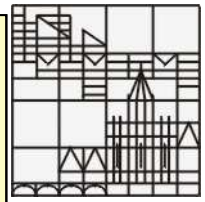
INNOMOL Workshop Proteomics
Ruder Boskovic Institute Zagreb
April 7th 2014



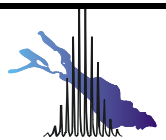
Online SAW- Bioaffinity- Mass Spectrometry: New Bioanalytical Tool for Detection, Structure Determination and Quantification of Protein-Ligand Interactions from Biological Material

Michael Przybylski

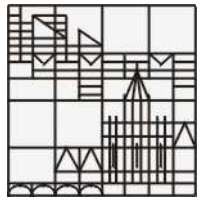
Laboratory of Analytical Chemistry and Steinbeis Research Centre Biopolymer Analysis University of Konstanz

www.uni-konstanz.de/agprzybylski/chemie

www.affinityms.de



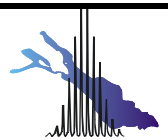
Why online Bioaffinity-MS ?



- Online HPLC-ESIMS: Standard for separation/ quantification and identification of biopolymer mixtures

- Bioaffinity/biosensor determination of binding stoichiometry and affinity quantification of biopolymer - ligand interactions - **but no molecular structure identification & characterisation**

- Mass spectrometry: **Identification of structures/interaction partners** of protein-ligand complexes



OVERVIEW

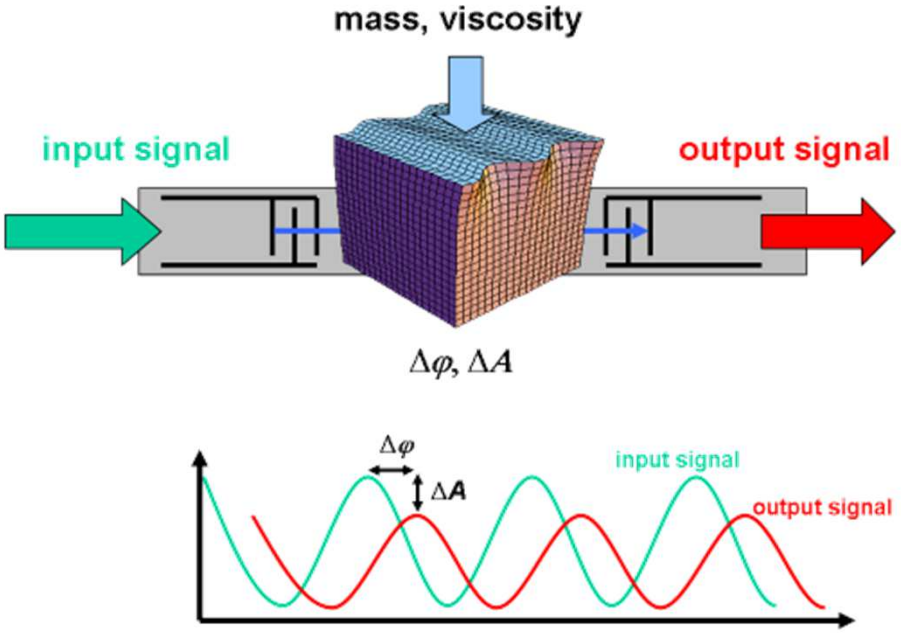
I Online SAW- Biosensor-MS Combination:

- **Analytical Development - Interface**
- **Application Examples**

II Oligomerisation - Aggregation of Parkinson's Disease Protein α-Synuclein:

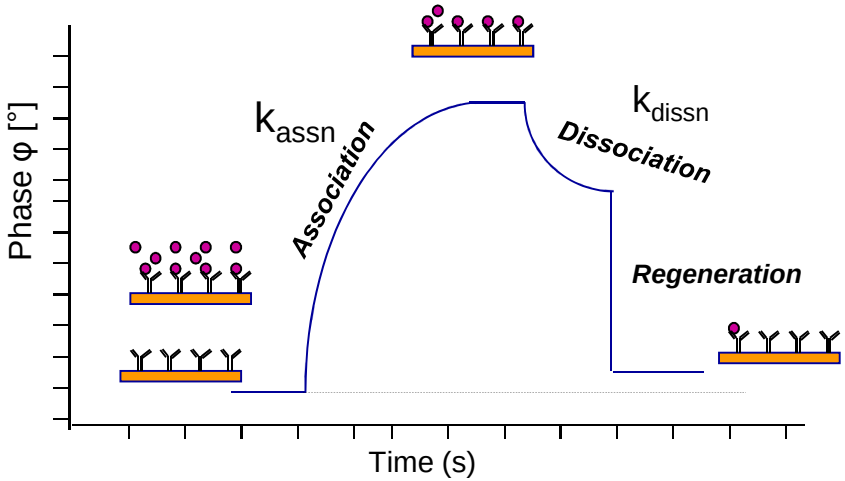
- **Identification of Oligomer Intermediates,**
- **Direct Analysis from Biological Material**

Principle of Surface Acoustic Wave biosensor (SAW)



Mass loading – **Δφ**

Viscosity change – **Δφ and ΔA**

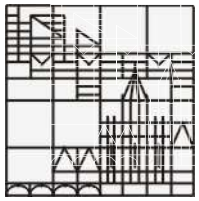


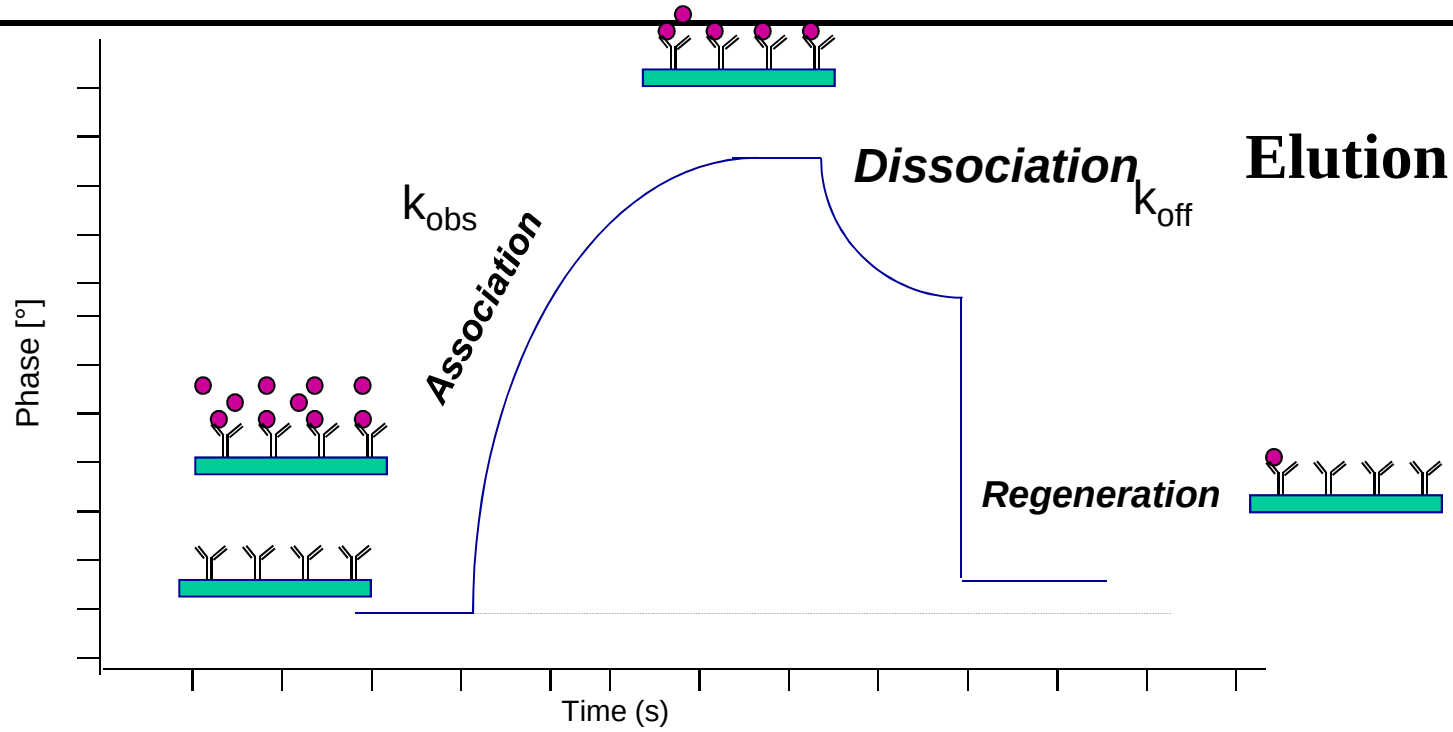
$$AB \underset{k_{assn}}{\overset{k_{dissn}}{\rightleftharpoons}} A + B$$

$$K_D = \frac{k_{dissn}}{k_{assn}}$$

Perpeet, M. et al. (2006), *Analytical Letters*, **39**: 1747–1757.

Affinity - MS:
Dissociation step: Elution interface needed



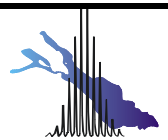


$$AB \underset{K_{on}}{\overset{K_{off}}{\rightleftharpoons}} A + B$$

$$Kd = \frac{K_{off}}{K_{on}}$$

K_{on} - association rate constant
K_{off} - dissociation rate constant
K_{obs} - pseudo-first order kinetic constant

$k_{obs} = c * k_{on} - k_{off}$



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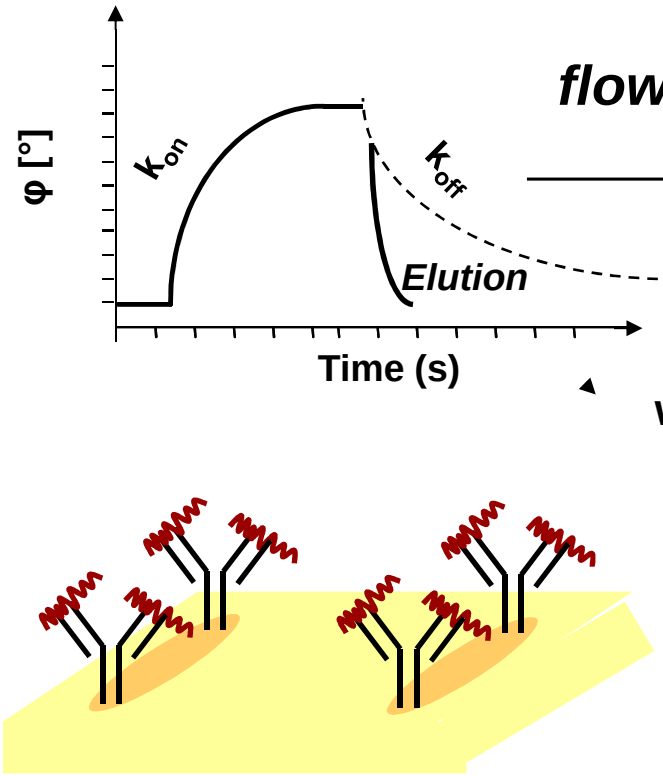
for Biopolymer Analysis &
Biomolecule Mass Spectrometry

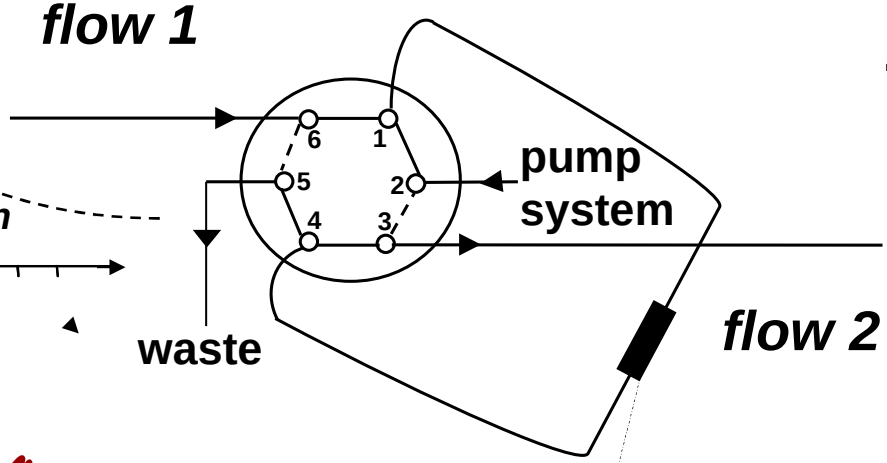
SAW- Bioaffinity- Mass Spectrometry Combination SAWMS – I

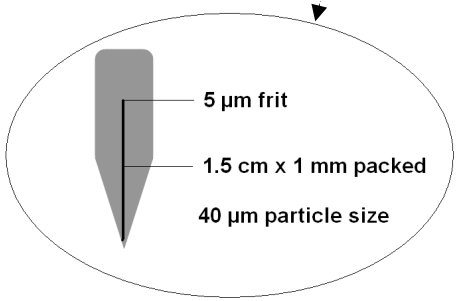
Interface

INTERFACE: Provides simultaneous desalting & concentration

Biosensor







ESI-MS

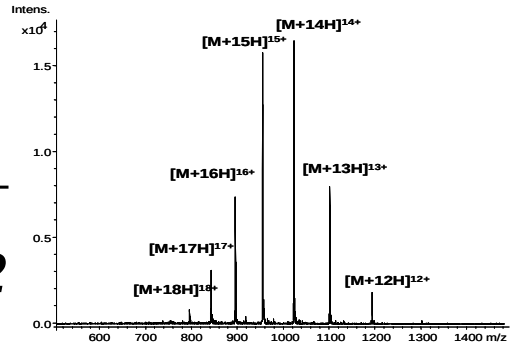
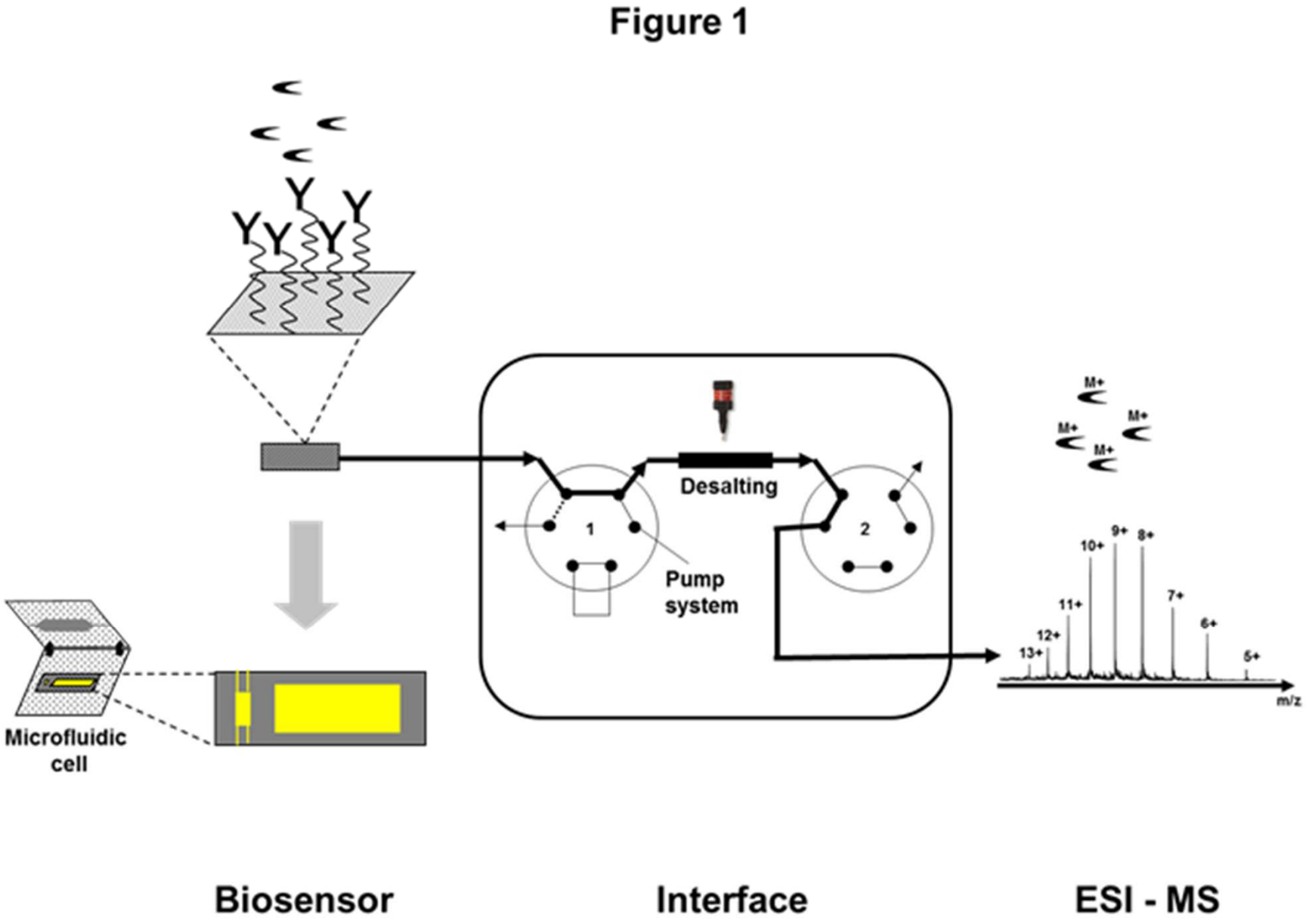
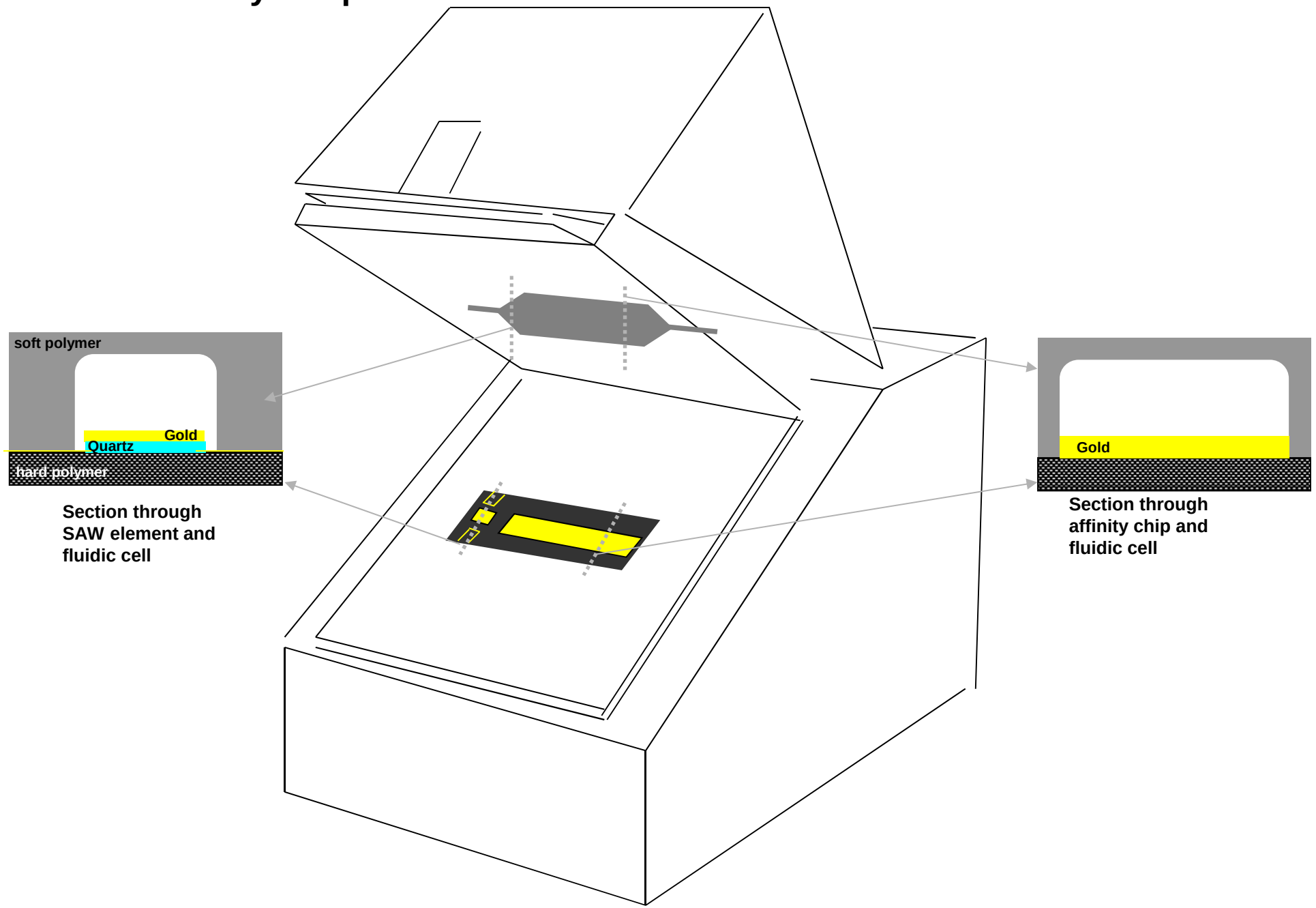


Figure 1 Scheme of SAW- affinity- MS interface



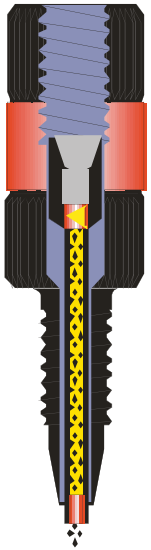
Affinity- Chip for online SAW-MS: Simultaneous Detection & Isolation



Section through SAW element and fluidic cell

Section through affinity chip and fluidic cell

Key integrating function between biosensor and MS



Functions:

- **Buffer desalting**
- **Flow rate equilibration**
- **Transfer of eluate to MS**

C_4, C_{18} /
Specific matrix

Online coupling of SAW- biosensor with ESI-MS

SAW-MSLife-I

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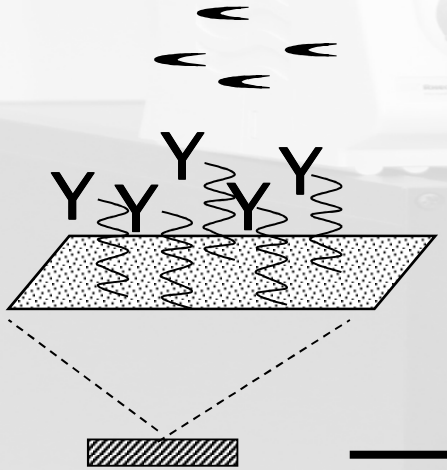
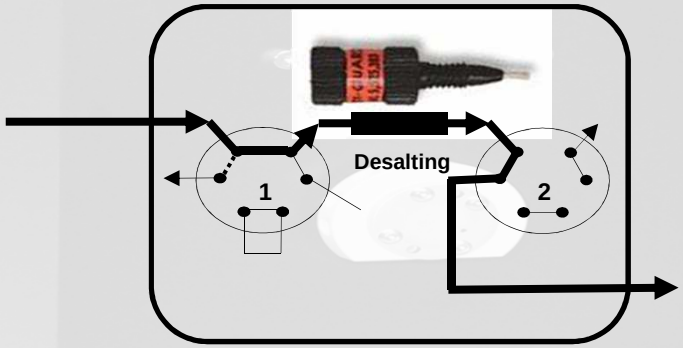
Analytical Scheme - automated interface operation, MS- Acquisition software for several ESI-MS systems

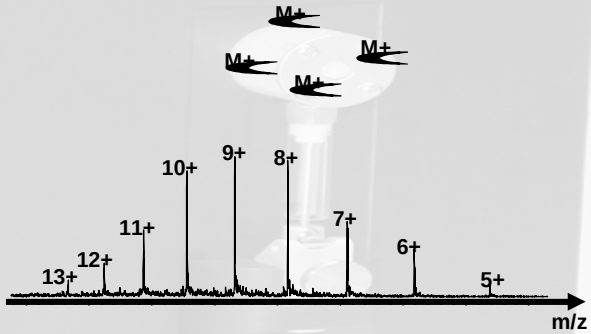
1st Installation: Nov. 2013 - Synapt-QTOF



Biosensor



Interface



ESI - MS

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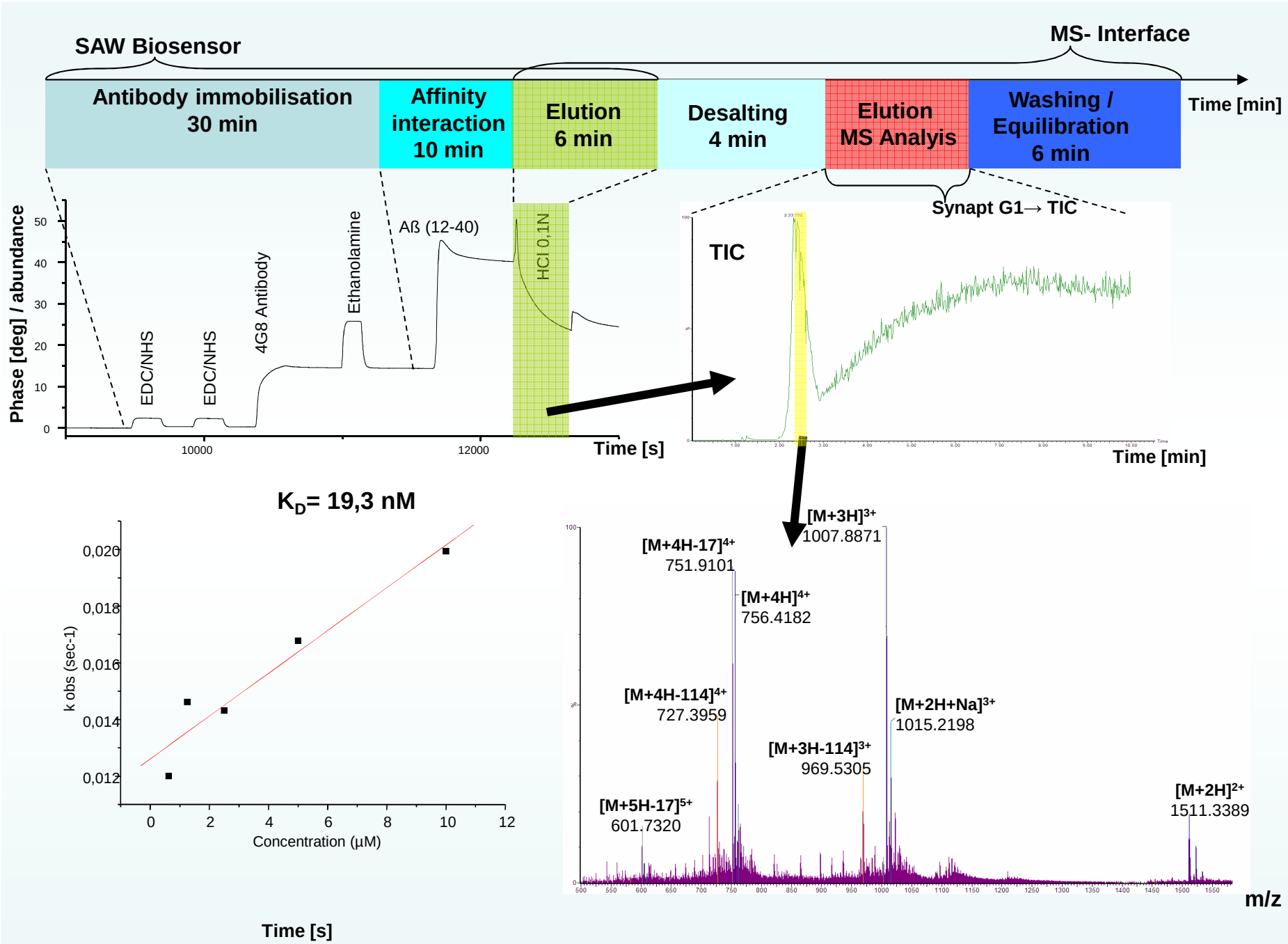
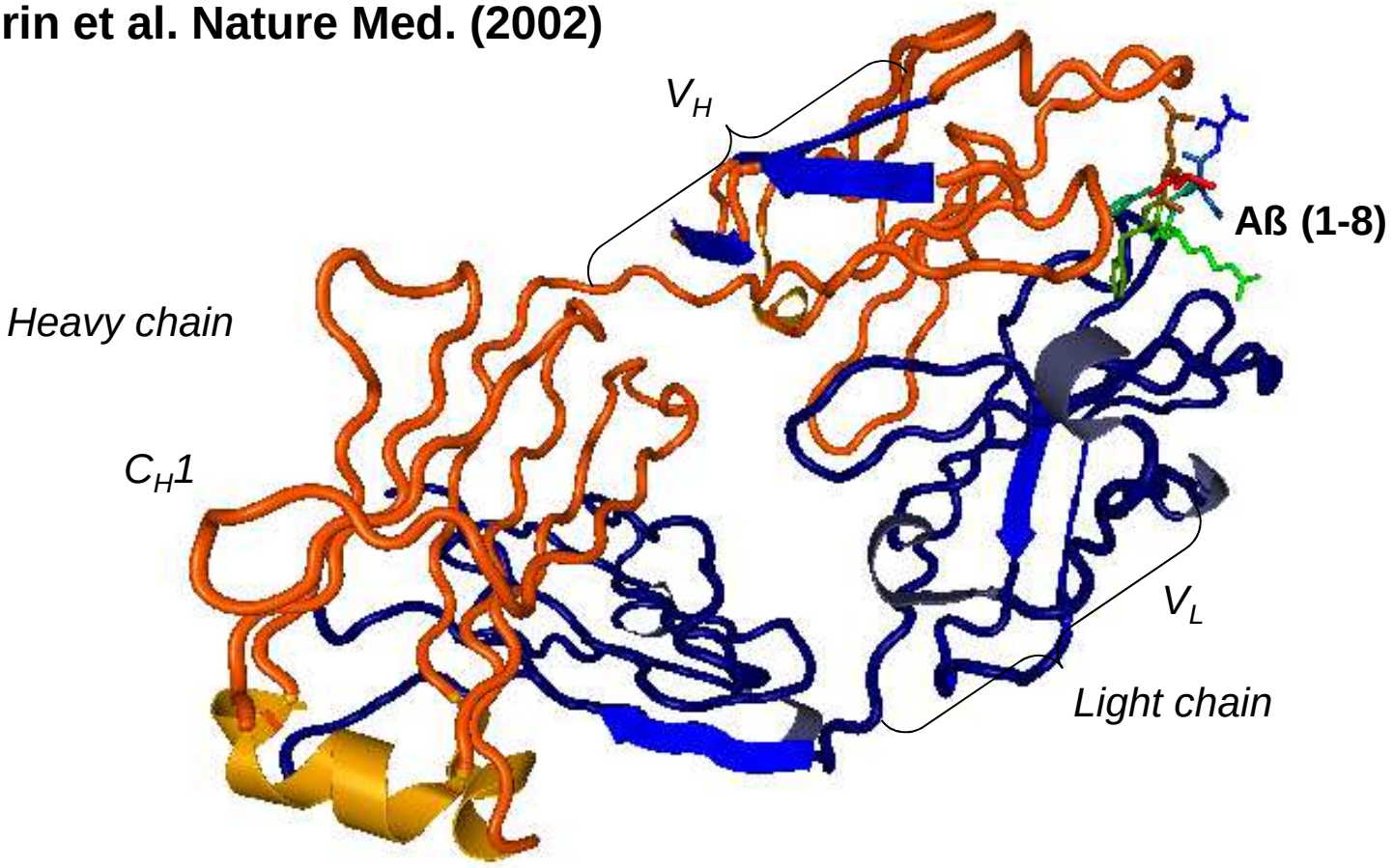


SAW-MS-SynaptG1 Coupling/Aß- Antibody/Aß40-Peptide (*courtesy Dr. M. Vilaseca, IRB* Barcelona)

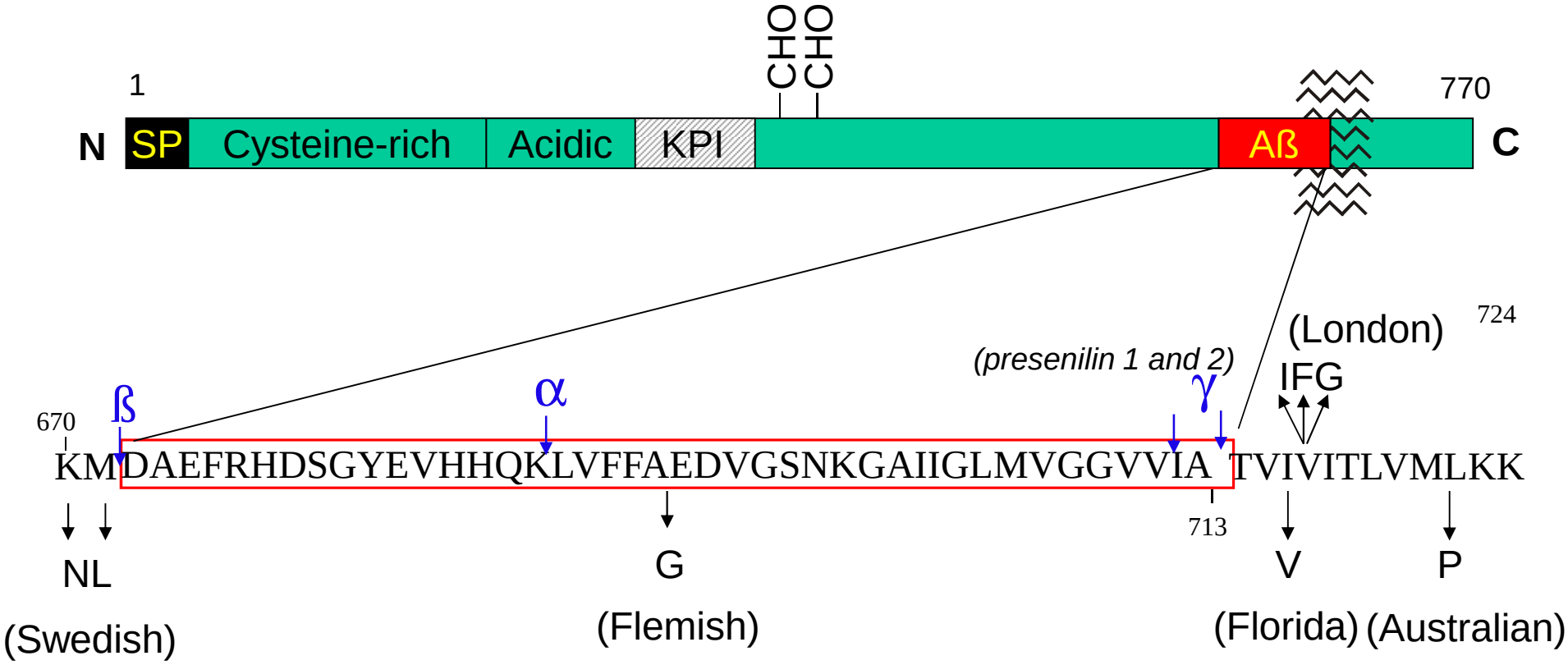
APPLICATION 1: Crystal structure of an Aß-plaque specific antibody complex with N-terminal Aß-epitope

J. McLaurin et al. Nature Med. (2002)



PDB access number: 2IPU - Gardberg, A.S., et al., (2007) Molecular basis for passive immunotherapy of Alzheimer's disease, PNAS, 104, 15659-15664

ß- Amyloid formation & „ß-and γ-secretase“ cleavage sites - Not well characterized



Selective proteolytic excision of antigens in immune complexes
-- Basis for mass spectrometric epitope identification

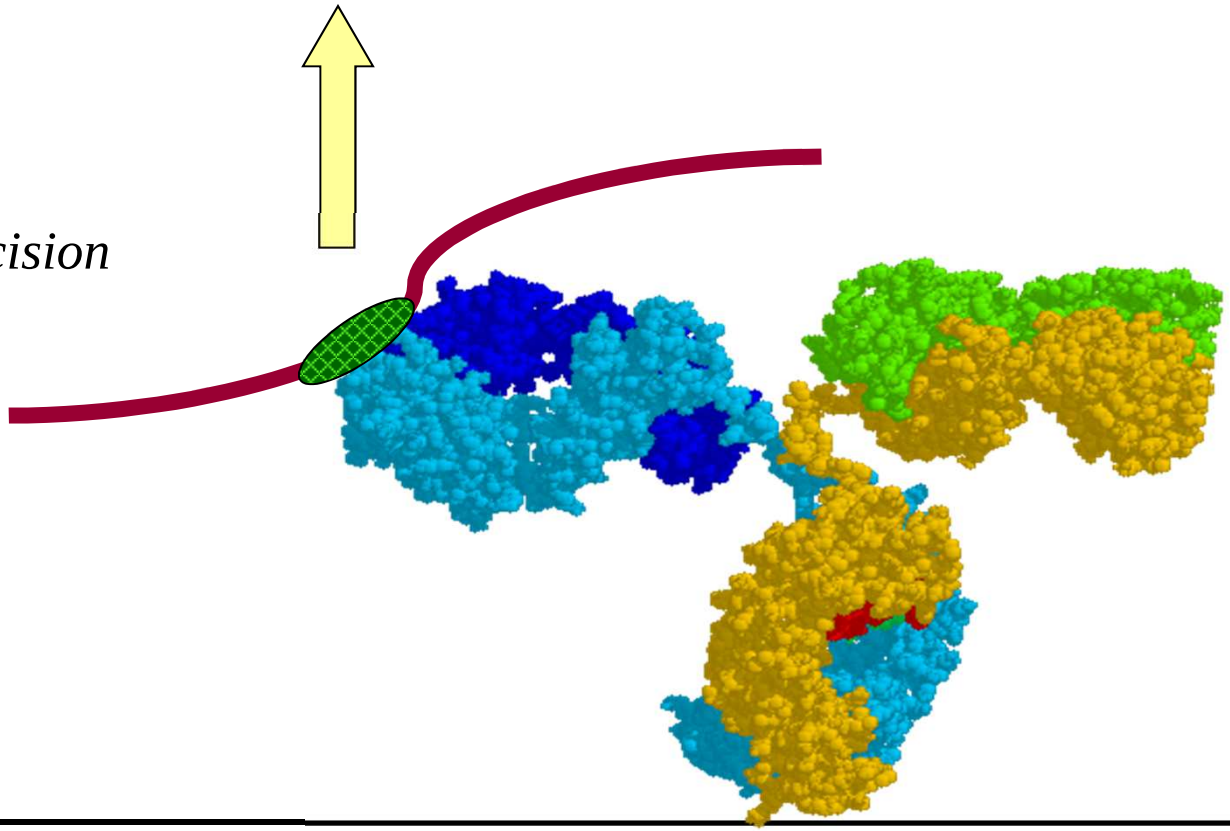
Preconditions:

Proteolytic stability of antibody

Epitope-Paratope Interaction shielded

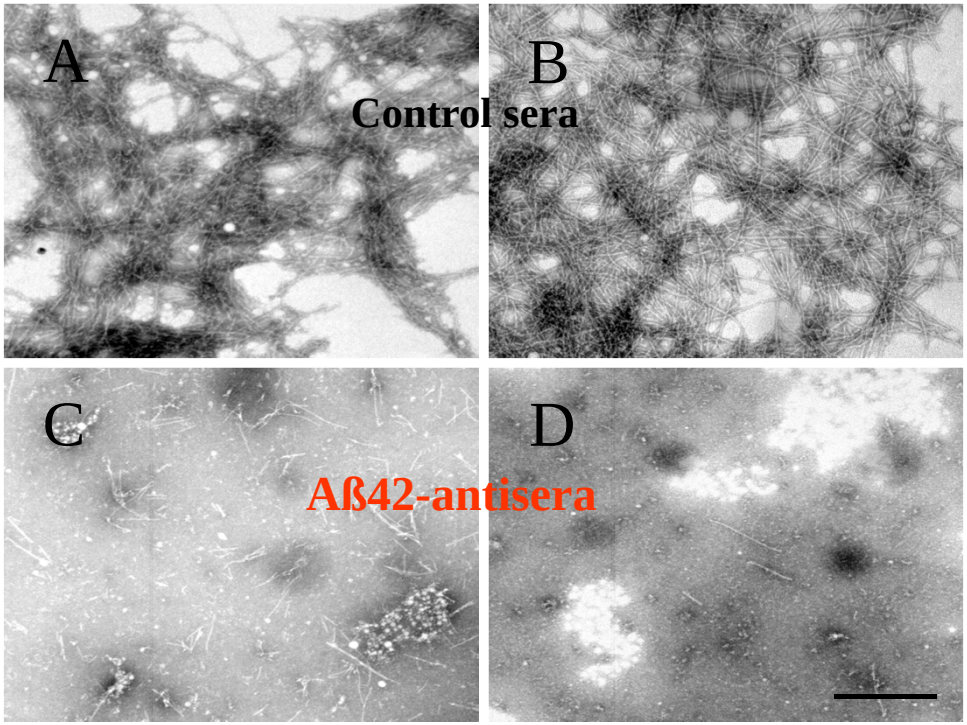
Epitope peptide

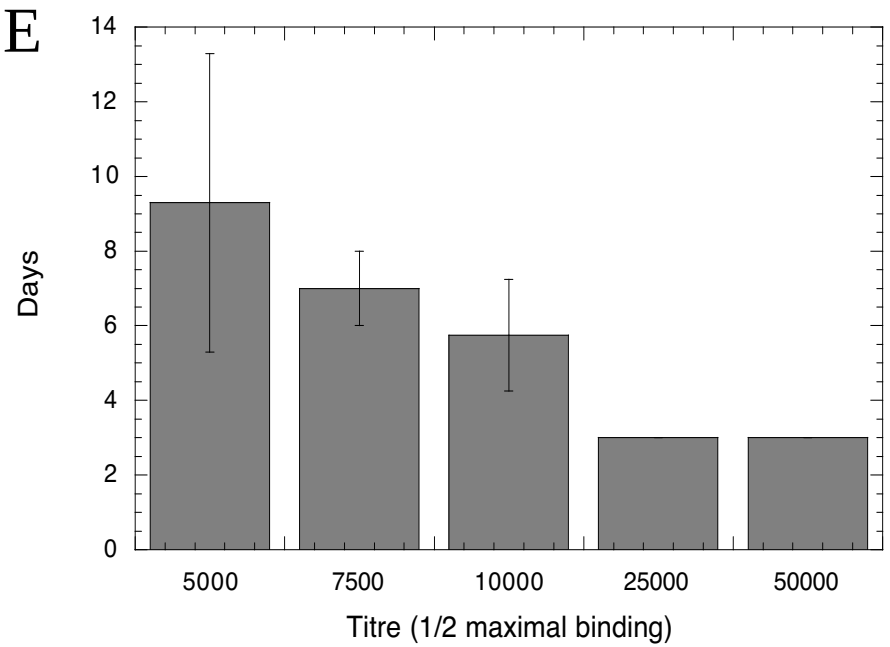
Epitope Excision



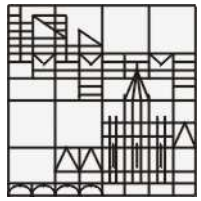
D. Suckau, M. Przybylski et al. *Proc. Natl. Acad. Sci USA* (1990)
M. Macht et al. *Biochemistry* (1996)

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



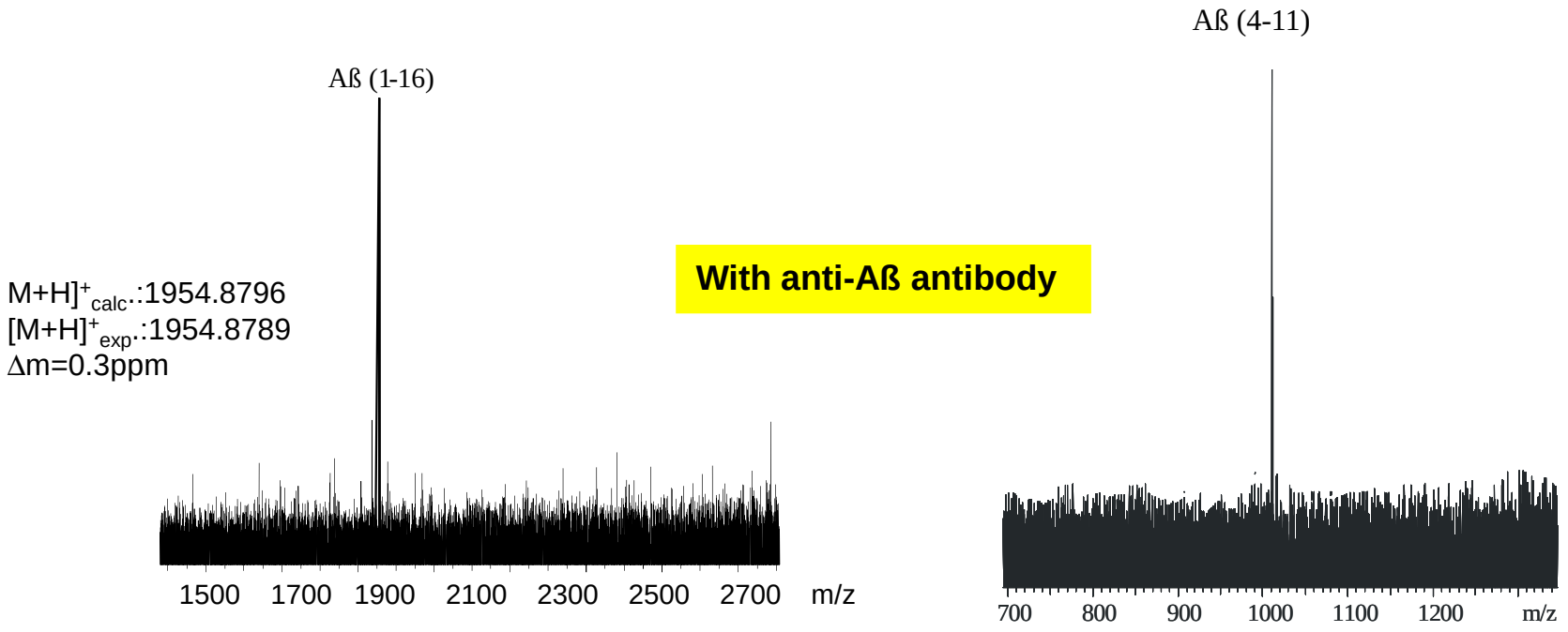


Epitope Identification of Amyloid Plaque Specific Antibody by MALDI-FTICR-MS



proteolytic digestion/trypsin, GluC, chymotrypsin

shielded from proteolytic digestion/trypsin, AspN



Trypsin **GluC**

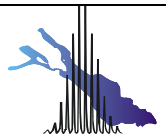
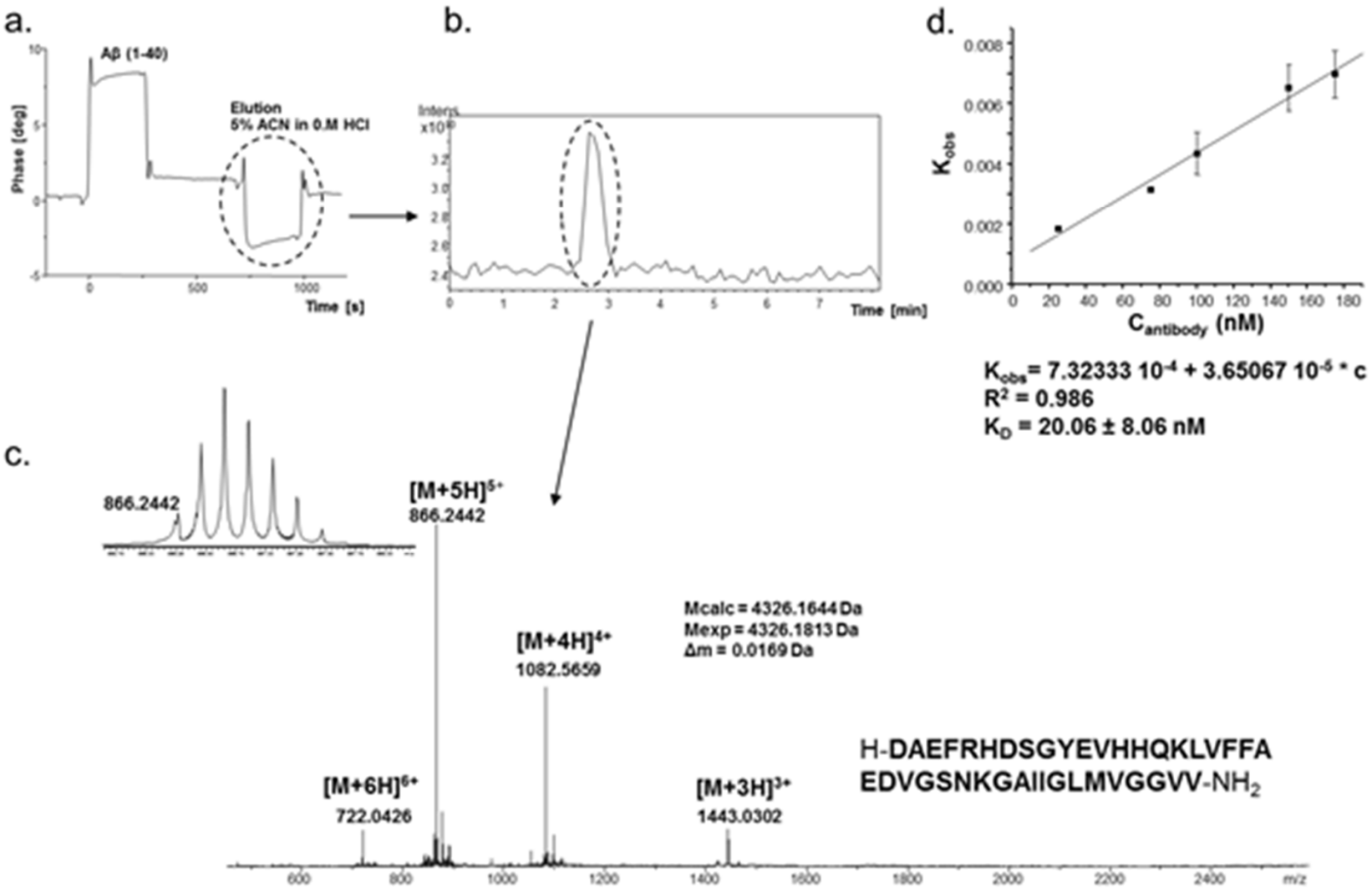
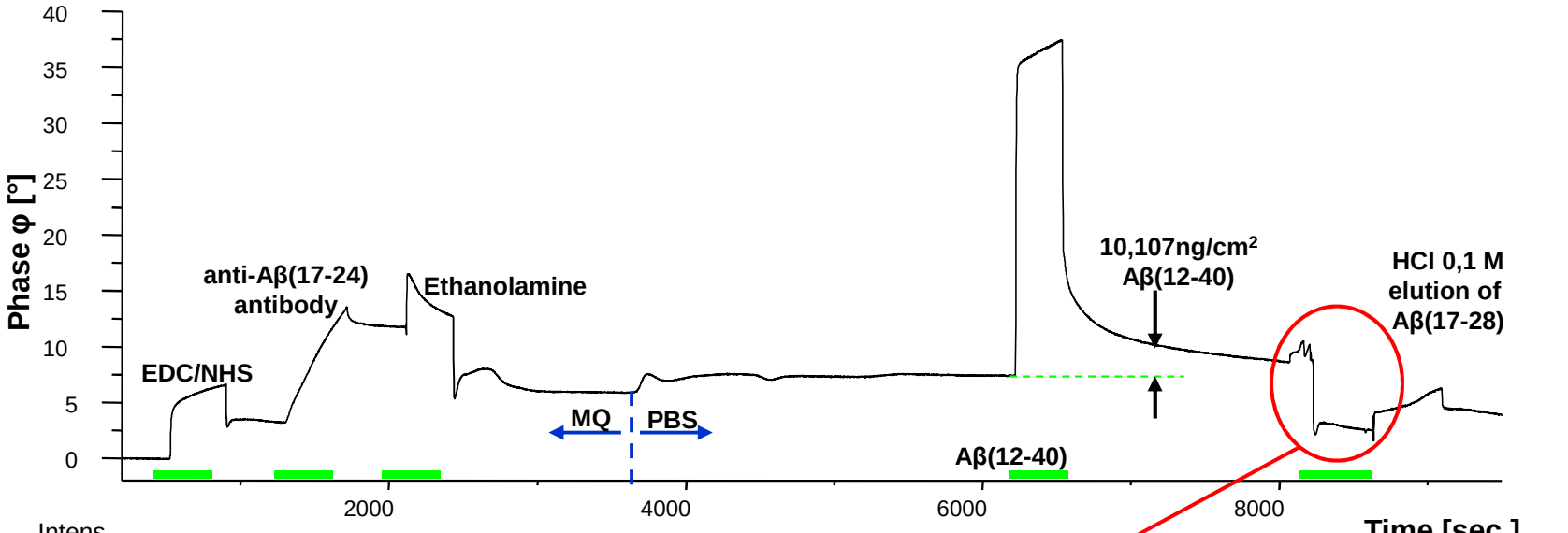
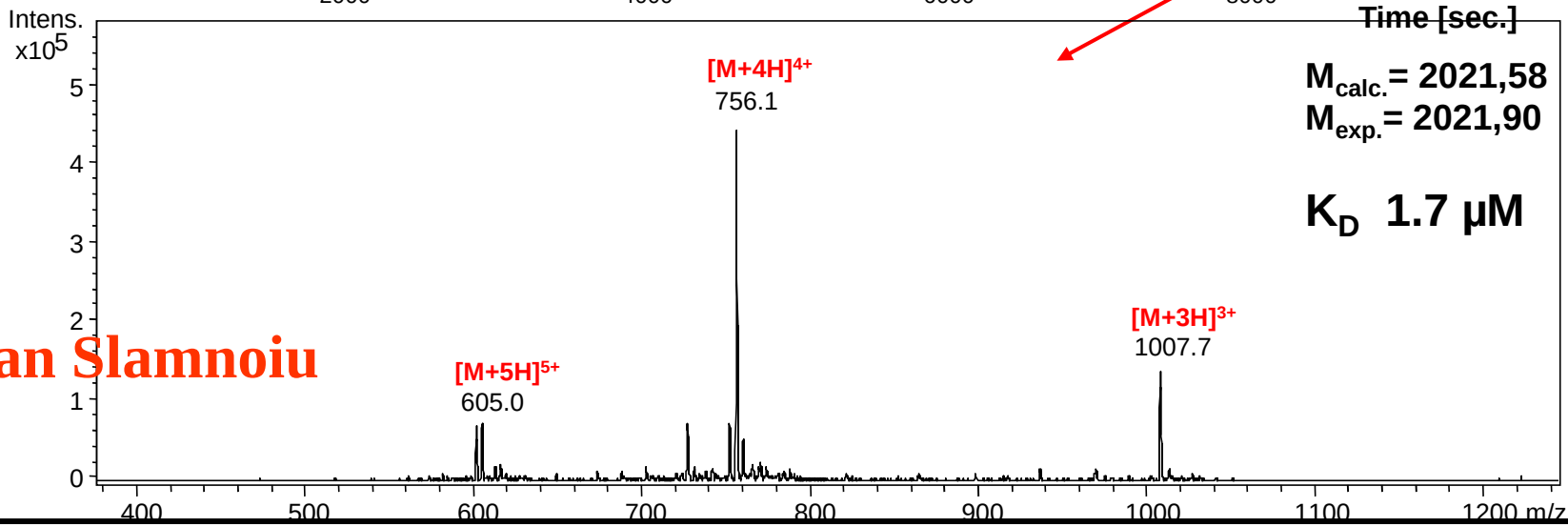


Figure 2 MS Determination and affinity quantification of Aß-specific antibody



Affinity of anti-Aβ antibody with Aβ(1 - 16) by direct coupling SAW biosensor – ESI ion trap MS





$M_{calc.}$ = 2021,58
$M_{exp.}$ = 2021,90

K_D 1.7 µM

Stefan Slamnoiu

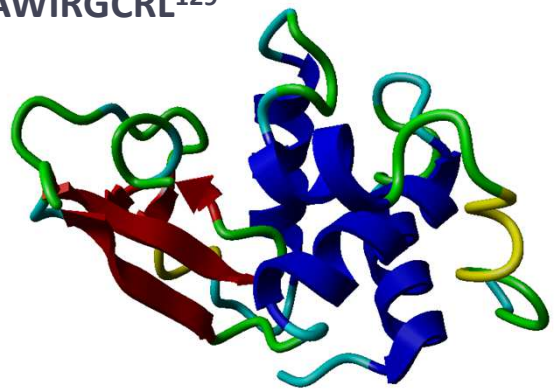
Analytical Chemistry & Biopolymer Structure Analysis
University of Konstanz

Application 2: Online Affinity-MS with FTICR-MS: Interaction of p- anti-Lysozyme Ab – HEL

Lysozyme

- strongly basic protein of 129 residues (14.3 kDa)
- small secretory enzyme that catalyzes hydrolysis of β-1-4 glycosidic bond
- bacteriostatic, bactericidal and bacteriolytic activity
- very stable and compact enzyme with four disulfide bonds

^{1}KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNTQATNRNTDGSTDY
GILQINSRWWCNDGRTPGSRNLCNIPCSALLSSDITASVNCAKKIVSDGNGMNAW
VAWRNRCKGTDVQAWIRGCRL^{129}

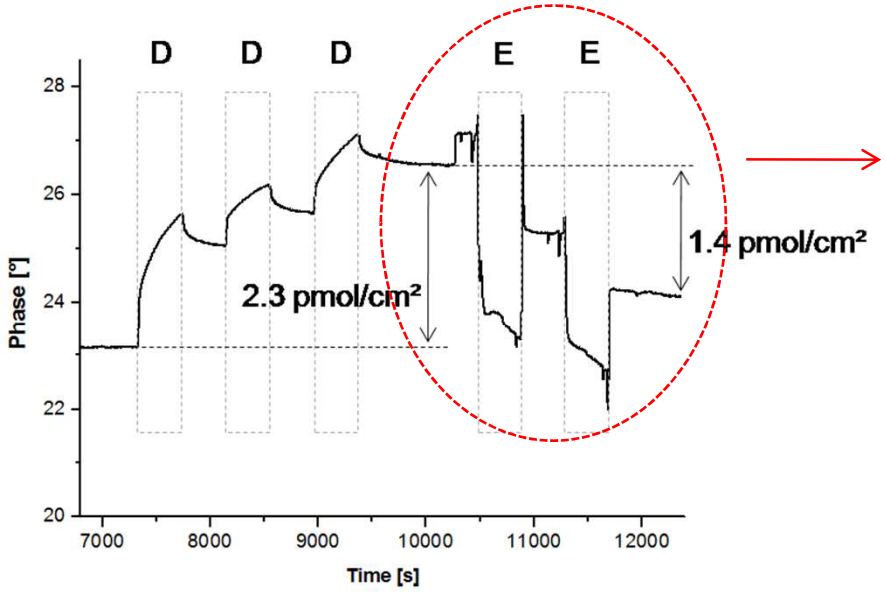


Ribbon structure, PDB 3IJV; E. Pechkova et al., **2010**, *to be published*.

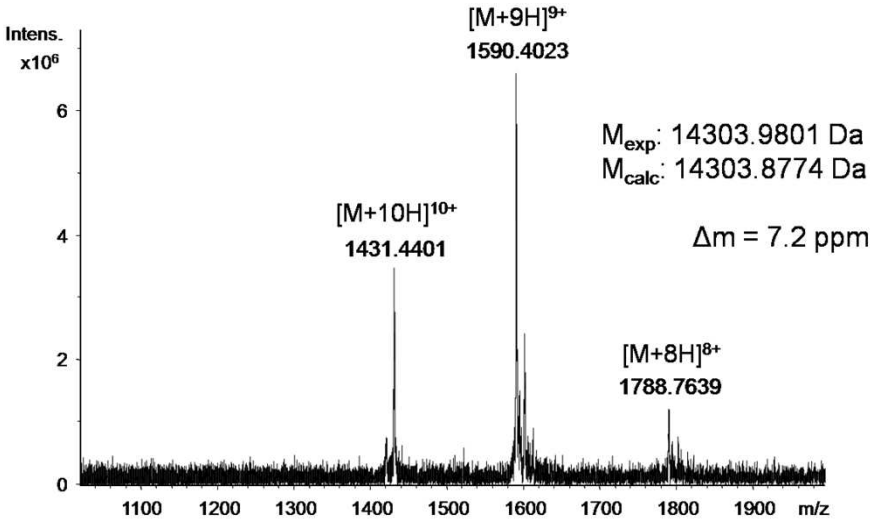
High resolution bioaffinity- MS of anti-HEL- antibody - Lysozyme interaction: "Top-Down" MS

K_D: 360 nM; Elution 0.3 % HCOOH / 80 % MeCN

Affinity Lysozyme

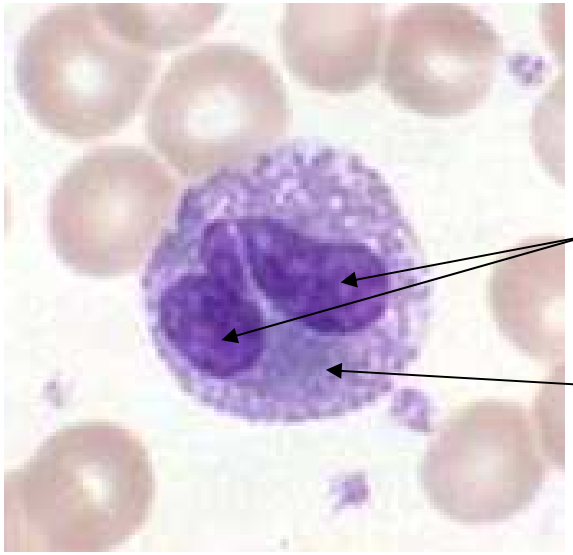


FT-ICRMS of elution fraction

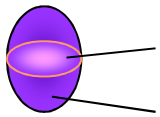


desalting: 200 µl solvent A, flow rate 20 µl/min
elution: solvent B, flow rate 30 µl/min

Application 3: Posttranslational Modifications - Tyrosine Nitration

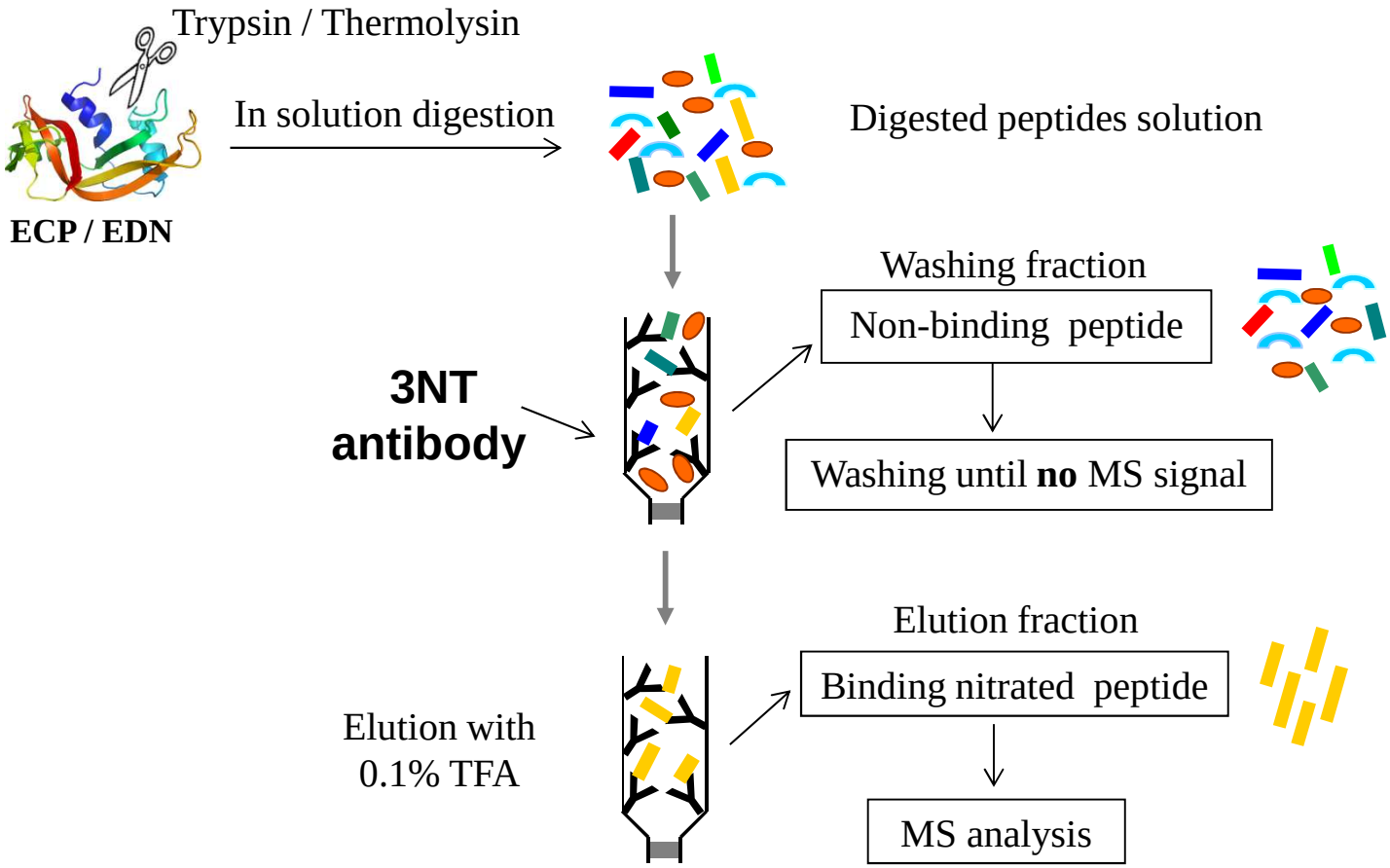


Crystalloid granule



- **Eosinophils: protection against infections**
- **EPO - in presence of H_2O_2 and halide catalyzes the formation of oxidants.**
- **EPO - catalyzes nitration with nitrite (NO_2^-) and H_2O_2 as co-substrate**
- **ECP/EDN - are cytotoxic to bacteria and parasites**
  - **promotes degranulation of mast cell**

Affinity MS identification of nitration sites by proteolytic- Affinity-Extraction - PROFINEX -



A. Petre et al. J. Biol. Chem. (2008)
A. Petre et al. (2012) JASMS CI

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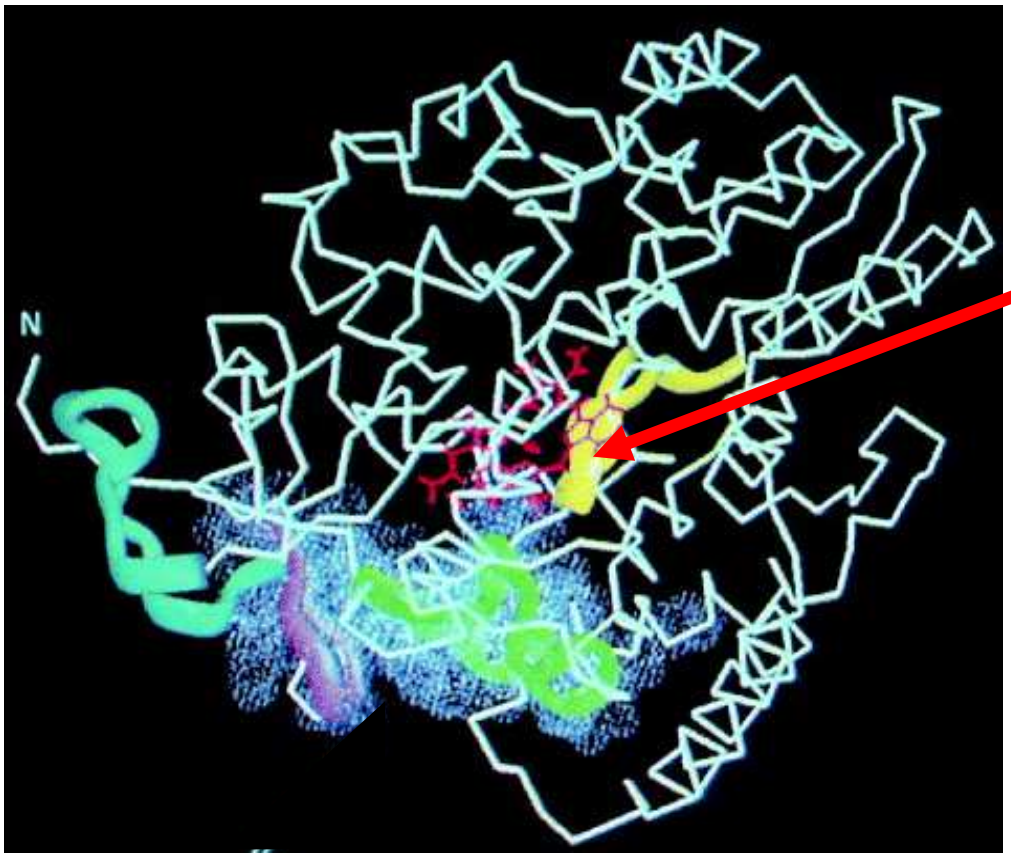
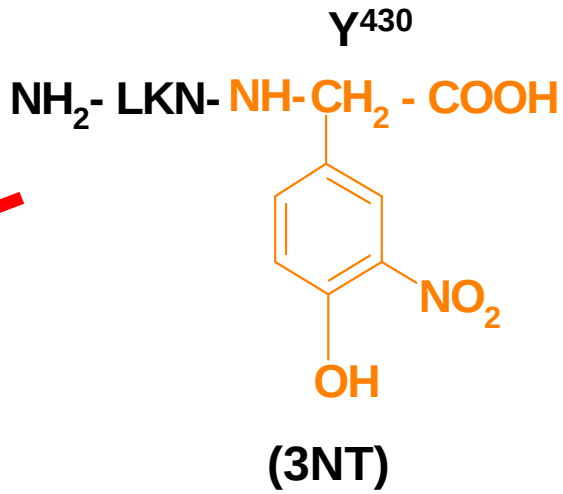
Prostacyclin Synthase - nitration of Tyr-430 in the catalytic center





(3NT)

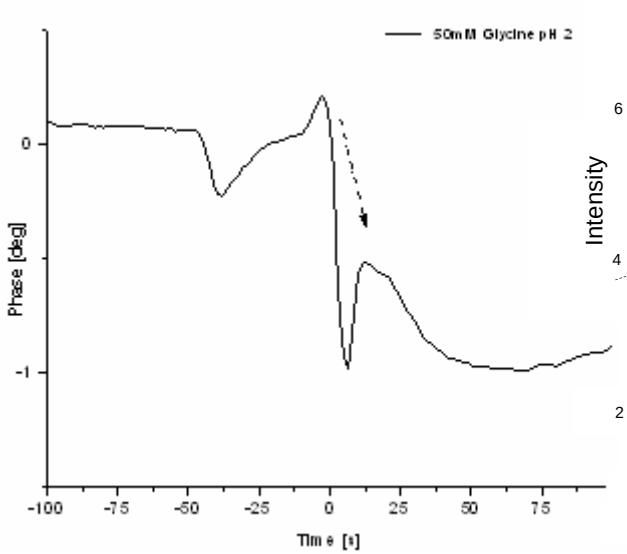
P. Schmidt, et al J. Biol. Chem. (2003) 278: 12813

Online SAW- MS: Identification of Tyr430-nitrated PCS peptide

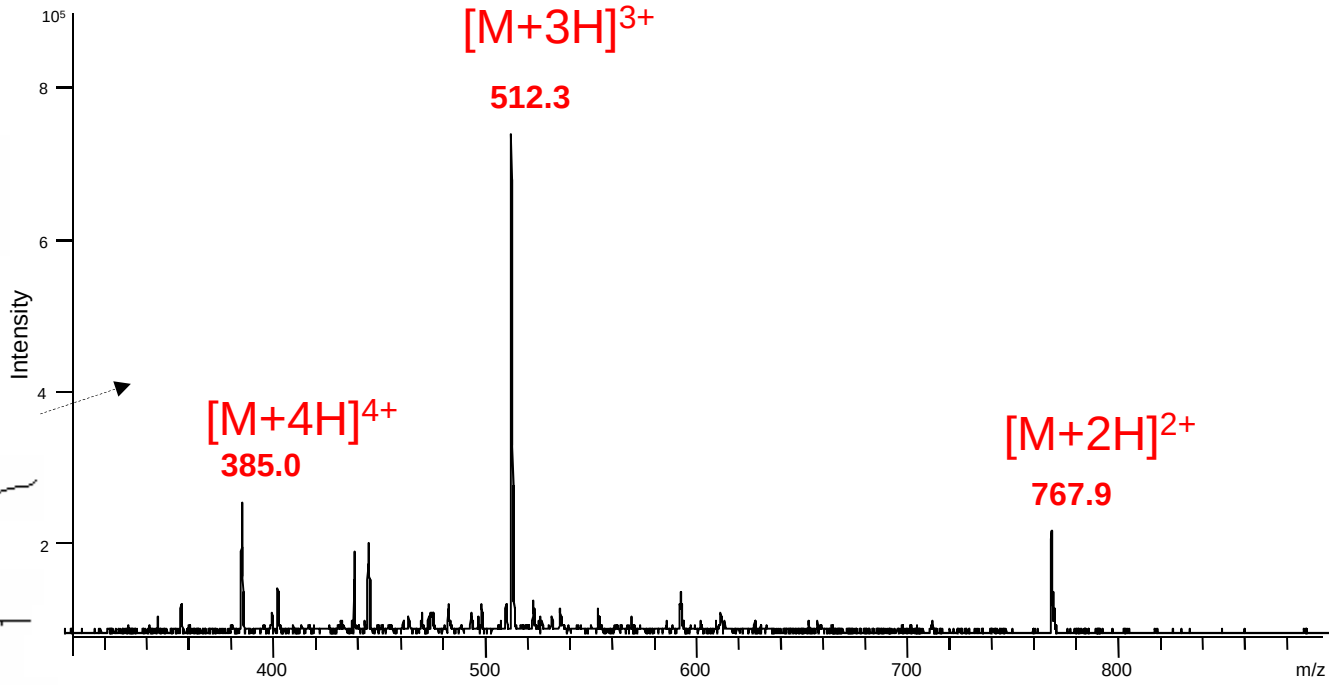
H-GKRLKNY(NO_2)SLPWGA-OH $[M+H]^+$ 1533.813

K_D : 25 nmol

Spectra of elution-fraction



ESI- MS of elution fraction



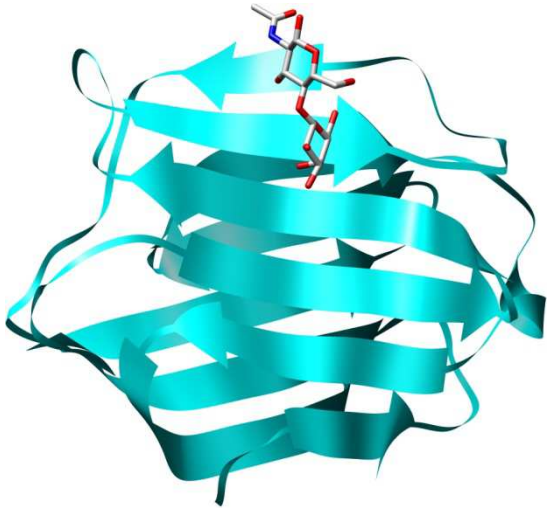
Application 4: Lectin- Carbohydrate Ligand Epitopes CREDEX-MS

Galectins: β-galactosides-binding ability.

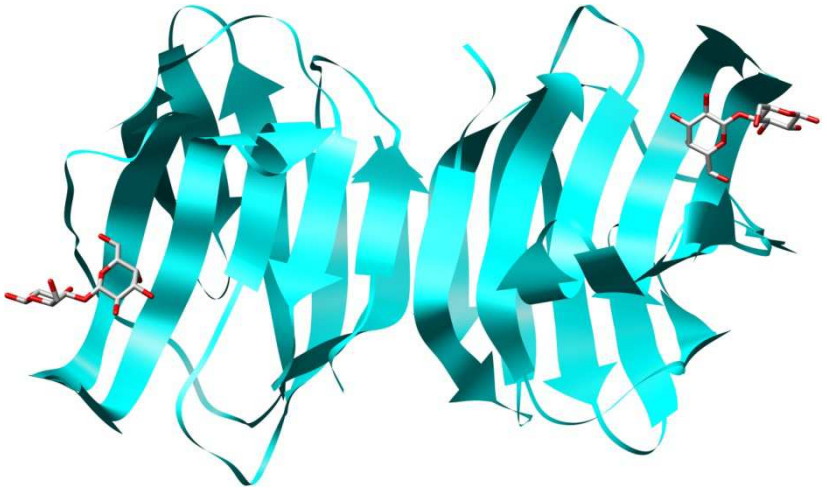
Highly conserved carbohydrate binding sites.

Metal-ion independent activity.

Do not form disulfide bridges.

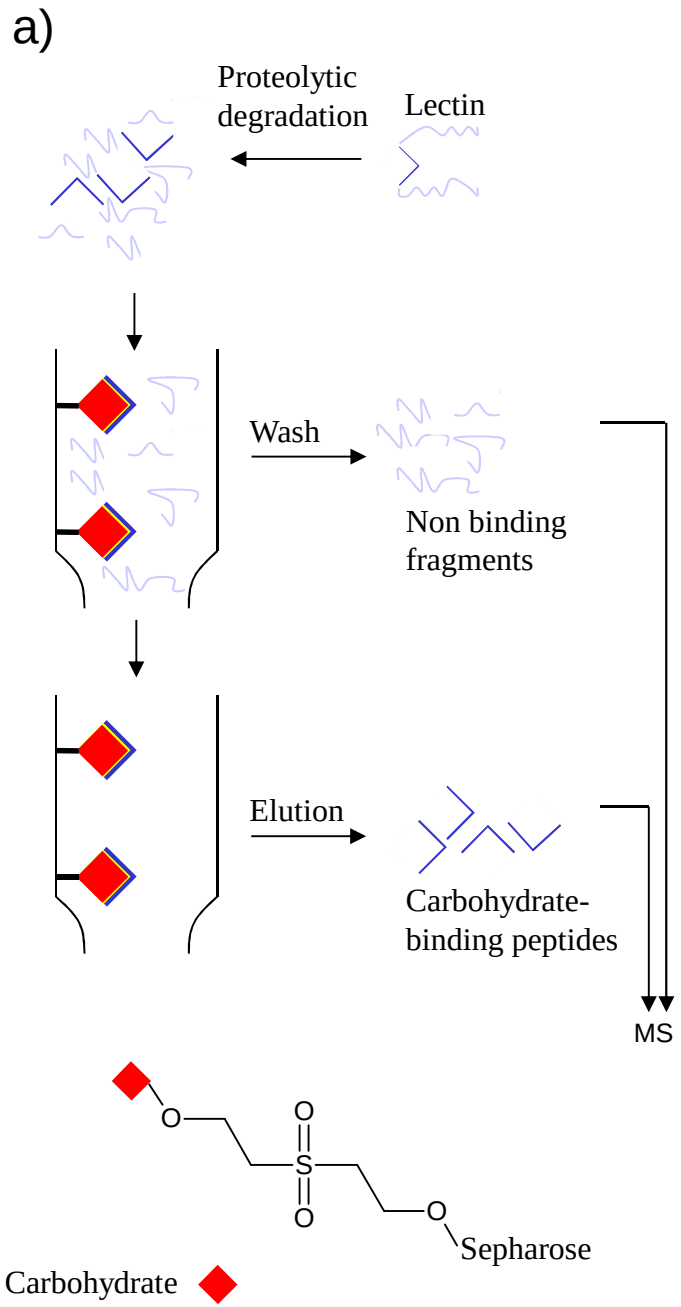


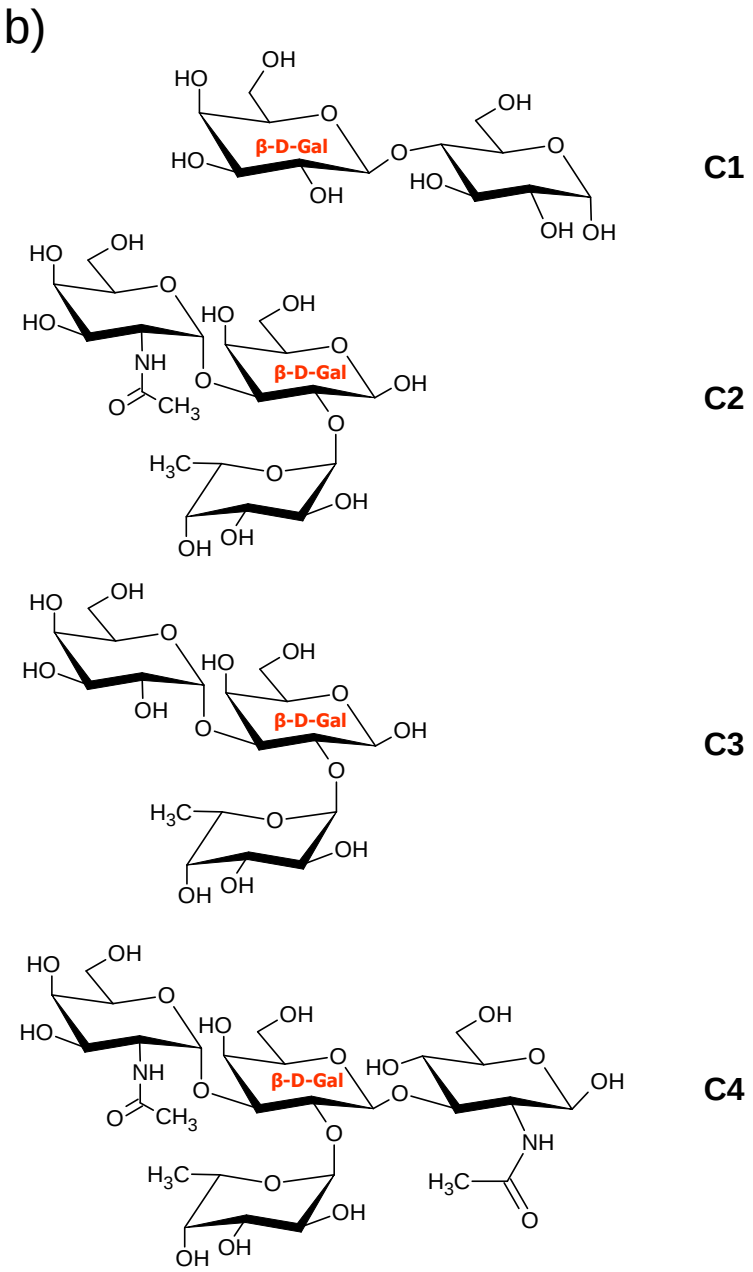
Galectin-3-LacNAc complex



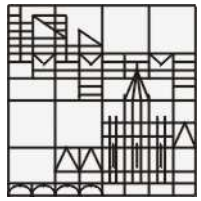
Galectin-1-Lac complex

Analytical concept of CREDEX-MS: Excision or Extraction

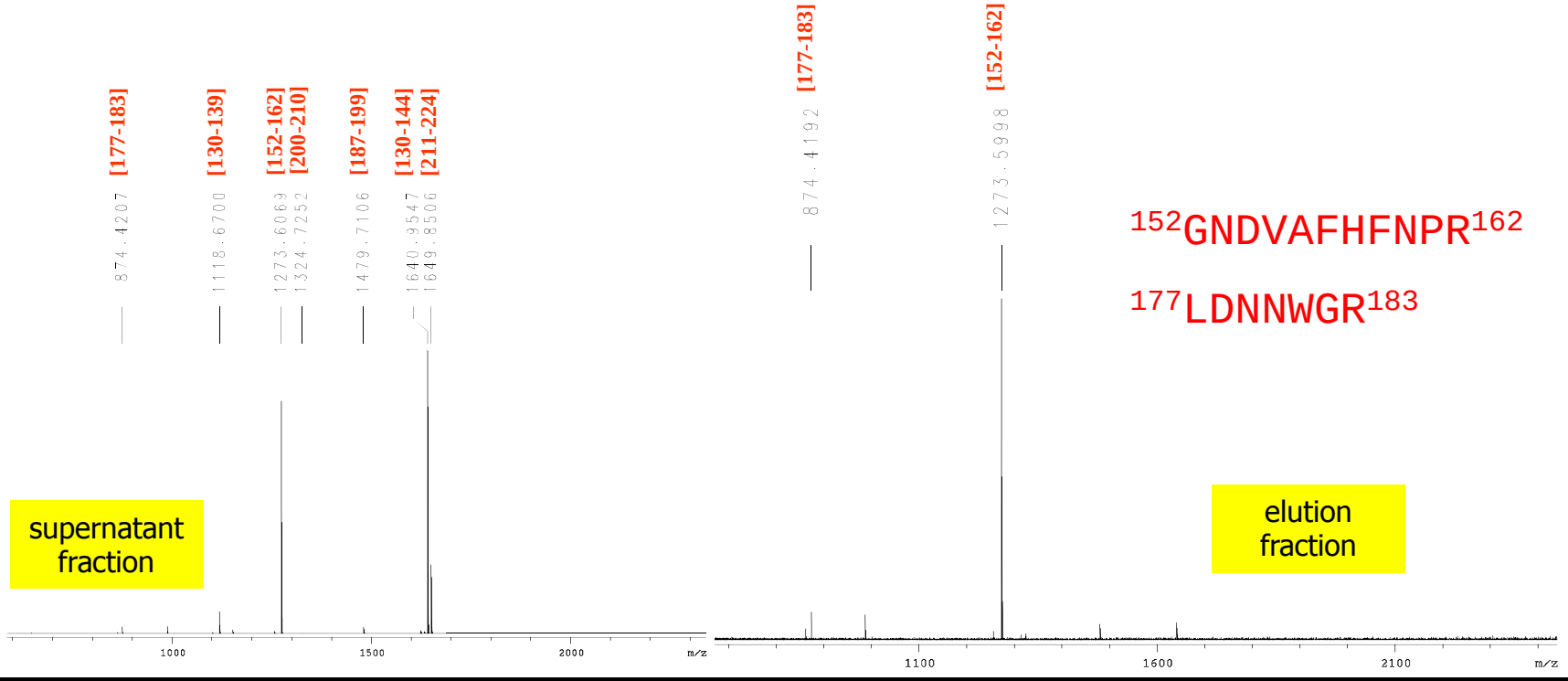




CREDEX-MS of galectin-3 interaction with lactose provides two specific CRD peptides (152-162) and (177-183)

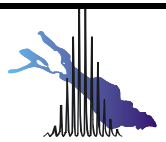


```
1MADNF SLHDA LSGSG NPNPQ  21GWPGA WGNQP AGAGG YPGAS  41YPGAY PGQAP
51PGAYP GQAPP GAYHG APGAY  71PGAPA PGVYP GPPSG PGAYP  91SSGQP SAPGA
101YPATG PYGAP AGPLI VPYNL 121PLPGG VVPRM LITIL GTVKP 141NANRI ALDFQ
151RGNDV AFHFN PRFNE NNRRV 171IVCNT KLDNN WGREE RQSVF 191PFESG KPFKI
201QVLVE PDHFK VAVND AHLLQ 221YNHRV KKLNE ISKLG ISGDI 241DLTSA SYTMI
```



^{152}GNDVAFHFNPR^{162}

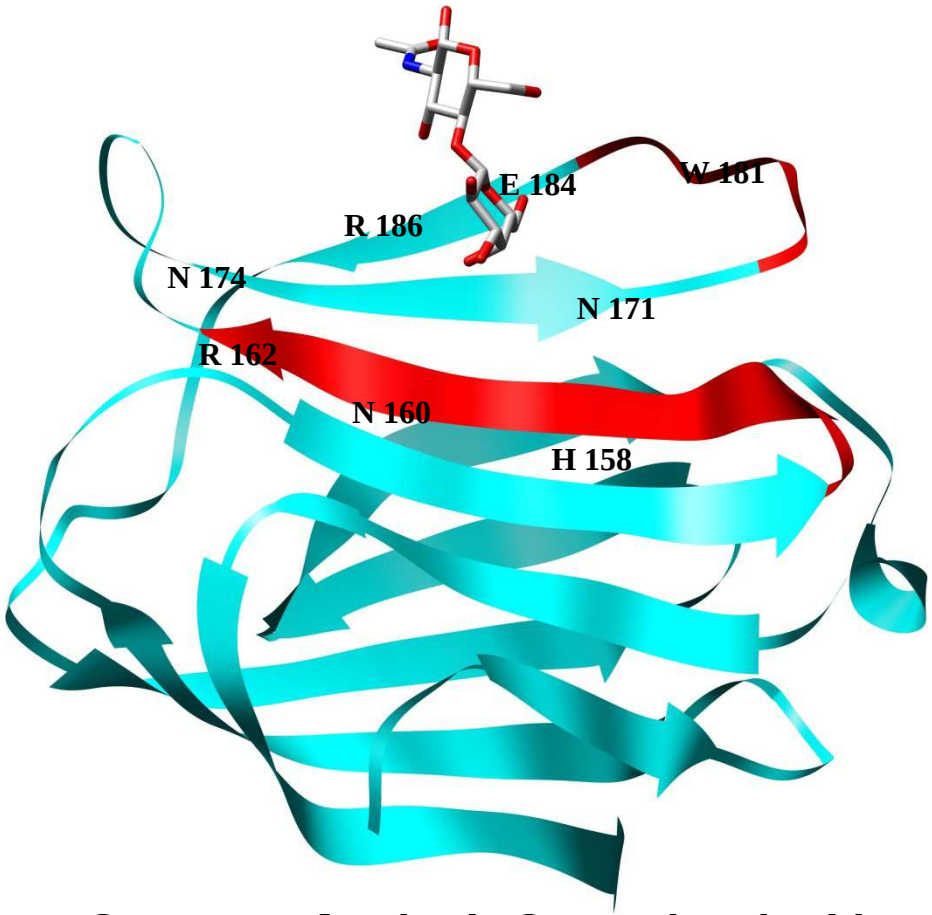
^{177}LDNNWGR^{183}



Analytical Chemistry & Biopolymer Structure Analysis
University of Konstanz

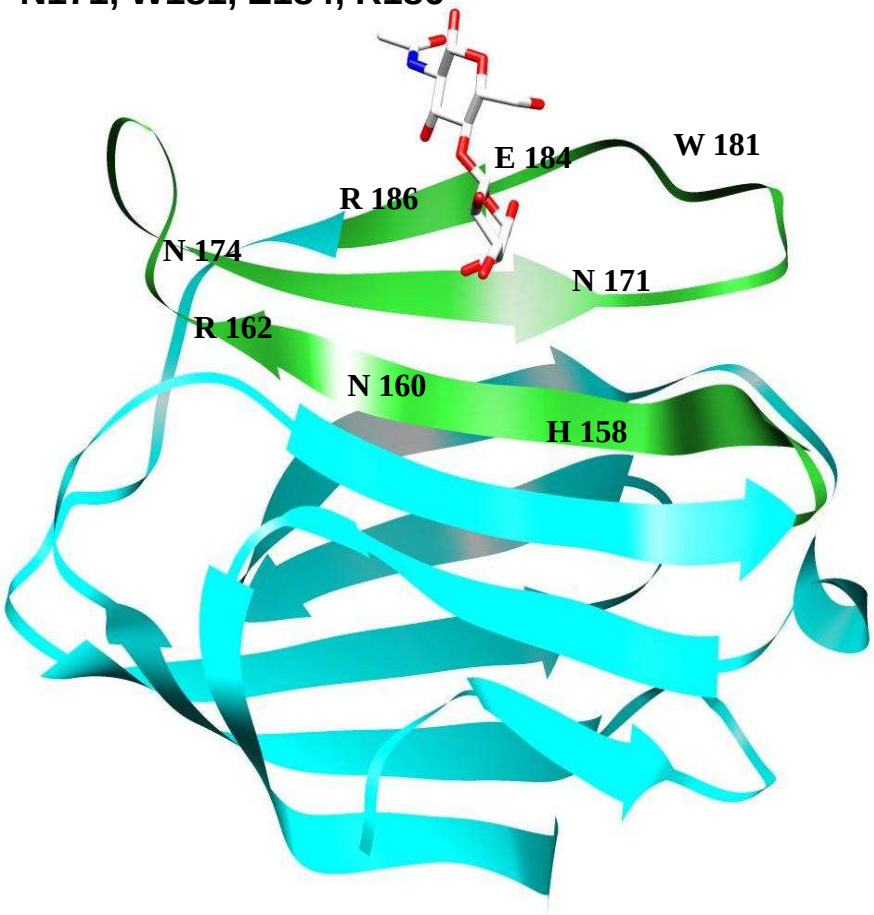
CRD Peptides from CREDEX-MS in galectin-3
- COMPLETE AGREEMENT WITH CRYSTAL STRUCTURE

Credex-MS: **(152-162) (177-183)**



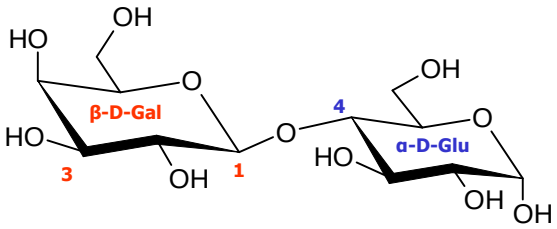
Structure of galectin-3 complexed with LacNAc (pdb file 1A3K).

Crystal structure: **H158, N160, R162, N174, N171, W181, E184, R186**

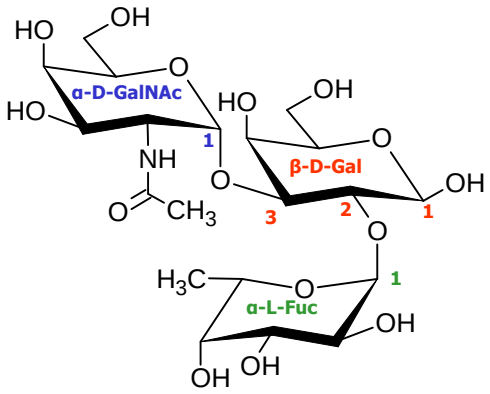


Galectin-3 in complex with LacNAc (pdb file 1A3K).

Lactose and blood group A trisaccharide

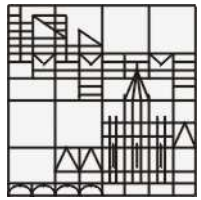


Lactose: β-D-Gal-(1-4)-α-D-Glu



Blood group A trisaccharide: α-L-Fuc-(1-2)-[α-D-GalNAc-(1-3)]-β-D-Gal

Comparison of CRD Peptides by CREDEX-MS of galectin-3 with lactose and A-Tri

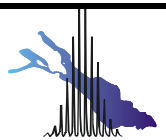


Galectin-3 binding sites for **A-Tri**: (152-162), (130-144)

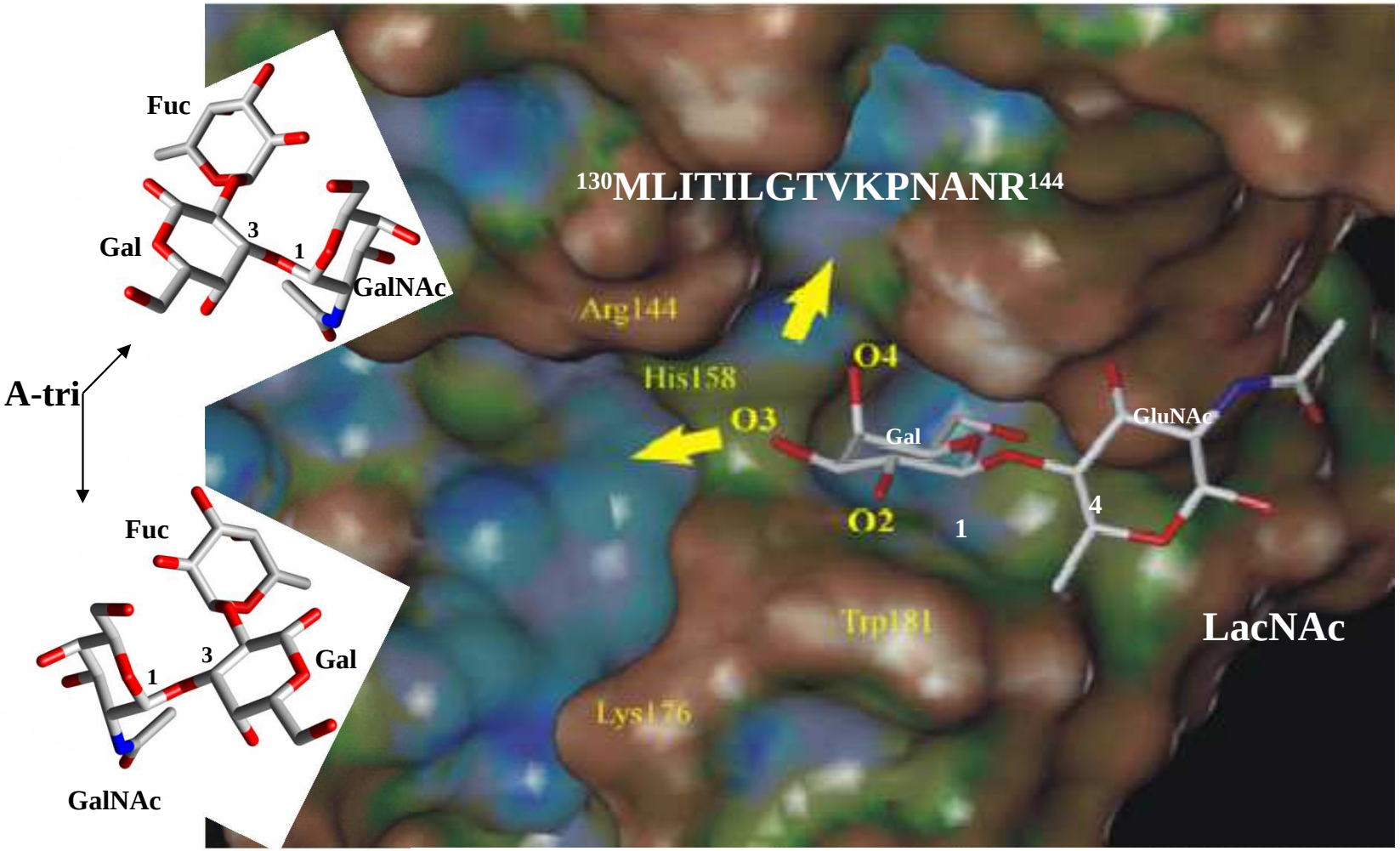
^{1}MADNF SLHDA LSGSG NPNPQ ^{21}GWPGA WGNQP AGAGG YPGAS ^{41}YPGAY PGQAP
^{51}PGAYP GQAPP GAYHG APGAY ^{71}PGAPA PGVYP GPPSG PGAYP ^{91}SSGQP SAPGA
^{101}YPATG PYGAP AGPLI VPYNL ^{121}PLPGG VVPR**M** **LITIL** **GTVKP** ^{141}**NANR**I ALDFQ
^{151}R**GNDV** **AFHFN** **PR**FNE NNRRV ^{171}IVCNT KLDNN WGREE RQSVF ^{191}PFESG KPFKI
^{201}QVLVE PDHFK VAVND AHLLQ ^{221}YNHRV KKLNE ISKLG ISGDI ^{241}DLTSA SYTMI

Galectin-3 binding sites for **lactose**: (152-162), (177-183)

^{1}MADNF SLHDA LSGSG NPNPQ ^{21}GWPGA WGNQP AGAGG YPGAS ^{41}YPGAY PGQAP
^{51}PGAYP GQAPP GAYHG APGAY ^{71}PGAPA PGVYP GPPSG PGAYP ^{91}SSGQP SAPGA
^{101}YPATG PYGAP AGPLI VPYNL ^{121}PLPGG VVPRM LITIL GTVKP ^{141}NANRI ALDFQ
^{151}R**GNDV** **AFHFN** **PR**FNE NNRRV ^{171}IVCNT K**LDNN** **WGR**EE RQSVF ^{191}PFESG KPFKI
^{201}QVLVE PDHFK VAVND AHLLQ ^{221}YNHRV KKLNE ISKLG ISGDI ^{241}DLTSA SYTMI

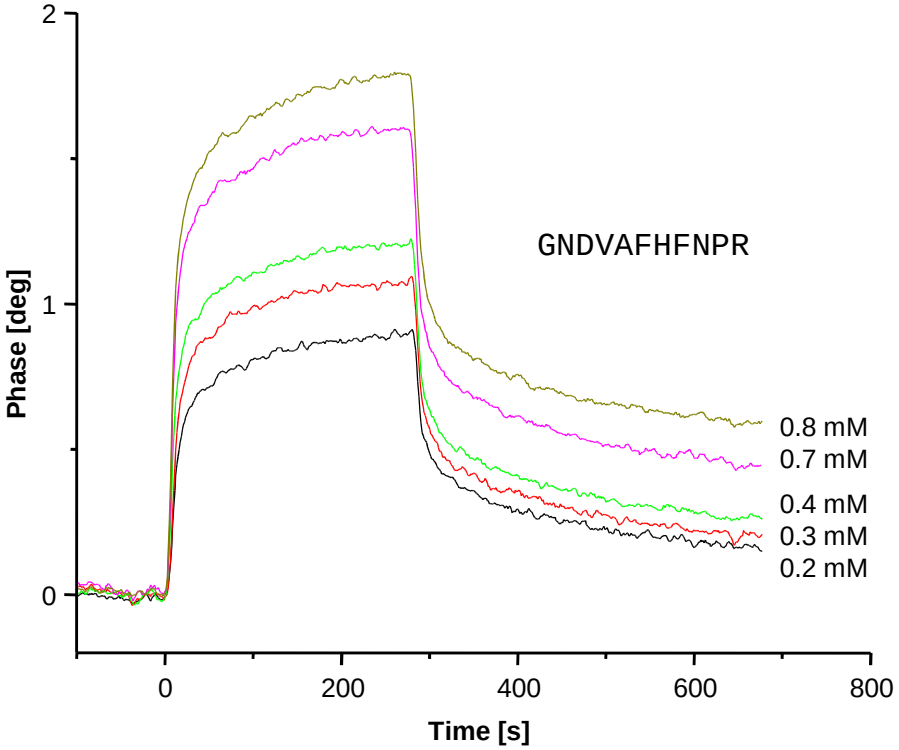


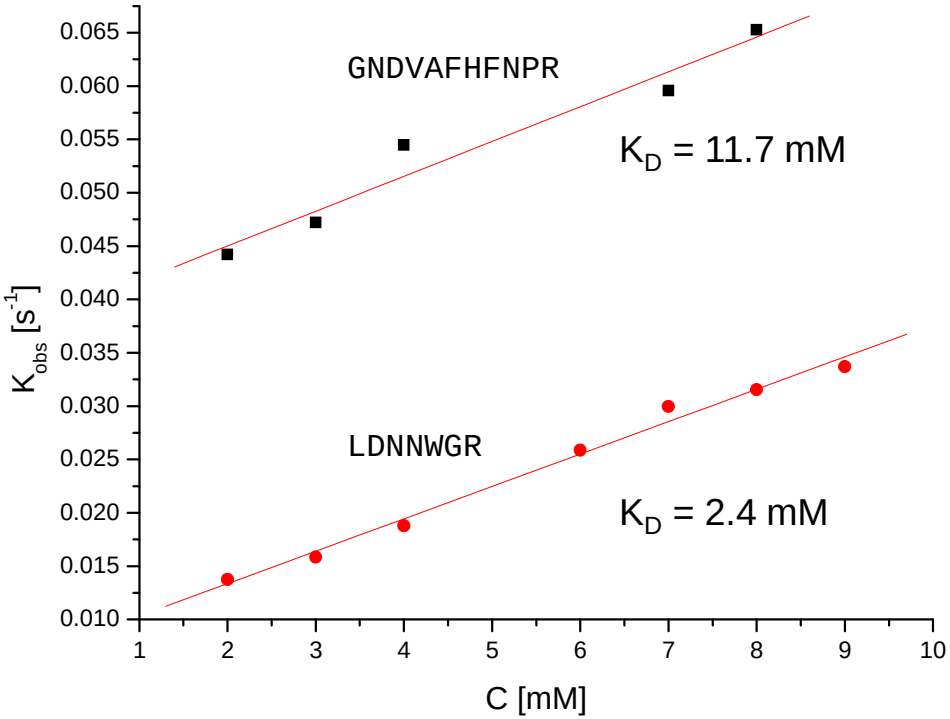
Gal-3 carbohydrate binding sites: CREDEX-MS provides orientation for A-tri



The arrows indicate the possibilities of extended binding sites. Blue indicates concave and brown convex surfaces.

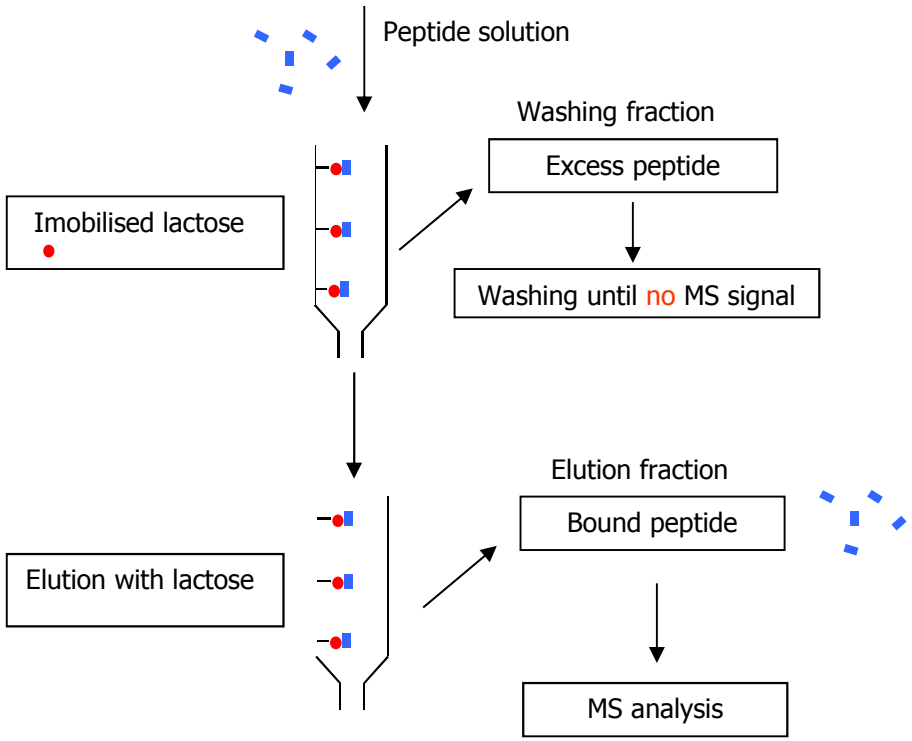
Binding curves of SAW sensogram and K_D for CRD peptides

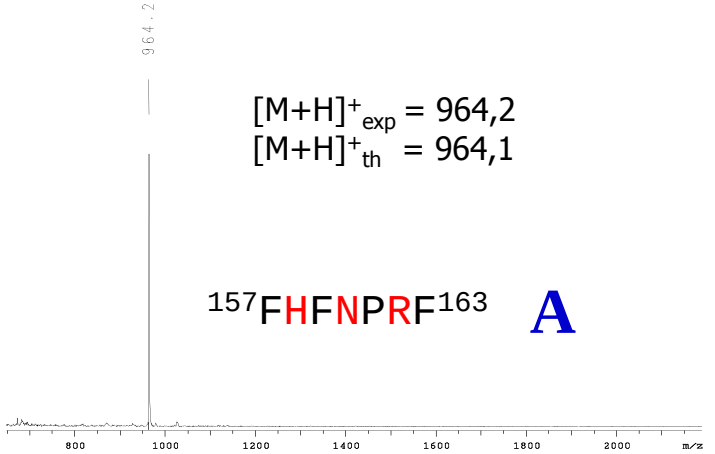




Synthetic CRD peptides show specific affinity to lactose and inhibit Gal-3 tumor cell recognition

Peptides A, B : inhibition of Gal-3 on 2 carcinoma cell lines

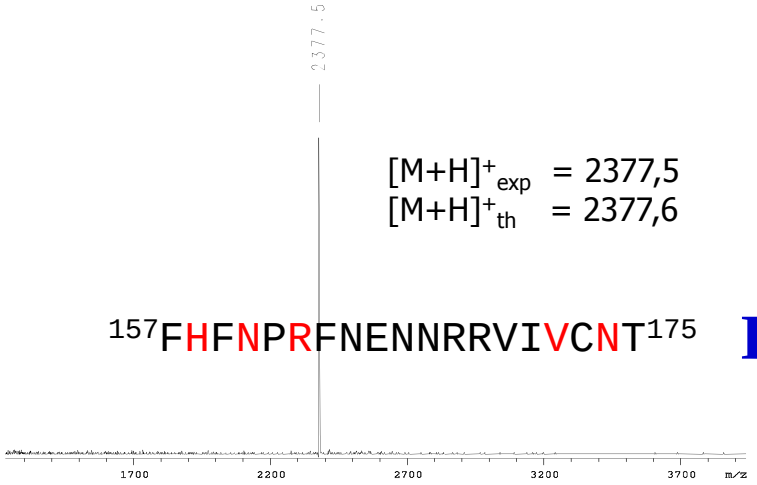




$[M+H]^+_{exp}$ = 964,2
$[M+H]^+_{th}$ = 964,1

^{157}FHFNPRF^{163} **A**

MALDI-MS of the Gal3 (157-163) peptide elution fraction.



$[M+H]^+_{exp}$ = 2377,5
$[M+H]^+_{th}$ = 2377,6

^{157}FHFNPRFNENNRRVIVCNT^{175} **B**

A. Moise et al. (2013) submitted

M. Przybylski et al. Eur. & PCT Patent Appl. (2010)

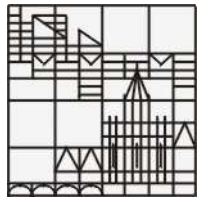
Analytical Chemistry & Biopolymer Structure Analysis
University of Konstanz

Steinbeis Center
for Biopolymer Analysis &
Biomolecule Mass Spectrometry

SAW- Bioaffinity- Mass Spectrometry Combination
SAWMS – I

Details & Applications: michael.przybylski@uni-konstanz.de
www.affinity-ms.de

OVERVIEW

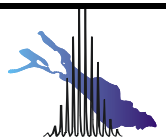


I Online SAW- Biosensor-MS Combination:

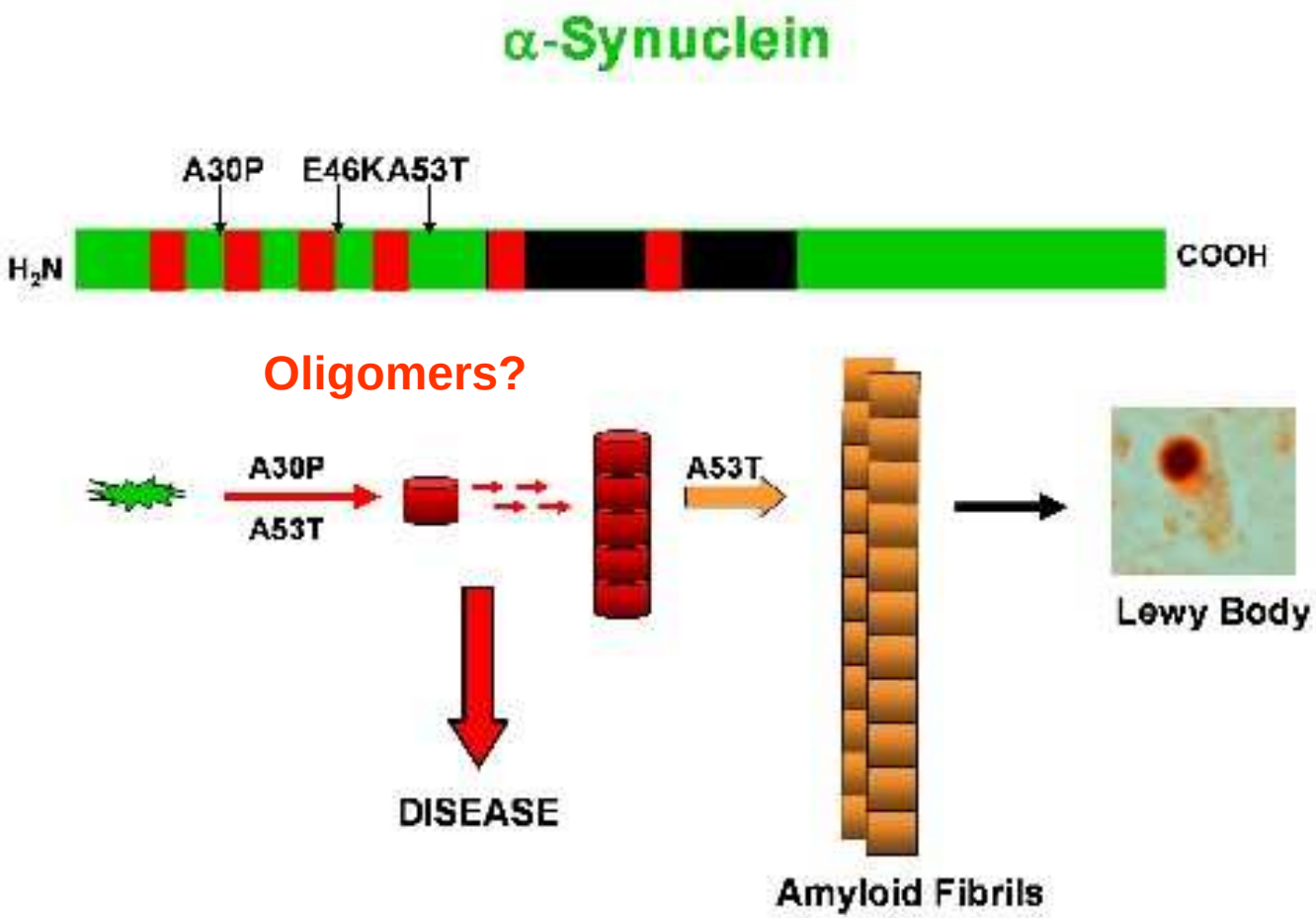
- **Analytical Development - Interface**
- **Application Examples**

II Oligomerisation - Aggregation of Parkinson's Disease Protein α-Synuclein:

- **Identification of Oligomer Intermediates**
- **Ion Mobility-, HDX- MS**
- **Affinity-MS: Direct Analysis from Biological Material**



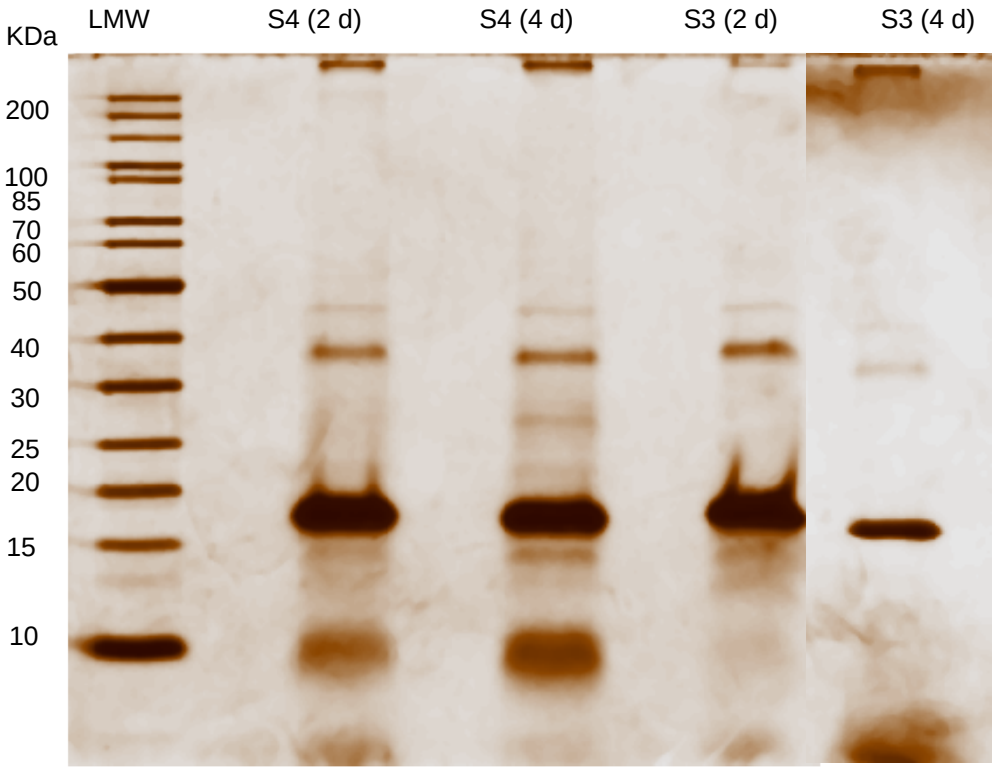
Aggregation of α-Synuclein - key protein in Parkinson‘s disease -



Conway, et al Nat Med 1998 *Conway et al PNAS 2000* *Goldberg and Lansbury, Nat Cell Biol 2001*

Alpha- Synuclein shows “oligomers” AND degradation products
Direct mass spectrometry unsuccessful

2 – 6 days



- ü **10 µg aggregates**
- ü **15 % Separation gel**
- ü **5 µl LMW marker**

S3 **α-Syn in ammonium acetate**
S4 **α-Syn in ammonium acid carbonate**

[ChemBioChem 2011]

Autoproteolytic Fragments are Intermediates in the Oligomerization-Aggregation of Parkinson's Disease Protein Alpha-Synuclein as Revealed by Ion Mobility Mass Spectrometry

Camelia Vlad,[a] Kathrin Lindner,[a] Christiaan Karreman,[b] Stefan Schildknecht,[b] Marcel Leist,[b] Nick Tomczyk,[c] John Rontree,[c] James Langridge,[c] Karin Danzer,[d] Thomas Ciossek,[d] Alina Petre,[a,e] Michael L. Gross,[e] Bastian Hengerer,[d] and Michael Przybylski[a]*

[a] *Dr. C. Vlad, MSc K. Lindner, Dr. A. Petre, Prof. Dr. M. Przybylski Department of Chemistry, University of Konstanz 78457 Konstanz (Germany)*
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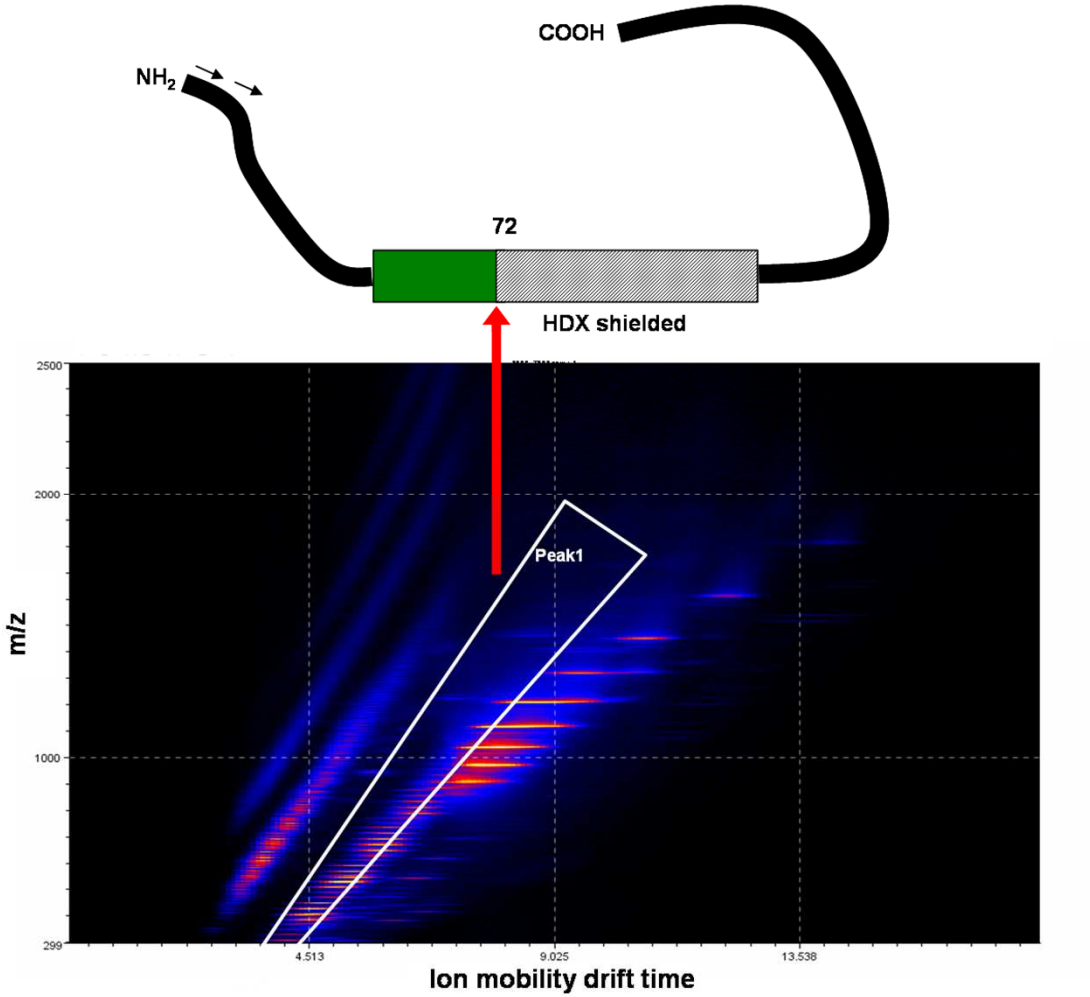
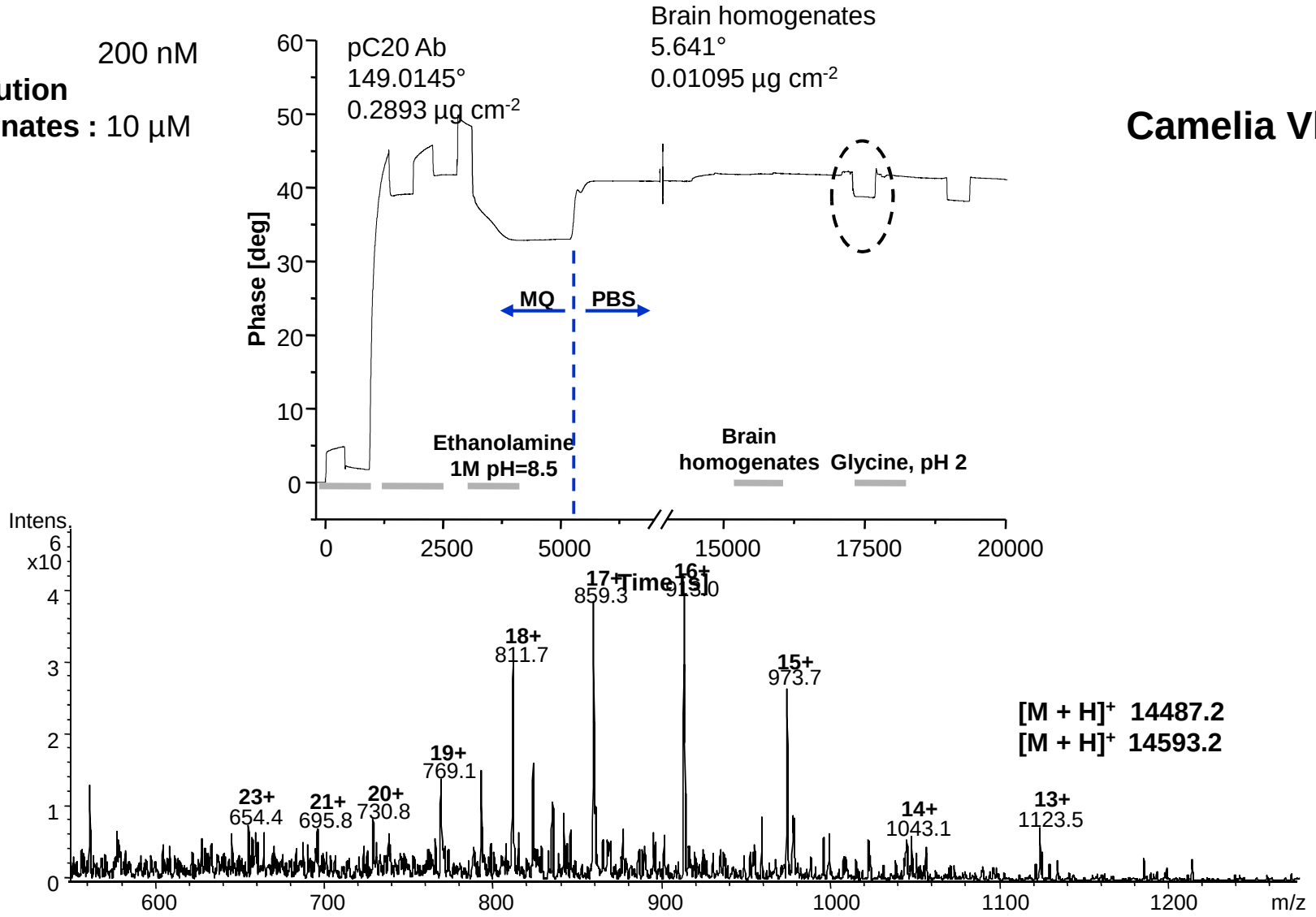
[c] *N. Tomczyk, Dr. J. Rontree, Dr. J. Langridge, Waters Ltd., Micromass Manchester, (UK)*
[d] *Dr. K. Danzer, Dr. T. Ciossek, Prof. Dr. B. Hengerer ZNS Research,Böhringer Ingelheim Pharma,Biberach/Riß (Germany)*
[e] *Dr. A. Petre, Prof. Dr. M. Gross, Department of Chemistry Washington University St. Louis (USA)*



Online Affinity-MS of transgenic alpha-Syn-m-130 directly from brain homogenate: New structure modification

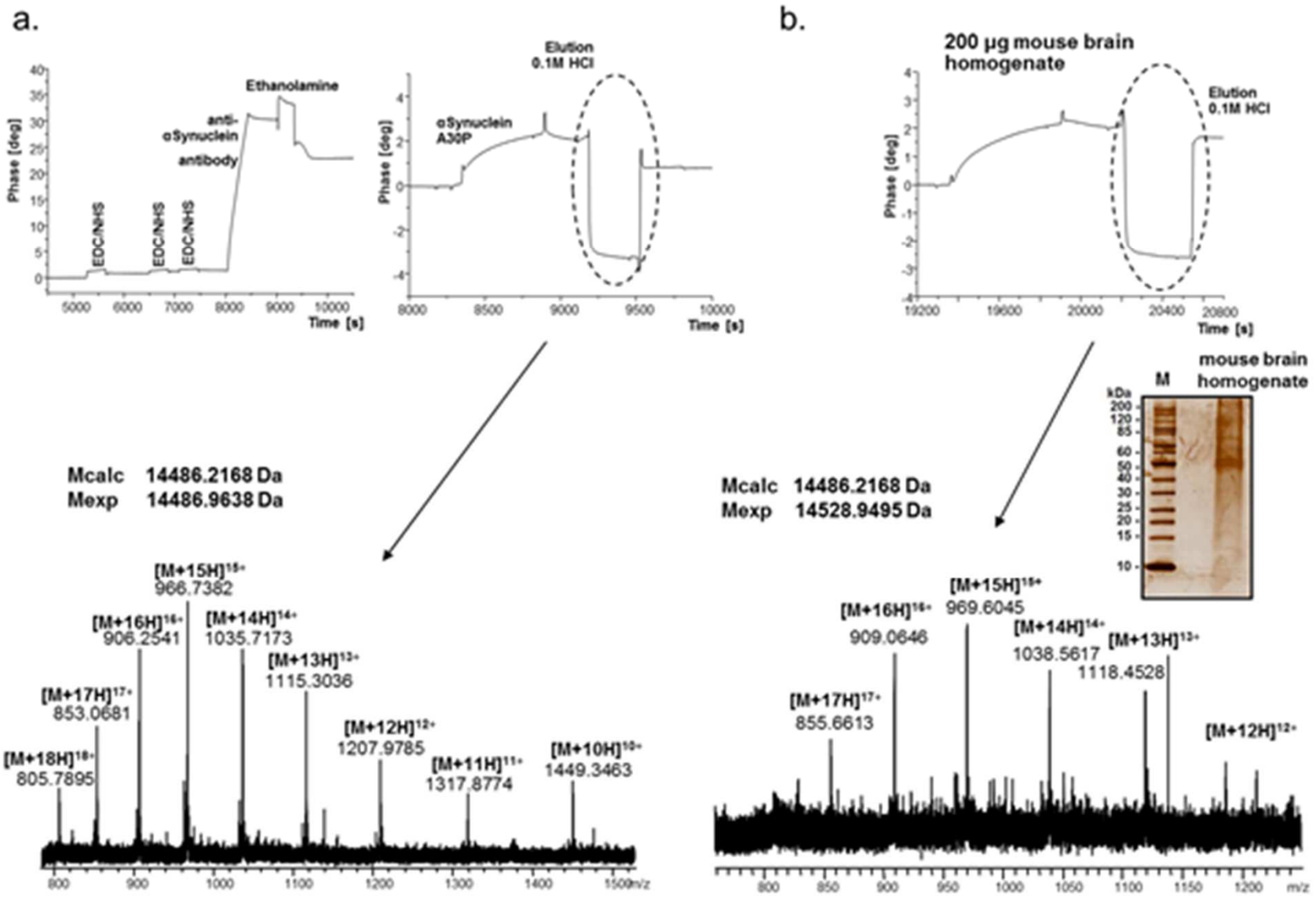
pC20: 200 nM
Brain elution homogenates : 10 µM



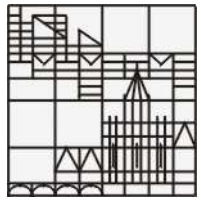
Camelia Vlad

Online SAW-affinity-MS of wt-aSyn in vitro (a) and from mouse brain homogenate (b)

Figure 3

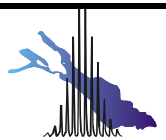


Perspectives for affinity- mass spectrometry /

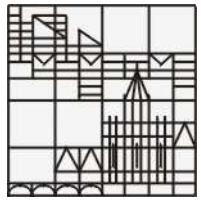


Online Affinity- Mass spectrometry

- Identification of antigen epitopes - vaccine lead structures
Biomarker identification
Ligand- binder recognition & interaction
Protein – carbohydrate ligand epitopes
Protein- DNA, -RNA interactions
Conformational/topography characterisation
Reactive intermediates in misfolding & aggregation



THANKS TO THE MAJOR PLAYERS...
… Coworkers, Collaborators, €€€...



Coworkers

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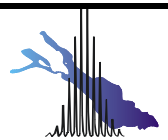
Nick Pierson
Adrian Moise
Frederike Eggers
Dr. Marilena Manea
Stefan Slamnoiu
Mihaela Dragusanu
Gabriela Paraschiv
Madalina Maftei
Marius Iurascu
Nicole Engel

Collaborators

Bastian Hengerer, Boehringer Ingelheim
Michael Gross, Washington Univ. St.Louis
Marcel Leist, Martin Scheffner, Konstanz
David Clemmer, Indiana University
SAW- Instruments, Bonn

€€€

DFG	Biopolymer-MS & ChemBio Grad School
EU	Antibodies to Human Proteome; RUBICON
Boehringer – Ingelheim,	Parkinson/ Synuclein
Univ. Konstanz	Research Center Proteostasis
BMWI	Affinity-MS



ANALYTICAL CHEMISTRY & BIOPOLYMER STRUCTURE ANALYSIS LAB