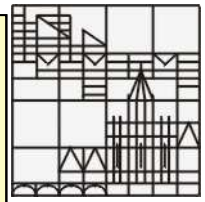
European Bioanalysis Forum
7th Open Symposium "Beyond the Horizon"
Barcelona November 20th 2014



Online Biosensor- Mass Spectrometry: Simultaneous Detection, Structure Determination and Affinity Quantification of Protein-Ligand Interactions

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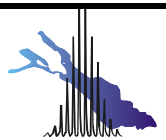
Steinbeis Centre for Biopolymer Analysis & Biomedical Mass Spectrometry

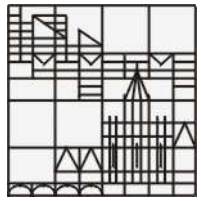
University of Konstanz & Rüsselsheim GERMANY

www.uni-konstanz.de/agprzybylski/chemie

www.affinityms.de



Why online Bioaffinity-MS ?



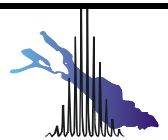
Ø Online HPLC-ESIMS:

1980: Almost unknown;

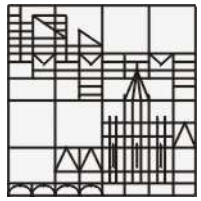
2014: Standard for separation/ quantification and identification of biopolymer mixtures

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

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



OVERVIEW

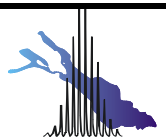


I Online Biosensor/SAW-MS Combination:

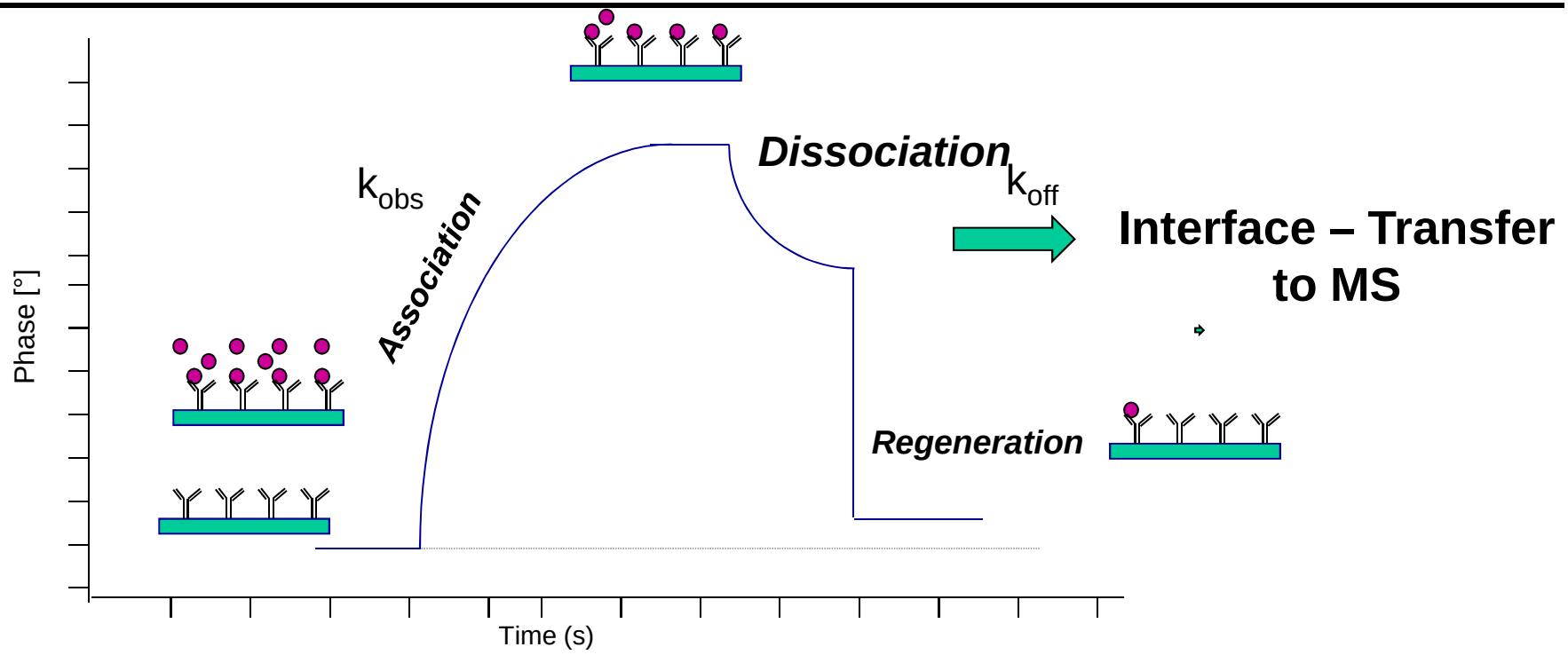
Analytical Development - Interface

II Application Examples:

Protein-antibody
Protein-carbohydrate
Parkinson's Protein α-Synuclein



Biosensor - dissociation step: Interface required in place of waste elution



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

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

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

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

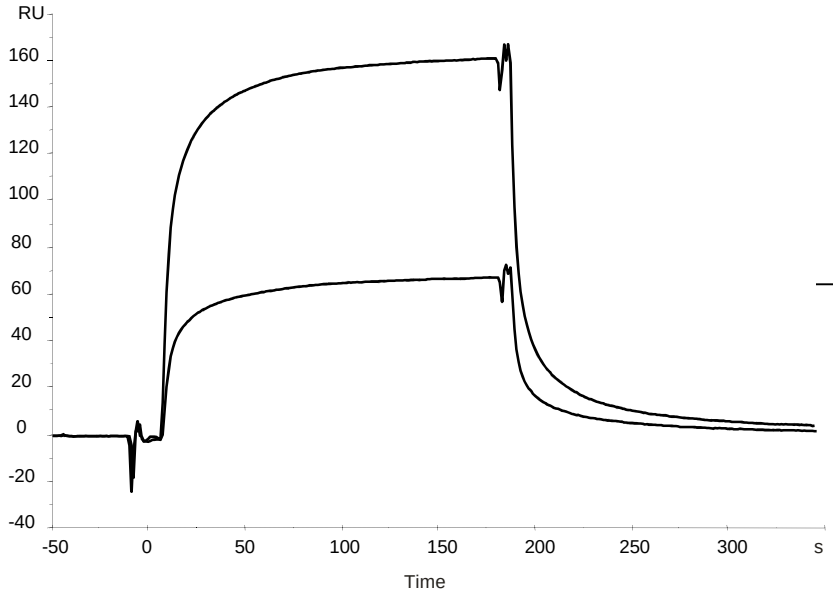
Principles & goals of online Biosensor-MS combination

Screening of analytes at native conditions possible

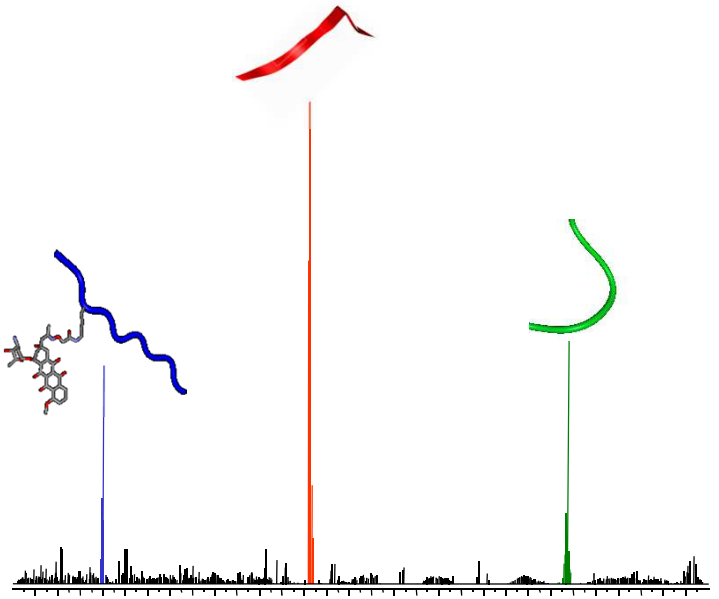
Identical analysis conditions for proteins, glycans, nucleic acids, lipids etc.

Correlation of bioaffinity (SPR, SAW) and molecular structure (MS) information

Automated and unsupervised MS analysis

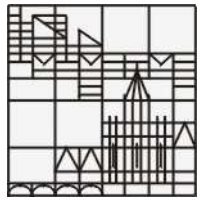


affinity binding



Epitope ligand mapping
Peptide ligand molecular differentiation
mixture analysis

HPLC vs. SPR-MS features comparison



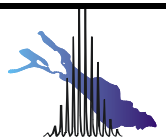
A) Sample composition

HPLC-MS

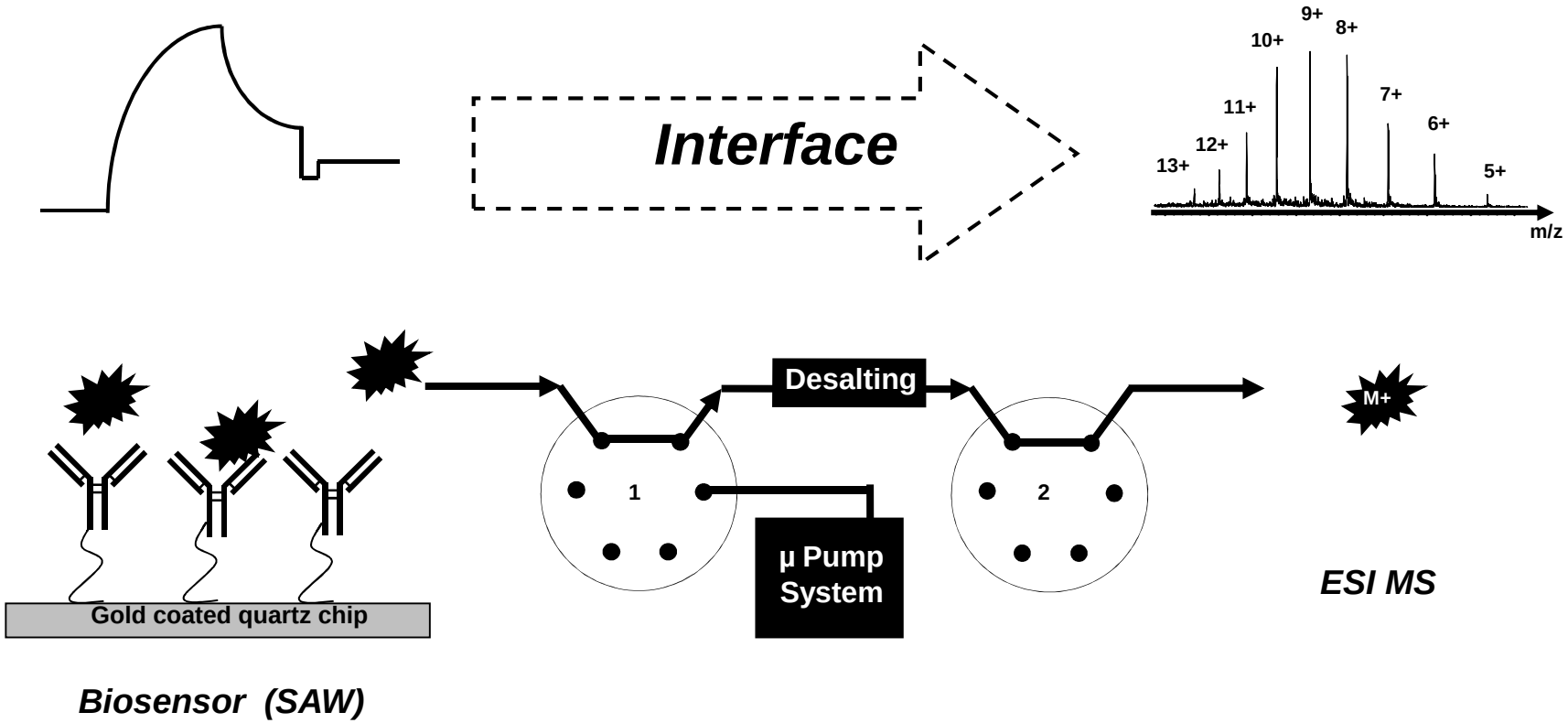
- Needs soluble homogenous samples
- Needs precise protocols for sample prep

Biosensor-MS

- Can handle complex biological materials
- Needs little or no sample prep

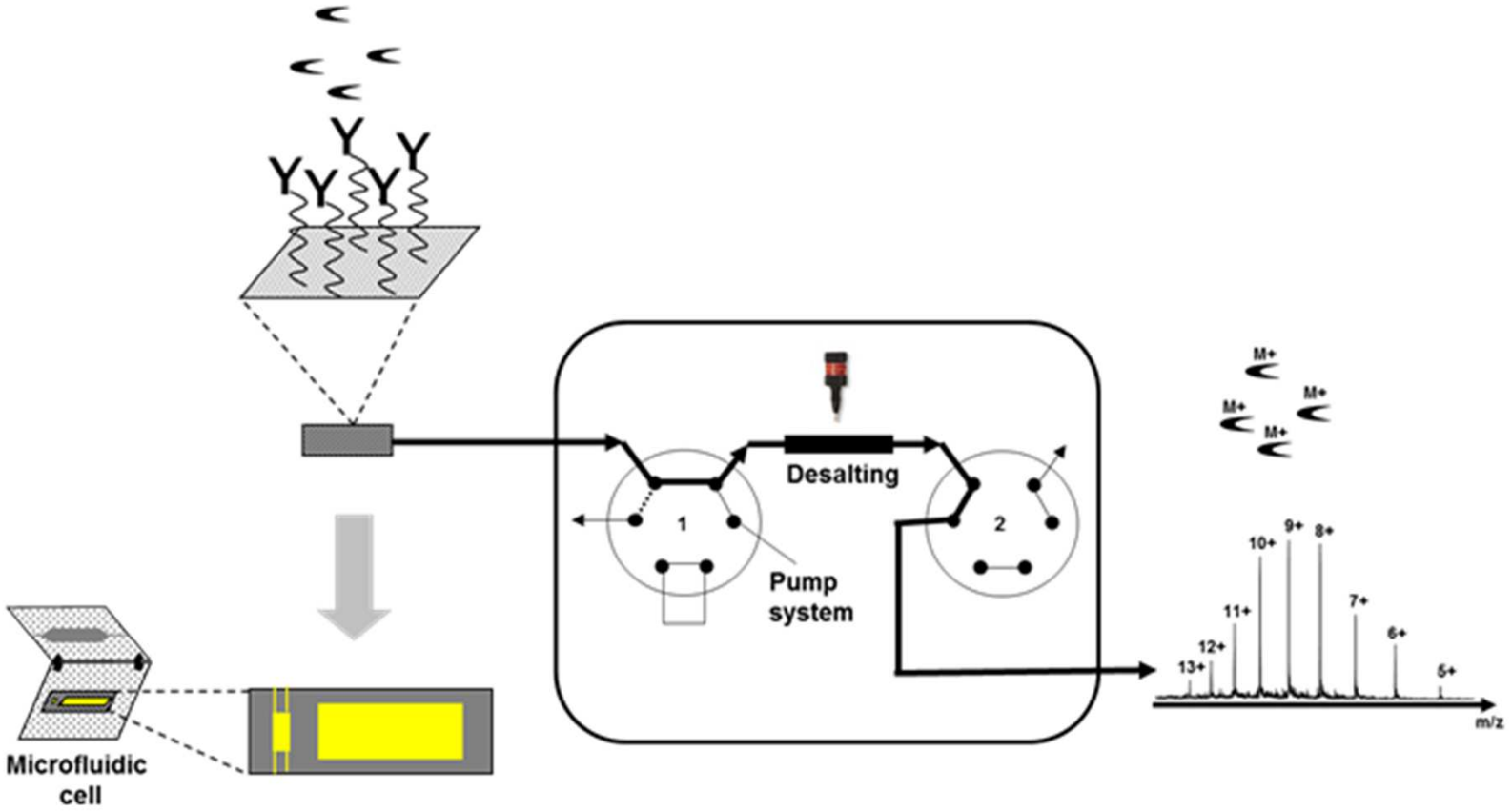


AFFYMS-I: Interface for desalting/affinity-capture & microfluidic transfer enables online-biosensor-MS



Scheme of Biosensor- MS interface

Figure 1



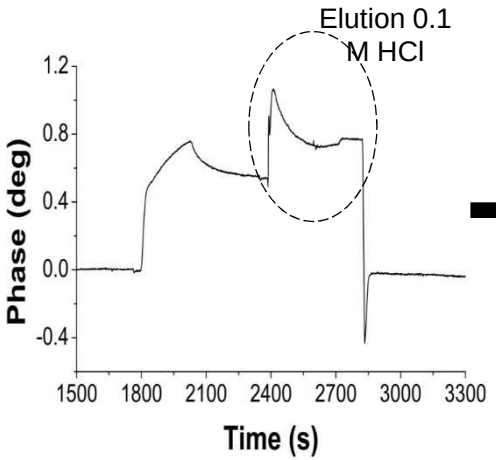
Biosensor Interface ESI - MS

M. Przybylski, S. Slamnoiou, M. Moise, (2014) patent pending

Online SAW-Biosensor- MS:
Avastin- Antibody Complex with Vacsular Endothelial Growth Factor (VEGF)

VEGF
Mcalc = 23250.78 Da
Mexp = 23250.19 ± 0.34 Da

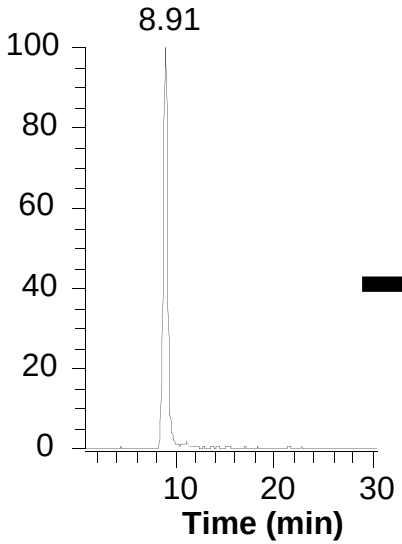
A



SAW Sensorgram
K_D VEGF-Avastin: 15,5 nM

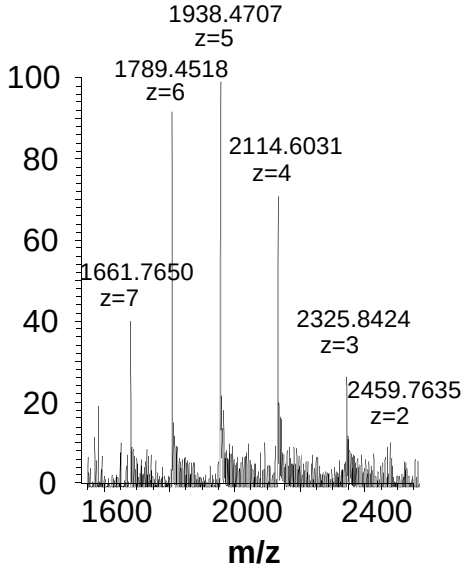
Interface MS-I Concentration and desalting

B



RIC Biosensor Eluate

C



ESI-MS

OVERVIEW

I Online Biosensor/SAW-MS Combination:

Analytical Development - Interface

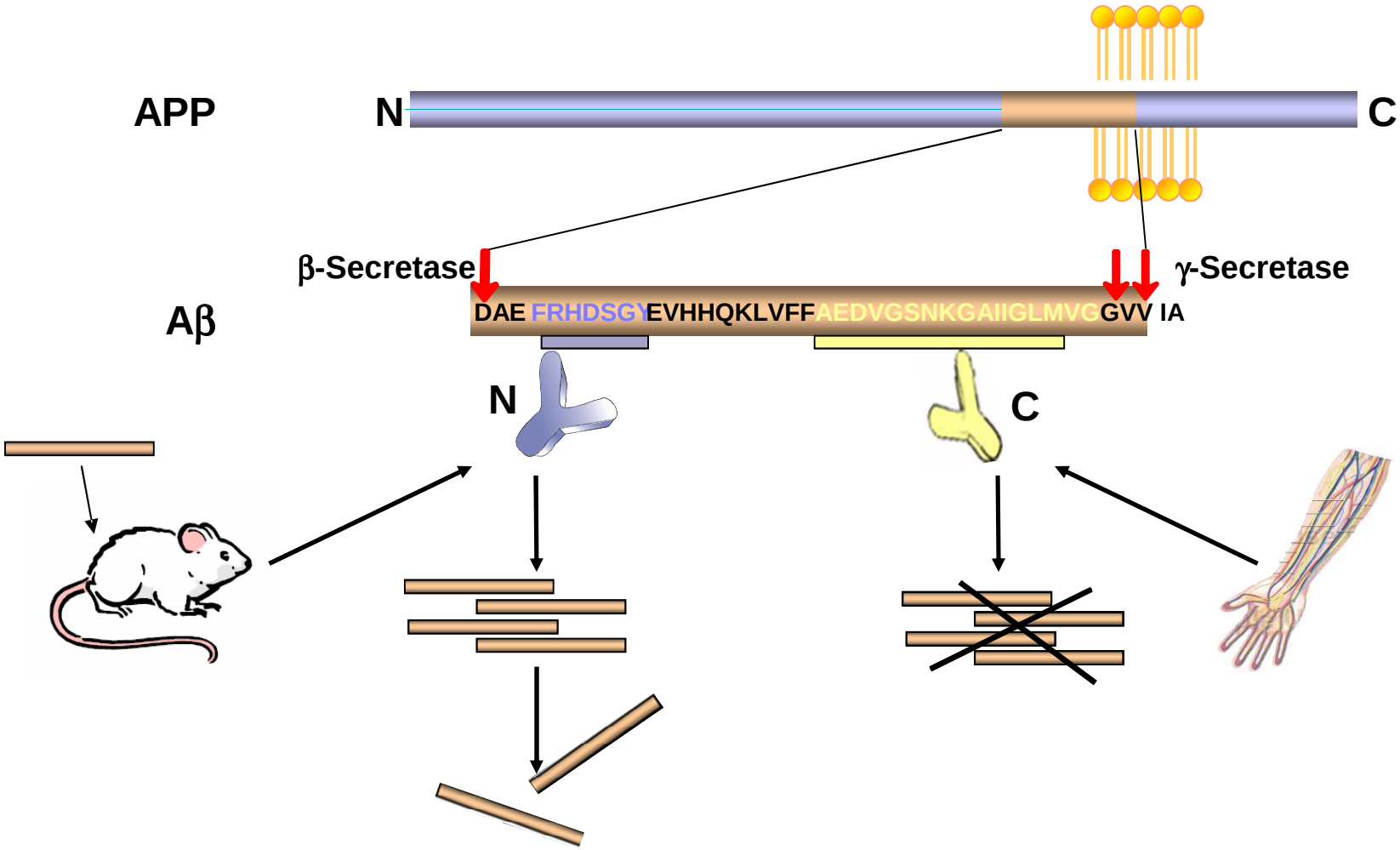
II Application Examples:

Aß- antibody / Epitope determination
Lectin- carbohydrate / CRD peptides
Parkinson‘s Protein α-Synuclein / in vivo

„ APPLICATION 1 Epitope Analysis 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 1 Antibody- Epitope Identification

Shielded proteolytic excision - basis for mass spectrometric epitope identification

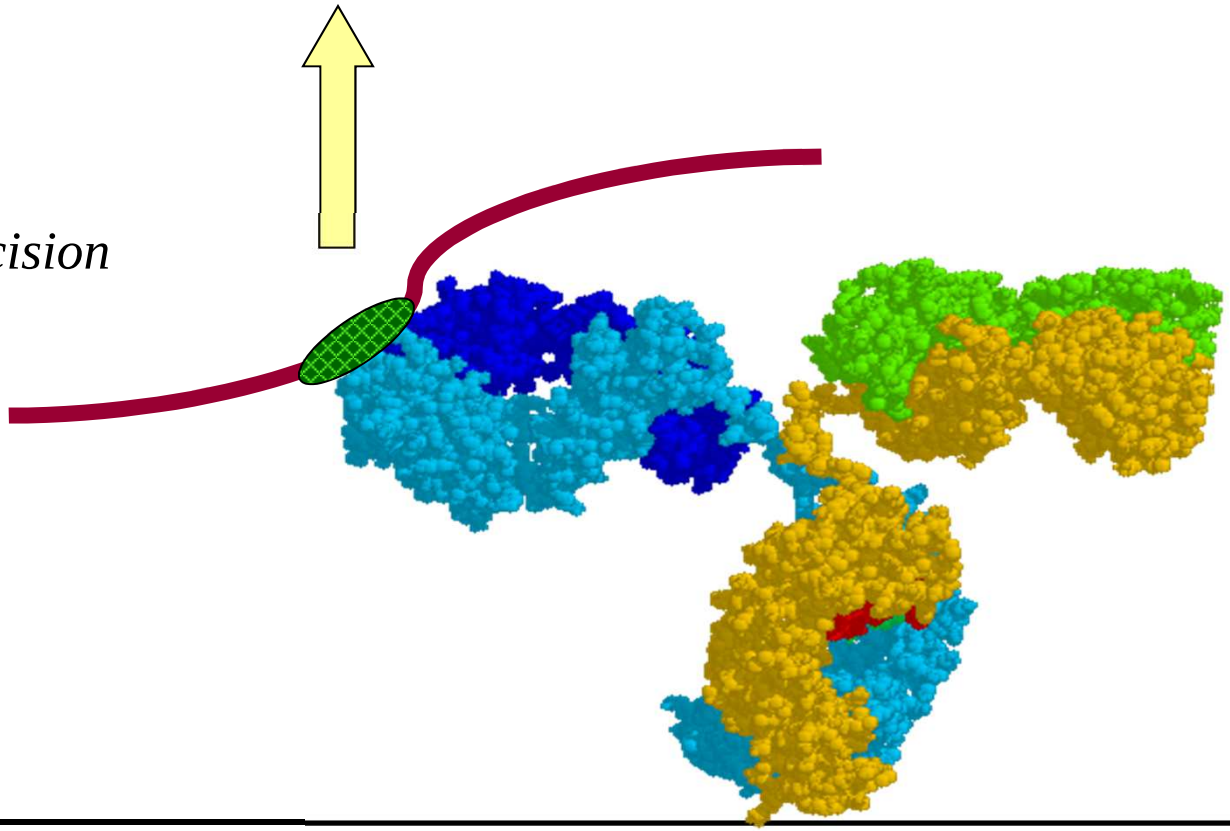
Preconditions:

Proteolytic stability of antibody

Epitope-Paratope Interaction shielded

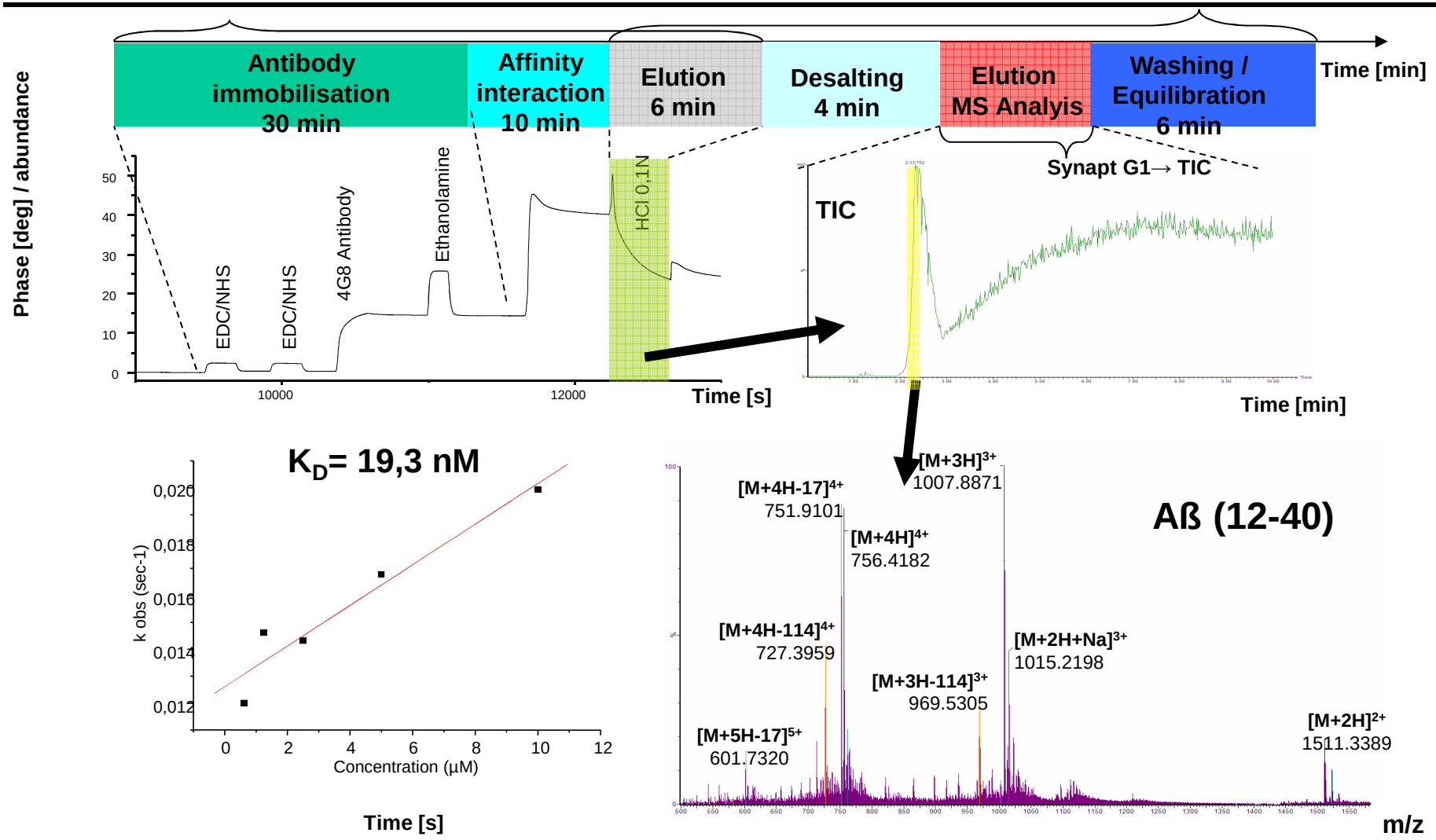
Epitope peptide

Epitope Excision



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

Aß- Autoantibody/ Aß- Epitope Determination & Quantification using online- SAW- biosensor MS



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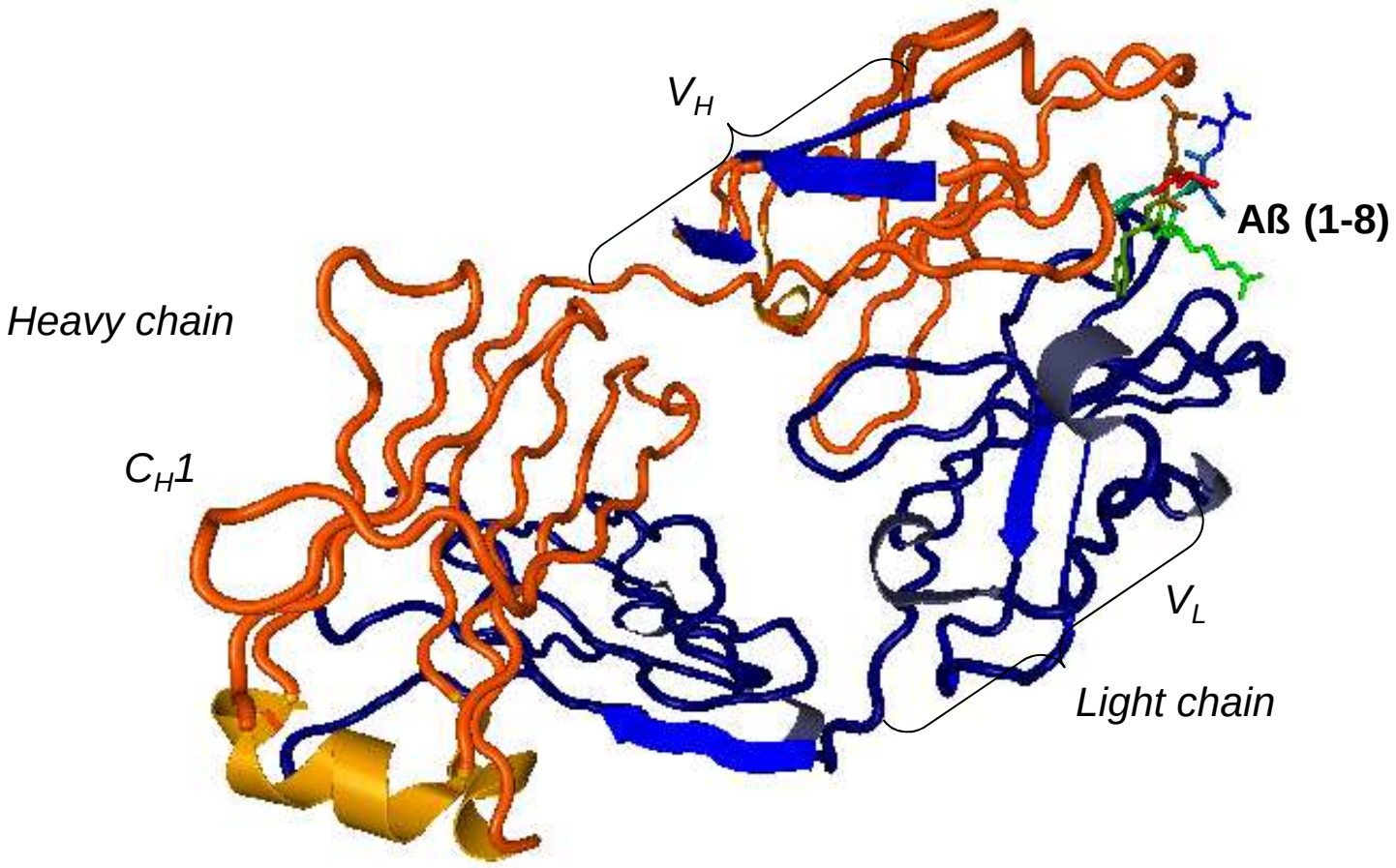
University of Konstanz

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

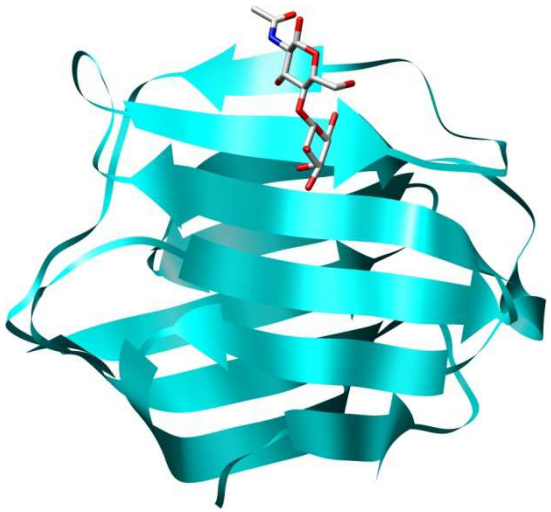


PDB access number: 2IPU - Gardberg, A.S., et al., (2007) Molecular basis for passive immunotherapy of Alzheimer's disease, PNAS, 104, 15659-15664

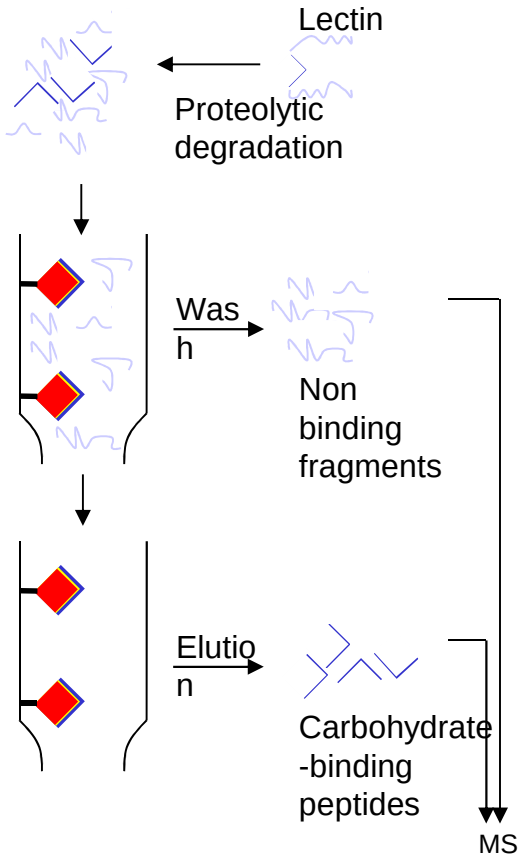
Application 2: Lectin- Carbohydrate Ligand Epitopes CREDEX-MS

Galectins: β-galactosides-binding ability

Highly conserved carbohydrate binding sites.

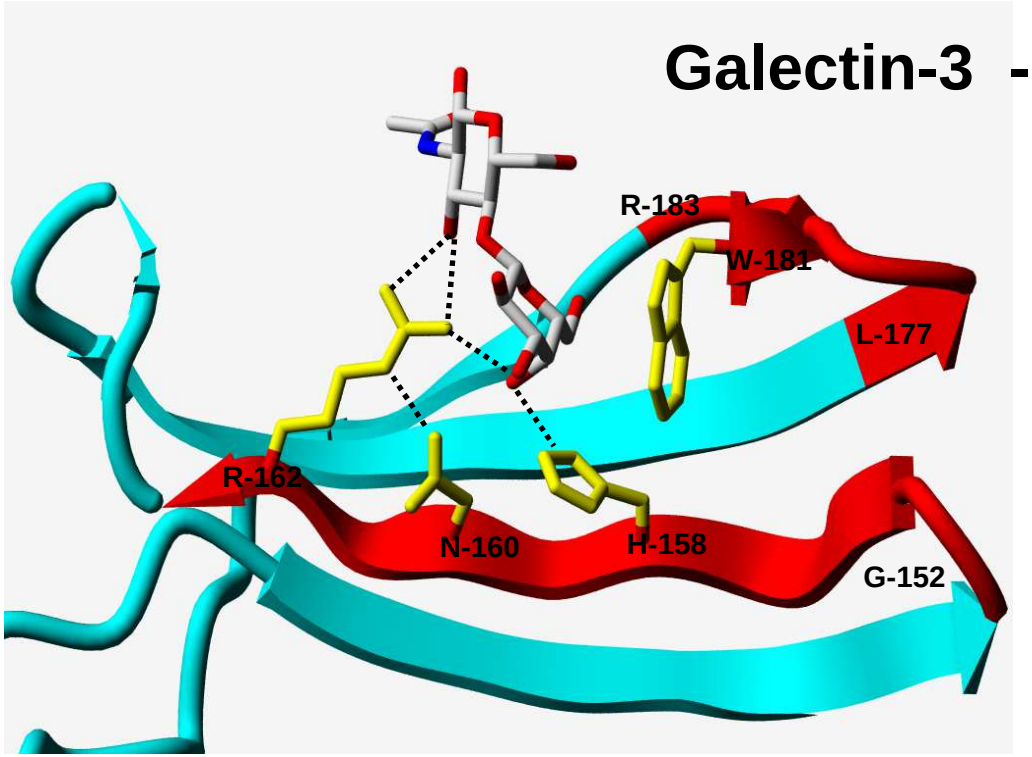


Galectin-3-LacNAc complex

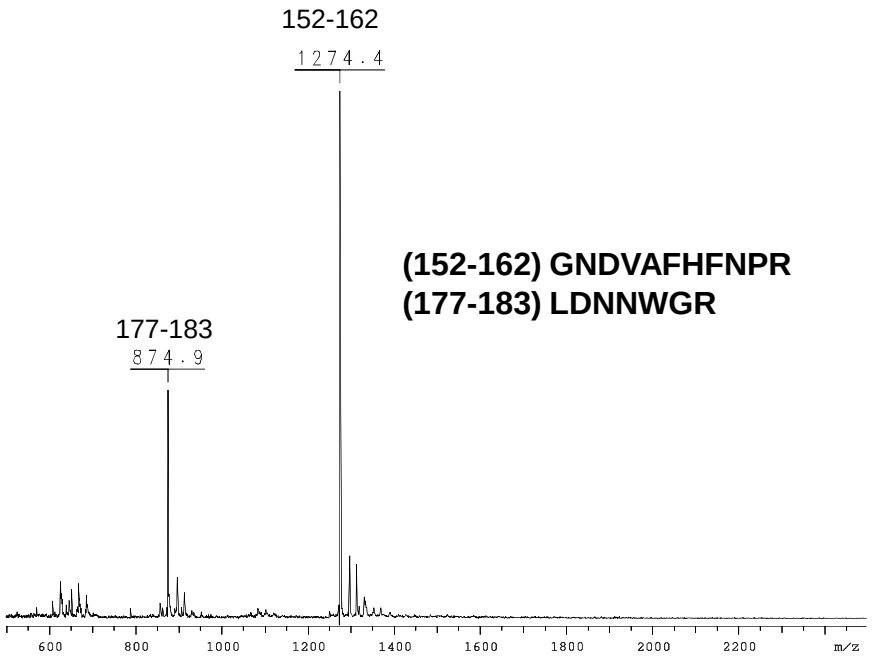


Identification of Galectin- carbohydrate interaction structures by proteolytic excision- biosensor-MS (CREDEX-MS)

Galectin-3 - Lactose complex



CRD Epitopes

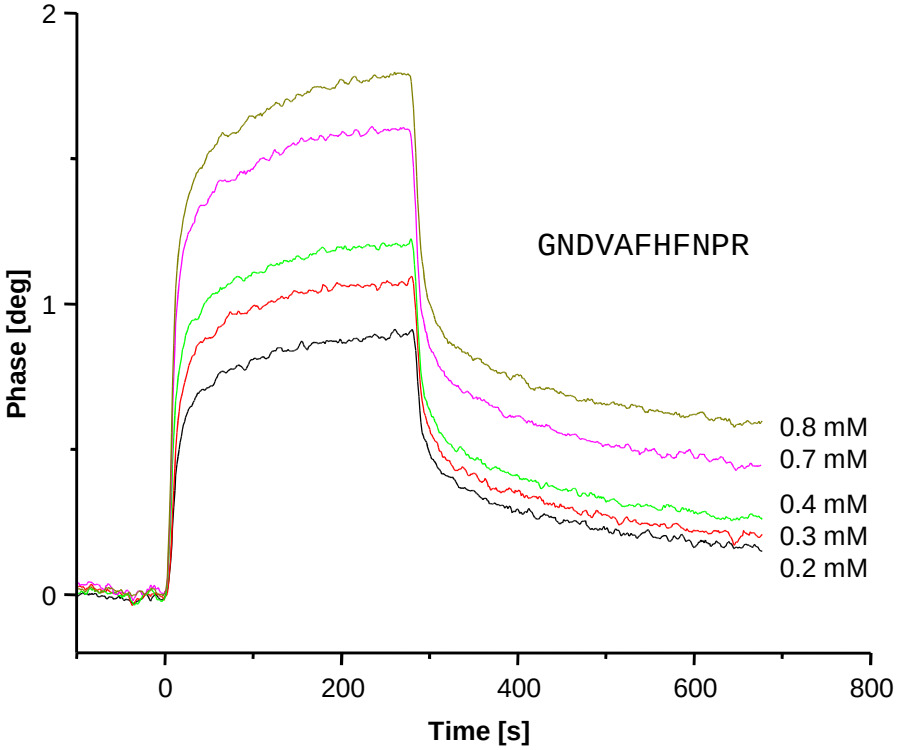


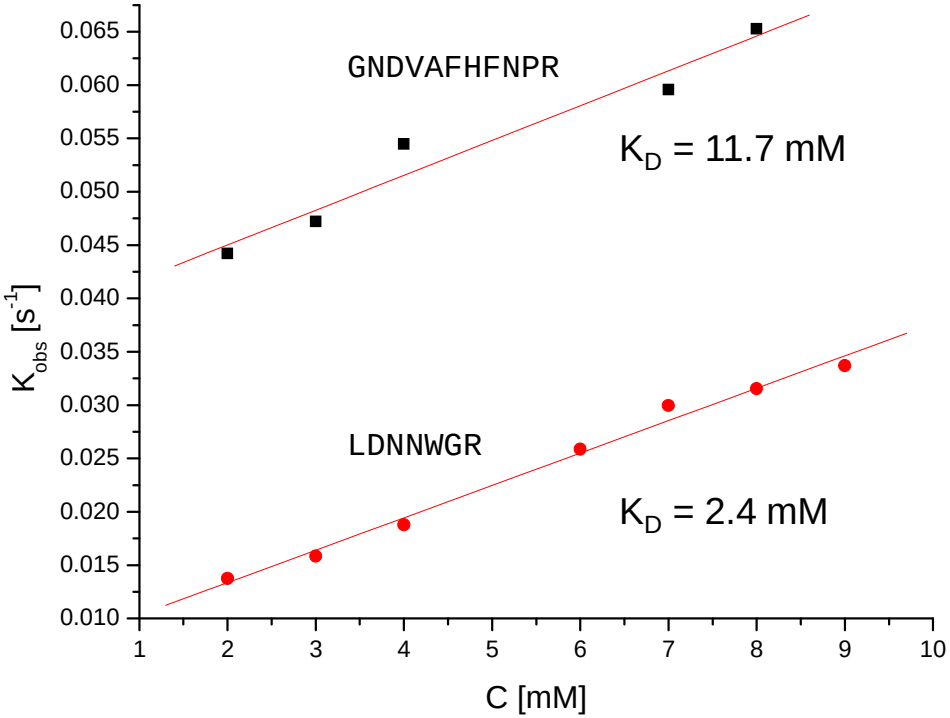
(152-162) GNDVAFHFNPR
(177-183) LDNNWGR

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

Analytical Chemistry & Biopolymer Structure Analysis
University of Konstanz

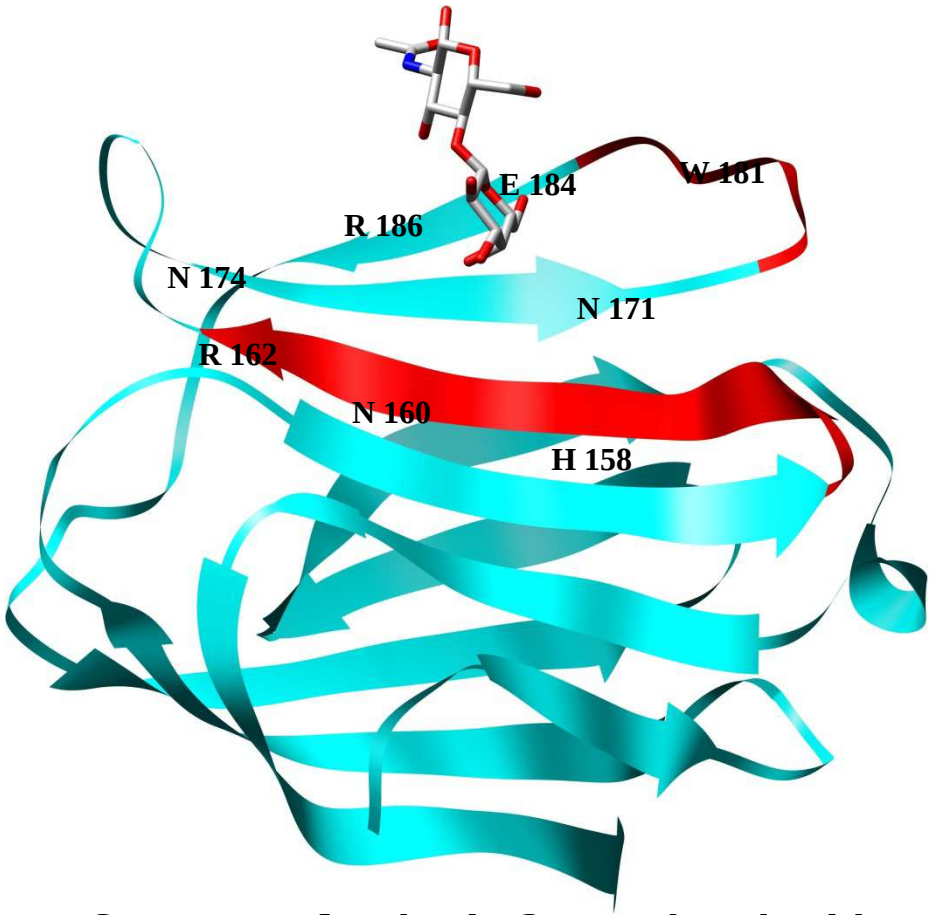
Binding curves of SAW sensorgram and K_D for CRD peptides





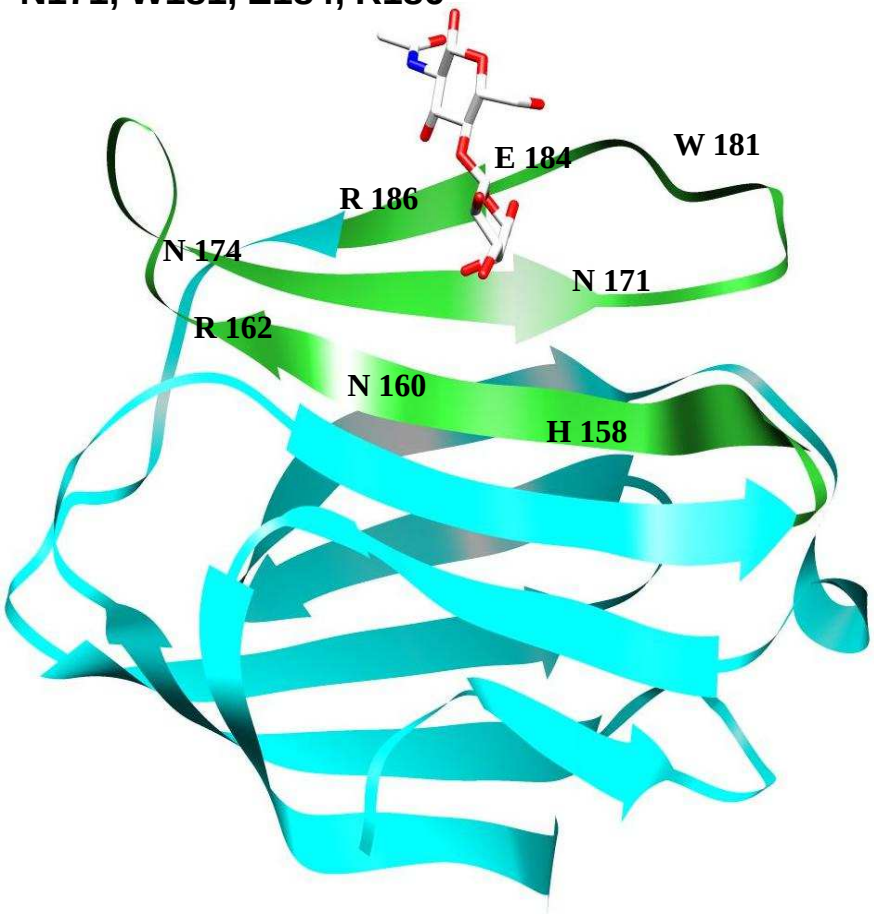
CRD Peptides from CREDEX-MS in galectin-3
- COMPLETE AGREEMENT WITH CRYSTAL STRUCTURE

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

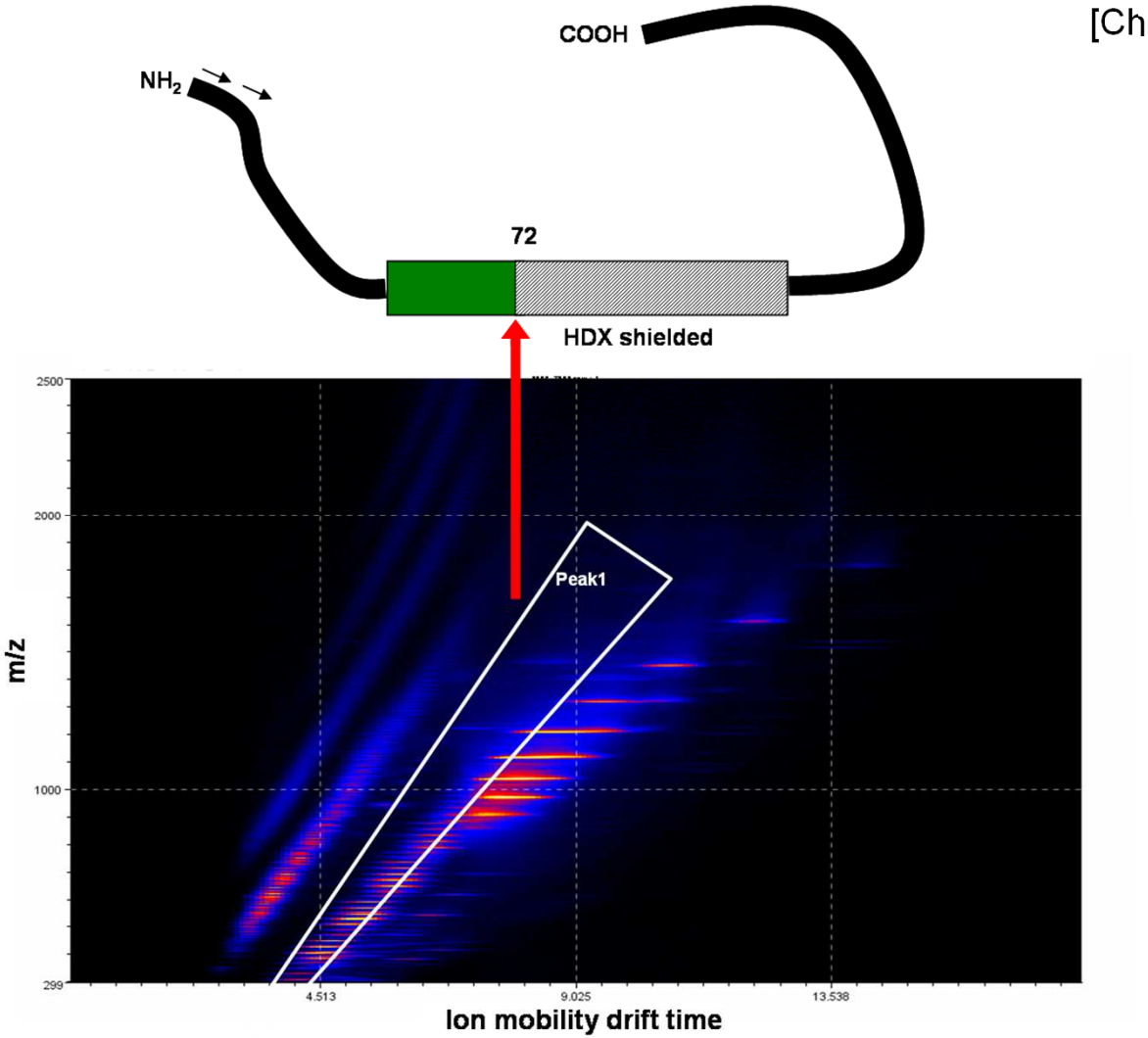


Galectin-3 in complex with LacNAc (pdb file 1A3K).

Application 3 [Autoproteolytic Fragments are Intermediates in the Oligomerization- Aggregation of Parkinson's Disease Protein Alpha-Synuclein

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]*

[ChemBioChem 2011, 12, 2740]

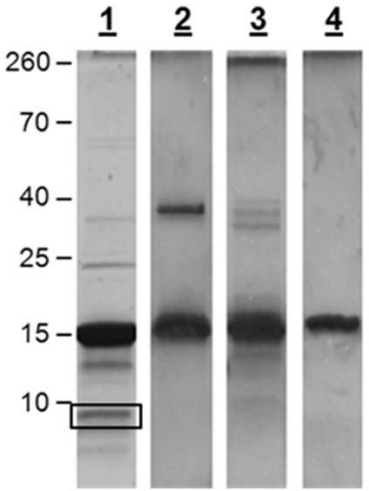


Fragmentation & Aggregation of physiological and pathological Synucleins: The αSyn tripeptide VVT(70-72)

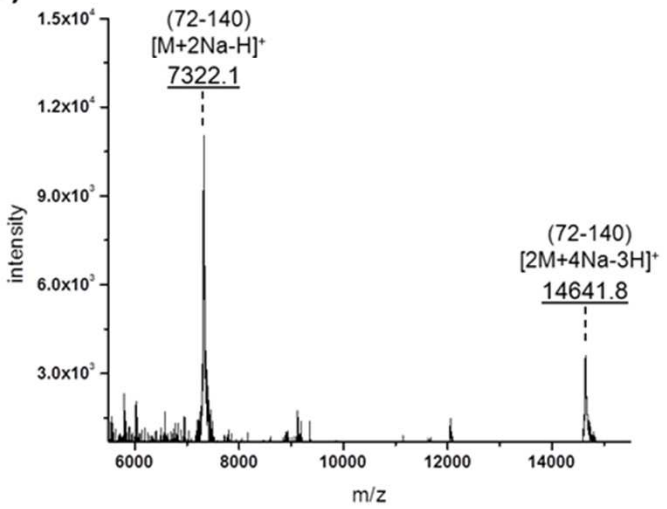
A)

1 αSyn wt	^{1}M…K^{61}EQVTNVGGA^{70}**VVT**^{73}GVTAVAQKTVEGAGSIA^{90}A…^{140}A
2 αSyn NAN	^{1}M…K^{61}EQVTNVGGA^{70}**NAN**^{73}GVTAVAQKTVEGAGSIA^{90}A…^{140}A
3 αSyn VFS	^{1}M…K^{61}EQVTNVGGA^{70}**VFS**^{73}GVTAVAQKTVEGAGSIA^{90}A…^{140}A
4 βSyn	^{1}M…K^{61}EQASHLGGA^{70}**VFS** ----------^{73}GAGNIA^{79}A…^{134}A

B)



C)

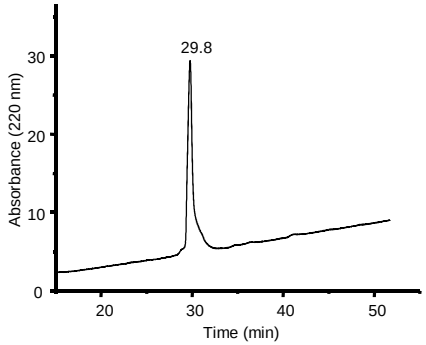


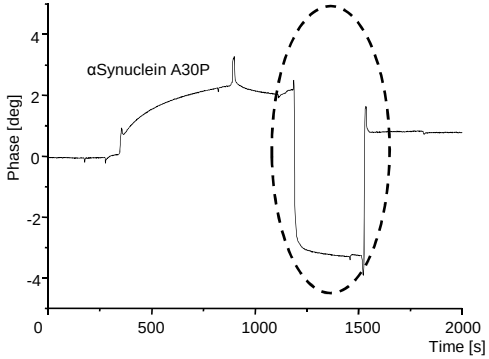
HPLC-MS & Biosensor- MS features / Experimental comparison /
Example: α-Synuclein – Parkinson's Disease Biomarker

	HPLC-MS	Biosensor/SAW-MS
Sample	Pure protein required	Biological samples feasible
Separation material	column specific for proteins needed	Antibody chip
Run time	ca. 50 minutes	< 30 minutes
Total time / costs	50 + 30 min (reconditioning) /x 100	30 + 0*

*** Interface washing and equilibration (10 min) is performed during next sample injection**

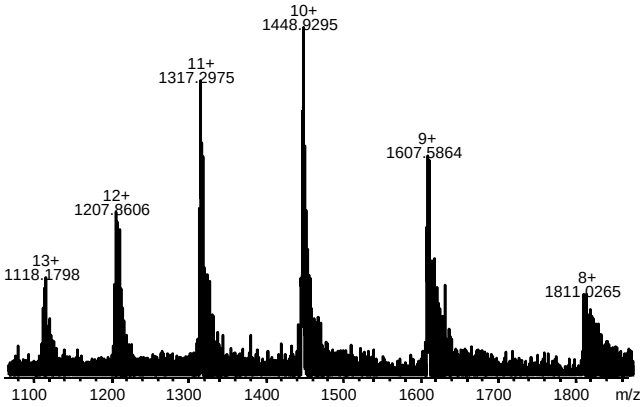
Chromatogram / Sensorgram

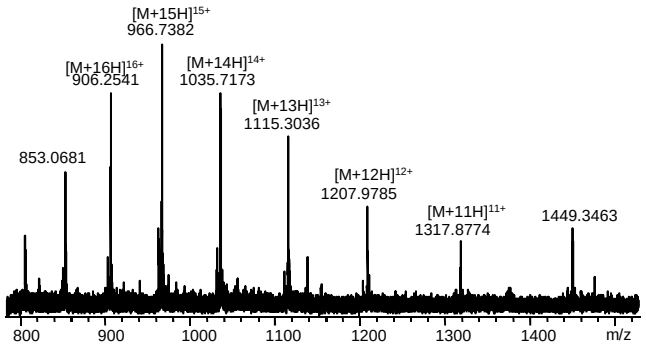




Mass spectrum

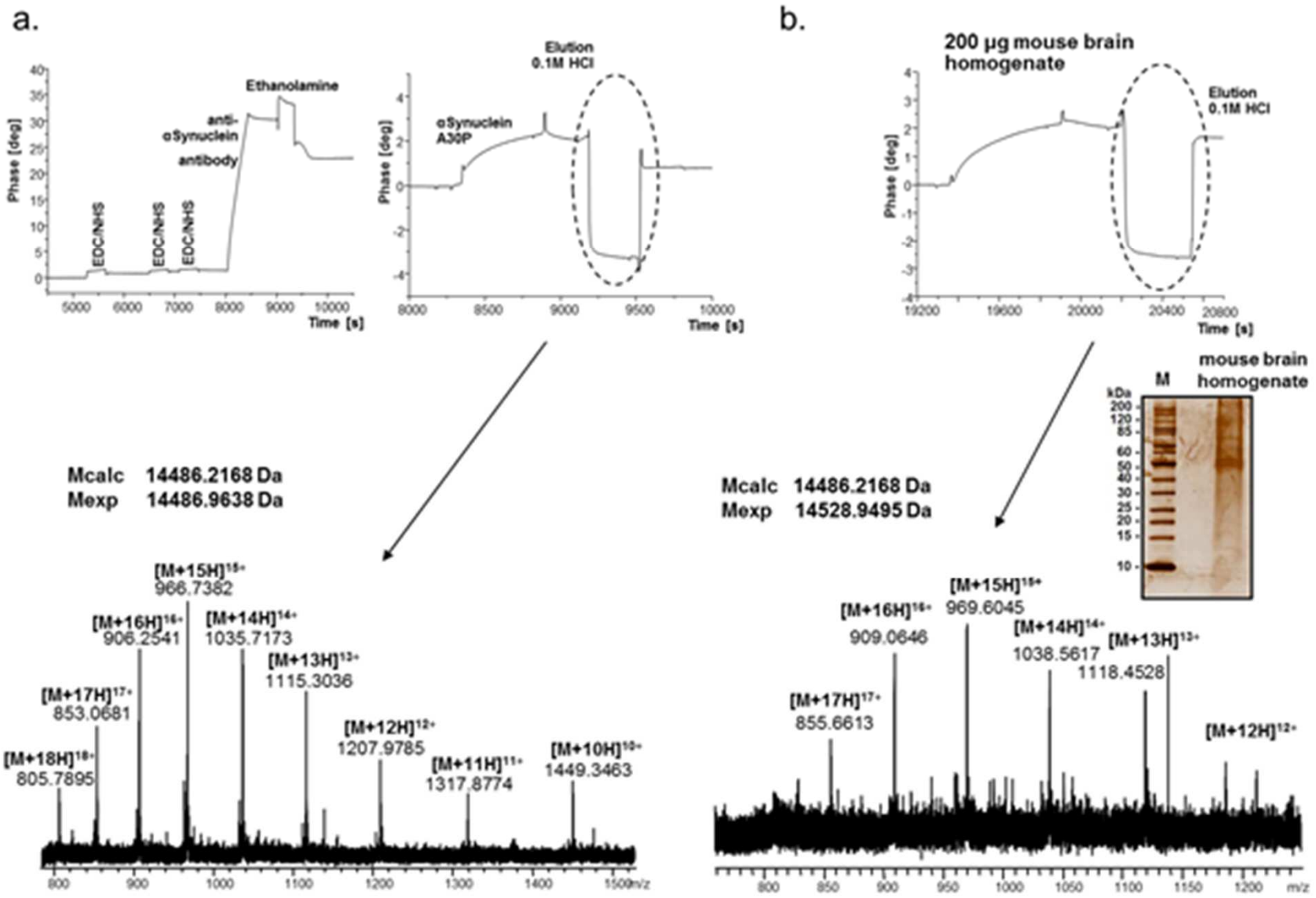
-molecular structure



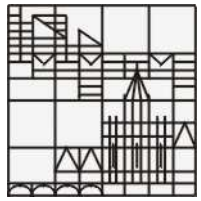


Online SAW-affinity-MS of wt-aSyn in vitro (a)
(b) In Vivo - mouse brain homogenate

Figure 3



Summary -
online- Biosensor-mass spectrometry

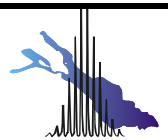


- Biosensor analysis: Well-established technique for sensitive detection, real-time monitoring, quantification of biomolecular interactions
- - No chemical structure determination
- Proteins bound to molecules immobilized on the biosensor chip can be recovered but need to be identified separately (time consuming)

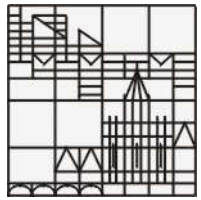
Biosensor- MS: Provides Chemical structure determination

- Biosensor/SAW-MS Interface: Essential for [1] concentration, [2] desalting , [3] microfluidic online transfer from biosensor to mass spectrometer

- Biosensor-MS combination reveals biomolecular interactions and identification of binding partners in complex samples



THANKS TO
… Coworkers, Collaborators, €€€...



Coworkers

Dr. Camelia Vlad
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Frederike Eggers
Stefan Slamnoiu
Dr. Mihaela Stumbaum
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Collaborators

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SAW-Instruments Bonn
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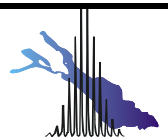
David Clemmer, Indiana University

€€€

DFG
Boehringer – Ingelheim,
Univ. Konstanz
BMWI

Biopolymer-MS & ChemBio Grad School
Parkinson/ Synuclein
Research Center Proteostasis
Affinity-MS



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Biopolymer Analysis and Biomedical Mass Spectrometry

MMS LifeChem

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Opening Workshop 24-25th November 2014