Identification & Analysis of Protein Complexes Mediating Synapse Formation

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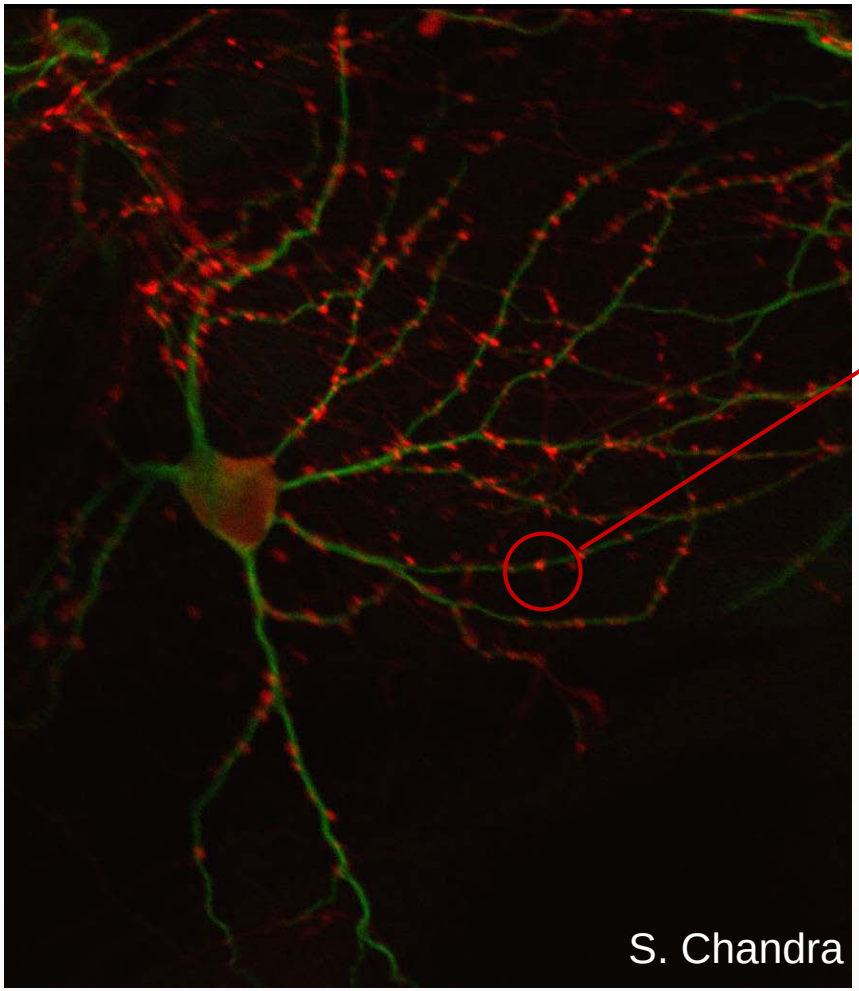
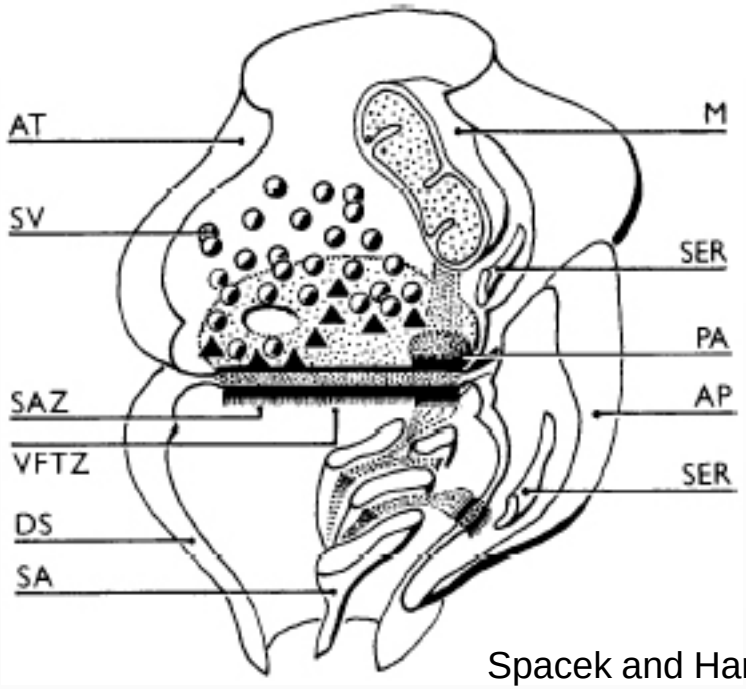
NIH/NIDA and March of Dimes Foundation

Synapses are specialized cell junctions





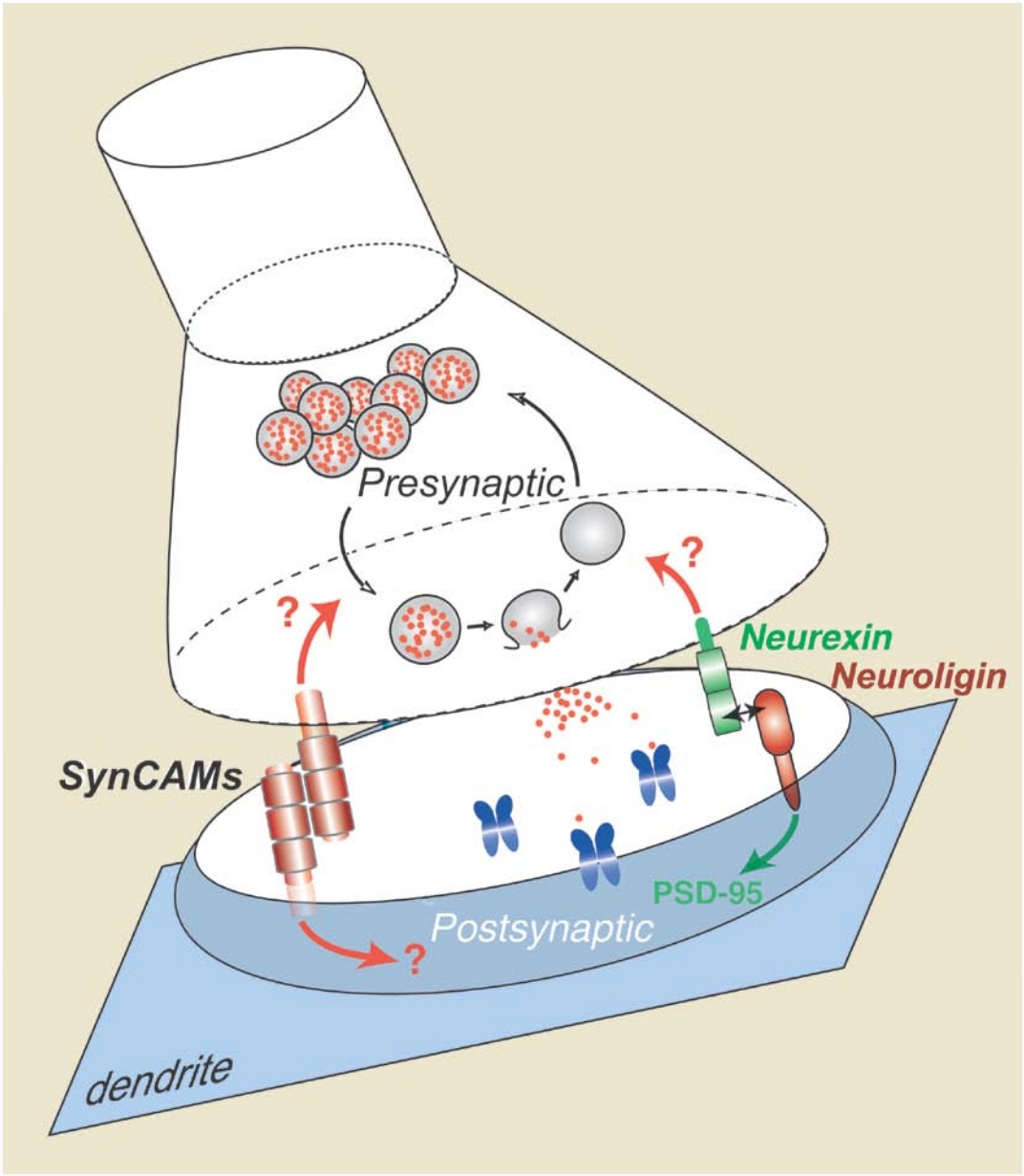
Spacek and Harris (1998)
J Comp Neurol. 393:58-68.

motivation to understand synapse organization:

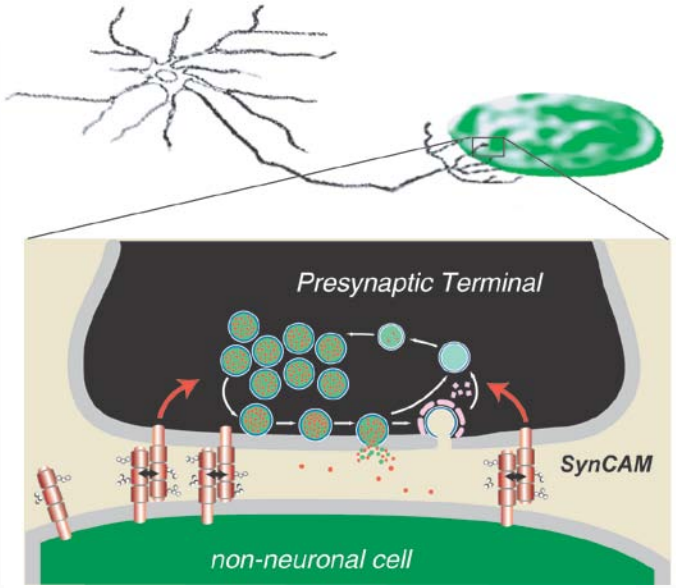
neurobiological motivation: role in brain development and plasticity
biomedical: neurodevelopmental and degenerative disorders, synaptic plasticity including learning and addiction

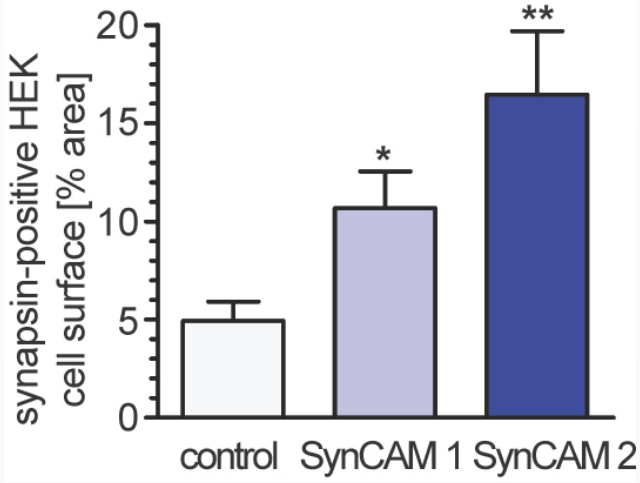
- biochemical: membrane assembly

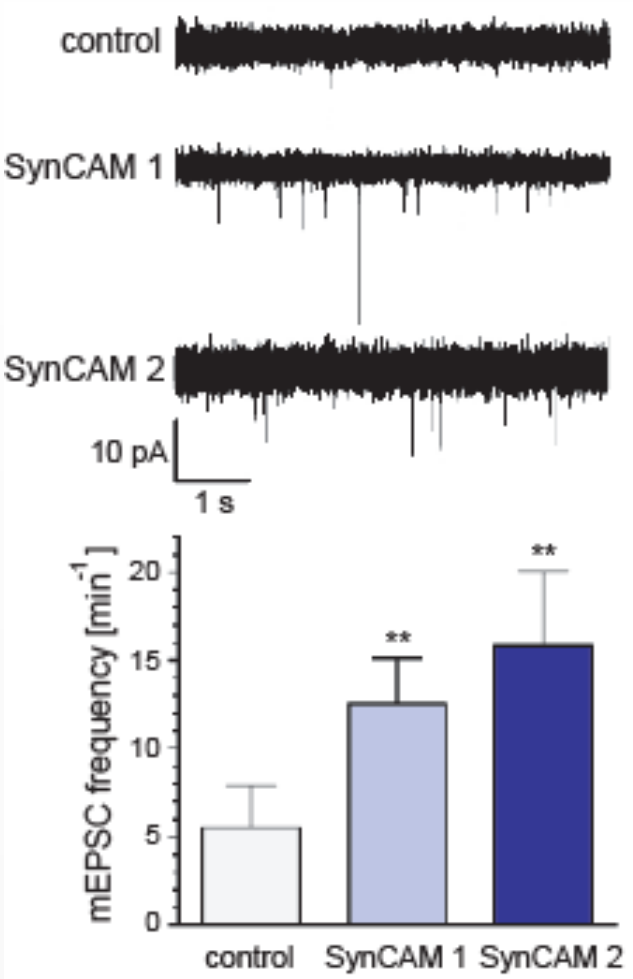
Trans-synaptic adhesion and synapse organization



Both SynCAM 1 and 2 are sufficient to induce presynaptic specializations

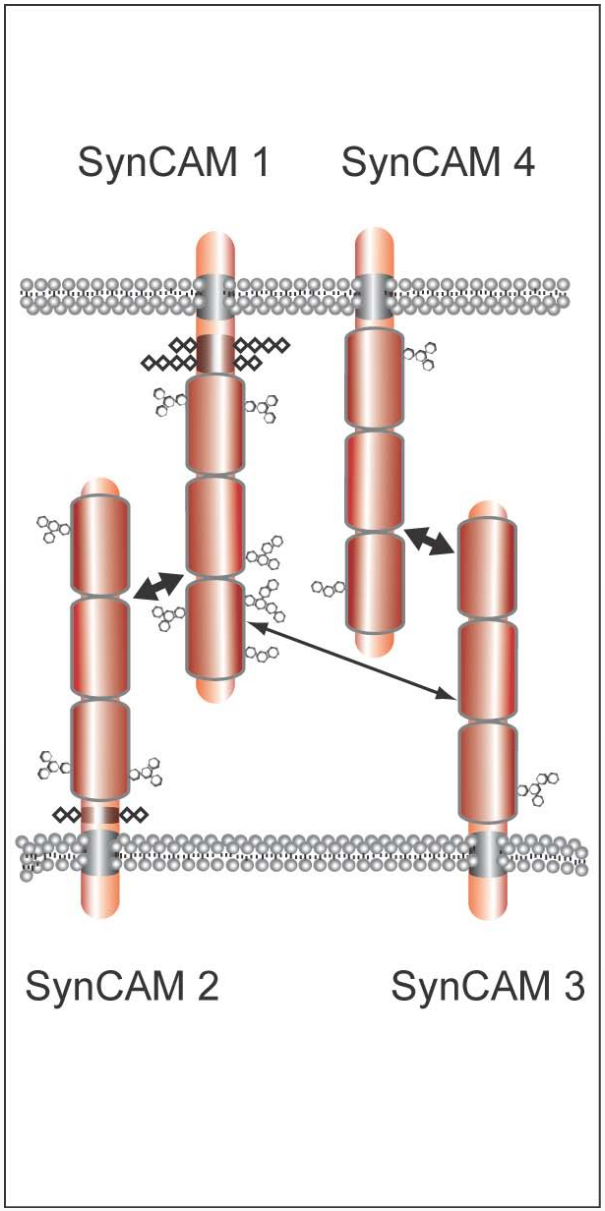






Adam Fogel and Alexander Krupp

SynCAM proteins engage each other in specific heterophilic interactions



Fogel et al., J. Neuroscience (2007) *27*:12516

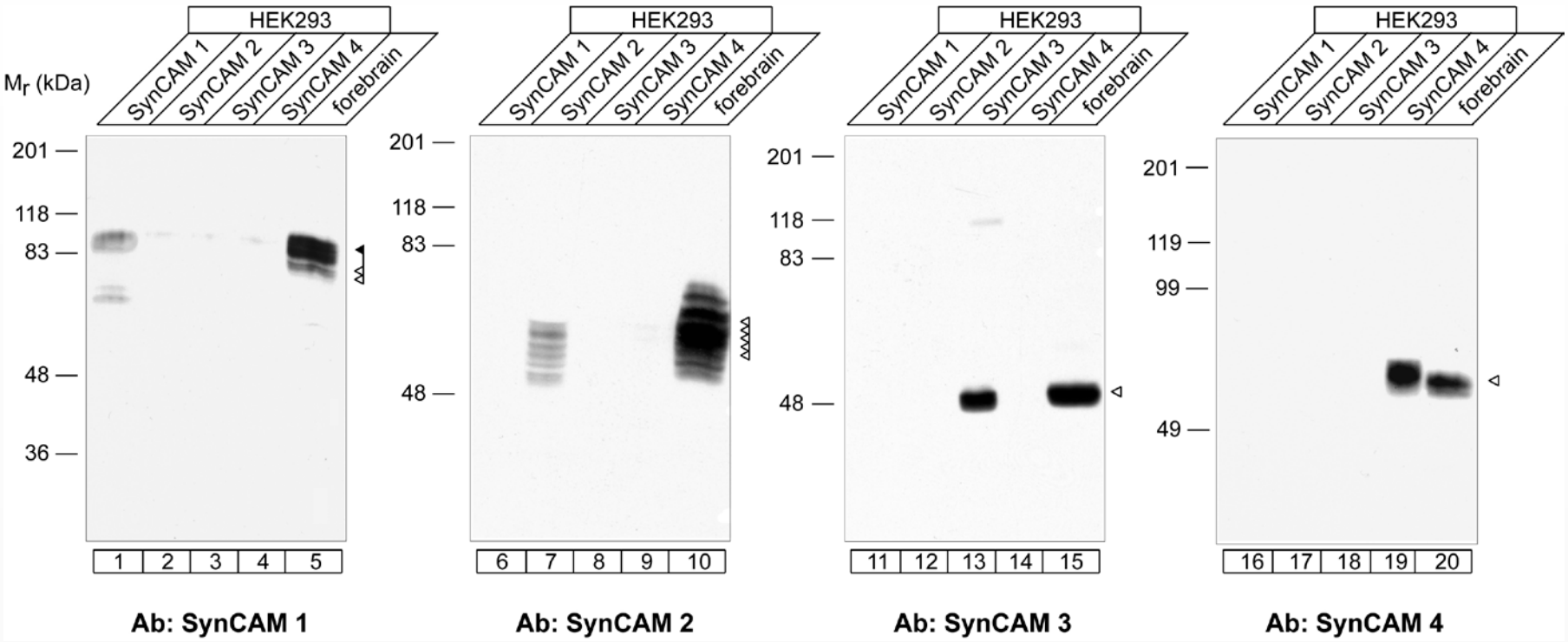
Aim 1: To analyze molecular SynCAM properties

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



Adam Fogel

Glycosylation analysis by light scattering

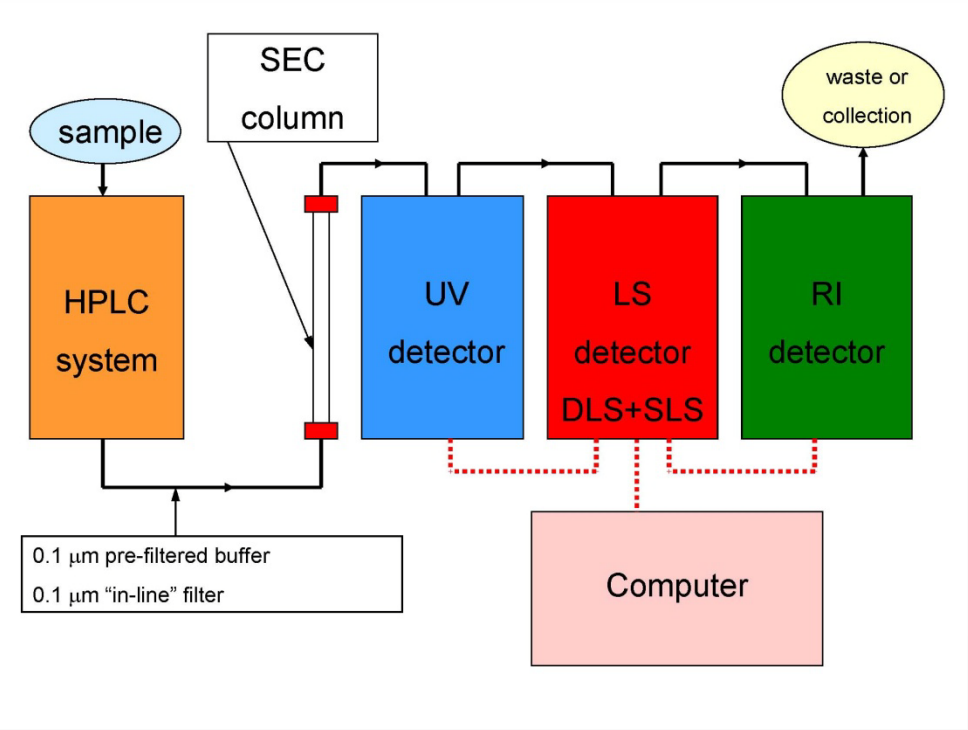
light scattering by a protein is a function of the specific refractive index, the concentration, and the molecular weight of the protein

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

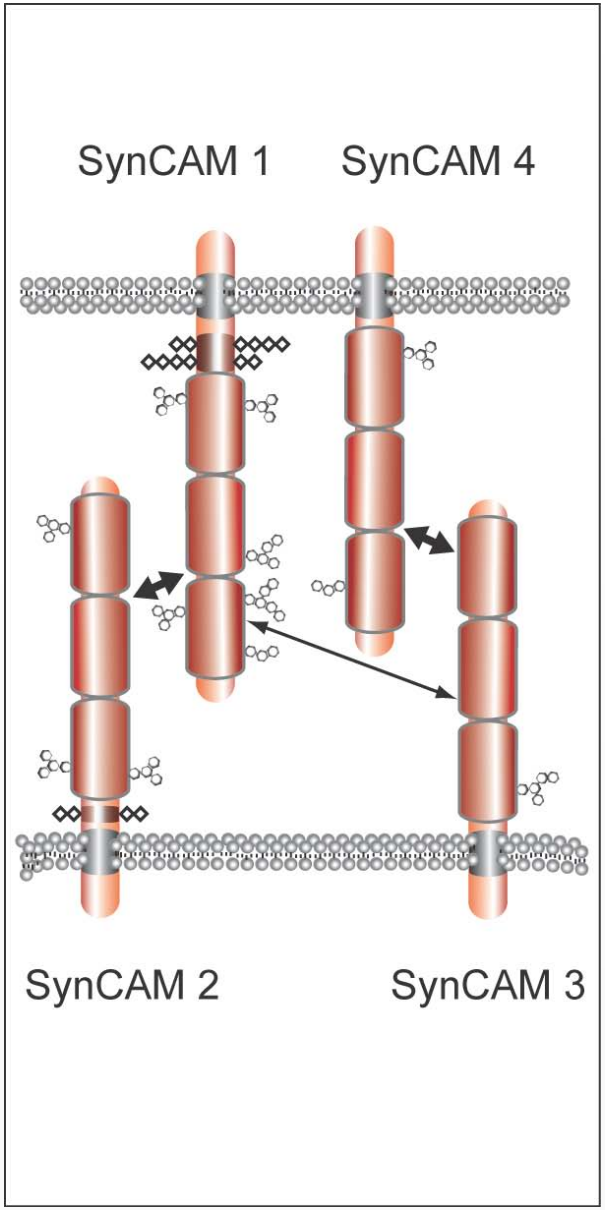
size exclusion chromatography / static light scattering:

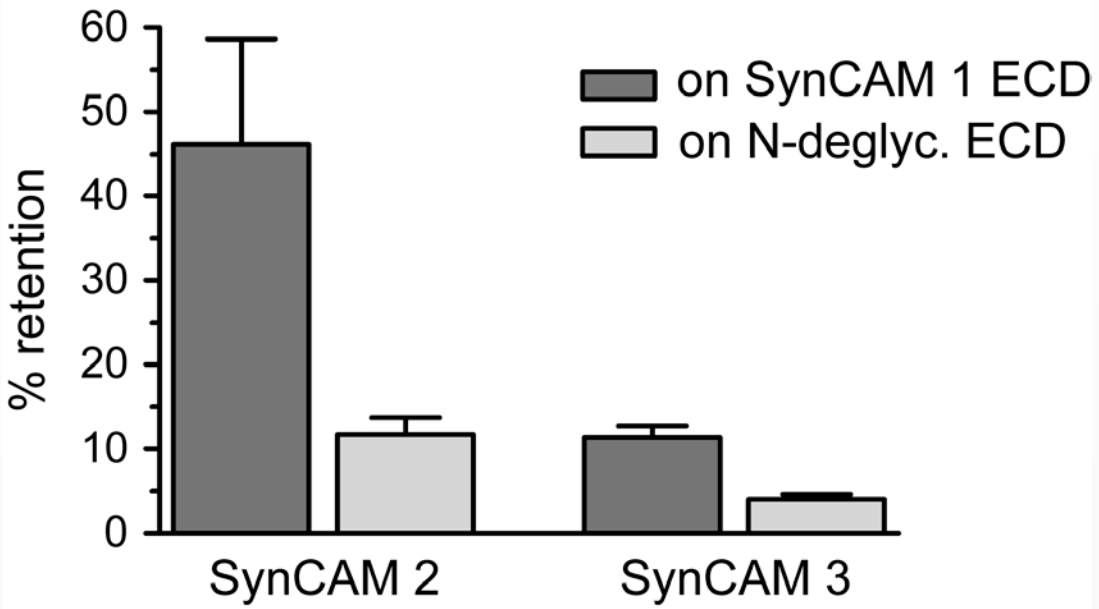
SynCAM 1 is heavily glycosylated

SynCAM 1	0.53 gram sugar/ gram of protein
SynCAM 2	0.15 gram sugar/ gram of protein
SynCAM 3	0.04 gram sugar/ gram of protein

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

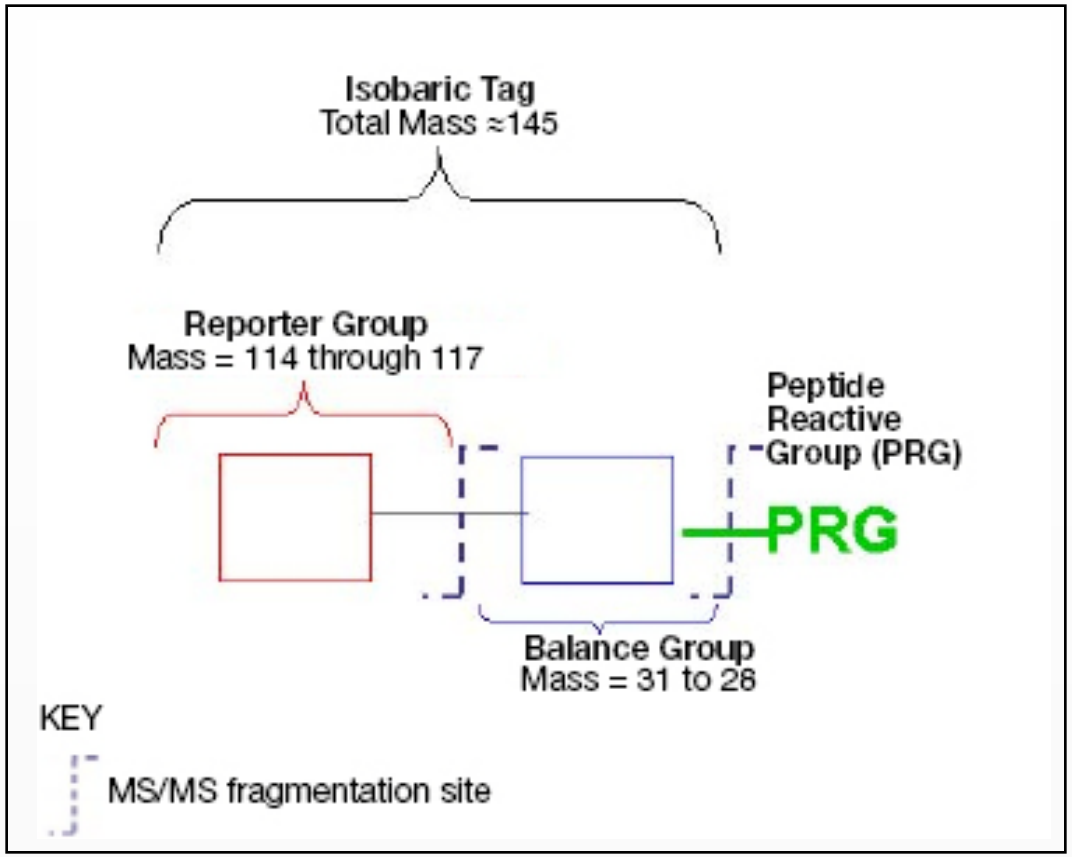
Analysis of synaptic composition by relative iTRAQ protein quantification

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

YPED

ITRAQ Results for Sample: iTRAQ SPM KO, SPM WT ProGroup IPI_mouse

Execution Date	Program Version	Database	Search Engine
08 Oct 2007 10:32	ProteinPilot 2.0	IPI_mouse	ProGroup

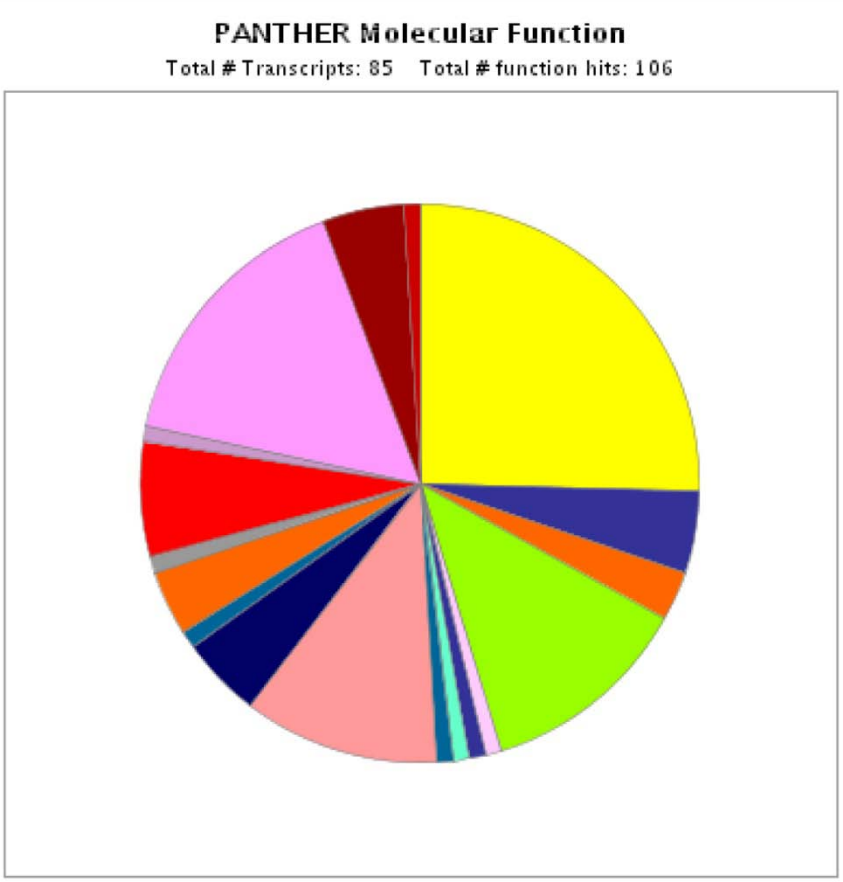
Summary Statistics Protein Score >=0.3	iTRAQ SPM KO, SPM WT ProGroup
#of proteins with 1 peptide identified	162
#of proteins with >= 99% confidence (protein score 2.0)	411
#of proteins with >= 95% confidence (protein score 1.3)	436
#of proteins with >= 90% confidence (protein score 1.0)	450
Range of 115/114 ratios	0.0361 - 24375.5605
Range of 117/114 ratios	0.2137 - 3.0512

Ratio	115/114	117/114
Observed Bias Correction	0.0494	1.564

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



Color	Panther Category	Transcripts(Trans)	% Trans to Total Trans
	Cell adhesion molecule (MF00040)	27	31.8%
	Cell junction protein (MF00276)	5	5.9%
	Chaperone (MF00077)	3	3.5%
	Cytoskeletal protein (MF00091)	13	15.3%
	Hydrolase (MF00141)	1	1.2%
	Ion channel (MF00024)	1	1.2%
	Isomerase (MF00166)	1	1.2%
	Membrane traffic protein (MF00267)	1	1.2%
	Miscellaneous function (MF00197)	12	14.1%
	Molecular function unclassified (MF00208)	5	5.9%
	Oxidoreductase (MF00123)	1	1.2%
	Phosphatase (MF00113)	4	4.7%
	Protease (MF00153)	1	1.2%
	Receptor (MF00001)	7	8.2%
	Select calcium binding protein (MF00188)	1	1.2%
	Select regulatory molecule (MF00093)	17	20.0%
	Transferase (MF00131)	5	5.9%
	Transporter (MF00082)	1	1.2%

THE NEXT SLIDES WERE REMOVED - DATA NOT FOR WEB POSTING

Future Aims

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

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