Structural Proteomics

Hydrogen/deuterium exchange

Petr Man

Hydrogen/deuterium exchange…

…is good for what?

Conformational characterization

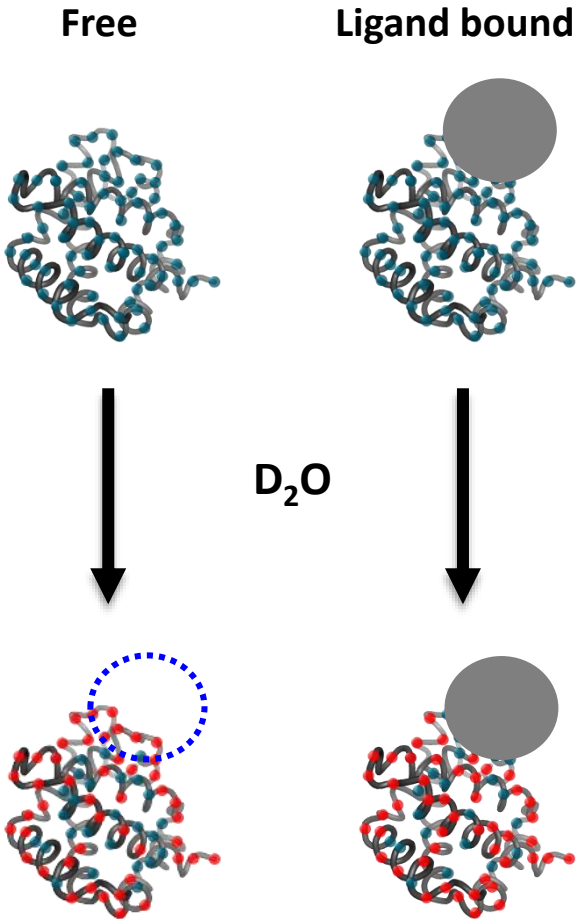
protein fold (structured regions)
comparability of proteins (biosimilars)
stability
influence of pH, ionic strength, temperature, mutation

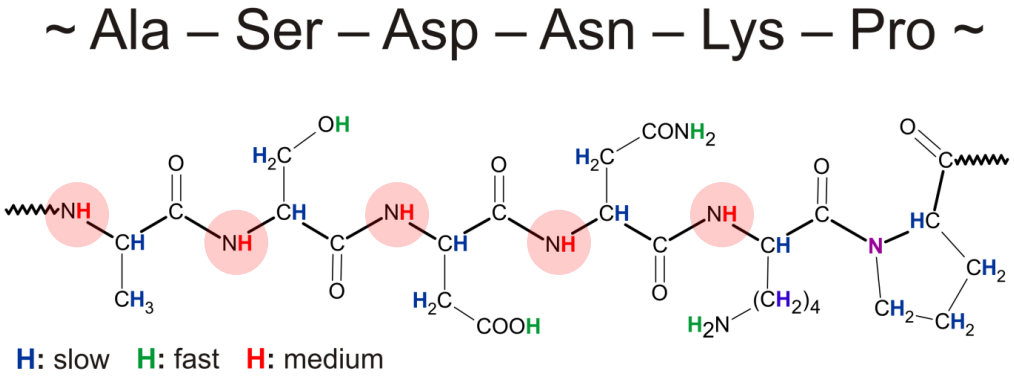
Protein-ligand interactions

lipids / membranes
nucleic acids
small molecules, cofactors, ions
proteins (epitope mapping)

Hydrogen/deuterium exchange

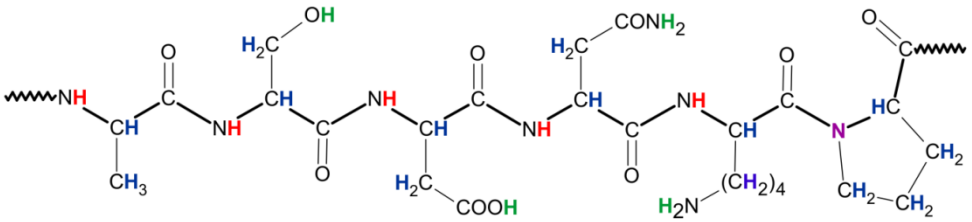
How it works?





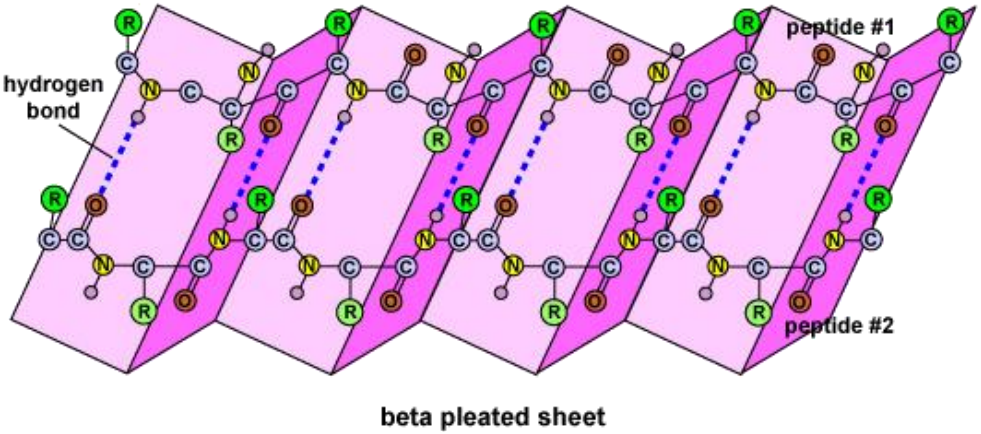
Factors affecting H/D exchange

~ Ala – Ser – Asp – Asn – Lys – Pro ~

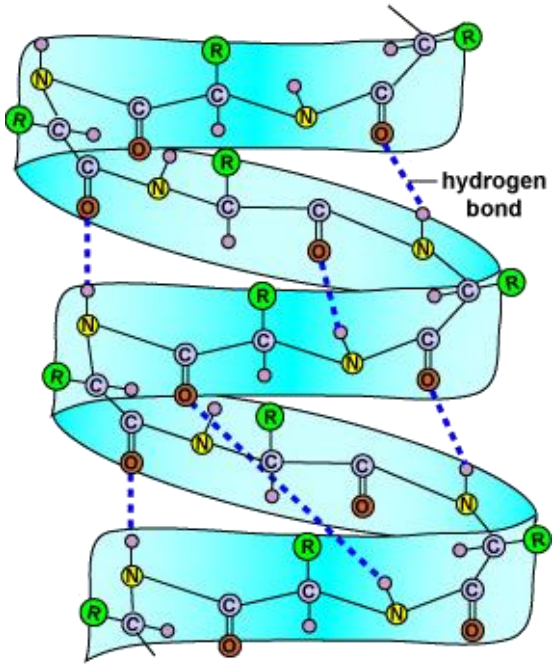


H: slow **H**: fast **H**: medium

Changes in **hydrogen bonding** and **solvent accessibility** accessed through **H/D exchange of protein backbone amide hydrogens.**

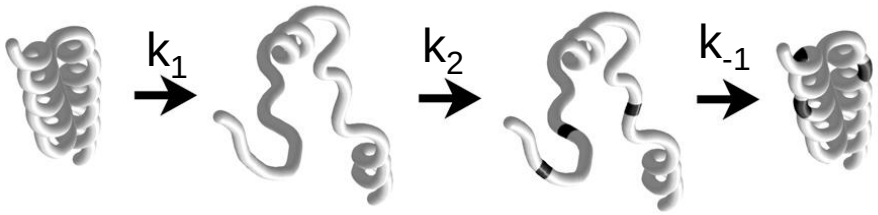


beta pleated sheet



H/D exchange mechanism / kinetics

$$F_H \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} U_H \xrightarrow[D_2O]{k_2} U_D \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} F_D$$



EX2

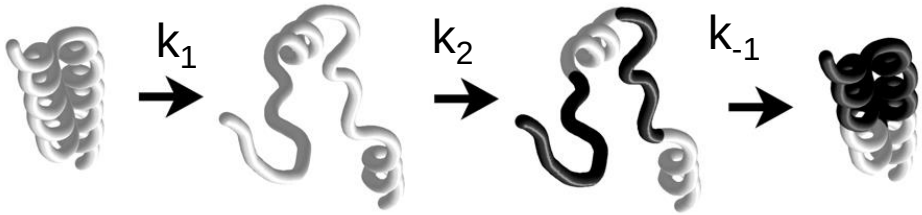
$k_{-1} >> k_2$

uncorrelated

EX1

$k_{-1} << k_2$

correlated



Factors affecting H/D exchange

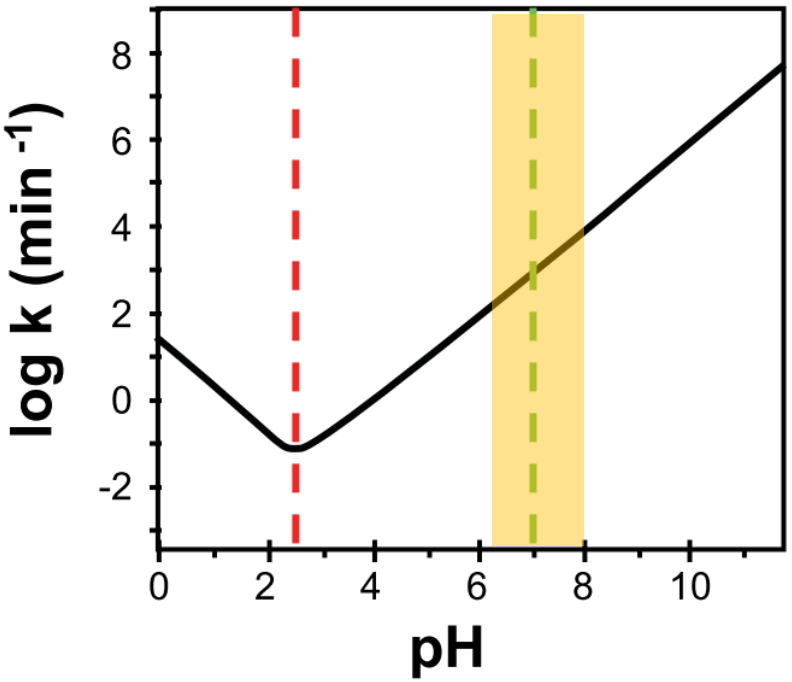
Temperature

$$k(T_2) = k(T_1)exp^{-\frac{E_a}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right)}$$

1 ΔpH ... 10×
10°C ... 3×

pH

$$pD = pH_{read} + 0.4$$



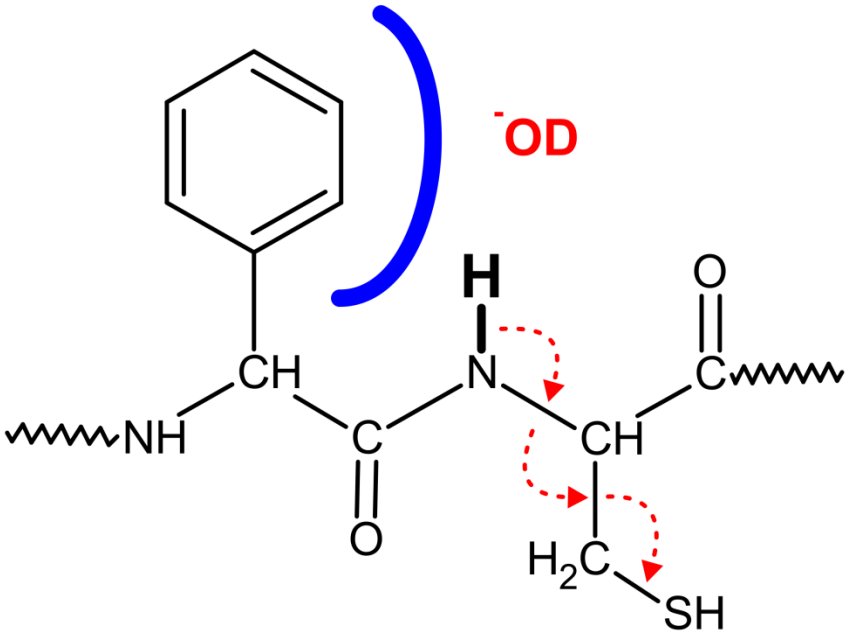
Bai *et al.*: Proteins (1993)

Glasoe, Long: J. Phys. Chem. (1960)

Factors affecting H/D exchange

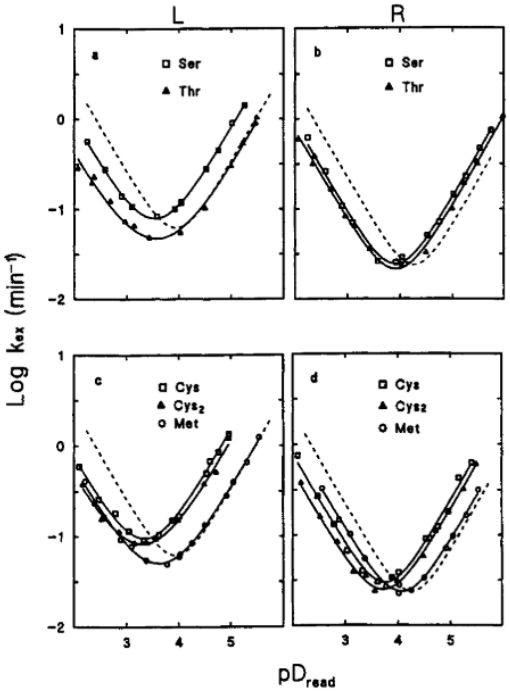
Side chains

(acidity, steric shielding)

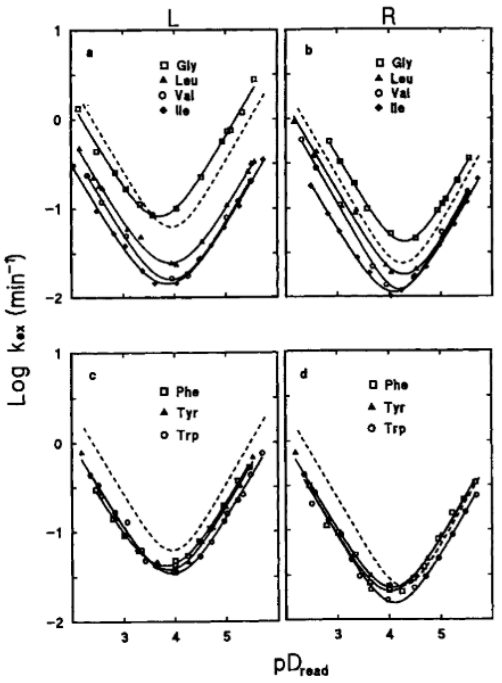


Bai *et al.*: Proteins (1993)

Factors affecting H/D exchange – side chain effects



Inductive effect – electron density is withdrawn from peptide bond (S, O). Increasing base catalyzed and decreasing acid catalyzed rates



Downward shift due to steric hindrance effect of aliphatic and aromartic side chains. Aromatics also shows inductive effect

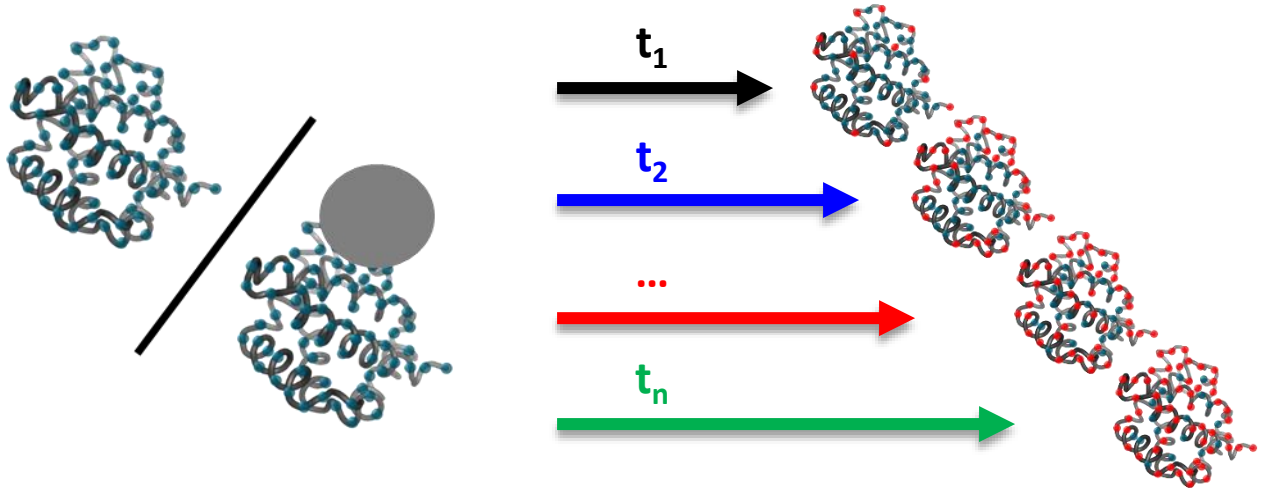
TABLE II. Effects of Amino Acid Side Chains on the HX Rates of Neighboring Peptides*

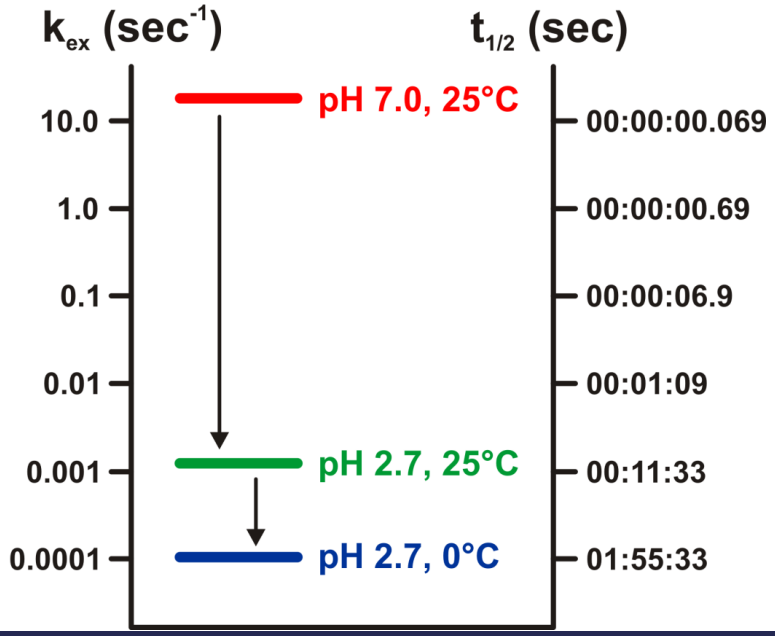
	$Logk_{ex}(X) - Logk_{ex}(Ala)$			
	Acid catalysis		Base catalysis	
Side chain (X)	L	R	L	R
Ala	0.00	0.00	0.00	0.00
Arg	−0.59	−0.32	0.08	0.22
Asn	−0.58	−0.13	0.49	0.32
Asp(COO^-)	(0.9)	0.58	−0.30	−0.18
Asp(COOH)	(−0.9)	−0.12	0.69	(0.6)
Cys	−0.54	−0.46	0.62	0.55
Cys_2	−0.74	−0.58	0.55	0.46
Gly	−0.22	0.22	0.27	0.17
Gln	−0.47	−0.27	0.06	0.20
Glu(COO^-)	(−0.9)	0.31	−0.51	−0.15
Glu(COOH)	(−0.6)	−0.27	0.24	0.39
His			−0.10	0.14
His^+	(−0.8)	−0.51	(0.8)	0.83
Ile	−0.91	−0.59	−0.73	−0.23
Leu	−0.57	−0.13	−0.58	−0.21
Lys	−0.56	−0.29	−0.04	0.12
Met	−0.64	−0.28	−0.01	0.11
Phe	−0.52	−0.43	−0.24	0.06
Pro(*trans*)		−0.19		−0.24
Pro(*cis*)		−0.85		0.60
Ser	−0.44	−0.39	0.37	0.30
Thr	−0.79	−0.47	−0.07	0.20
Trp	−0.40	−0.44	−0.41	−0.11
Tyr	−0.41	−0.37	−0.27	0.05
Val	−0.74	−0.30	−0.70	−0.14
N-term (NH_3^+)		−1.32		1.62
C-term (COO^-)	0.96		(−1.8)	
C-term (COOH)	(0.05)			

*L and R refer to peptide groups to the left and right, respectively, of the side chain indicated (replacing the λ and ρ terminology of Molday et al.[6]). Values are listed in logarithmic form for use with Eq. (2). Values in parentheses are less well determined.

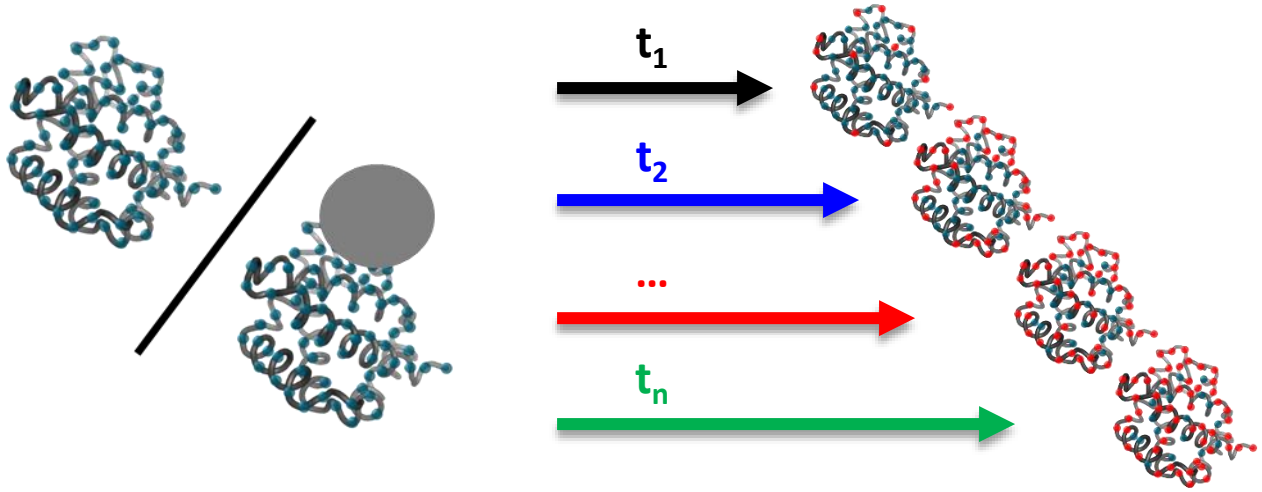
Bai *et al.*: Proteins (1993)

H/D exchange workflow





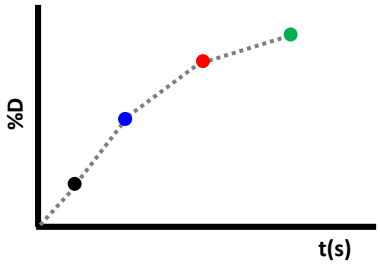
H/D exchange workflow



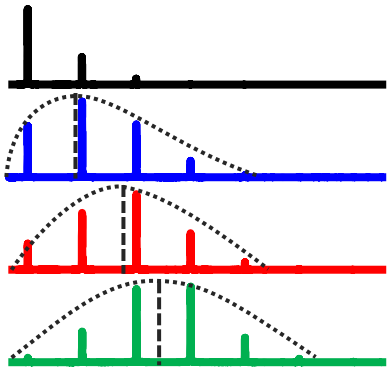
Stop the exchange
pH (2,5)
T (0°C)

MS analysis
?

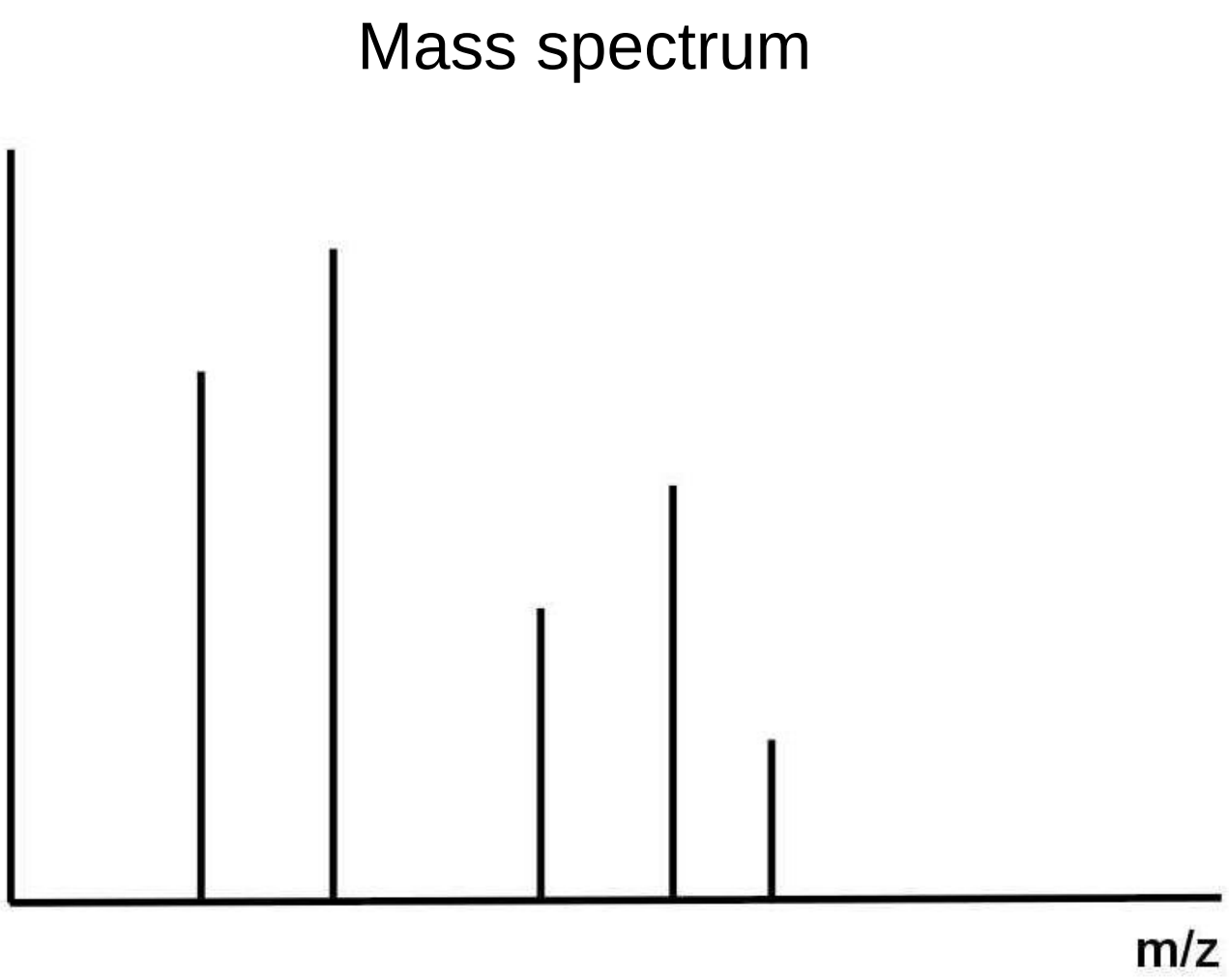
Interpretation



LC-MS

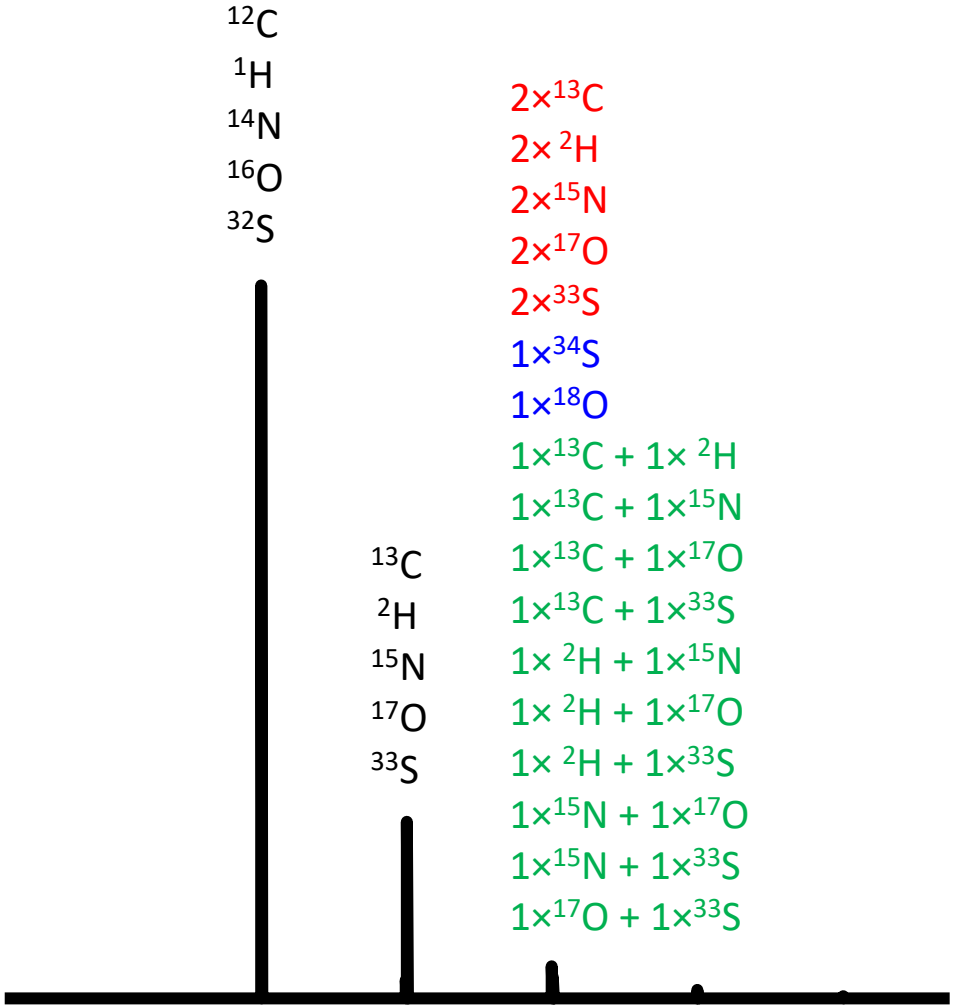


H/D exchange affects MS spectrum – isotopic pattern



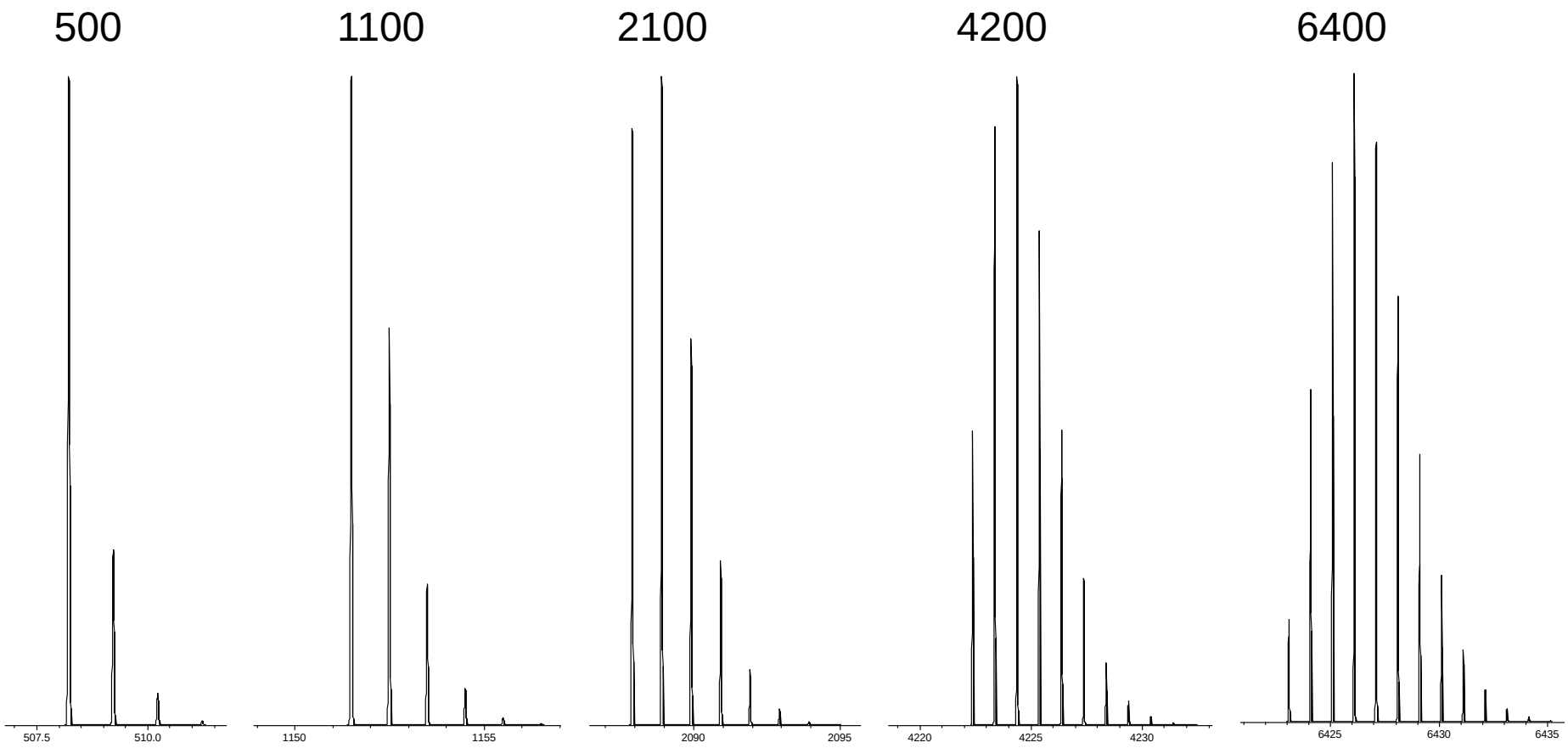
H/D exchange affects MS spectrum – isotopic pattern

Mass spectrum in detail

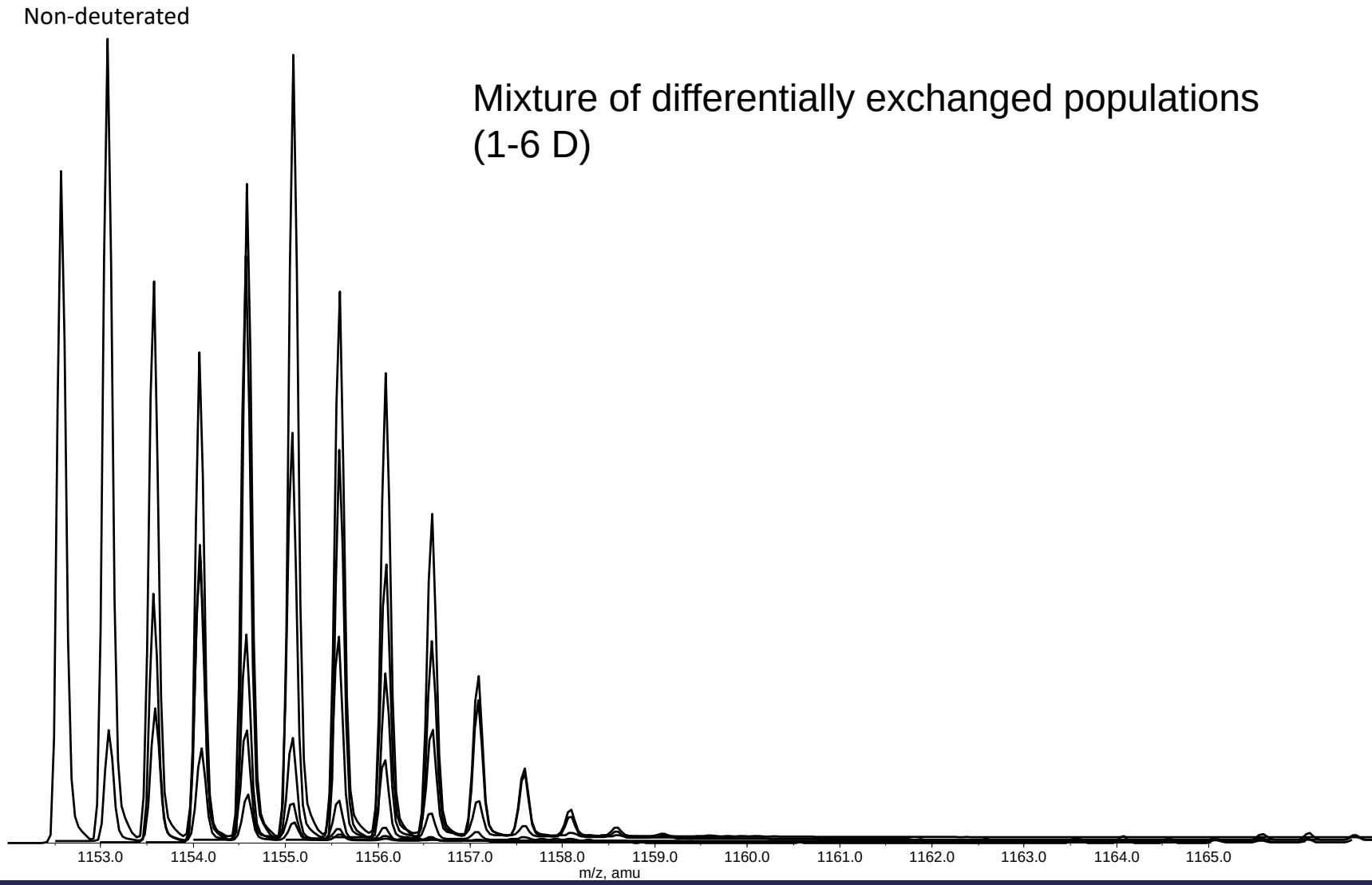


Symbol	M_{nom}	M_{mono}	%
C	12	12.00000	98.9300
	13	13.00336	1.0700
H	1	1.00783	99.9885
	2	2.01411	0.1150
N	14	14.00307	99.6320
	15	15.00011	0.3680
O	16	15.99492	99.7570
	17	16.99913	0.0380
	18	17.99916	0.2050
S	32	31.97207	94.9300
	33	32.97146	0.7600
	34	33.96787	4.2900
	36	35.96708	0.0200
P	31	30.97376	100.0000

Isotope pattern at increasing mass

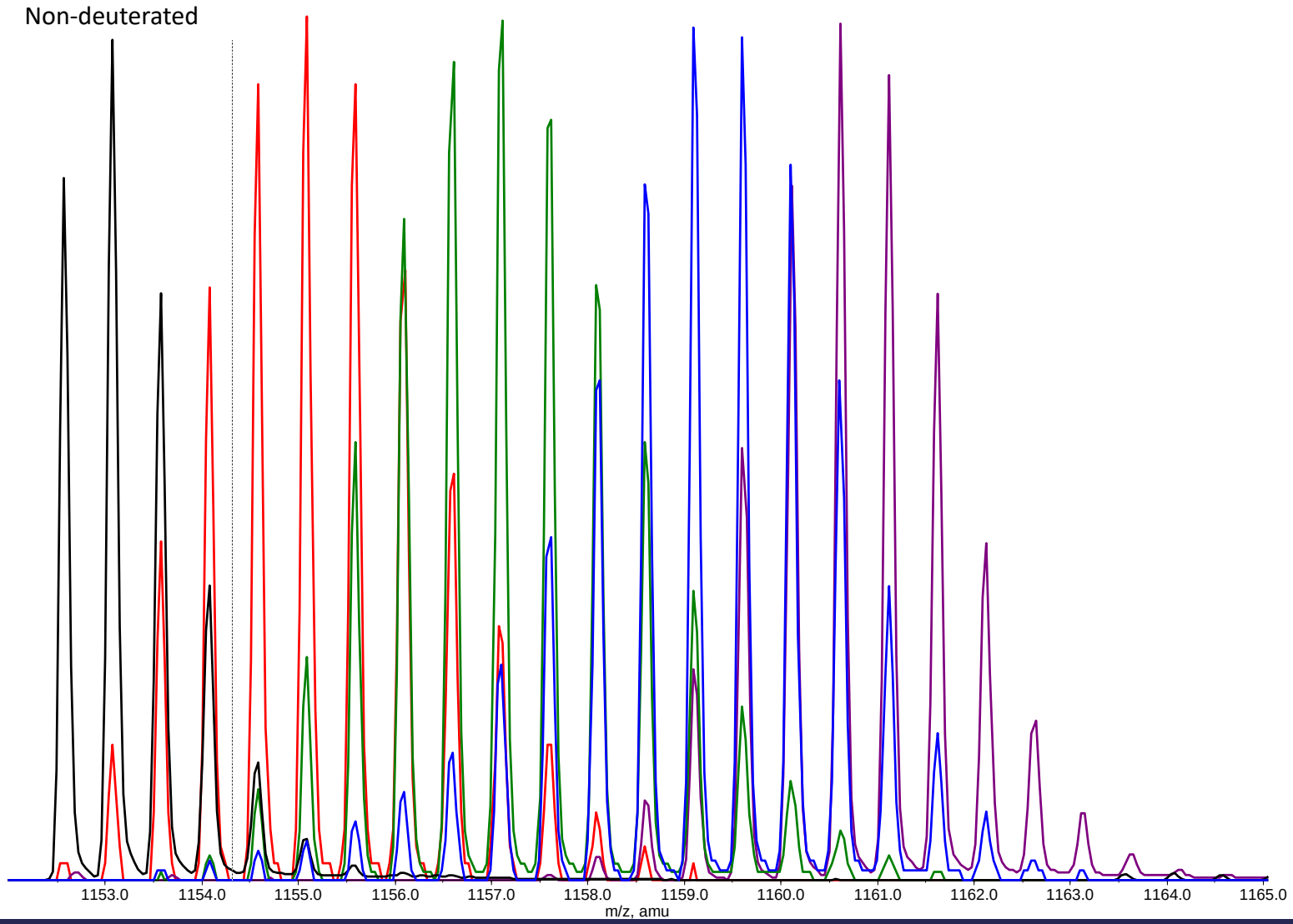


Isotope pattern changes through isotope exchange

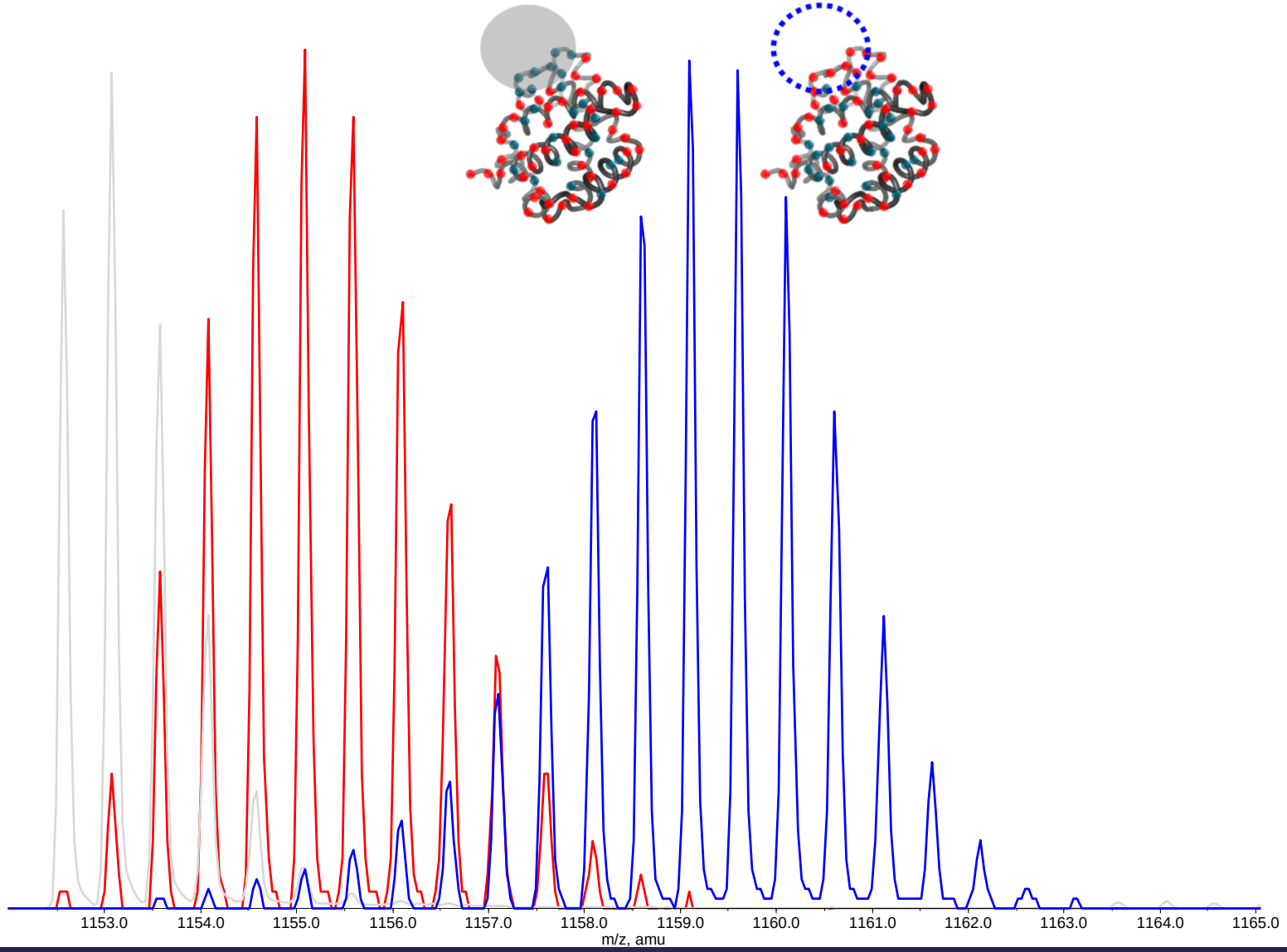


Mixture of differentially exchanged populations (1-6 D)

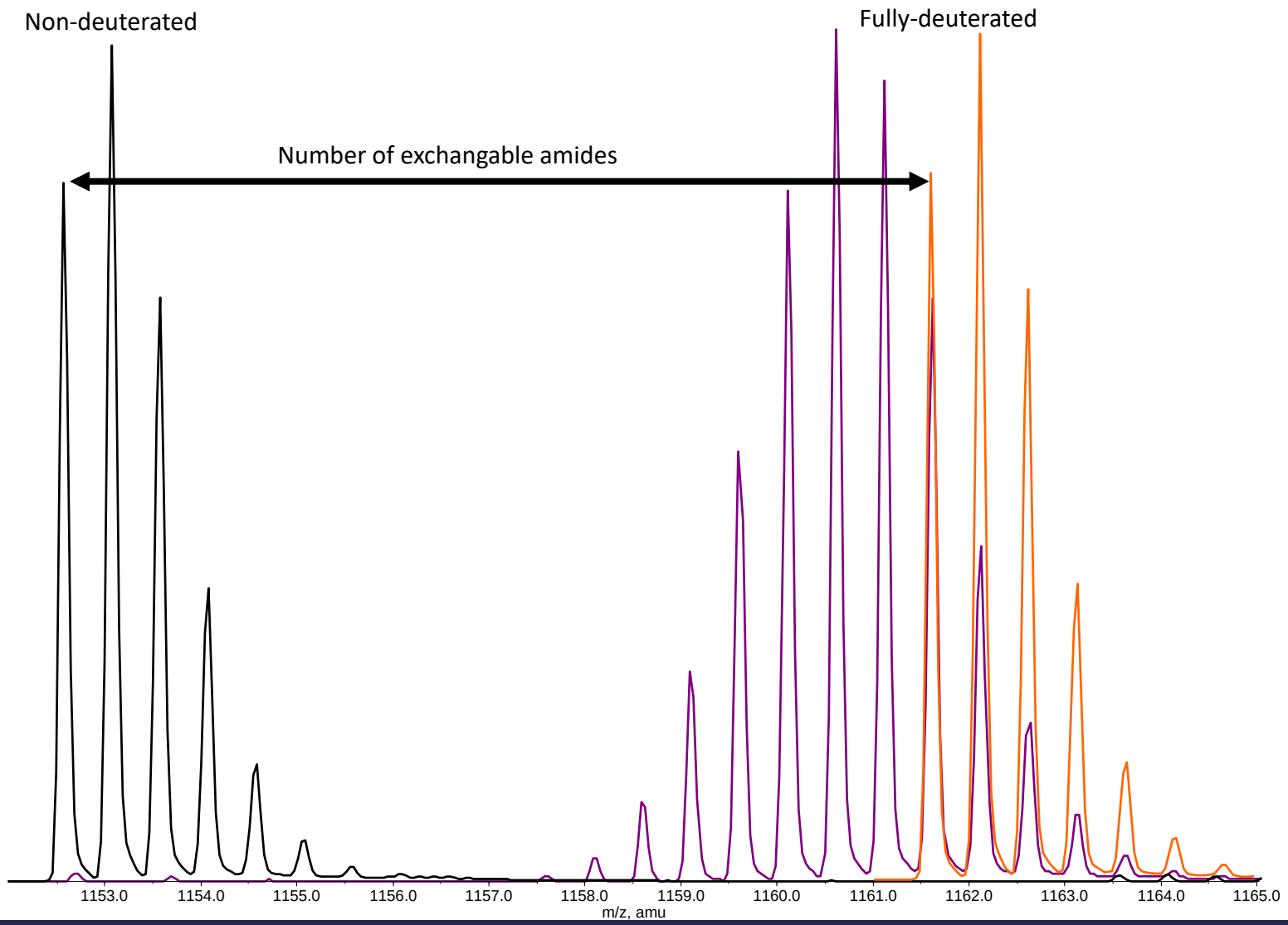
Time resolved deuteration changes



Comparison of free vs ligated protein state

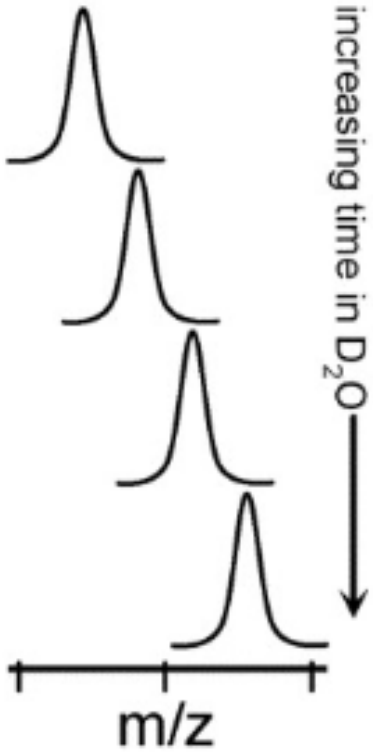


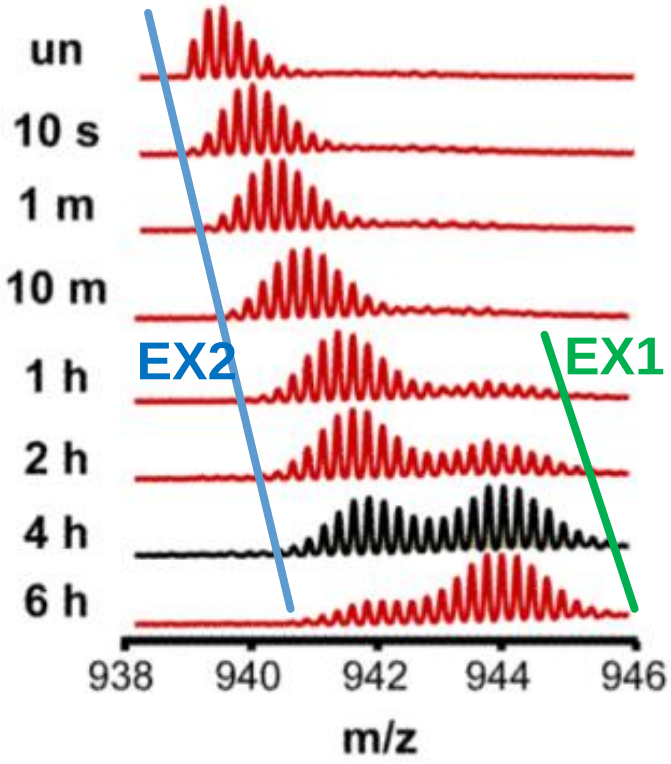
Back-exchange!



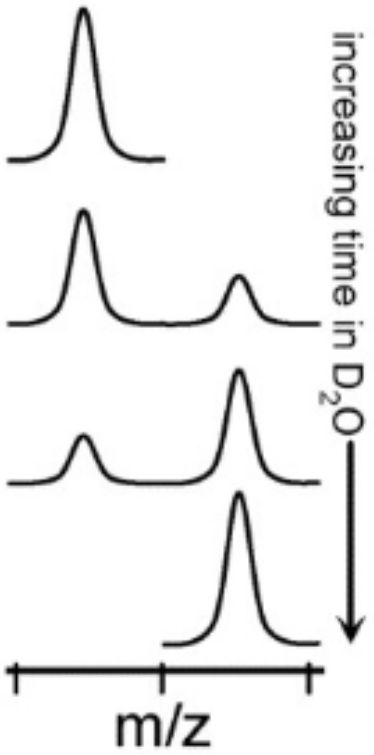
H/D exchange mechanism / kinetics

EX2: $k_{cl} >> k_{ex}$



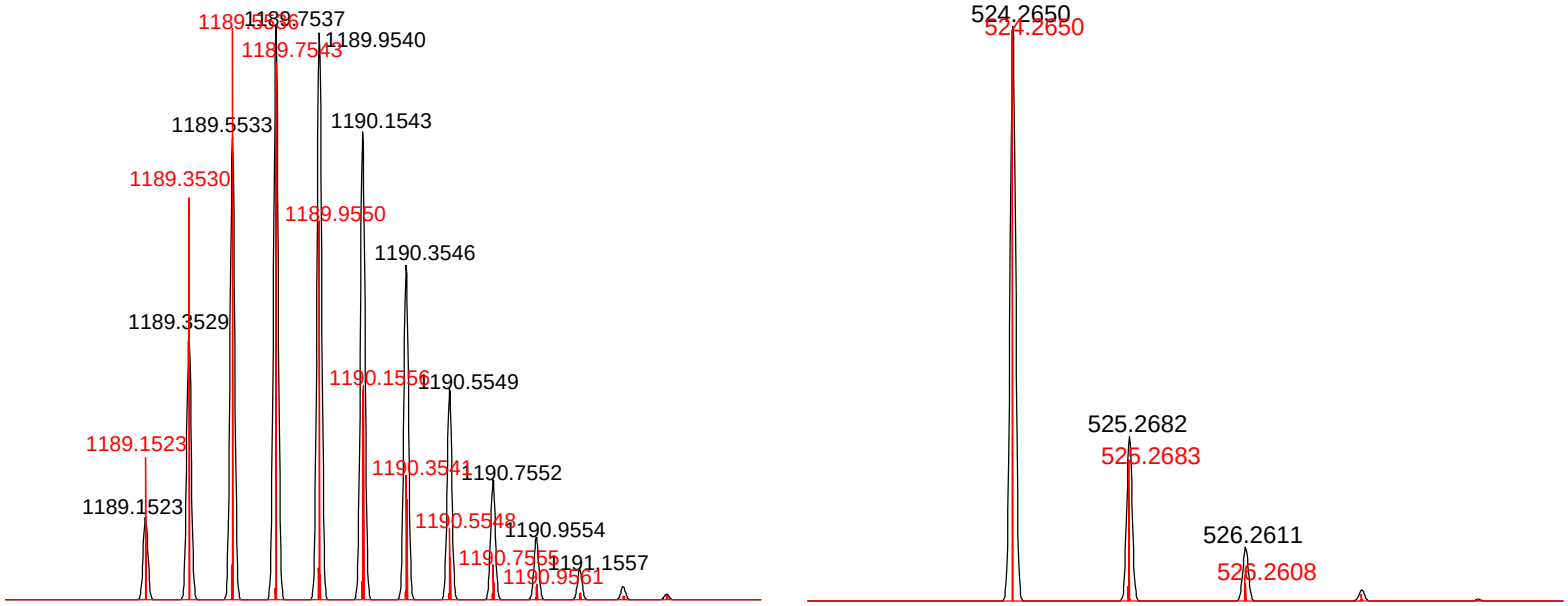


EX1: $k_{cl} << k_{ex}$



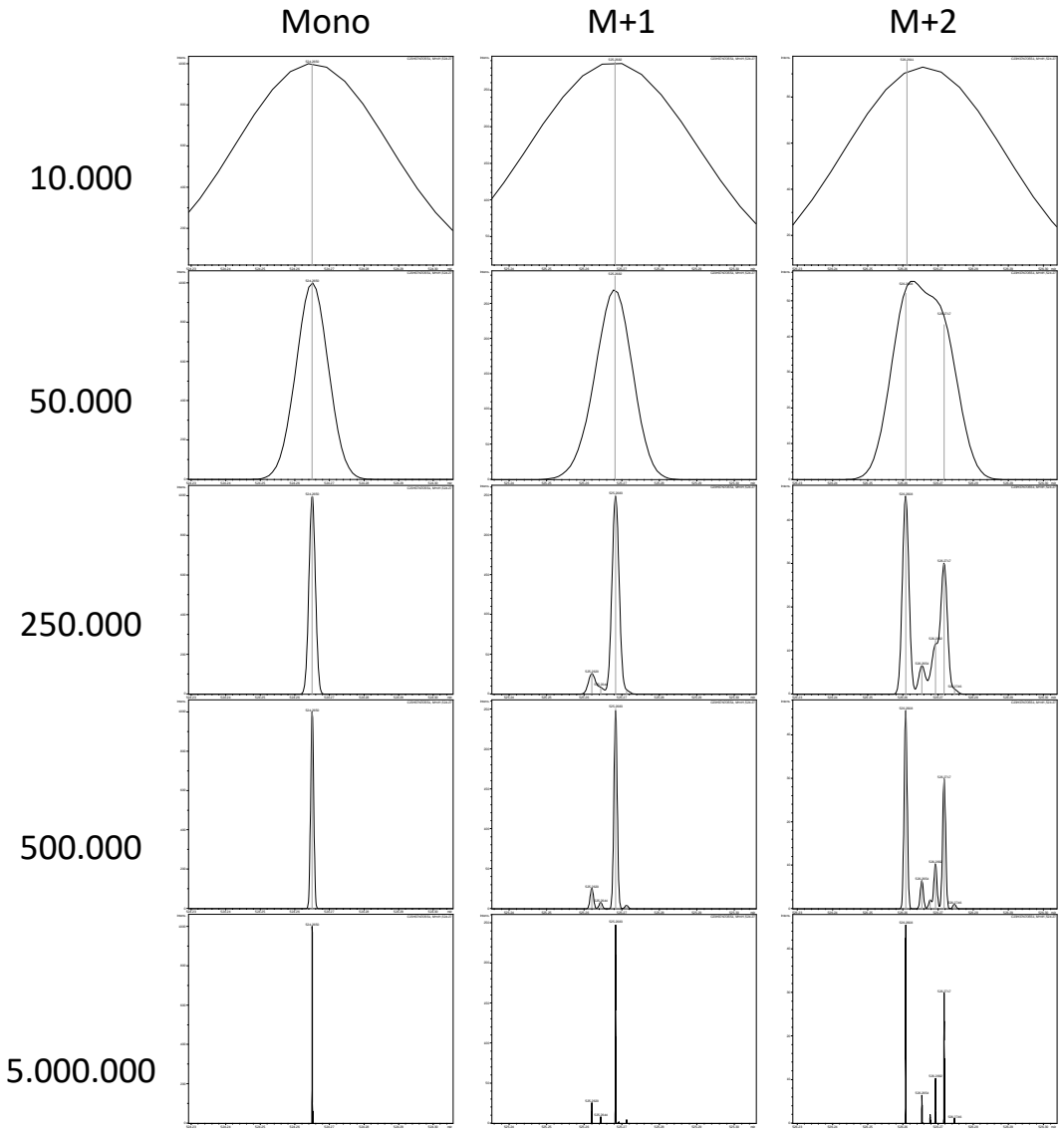
Weis DD *et al.*: J. Am. Soc. Mass Spectrom. (2006) Fang J *et al.*: Biochemistry (2011)

Isotope pattern at increasing resolving power



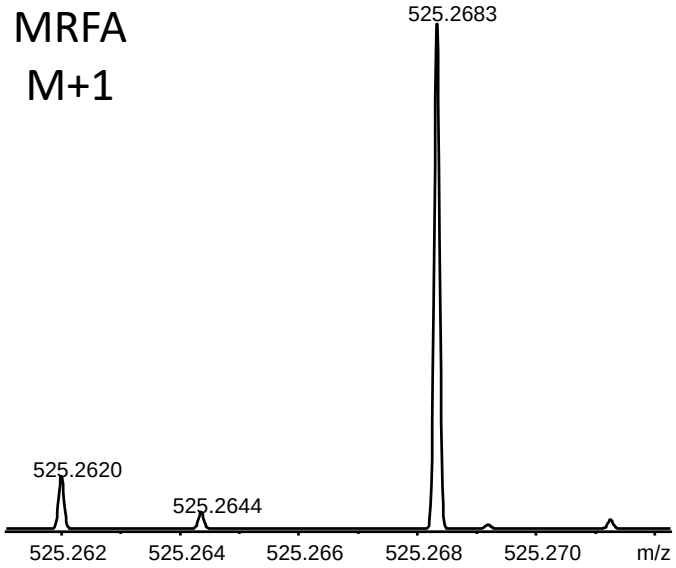
Theoretical models for 5+ insulin and 1+ MFRA @ 50.000 vs 5.000.000

Fine isotopic structure



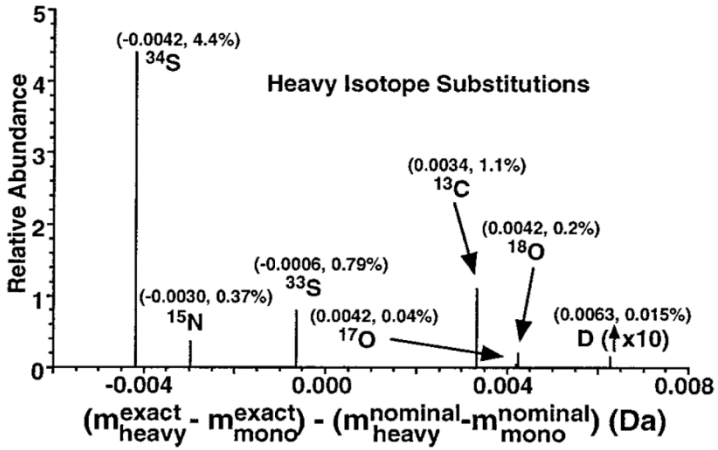
Fine isotopic structure

MRFA
M+1



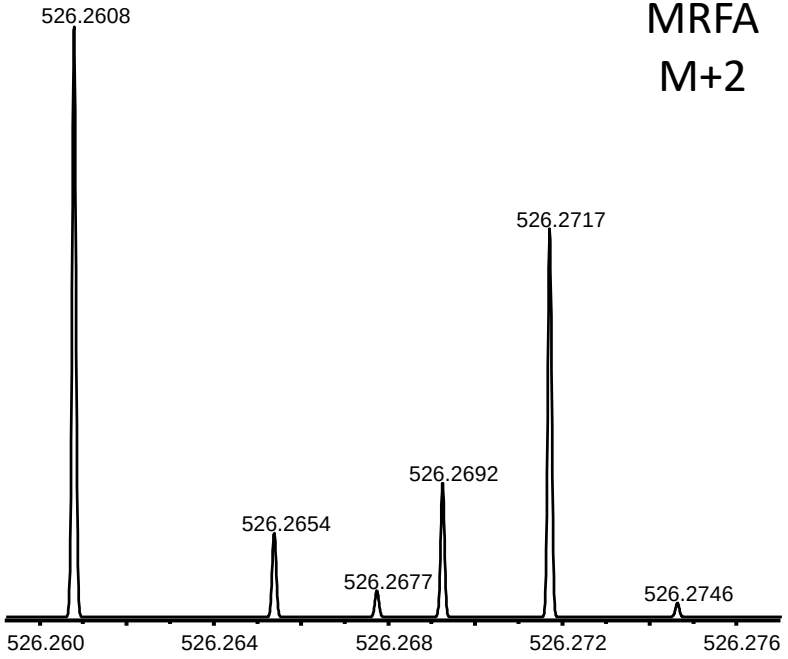
Distance between isotopes

^{13}C-^{12}C	1,003355
^{2}H- ^{1}H	1,006280
^{15}N-^{14}N	0,997035
^{17}O-^{16}O	1,004217
^{33}S-^{32}S	0,999387



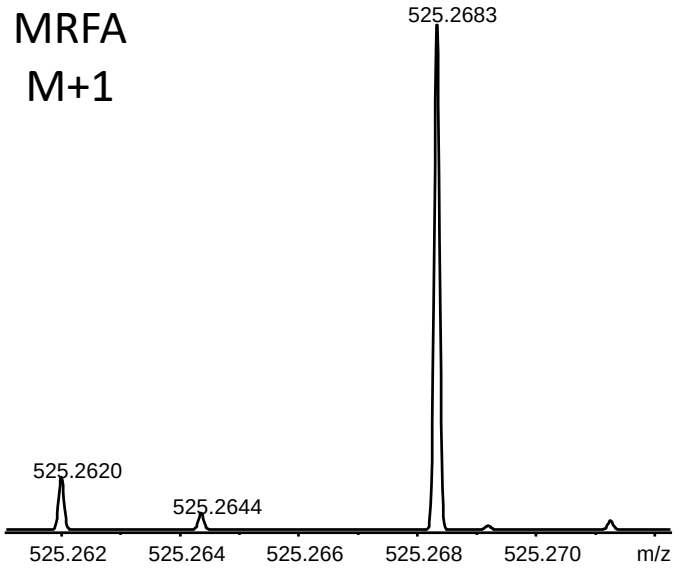
Shi DH et al Proc. Natl. Acad. Sci USA 1998, 95, 11532.

MRFA
M+2



Fine isotopic structure

MRFA
M+1



Distance from nominal mass +1

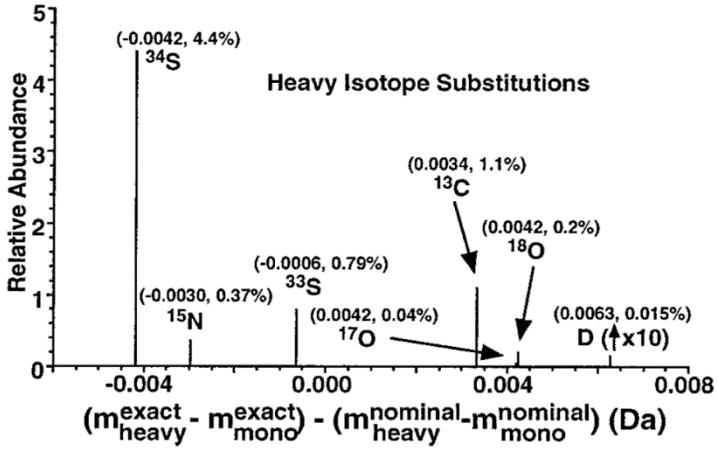
525.2620 = −0.0030 ~ ^{15}N

525.2644 = −0.0006 ~ ^{33}S

525.2683 = +0.0033 ~ ^{13}C

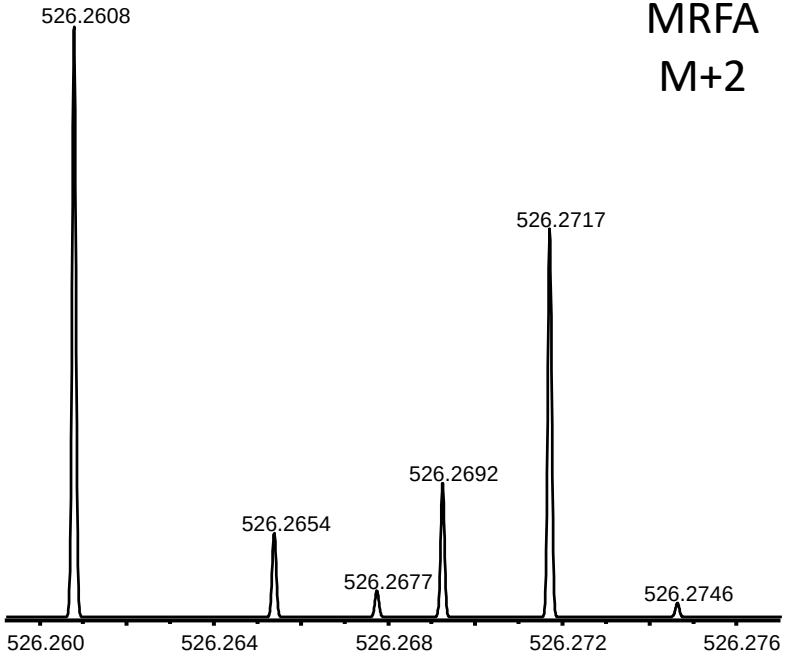
525.2692 = +0.0042 ~ ^{17}O

525.2713 = +0.0063 ~ ^{2}H



Shi DH et al Proc. Natl. Acad. Sci USA 1998, 95, 11532.

MRFA
M+2



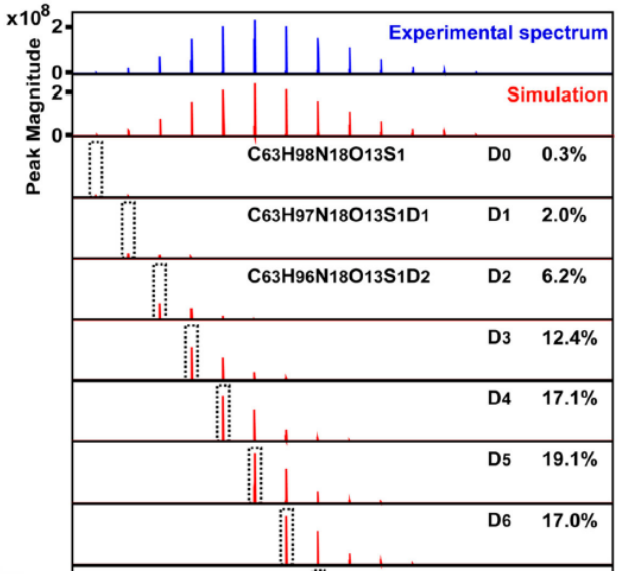
Utility of fine isotopic structure in HDX

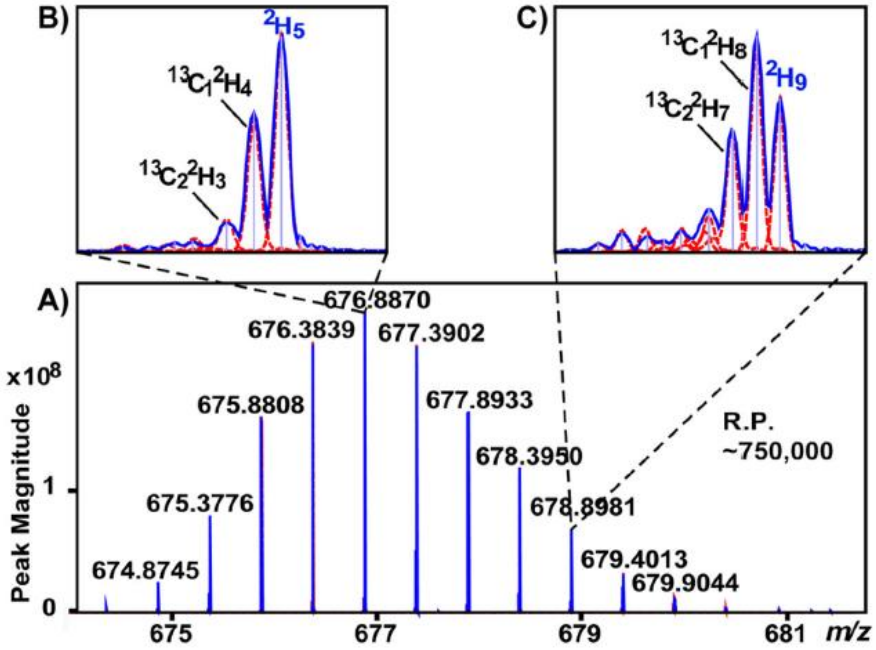
Ultra high resolution required! ^{12}C-^{13}C vs ^{1}H-^{2}H ~ 3mDa

Direct readout of the deuteration level (ND spectrum not needed) + “deconvolution” of differentially deuterated species – exchangeomer distribution

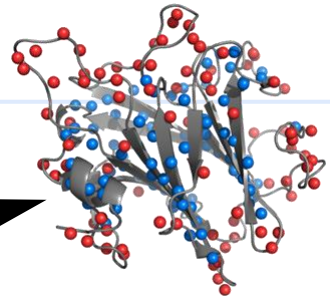
Pseudomonoisotopic peak is defined – monoisotopic undergoing just H/D exchanges. No other isotopes.

Claimed to be more accurate…

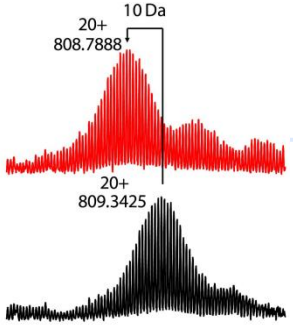




Global exchange



ESI / MALDI



labeling

doi:10.1016/j.jmb.2007.02.014 *J. Mol. Biol.* (2007) **368**, 464–472

JMB

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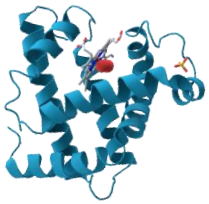
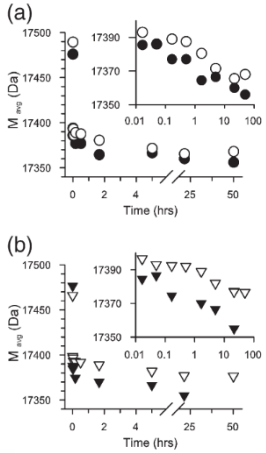
Defining the Interacting Regions between Apomyoglobin and Lipid Membrane by Hydrogen/ Deuterium Exchange Coupled to Mass Spectrometry

Petr Man[1], Caroline Montagner[2], Grégory Vernier[2], Bernard Dublet[1] Alexandre Chenal[2], Eric Forest[1]* and Vincent Forge[2]*



D/H exchange (to diminish effect of side-chains) using MALDI-TOF



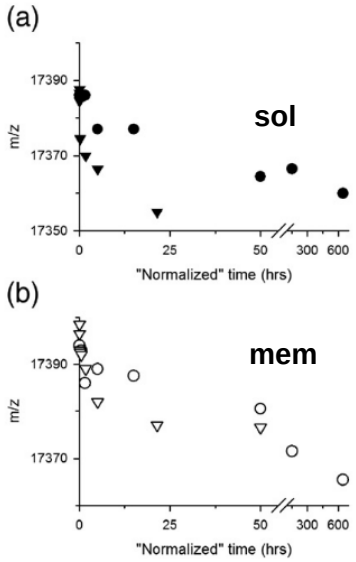
aMb free (black) / + membrane (white)

pH 5.5

pH 4.0

Bigger protection @ pH 4.0
Stronger interaction

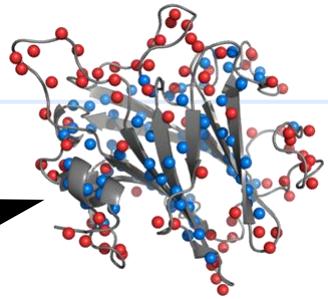
Comparison at different pH values (correction by $10^{1.5}$)
aMb free (black) / + membrane (white)



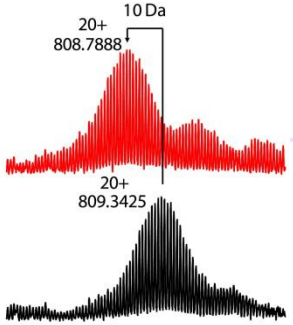
Faster exchange at pH 4.0
=
protein opens (molten globule)

Faster exchange at pH 4.0
but stops at certain moment
=
protein opens (molten globule)
but is protected by the membrane

Global exchange



ESI / MALDI



labeling

prokaryotic hydrophobic amino acid transporter, LeuT

Moeller *et al.*: J Proteomics (2020)

Contents lists available at ScienceDirect

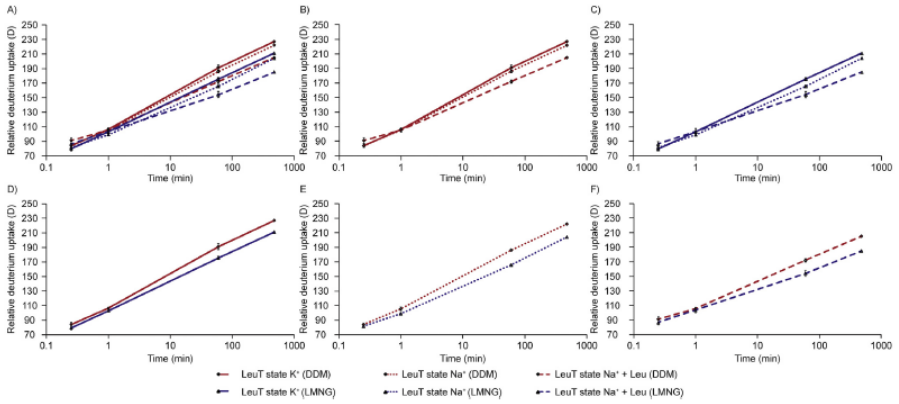
Journal of Proteomics

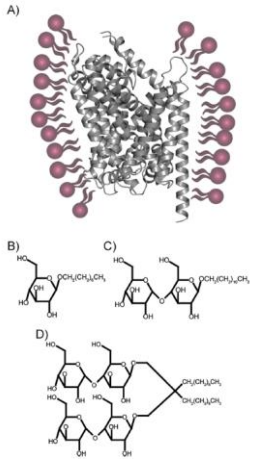
journal homepage: www.elsevier.com/locate/jprot

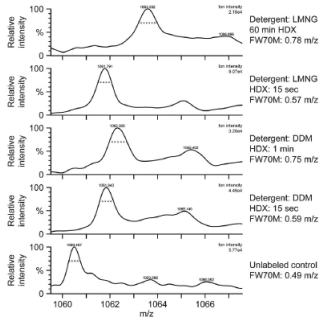
Probing the conformational impact of detergents on the integral membrane protein LeuT by global HDX-MS

Ingvar R. Möller[a,1], Patrick S. Merkle[a,1], Dionisie Calugareanu[a], Gerard Comamala[a], Solveig Gaarde Schmidt[b], Claus J. Loland[b], Kasper D. Rand[a,*]

Detergent DM/LMNG and ions K+/Na+

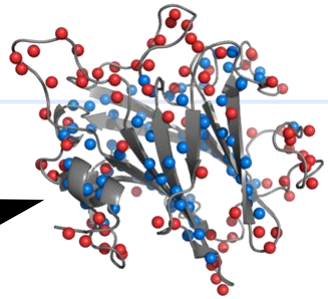




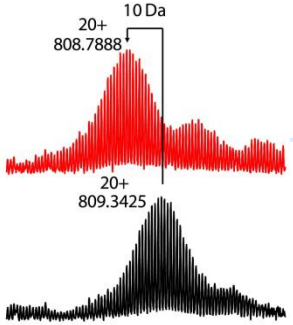


Protein dynamics (EX1/EX2) assessed through peak width

Global exchange



ESI / MALDI



labeling

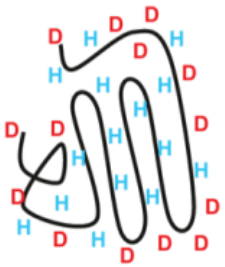
Local exchange

Rand *et al.*: Acc. Chem. Res. (2014)

H/D exchange

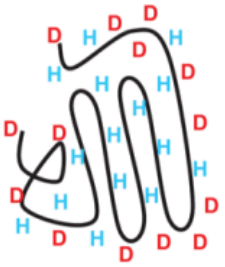
t_1 t_2 t_3 t_4

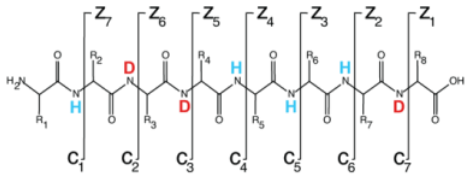
D_2O



Quench

(pH 2.5, 0°C)

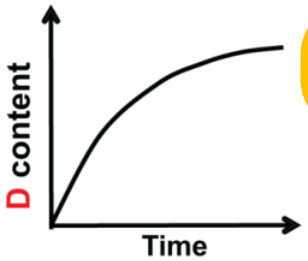




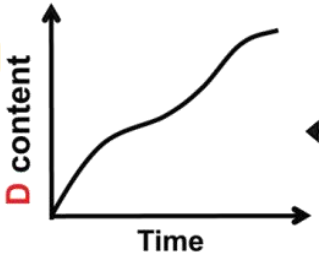
Gas-phase cleavage (protein)

Solution-phase **cleavage (pepsin, pH 2.5, 0°C)**

Gas-phase **cleavage (peptide)**

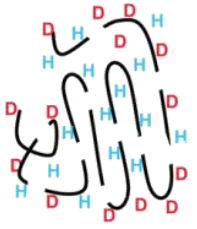


HDX of individual residues



HDX of peptide segments

Cooled LC-MS



CID vs Electron Capture/Transfer Dissociation (ECD/ETD)

CID – collision-induced dissociation

Slow, leads to H/D movement = scrambling

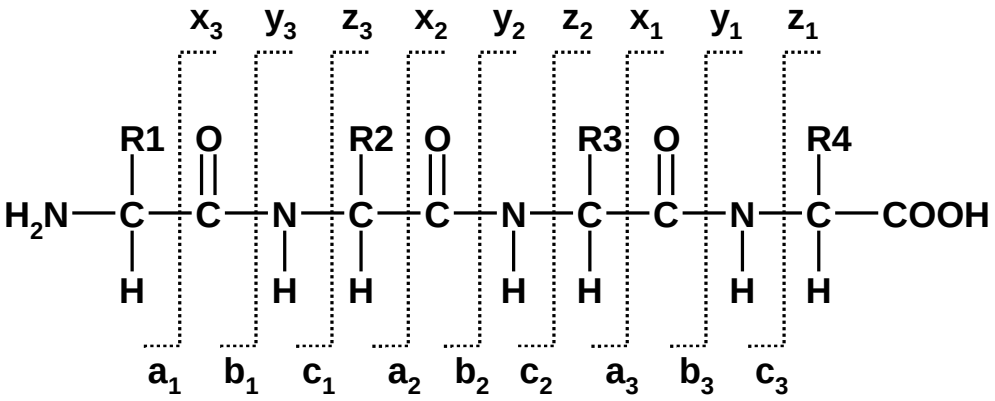
Spatial information is lost

ECD / ETD – electron capture/transfer dissociation

Capture of electron or transfer from radical anions

Faster fragmentation, no scrambling!

Side effect – poor yield, charge neutralization (needs multiply charged precursors)



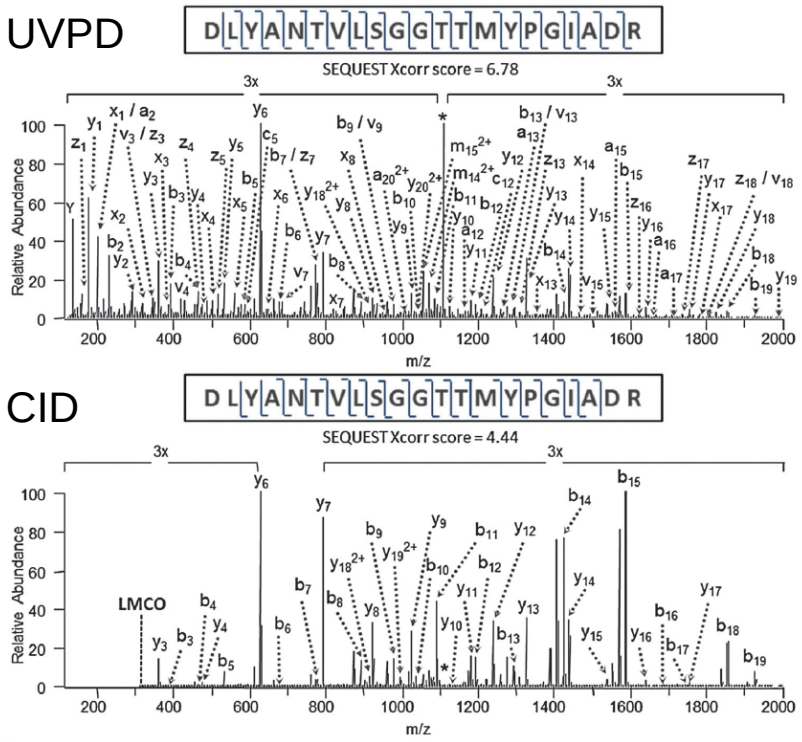
UltraViolet PhotoDissociation (UVPD)

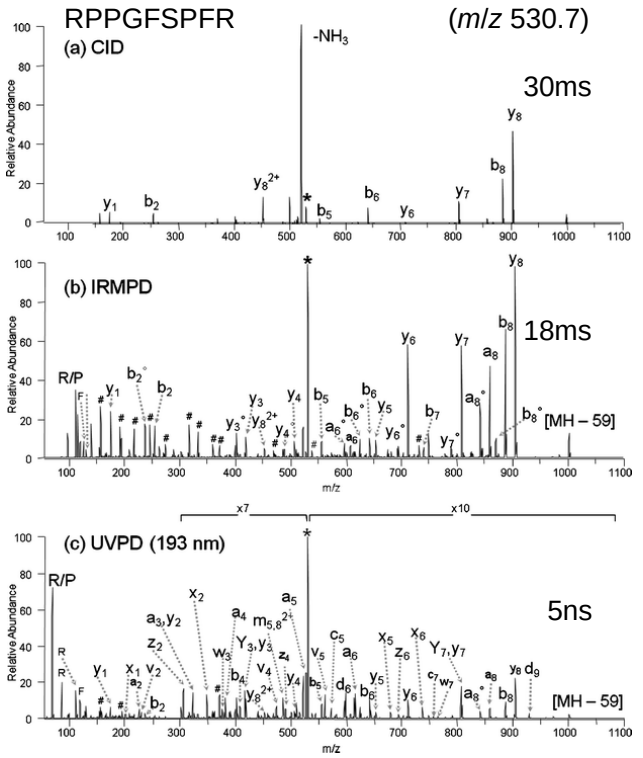
UV lasers – Nd:YAG a excimer ArF, KrF,..

wavelength: 157nm, 193nm (peptide bond), 213nm, 266nm,…
wavelength close to 280nm – fragmentation close to aromatics

Very fast fragmentation – no/minimal scrambling

a/x, c/z, but also b/y ions and side chain fragments

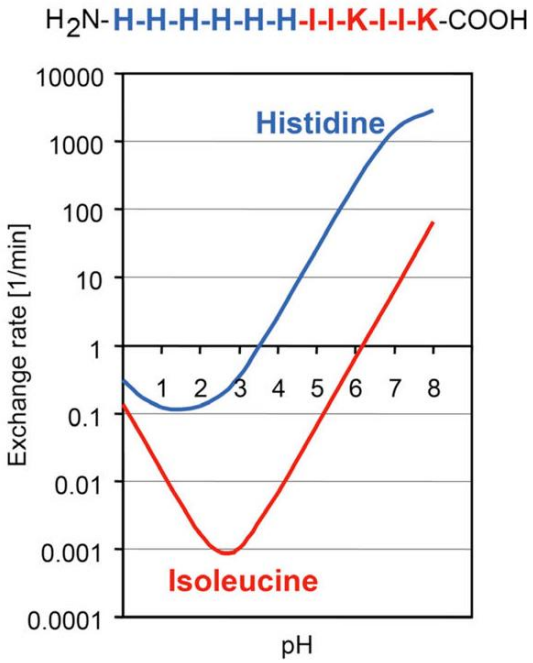




EU FT-ICR MS

Scrambling test

Kaltashov, Eyles: J. Mass Spectrom. (2002)
Rand *et al.*: Acc. Chem. Res. (2014)



HHHHHHIIKIIK

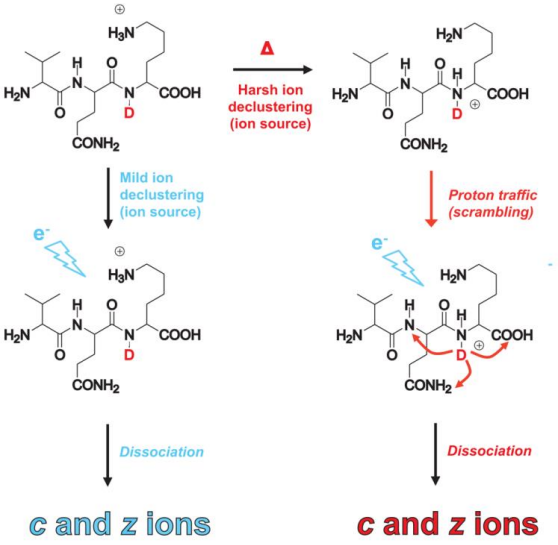
Fully deuterate

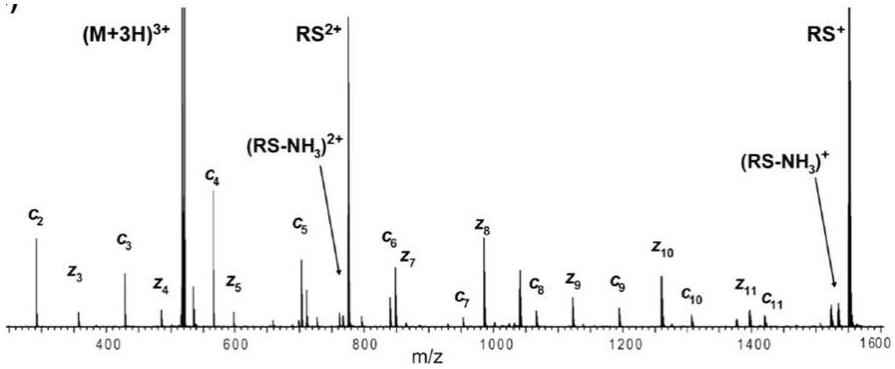
⬇

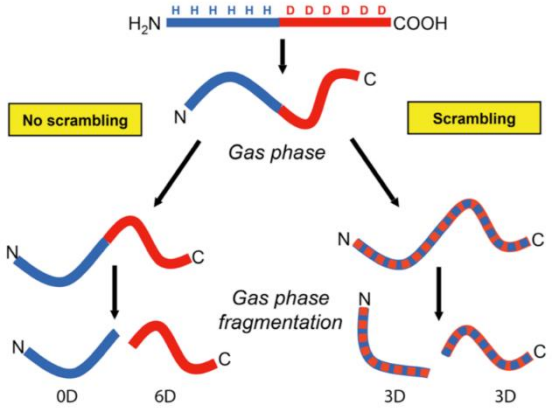
Quench

⬇

Infuse

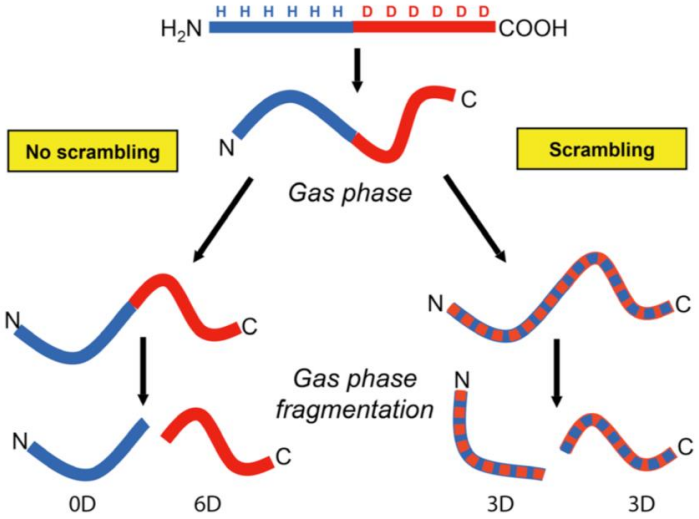




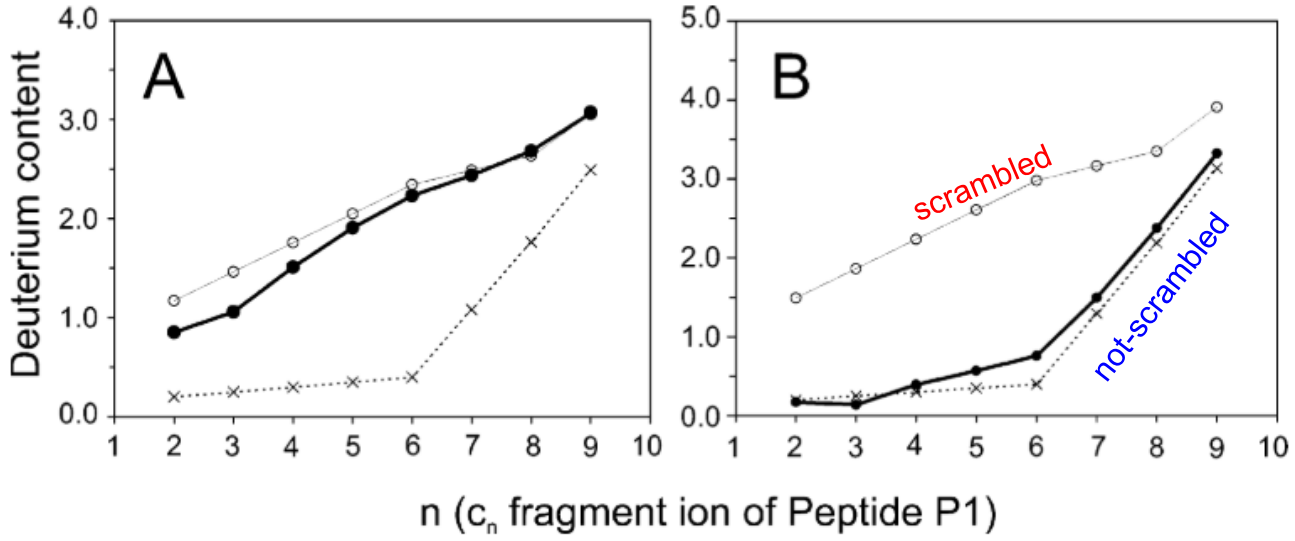


Scrambling – ETD/ECD

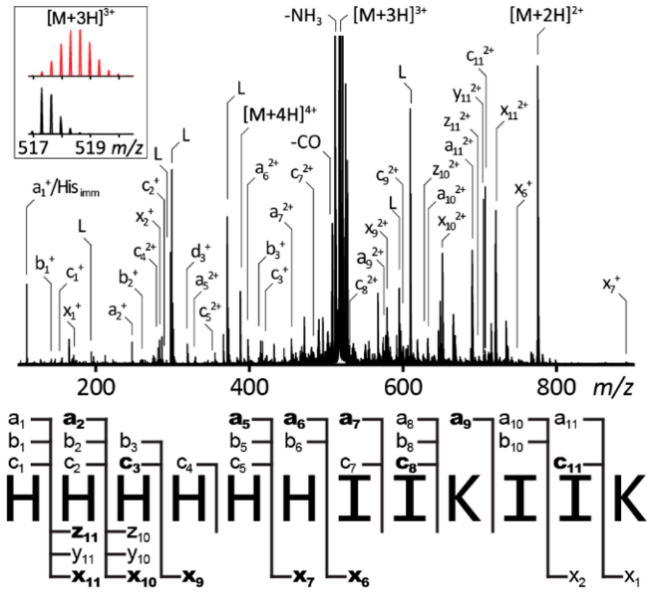
Kaltashov, Eyles: J. Mass Spectrom. (2002)
Rand *et al.*: Acc. Chem. Res. (2014)



Activation via dissociation but also during ionization and ion transfer



Scrambling - UVPD

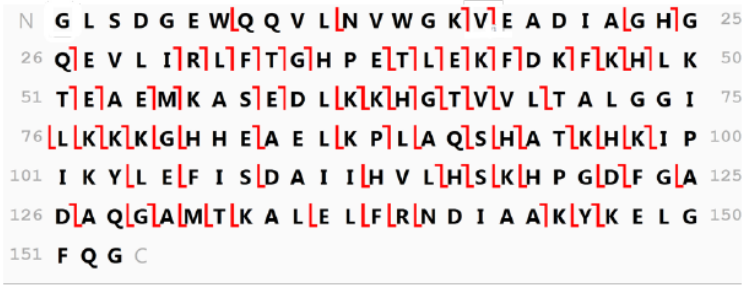


Mistraz U.H. et al Anal Chem 2018
Brodie N.I. et al Anal Chem 2018

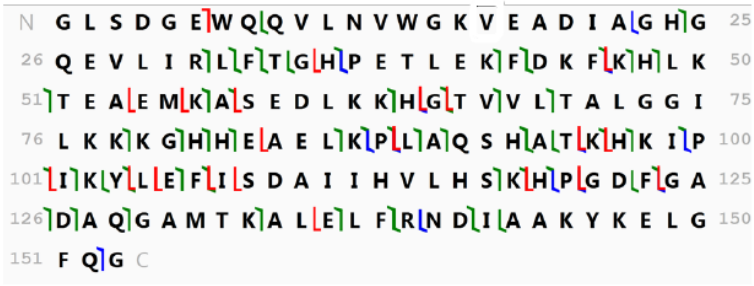
UVPD is applicable (similarly to ExD)

More fragments produced (P1 on the *left* and Mb top-down *bottom*)

ETD



UVPD



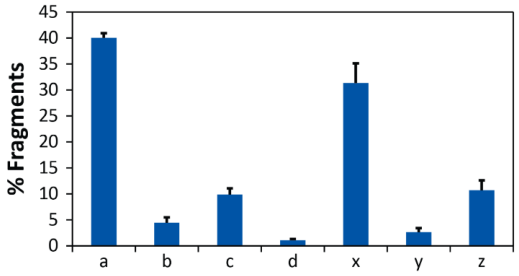


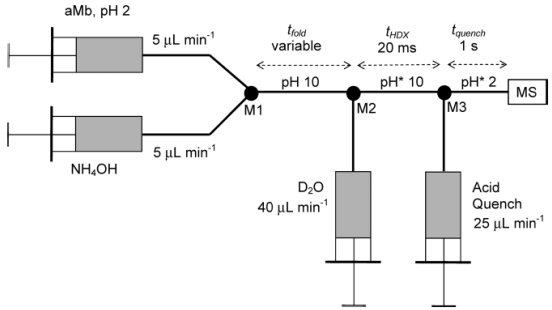
Figure S-2. Abundance of fragment ion types observed during 213 nm UVPD of peptide P1.

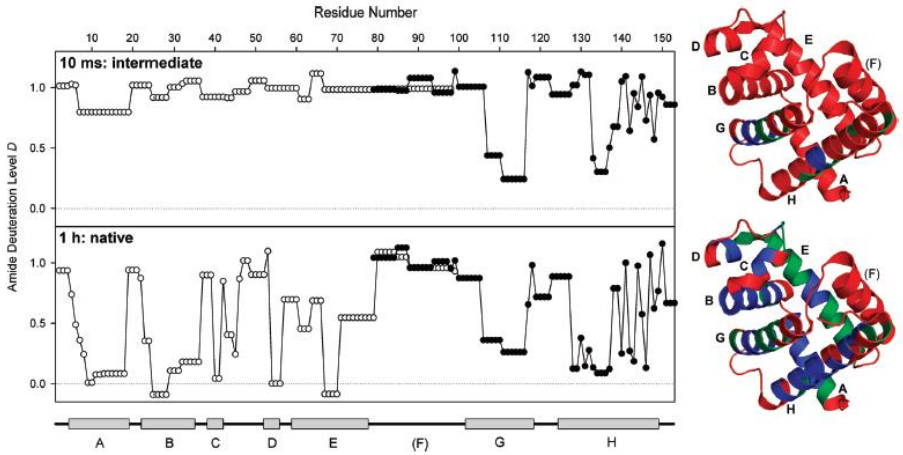
Top-Down HDX-MS

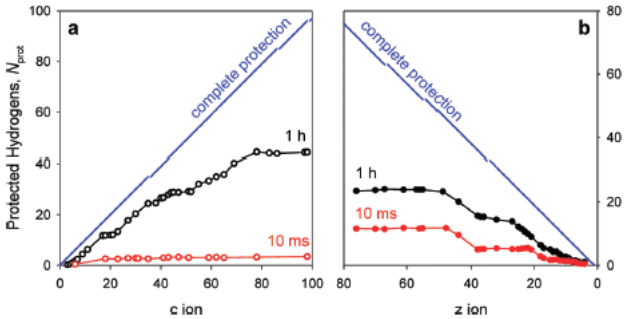


Number of reports rather limited (C.H. Borchers, L. Konermann, I.A. Kaltashov,…).

Mostly on smaller, well behaving, well characterized proteins – e.g. Mb folding, histones,…





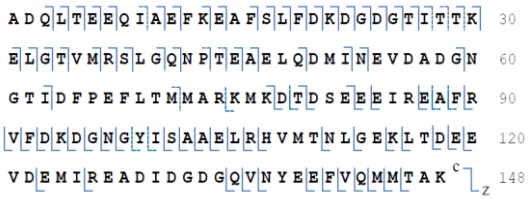


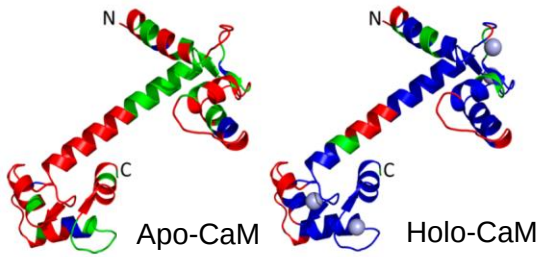
Pan J et al Anal Chem 2010
c ions (open)
z ions (closed)

Top-Down HDX-MS

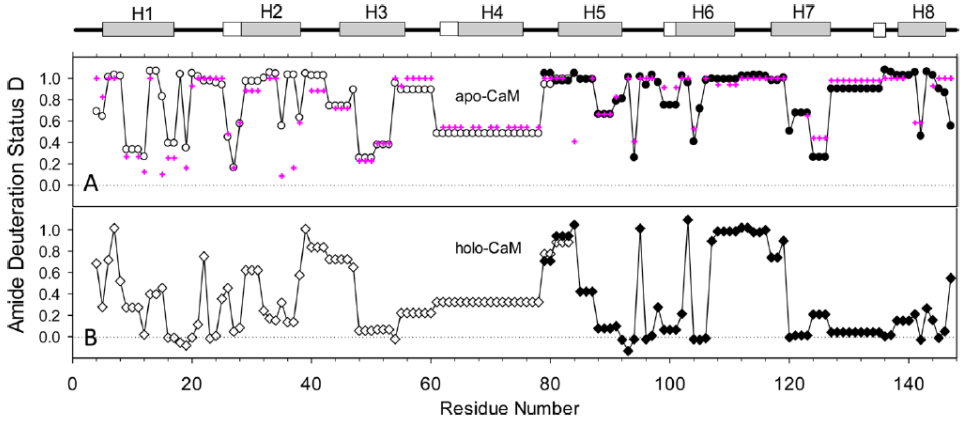
Number of reports still limited (C.H. Borchers, L. Konermann, I.A. Kaltashov,…).

Mostly on smaller, well behaving, well characterized proteins – e.g. CaM





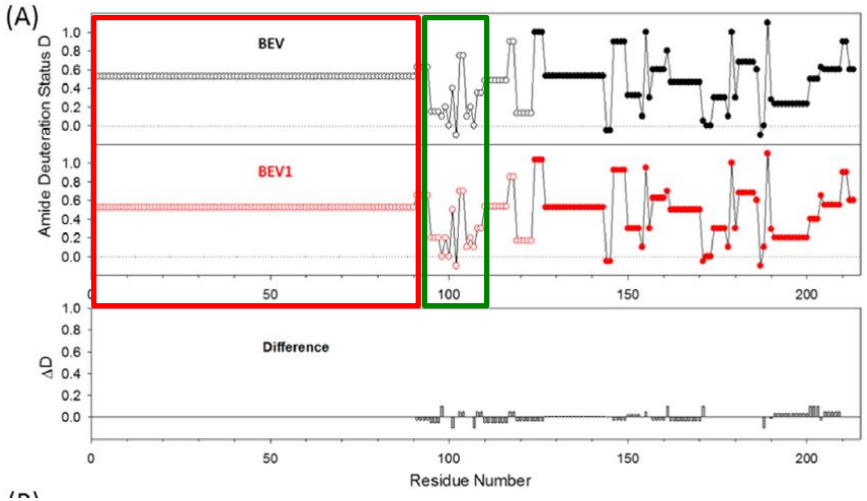
D-level
<0.33
0.33-0.66
>0.66



Pan J et al JACS 2014
c ions (open)
z ions (closed)

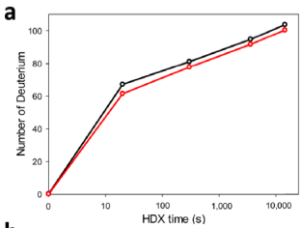
Top-Down HDX-MS

Ab studies also reported, however, Ab (150kDa) is in fact composed of 50kDa and 25kDa proteins (HC and LC, respectively) + HC is “not interesting”



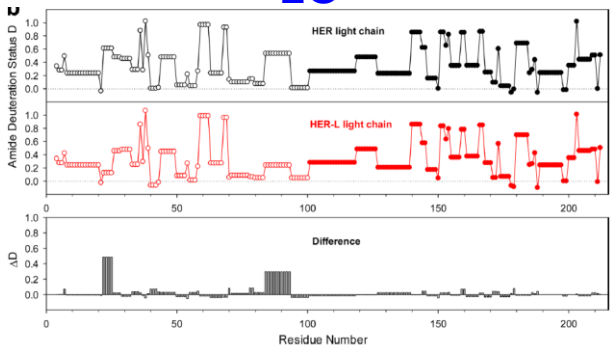
Fragmentation can go to “residue level” but has also spans over large portion of the protein – BEV = Bevacizumab (Avastin) = Ab against VEGF – light chain shown here

Pan J et al BBA 2016
c ions (open)
z ions (closed)

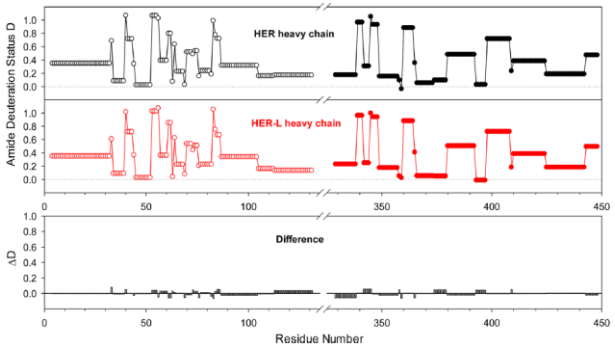


Pan J et al JACS 2014
c ions (open)
z ions (closed)

LC



HC – 1-132 + 328-449



Top-Down HDX-MS

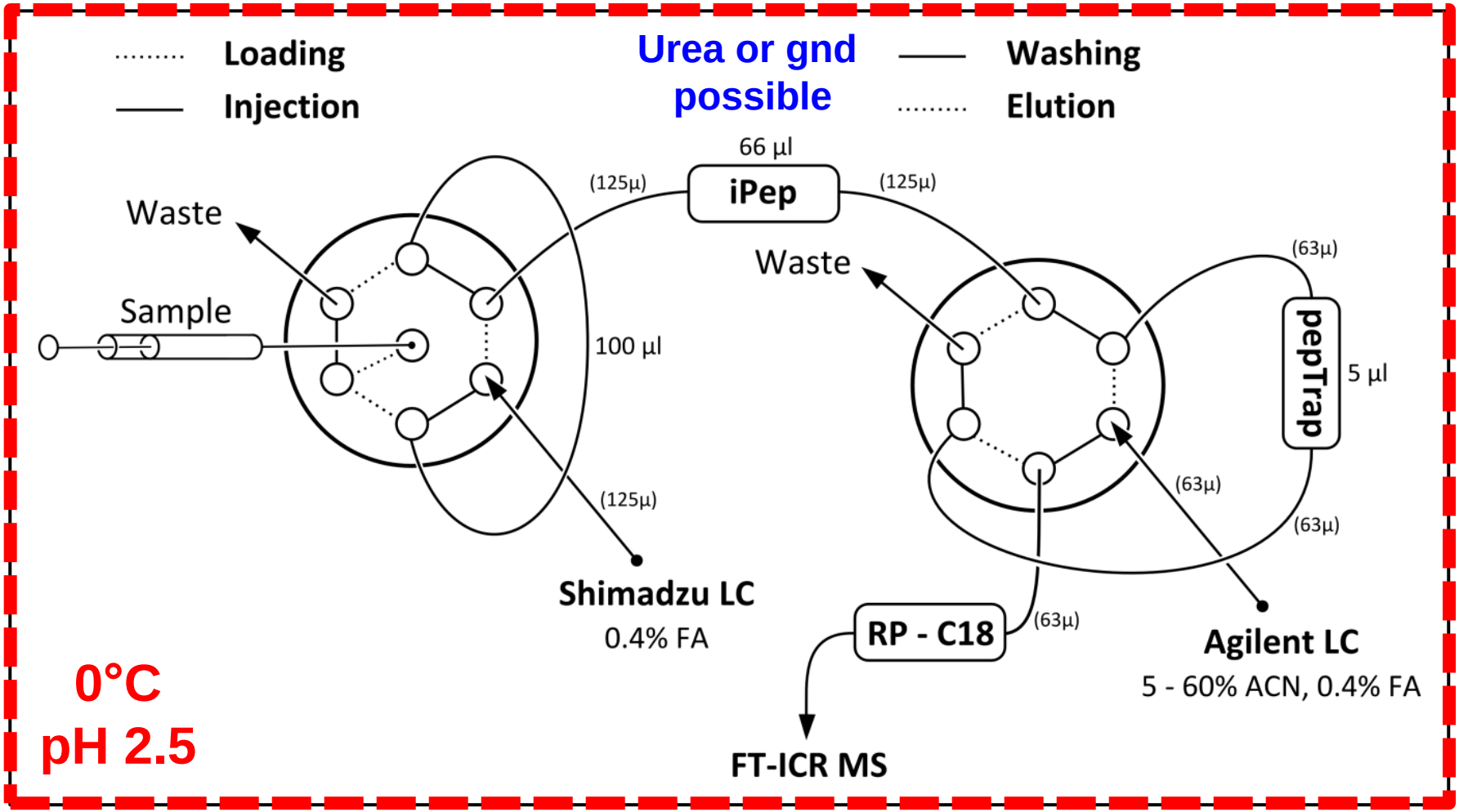
Problem with ionization/fragmentation of some/larger proteins, not likely applicable (yet) to complexes.

Poor fragmentation yield - coverage/resolution. Fragments mostly at the termini.

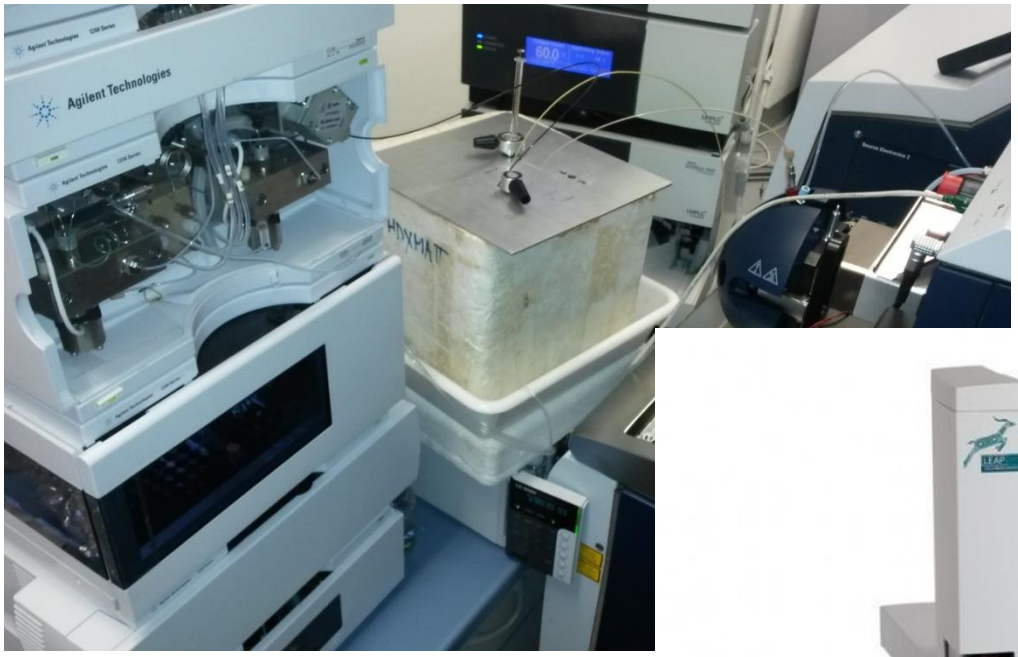
Motivation/advantages – sequence coverage sometimes not complete – already two complementary fragment ions do the job!

Digestion is often not easy, more handling=bigger D-loss (BE in bottom-up 15-35%, in top-down approx 2% using sub-zero LC – Pan J et al. JACS 2014)

H/D exchange setup for online digestion and LC



H/D exchange setup for online digestion and LC



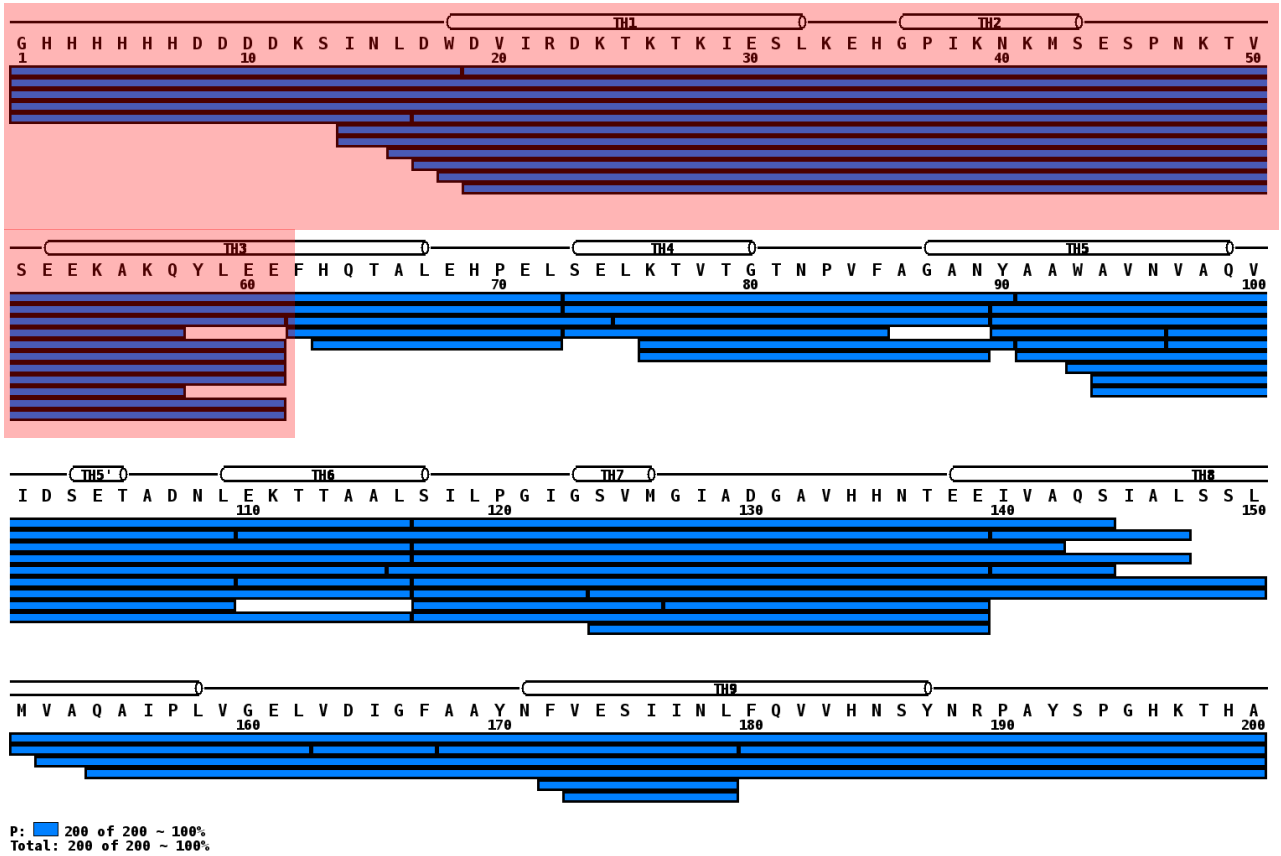
Bottom-up HDX - proteolysis

- defines spatial resolution of the method / full sequence coverage wanted
- Golden standard – porcine pepsin A
  not all proteins can be efficiently by pepsin digested under HDX-MS conditions
- poor choice of commercial proteases suitable for HDX-MS
  type XIII – Aspergillopepsin, type XVIII – Rhizopuspepsin
  – both not very well defined crude extracts
- *Immobilized protease – high local protein-enzyme ratio, tunable via column size, flow, pressure, temperature*

Bottom-up HDX - proteolysis

Proteolysis

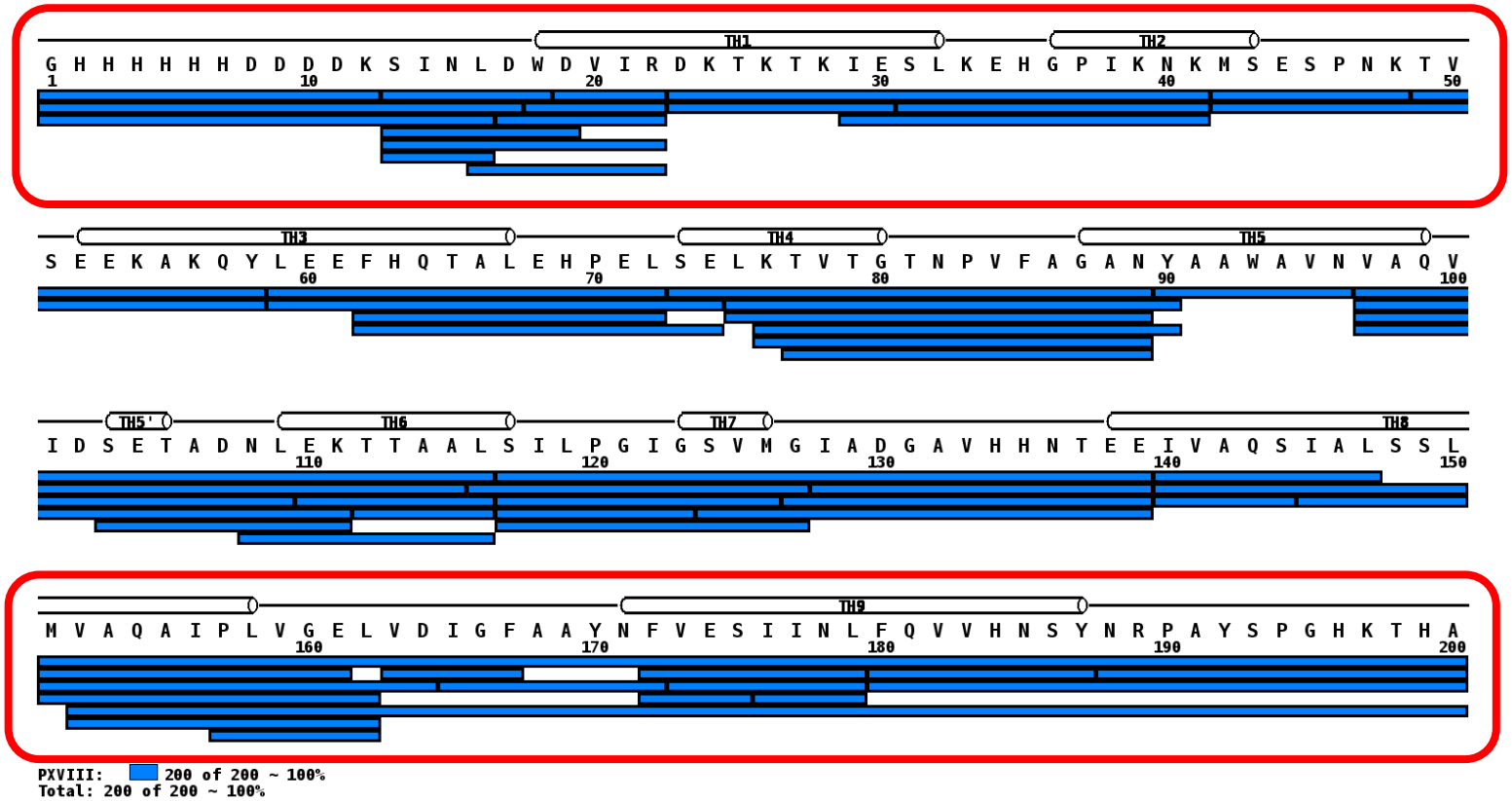
- defines spatial resolution of the method
- not all proteins can be efficiently by pepsin digested under HX-MS conditions



Bottom-up HDX - proteolysis

Try different proteases (commercial extracts – protease type XIII, type XVIII)

Digestion by pepsin+rhizopuspepsin (type XVIII)



Acidic proteases in HDX – overview

2017 – Tsiatsiani et al – An-PEP + Nirudodhi et al – dual proteases (XIII+pepsin)

2016 – Rey et al – neprosin (not yet for H/D)

2015 – Yang et al – nepenthesin-2

2014 – Kadek et al – nepenthesin-1

2013 – Ahn et al – rice field eel

2013 – Rey et al – nepenthes pitcher fluid

2010 – Marcoux et al – plasmepsin 2

2009 – Rey et al – recombinant rhizopuspepsin
(protease type XVIII)

2007 – Brier et al – pepsins from Antarctic rock cod

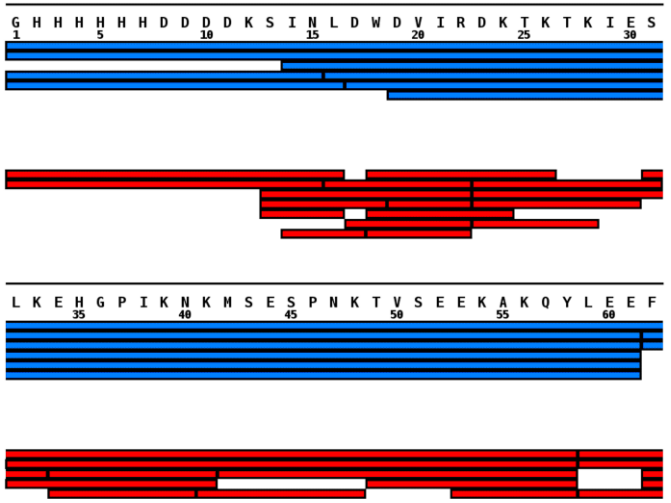
2003 – Cravello et al – protease type XIII and XVIII

Dykstra et al – endothiapepsin

2002 – Wang et al – pepsin immobilization

1993 – Zhang&Smith – MS + protein digestion (pepsin)

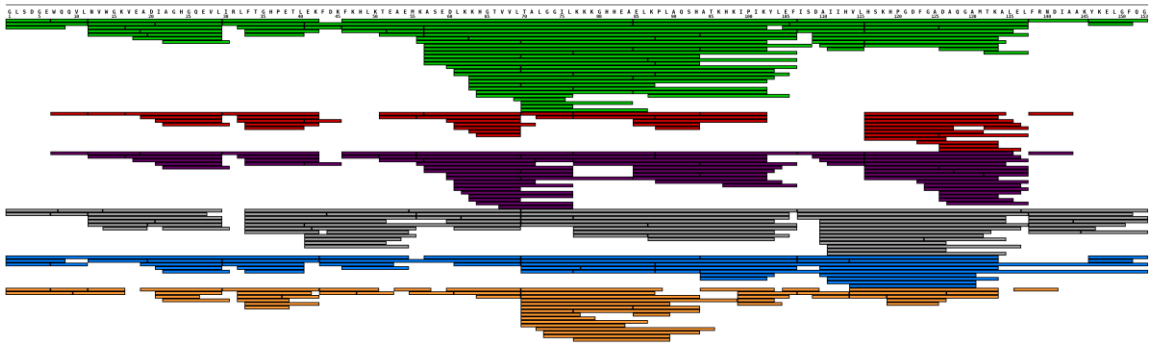
1979 - Rosa&Richards – protein digestion (pepsin, rhizopuspepsin)



Proteases – what is available

Nepenthesin-1
Nepenthesin-2
Rhizopuspepsin (XVIII)
Aspergillopepsin (XIII)
Pepsin
AN-PEP
Orzyasin
Neprosin

Comparisons of peptide maps

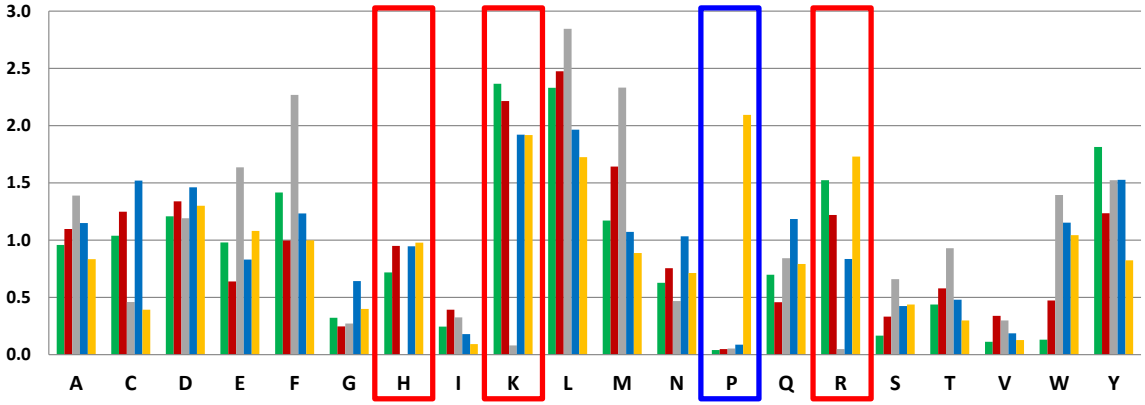


GOALS

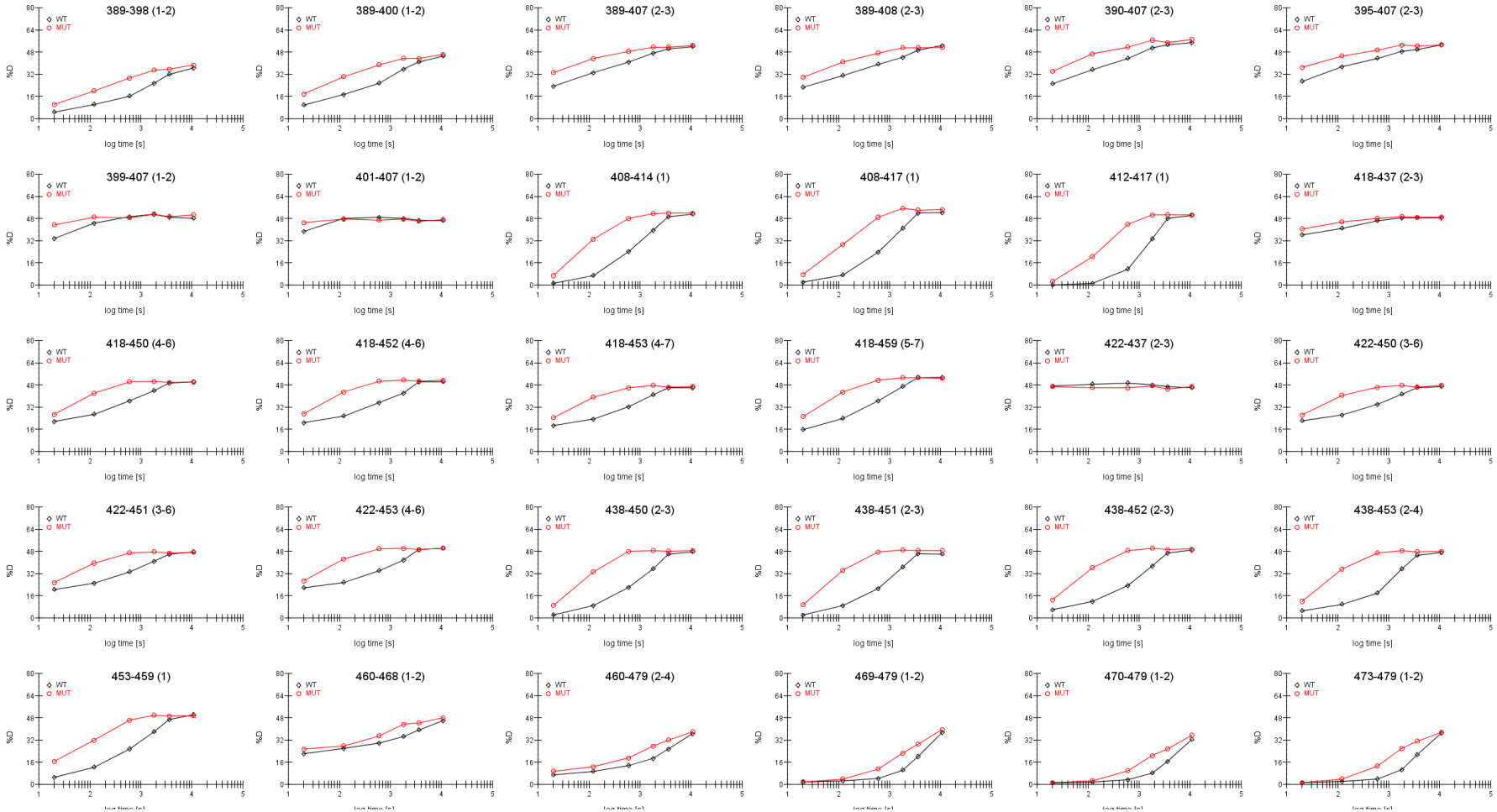
Digest every protein

Care about average peptide length, redundancy, reproducibility, suitability for ETD,…

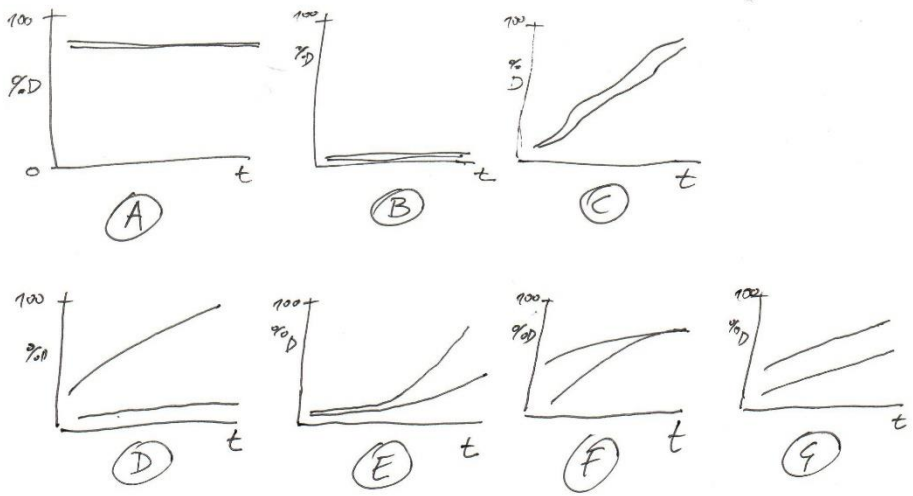
Cleavage after…



Output and data presentation – uptake plots



Output and data presentation – uptake plots



A: fast deuteration and remains constant - fast exchanging region, most likely no secondary structure

B: most probably deep in the structure - virtually no exchange. Can be also fast exchanger

C: structured region - deuterium uptake plots are somehow evolving

D-G: Various types of differences in deuteration

D: huge difference that appears already in the beginning and is getting even bigger during the exchange - clear difference is accessibility and H-bonding + signature of extremely stable binding of a ligand - the lower curve simply does not change while the upper one (for free protein) evolves

E: in contrast to D, this is poorly exchanging region with stable change upon ligand binding - it takes some time for the differences to appear but once they are there, the curves are separating

F: ligand binding occurs but is not so stable. so during the time of exchange we can observe dissociation events during which the protein has chance to gets deuterated.

G: stable change, similar to D

Back-exchange correction

Correction for back-exchange

$$\text{Corr \%D} = \frac{Mp - Mn}{Mf - Mn} * 100$$

$$\text{Corr No D} = \frac{Mp - Mn}{Mf - Mn} * Ns$$

Mp - MW partially deuterated
Mn - MW nondeuterated
Mf - MW fully deuterated (equilibrium)
Ns - number of exchangeable sites (Σamide bonds-Pro)

Important for comparison of different sequences, mutants

Not needed in typical comparison experiment

Needed when conclusions about protein folding or fold are made – helps to distinguish fast exchanging (in- as well as out- exchangers) and poor accessibility/strong hydrogen bodning

Protein incubated in D2O (under denaturing, heating,… conditions) and analyzed like the samples. Alternative – pre-digestion and deuteration of peptides!

In general between 15-25%

Back-exchange correction

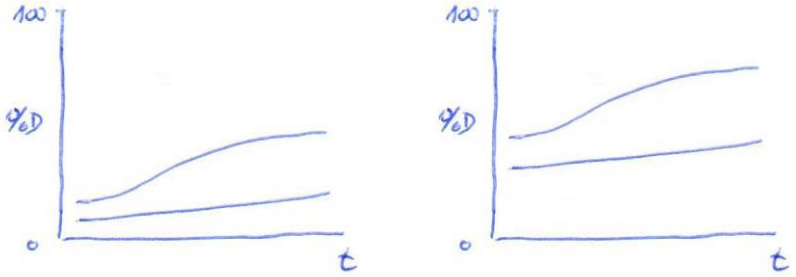
Correction for back-exchange

$$\text{Corr \%D} = \frac{Mp-Mn}{Mf-Mn} * 100$$

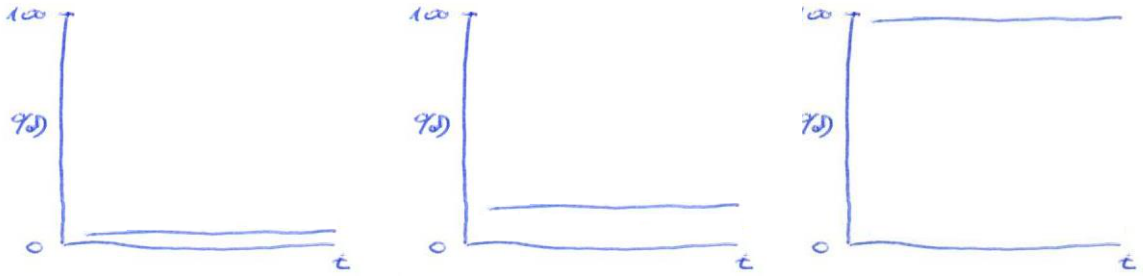
$$\text{Corr No D} = \frac{Mp-Mn}{Mf-Mn} * Ns$$

Mp - MW partially deuterated
Mn - MW nondeuterated
Mf - MW fully deuterated (equilibrium)
Ns - number of exchangeable sites (Σamide bonds-Pro)

Examples – before (left) and after (right) correction to BE – overall level raises + difference is slightly bigger (due to bigger y-scale)

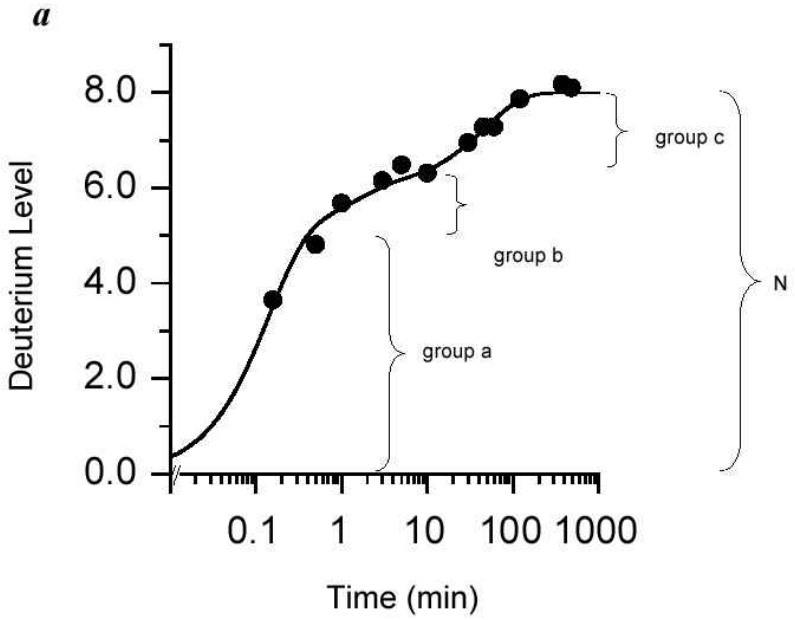


Examples – slow and fast exchanger
left – before BE correction hard to judge; middle – slow exchanger (deep in the structure) after correction; right – fast exchanger – e.g. HisTag - after correction



Datamining

a



If fine sampling in HDX is used, the curve can be fitted and number of fast, slow and intermediate exchanging amides can be deduced

b

$$D = N - \sum_{i=1}^{N} \exp^{-k_i t}$$

c

$$D = 8 - \left[\underbrace{(5\,(e^{-(m1)\cdot t}))}_{\text{group a}} + \underbrace{(1\,(e^{-(m2)\cdot t}))}_{\text{group b}} + \underbrace{(2\,(e^{-(m3)\cdot t}))}_{\text{group c}} \right]$$

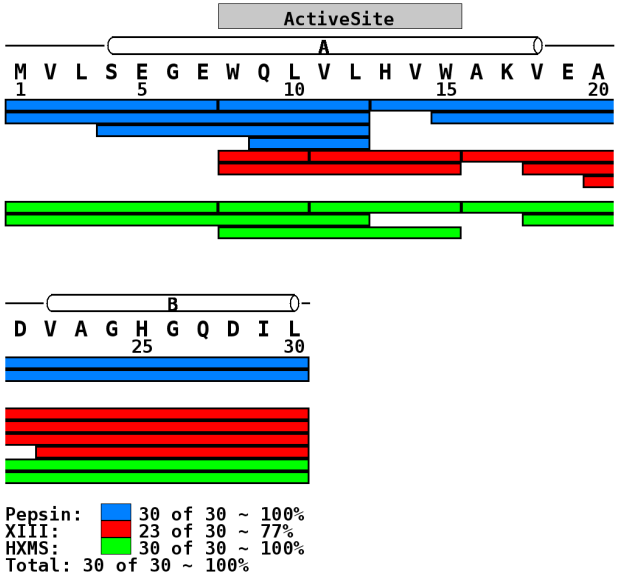
group a
m1=7.21 ± 0.82 min^{-1}
$t_{1/2}$=0.096 min

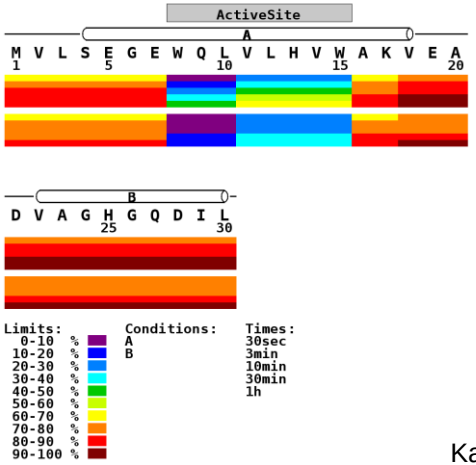
group b
m2=0.77 ± 0.32 min^{-1}
$t_{1/2}$=0.90 min

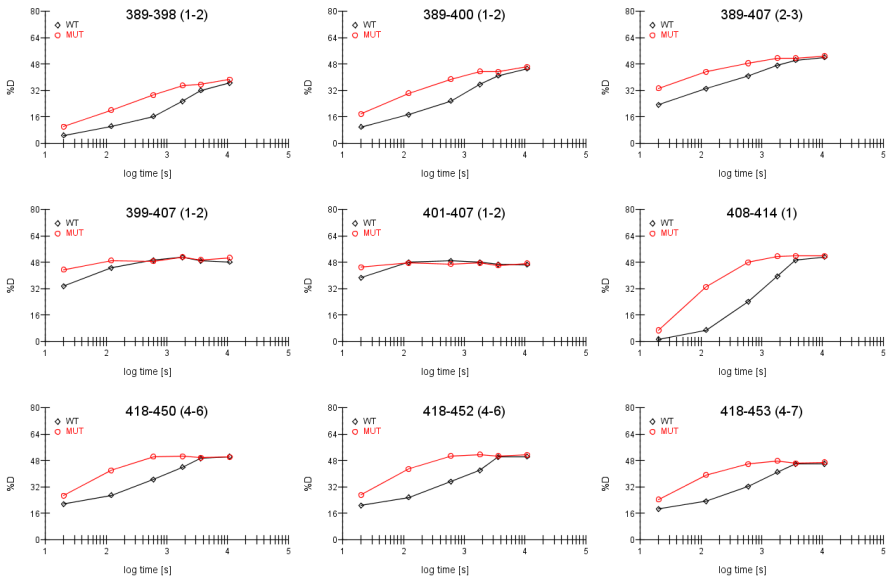
group c
m3=0.021 ± 0.003 min^{-1}
$t_{1/2}$=33.0 min

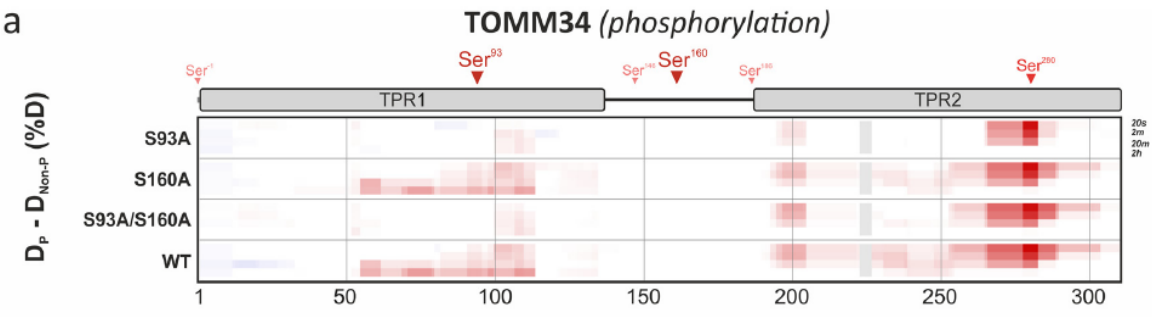
Data presentation

http://peterslab.org/MSTools/



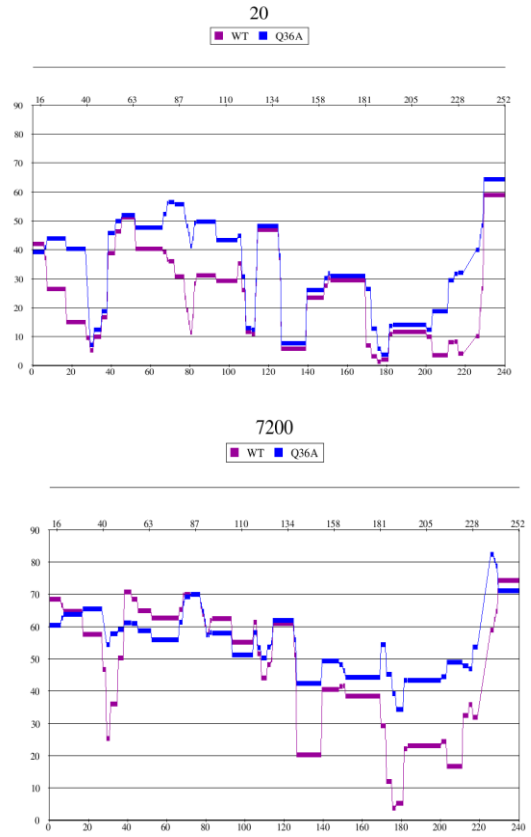


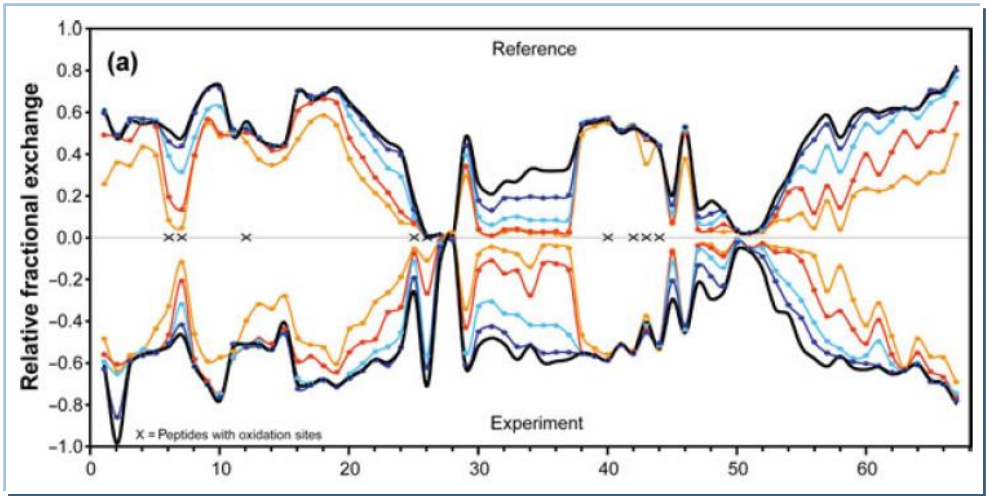


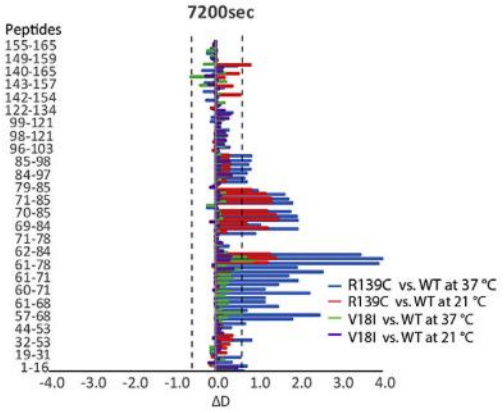


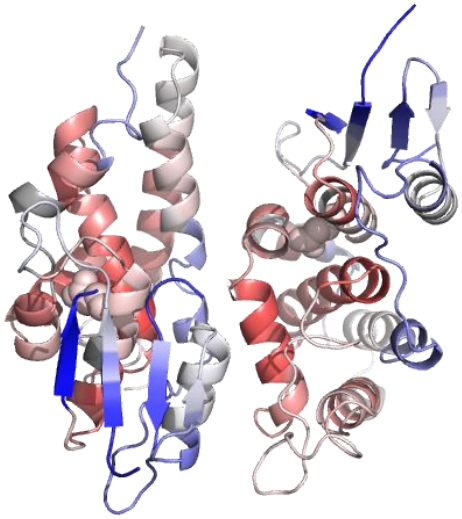
Kavan, D. and Man, P. Int. J. Mass Spectrom. 2011, 302: 53-58. http://dx.doi.org/10.1016/j.ijms.2010.07.030.

Data presentation

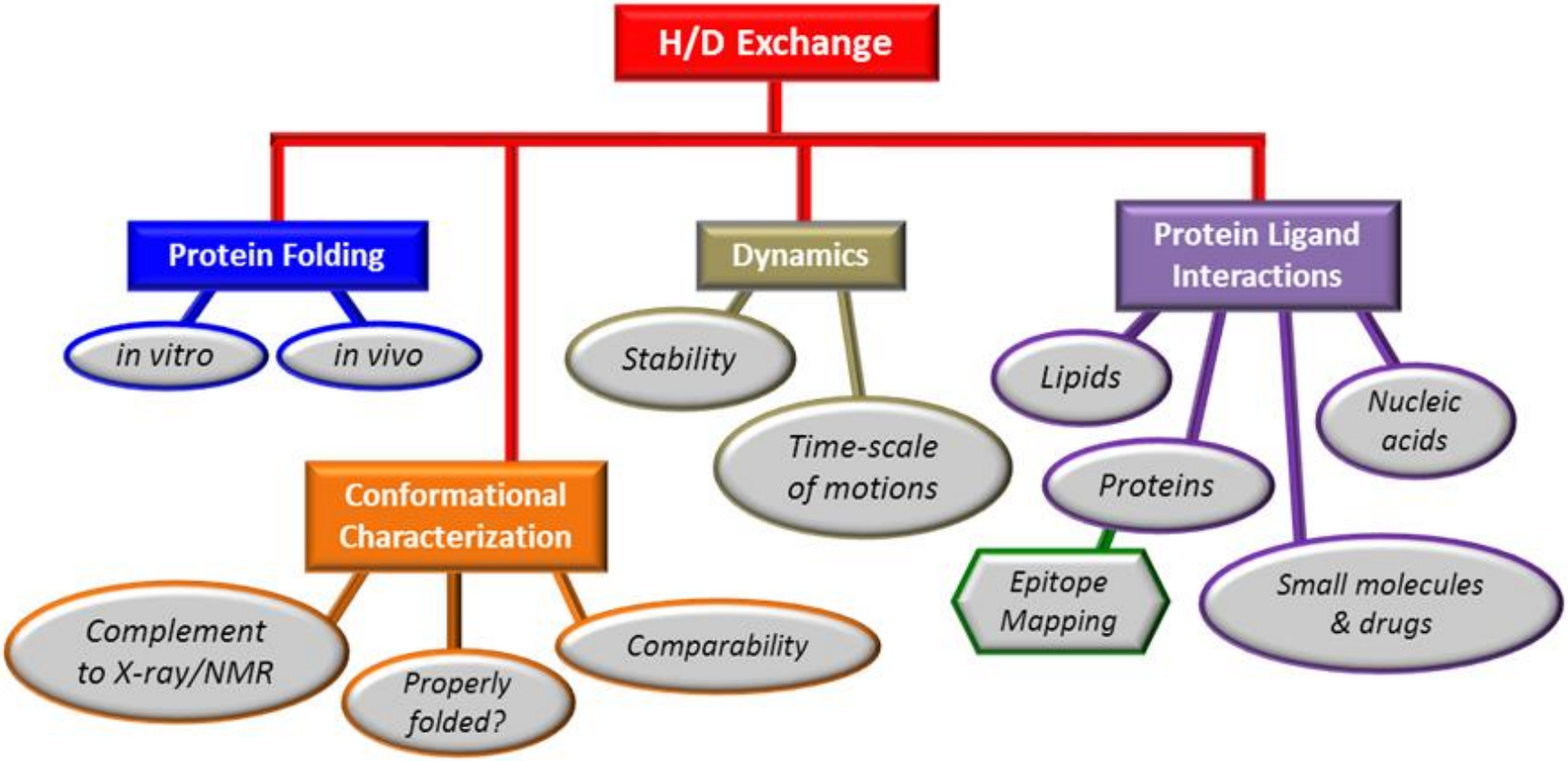








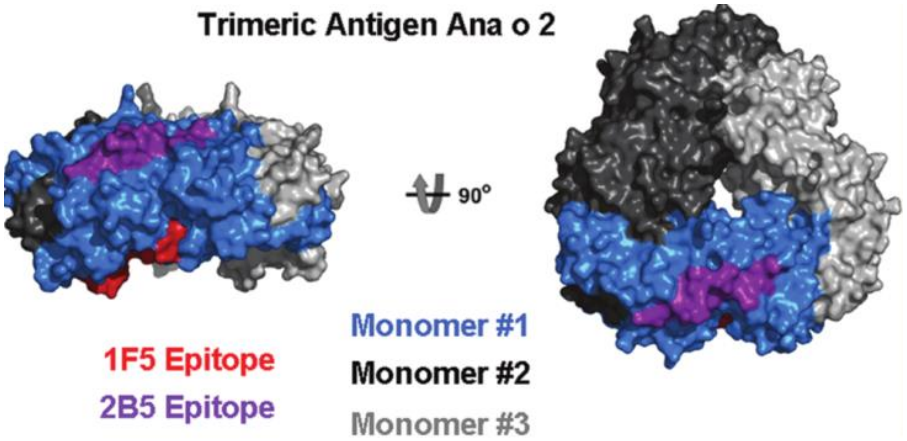
What is it good for?

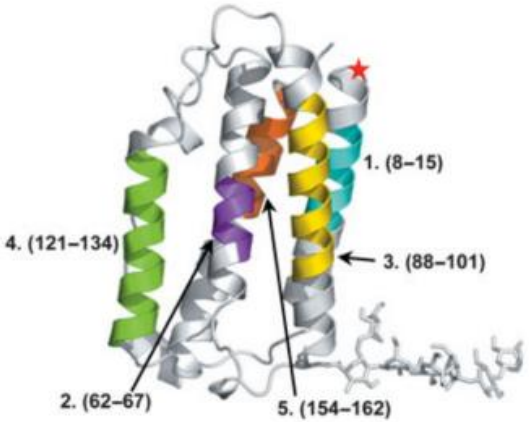


Engen et al. (2011). *Ency. Anal. Chem.* ISBN 978-04-700-2731.

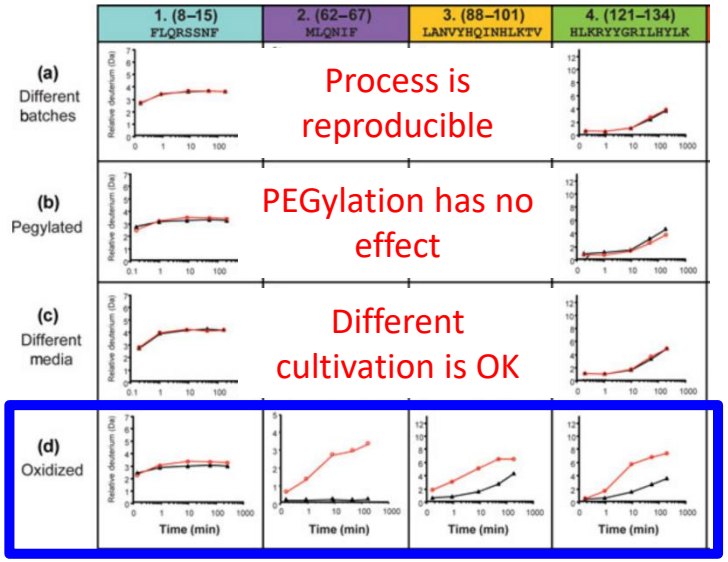
Biologicals, anitbodies, epitope mapping

EPITOPE MAPPING





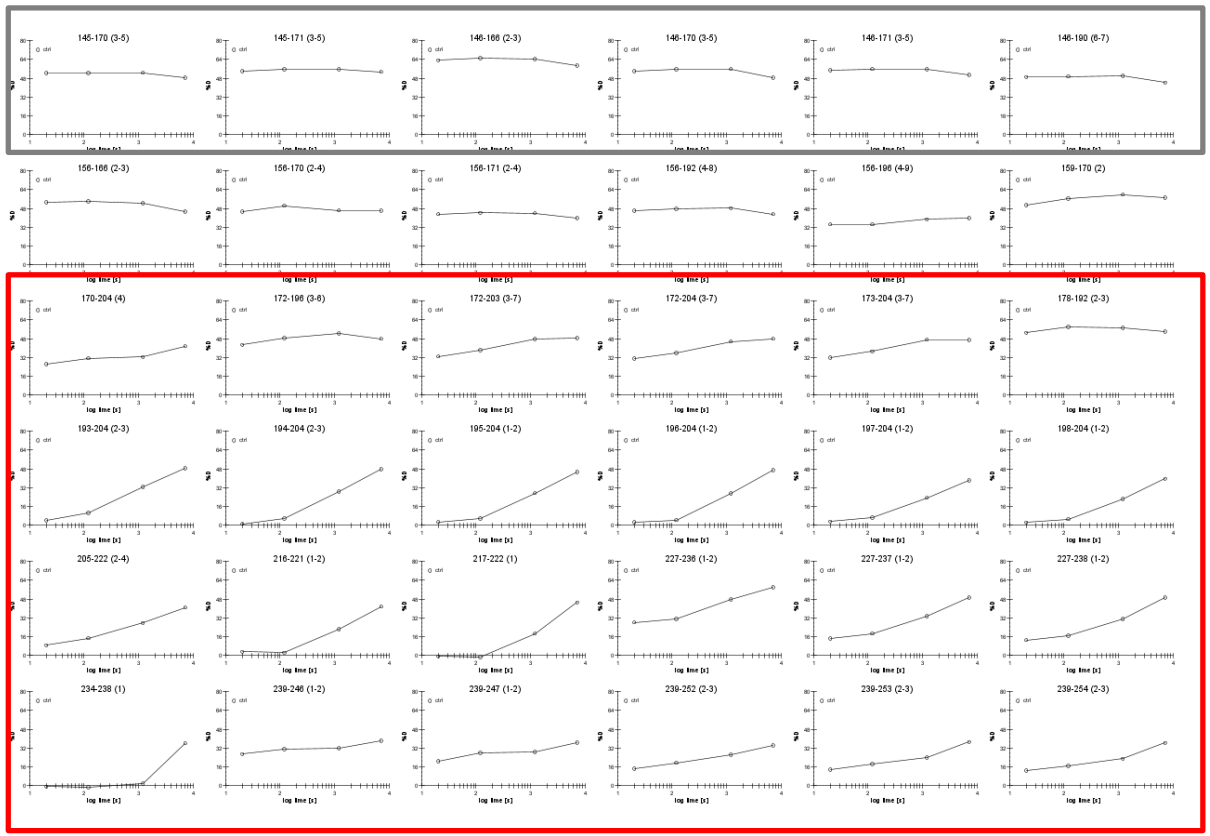
BIOSIMILARS



!oxidation!

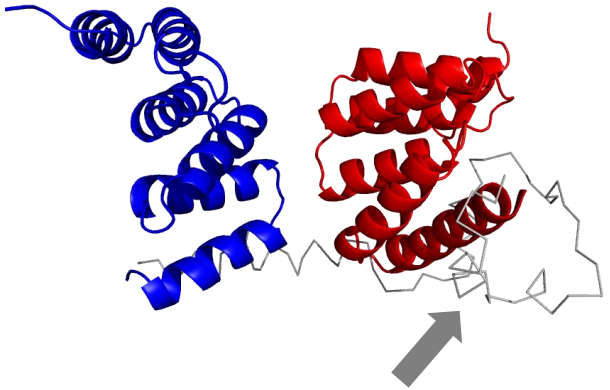
Zhang *et al.*: Anal. Chem. (2011) Houde *et al.*: J. Pharm. Sci. (2011)

Protein structuration



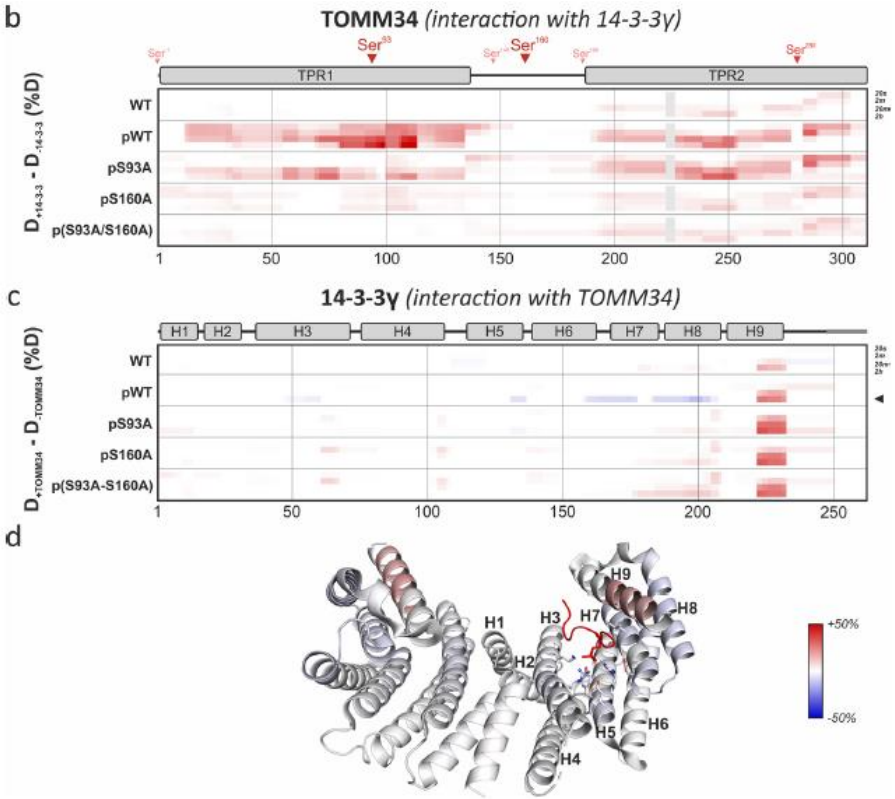
The easiest and simplest setup + data interpretation – HDX profile of a single protein

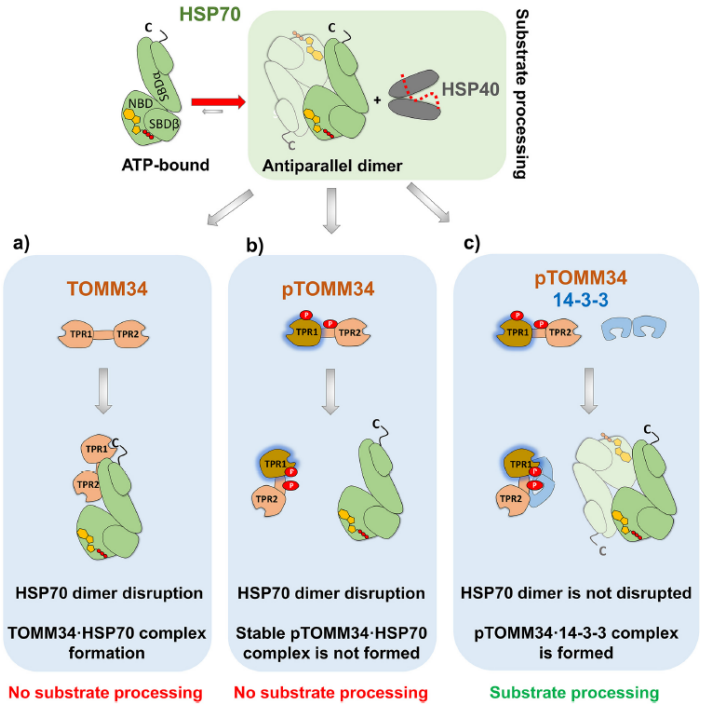
Tomm34 – structured vs unstructured regions



Protein:protein interactions + effect of modifications

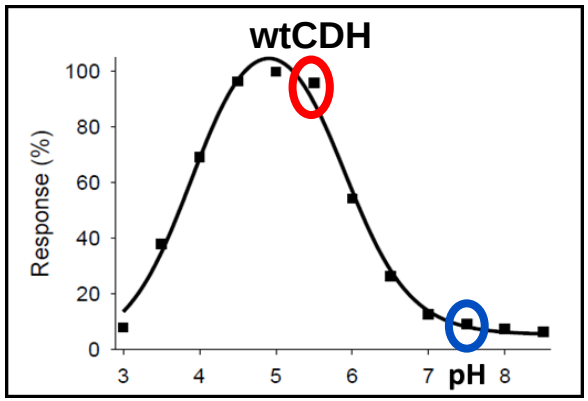
Phosphorylated TOMM34 interacts with 14-3-3 through its unstructured linker (no change in HDX detected) but it leads to overall structure “opening”. Weak protection is observed on14-3-3.

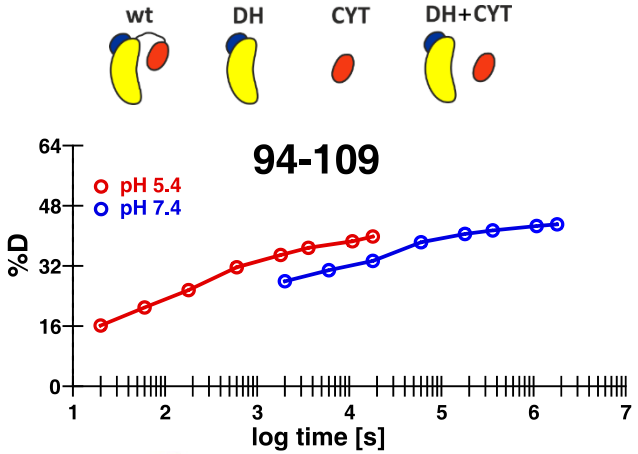




Intraprotein interaction

Cellobiose dehydrogenase – pH dependent intraprotein domain-domain interaction = protection. But it is all different!

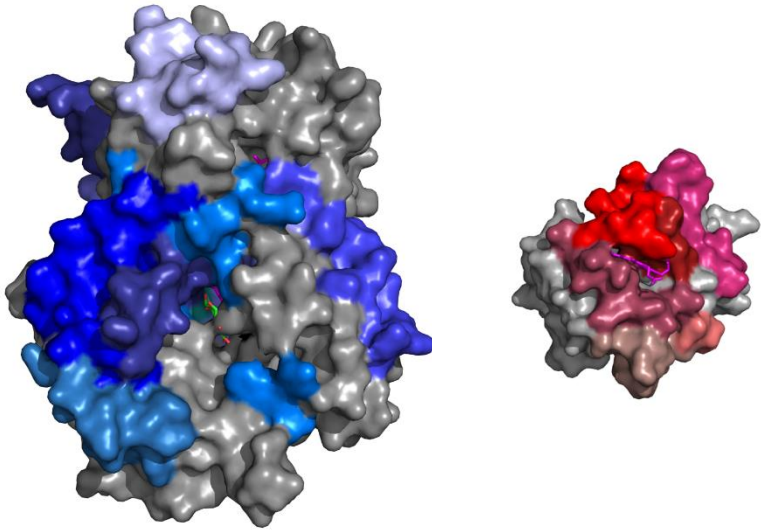




No protection upon the interaction

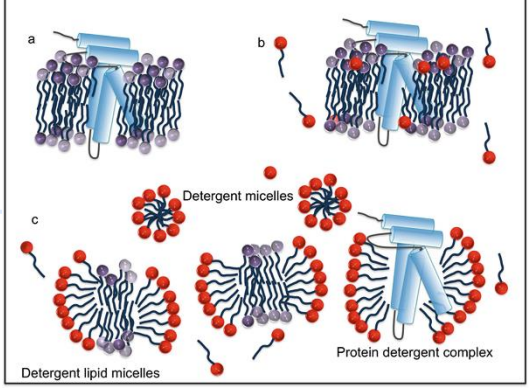
Deprotection around the proposed interacting surfaces

Protonation (charge neutralization) enables the interaction…



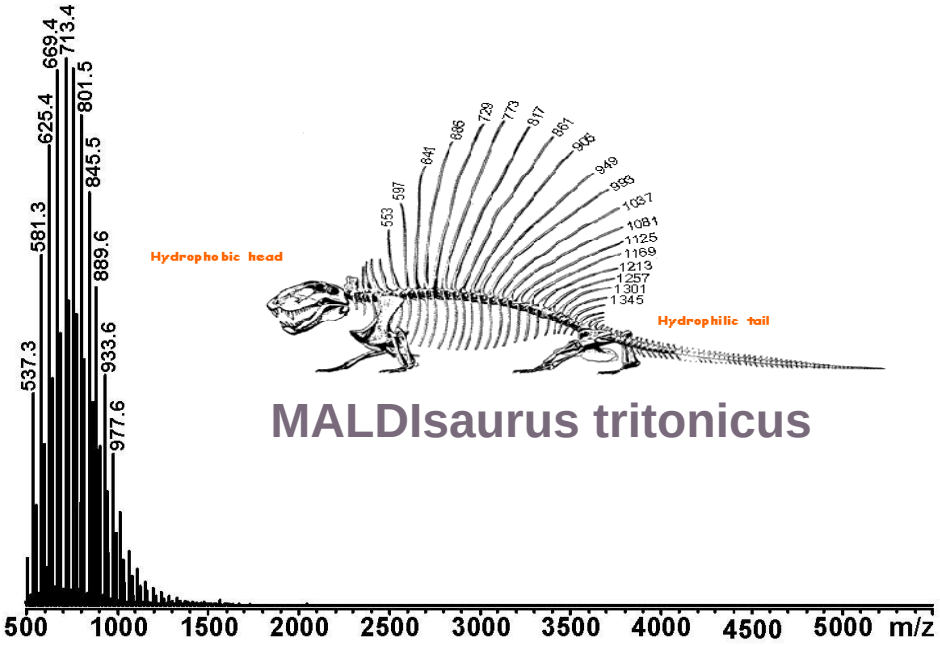
Kadek A et al BBA 2017

Membrane proteins



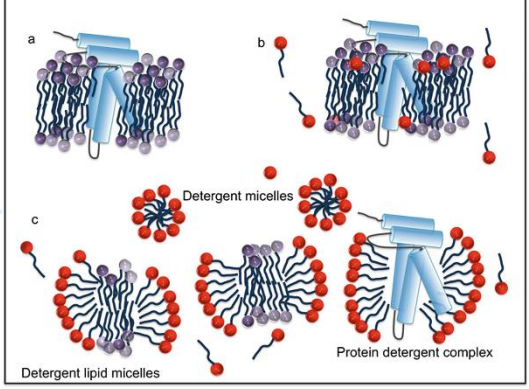
Detergent solubilized

- not all detergents are MS-friendly (CHAPS, cholate, SDS)
- not all detergents can be removed prior to the MS
- fancy detergents with very low CMC likely have “issues”



Rey M. et al. Anal. Chem. 2010

Membrane proteins



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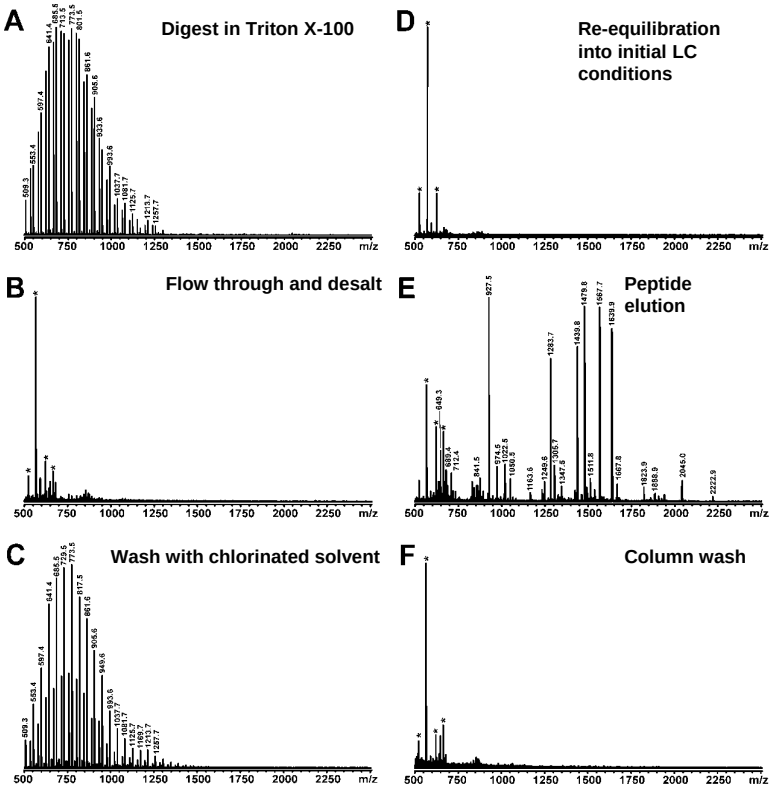
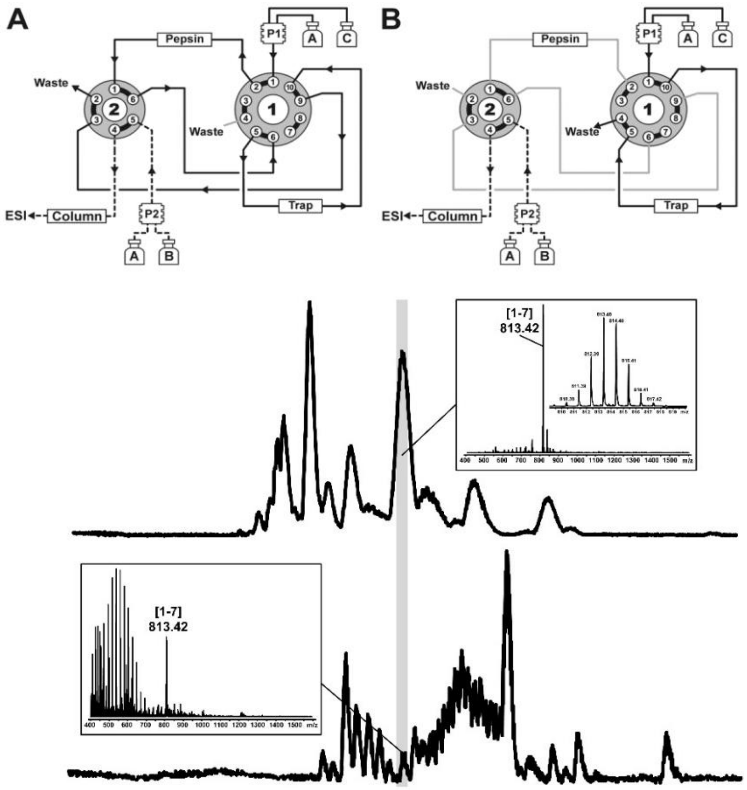


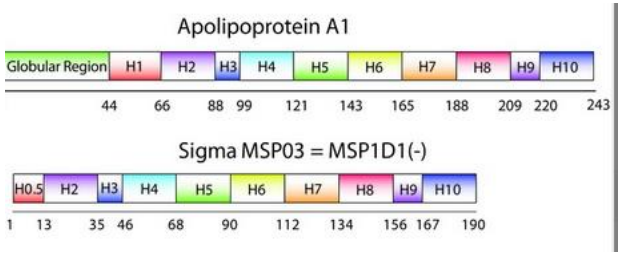


Rey M. et al. Anal. Chem. 2010

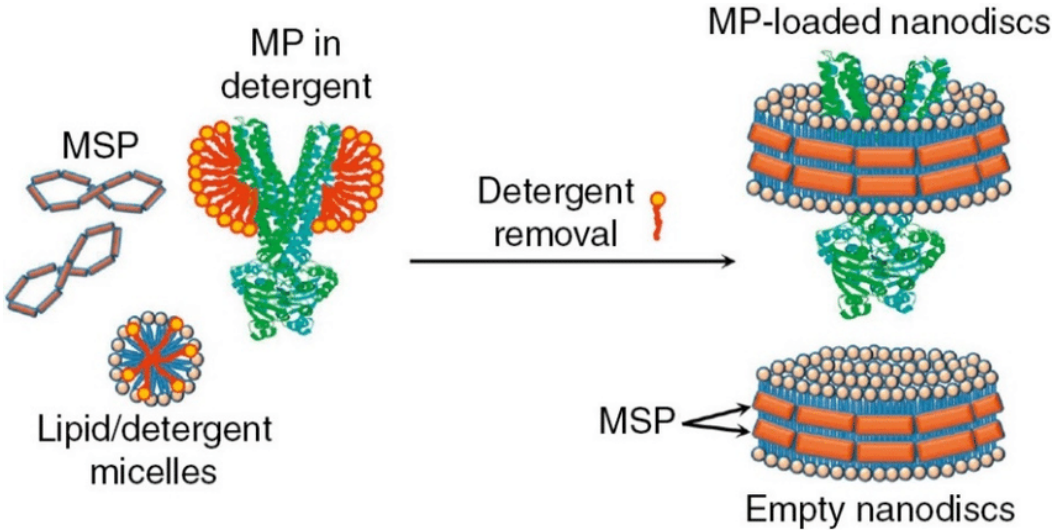
EU FT-ICR MS

Membrane proteins

Nanodiscs – membrane scaffolding protein + lipids



Many different (size/no of repeats) MSPs, various lipid compositions



Alternatives to MSP

SMALP (styrene maleic acid/anhydride copolymer)

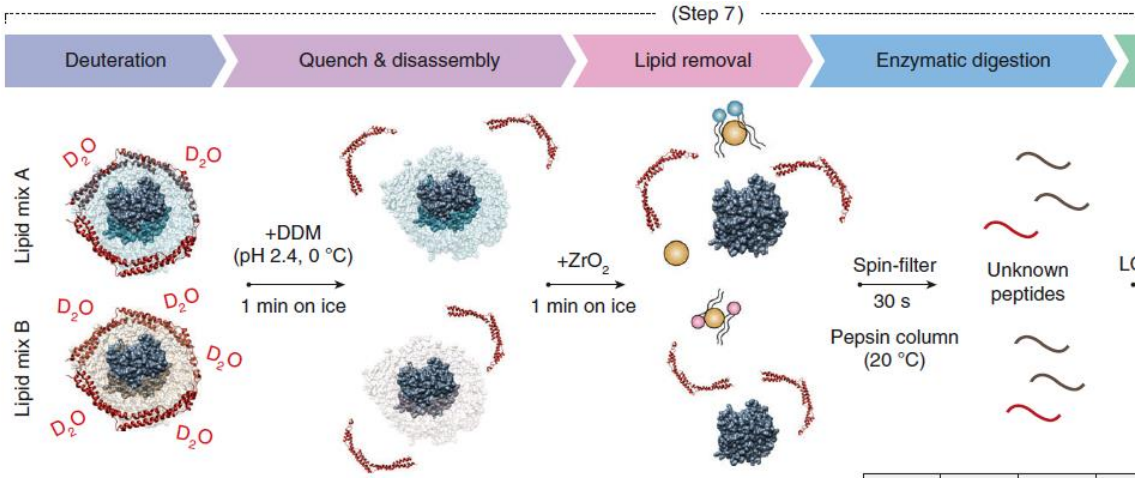
SaposinA

Hebling CM et al Anal Chem 2010
Harrison RA et al Curr Opin Struct Biol 2016
Martens C et al Nat Protocols 2019

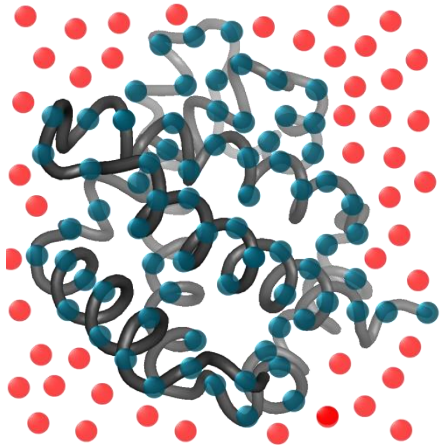
Membrane proteins

Nanodiscs – protocol *(Martens C et al Nat Protocols 2019)*

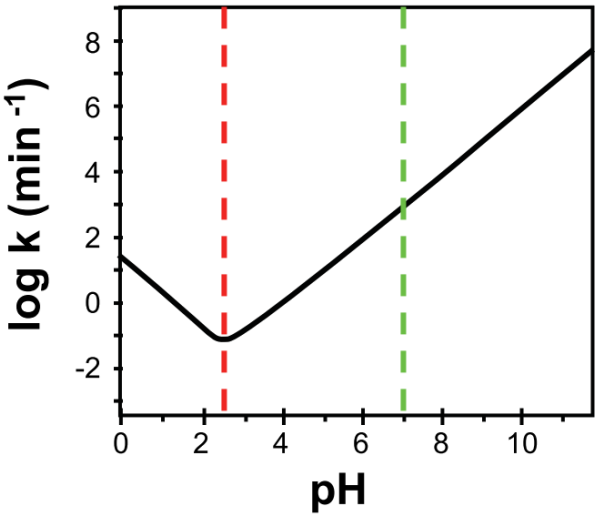
- Quench + disassembly (detergent)
- Lipid removal by ZrO2 coated resin
- ZrO2 particles filtering
- Digestions (eventually done during lipid removal)



Pros and cons of HDX-MS



D_2O buffer



$$k(T_2) = k(T_1)exp^{-\frac{E_a}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right)}$$

1 ΔpH … 10×
10°C … 3×

Buffer – can be whatever
Protein size – does not matter
Protein concentration – can be very low
Mixture compatible – many proteins
Temperature or pH – can be whatever
(correction must be done - H/D depends on pH and T)
Commercial solutions available

Dilutions – into D_2O and to lower the pH
Protein concentration – not all complexes are formed at given concentration (K_D)
Comparison – at least two states required

H2020

EUROPEAN NETWORK OF FOURIER-TRANSFORM ION-CYCLOTRON-RESONANCE MASS SPECTROMETRY CENTERS

PROJECT AGREEMENT NO.731077