Structural Proteomics: The in and the out of a protein

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EU FT-ICR_MS End User School
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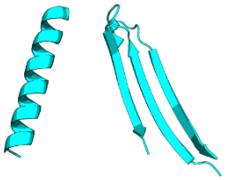
PRIMARY

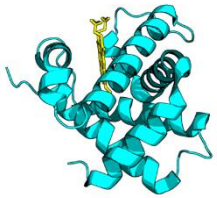
NIDDEGSAFYGVSSQYES

SECONDARY



STRUCTURE

TERTIARY



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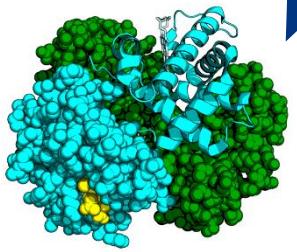

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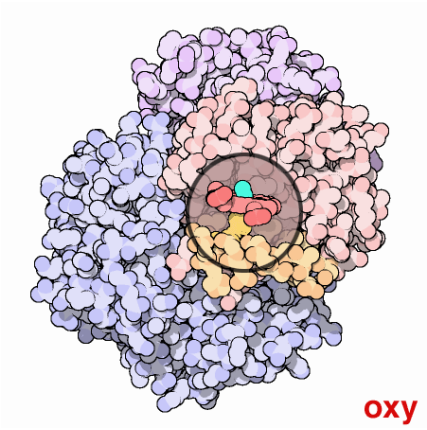
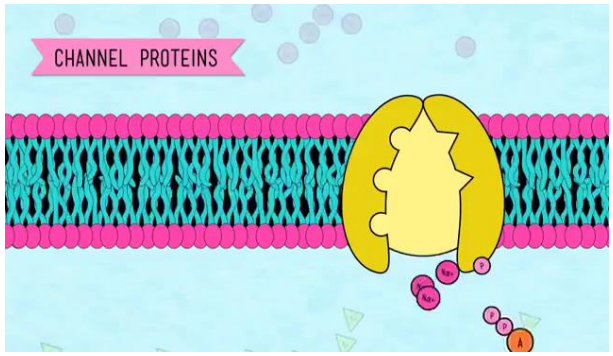


FUNCTION



Six Blind Men and the Protein of Unknown Structure

"It was six men of Indostan
To learning much inclined,
Who went to see the Elephant
(Though all of them were blind)
That each by observation
Might satisfy his mind..."
John Godfrey Saxe
(1816-1887)

Structural Mass Spectrometry…

Protein covalent labeling

Chemical cross-linking

H/D exchange

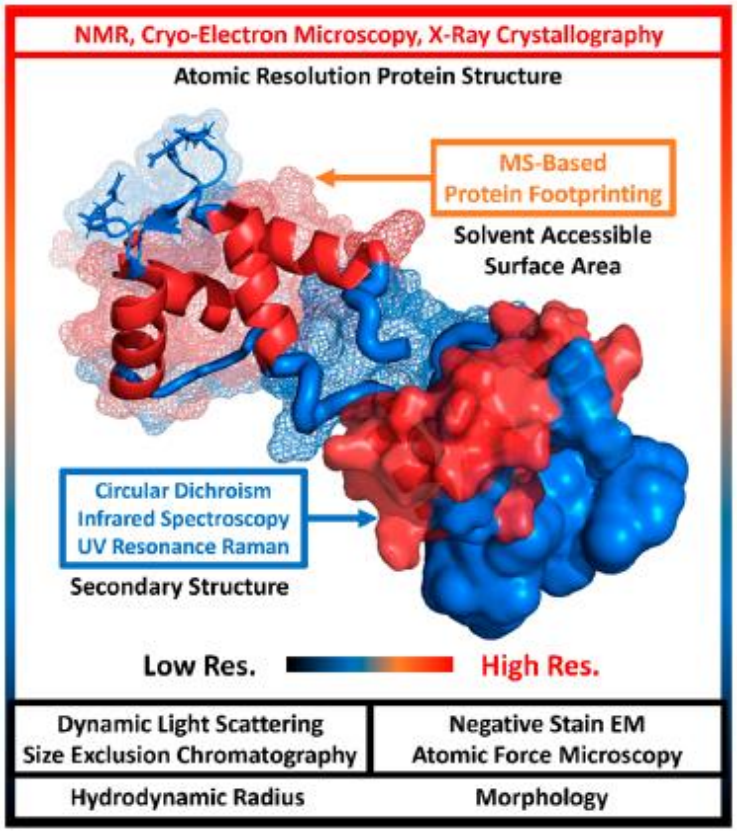
Disulfide bonds mapping

Native mass spectrometry and Ion mobility

Fast photochemical oxidation of proteins

ETD/ECD fragmentation

Limited proteolysis



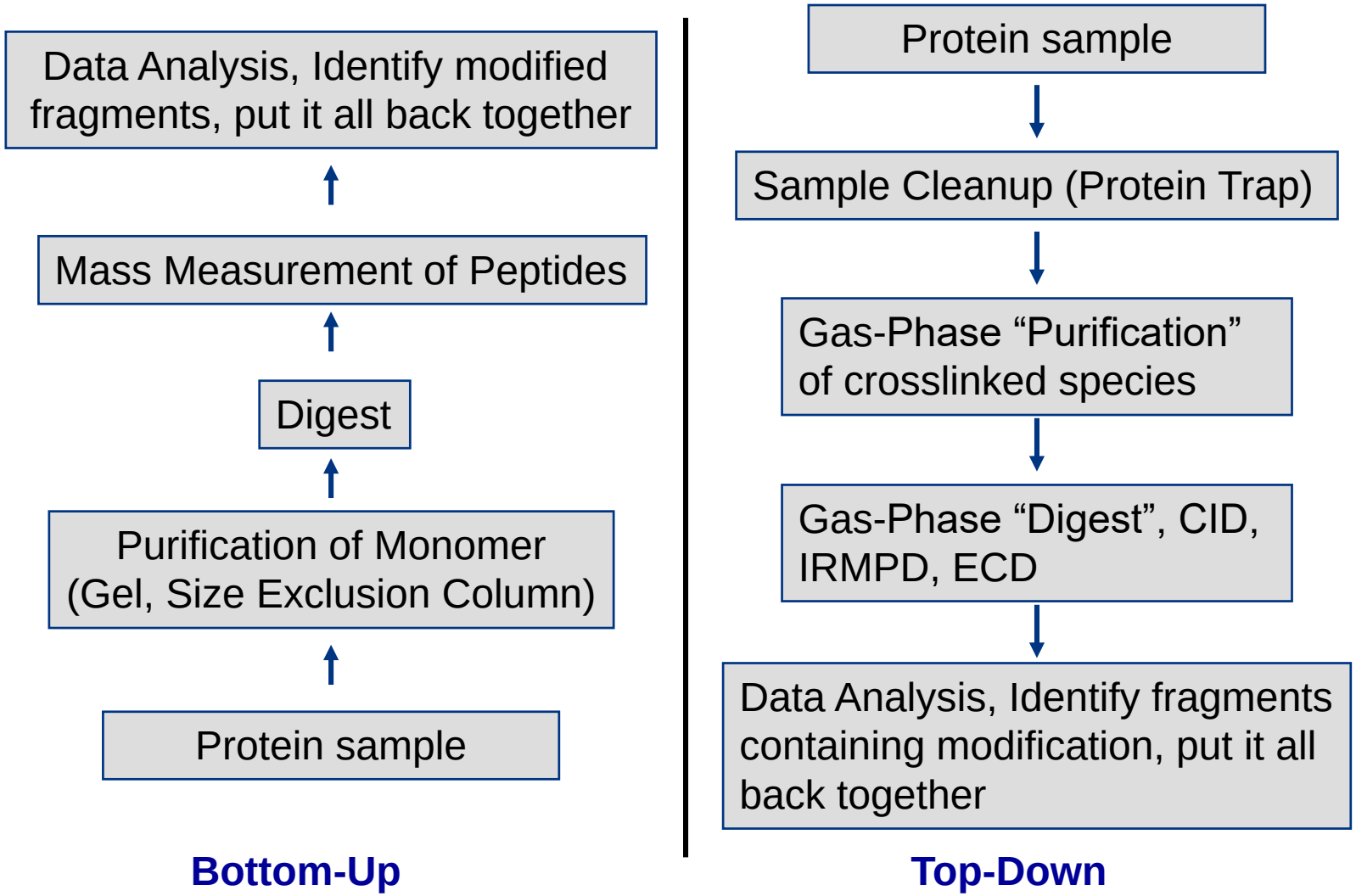
Special Issue on Mass Spectrometry in Structural Biology (2015) Protein Science *24*, 1173-1332

Mass Spectrometry-Based Protein Footprinting for Higher-Order Structure Analysis: Fundamentals and Applications. Chem Rev. 2020; 120: 4355-4454.

Structural Mass Spectrometry…

Liu XR, Zhang MM, Gross ML. Mass Spectrometry-Based Protein Footprinting for Higher-Order Structure Analysis: Fundamentals and Applications. Chem Rev. 2020; 120: 4355-4454.

Mass Spectrometry: Goal in Protein Structure Characterization



Footprinting

Assay examining higher structures of biomacromolecules by monitoring surface accessibility of their regions

- Single molecule conformation / Conformational changes
- Ligand binding / biomacromolecular interactions

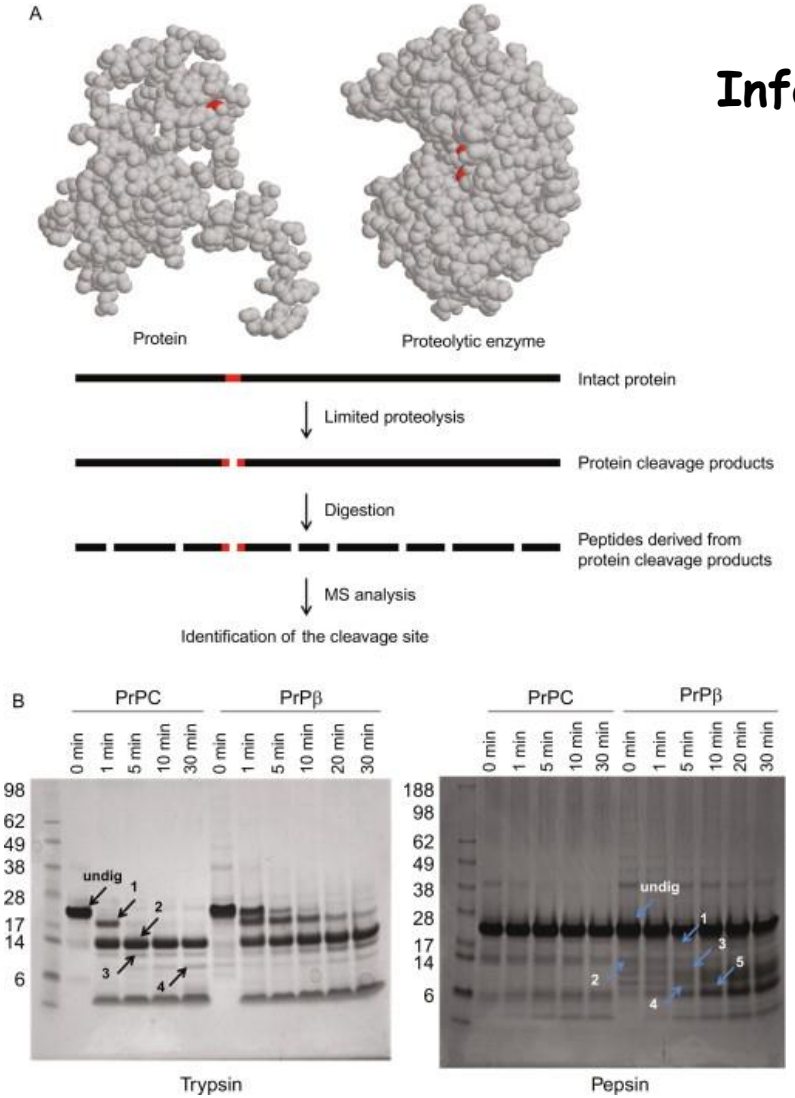
Different techniques

- Enzymatic / chemical cleavage
- Covalent labeling

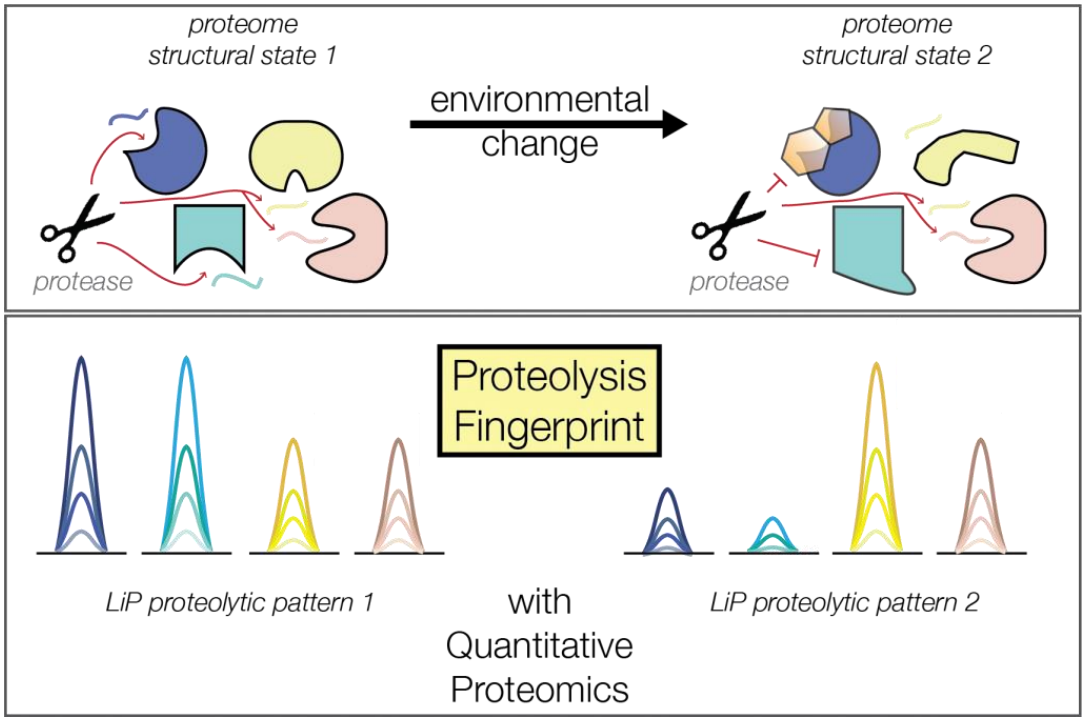
Covalent labeling

- Hydrogen-deuterium Exchange
- Stable covalent labeling – Chemical or Radical footprinting and cross-linking

Enzymatic cleavage – Limited proteolysis



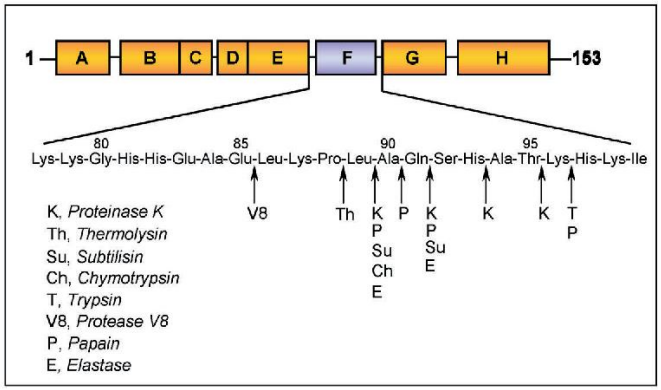
Information about the accessible surface area

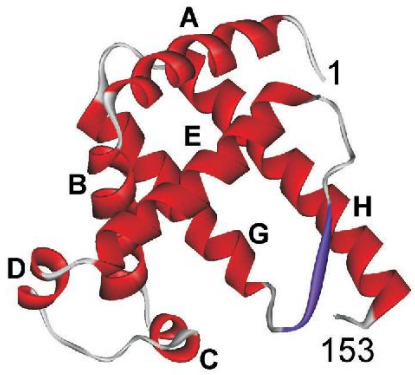


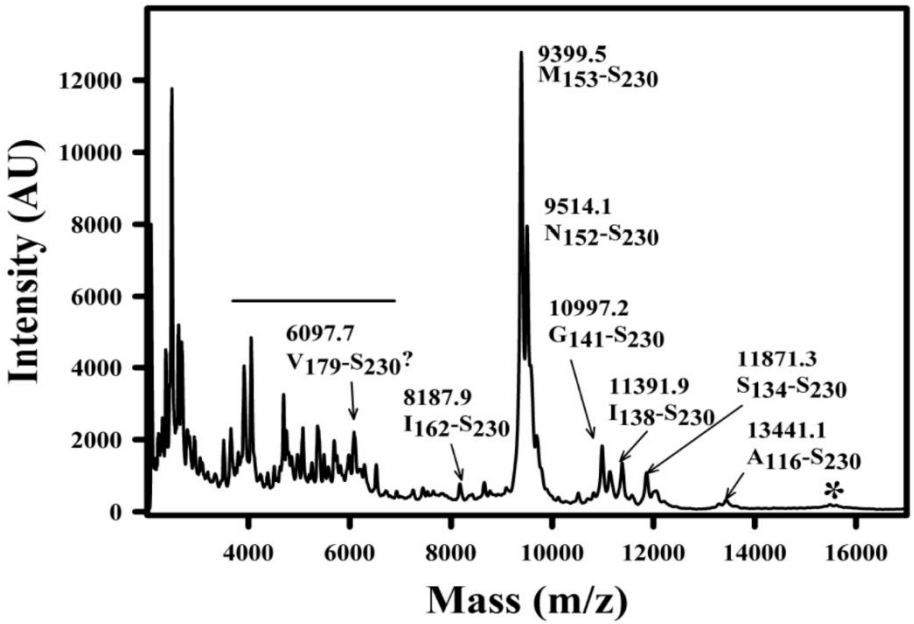
E. V. Petrotchenko, C. H. Borchers, ***Modern Mass Spectrometry-Based Structural Proteomics***, **Elsevier Inc., 2014.**

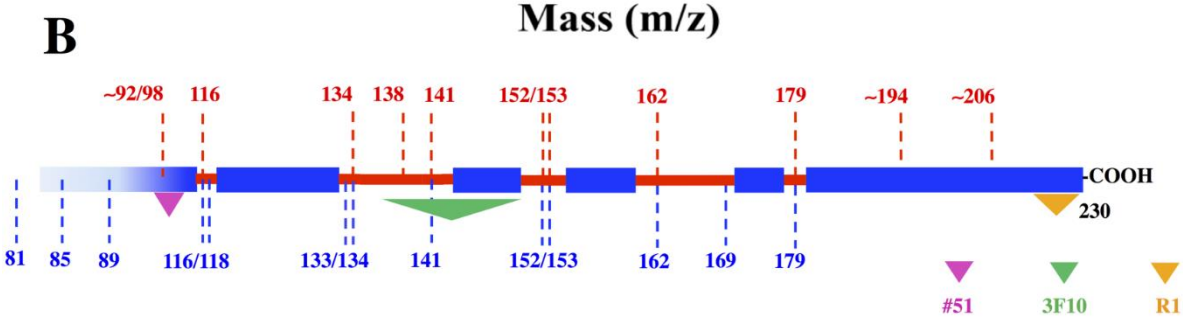
Limited proteolysis

- protease serves as probe
- controlled cleavage time
- first cleavage of outermost regions
- SDS-PAGE → MS

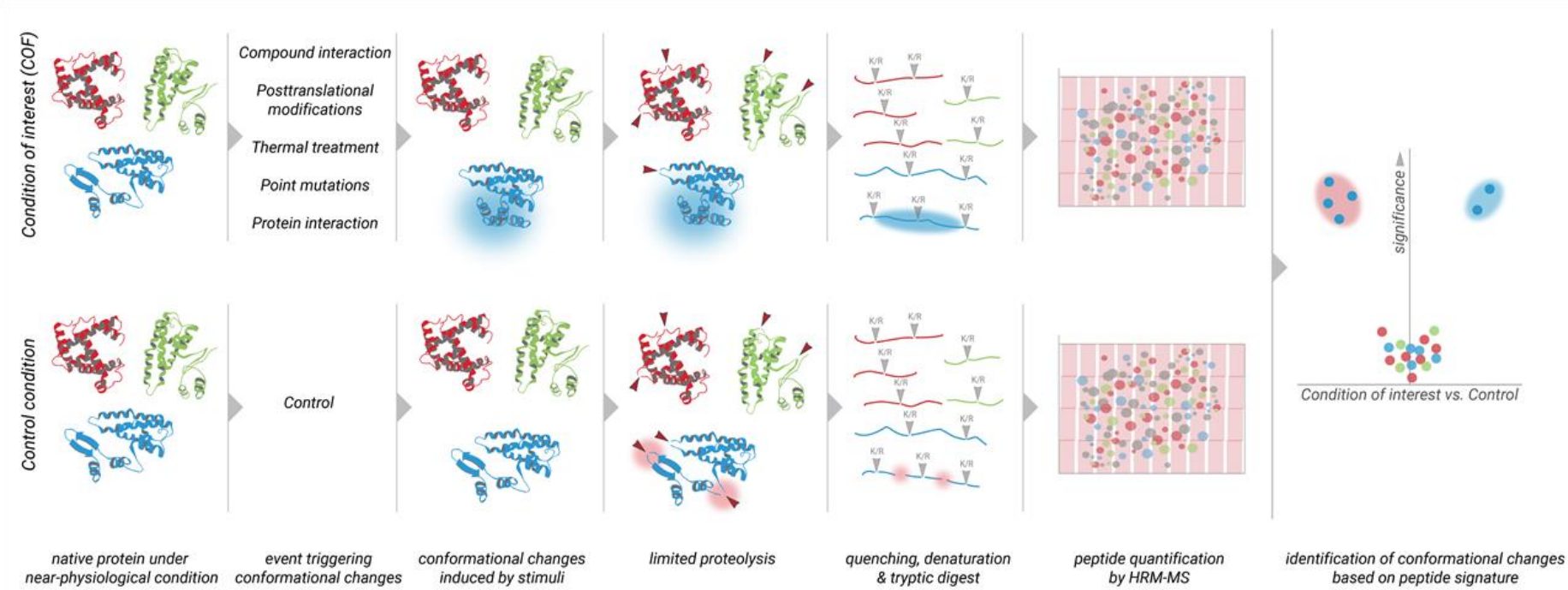








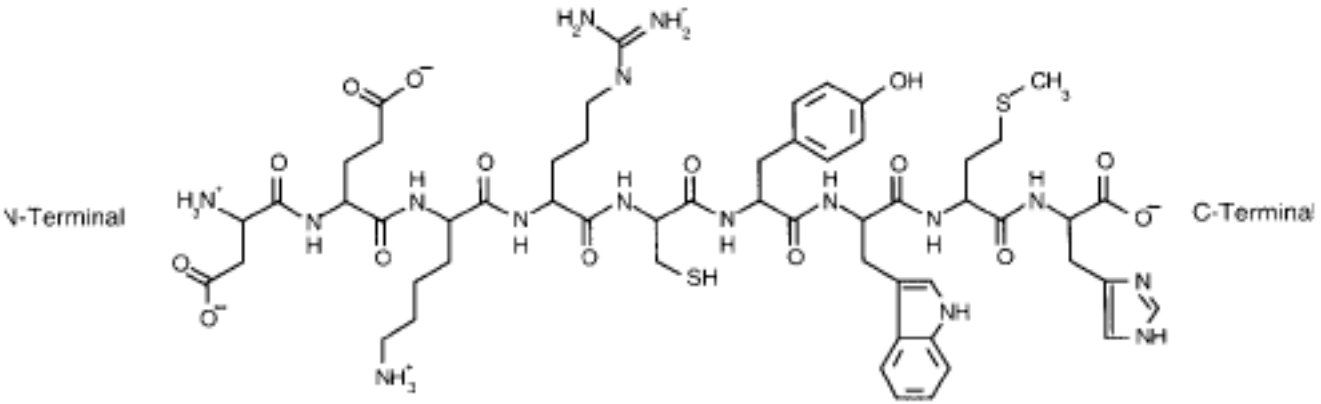
Limited proteolysis – identification of fragments (“Bottom up”)



Schopper S, Kahraman A, Leuenberger P, et al. Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry. Nat Protoc. 2017;12(11):2391-2410. doi:10.1038/nprot.2017.100

Protein covalent labeling and chemical cross-linking

Available amino acid sidechains for covalent modification



Carboxy groups – Asp, Glu, C-term, pKa (3.8, 4.3, 2.3) pH≥7 » deprotonation

Amino groups – Lys, Arg, His, N-term, pKa (9.4, 12, 6.8, 7.8) 7≥pH » protonation

Sulfhydryl groups - Cys . pKa 8.9 pH≥7 » -SH

Aromatic groups– Trp (indol), Tyr (hydroxyfenyl, pKa 9.9)

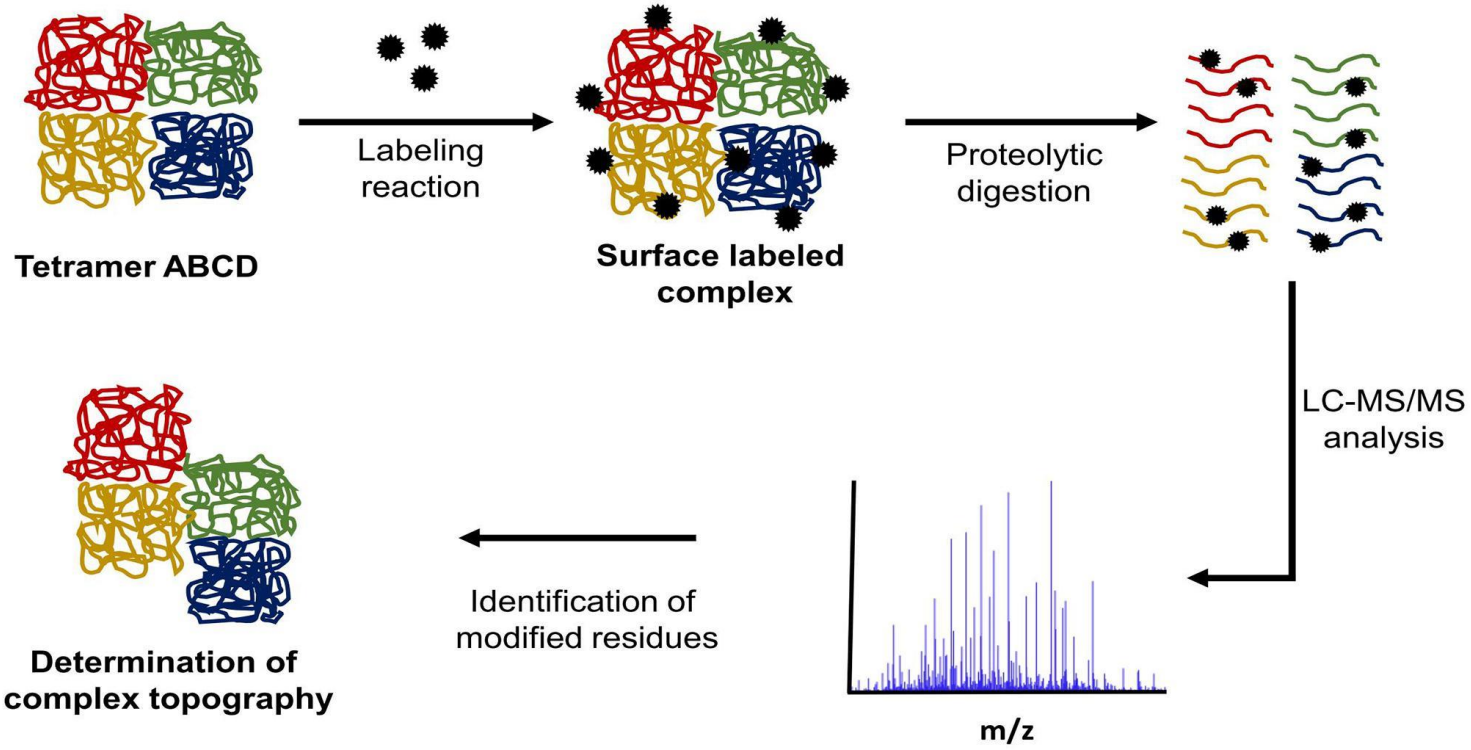
~ 23% of amino acid can be covalently modified

Klapper et. al. Biochem. Biophys. Res. Commun. 1977, 78, 1018.

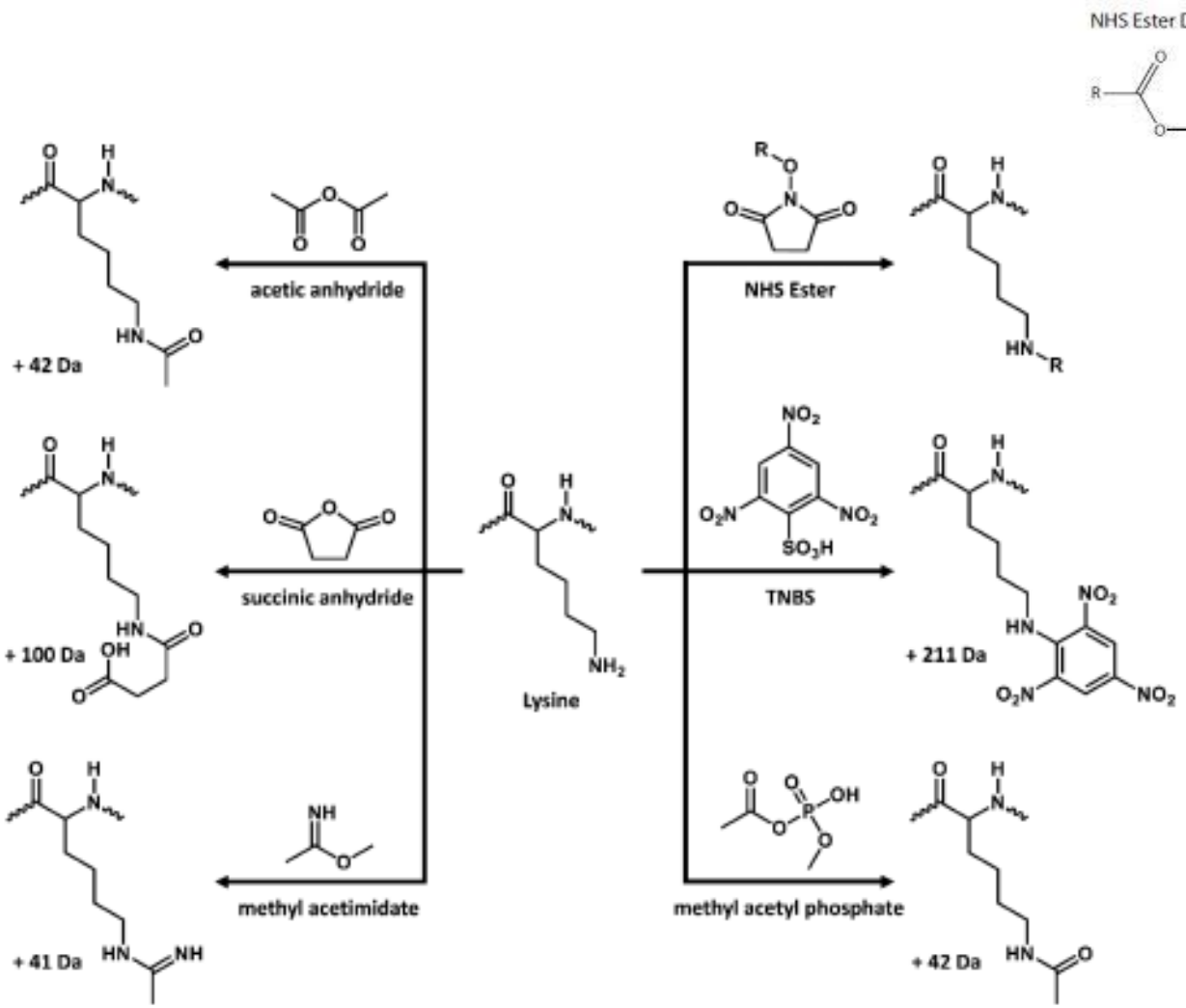
Stable covalent labeling

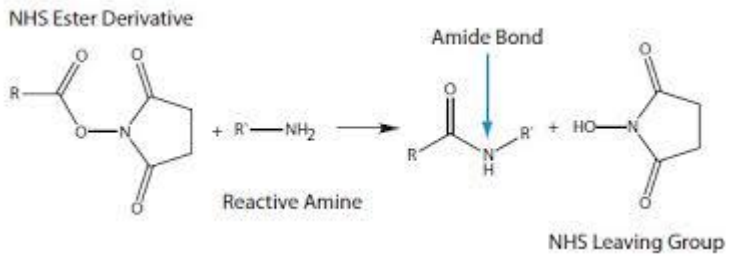
Chemical probes

- amino acids functional groups (-COOH, -SH, -NH_2, -OH, $CONH_2$, aromatic ring)
- **reactivity, specificity** × solvent-accessible surface area (SASA)
- reaction time - minutes to hours
- bifunctional footprinters = **cross-linkers**



Covalent modification of amino acid side chains - lysine

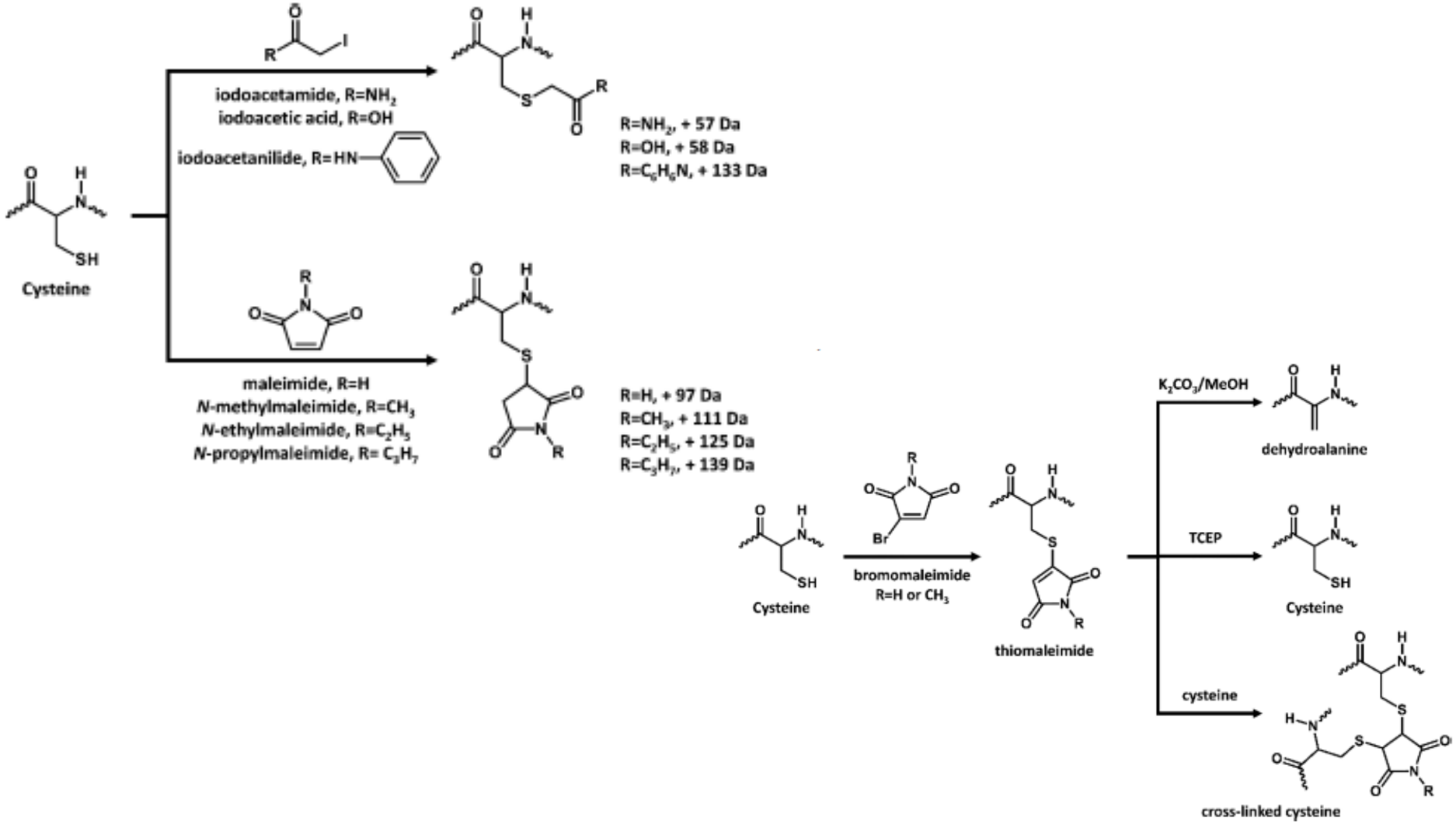




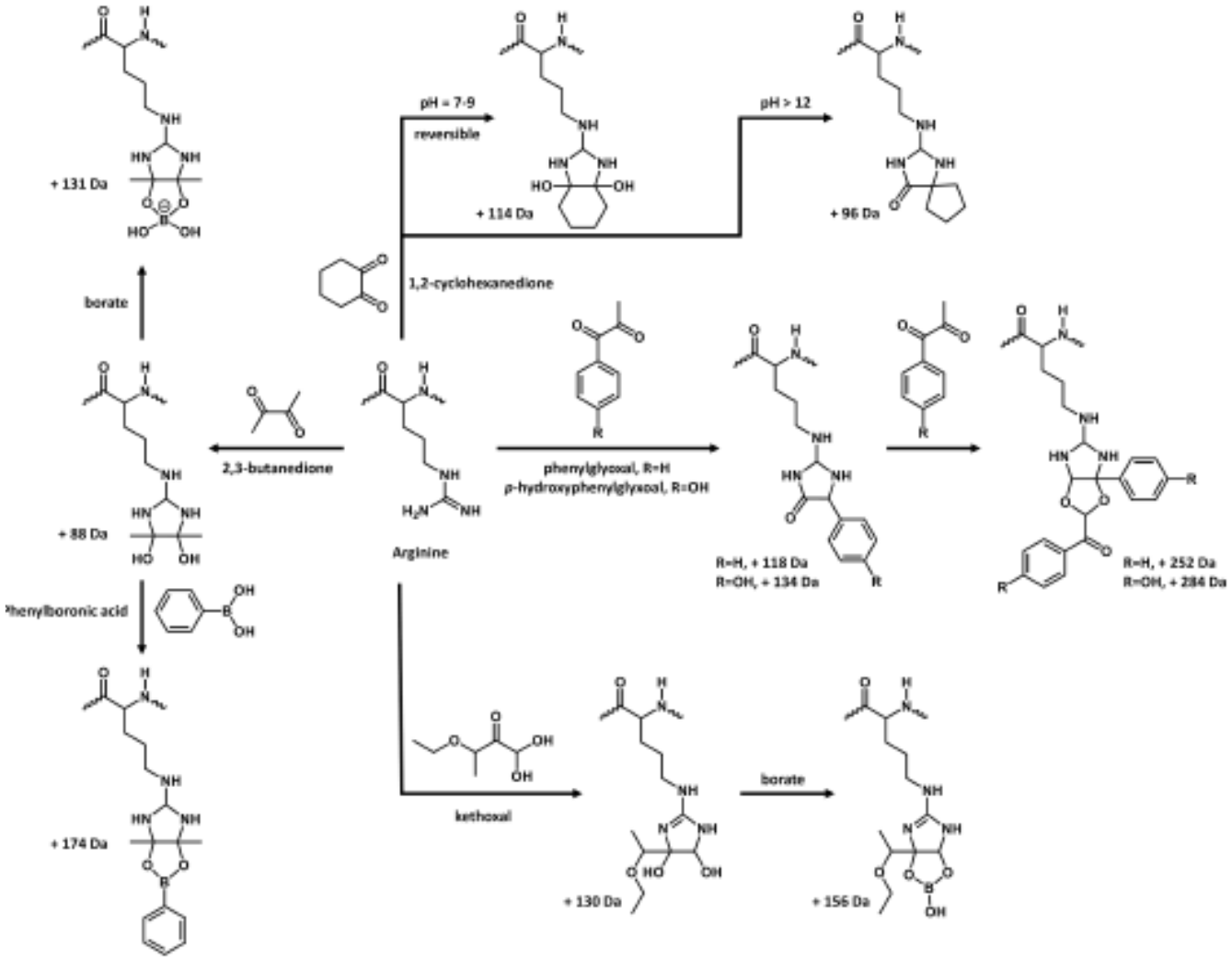
Acetylation

(N-hydroxysuccinimide esters)

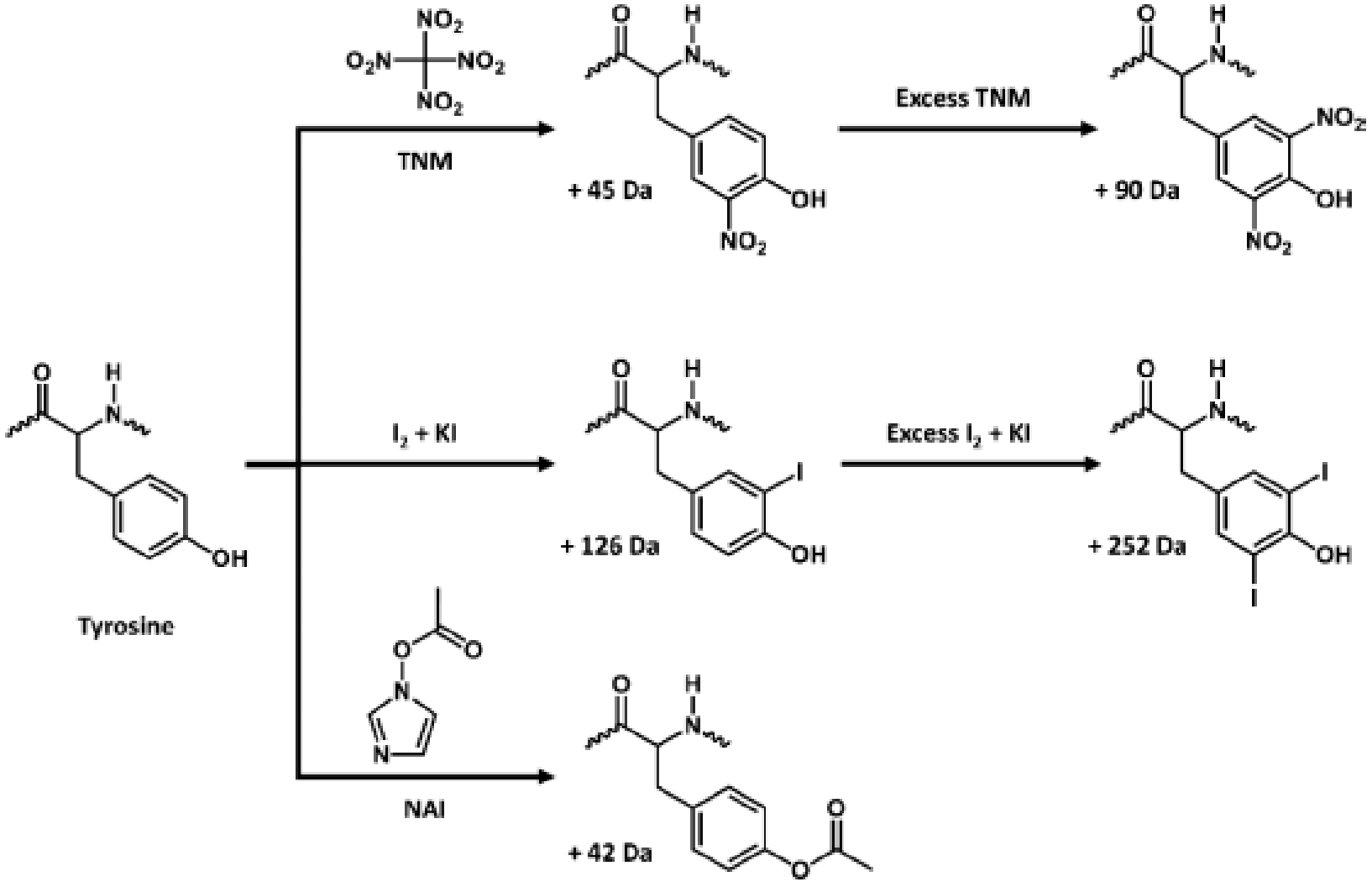
Covalent modification of amino acid side chains - cysteine



Covalent modification of amino acid side chains - arginine

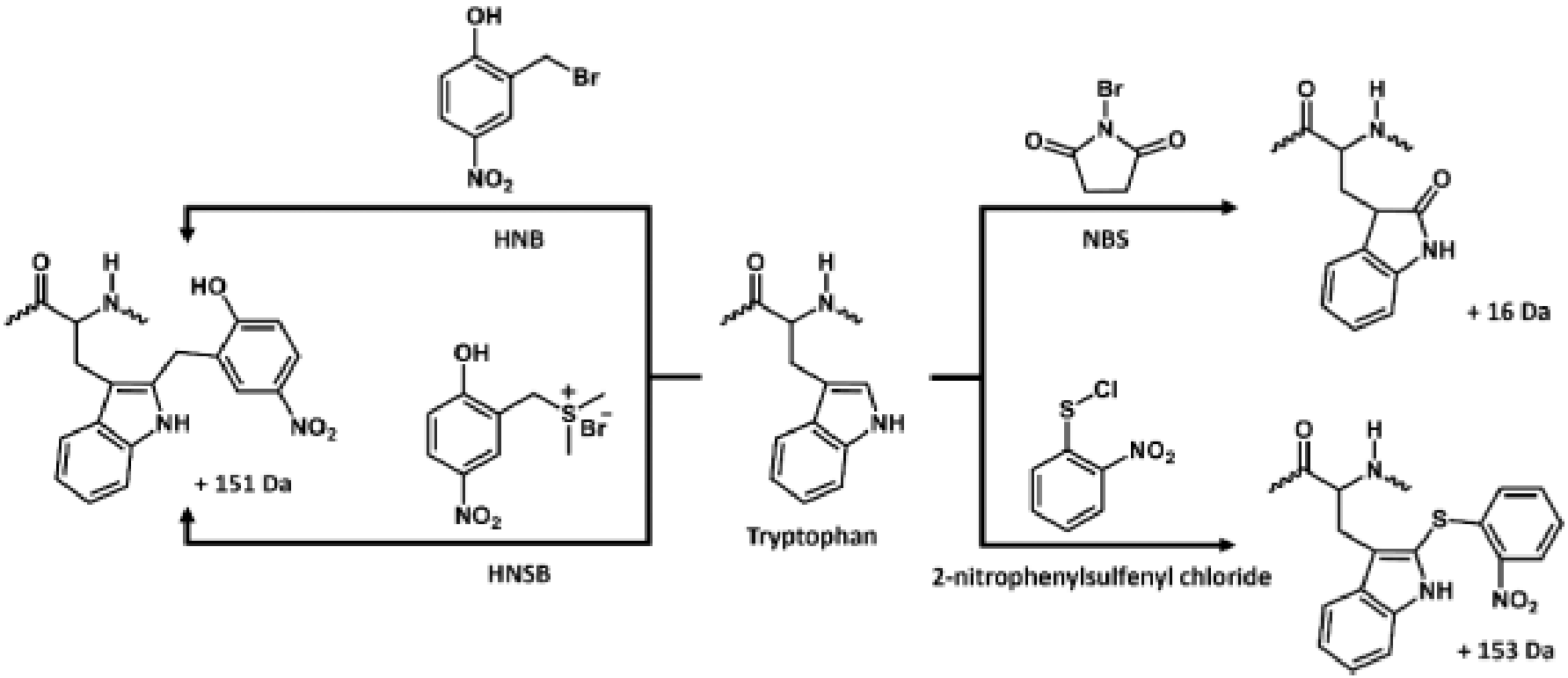


Covalent modification of amino acid side chains - tyrosine

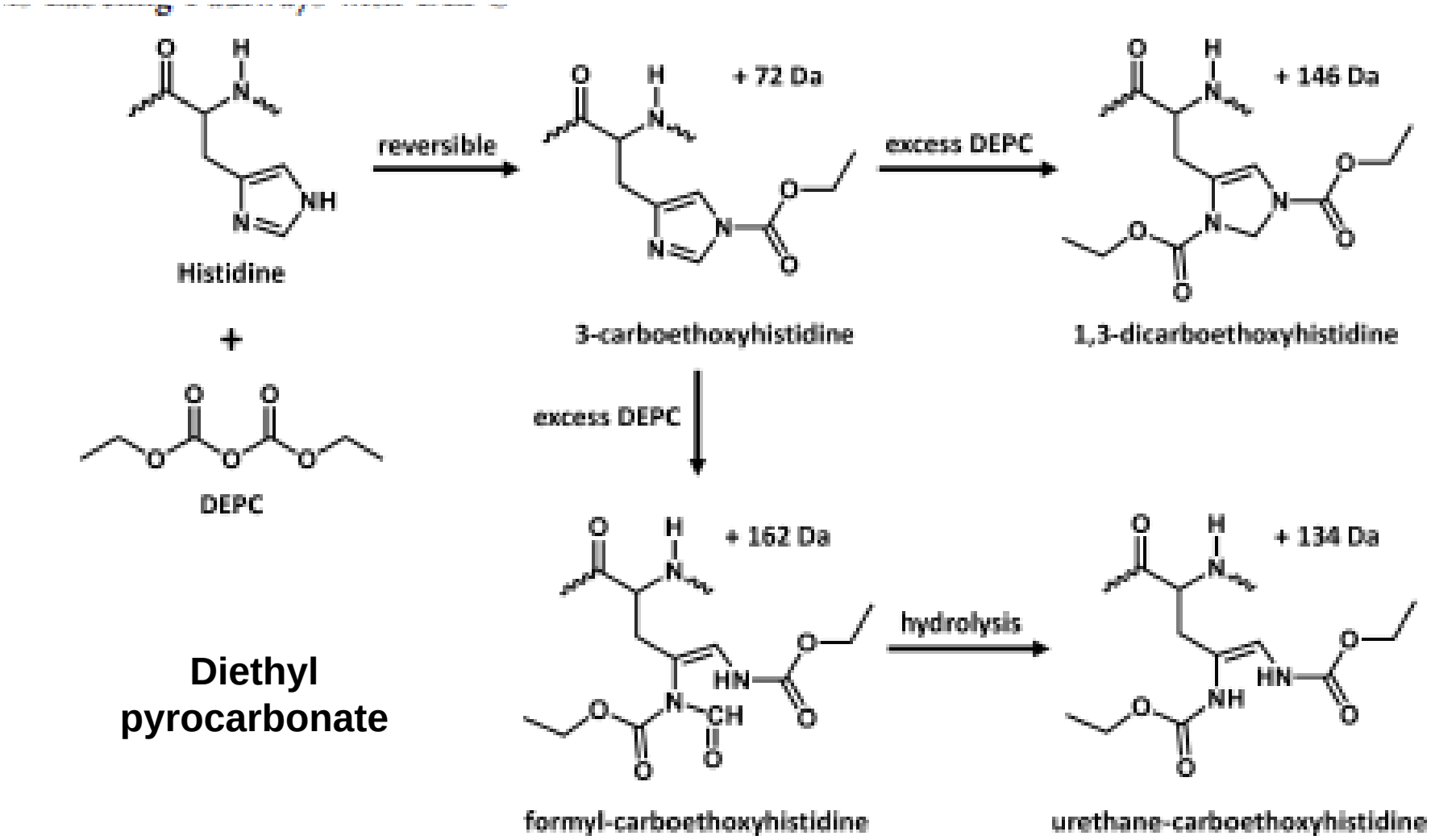


Covalent modification of amino acid side chains - tryptophan

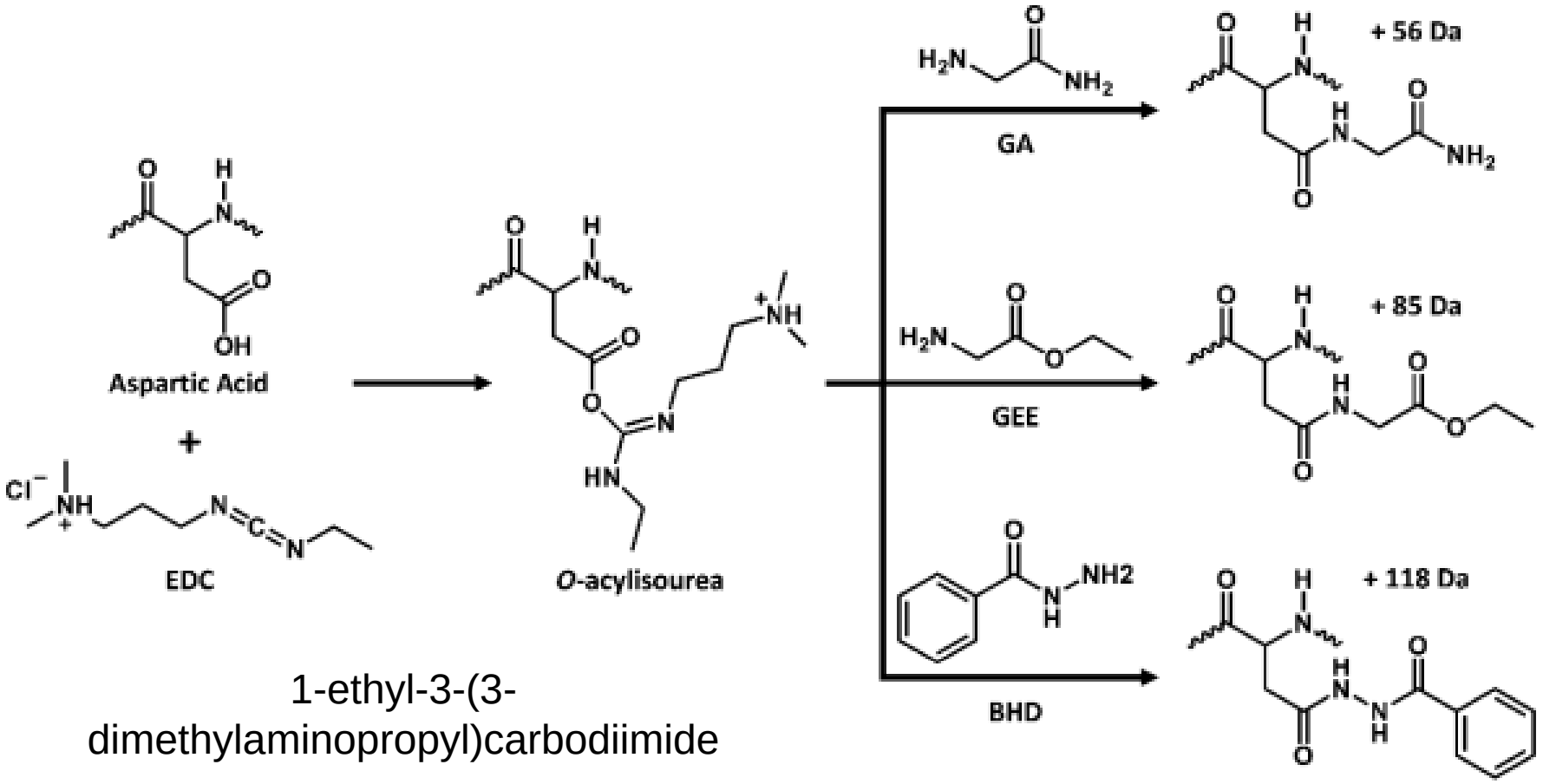
2-hydroxy-5-nitrobenzyl bromide



Covalent modification of amino acid side chains - histidine

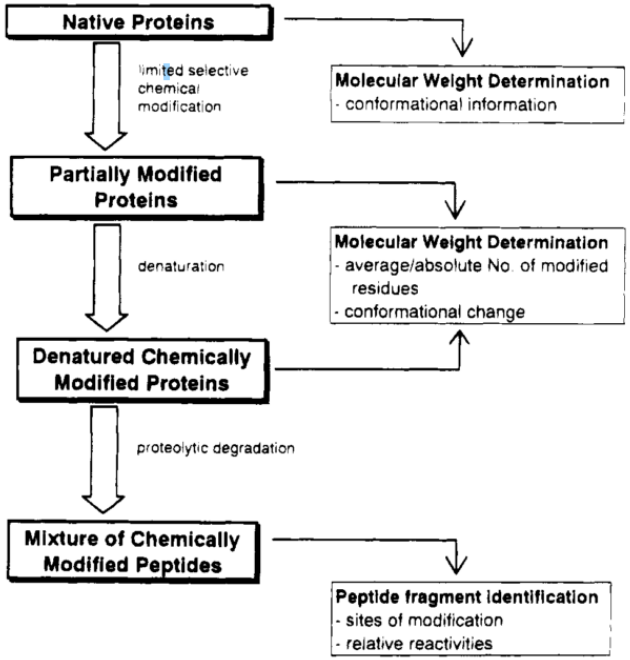


Covalent modification of amino acid side chains – Asp, Glu



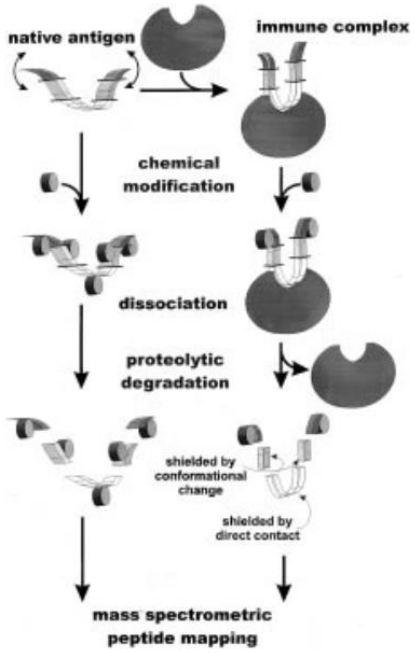
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

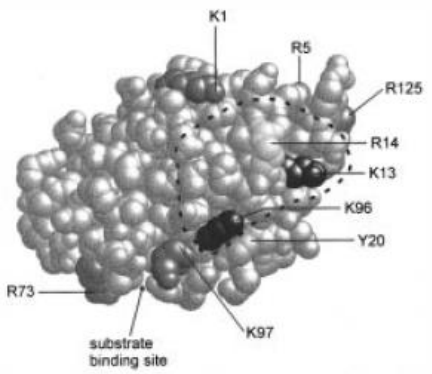
Protein covalent labeling: Lys, Tyr, Arg

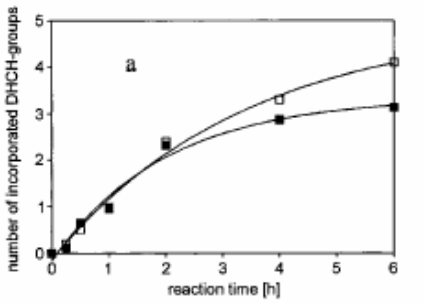


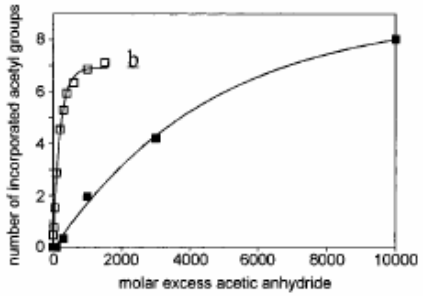
relative reactivity NH_2 groups[a]	lysine residues, ϵ-amino groups		
	RNAse A	HEL	myoglobin
	α-NH_2		α-NH_2
1	41, 104	97, 33 α-NH_2	45, 63, 77, 79, 145, 147
2	1, 7, 37	1	16, 42, 87
3	31, 61, 91	13, 116	56, 50, 62, 78, 102
4	66, 98	96	96, 47, 87, 133,[b] 118[b]

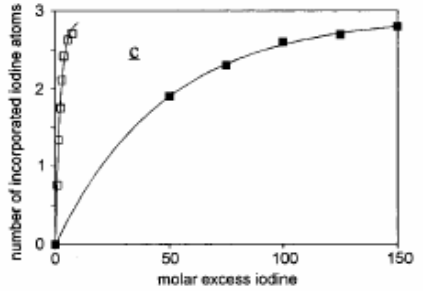
Suckau et. al. *PNAS 1992, 89, 5630*
and ***Glocker et. al.*** *Bioconj. Chem. 1994, 5, 583*





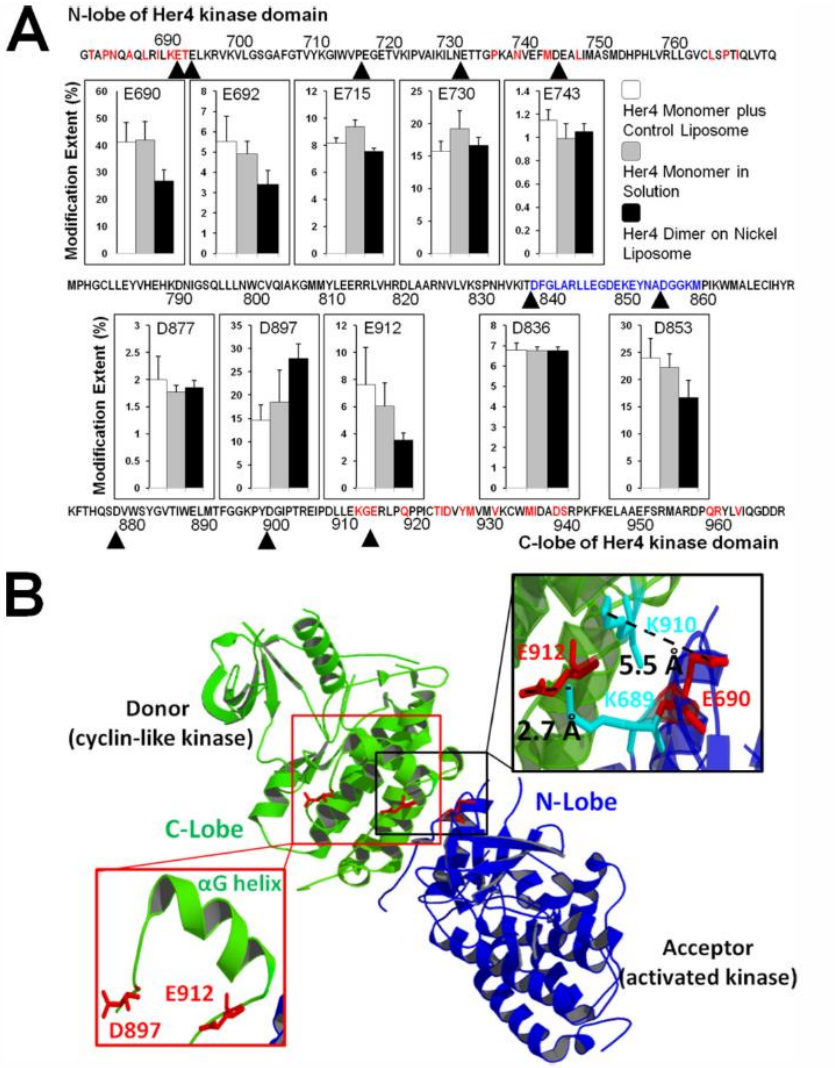




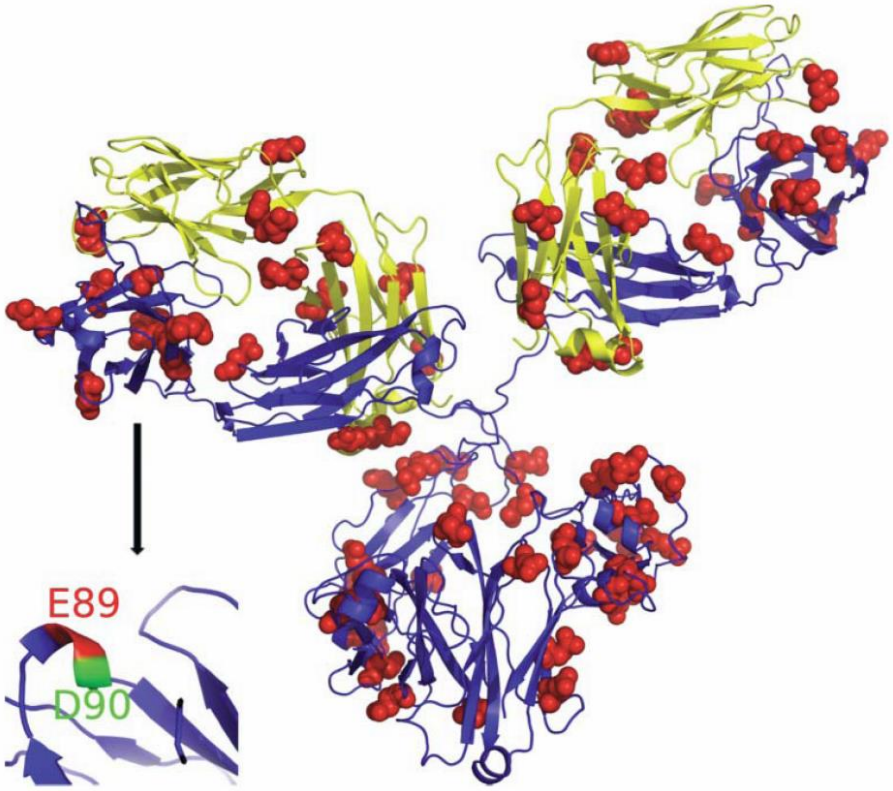


Fiedler et. al. *Bioconj. Chem. 1998, 9, 236*

Protein covalent labeling: Asp, Glu

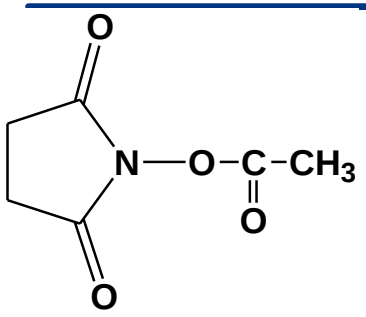


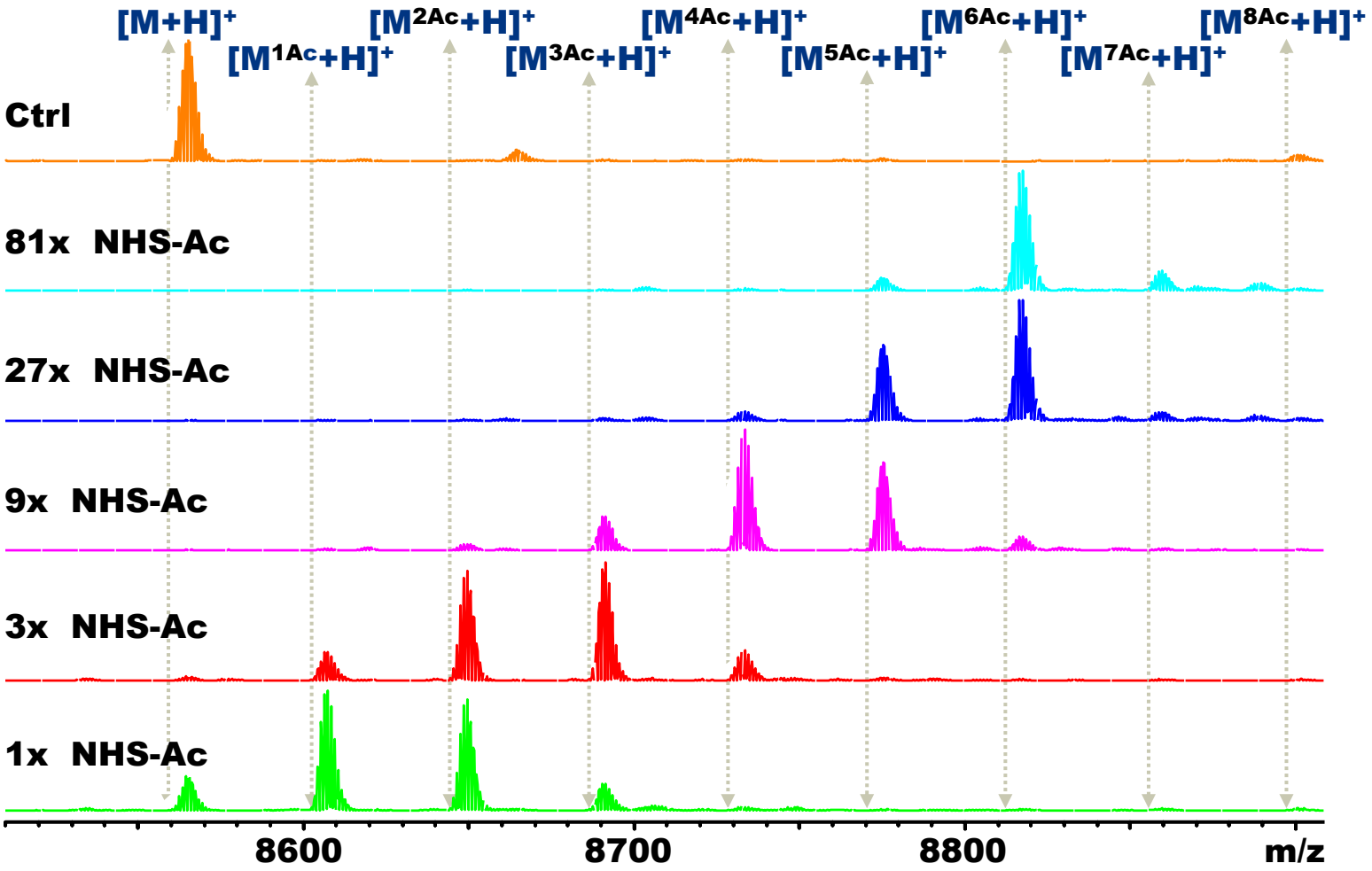
Zhang H. et al. MCP 2011



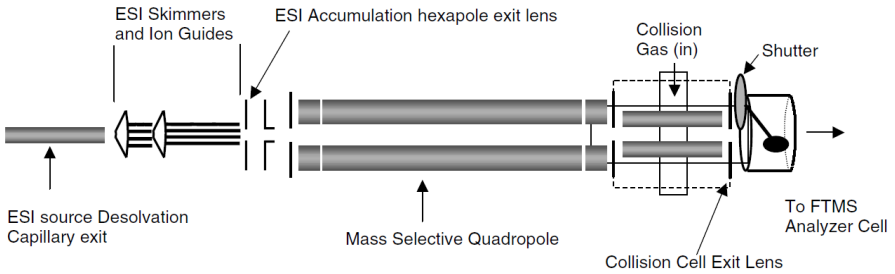
Kaur P. et al. mAb 2015

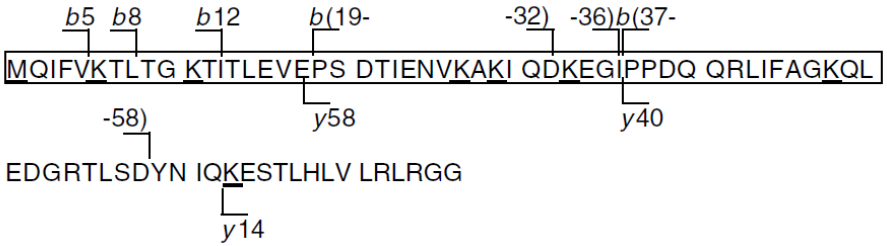
Protein covalent labeling: Top down

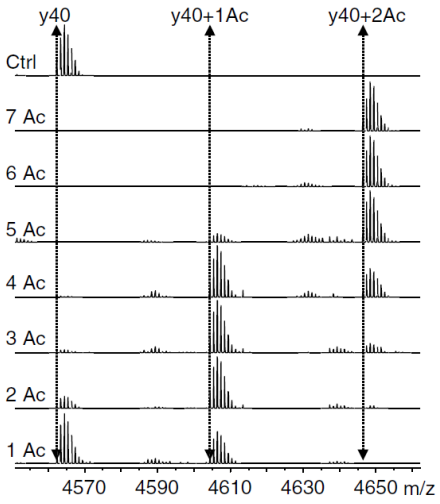


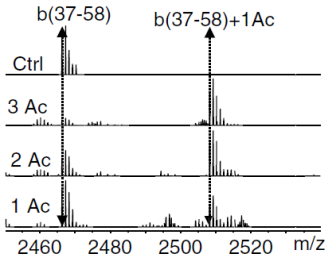


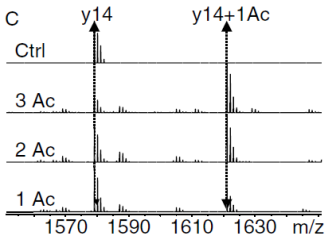
Protein covalent labeling: Top down

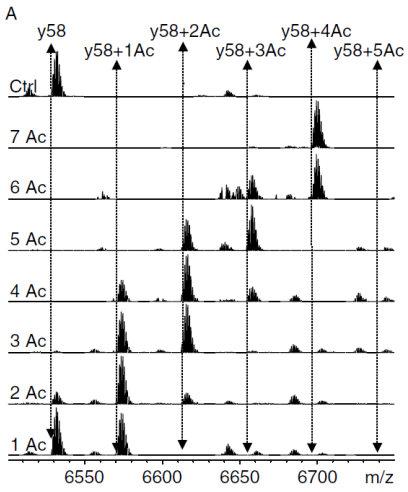


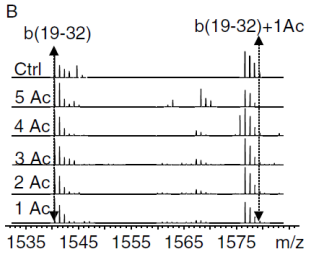


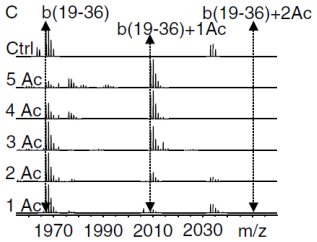












Protein covalent labeling: a reactivity of lysine in an issue

^{1}MQIFVKTLTG ^{11}KTITLEVEPS ^{21}DTIENVKAKI ^{31}QDKEGIPPDQ
^{41}QRLIFAGKQL ^{51}EDGRTLSDYN ^{61}IQKESTLHLV ^{71}LRLRGG

(^{1}M~K6~K48~K63) > K33 > K11 > (K27,K29)

- In agreement with NMR data, which shows.
  - K11 interacts with E34; K29 interacts with D21 A
- Crystal structure indicates K27 H-bonds to D52.
- More reactive lysines don't H-bond (K63) or H-bond to backbone carbonyls (K48, K33).
- K48 and K63 participate in formation of polyubiquitin.

Novak et. al. *J. Mass Spectrom. 2004, 39, 322*

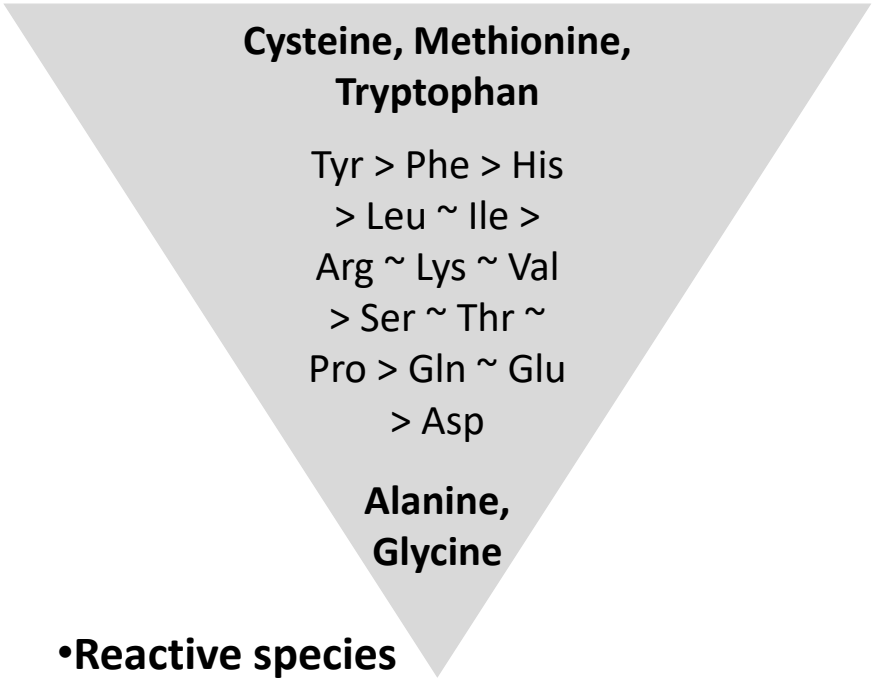
Hydroxyl Radical Footprinting

Products of water or hydrogen peroxide molecule homolytic bond cleavage

Hydroxyl radicals can be generated by various means:

- **Fenton reaction**
- **Irradiation of water by x-rays or electron beams**
- **Photolysis of hydrogen peroxide FPOP (fast photochemical oxidation of proteins)**
- **Other radicals available $^{\bullet}OH$, $^{\bullet}I$, $^{\bullet}CF3$**

The relative reactivity of the amino acid side chains



- **Reactive species**
- **React efficiently with most AA side chains**
- **Form STABLE oxidation products**

Takamoto K . et al. *Annu Rev Biophys Biomol Struct.* 2006, 35, 251-276

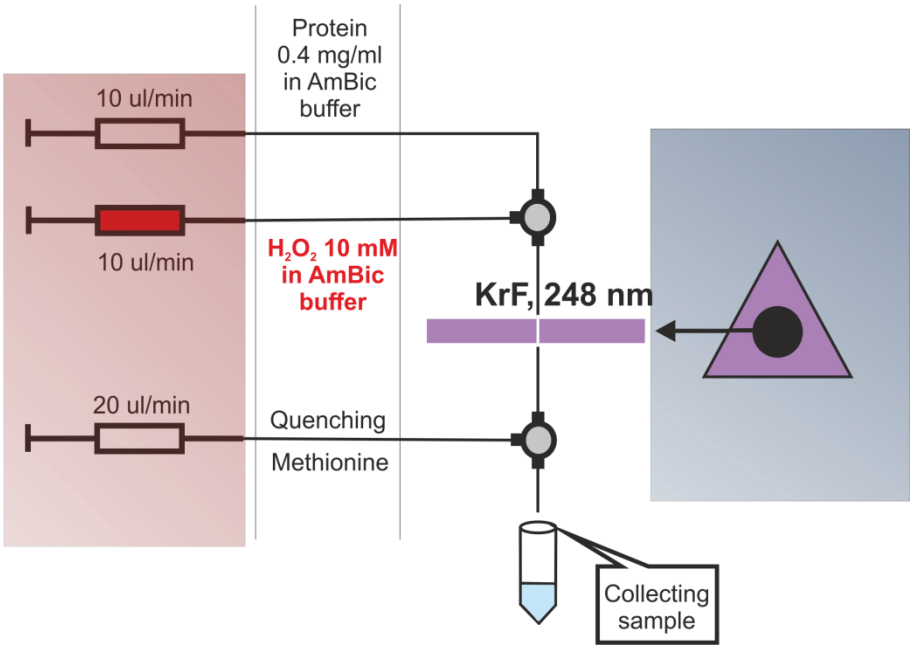
Conditions for radical labeling

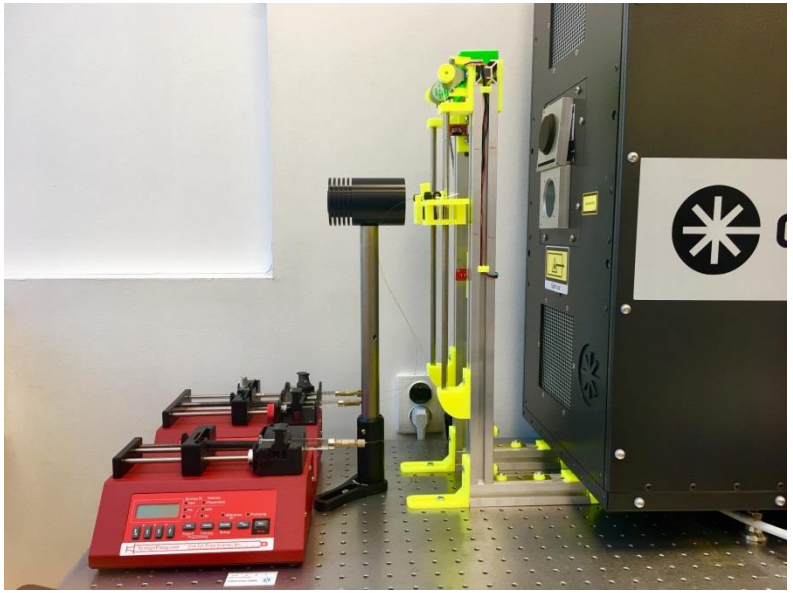
- Electron pulse radiolysis:
  - reproducible 1-100 ns pulses; MeV energy range on linear accelerator
- Synchrotron radiolysis:
  - X-ray; 3-30 keV @ beam current ~ 250 mA
- Laser H_2O_2 photolysis:

  1% - 0,04% H_2O_2 (mixing by stopped-flow device or just before irradiation); quench and removal of residual peroxide is vital
  - Nd:YAG; 2 mJ/pulse @ 266 nm; 3-5 ns pulse; 1-100 shots
  - 17 ns KrF excimer laser; 50 mJ/pulse @ 248 nm
  - 18 ns KrF excimer laser; 62,5 mJ/pulse @ 248 nm; 16 Hz

Fast photochemical oxidation of proteins

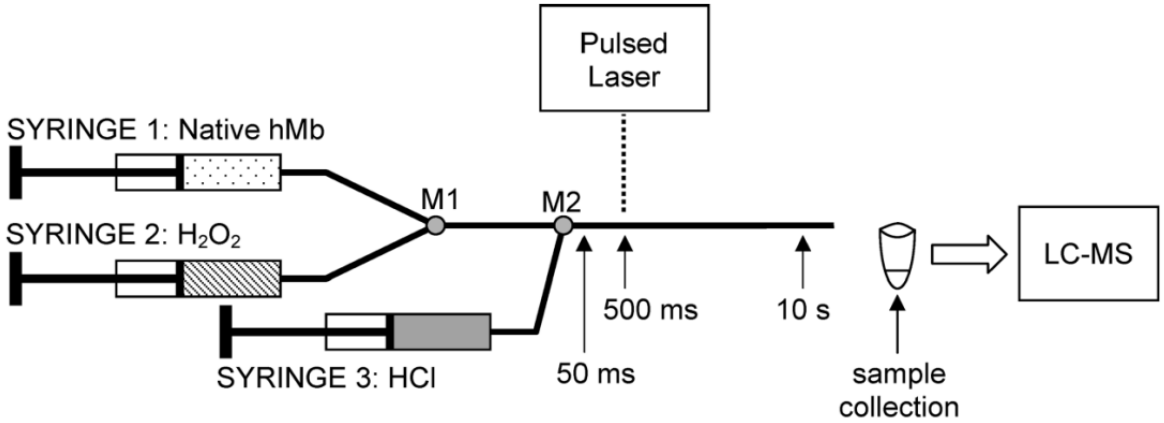
- **Covalent modification preserves the primary sequence of modified residues**
- **High reactivity of •OH the modifications of more than half of amino acid side-chains, providing a higher coverage**





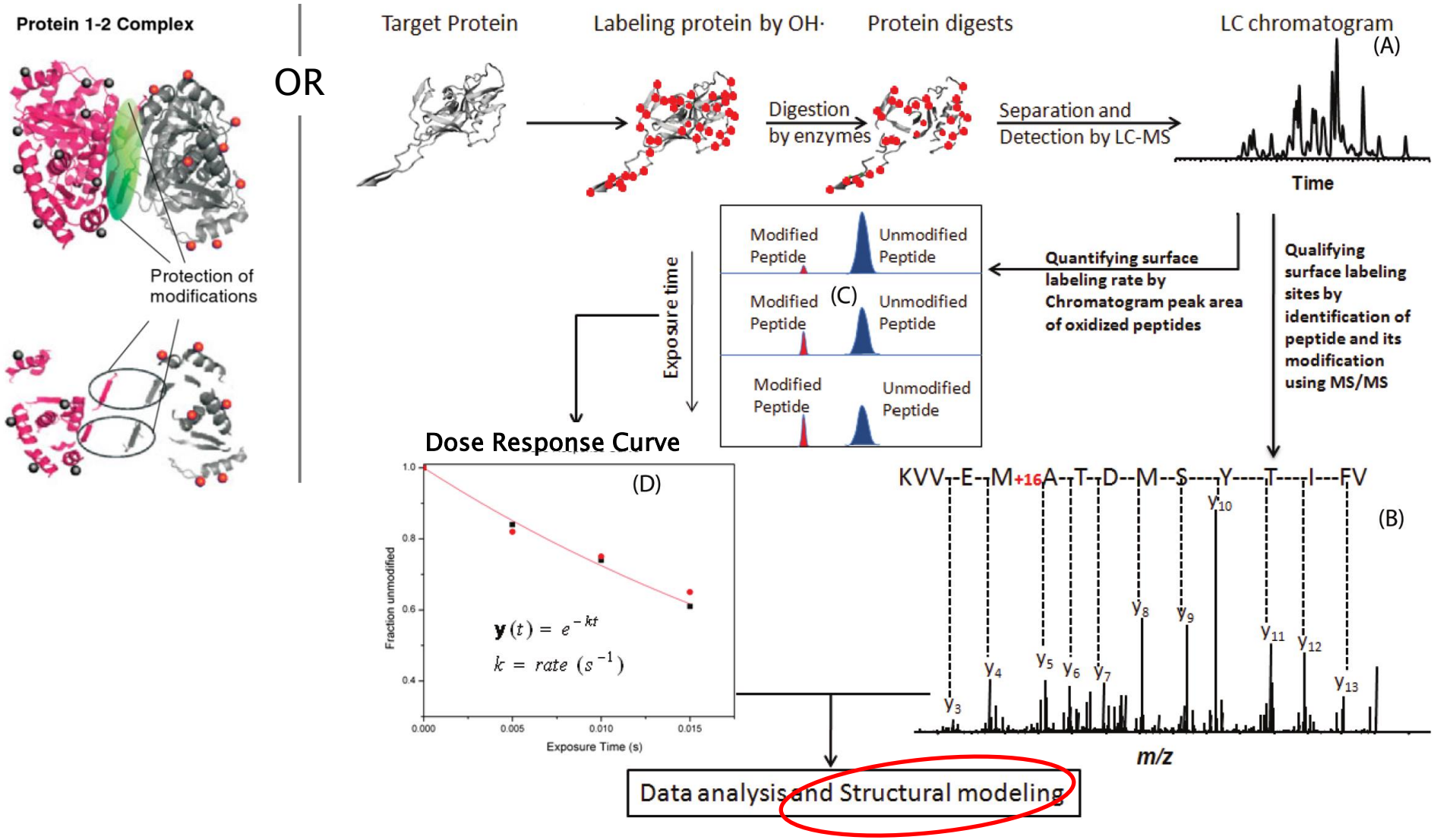
Experimental setup

- Sample mixed and irradiated in
  - **µtubes** (sample volume ~ 15 µl) or in
  - **stopped-flow microfluidic mixing device** - essential for folding / kinetic studies (capillary flow ~ 20 µl.min^{-1})



• Short pulses with high energy are needed to create sufficient concentration of radicals on very short (sub-microsecond) timescales to avoid conformational changes of protein during labeling.
• Possible protein conformational changes occur mostly on a longer than milisecond timescale.

Hydroxyl radical footprinting – work-flow



Hydroxyl radical footprinting – sample complexity

Benefits of Ion Mobility Separation and Parallel Accumulation–Serial Fragmentation Technology on timsTOF Pro for the Needs of Fast Photochemical Oxidation of Protein Analysis

Dmitry S. Loginov,* Jan Fiala, Josef Chmelik, Peter Brechlin, Gary Kruppa, and Petr Novak*

Cite This: https://doi.org/10.1021/acsomega.1c00732 Read Online

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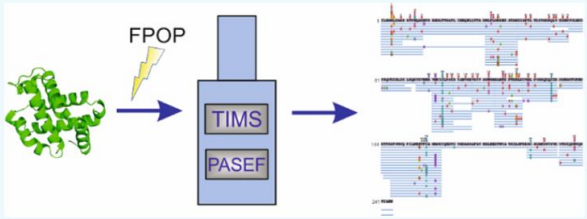
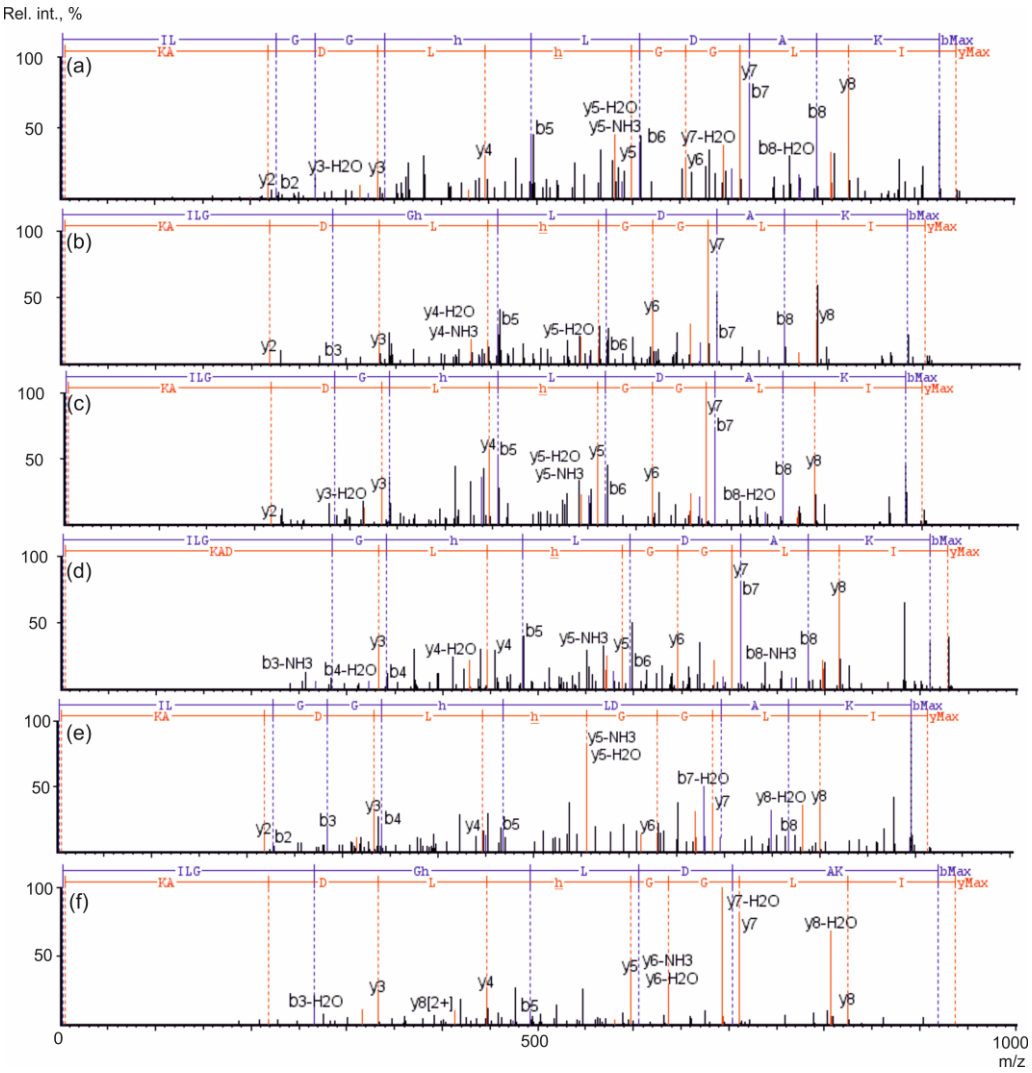


ABSTRACT: Fast photochemical oxidation of proteins (FPOP) is a recently developed technique for studying protein folding, conformations, interactions, etc. In this method, hydroxyl radicals, usually generated by KrF laser photolysis of H_2O_2, are used for irreversible labeling of solvent-exposed side chains of amino acids. Mapping of the oxidized residues to the protein's structure requires pinpointing of modifications using a bottom-up proteomic approach. In this work, a quadrupole time-of-flight (QTOF) mass spectrometer coupled with trapped ion mobility spectrometry (timsTOF Pro) was used for identification of oxidative modifications in a model protein. Multiple modifications on the same residues, including six modifications of histidine, were successfully resolved. Moreover, parallel accumulation–serial fragmentation (PASEF) technology allows successful sequencing of even minor populations of modified peptides. The data obtained indicate a clear improvement of the quality of the FPOP analysis from the viewpoint of the number of identified peptides bearing oxidative modifications and their precise localization. Data are available via ProteomeXchange with identifier PXD020509.

- **Reproducibility**
- **Sensitivity**
- **Precise localization of modification**

Peptide map of haptoglobin β

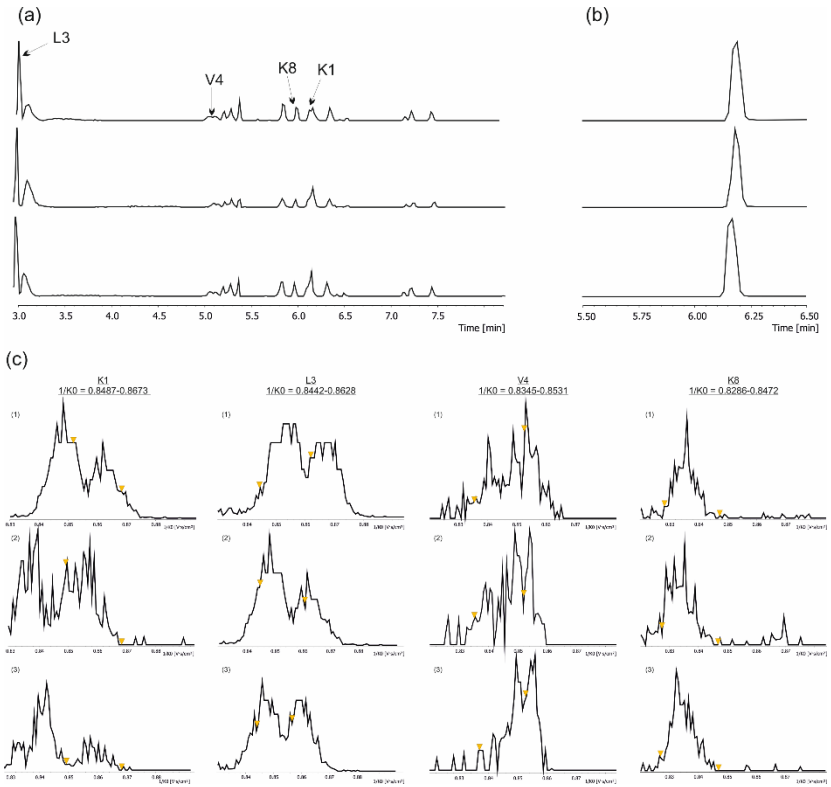
Hydroxyl radical footprinting – histidine modifications



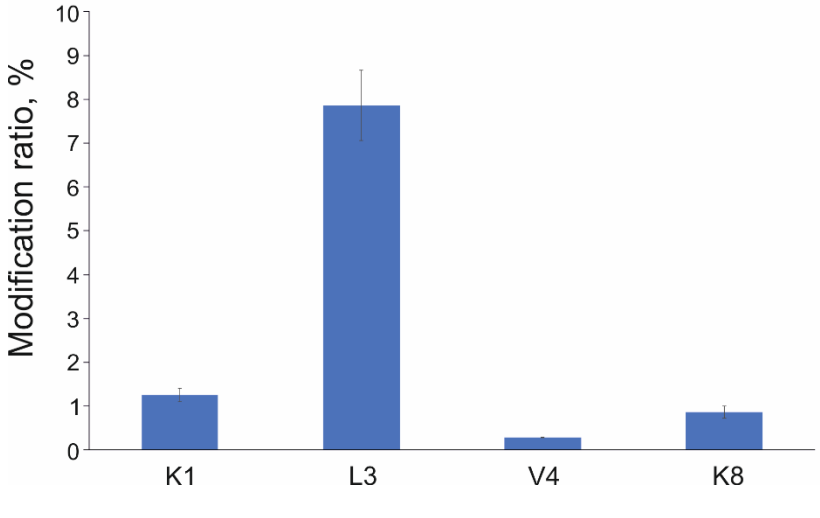
Peptide with the sequence ILGG**H**LDAK

Modification	Intensity (no laser/laser)	Contribution to the overall oxidation of the peptide (no laser/laser), %
+14 Da	13852 / 22520	1.2 / 0.6
His-> Asn	- / 41104	- / 1.2
His-> Asp	- / 27527	- / 0.8
-10 Da	8763 / 11365	0.7 / 0.3
+5 Da	- / 21471	- / 0.6
+16 Da	6025 / 505579	0.5 / 14.5

Hydroxyl radical footprinting – several modifications for a single peptide

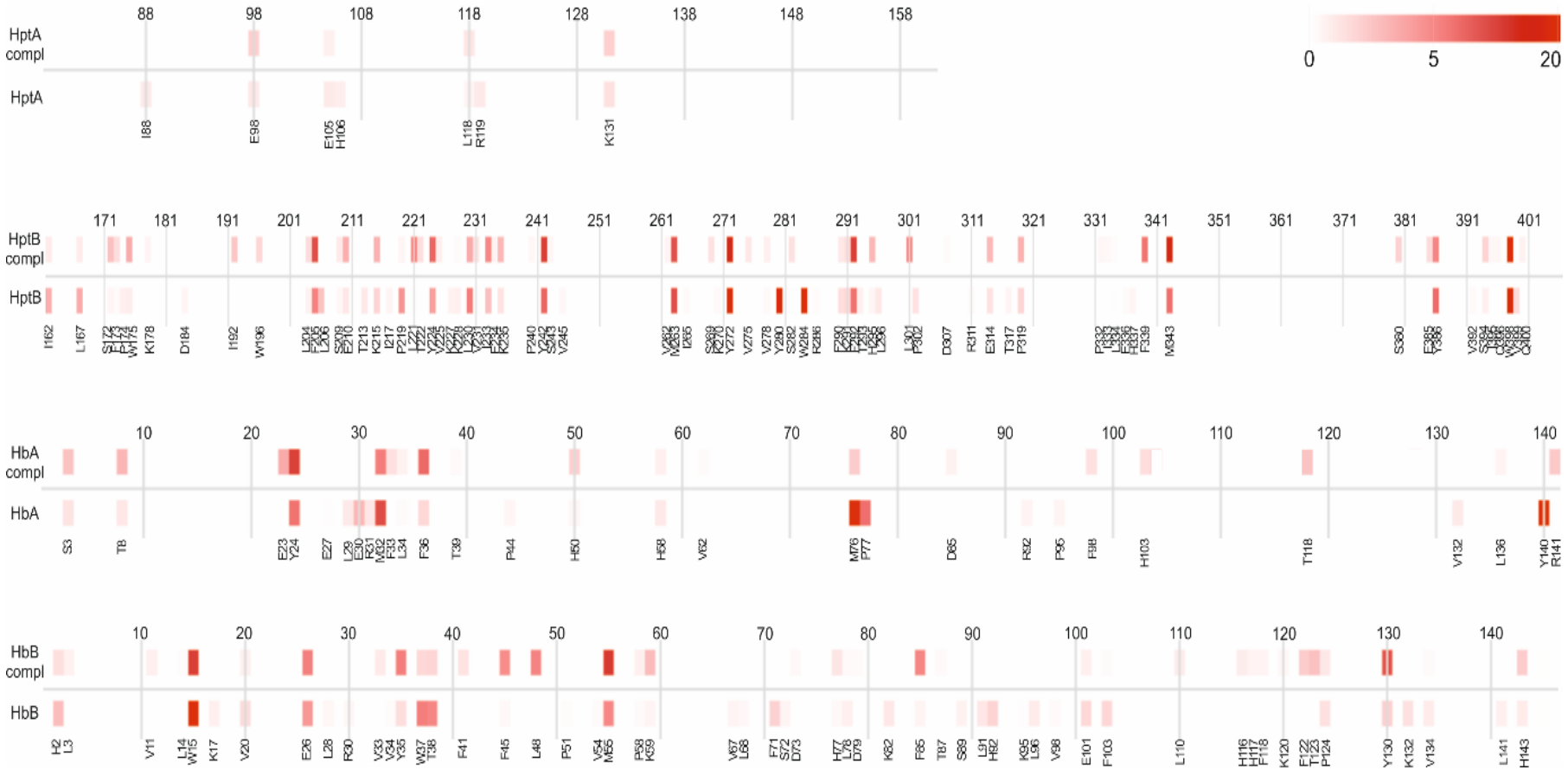


Peptide **K**Q**LV**EIE**K** appearing 2^+ at m/z 501.78



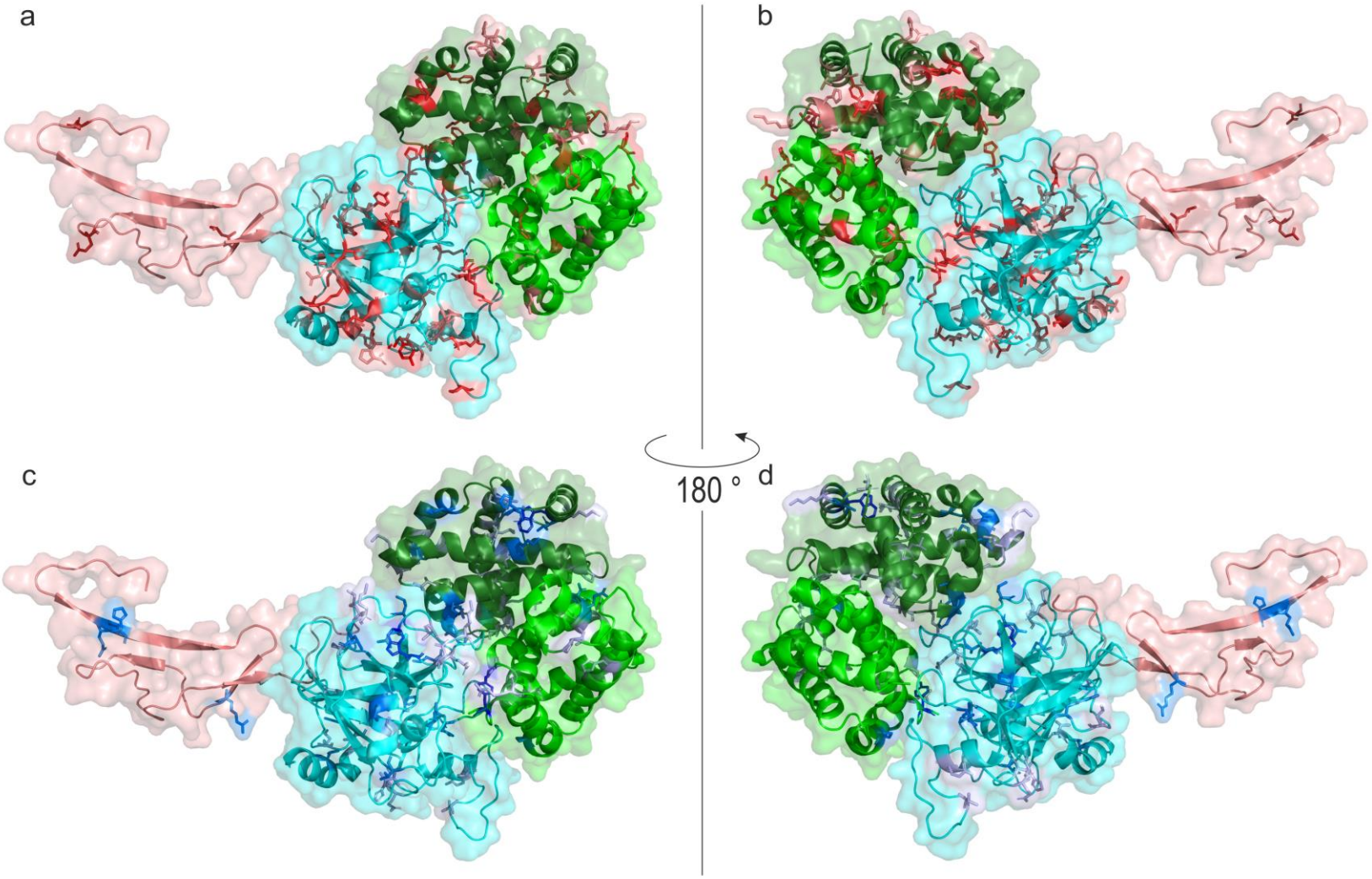
−10lgP	z	RT	1/k_0 range	intensity	oxidation site	AScore
38.14	2	2.97	0.8442–0.8628	147,992	L3	26.31
41.03	2	3.79	0.8345–0.8531	1224	V4	30.46
36.83	2	5.83	0.8286–0.8472	16,835	K8	26.31
43.59	2	6.12	0.8487–0.8673	25,697	K1	36.05
38.07	2	7.15	0.8717–0.8903	5250	I6	32.28
53.33	2	6.31	0.8583–0.8769	1,479,460	NA	NA

Hydroxyl radical footprinting – human hemoglobin-haptoglobin complex

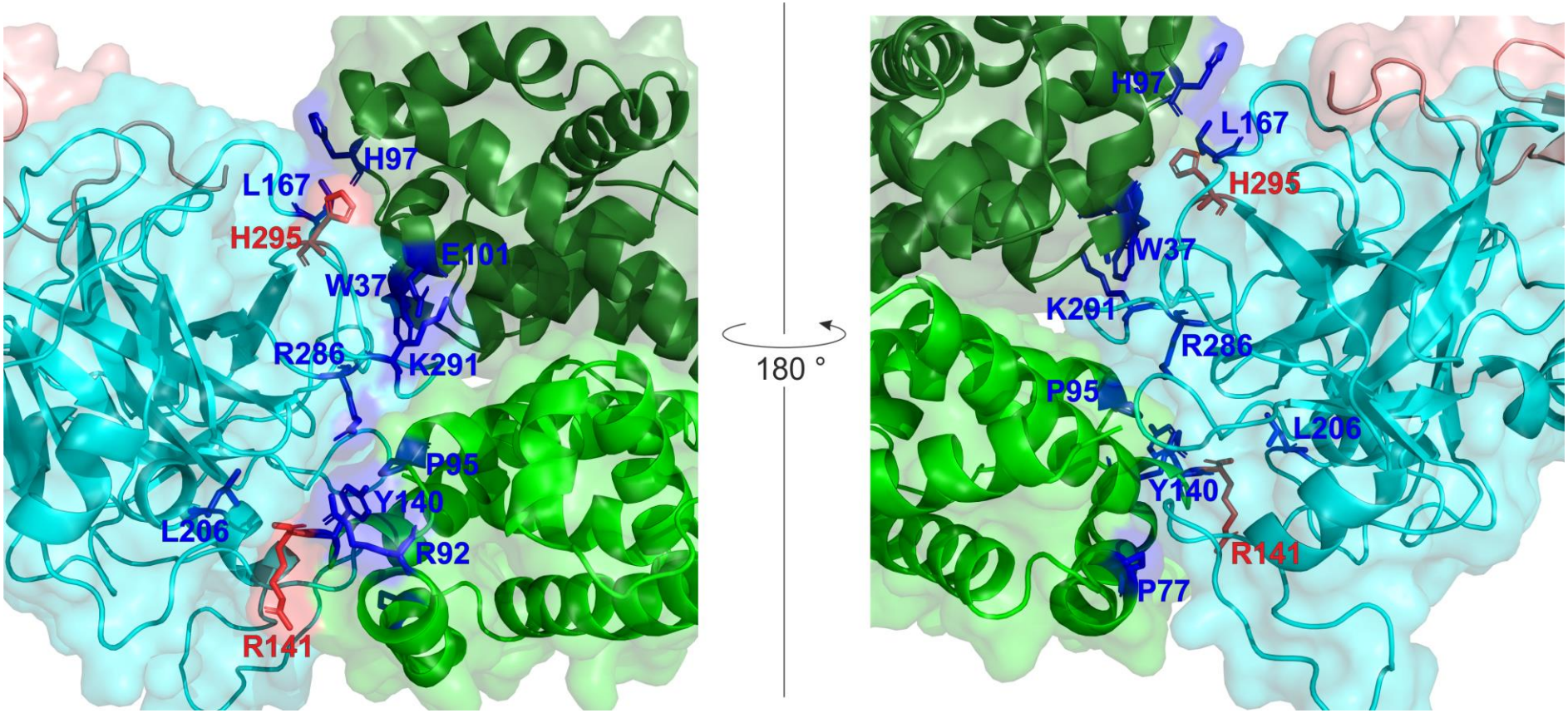


An oxidation rate was determined for 7, 77, 30 and 56 residues in Hpα, Hpβ, Hbα and Hbβ, respectively

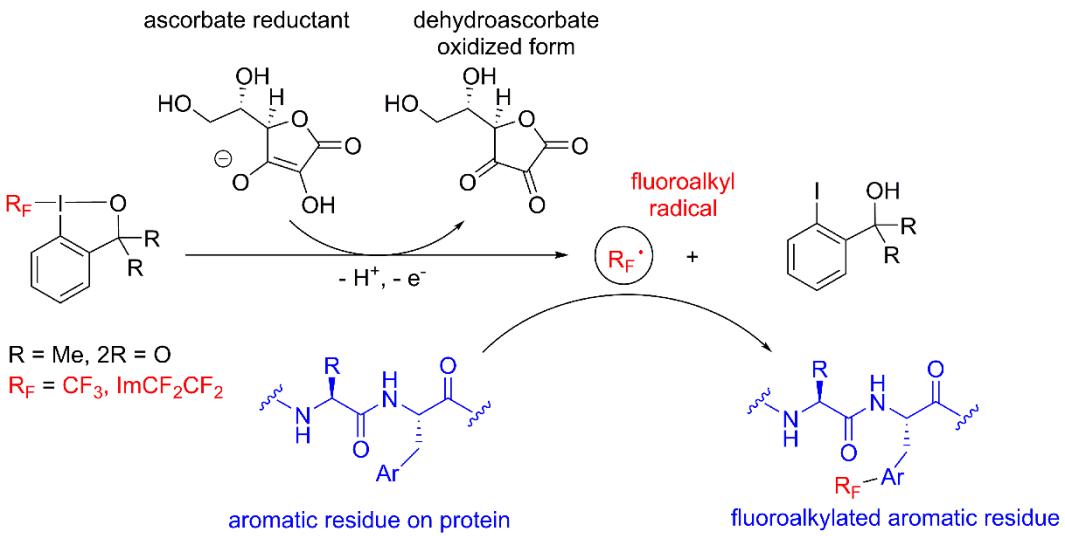
Structural model of a tetrameric Hb – Hp complex

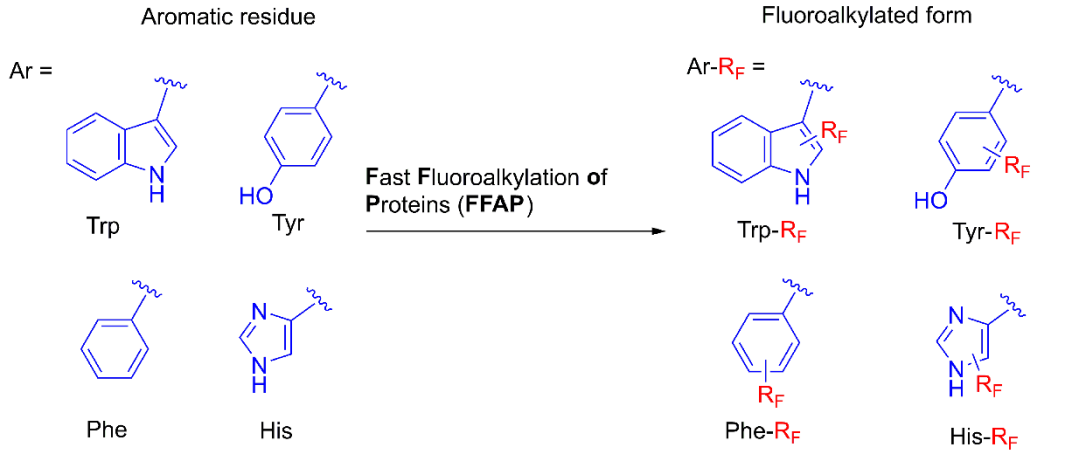


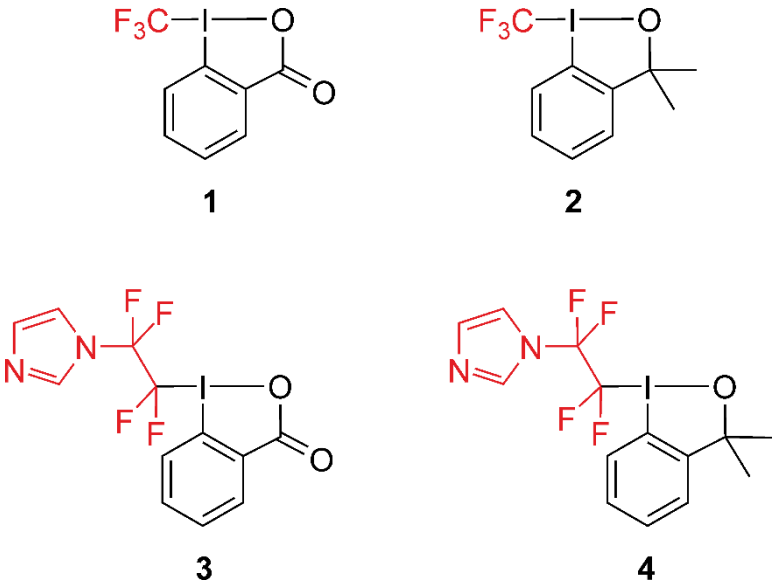
Interacting interface of a tetrameric Hb – Hp complex



Selective radical chemistry







ChemPubSoc Europe
DOI: 10.1002/chem.201902944
CHEMISTRY A European Journal Full Paper

Fluoroalkylation

Reductant-Induced Free Radical Fluoroalkylation of Nitrogen Heterocycles and Innate Aromatic Amino Acid Residues in Peptides and Proteins

Kheironnesae Rahimidashaghoul,[a, b] Iveta Klimánková,[a] Martin Hubálek,[a] Michal Korecký,[a] Matúš Chvojka,[c] Daniel Pokorný,[c] Václav Matoušek,[c] Lukáš Fojtík,[d] Daniel Kavan,[d] Zdeněk Kukačka,[d] Petr Novák,*[d] and Petr Beier*[a]

Uncovering amino acid side chains sensitive to Togni chemistry

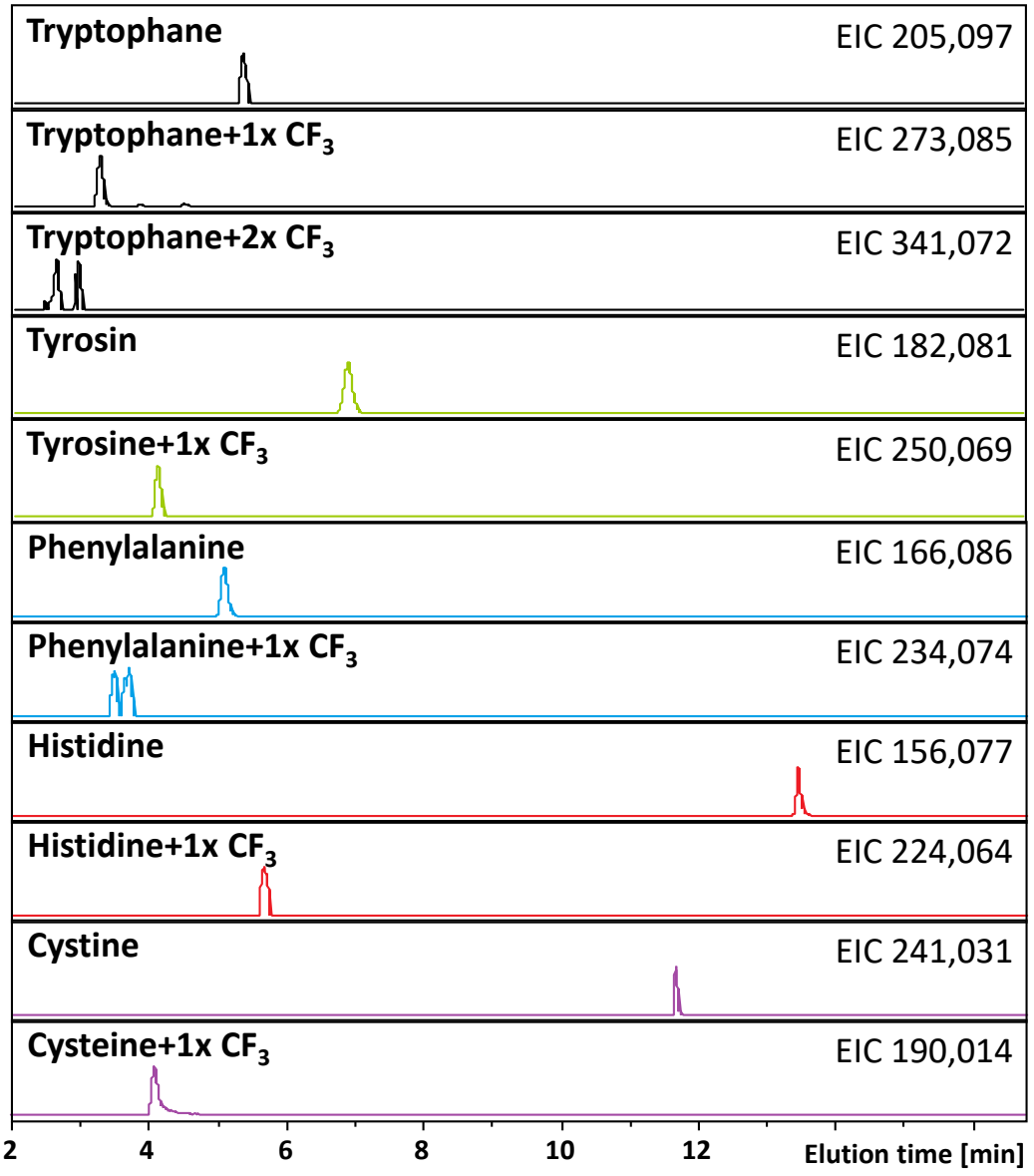
Mixture of 20 amino acids + Togni reagent

HILIC column (Imtakt, 2.1x150 mm)

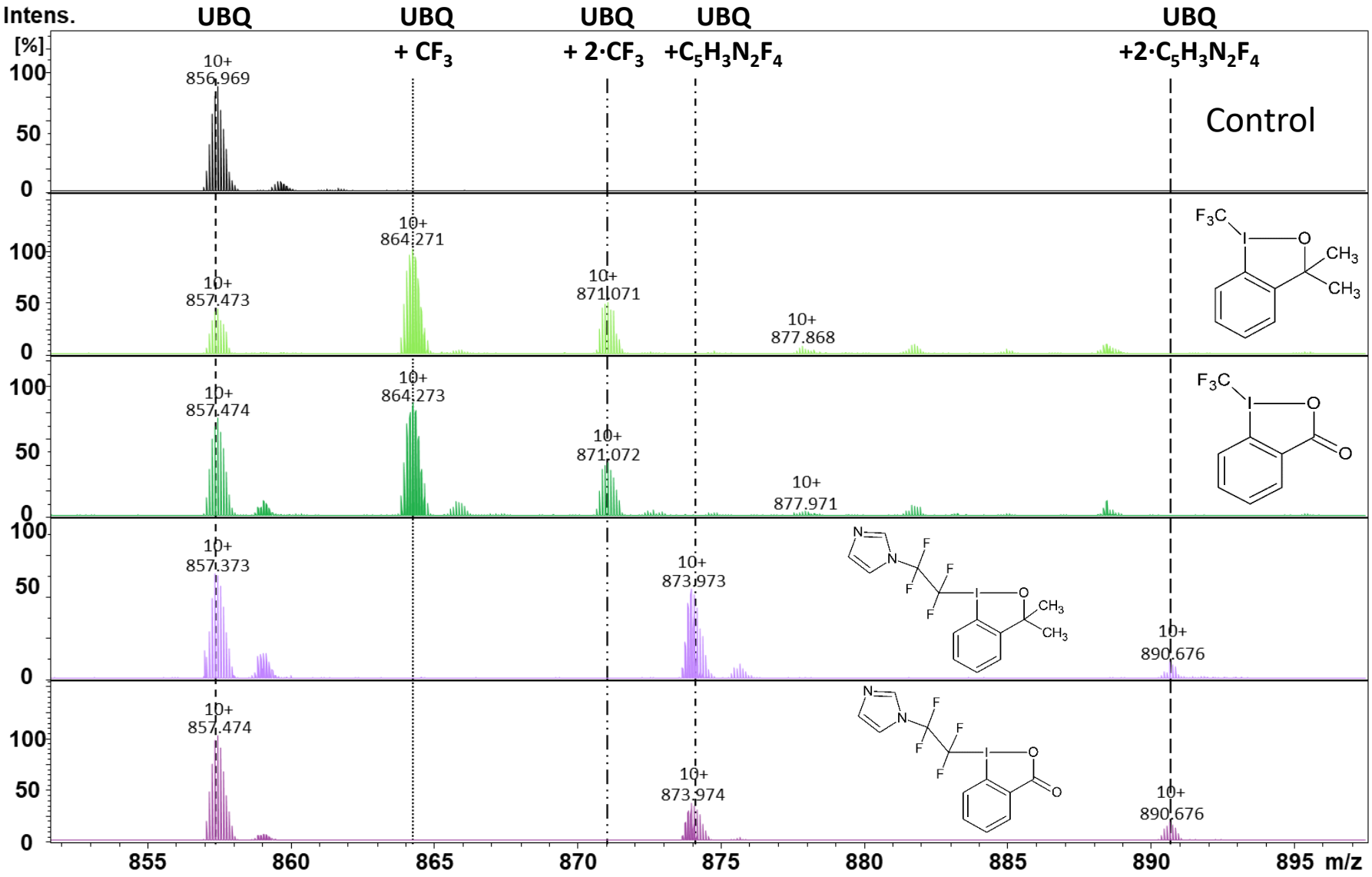
Gradient:
from 80% Buffer B (0.1% formic acid in AcN) to 100% Buffer A (100mM ammonium acetate)

maXis II QTOF

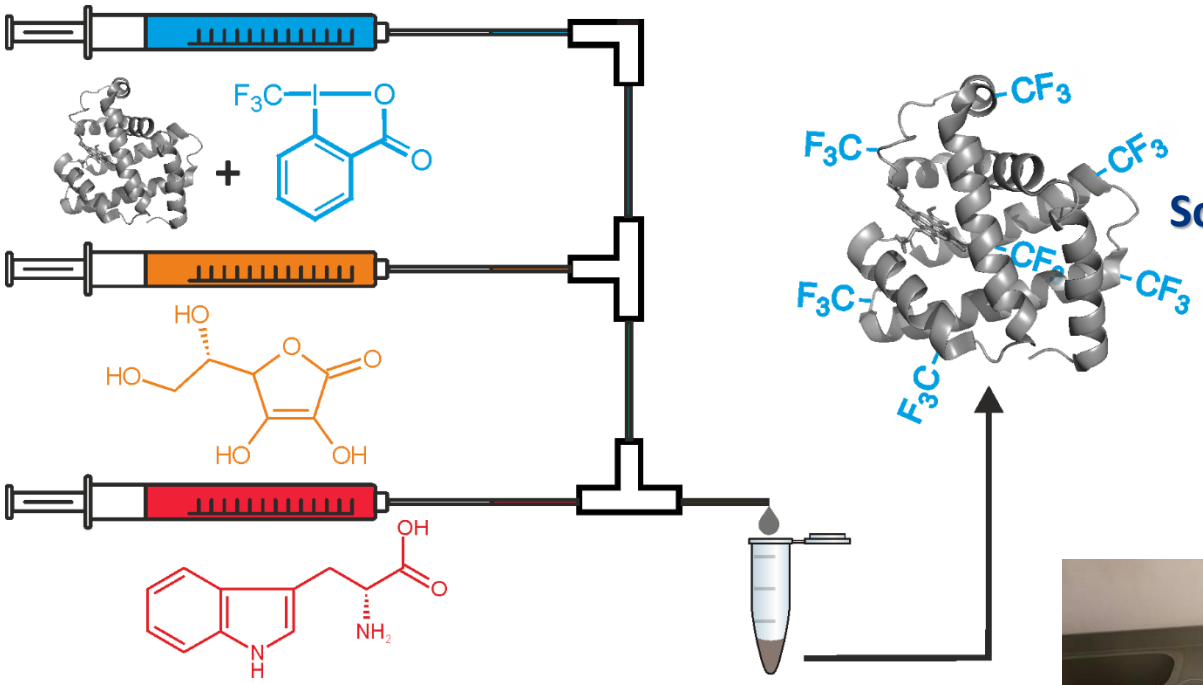
Reactive residues: Trp, Tyr, Phe, His, Cys



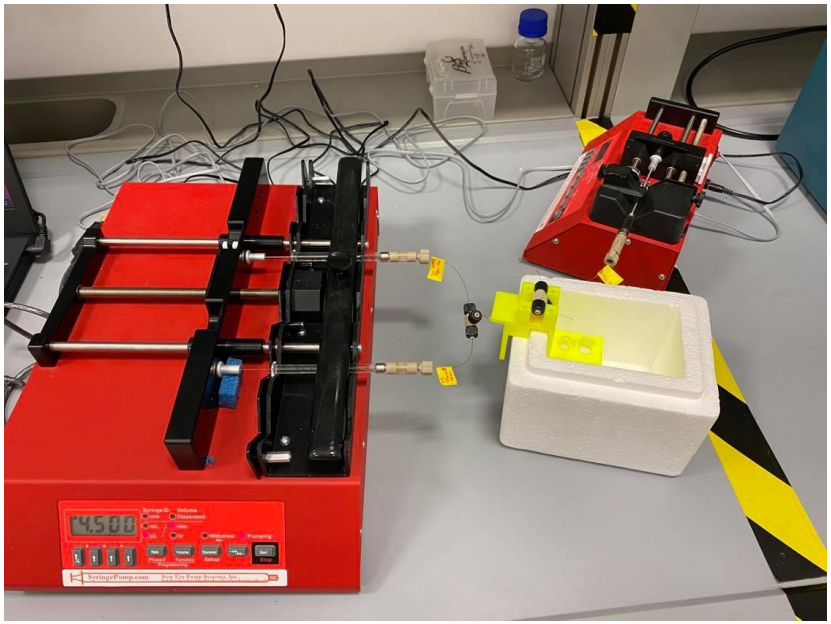
Does Togni work for proteins as well? The case of Ubiquitin



Fast Fluor-Alkylation of Proteins (FFAP)



So let's move to quench flow set up



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Article

Fast Fluoroalkylation of Proteins Uncovers the Structure and Dynamics of Biological Macromolecules

Lukáš Fojtík, Jan Fiala, Petr Pompach, Josef Chmelík, Václav Matoušek, Petr Beier, Zdeněk Kukačka,* and Petr Novák*

Cite This: https://doi.org/10.1021/jacs.1c07771
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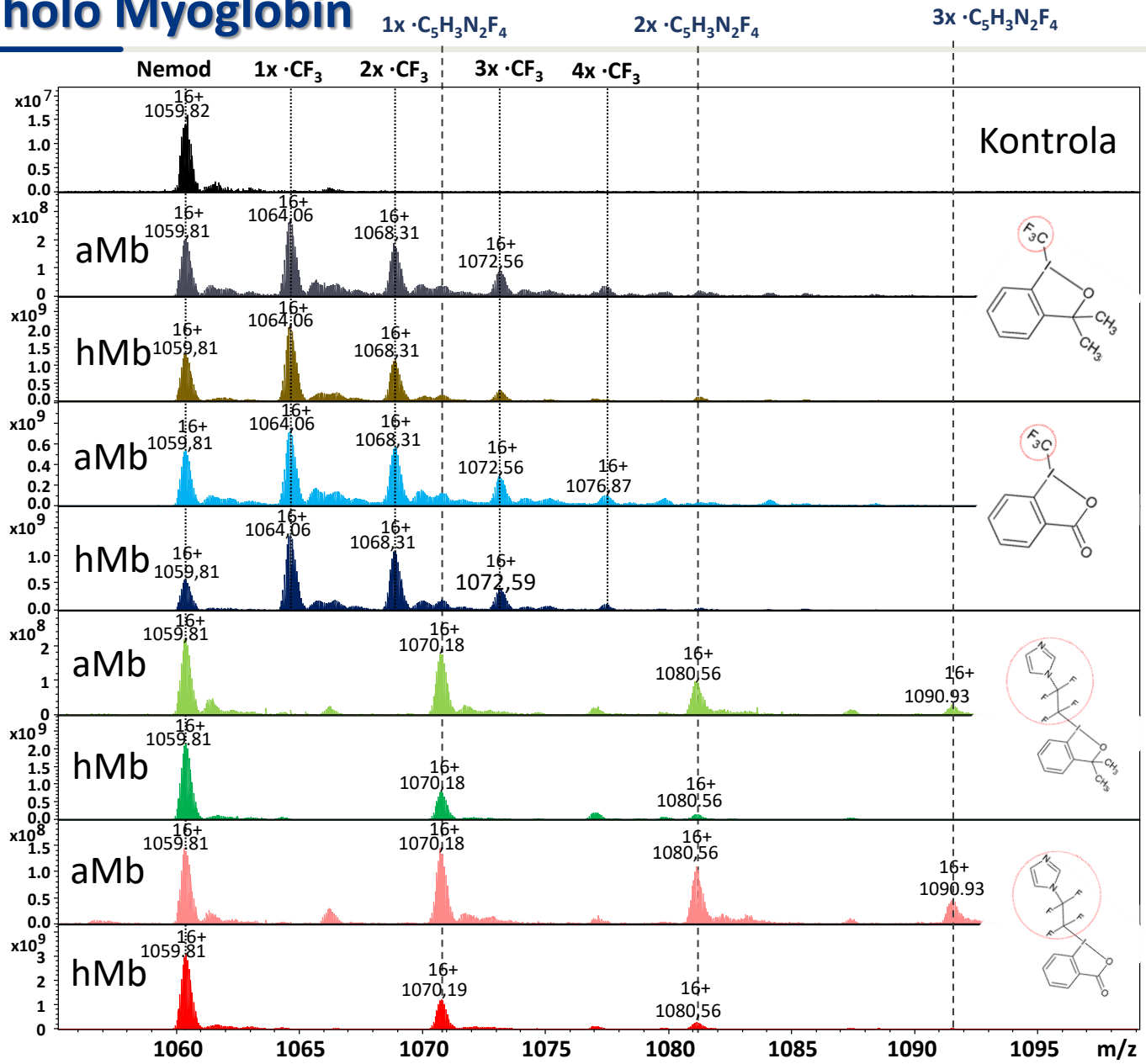
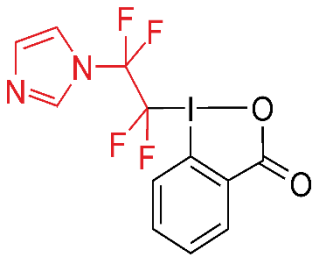
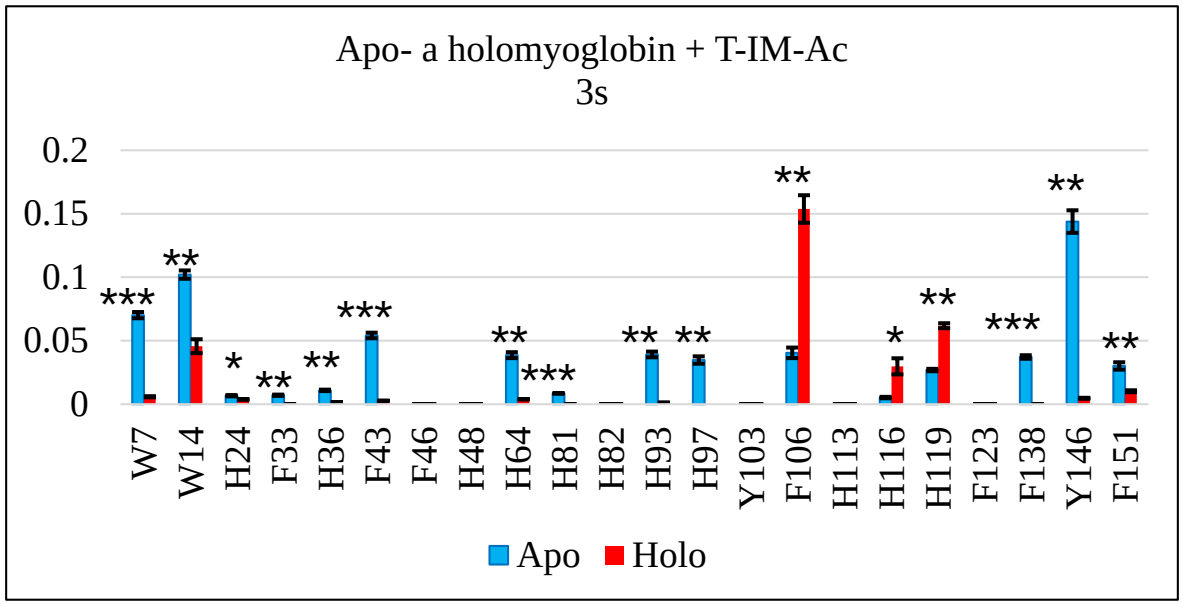
Apo and holo Myoglobin



Quatifying the extend of modification for each residue on Myoglobin

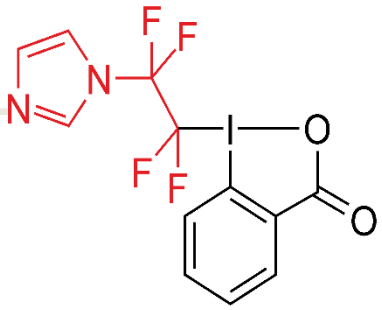
$$[X] = \frac{I_{monoisotopic\ mass\ of\ the\ modified\ AA}}{I_{monoisotopic\ mass\ of\ non\ modified\ AA} + I_{monoisotopic\ mass\ of\ all\ modiefied\ AA}}$$



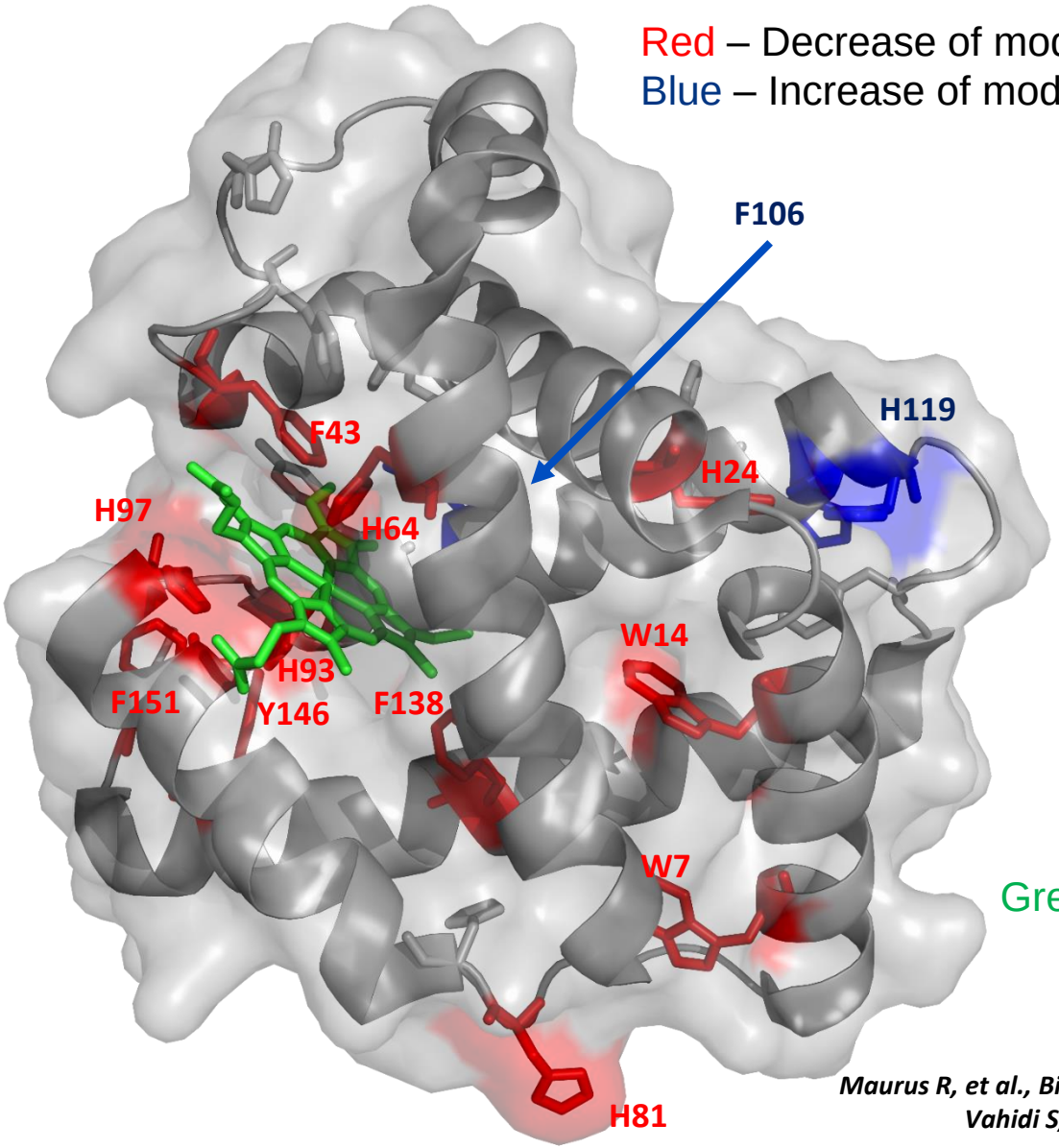


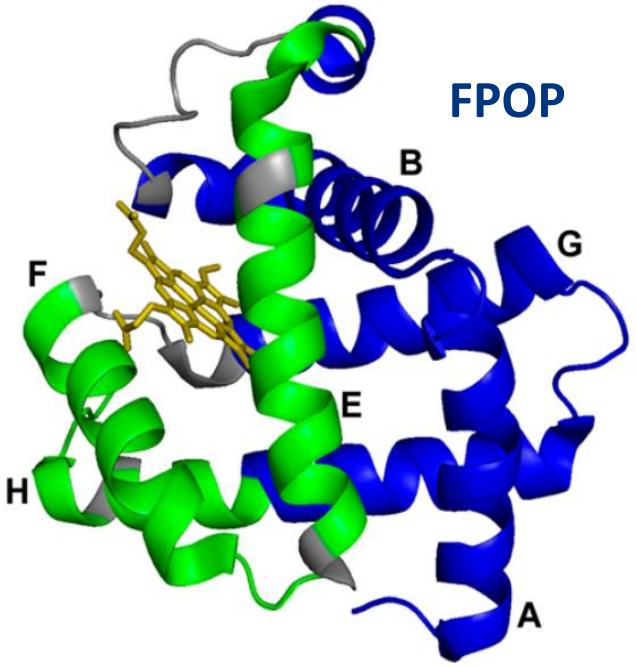
T-test = * - P < 0,005; ** - P < 0,01; * - P < 0,05**

Does the fooprinting reflect the Myolobin structure?



Red – Decrease of modification in hMb
Blue – Increase of modification in hMb





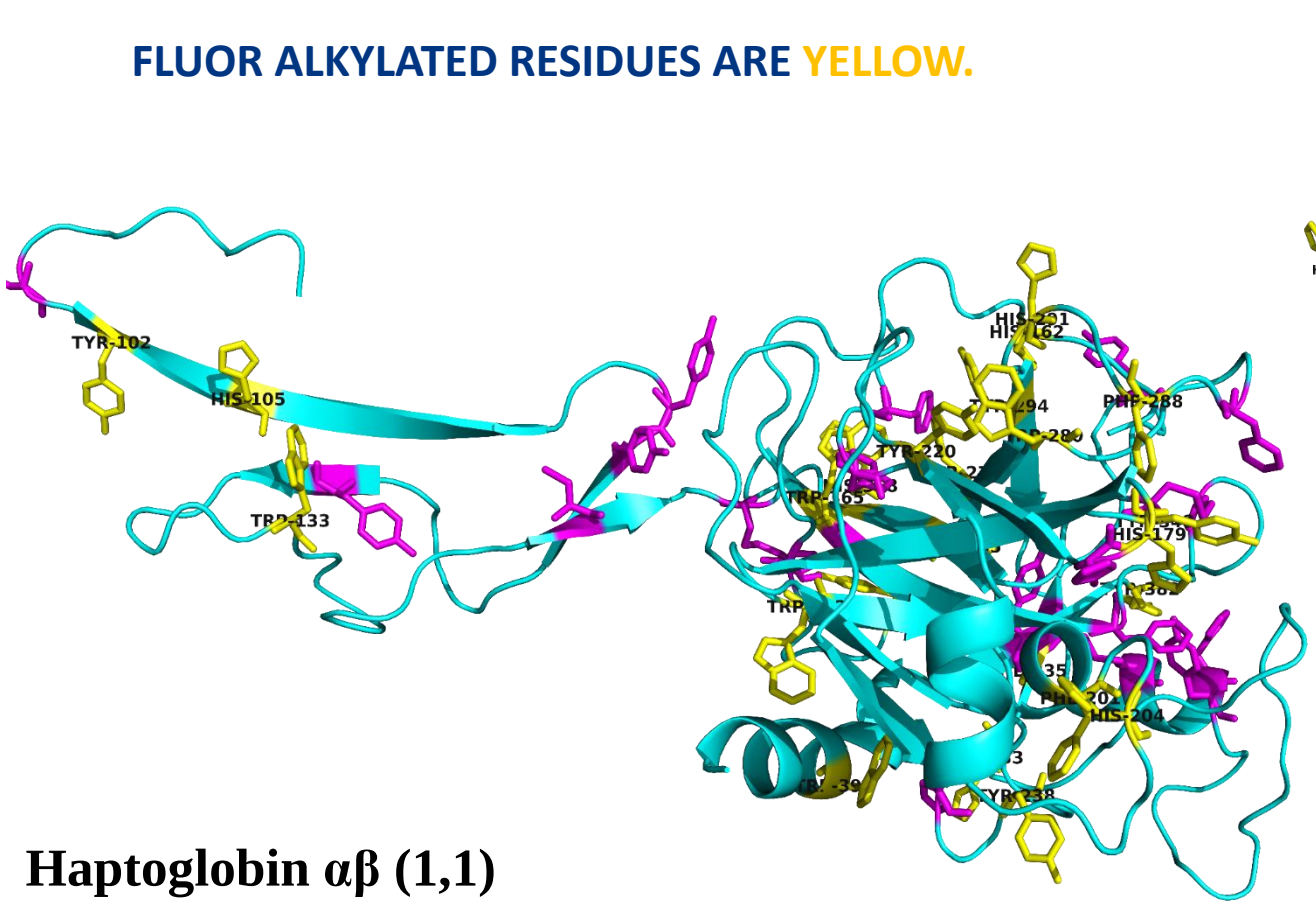
Green - Decrease of modification in hMb

PDB: 1WLA

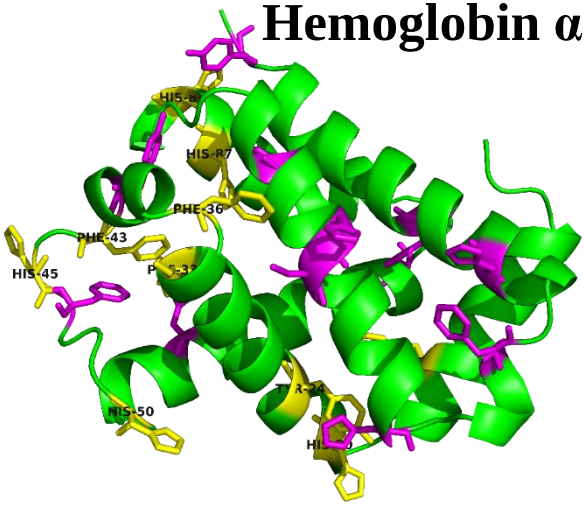
Maurus R, et al., Biochim Biophys Acta - Protein Struct Mol Enzymol. 1997
Vahidi S, et. Al, Anal Chem. 2012;84(21):9124-9130.

Can we reach a reasonable spatial resolution?

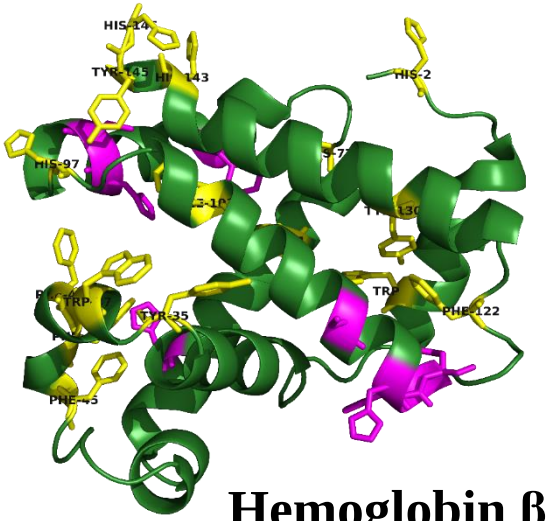
FLUOR ALKYLATED RESIDUES ARE YELLOW.



Haptoglobin αβ (1,1)

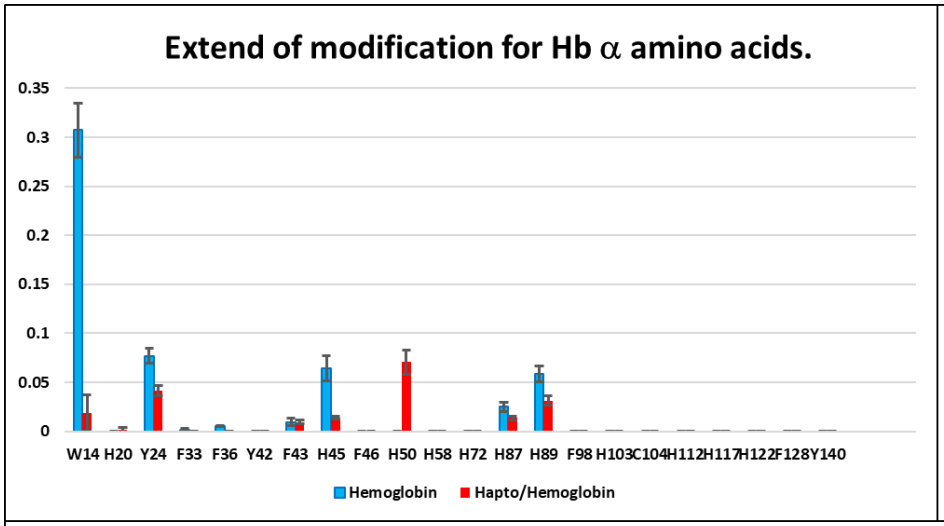


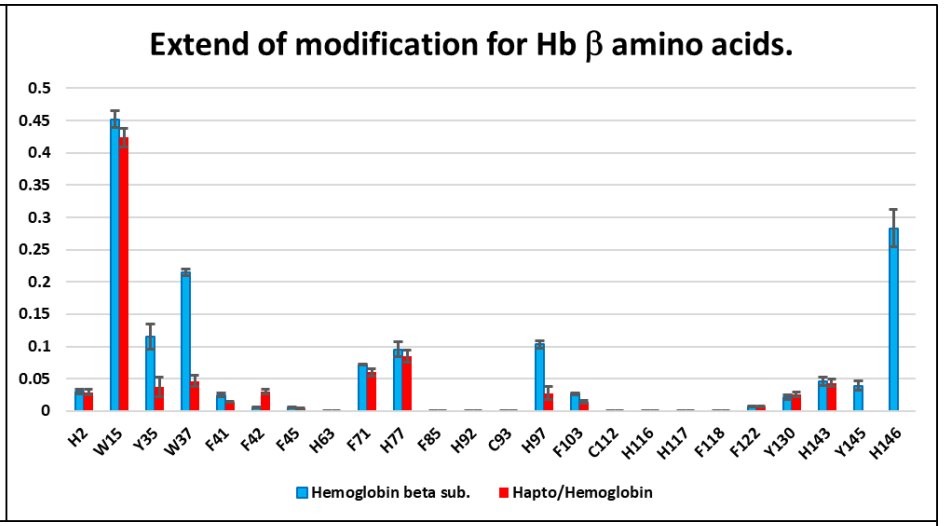
Hemoglobin α

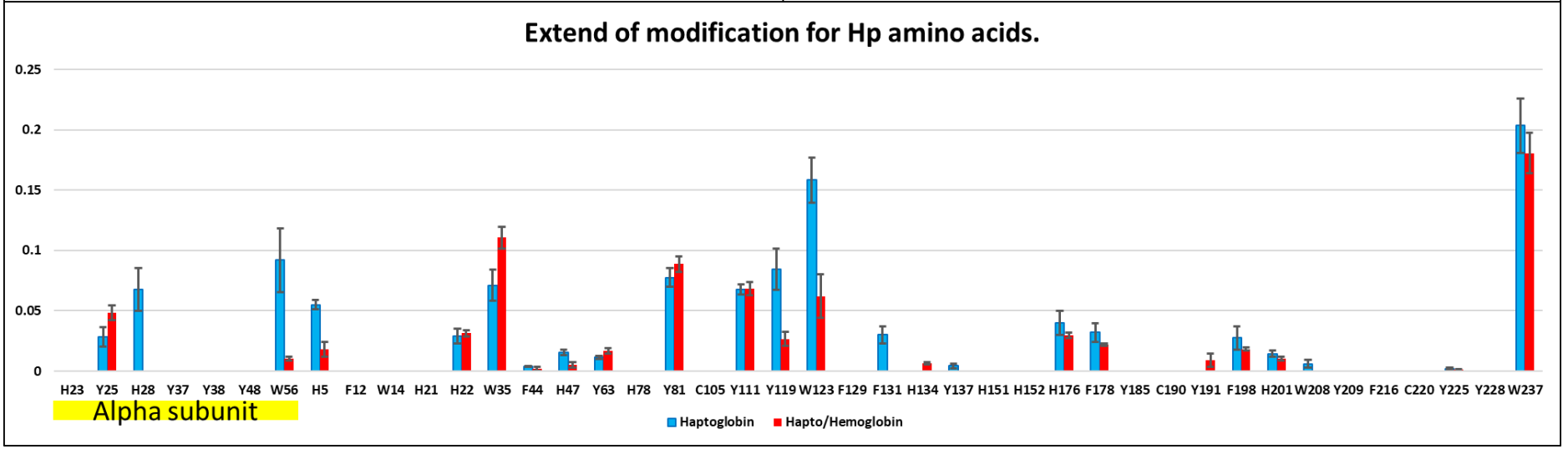


Hemoglobin β

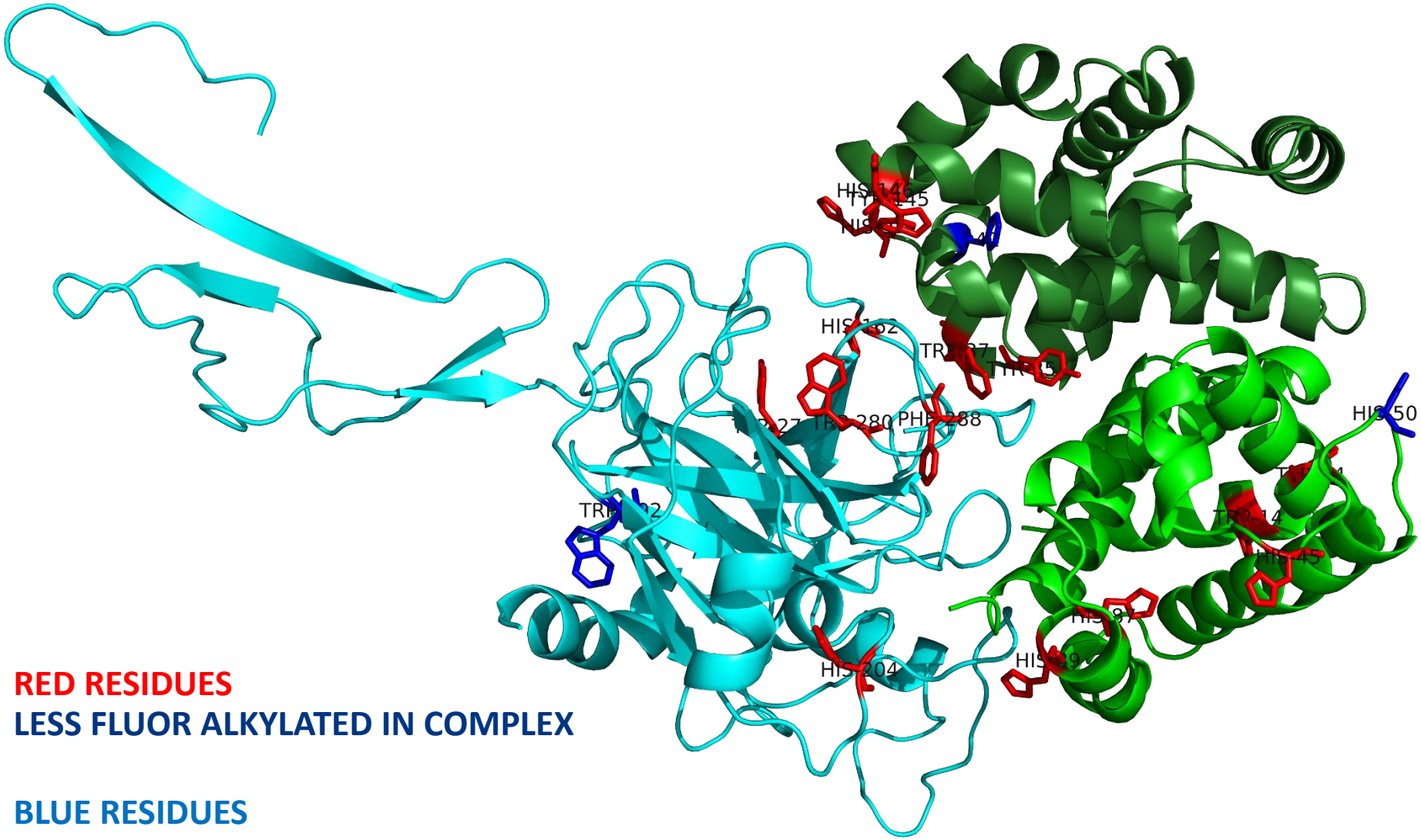
The extend of modification for each residue on Hp-Hb complex







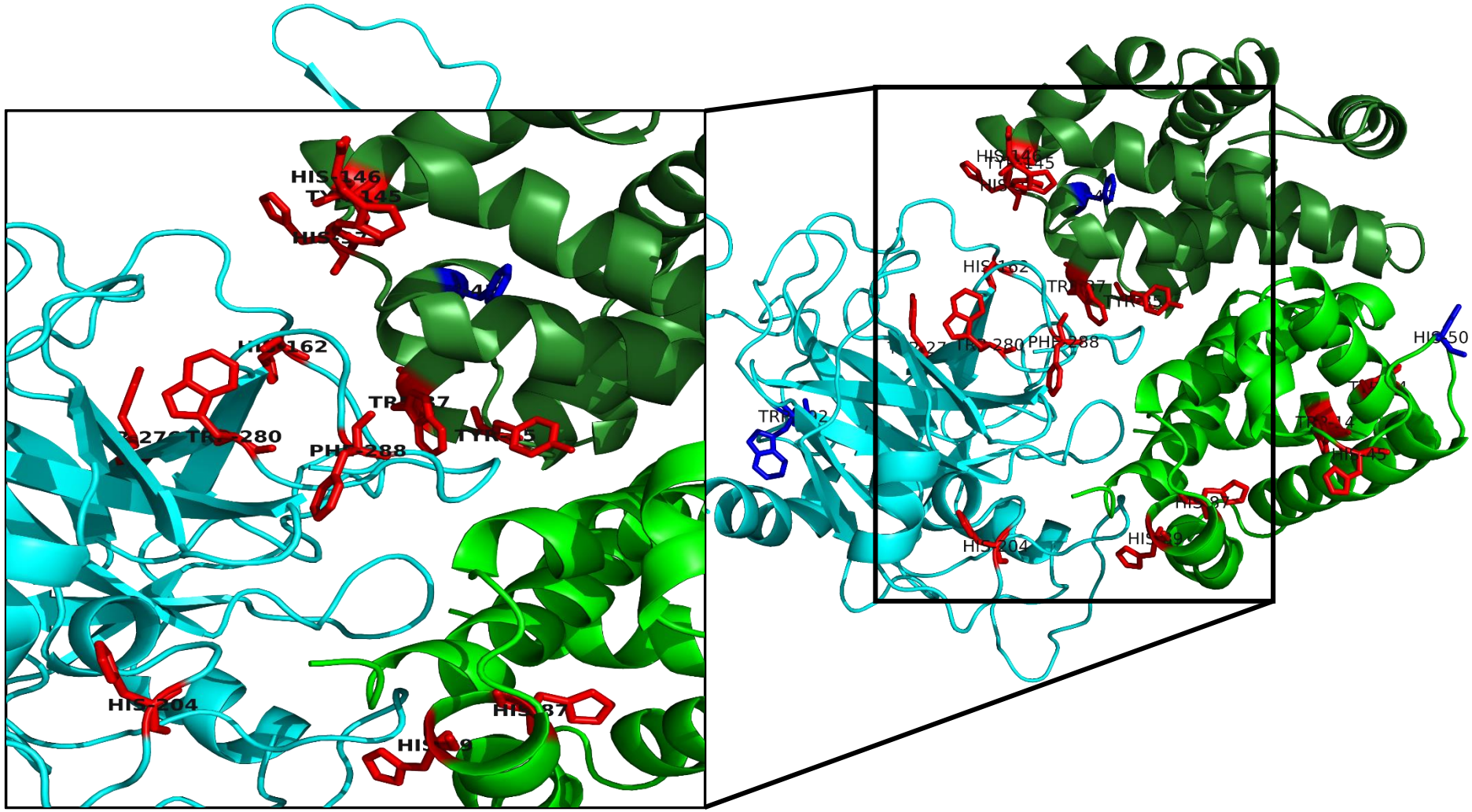
Complex of Hemoglobin α, β subunits and Haptoglobin α, β (1,1)



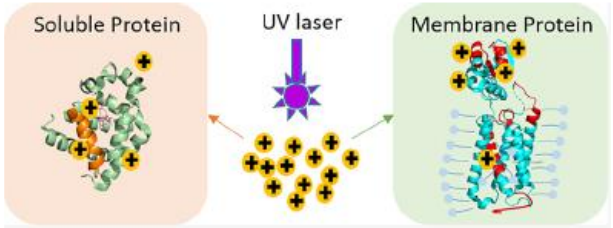
RED RESIDUES
LESS FLUOR ALKYLATED IN COMPLEX

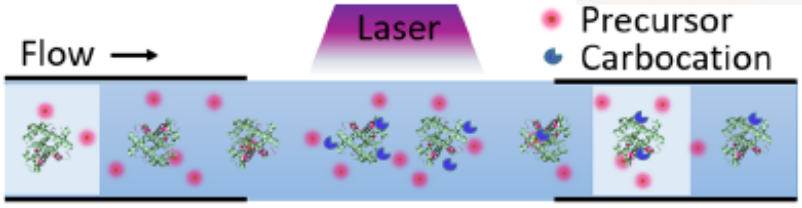
BLUE RESIDUES
MORE SENSITIVE TO FLUOR ALKYLATION IN COMPLEX

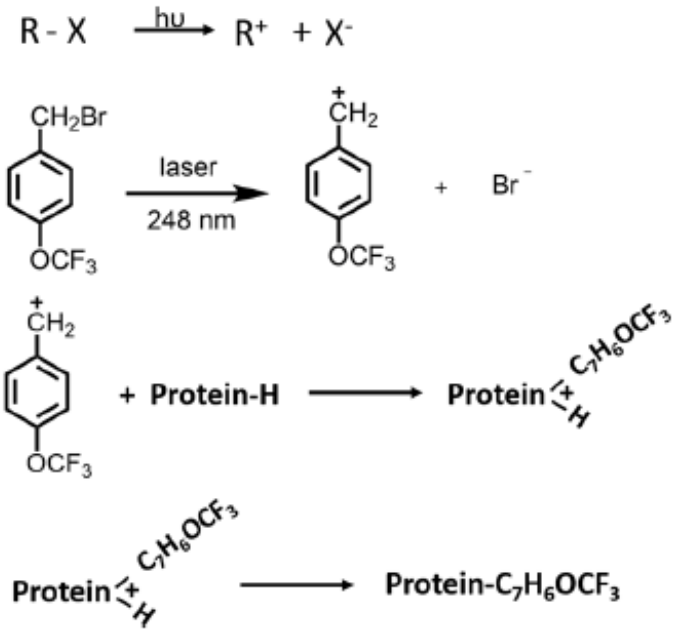
Interaction interface of the Hb α, β and Hb α, β (1,1) complex

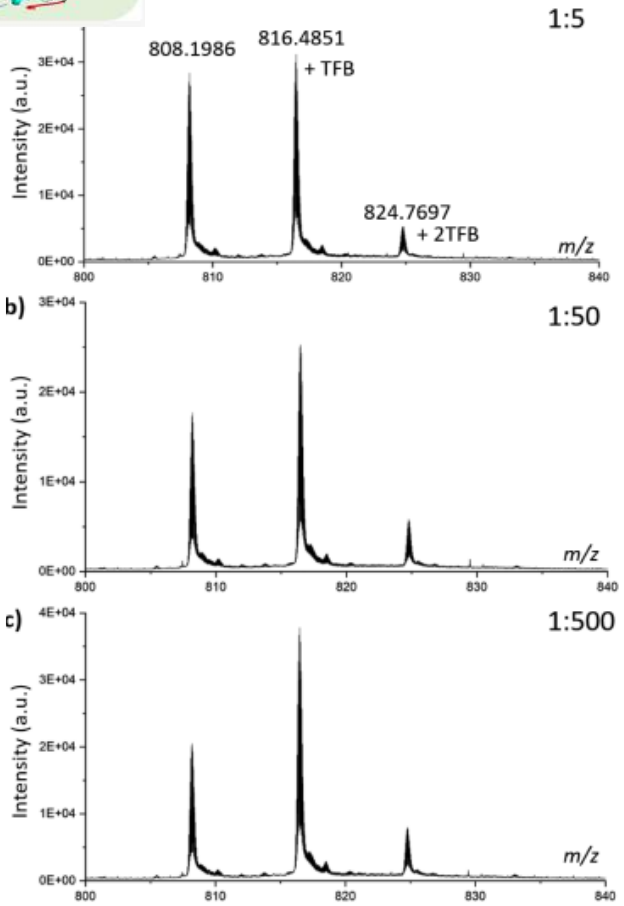


Carbocation Footprinting



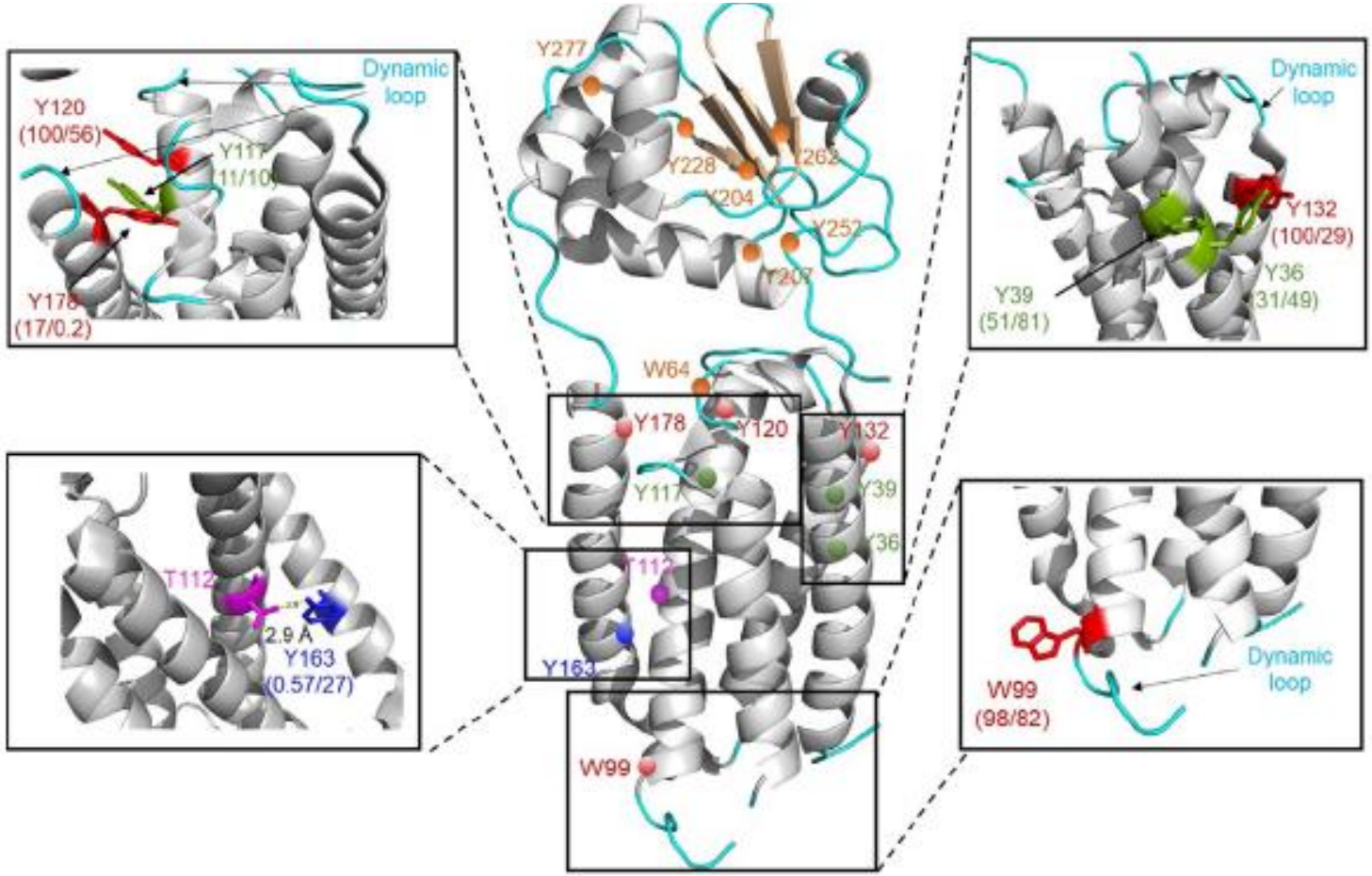






Sun J, Li S, Li W, Gross ML. Carbocation Footprinting of Soluble and Transmembrane Proteins. Anal Chem. 2021; 93:13101-13105.

Recent development for membrane proteins



Cheng M, Guo C, Li W, Gross ML. Free-Radical Membrane Protein Footprinting by Photolysis of Perfluoroisopropyl Iodide Partitioned to Detergent Micelle by Sonication. Angew Chem Int Ed Engl. 2021;60(16):8867-8873.

Fast Photochemical Iodination and Top down

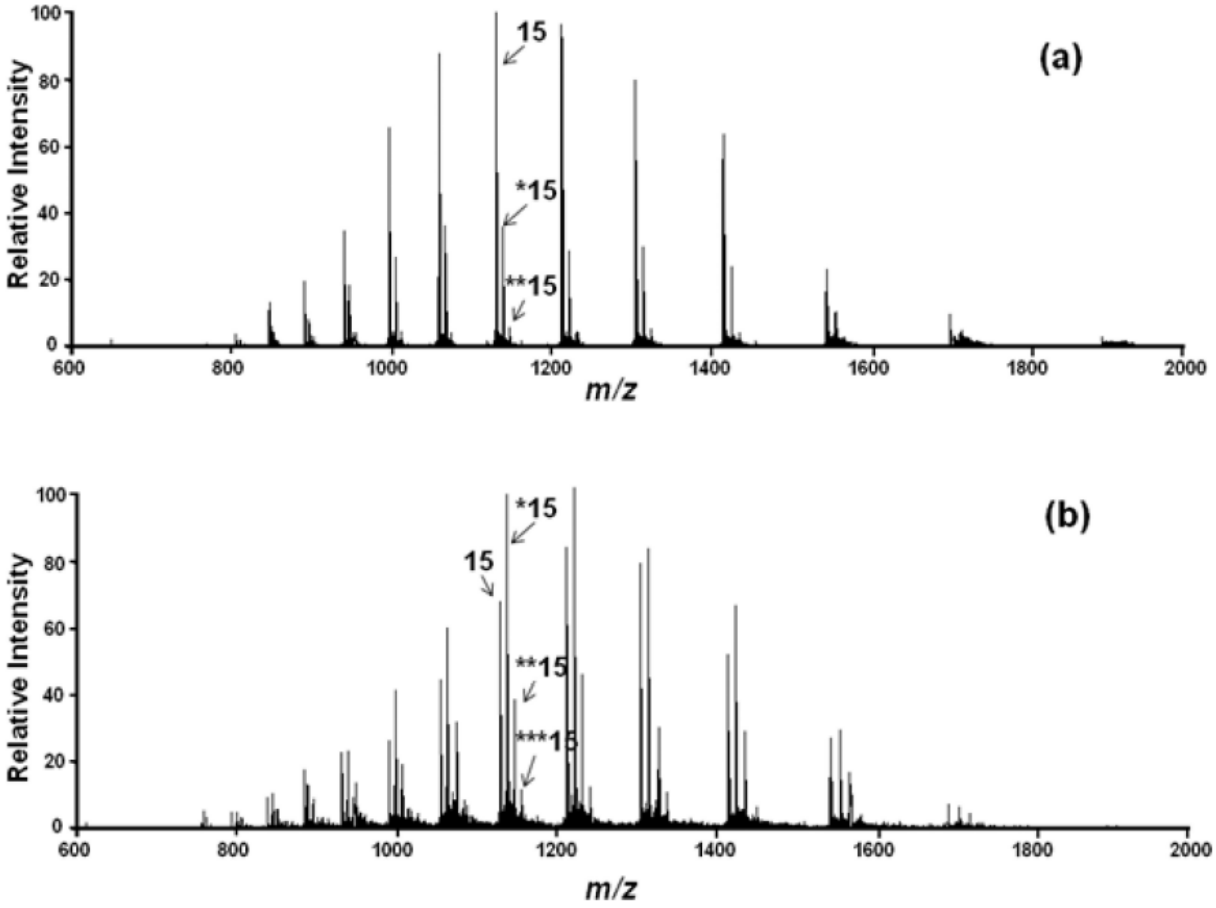
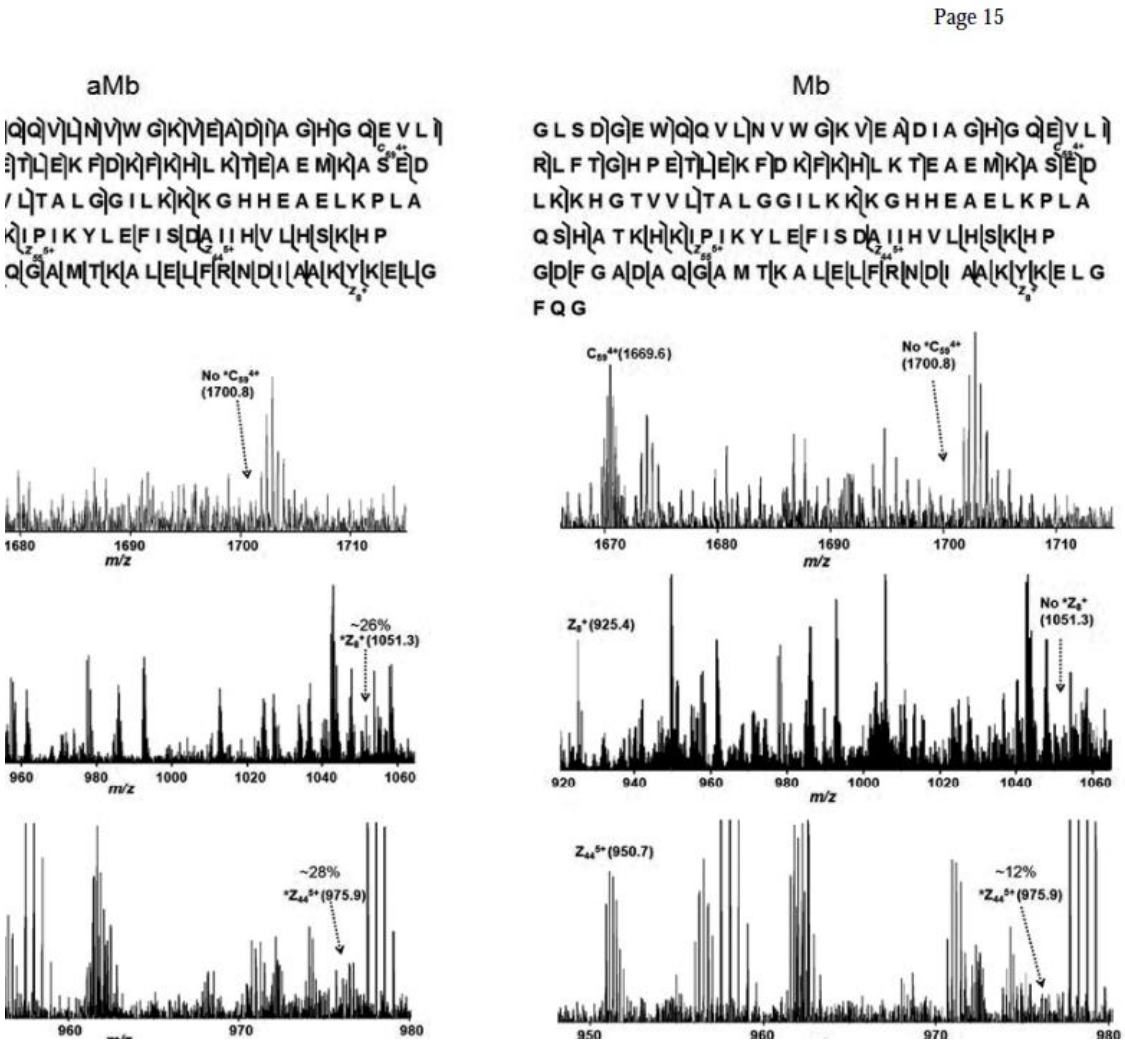


Figure 1.
(a) Full ESI mass spectrum of iodinated myoglobin (Mb). **(b)** Full ESI mass spectrum of iodinated apomyoglobin (aMb). The unmodified, mono-, di- and tri-iodinated species of the 15^{th} charge state are indicated by the number of stars.

Chen J, Cui W, Giblin D, Gross ML. New protein footprinting: fast photochemical iodination combined with top-down and bottom-up mass spectrometry. J Am Soc Mass Spectrom. 2012;23(8):1306-1318. doi:10.1007/s13361-012-0403-1

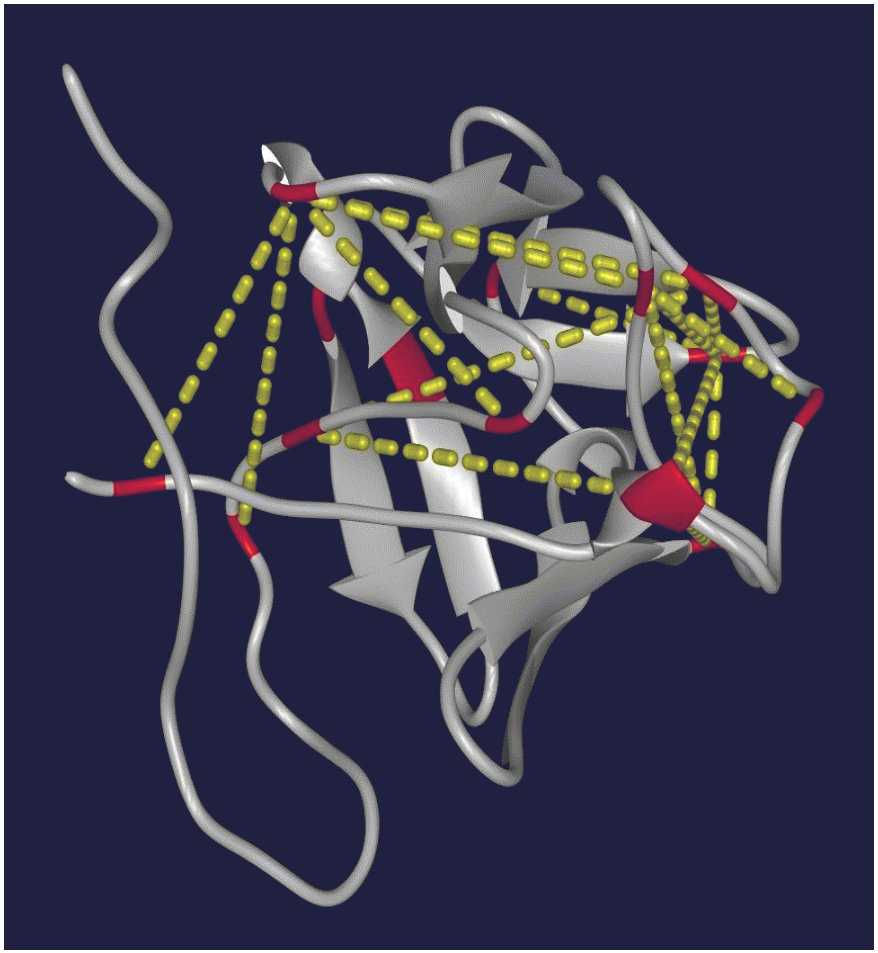
Fast Photochemical Iodination and Top down



Chen J, Cui W, Giblin D, Gross ML. New protein footprinting: fast photochemical iodination combined with top-down and bottom-up mass spectrometry. J Am Soc Mass Spectrom. 2012;23(8):1306-1318. doi:10.1007/s13361-012-0403-1

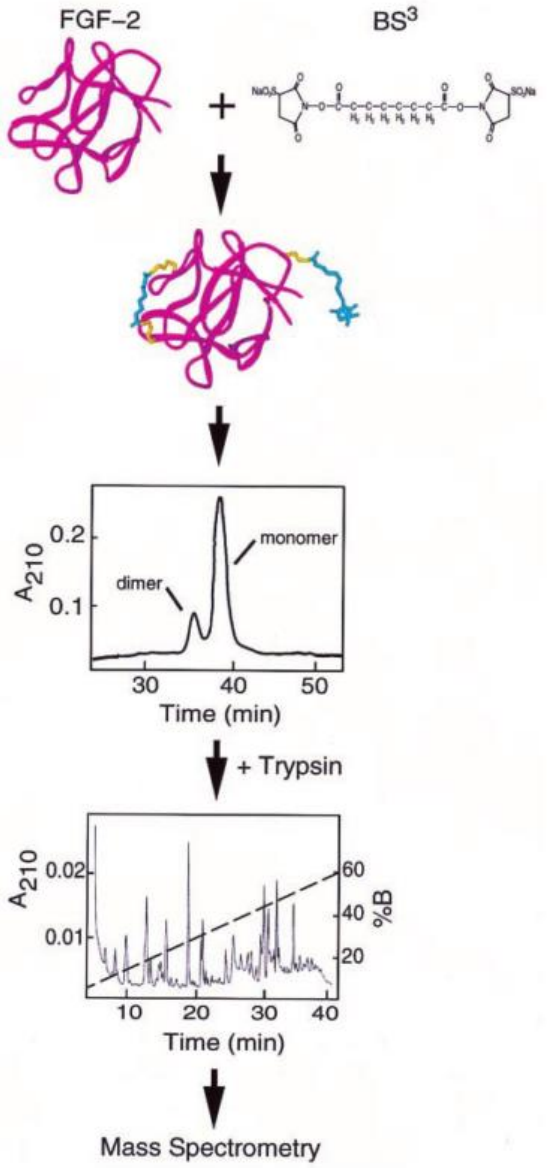
Chemical cross-linking

Chemical cross-linking combined with MS has been proposed as a method to obtain distance constraints (MS3D)

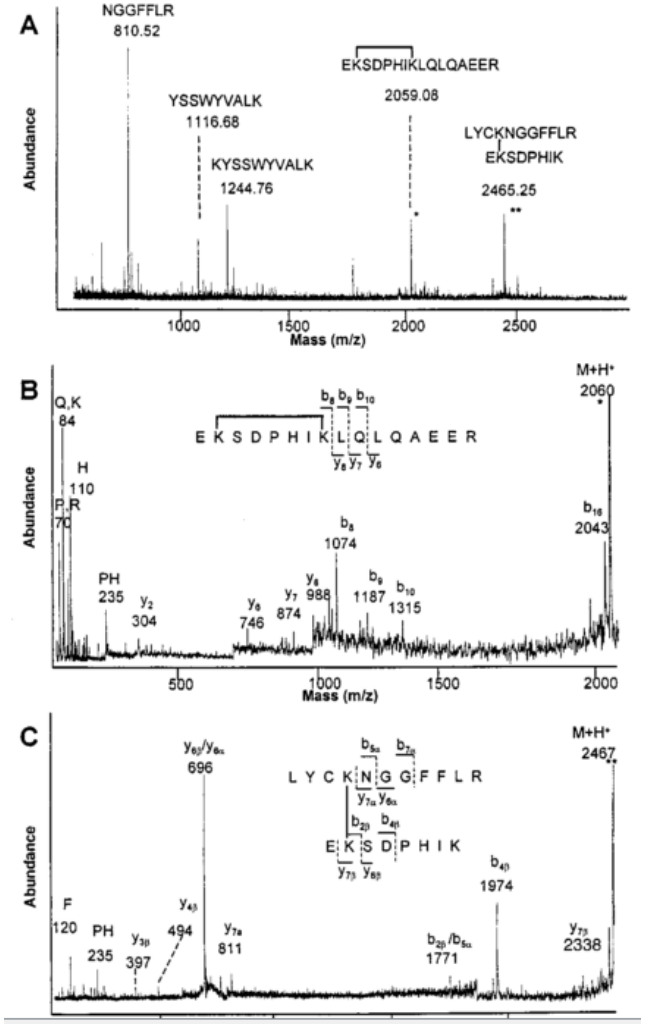


Young, Tang et al. *PNAS* 97(11):5802-6. 2000.

Chemical cross-linking: the first 3-D structure



Fibroblast grow factor 2



Top 20 threading models ranked by constraint error

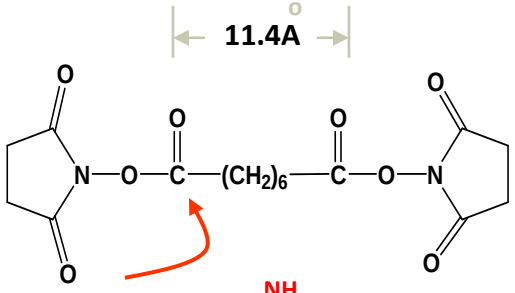
Name	Fold family	% Sequence identity	Threading rank	Constraint error, Å*	Number of violations†
FGF-2	β-Trefoil	98.6	1	0.0	0
IL-1β	β-Trefoil	12.7	5	0.0	0
Gastrotropin	Lipocalin	7.1	8	2.9	1
Hisactophilin	β-Trefoil	8.6	12	5.5	2
Guanylate kinase	P-loop	12.4	9	7.4	4
NTP pyrophosphohydrolase	NTP pyrophosphohydrolase	9.3	6	14.5	3
Glutathione peroxidase	Thioredoxin	11.1	14	16.6	5
Retinol-binding protein	Lipocalin	9.1	18	17.1	3
Nucleoside diphosphokinase	Ferridoxin-like	8.8	20	18.6	2
Cytochrome c_4	Cytochrome c	12.6	11	21.4	5
Aspartate carbamoyltransferase	Ferridoxin-like	9.8	13	22.6	4
D-UTPase	β-Clip	7.8	2	27.5	7
Disulfide bond formation protein	Thioredoxin	8.4	15	28.1	8
ASV integrase	Ribonuclease H-like	7.8	19	28.6	5
Endoglucanase C	Galactose binding	11.6	4	33.8	6
TATA-box-binding protein	TATA-box-binding protein-like	10.3	7	40.0	8
Phospholipase A2	Phospholipase A2	9.5	16	55.4	7
PRD paired domain	3-Helix bundle	12.7	17	143.4	8

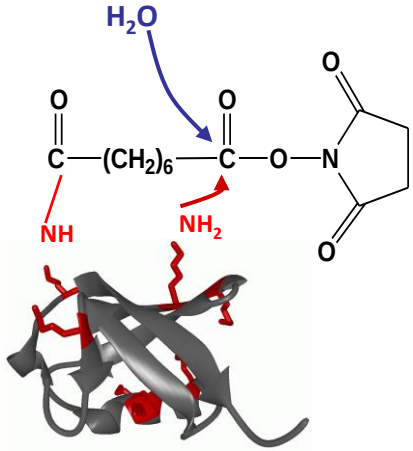
Young et. al. PNAS 2000, 97, 5802

Chemical cross-linking: the chemistry behind…

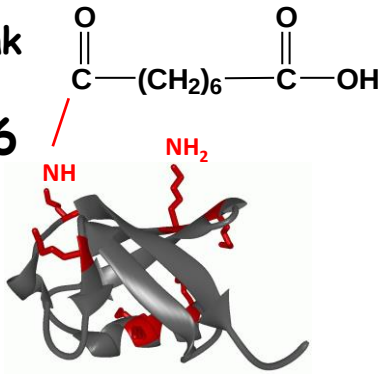
Primary amine reactive cross-linker

Disuccinimidyl Suberate (DSS)
Cross-linker "arm length"

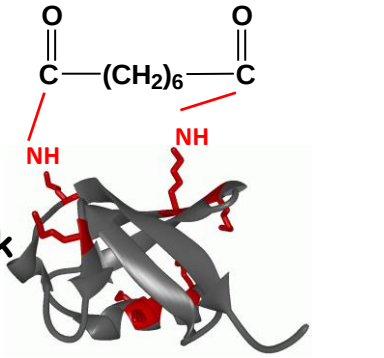


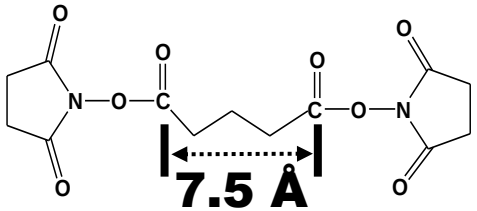


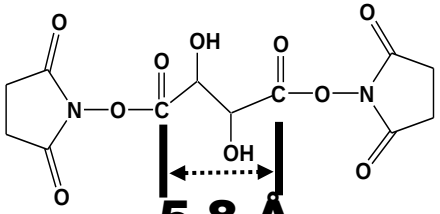
"Hanging" Cross-link
Add $C_8H_{12}O_3$,
Δm = 156.0786



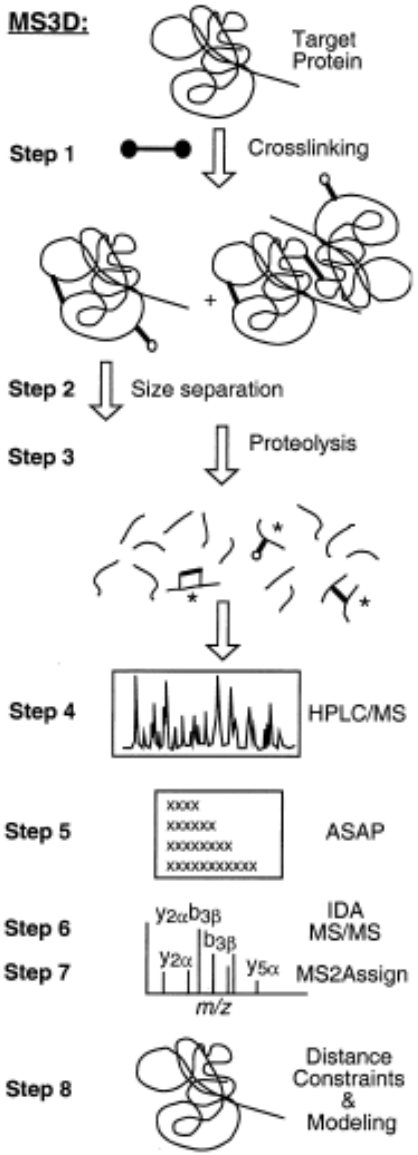
"Internal" Cross-link
Add $C_8H_{10}O_2$,
Δm = 138.0861

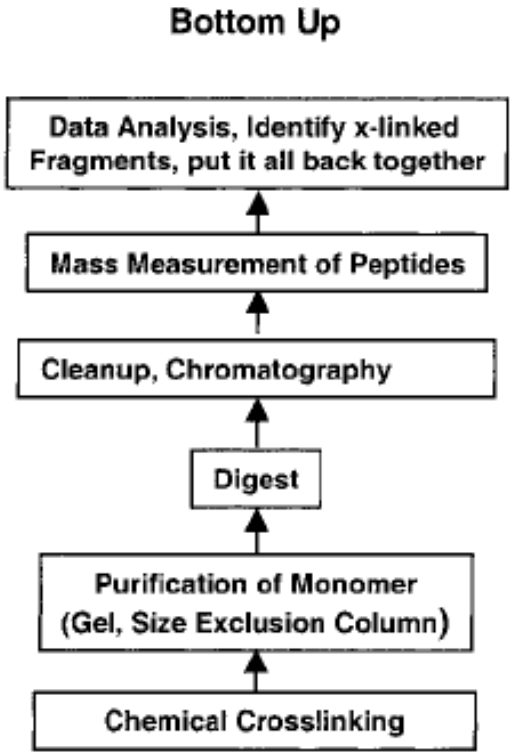


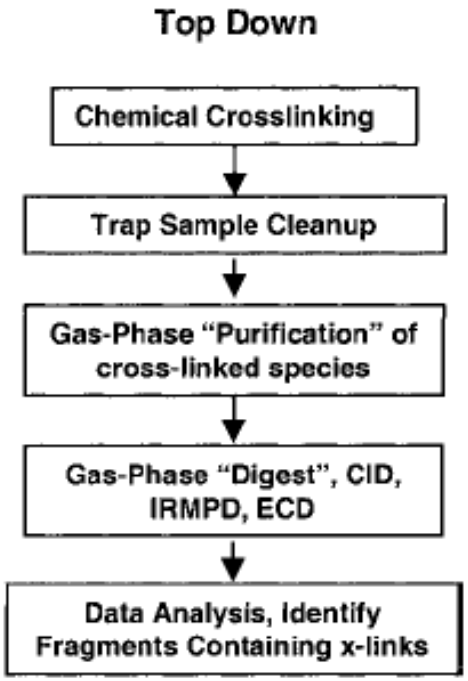




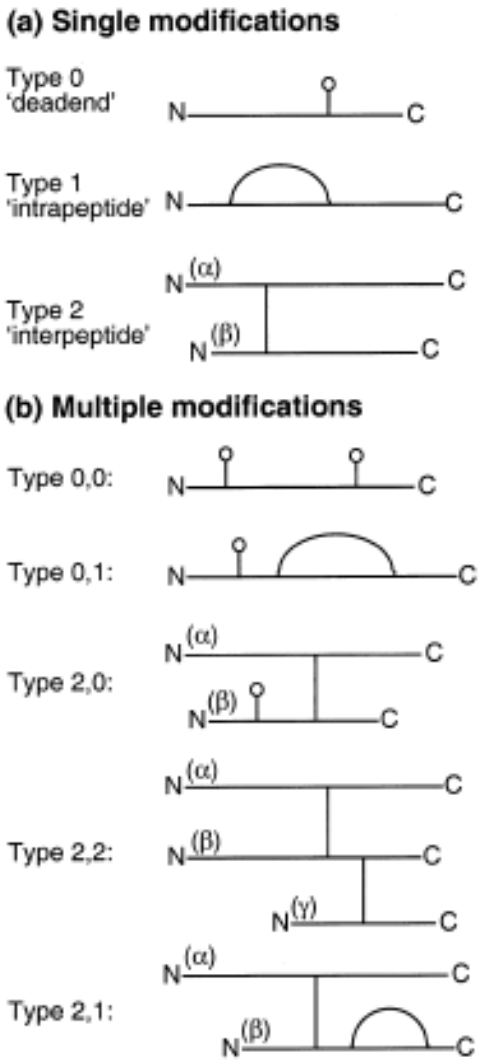
CXMS experiment



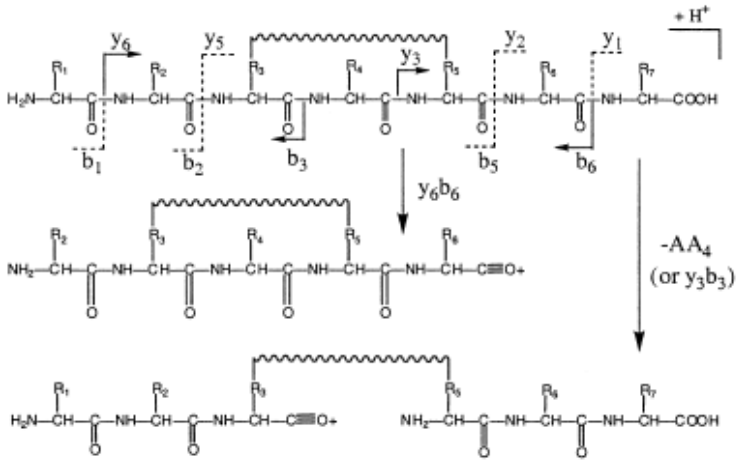




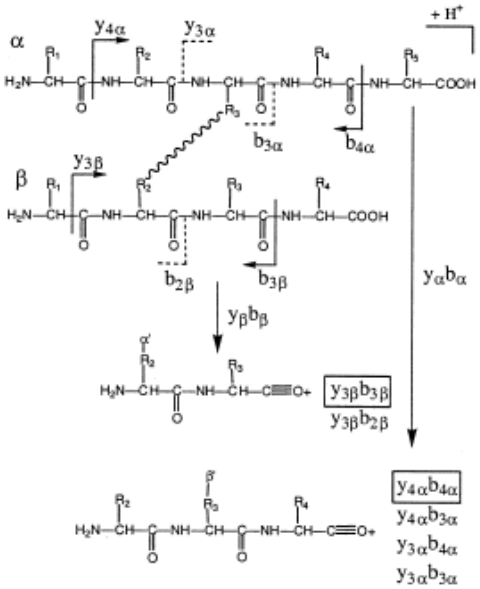
Nomenclature of peptide cross-linked fragments



Type 1

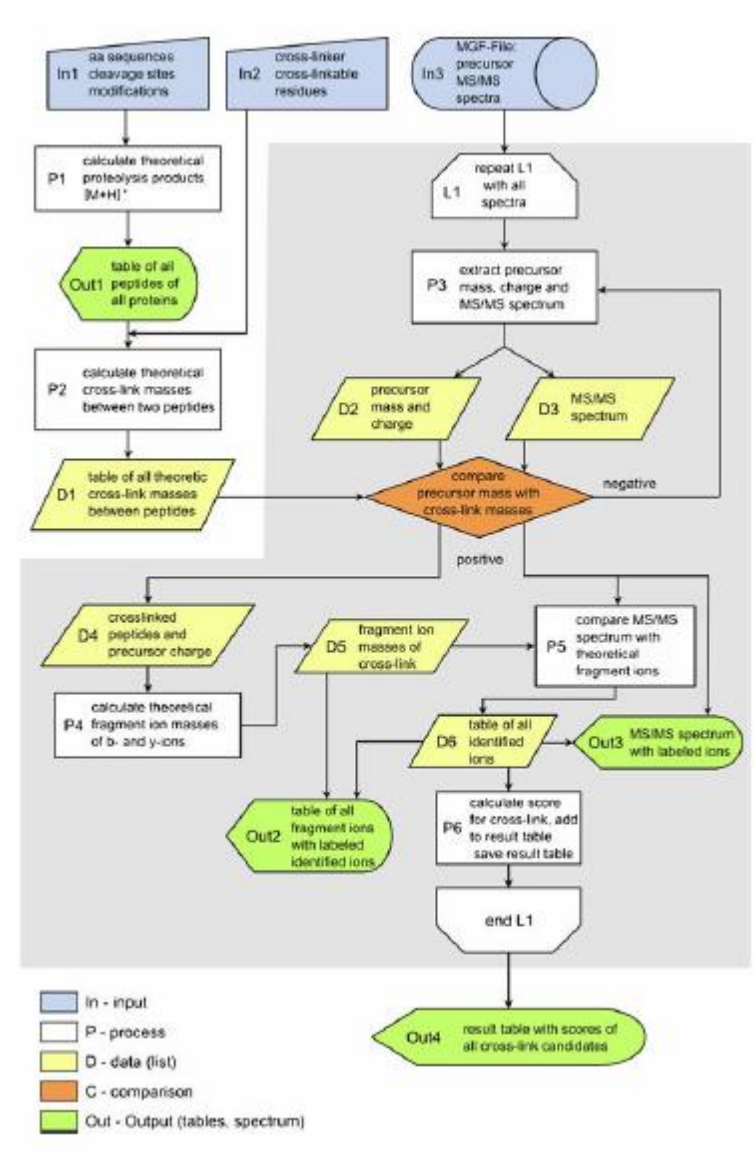


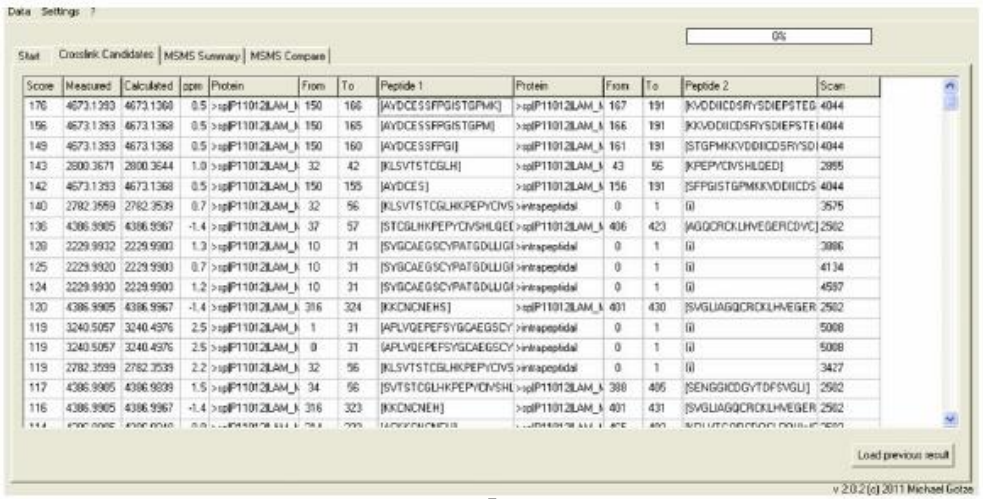
Type 2

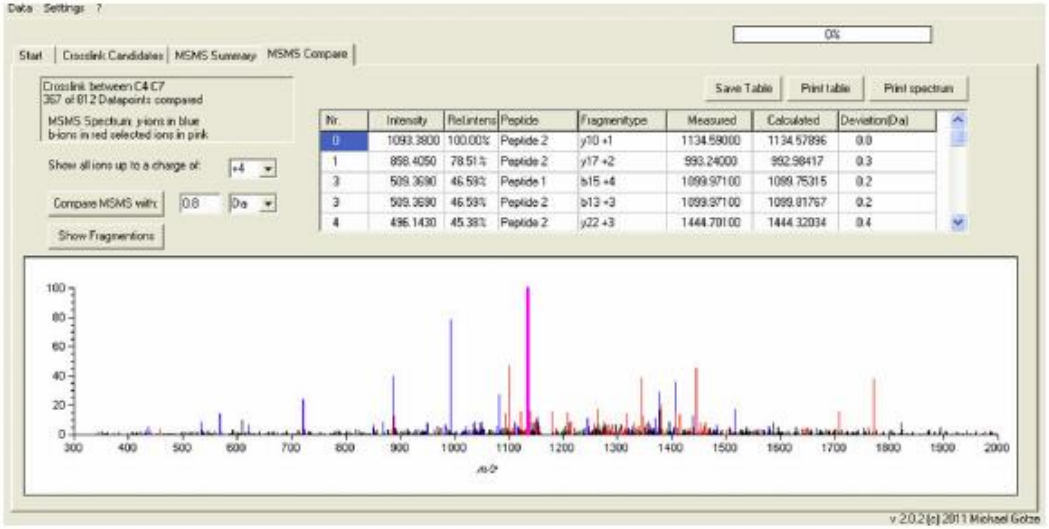


Schilling B, Row RH, Gibson BW, Guo X, Young MM. MS2Assign, automated assignment and nomenclature of tandem mass spectra of chemically crosslinked peptides. J Am Soc Mass Spectrom. 2003;14(8):834-850.

Data analysis: STAVROX



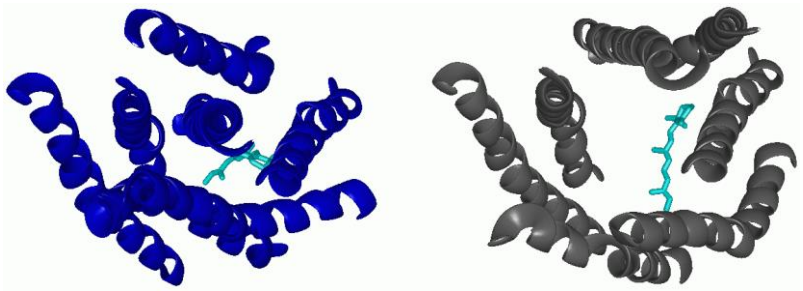




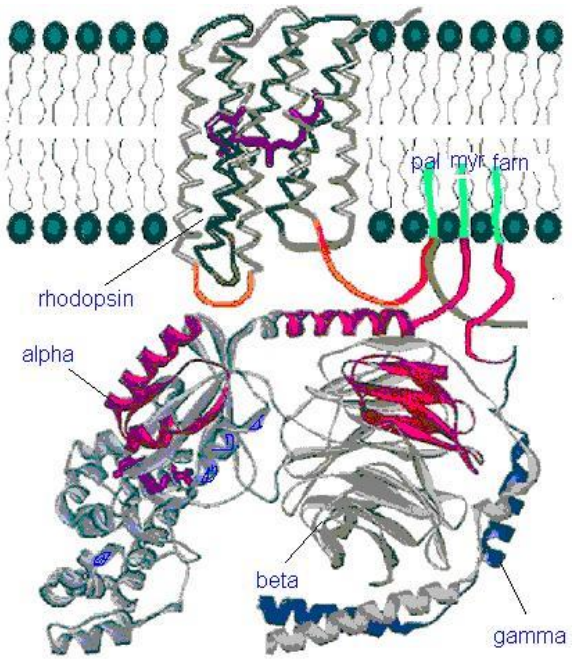
Gotze M. et al. JASMS 2012

Middle down - *Rhodopsin*

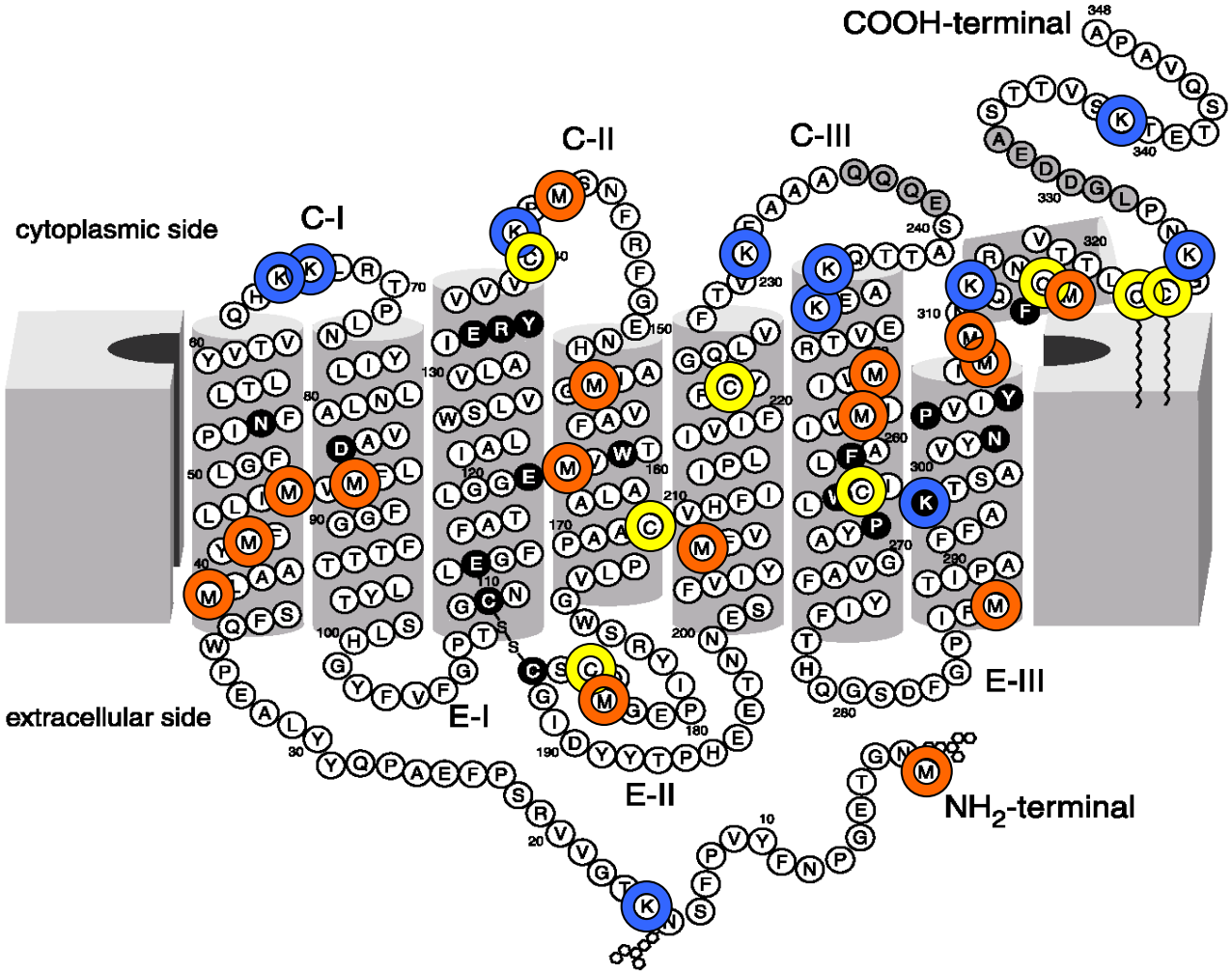
Rhodopsin has open structure/function questions



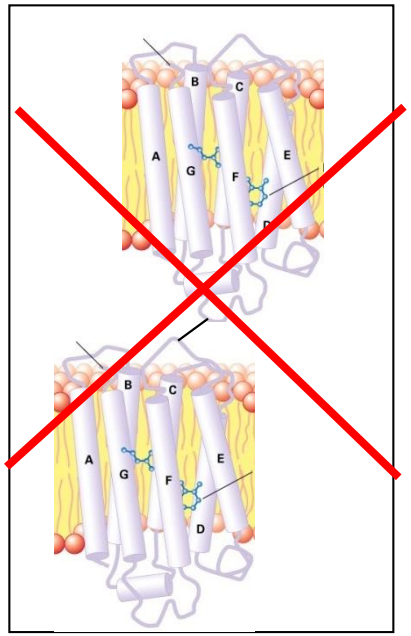
- **What is the conformational change that occurs upon light activation?**
- **What is the configuration of loops involved in Gi binding (not visible on X-ray)?**



Rhodopsin Has Many Potential Targets for Cross-linking

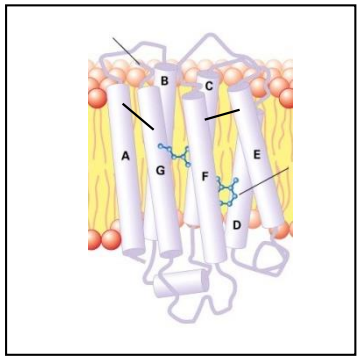


Proteoliposomes/Detergent



Crosslink Protein

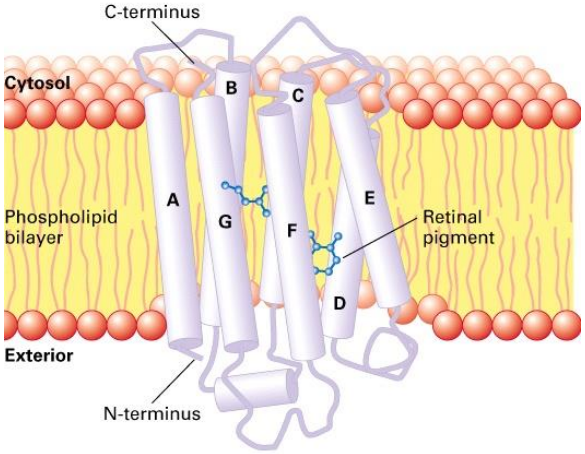
Purify Monomer



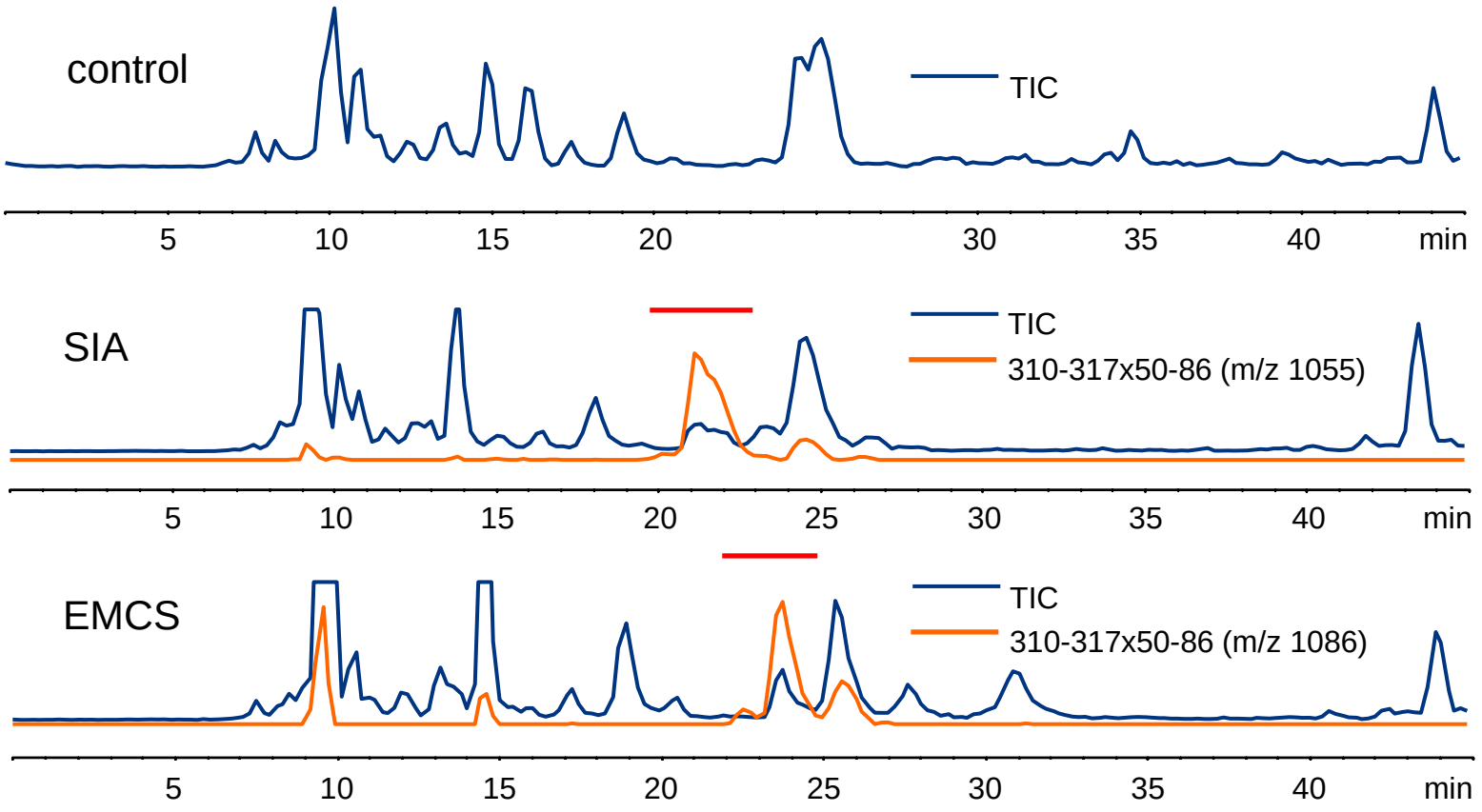
Protein Digestion

LC/MS Analysis

Data Analysis

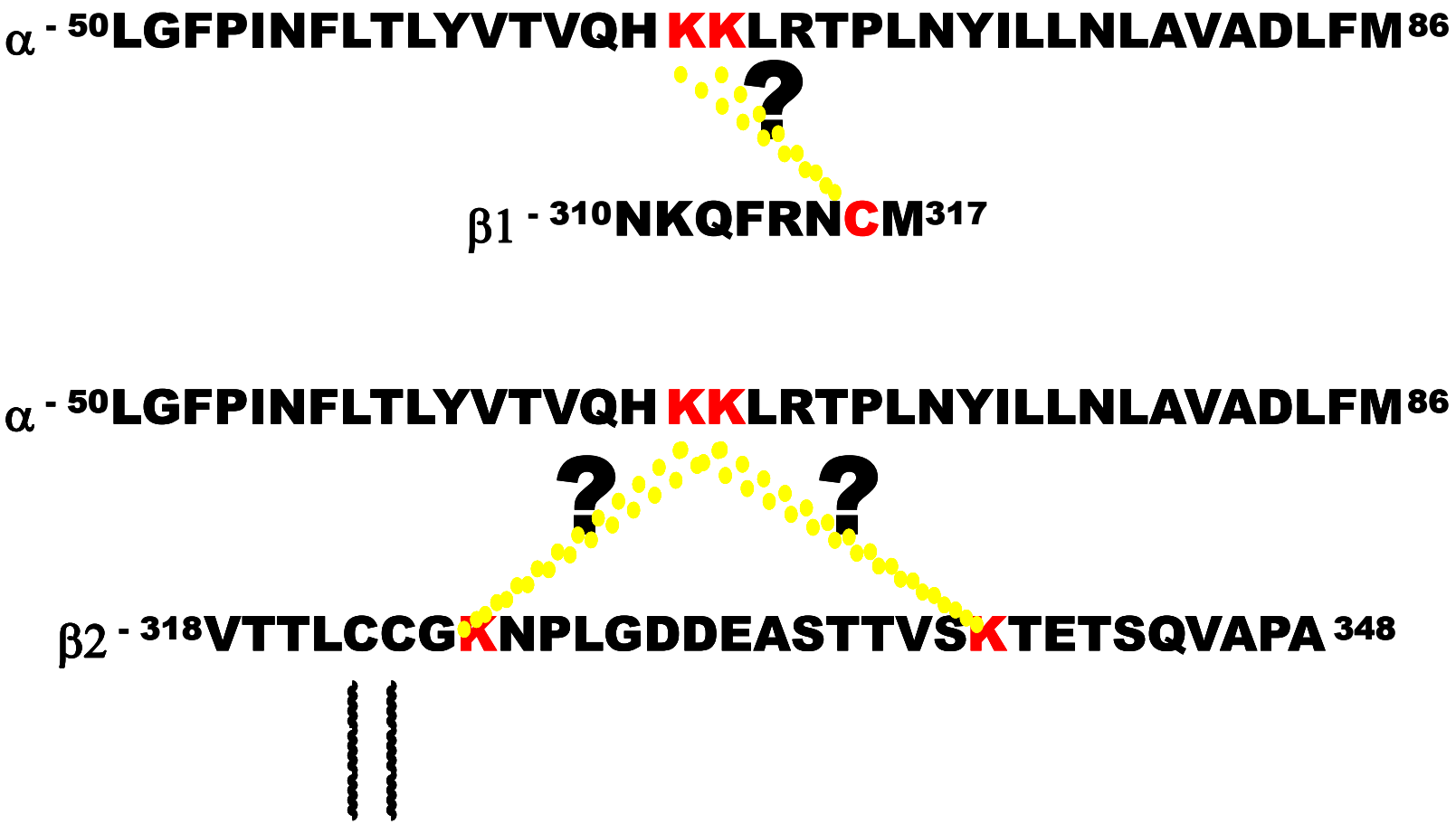


LCMS analysis of Rhodopsine CNBr digest

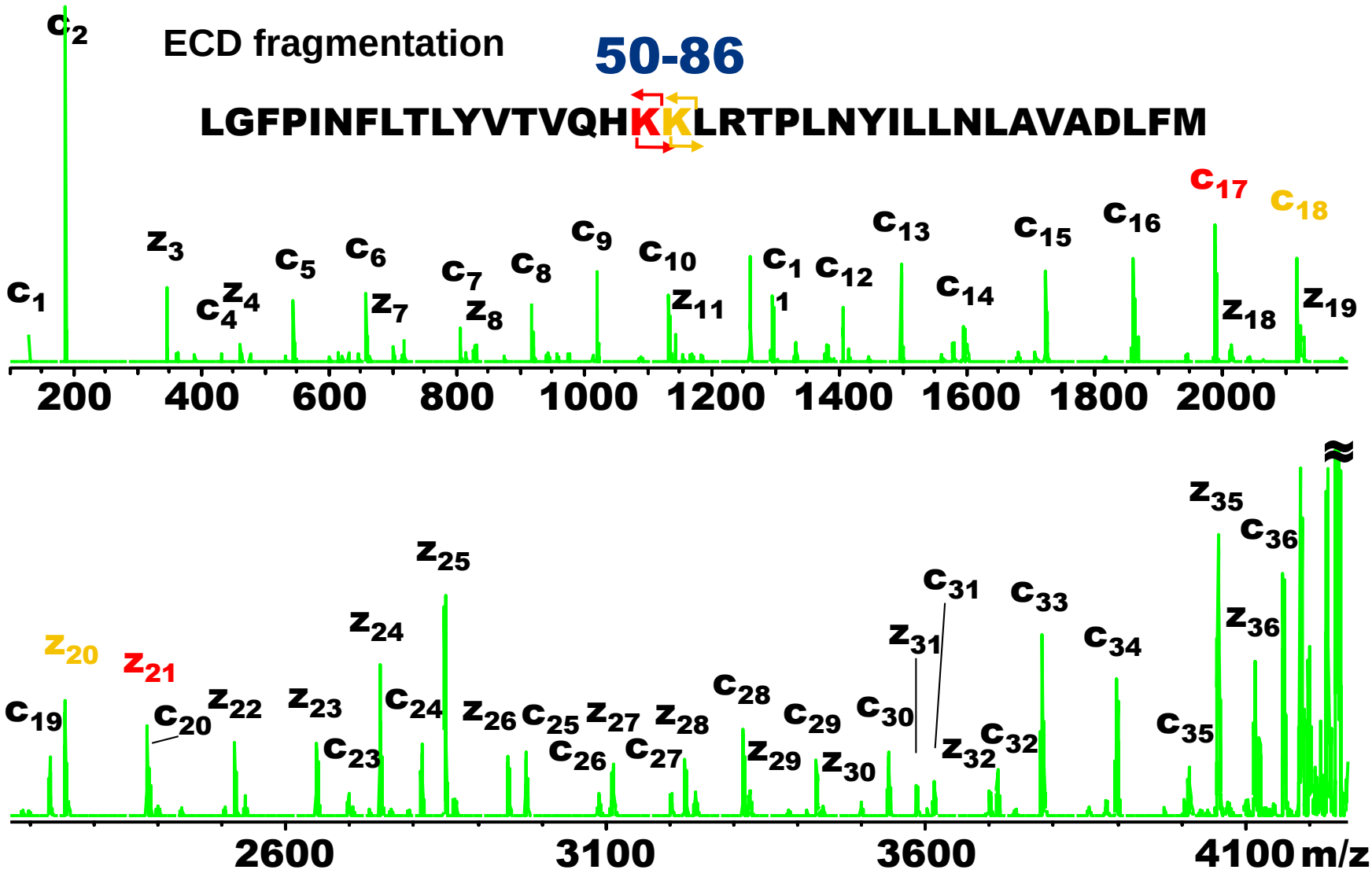


Red line corresponds to extracted ion chromatograms of selected cross-linked peptides.

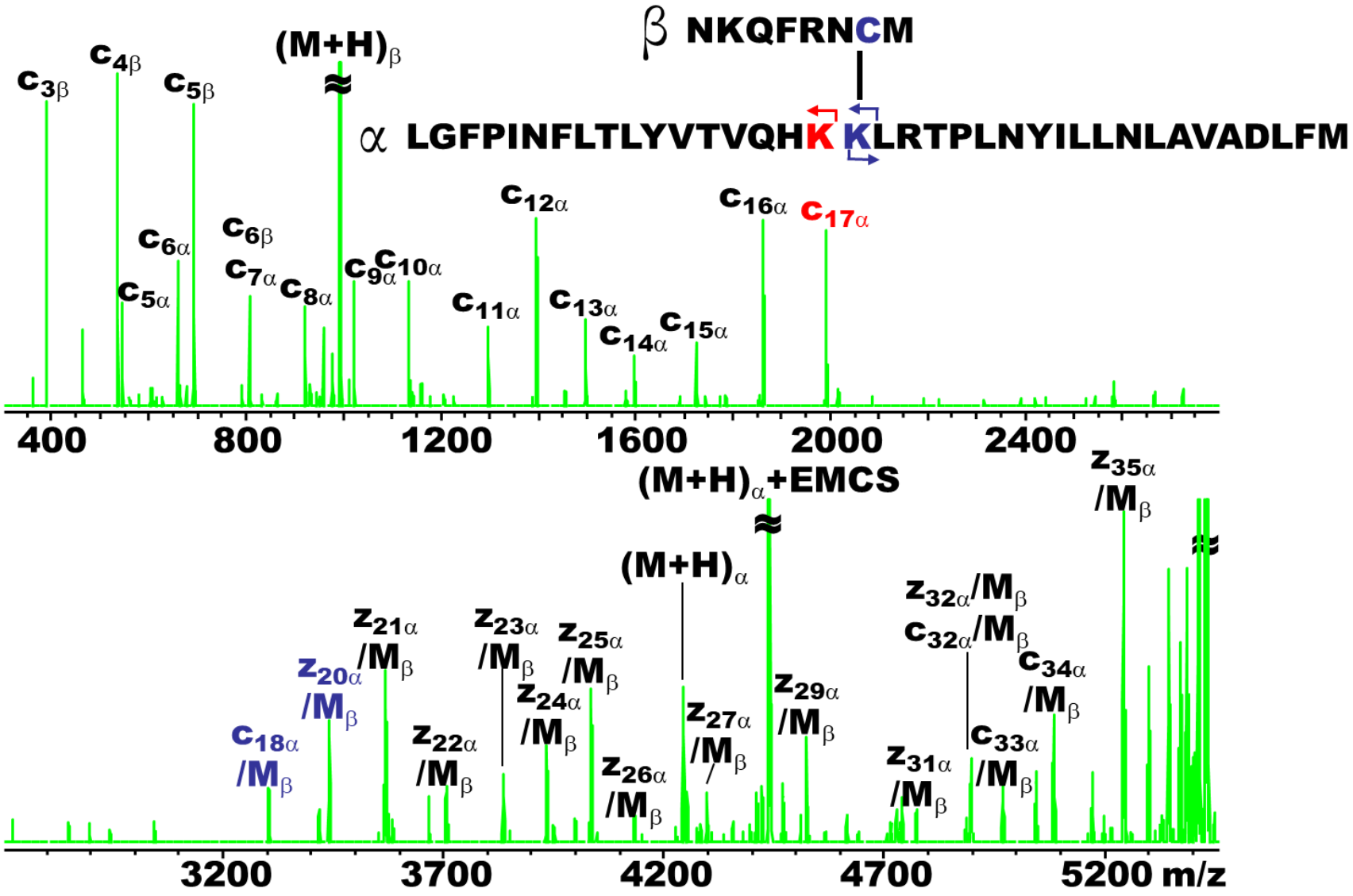
What's wrong? Too many possibilities….



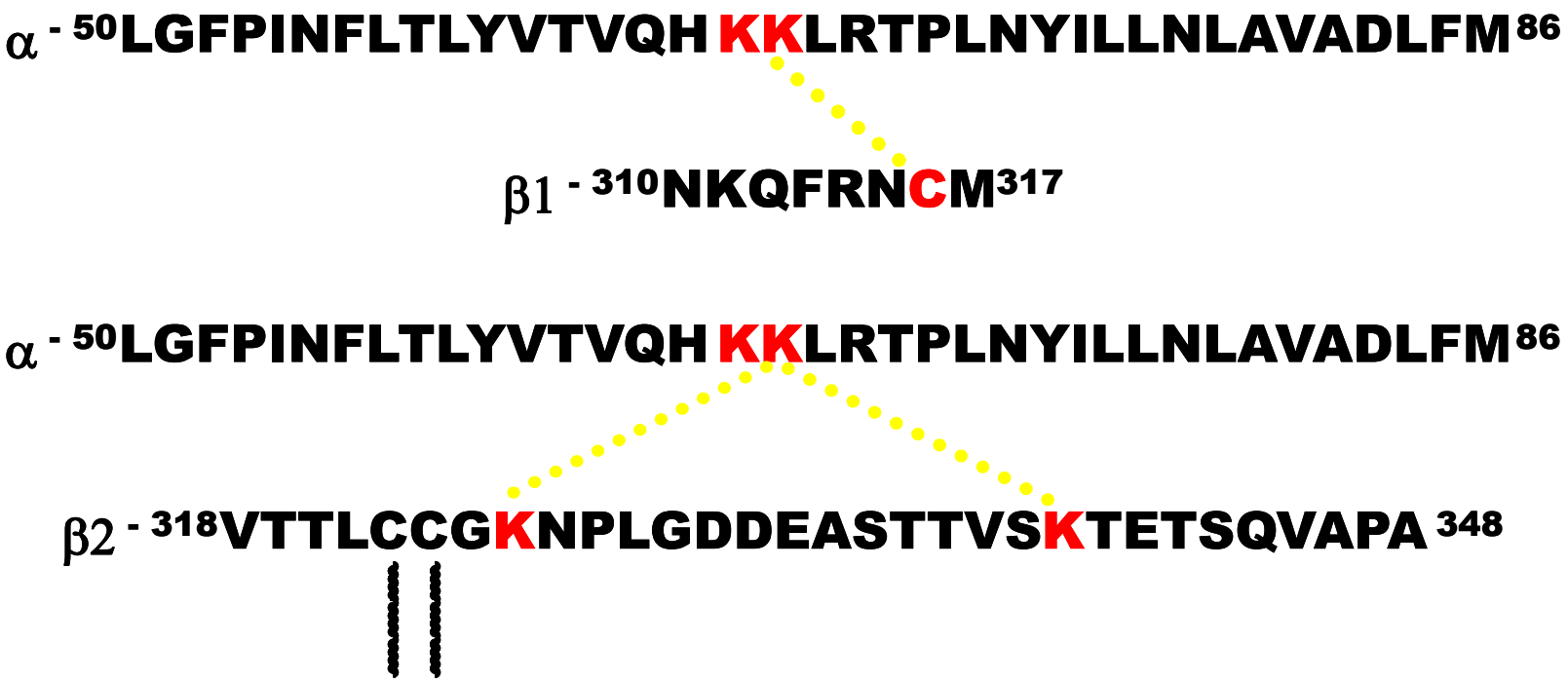
Can We Resolve the Cross-link at K66/K67?



ECD fragmentation of cross-linked peptides

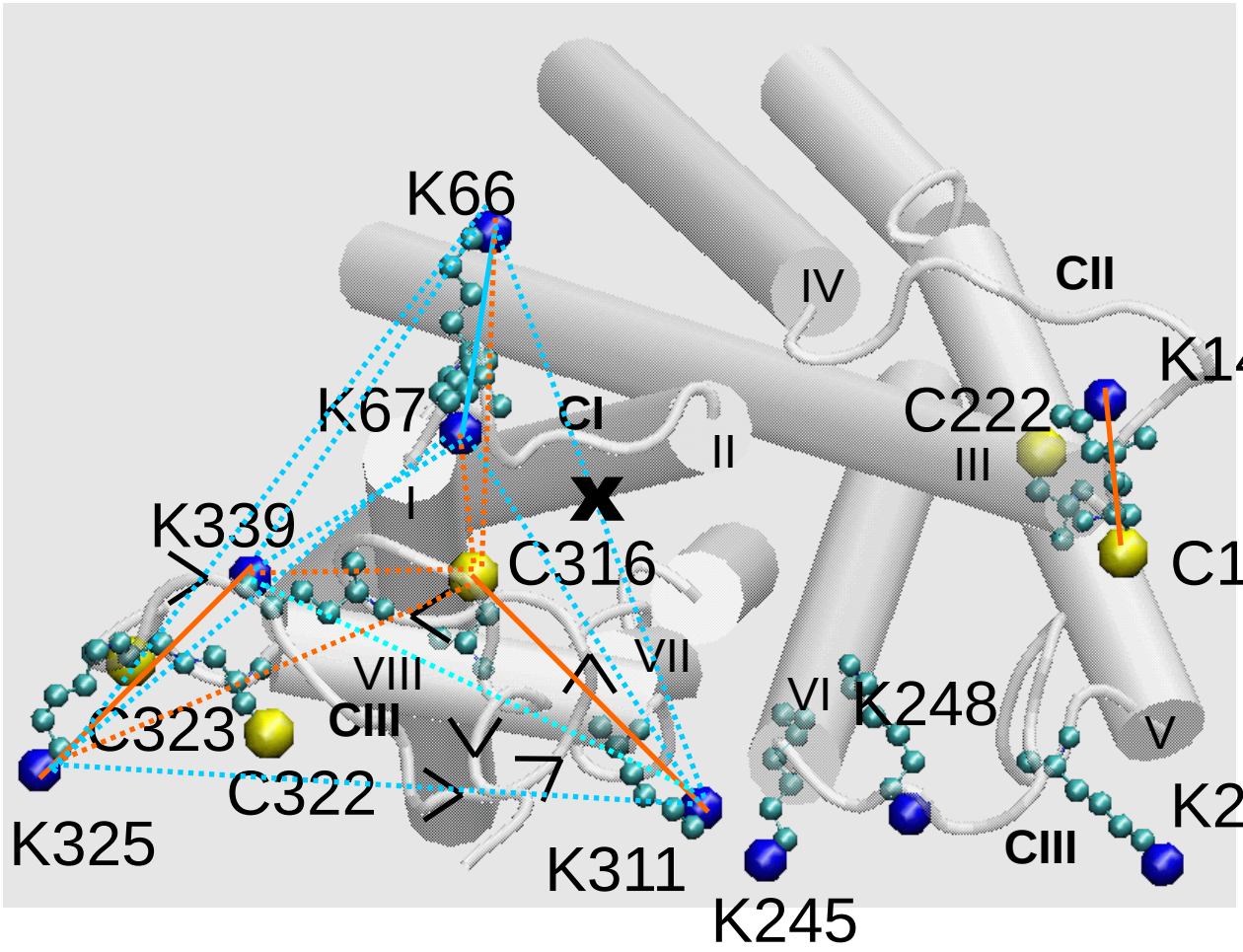


ECD enables single residue resolution!



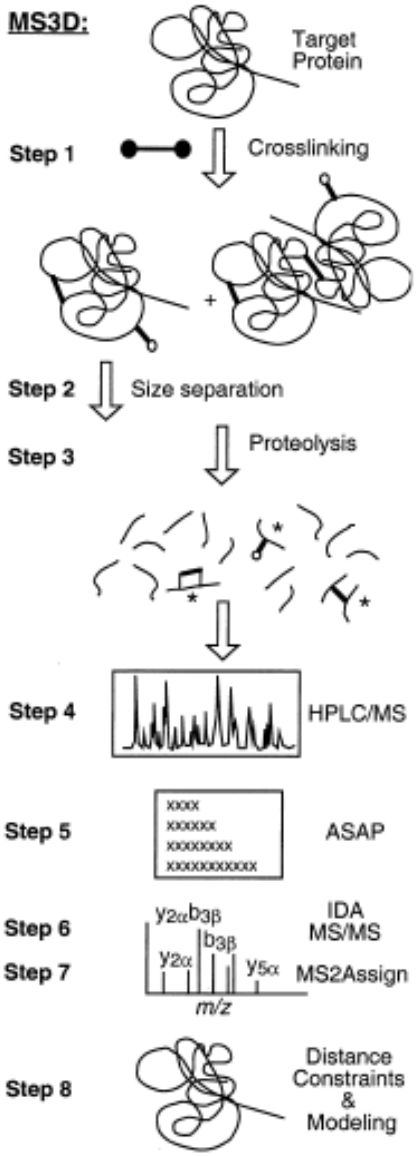
Novak P, Haskins WE, Ayson MJ, et al. Unambiguous assignment of intramolecular chemical cross-links in modified mammalian membrane proteins by Fourier transform-tandem mass spectrometry. Anal Chem. 2005;77(16):5101-5106. doi:10.1021/ac040194r

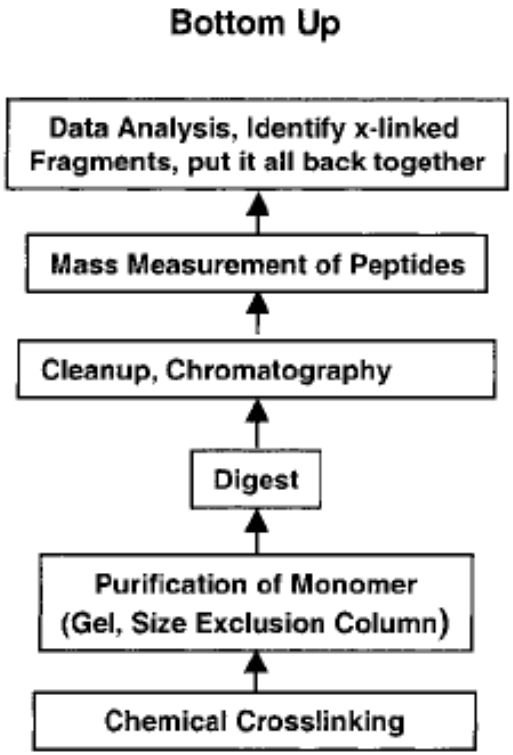
The Cytoplasmic Face of Rhodopsin

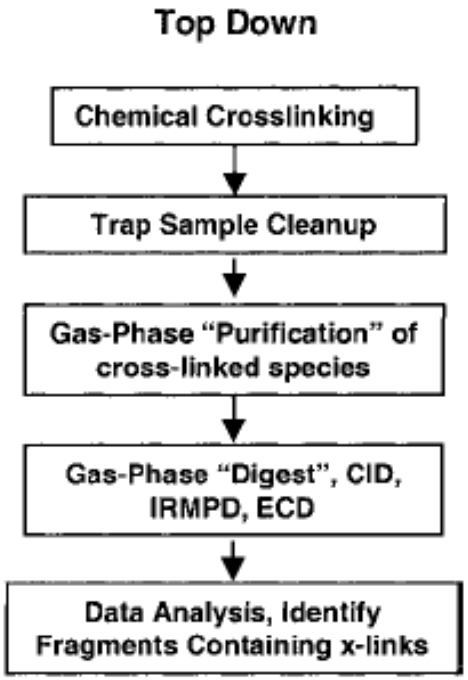


Jacobsen RB, Sale KL, Ayson MJ, et al. Structure and dynamics of dark-state bovine rhodopsin revealed by chemical cross-linking and high-resolution mass spectrometry. Protein Sci. 2006;15(6):1303-1317. doi:10.1110/ps.052040406

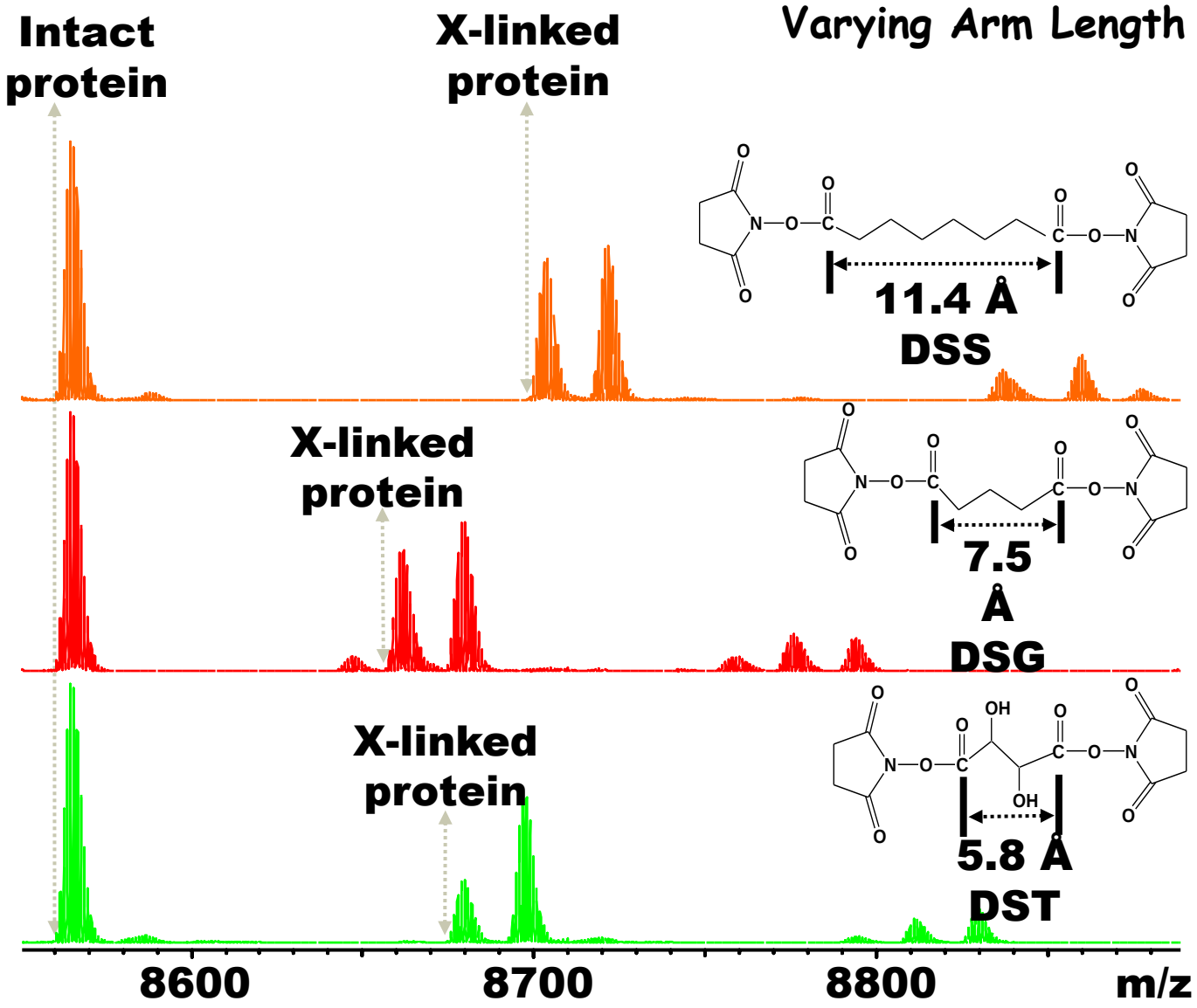
CXMS experiment: Top-down approach



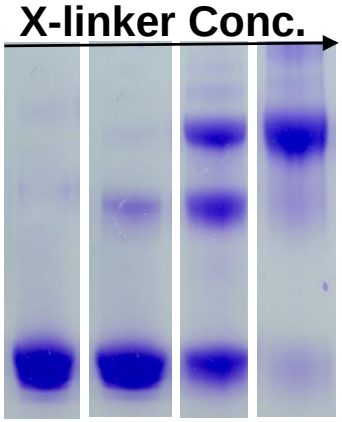


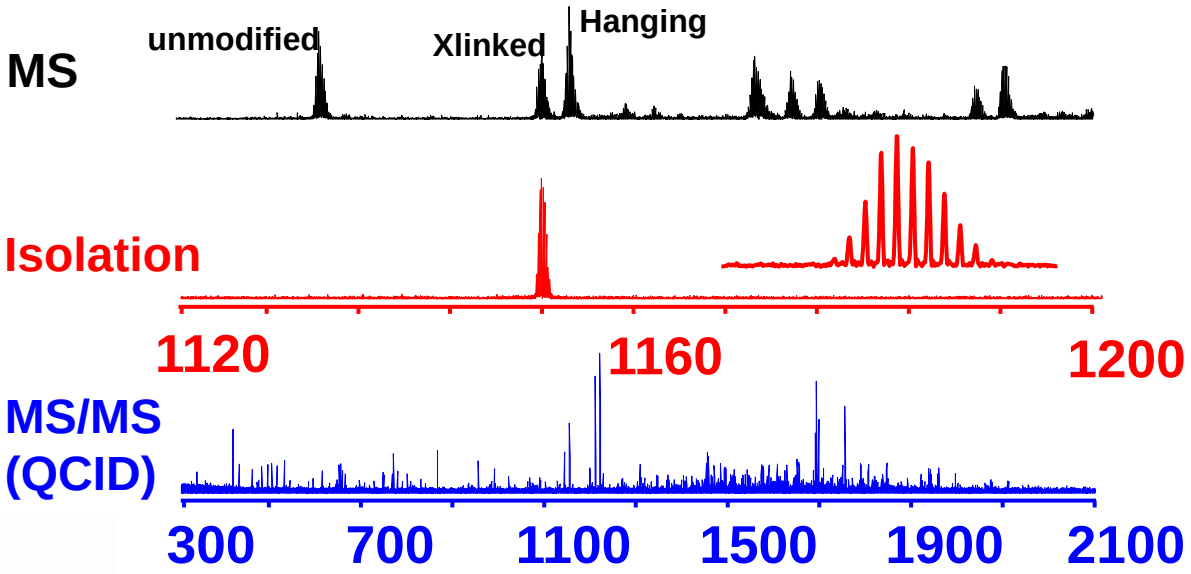


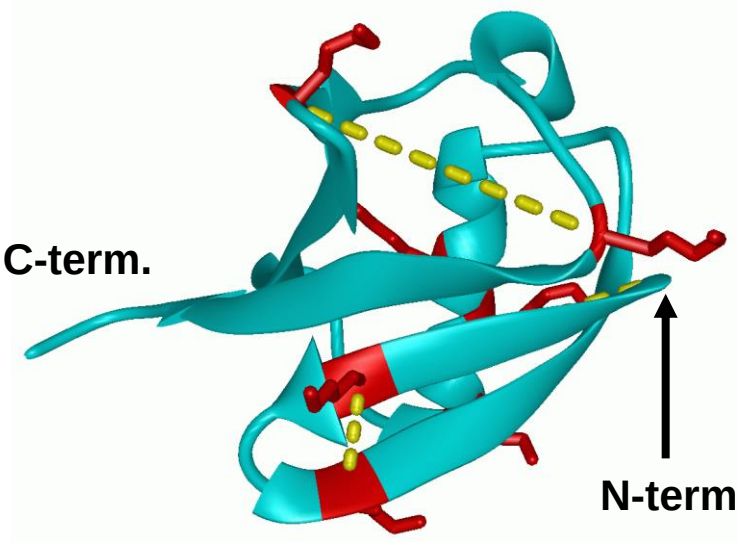
Top down: Cross-linked Ubiquitin with a Series of Cross-linkers



Chemical cross-linking: the identification of cross-link







Cross-Link	DSS	DSG	DST	Constraint (N_e-N_e)
M1 – K6	Yes	Yes	Yes	Distance < 5.8 Å
K6-K11	Yes	Yes	Yes	Distance < 5.8 Å
K48-K63	Yes	Yes	No	5.8 Å < Distance < 7.5 Å

Novak et. al. *Eur. J. Mass Spectrom. 2003, 9, 623*

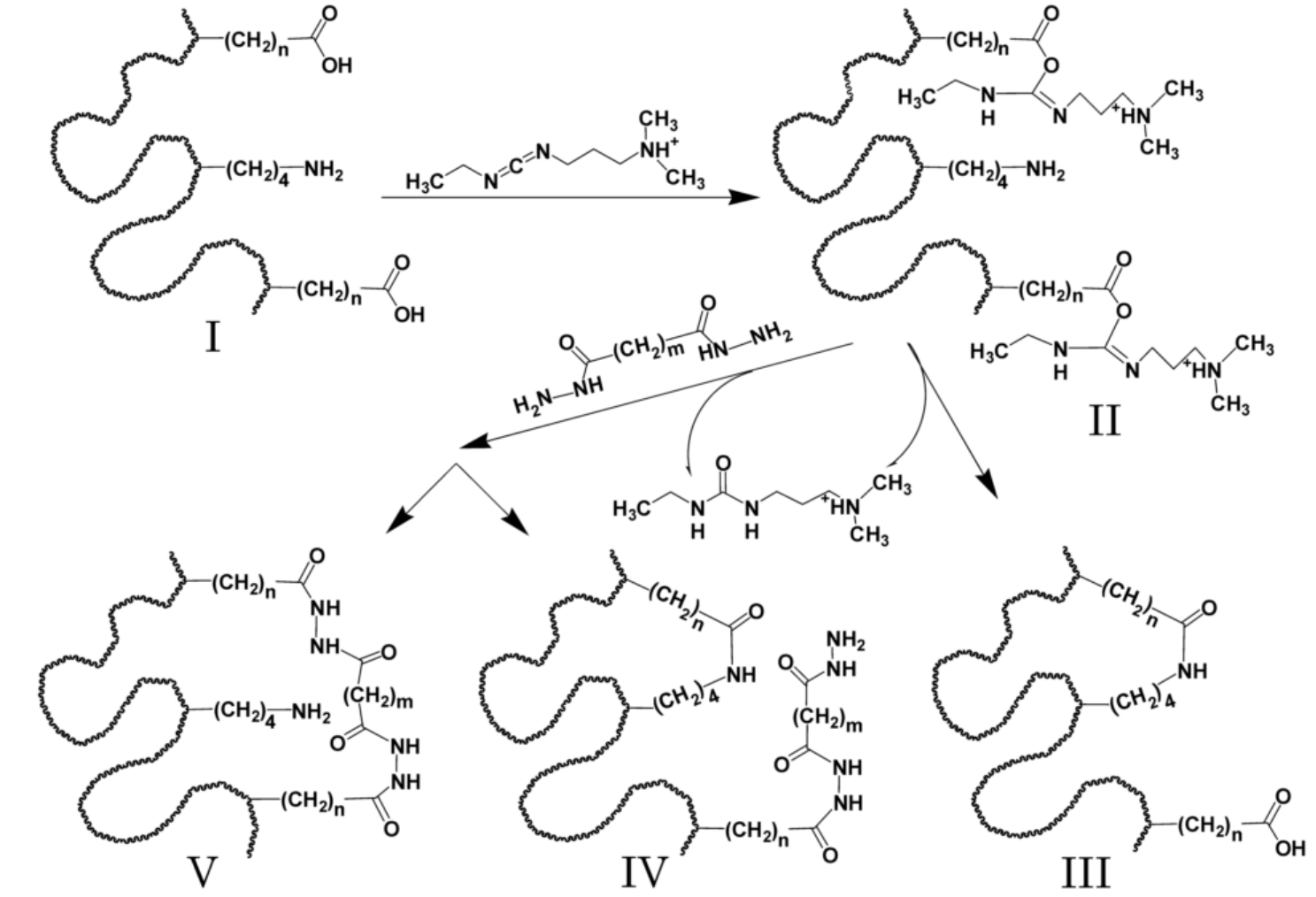
Zero-Length and Carboxy-Carboxy Cross-linking

- “Zero-Length” Cross-linking
  - **No cross-linker used.**
  - **Activate carboxylic acid groups with EDC.**
  - **Activated acid side-chains react with primary amine side-chains (DEO-XK).**
  - **Cross-link formed via new amide linkage.**

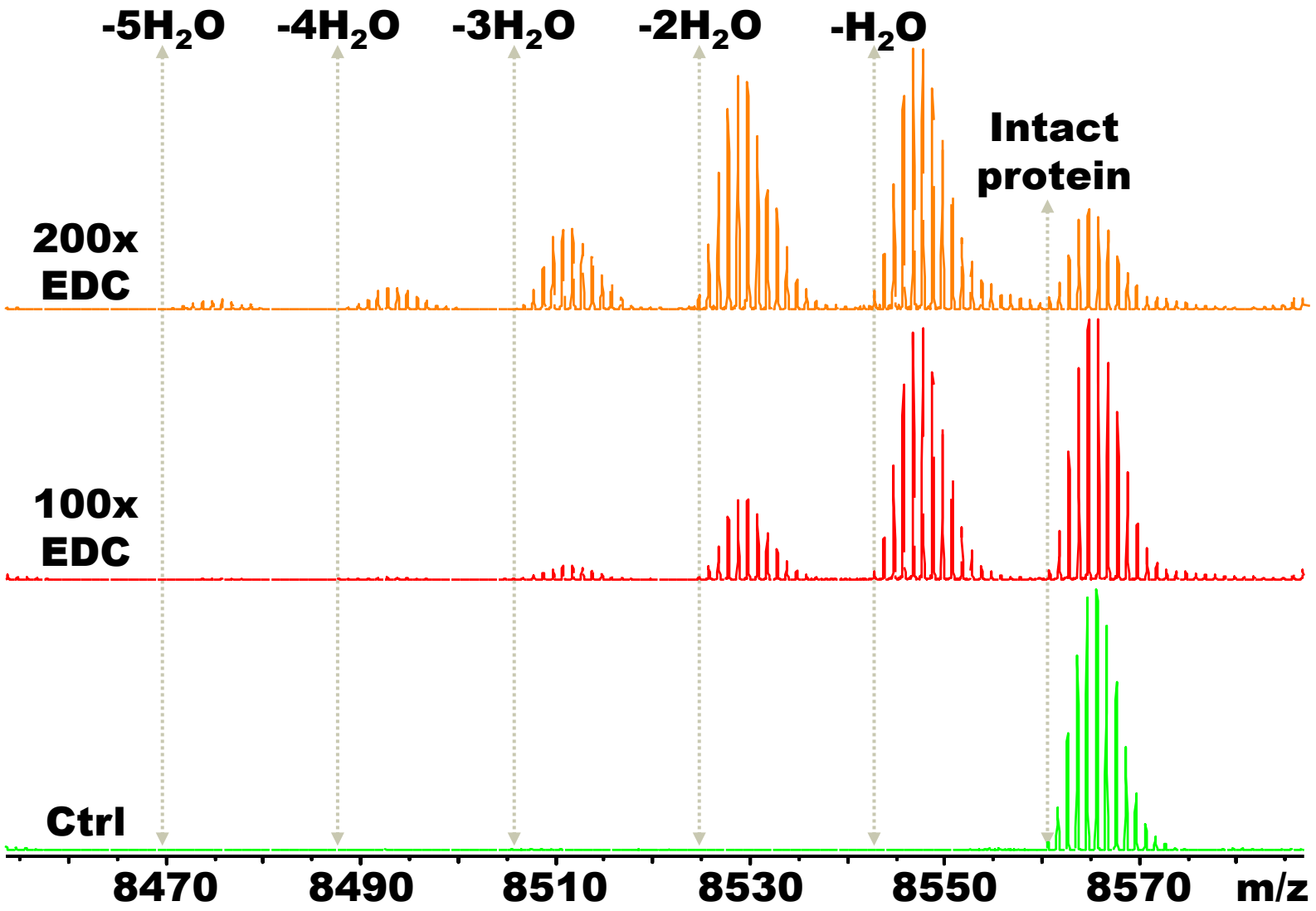
- EDC activation can also be used to cross-link acidic side-chains to each other (DEO-DEO)
  - **Use dihydrazides as the cross-linking reagent.**

Chemical cross-linking: an alternative chemistry

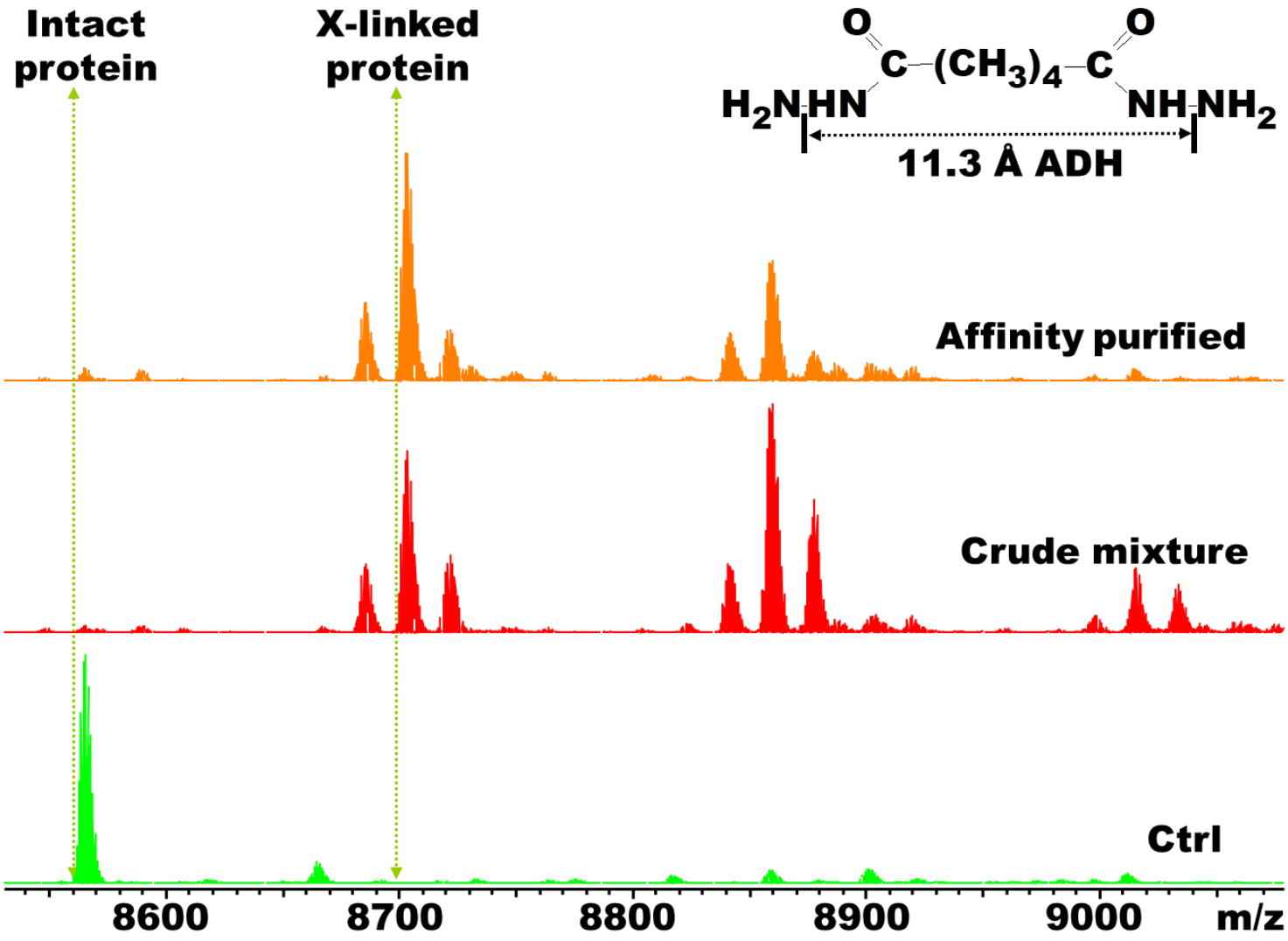
Carboxylic group reactive cross-linkers



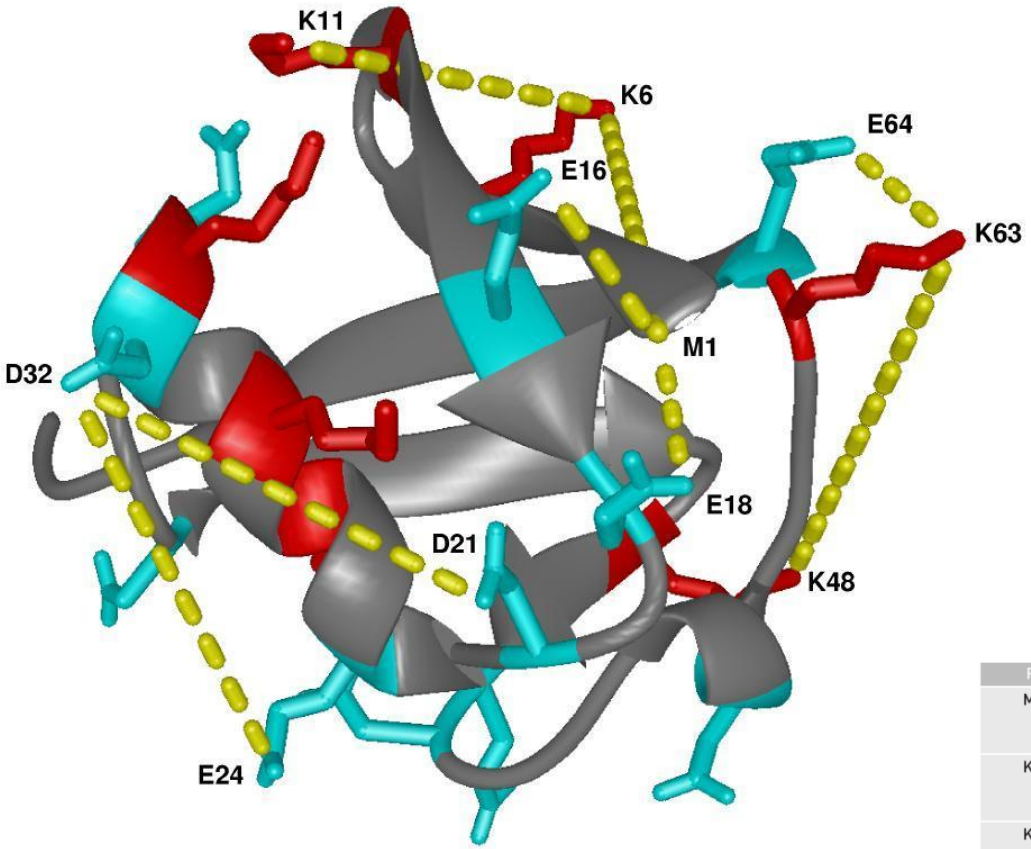
Zero-Length Cross-linking



Carboxy-Carboxy Cross-linking



Chemical cross-linking: an alternative chemistry

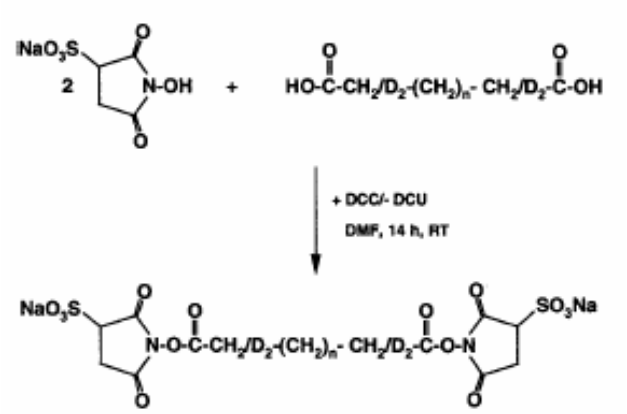


Residue	Cross-linker	Cross-link	Constraint (X-ray constraint)	Type
M1–K6	DSS	Yes	5.8 Å < Distance < 7.5 Å (20.0 Å)	N_α–N_ε
	DSG	Yes		
	DST	No		
K6–K11	DSS	Yes	Distance < 5.8 Å (14.0 Å)	N_ε–N_ε
	DSG	Yes		
	DST	Yes		
K48–K63	DSS	Yes	5.8 Å < Distance < 7.5 Å (19.8 Å)	N_ε–N_ε
	DSG	Yes		
	DST	No		
M1–E16	EDC	Yes	Distance < 1.5 Å (6.2 Å)	N_α–C_δ
M1–E18	EDC	Yes	Distance < 1.5 Å (4.4 Å)	N_α–C_δ
K63–E64	EDC	Yes	Distance < 1.5 Å (4.8 Å)	N_ε–C_δ
D21–D32	ADH	Yes	5.8 Å < Distance < 7.5 Å (12.9 Å)	C_γ–C_γ
	SDH	No		
E24–D32	ADH	Yes	5.8 Å < Distance < 7.5 Å (14.0 Å)	C_δ–C_γ
	SDH	No		

Novak et. al. *Eur. J. Mass Spectrom. 2008, 14, 355*

Introduction of isotopically labeled probes

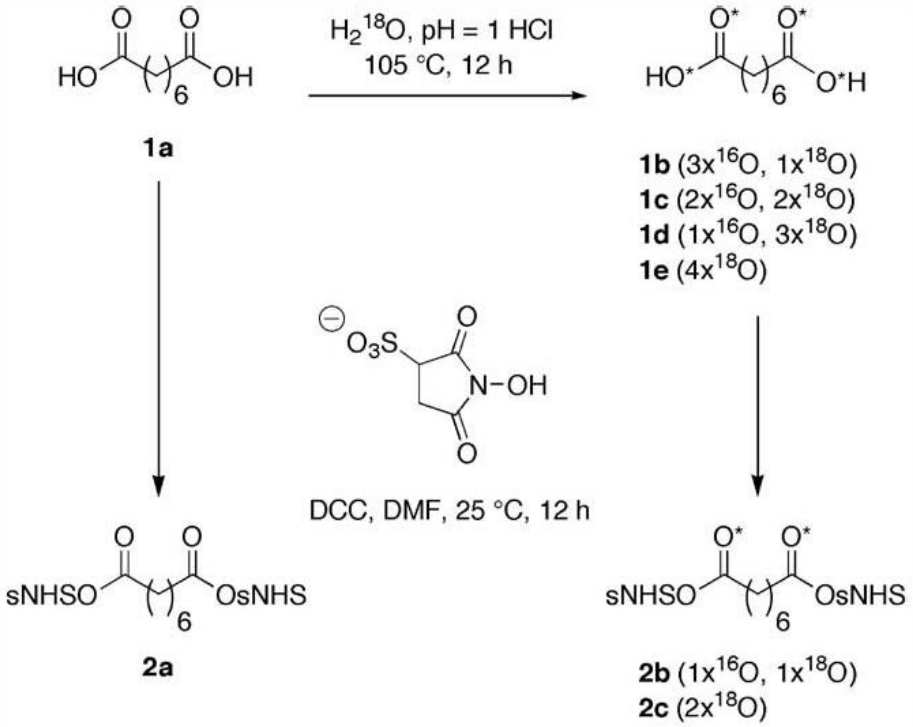
Incorporation of stable isotopes (deuterium) to the linker



^{13}C available as well

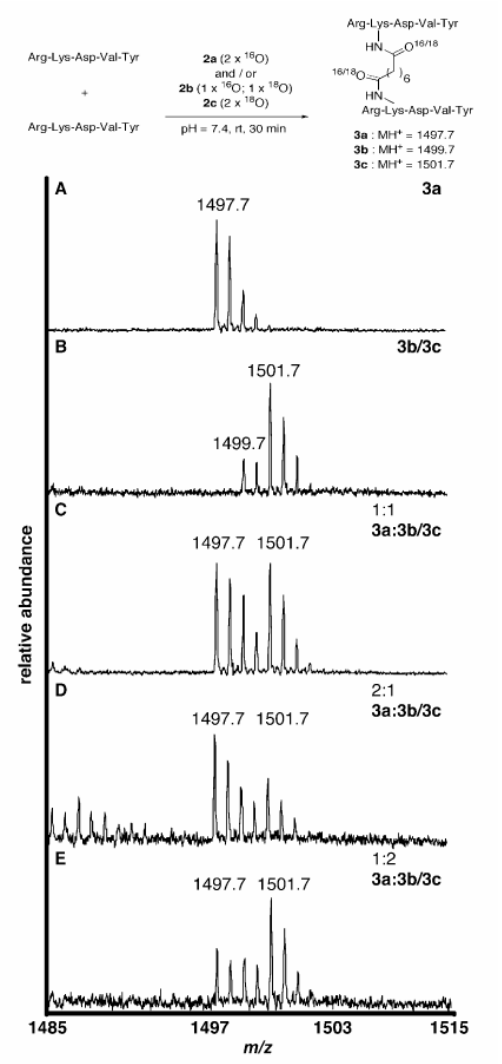
Muller DR. et al. Anal. Chem. 2001

Incorporation of stable isotopes (oxygen, ^{18}O) to the linker



Collins CJ. et al. Bioorg. Med. Chem. Lett. 2003

Introduction of isotopically labeled probes



Simplifies data analysis

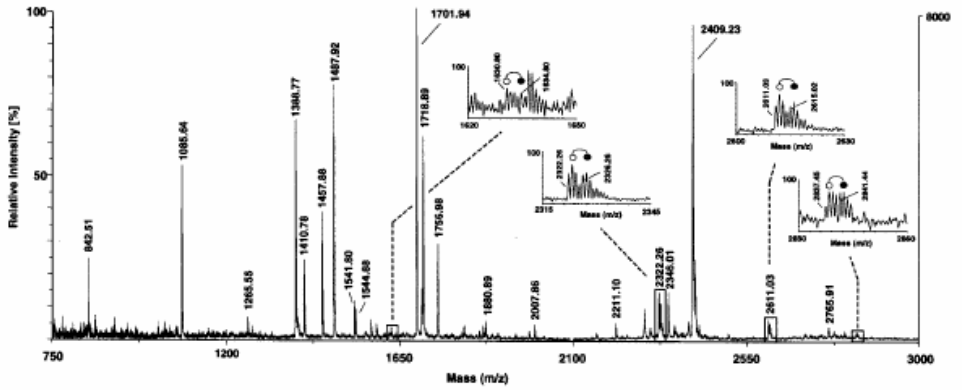
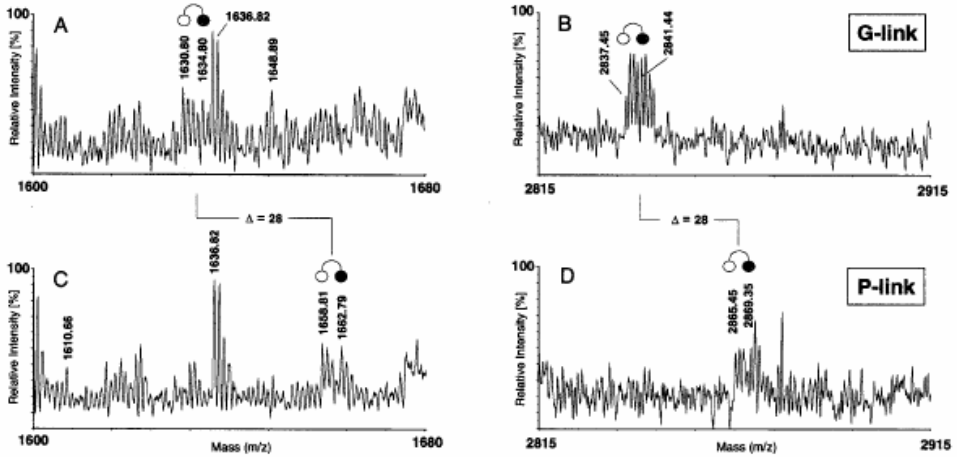


Figure 4. MALDI mass map obtained from G-linked Op18–tubulin complexes with doublet regions expanded in insets.

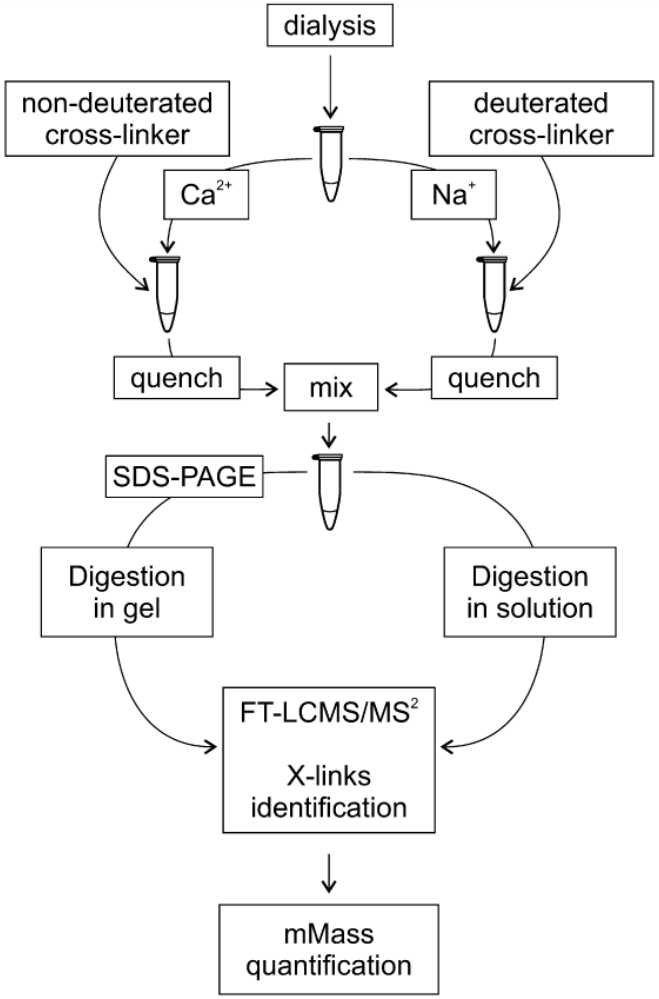


Collins CJ. et al. Bioorg. Med. Chem. Lett. 2003

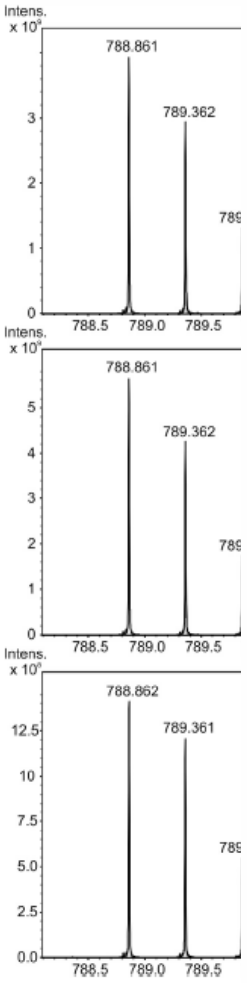
Muller DR. et al. Anal. Chem. 2001

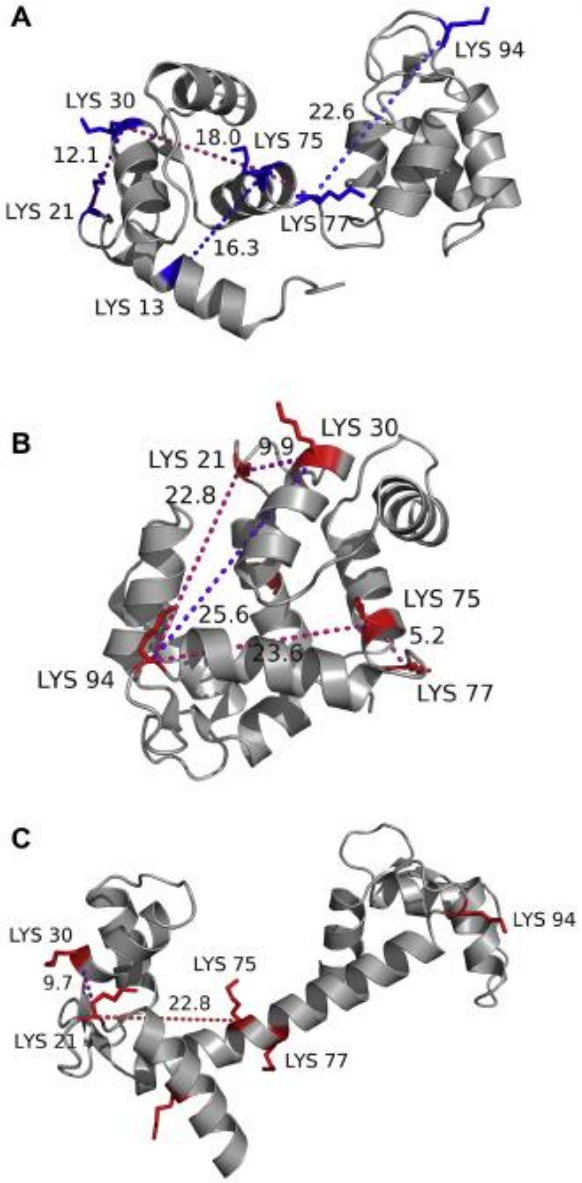
Quantitative chemical cross-linking

Protein dynamics and conforma



Kukacka Z. et al. Methods 2015





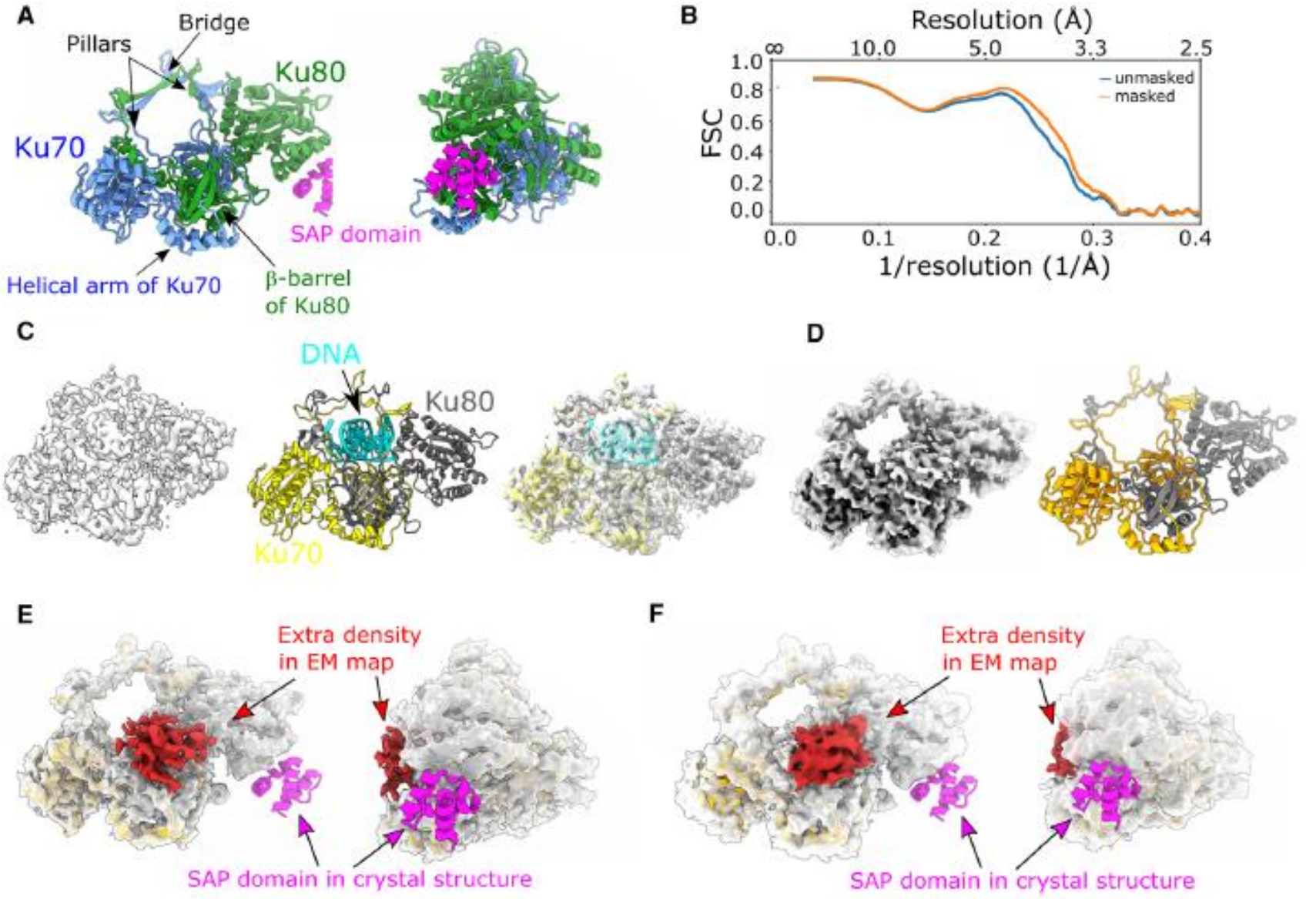
er

Na+

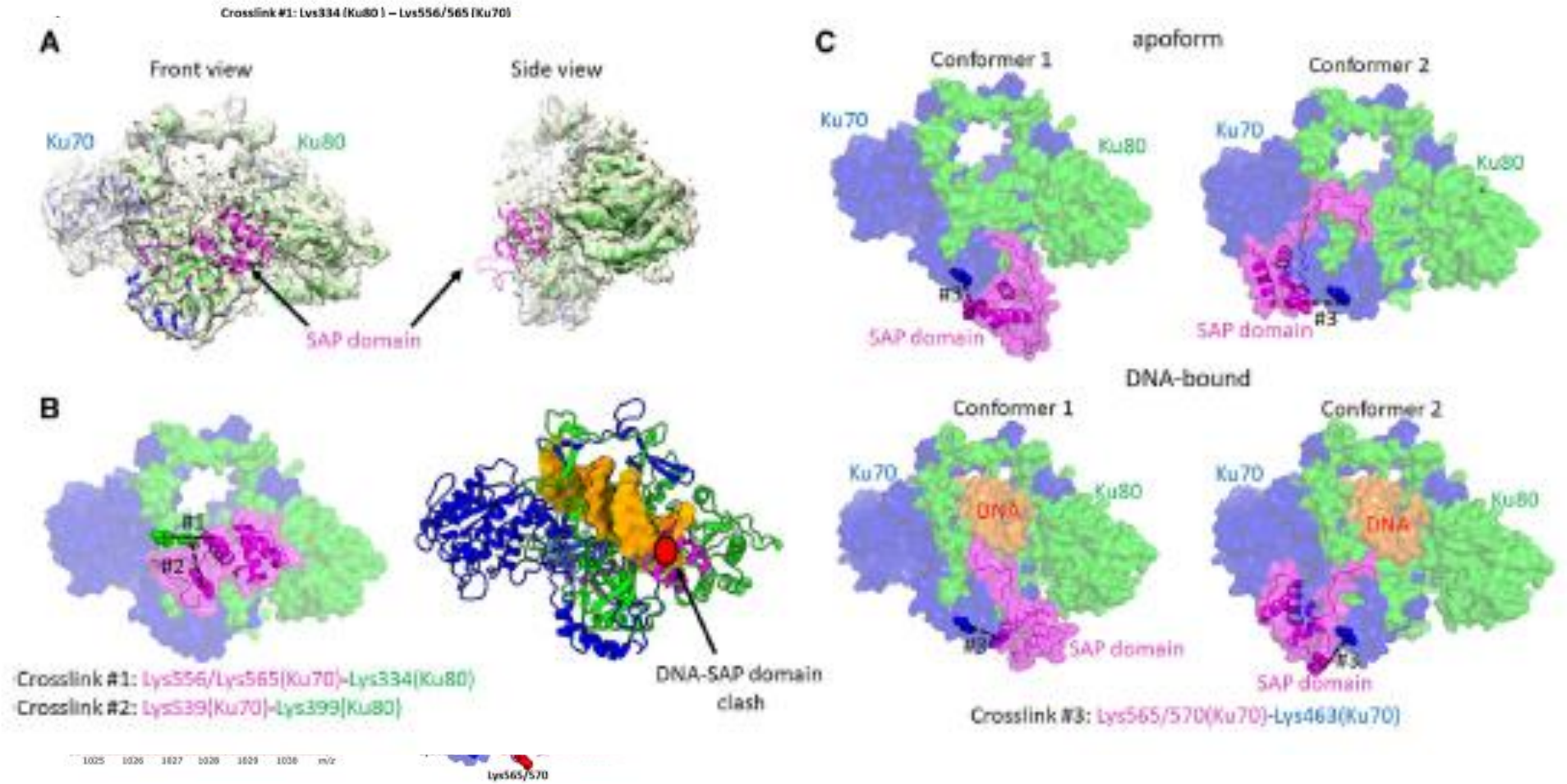
ers

er

Combination of cryo-EM and chemical cross-linking



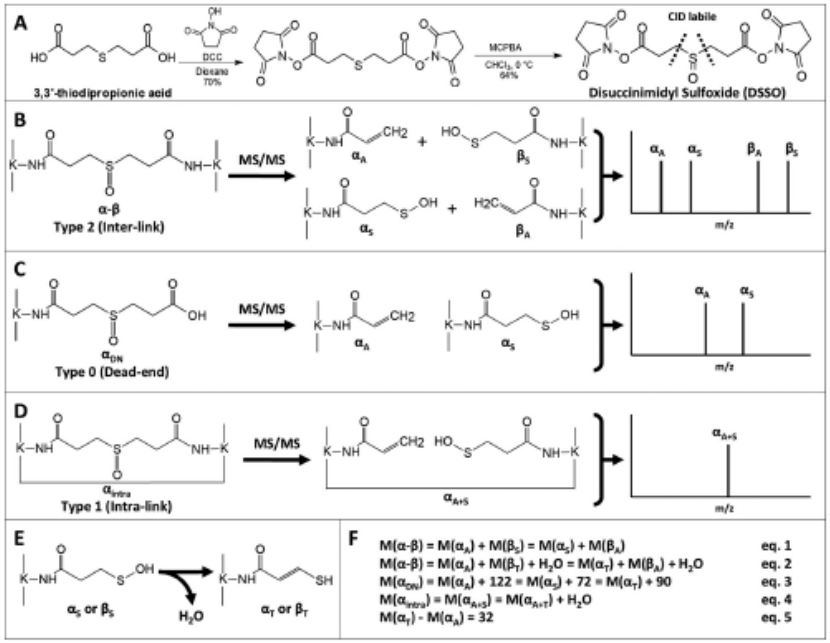
Ku70/Ku80 with and without DNA complexes



Hnízda A, Tesina P, Nguyen TB, Kukačka Z, Kater L, Chaplin AK, Beckmann R, Ascher DB, Novák P, **Blundell TL.** SAP domain forms a flexible part of DNA aperture in Ku70/80. FEBS J. 2021 Jul;288(14):4382-4393. doi: 10.1111/febs.15732.

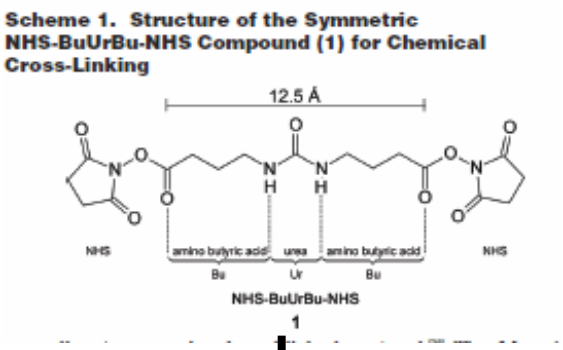
New generation of cleavable cross-linkers

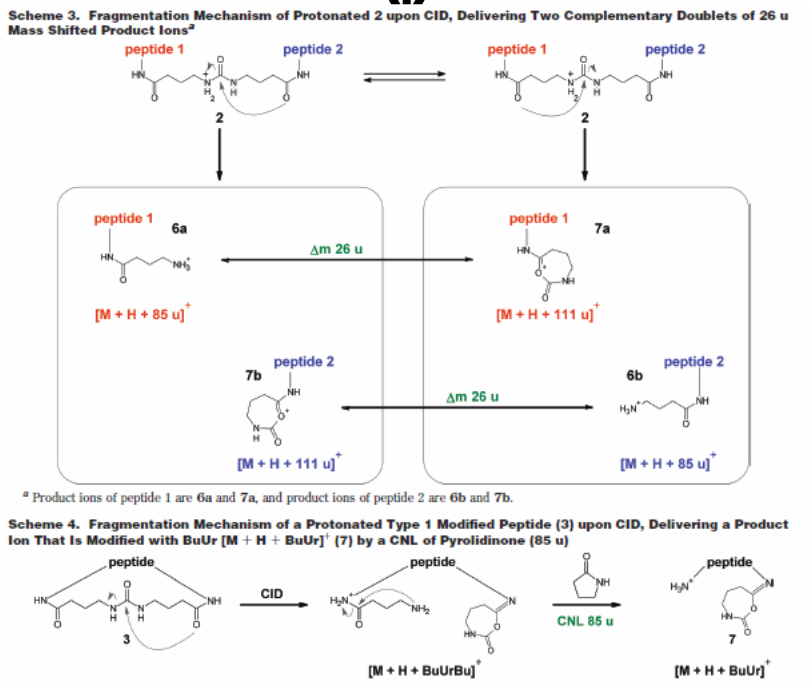
DSSO



Kao MQ. et al. Mol. Cell Prot. 2010

DSBu





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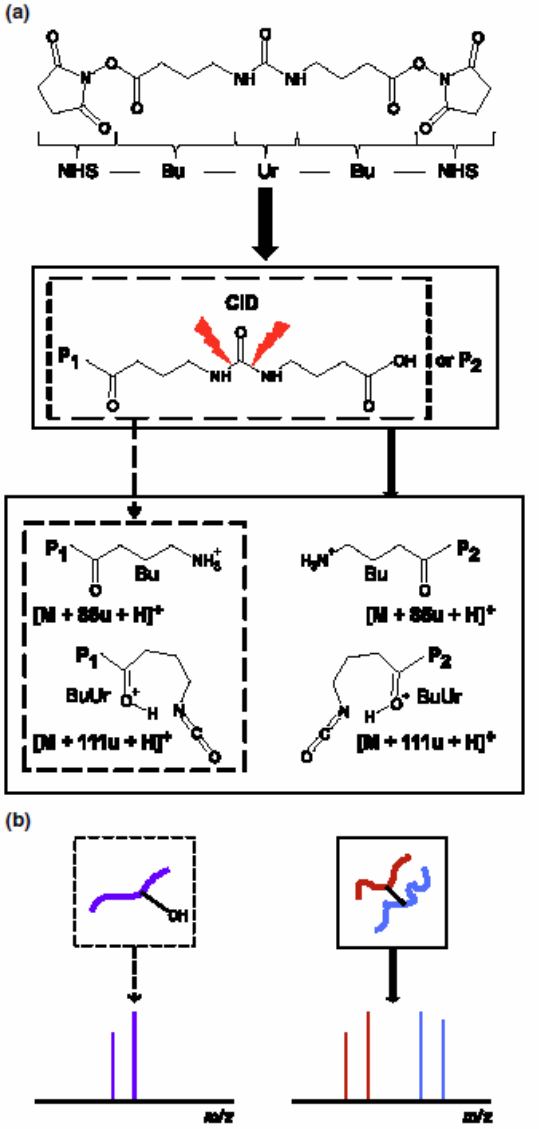
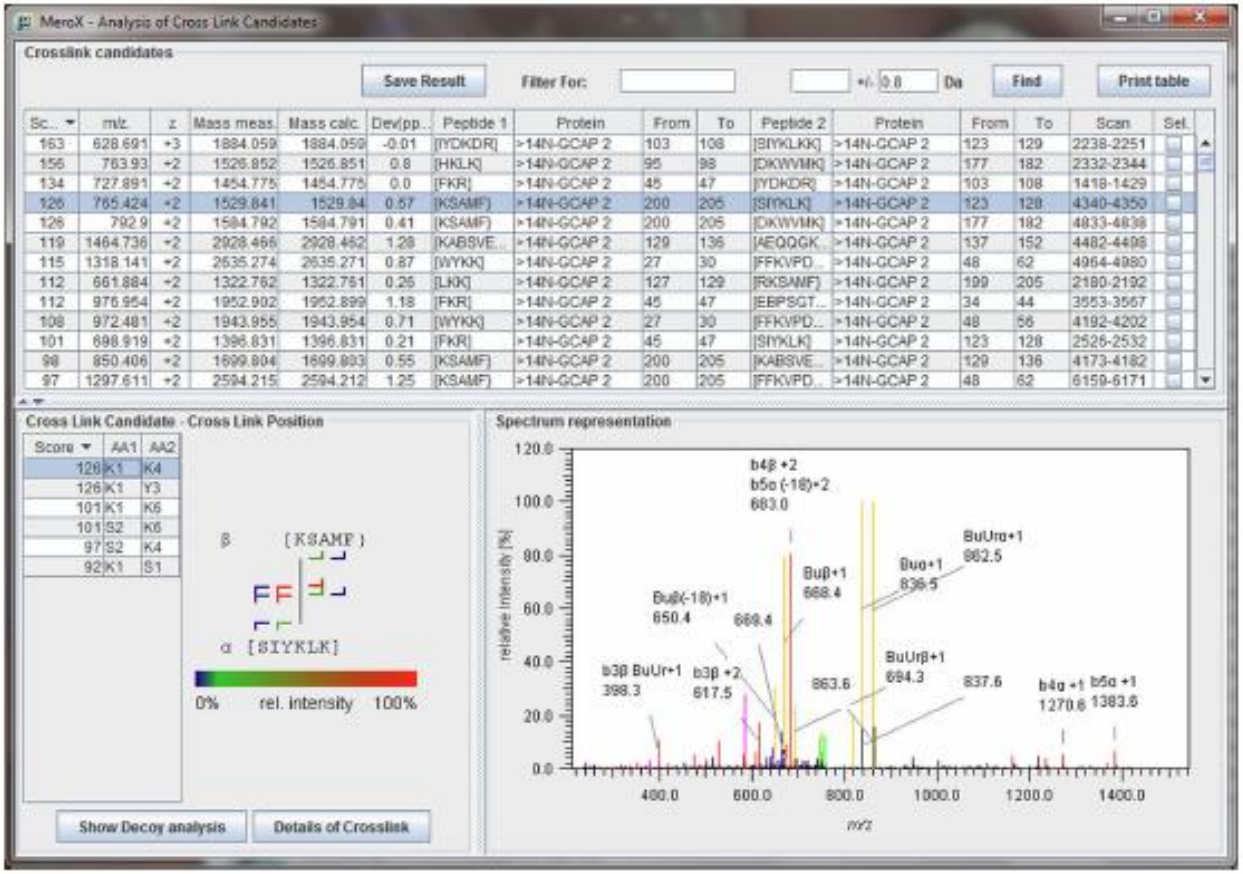
Data analysis: MEROX





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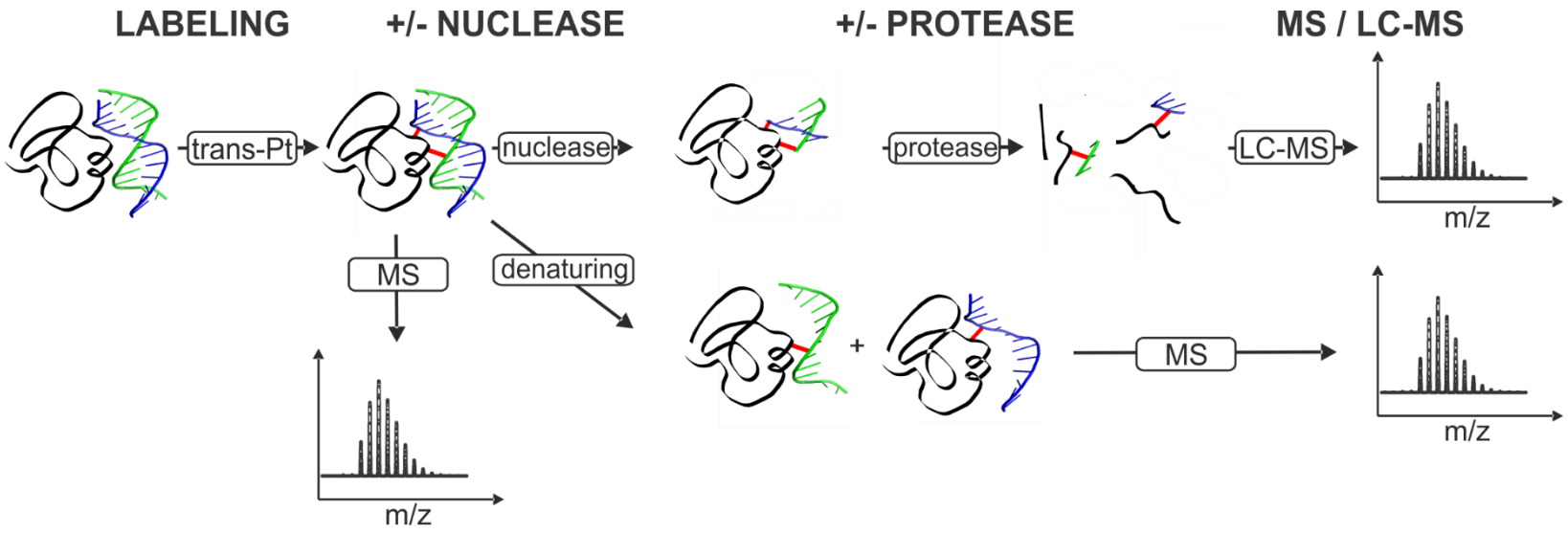
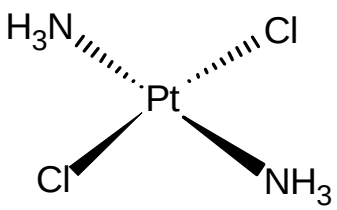
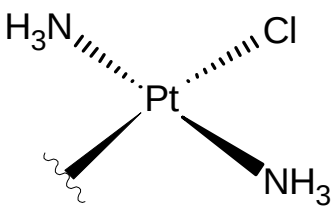
Protein-nucleic acid cross-linking

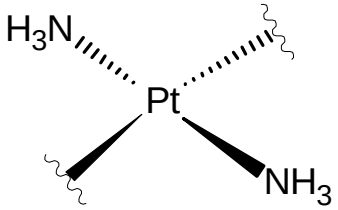




Trans-platinum



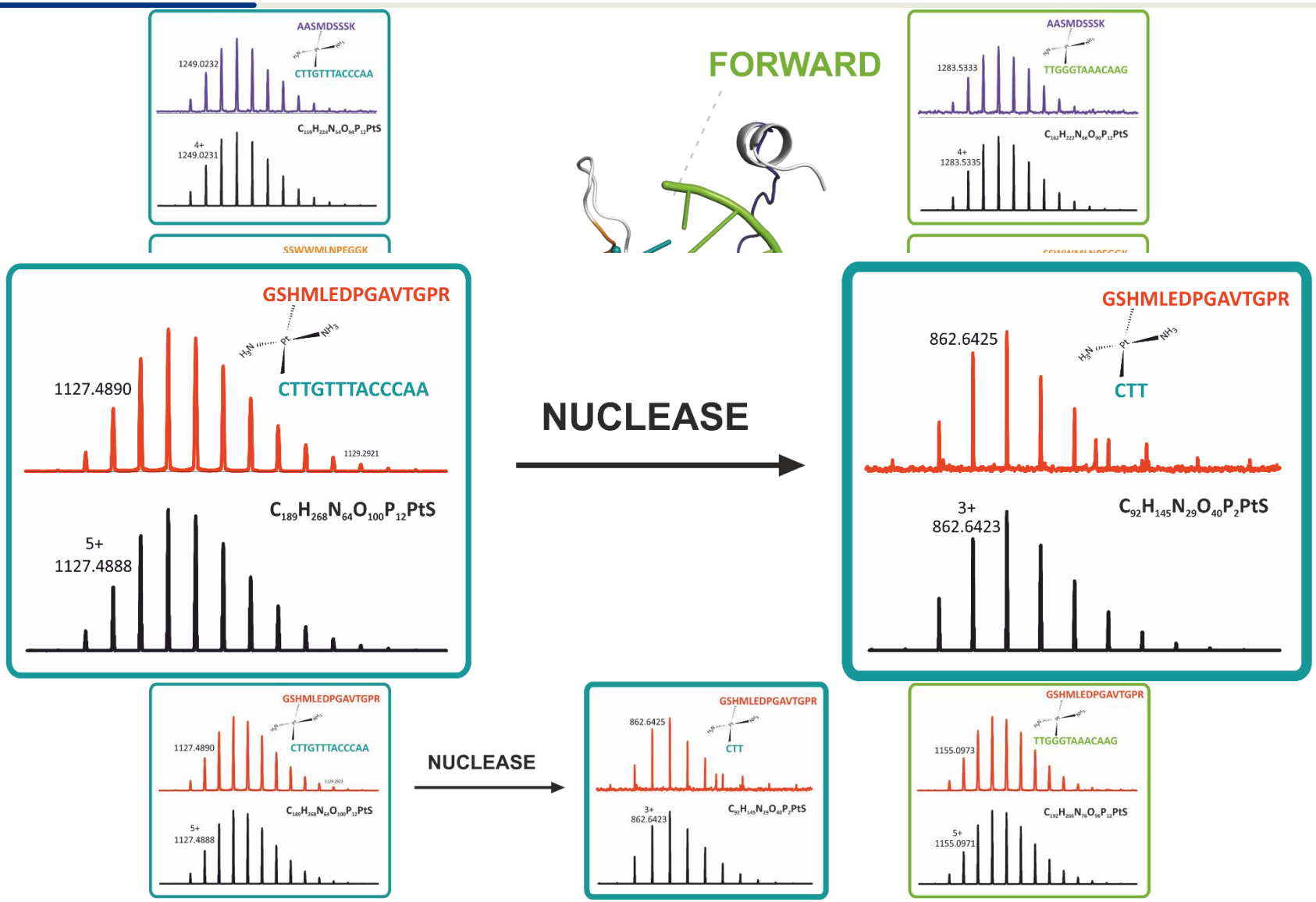
Trans-platinum (monofunctional)



Trans-platinum (bifunctional)

Slavata, L. et al Biomolecules 2019, 9(10), 535

Protein – DNA cross-links



Kukačka Z et al J Proteome Res. 2021 Apr 2;20(4):2021-2027

Thanks for your attention!

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