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Medizinische Fakultät Mannheim
der Universität Heidelberg

Universitätsklinikum Mannheim

Decay-markers for quality assesment of plasma- und serum specimens

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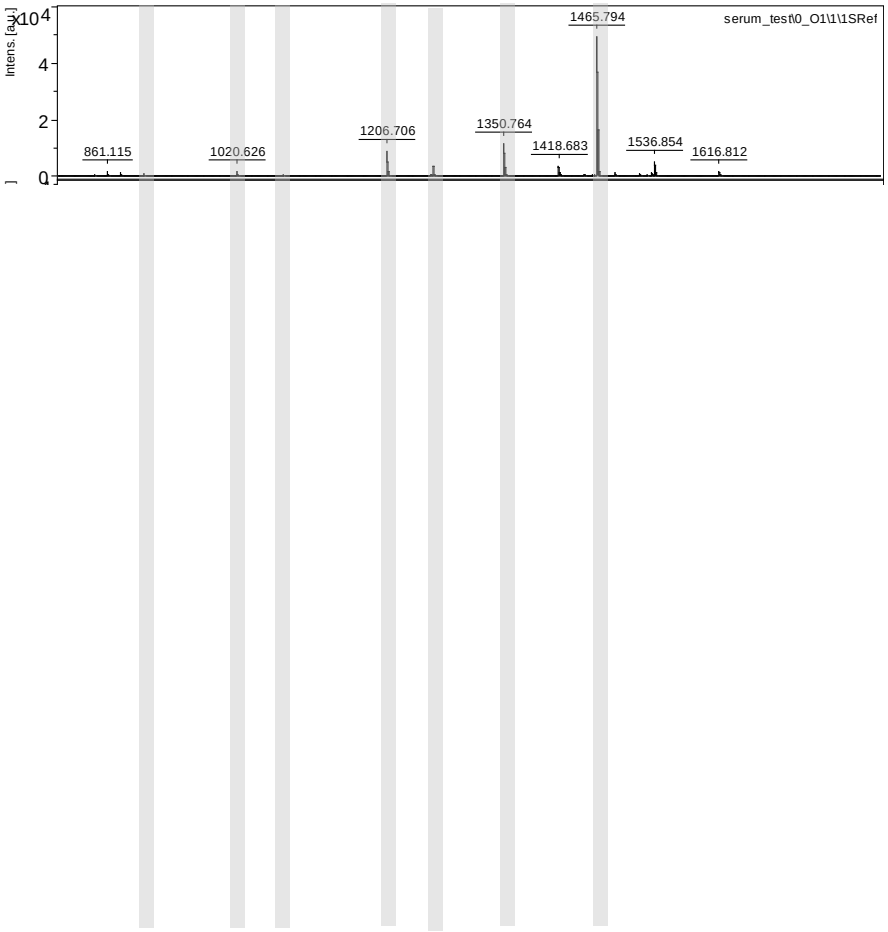
Limitations of MALDI-TOF MS Profiling

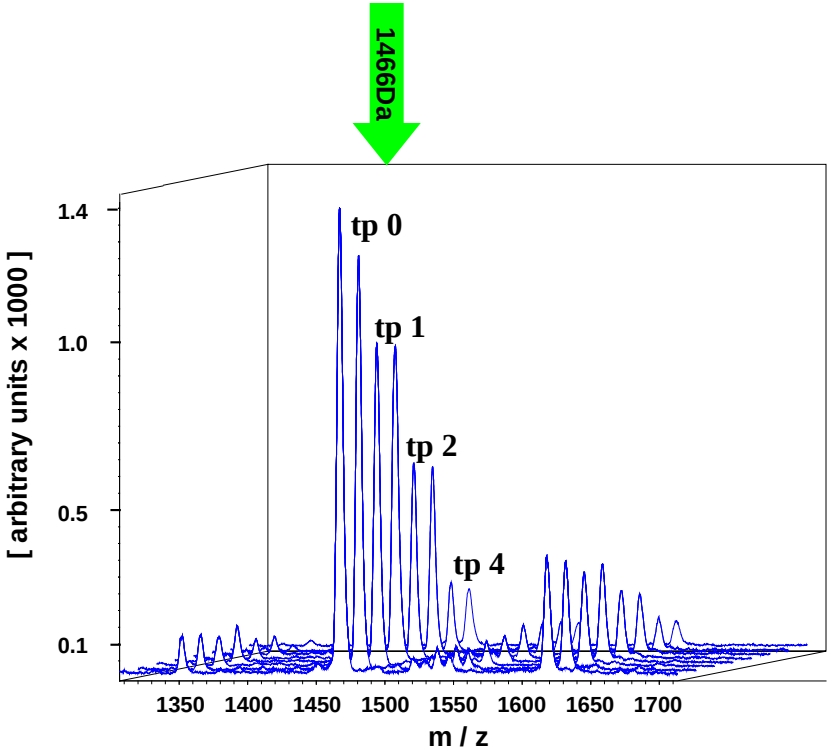
1h

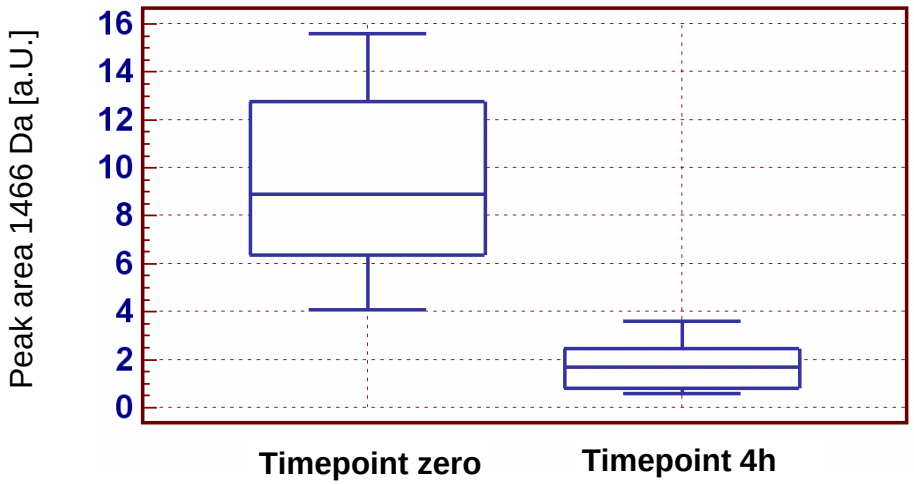


1466:	DSGEGDFLAEGGGVR
1351:	SGEGDFLAEGGGVR
1263:	GEGDFLAEGGGVR
1207:	EGDFLAEGGGVR
1077:	GDFLAEGGGVR
1021:	DFLAEGGGVR
906:	FLAEGGGVR

Findeisen et al. Clin Chem 2005.51(12):2409-11

Preanalytical classification





Findeisen et al, Clin.Chem. 2005

11 Plasmaproteins

Selection of 62 endogenous decy markers

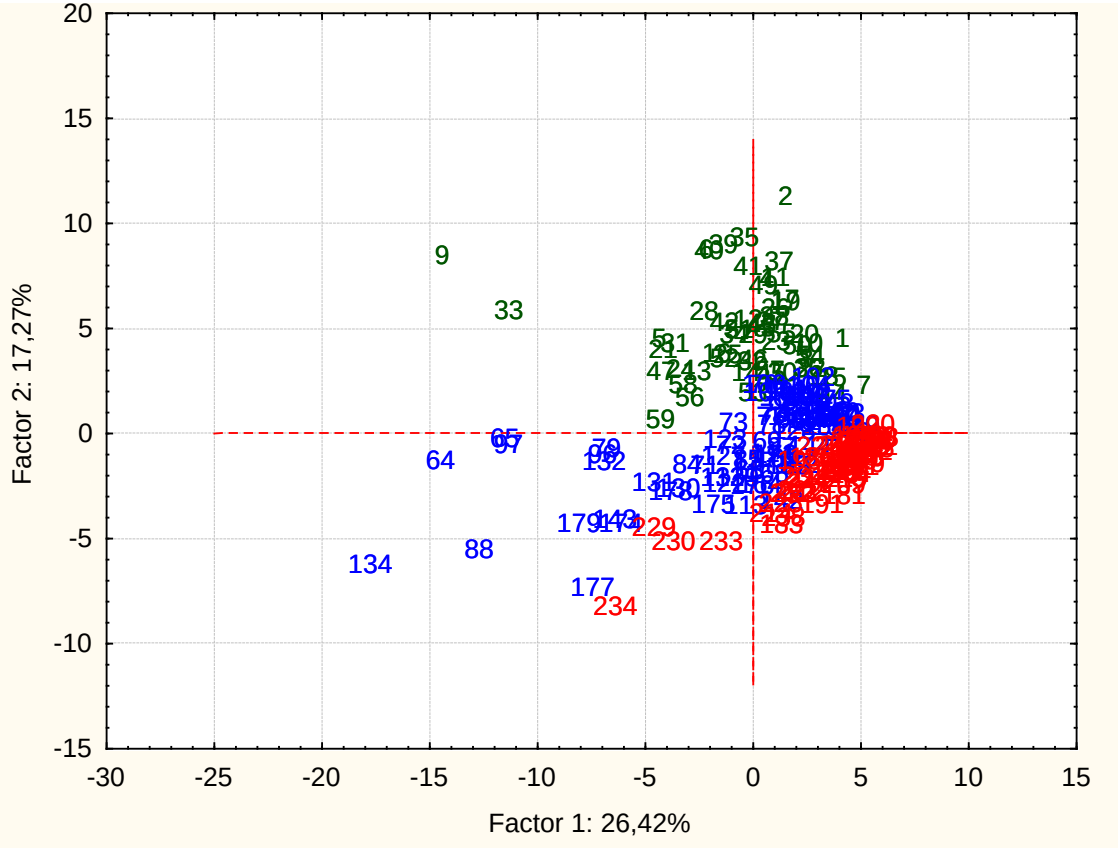
µmol/L		n		n
500-800[a]	Alb	009		001
2-5[f]	C4A	005		000
5-10[a]	C3	005		002
0.2-0.4[e]	F13	004		002
3 – 5[b]	F2	006		004
6-12[a]	FGA	100		031
- // -	FGB	019		013
2 - 4[a]	ITIH4	015		003
1 - 3[d]	KNG1	008		003
0.3-0.6[C]	TMSB4	012		002
	FUK	003		001
	Σ	**186**	selection*	**62**

[a]*Hortin et al. Clin Chem. 2006 Jul;52(7):1223-37*
[b]*Koomen et al. J Proteome Res. 2005 May-Jun;4(3):972-81.*
[c]*Hannappel J Chromatogr. 1987 Jun 26;397:279-85.*
[d] *Kleniewski et a. Thromb Haemost. 1979 Oct 31;42(3):1046-55.*
[e] *Katona et al. Thromb Haemost. 2000 Feb;83(2):268-73.*
[f] *Gorski et al. J Immunol Methods. 1981;47(1):61-73.*

*highS/N ratio
highly reproducible ionisation status

Principle Component Analysis

Hauptkomponentenanalyse
62 Variablen (Marker); 244 Proben, 3 Qualitätsklassen



High: 0-2h

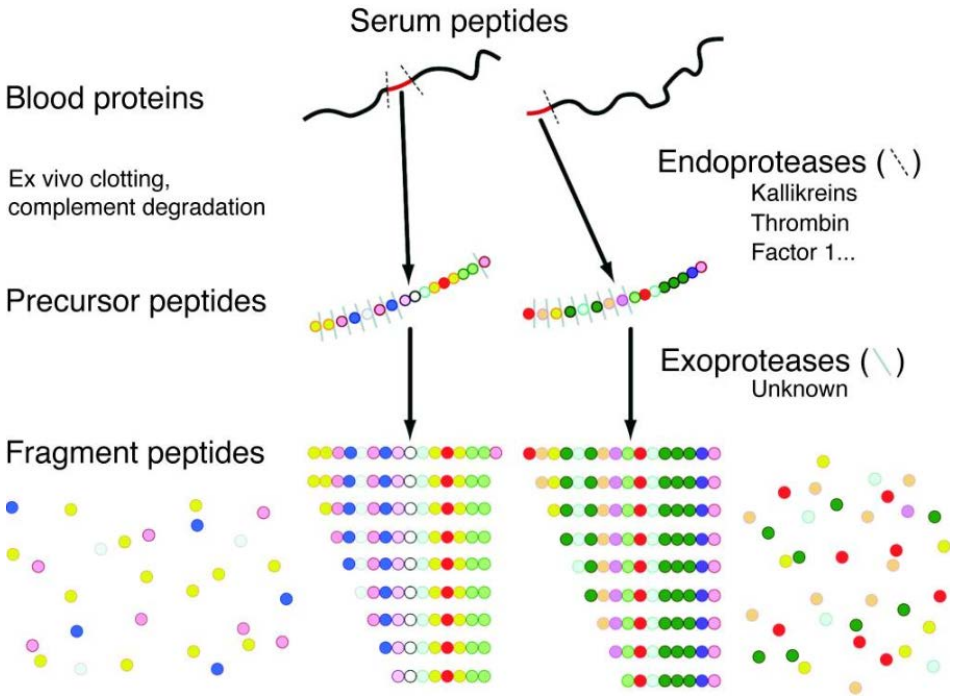
Medium: 4-9h

Low: 22-30h

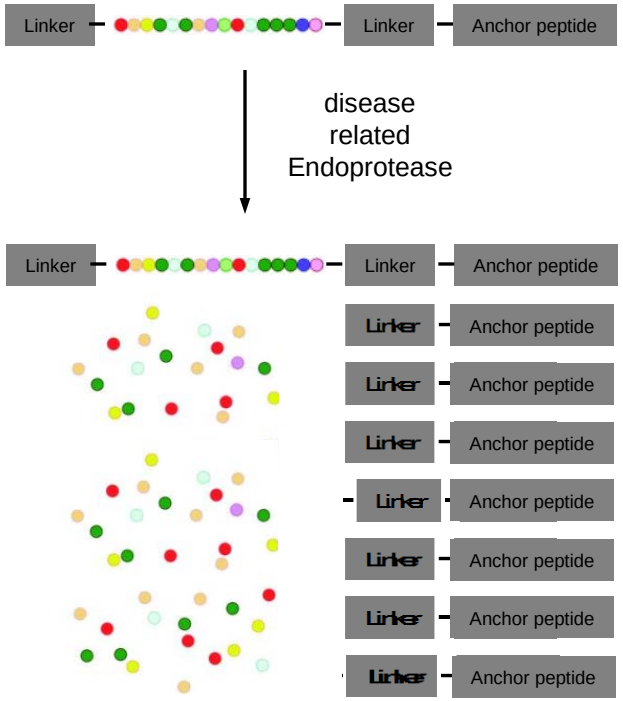
Monitoring proteolytic activity

endogenous verus exogenous decay markers

Endogenous reporter peptides



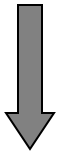
Exogenous reporter peptides



Adopted from Villanueva et al. J Clin Invest. 2006;116(1):271-84

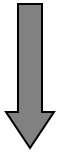
Study Design

15 Healthy Controls
15 CRC Tumor Patients



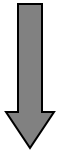
Serum and EDTA Plasma

Reporter Peptide was added prior to blood withdrawal



Storage at room temperature

2h 5h 8h 24h



Liquid chromatography / mass spectrometry

Quantification of RP-fragments

Exogenous Decay Marker (Reporter Perptide)

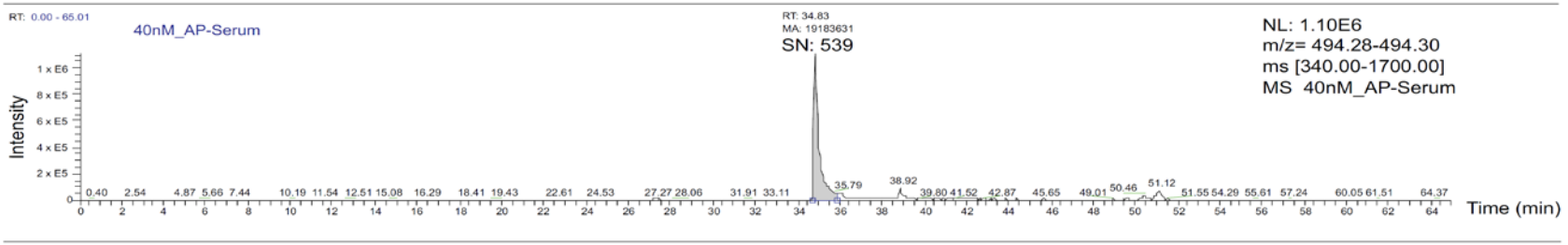
The `proteomic degradation clock`

Name	Sequence	m/z $[M+2H]^{2+}$
Reporter Peptide (RP)	LSLQKPRL-Ahx-ateelkal	
	SLQKPRL-Ahx-ateelkal	
	LQKPRL-Ahx-ateelkal	
	QKPRL-Ahx-ateelkal	
Intermediate Fragment (RP-4)	KPRL-Ahx-ateelkal	741,46
	PRL-Ahx-ateelkal	
	RL-Ahx-ateelkal	
	L-Ahx-ateelkal	
Anchor Peptide (AP)	Ahx-ateelkal	494,29

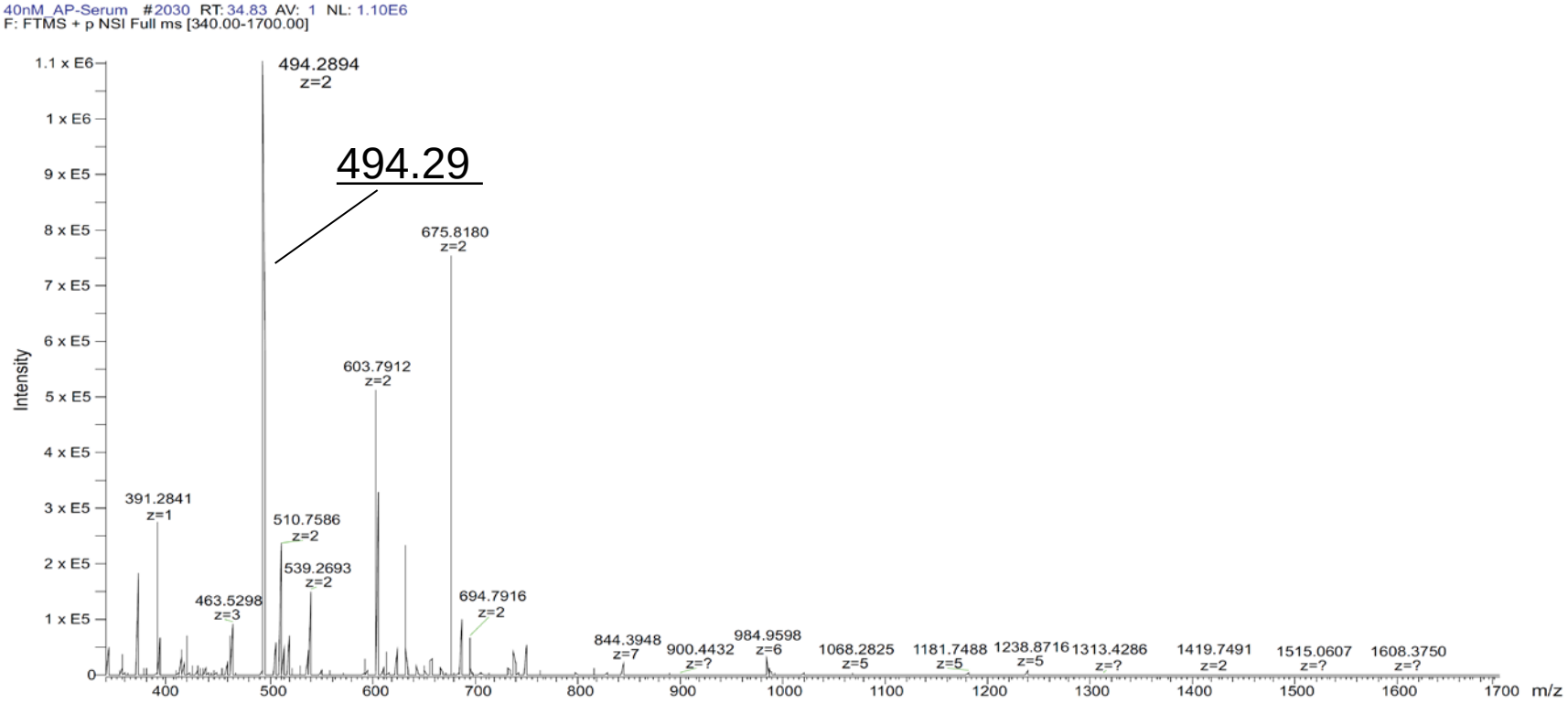
Findeisen et al. 2013. Am J Clin Pathol 2013;140:314-323

LC-MS of anchor peptide

A



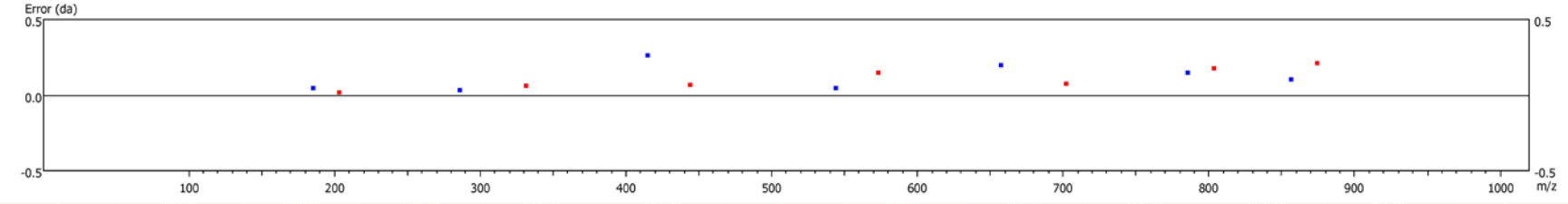
B

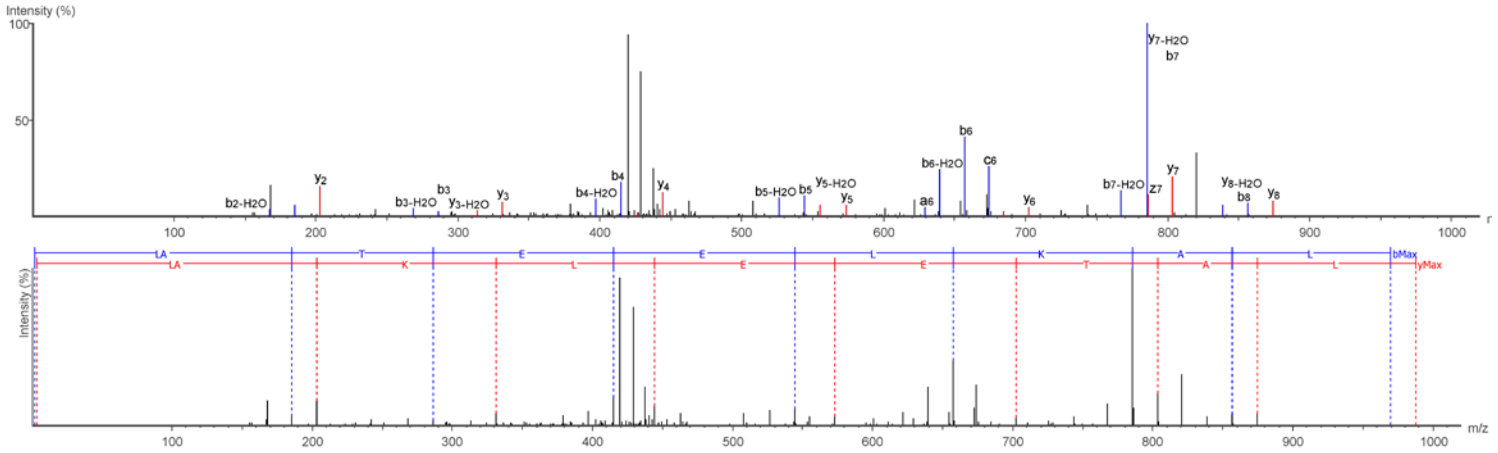


Sequence confirmation

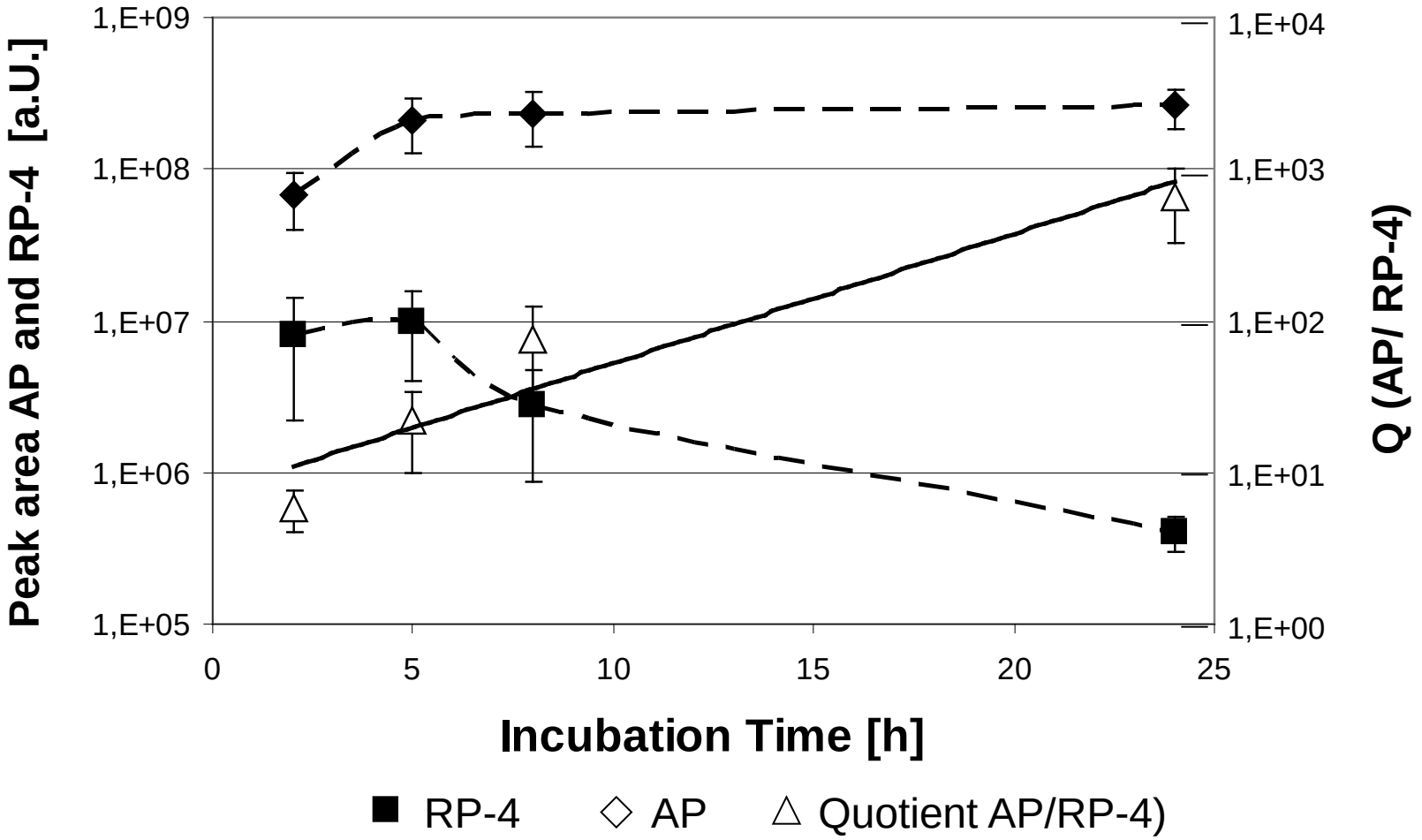
Anchor peptide: AHX-ateelkal

#	Immonium	b	b-H2O	a	c	Seq	y	y-H2O	z	z'	y (2+)	#
1	86.10	114.09	96.08	86.10	131.12	L						9
2	44.05	185.08	167.14	157.13	202.16	A	874.27	856.37	857.46	858.46	437.74	8
3	74.06	286.14	268.22	258.18	303.20	T	803.27	785.29	786.46	787.42	402.23	7
4	102.06	414.95	397.14	387.22	432.25	E	702.32	684.39	685.38	686.38	351.70	6
5	102.06	544.21	526.14	516.27	561.29	E	573.21	555.27	556.33	557.33	287.18	5
6	86.10	657.14	639.32	629.33	674.30	L	444.25	426.60	427.29	428.29	222.66	4
7	101.11	785.29	767.32	757.45	802.47	K	331.17	313.29	314.21	315.21	166.12	3
8	44.05	856.37	838.40	828.48	873.51	A	203.12	185.08	186.11	187.11	102.07	2
9	86.10					L	132.10	114.09	115.07	116.07	66.55	1



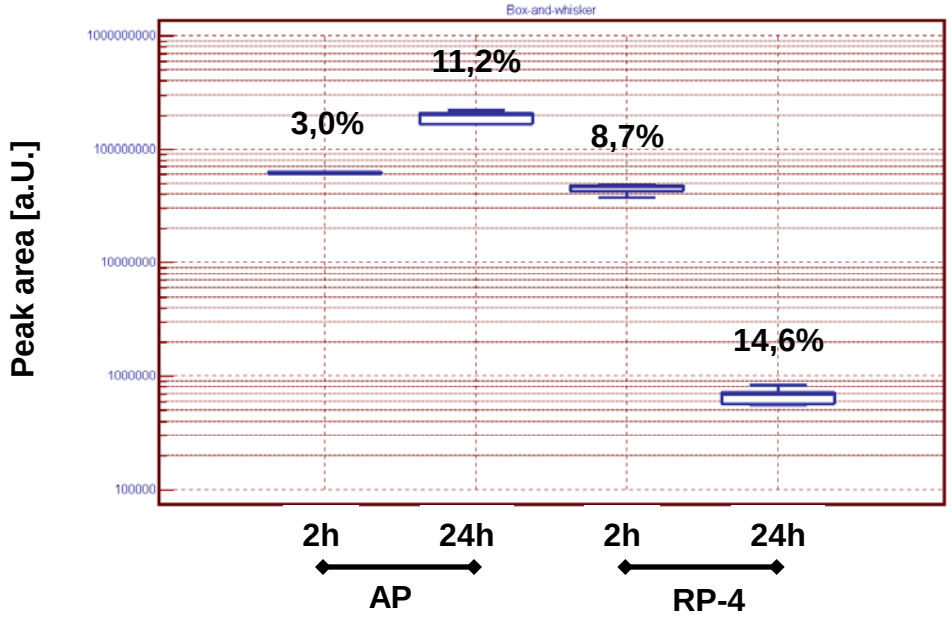


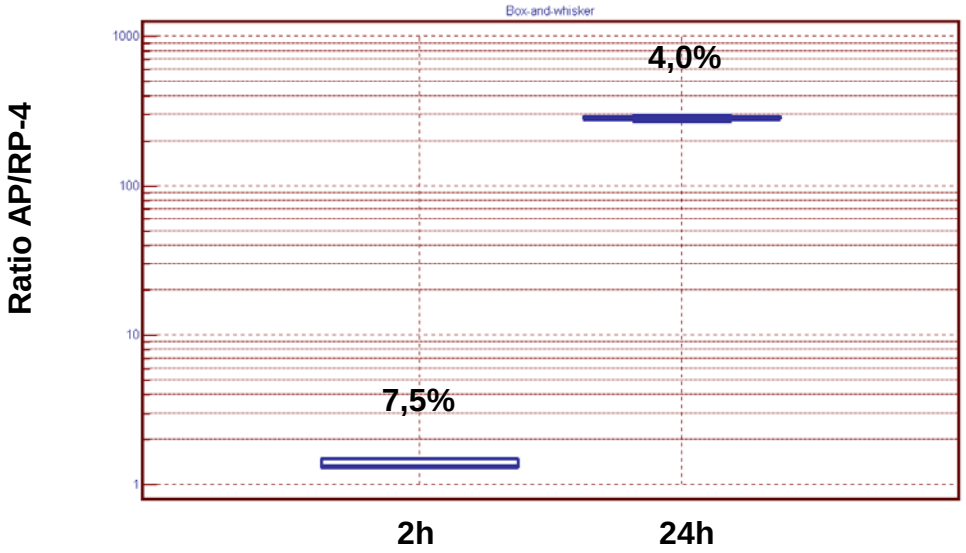
Decay Kinetics



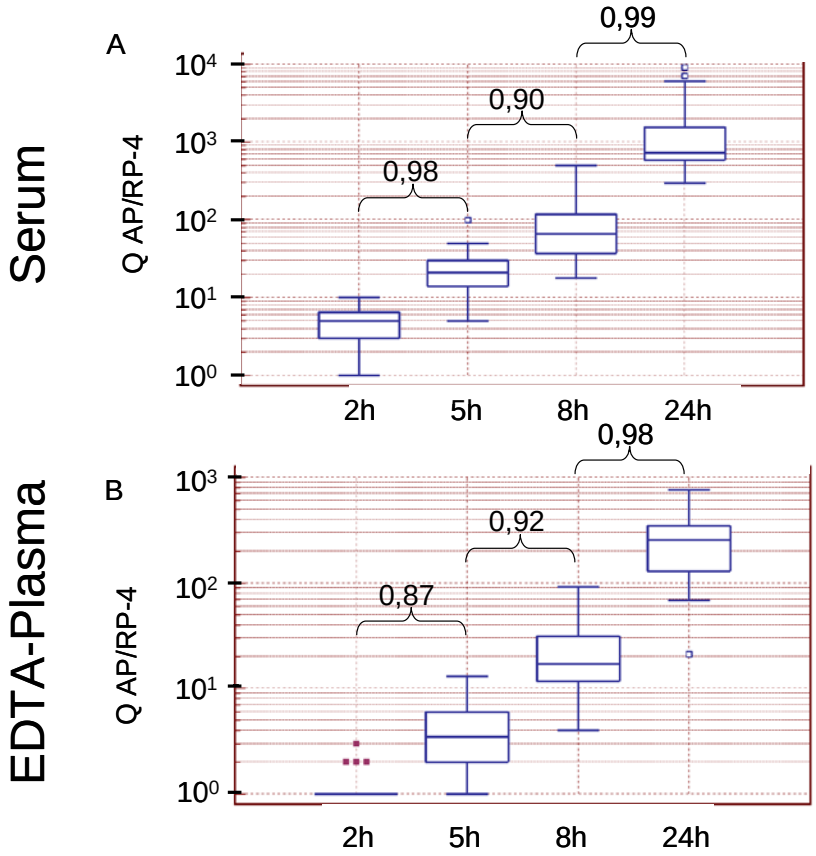
Findeisen et al. 2013. Am J Clin Pathol 2013;140:314-323

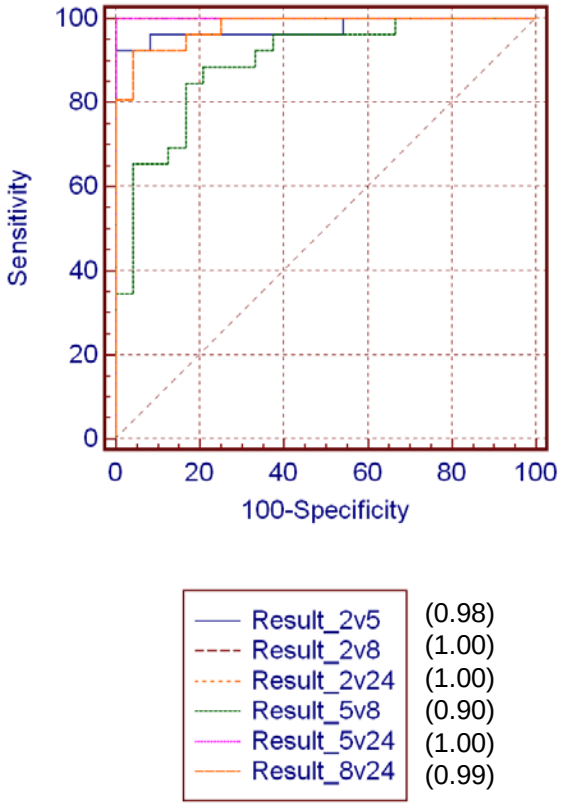
Reproducibility





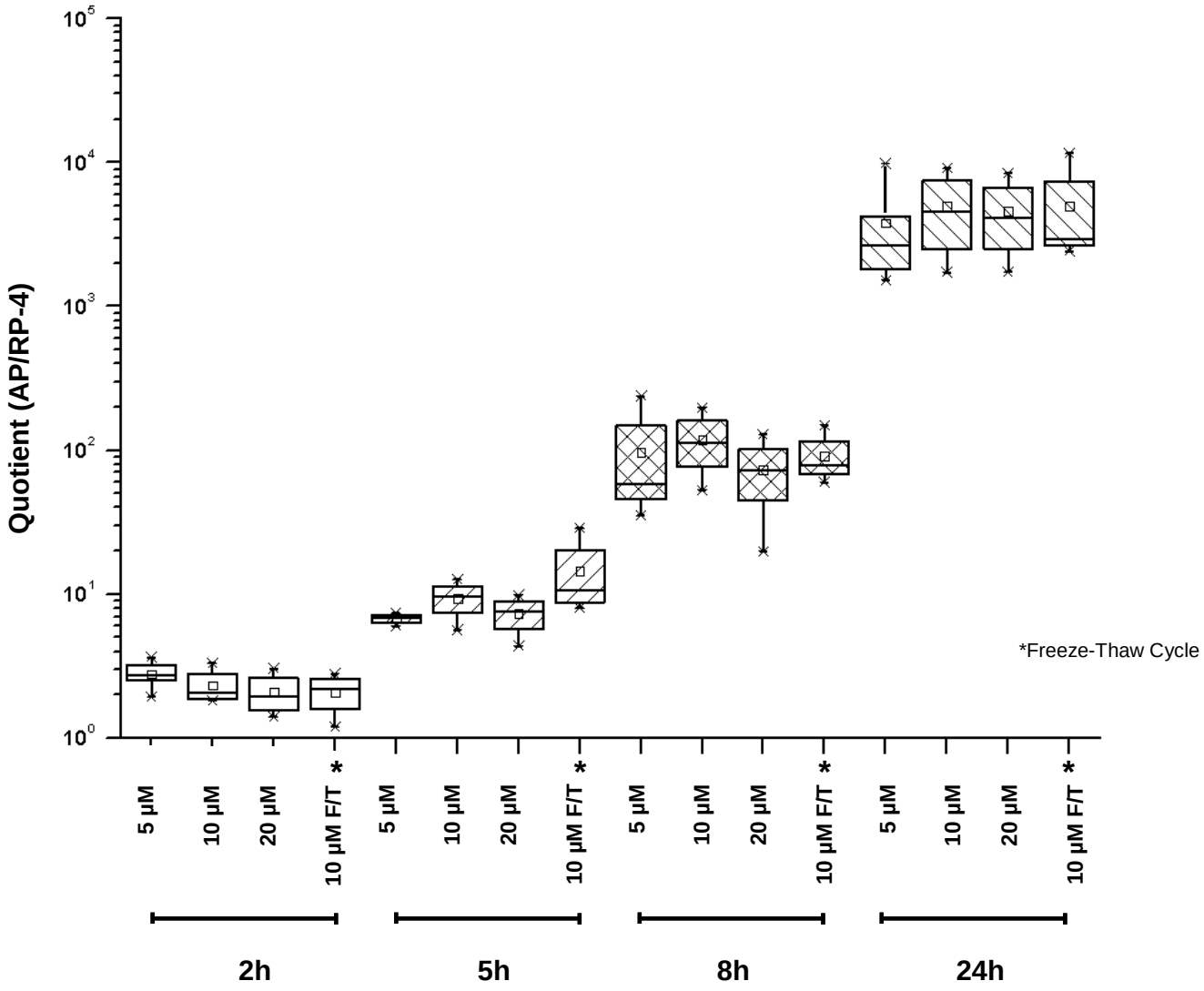
Classification



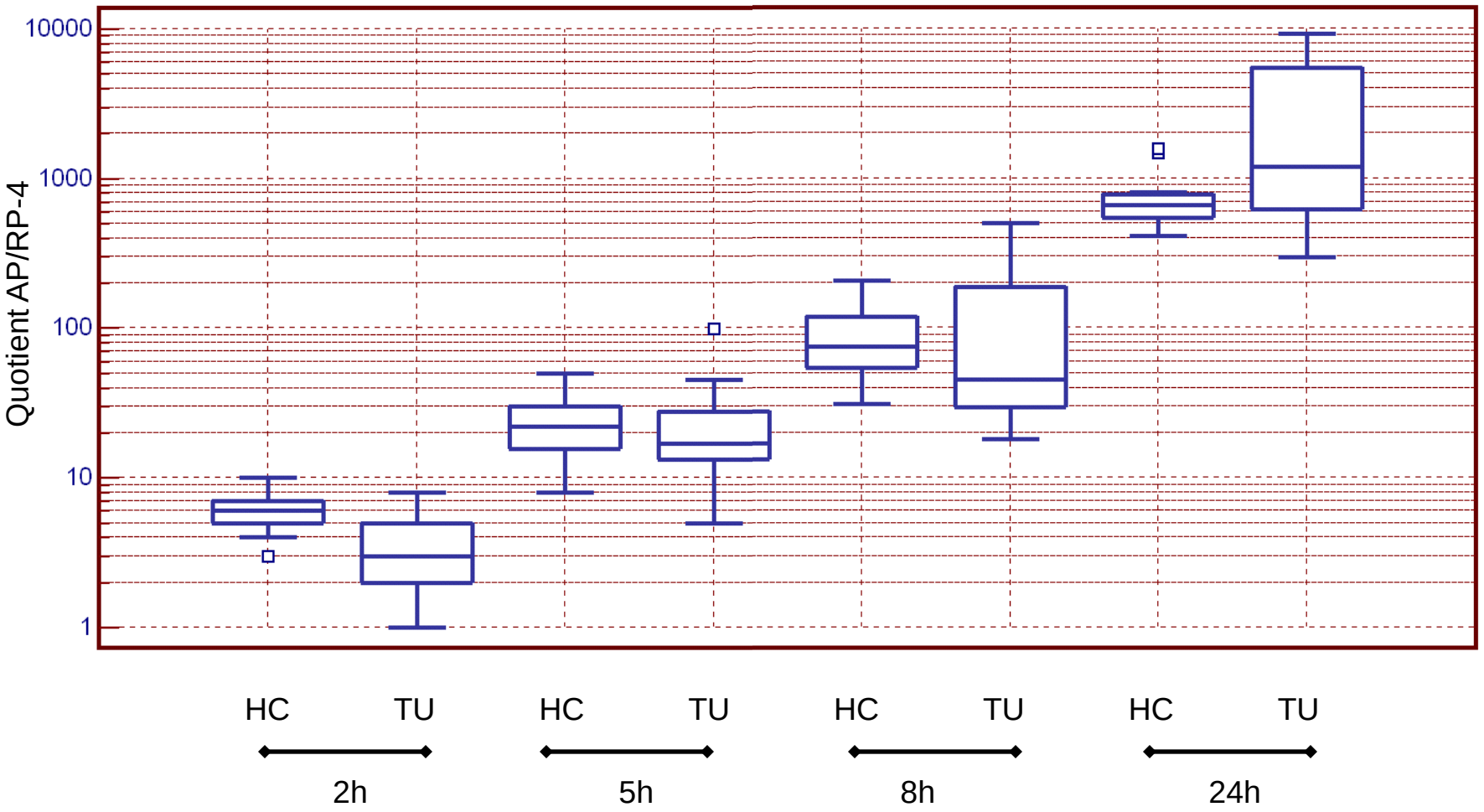


Findeisen et al. 2013. Am J Clin Pathol 2013;140:314-323

RP concentration and freeze-thaw Cycle

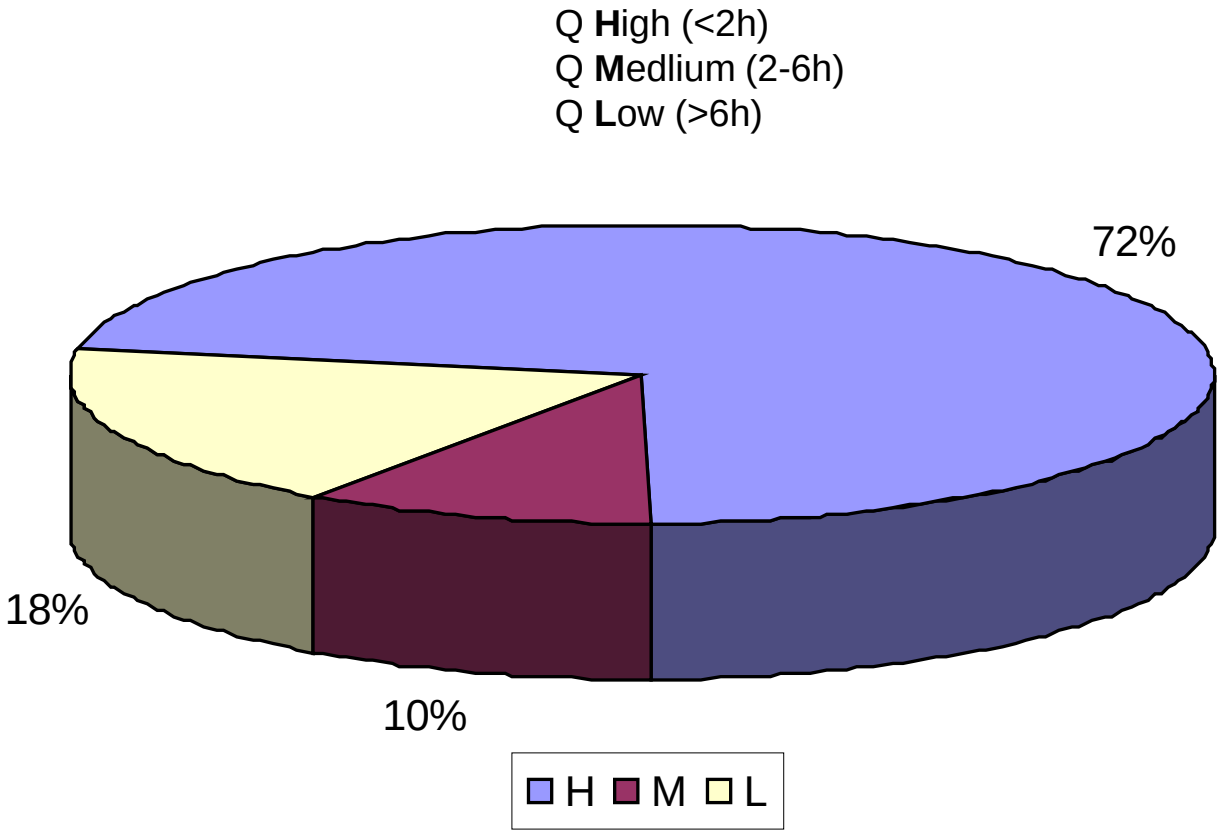


Healthy controls versus tumor patients



Exemplary application

Exclusion of samples with low quality prior to IL6 measurements



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Weh et al. Diagn Microbiol Infect Dis. 2013;77(1):79-84

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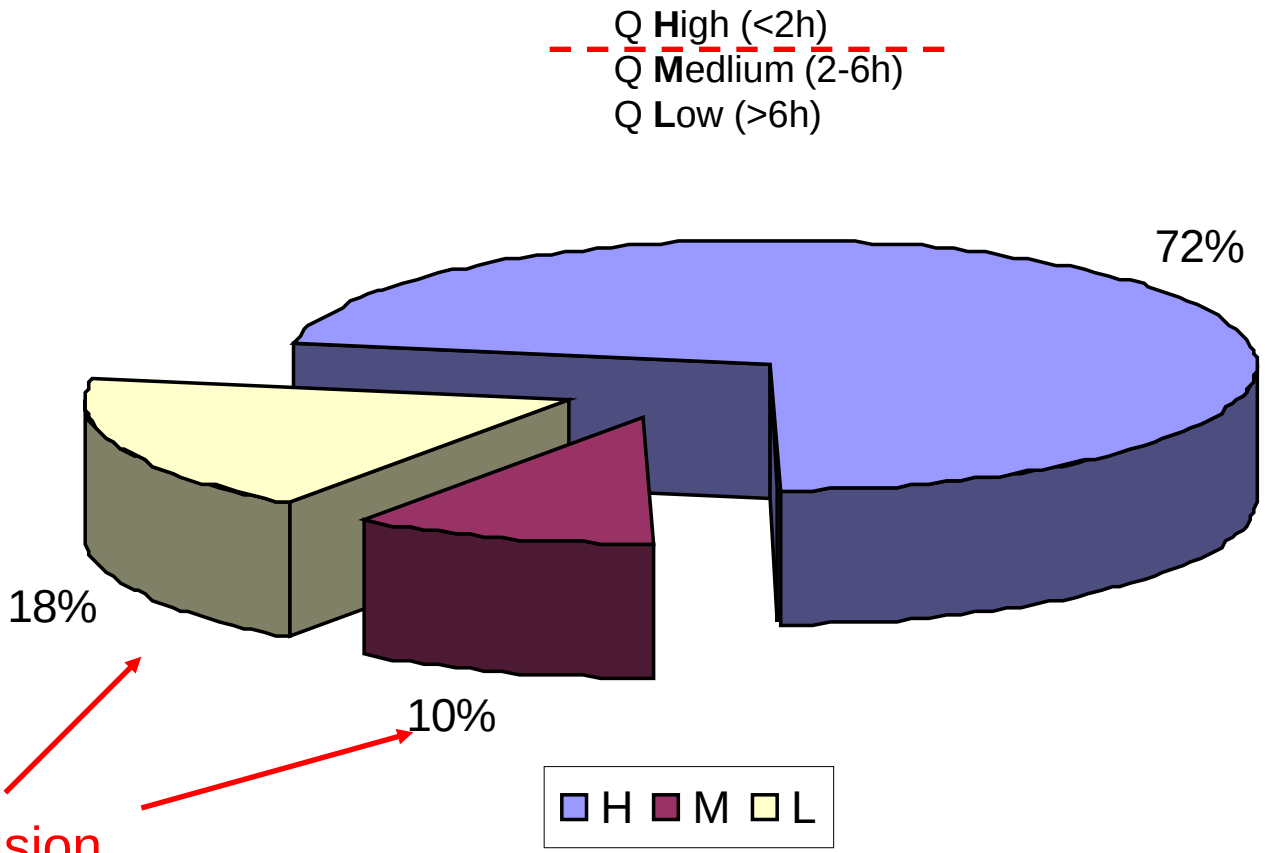

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Conclusion

PROS

- Endo– and exogenous reporter peptides can be used as decay markers
- Targeted peptidomics of proteolytic fargemts via LC-MS
- Preanalytical monitoring of serum and plasma specimens is feasible
- RP decay is the effect of housekeeping aminopeptidases (no relation to disease status)
- Aminopeptidases are not sensitive to freeze-thaw cycles
- External quality assesment for Biobanks

CONS

- High interindividual variabilizy of endogenous decay markers
- Only prospective study design (RP spiking of tubes before blood withdrawal)
- Time resolution might not be sufficient
- Possible interference of exogenous RPs with downstream assays

- Long term storage?

Acknowledgement

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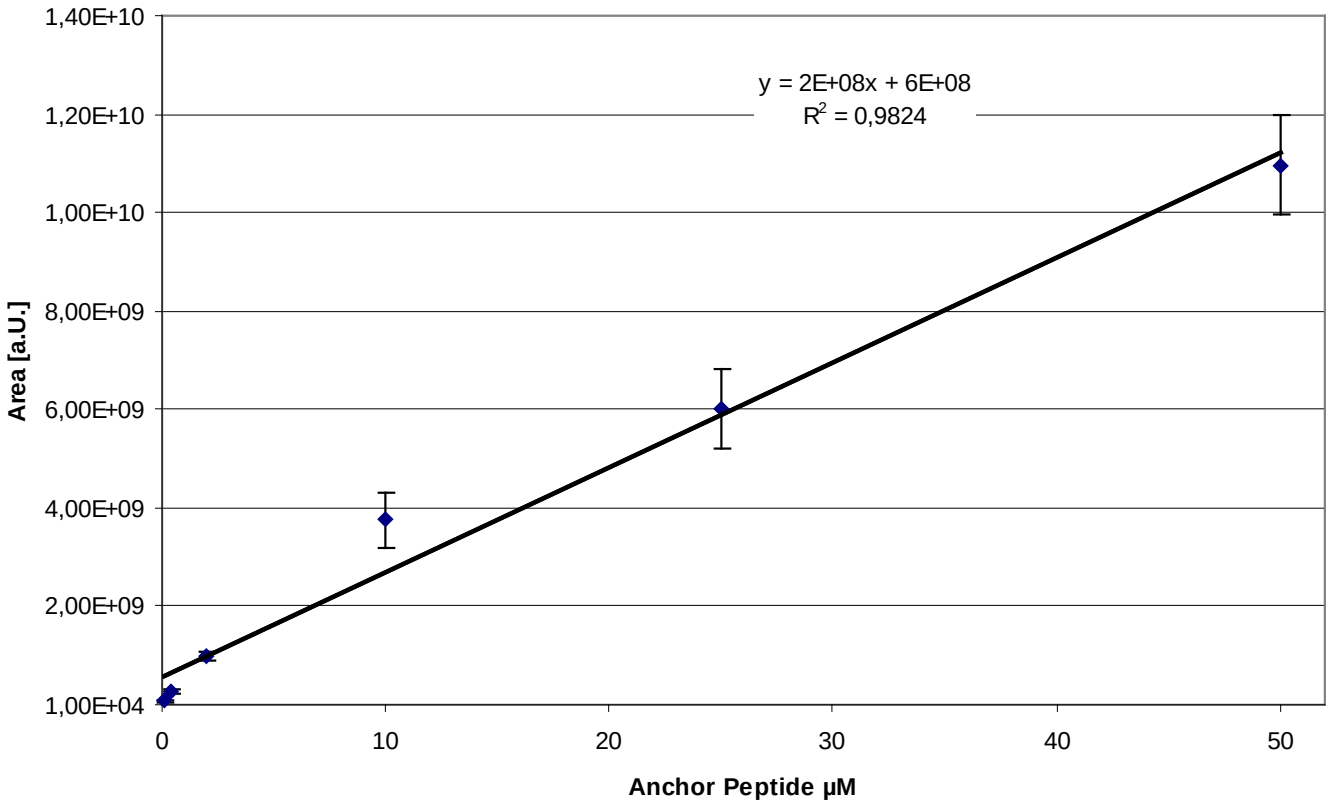
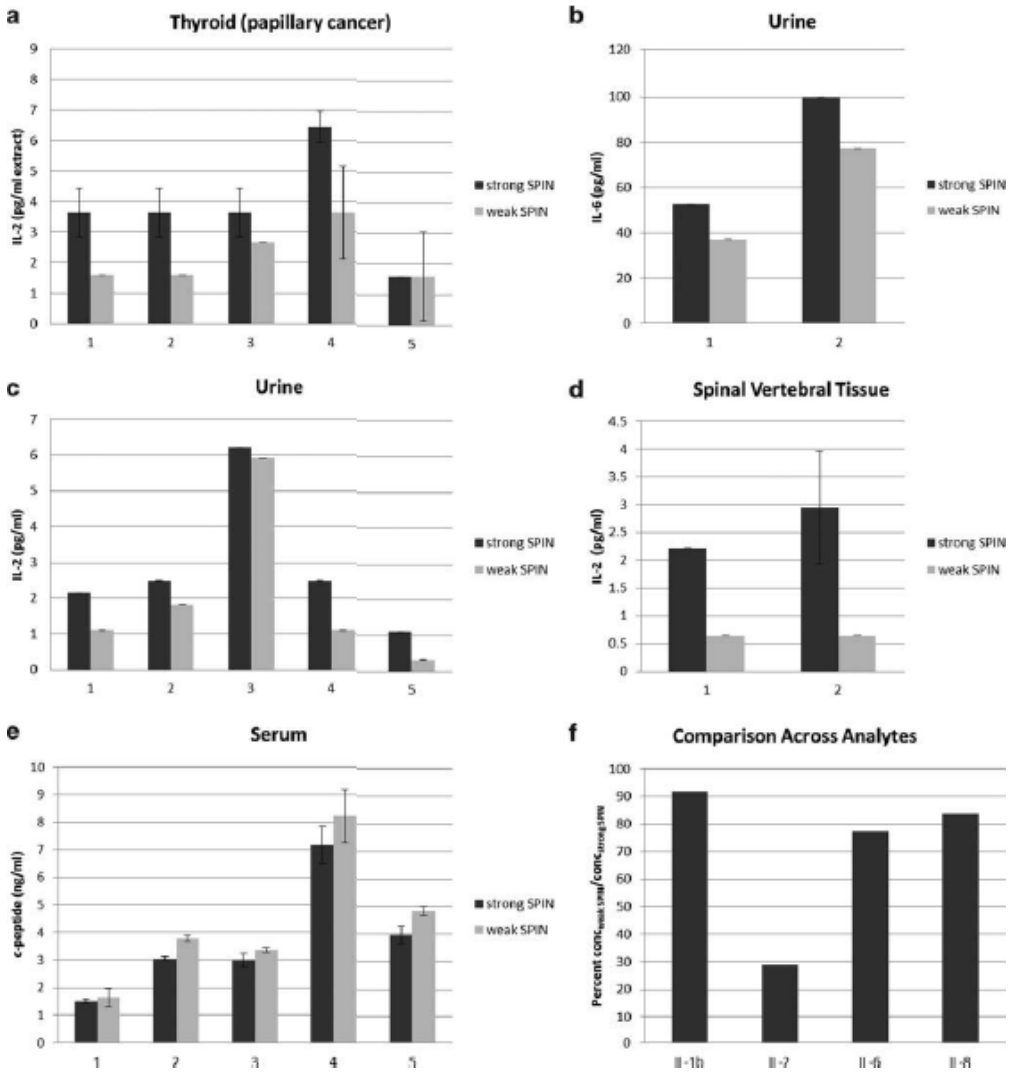
Bundesministerium für Bildung und Forschung

DFG Deutsche Forschungsgemeinschaft

Linearity


y = 2E+08x + 6E+08
R2 = 0,9824
1,40E+10
1,20E+10
1,00E+10
8,00E+09
6,00E+09
4,00E+09
2,00E+09
1,00E+04
Area [a.U.]
0
10
20
30
40
50
Anchor Peptide µM

Sample Quality ~ Analyte Concentration



Geddes et al. 2013. Biopreservation and Biobanking.11(1):25-32

Beispiel Peptid-Degradation FGA

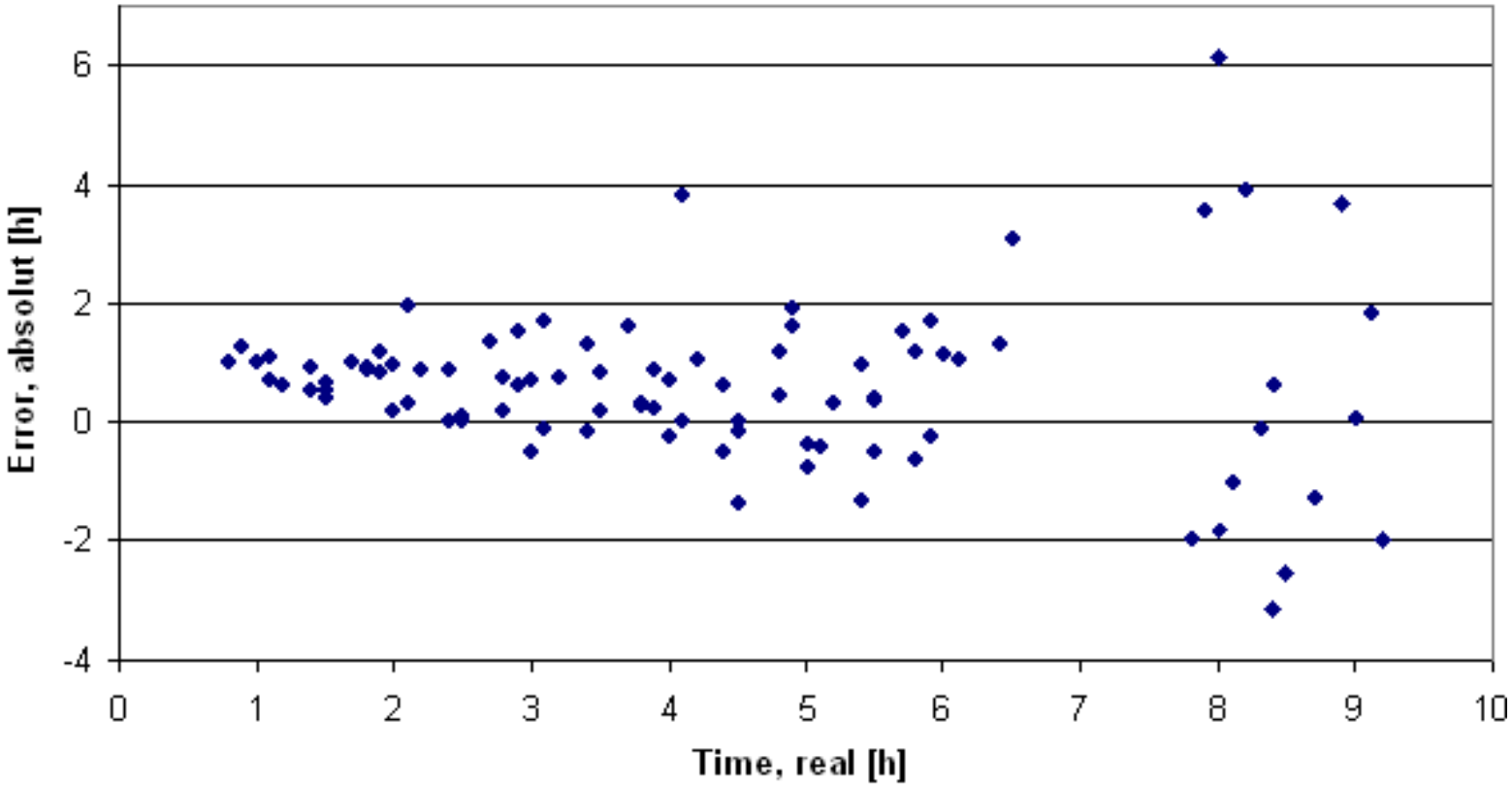
aa pos Anfang	aa pos ende	Protein	AA-Sequenz	M [Da]
21	35	FGA	A.DSGEGDFLAEGGGVR.G	1466,7
22	35	FGA	D.SGEGDFLAEGGGVR.G	1351,6
23	35	FGA	S.GEGDFLAEGGGVR.G	1264,6
24	35	FGA	G.EGDFLAEGGGVR.G	1207,6
26	35	FGA	E.GDFLAEGGGVR.G	1078,5
27	35	FGA	G.DFLAEGGGVR.G	1021,5
28	35	FGA	D.FLAEGGGVR.G	906,5
29	35	FGA	F.LAEGGGVR.G	759,4

N-Terminus

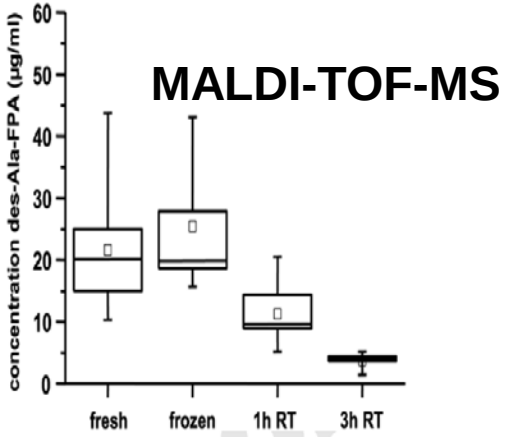
576	598	FGA	K.SSSYSKQFTSSTSYNRGDSTFES.K	2555,1
576	595	FGA	K.SSSYSKQFTSSTSYNRGDST.F	2192,0
576	594	FGA	K.SSSYSKQFTSSTSYNRGDS.T	2090,9
576	593	FGA	K.SSSYSKQFTSSTSYNRGD.S	2002,9
576	592	FGA	K.SSSYSKQFTSSTSYNRG.D	1887,9
576	590	FGA	K.SSSYSKQFTSSTSYN.R	1674,7
576	589	FGA	K.SSSYSKQFTSSTSY.N	1560,7
576	588	FGA	K.SSSYSKQFTSSTS.Y	1397,6
576	587	FGA	K.SSSYSKQFTSST.S	1310,6
576	586	FGA	K.SSSYSKQFTSS.T	1209,5
576	585	FGA	K.SSSYSKQFTS.S	1122,5
576	583	FGA	K.SSSYSKQF.T	934,4

C-Terminus

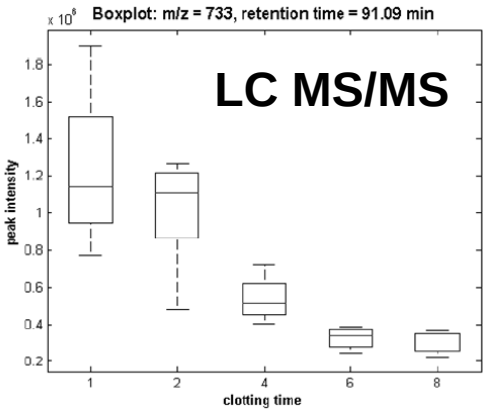
Cross Validation



Validierung des endogenen Decay Markers



Baechle et al. Clin Proteomics 2007



Govorukhina et al. RCMS 2009