ATTR, SSA	Transthyretin	S	Familial, Senile
AA	(Apo)serum AA	S	Secondary/reactive
$A\beta_2M$	β_2-Microglobulin	S	Chronic microdialysis
AApoA1	Apolipoprotein A1	S	Familial
AGel	Gelsolin	S	Familial
ALys	Lysozyme	S	Familial
AFib	Fibrinogen α-chain	S	Familial
ACys	Cystatin C	S	Familial
Aβ	Aβ precursor AβPP	L	Alzheimer's Disease
$APrP^{sc}$	Prion protein	L	Spongiform enceph.
AIAPP	Islet amyloid polypep.	L	Islets of Langherhans
AIns	Insulin	L	Iatrogenic
APro	Prolactin	L	Aging pituitary

Crystal structure of TTR. The TTR tetramer is shown in green, retinol binding protein in red, and vitamin A in yellow.

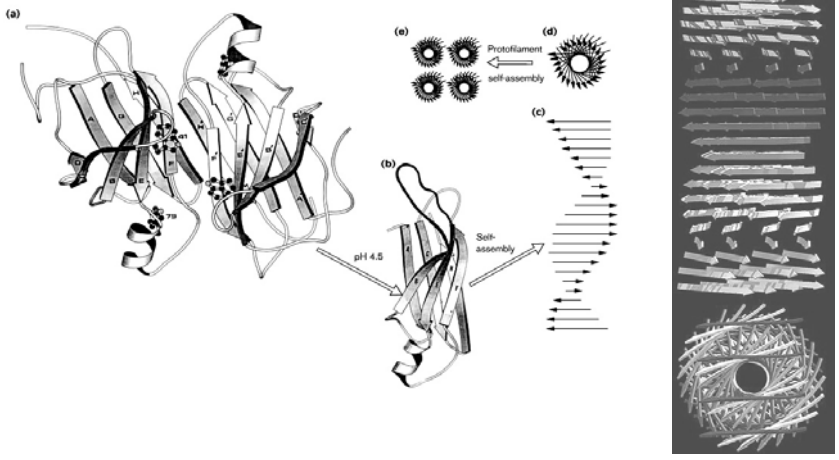


Proteins: Structure, Function, and Genetics, **1998**, *33*, 3-11



NanoESI mass spectra of TTR monomer (T1) and TTR tetramer (T4) and complexes of retinol-binding protein (RBP) and of TTR with one (TR1) and two (TR2) RBP and retinol

Rostom & Robinson, *Curr Op Struct Biol*, **1999**, *9, 135-141*



Formation of TTR amyloid fibrils from its amyloidogenic intermediate. A model of the TTR protofilament is shown on the right. *Curr Opin Struct Biol* **1998**, *8,*101-106 and *Structure* **1996**, 4, 989-998

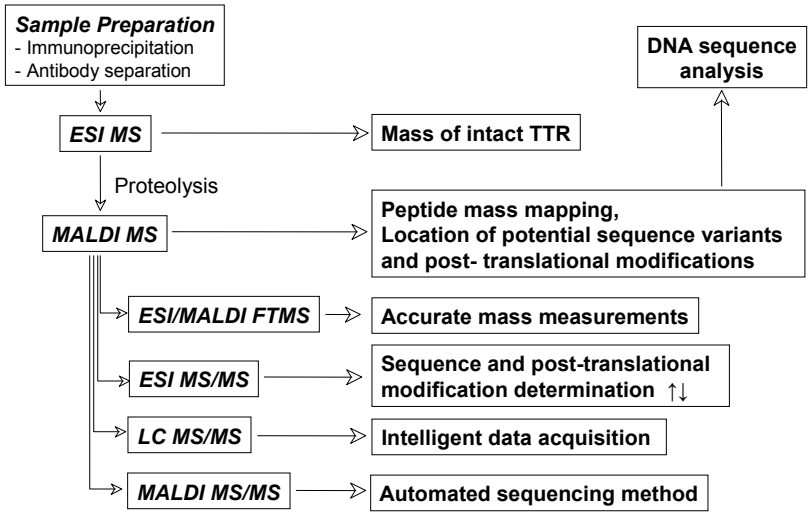
Methods for the clinical diagnosis of ATTR

- Molecular genetic analysis
  - Direct DNA sequence analysis → indeterminate results
  - Restriction fragment length polymorphism → multiple restriction enzymes
  - Single strand conformation polymorphism → false positives

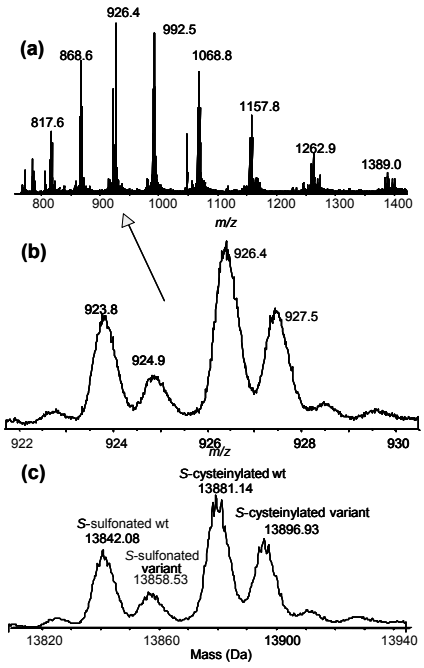
  Current methods → Time consuming

- Mass spectrometry
  - Speed
  - Sensitivity
  - Direct protein characterization
  - Post-translational modifications
  - Unambiguous sequence determination

Method development for analysis of TTR variants



R Theberge *et al, Anal Chem,* **1999**, *71*, 452-459
R Theberge *et al, J Am Soc Mass Spectrom,* **2000,** *11*, 172-175
A Lim *et al, Anal Chem,* **2002,** *74*, 741- 751
J Kingsbury *et al*, *Anal Chem*, **2007**, *79*, 1990-1998



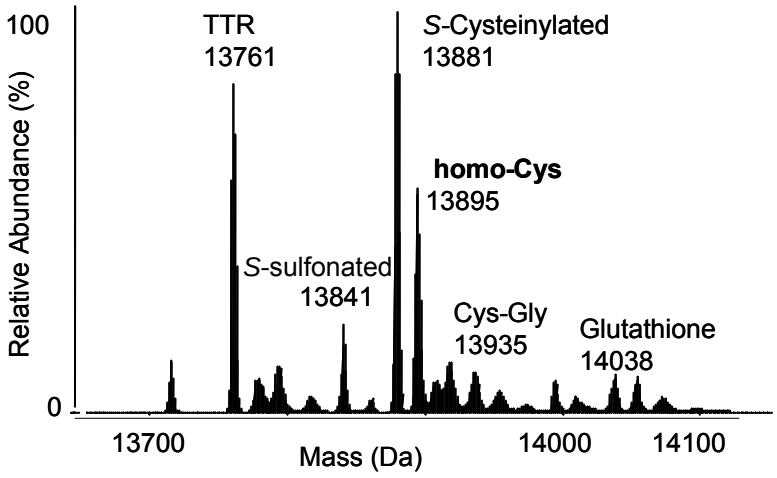
(a) 15+ charge state at expanded scale
(b) ESI-QoTOF mass spectrum of a TTR sample
(c) neutral mass reconstruction

S-Sulfonation at cysteine:

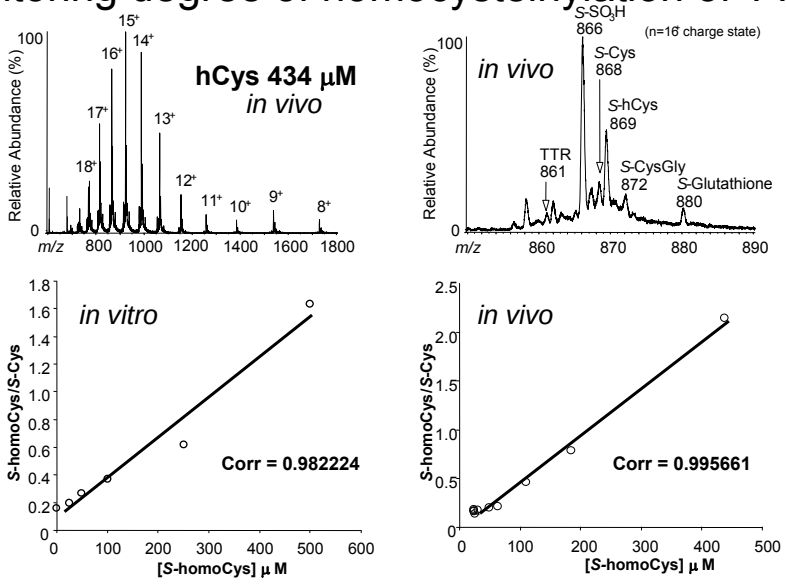
$$\text{CysS-H} \xrightarrow{???} \text{CysS-SO}_3\text{H}$$

$$\text{CysS-SR} + \text{H-SO}_3\text{H}(l) \rightarrow \text{CysS-SO}_3\text{H} + \text{RS-H}$$

Deconvoluted ESI-QoTOF mass spectrum of intact TTR exposed *in vitro* to 250 μM homocysteine



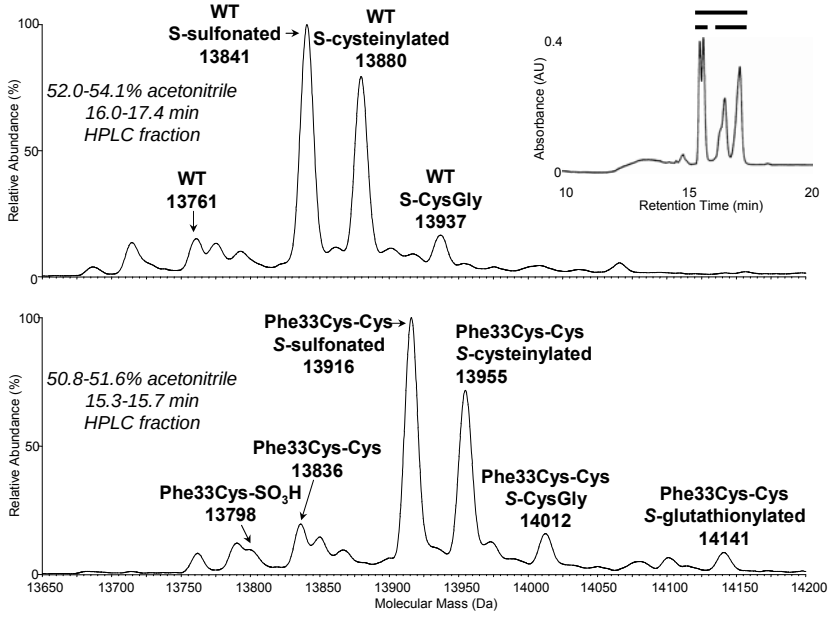
Monitoring degree of homocysteinylation of TTR



- Degree of incorporation ≅level of *S*-homocysteinylation.
- TTR is a major sink for homocysteine.

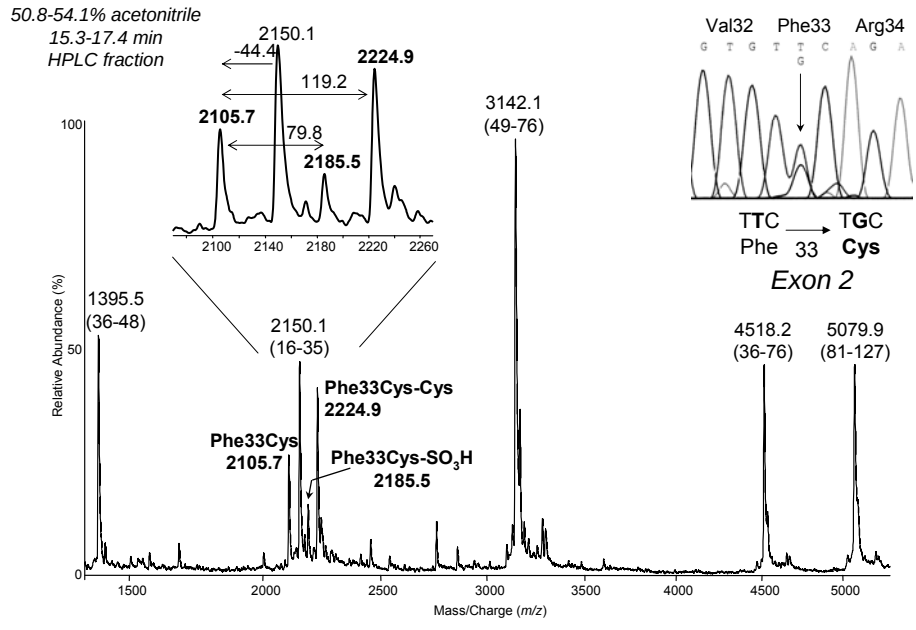
Deconvoluted ESI MS of intact TTR

Patient TTR-00084

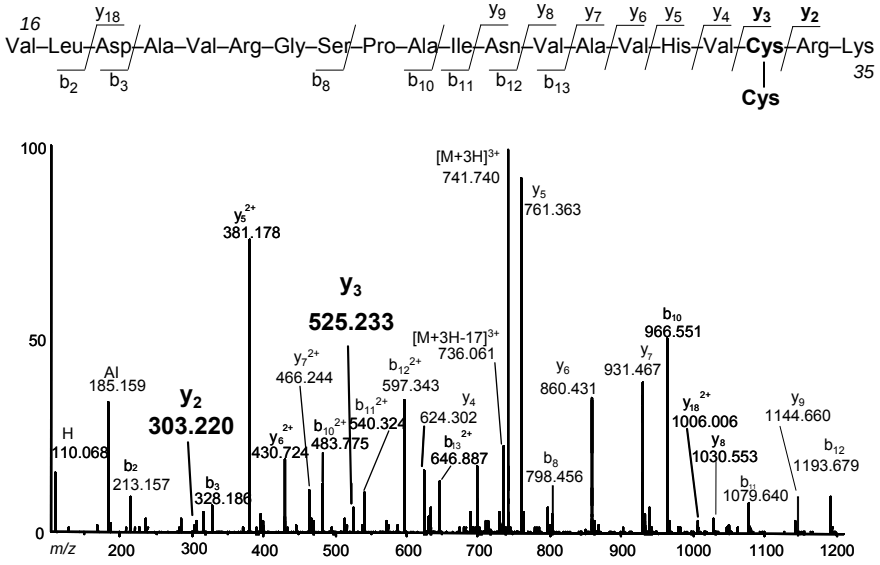


MALDI-TOF MS of Lys-C digest of TTR sample

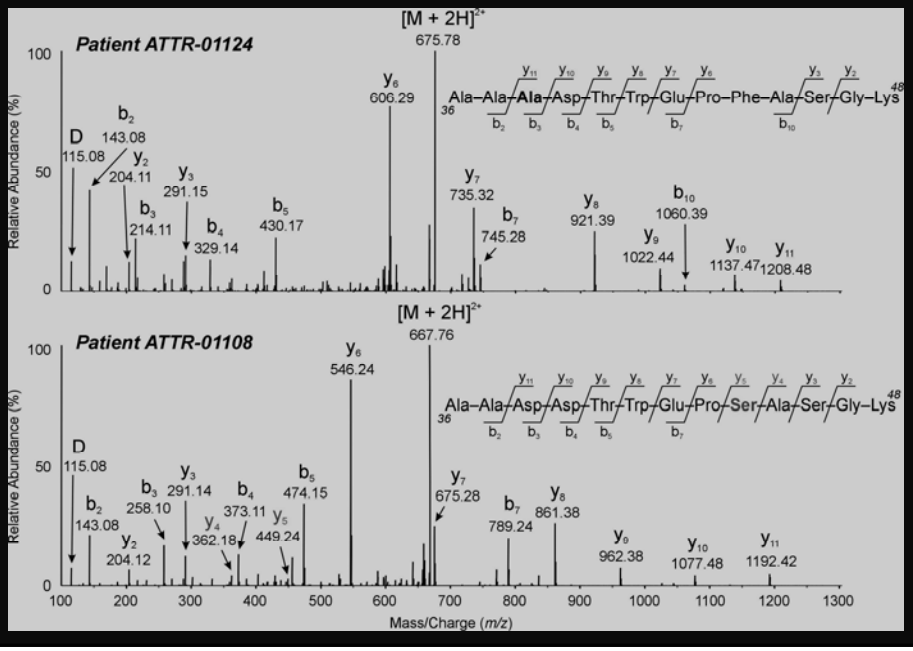
Patient TTR-00084



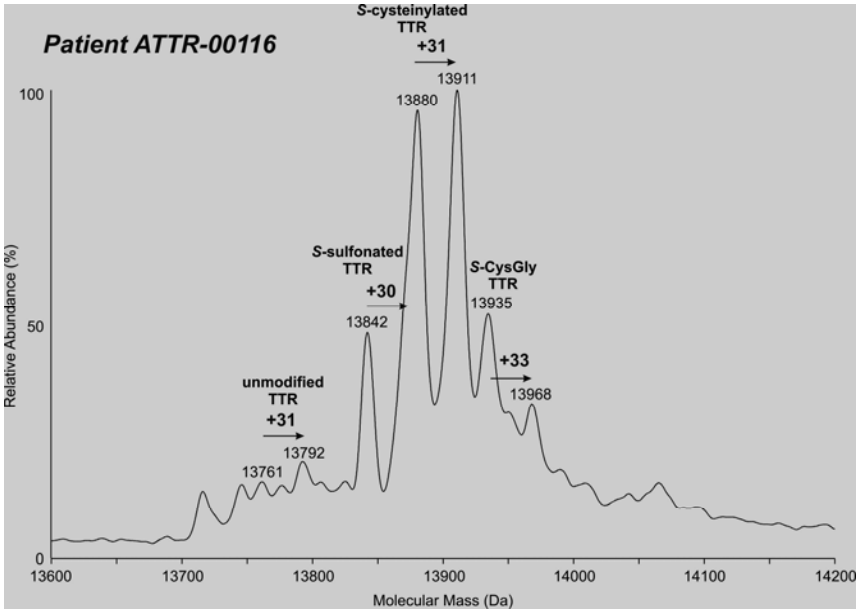
ESI-QoTOF CID MS/MS of the $[M+3H]^{3+}$ *m/z* 741.40 of the variant peptide (*S*-Cysteinylated) from the Lys-C digest of Phe33Cys TTR



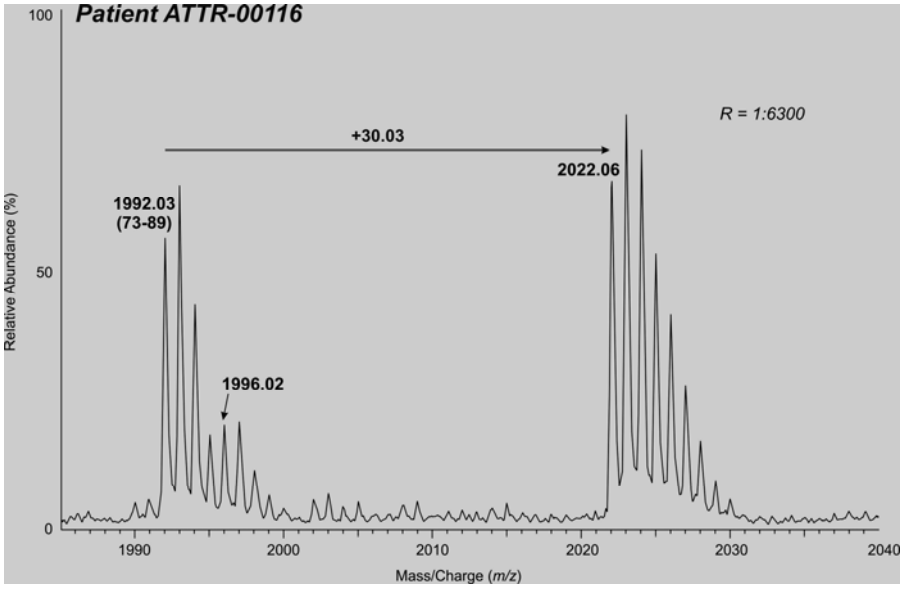
ATTR Asp38Ala and Phe44Ser variants



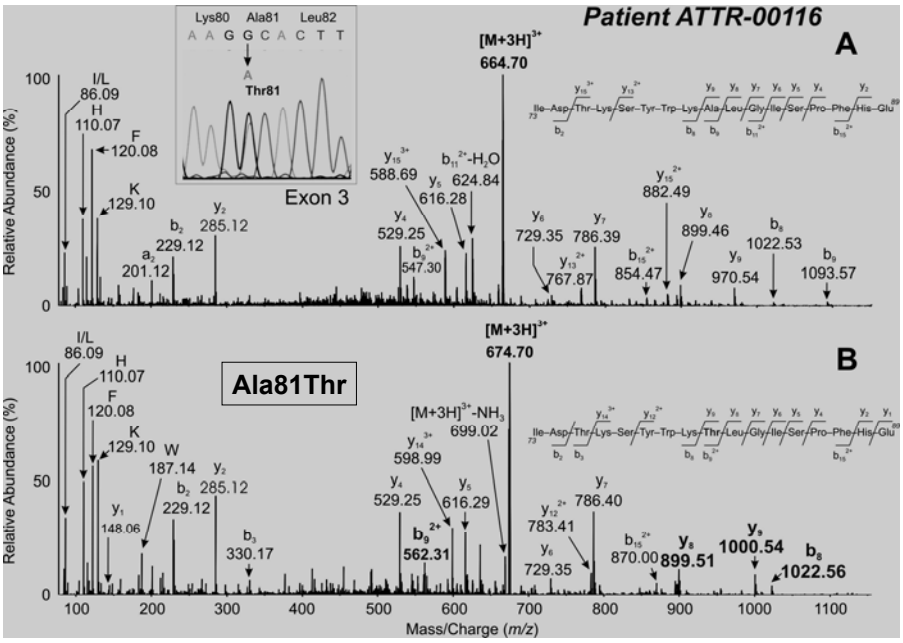
Deconvoluted ESI MS of intact TTR



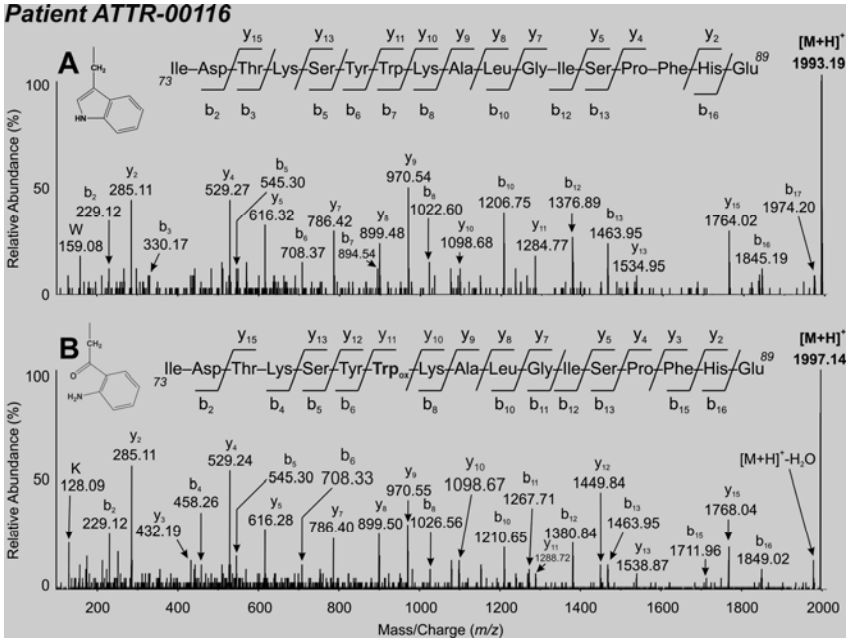
MALDI MS of the Glu-C digest of the TTR

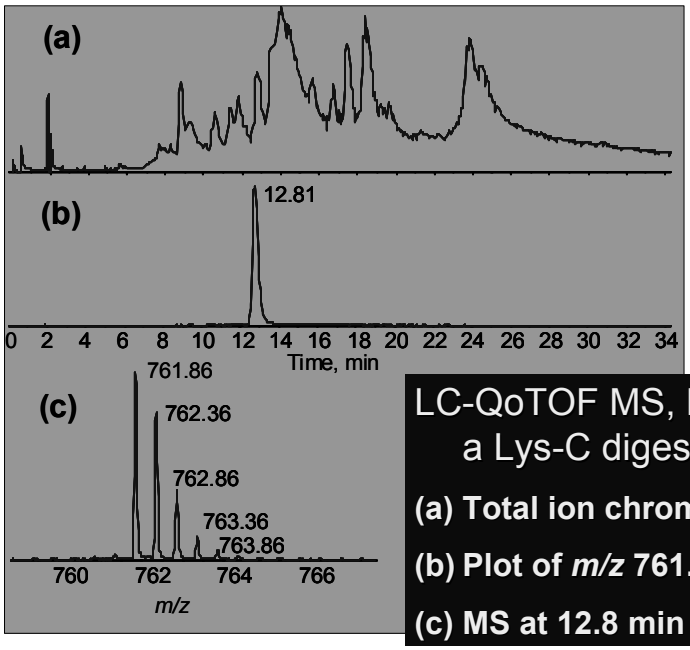


ESI-CID MS/MS of the $[M+3H]^{3+}$ TTR Glu-C peptides



MALDI-CID MS/MS of the $[M+H]^{+}$ TTR Glu-C peptides





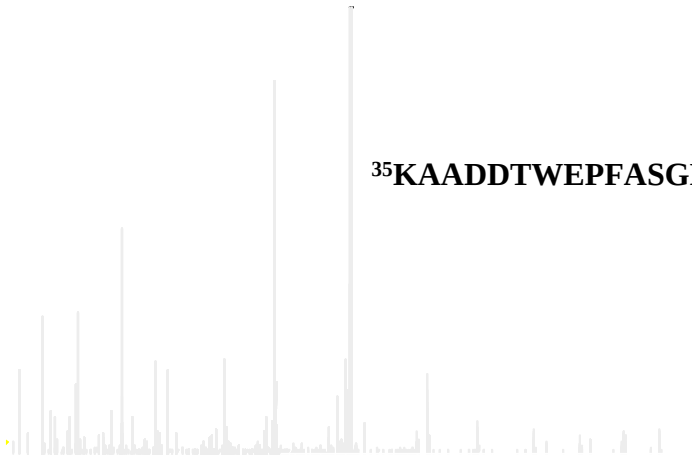
LC-QoTOF MS, MS/MS of a Lys-C digest of TTR

(a) Total ion chromatogram

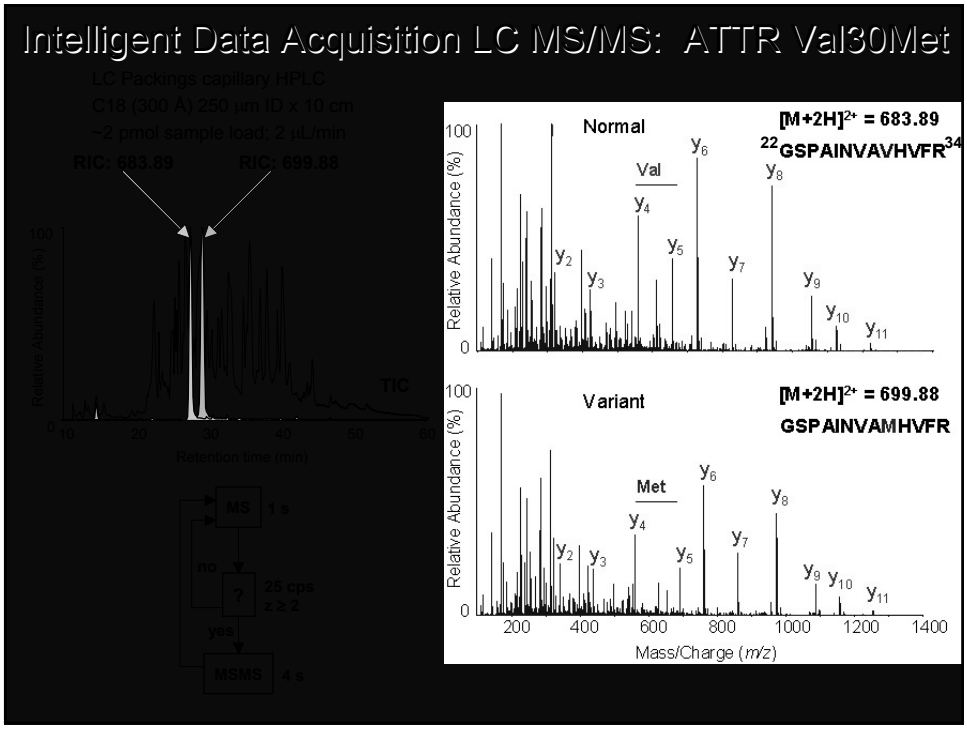
(b) Plot of *m/z* 761.8

(c) MS at 12.8 min

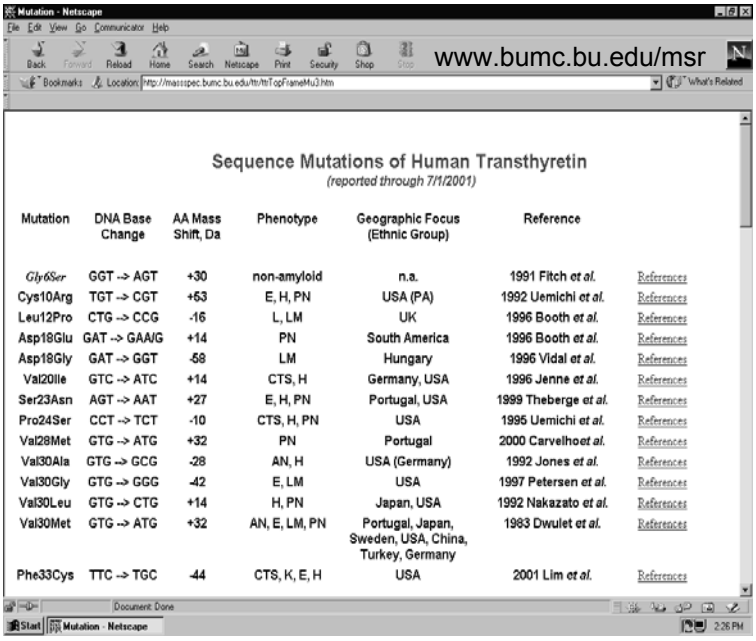
ESI-QoTOF CID MS/MS spectrum, *m/z* 761.86, eluting at 12.8 min during the capillary HPLC of a TTR digest



^{35}KAADDTWEPFASGK$_{48}$



Tabulation of transthyretin variants

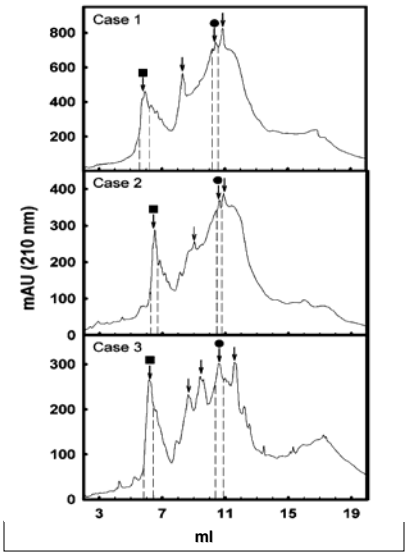


Sequence Mutations of Human Transthyretin

(reported through 7/1/2001)

Mutation	DNA Base Change	AA Mass Shift, Da	Phenotype	Geographic Focus (Ethnic Group)	Reference	
Gly6Ser	GGT -> AGT	+30	non-amyloid	n.a.	1991 Fitch *et al.*	References
Cys10Arg	TGT -> CGT	+63	E, H, PN	USA (PA)	1992 Uemichi *et al.*	References
Leu12Pro	CTG -> CCG	-16	L, LM	UK	1996 Booth *et al.*	References
Asp18Glu	GAT -> GAA/G	+14	PN	South America	1996 Booth *et al.*	References
Asp18Gly	GAT -> GGT	-58	LM	Hungary	1996 Vidal *et al.*	References
Val20Ile	GTC -> ATC	+14	CTS, H	Germany, USA	1996 Jenne *et al.*	References
Ser23Asn	AGT -> AAT	+27	E, H, PN	Portugal, USA	1999 Theberge *et al.*	References
Pro24Ser	CCT -> TCT	-10	CTS, H, PN	USA	1995 Uemichi *et al.*	References
Val28Met	GTG -> ATG	+32	PN	Portugal	2000 Carvelhoet *al.*	References
Val30Ala	GTG -> GCG	-28	AN, H	USA (Germany)	1992 Jones *et al.*	References
Val30Gly	GTG -> GGG	-42	E, LM	USA	1997 Petersen *et al.*	References
Val30Leu	GTG -> CTG	+14	H, PN	Japan, USA	1992 Nakazato *et al.*	References
Val30Met	GTG -> ATG	+32	AN, E, LM, PN	Portugal, Japan, Sweden, USA, China, Turkey, Germany	1983 Dwulet *et al.*	References
Phe33Cys	TTC -> TGC	-44	CTS, K, E, H	USA	2001 Lim *et al.*	References

HPLC of extracted TTR fibrils

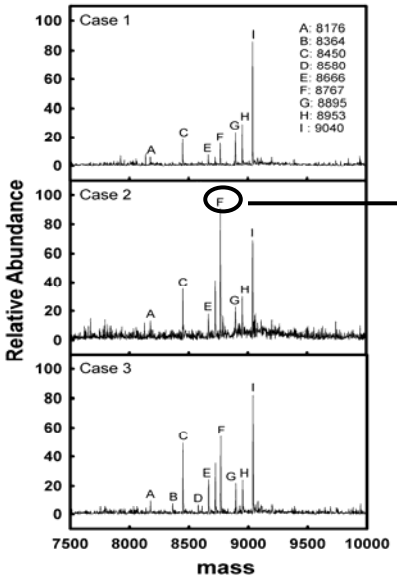


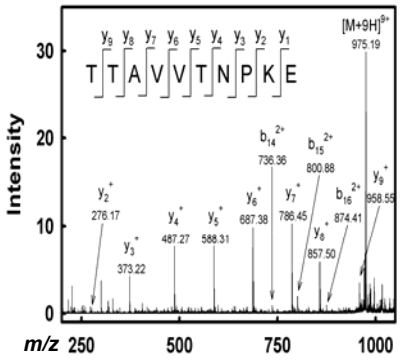
RP-HPLC chromatograms of PBS-soluble amyloid fractions extracted from cardiac fibrils in three cases of SSA.

Zorbax Poroshell 300SB-C8 HPLC column pre-equilibrated in 20% buffer B and eluted over 20 min with a 20-50% linear increase in buffer B.

Arrows indicate the major peaks that were collected and screened for TTR by SDS-PAGE/Western blot analysis and nanospray mass spectrometry. The two TTR-containing isolations (bordered by dashed lines) were termed the early (9) and late (b) fractions.

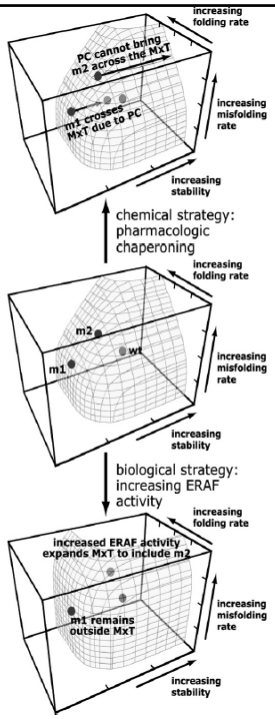
Top-down characterization of truncated TTR in fibrils





ESI-QoTOF CID MS/MS of component F, M_r 8767

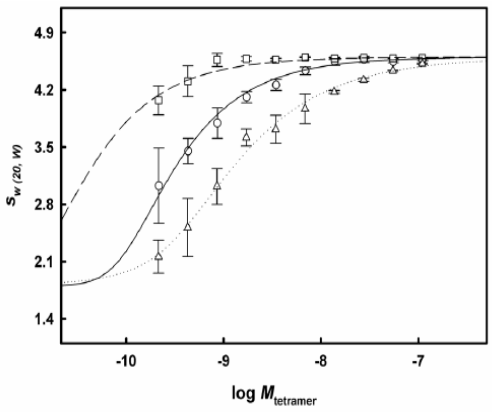
JS Kingsbury, R Théberge, JA Karbassi, A Lim, CE Costello, LH Connors. *Anal Chem*, **2007**, *79*, 1990-1998



Increasing ERAF and pharmacologic chaperoning have distinct influences on different loss-of-function mutations

RL Wiseman, ET Powers, JN Buxbaum, JW Kelly, WE Balch, *Cell*, **2007**

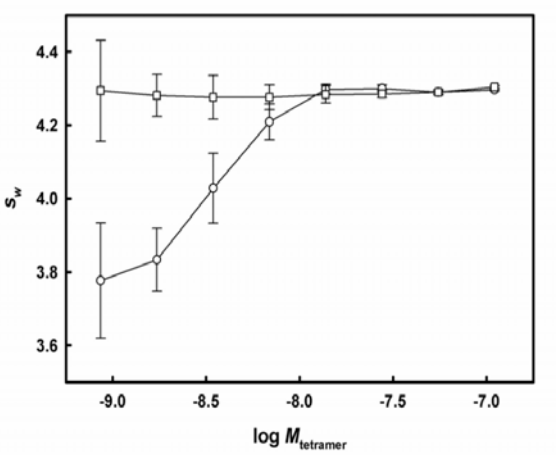
Fluorescence-detected analytical ultracentrifugation of diflunisal-stabilized rTTR in human serum



Sedimentation velocity isotherms for F-rTTR as unmodified (○), Cys10 SO3H (□), Cys10-Cys (Δ)

JS Kingsbury, TM Laue, ES Klimtchuk, R Théberge, JA Karbassi, A Lim, CE Costello, LH Connors. *J Biol Chem*, **2008,** *283*, 11887-11896

Fluorescence-detected analytical ultracentrifugation of diflunisal-stabilized rTTR in human serum



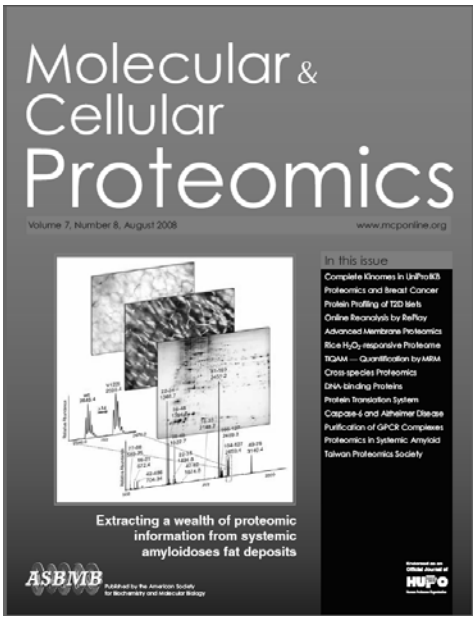
Sedimentation velocity isotherms for F-rTTR in the absence (○) and presence (□) of diflunisal (5-(2,4-difluorophenyl) -2-hydroxy-benzoic acid)

JS Kingsbury, TM Laue, ES Klimtchuk, R Théberge, JA Karbassi, A Lim, CE Costello, LH Connors. *J Biol Chem*, **2008,** *283*, 11887-11896

2D-gel and MS analysis of fat biopsies for amyloid diagnosis and study

MS Resource: R Théberge, Y Jiang, DH Perlman, CE Costello

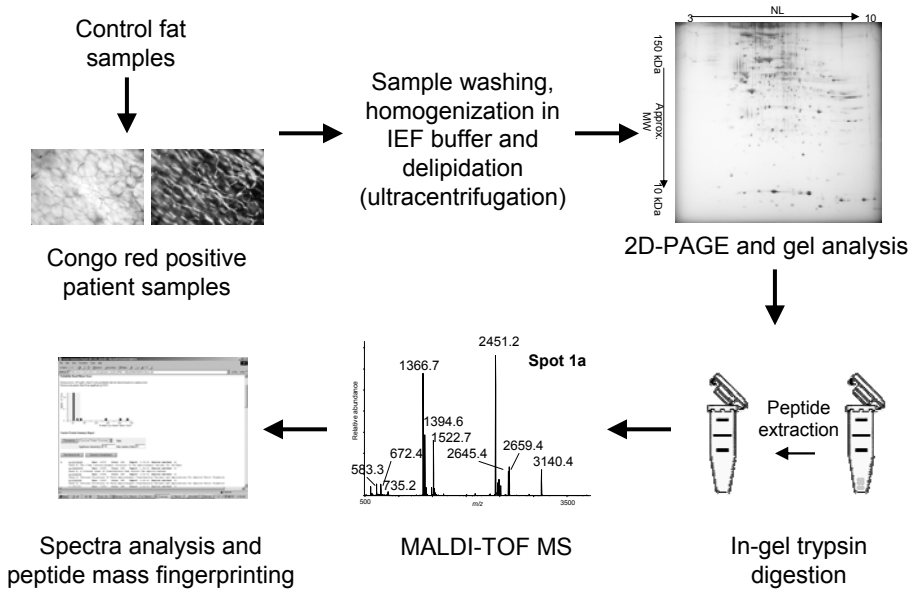
Collaborators:
BUSM Amyloid: M Skinner, D Seldin, L Connors
Univ. Pavia: F Lavatelli, G Merlini



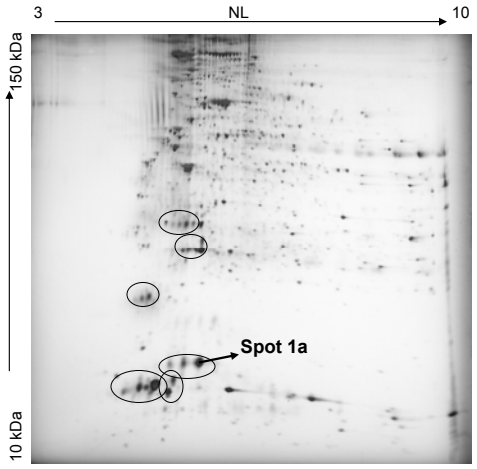
2D-gel and peptide mass fingerprinting for amyloid protein analysis in fat biopsies

F Lavatelli, DH Perlman, B Spencer, T Prokaeva, ME McComb, R Théberge, LH Connors, V Bellotti, DC Seldin, G Merlini, M Skinner, CE Costello. *Mol. Cell. Proteomics* **2008**, *7*, 1570-1583

Fat tissue protein analysis



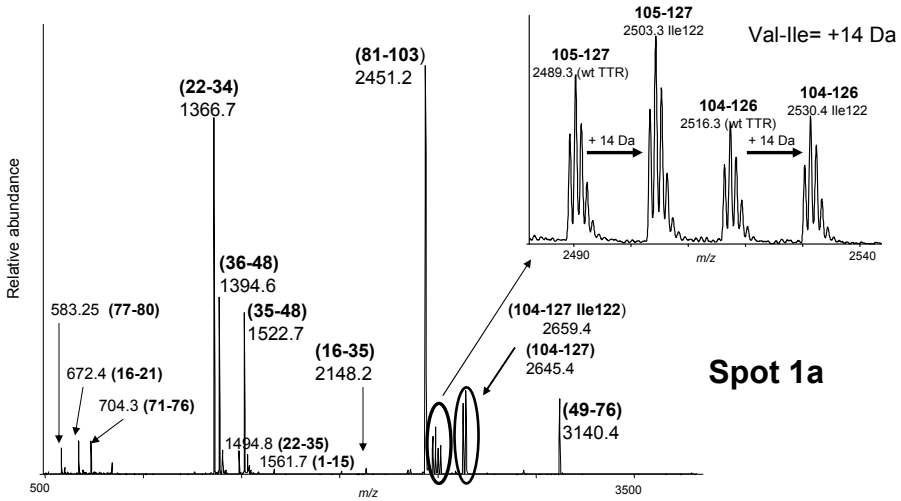
Transthyretin amyloidosis



In this patient, the amino acid substitution **Val122Ile** caused TTR to be amyloidogenic

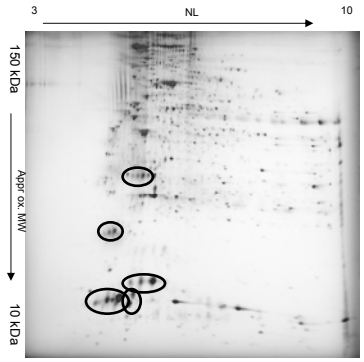
Circled regions: spots not visible in controls

ATTR Ile122: Spot identification



MALDI-TOF spectrum after tryptic digestion of spot 1a

ATTR Ile122: gel characterization



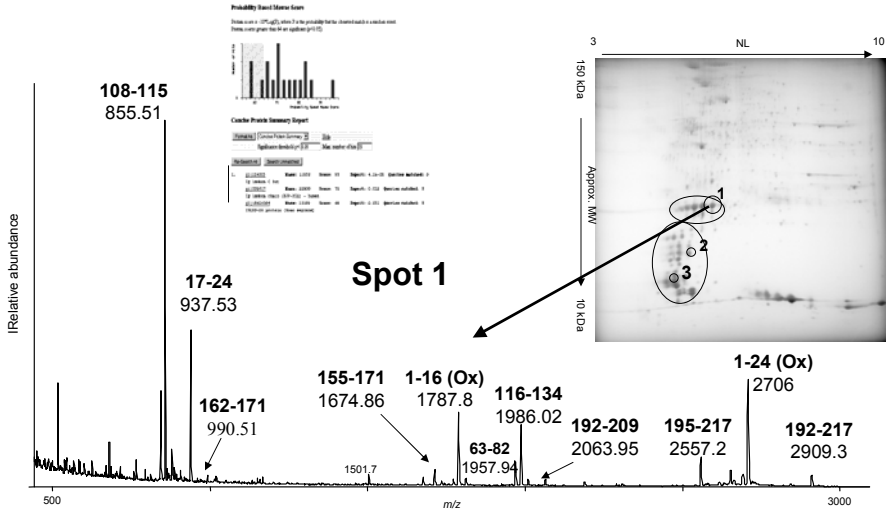
Spots in red circle: TTR (wild-type + Val122Ile)

Spots in blue circles: TTR (wild-type + Val122Ile), possibly truncated

Spots in green circles: TTR (wild-type + Val122Ile) multimers

In all the spots where TTR is found, wild-type protein and the variant coexist

AL λ: spot identification

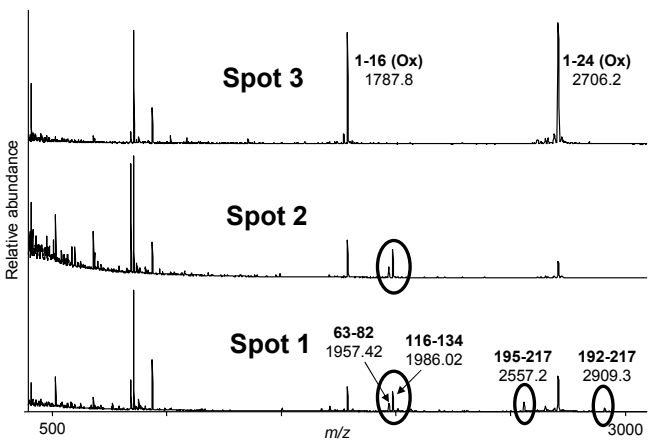


Spots 1 contain the amyloidogenic λ light chain

AL λ: gel characterization

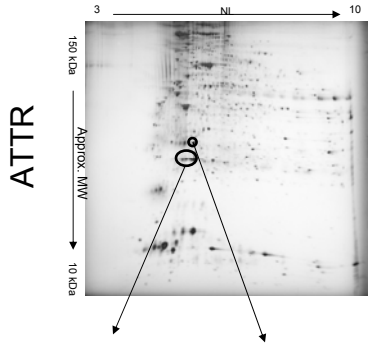
Overlay of spectra from spots in different regions of the circled areas.

Spots contain the same protein (λ light chain)



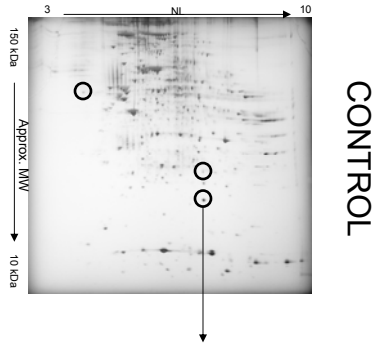
As the molecular weight of the spots decreases, peaks from the C-terminus of the λ light chain progressively disappear

Other proteins found on gels



SAP TTR + **Apo E**

Proteins known to be associated with amyloid deposits



αB-crystallin

Examples of spots whose intensity apparently changes in some patients

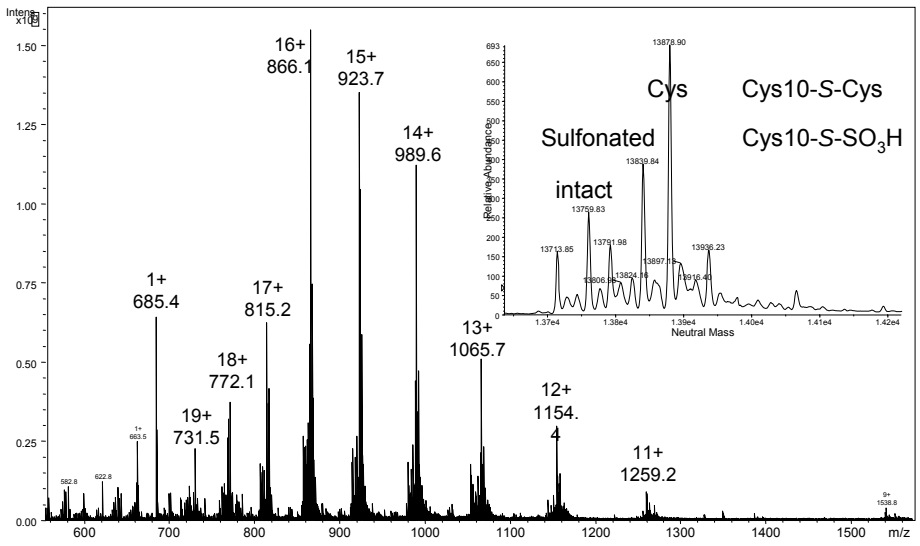
Conclusions on fat biopsy studies

- A proteomic approach (2D-PAGE, MALDI-TOF MS, PMF), allowed identification/characterization of amyloidogenic proteins and their deposited fragments in fat tissue biopsies from patients with different types of systemic amyloidoses.

- Comparison with control fat tissue maps allowed isolation of apparently up- and down-regulated proteins.

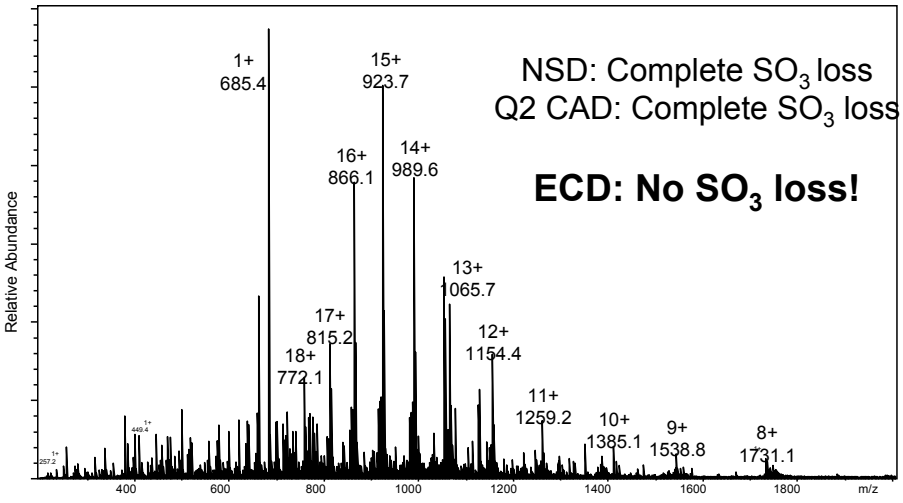
- Proteomics applied to systemic amyloidosis could be a novel diagnostic tool and help cast insights into the mechanism of tissue damage.

ESI-FTMS of human TTR (immunoppt from pooled serum)



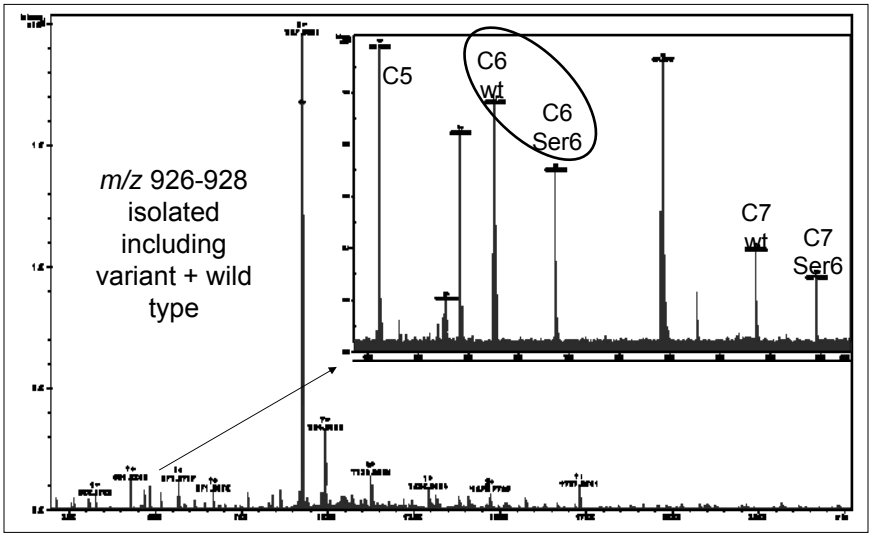
R Théberge *et al*., CBMS, **2009**, unpublished data

Top-down MS/MS of transthyretin from pooled serum



R Théberge *et al*., CBMS, **2009**, unpublished data

ECD of TTR fragment containing variant position Ser6



R Théberge *et al*., CBMS, **2009**, unpublished data

Ongoing BUSM MS studies of ATTR

- Diagnosis of variants in patients referred to BUSM amyloid program
- Investigation of *S*-sulfonation and *S*-homocysteinylation in TTR amyloidogenesis
- Determination of contribution of Val122Ile to heart disease in African Americans (LH Connors, T Prokaeva, A Lim, R Théberge, RH Falk, G Doros, A Berg, CE Costello, C O'Hara, DC Seldin, M Skinner, M., *Am Heart J*, **2009**, *158*, 607-14)
- Investigation of correlation of PTMs of TTR and its amyloidogenesis in SSA
- Development of automated sequencing methods

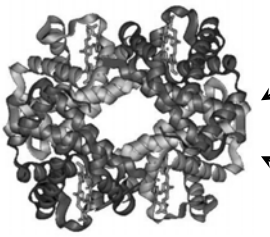
  -Online LC/MS and MS/MS, MALDI-MS/MS-immunoassay, top-down sequencing
- Elucidation of the molecular mechanism of amyloid fibrillogenesis
  - Investigation of noncovalent complexes of native TTR tetramer
  - Determination of relative stability of tetramers of TTR variants
  - Documentation of effects of drug-based stabilization of TTR tetramers
  - Correlation of AFM images of amyloid fibrils with MS protein variant and PTM analysis

Proteomics approaches for identification of hemoglobin variants and post-translational modifications

CBMS: ME McComb, R Théberge, CE Costello (previous staff: H Huang, DH Perlman, BA Budnik, P Kaur, PB O'Connor)

Collaborators:
BUSM Sickle Cell Center: ES Klings, MH Steinberg, DHK Chui

Human hemoglobin tetramer and known structural changes



Stable/unstable variants
>1200 known
responsible for many diseases

↔ Gene-base DNA analysis

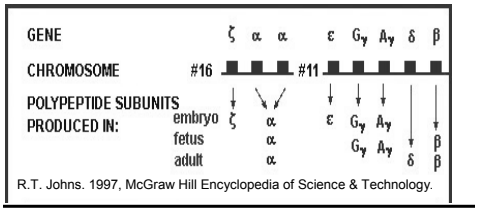
correlate

Stable variants

PTMs
100s known
potential biomarkers

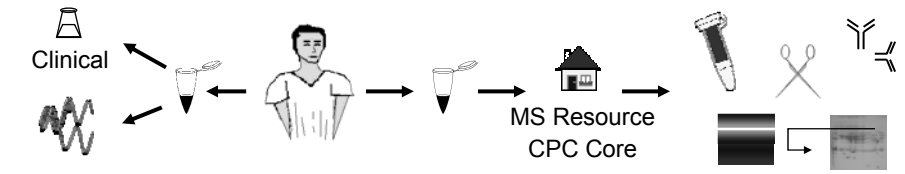
↔ Protein-based mass spectrometry

hemoglobin	name	formula
embryo	Gower I	$\xi_2\varepsilon_2$
	Gower II	$\alpha_2\varepsilon_2$
	Portland I	$\xi_2\gamma_2$
fetus	A	$\alpha_2\beta_2$
	F	$\alpha_2\gamma_2$
adult	A	$\alpha_2\beta_2$
	A_2	$\alpha_2\delta_2$
	A_{Ic}	$\alpha_2\beta_2^{glucose}$



R.T. Johns. 1997, McGraw Hill Encyclopedia of Science & Technology.

BUSM flexible methodology for MS-based proteomics



MS Results

ESI + MALDI MS ↔ Mass of intact protein

ESI + MALDI MS ↔ Peptide mass mapping

ESI + MALDI MS/MS ↔ Sequence/ PTM id.

LC MS/MS ↔ Automated sequencing/ id.

MRM ↔ High throughput

Protein Processing → MS + MS/MS

Different approaches yield increasingly accurate results.
Speed + Sensitivity, direct protein characterization
Post-translational modifications and unambiguous sequence determination
Correlate MS and MS/MS data with other analyses.

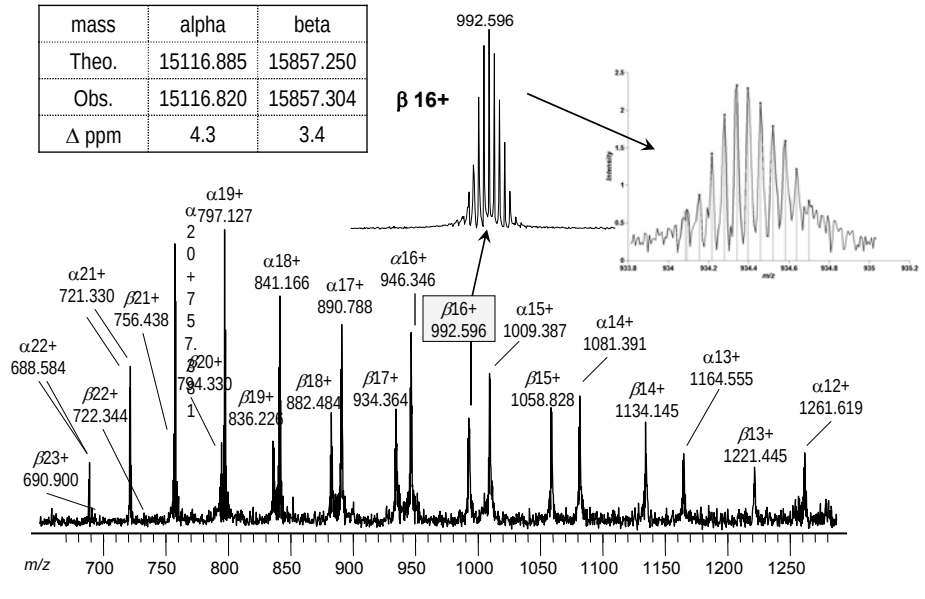
Targeted Database: Human Hemoglobins

```
>sp|P01922|HBA_HUMAN Hemoglobin alpha chain - Homo sapiens.
>sp|P02023|HBB_HUMAN Hemoglobin beta  chain - Homo sapiens.
>sp|P02042|HBD_HUMAN Hemoglobin delta chain - Homo sapiens.
>sp|P02096|HBG_HUMAN Hemoglobin gamma-A/G chains - Homo sapiens.

a    VLSPADKTNV KAAWGKVGAH AGEYGAEALE RMFLSFPTTK TYFPHFDLSH GSAQVKGHGK
b    VHLTPEEKSA VTALWGKVNV DEVGGEALGR LLVVYPWTQR FFESFGDLST PDAVMGNPKV
d    VHLTPEEKTA VNALWGKVNV DAVGGEALGR LLVVYPWTQR FFESFGDLSS PDAVMGNPKV
g    VHLTPEEKSA VTALWGKVNV DEVGGEALGR LLVVYPWTQR FFESFGDLST PDAVMGNPKV

61   KVADALTNAV AHVDDMPNAL SALSDLHAHK LRVDPVNFKL LSHCLLVTLA AHLPAEFTPA
     KAHGKKVLGA FSDGLAHLDN LKGTFATLSE LHCDKLHVDP ENFRLLGNVL VCVLAHHFGK
     KAHGKKVLGA FSDGLAHLDN LKGTFSQLSE LHCDKLHVDP ENFRLLGNVL VCVLARNFGK
     KAHGKKVLGA FSDGLAHLDN LKGTFATLSE LHCDKLHVDP ENFRLLGNVL VCVLAHHFGK

121  VHASLDKFLA SVSTVLTSKYR
     EFTPPVQAAY QKVVAGVANA LAHKYH
     EFTPQMQAAY QKVVAGVANA LAHKYH
     EFTPPVQAAY QKVVAGVANA LAHKYH
```

~ protein redundancy reduces positive matches and limits % coverage
~ single protein data bases force positive matches
~ sequence homology and multiple trypsin cleavage sites present a challenge

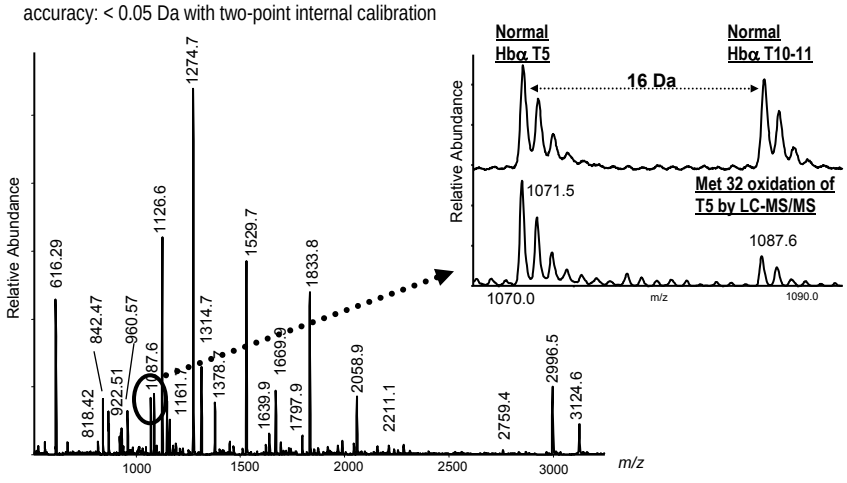
ESI-FT-MS: high accuracy assures unambiguous protein identification

mass	alpha	beta
Theo.	15116.885	15857.250
Obs.	15116.820	15857.304
Δ ppm	4.3	3.4



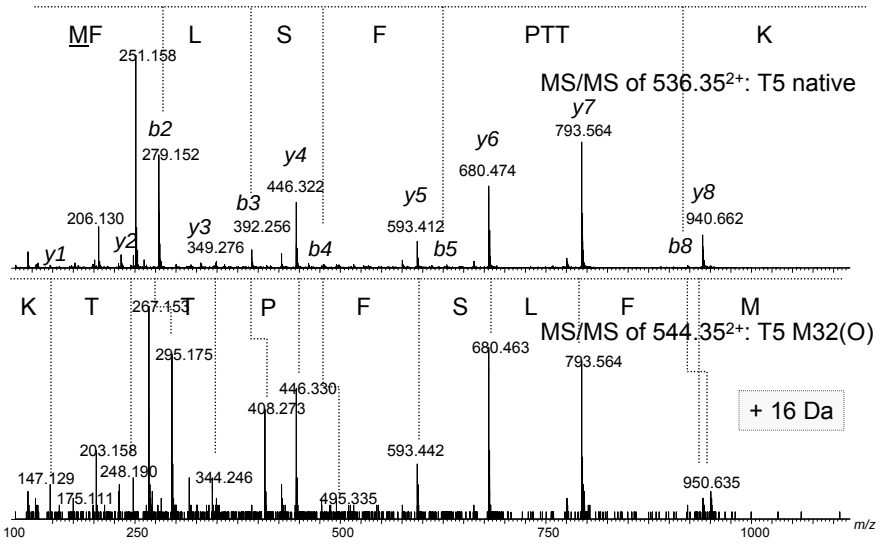
MALDI-TOFMS peptide mapping: fast, easy - tentative identification

~ 90 peaks detected
> 90% sequence coverage
accuracy: < 0.05 Da with two-point internal calibration



MALDI-TOF-MS for the tryptic digest of a blood sample. Matrix 2,5-DHB.

LC-MS/MS identification of Met oxidation on Hbα T5



False variant match of Hbα T5 (MFLSFPTTK) Met substituted by Phe

PLGS 2.2 automatic search

↓

alpha 32-40 (R)**F**FLSFPTTK(T)
substitution M32 to F (+16 Da)

--- ATG (Met) to TTT/TTC (Phe): genetically unlikely
--- Not observed by DNA analysis

↓

Met 32 oxidation

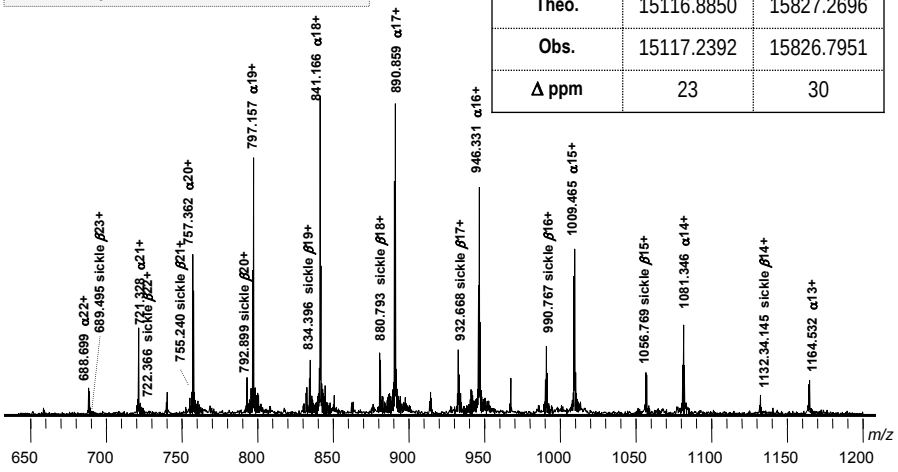
peptide map: tentative identification only ⇒ NO conclusive ID/PTMs; LC-MS/MS ⇒ detailed structure

unambiguous results are data/knowledge/experience dependent

Intact mass measurement of sickle beta chain

DNA sequencing: beta CD 6 GAG → GTG
Protein expressed: Glu → Val: -29.974 Da

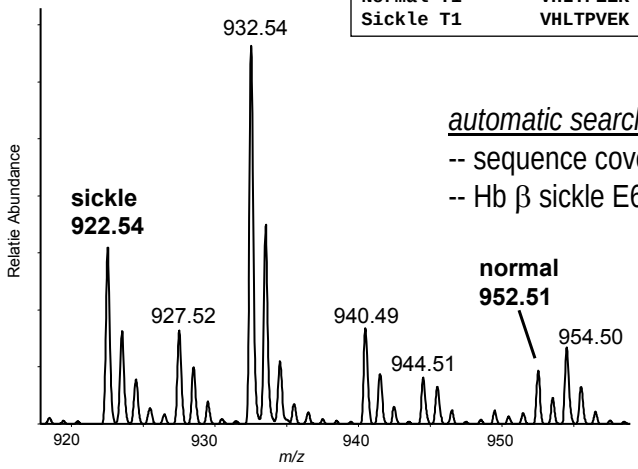
Mono mass	alpha	sickle beta
Theo.	15116.8850	15827.2696
Obs.	15117.2392	15826.7951
Δ ppm	23	30



ESI-FT-MS spectrum of sickle hemoglobin β E6V

Mass fingerprint assignment of sickle Hb β peptide

AA (Hbβ chain)	Sequence	[M+H]	Δmass
Normal T1	VHLTPEEK	952.51	-29.98
Sickle T1	VHLTPVEK	922.55	



automatic search results:

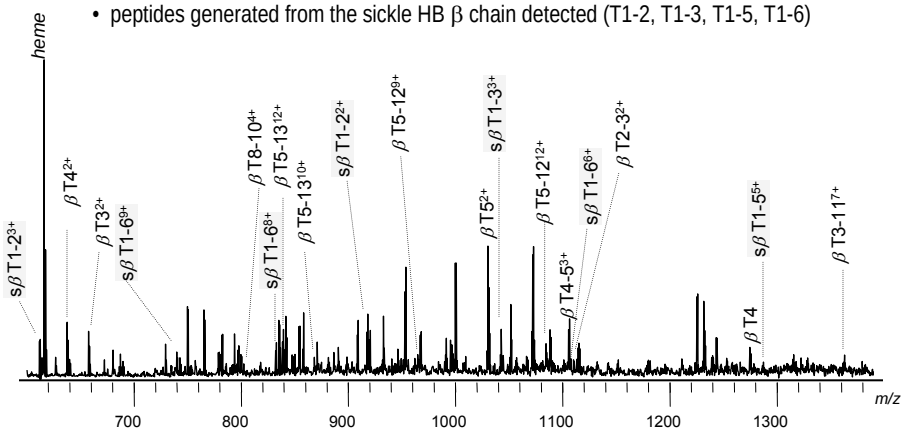
-- sequence coverage: 95%

-- Hb β sickle E6V found

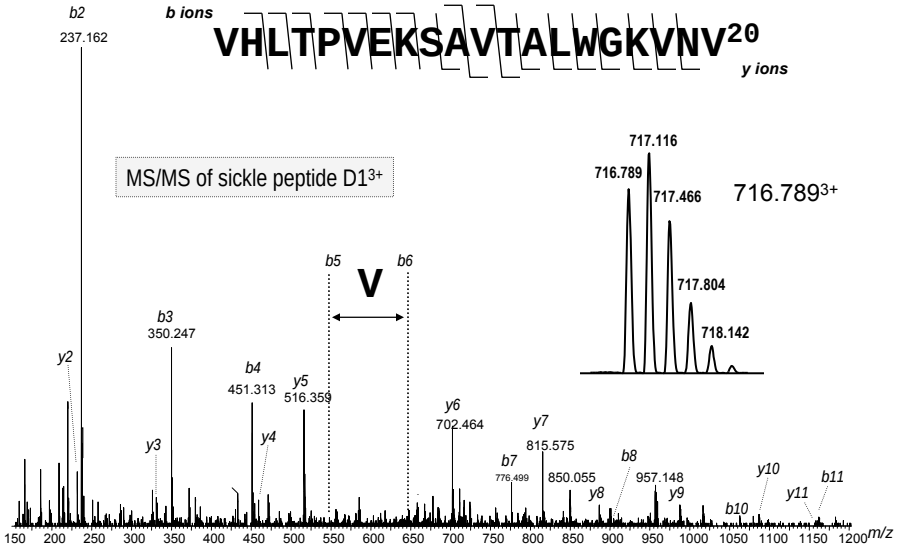
MALDI-TOF MS of sickle blood tryptic digest and search results

ESI-FTMS accurate mass fingerprint match of sickle Hb β tryptic peptides to multiple variants

- 93% coverage of sickle Hb β chain
- peptides generated from the sickle HB β chain detected (T1-2, T1-3, T1-5, T1-6)



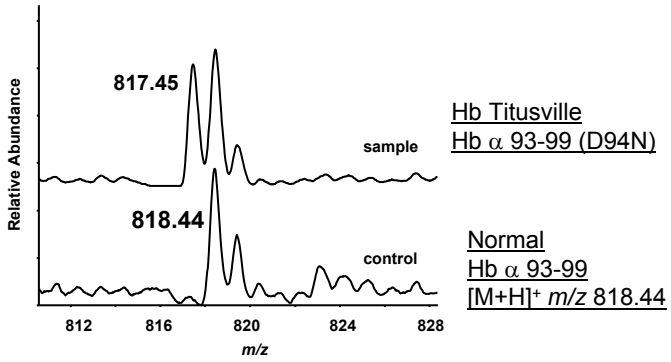
Hb β sickle confirmation by LC-MS/MS sequencing



Identification of sickle Hb β chain E6V

- ✓ **DNA sequencing: Hb β codon 6 GAG → GTG**
  - ☞ ***Hb β Glu 6 → Val***
- ✓ **ESI-FT MS for whole protein:**
  - ☞ ***match of intact mass***
- ✓ **MALDI-TOF MS for tryptic digest:**
  - ☞ ***easy/fast match of sickle peptides***
- ✓ **ESI-FT MS for tryptic digest:**
  - ☞ ***accurate match of sickle peptides***
- ✓ **LC-MS and MS/MS peptide sequencing:**
  - ☞ ***sickle peptide sequencing***

Sample 67 Hb Titusville: MALDI-TOF-MS of variant Hb α D94N in peptide 93-99 V<u>N</u>PVNFK, $[M+H]^+$ *m/z* 817.45



Sample 67 search results report for LC-MS/MS. Hb Titusville (D94N) identified and PTM found

Chain	m/z	z	Expt Mass	mW	Delta (Da)	ppm	Probability	L-Score	Start	End	Sequence	Mods/Subs
beta	476.761	2	951.506	951.503	-0.003	-3.5	75	100	1	8	(-)VHLTPEEK(S)	
beta	466.763	2	931.510	931.513	0.003	2.8	114	95	9	17	(K)SAVTALWGK(V)	
beta	438.890	3	1313.647	1313.658	0.011	8.7	129	85	18	30	(K)VNVDEVGGEALGR(L)	
beta	637.873	2	1273.730	1273.718	-0.012	-9.7	69	88	31	40	(R)LLVVYPWTQR(F)	
beta	1029.974	2	2057.932	2057.940	0.008	3.7	132	86	41	59	(R)FFESFGDLSTPDAVMGNPK(V)	
beta	600.003	3	1796.986	1796.979	-0.007	-3.6	78	100	66	82	(K)KVLGAFSDGLAHLDNLK(G)	
beta	835.451	2	1668.886	1668.884	-0.002	-1.4	101	94	67	82	(K)VLGAFSDGLAHLDNLK(G)	
beta	474.563	3	1420.666	1420.666	0.000	0.3	124	76	83	95	(K)GTFATLSELHCDK(L)	
beta	633.059	4	2528.205	2528.212	0.007	2.9	19	49	83	104	(K)GTFATLSELHCDKLHVDPENFR(L)	
beta	430.746	4	1718.953	1718.965	0.012	7.1	113	69	105	120	(R)LLGNVLVCVLAHHFGK(E)	
beta	689.860	2	1377.704	1377.693	-0.011	-8.2	105	92	121	132	(K)EFTPPVQAAYQK(V)	
beta	483.937	3	1448.788	1448.789	0.001	1.0	2	78	133	146	(K)VVAGVANALAHKYH(-)	
alpha	391.225	3	1170.652	1170.661	0.009	8.1	86	89	1	11	(-)VLSPADKTNVK(A)	
alpha	765.372	2	1528.728	1528.727	-0.001	-0.9	140	100	17	31	(K)VGAHAGEYGAEALER(M)	
alpha	536.284	2	1070.552	1070.547	-0.005	-5.0	26	74	32	40	(R)MFLSFPTTK(T)	
alpha	611.969	3	1832.884	1832.885	0.001	0.8	132	80	41	56	(K)TYFPHFDLSHGSAQVK(G)	
alpha	781.897	4	3123.557	3123.577	0.020	6.5	77	60	61	90	(K)KVADALTNAVAHVDDMPNALSALSDLHAHK(L)	
alpha	749.874	4	2995.465	2995.482	0.017	5.8	178	54	62	90	(K)VADALTNAVAHVDDMPNALSALSDLHAHK(L)	
alpha	**750.122**	**4**	**2996.457**	**2996.466**	**0.009**	**3.1**	**81**	**54**	**62**	**90**	(K)VADALTNAVAHVDDMPNALSALSDLHAHK(L)	**Deamidation N (17)**
alpha	**409.225**	**2**	**816.434**	**816.449**	**0.015**	**17.9**	**111**	**61**	**93**	**99**	**(R)VNPVNFK(L)**	**N for D (2)**
alpha	742.656	4	2966.593	2966.605	0.012	4.1	78	56	100	127	(K)LLSHCLLVTLAAHLPAEFTPAVHASLDK(F)	
alpha	626.868	2	1251.720	1251.707	-0.013	-10.7	87	90	128	139	(K)FLASVSTVLTSK(Y)	
delta	480.273	2	958.530	958.524	-0.006	-6.6	69	100	9	17	(K)TAVNALWGK(V)	

beta 96%

```
1    VHLTPEEKSA  VTALWGKVNV  DEVGGEALGR  LLVVYPWTQR  FFESFGDLST
51   PDAVMGNPKV  KAHGKKVLGA  FSDGLAHLDN  LKGTFATLSE  LHCDKLHVDP
101  ENFRLLGNVL  VCVLAHHFGK  EFTPPVQAAY  QKVVAGVANA  LAHKYH
```

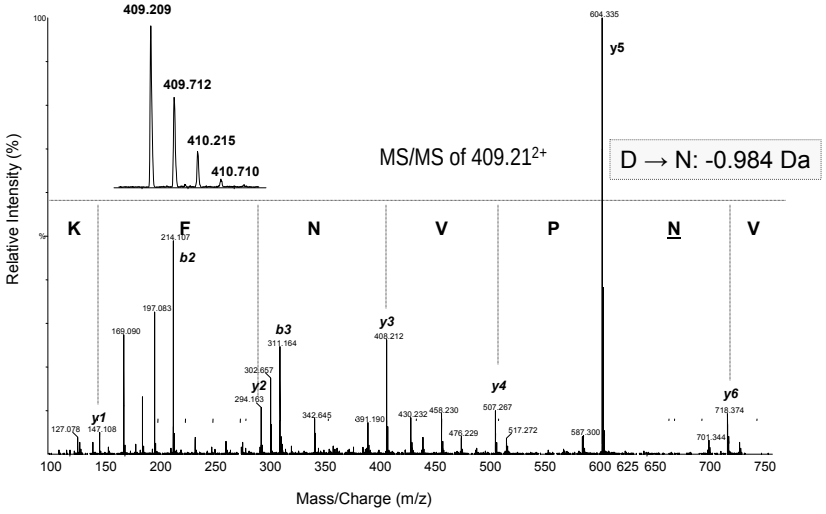
alpha 91%

```
1    VLSPADKTNV  KAAWGKVGAH  AGEYGAEALE  RMFLSFPTTK  TYFPHFDLSH
51   GSAQVKGHGK  KVADALTNAV  AHVDDMPNAL  SALSDLHAHK  LRVDPVNFKL
101  LSHCLLVTLA  AHLPAEFTPA  VHASLDKFLA  SVSTVLTSKY  R
```

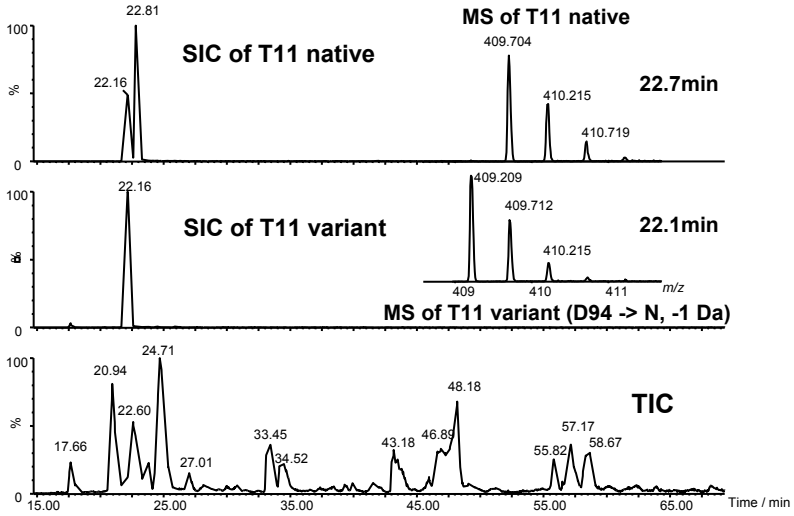
small peptides missed in the trap column

Purple: matched to a peptide; Pink: matched to a partial peptide; green: matched to a modified peptide; yellow: matched to a partial modified peptide

Sample 67: MS and MS/MS of variant alpha D94N at T11
Peptide 93-99 V<u>N</u>PVNFK, native not found in DDA



The MS/MS for native T11 was excluded in DDA due to the <1min difference in elution time. The MS/MS scan was then obtained by adding native T11 to "include list".



Conclusions on amyloid proteins and hemoglobins

- AA sequence variations can easily be detected by MS methods.
- PTMs must be considered for amyloid proteins.
- Novel PTMs may be encountered.
- Multiple MS approaches increase coverage.
- Targeted databases increase assignment efficiency.

Virus capsid maturation

BUSM MS Resource:

- EA Berg, Z Hong, PB O'Connor, CE Costello

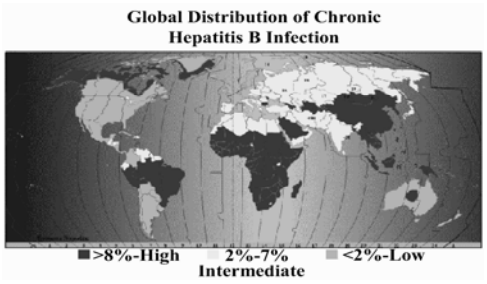
Collaborators:

- BUSM Microbiology: J Hu, DH Perlman

DH Perlman, EA Berg, PB O'Connor, CE Costello, J Hu, *Proc Natl Acad Sci USA*, **2005**, *102*, 9020-9025

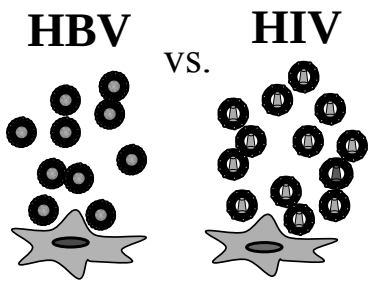
Hepatitus B Virus: a significant health threat

Prevalence



- 350 million chronic HBV
- 1 million deaths/year:
  - hepatocellular carcinoma
  - cirrhosis
- >50 million new infections/year

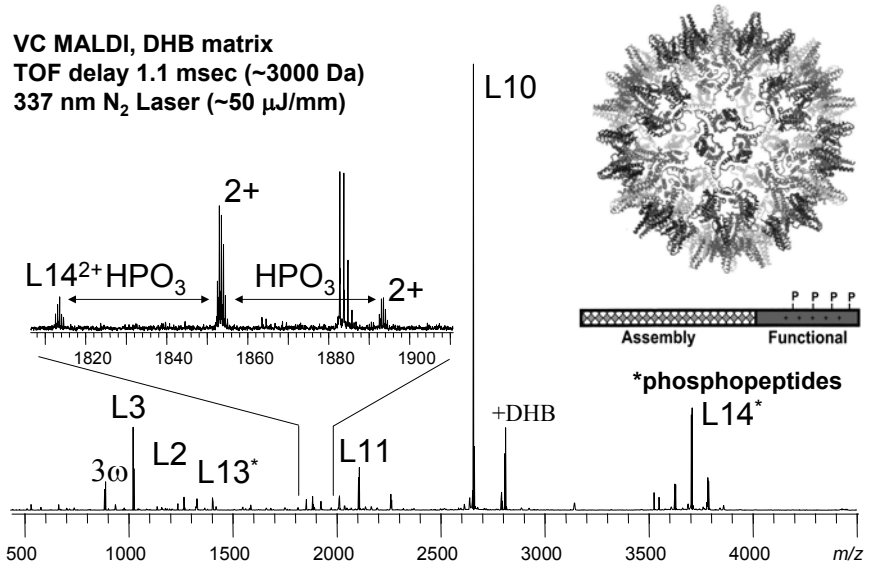
Infectiousness



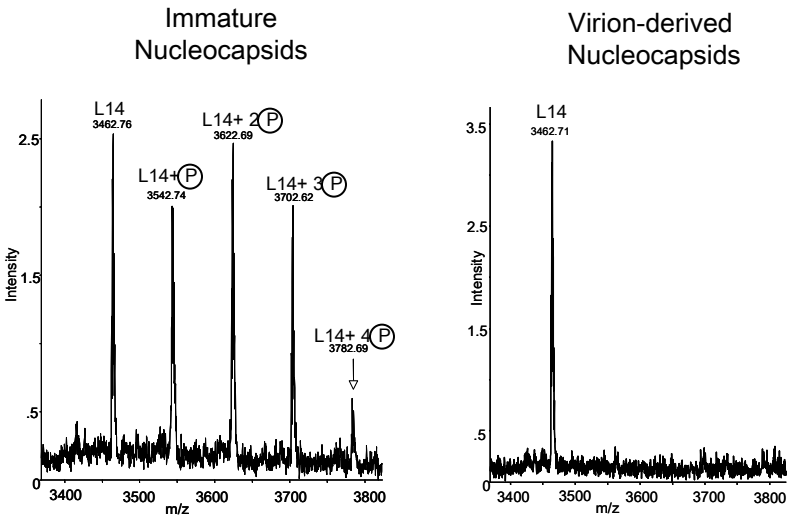
Almost every secreted particle infectious

Only ~ 1 in 10^5 secreted particles infectious

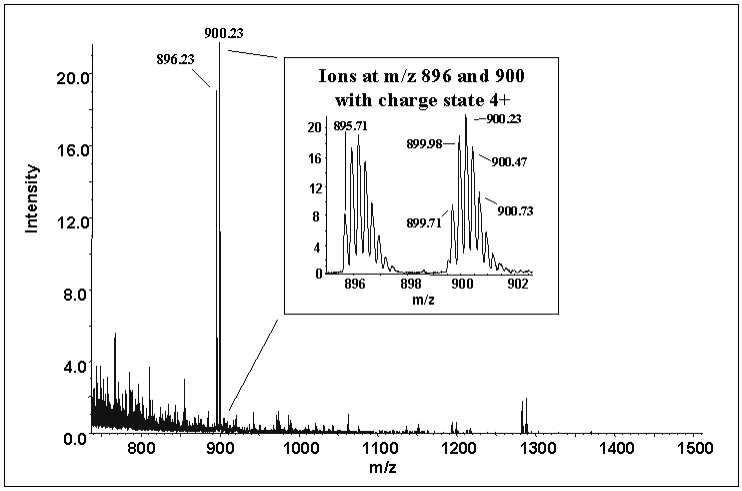
Lys-C digest of Hepatitis viral capsid protein



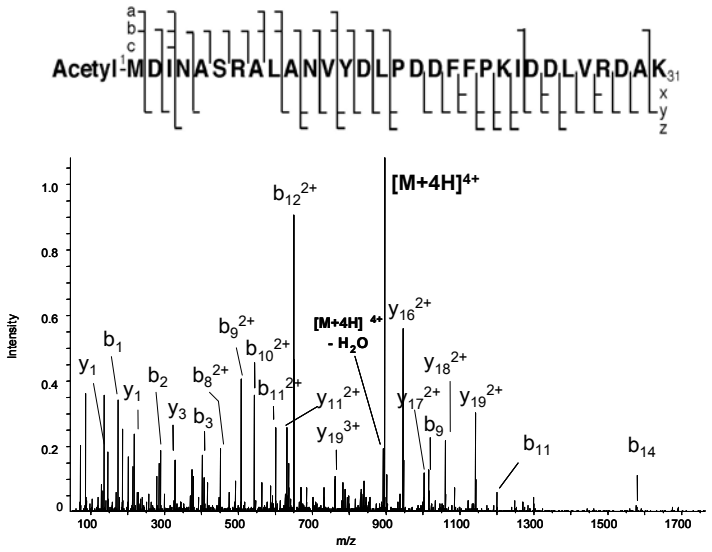
Phosphorylation state of C-terminal L14 peptide: Dramatic changes during nucleocapsid maturation



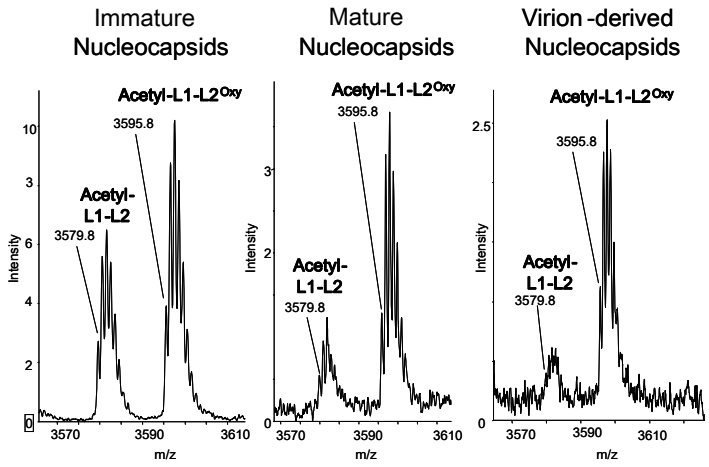
N-terminal capsid peptide dominates electrospray mass spectrum of Lys-C digested RNA capsids



Capsid N-terminal acetylation evident by tandem-MS sequencing of *m/z* 896 4^+ ion



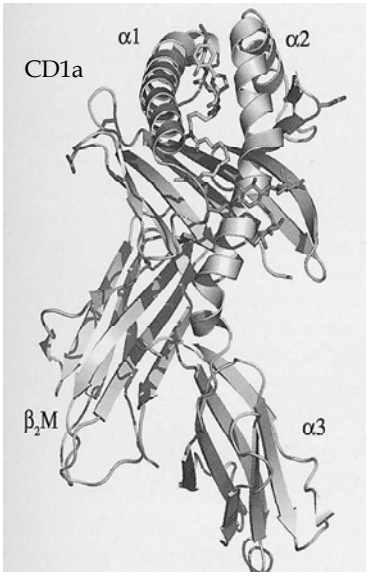
N-terminal acetylation remains unchanged throughout nucleocapsid maturation

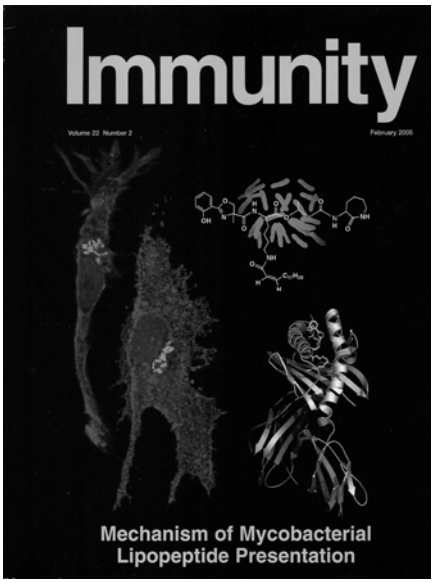


Conclusions on DHB Virus maturation

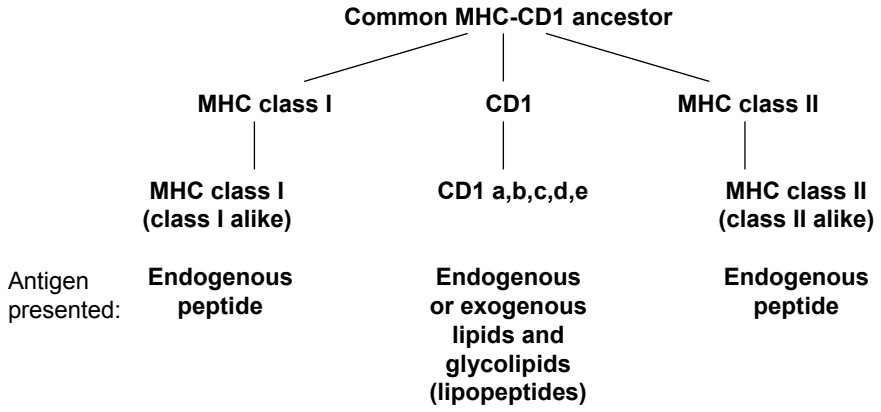
- All known sites of capsid C-terminal phosphorylation are dephosphorylated during nucleocapsid maturation.
- Additional novel sites of phosphorylation are also dephosphorylated during nucleocapsid maturation.
- Capsid N-terminus is acetylated; this modification does not change during nucleocapsid maturation.
- AFM detects remarkable changes in capsid size during maturation

Lipids may lead us to a new understanding of immunity.

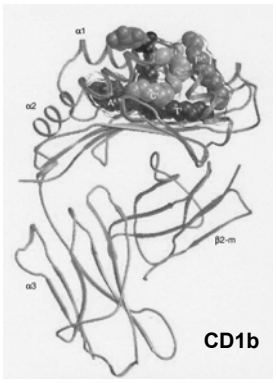


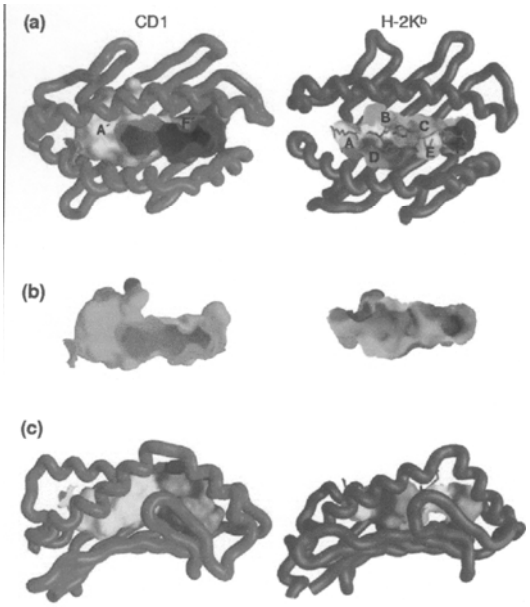


CD1 presentation of antigenic lipids: Proposed evolution of antigen presentation molecules



M Brenner and S Porcelli, *Science*, **1997,** *277*, 332

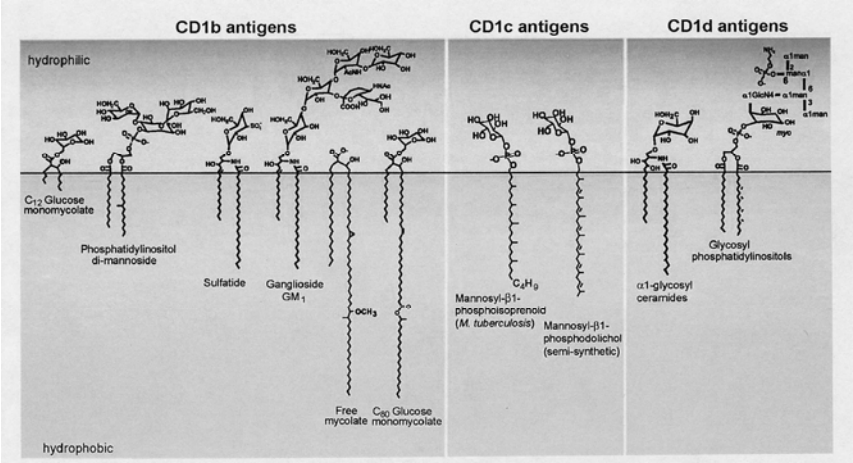




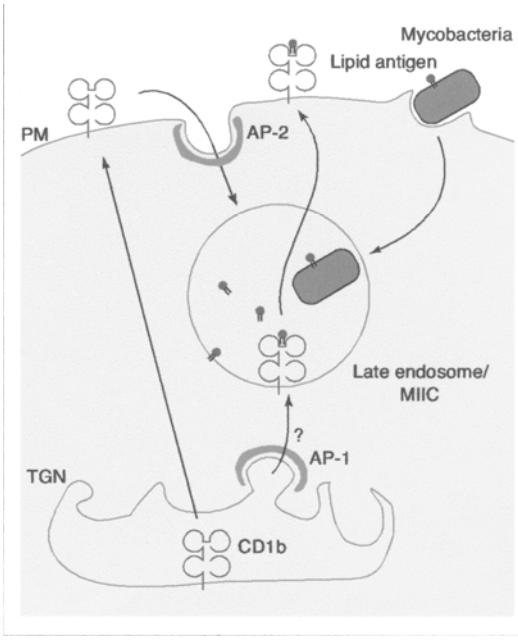
Comparison of the ligand-binding grooves of CD1 and MHC Class I molecules

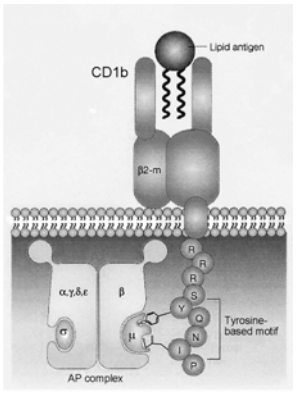
S Porcelli *et al*, *Immunol Today*, ***1998***, *19,* 362

Antigens presented by CD1b, c and d:



DB Moody, *Nat Rev Immunol*, **2003**, *3*, 11-22

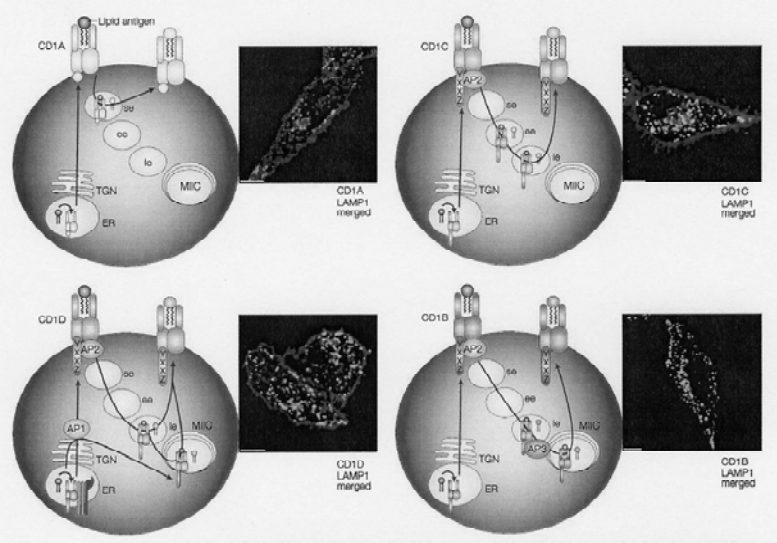




Proposed intracellular trafficking and complexation of CD1b

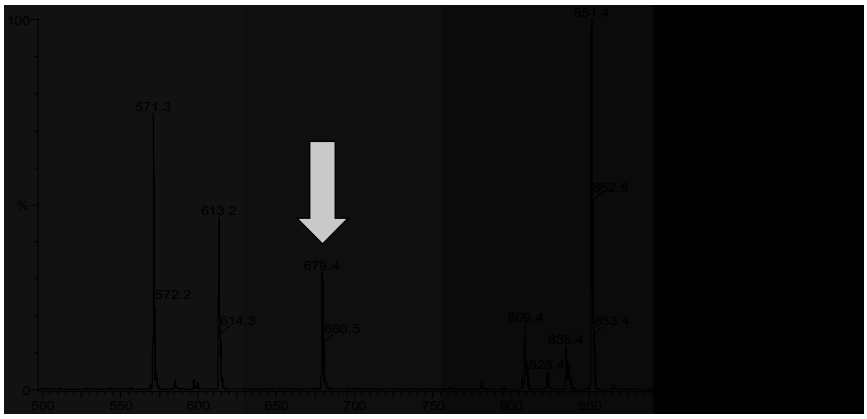
Porcelli *et al, Immunol Today,* **1998,** *19*, 362; Moody & Porcelli, *Nat. Rev. Immunol.* **2003,** *3*, 11

Proposed intracellular trafficking and complexation of CD1s



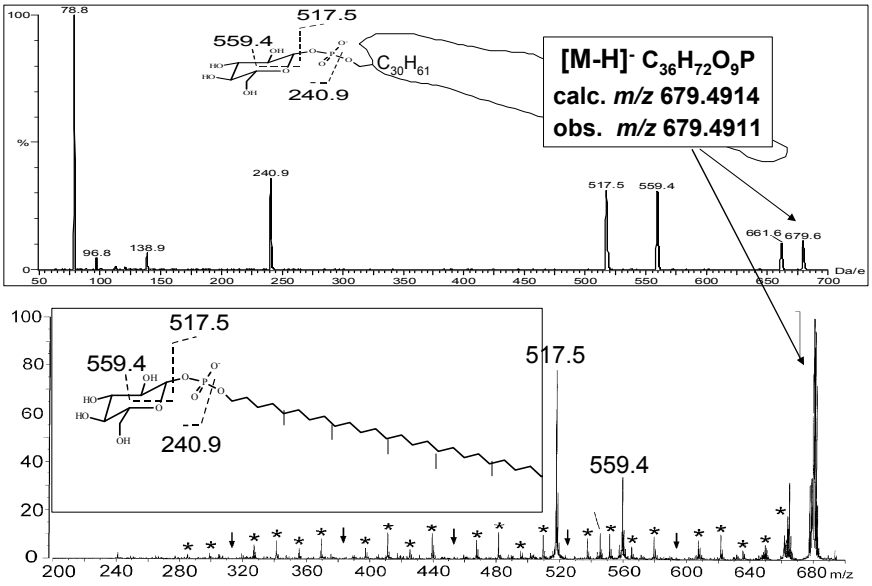
DB Moody and SA Porcelli, *Nat Rev Immunol,* **2003,** *3*, 11-22

(-) ESI MS of M. avium active fraction from 2D TLC of antigen presented by CD1c



DB Moody *et al*, *Nature*, **2000**, *404*, 884-888

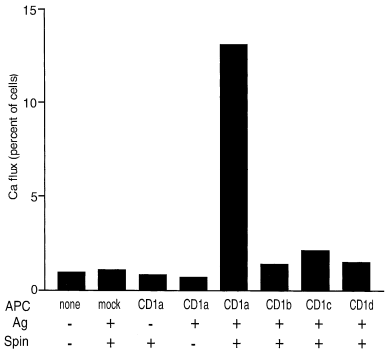
ESI CID MS/MS *m/z* 679 of *M. avium* active fraction



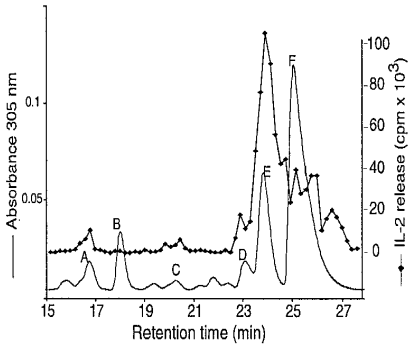
DB Moody *et al*, *Nature*, **2000**

Biosynthesis: Matsunaga, I et al, *J Exp Med* **2004**, *200*, 1559-1569

CD1a presentation of novel *M. tuberculosis* antigens



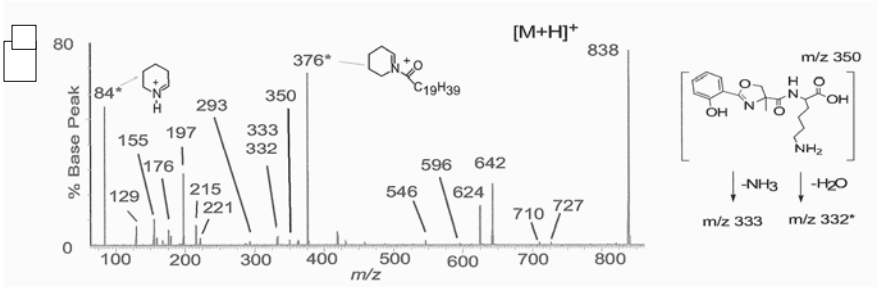
Ca flux of J.RT3.CD8.-2 reporter cells exposed to C1R B lymphoblastoid cells transfected with CD1 constructs and incubated with antigen-containing *M. tuberculosis* lipid fractions

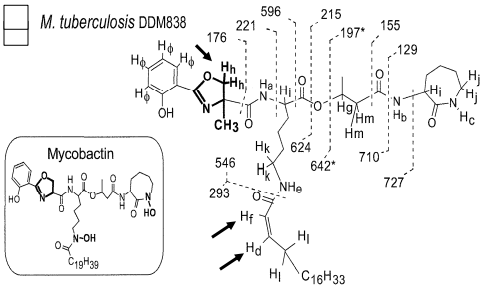


HPLC separation of CD1a-presented antigen active fractions

DB Moody *et al, Science*, **2004**, *303*, 527-531

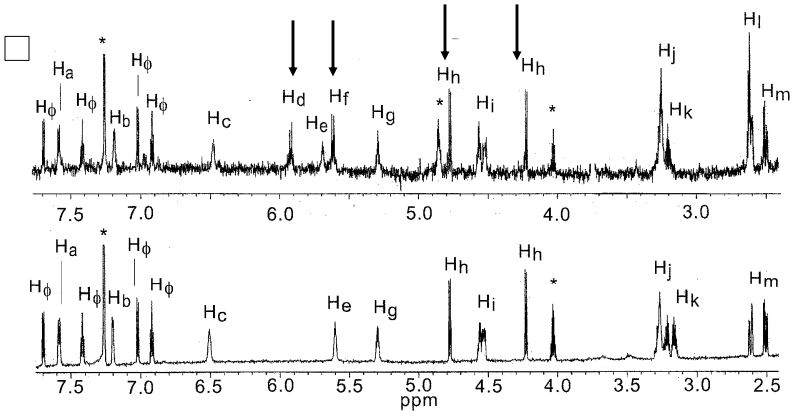
MS analysis of antigen presented by CD1a:





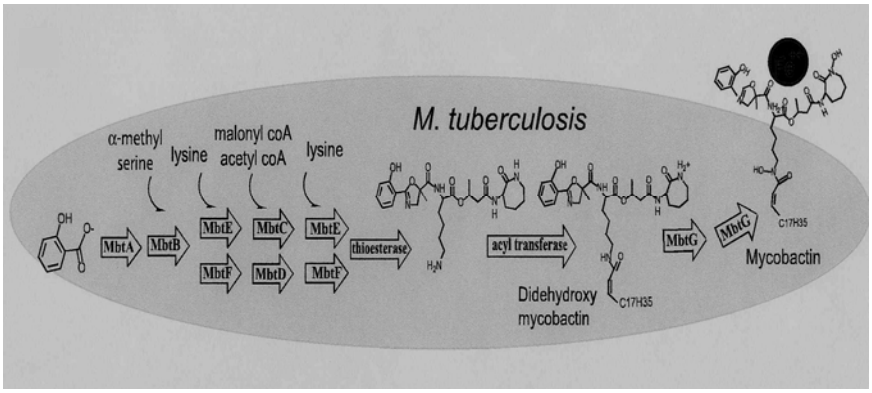
m/z obs	*m/z* calc.	El. Comp.
84.0813	84.0808	$C_5H_{10}N$
197.1286	197.1285	$C_{10}H_{17}N_2O_2$
332.1613	332.1605	$C_{17}H_{22}N_3O_4$
376.3571	376.3574	$C_{25}H_{46}NO$
642.4488	642.4477	$C_{37}H_{60}N_3O_6$
838.5684	838.5688	$C_{47}H_{76}N_5O_8$

NMR spectra of novel antigens presented by CD1a:



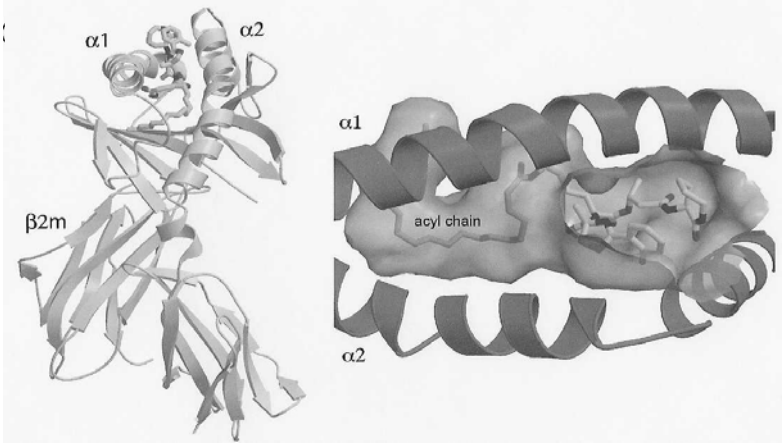
DB Moody *et al, Science*, **2004**

Biosynthetic pathway of mycobactin?



DB Moody *et al, Science*, **2004**

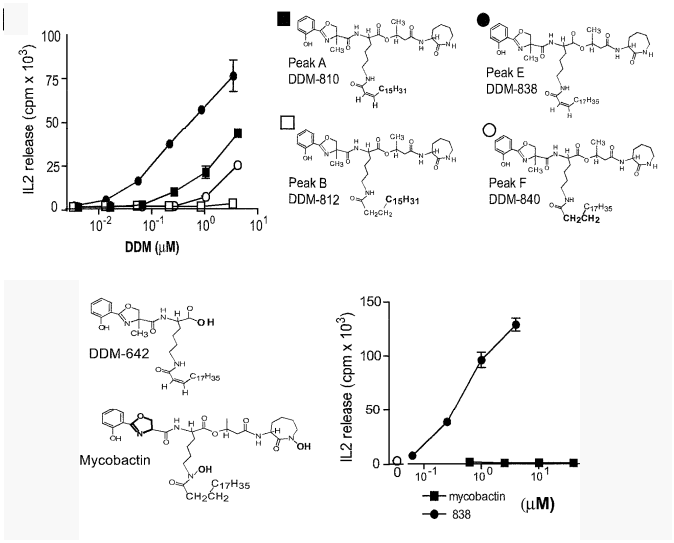
Presentation of dideoxy mycobactin by CD1a:



DB Moody *et al, Science*, **2004**

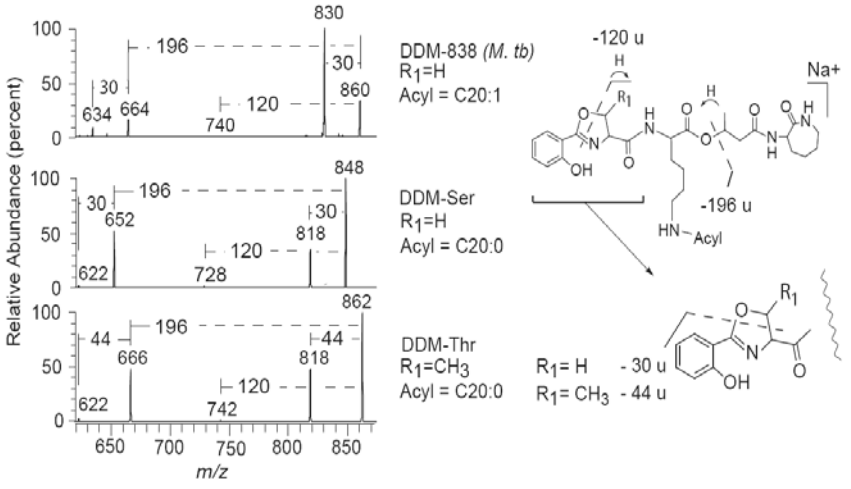
X-ray analysis: Zajonc, DM et al, *Immunity* **2005** *22*, 209-219.

Activities of fractions of DDM, the first known CD1a antigen:



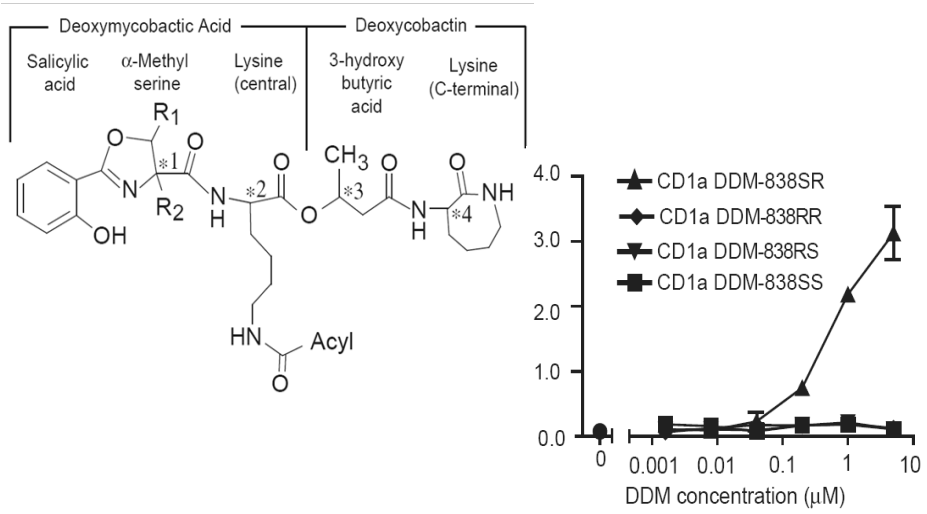
DB Moody *et al, Science*, **2004**

LC/ESI-CID MS/MS of dideoxymycobactin analogs $[M + Na]^+$



DC Young *et al, J Biol Chem,* **2009**, *284*, 25087-25096

Peptide stereochemical specificity of DDM activity

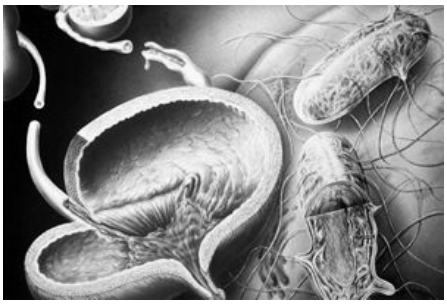


DC Young *et al, J Biol Chem,* **2009**, *284*, 25087-25096

Summary of CD1 presentation of lipoconjugates

- CD1 presentation may be as important as MHC presentation.
- CD1s can present multiple types of lipid conjugates.
- Lipid specificity for presentation depends on fit into grooves.
- Antigenic activity also depends on the fine structure of epitopes.

Urinary infections –



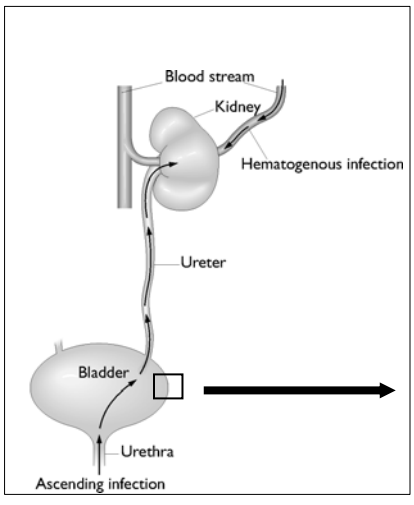
- Single most important cause of urinary disease
- Millions of cases/year ⇒⇒⇒ health, economic impacts
- Major organism: type 1-fimbriated *Escherichia coli*

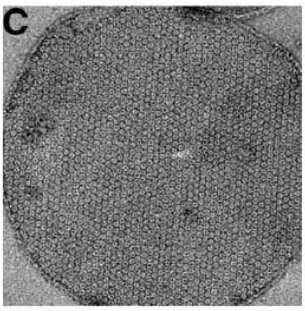
MS Resource: B Xie and CE Costello
Collaborators: TT Sun *et al*, NY Univ School of Medicine

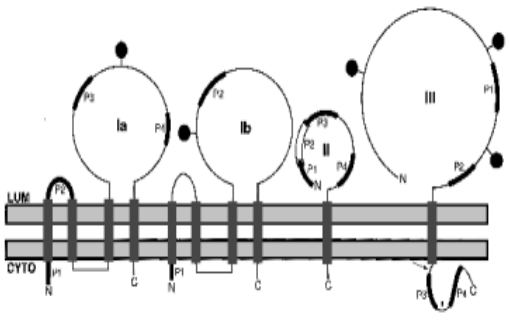
B Xie, G Zhou, S-Y Chan, E Shapiro, XP Kong, X-R Wu, T-T Sun, CE Costello, *J Biol Chem*, **2006**, *281*, 14644-14653

What binds the *Escherichia coli ?*

Uroplakins







UPIa and UPIb predicted from cDNA sequences

Murine

UP Ia: (29 KDa)
MASAATEGEKGSPVVVGLLVVGNIIILLSGLALFA
ETVWVTADQYRVYPLMGVSGKDDVFAGAWIAIF
CGFSFFVVASFGVGAALCRRRYMILTYLLLMLIV
YIFECASCITSYTHRDYMVSNPSLITKQMLTYYSA
DTDQGQELTRLWDRIMIEQECCGTSGPMDWVN
YTSAFRAATPEVVFPWPPLCCRRTGNFIPINEDG
CRVGHMDYLFTKGCFEHIGHAIDSYTWGISWFG
FAILMWTLPVMLIAMYFYTTL -257

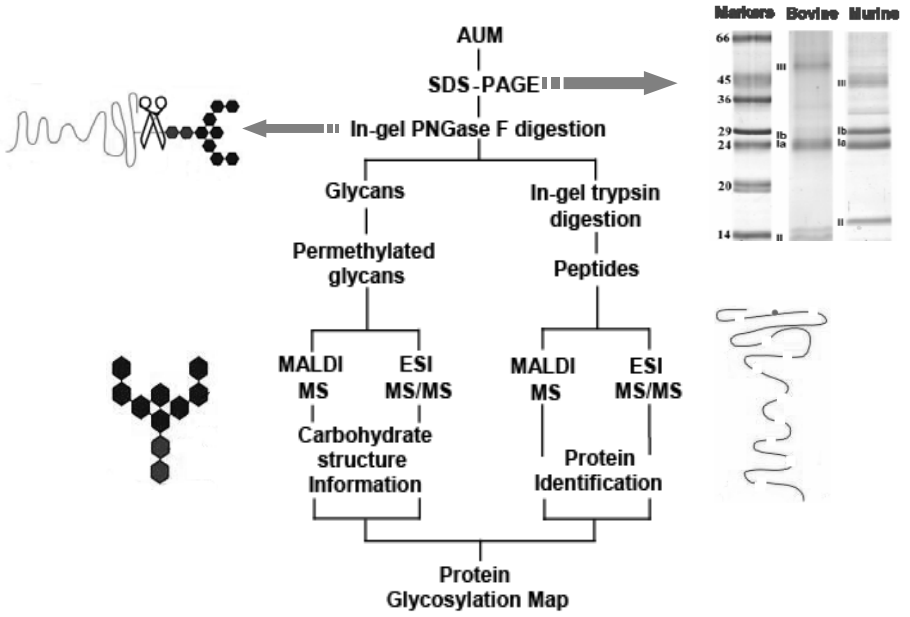
UP Ib: (30 KDa)
MAKDDSTVRCFQGLLIFGHVIVGMCGIALTAECIF
FVSDQHSLYPLLEATNNDDIFGAAWIGMFVGICL
FCLSVLAIVGIMKSNRKILLAYFIMMFIVYGFEVAS
CITAATQRDFFTTNLFLKQMLMRYQNNSPPTND
DEWKNNGVTKTWDRLMLQDHCCGVNGPSDWQ
KYTSAFRVENNDADYPWPRQCCVMDKLKEPLN
LDACKLGVPGYYHSQGCYELISGPMDRHAWGV
AWFGFAILCWTFWVLLGTMFYWSRIEY -260

Bovine

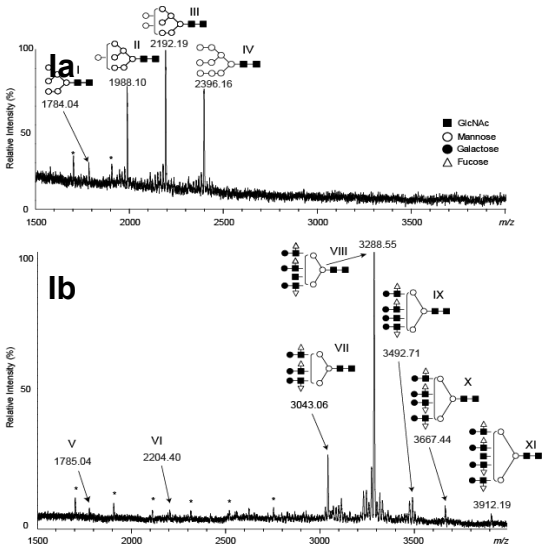
UP Ia: (29 KDa)
MASAAAATTEKGSPVVVGLLVMGNIIILLSGLALFA
ETVWVTADQYRIYPLMGVSGKDDVFAGAWIAIFCG
FSFFVVASFGVGAALCRRRSMILTYLILMLIIYIFECA
SCITSYTHRDYMVSNPSLITKQMLTFYSADSNQGR
ELTRLWDRIMIEQECCGTSGPMDWVNFTSAFRATT
PEVVFPWPPLCCRRTGNFIPVNEEGCRLGHLDYLF
TKGCFEHIGHAIDSYTWGISWFGFAILMWTLPVMLI
AMYFYTTL -258

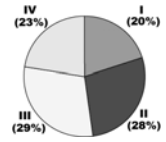
UP Ib: (30 KDa)
MAKDDSTVRCFQGLLIFGNVIIGMCSIALMAECIFFV
SDQNSLYPLLEATNNDDIYAAAWIGMSVGICLFCLS
VLGIVGIMKSNRKILLVYFILMFIVYAFEVASCITAAT
QRDFFTPNLFLKQMLERYQNNSPPNNDDQWKNN
GVTKTWDRLMLQDNCCGVNGPSDWQKYTSAFRT
ENSDADYPWPRQCCVMNSLKEPLNLDACKLGVP
GYYHSHGCYELISGPMNRHAWGVAWFGFAILCWT
FWVLLGTMFYWSRIDY –260

Methods design

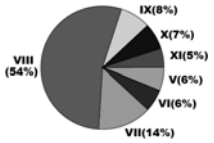


MALDI-TOF MS glycan profiles from murine UP Ia,Ib



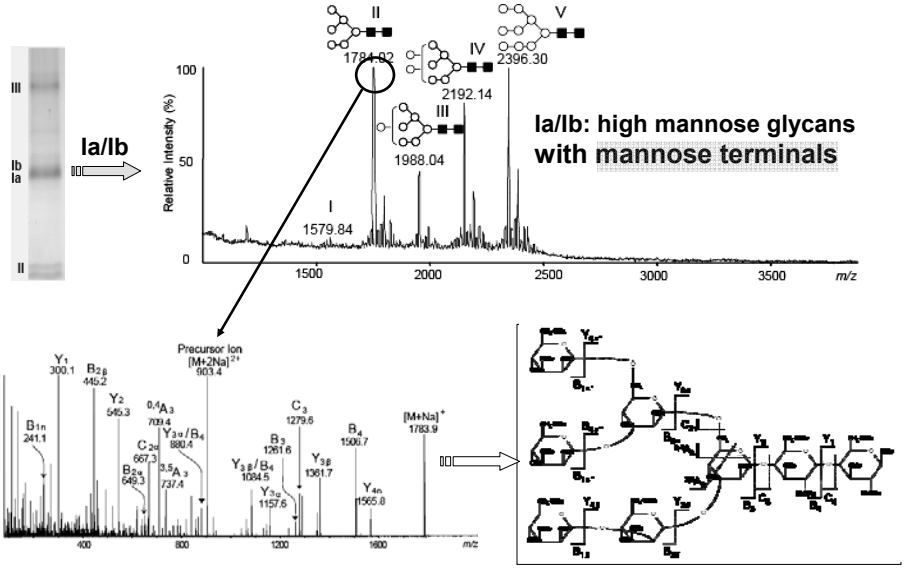


Ia: High mannose glycans with mannose terminals



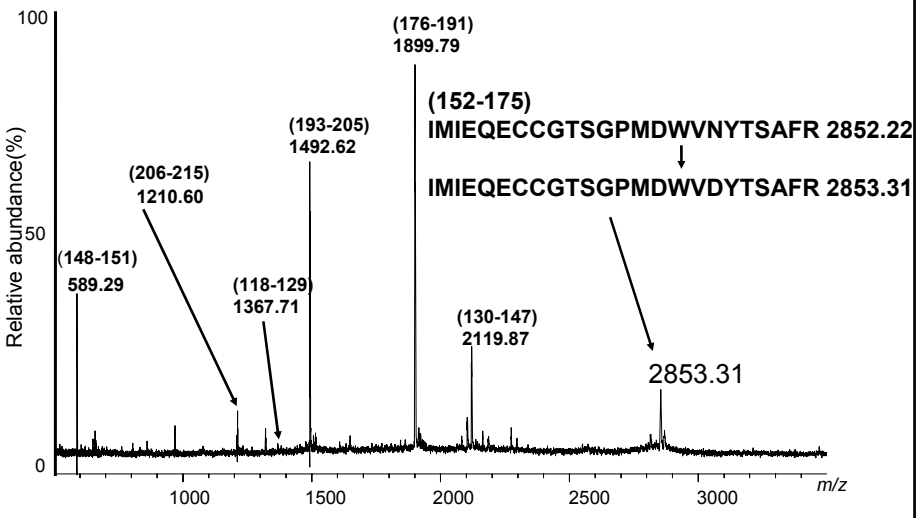
Ib: Complex glycans with galactose/fucose terminals

MALDI-MS/MS of bovine UP Ia/Ib glycans

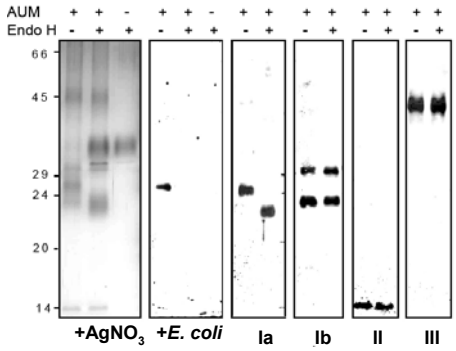


Ia/Ib: high mannose glycans with mannose terminals

MALDI-TOF MS spectrum of mouse UP Ia peptides after in-gel PNGase F digestion, in-gel trypsin digestion



Results on human UPIa & Ib



- **UP Ia is the receptor for bacteria**
  The binding is glycan-dependent
- **What type of glycans (Specificity of enzymes)**
  UPIa: 100% Endo-H sensitive glycans
  UPIb: 100% PNGase F sensitive glycans

Conclusions and clinical implications

- **Glycan profiles and structures directly related with Urinary Tract Infections from UP Ia and UPIb from bovine, mice and human were determined.**
- **Protein sequences were identified, glycosylation sites located.**
- **Results shed light on the molecular basis of urinary tract infections in different species.**
- **Information should aid in the design of glyco-mimetic inhibitors for preventing and treating this disease.**

Xie, Zhou, Chan, Shapiro, Kong, Wu, Sun, Costello, *J Biol Chem*, **2006**, *281*, 14644-14653

Summary of MS Course

- General features of mass spectra
- Major ionization techniques, mass analyzers
- Sample selection and preparation
- Characteristics of MALDI, ESI mass spectra and tandem mass spectra
  - Proteins, peptides, carbohydrates, glycoconjugates, lipids, nucleic acids
- Data processing and database searching