“Seeing is Believing: Analyzing Protein Complexes Assembled on and Released from Biolayer interferometry Biosensor Surfaces using Mass Spectroscopy and Electron Microscopy”

Mark Fisher
Professor
Department of Biochemistry and Molecular Biology
University of Kansas Medical Center
Kansas City KS
Analyzing protein complexes assembled on and isolated from BLI biosensor using EM and MS

1. Load biosensor onto the Blitz
2. Hydrate and/or activate biosensor
3. Assemble protein complex
4. Elute complexes from biosensor
5. Perform negative staining and observe under EM
6. Perform Mass Spectroscopy
Protein Toxin Stabilizer validation

Therapeutic Protein Testing and Stabilizer Validation

Stabilizer Identification and Validation For Folding Diseases
Preparing for Mass Spectroscopy

Removing Biosensor tip
After assembly the two or three Component LFN-PA pore or LFN-PA prepore-CMG2 complex

Dip into 2ul of refolding buffer containing 10mM DTT to release LFN-PA prepore-CMG2 complexes

Repeat 5 times and pool all the samples and submit for MS
Output Protein discoverer
Search engine set up

From-20131101-LFn-PA-CMG2-complex-all
Fisher-db-2-Trypsin-full-2 missed cleavages
Precursor mass tolerance 50ppm
Fragment mass tolerance 0.8Da

4. Dynamic Modifications:

Max. Modifications Per Peptide: 4
2. Dynamic Modification: Deamidated / +0.984 Da (N, Q)
3. Dynamic Modification: Oxidation / +15.995 Da (M)

5. Static Modifications:

1. Static Modification: Carbamidomethyl / +57.021 Da (C)

Protein identifications

<table>
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<tr>
<th>Accession</th>
<th>FROM-20131101-LFn-PA-CMG2-complex-all</th>
<th>Score</th>
<th>Coverage</th>
<th>Proteinique Pept</th>
<th># Peptides</th>
<th># PSMs</th>
<th># AAs</th>
<th>MW (Da)</th>
<th>calc. pl</th>
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<tbody>
<tr>
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Peptide identifications
Mass Spectroscopy peptide identification of Protective Antigen (PA) – Lethal Factor (LF_N) complex

Lethal Factor N termini (LF_N)

Anthrax Protective Antigen (PA) monomer
GroEL–LOX (lactose oxidase) complex  Both GroEL and LOX detected in 2 ul volumes
Mass Spectroscopy data of $L_{F_N}$-PA:

Identified peptide regions within released $L_{F_N}$ – PA complexes

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Chain</th>
<th>Sequence</th>
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<tr>
<td>3KWW</td>
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<tr>
<td>1J7N</td>
<td>A</td>
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Lethal Factor N termini ($L_{F_N}$)

Protective Antigen Oligomer
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6. Perform Mass Spectroscopy
Perform negative staining and observe under EM

Perform Mass Spectroscopy

Load biosensor onto the Blitz

Hydrate and/or activate biosensor

Assemble protein complex

Elute complexes from biosensor

Example: Anthrax Toxin Transition

Analyzing protein complexes assembled on and isolated from BLI biosensor using EM and MS
Monitoring Anthrax Toxin Prepore to Pore Transition

Activate tip

Anthrax Prepore on BLI biosensor tip

Prepore to pore conversion

PA micelle formation

1M Urea 37 °C

MSP, lipid, cholate Mixture

Graph showing BLI Signal over time.
Analyzing protein complexes assembled on and isolated from BLI biosensor using EM and MS

Example: Partially Folded States

1. Load biosensor onto the Blitz
2. Hydrate and/or activate biosensor
3. Assemble protein complex
4. Elute complexes from biosensor
5. Perform negative staining and observe under EM
6. Perform Mass Spectroscopy
Denaturant Pulse: Detect Partially Folded States

BLI Δ nm amplitude

Time (s)
Analyzing protein complexes assembled on and isolated from BLI biosensor using EM and MS

1. Load biosensor onto the Blitz
2. Hydrate and/or activate biosensor
3. Assemble protein complex
4. Elute complexes from biosensor
5. Perform negative staining and observe under EM
6. Perform Mass Spectroscopy
Method to knock off the immobilized biological molecules on biosensor tip and visualized them by Electron Microscopy

- Removing Biosensor tip containing protein complexes
- Dip into 2µl buffer + decouple reagent
- Preparing EM grid
Removal from solid surface (BLItz tip) indicating that insertion of PA in to lipid nanodisc, confirmed by TEM
TEM of Biotinylated GroEL (+/- heated mAb) Released from BLI Biosensor

Panel A: GroEL alone
Top view
Side view

Panel B: GroEL + heated mAb:

TEM: vWF + GroEL

http://www.cryst.bbk.ac.uk/PPS2/course/section12/ellis.html
IPET

- Individual Particle Electron Tomography
IPET

- Individual Particle Electron Tomography
- Multiple angles
- Idea of 3D structure
IPET

- Individual Particle Electron Tomography

- 7 angle tilt to envelope
- Compares well to crystal
IPET

- **Individual Particle Electron Tomography**
- Regions of flexibility
- No class averaging
IPET

- Individual Particle Electron Tomography

TeNT bound

Side view of GroEL