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

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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
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

\[ AB \xrightarrow{K_{off}} A + B \]

\[ K_d = \frac{K_{off}}{K_{on}} \]

\[ k_{obs} = c \cdot k_{on} - k_{off} \]

\[ K_{on} \] - association rate constant

\[ K_{off} \] - dissociation rate constant

\[ K_{obs} \] - pseudo-first order kinetic constant

Analytical Chemistry & Biopolymer Structure Analysis
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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

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

Biosensor (SAW)

Gold coated quartz chip

Desalting

µ Pump System

ESI MS

m/z

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

SAW Sensorgram
$K_b$ VEGF-Avastin: 15.5 nM

RIC Biosensor Eluate

ESI-MS

VEGF
$M_{calc} = 23250.78$ Da
$M_{exp} = 23250.19 \pm 0.34$ Da

M. Diaz et al., HUPO- Conf. (2014)
OVERVIEW

I  Online Biosensor/SAW-MS Combination:

Analytical Development - Interface

II  Application Examples:

  Aβ- antibody / Epitope determination
  Lectin- carbohydrate / CRD peptides
  Parkinson’s Protein α-Synuclein / in vivo
APPLICATION 1 Epitope Analysis of Aβ- Antibodies

Plaque-specific Aβ-antibodies: Recognition of N-terminal epitope

Plaque-protective Aβ-autoantibodies: C-terminal, oligomer-specific epitope

APPLICATION 1  Antibody- Epitope Identification
Shielded proteolytic excision - basis for mass spectrometric epitope identification

Epitope peptide

Preconditions:
Proteolytic stability of antibody
Epitope-Paratope Interaction shielded

Epitope Excision

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

#### Phase [deg] / abundance

<table>
<thead>
<tr>
<th>Time [s]</th>
<th>EDC/NHS</th>
<th>EDC/NHS 4G8 Antibody</th>
<th>Ethanolamine</th>
</tr>
</thead>
</table>

#### Time [s] (for antibody immobilisation, affinity interaction, elution, desalting, elution MS analysis, washing, and equilibration)

- Antibody immobilisation: 30 min
- Affinity interaction: 10 min
- Elution: 6 min
- Desalting: 4 min
- Elution MS analysis: 6 min
- Washing / Equilibration: 6 min

#### K<sub>D</sub> = 19.3 nM

#### m/z

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>751.9101</td>
<td>1007.8871</td>
<td></td>
<td></td>
<td>756.4182</td>
<td></td>
<td>1015.2198</td>
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<tr>
<td>727.3959</td>
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<td></td>
<td></td>
<td>969.5305</td>
<td></td>
<td>1511.3389</td>
</tr>
</tbody>
</table>

#### k<sub>obs</sub> (sec<sup>-1</sup>) vs Concentration (µM)

- K<sub>D</sub> = 19.3 nM

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**Analysis:**

- Antibody immobilisation:
- Affinity interaction:
- Elution:
- Desalting:
- Elution MS analysis:
- Washing / Equilibration:

**m/z Analysis:**

- [M+3H]<sup>3+</sup> 751.9101
- [M+2H]<sup>2+</sup> 1007.8871
- [M+3H-114]<sup>3+</sup> 756.4182
- [M+4H]<sup>4+</sup> 969.5305
- [M+4H-17]<sup>4+</sup> 1015.2198
- [M+2H+Na]<sup>2+</sup> 1511.3389
Crystal structure of an Aβ-plaque specific antibody complex with N-terminal Aβ-epitope

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

A. Moise et al. J. Am. Chem. Soc. (2011) 133, 14844
Binding curves of SAW sensorgram and $K_D$ for CRD peptides

$K_D = 11.7$ mM

$K_D = 2.4$ mM

GNDVAFHFNPR

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

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

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

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

**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


[ChemBioChem 2011, 12, 2740]
Fragmentation & Aggregation of physiological and pathological αSynucleins: The αSyn tripeptide VVT(70-72)

A)  
1. αSyn wt  
   1M...K^61EQVTNVGGA^70VVT^73GVTAVAQKTVEGAGSIA^90A...^140A

2. αSyn NAN  
   1M...K^61EQVTNVGGA^70NAN^73GVTAVAQKTVEGAGSIA^90A...^140A

3. αSyn VFS  
   1M...K^61EQVTNVGGA^70VFS^73GVTAVAQKTVEGAGSIA^90A...^140A

4. βSyn  
   1M...K^61EQASHLGGGA^70VFS  - - - - - - - 73GAGNIA^79A...^134A

B)  

C)  
(72-140)  
[M+2Na-H]^+  
7322.1

(72-140)  
[2M+4Na-3H]^+  
14641.8
# HPLC-MS & Biosensor-MS features / Experimental comparison

## Example: α-Synuclein – Parkinson's Disease Biomarker

<table>
<thead>
<tr>
<th>HPLC-MS</th>
<th>Biosensor/SAW-MS</th>
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<tbody>
<tr>
<td><strong>Sample</strong></td>
<td>Pure protein required</td>
</tr>
<tr>
<td><strong>Separation material</strong></td>
<td>column specific for proteins needed</td>
</tr>
<tr>
<td><strong>Run time</strong></td>
<td>ca. 50 minutes</td>
</tr>
<tr>
<td><strong>Total time / costs</strong></td>
<td>50 + 30 min (reconditioning) /x 100</td>
</tr>
</tbody>
</table>

### Chromatogram / Sensorgram

**Mass spectrum - molecular structure**

![Chromatogram](image1.png)

![Sensorgram](image2.png)
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-MS combination reveals biomolecular interactions and identification of binding partners in complex samples
THANKS TO
... Coworkers, Collaborators, €€€...

Coworkers

Dr. Camelia Vlad
Dr. Kathrin Lindner
Adrian Moise
Claudia Cozma
Frederike Eggers
Stefan Slamnoiu
Dr. Mihaela Stumbaum
Dr. Marilena Manea
Nicole Engel

Collaborators

Bastian Hengerer, Boehringer Ingelheim
Marcel Leist, Stefan Schildknecht, Konstanz
Michael Gross, Washington Univ. St.Louis
Marta Vilaseca, IRB Barcelona
SAW-Instruments Bonn
Waters Ltd, Manchester
David Clemmer, Indiana University

€€€

DFG
Boehringer – Ingelheim, Univ. Konstanz
BMWI

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

Analytical Chemistry & Biopolymer Structure Analysis
University of Konstanz
NEW LABORATORY - RESEARCH CENTER - RÜSSESHEIM
Opening Workshop 24-25th November 2014