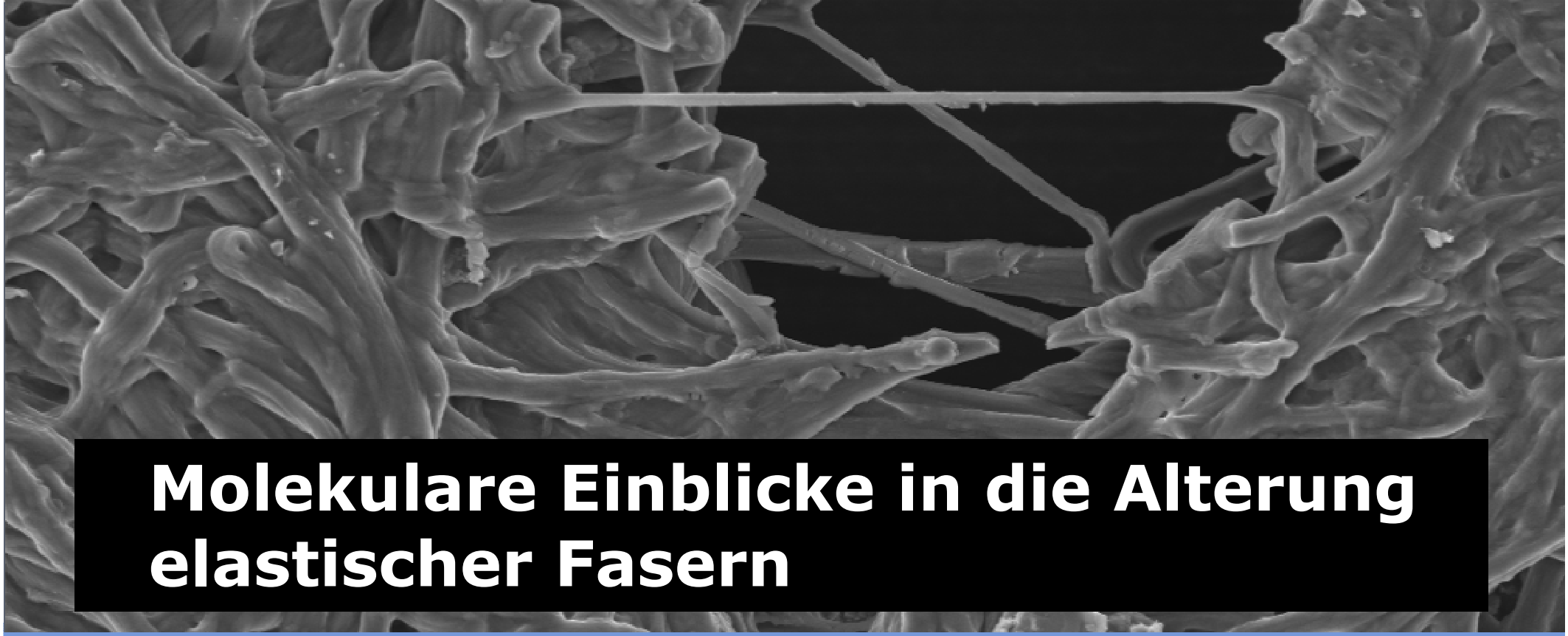


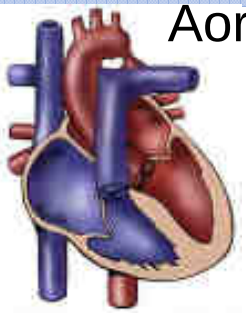
Molekulare Einblicke in die Alterung elastischer Fasern

www.elastin-forschung.de

Christian Schmelzer

Martin Luther University
Halle-Wittenberg

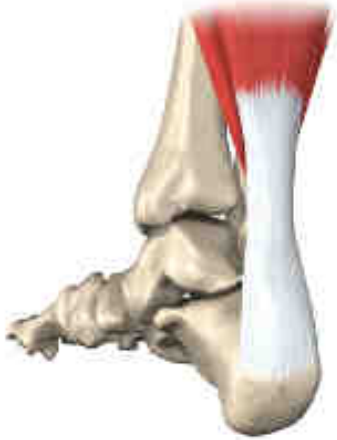
Aorta



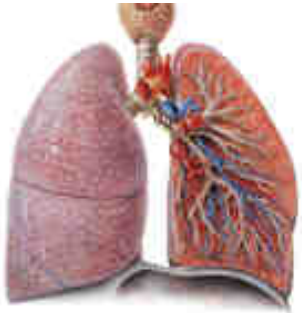
Skin

Intervertebral disc

Elastic Fibers



Tendon

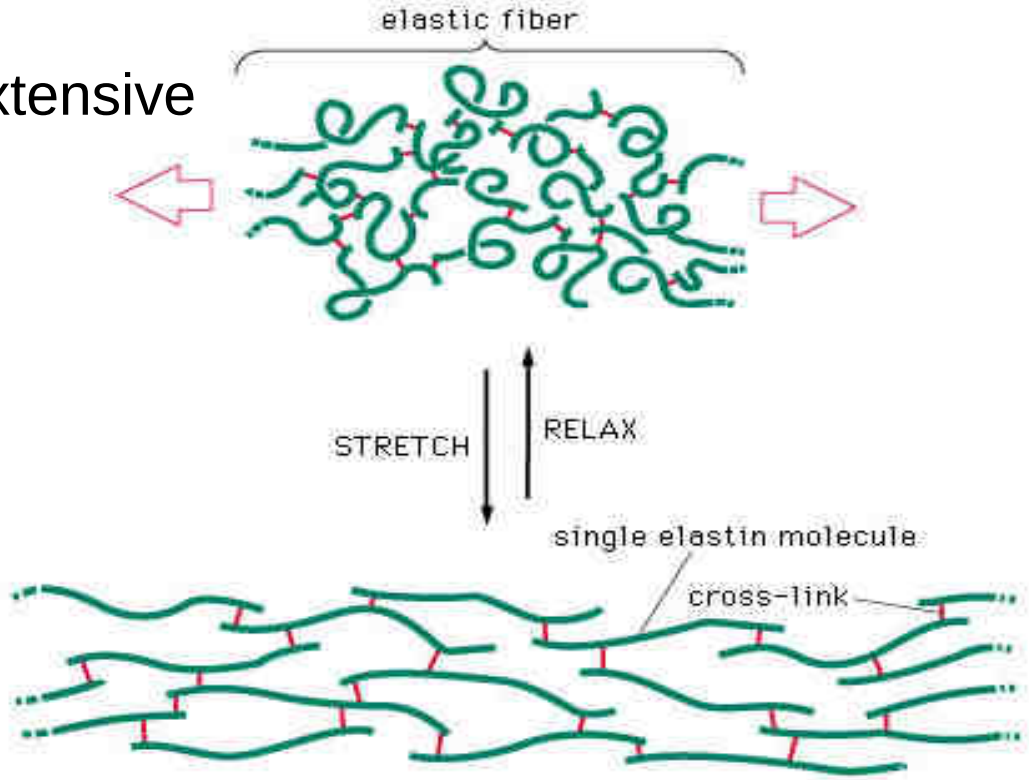


Lung

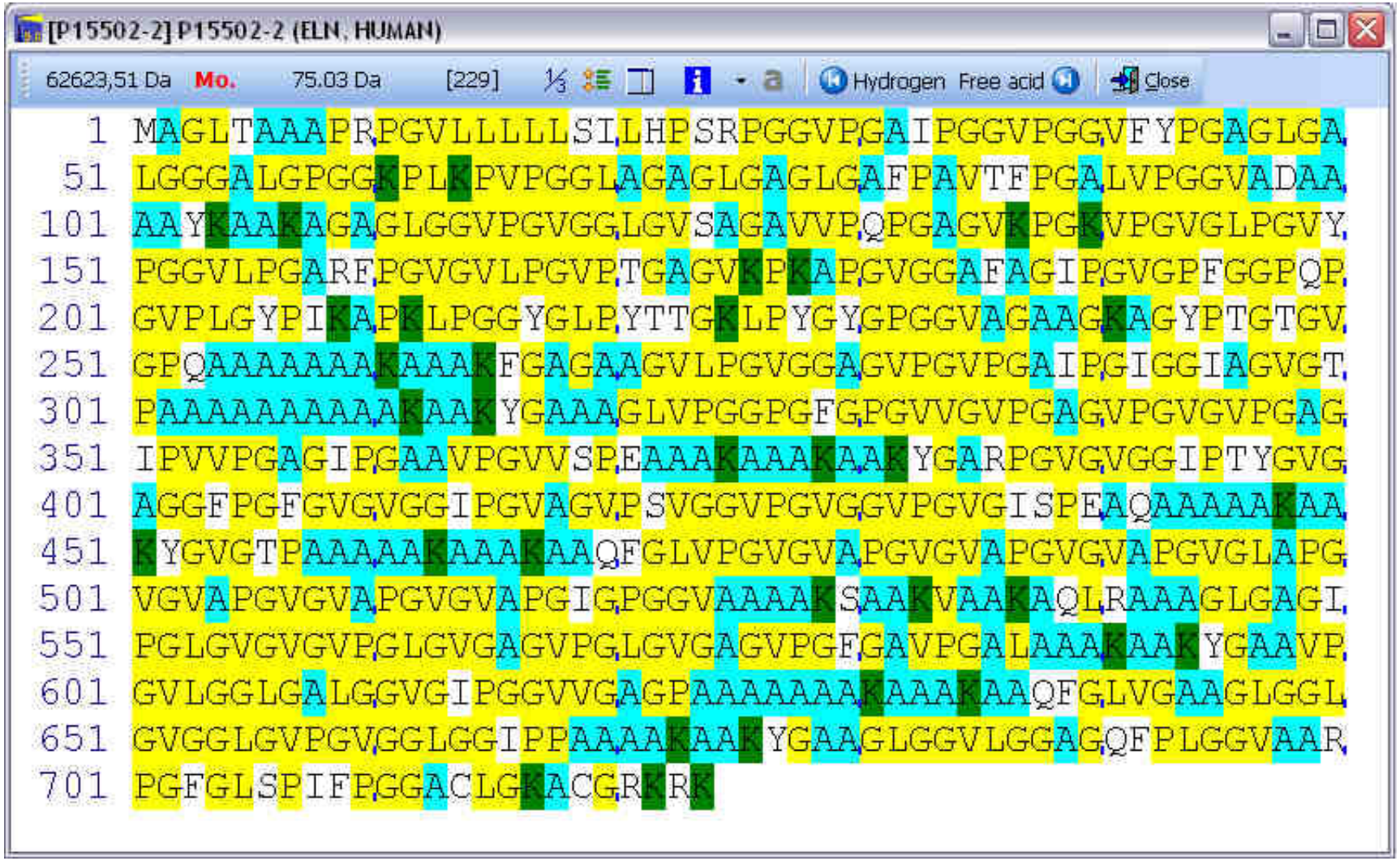
Blood vessels

Core protein of elastic fibers → Elastin

- Very hydrophobic and highly cross-linked fiber protein, insoluble in any solvent
- Soluble precursor: tropoelastin (60 kDa – 70 kDa), undergoes extensive alternative splicing
- High reversible extensibility
- Half-life: ~70 years



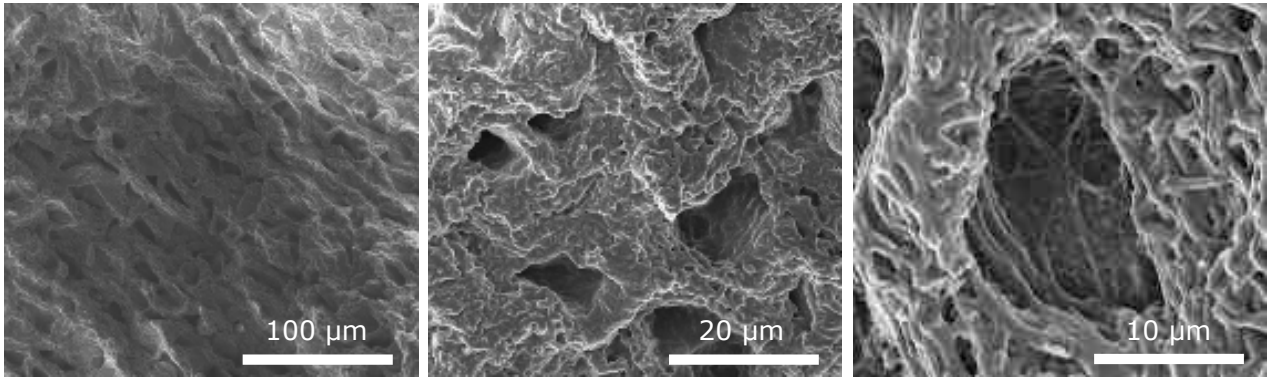
Tropoelastin



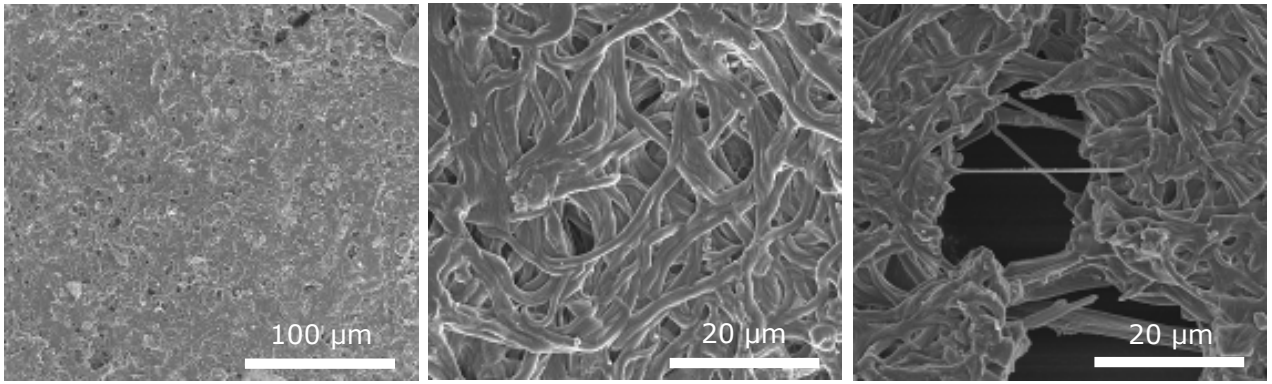
A GPVL K

Structure of elastic fibers

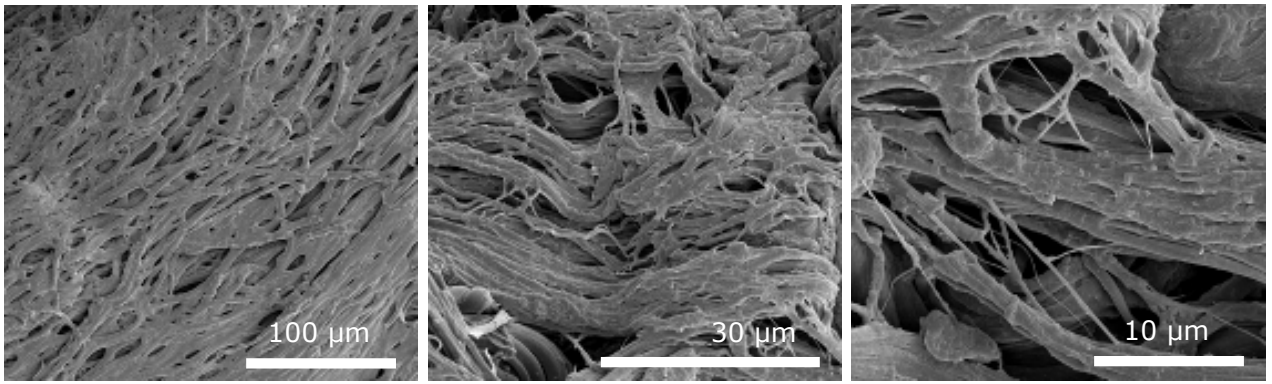
Cartilage



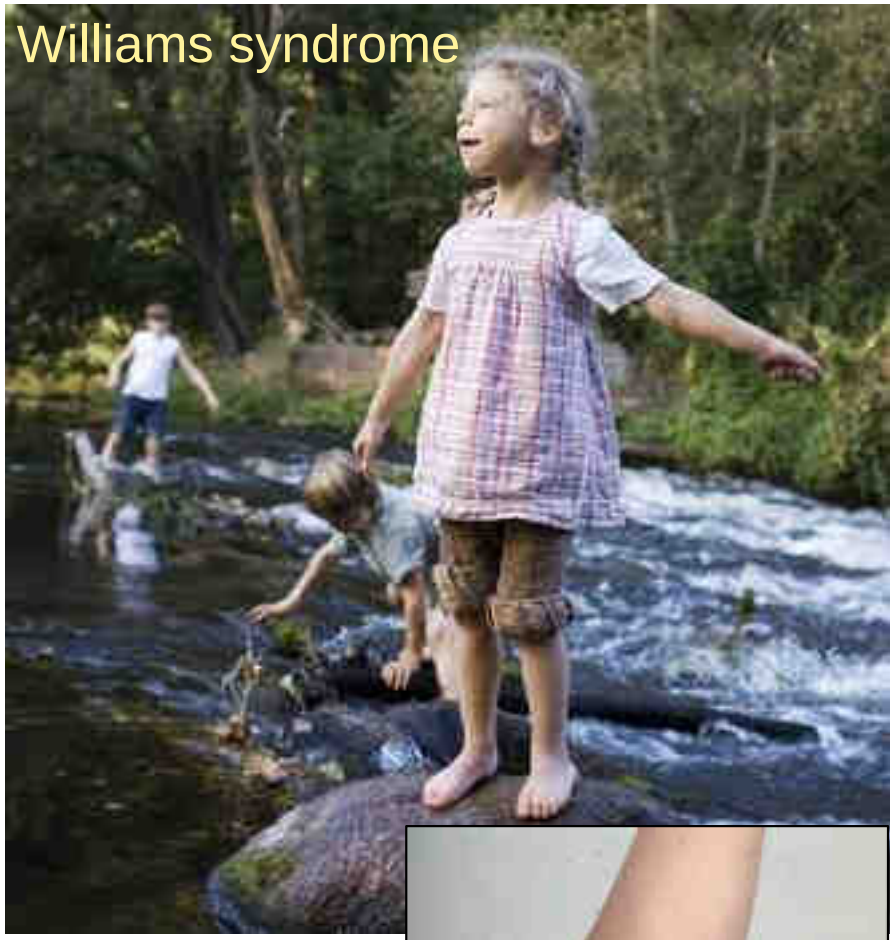
Skin

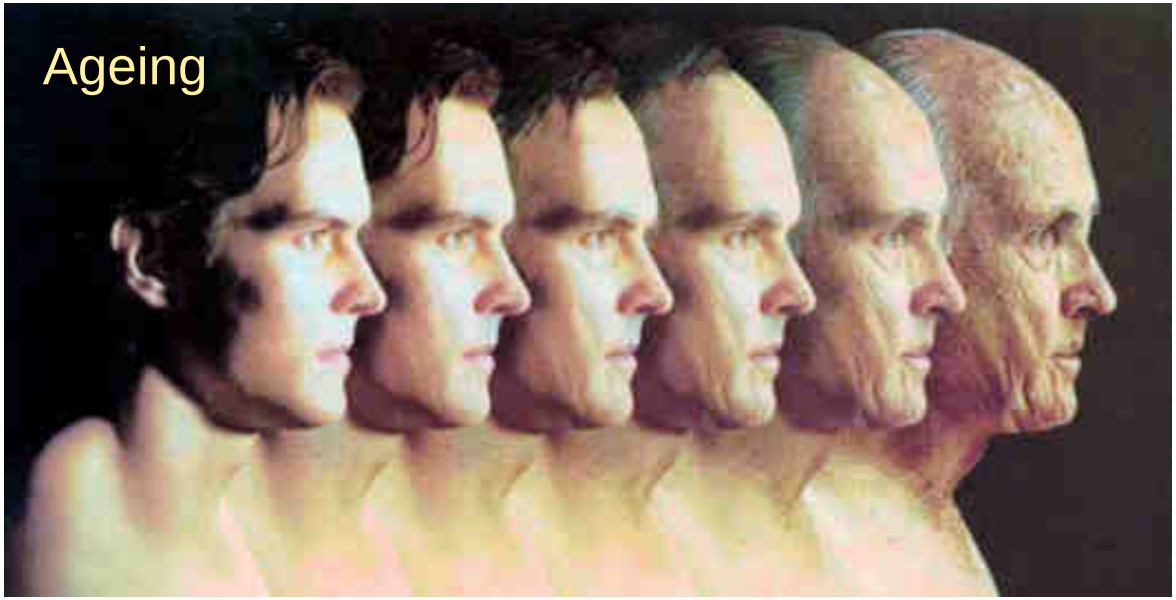


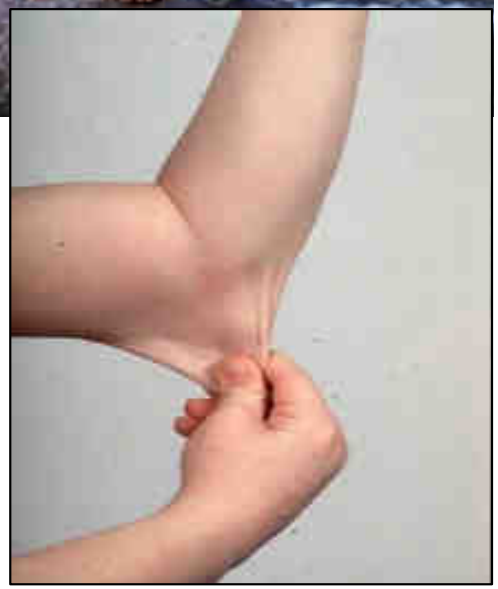
Aorta




Solar elastosis


Williams syndrome

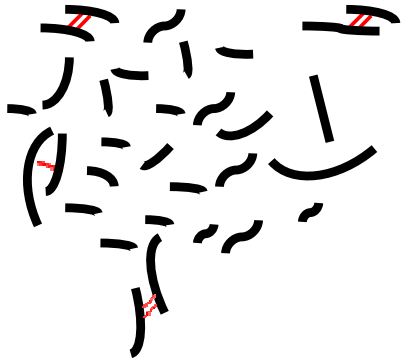

Ageing


Cutis Laxa

Elastin – a mere structure protein?

Cross-linked elastin

Elastases
(serine proteases,
matrix metalloproteinases)



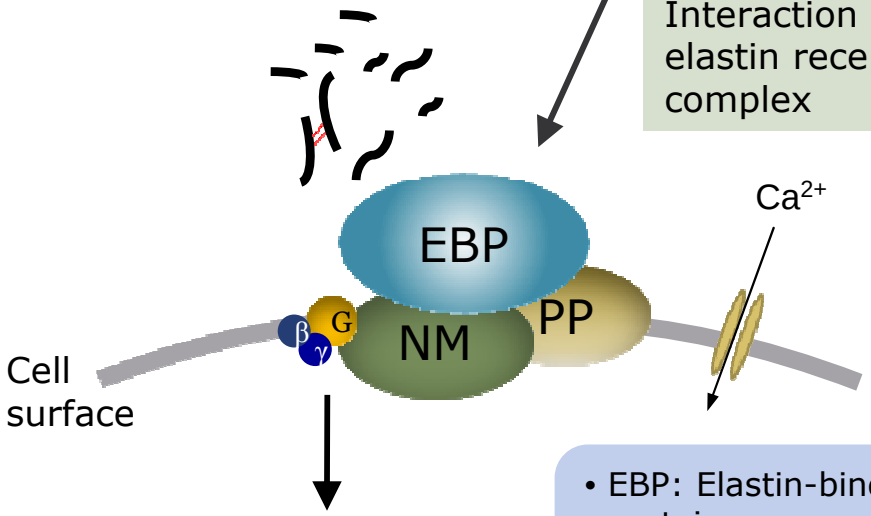
Elastin peptides, some containing the **xGxxPG** motif

Matrikines
bioactive ligands that exist as part of an extracellular matrix protein

Biological effects of matrikines

- Expression of MT-MMP-1, MMP-1 and MMP-2
- Chemotaxis
- Proliferation
- Induction of apoptosis
- Induction of angiogenesis
- Vasorelaxation and NO liberation
- Osteogenesis
- Th-1 polarization

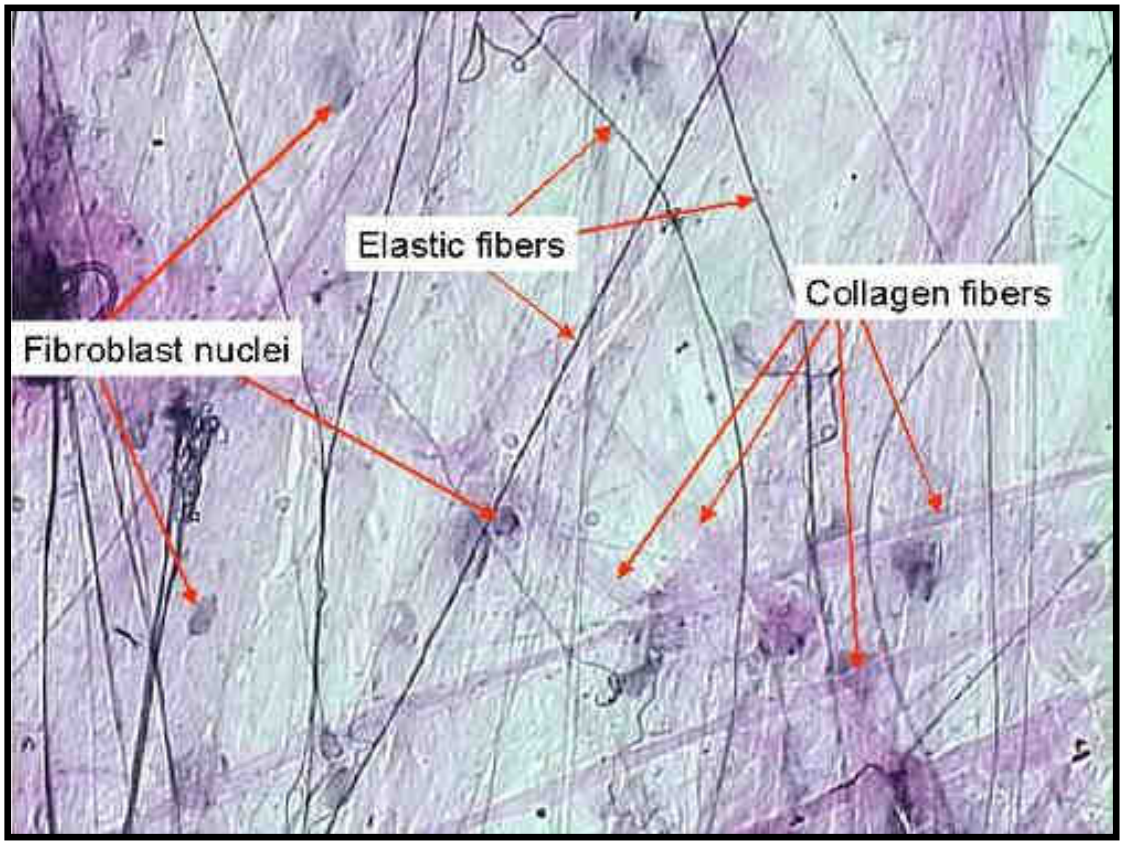
Interaction with elastin receptor complex



- EBP: Elastin-binding protein
- PP: Protective protein
- NM: Neuraminidase

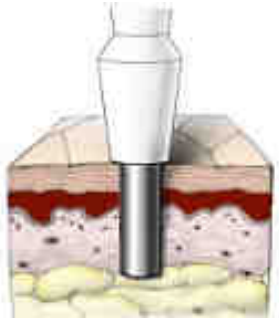
Cell types: **fibroblasts, endothelial cells, lymphocytes, monocytes, smooth muscle cells**

Isolation of elastin



Isolation of elastin

→ Intact elastin free from contaminants and remnants of the ECM



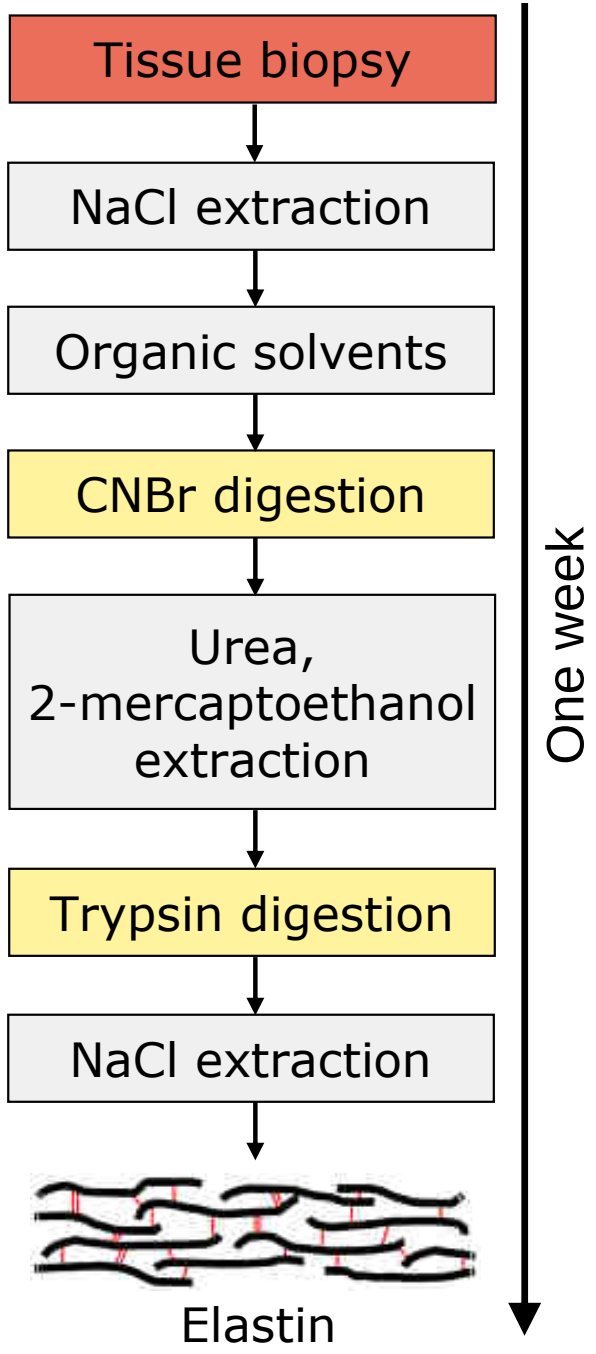
Skin biopsy

Hot alkali treatment (NaOH, 98 °C)

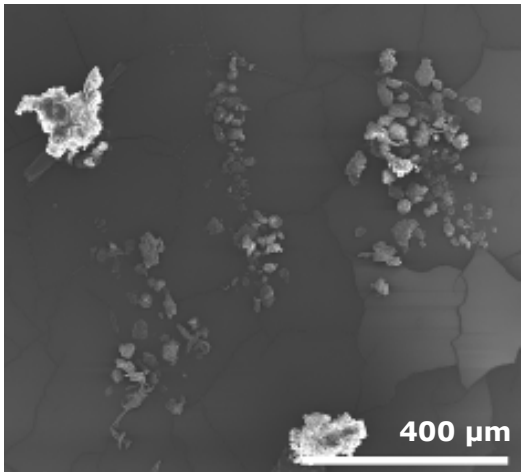
Autoclave treatment

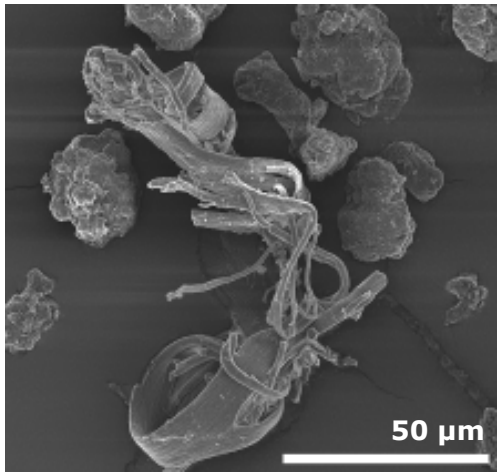
Treatment with reducing and chaotropic agents (mercaptoethanol, guanidine-HCl, urea)

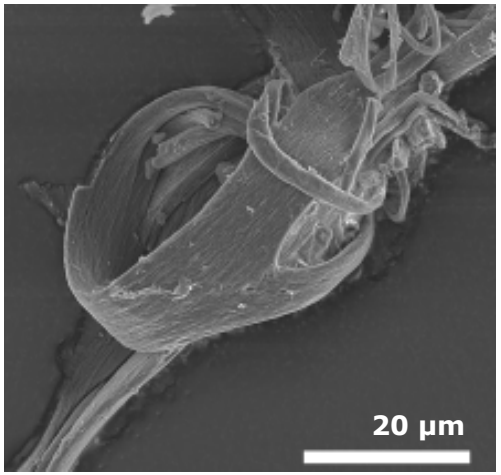
Treatment with enzymes (collagenase, trypsin)

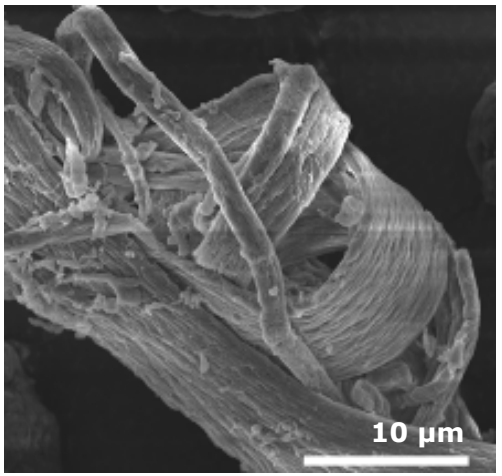


Morphological characterization

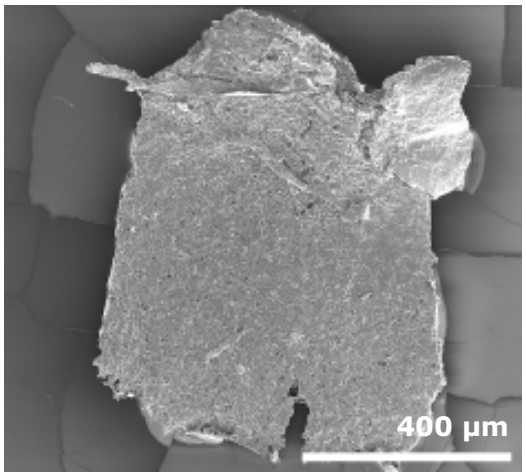


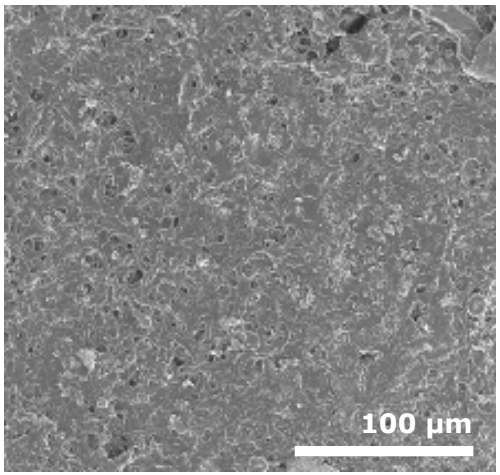


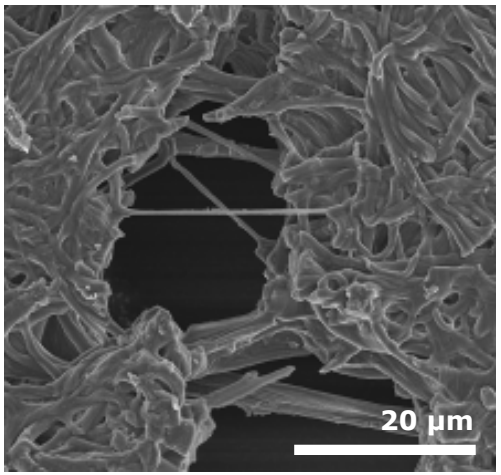


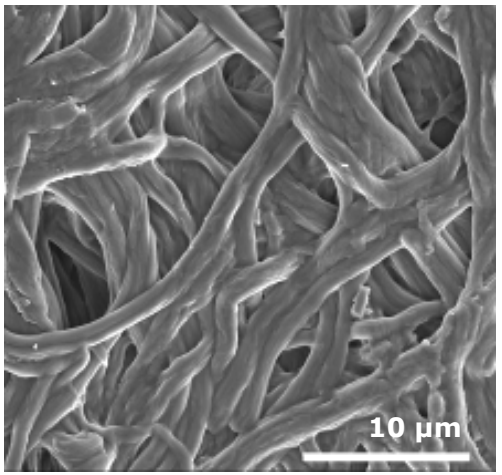


Human skin elastin isolated after Starcher and Galione









Human skin elastin isolated by a less destructive method

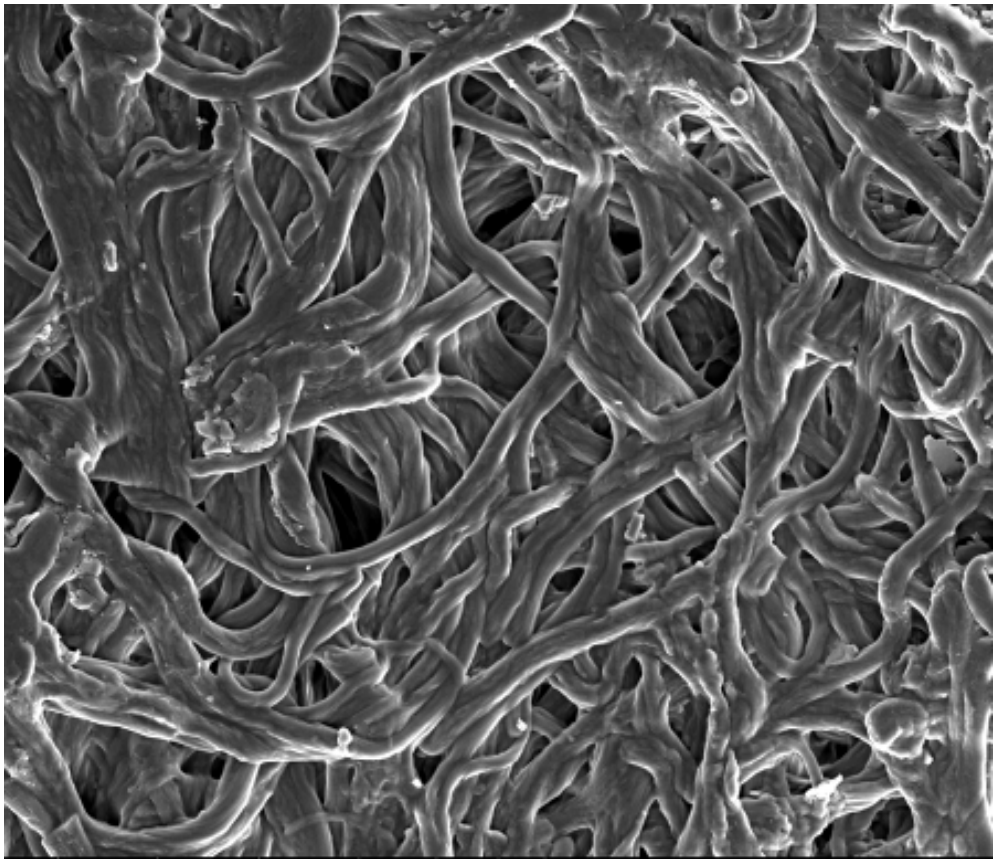
Molecular-level characterization

- Degradation of isolated elastin by porcine pancreas elastase
- Qualitative analysis by nanoHPLC-MS/MS and nanoLC/MALDI-TOF/TOF

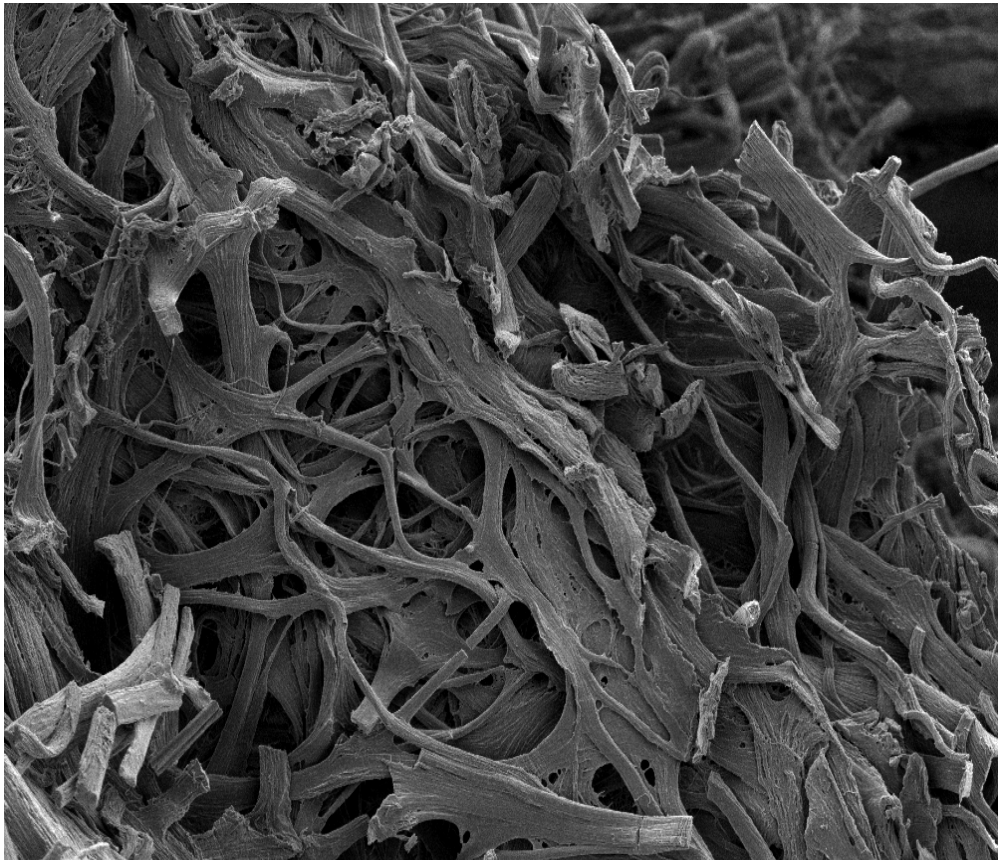
Elastin from human skin *(after Starcher and Galione)*		**Elastin from human skin** *(isolated using a less destructive method)*	
Identified precursors	**InChorus Score**	**Identified precursors**	**InChorus Score**
Tropoelastin	**99 %**	**Tropoelastin**	**99 %**
Collagen Type I α-1	**99 %**		
Collagen Type I α-2	**99 %**		
Collagen Type III	**99 %**		

S. Taddese, M. C. Jung, C. Ihling, A. Heinz, R. H. H. Neubert, and C. E. H. Schmelzer, MMP-12 catalytic domain recognizes and cleaves at multiple sites in human skin collagen type I and type III, Biochim Biophys Acta 1804 (2010) 731-739.

The turnover of elastic fibers



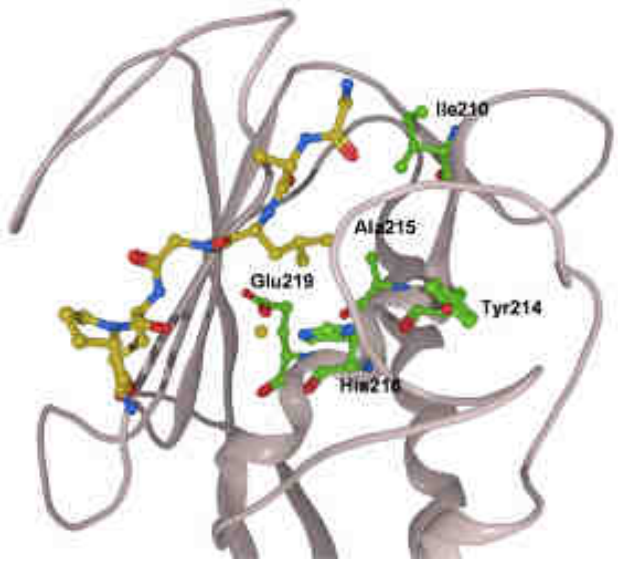
~35 years



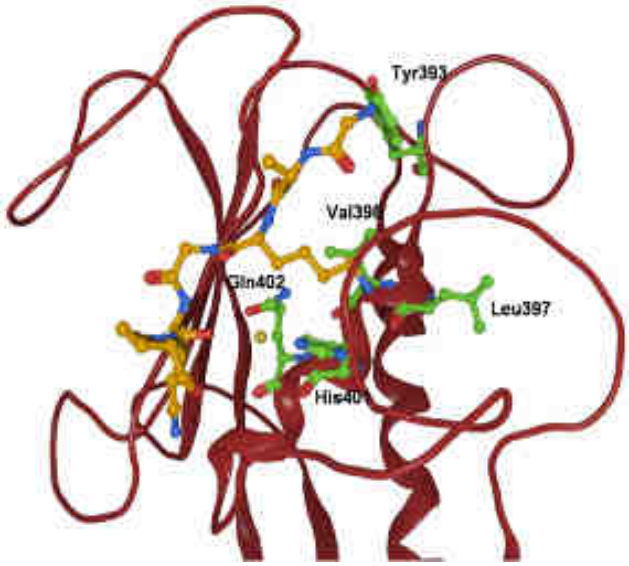
~90 years

Elastases

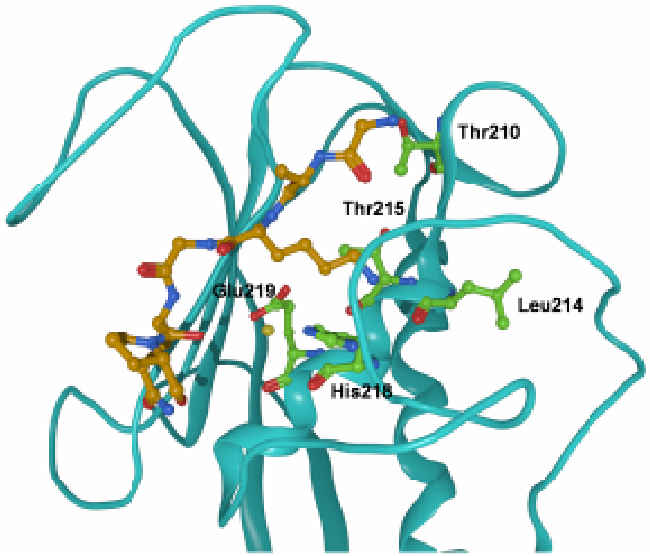
Matrix metalloproteinases



MMP-7



MMP-9



MMP-12

MMP-7: epithelial cells

MMP-9: neutrophils, macrophages

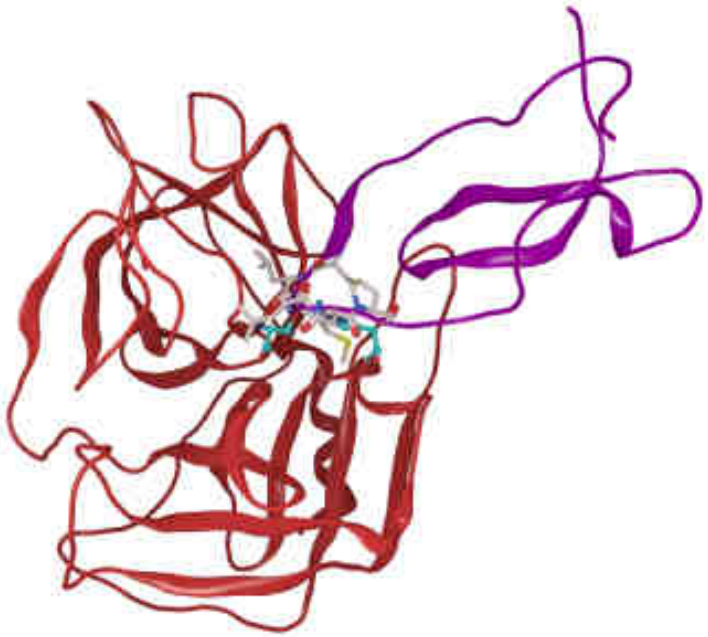
MMP-12: macrophages

→ Connective tissue remodeling processes →

- Aortic stenosis
- Aortic aneurysm
- Photoaging of skin
- Tumor progression
- Pulmonary emphysema

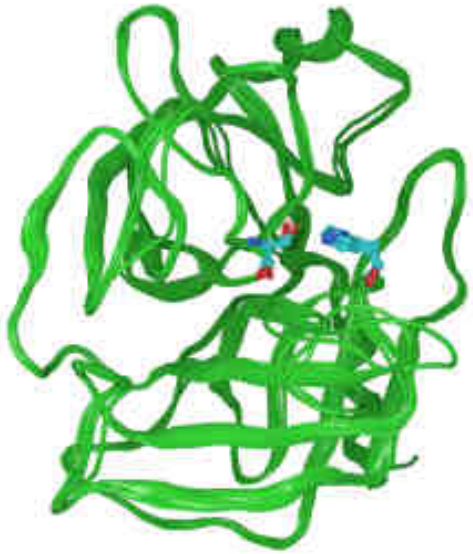
Elastases

Serine proteases



Human leukocyte elastase (HLE)

Proteinase 3 (PR3)



Cathepsin G (CG)

Stored in azurophilic granula of human neutrophils

→

Released in response to inflammation processes, degradation of pathogens

→

- Chronic obstructive pulmonary disease
- Pulmonary emphysema
- Atherosclerosis
- Tumor progression

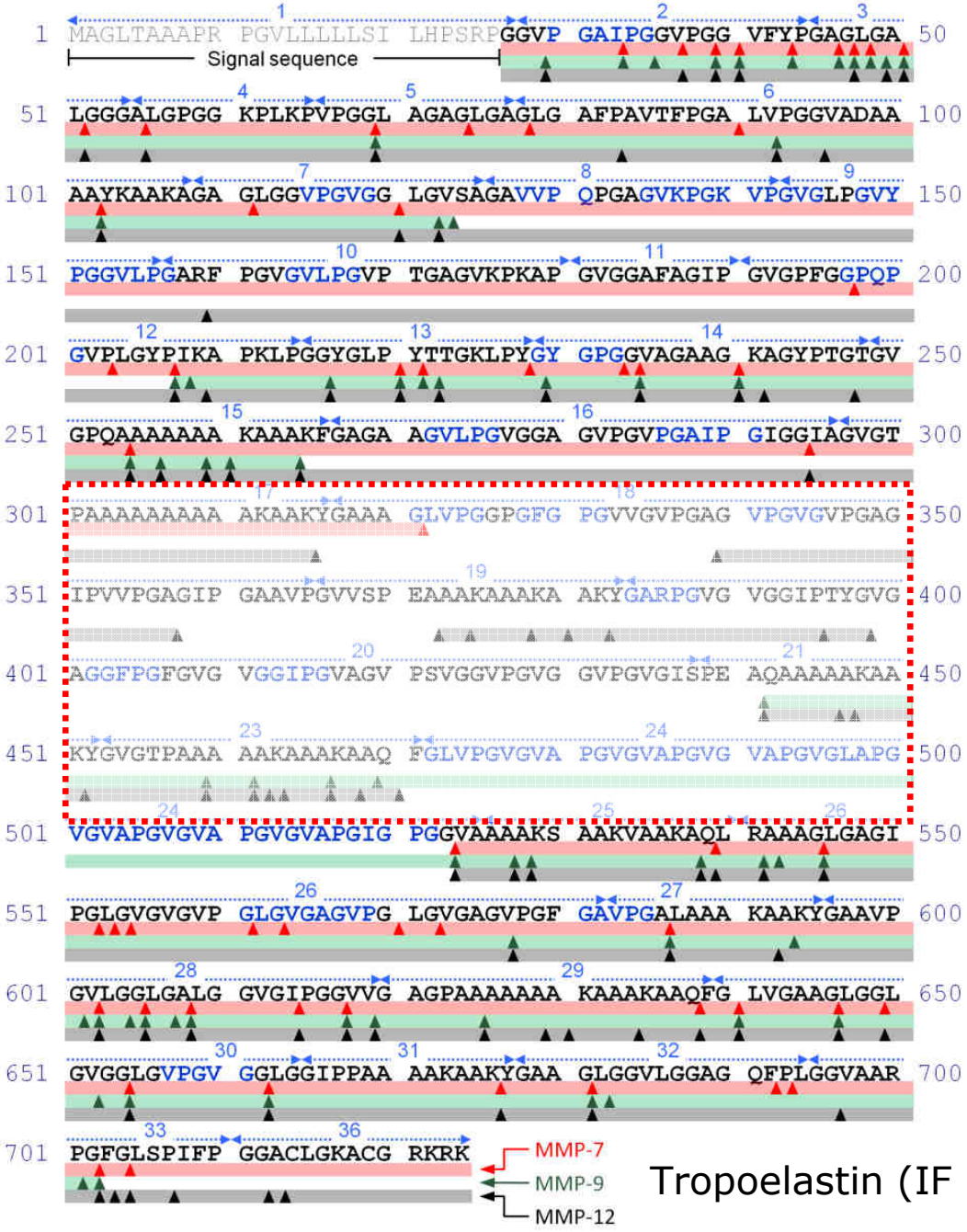
Enzymatic degradation of elastin

How do the enzymes act on elastin and tropoelastin?
- Cleavage site specificities
- Cleavage behavior

Are there any differences between elastin isolated by different methods?

Which peptides are released during degradation of elastin?
- Bioactive motifs?

Cleavage behavior



Tropoelastin (IF 2)

- MMP-7: 84 peptides
- MMP-9: 74 peptides
- MMP-12: 132 peptides

Sequence coverages

MMP-7: 71 %

MMP-9: 60 %

MMP-12: 81 %

Cleavage site preferences

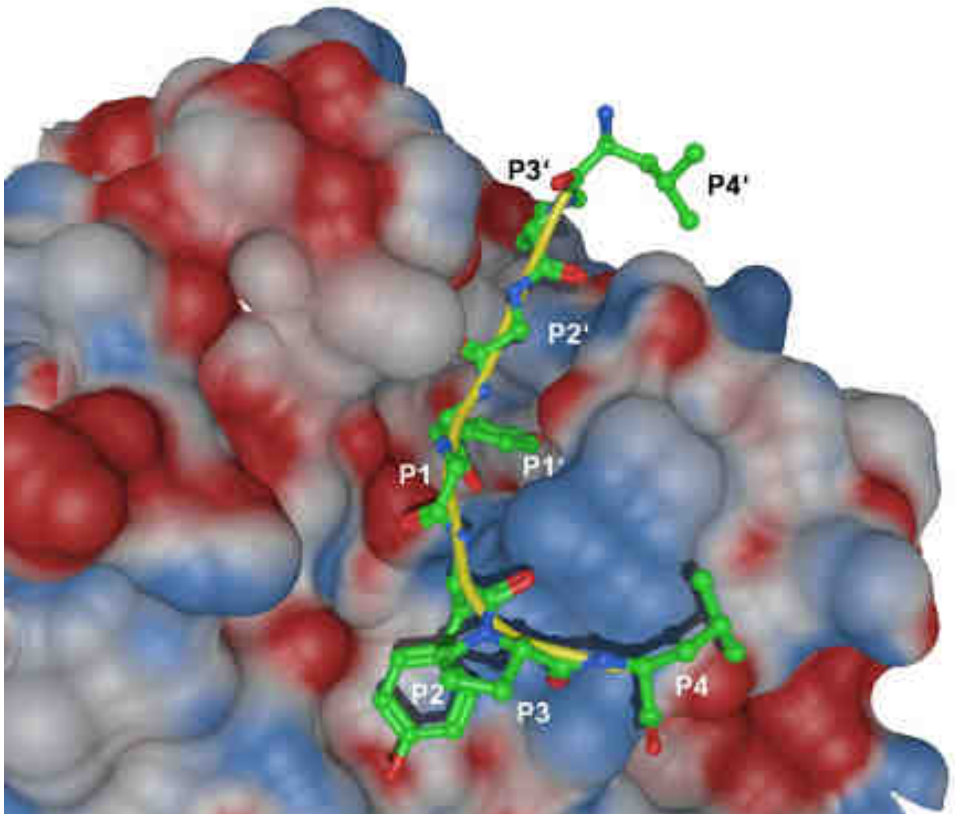
NH_2 ← - · - · - · - · - · - · - ✂ - · - · - · - · - → COOH

Amino acid	P_4 / %			P_3 / %			P_2 / %			P_1 / %			P_1' / %			P_2' / %			P_3' / %			P_4' / %		
G	38	39	27	28	14	15	38	31	32	55	38	33	12	14	7	53	31	34	39	41	24	32	23	21
A	18	27	27	17	30	41	27	30	40	15	33	39	3	19	26	13	29	30	29	34	42	22	36	35
V	8	9	10	13	9	6	2	2	1	3	11	7	12	9	9	13	8	7	12	3	5	17	6	11
L	5	6	6	10	13	6	7	11	6	5	3	1	48	22	20	3	9	4	5	5	5	7	14	7
I	3	2	1	3	2	1	0	0	2	3	3	1	3	3	2	0	0	1	0	0	0	3	3	3
F	2	0	1	2	0	0	8	5	4	2	0	1	3	0	4	2	8	2	2	3	3	2	0	1
Y	5	3	3	0	2	0	2	5	4	5	3	0	5	6	9	2	6	1	3	2	4	3	3	2
K	5	3	11	2	3	1	0	2	4	2	0	8	2	11	12	5	8	11	0	2	3	2	5	7
P	15	5	8	18	23	24	13	8	2	7	6	4	10	8	5	0	0	2	9	6	5	9	8	8
MMP-	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12

Occurrence of different amino acids at the substrate positions P_1 - P_4 and P_1' - P_4' after digestion with MMP-7, MMP-9, and MMP-12. Values are based on the number of cleavage sites identified upon mass spectrometric analysis of the digests.

Molecular modeling

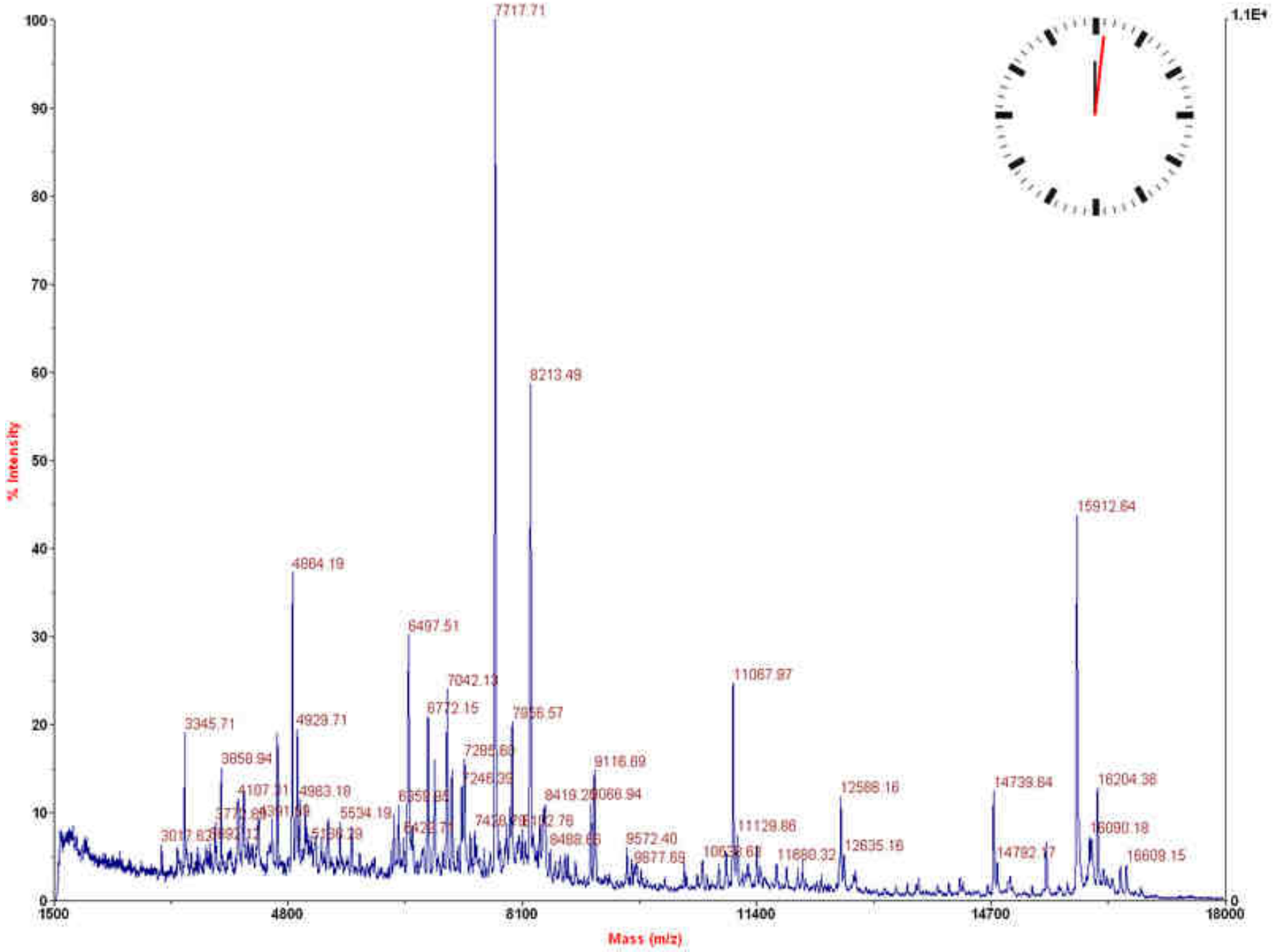
Tropoelastin (isoform 2)



Interaction of the natural substrate **LPYGYGPG** (residues 226-233 from tropoelastin isoform 2) and the MMP-12 active site. The molecular surface of the binding pocket is colored according to electrostatic potential (red: negative electrostatic potential, blue: positive electrostatic potential).

Does HLE degrade elastin?

Digestion of tropoelastin by HLE



1 min

3 min

10 min

20 min

30 min

45 min

60 min

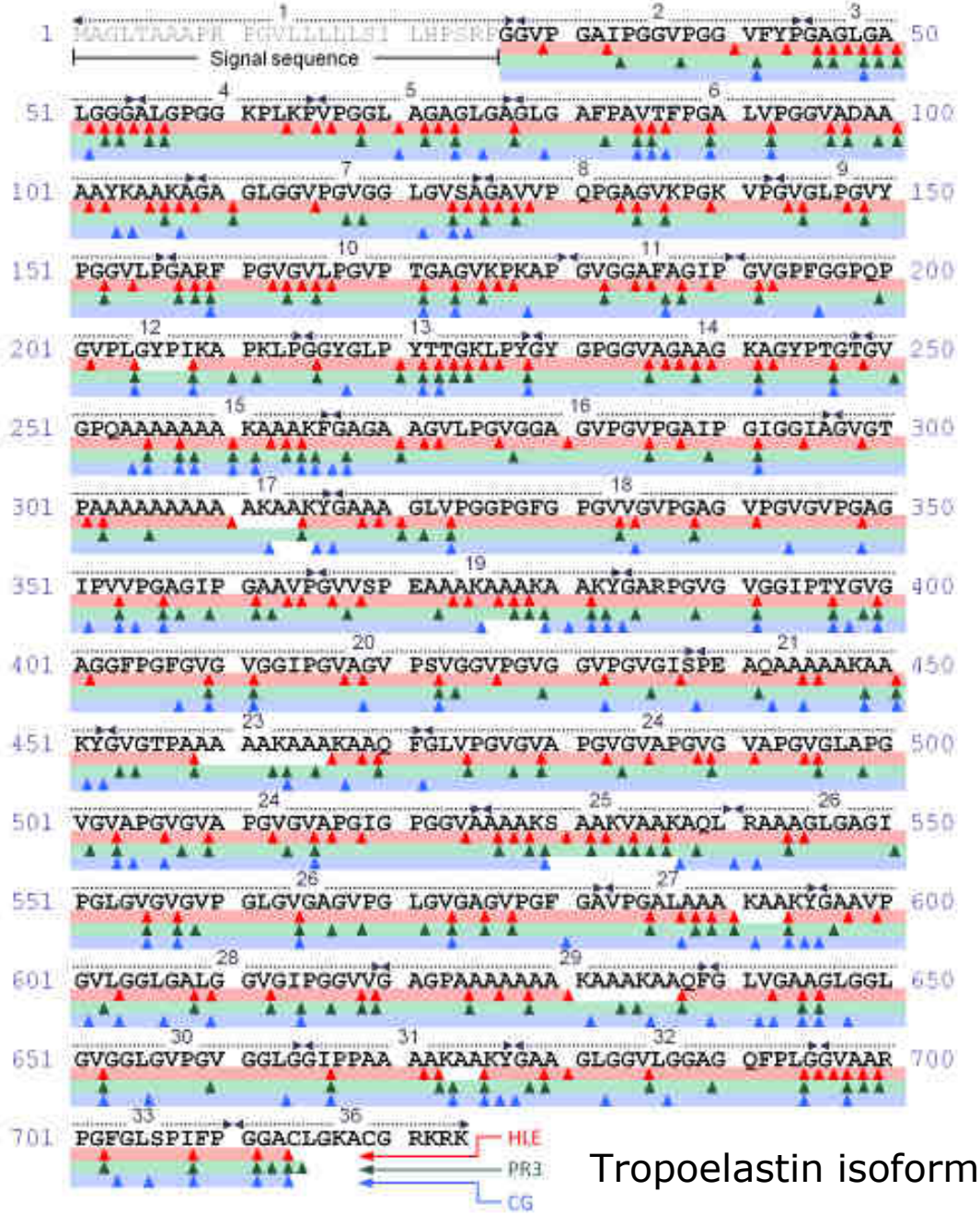
120 min

180 min

300 min

600 min

Cleavage behavior of NSPs - tropoelastin



Tropoelastin isoform 2

- HLE: 408 peptides
- PR3: 305 peptides
- CG: 197 peptides

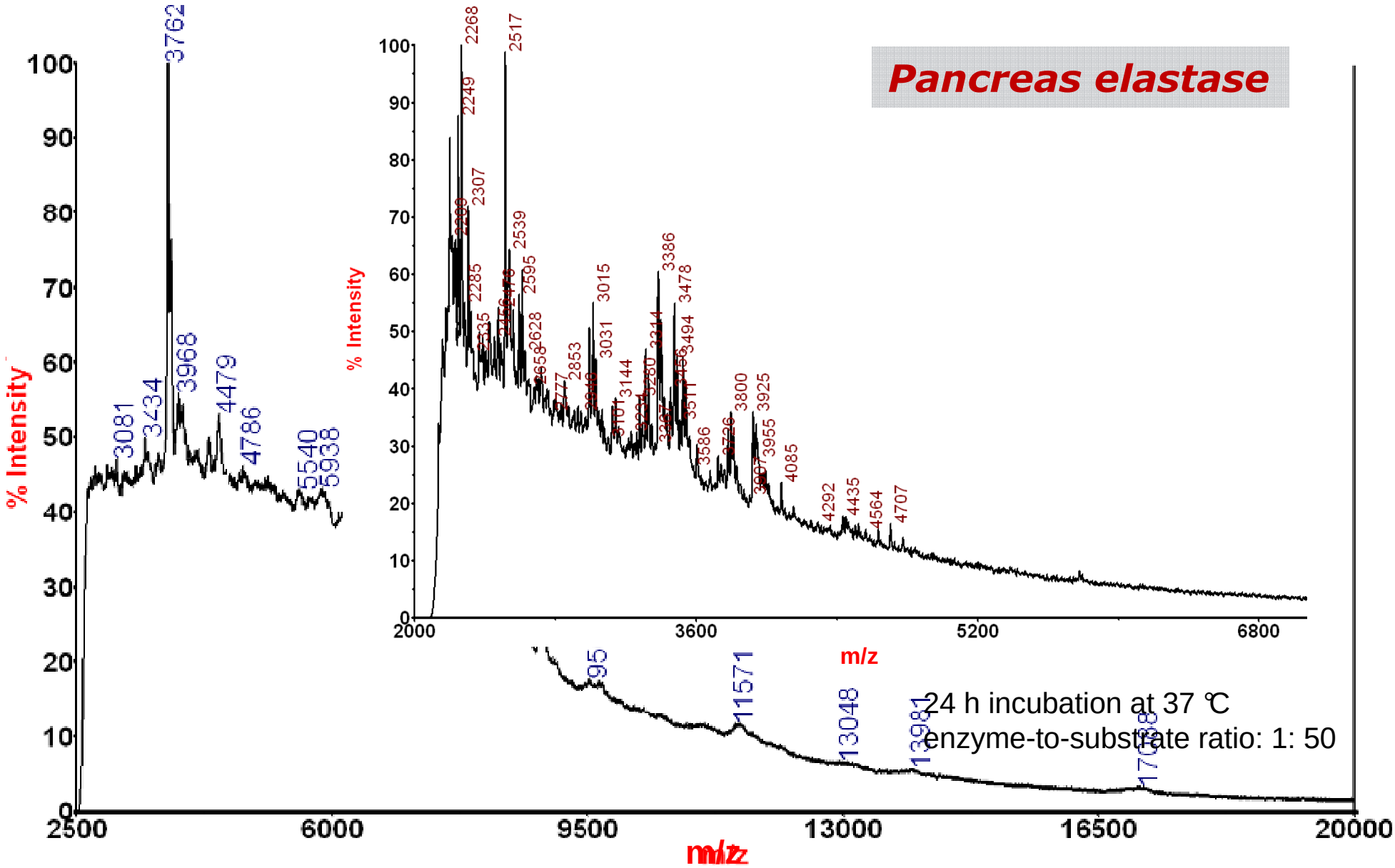
Sequence coverages

HLE: 94 %

PR3: 99 %

CG: 96 %

Digestion with HLE



Characterization of elastin from different tissues

Challenges

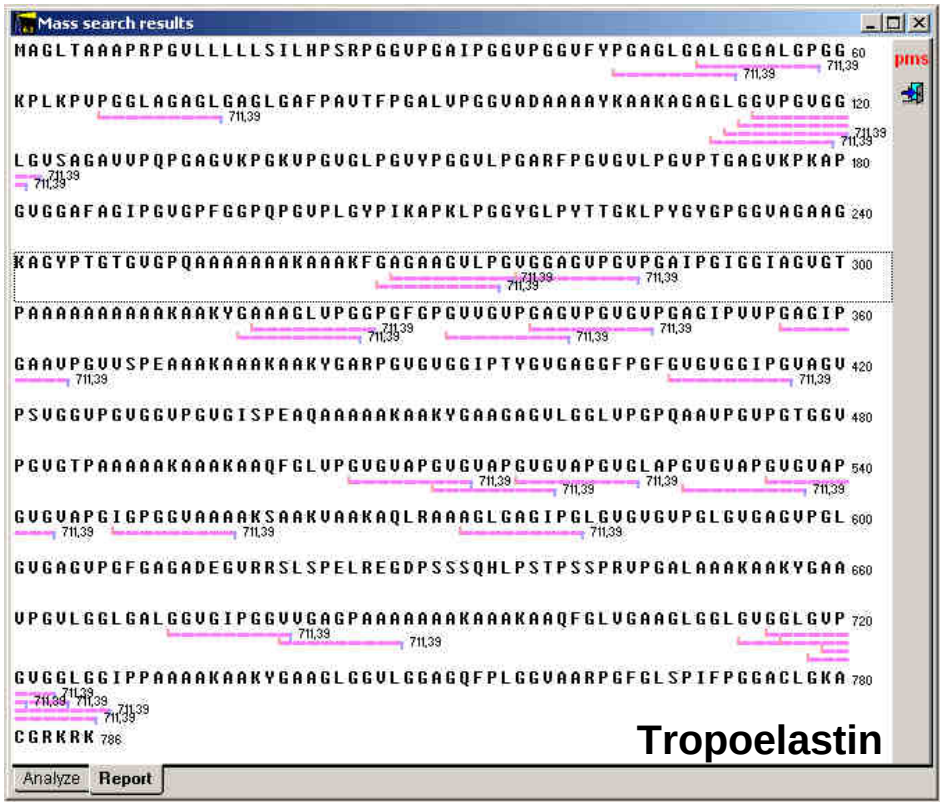
… with elastin peptides

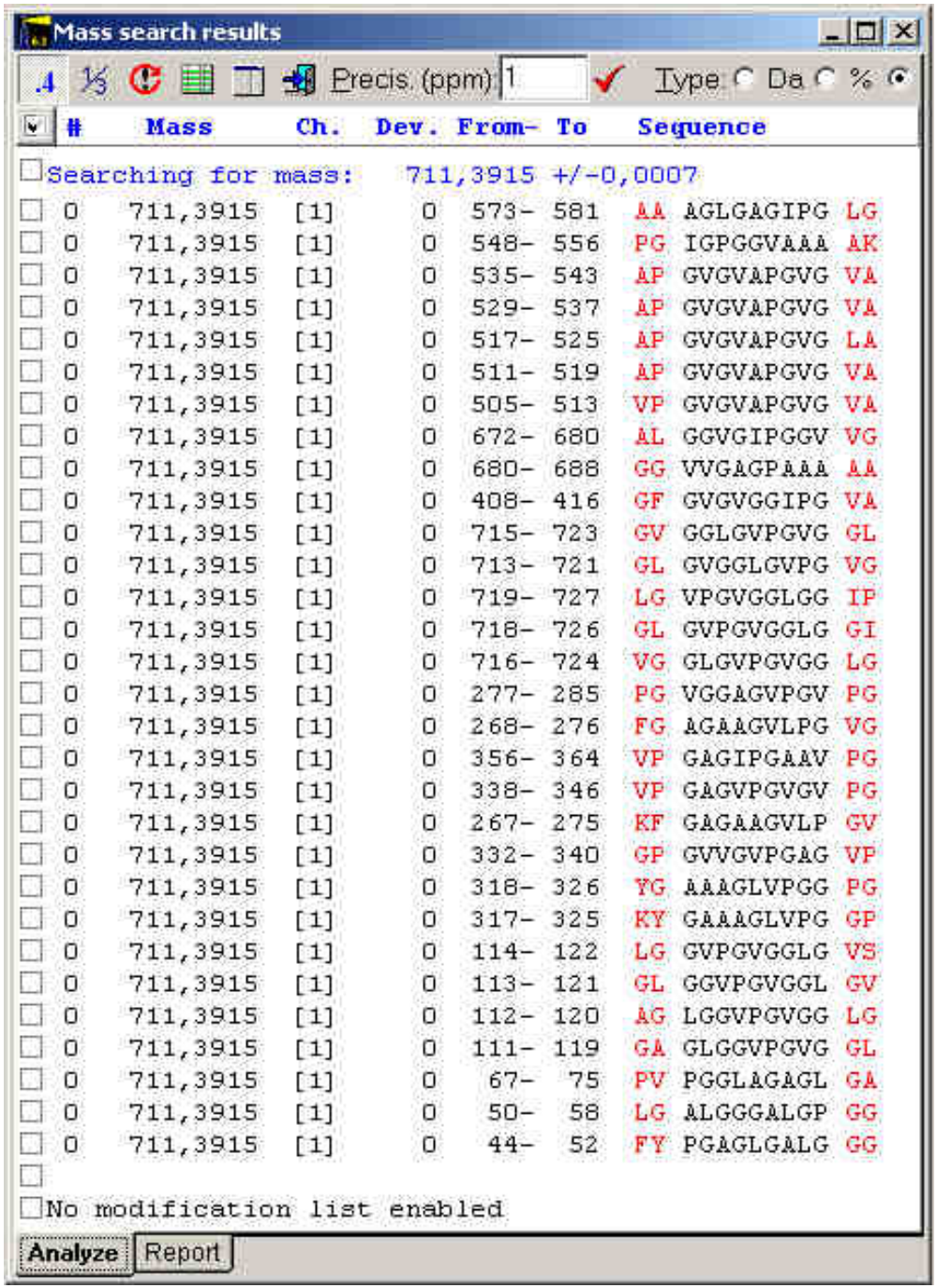
(1) Peptides mainly hydrophobic

(2) Proteolysis time consuming, not digestable with specific proteases

(3) Many of the peptides have equal or almost equal masses

Search for M_r = 711.3915





Challenges

… with elastin peptides

(4) Many repetitive regions

..GL**GVPGVGG**.. 711.39 Da

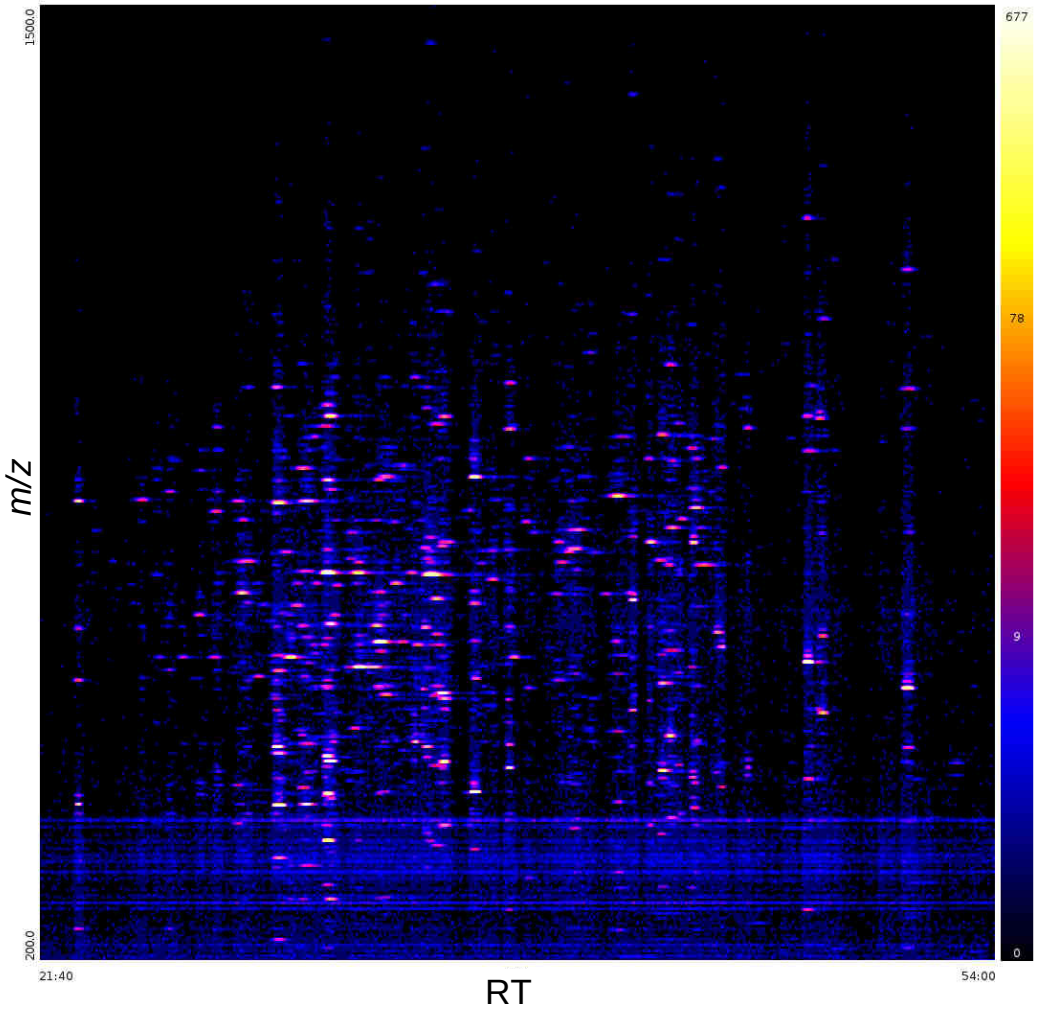
⇒ High similarity between peptides and thus between fragmentation patterns

..LG**GVPGVGG**.. 711.39 Da

..G**GVPGVGG**L.. 711.39 Da

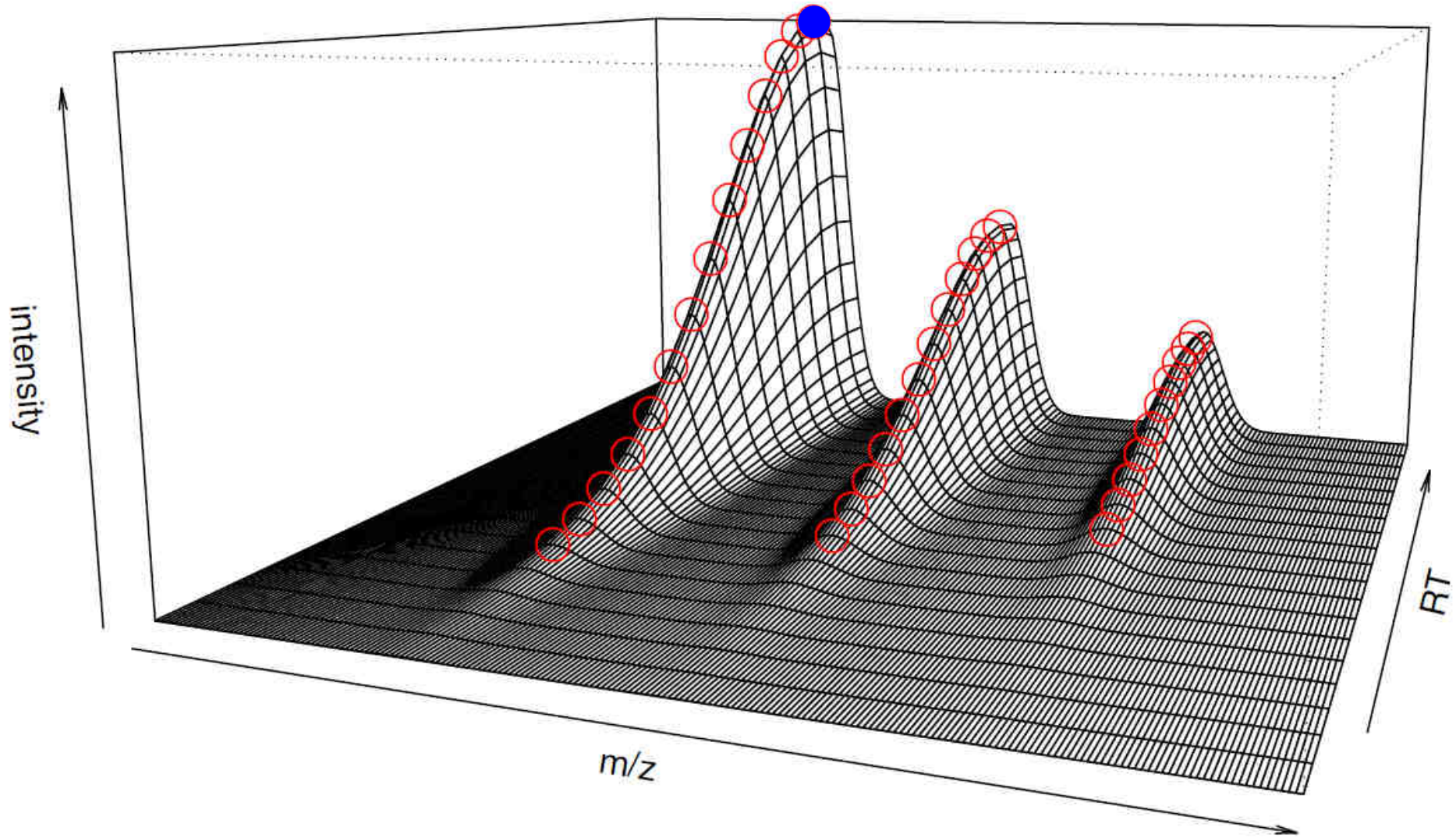
(5) Cross-links and other PTMs

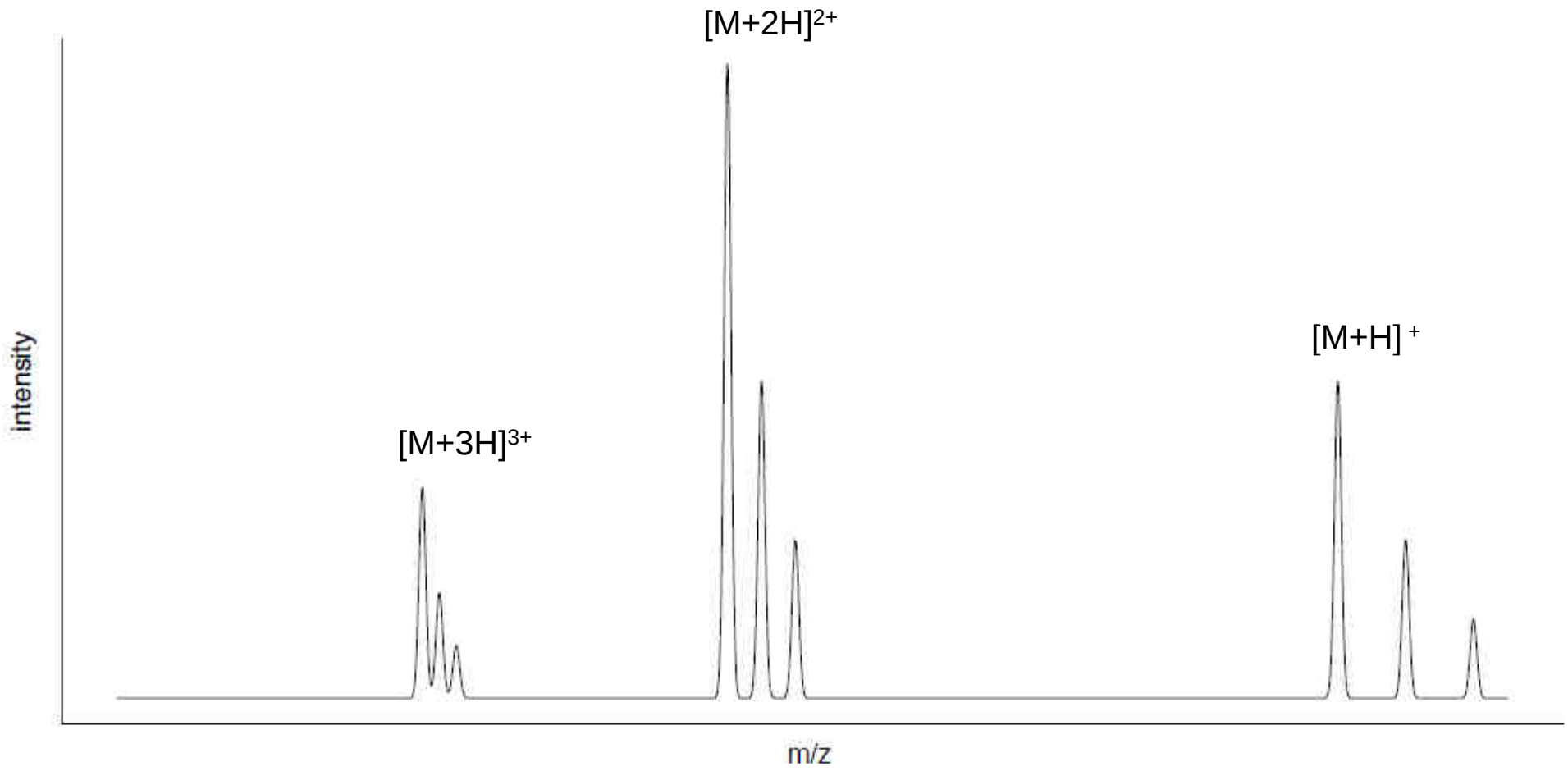
Initial situation



x 150 samples x 7 = 1050

- Skin
- Aorta
- Cartilage
- Intervertebral disc
- WBS skin
- WBS aorta

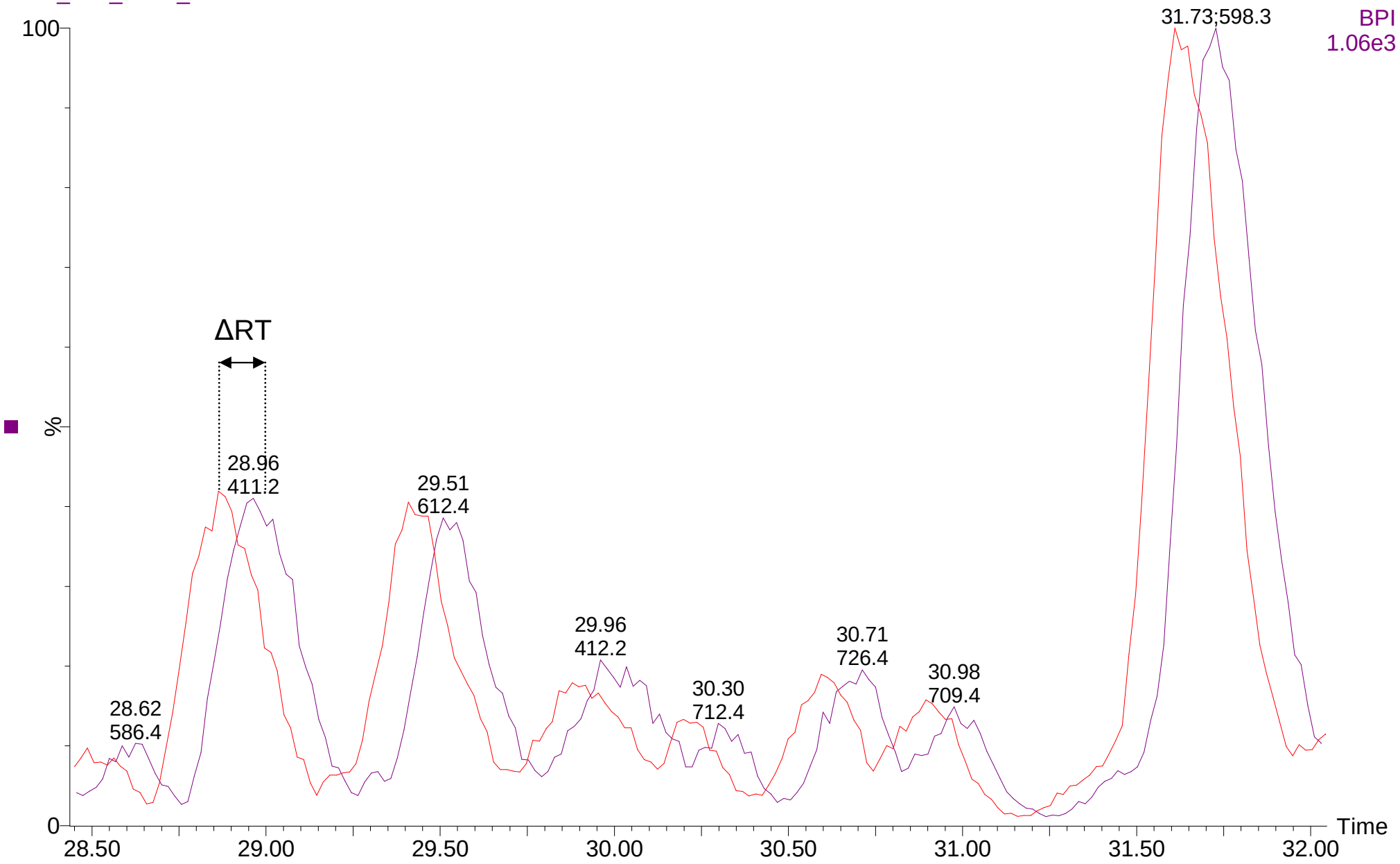

intensity
m/z
RT


[M+2H]2+
[M+H] +
[M+3H]3+
intensity
m/z

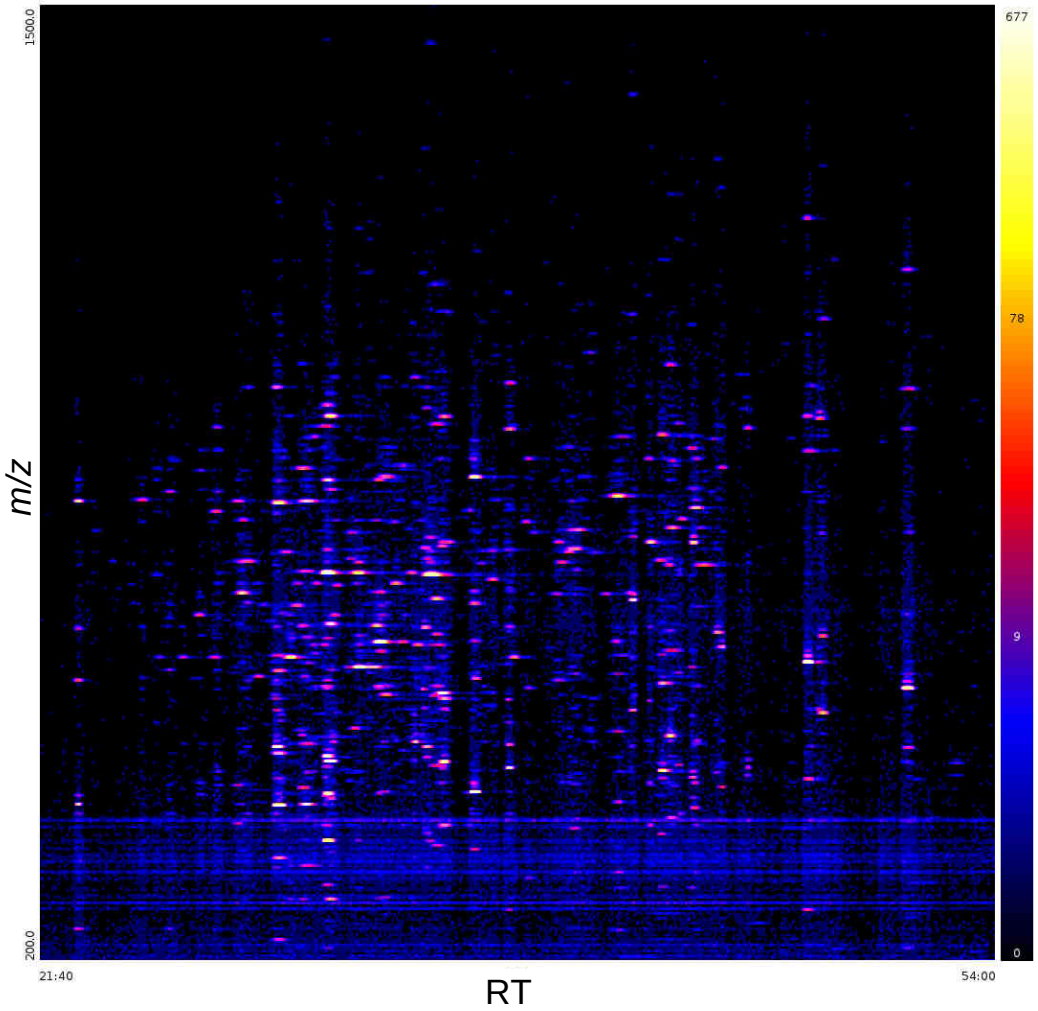
18-Sep-2011 **Kontrollprobe WR, PE nach 24h, 1 : 8.75 verdünnt mit Wasser, 10 min beladen** **07:42:54**

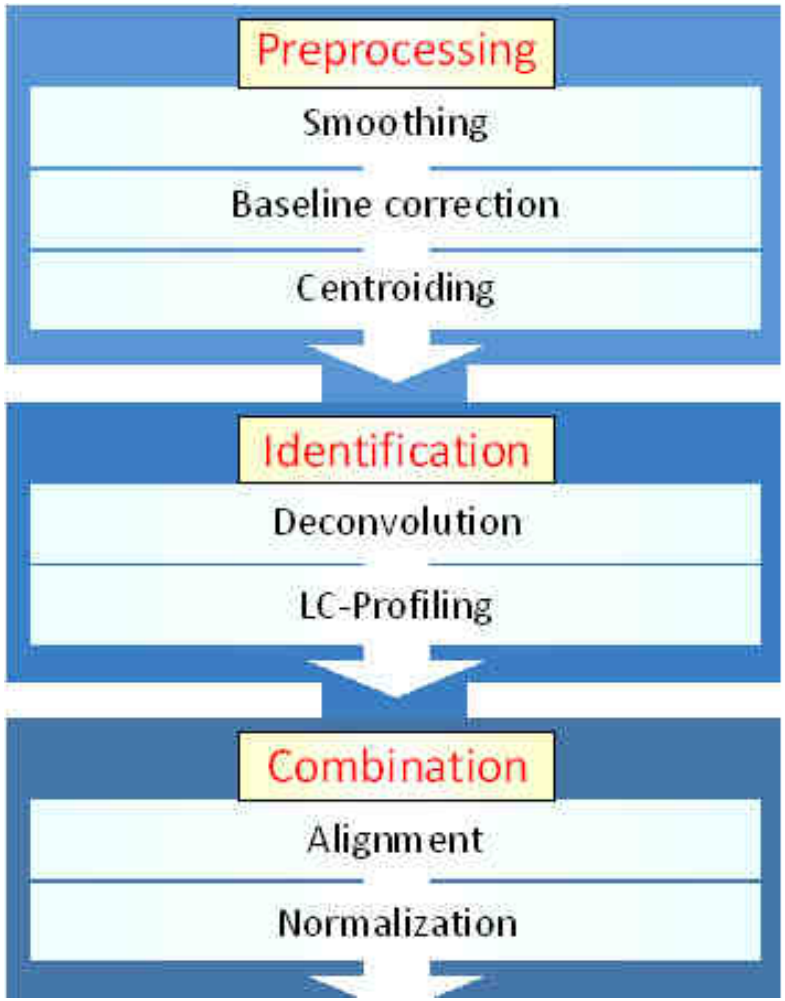
WBS_WR_PEk2_3

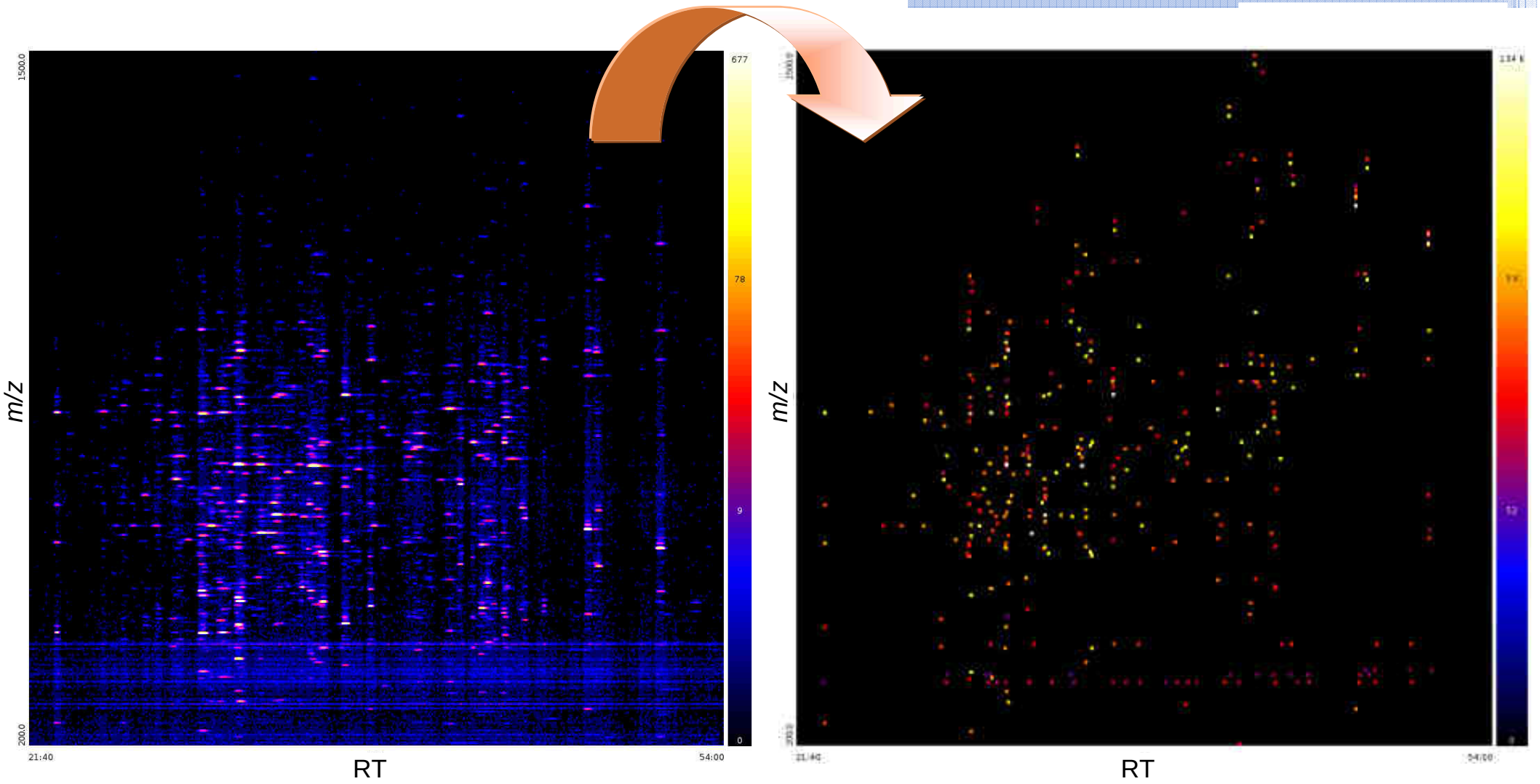
TOF MS ES+
BPI
1.06e3

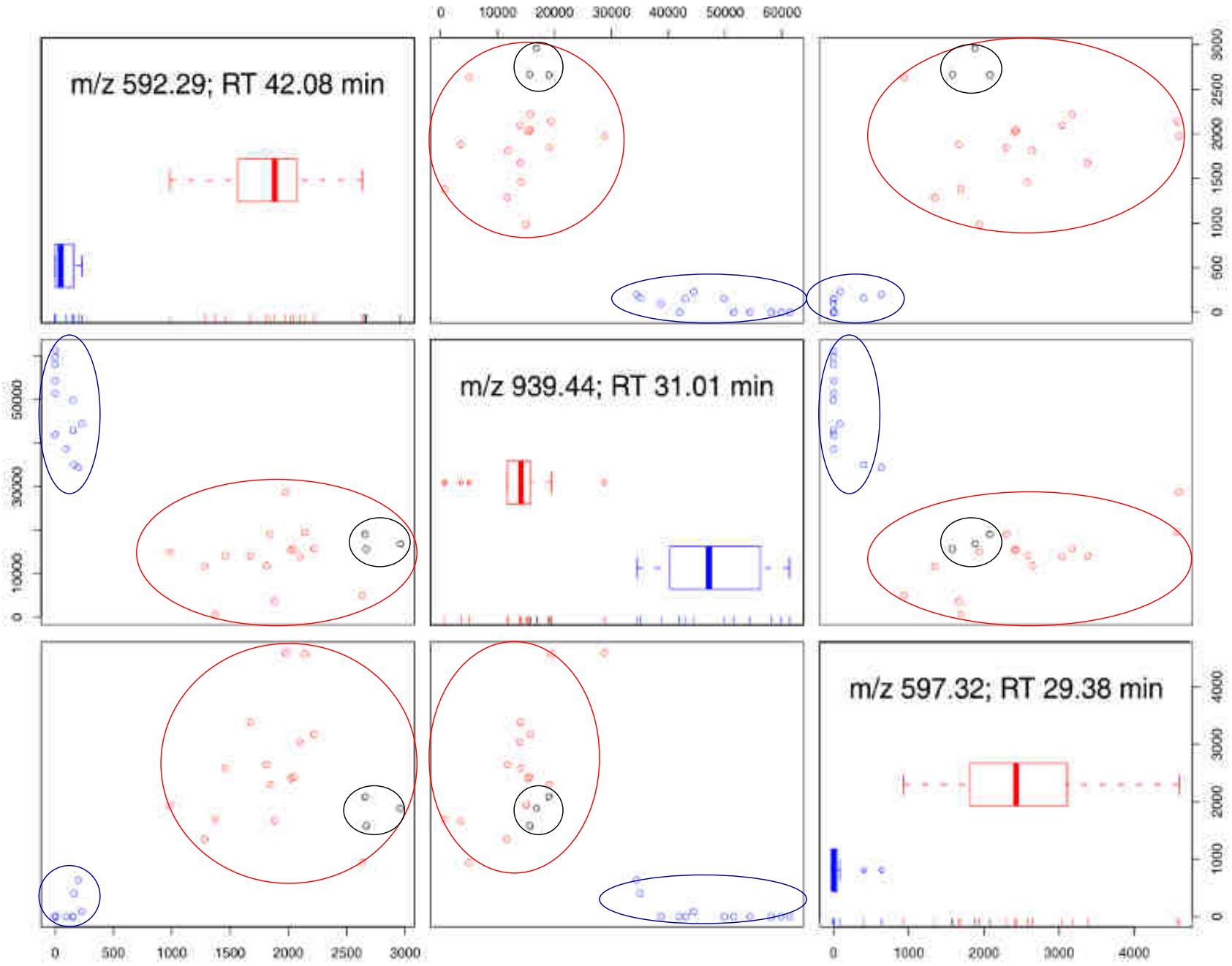


IonHunter


1500.0
200.0
m/z
677
78
9
0
21:40
54:00
RT


Preprocessing
Smoothing
Baseline correction
Centroiding
Identification
Deconvolution
LC-Profiling
Combination
Alignment
Normalization

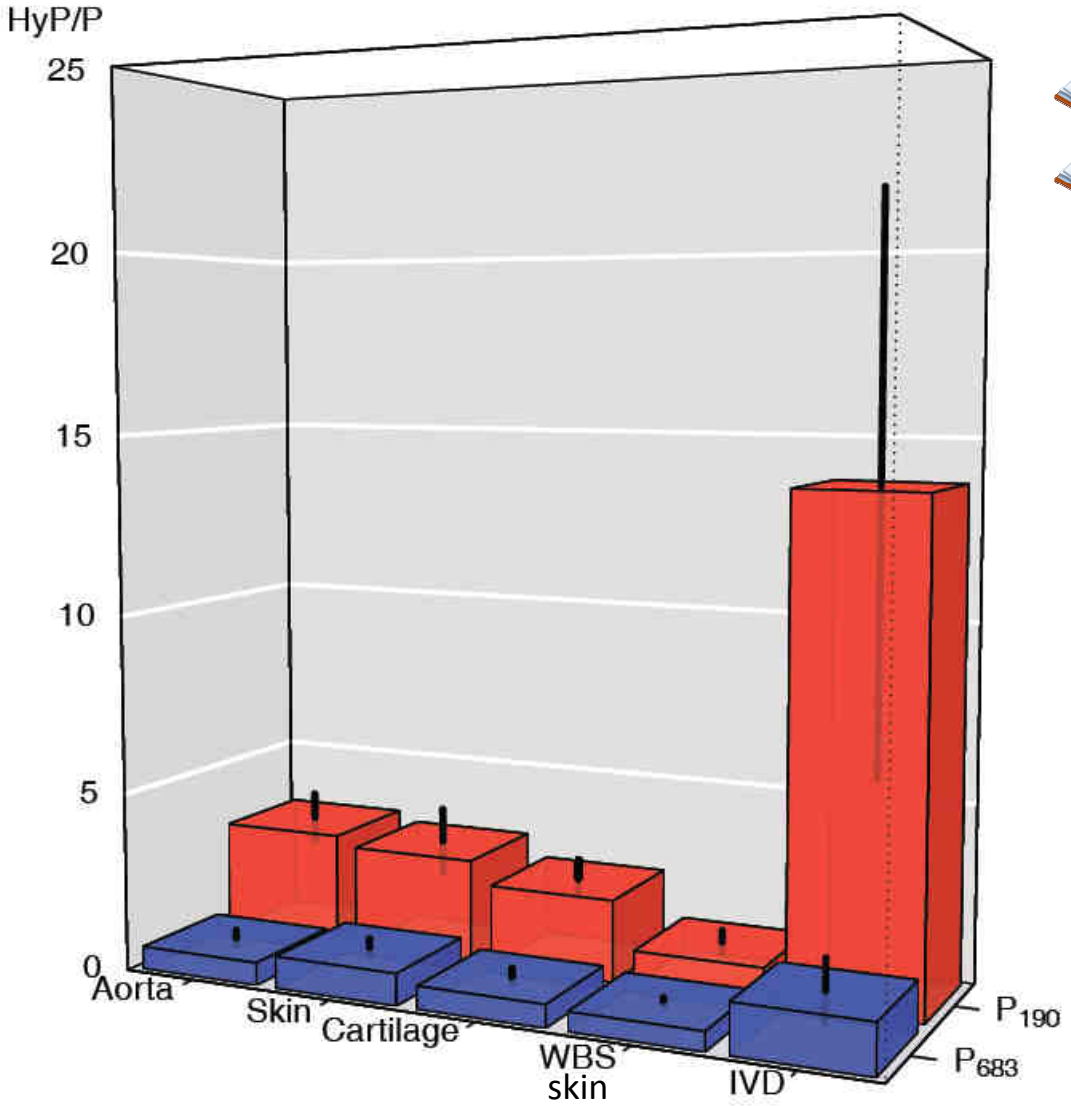

1500.0
200.0
m/z
677
78
9
0
21:40
54:00
RT
m/z
RT
Analysis
Supervised and unsupervised
biomarker identification



Aorta (40 - 83 years)

Skin (4 - 13 years)

Skin (90 years)



C. E. H. Schmelzer, M. Getie, and R. H. H. Neubert, J. Chromatogr. A 1083 (2005) 120-126.

S. Taddese, A. S. Weiss, R. H. H. Neubert, and C. E. H. Schmelzer, Matrix Biol 27 (2008) 420-428.

Proline hydroxylation

$[M+H]^+$	Sequence	Start/Stop residue (IF 9)	Pro residue (IF 9)	Ratio
814.45 Da	F.**AGIPGVGPF**.G	187 - 195	190	$AUC_{830.44}/AUC_{814.45}$
830.44 Da	F.**AGIpGVGPF**.G	187 - 195	190	$= HyP_{190}/P_{190}$
939.53 Da	L.**GGVGIPGGVVGA**.G	678 - 689	683	$AUC_{955.52}/AUC_{939.53}$
955.52 Da	L.**GGVGIpGGVVGA**.G	678 - 689	683	$= HyP_{683}/P_{683}$

Identified marker peptides

About 20 identified marker peptides that give insight into specific features of elastin such as

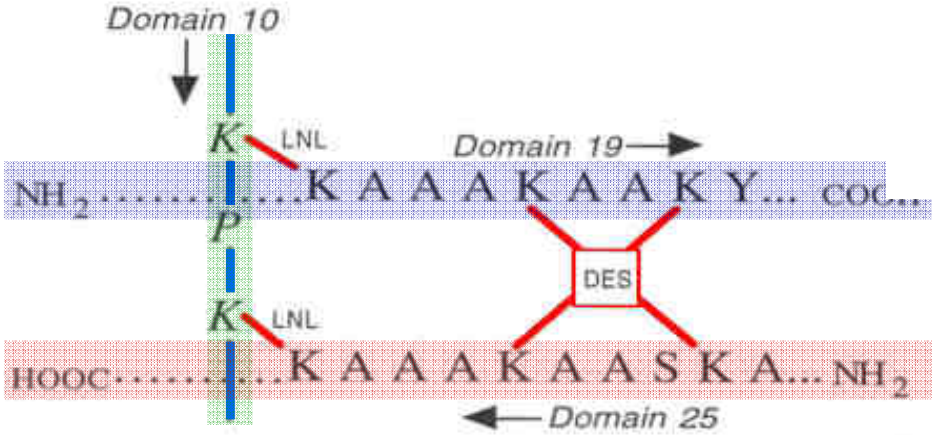
- Age and/or degeneration status
- Type of tissue
- Existence and level of PTMs
- Presence of absence of certain domains
- Status of known point mutations

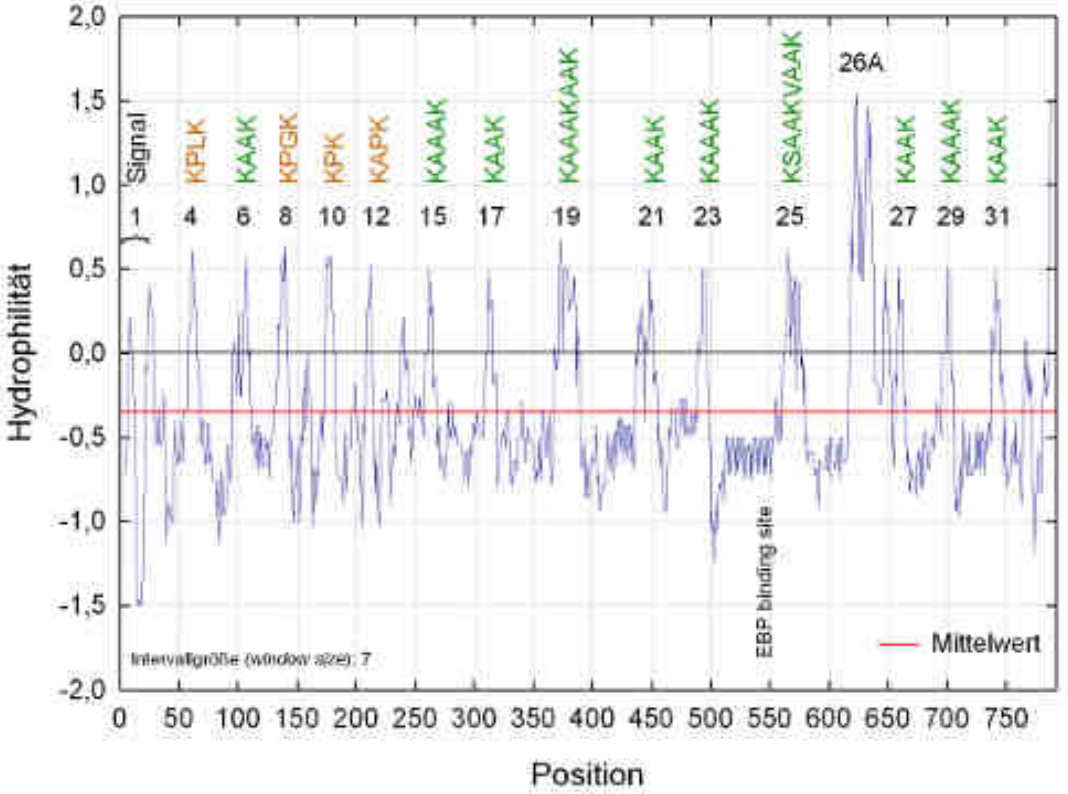
Characterization of cross-linking pattern of elastin

Recovered peptides from degradation with elastases

Cross-linking in elastin

Brown-Augsburger, P., Tisdale, C., Broekelmann, T., Sloan, C. and Mecham, R. P. (1995) *J Biol Chem* **270** (30): 17778-17783.

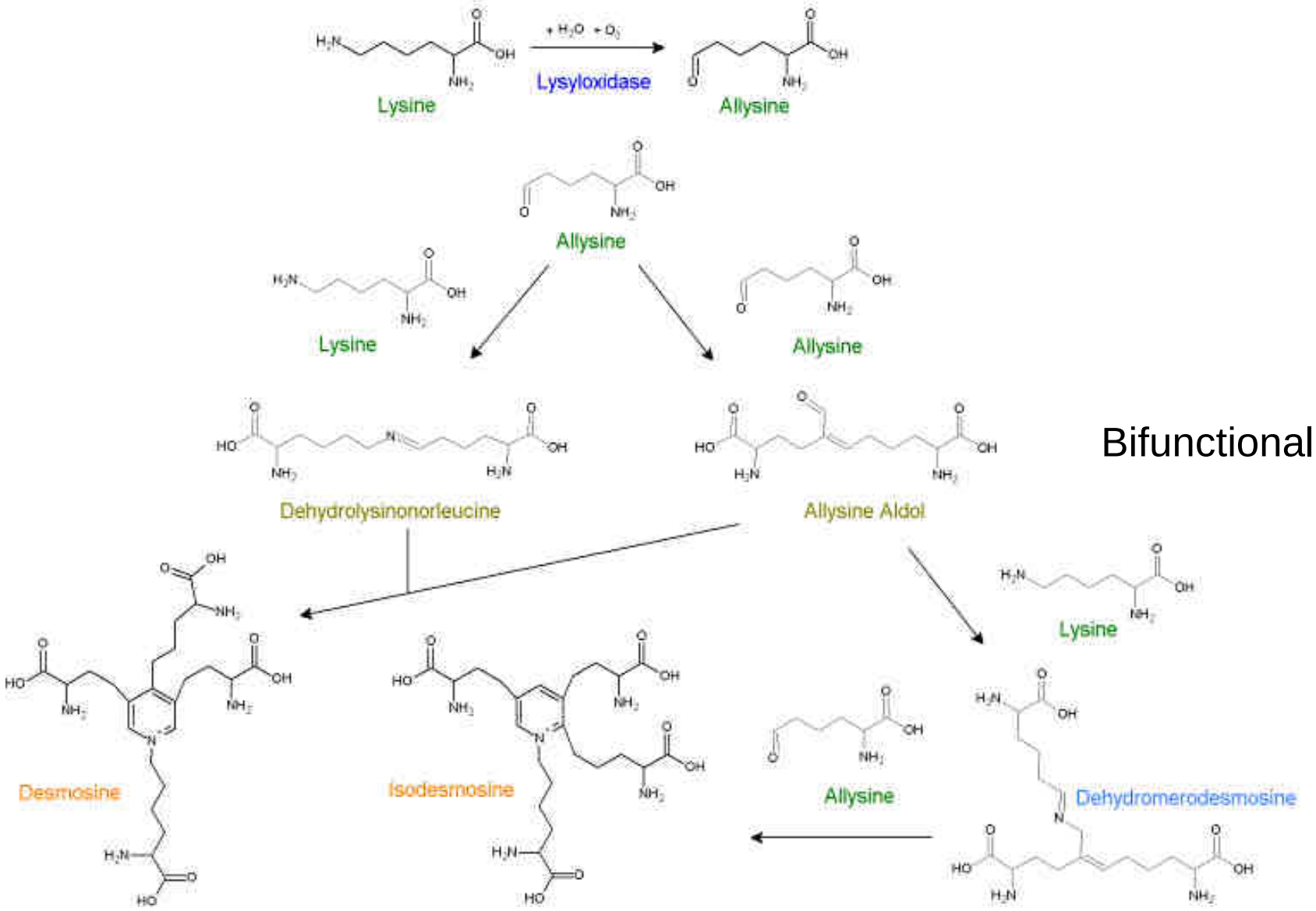


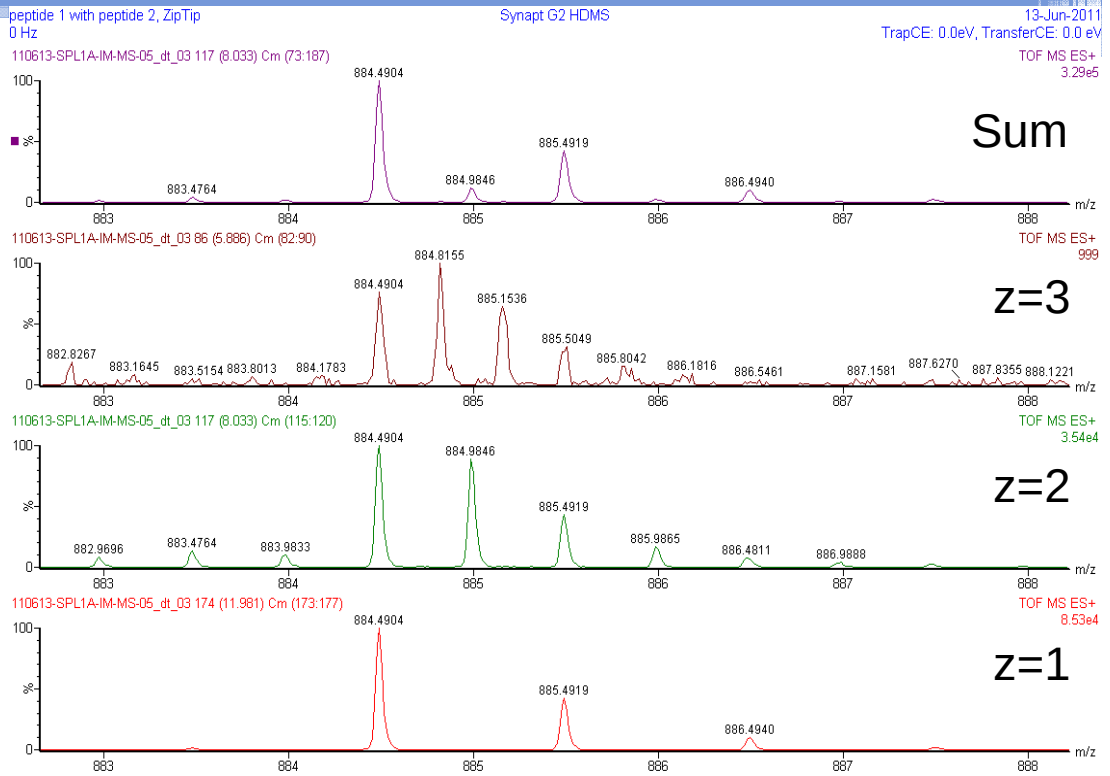


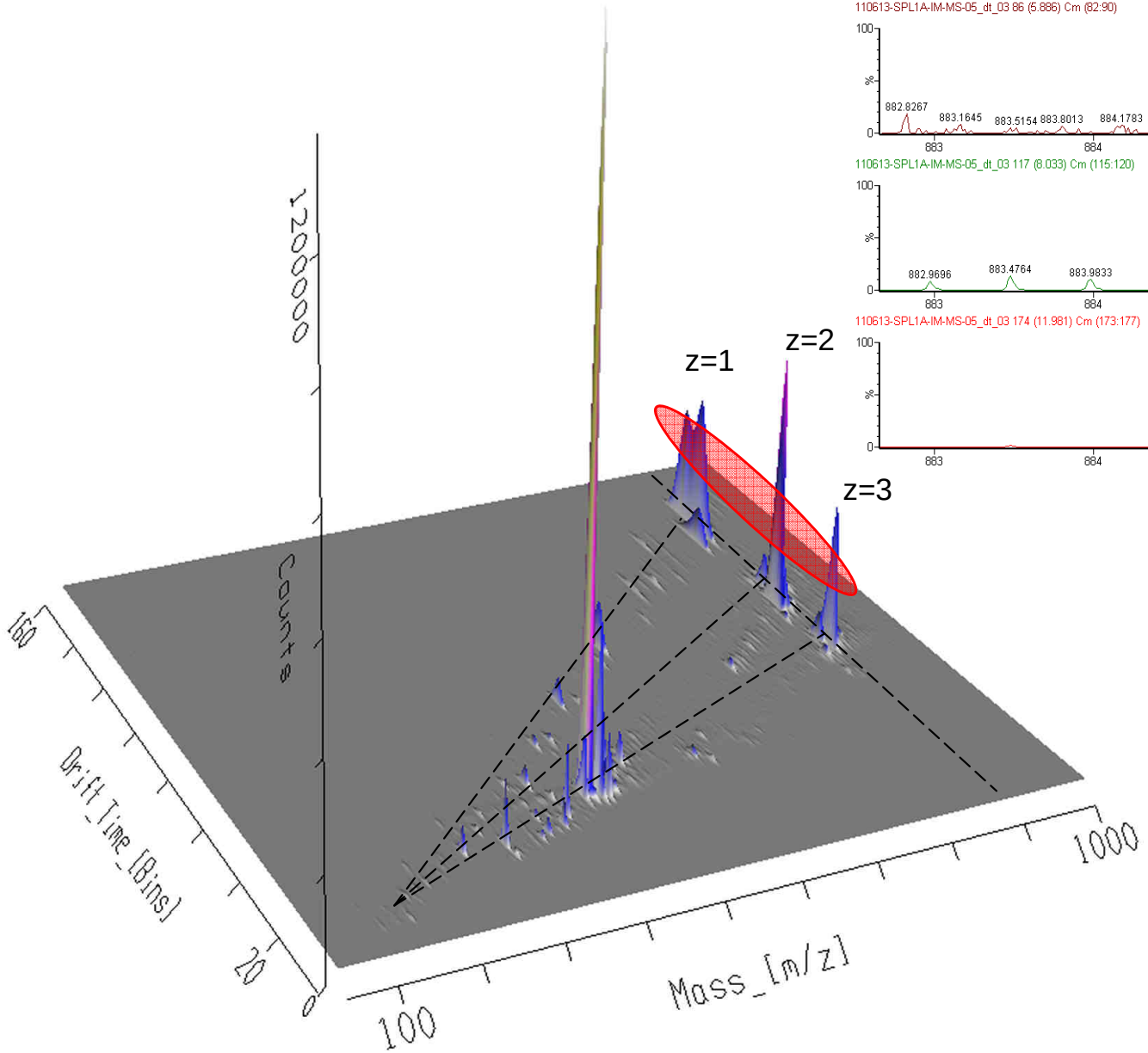
Hydrophobicity/Hydrophilicity plot of human tropoelastin (Swiss-Prot accession number P15502, Isoform 9) calculated by the method of Hopp and Woods (1981).

Cross-linking of three tropoelastin molecules at domains 10, 19 and 25

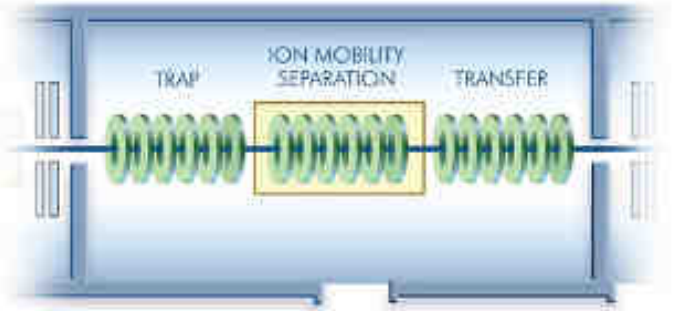
Cross-linking in elastin

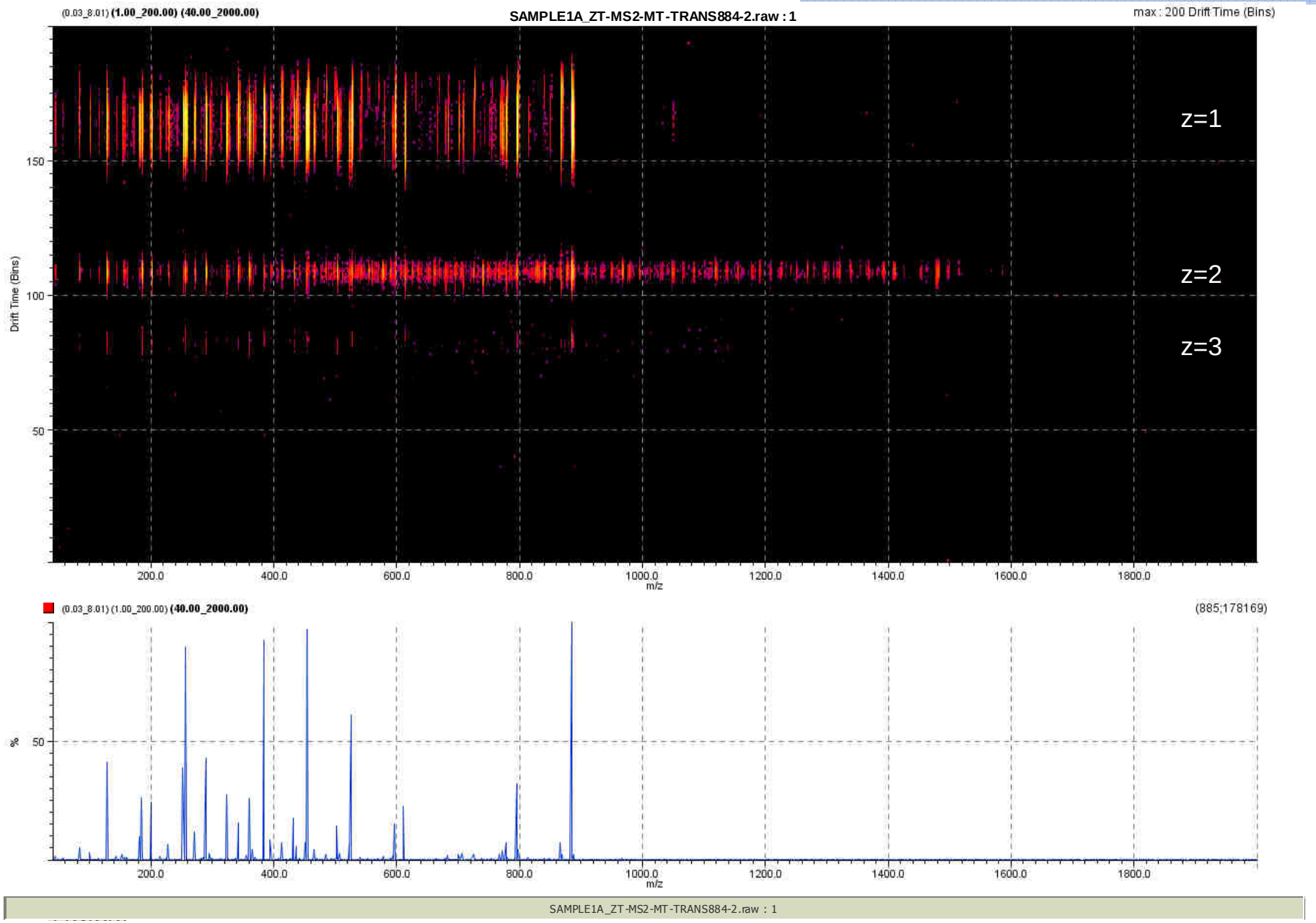




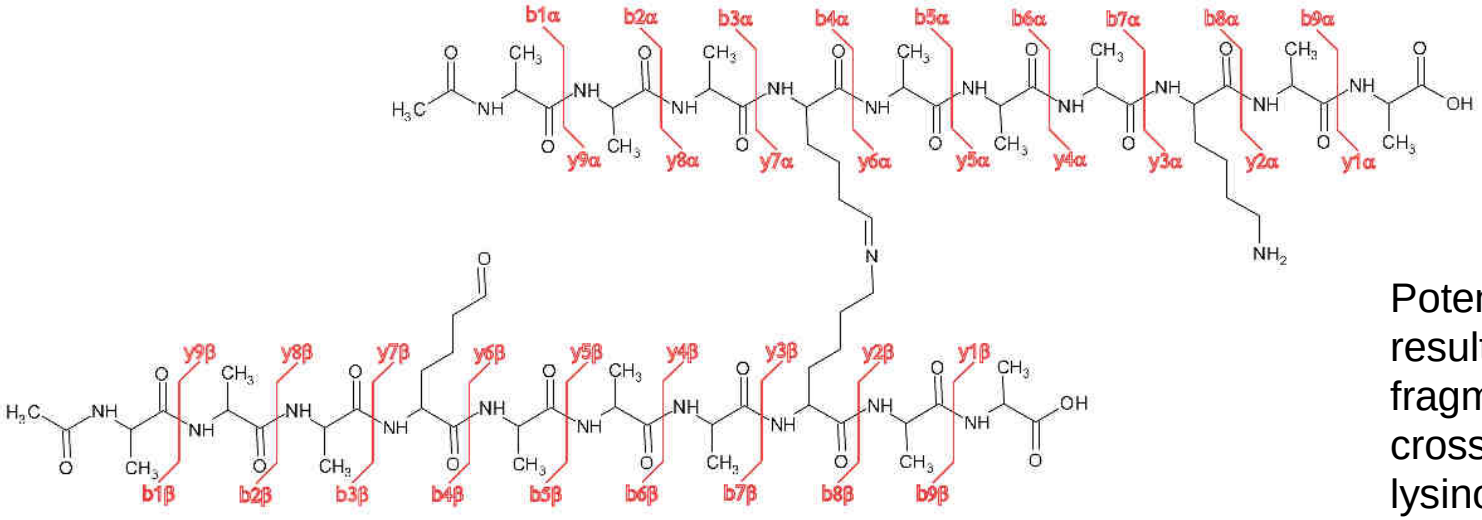


Travelling wave ion mobility

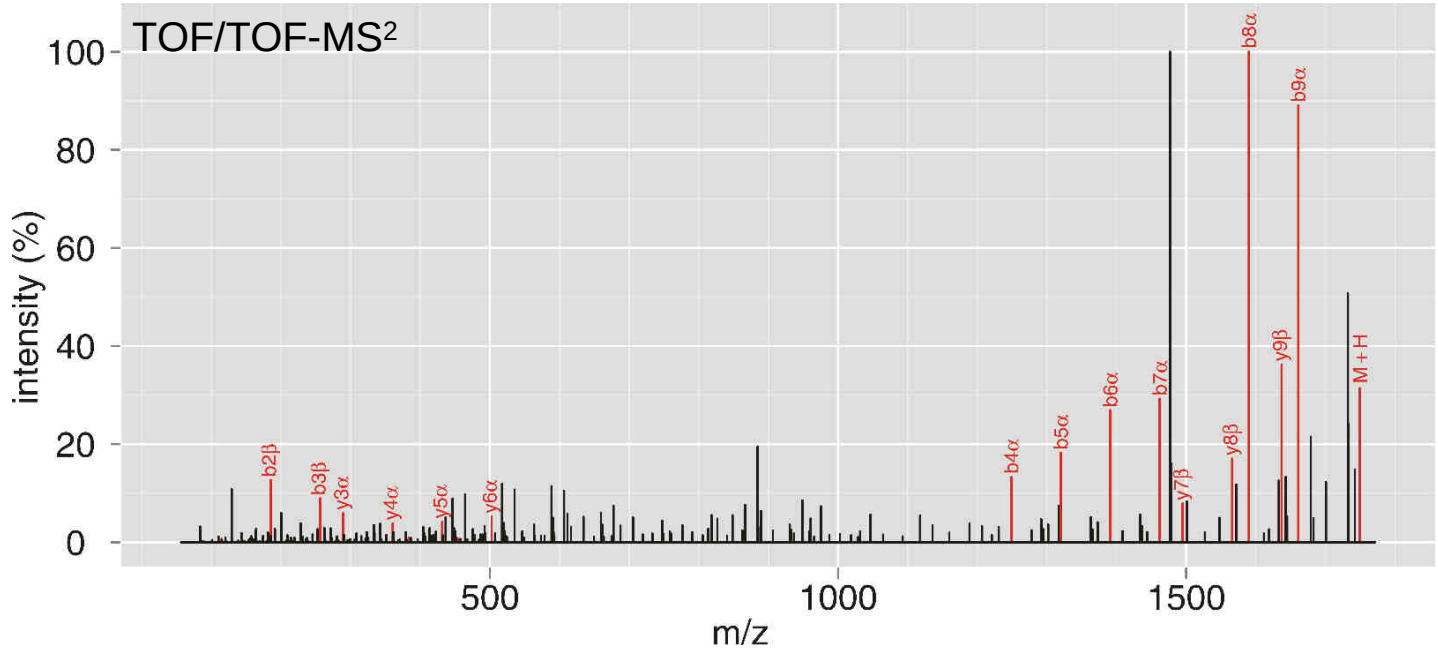




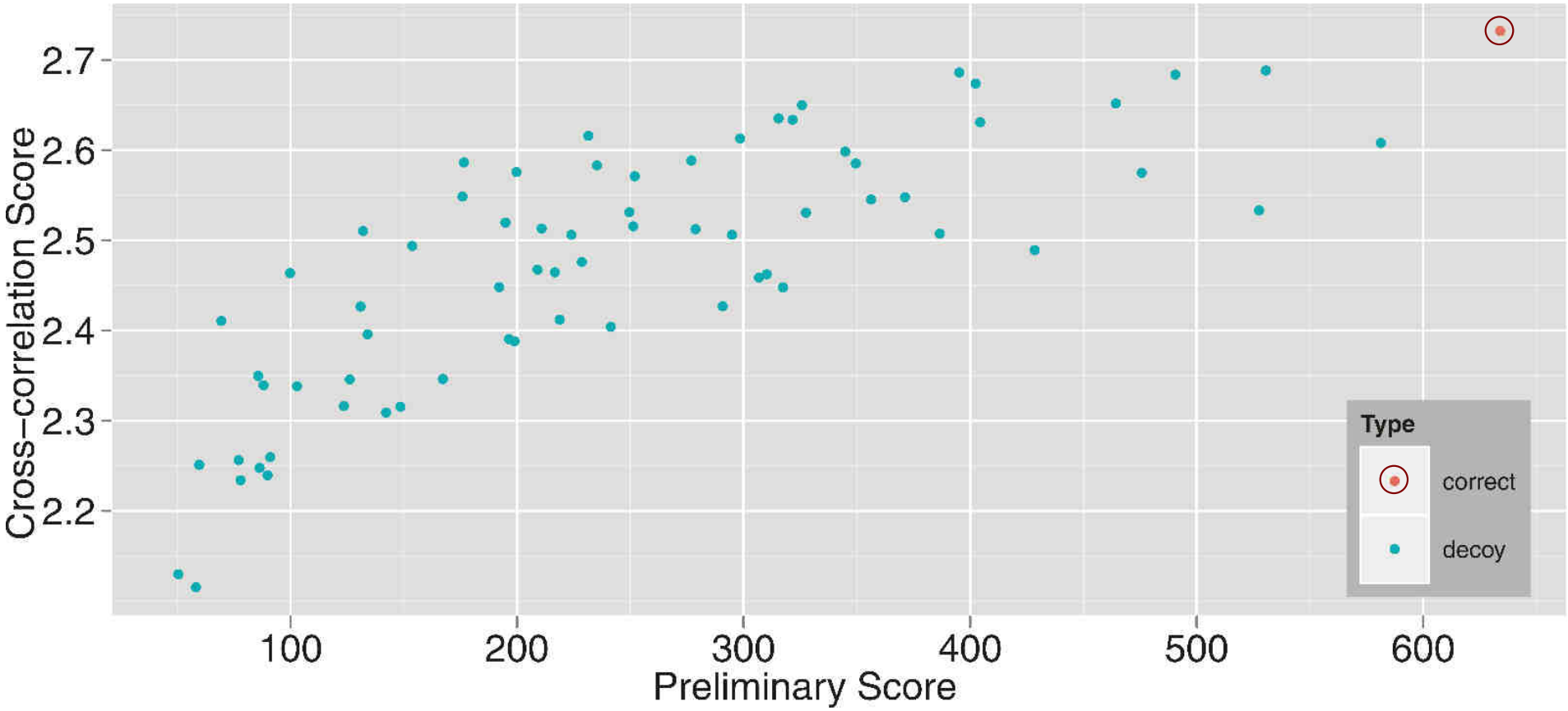
Transfer-Coll. 50 eV, Mobility TOF, MS/MS 884.55



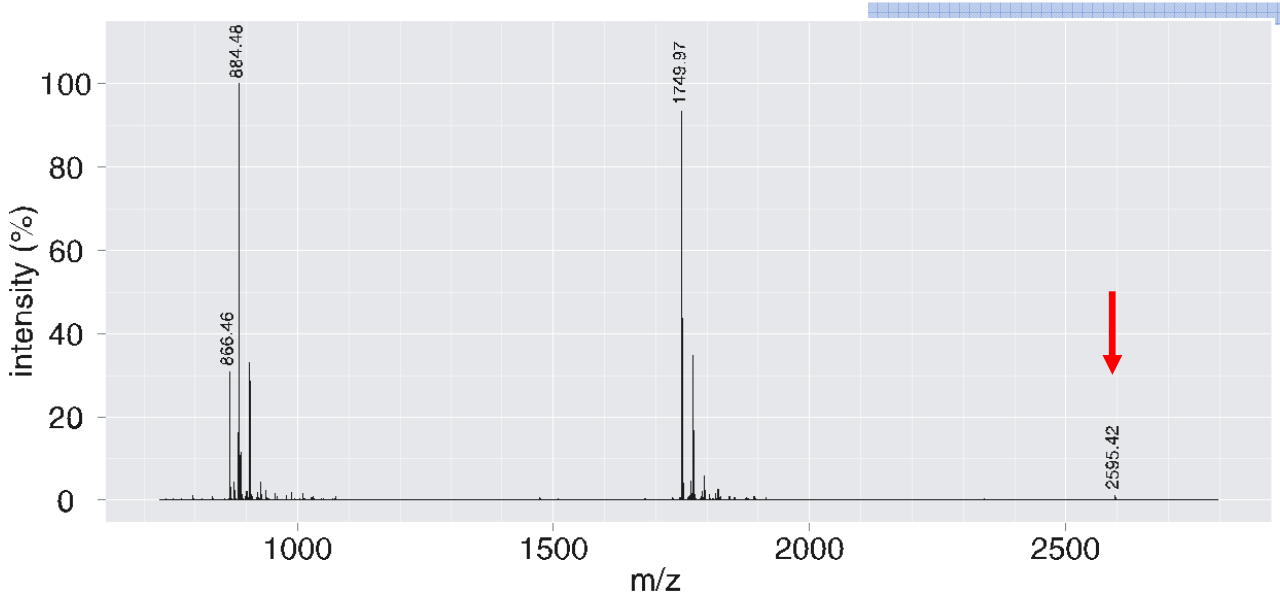
Potential b and y ions resulting from the fragmentation of a peptide cross-linked via dehydro-lysinonorleucine.



Labeled TOF/TOF fragment spectrum of cross-linked species containing dehydroly-sinonorleucine with a precursor mass of 1749.94 Da.

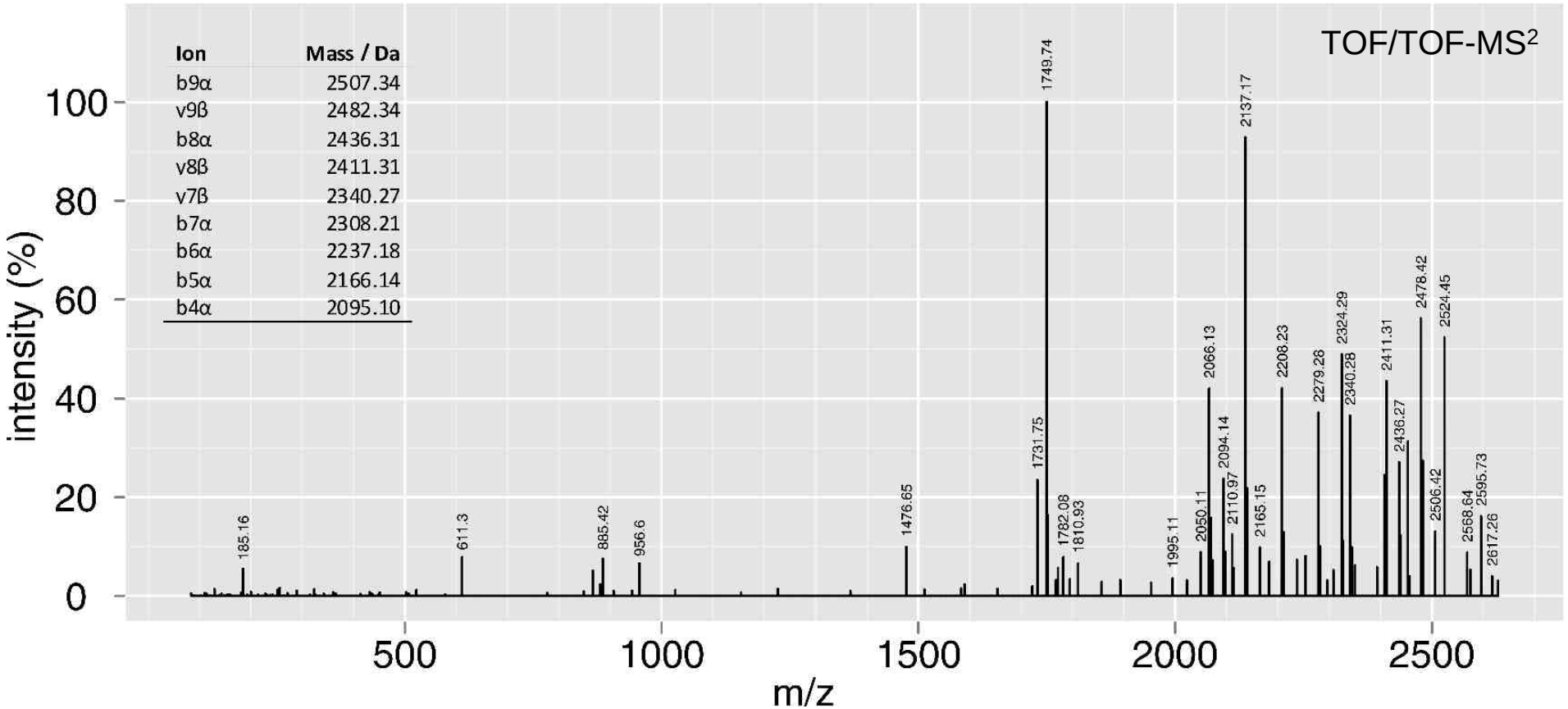


Scores for different cross-linked species containing dehydrolysinonorleucine. The score obtained for the correct peptide sequence is compared with scores obtained for its permutated decoy sequences.

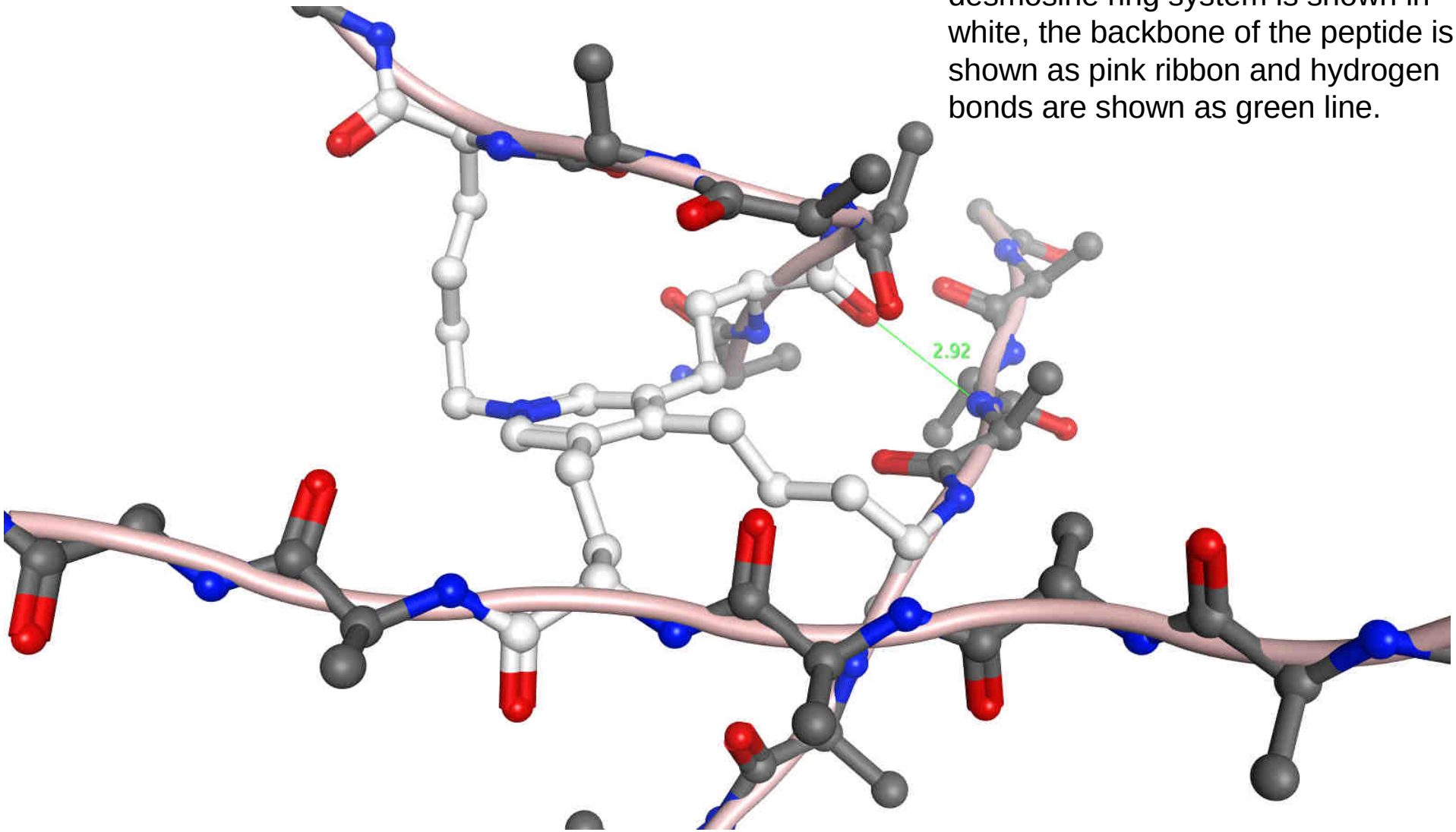


TOF/TOF-MS^2

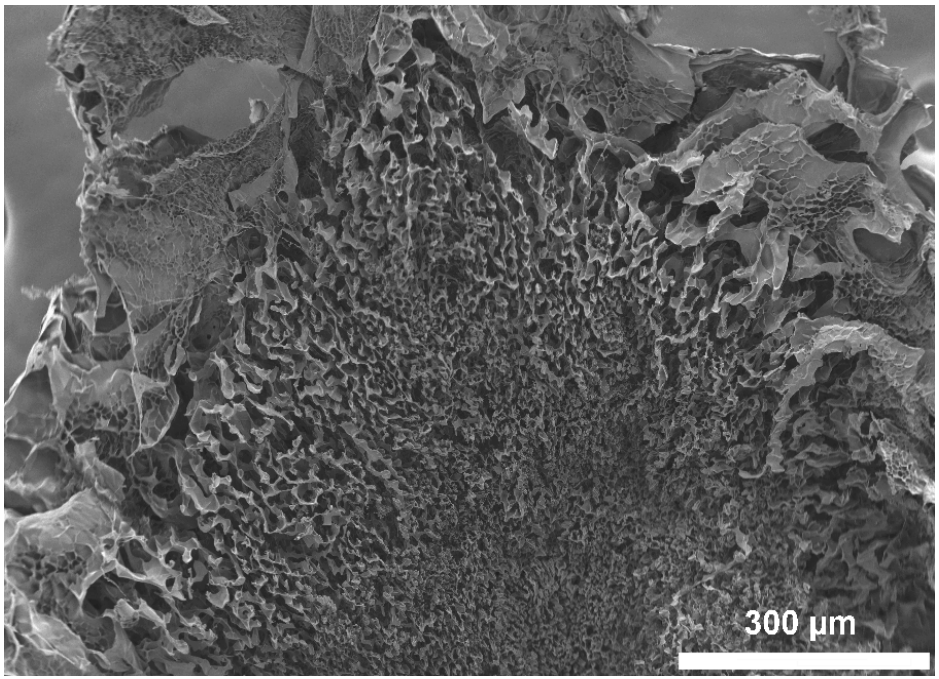
Ion	Mass / Da
b9α	2507.34
v9β	2482.34
b8α	2436.31
v8β	2411.31
v7β	2340.27
b7α	2308.21
b6α	2237.18
b5α	2166.14
b4α	2095.10

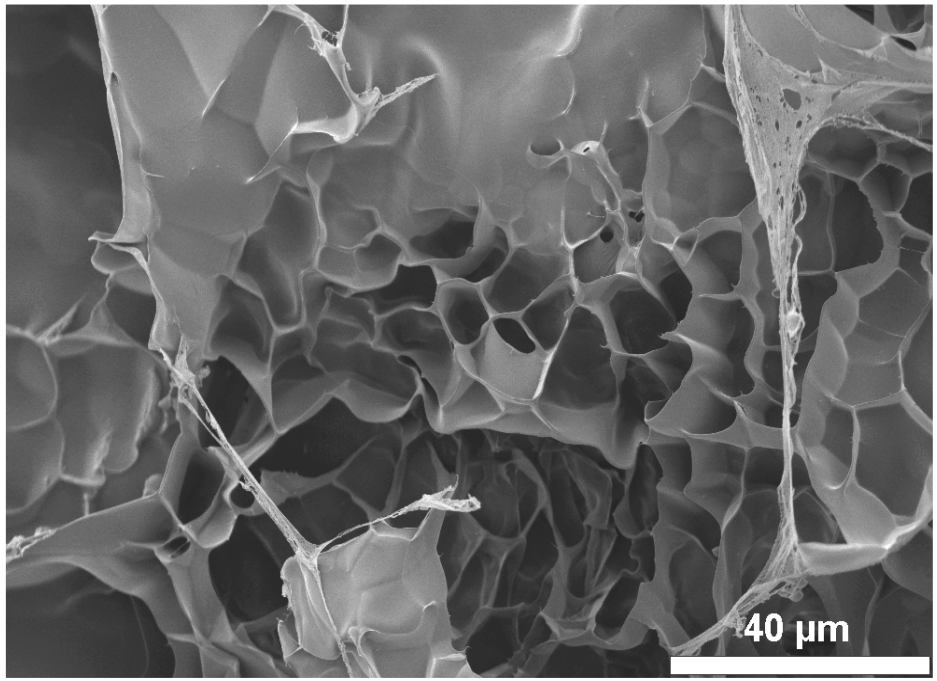


Minimized structure of a desmosine-containing peptide after 20 ns of molecular dynamics simulations using the AMBER program. The central desmosine ring system is shown in white, the backbone of the peptide is shown as pink ribbon and hydrogen bonds are shown as green line.

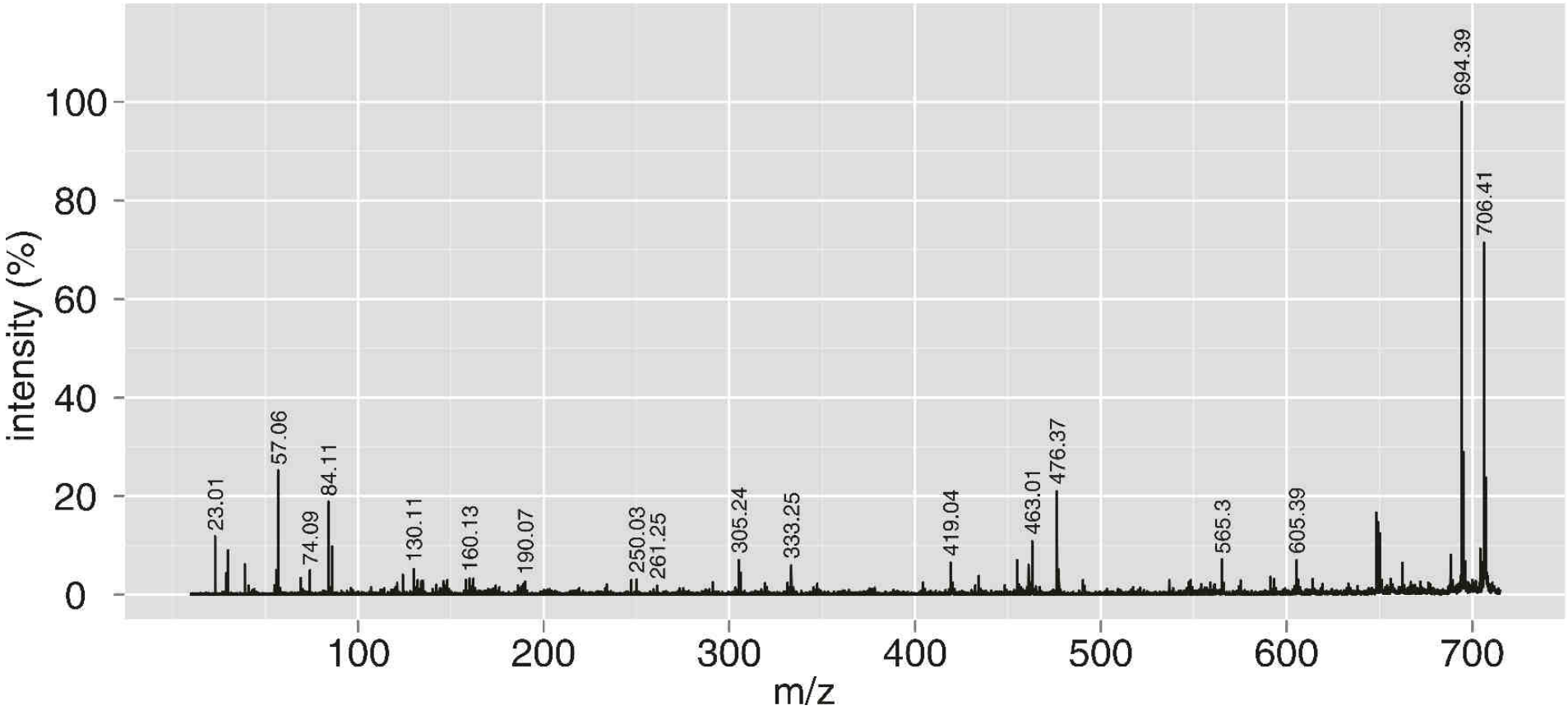


Cross-linked tropoelastin





A. P. McGrath, S. M. Mithieux, C. A. Collyer, J. G. Bakhuis, M. van den Berg, A. Sein, A. Heinz, C. Schmelzer, A. S. Weiss, and J. M. Guss, Biochemistry 50 (2011) 5718-5730.



MALDI TOF/TOF tandem mass spectrum of propionylated demosine ([M+H]+ 750.39) detected in ANAO cross-linked tropoelastin.

Summary

- Isolation of intact and pure elastic fibers from single biopsies allows for elastolytic studies *in vitro*
- Intact elastin from young individuals (< 40 years) cannot be degraded by human leukocyte elastase
- However, elastin from older patients is susceptible to HLE

With increasing age, elastic fibers lose their resistance against proteolytic degradation → increased turnover

- Clear differences in the cleavage pattern of elastin from patients of different ages

Molecular clocks

- Increased hydroxyproline levels for IVD elastin and decreased levels for elastin from WBS patients

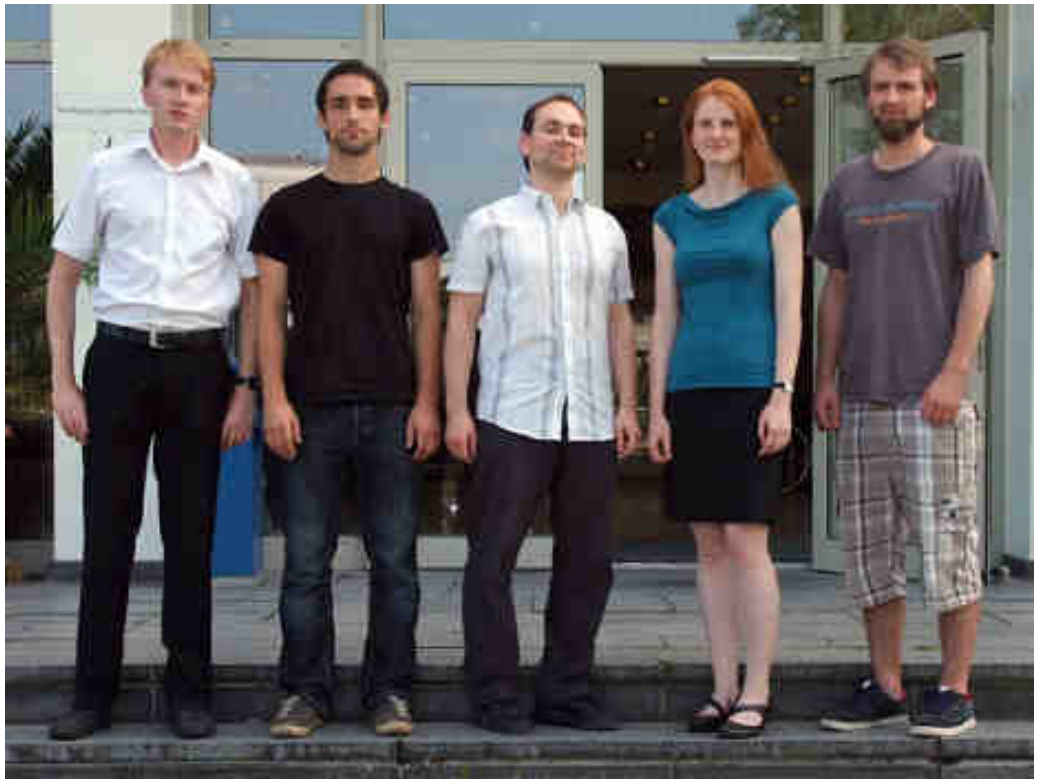
Summary

- Development of new algorithms for biomarker discovery taking into account common limitations and features of LC-MS measurements such as non-linear retention time shifts
- *In vitro* formation of dehydrolysinonorleucine and desmosine containing peptides

Scoring designed for the specific demands of the fragmentation pattern of cross-linked elastin peptides

- ➢ Insights into the cross-linking of tropoelastin monomers and elastin-degrading processes
- ➢ Development of directed therapies against elastin-degrading diseases
- ➢ Biomaterials (stents, soft tissue repair matrix)

Acknowledgements



Ch. Ruttkies Dr. A. Heinz M. Jung M. Scharm

- **Prof. Reinhard Neubert**
- **Prof. Johannes Wohlrab** (University Hospital, Halle)
- **Prof. Laurent Debelle, Dr. Laurent Duca** (Université de Reims)
- **PD Dr. Günther Jahreis** (Max Planck Research Unit for Enzymology of Protein Folding)
- **Prof. Wolfgang Sippl** (MLU Halle)
- **Prof. Anthony Weiss** (University of Sydney)
- **Prof. Rainer Pankau** (Heidekreis Hospital)
- **Dr. Sarit Sivan** (Israel Institute of Technology)

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