High Pressure Proteolysis - a highly efficient technique for antibody epitope elucidation by proteolytic excision affinity- mass spectrometry

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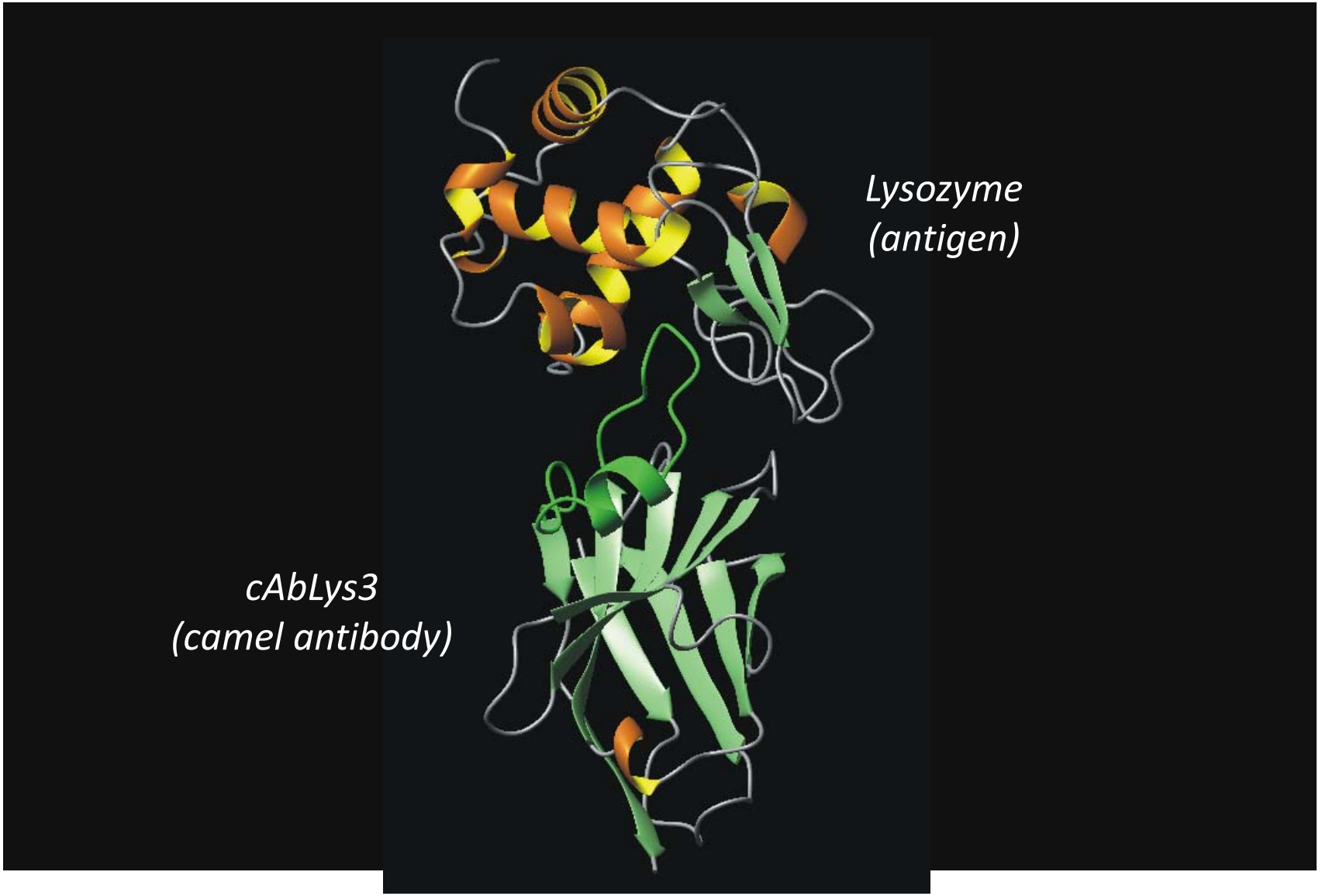
www.affinityms.de

http://AffyMS*Life*Chem.de

Biomolecular Recognition „FIT“ in Antigen- Antibody Complex

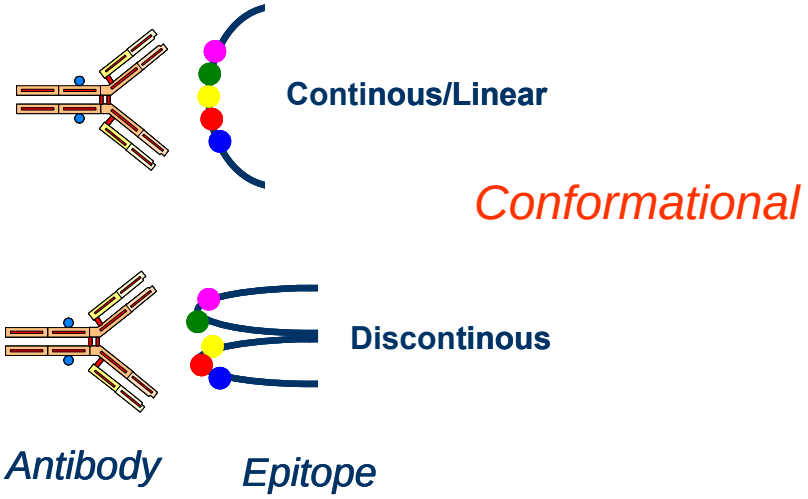


Antigen Epitopes and Antibody Paratopes

- Antigens (Ags) are molecules (e.g., proteins) which are in an *immune complex* bound to antibodies (Abs).

- The partial structure of an antigen which is in direct contact to the antibody is called *epitope*.
  Epitopes typically comprise partial amino acid sequence of 5 – 25 amino acids.
  The epitope-binding region of the antibody is called *paratope*.

- The two main types of epitopes:



Methods for Biomolecular Interaction Analysis

Affinity methods for a wide range of biomolecules

- **Affinity Determination /Biosensor**
  - **Surface Plasmon Resonance (SPR), Surface-Acoustic Wave (SAW)**

- **Immunological Methods (only for antibodies and antigens)**
  - **Immunoprecipitation**
  - **Western Blotting**
  - **ELISA**
  - **Epitope „mapping“ (pepscan; predictive not molecular)**

- **Mass Spectrometry**

Combining Epitope Identification and Affinity Determination

OVERVIEW

I MS Methods for studying biomolecular interactions

- Hydrogen-deuterium exchange (HDX)
- Fast Photochemical Oxidation (FPOP)
- Proteolytic excision / extraction (PROTEX)

II Biosensor – Mass Spectrometry Combination

III Application Examples

- Antibody- Epitope Determination
- Protein- Carbohydrate Interactions
- Peptide Epitopes in lysosomal therapy

PROTEX - Proteolytic excision of immune complexes - Basis for mass spectrometric epitope identification

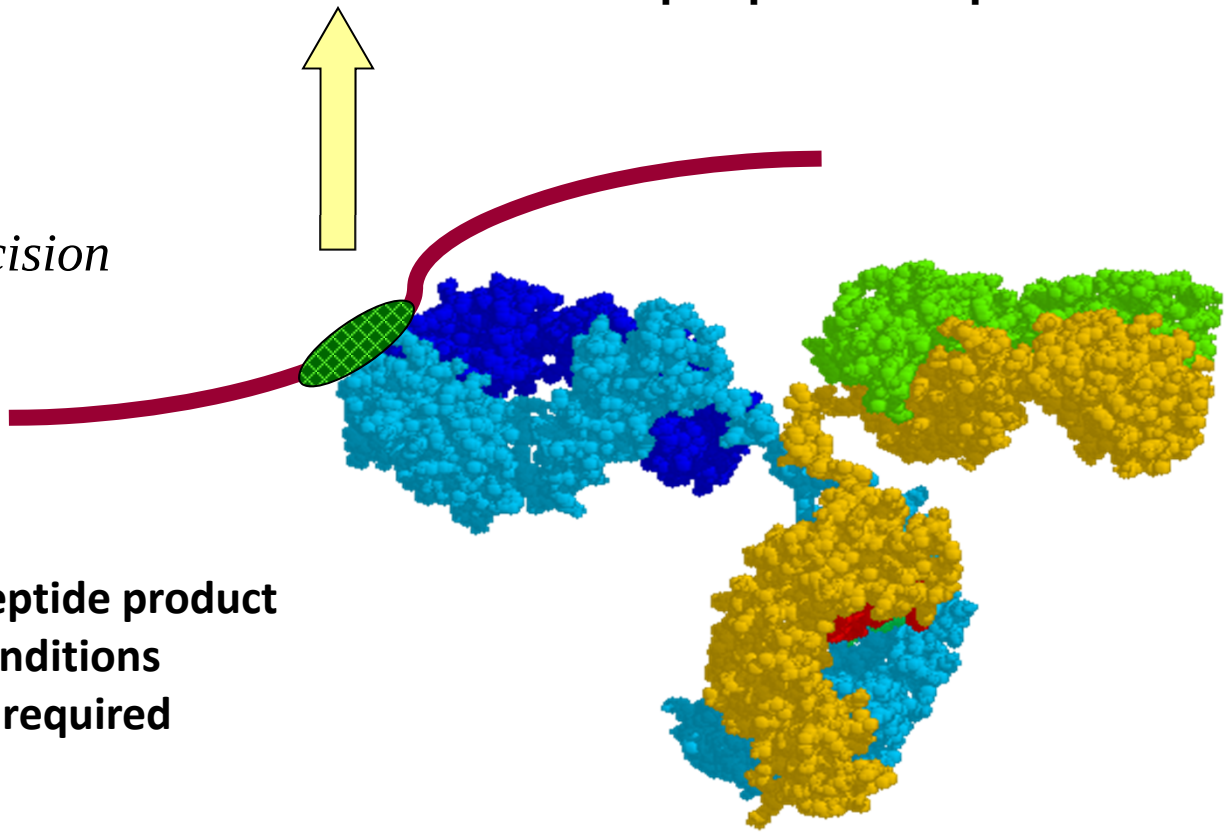
Preconditions:

Proteolytic stability of antibody

Epitope-Paratope Interaction shielded

Epitope peptide

Epitope Excision



- **Defined peptide product**
- **Flexible conditions**
- **Chemistry required**

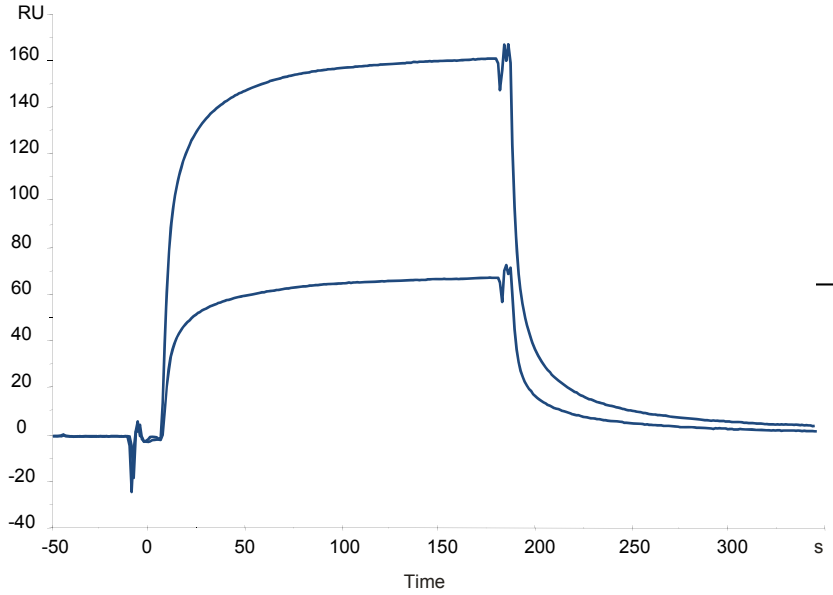
D. Suckau, M. Przybylski et al. *Proc. Natl. Acad. Sci USA* (1992)
M. Macht et al. *Biochemistry* (1996)

Why online Biosensor-MS combination? Principles

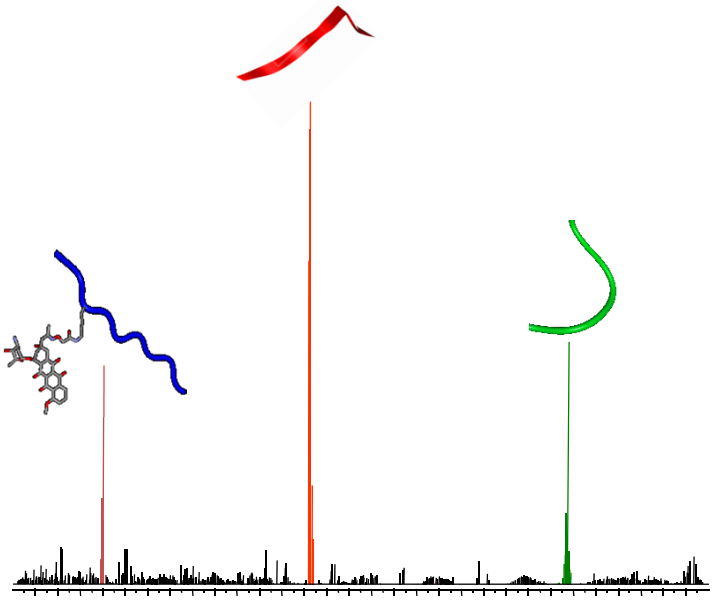
Screening of analytes at native conditions

Identical analysis conditions for proteins, glycans, nucleic acids, lipids

Correlation of bioaffinity and molecular structure

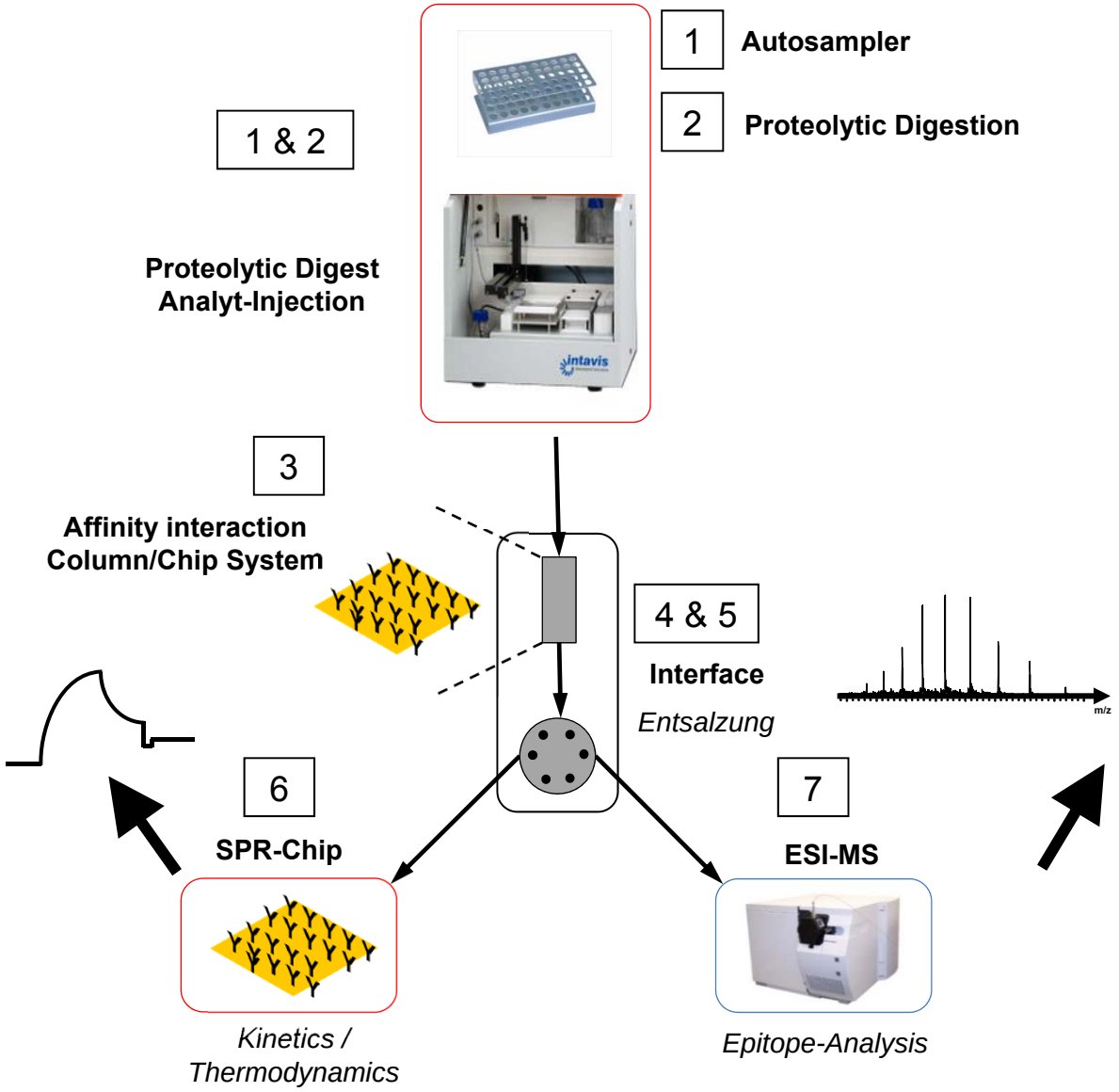


affinity binding

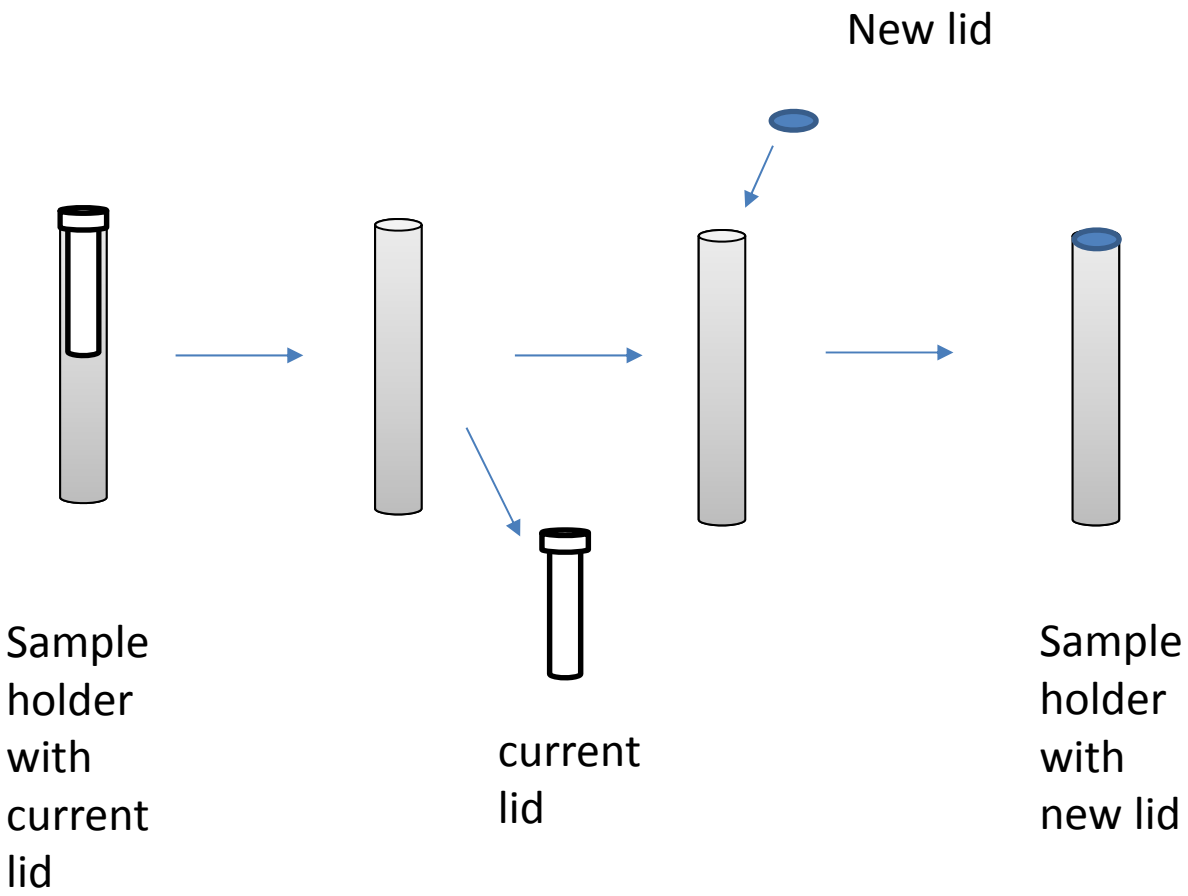


Epitope ligand mapping
Peptide ligand molecular differentiation
mixture analysis

DIGEST-PROTEX- MS (Intavis):
Proteolytic Digestion combined with SPR-MS



High pressure proteolytic digestion

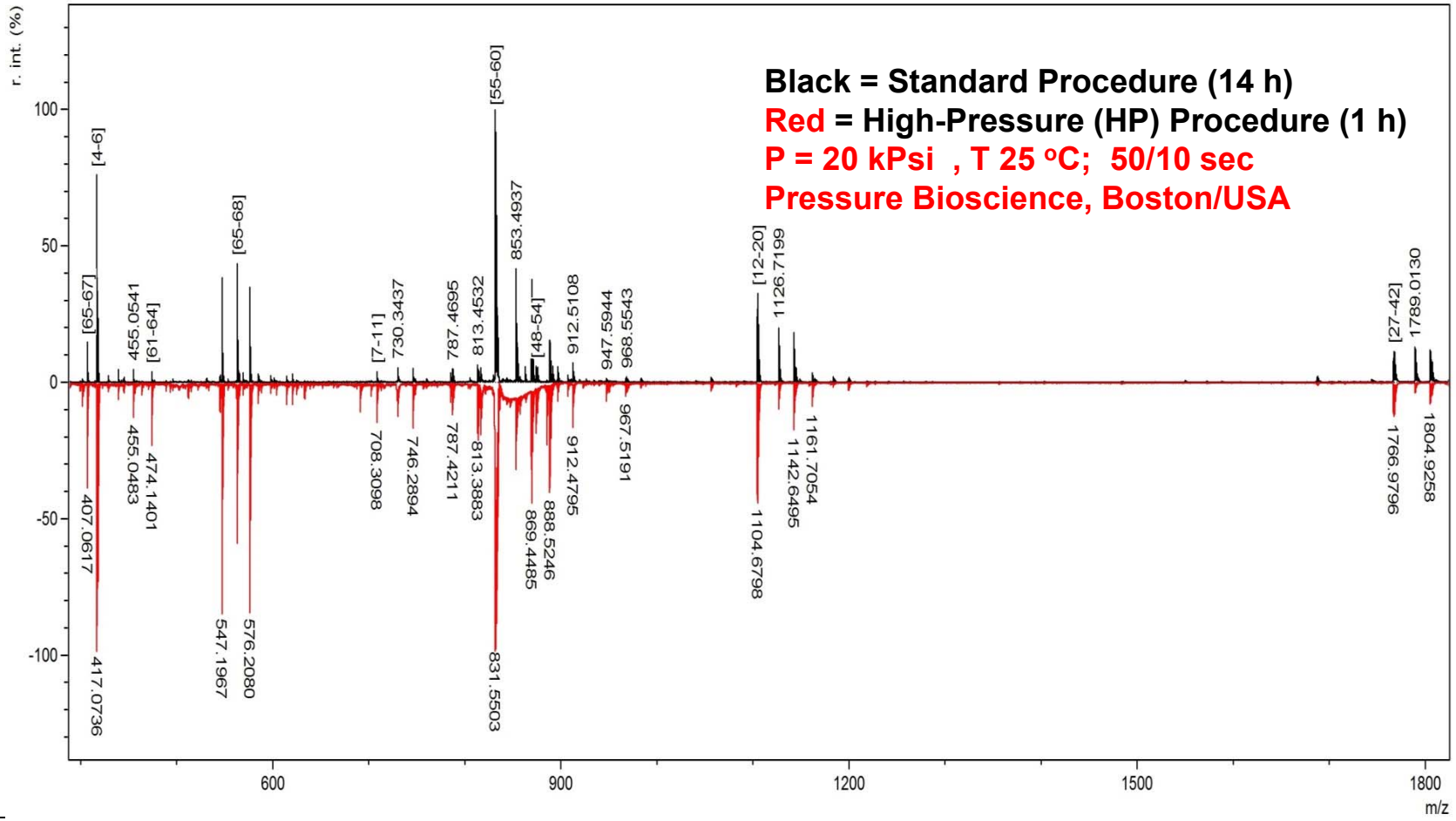


The sample will be put into the normal holders. After the high-pressure digest the lids will be replaced and the holder relaced for an autosampler

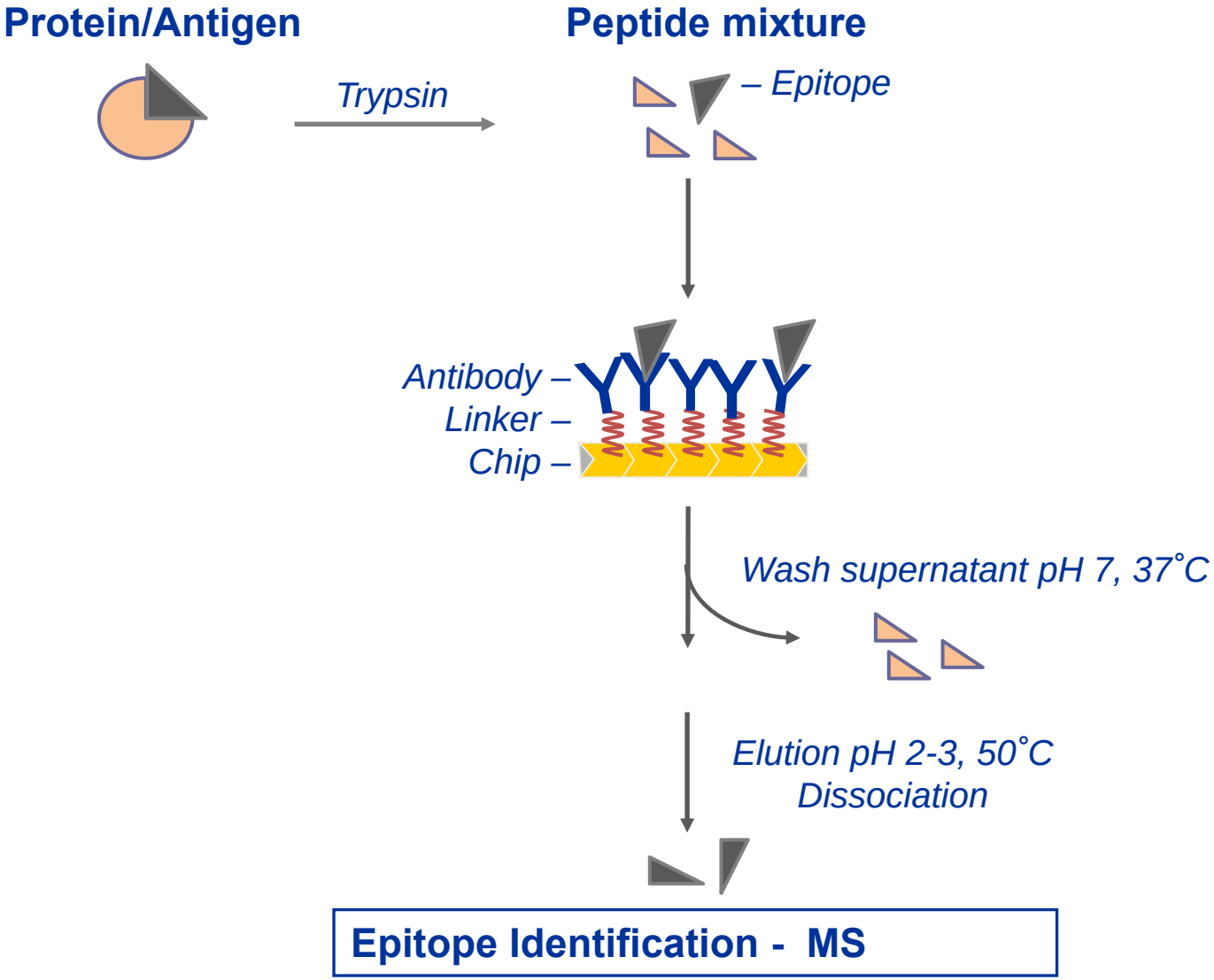
Tryptic Digestion of IL8: Standard vs. high pressure proteolysis

SAKELRCQCI KTYSKPFHPK FIKELRVIES GPHCANTEII VKLSDGRELC LDPKENWVQR VVEKFLKRAE NS

1 11 21 31 41 51 61 71

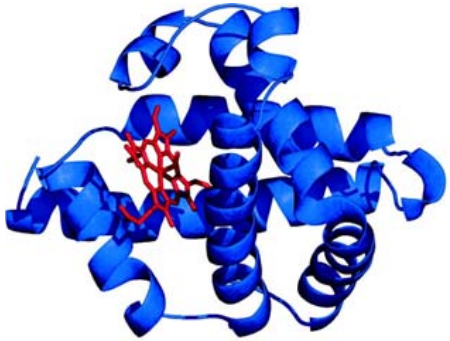


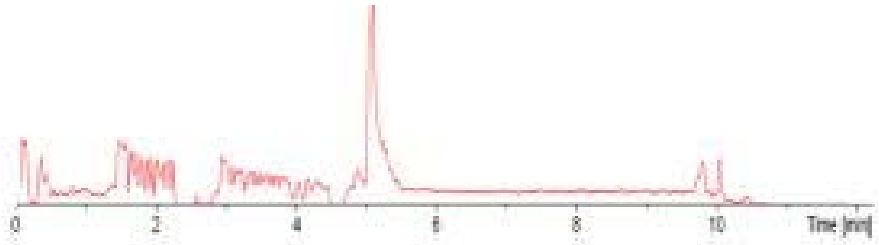
Epitope identification by proteolytic extraction- MS



Native Horse Heart Myoglobin and Unfolded Apomyoglobin by SPR-MS

Myoglobin

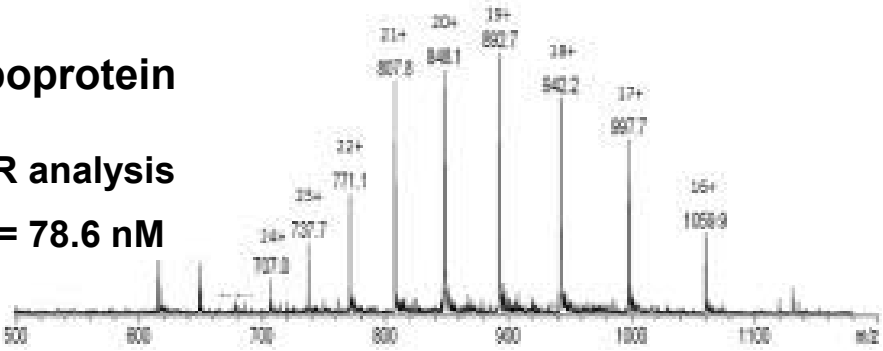




Apoprotein

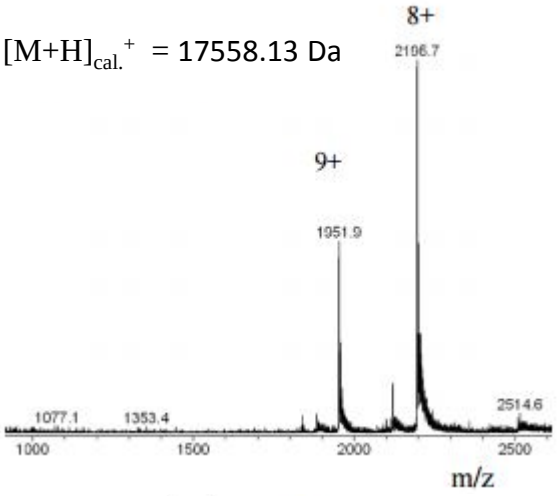
SPR analysis

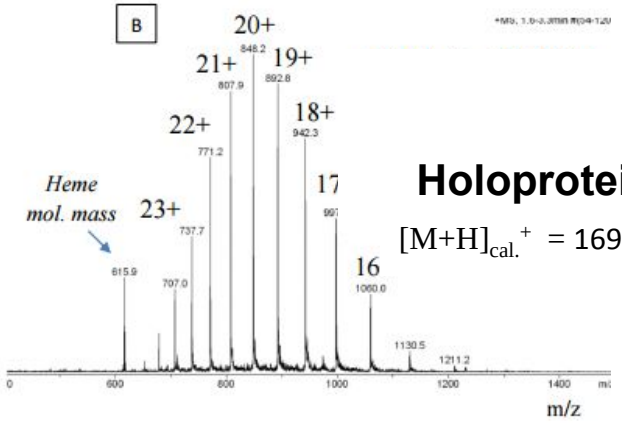
K_D = 78.6 nM



MS analysis

$[M+H]_{cal.}^{+}$ = 17558.13 Da





Holoprotein

$[M+H]_{cal.}^{+}$ = 16941.95 Da

OVERVIEW

Application Examples

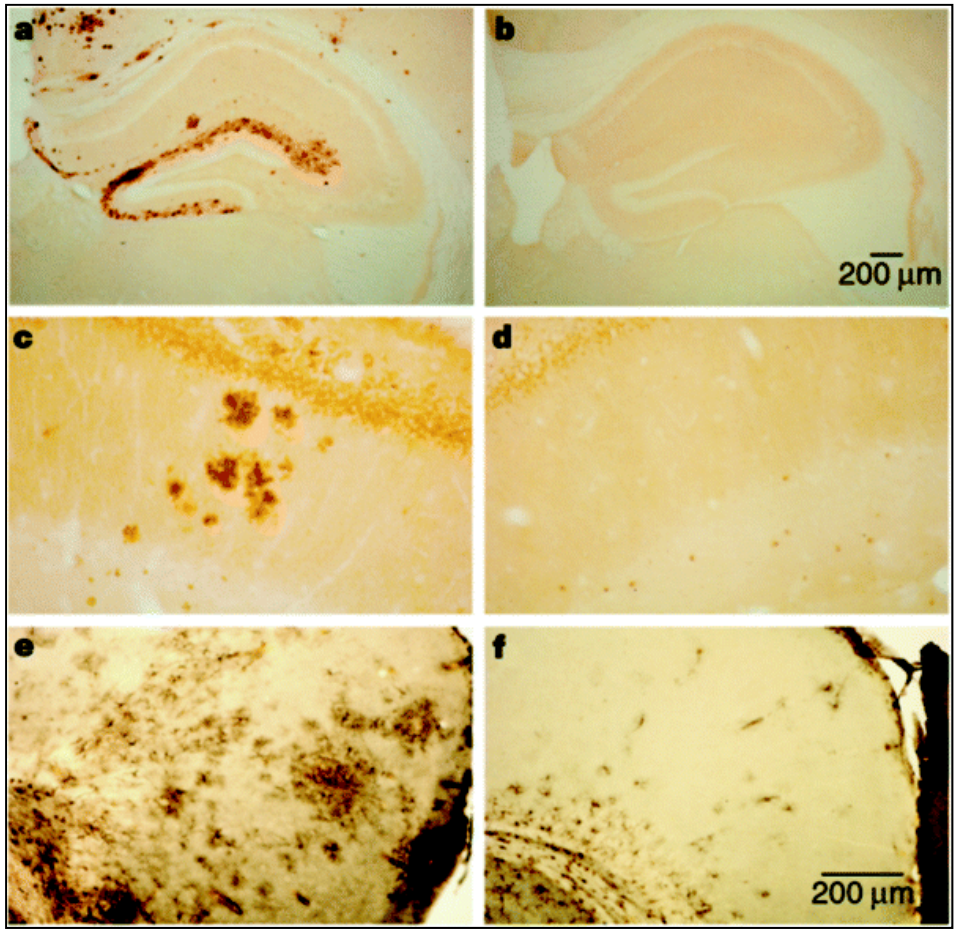
- Antibody- Epitope Determination
- Protein- Carbohydrate Interactions
- Peptide Epitopes in lysosomal therapy

Bundestag Decision 11th Nov. 2016

Immunization with amyloid-β attenuates Alzheimer-disease-like pathology in the PDAPP mouse

Dale Schenk, Robin Barbour, Whitney Dunn, Grace Gordon, Henry Grajeda, Teresa Guido, Kang Hu, Jiping Huang, Kelly Johnson-Wood, Karen Khan, Dora Kholodenko, Mike Lee, Zhenmei Liao, Ivan Lieberburg, Ruth Motter, Linda Mutter, Ferdie Soriano, George Shopp, Nicki Vasquez, Christopher Vandevert, Shannan Walker, Mark Wogulis, Ted Yednock, Dora Games & Peter Seubert

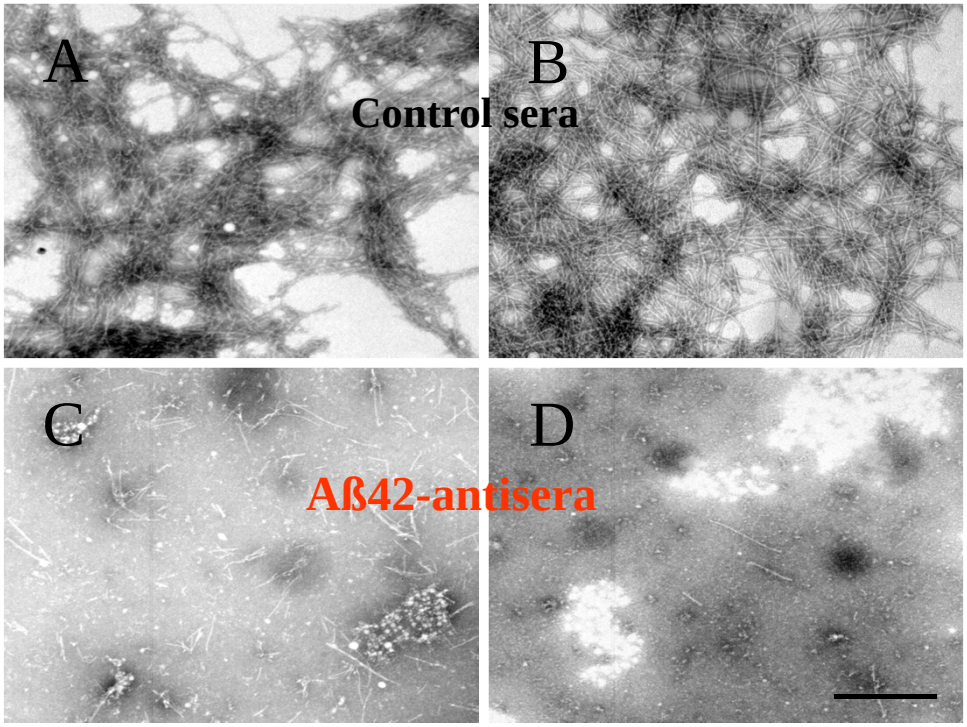
Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, California 94080, USA

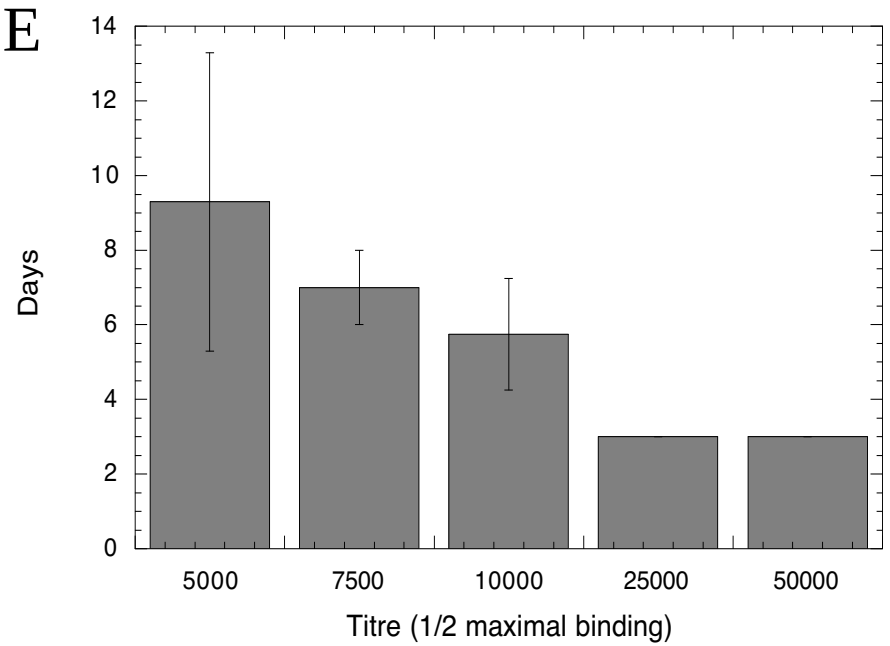


Control Immunisation

Schenk, D. et al. (1999) Nature 400.
Bard, F. et al. (2000) Nat Med. 6.
Weiner, H.L. et al. (2000) Ann Neurol. 48.
Morgan, D. et al. (2000) Nature 408.
DeMattos, R.B. et al. (2001) PNAS USA. 98.

Disaggregation of Aß42-fibres by therapeutically active antibodies correlates with antibody titres





J. McLaurin, M. Przybylski et al.
Nature Med. (2002) 8: 1263

Epitope Identification of Amyloid Plaque Specific Antibody by Affinity-MS

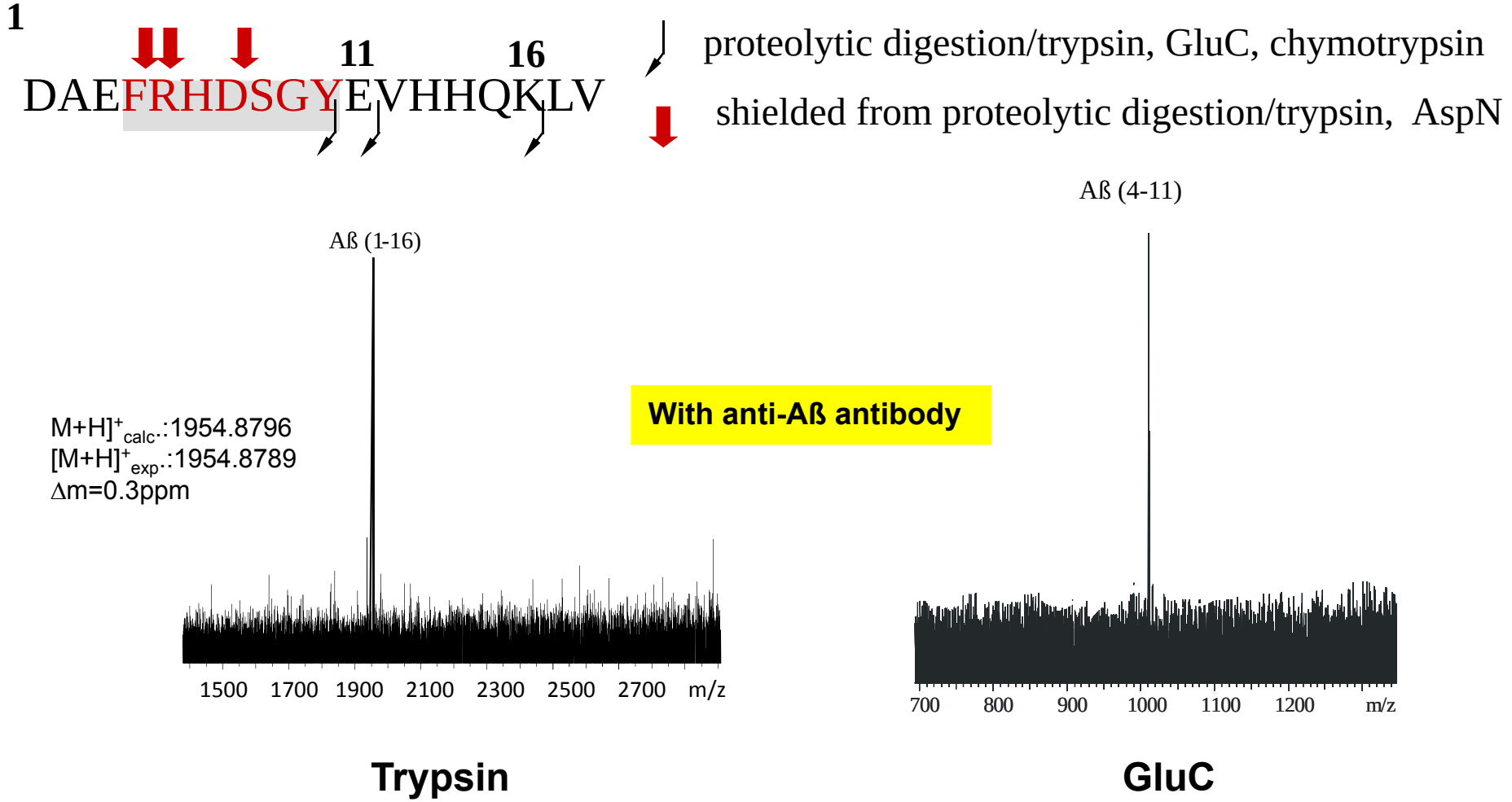
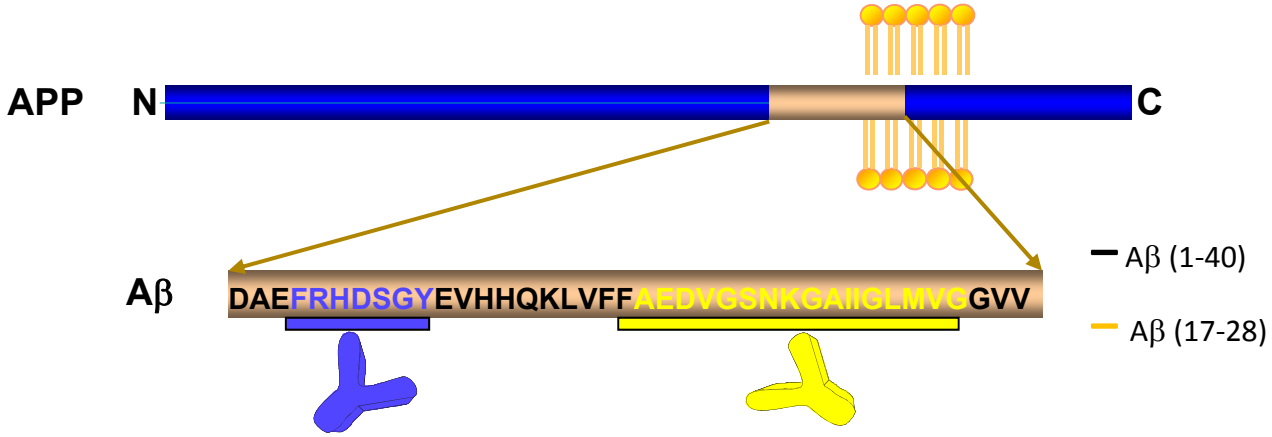
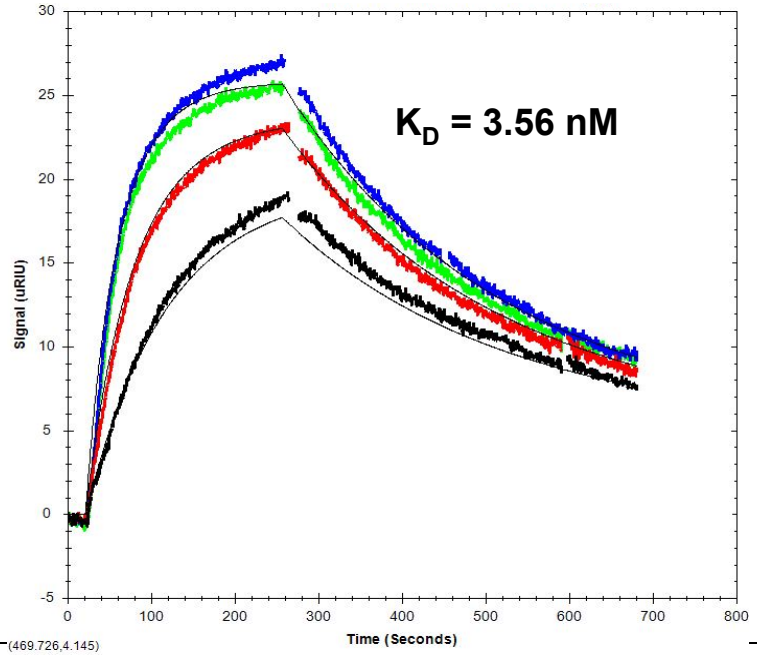


Figure 2

Epitope Identification and Affinity Quantification of β-Amyloid Antibody



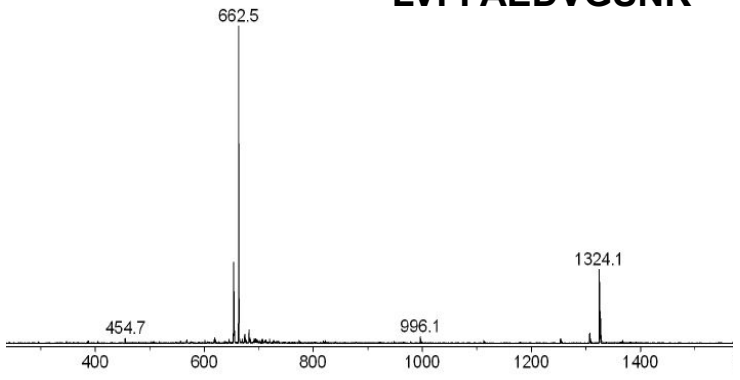
SPR



MS Epitope

Aβ (17-28) Epitope

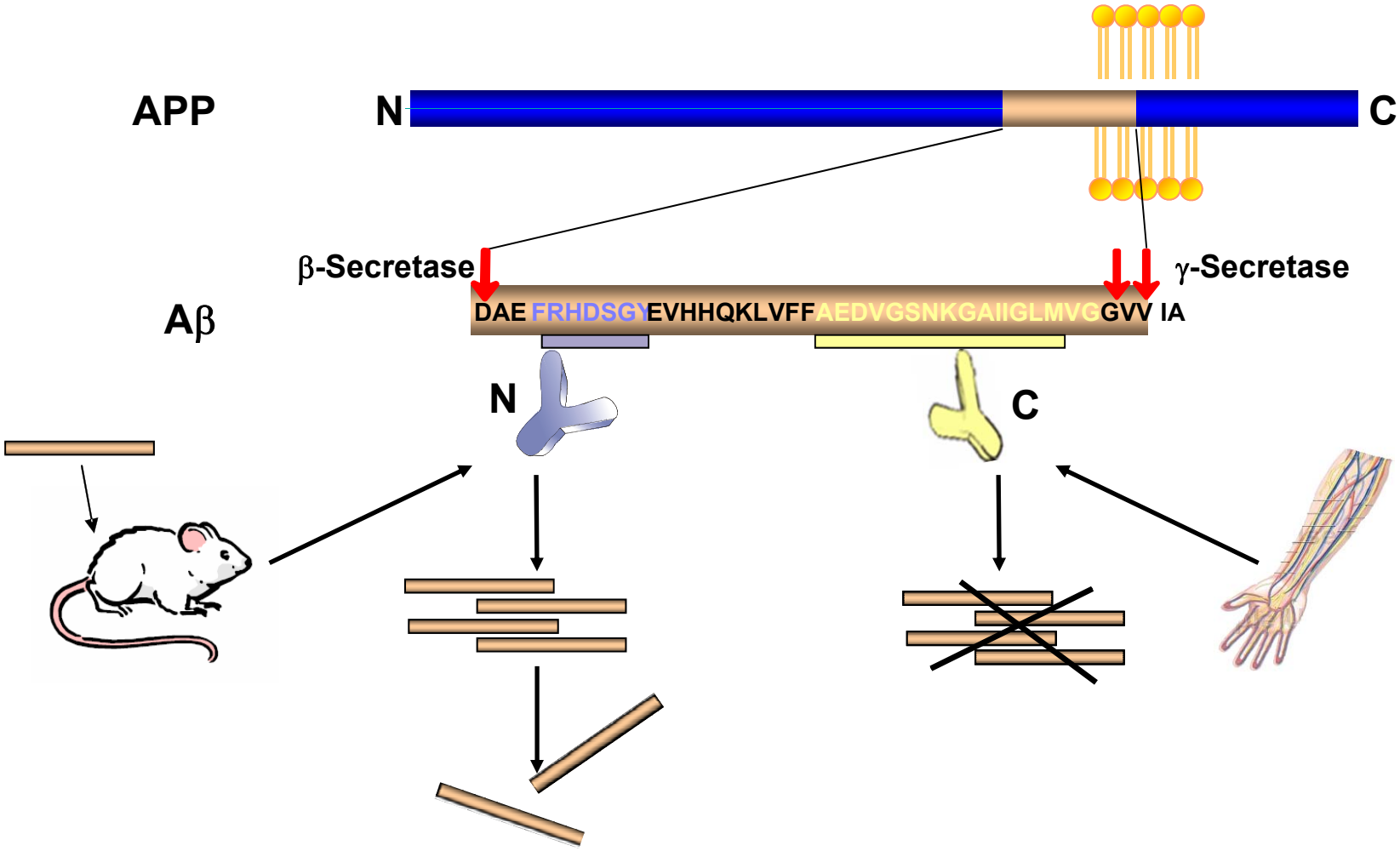
LVFFAEDVGSNK



Epitope Specificity define Function of Aß- Antibodies

Plaque- specific“ Aß-antibodies: Recognition of N-terminal epitope

„Plaque-protective“ Aß-autoantibodies: C-terminal, oligomer-specific epitope



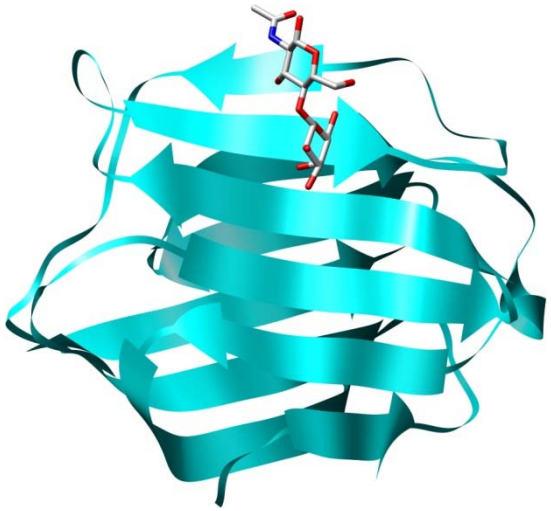
J. McLaurin et al, Nature Med (2002)
M. Przybylski et al. EPA, US Patent (2010)

Application 2: Lectin- Carbohydrate Ligand Epitopes

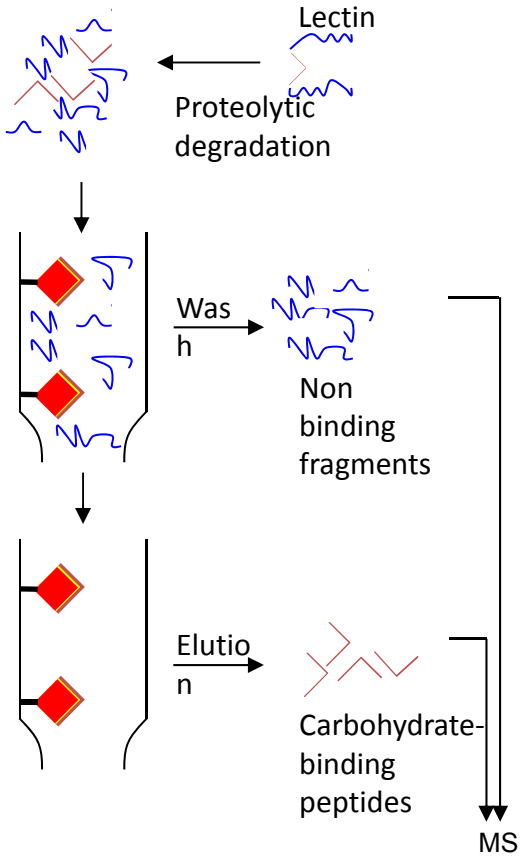
HOW DO GLYCANS INTERACT WITH PROTEINS?

Galectins: β-galactosides-binding ability

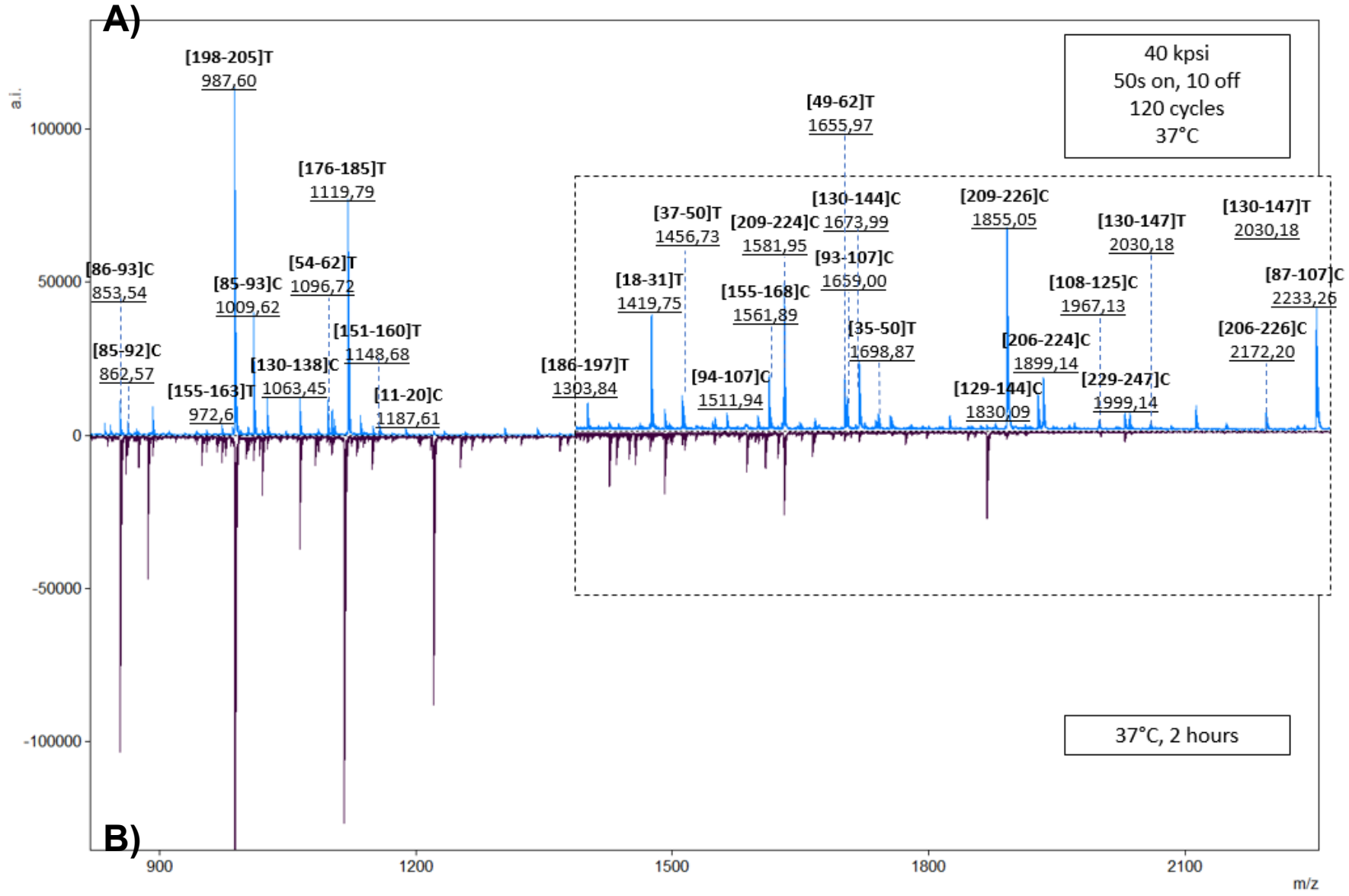
Highly conserved carbohydrate binding sites.



Galectin-3-LacNAc complex

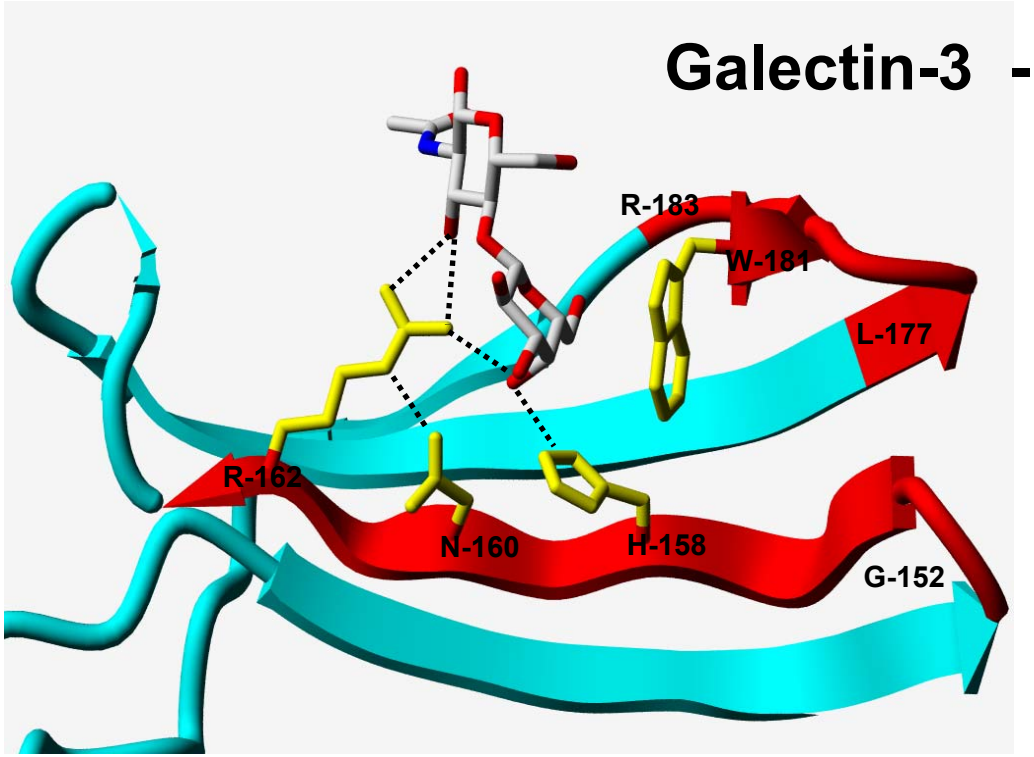


Galectin- 3 Digestion: Trypsin + Chymotrypsin

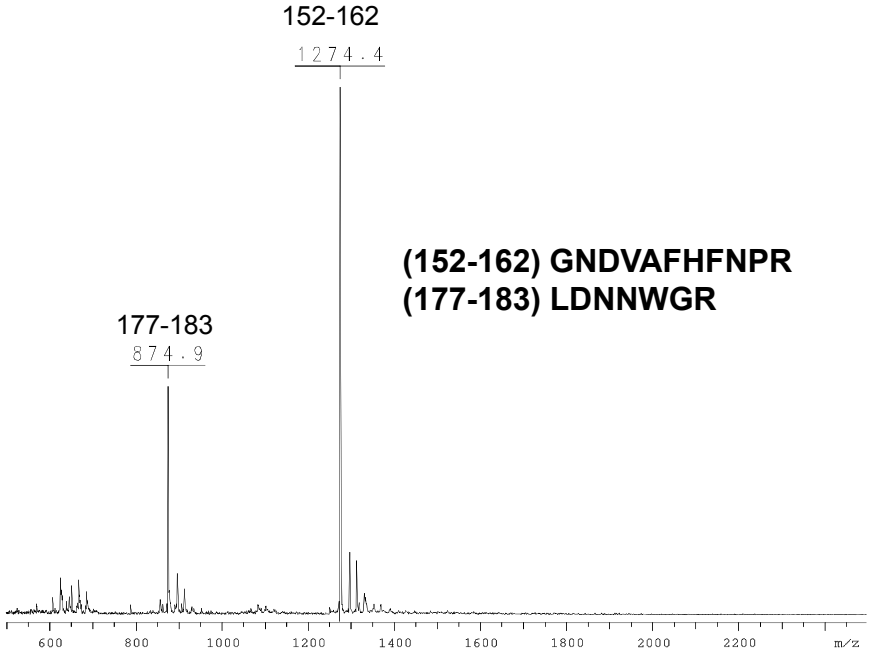


Galectin- carbohydrate interaction structures Revealed by proteolytic excision- biosensor-MS (CREDEX-MS)

Galectin-3 - Lactose complex



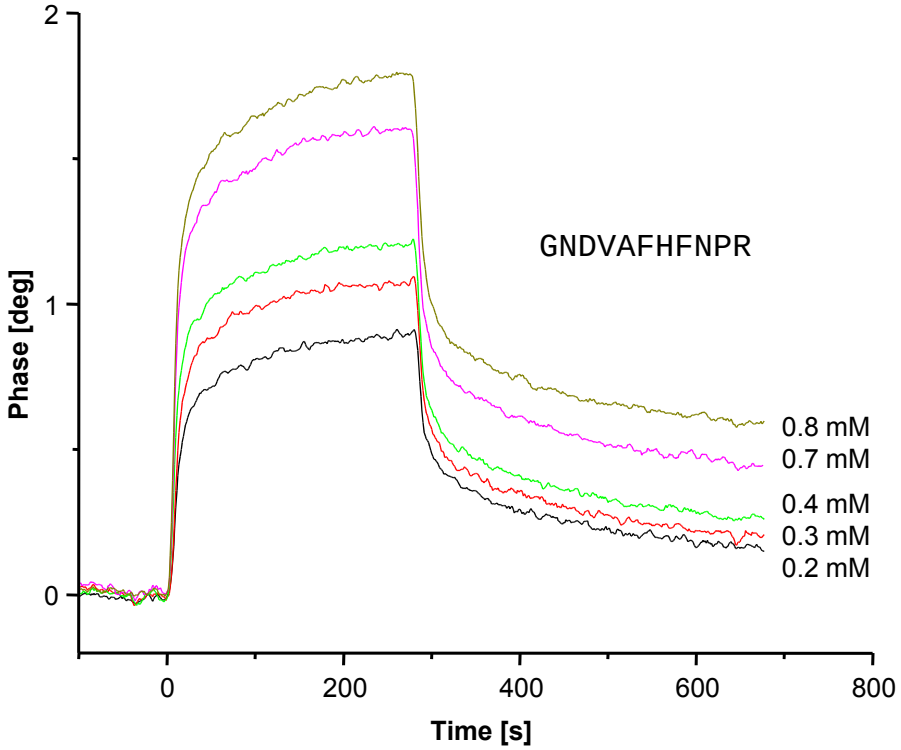
CRD Epitopes

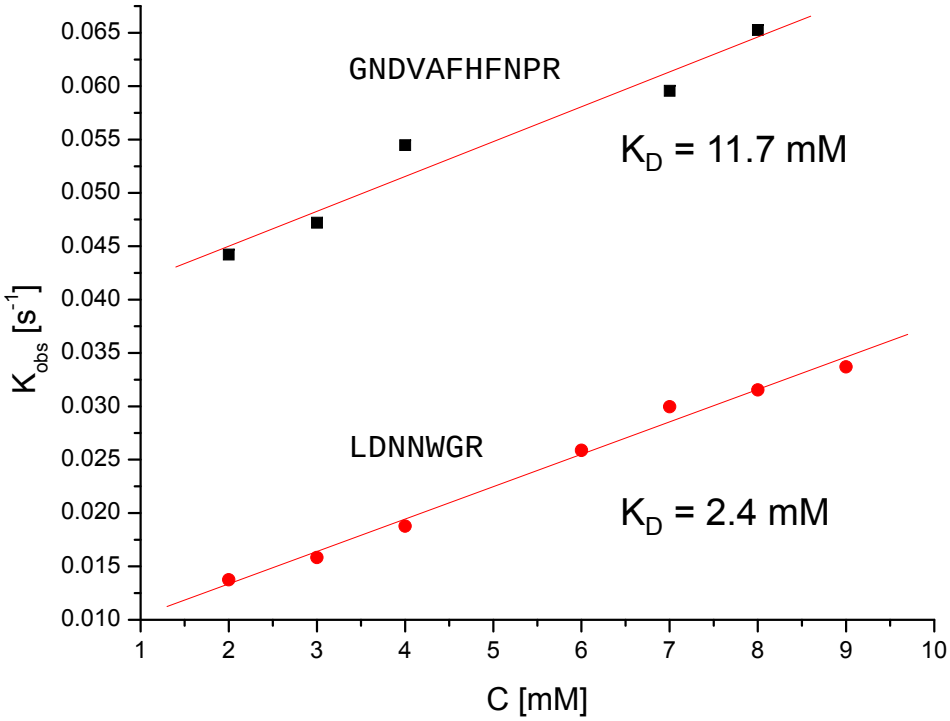


(152-162) GNDVAFHFNPR
(177-183) LDNNWGR

A. Moise et al. J. Am. Chem. Soc. (2011) 133, 14844

SPR Binding curves and K_D for CRD peptides





IMMUNERT:
Antibody Epitope Analysis provides new Approaches in Lysosomal Enzyme Replacement Therapy

Zdenek Kukacka[1], Hendrik Rusche[1], Loredana Lupu[1], Stefan Maeser[1], Fabio Borri[2], Anna Maria Papini[2], Julia Hennermann[3], Michael Przybylski[1]

[1]Steinbeis Research Center of Biopolymer Analysis & Biomedical Mass Spectrometry, Rüsselsheim am Main, Germany

[2]University of Florence, Department of Peptide Chemistry & Biology, Florence, Italy

[3]Universitätsmedizin Mainz, Department of Pediatrics, Mainz, Germany

What are Lysosomal Storage Diseases?

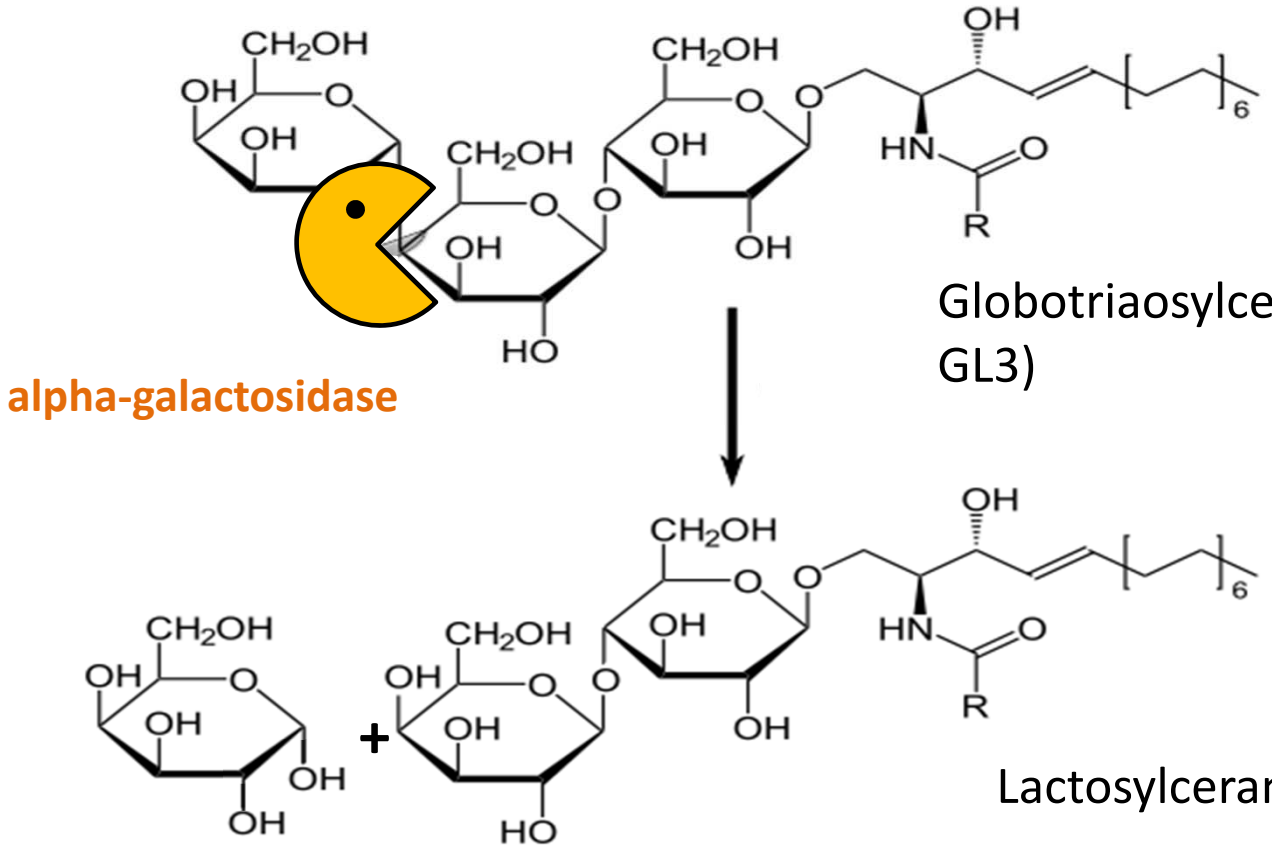
- Group of >70 genetic & partially biochemical disorders
  - A Specific lysosomal protein/enyme is deficient
  - Usually a hydrolase enzyme
    - Residual enzyme activity can range from 0% to ~30%

- Enzyme deficiency leads to substrate accumulation
  - Lysosomes become engorged
  - Leads to cellular dysfunction and irreversible organ damage

α-galactosidase A – defect in Fabry's Disease

- function: hydrolysis of terminal Gal

  - gangliosides and cerebrosides



Fabry disease treatment

- enzyme replacement therapy (ERT) with Fabrazyme or Replagal
- life long infusions of recombinant enzyme
- vast majority of male patients – development antibodies which cause allergic reactions and can neutralize the infused enzyme

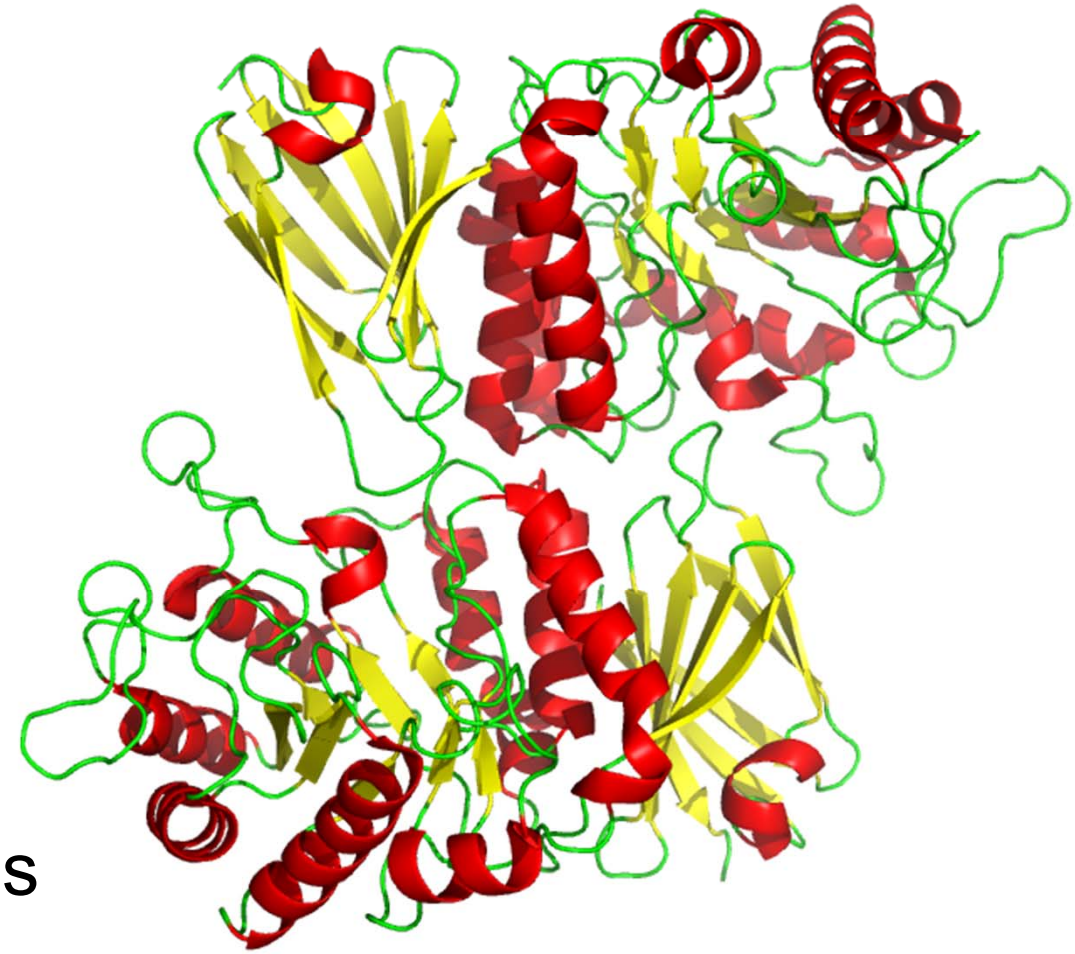
Main Complication: Immunoreactivity
- Antibody formation -

4 years

16 years

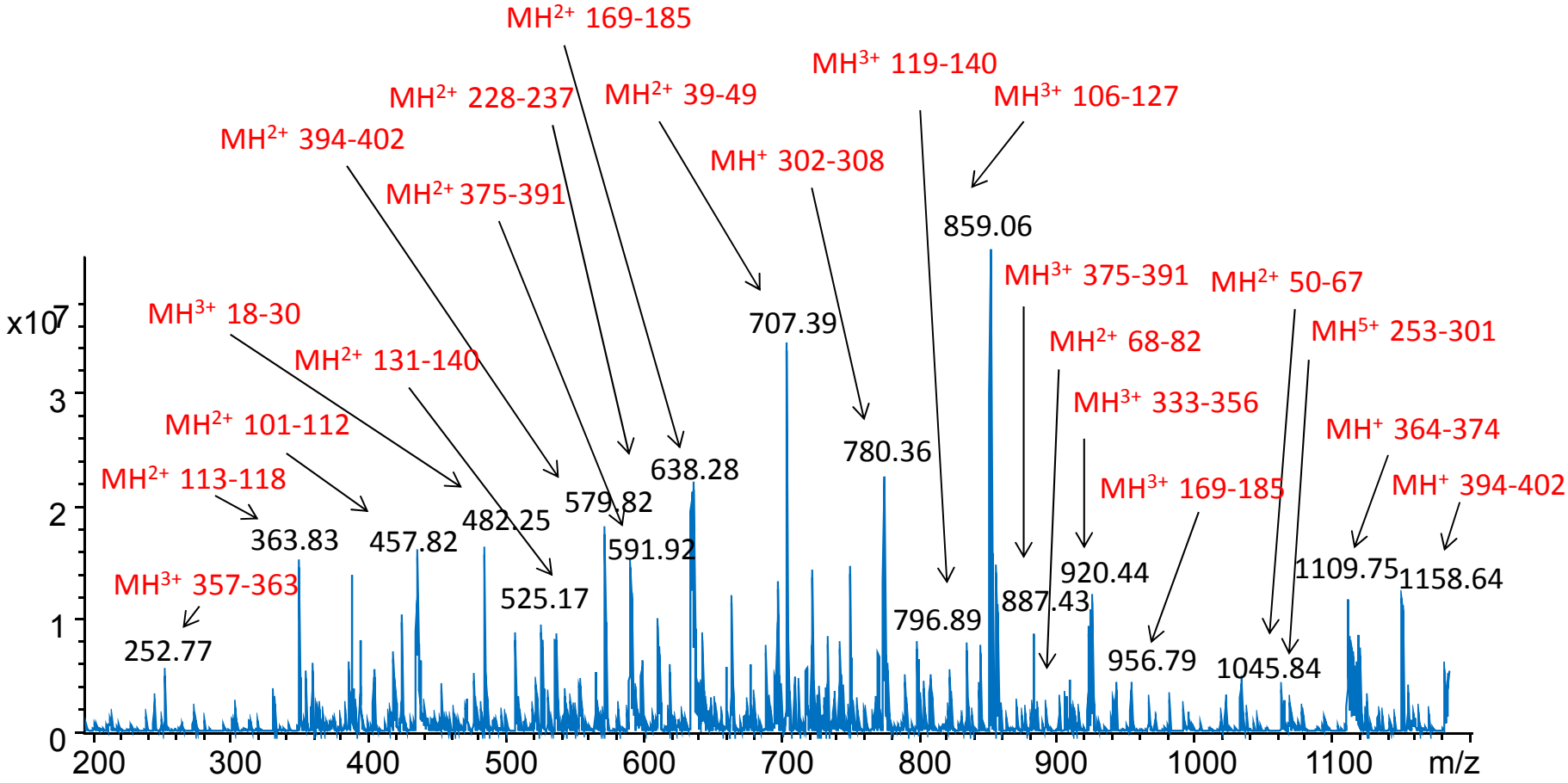
Human alpha-galactosidase

- lysosomal enzyme
- homodimer
- 429 AA, 50 kDa
- glycoprotein
- GH27 family
- 3 glycosylation sites



crystal structure of human α−galactosidase; PDB:4NXS

α-Gal A epitope determination

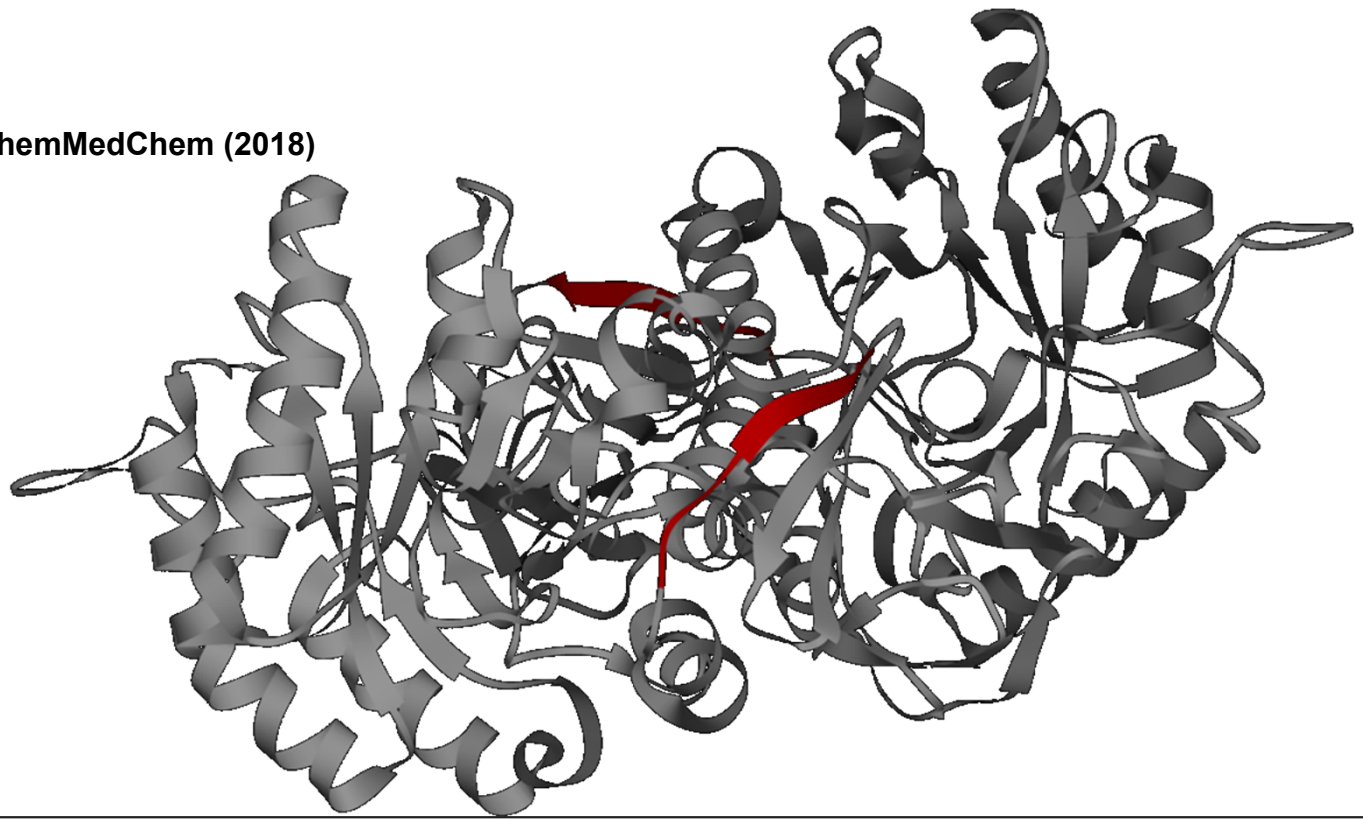


mass spectrum of supernatant fraction after α−Gal A tryptic digestion
Trypsin 1 hr

1	MQLRNPELHL	GCALALRFLA	LVSWDIPGAR	ALDNGLARTP	TMGWLHWERF	50
51	MCNLDCQEEP	DSCISEKLFM	EMAELMVSEG	WKDAGYEYLC	IDDCWMAPQR	100
101	DSEGRLQADP	QRFPHGIRQL	ANYVHSKGLK	LGIYADVGNK	TCAGFPGSFG	150
151	YYDIDAQTFA	DWGVDLLKFD	GCYCDSLENL	ADGYKHMSLA	LNRTGRSIVY	200
201	SCEWPLYMWP	FQKPNYTEIR	QYCNHWRNFA	DIDDSWKSIK	SILDWTSFNQ	250
251	ERIVDVAGPG	GWNDPDMLVI	GNFGLSWNQQ	VTQMALWAIM	AAPLFMSNDL	300
301	RHISPQAKAL	LQDKDVIAIN	QDPLGKQGYQ	LRQGDNFEVW	ERPLSGLAWA	350
351	VAMINRQEIG	GPRSYTIAVA	SLGKGVACNP	ACFITQLLPV	KRKLGFYEWT	400
401	SRLRSHINPT	GTVLLQLENT	MQMSLKDLLA	HHHHHHHHHH		440

A

Kukacka et al ChemMedChem (2018)

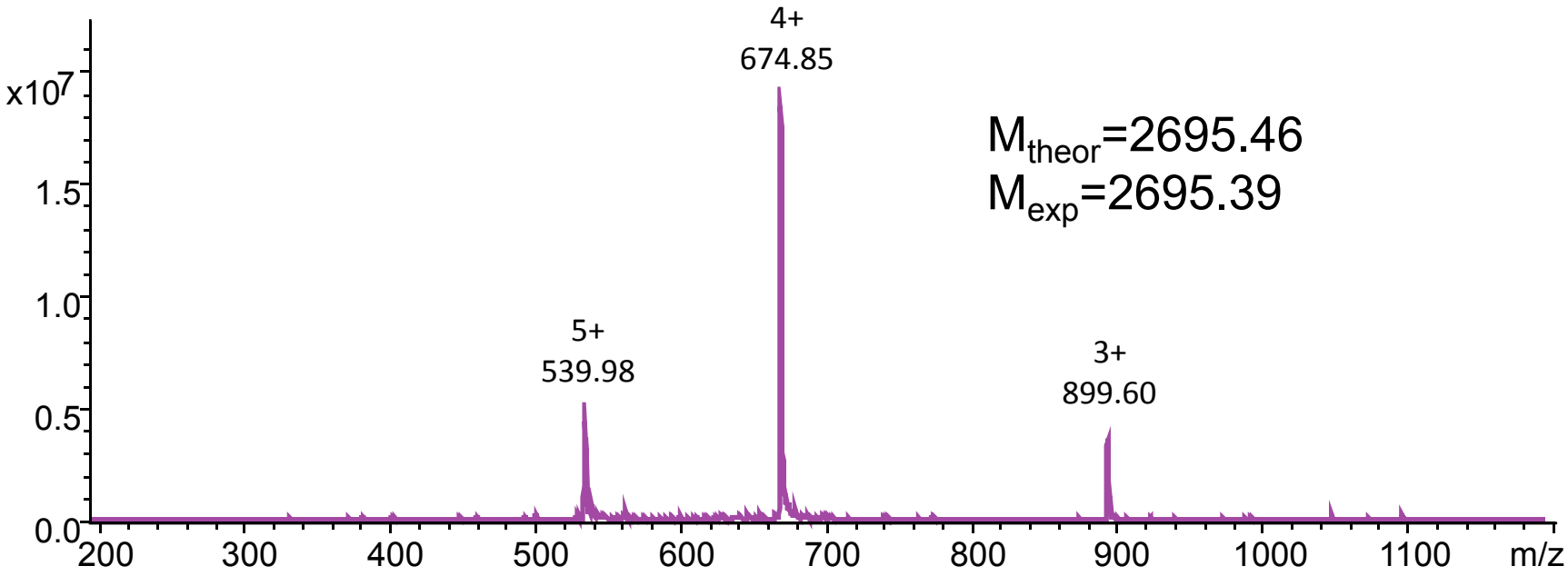


B

α-Gal epitope identication by ESI-MS
Sera of 2 Patients

epitope peptide
α-Gal (309-332)

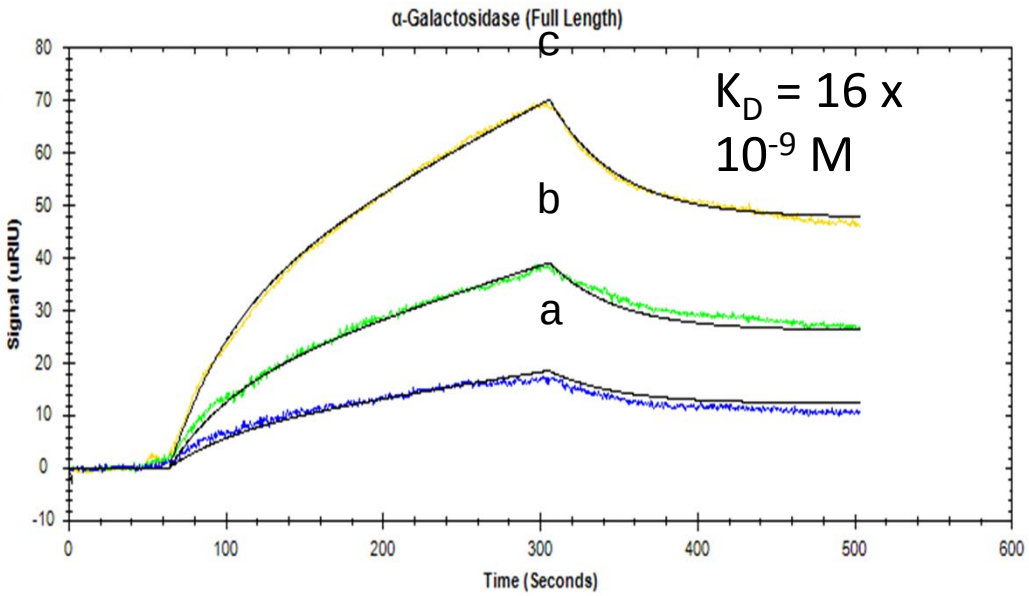
^{309}ALLQDKDVIAINQDPLGKQGYQLR^{332}



mass spectrum of elution fraction of α-Gal tryptic epitope extraction

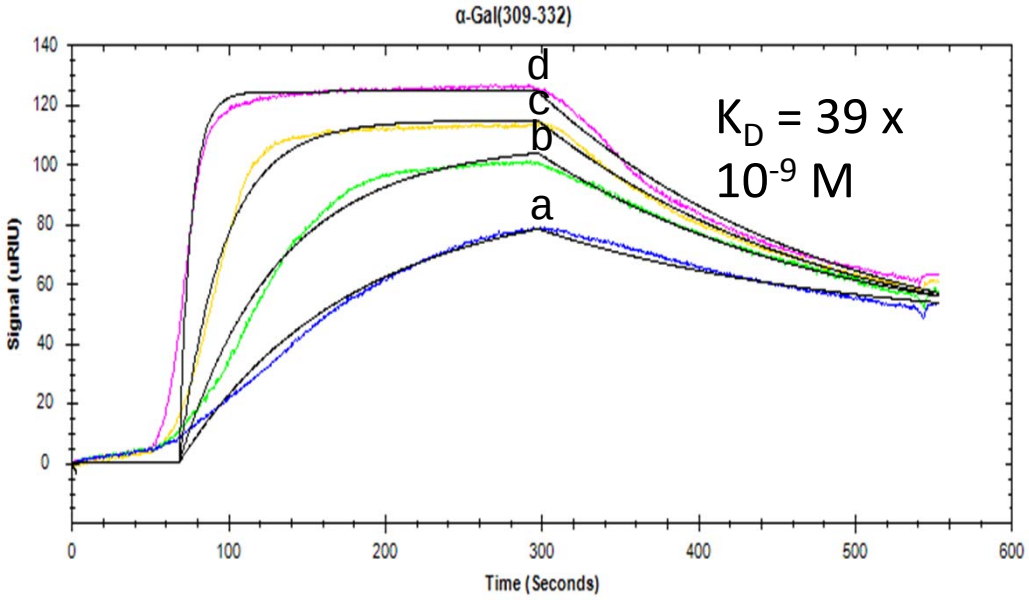
K_D determination of complete α-Gal and (309-332) epitope are comparable

A



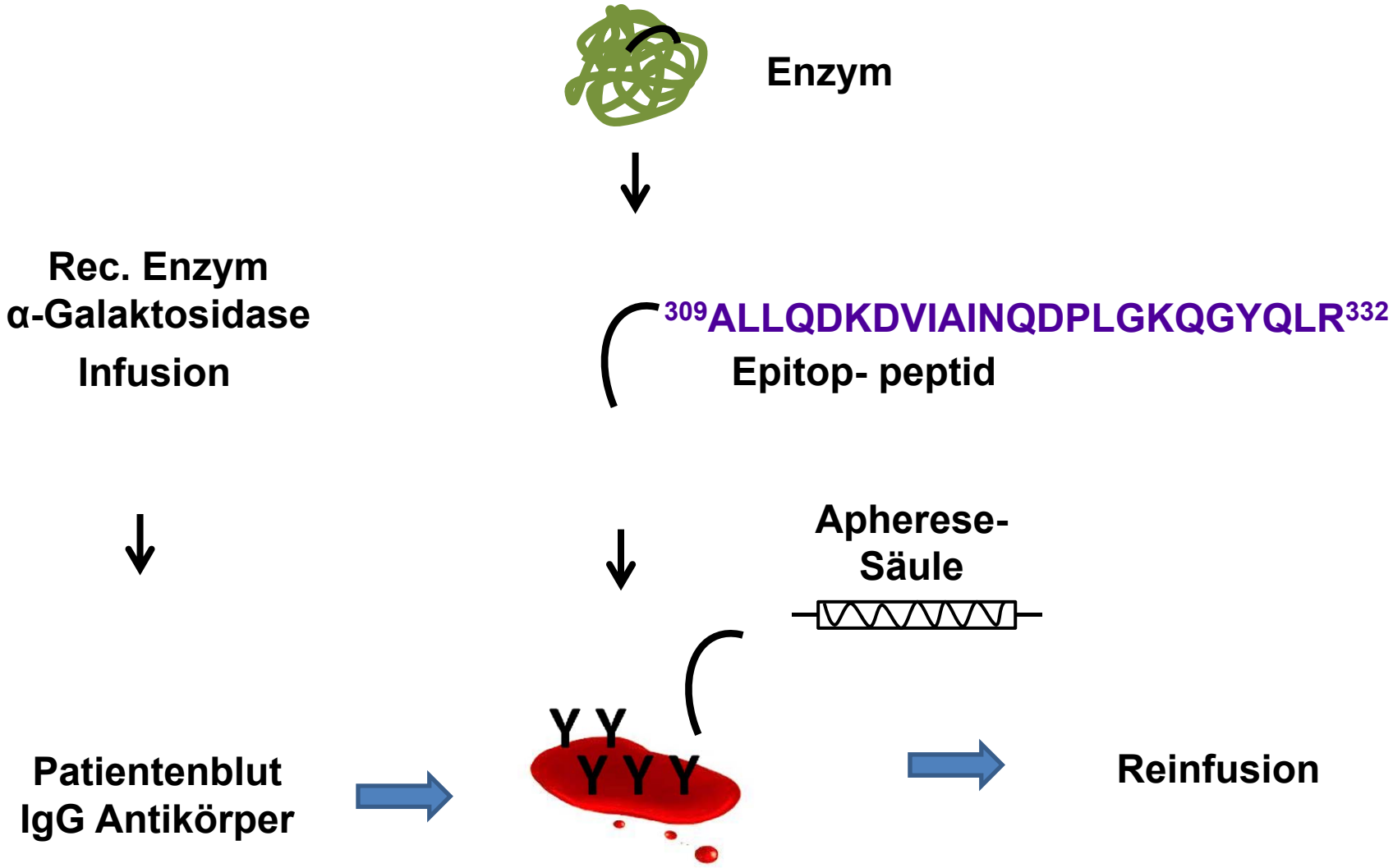
- a = 5 µM
- b = 10 µM
- c = 20 µM

B



- a = 50 nM
- b = 100 nM
- c = 250 nM
- d = 1 µM

Neutralisierung von Antikörpern nach Enzymersatztherapie durch molekulare Apherese mit Epitop- Peptid



Conclusions - Perspectives

Molecular Antibody Epitope analysis enables new Applications for:

- **Neutralizing immunogenic therapeutic proteins**
- **Characterization of therapeutic antibodies - individual medicine**
- **Neutralization of pathophysiological antibodies in transplantation medicine**

- **Key tools: Proteolytic Digestion techniques**

Int. Workshop

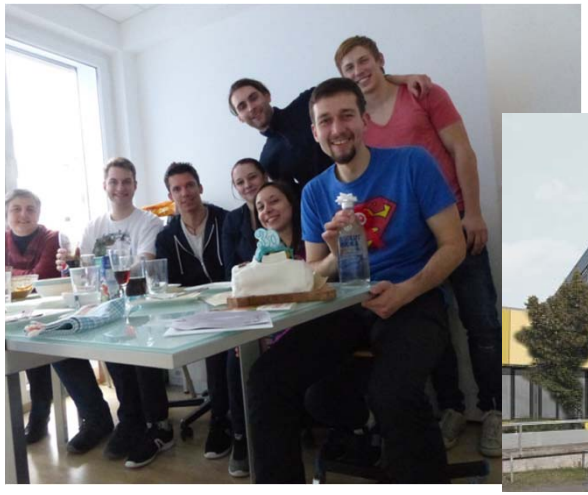
“Affinity – Mass Spectrometry – Clinical Method Development and Applications”

August 19 - 24th 2018

Rüsselsheim am Main, Hochschule Rhein-Main

Topics include:

Antibody Characterization - Peptide & Protein Epitope Identification – Biosensor- Mass Spectrometry – Affinity-Proteomics – Clinical Immunoassays – New MS Technologies

Organizers:

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