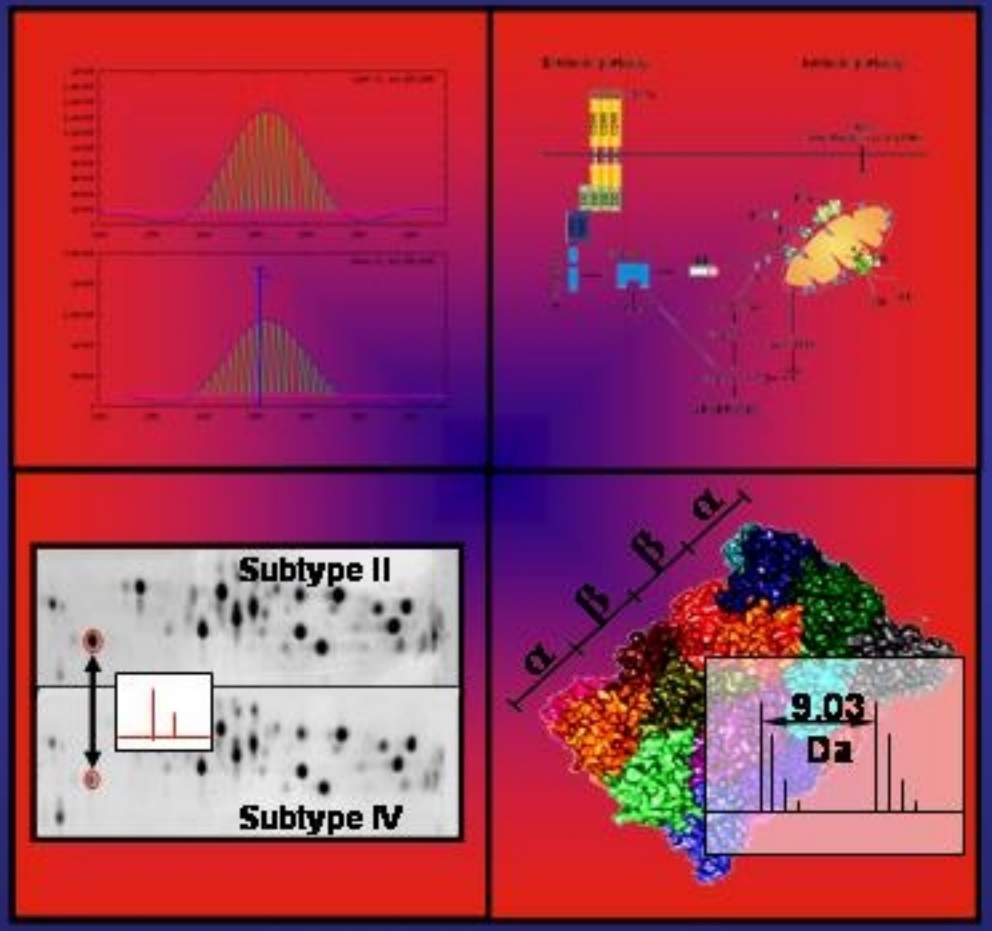
Quantitative proteome analysis of 20S proteasome by ICAT- or SILAC based LC and 2-DE approaches



Dr. Frank Schmidt

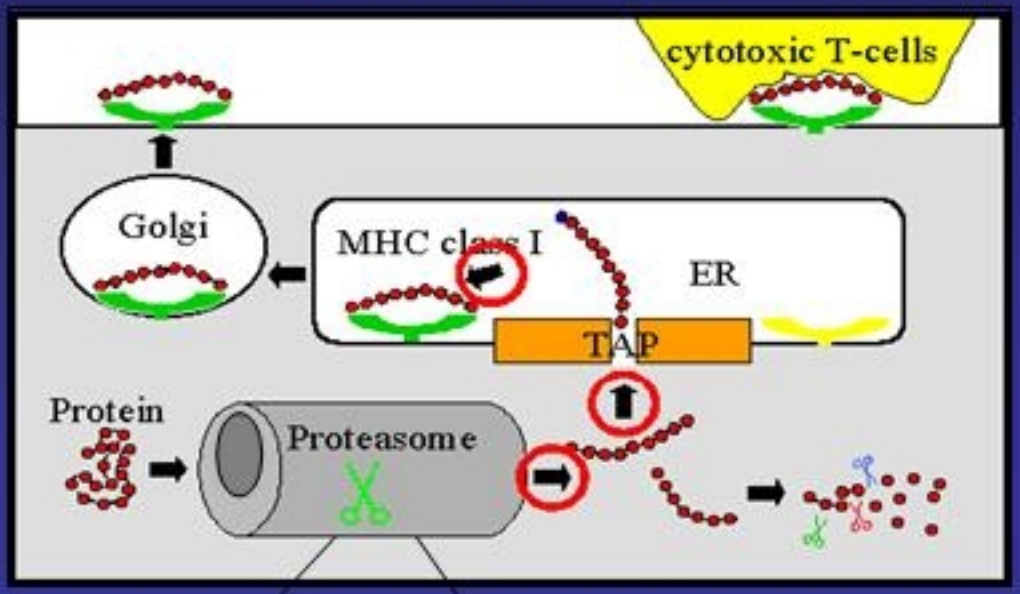
Overview:

1. Quantification of 20S proteasome subtypes by ICAT and 2-DE
2. Quantification of 20S proteasome by SILAC and SDS-PAGE

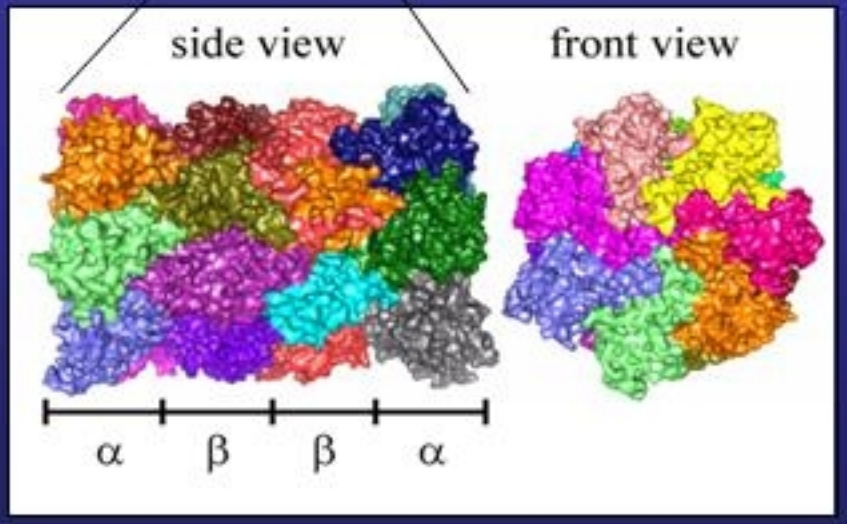
Aim of study:

- Generation of a 2-DE standard pattern of 20S proteasome from rat liver
- Find out new PTM's
- Comparison of 20S proteasome vs. 20S immunoproteasome using ICAT and 2-DE
- Comparison of four common quantification techniques

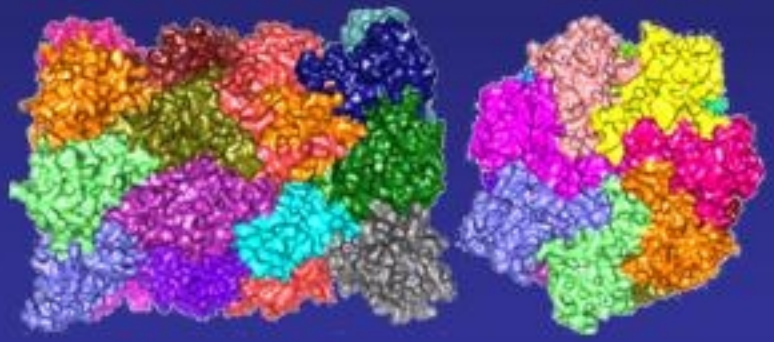
1 The Proteasome



20S Core Complex of the Proteasome



1.1 20S Core Complex and its Cleavage Motifs



- 20S proteasome is composed of 7 α- and 7 β-subunits
- Barrel-shaped heterodimeric configuration: α-β-β-α
- Three nominal activity sites in 20S core are known
- Postglutamyl cleavage activity, catalysed by subunit β-1
- Trypsin-like cleavage, catalysed by subunit β-2
- Chymotrypsin-like cleavage, catalysed by subunit β-5

Nussbaum, A.K. et al. Proc.natl.Acad.Sci USA 95, 12504-12509 (1998), Peters, B. et al. J.Mol. Biol. 318, 847-862 (2002)

1.2 Replacement of β 1, 2 and 5 in Immunoproteasome

- 3 of 7 β-subunits were replaced in the immunoproteasome
- Transformation of β-1 into β-1i, β-2 into β-2i and β-5 into β-5i
- β-i forms are different in its AA composition and leads to a new conformation and change in the proteasome netto charge
- Changes are detectable by Ion-exchange chromatography

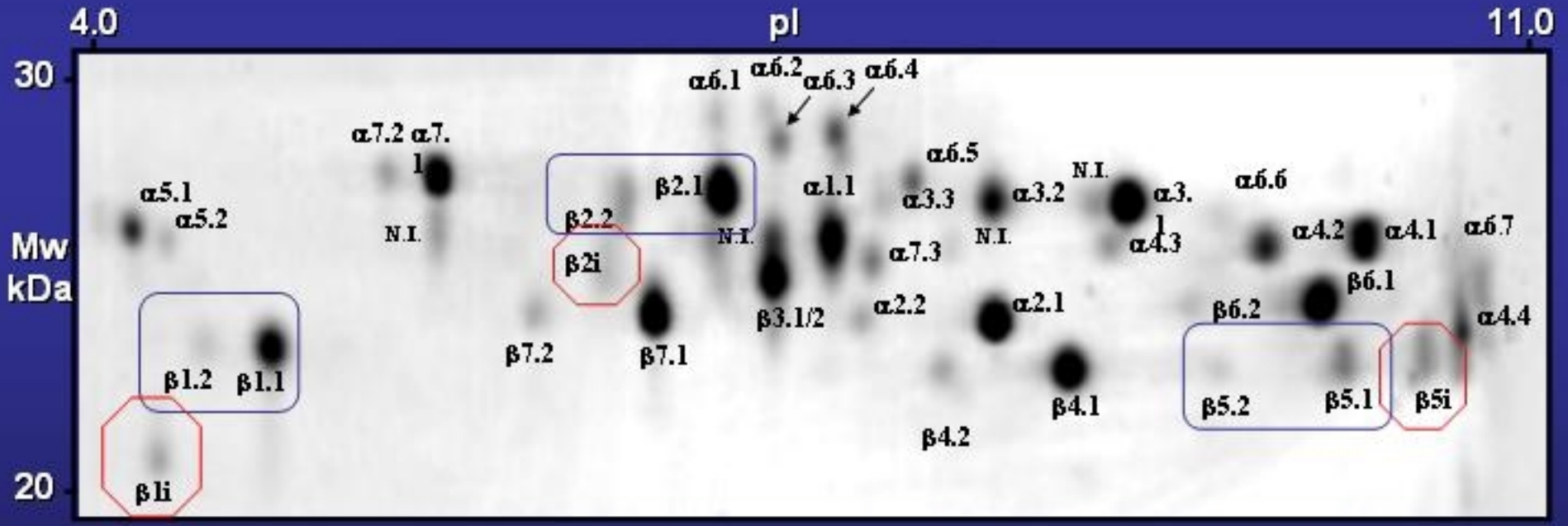
Dahlmann, R., Ruppert, T., Kloetzel, P.-M. and Kühn, L. Biochimie 83, 295-299 (2001)

1.3 2DE Standard Pattern of the 20S Core Complex

Gel-chromatography

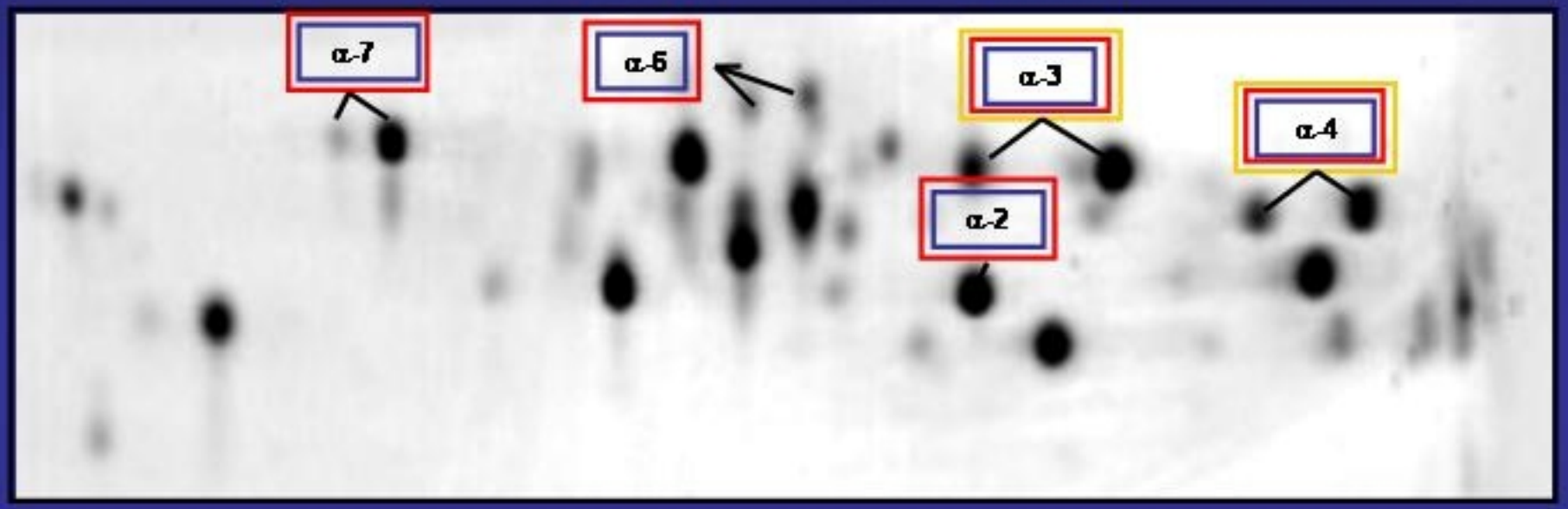
Ion-exchange chromatography

72 protein species separated by 2-DE



http://www.mpiib-berlin.mpg.de/2D-PAGE/

1.4 Known Post-translational Modifications



Phosphorylation

N-Acetylation

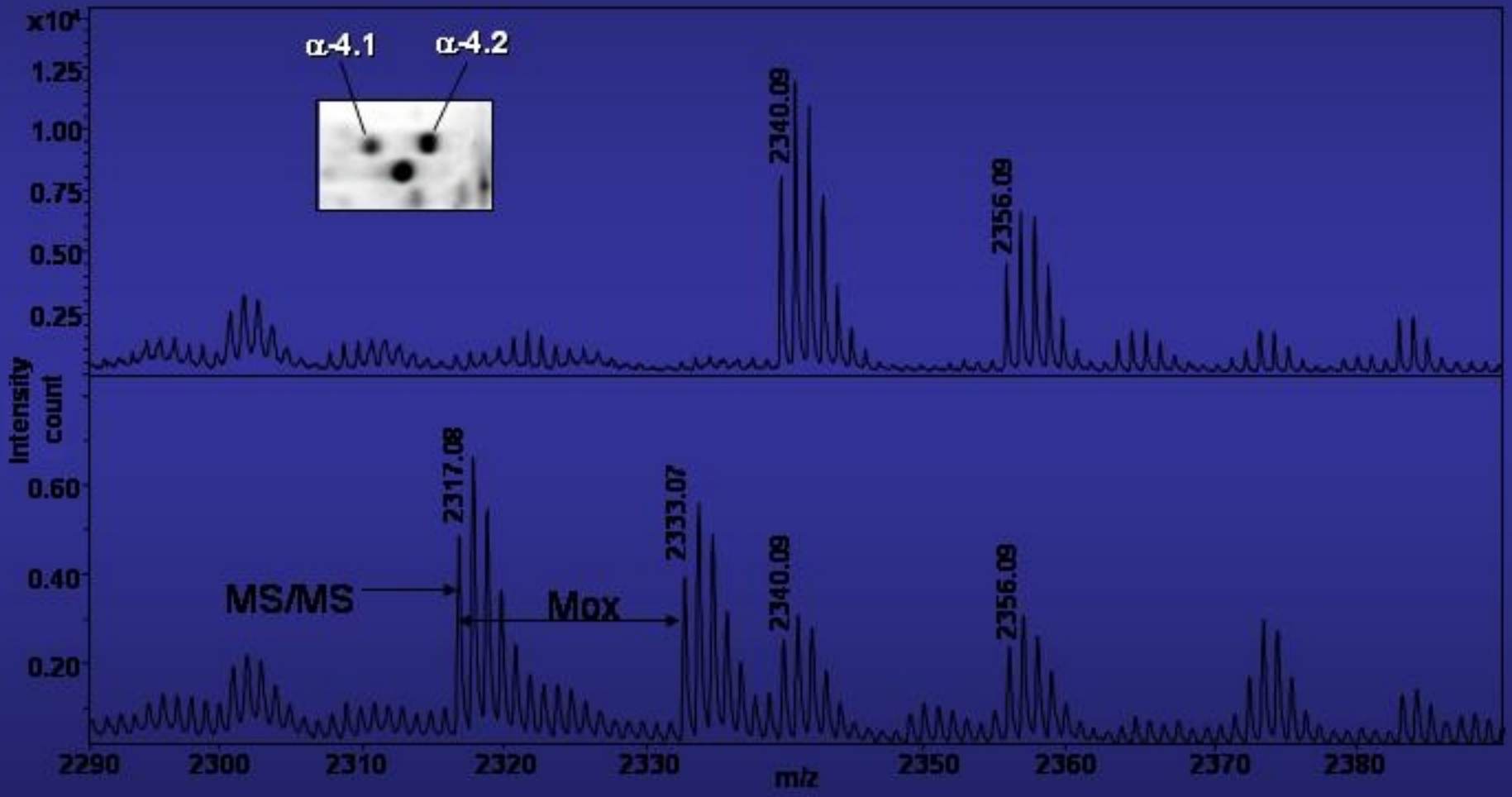
Glycosylation

Deamidation: α-7

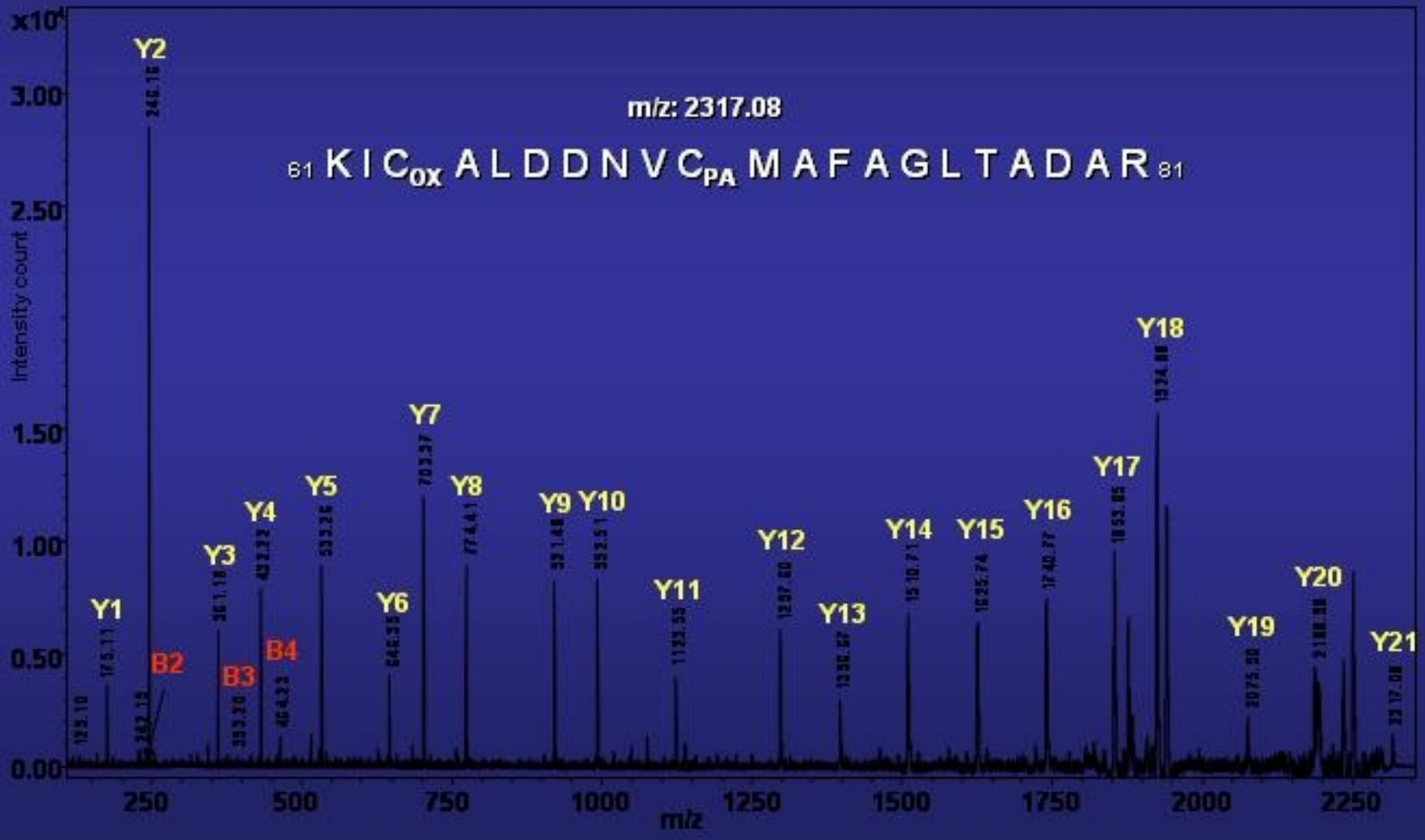
Claverol et al., MCP, 2002, 26, 567-578,
Schmid et al., Biochimie, 1993, 905-910,

Kimura et al.,1999, JBC, 7, 4635-4639,
Bulteau et al., 2001, JBC, 267, 300057-63

1.5 New PTM: Cysteine Oxidation at Subunit α – 4

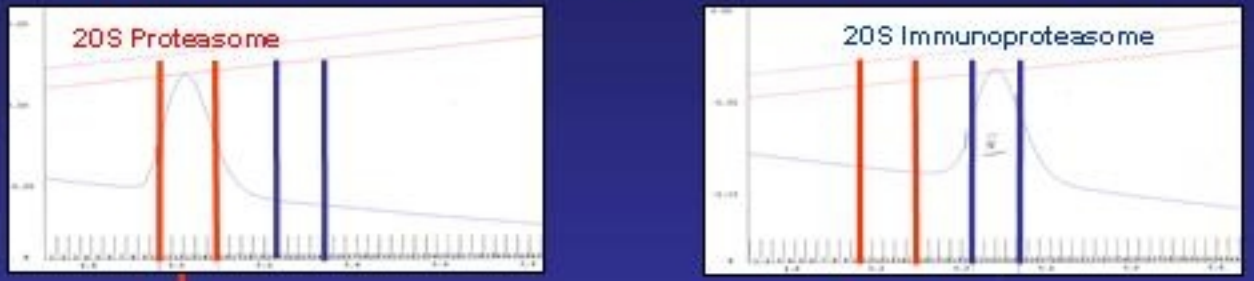


1.6 New PTM: Cysteine Oxidation at Subunit α – 4



1.8 Analytical Course of Action: Quantification

Ion-exchange chromatography



Labelling cICAT-^{12}C

Labelling cICAT-^{13}C

Combination

LC/ESI-MS/MS

LC/MALDI-MS/MS

2-DE Gel MALDI-MS

2-DE Gel MALDI-MS

Identification
Quantification

SEQUEST Xpress

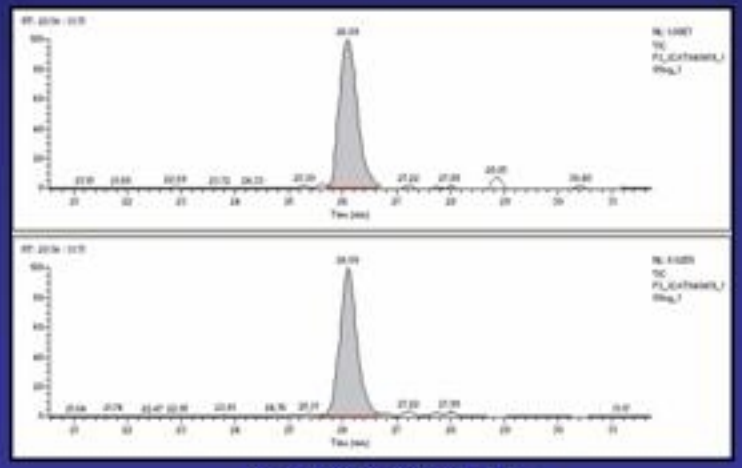
Mascot MS-Screener

Mascot MS-Screener

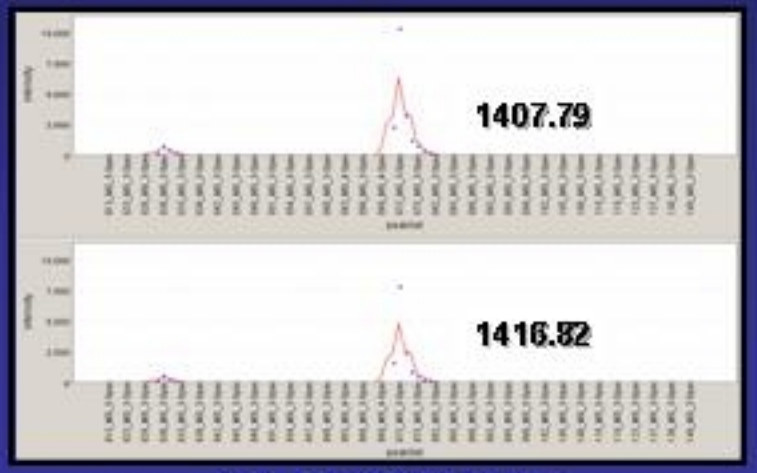
Mascot Topspot

LIMS

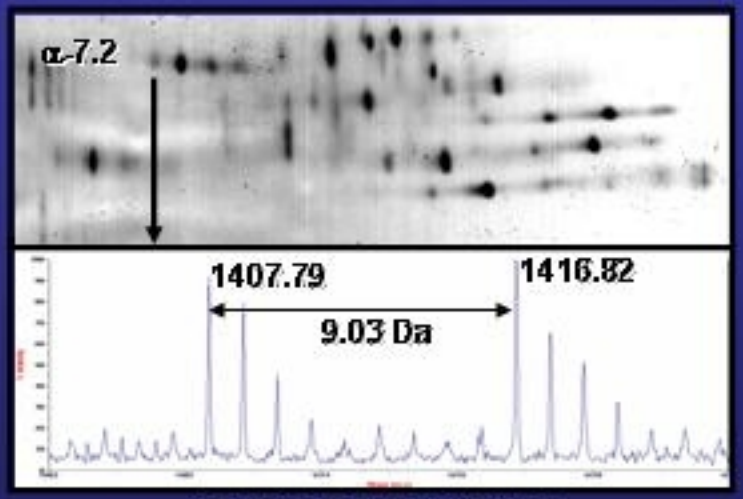
1.9 Quantification Techniques



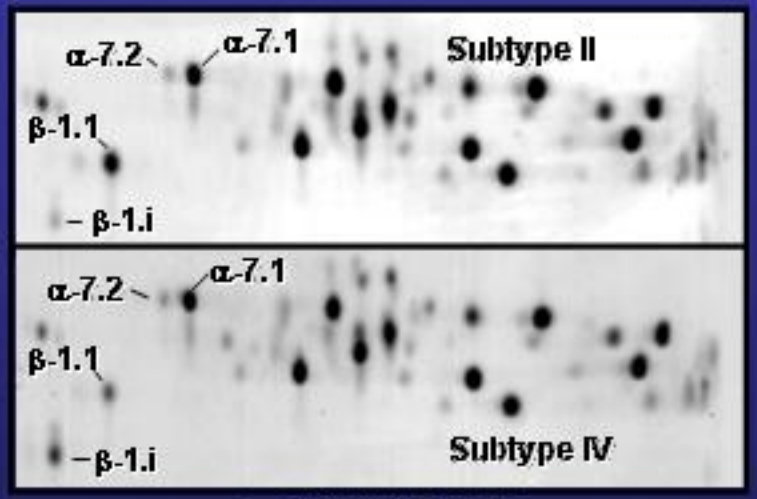
cICAT/LC/ESI-MS



cICAT/LC/MALDI-MS



cICAT/2-DE/MALDI-MS



2-DE/MALDI-MS

1.10 Results Quantification Techniques

Criteria\Methods	ICAT based			
	LC-ESI	LC-MALDI	2-DE/ MALDI	2-DE/ MALDI
Time effort	~5h	~20h	~14d	~14d
Sensitivity	200ng	200ng	30µg	30µg
Protein coverage	70%	53%	100%	100%
Quantitative standard deviation	+/- 20%	+/- 5%	n.a.	+/- 20%
Sequence coverage proteins	10-20%	10-20%	30-70%	30-70%
PTM's	N	N	56	56

Conclusion

- Development of an 2-DE standard pattern of 20S rat liver proteasome
- Identification of all protein species only by 2-DE
- PTM's were only detected by 2-DE (56)
- New cysteine oxidation at α-4.1 discovered
- ICAT and 2-DE are complementary approaches
- 2-DE spots with more than one protein species can only be quantified at peptide level (isotopic labelling)

- Replacement of β-subunits 1 were detected by all approaches
- Replacement of β-subunits 2 and 5 were only detected by 2-DE
- ICAT/LC-MALDI was the most accurate method (+/- 5% StDv)

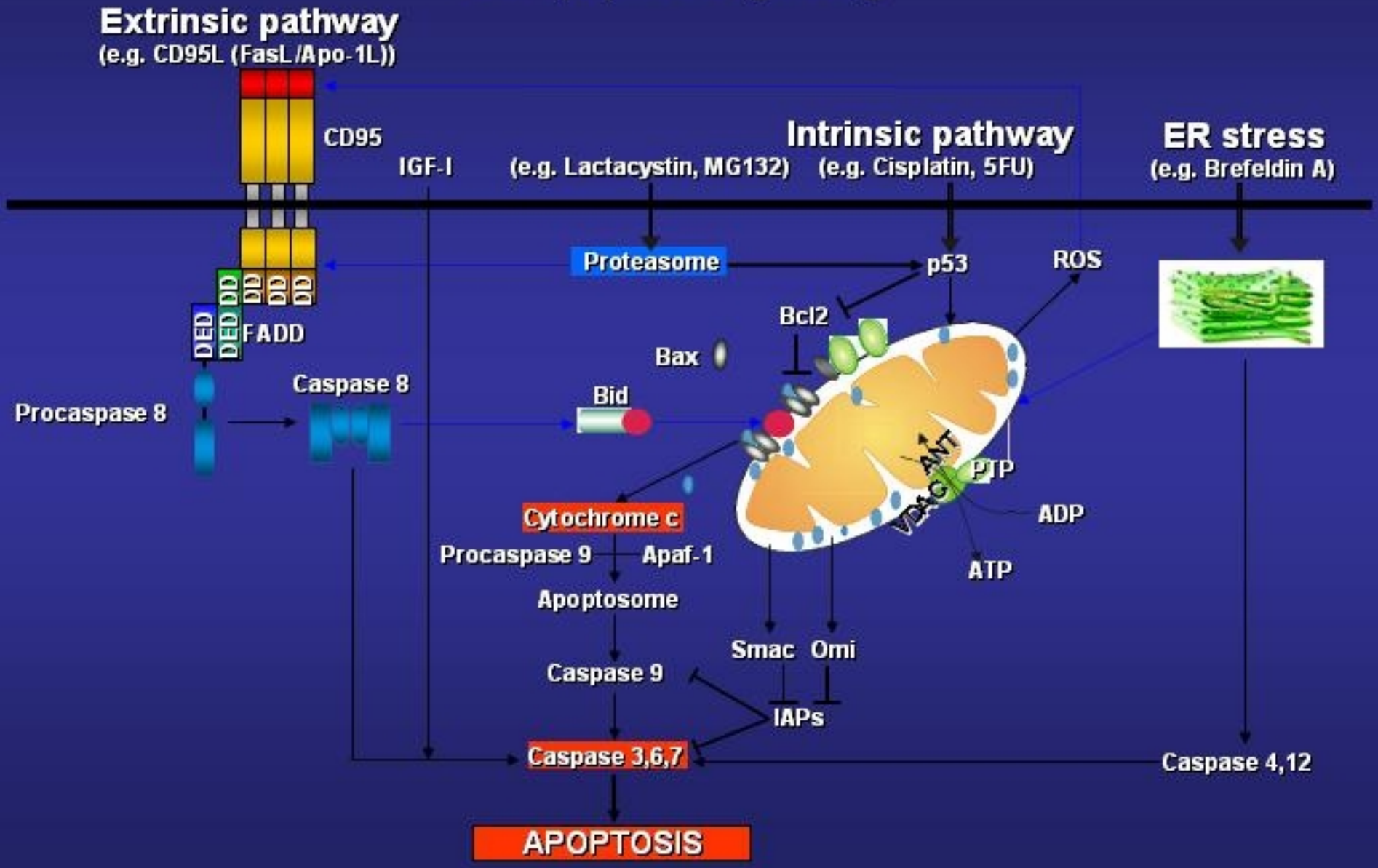
Schmidt et al. (2006) *Proteomics* 3: 24-32.
Schmidt et al. (2003) Mol Cell Proteomics 3: 24-42.
Schmidt et al. (2003) J Am Soc Mass Spectrom 14: 943-956.

2. Quantification of 20S Proteasome by SILAC and SDS-PAGE

Aim of study:

-Quantification of 20S proteasome from apoptosis induced vs. healthy cells by SDS-PAGE and LC-MS

2.1 Apoptosis Signalling



2.2 20S Proteasome Quantification of 5FU Induced Apoptosis

Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC)

$^{12}C_6$ Arginine/ Lysine (light)

$^{13}C_6$ Arginine/ Lysine (heavy)

Jurkat T-Cells

"Health" vs. Cisplatin

Gel-chromatography

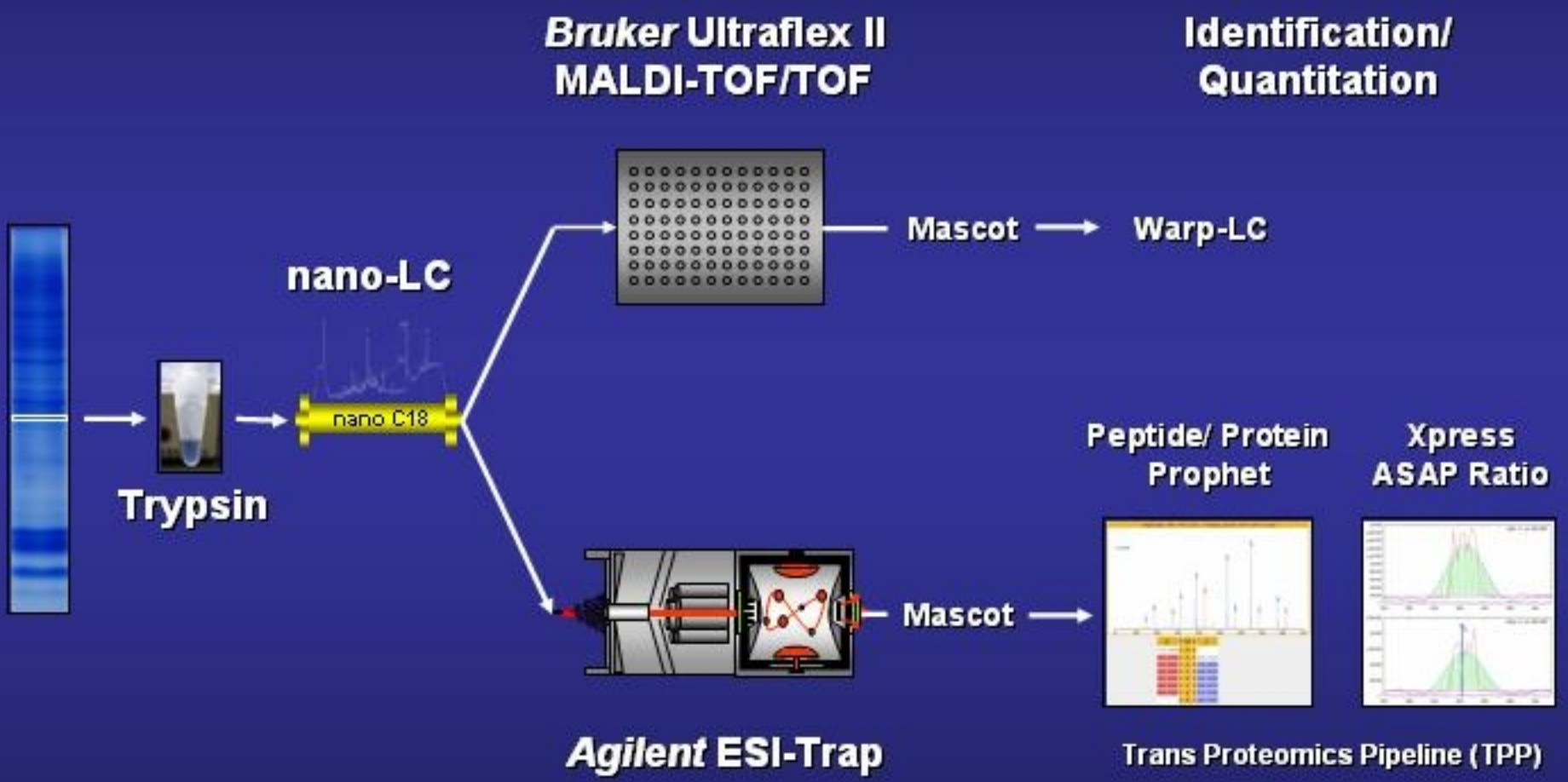
↓

Protein separation

↓

SDS-PAGE

2.3 Experimental Course of Action



Kaiser J. Science. 2002 3;296(5569):827

Schmidt et al., Bruker Technical Note: MT-86 SILAC

2.4 Quantification Results LC-MALDI-MS
Health vs. Apoptotic

Subunit	Acc. #	Quantified Peptides	Ratio H/L	StDv
PSA1	P25786	6	1,00	0,07
PSA2	P25787	6	1,16	0,12
PSA3	P25788	4	0,64	0,06
PSA4	P25789	5	1,00	0,09
PSA5	P28066	4	1,03	0,06
PSA6	P60900	4	1,00	0,01
PSA7	O14818	6	1,01	0,06
PSB1	P20618	3	1,06	0,10
PSB2	P49721	4	1,05	0,06
PSB3	P49720	4	1,03	0,17
PSB5	P28074	7	0,99	0,16
PSB6	P28074	3	0,99	0,16
PSB7	Q99436	2	0,98	0,01

2.5 Quantification Results LC-ESI-MS
Health vs. Apoptotic

Subunit	Acc. #	Quantified Peptides	Ratio H/L	StDv
PSA1	P25786	6	0.79	0,22
PSA2	P25787	8	1.00	0,24
PSA4	P25789	2	1.00	0,02
PSA5	P28066	6	0.97	0,15
PSA6	P60900	9	0.83	0,32
PSA7	O14818	15	0.92	0,25
PSB1	P20618	10	1.09	0,14
PSB2	P49721	6	0.96	0,38
PSB3	P49720	9	1.04	0,29
PSB4	P28070	10	0.92	0,31
PSB5	P28066	15	0.87	0,36
PSB6	P280721	8	1.15	0,24
PSB7	Q994361	4	1.24	0,26
PSB8	P280621	5	1.05	0,18
PSB9	P280651	6	0.95	0,34
PSB10	P403061	1	0.48	0,06

2.4 Conclusion

LC-MALDI-MS

- 13 of 17 subunits detected
- Average StDv +/- 9%

LC-ESI-MS

- 16 of 17 subunits detected
- Average StDv +/- 23%

- Decreased amount of PSA3 in apoptotic cells
- Immunoproteasome subunits PSB1i,2i and 5i only by LC-ESI-MS
- LC-ESI showed a higher number of lysine containing peptides
- LC-MALDI showed higher number for arginine containing peptides
- Protein species not distinguishable (Quantification: sum of protein species)
- low number of PTM's
- LC-MALDI showed the most accurate quantification

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