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

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Why online Bioaffinity-MS?

Ø Online HPLC-ESIMS: 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 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 $\Delta \varphi$  
Visibility change $\Delta \varphi$ and $\Delta A$


$$AB \xrightleftharpoons[k_{dissn}]{k_{assn}} A + B$$

$$K_D = \frac{k_{dissn}}{k_{assn}}$$
Affinity - MS:
Dissociation step: Elution interface needed

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

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

K\text{on} - association rate constant
K\text{off} - dissociation rate constant
K\text{obs} - pseudo-first order kinetic constant
k\text{obs} = c \cdot k\text{on} - k\text{off}
SAW- Bioaffinity- Mass Spectrometry Combination
SAWMS – I

Interface
INTERFACE: Provides simultaneous desalting & concentration

Biosensor

- 

- Elution

- Time (s)

- $k_{on}$

- $k_{off}$

- Flow 1

- Flow 2

- Pump system

- Waste

- 5 μm frit

- 1.5 cm x 1 mm packed

- 40 μm particle size

ESI-MS

- $[M+12H]^{12+}$

- $[M+13H]^{13+}$

- $[M+14H]^{14+}$

- $[M+15H]^{15+}$

- $[M+16H]^{16+}$

- $[M+17H]^{17+}$

- $[M+18H]^{18+}$

- m/z

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


\( \beta^- \) Amyloid formation & „\( \beta^- \) and \( \gamma^- \) 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

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

Figure 2

(a) Aβ (1-40)

(b) Elution 5% ACN in 0.1 M HCl

(c) [M+5H]^{5+} 866.2442

(d) $K_{\text{obs}} = 7.3233 \times 10^{-4} + 3.65067 \times 10^{-5} \times c$

$R^2 = 0.986$

$K_D = 20.06 \pm 8.06 \text{ nM}$

H-DaEFRHDSGVEYHVHQLVFFA

EDVGSNKGAIGLMVGGV-NH$_2$
Affinity of anti-Aβ antibody with Aβ(1-16) by direct coupling SAW biosensor – ESI ion trap MS

Stefan Slamnoiu
**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

1KVFRGCEAAAMKRHGLDNRYGISLGNWVCAAKFESNFNTQATNRNTDGSTDY
GILQINSRWWCNDGRTPGSRNLICSNPSALLSDITASVNCACKIVSDGNMN
VAWRNRCKGTVDQAWIRGCRL129

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

Affinity Lysozyme

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

FT-ICR-MS of elution fraction

Solvent A: 0.3 % HCOOH; solvent B: 0.3 % HCOOH / 80 % MeCN
Application 3: Tyrosine Nitration of Eosinophils

- Eosinophils: protection against infections
- EPO - in presence of H₂O₂ and halide catalyzes the formation of oxidants.
- EPO - catalyzes nitration with nitrite (NO₂⁻) and H₂O₂ 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 -

Trypsin / Thermolysin → In solution digestion → Digested peptides solution

ECP / EDN → 3NT antibody

Washing fraction → Non-binding peptide

Washing until no MS signal

Elution with 0.1% TFA → Elution fraction → Binding nitrated peptide

MS analysis

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

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

H-GKRLKNY(NO$_2$)SLPWGA-OH  [M+H]$^+$  1533.813

K$_D$ : 25 nmol

ESI-MS of elution fraction

\([M+4H]^4+\)  385.0
\([M+3H]^3+\)  512.3
\([M+2H]^2+\)  767.9
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

Carbohydrate

Lectin

Proteolytic degradation

Wash

Non binding fragments

Elution

Lectin epitopes
CREDEX-MS of galectin-3 interaction with lactose provides two specific CRD peptides (152-162) and (177-183)
CRD Peptides from CREDEX-MS in galectin-3
- COMPLETE AGREEMENT WITH CRYSTAL STRUCTURE

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).
Online SAW-MS:
Identification and affinity quantification of Galectin-5 carbohydrate binding peptide

**G5B**

Elution:
10% $\text{H}_3\text{COOH}$ in $\text{H}_2\text{O}$

\[
K_{\text{obs}} = k_{\text{off}} + C \times k_{\text{on}}
\]

\[
K_D = k_{\text{off}} / k_{\text{on}} = 192.7 \, \mu\text{M}
\]
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II  Oligomerisation - Aggregation of Parkinson's Disease Protein α-Synuclein:
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   - Ion Mobility-, HDX- MS
   - Affinity-MS: Direct Analysis from Biological Material
Aggregation of α-Synuclein - key protein in Parkinson’s disease -

α-Synuclein

Oligomers?

DISEASE

Amyloid Fibrils

Lewy Body

Conway et al PNAS 2000
Goldberg and Lansbury, Nat Cell Blol 2001
Alpha- Synuclein shows “oligomers” AND degradation products
Direct mass spectrometry unsuccessful

2 – 6 days

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

10 µg aggregates
15 % Separation gel
5 µl LMW marker
Autoproteolytic Fragments are Intermediates in the Oligomerization-Aggregation of Parkinson’s Disease Protein Alpha-Synuclein as Revealed by Ion Mobility Mass Spectrometry


[ChemBioChem 2011]
Fragmentation & Aggregation of Synucleins differentiate by the beta-breaking triplett (70-72)

Primary structure of human αSyn wt 1, αSyn triplet -mutants (αSyn NAP 2, αSyn VFS 3) and βSyn 4, and their structural behavior with particular emphasis on the analysis of aggregation process after 4 days of in vitro incubation, followed by B) gel electrophoresis. The boxed gel band (~9 kDa) of αSyn wt 1 was eluted and measured by MALDI

B) Autopreolytic αSyn fragment αSyn (72-140).
Selective mutation of key sequence in $\alpha$-syn

$\alpha$-syn wt

$1^{\text{MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKT}}$
$\text{KEGVVHGVAETVAEKTKEQVTNVGGAG}^{70} \text{VVT}^{72} \text{GVTAVAQKTVEGAG}$
$\text{SIAAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEE}$
$\text{GYQDYEPEA}^{140}$

$\alpha$-syn $70^{\text{NAN}}^{72}$

$1^{\text{MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKT}}$
$\text{KEGVVHGVAETVAEKTKEQVTNVGGAG}^{70} \text{NAN}^{72} \text{GVTAVAQKTVEGA}$
$\text{GSIAAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNEAYEMPSEE}$
$\text{EGYQDYEPEA}^{140}$
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)
Perspectives for affinity- mass spectrometry /

Online Affinity- Mass spectrometry

- Identification of antigen epitopes - vaccine lead structures
  Biomarker identification
  Ligand- binder recognition & interaction
  Conformational/topography characterisation
  Reactive intermediates in misfolding & aggregation
THANKS TO THE MAJOR PLAYERS...
... Coworkers, Collaborators, €€€...

Coworkers

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€€€

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EU
Boehringer – Ingelheim,
Univ. Konstanz
BMWI

Biopolymer-MS & ChemBio Grad School
Antibodies to Human Proteome; RUBICON
Parkinson/ Synuclein
Research Center Proteostasis
Affinity-MS

Analytical Chemistry & Biopolymer Structure Analysis
University of Konstanz
SAW- Bioaffinity- Mass Spectrometry Combination
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