Structural mass spectrometry

hydrogen/deuterium exchange

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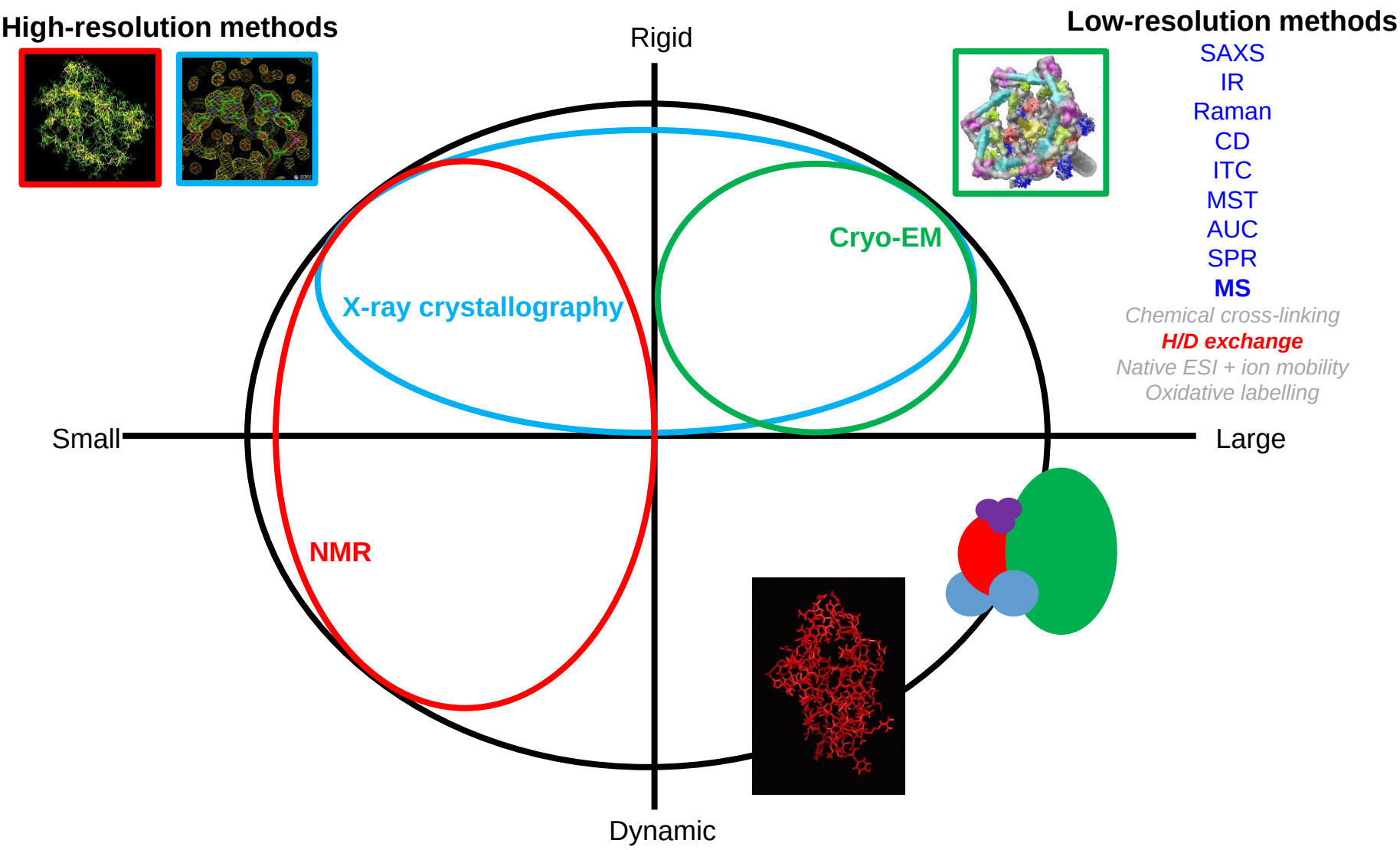
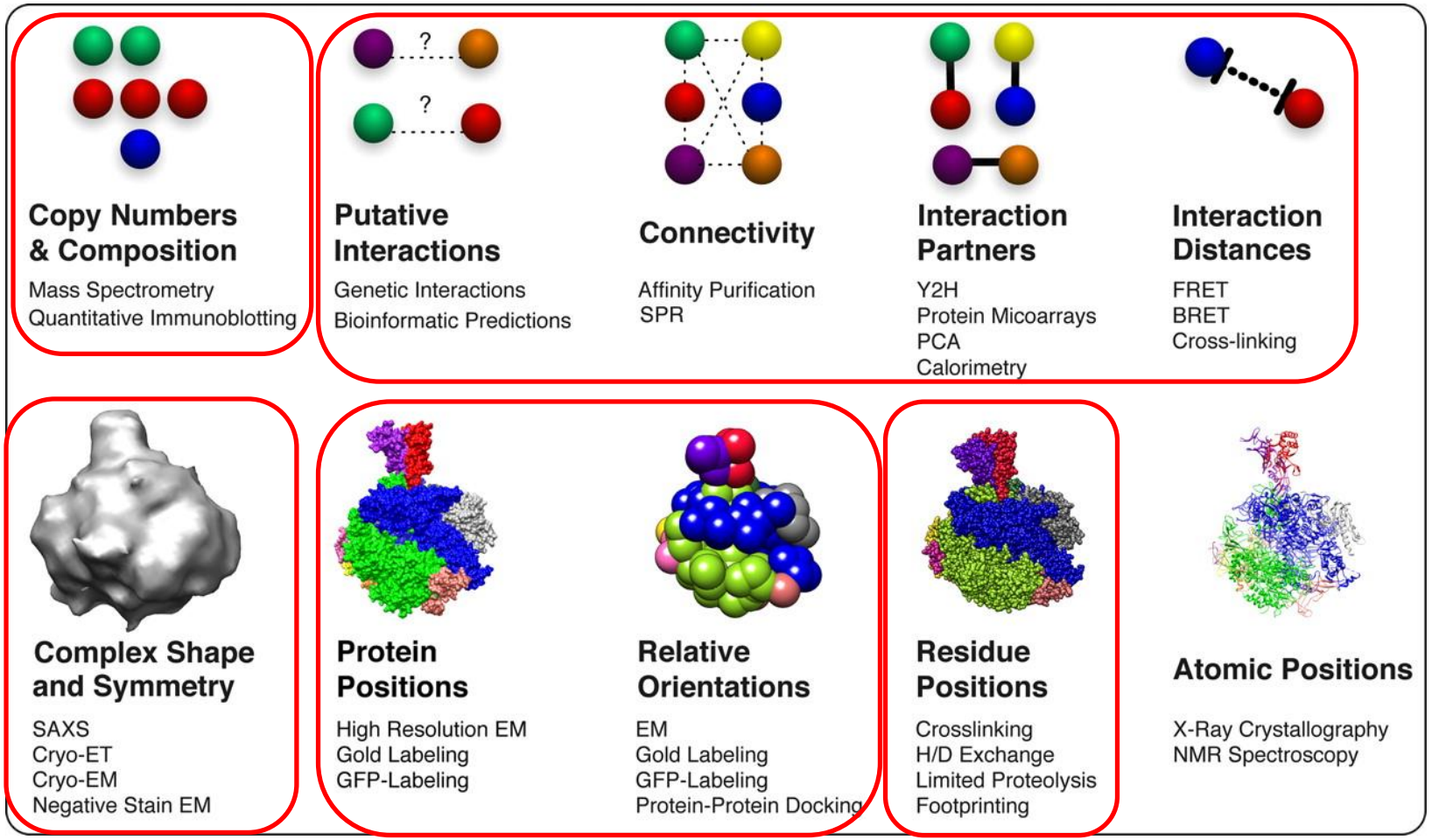
Structural biology methods



Structural biology approaches

Simple MS, quantitative MS

Cross-linking, top-down, native MS+dissociation

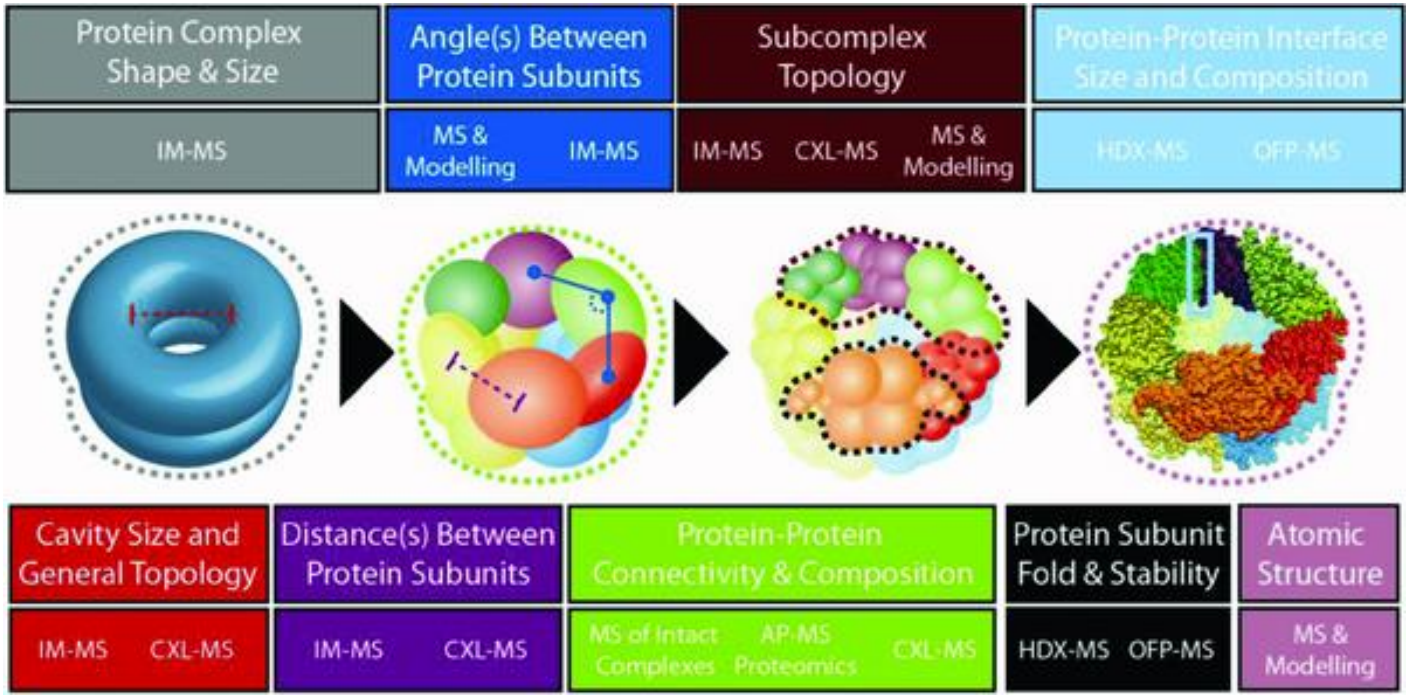


native MS+ion mobility

Cross-linking

Structural MS

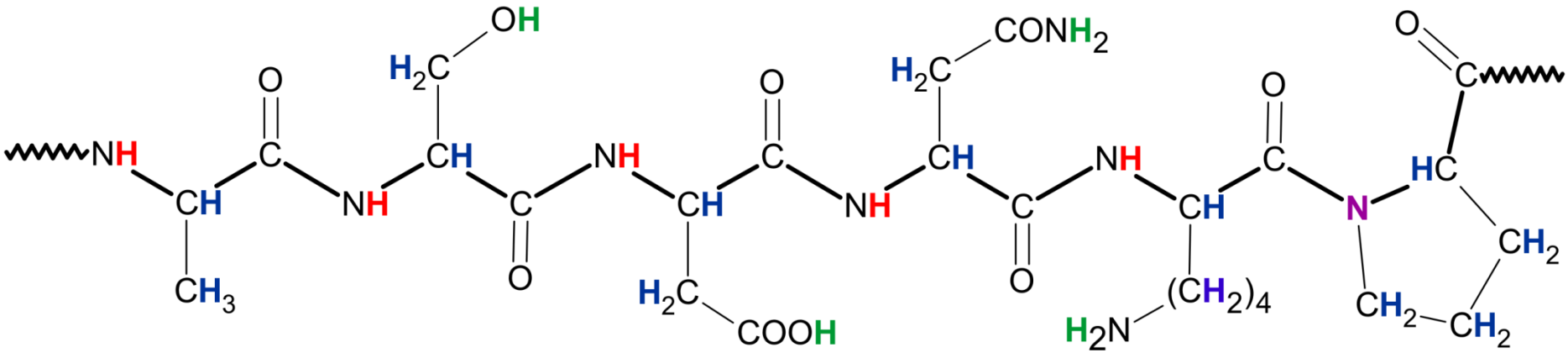
What can we get using mass spectrometry



IM – ion mobility
CXL – chemical cross-linking
AP – afinity purification
OFP – oxidative footprinting
HDX – hydrogen/deuterium exchange

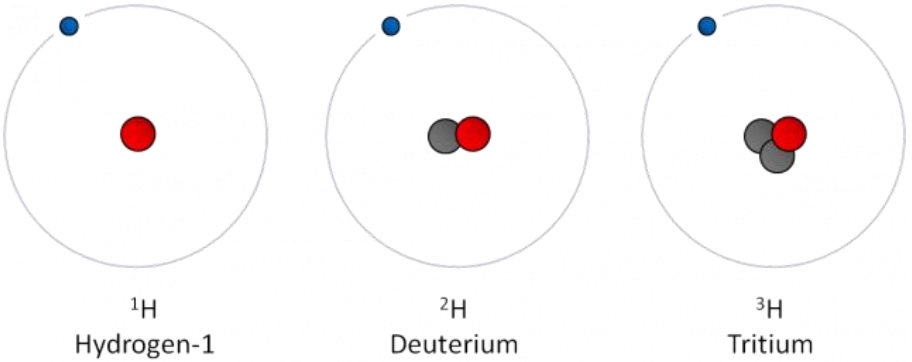
ISOTOPE EXCHANGE IN PROTEINS

~ Ala – Ser – Asp – Asn – Lys – Pro ~



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

	^{1}H	^{2}H	^{3}H
occurence [%]	99.9885	0.0115	trace

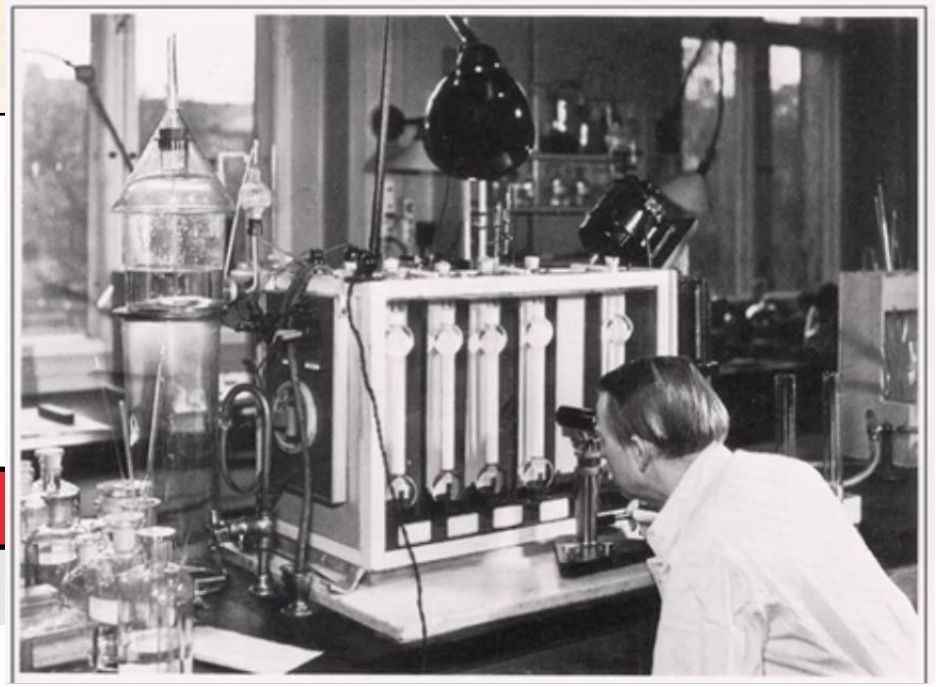


…Kaj Ulrik Linderstrøm-Lang

EXCHANGE OF HYDROGEN ATOMS IN INSULIN WITH DEUTERIUM ATOMS IN AQUEOUS SOLUTIONS

by

AASE HVIDT AND K. LINDERSTRØM-LANG
Carlsberg Laboratorium, Copenhagen (Denmark)

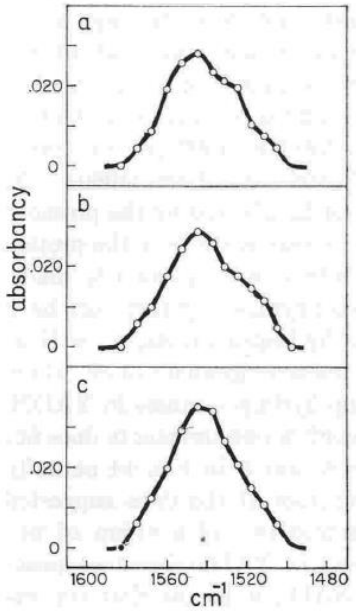


„Cartesian diver“
Proteins are migrating in tubes with density gradient until they stop at the point where the densities are equal

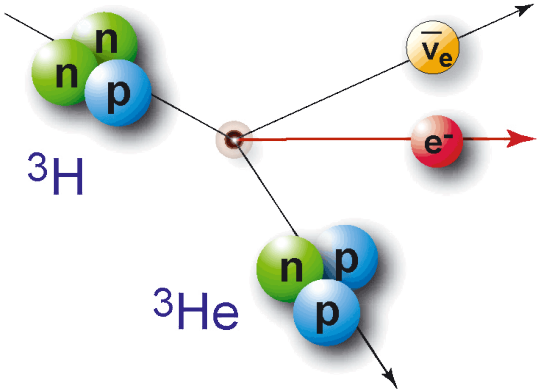
	^{1}H	^{2}H	^{3}H
%	99.9885	0.0115	trace
density [g/cm^3]	1.000	1.106	1.215

Methods of detection

IR:



β-:



NMR:

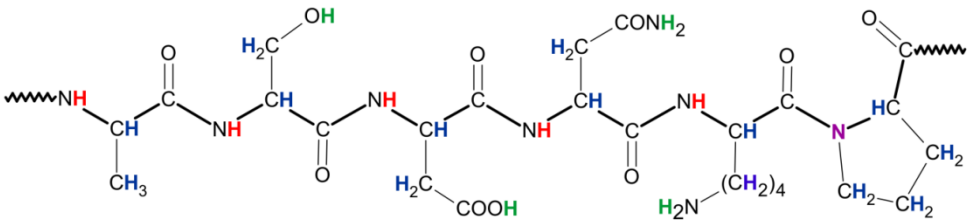
1 n = 1.6749 × 10^{-27} kg

MS:

	^{1}H	^{2}H	^{3}H
%	99.9885	0.0115	trace
density [g/cm^3]	1.000	1.106	1.215
spin	½+	1+	½+
mass [u]	1.00783	2.01410	3.01605

Factors affecting H/D exchange

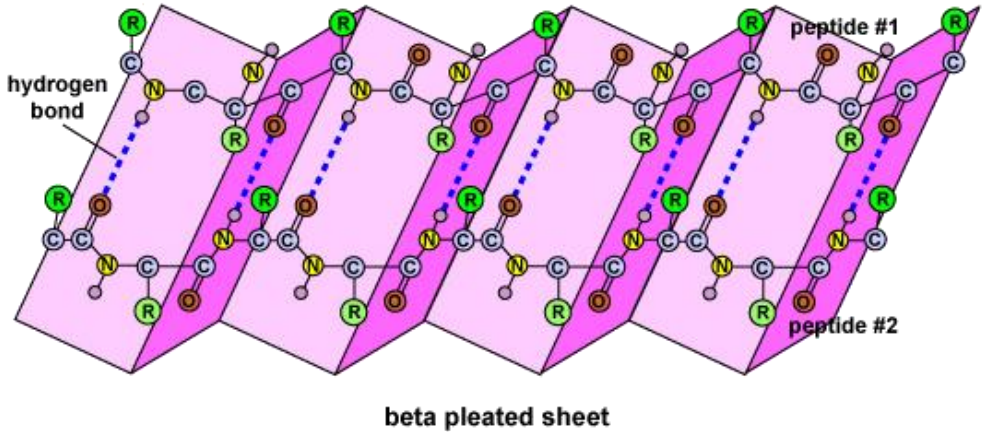
~ Ala – Ser – Asp – Asn – Lys – Pro ~



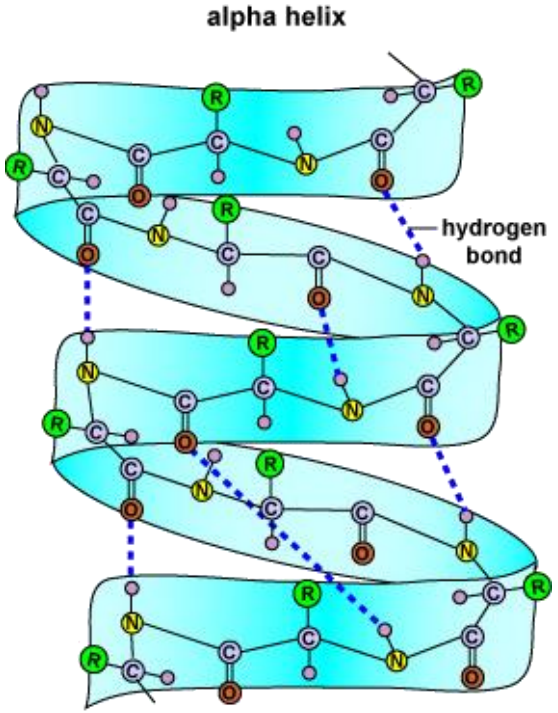
H: slow **H**: fast **H**: medium

hydrogen bonding

solvent accessibility



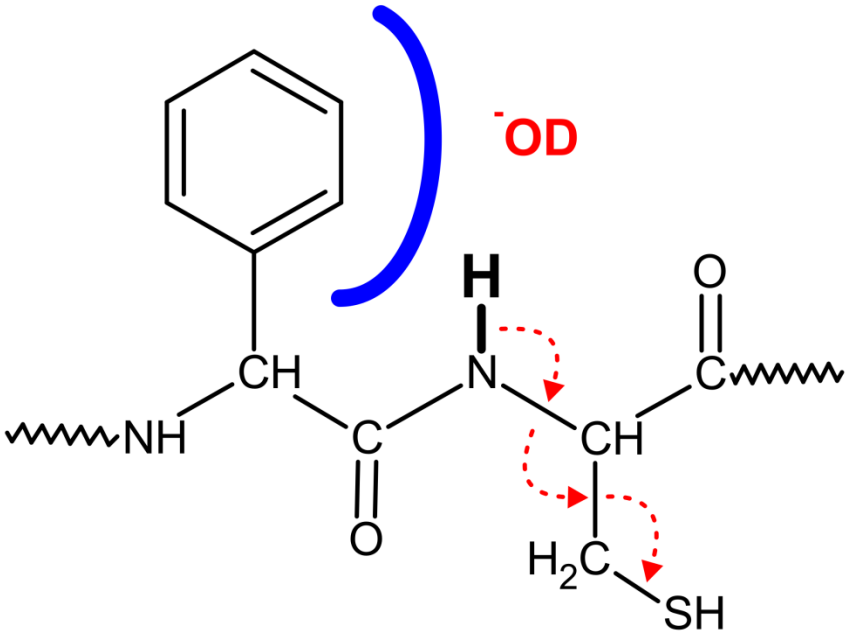
beta pleated sheet



Factors affecting H/D exchange

Side chains

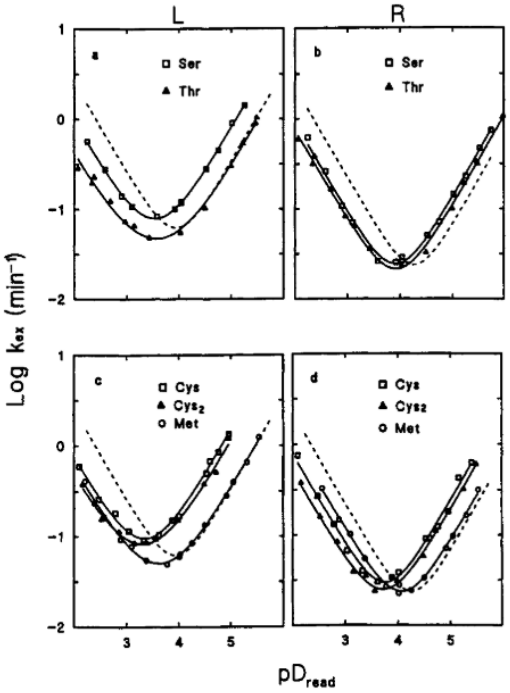
(acidity, steric shielding)



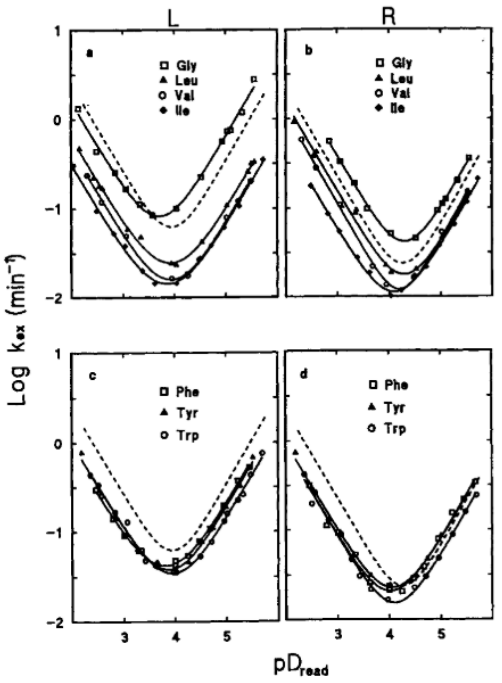
Bai *et al.*: Proteins (1993)

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

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)

Factors affecting H/D exchange

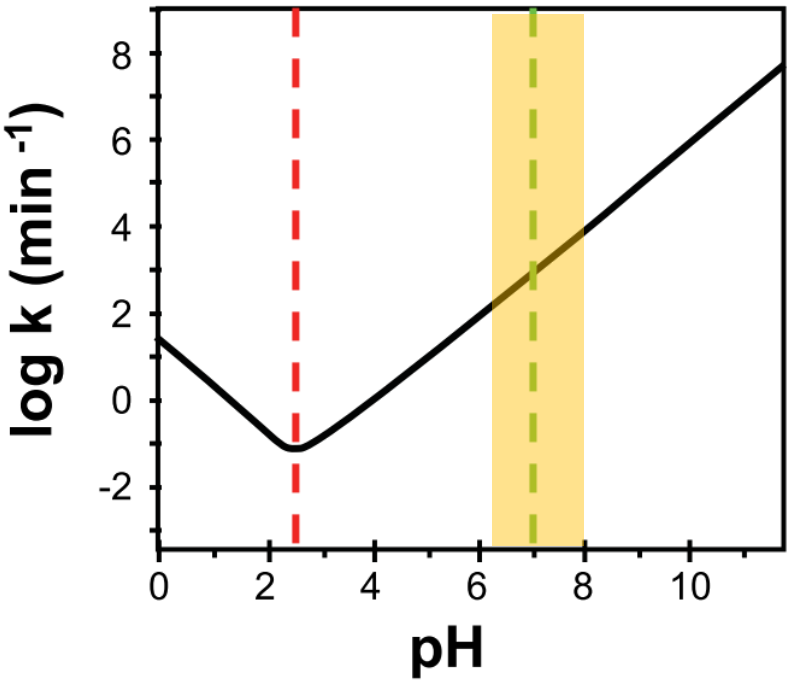
Temperature

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

1 ΔpH ... 10×
10°C ... 3×

pH

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

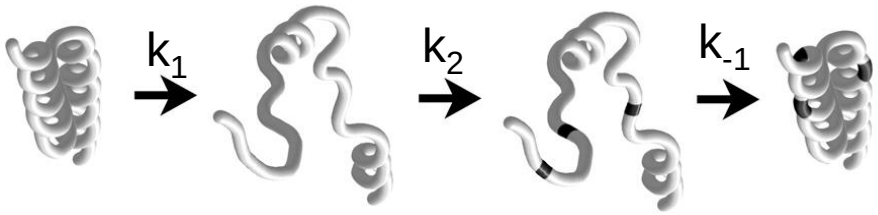


Bai *et al.*: Proteins (1993)

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

H/D EXCHANGE 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

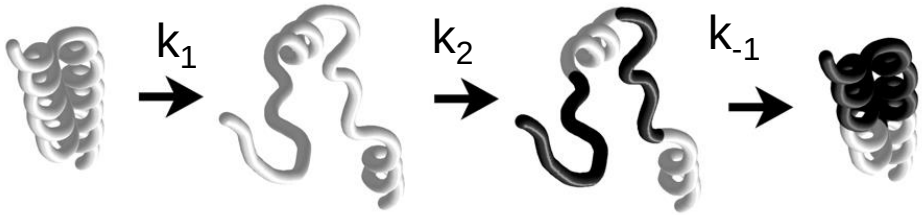
$k_{-1} >> k_2$

uncorrelated

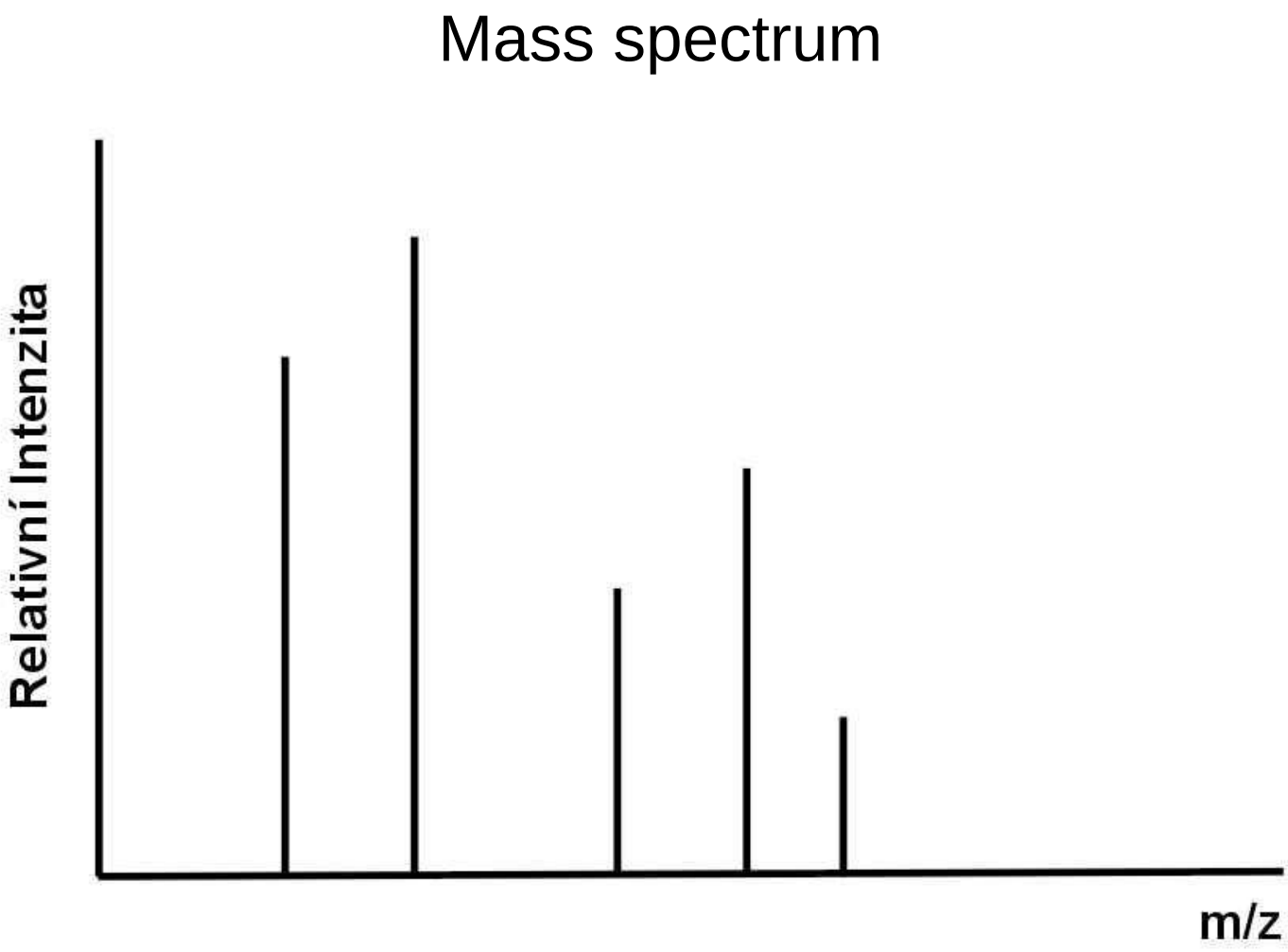
EX1

$k_{-1} << k_2$

correlated

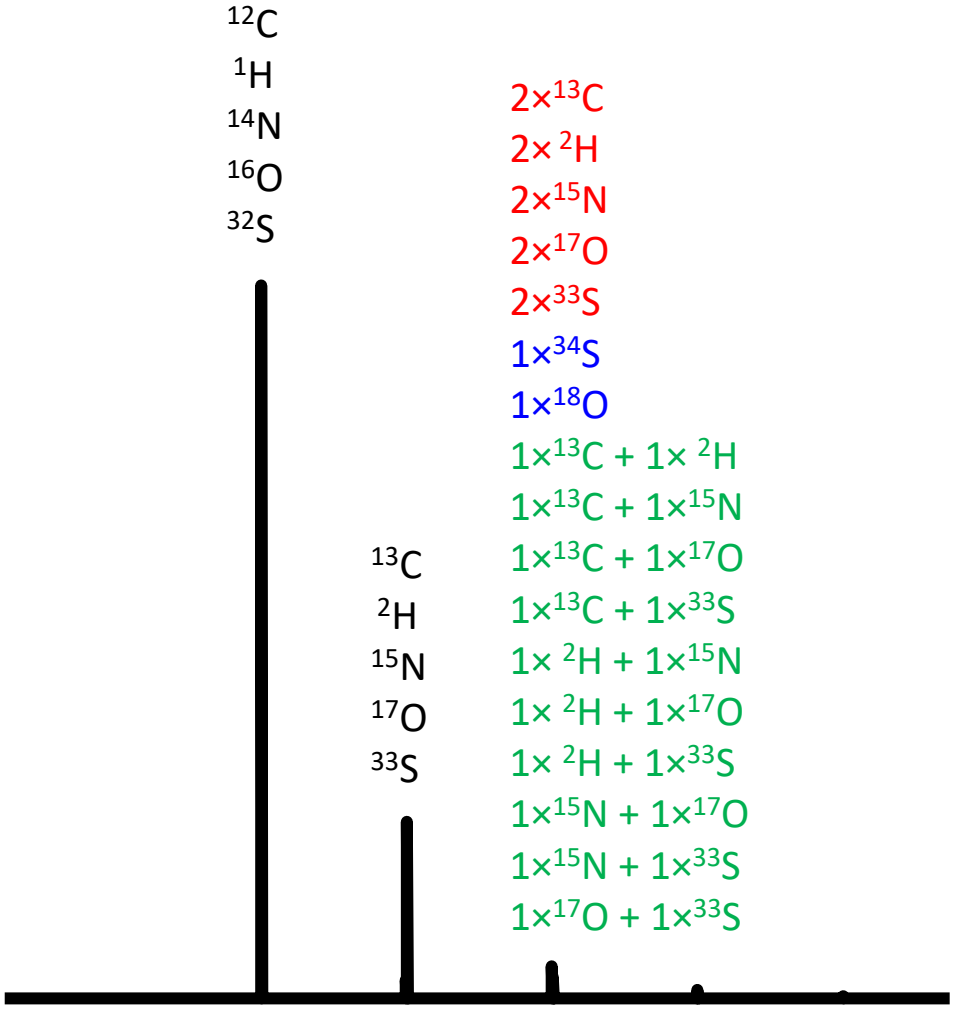


Isotope pattern



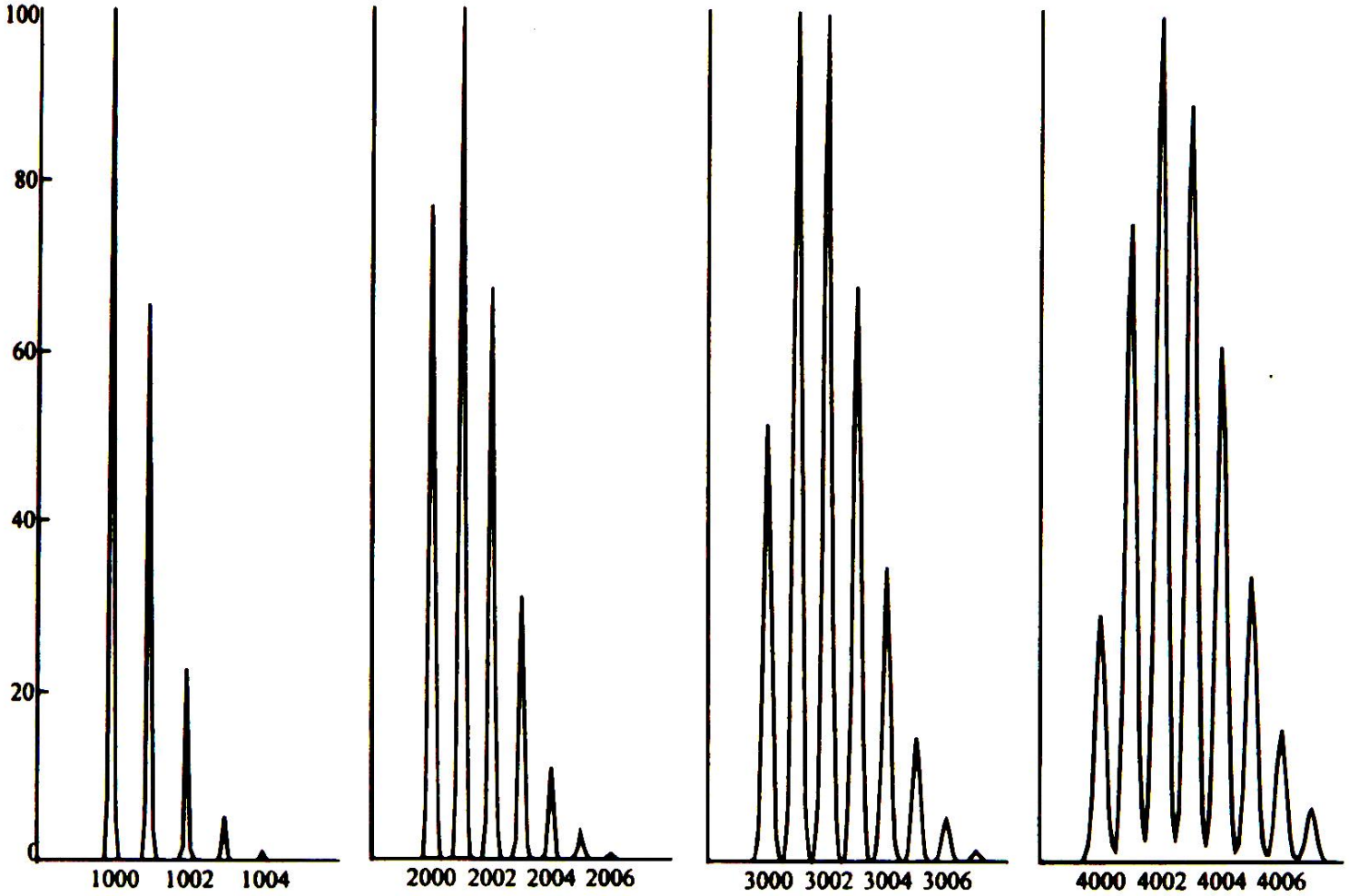
Isotope pattern

Mass spectrum in detail

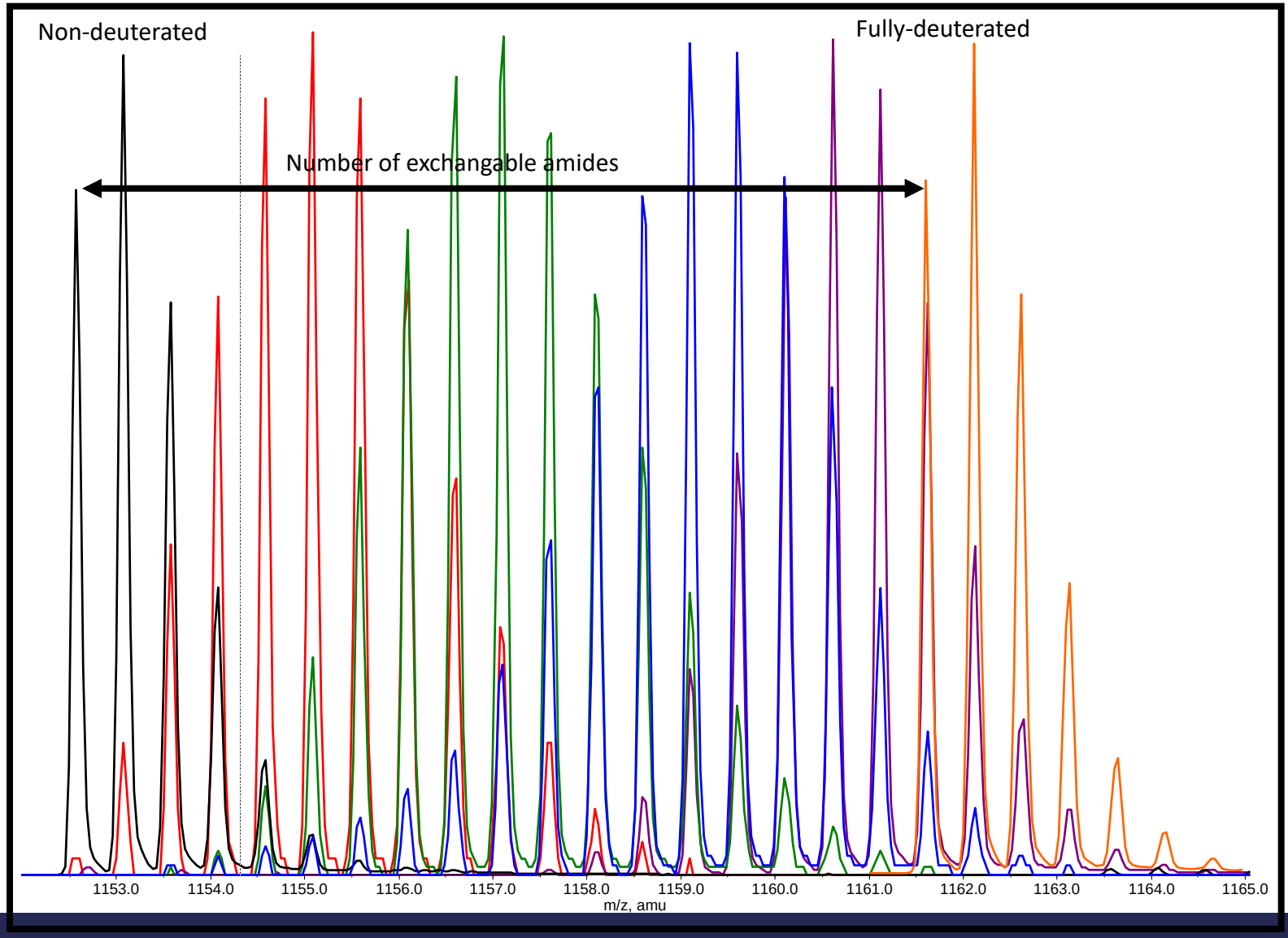


Symbol	M_{nom}	M_{mono}	%
C	12	12.00000	98.9300
	13	13.00336	1.0700
H	1	1.00783	99.9885
	2	2.01411	0.1150
N	14	14.00307	99.6320
	15	15.00011	0.3680
O	16	15.99492	99.7570
	17	16.99913	0.0380
	18	17.99916	0.2050
S	32	31.97207	94.9300
	33	32.97146	0.7600
	34	33.96787	4.2900
	36	35.96708	0.0200
P	31	30.97376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

Isotope pattern at increasing mass

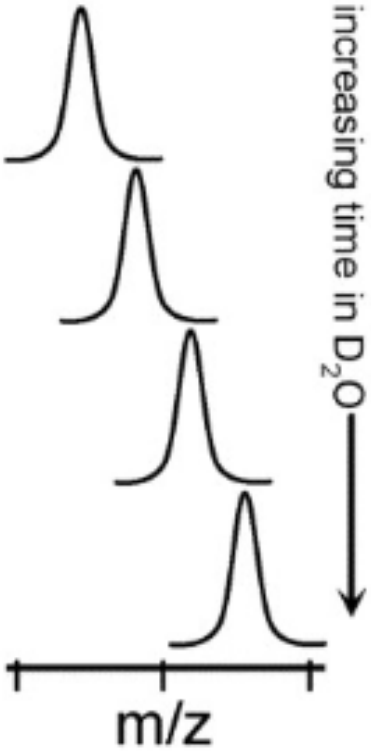


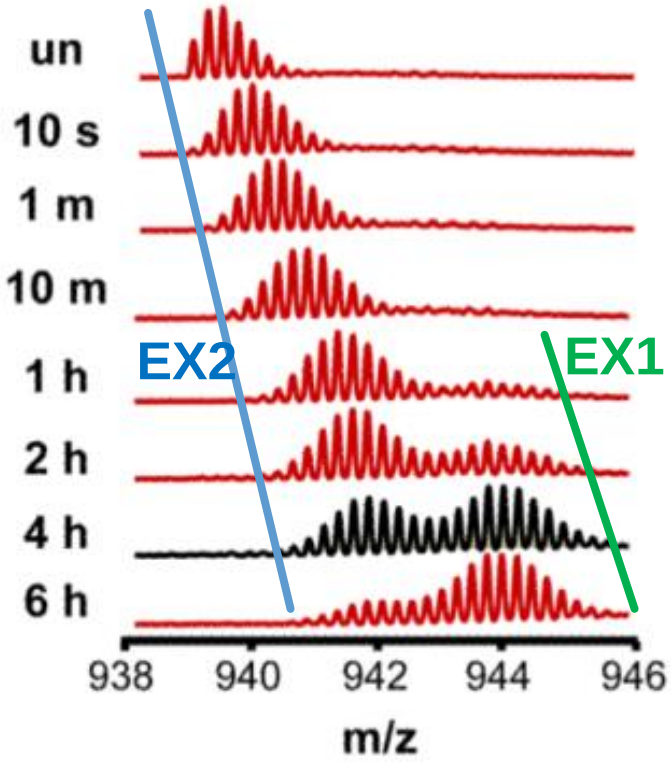
Isotope pattern changes



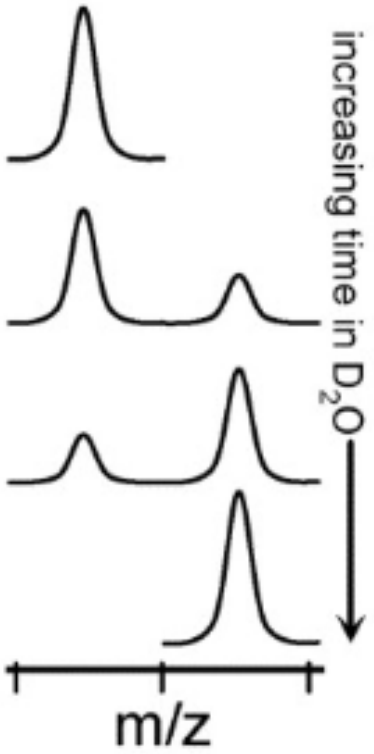
H/D EXCHANGE KINETICS

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



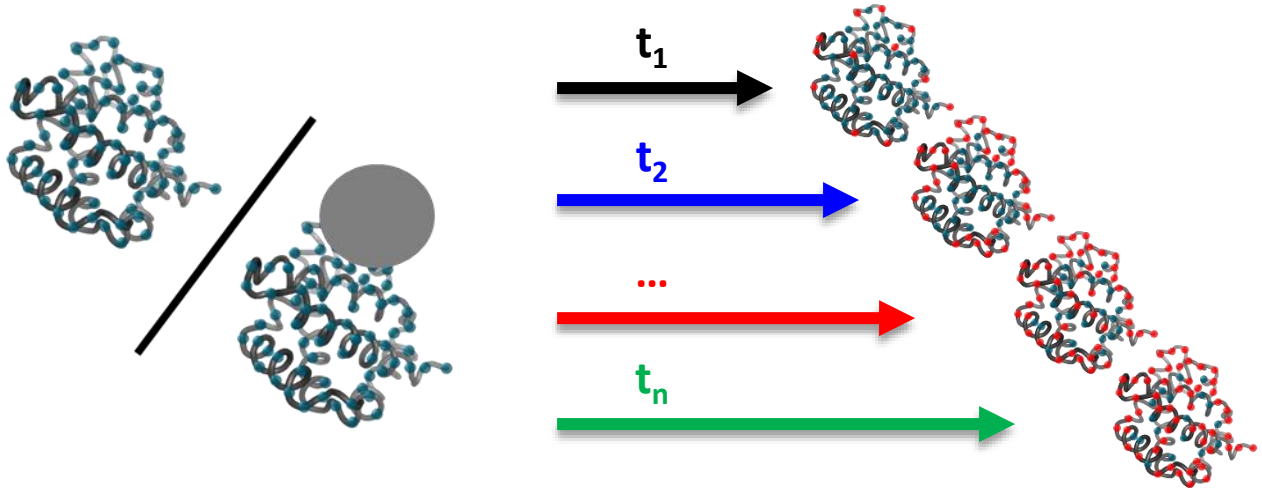


EX1: $k_{cl} << k_{ex}$



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

H/D exchange workflow

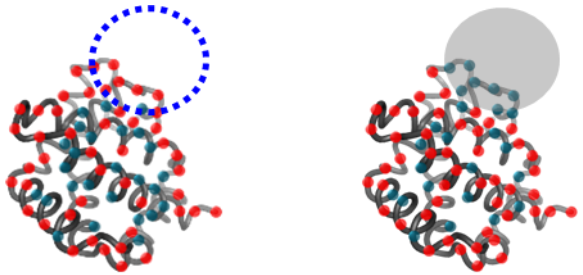


Stop the exchange

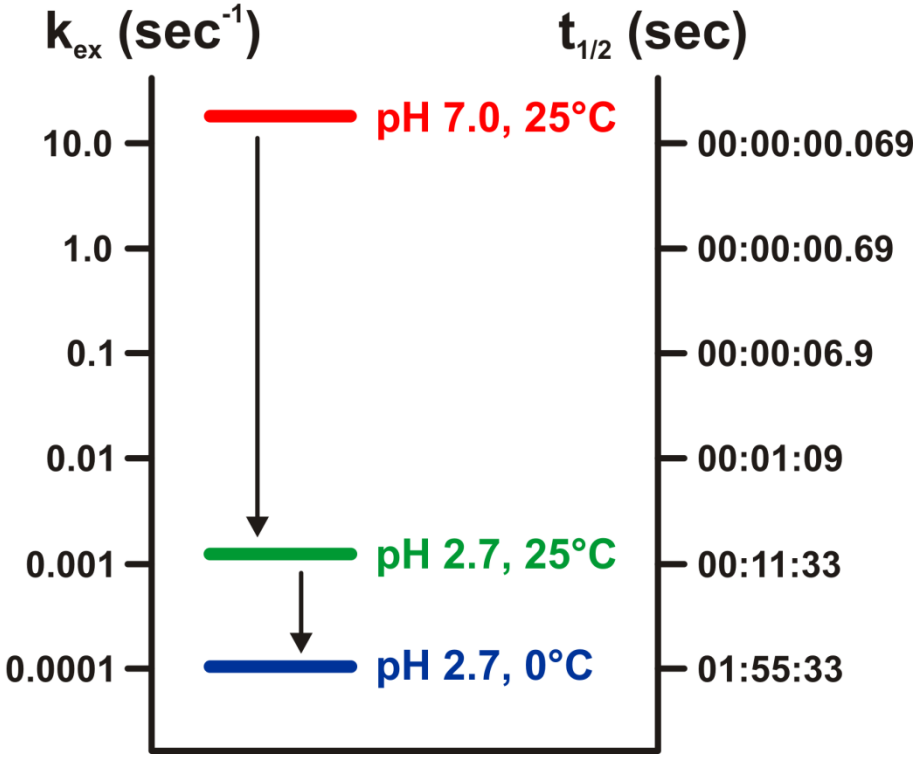
?

MS analysis

?



H/D exchange can be “stopped”



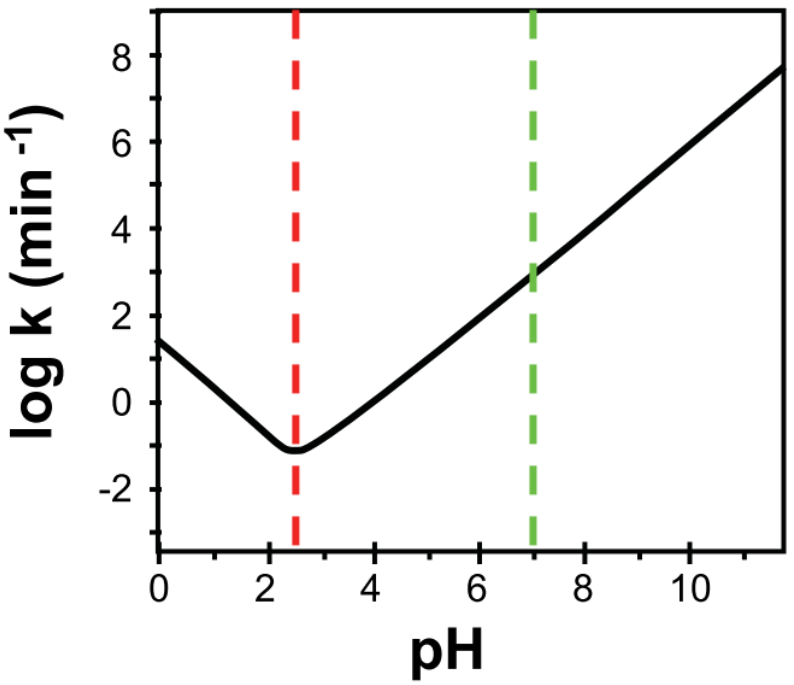
1 ΔpH … 10×
10°C … 3×

Temperature

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

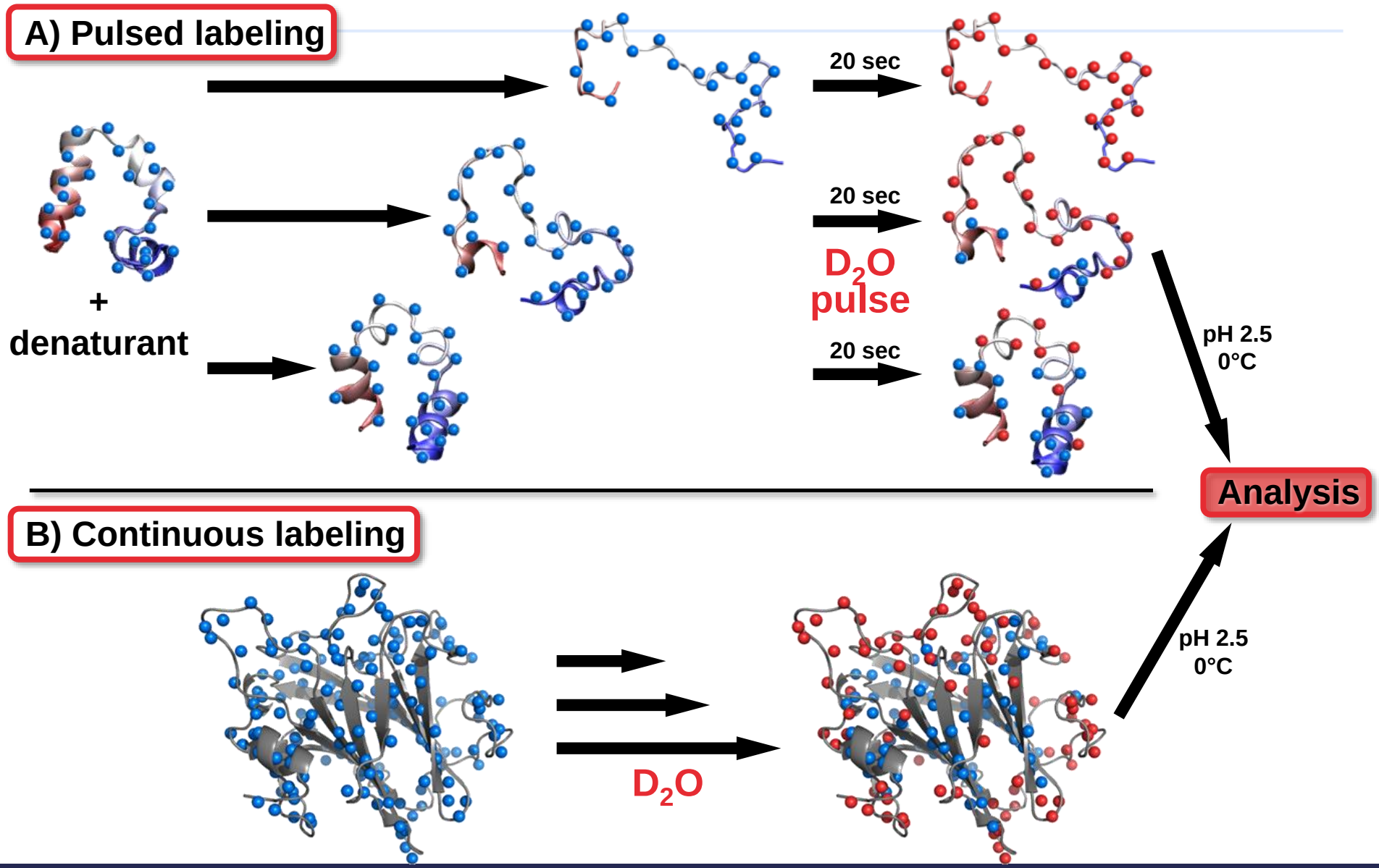
pH

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



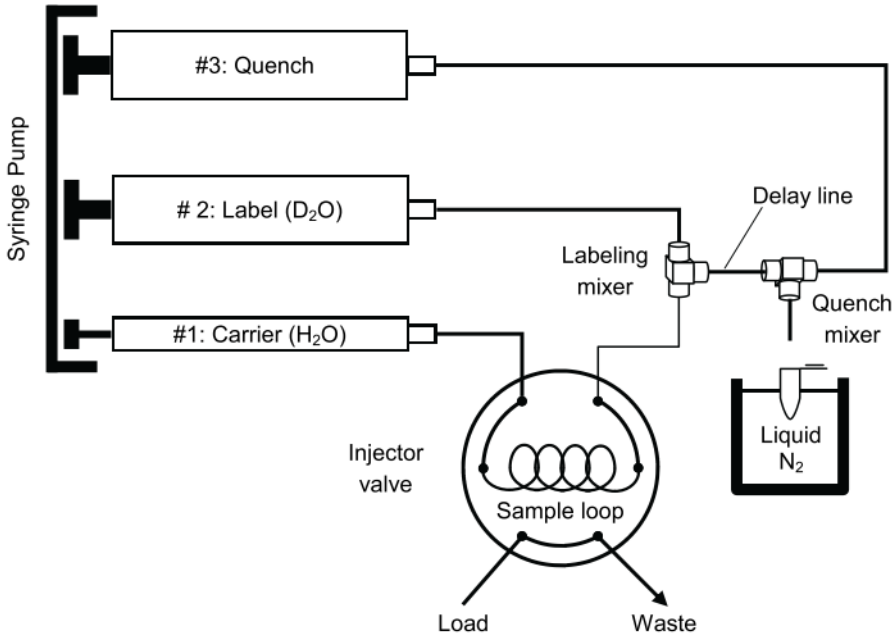
Bai *et al.*: Proteins (1993) Glasoe, Long: J. Phys. Chem. (1960) www.hxms.com

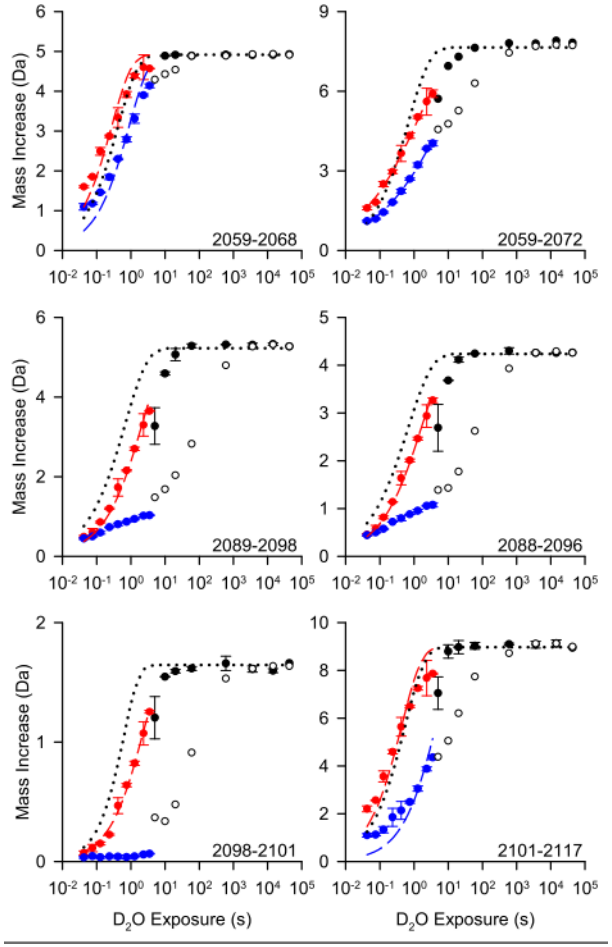
EXPERIMENTAL SETUP



EXPERIMENTAL SETUP

C) Quenchflow – millisecond HDX

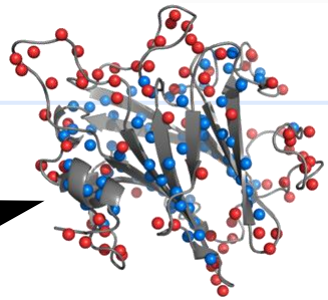




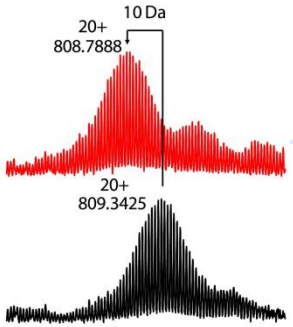
Microfluidics – mixing issues / flow

Keppel *et al.*: Anal Chem (2013)

1) Global exchange



ESI / MALDI



labeling

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

JMB

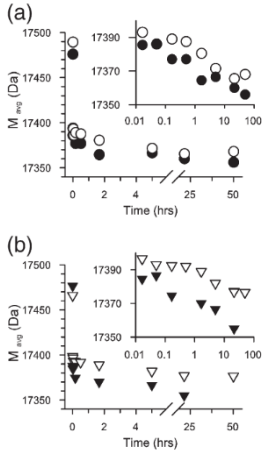
Available online at www.sciencedirect.com
ScienceDirect

ELSEVIER

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

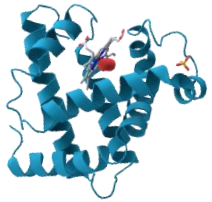


aMb free (black) / + membrane (white)

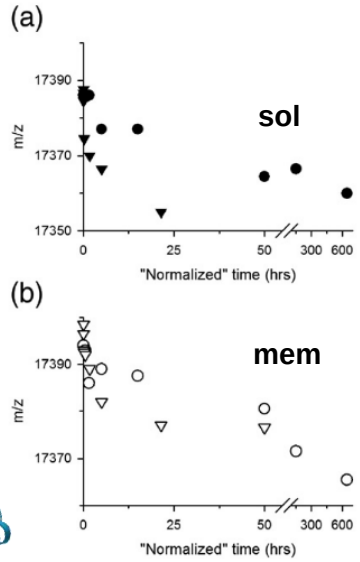
pH 5.5

pH 4.0

Bigger protection @ pH 4.0
Stronger interaction



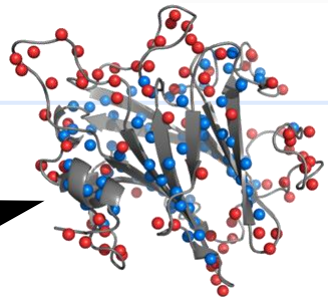
Comparison at different pH values (correction by 10e1.5)
aMb free (black) / + membrane (white)



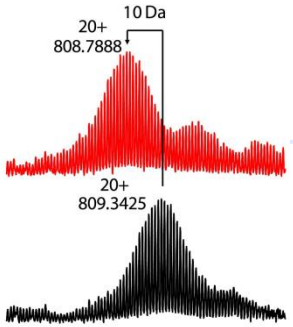
Faster exchange at pH 4.0
=
protein opens (molten globule)

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

1) Global exchange



ESI / MALDI



labeling

prokaryotic hydrophobic amino acid transporter, LeuT

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

Contents lists available at ScienceDirect

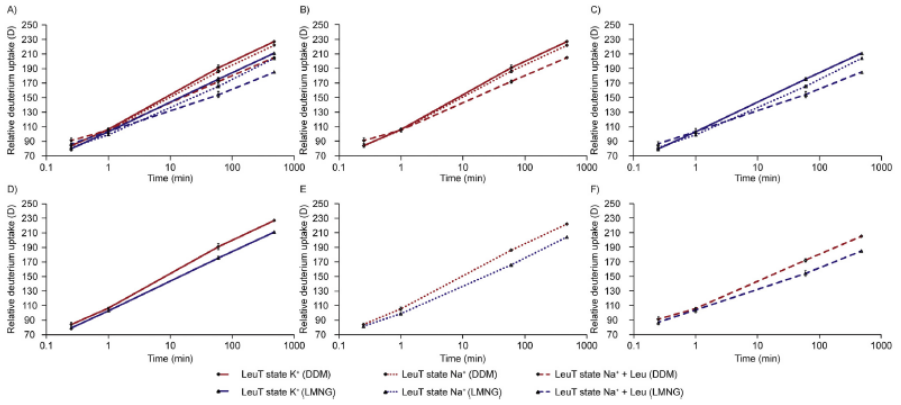
Journal of Proteomics

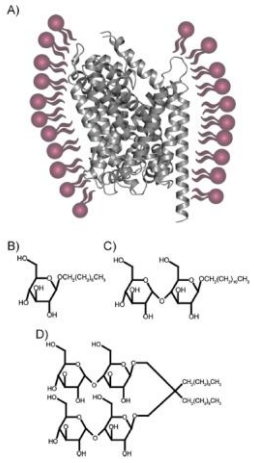
journal homepage: www.elsevier.com/locate/jprot

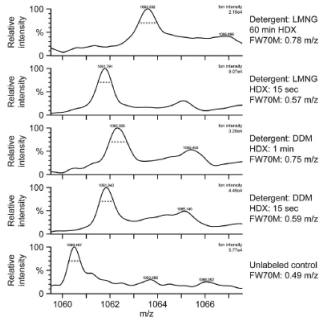
Probing the conformational impact of detergents on the integral membrane protein LeuT by global HDX-MS

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+

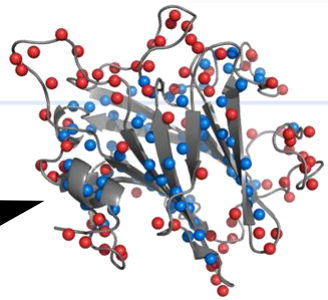




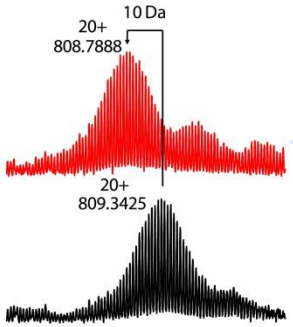


Protein dynamics (EX1/EX2) assessed through peak width

1) Global exchange



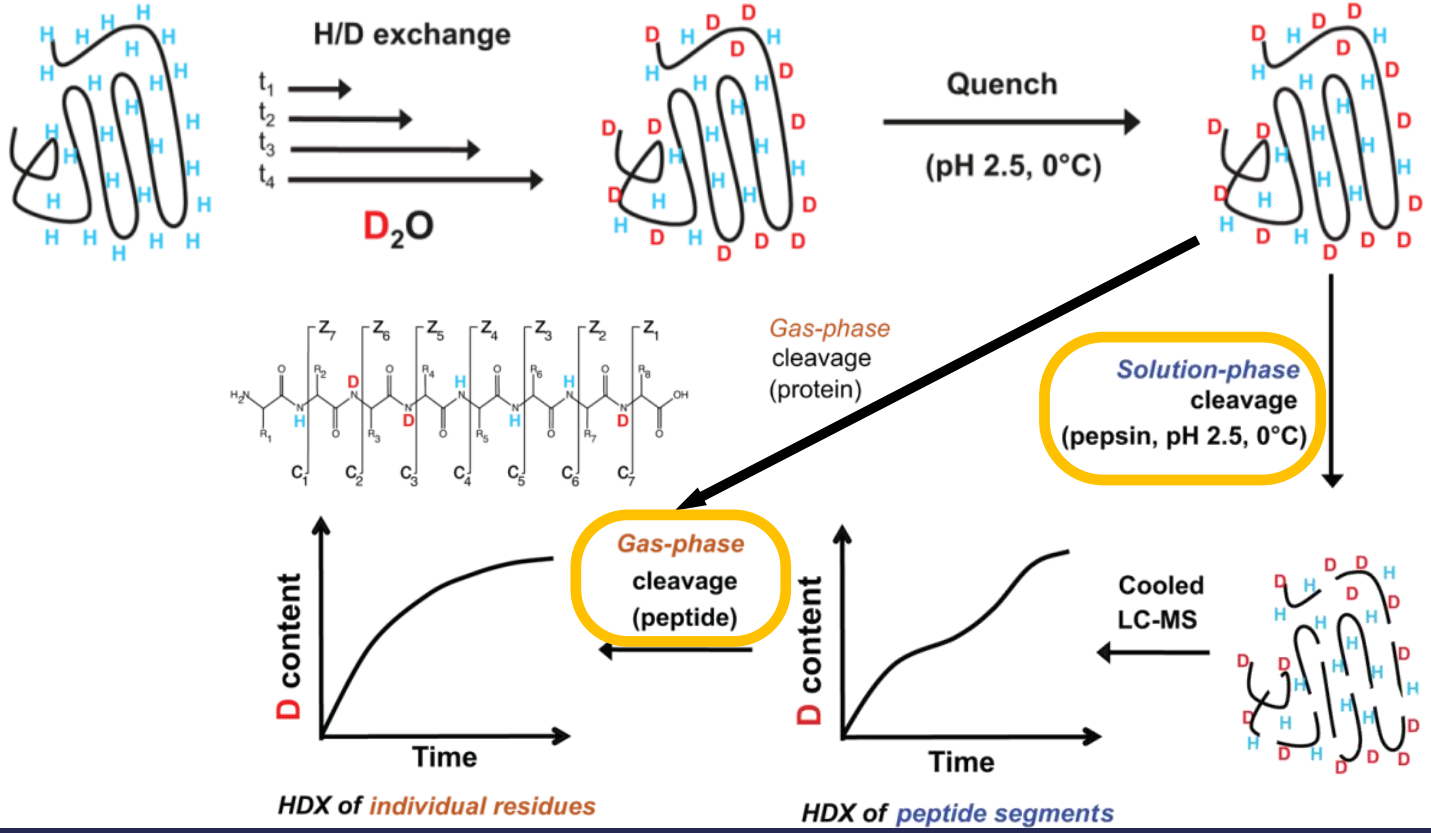
ESI / MALDI



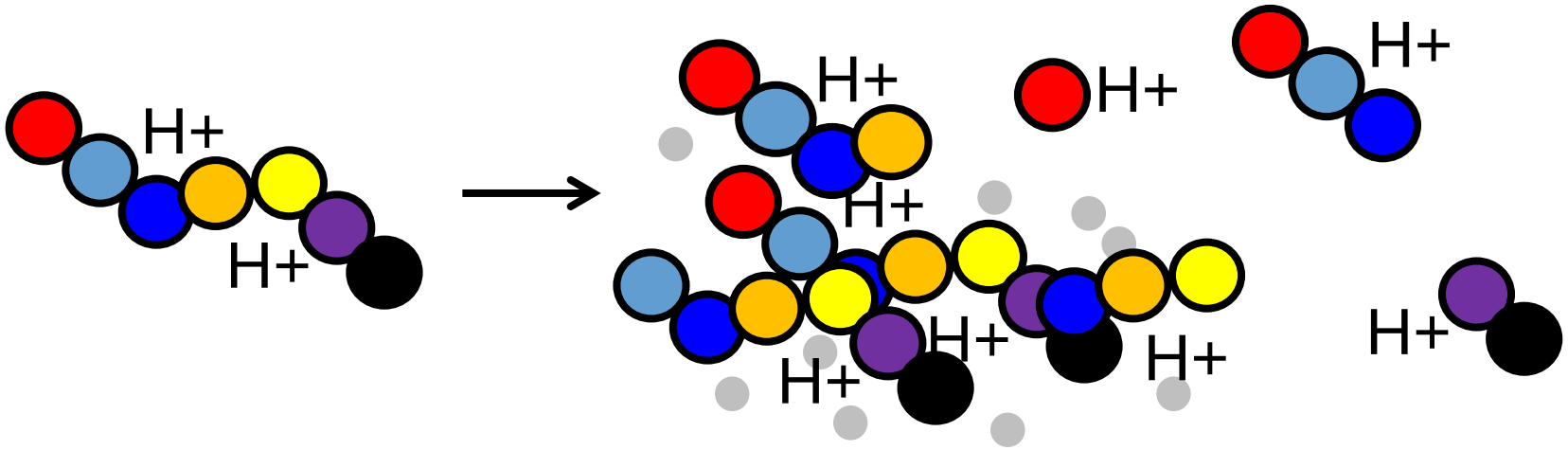
labeling

2) Local exchange

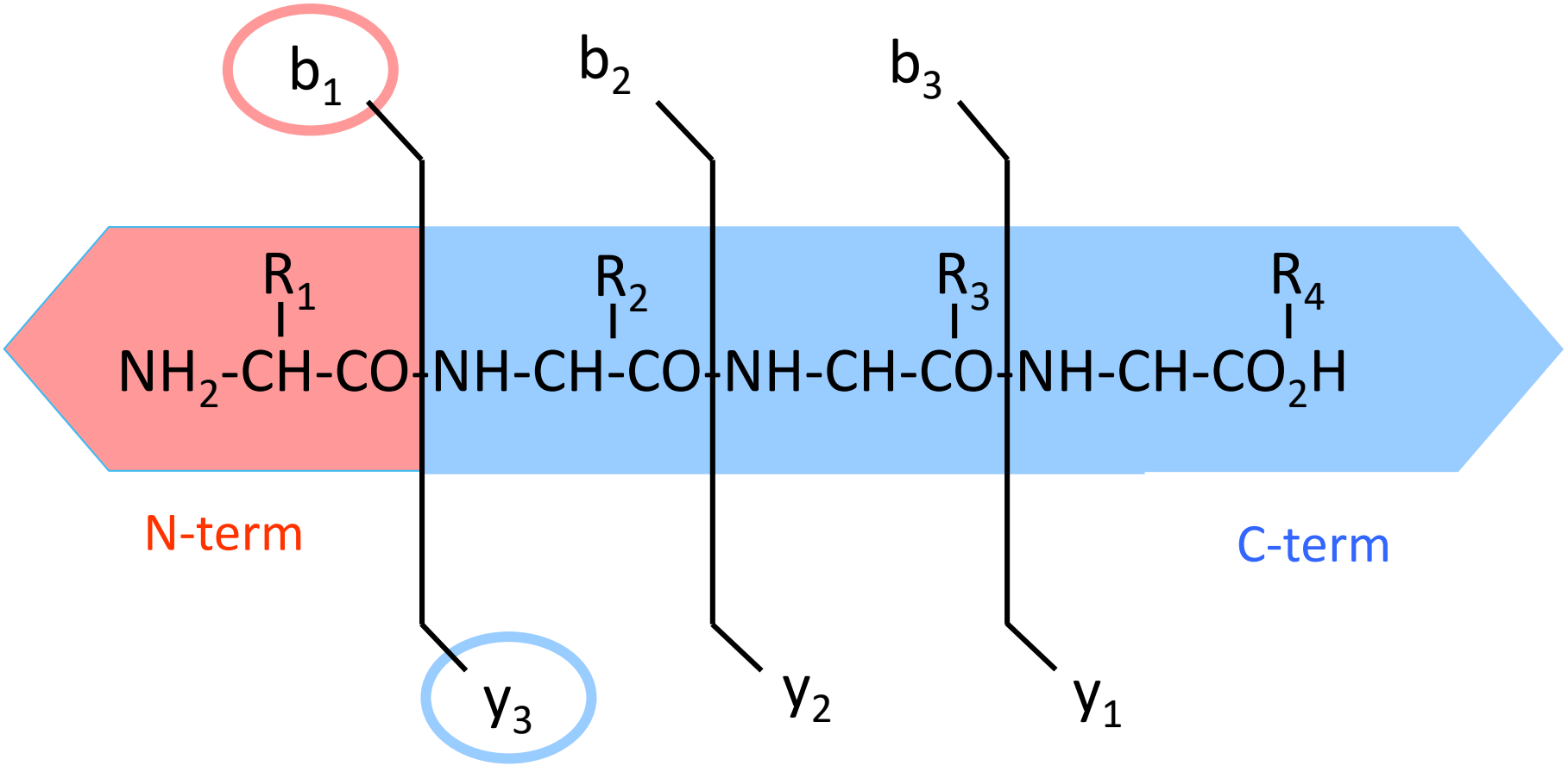
Rand *et al.*: Acc. Chem. Res. (2014)



CID – collision induced dissociation



Peptide fragmentation



Peptide fragmentation

S-P-A-F-D-S-I-M-A-E-T-L-K MH^+ = 1410.6

b-ions$^+$			y-ions$^+$
88.1	S	PAFDSIMAETLK	1323.6
185.2	SP	AFDSIMAETLK	1226.4
256.3	SPA	FDSIMAETLK	1155.4
403.5	SPAF	DSIMAETLK	1008.2
518.5	SPAFD	SIMAETLK	893.1
605.6	SPAFDS	IMAETLK	806.0
718.8	SPAFDSI	MAETLK	692.3
850.0	SPAFDSIM	AETLK	561.7
921.1	SPAFDSIMA	ETLK	490.6
1050.2	SPAFDSIMAE	TLK	361.5
1151.3	SPAFDSIMAET	LK	260.4
1264.4	SPAFDSIMAETL	K	147.2

Electron Capture/Transfer Dissociation (ECD/ETD)

Electron capture (E ≤ 0.1 eV) - on S-S bonds and N-C_α bonds

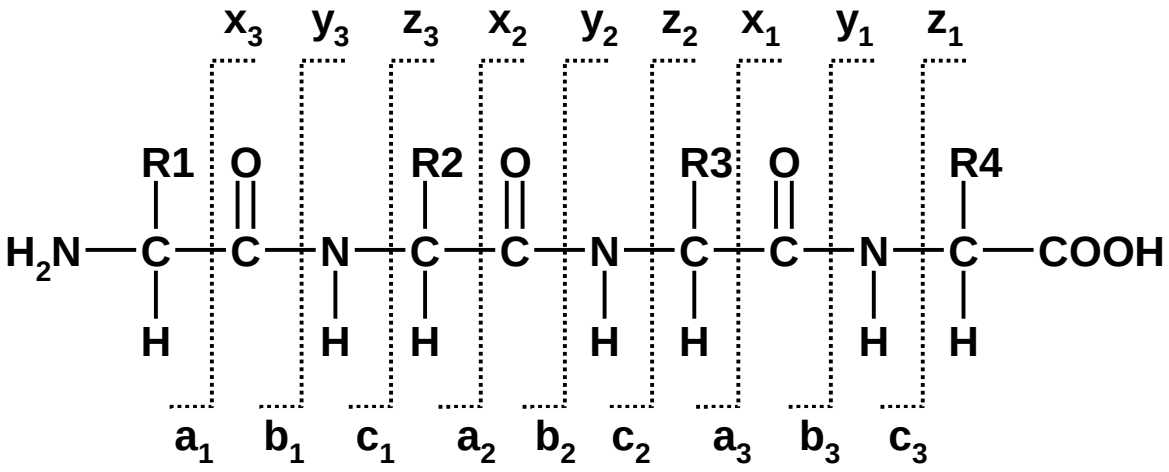
Neutralization H^+; very fast process but requires multiply charged ions

c- and *z*-ion series

Electron Transfer Dissociation (ETD)

ECD not applicable in Rf field analyzers (traps, quads)

Chemical ionization = electron is transferred by an anion (anthracene, methane, …)



UltraViolet PhotoDissociation (UVPD)

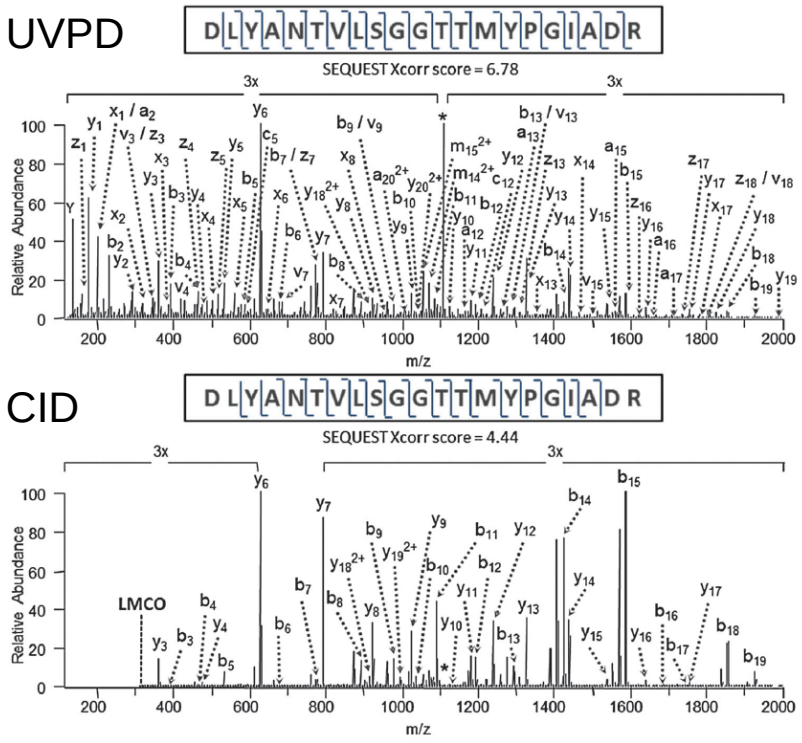
UV lasers – Nd:YAG a excimer ArF, KrF,..

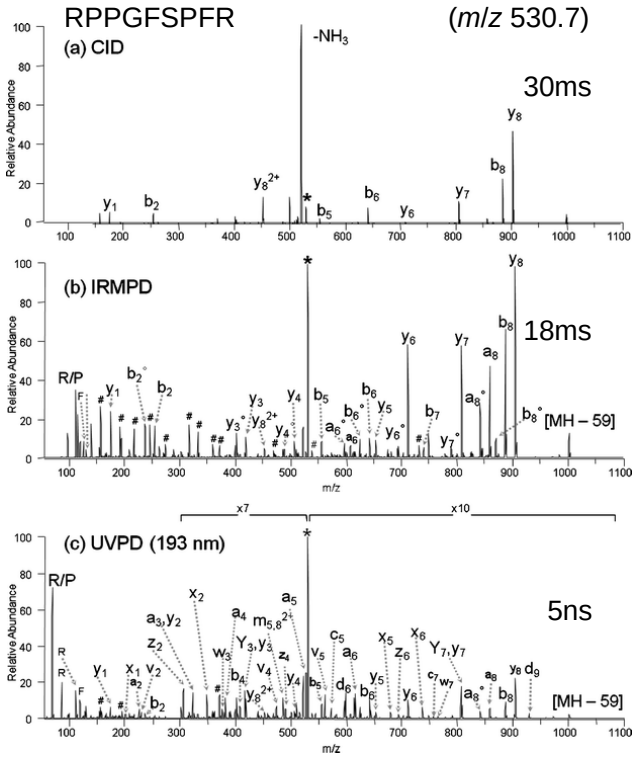
wavelength: 157nm, 193nm (peptide bond), 213nm, 266nm,…
wavelength close to 280nm – fragmentation close to aromatics

Very fast fragmentation - in psec – no scrambling

Similar to ExD but no charge reduction – also applicable to 1+ ions

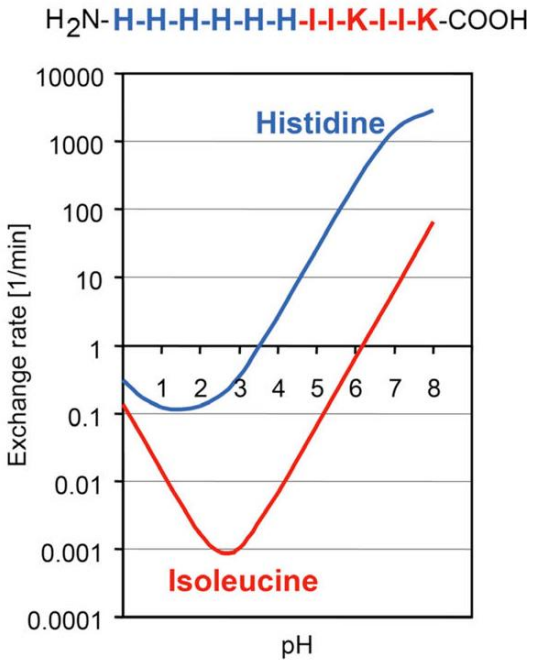
a/x, c/z, but also b/y ions and side chain fragments





Scrambling test

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



HHHHHHIIKIIK

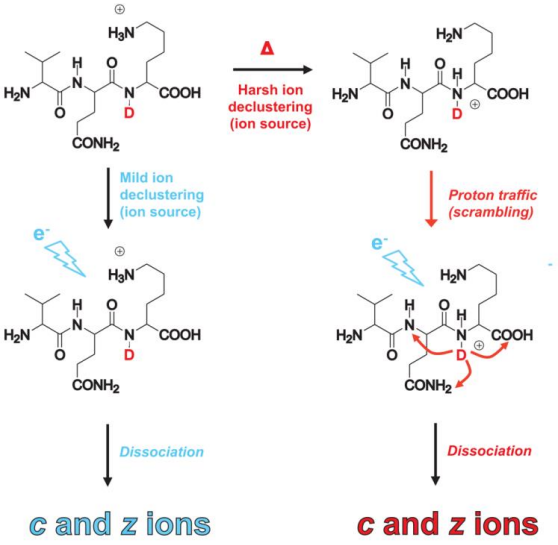
Fully deuterate

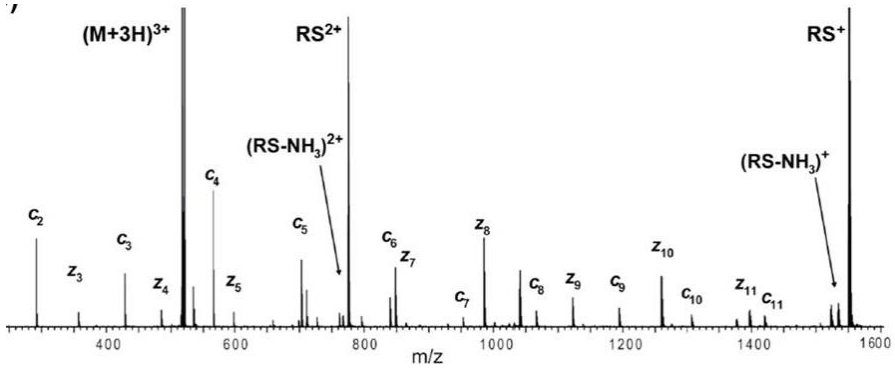
⬇

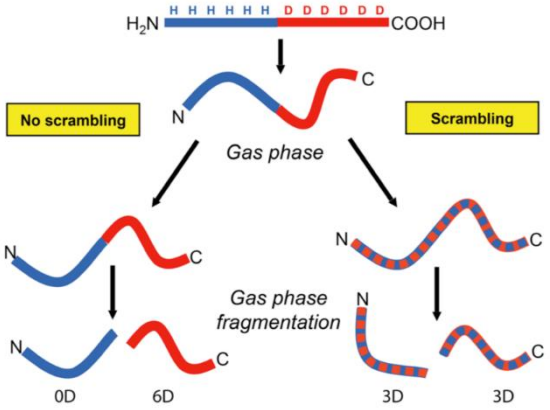
Quench

⬇

Infuse

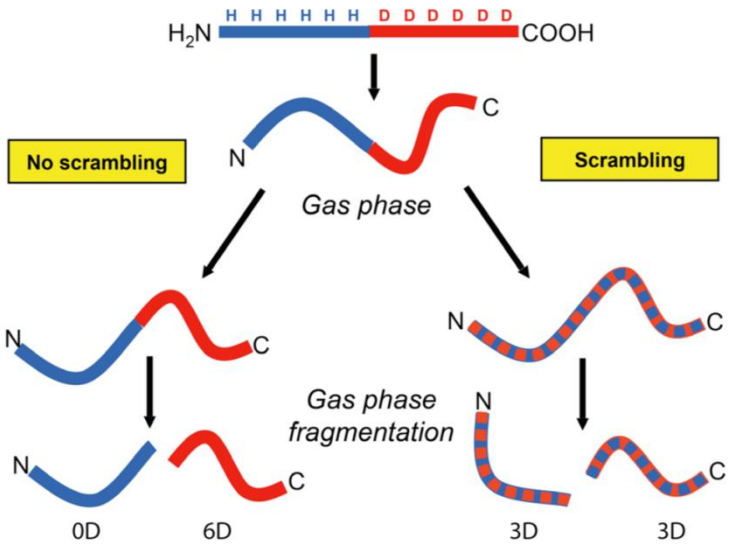




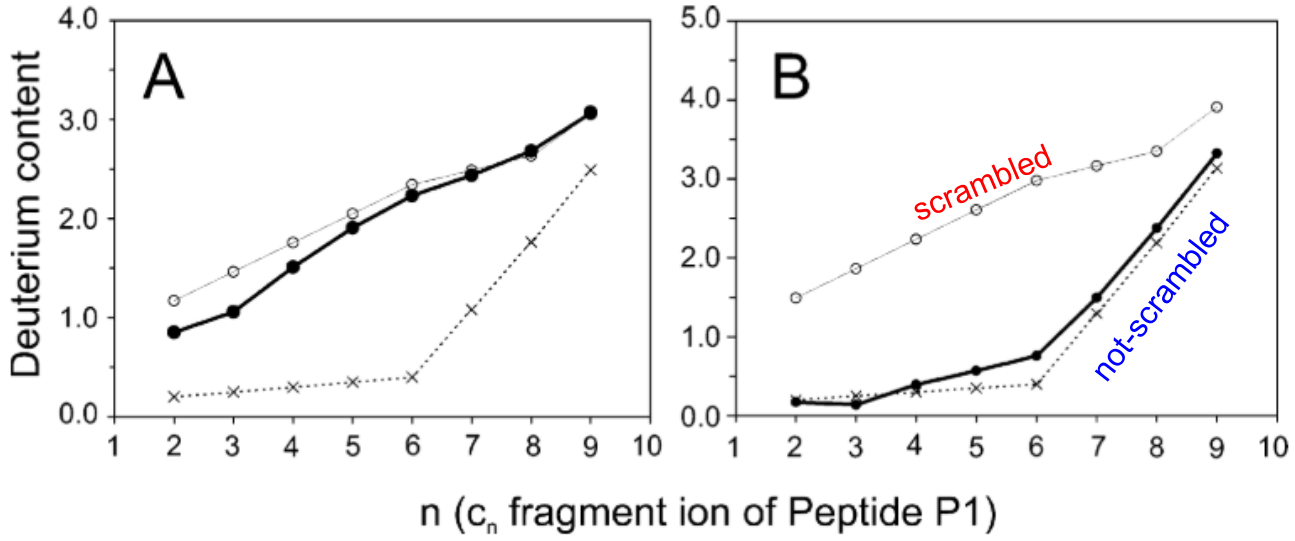


Scrambling – ETD/ECD

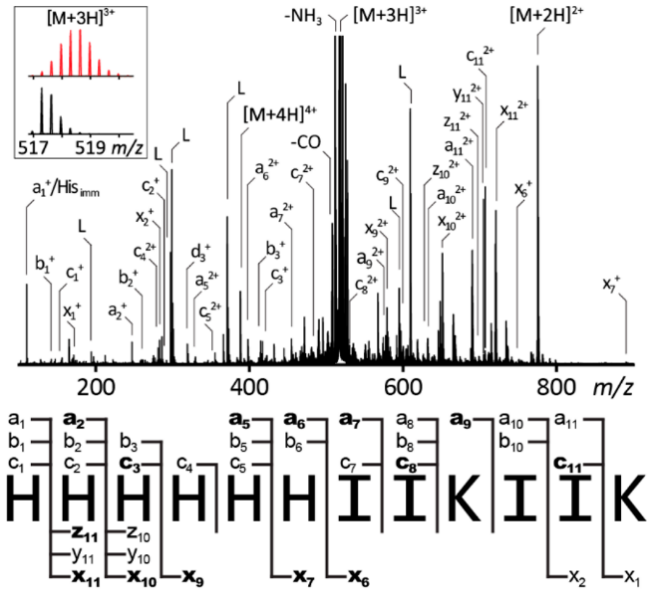
Kaltashov, Eyles: J. Mass Spectrom. (2002)
Rand *et al.*: Acc. Chem. Res. (2014)



Activation via dissociation but also during ionization and ion transfer



Scrambling - UVPD



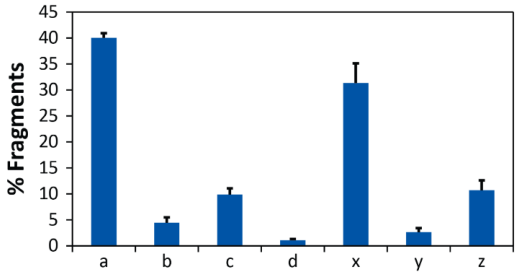


Figure S-2. Abundance of fragment ion types observed during 213 nm UVPD of peptide P1.

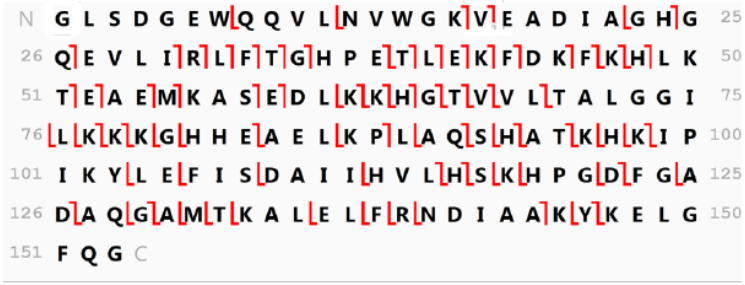
Mistraz U.H. et al Anal Chem 2018
Brodie N.I. et al Anal Chem 2018

UVPD is applicable (similarly to ExD)

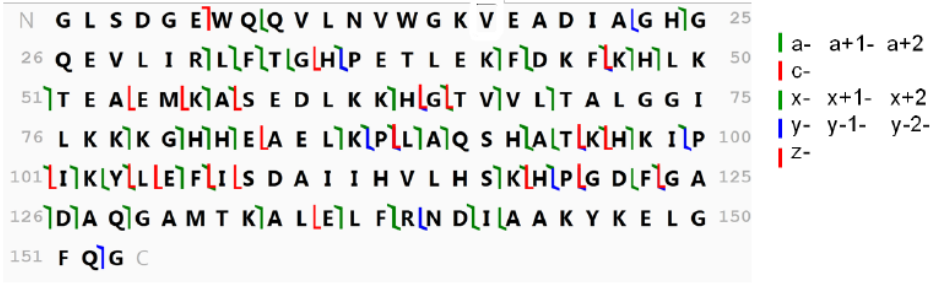
More fragments produced (P1 on the left and Mb top-down bottom)

No charge reduction/stripping

ETD



UVPD

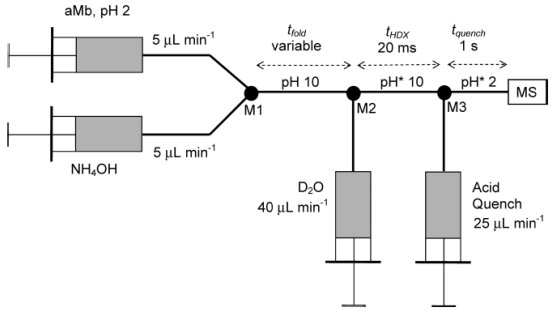


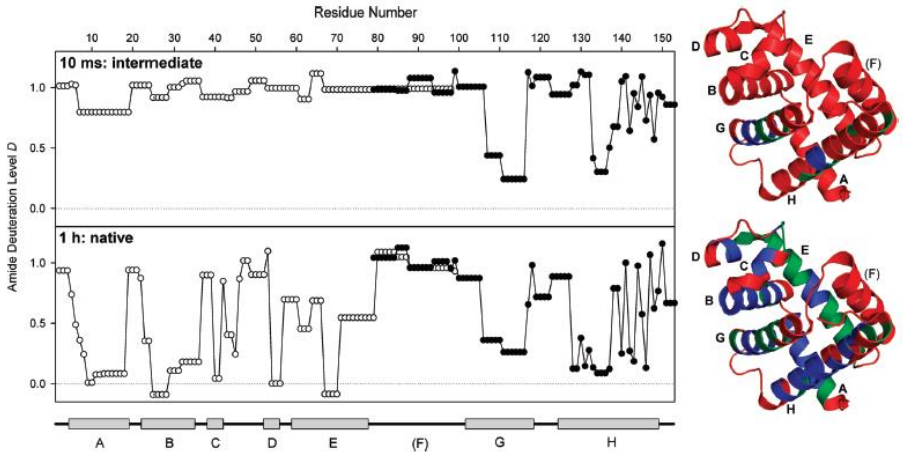
Top-Down HDX-MS

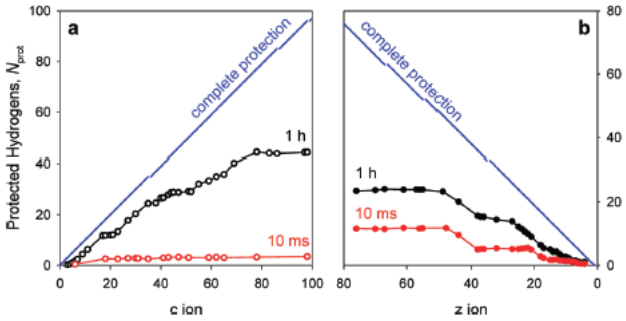


Number of reports still limited (C.H. Borchers, L. Konermann, I.A. Kaltashov,…).

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



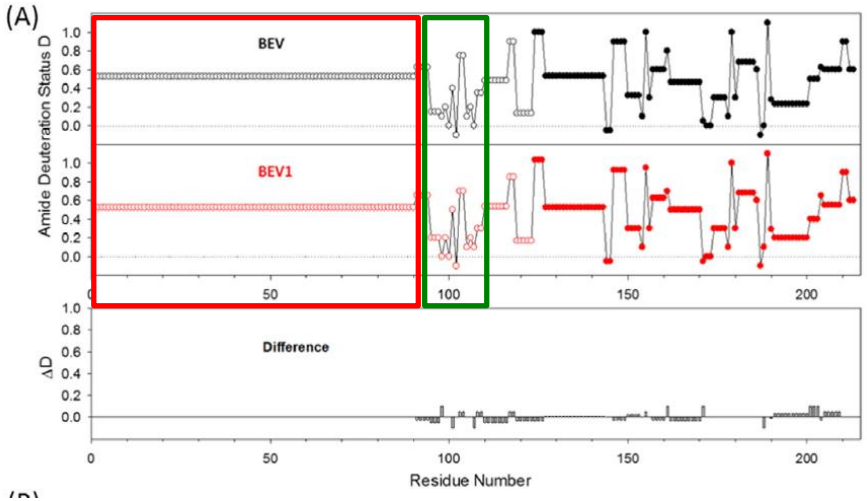




Pan J et al Anal Chem 2010
c ions (open)
z ions (closed)

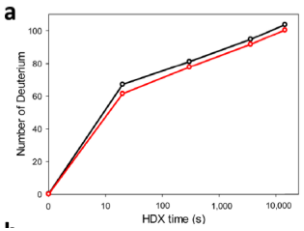
Top-Down HDX-MS

Ab studies also reported, however, Ab (150kDa) is not a big protein (HC 50kDa, LC 24kDa) + HC is “not interesting”



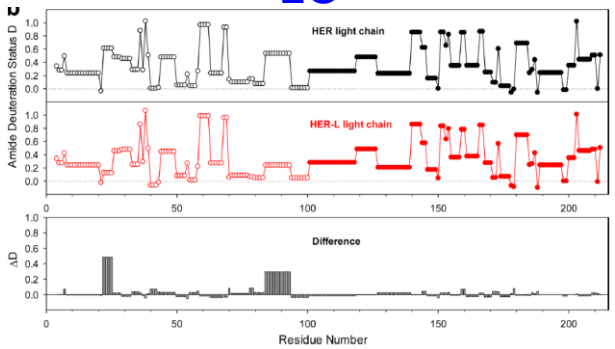
Fragmentation can go to “residue level” but has also spans over large portion of the protein – BEV = Bevacizumab (Avastin) = Ab against VEGF – light chain shown here

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

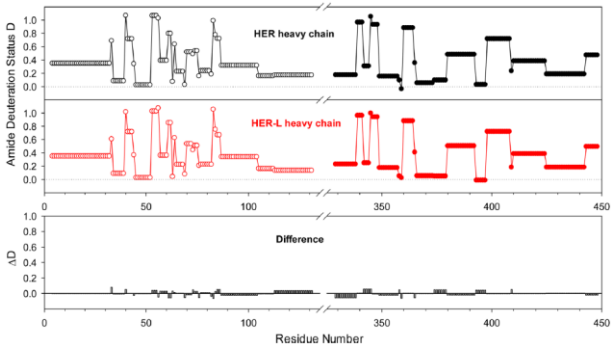


Pan J et al JACS 2014
c ions (open)
z ions (closed)

LC



HC – 1-132 + 328-449



Top-Down HDX-MS

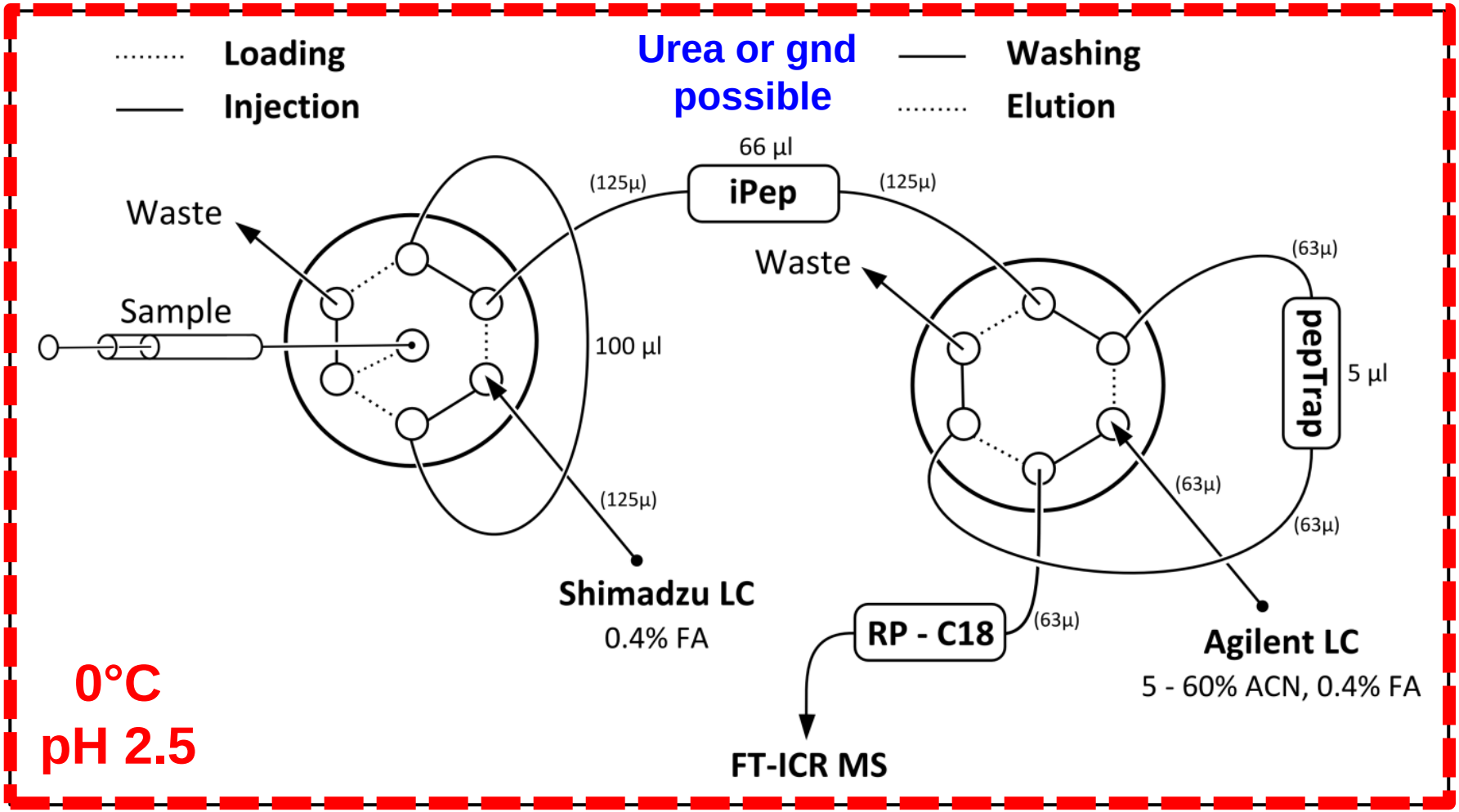
Problem with ionization/fragmentation of some/larger proteins, not likely applicable (yet) to complexes.

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

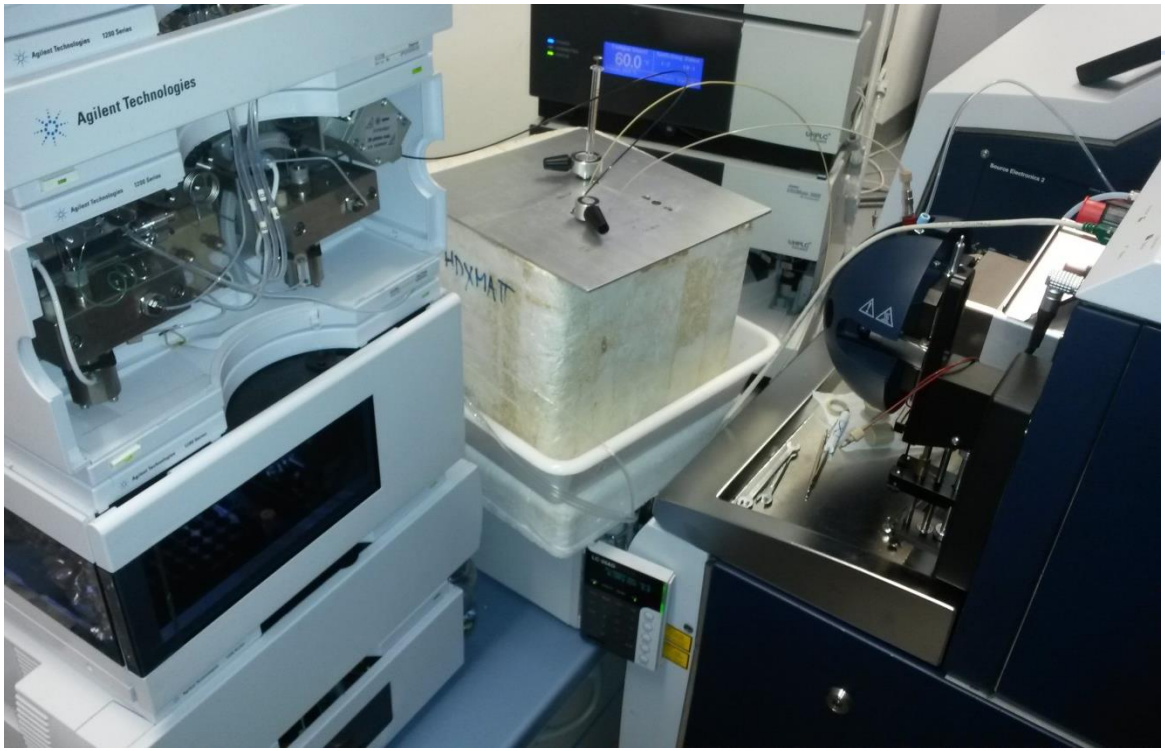
Motivation/advantages – sequence coverage sometimes not complete – already two complementary fragment ions do the job!

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)

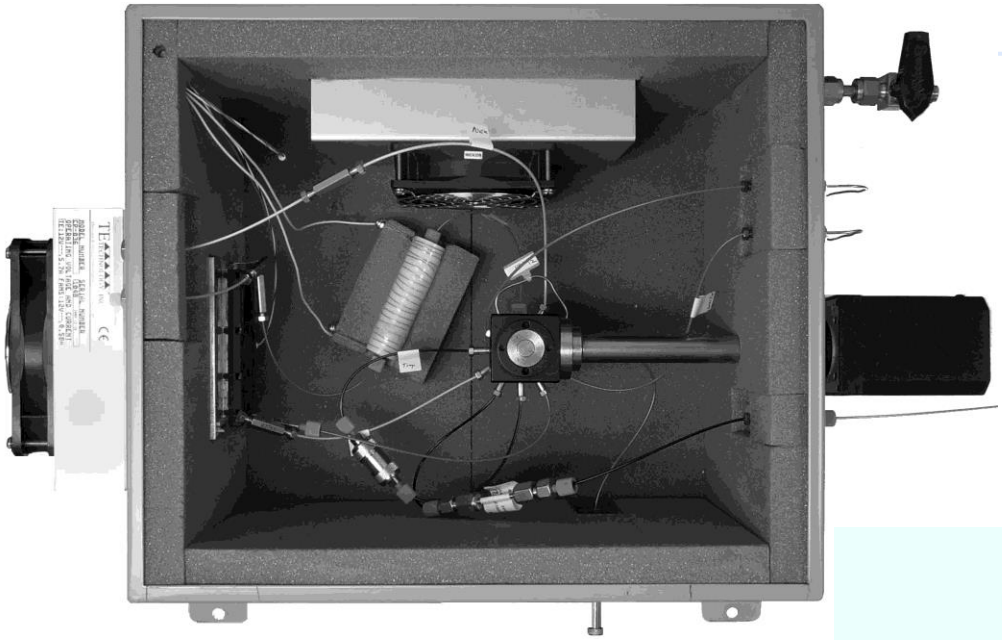
H/D exchange workflow



LAB SETUP



LAB SETUP



LAB SETUP

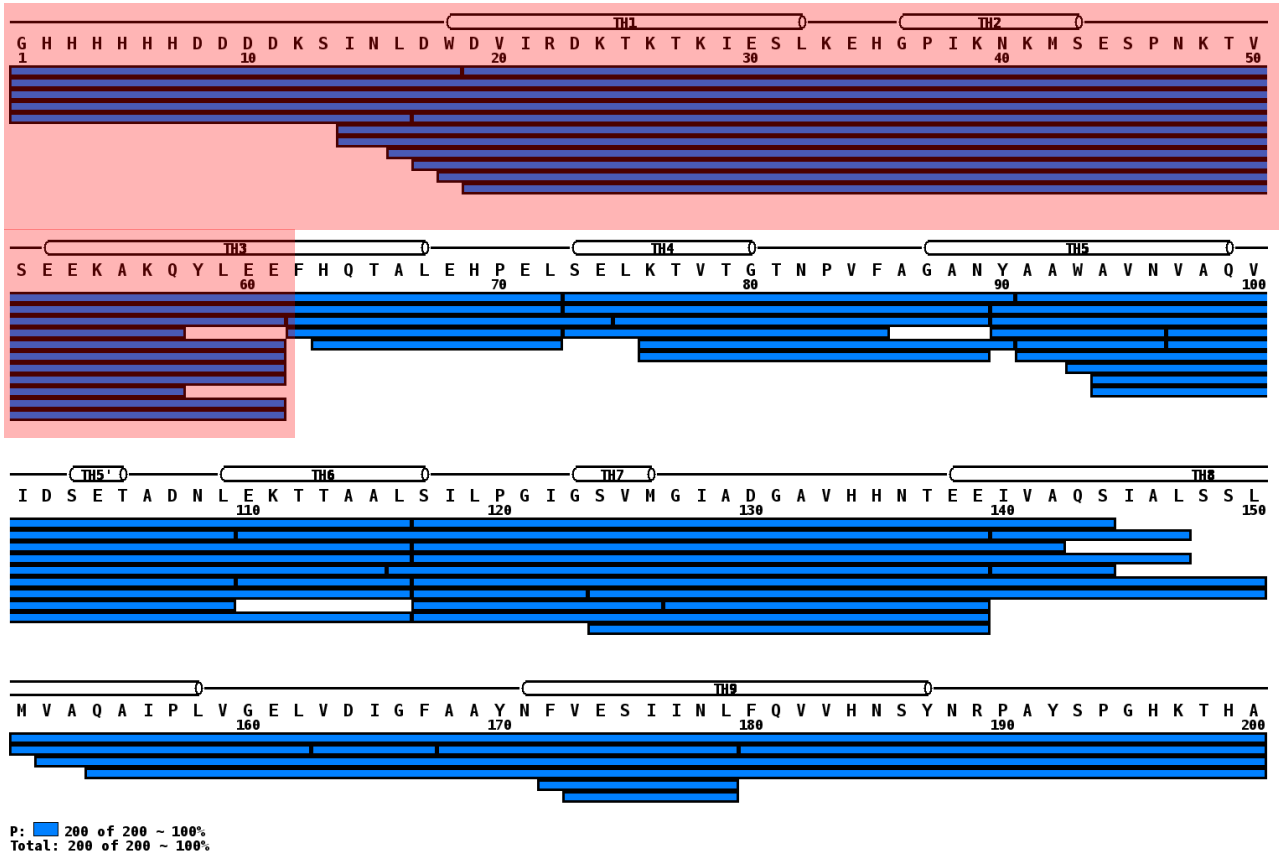
Focus on: Proteolysis

- 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*

T-domain - proteolysis

Proteolysis

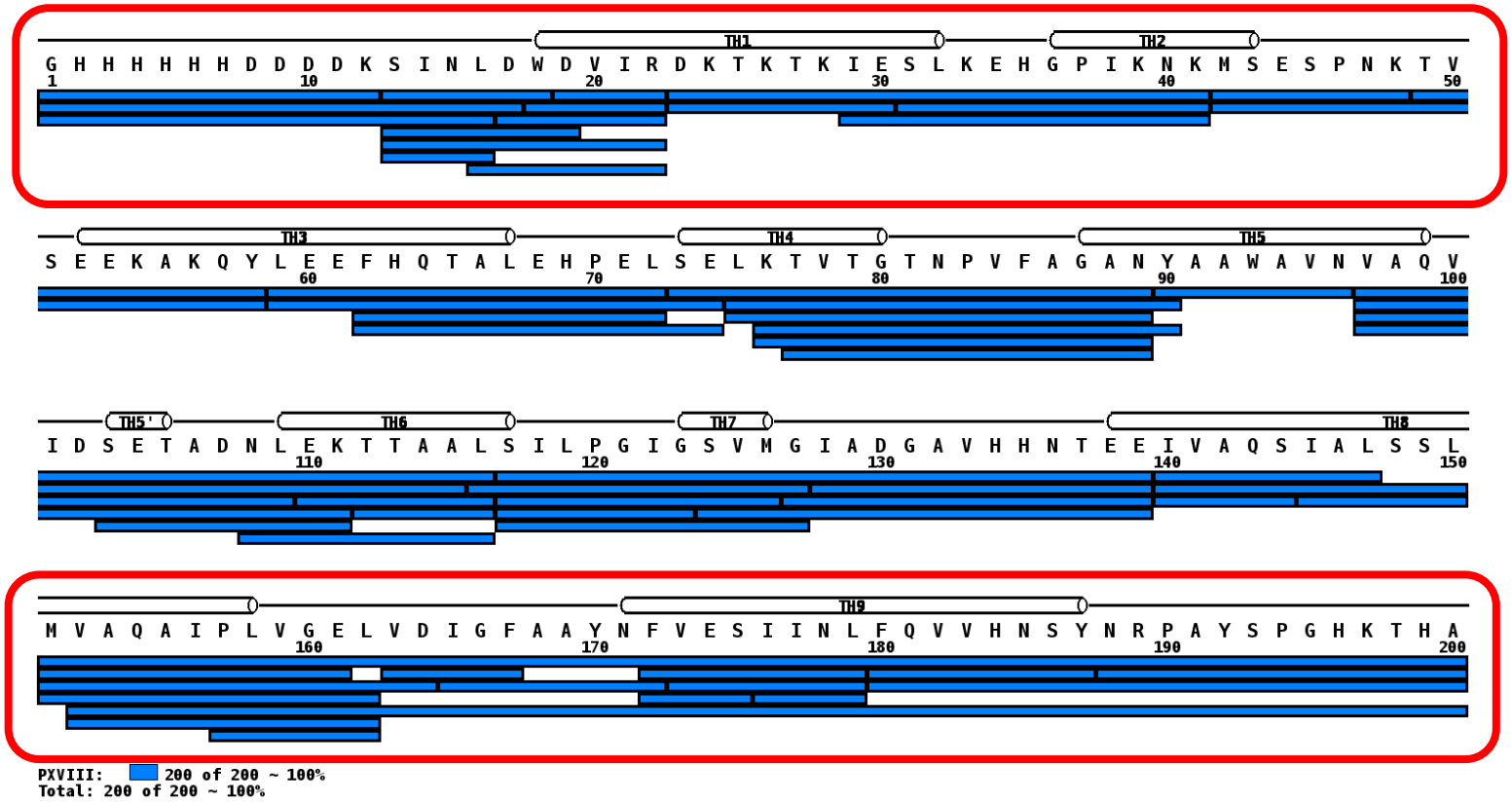
- defines spatial resolution of the method
- not all proteins can be efficiently by pepsin digested under HX-MS conditions



T-domain - proteolysis

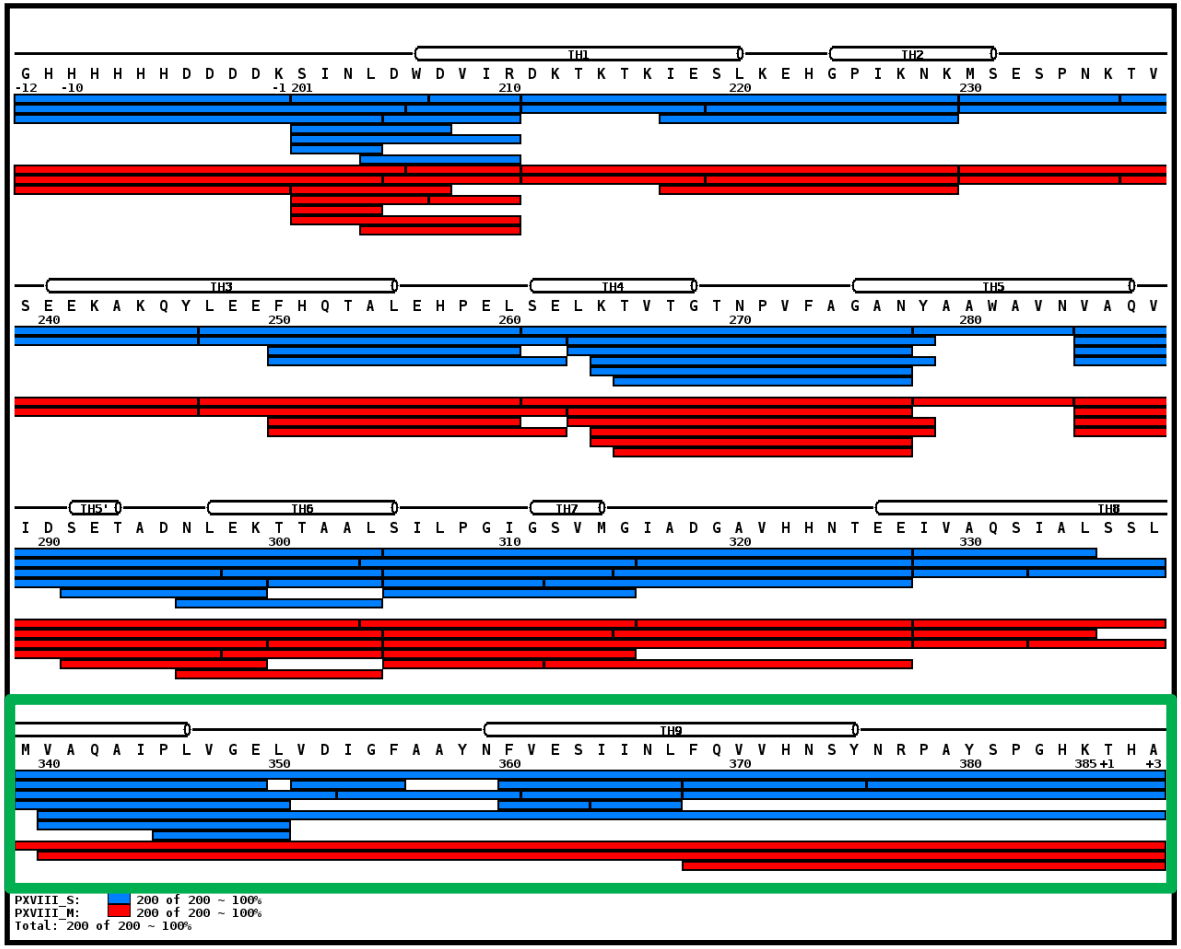
Try different proteases (commercial extracts – protease type XIII, type XVIII)

Digestion by pepsin+rhizopuspepsin (type XVIII)

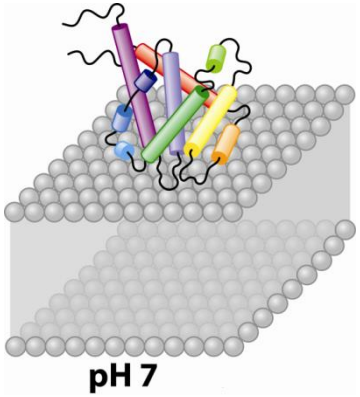


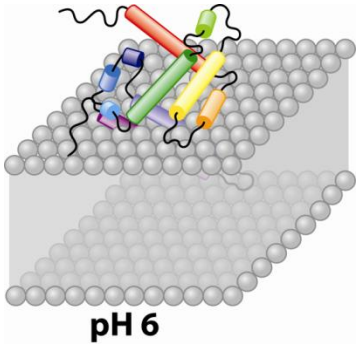
T-domain - mapping

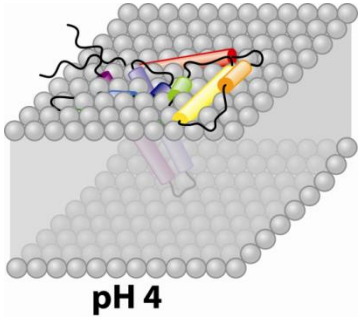
T-domain in solution , T-domain+LUVs



Summary for T-domain interacting with the membrane







pH 7 to pH 6 – still close to the native fold with just TH8 and TH9 inserting into the membrane (membrane bound state)

pH 6 to pH 4 – melting of the structure. TH8-9 deeply inserted, stabilized by TH5 and TH5’.

N-terminal part (mainly TH1 and TH3) stabilizes the membrane inserted state via ionic interactions.

Acidic proteases in HX – 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)

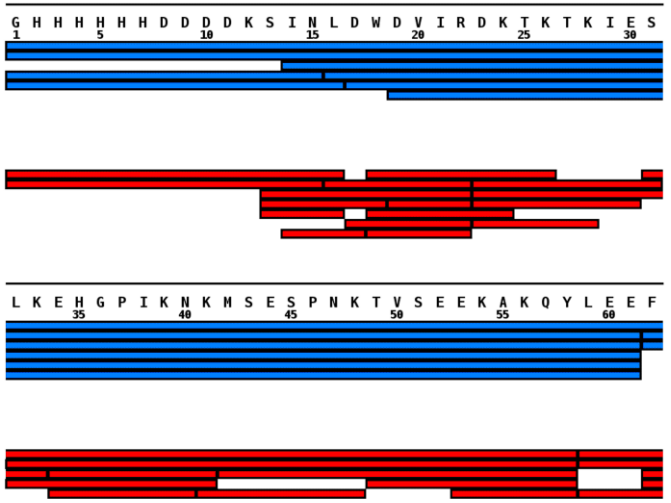
2007 – Brier et al – pepsins from Antarctic rock cod

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)

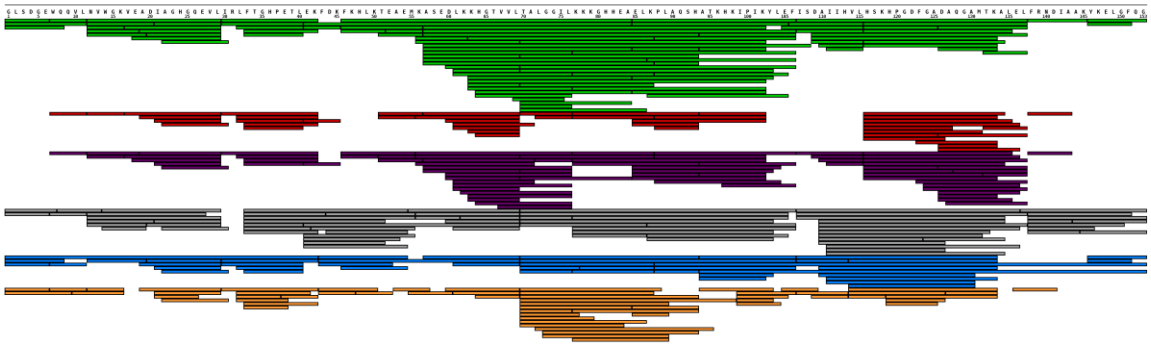
1979 - Rosa&Richards – protein digestion (pepsin, rhizopuspepsin)



Proteases – what is available (in our lab)

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

Comparisons of peptide maps

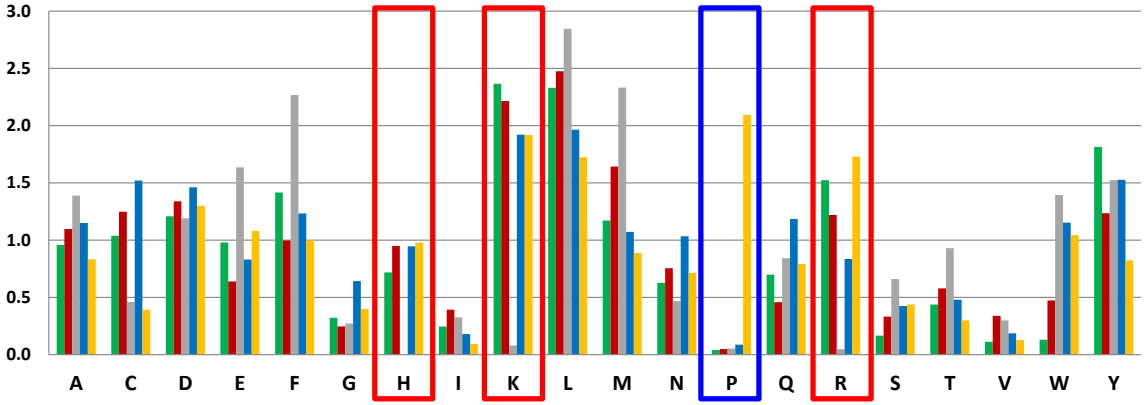


GOALS

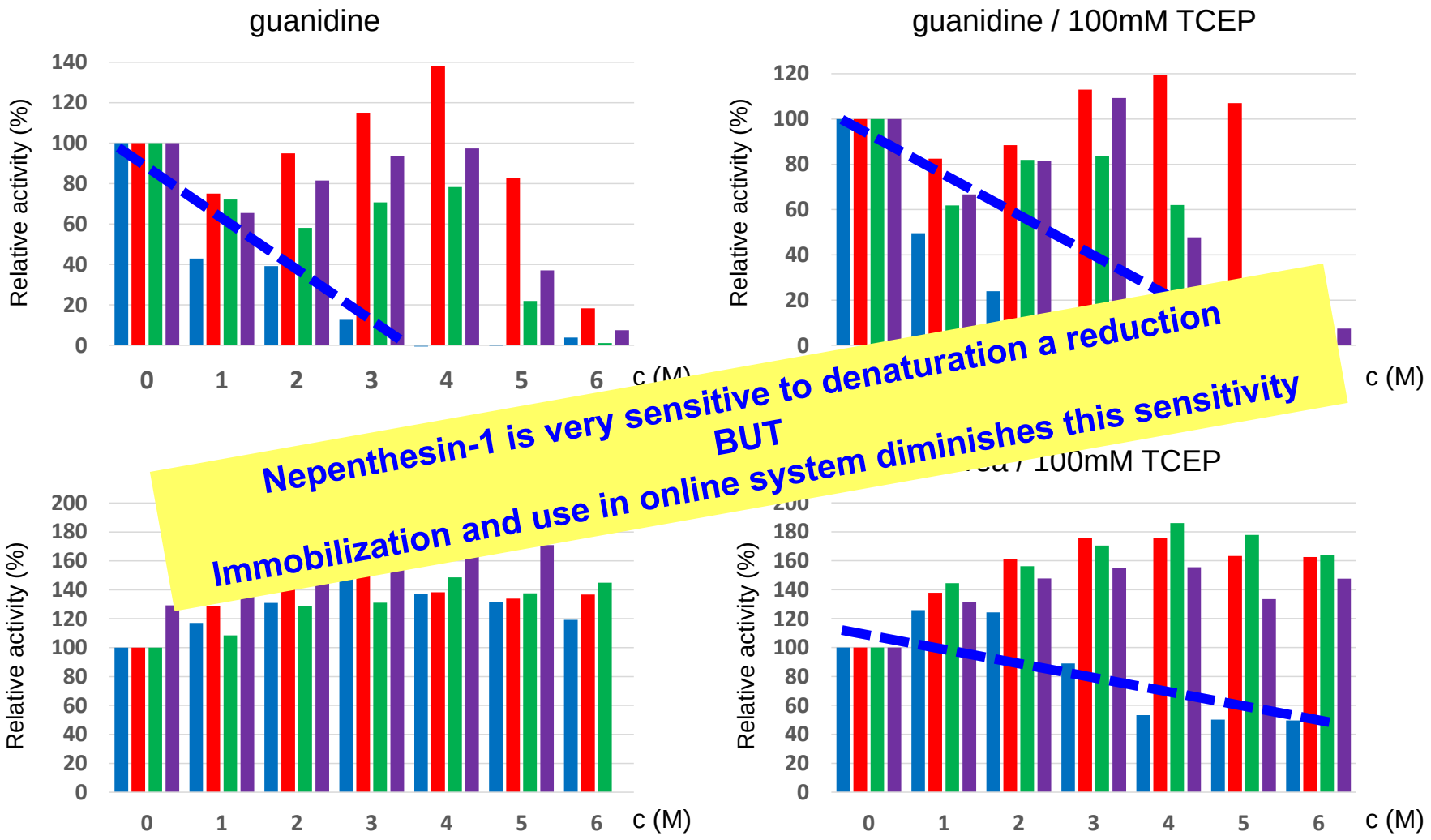
Digest every protein

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

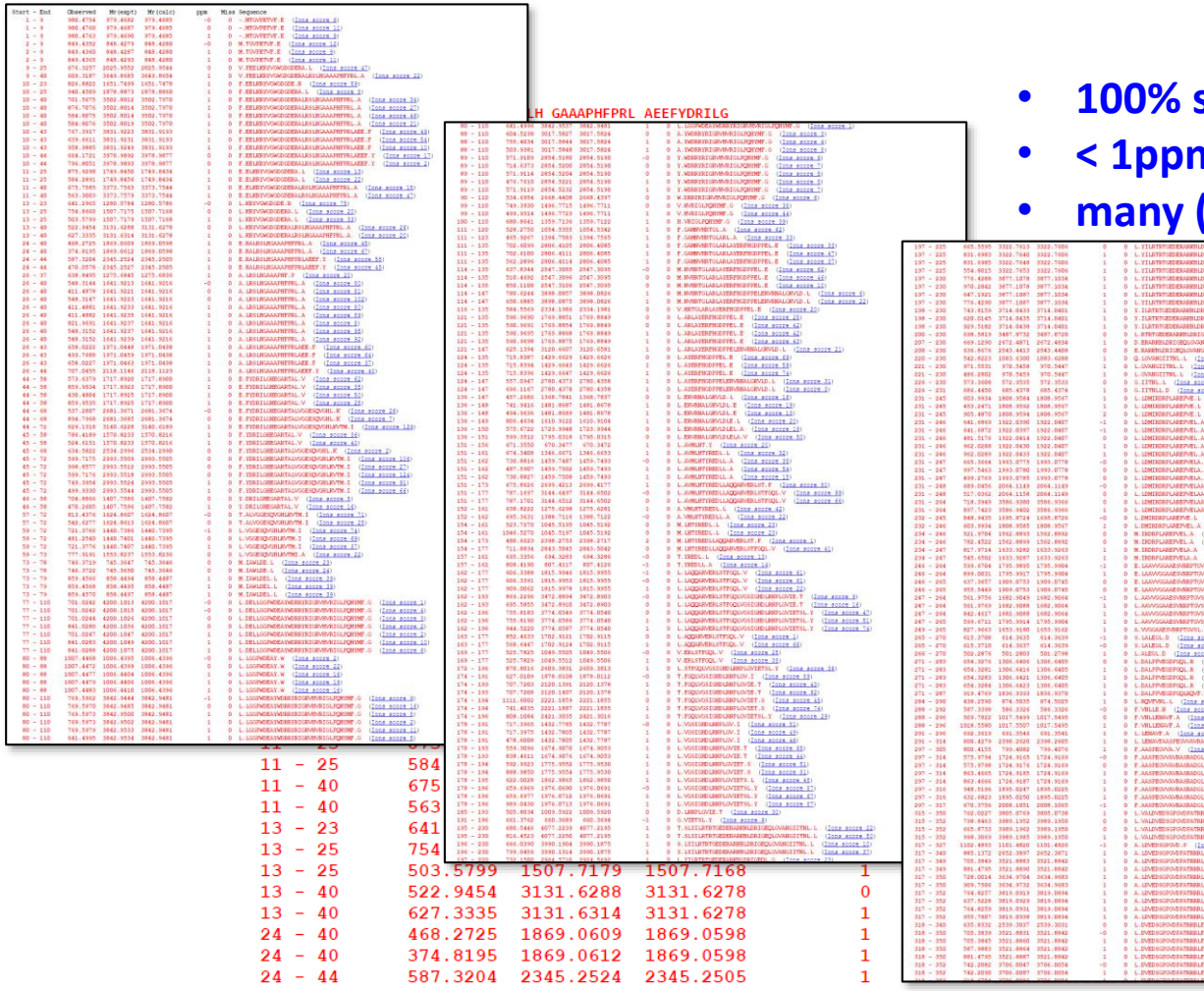
Cleavage after…



Proteases – effect of denaturing and reducing agents in solution

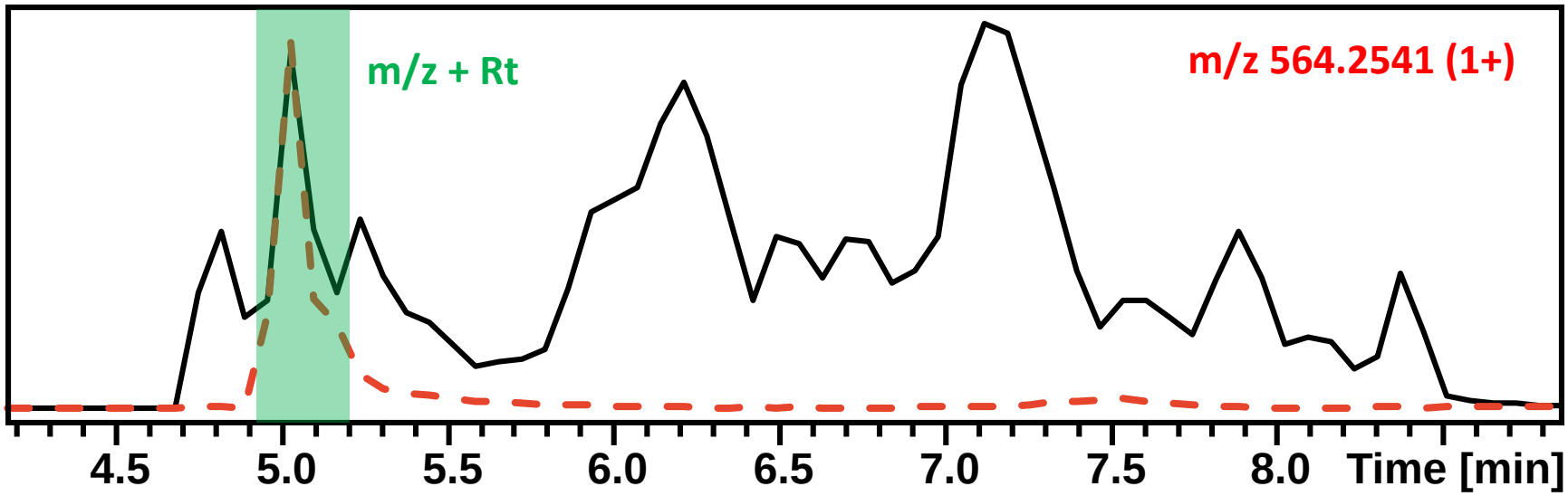


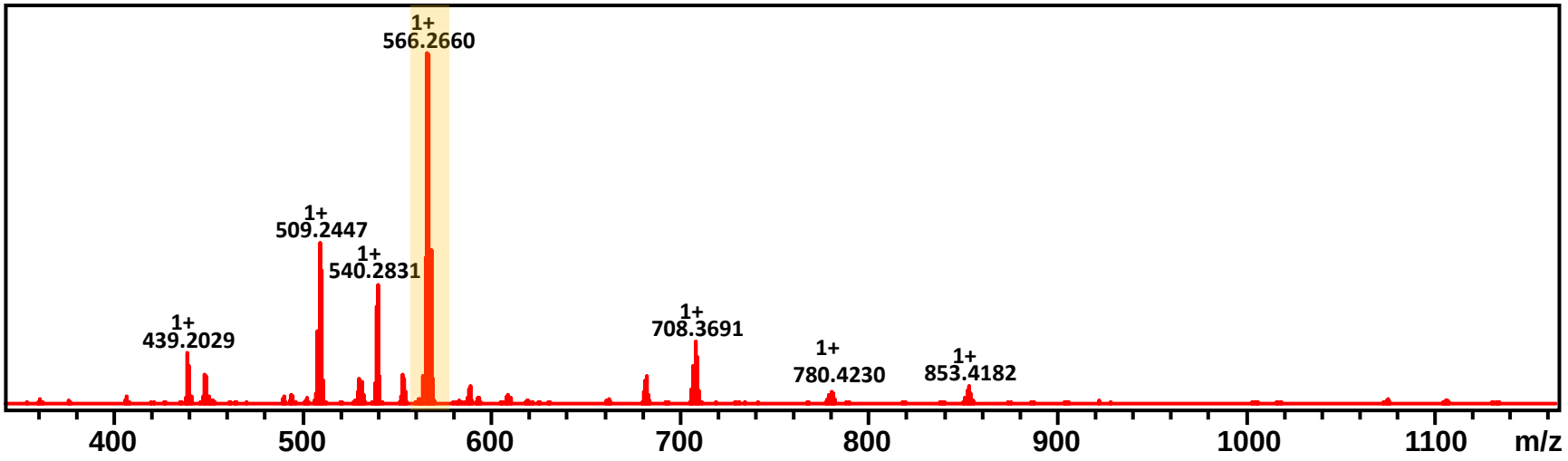
Focus on: Data interpretation



- 100% sequence coverage
- < 1ppm
- many (and overlapping) peptides

Focus on: Data interpretation – manual way





Focus on: Data interpretation – summary on the manual approach

Locating a peptide	**30s**	
Plotting XIC	**15s**	
Averaging a spectrum	**10s**	
Export	**20s**	
Opening file	**15s**	**(MagTran or mMass)**
Deconvolution	**10s**	
Writing down to xls	**10s**	

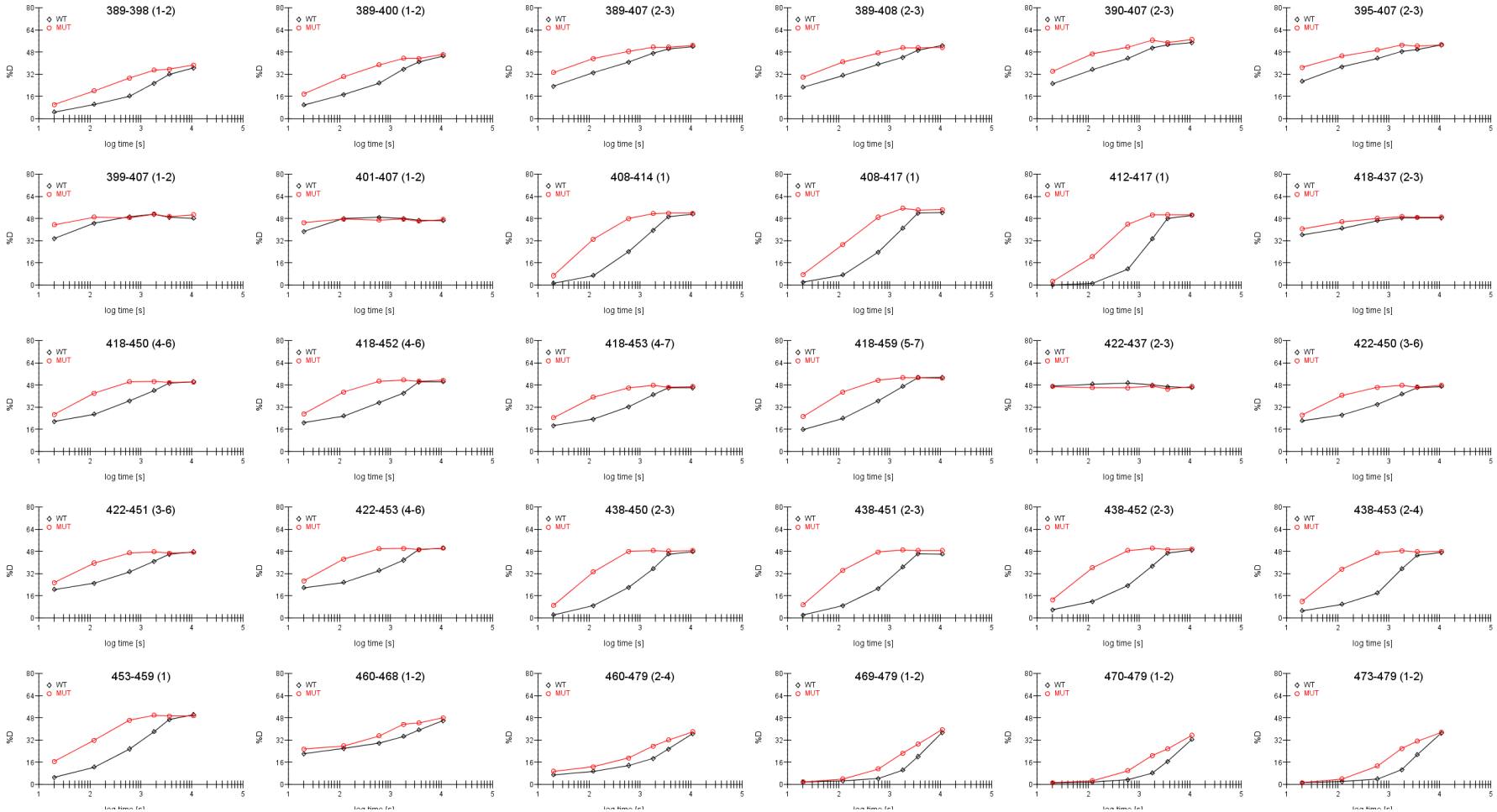
2-10min per peptide from one state

Software tools

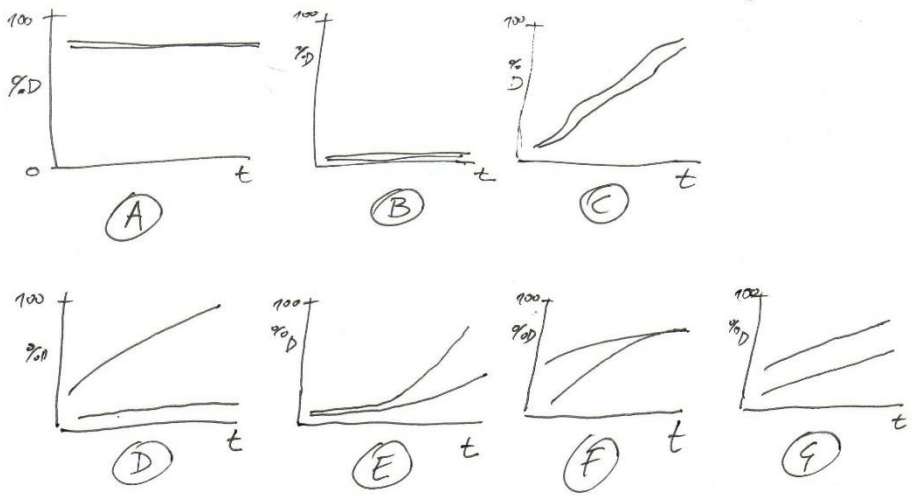
Waters – DynamX
LEAP / Sierra Analytics – HDEXaminer
OmicsX – HDX Workbench

Mike Guttmann – HX Express (Excel base for Waters users, EX1/EX2 deconvolution)

Output – uptake plots



Output – 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

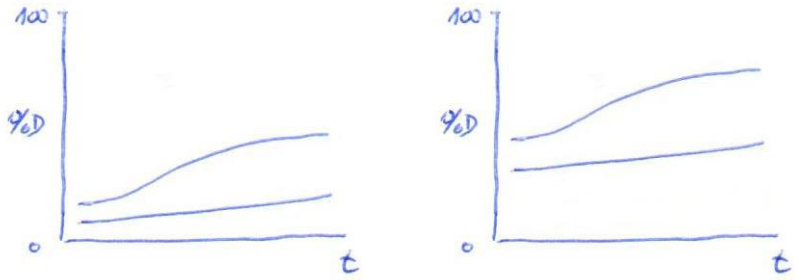
Correction for back-exchange

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

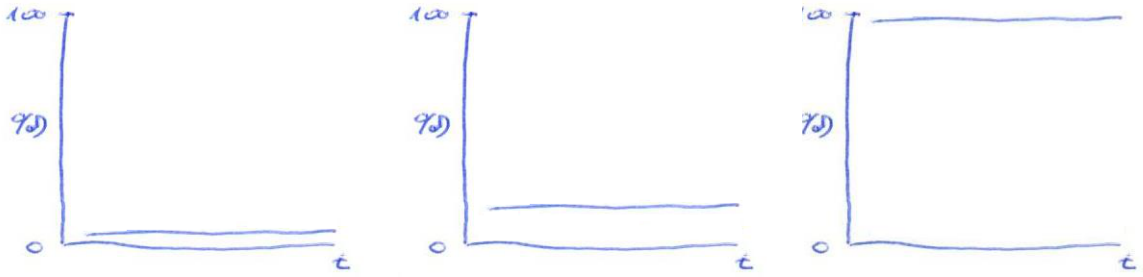
$$\text{Corr No D} = \frac{Mp-Mn}{Mf-Mn} * Ns$$

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

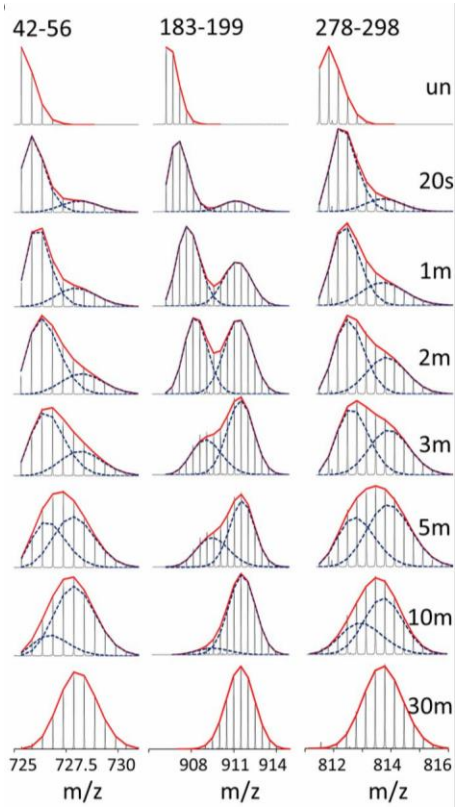
Examples – before (left) and after (right) correction to BE – overall level raises + difference is slightly bigger (due to bigger y-scale)

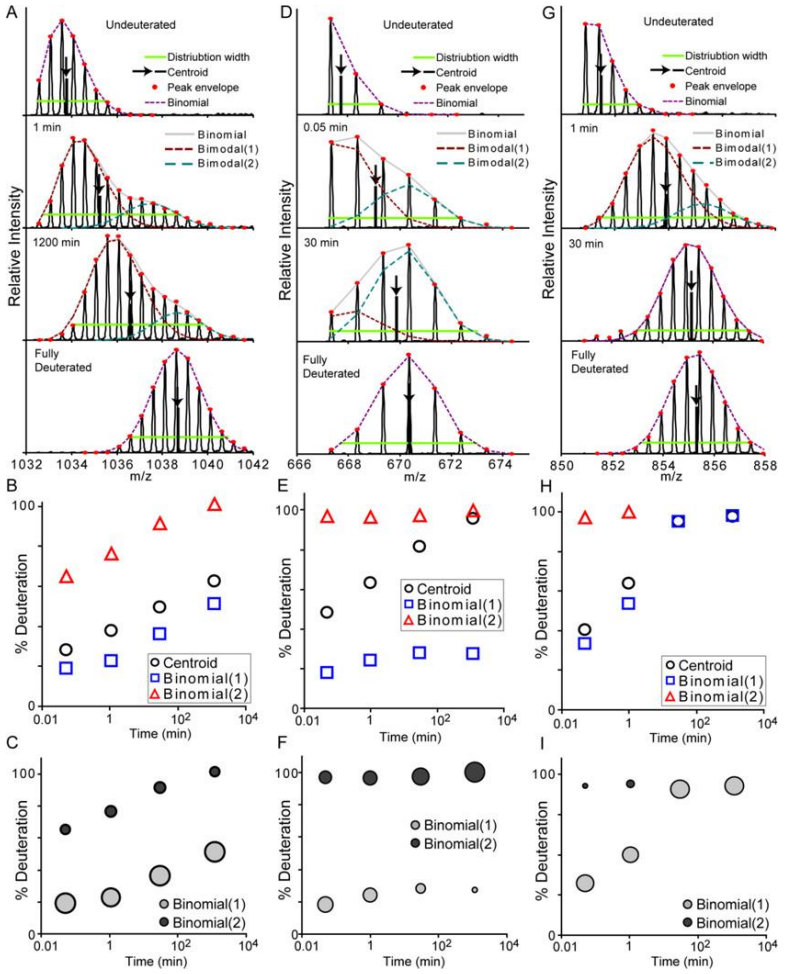


Examples – slow and fast exchanger
left – before BE correction hard to judge – middle – slow exchanger (deep in the structure) after correction; right – fast exchanger – e.g. HisTag - after correction



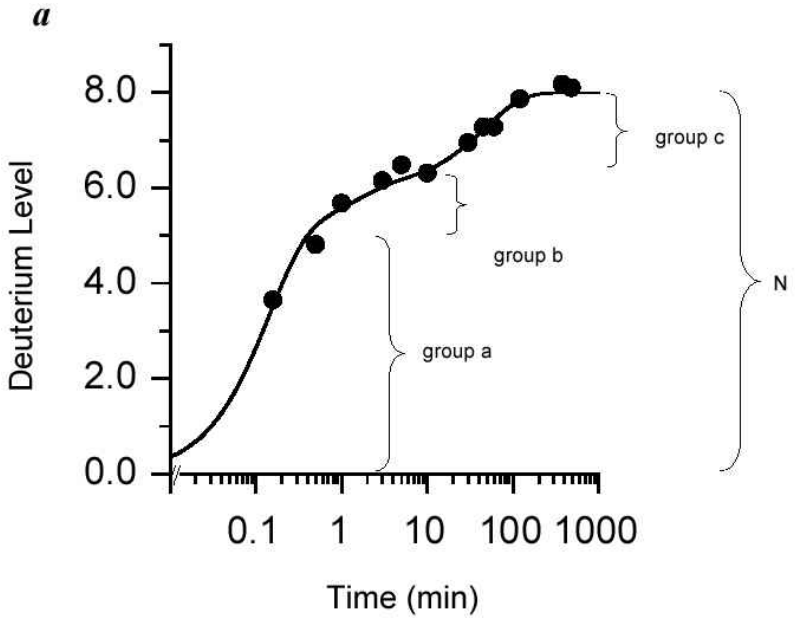
EX1/EX2 analysis





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

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

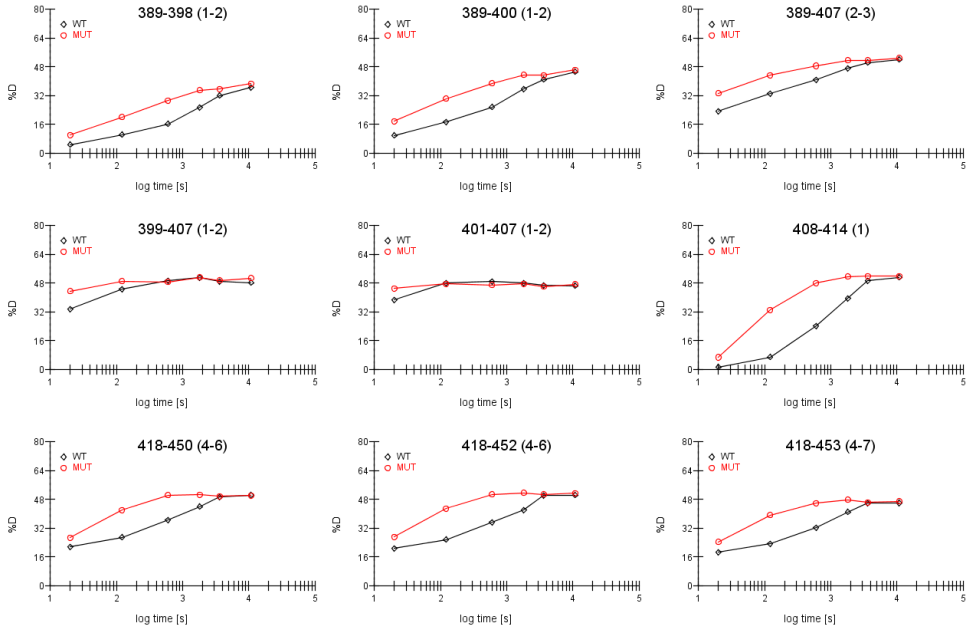
group a
m1=7.21 ± 0.82 min^{-1}
$t_{1/2}$=0.096 min

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

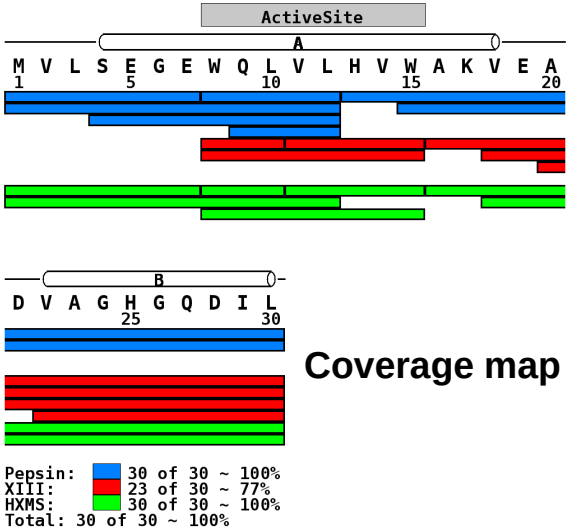
group c
m3=0.021 ± 0.003 min^{-1}
$t_{1/2}$=33.0 min

Data presentation

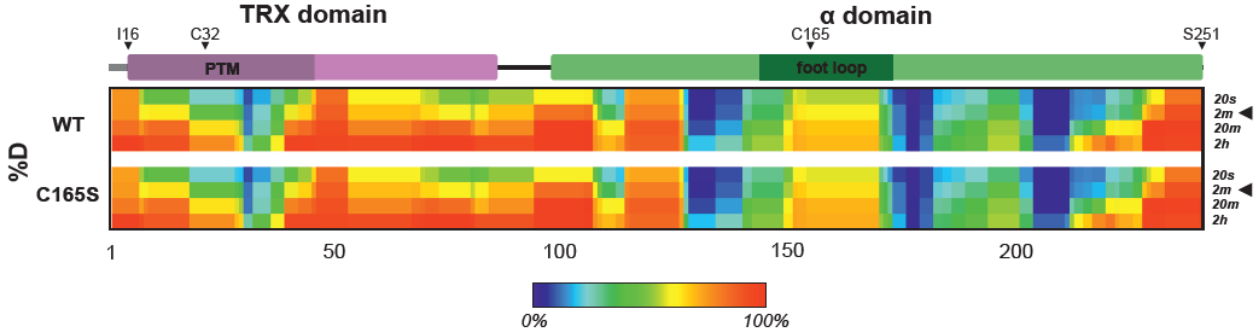
Uptake plots



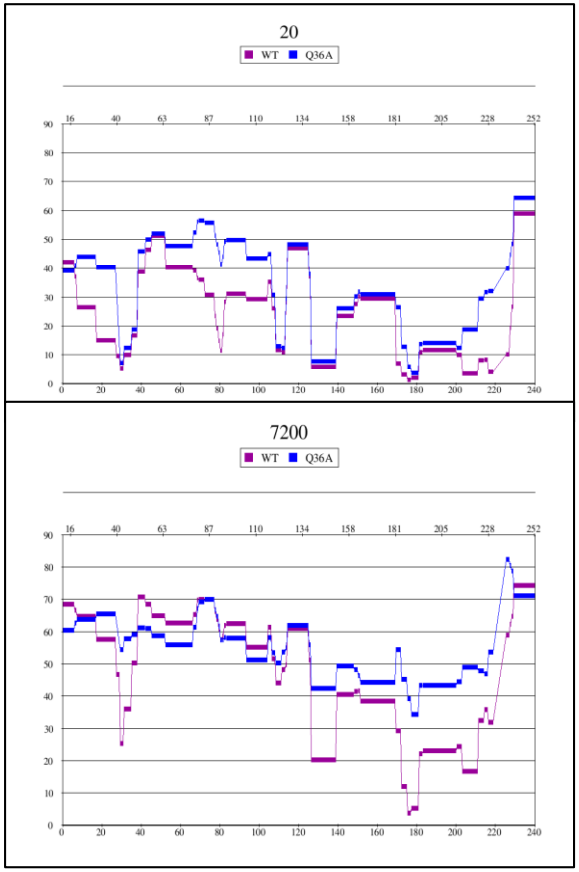
Coverage map



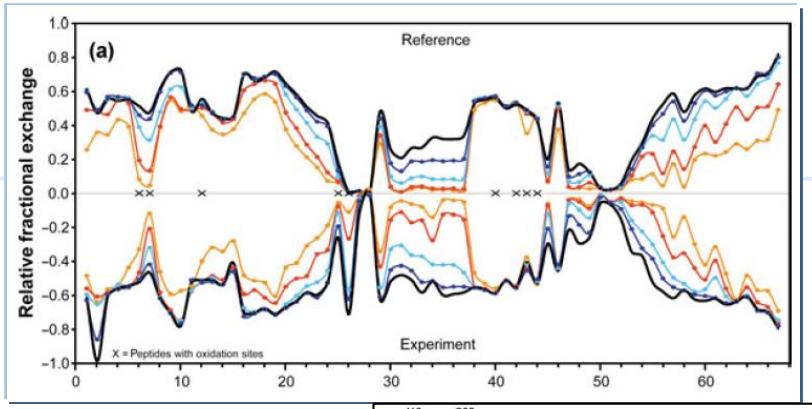
Heat map



Data presentation

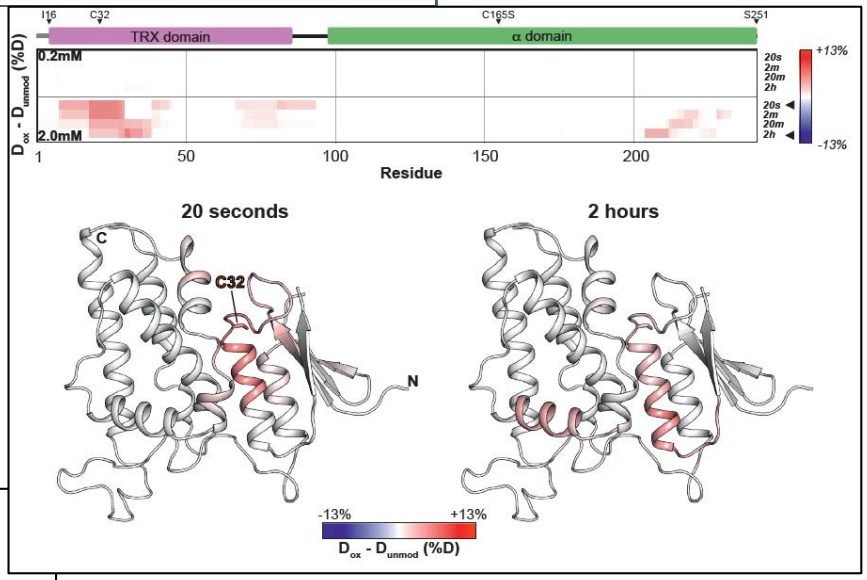


Protection plots

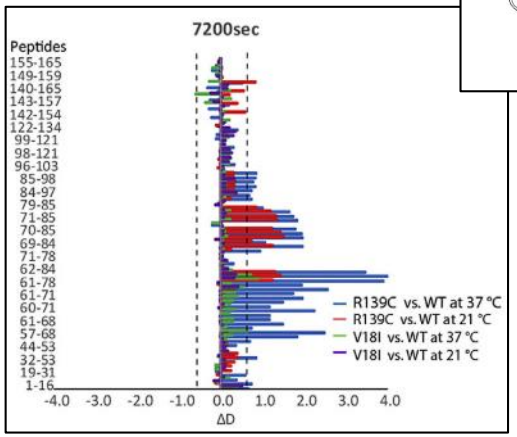


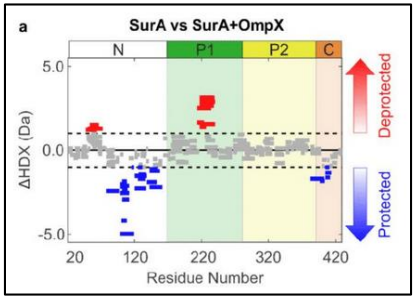
Mirror plot / Butterfly plot

Differential heat map
+
Structure visualization



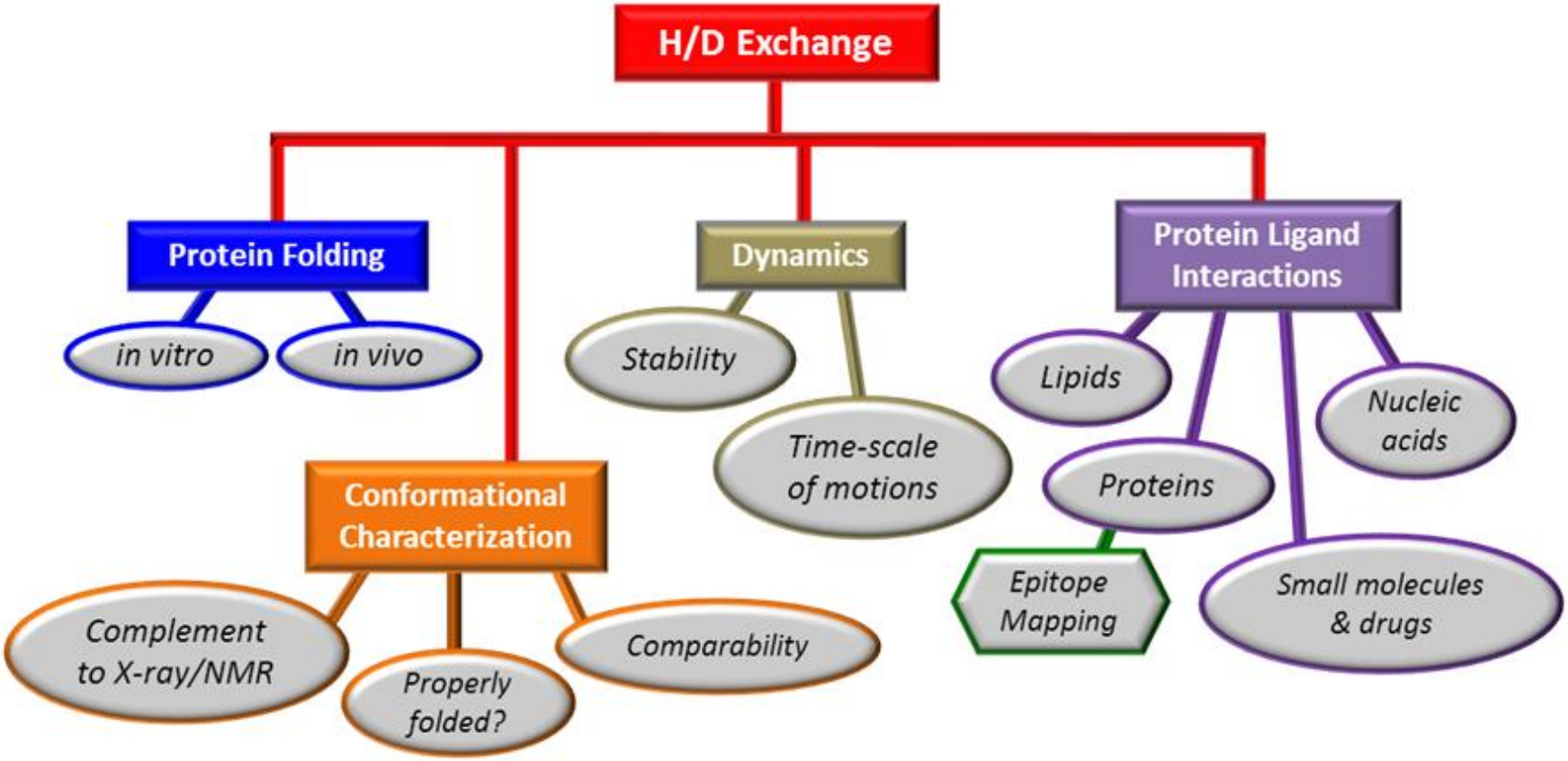
Bar plot





Wood's plot

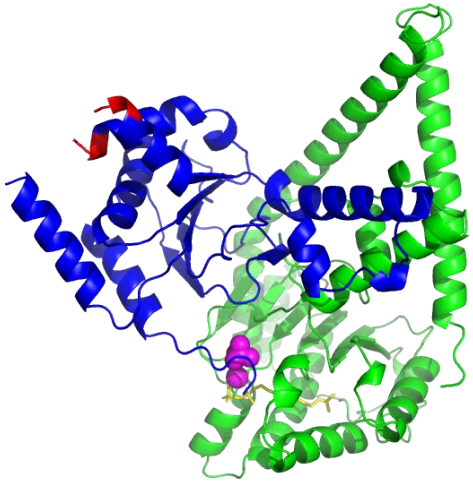
WHAT CAN BE DONE

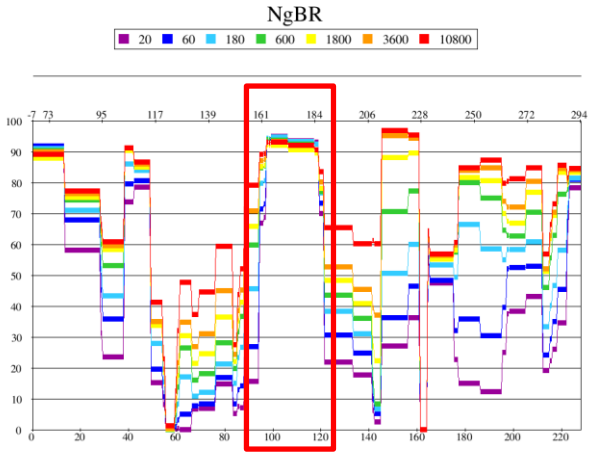


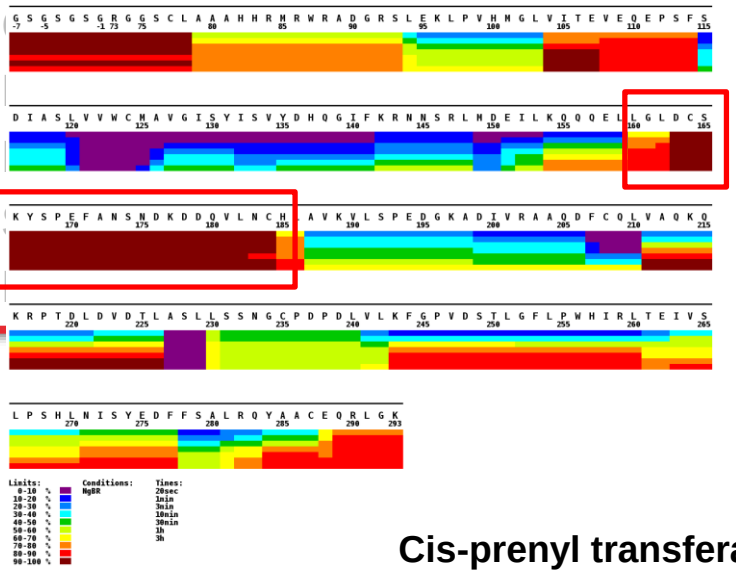
Engen et al. (2011). *Ency. Anal. Chem.* ISBN 978-04-700-2731.

PROTEIN FOLD – STRUCTURED REGIONS

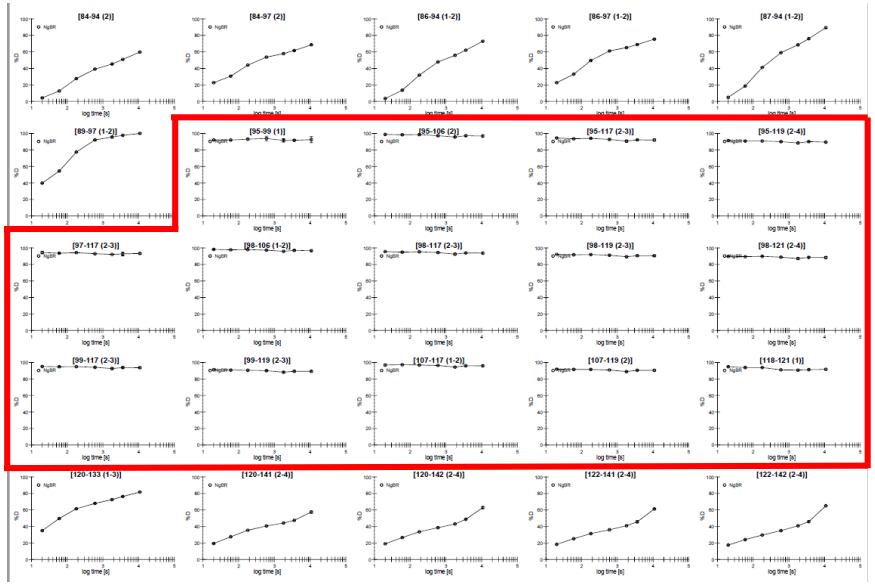
- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SURREY
- PROTEIN INTERACTIONS
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS





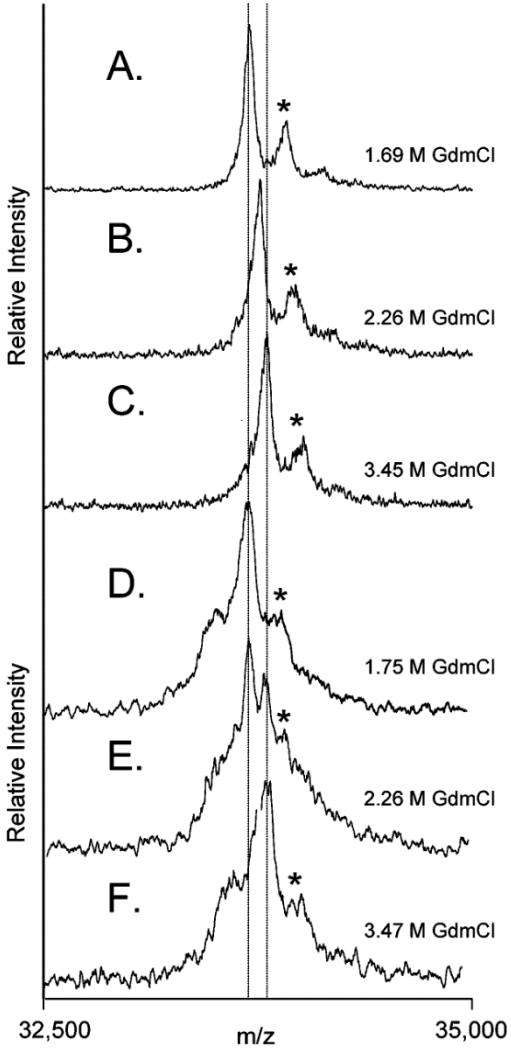


Cis-prenyl transferase

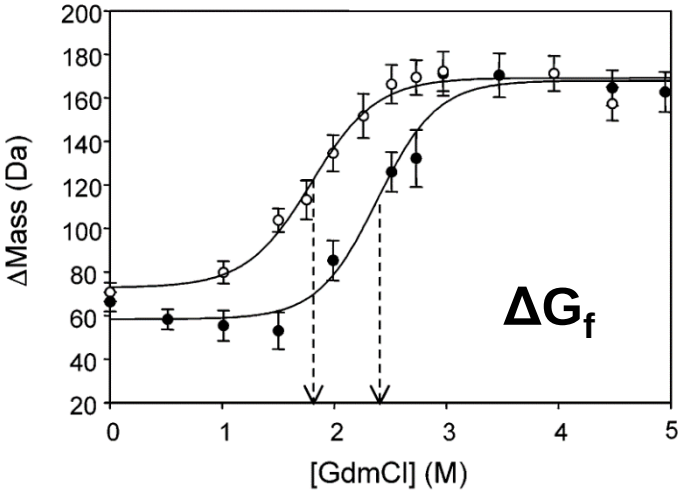


PROTEIN FOLDING

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - COFACTORS – PLIMSTEX
  - MEMBRANES
  - DNA
  - SMALL MOLECULES/GAS
  - LARGE COMPLEXES



SUPREX :
Stability of Unpurified Proteins from Rates of H/D Exchange



Roulhac *et al.*: Biochemistry (2004)

BIOLOGICALS

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SURREY
- PROTEIN INTERACTIONS
  - **ANTIBODIES**
  - OTHER PROTEINS
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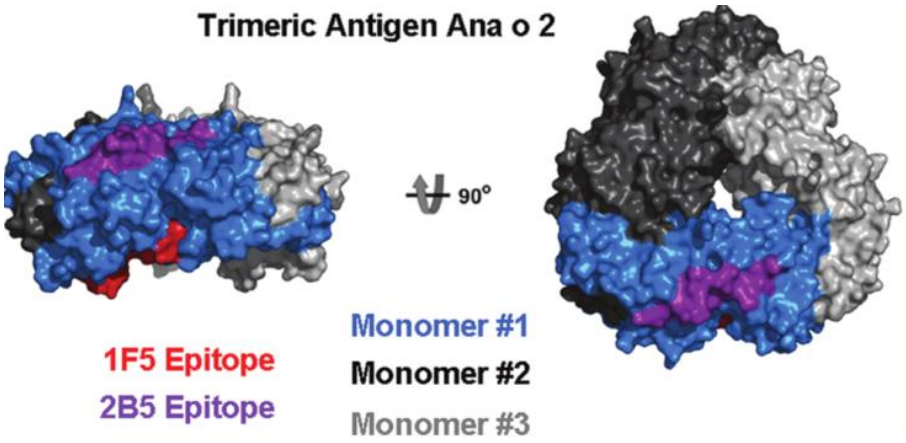
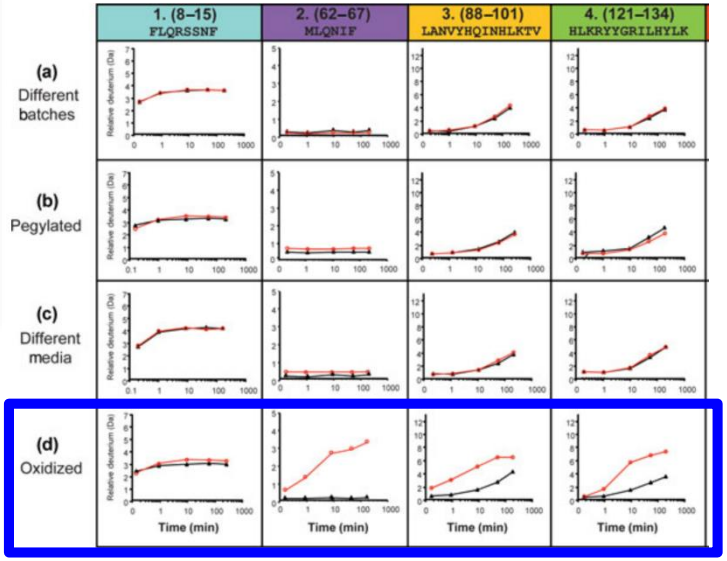
  - COFACTORS – PLIMSTEX
  - MEMBRANES
  - DNA
  - SMALL MOLECULES/GAS
  - LARGE COMPLEXES

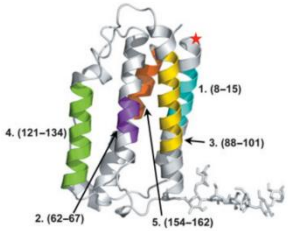
A) EPITOPE MAPPING



B) BIOSIMILARS



Process is reproducible



PEGylation has no effect

Different cultivation is OK

Watch for oxidation

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

PROTEIN-PROTEIN INTERACTIONS

- (UN)STRUCTURED PROTEINS
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- PROTEIN INTERACTIONS
  - ANTIBODIES
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  - MEMBRANES
  - DNA
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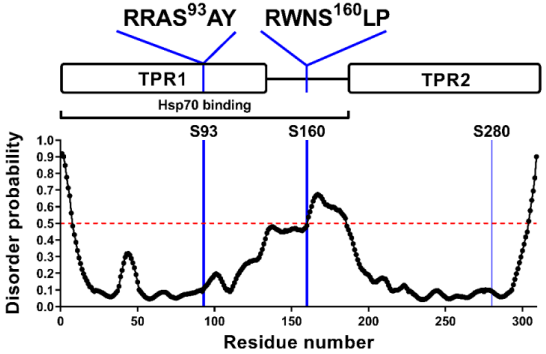
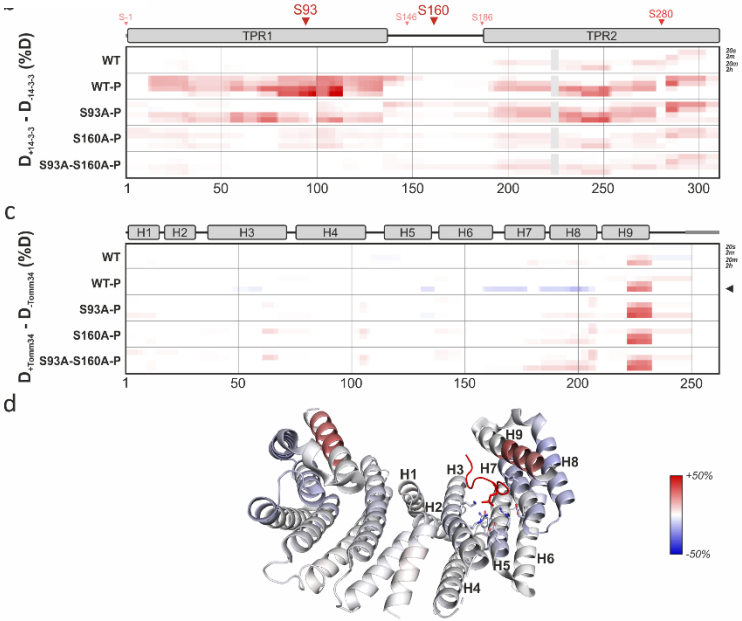
  - LARGE COMPLEXES

Phosphorylation dependent interaction between TOMM34 and 14-3-3





PROTEIN-PROTEIN INTERACTIONS

- (UN)STRUCTURED PROTEINS
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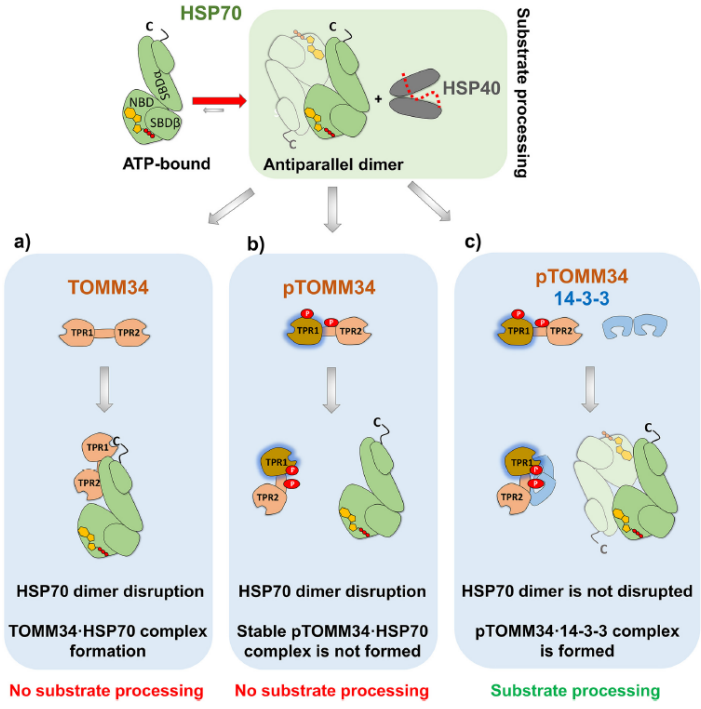
  - LARGE COMPLEXES

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.



PROTEIN-PROTEIN INTERACTIONS

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  - MEMBRANES
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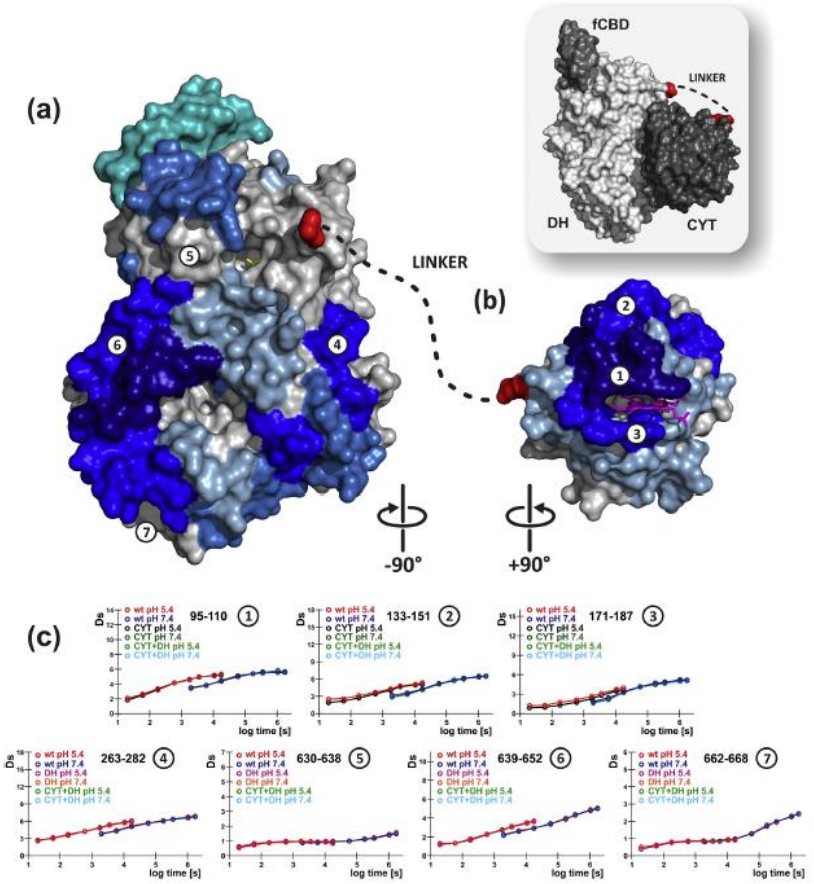
  - LARGE COMPLEXES

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



Kadek A et al BBA 2017

MEMBRANE PROTEINS

- (NE)STRUKTUROVANÉ PROTEINY
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - ANTIBODIES
  - OTHER PROTEINS
  - **MEMBRANE PROTEINS**
  - COFACTORS – PLIMSTEX
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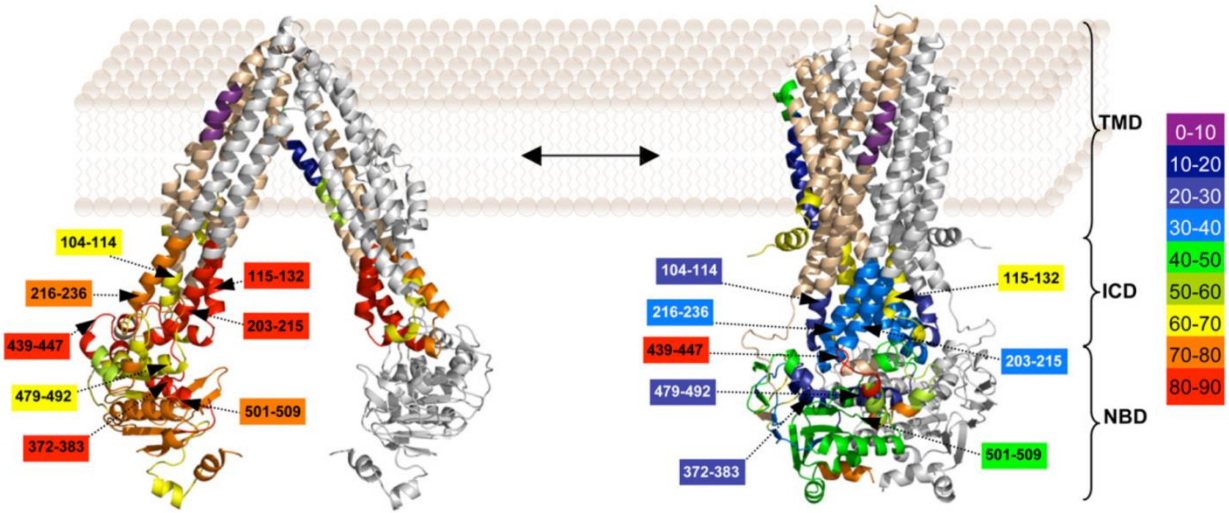
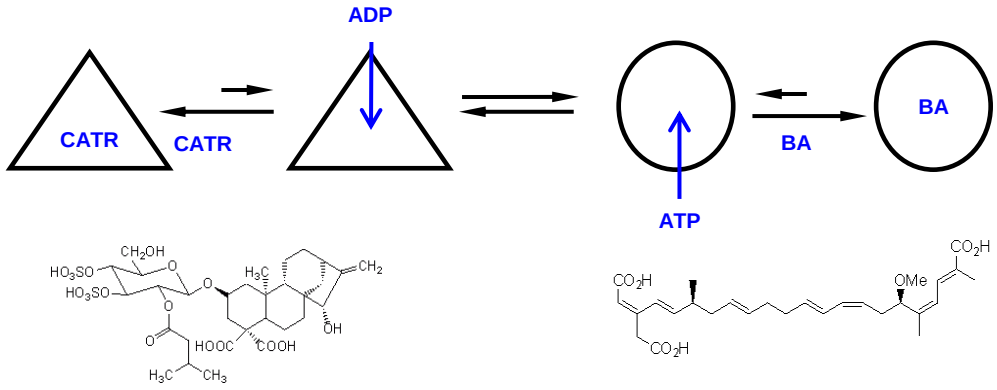


Mehmood *et al.*: PNAS (2012)

MEMBRANE PROTEINS IN NON-IONIC DETERGENTS

ANT, ADP/ATP TRANSPORTER

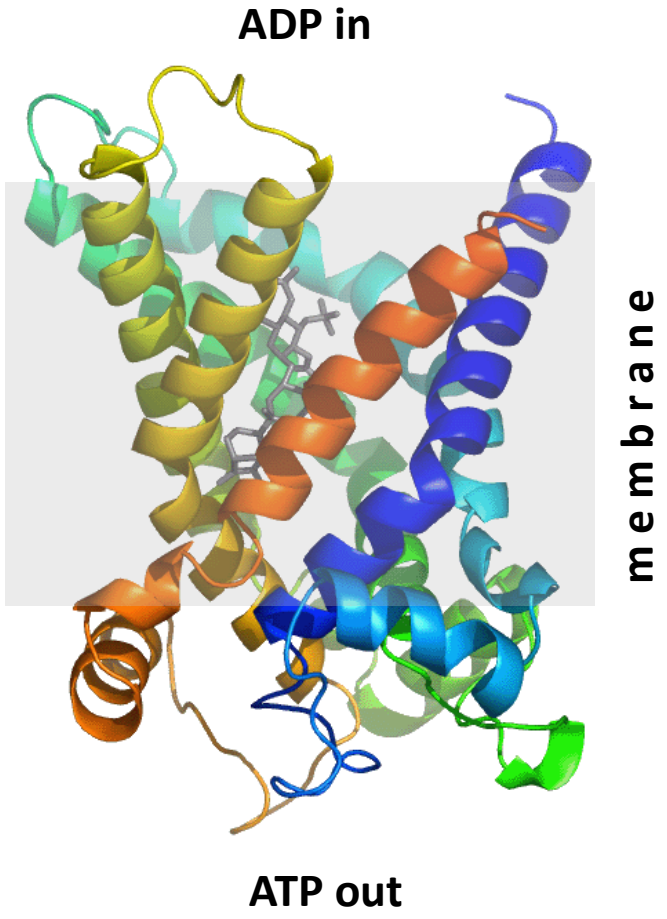
TWO STATES WHICH CAN BE BLOCKED BY TWO DIFFERENT INHIBITORS



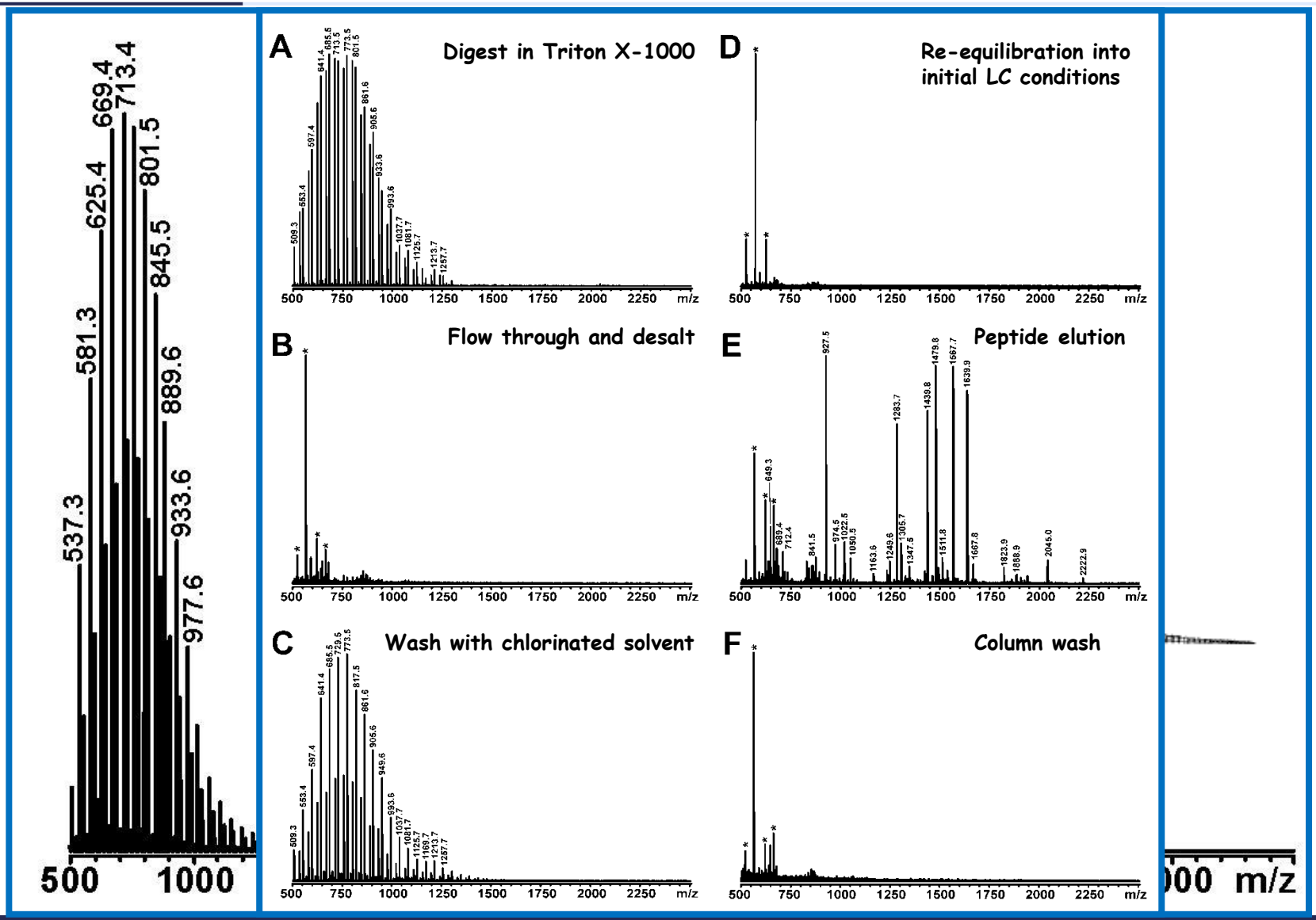
X-RAY STRUCTURE KNOWN FOR CATR

IMPOSSIBLE TO GET STRUCTURE FOR BA

REQUIRES PRESENCE OF DETERGENT (TRITON X-100)

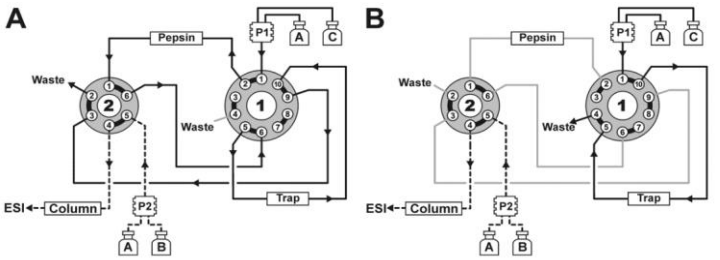


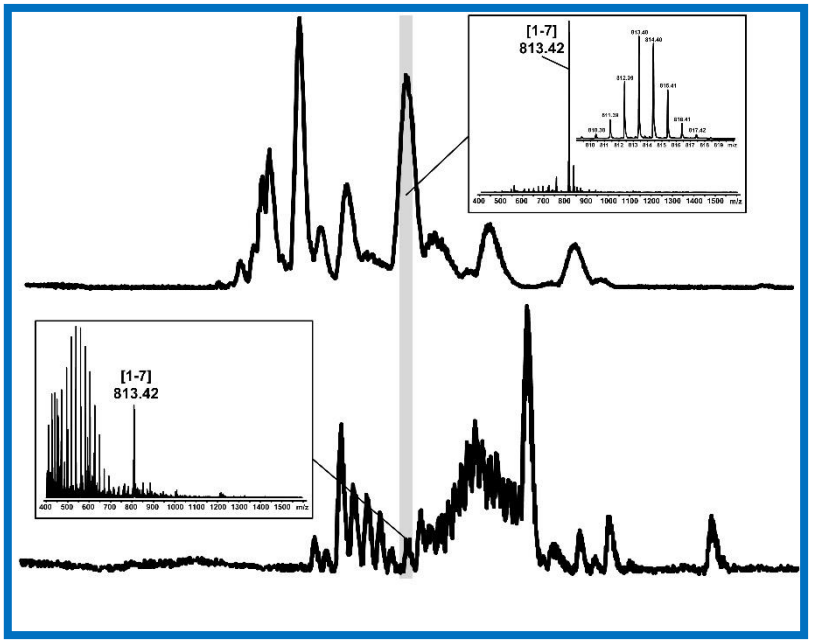
MEMBRANE PROTEINS IN NON-IONIC DETERGENTS

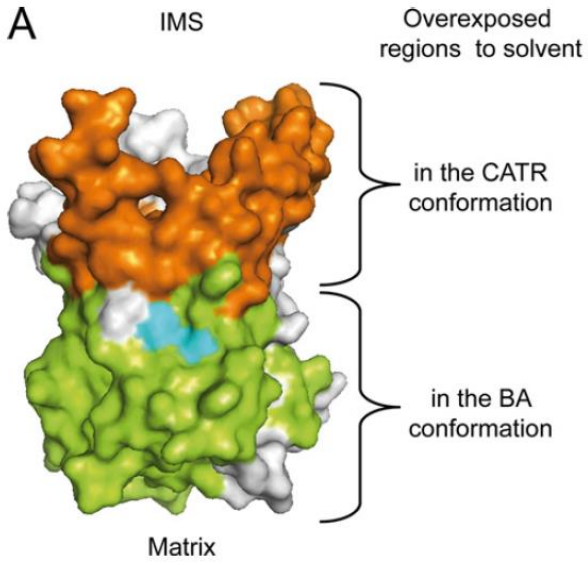


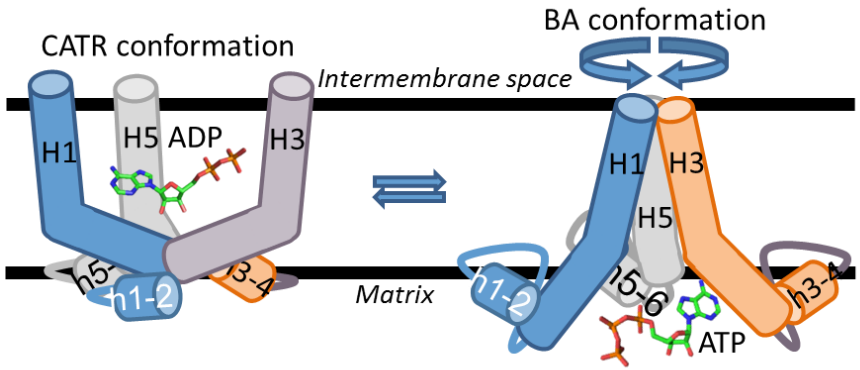
Rey M. et al. Anal. Chem. 2010

MEMBRANE PROTEINS IN NON-IONIC DETERGENTS







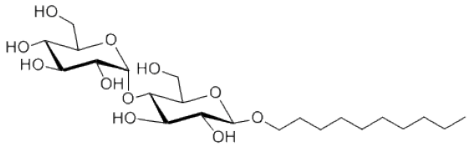


Rey, M. et al. J. Biol. Chem. 2010

MEMBRANE PROTEINS IN ALKYLGLYSIDES

DM – Decyl Maltoside

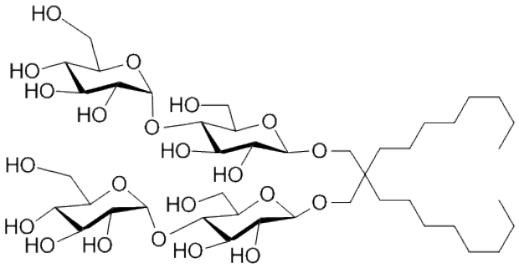
n-decyl-β-D-maltopyranoside



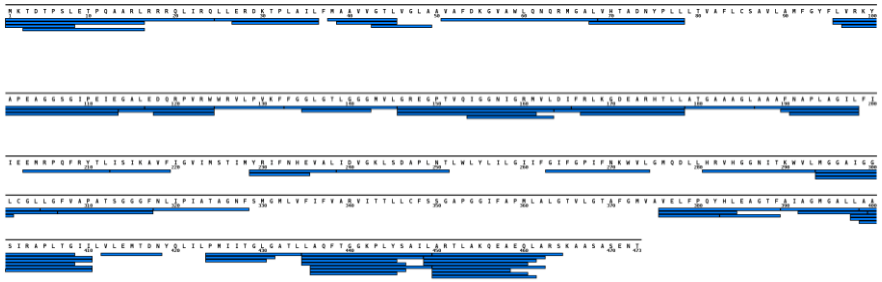
- $C_{22}H_{42}O_{11}$
- M_w: 482.6
- CMC (H_2O): 1.8mM

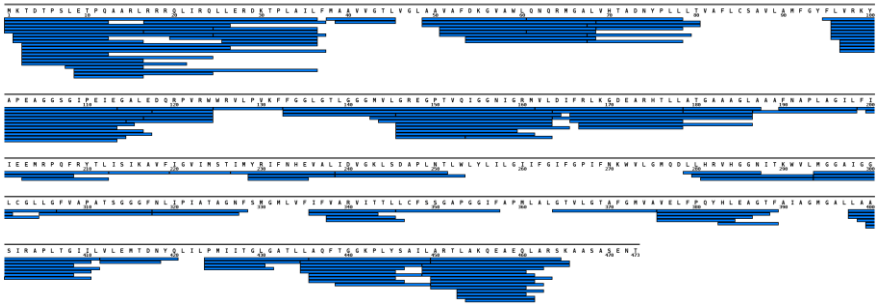
DMNG – Decyl Maltose Neopentyl Glycol

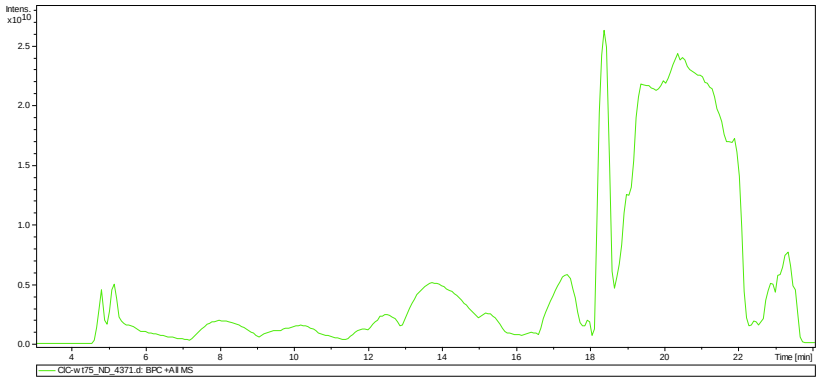
2,2-dioctylpropane-1,3-bis-β-D-maltopyranoside

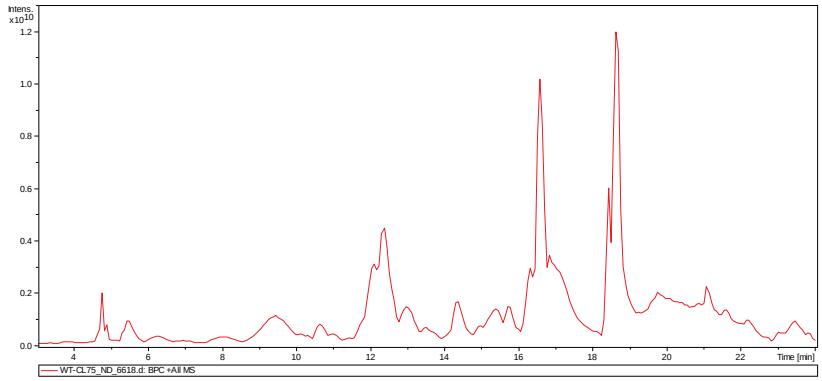


- $C_{43}H_{80}O_{22}$
- M_w: 949.08
- CMC (H_2O): 0.036mM



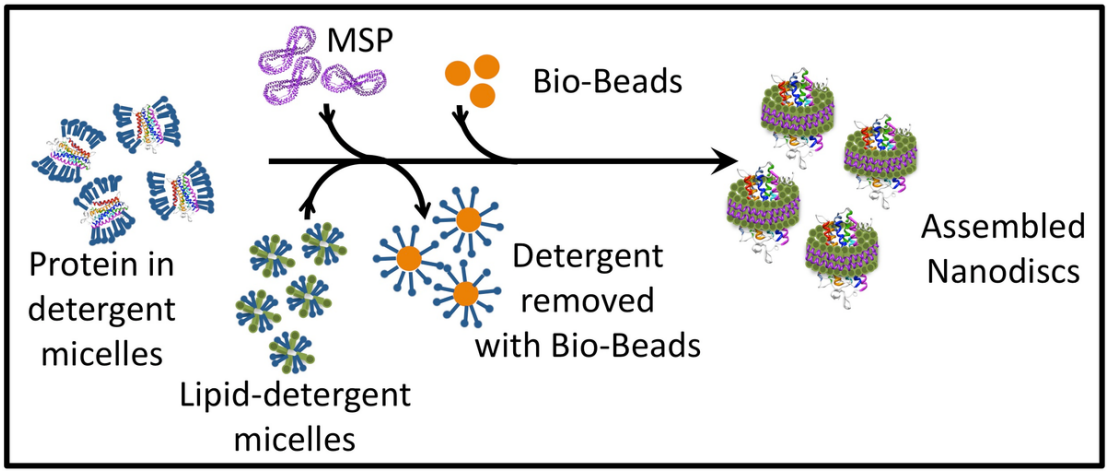


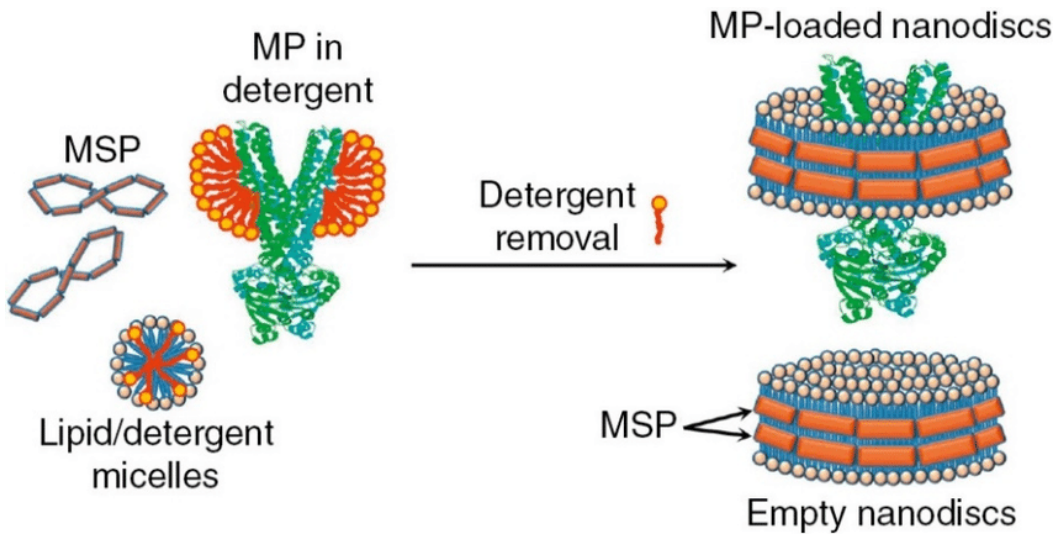




MEMBRANE PROTEINS IN NANODISCS

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - COFACTORS – PLIMSTEX
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - **MEMBRANES**
  - DNA
  - SMALL MOLECULES/IONS
  - LARGE COMPLEXES





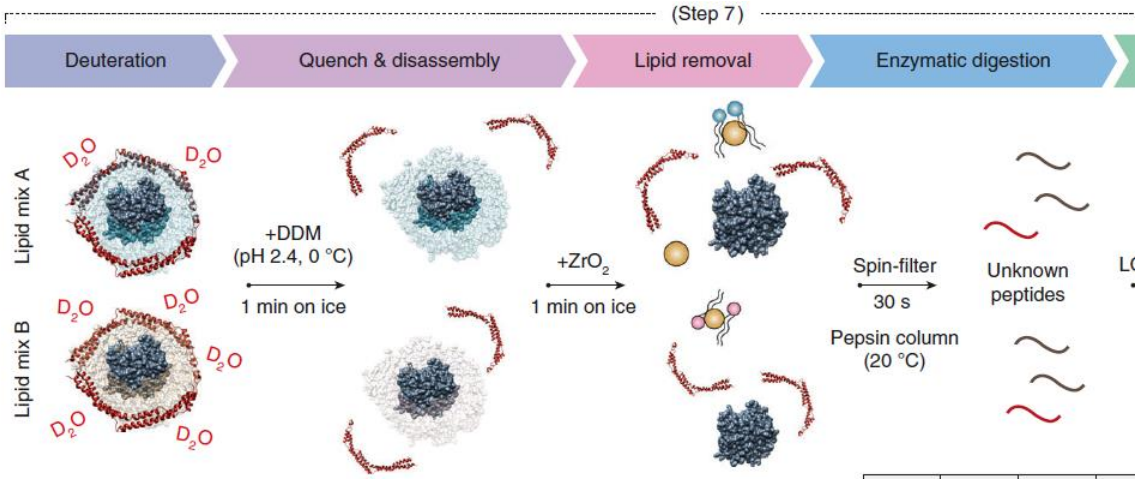
Hebling CM et al Anal Chem 2010
Harrison RA et al Curr Opin Struct Biol 2016
Martens C et al Nat Protocols 2019

MEMBRANE PROTEINS IN NANODISCS

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - COFACTORS – PLIMSTEX
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - **MEMBRANES**
  - DNA
  - SMALL MOLECULES/IONS
  - LARGE COMPLEXES

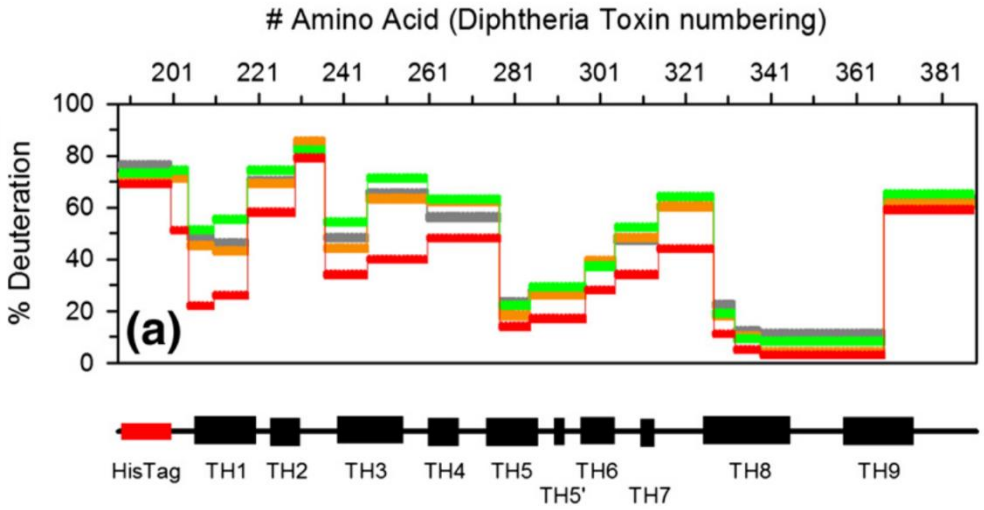
Nanodiscs – protocol *(Martens C et al Nat Protocols 2019)*

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

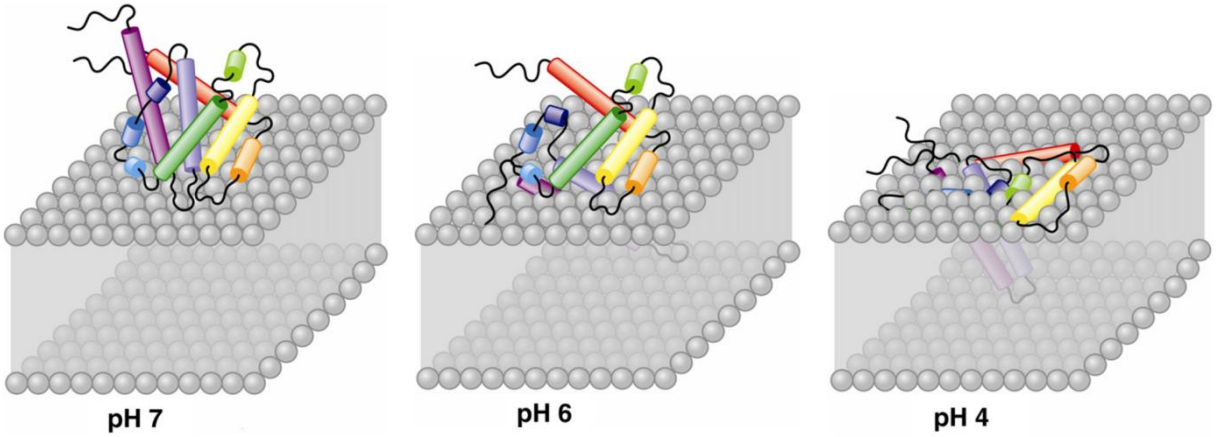


MEMBRANE INTERACTION

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SURREY
- PROTEIN INTERACTIONS
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - COFACTORS – PLIMSTEX
  - **MEMBRANES**
  - DNA
  - SMALL MOLECULES/GAS
  - LARGE COMPLEXES



G: - mem / - NaCl
R: + mem / - NaCl
O: + mem / + NaCl
Gr: - mem / + NaC

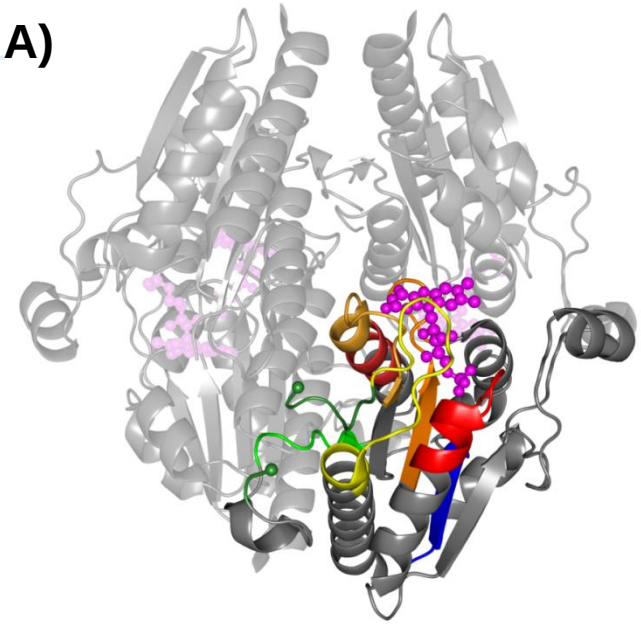


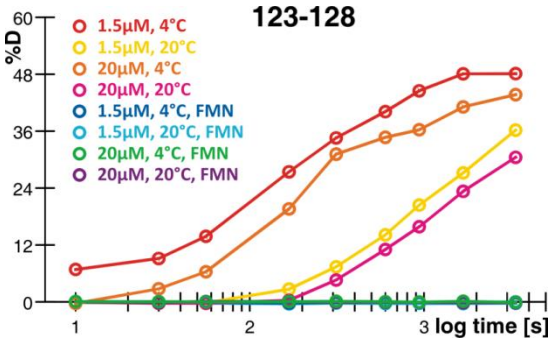
Man *et al.*: J. Mol. Biol. (2011)

COFACTOR BINDING

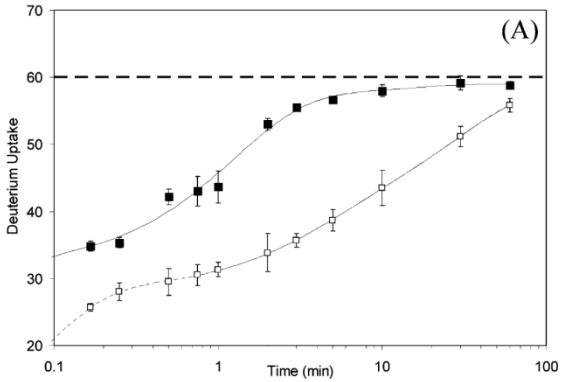
- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SURREY
- PROTEIN INTERACTIONS
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - **COFACTORS – PLIMSTEX**
  - MEMBRANES
  - DNA
  - SMALL MOLECULES/GAS
  - LARGE COMPLEXES

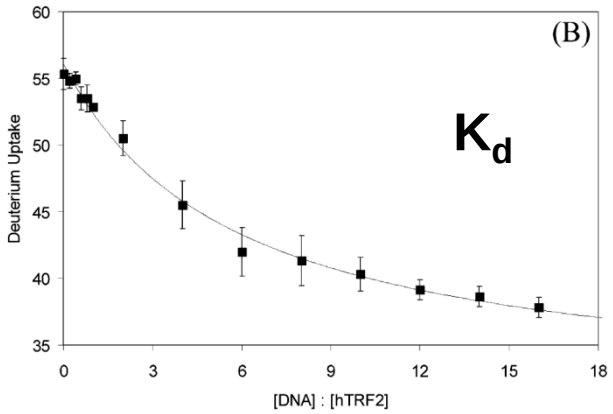
A)





B) PLIMSTEX : Protein-ligand interactions by mass spectrometry, titration and H/D exchange

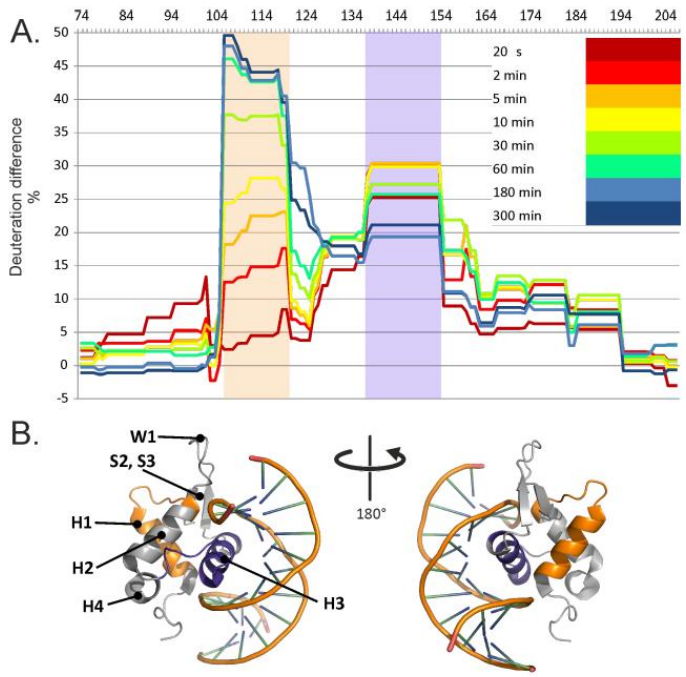




Zhu *et al.*: JACS (2003) Sperry *et al.*: Biochemistry (2008)

DNA BINDING

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - COFACTORS – PLIMSTEX
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - MEMBRANES
  - **DNA**
  - SMALL MOLECULES/IONS
  - LARGE COMPLEXES



DNA ISSUES!!!

DNA gets protonated upon quenching/acidification and may precipitate. Protein co-precipitate.
DNA affects LC separation on RP columns

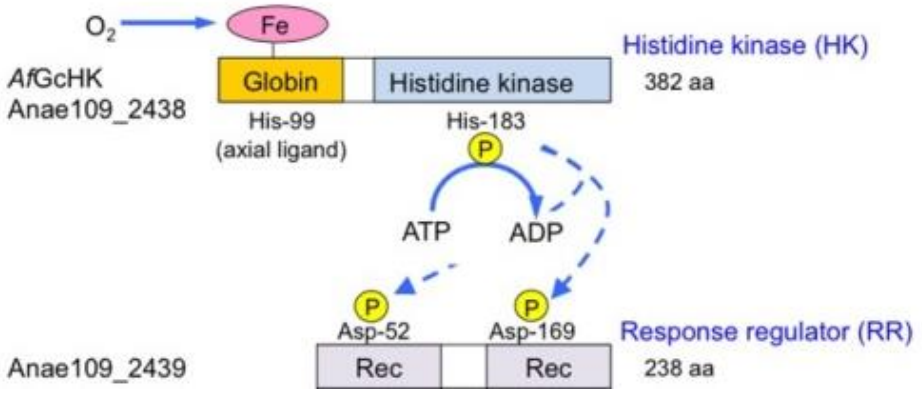
Tricks available (published)
- Strong anion exchange (DNA removal)
- Charged compounds added (protamine)
- Denaturation
- LC system washing (neutral pH+organics)

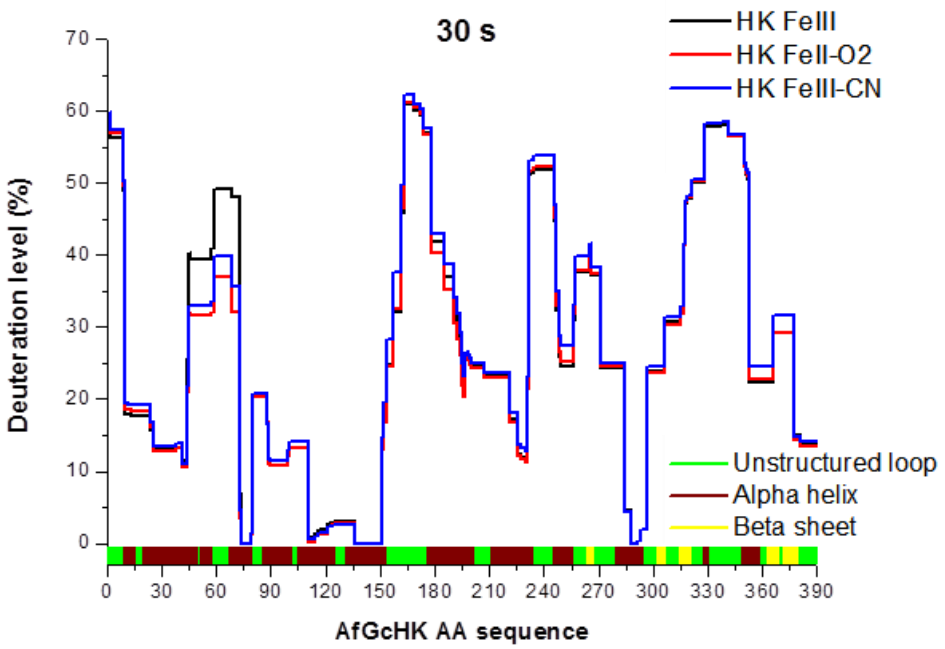
Slavata et al.: Biomolecules 2019

Sperry J.B. et al J Am Soc Mass Spectrom 2008
Poliakov A. et al Rapid Commun Mass Spectrom 2008
Roberts V.A. et al Nucleic Acid Res 2012
Graham B.W. et al J Biol Chem 2016

SMALL MOLECULES / GAS SENSING

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SURREY
- PROTEIN INTERACTIONS
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - COFACTORS – PLIMSTEX
  - MEMBRANES
  - DNA
  - **SMALL MOLECULES/GAS**
  - LARGE COMPLEXES

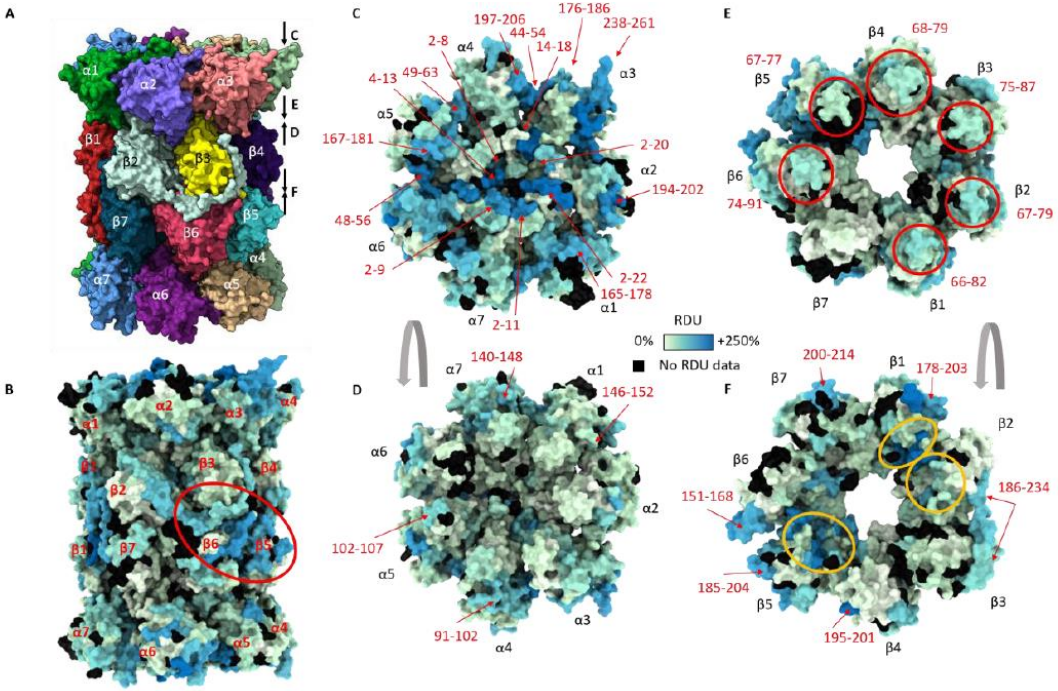


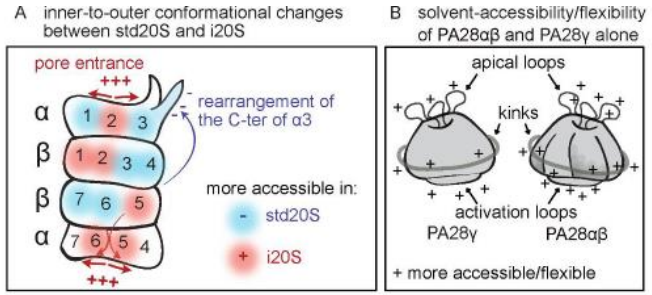


Martinkova, Kitanishi, Shimizu: J. Biol. Chem. (2013)

SUPRAMOLECULAR COMPLEXES

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - COFACTORS – PLIMSTEX
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - MEMBRANES
  - DNA
  - SMALL MOLECULES/GAS
  - **LARGE COMPLEXES**





Lesne J et al bioRXiv 2020

SUPRAMOLECULAR COMPLEXES

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
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  - COFACTORS – PLIMSTEX
  - ANTIBODIES
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  - MEMBRANE PROTEINS
  - MEMBRANES
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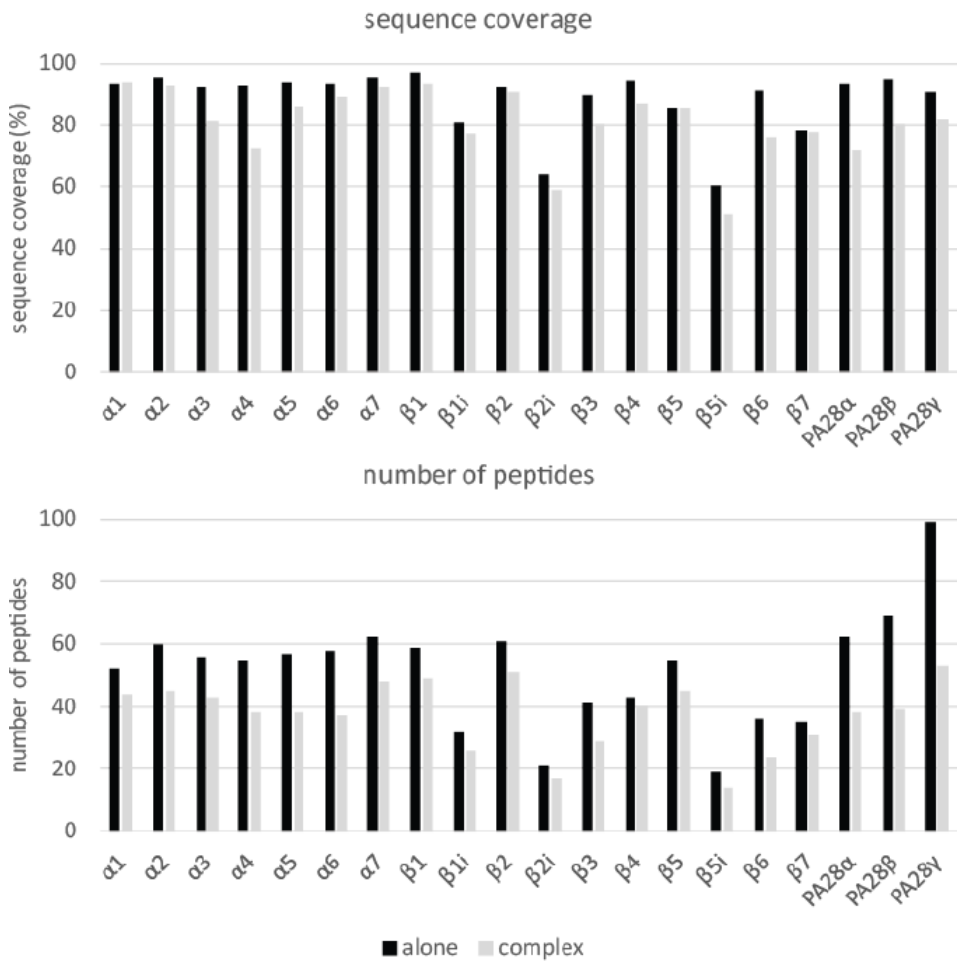
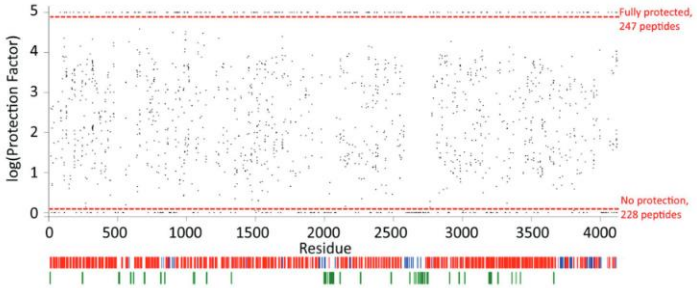


Lesne J et al bioRXiv 2020

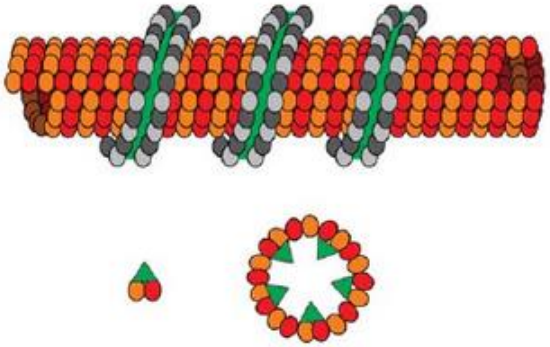
HUGE PROTEINS

- (UN)STRUCTURED PROTEINS
- FOLDING / UNFOLDING – SUPREX
- PROTEIN INTERACTIONS
  - COFACTORS – PLIMSTEX
  - ANTIBODIES
  - OTHER PROTEINS
  - MEMBRANE PROTEINS
  - MEMBRANES
  - DNA
  - SMALL MOLECULES/GAS
  - **LARGE COMPLEXES**

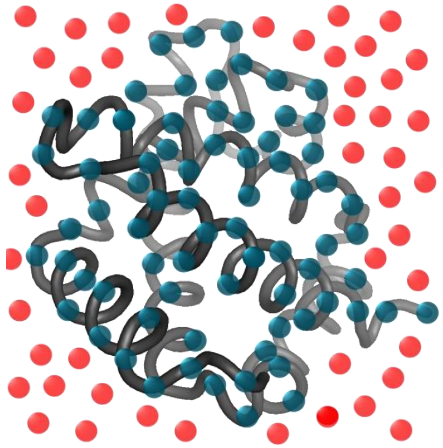
Sheff JG et al Analyst 2017 – DNA-PKc (469kDa)



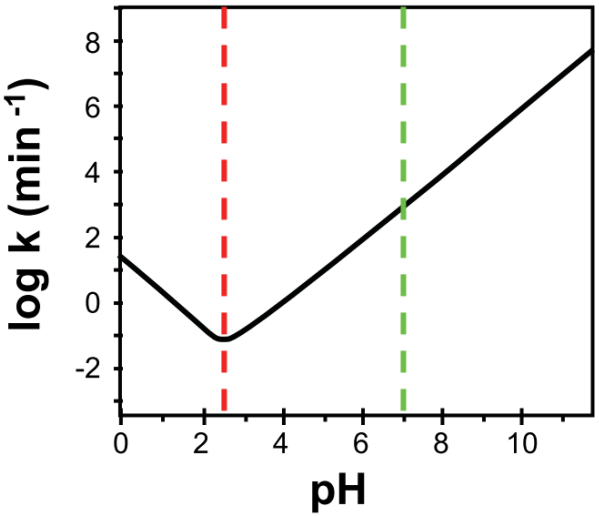
Burns KM et al Prot Sci 2015 – alpha/beta tubulin + dimeric kinesin 175kDa total sequence assembled >1GDa



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