Mapping the Proteome of the Synaptic Cleft Through Proximity Labeling

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Molecular organization of the excitatory synaptic cleft

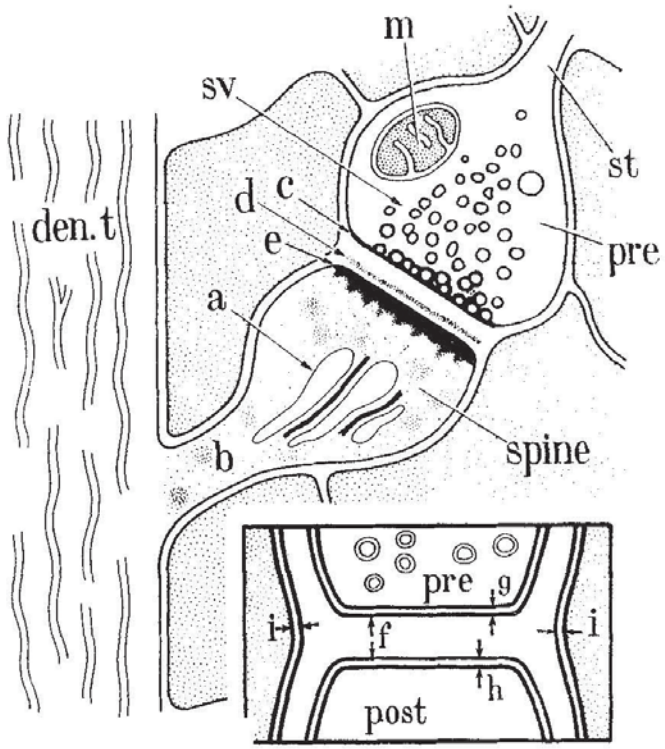
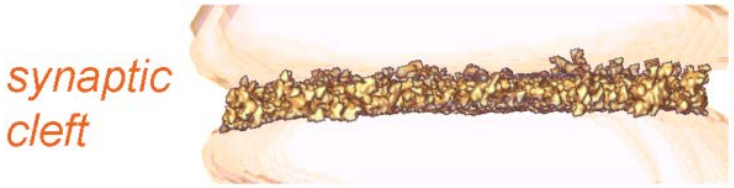
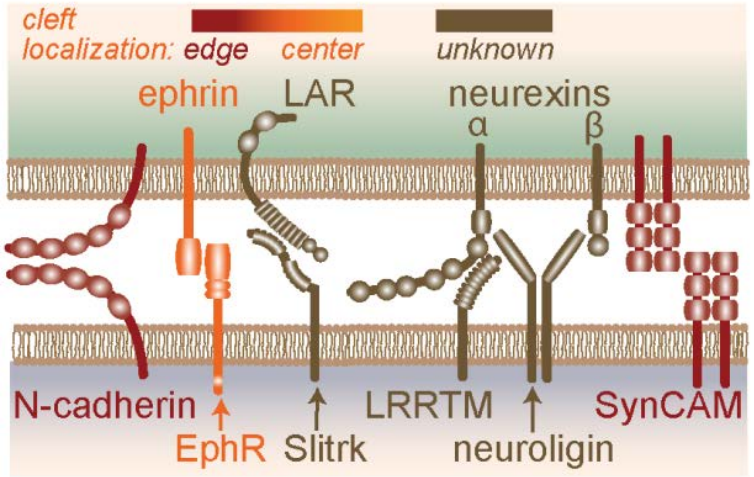


Fig. 1. Diagram of a synaptic contact on a dendritic spine, observed with the electron microscope after osmium tetroxide fixation. The stippled regions represent neuronal and glial processes of the neuropil

Inset. The opposed regions of the pre- and post-synaptic membranes seen after potassium permanganate fixation. The membranes (*i*) are of neighbouring processes of the neuropil

Gray (1959) *Nature* 183:1592-1593

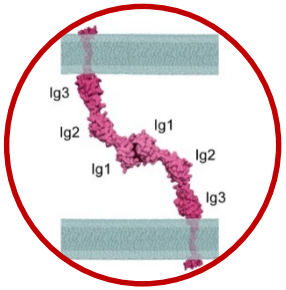


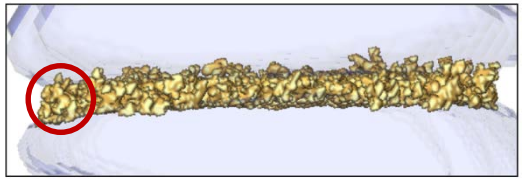


Biederer, Kaeser and Blanpied (2017) *Neuron* 96:680-696

Our approaches to investigate synapse development

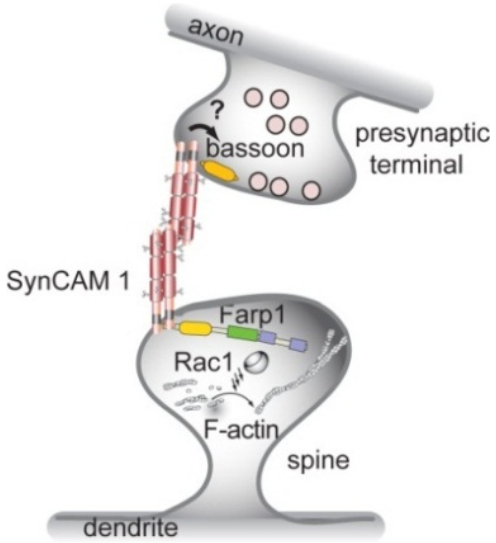
I. Molecular properties of synaptic adhesion





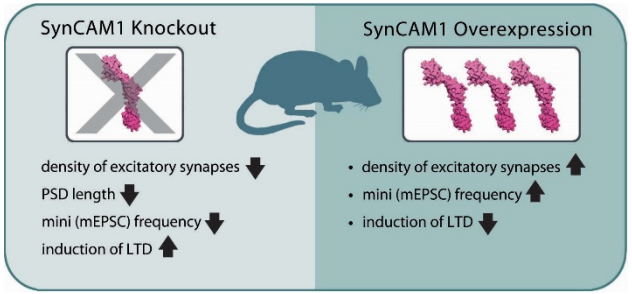
Biederer, *Genomics* 2006
Fogel et al. *J Neurosci* 2007
Thomas et al. *J Comp Neurol* 2008
Fogel et al. *J Biol Chem* 2010
Fogel et al. *EMBO J* 2011
Perez de Arce et al. *Neuron* 2015
Cijsouw et al. *Proteomes* 2018

II. Synaptogenic signaling

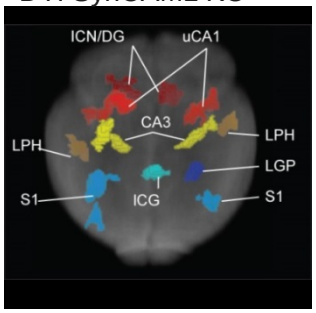


Stagi et al. *PNAS* 2010
Cheadle & Biederer *J Cell Biol* 2012
Cheadle & Biederer *J Neurosci* 2014

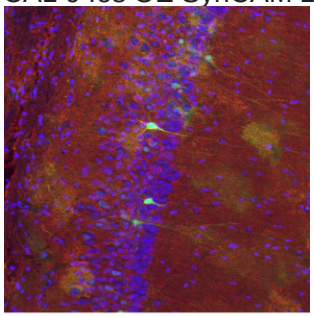
III. Wiring neurons into circuits



DTI SynCAM1 KO



CA1 c-fos OE SynCAM 1^{flag}

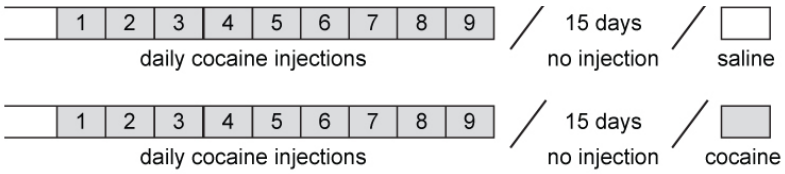


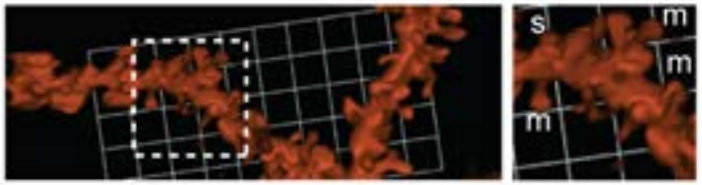
flag / SynCAM 1 / NeuN

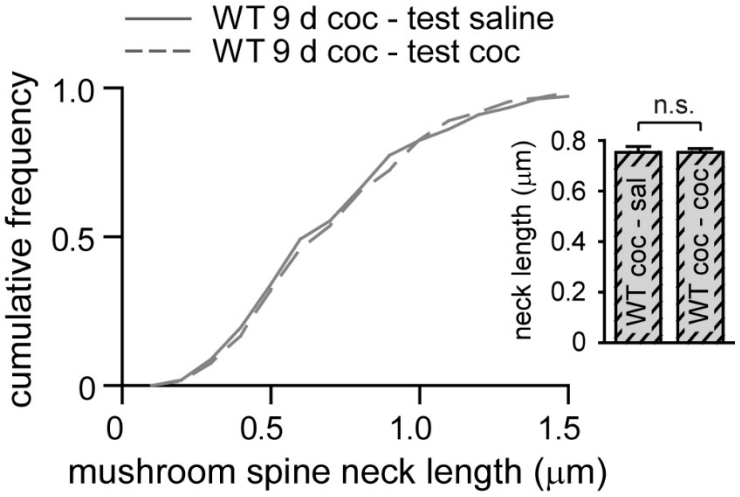
Robbins et al. *Neuron* 2010
Giza et al. *Neuropsychopharm* 2013
Ribic et al. *J Comp Neurol* 2014
Park et al. *J Neurosci* 2016
Ribic, Crair and Biederer *Cell Reports* 2019

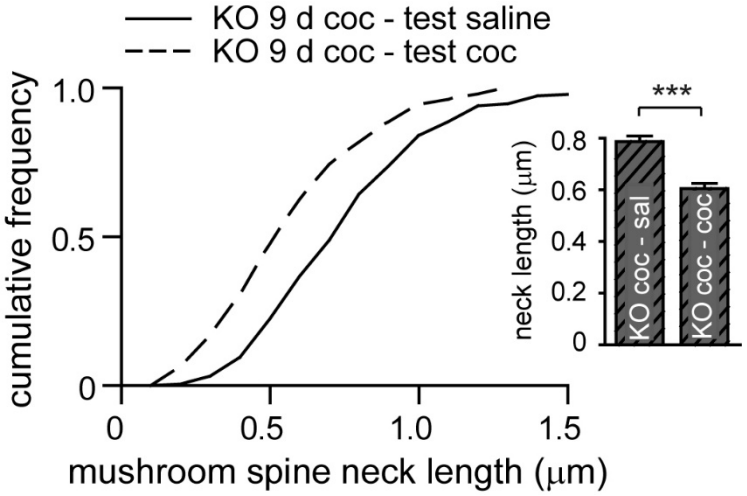
Trans-synaptic interactions impact synapse structure effects of psychostimulants

Loss of SynCAM 1 sensitizes in NAc medium spiny neurons
the mushroom spines to cocaine-induced shortening:









Giza, Picciotto, Biederer and colleagues (2013) *Neuropsychopharm* 38: 628-638

Unraveling the complexity and heterogeneity of synaptic composition

Resource

TRANSPARENT PROCESS

THE EMBO JOURNAL

Proteomic screening of glutamatergic mouse brain synaptosomes isolated by fluorescence activated sorting

Christoph Biesemann[1], Mads Grønborg[2,10,†], Elisa Luquet[3,4], Sven P Wichert[5,‡], Véronique Bernard[6,7,8], Simon R Bungers[1], Ben Cooper[1], Frédérique Varoqueaux[1], Liyi Li[1], Jennifer A Byrne[9], Henning Urlaub[10,11], Olaf Jahn[12], Nils Brose[1,*] & Etienne Herzog[1,3,4,6,7,8,**]

nature neuroscience

Altmetric: 20 More detail »

Resource

A multiregional proteomic survey of the postnatal human brain

Becky C. Carlyle, Robert R. Kitchen, Jean E. Kanyo, Edward Z. Voss, Mihovil Pletikos, André M. M. Sousa, TuKiet T. Lam, Mark B. Gerstein, Nenad Sestan & Angus C. Nairn

OPEN ACCESS Freely available online

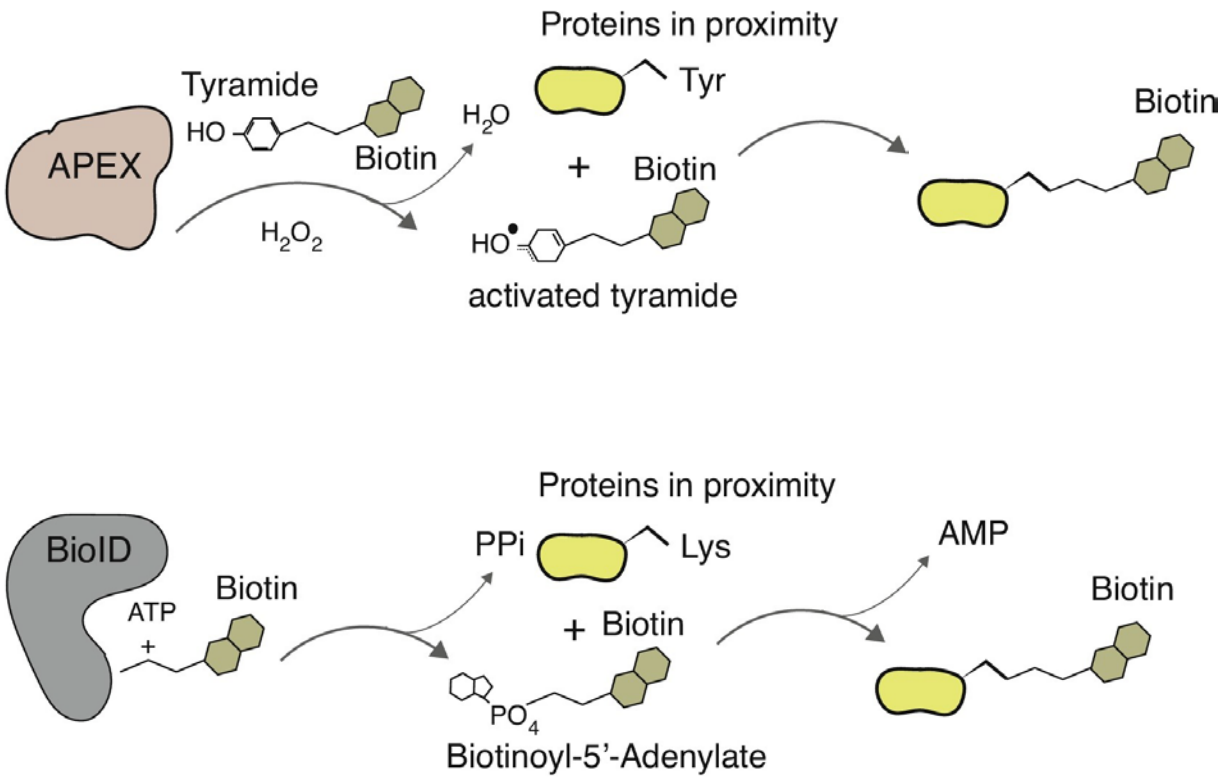
PLoS one

The Biochemical Anatomy of Cortical Inhibitory Synapses

Elizabeth A. Heller[1], Wenzhu Zhang[2], Fekrije Selimi[3], John C. Earnheart[1], Marta A. Ślimak[4], Julio Santos-Torres[4], Ines Ibañez-Tallon[4], Chiye Aoki[5], Brian T. Chait[2], Nathaniel Heintz[1]*

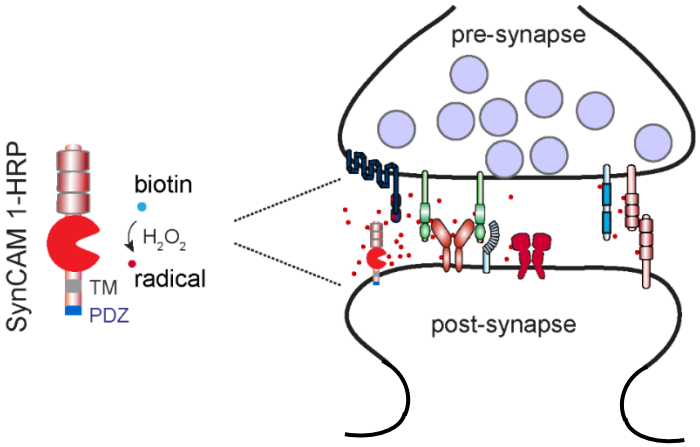
1 Howard Hughes Medical Institute, Laboratory of Molecular Biology, The Rockefeller University, New York, New York, United States of America, **2** Laboratory for Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, New York, United States of America, **3** CIRB, Collège de France, Paris, France, **4** Molecular Neurobiology Group, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany, **5** Center for Neural Science, New York University, New York, New York, United States of America

Proximity labeling: a method to tag proximal proteins with biotin



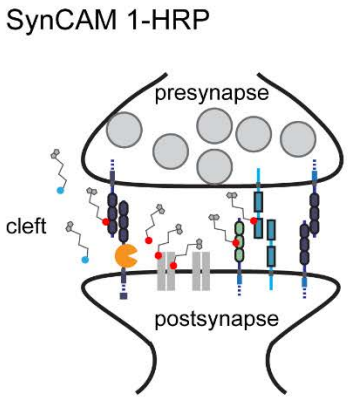
Adapted from Schreiner et al. (2017) *Current Opinion in Neurobiology* 42:102-110

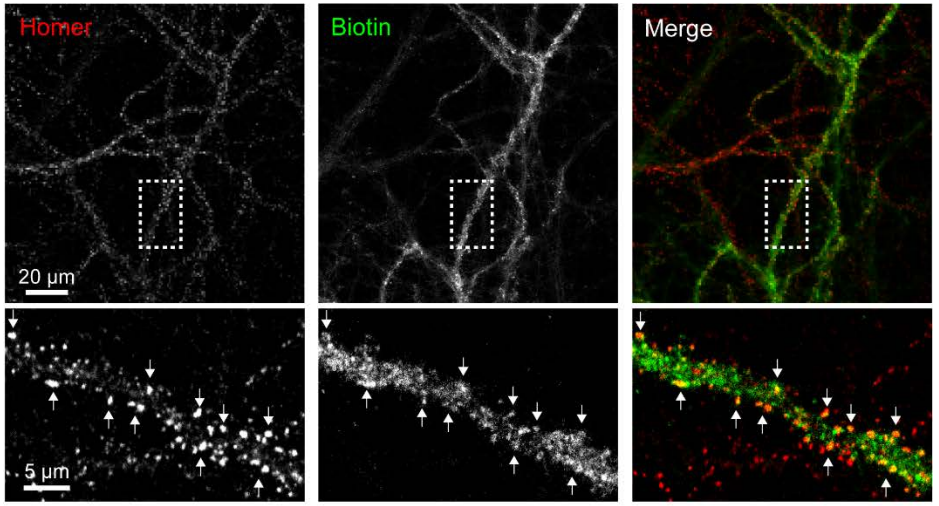
A SynCAM 1-HRP reporter to target the excitatory synaptic cleft



Based on proximity labelling approach developed by Alice Ting and colleagues.

Synaptic protein labeling by the SynCAM1-HRP reporter *vs* dendritic control





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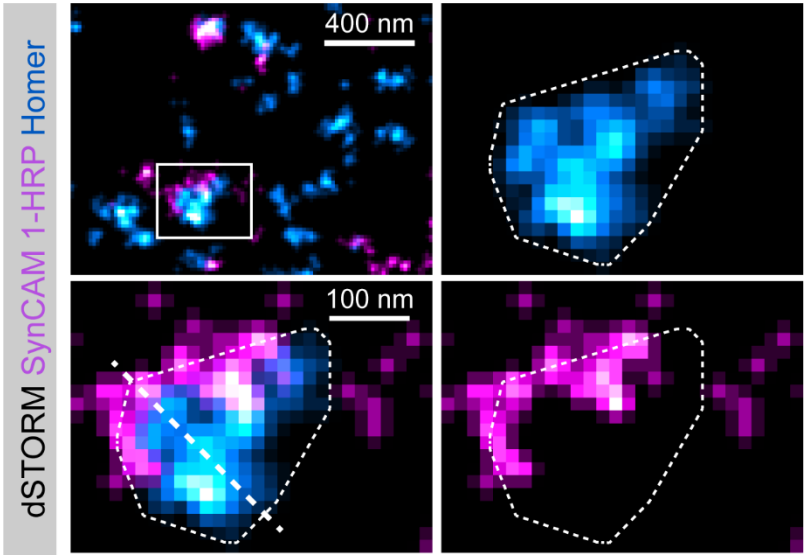
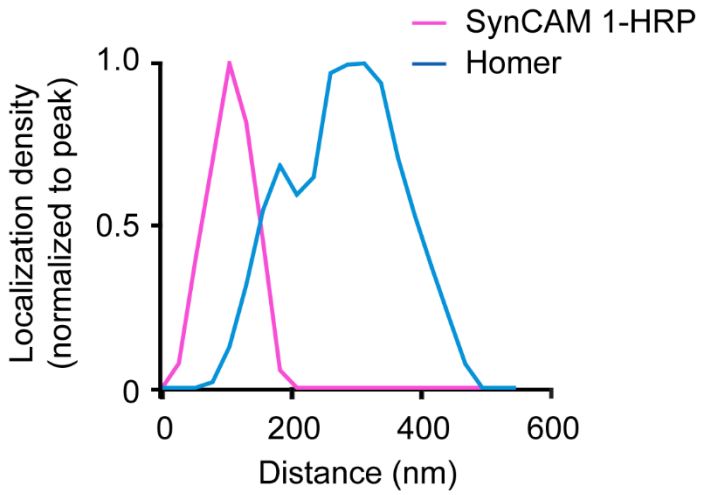
Sub-synaptic localization of the SynCAM 1-HRP reporter at the synaptic edge

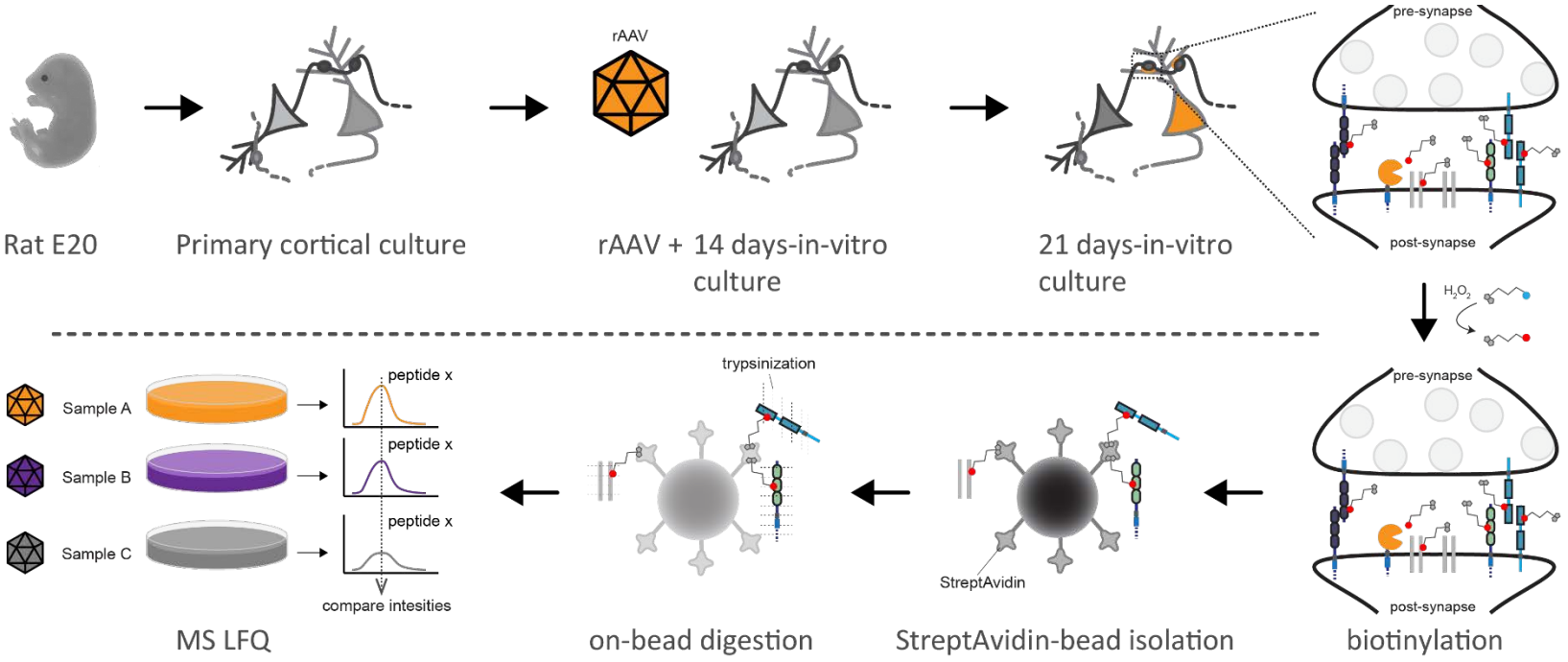


3D dSTORM imaging



Austin Ramsey and Tom Blanpied

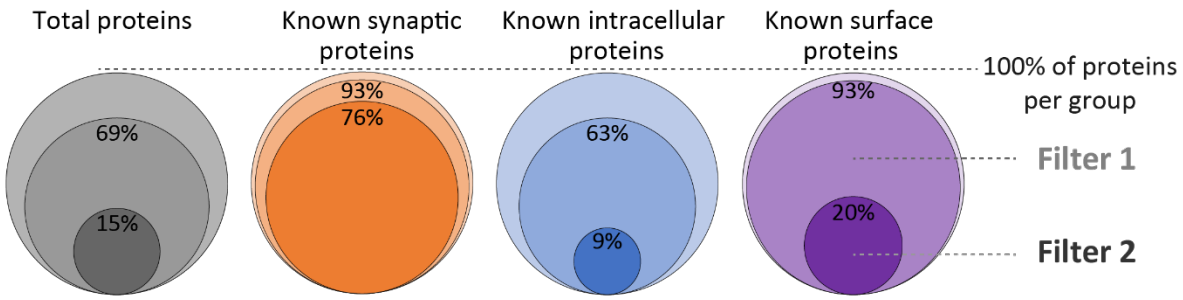
Experimental design and workflow



Tony Cijsouw with TuKiet Lam

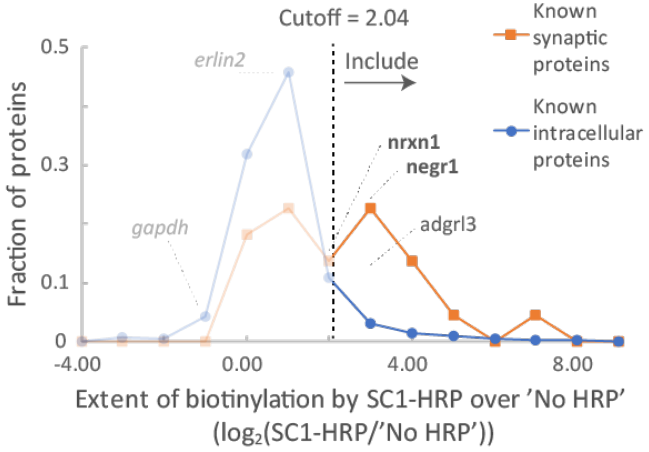
Data analysis steps for selection of putative cleft proteins

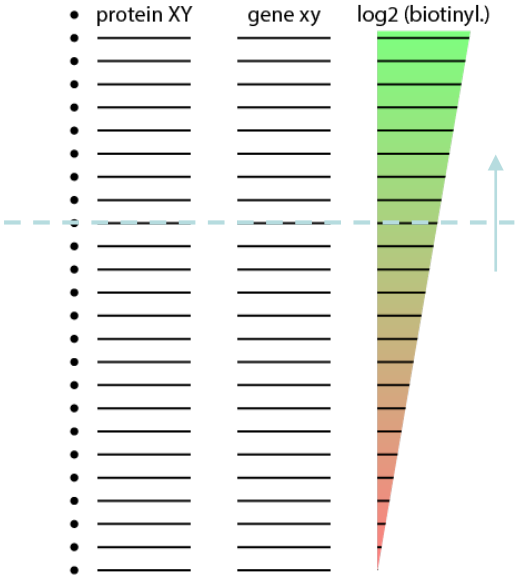
	All biological replicates
# detected proteins	894 proteins
Filter 1 ↓	
# biotinylated proteins	614 proteins
Filter 2 ↓	
# cleft-enriched proteins	137 proteins
↓	
111 proteins	



Tony Cijsouw

A ‘true positive – false positive’ filter for synaptic cleft proteins





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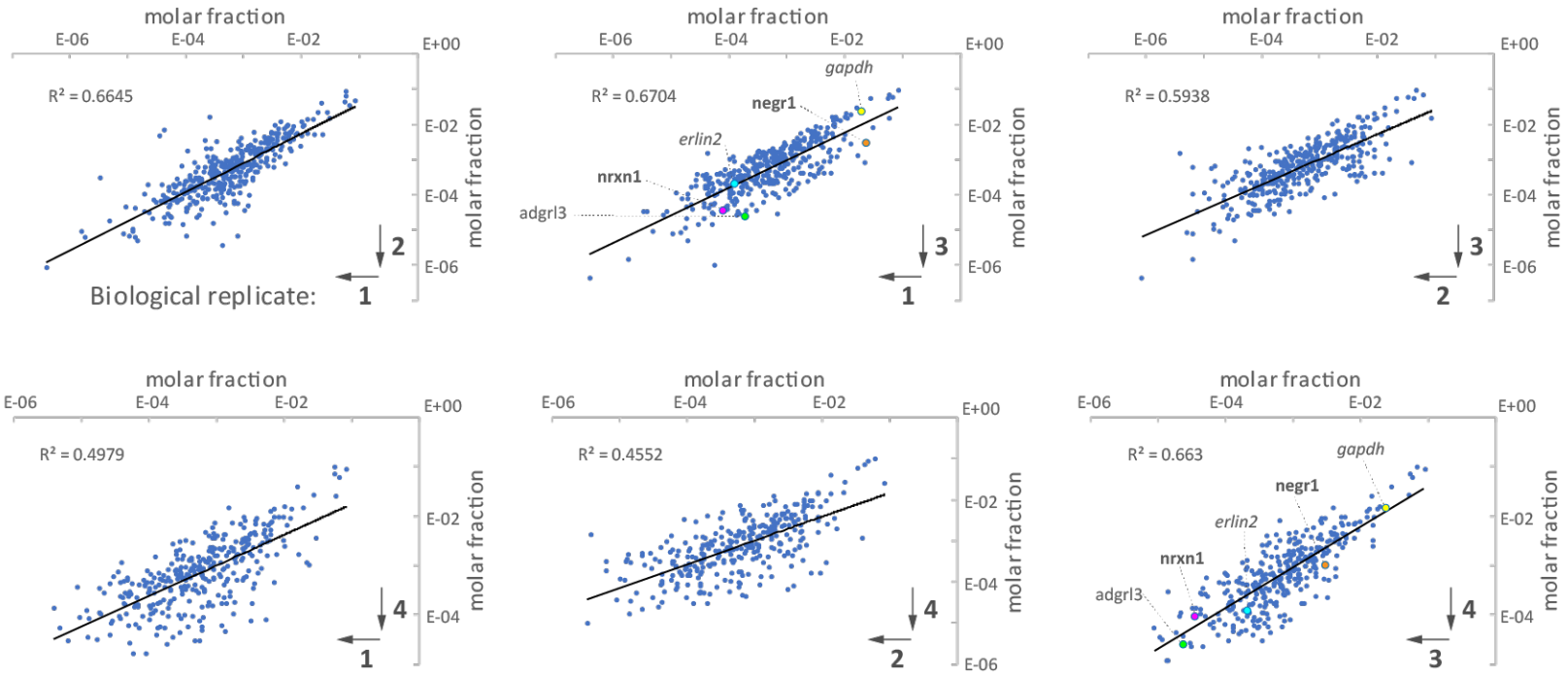
Identified proteins correlate well across biological replicates



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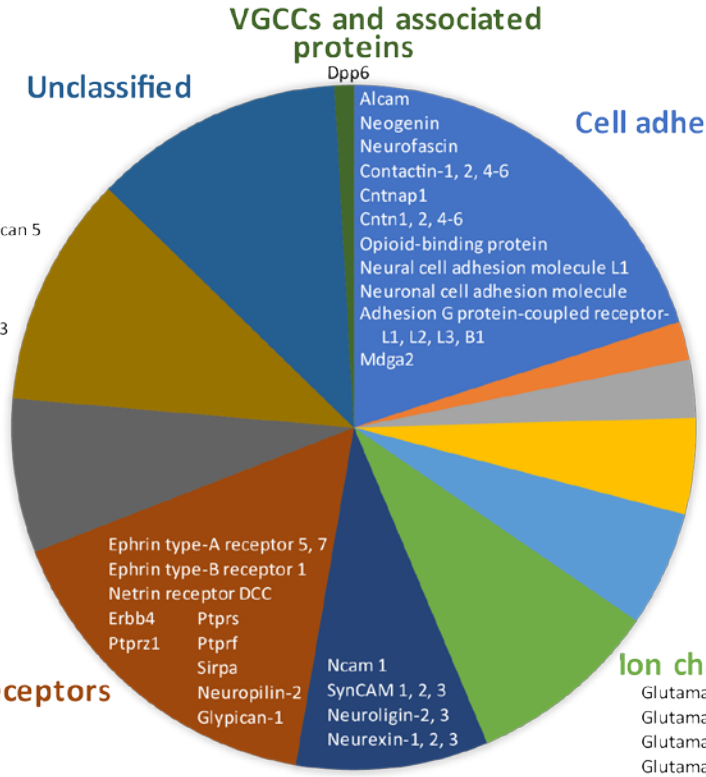
Synaptic cleft candidates are found across protein classes



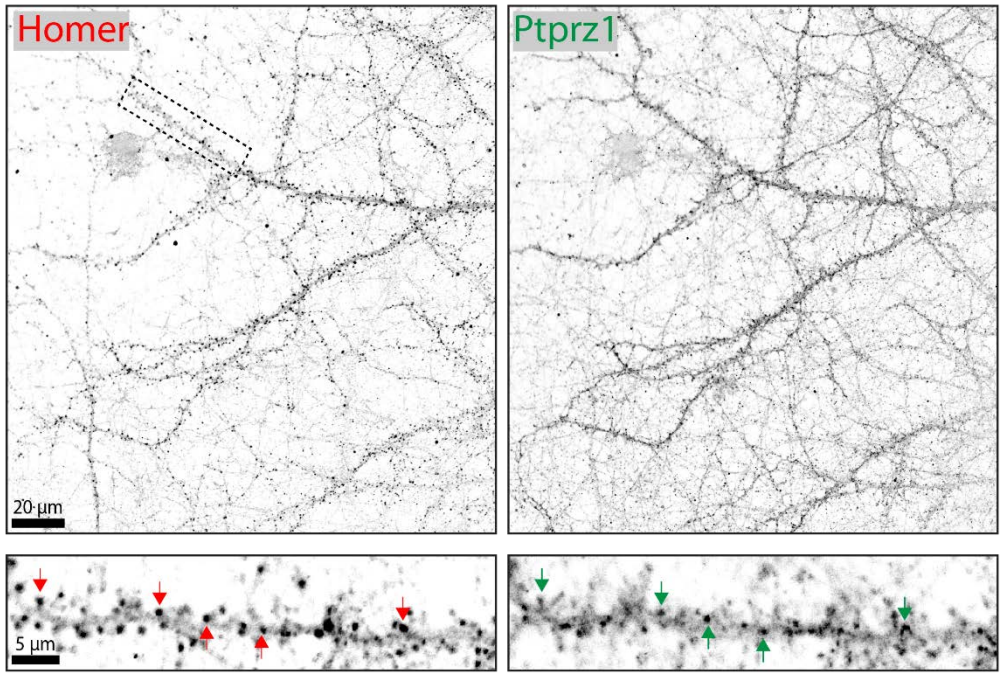
Cijsouw et al. (2018) *Proteomes* 6, 48

Tony Cijsouw

Validation: The tyrosine phosphatase Ptprz1 colocalizes with excitatory synapses

Protein-Tyrosine Phosphatase, Receptor-type, Zeta-1:

Ptprz1 expression is regulated by morphine in rodents (Garcia-Perez et al. (2017) *Mol Neurobiol* 54:495-510)

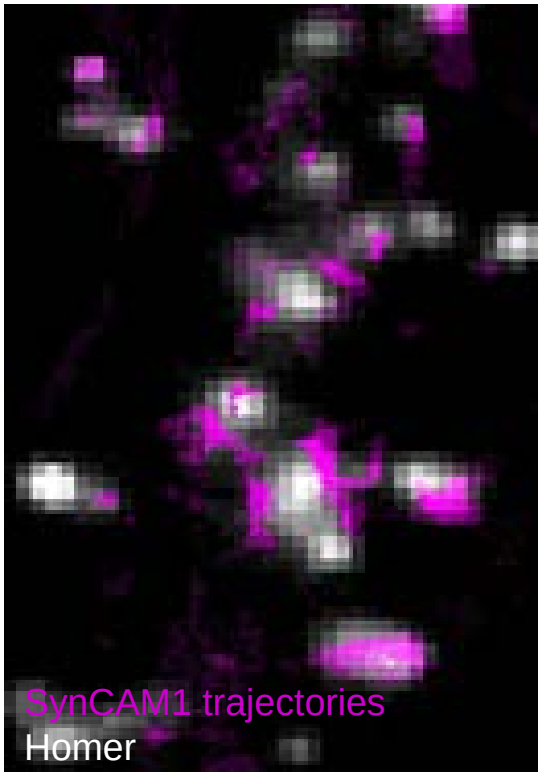


Detected using immunocytochemistry

Cijsouw et al. (2018) *Proteomes* 6, 48

The next step: Defining the dynamic activity-dependent remodeling of the synaptic cleft

Single particle tracking of super-resolved SynCAM 1 using the uPAINT approach (Daniel Choquet):



SynCAM 1-pHluorin with single molecule nanobody labeling
20 ms per frame

Sabbatical with Olivier Thoumine (Bordeaux)

Acknowledgements

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Katie Watters

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Andrew Paquette

Cami Gil Ramirez

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Tom Blanpied

Yale/NIDA Neuroproteomics Center

TuKiet Lam

National Institutes of Health

NIH/NIDA R01 DA018928

NIH/NIMH R01 MH119826

NIH/NINDS R21 NS10963

NIH/NIDA P30 DA018343

Initiative d'Excellence Université de Bordeaux

Mead Johnson Nutrition

Reckitt Benckiser Group