Identification & Analysis of Protein Complexes Mediating Synapse Formation

Thomas Biederer

Department of Molecular Biophysics and Biochemistry

Yale University

Funding Support:
NIH/NIDA and March of Dimes Foundation
Synapses are specialized cell junctions


motivation to understand synapse organization:

- neurobiological motivation: role in brain development and plasticity
- biomedical: neurodevelopmental and degenerative disorders, synaptic plasticity including learning and addiction
- biochemical: membrane assembly
Trans-synaptic adhesion and synapse organization
Both SynCAM 1 and 2 are sufficient to induce presynaptic specializations
SynCAM proteins engage each other in specific heterophilic interactions
Aim 1: To analyze molecular SynCAM properties

**Hypothesis:**
*The post-translational modification of SynCAM proteins is prominent and functionally relevant.*

**achieved aim:**
- determined biophysical properties of SynCAM extracellular domains and quantified high extent of glycosylation
Four SynCAM proteins are expressed in brain
Glycosylation analysis by light scattering

Light scattering by a protein is a function of the specific refractive index, the concentration, and the molecular weight of the protein.

Size exclusion chromatography, followed by measurement of light scattering and refractive index determination for native macromolecules in solution of:

- Absolute mass (MW)
- Size (radius of gyration)
- Extent of carbohydrate modification due to change in refractive index

Ewa Folta-Stogniew
Keck Facility and NIDA Neuroproteomics Center, Yale University
SynCAMs can be heavily N-glycosylated

starting material: heterologously expressed, purified SynCAM extracellular domains

note that expression in HEK 293 cells yields apparently identical posttranslational modification of SynCAMs as observed in adult brain

<table>
<thead>
<tr>
<th>SynCAM</th>
<th>Sugar/Protein (gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SynCAM 1</td>
<td>0.53</td>
</tr>
<tr>
<td>SynCAM 2</td>
<td>0.15</td>
</tr>
<tr>
<td>SynCAM 3</td>
<td>0.04</td>
</tr>
</tbody>
</table>

size exclusion chromatography / static light scattering:
SynCAM 1 is heavily glycosylated

Ewa Folta-Stogniew
Keck Facility and NIDA Neuroproteomics Center, Yale University
Structure-function analysis of N-glycosylation in heterophilic SynCAM interactions

Adam Fogel
Aim 2: To identify synaptic protein changes due to altered SynCAM 1 expression *in vivo*

**Hypothesis:**
The proteomic analysis of SynCAM 1 synapses will lead to the identification of downstream proteins in synaptogenic signaling.

**Achieved aim:**
- Prepare synaptic plasma membranes from SynCAM 1 KO or wild-type littermates
- Evaluate changes in protein composition by iTRAQ
- Raise specific antibodies and validate target protein changes

**Ongoing:**
- Test for biochemical and/or functional interactions of target proteins
Analysis of synaptic composition by relative iTRAQ protein quantification

synaptic plasma membrane proteins:

iTRAQ analysis of preparations from SynCAM 1 overexpressing brains vs. controls after isobaric tag labeling

multiplexing of four different samples in a single LC/MS/MS experiment

relationships can be quantified by comparing the MS peak area of one reporter group peak to another

from: Applied Biosystems iTRAQ Reference Guide
Analysis of synaptic composition in SynCAM 1 knock-out mice

identified 450 proteins with a protein score >0.3
239 reduced 117/114 <= 1.000
211 increased

Chris Colangelo
Keck Facility and NIDA Neuroproteomics Center, Yale University
Synaptic plasma membranes lacking SynCAM 1 display altered protein composition tabulated: molecular function down-regulated

<table>
<thead>
<tr>
<th>Panther Category</th>
<th>Transcripts(Trans)</th>
<th>% Trans to Total Trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell adhesion molecule (MF00040)</td>
<td>27</td>
<td>31.8%</td>
</tr>
<tr>
<td>Cell junction protein (MF00276)</td>
<td>5</td>
<td>5.9%</td>
</tr>
<tr>
<td>Chaperone (MF00077)</td>
<td>3</td>
<td>3.5%</td>
</tr>
<tr>
<td>Cytoskeletal protein (MF00091)</td>
<td>13</td>
<td>15.3%</td>
</tr>
<tr>
<td>Hydrolase (MF00141)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Ion channel (MF00024)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Isomerase (MF00166)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Membrane traffic protein (MF00267)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Miscellaneous function (MF00197)</td>
<td>12</td>
<td>14.1%</td>
</tr>
<tr>
<td>Molecular function unclassified (MF00208)</td>
<td>5</td>
<td>5.9%</td>
</tr>
<tr>
<td>Oxidoreductase (MF00123)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Phosphatase (MF00113)</td>
<td>4</td>
<td>4.7%</td>
</tr>
<tr>
<td>Protease (MF00153)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Receptor (MF00001)</td>
<td>7</td>
<td>8.2%</td>
</tr>
<tr>
<td>Select calcium binding protein (MF00188)</td>
<td>1</td>
<td>1.2%</td>
</tr>
<tr>
<td>Select regulatory molecule (MF00093)</td>
<td>17</td>
<td>20.0%</td>
</tr>
<tr>
<td>Transferase (MF00131)</td>
<td>5</td>
<td>5.9%</td>
</tr>
<tr>
<td>Transporter (MF00082)</td>
<td>1</td>
<td>1.2%</td>
</tr>
</tbody>
</table>
THE NEXT SLIDES WERE REMOVED - DATA NOT FOR WEB POSTING
Objective 1: Complete the proteomic analysis of synaptic membranes lacking and overexpressing SynCAM 1 from KO and transgenic mouse models.

Objective 2: Identify the direct binding partners of SynCAM 1 that organize synapse formation across the synaptic cleft.

aims:
- affinity purification of synaptic membrane proteins on SynCAM 1 and SynCAM 2 extracellular domains
- affinity purification of brain extract on GST-SynCAM 1 cytosolic tail
Acknowledgements

Adam Fogel
Elissa Robbins
Bingjie Han
Lucas Cheadle
Ananda Ghosh
Massimiliano Stagi
Yuling Lei

Ken Williams
Angus Nairn

*iTRAQ Protein Quantification*
Chris Colangelo and Kathrin Wilczak-Havill

*DIGE Profiling*
Terence Wu and Kathy Stone

*Biophysics Resource*
Ewa Folta-Stogniew

---

**Funding Support:**

NIH/NIDA P30 DA018343 (NIDA Neuroproteomics Center),
NIH/NIDA R01 DA018928 (T.B.) and March of Dimes (T.B.)