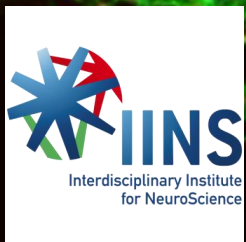


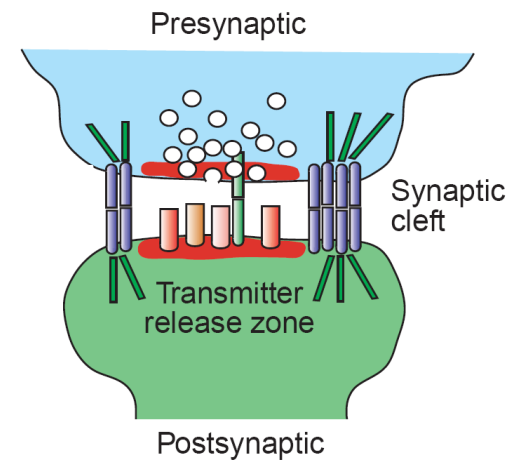
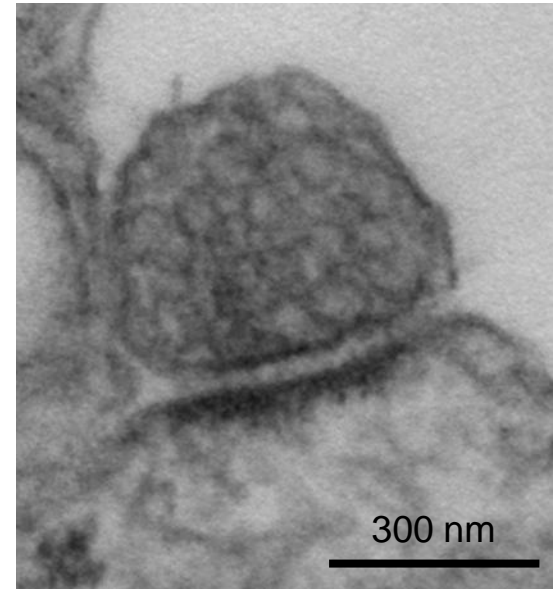
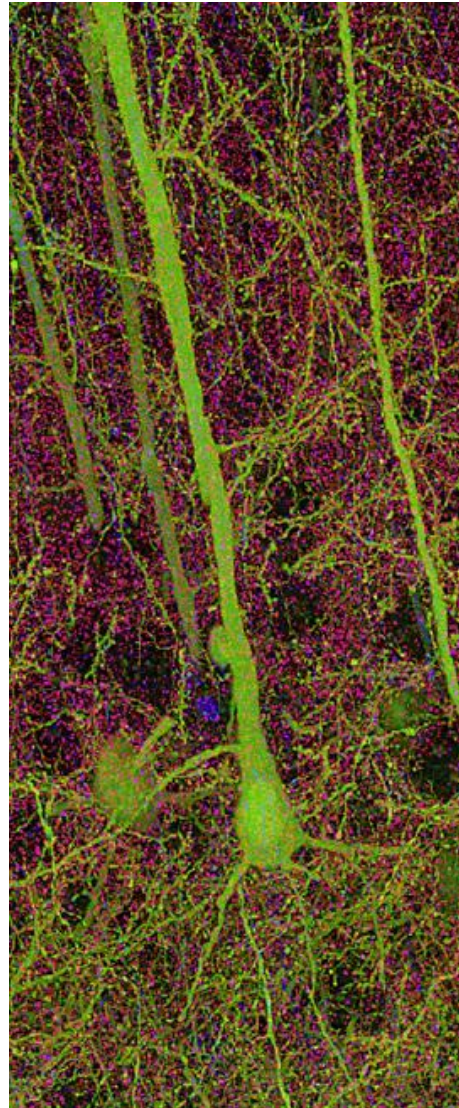
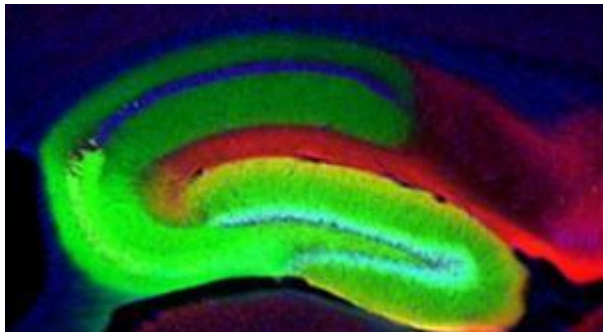
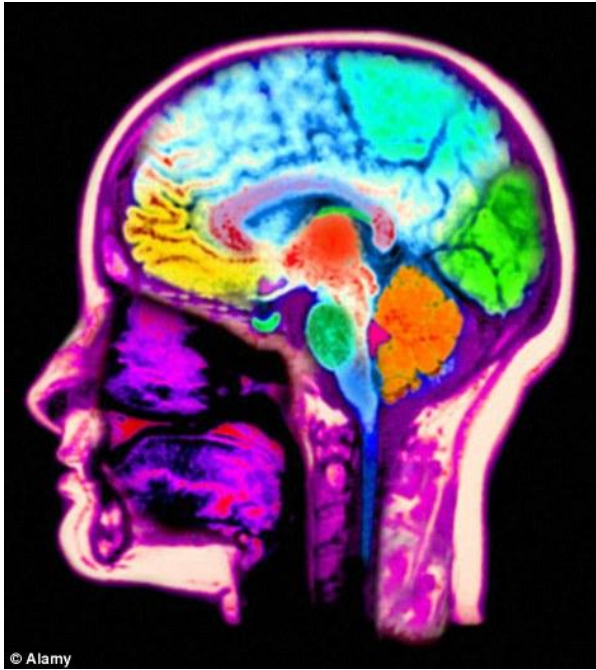
Assembly of synapses by neuronal adhesion molecules: single molecule studies

Olivier Thoumine

Interdisciplinary Institute of Neurosciences
CNRS - University of Bordeaux

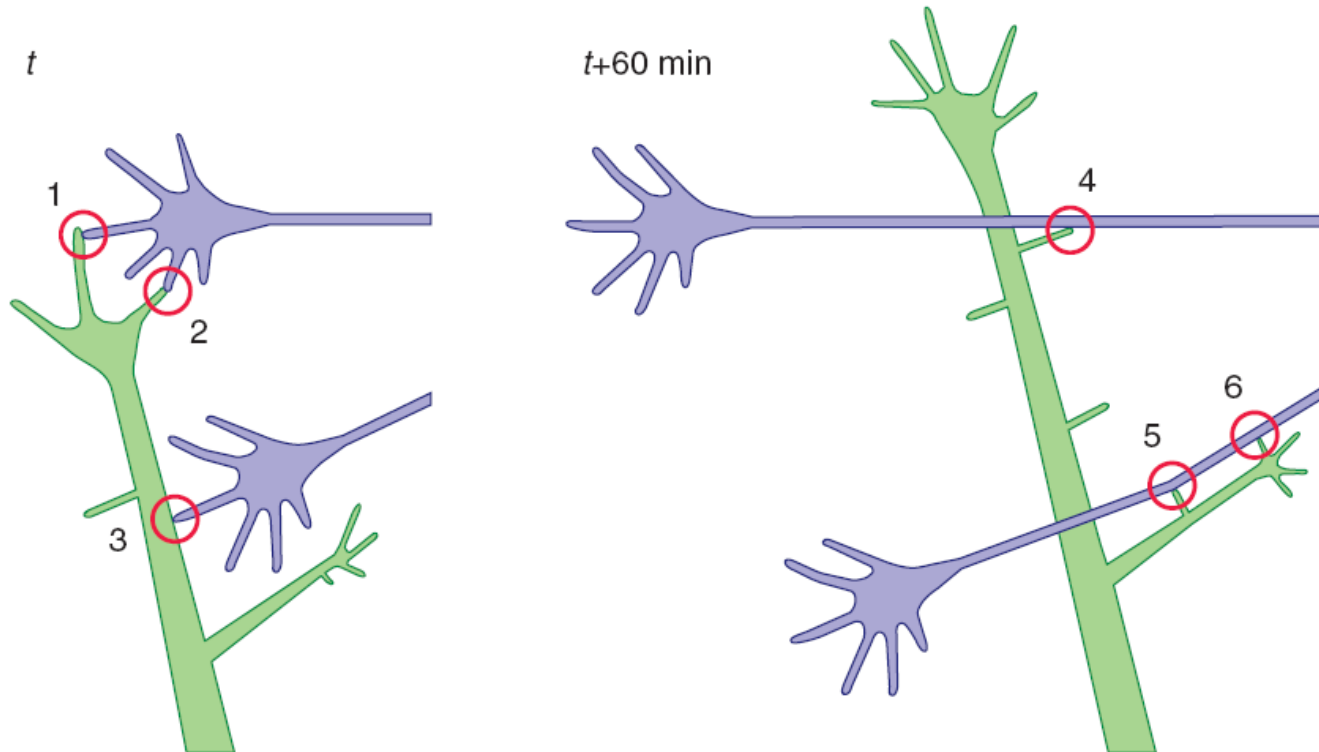


Connectivity in the brain



Micheva et al. *Neuron* 2010

Molecular determinants of synapse formation

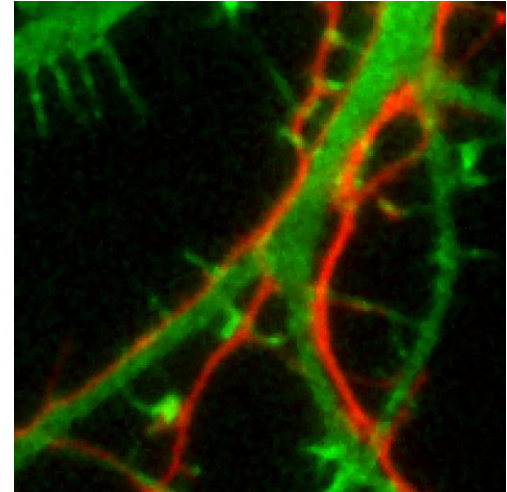


Ziv and Garner *Current Opinion in Neurobiology* 2001

Experimental approach

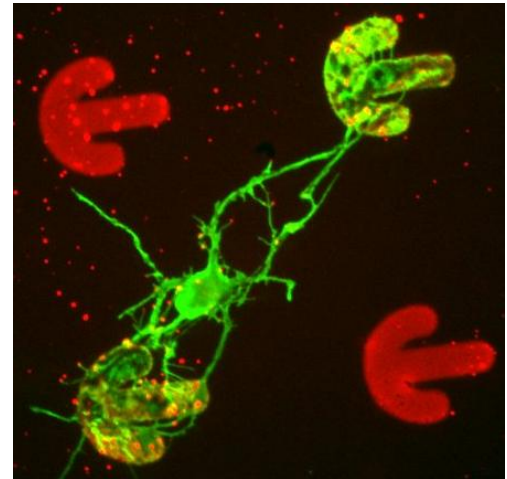
Spontaneous contacts

- Low spatial and temporal resolution
- Coexistence of multiple molecules
- Unknown stoichiometry



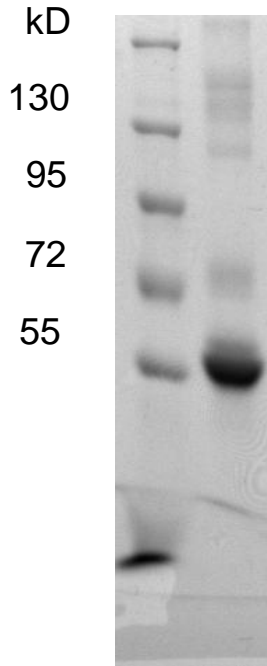
Biomimetic contacts

- Use purified proteins
- Control surface density
- Control position and time of contact



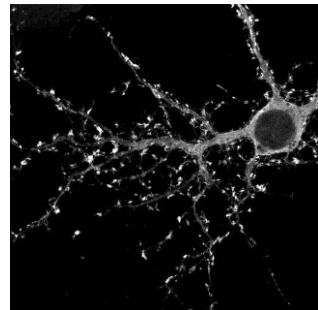
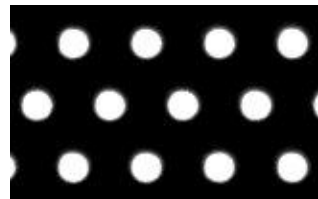
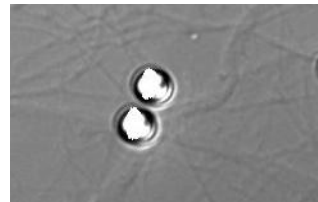
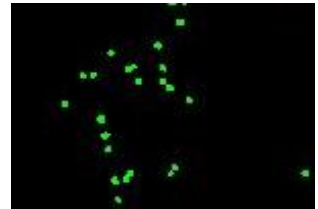
Biomimetic assays

Purified
Neurexin1 β -Fc



- Quantum dots (20 nm)
- Microspheres (1 μ m)
- Micro-patterns (2 μ m)

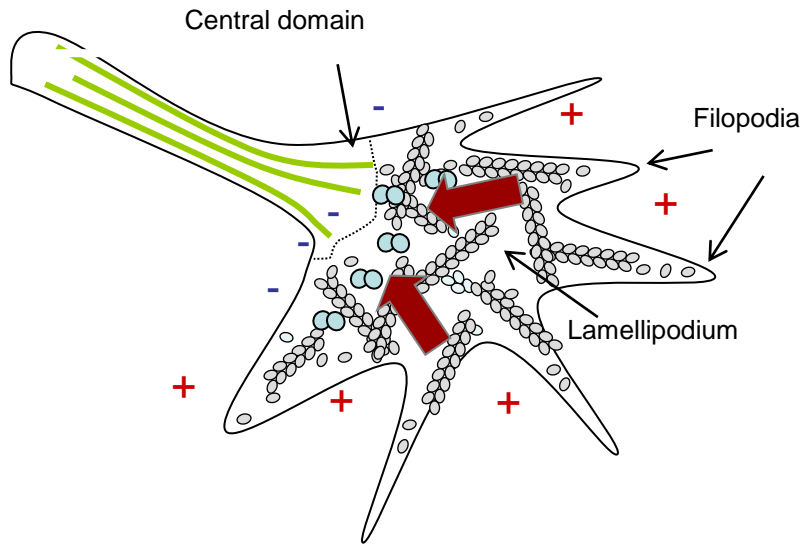
Primary hippocampal neurons







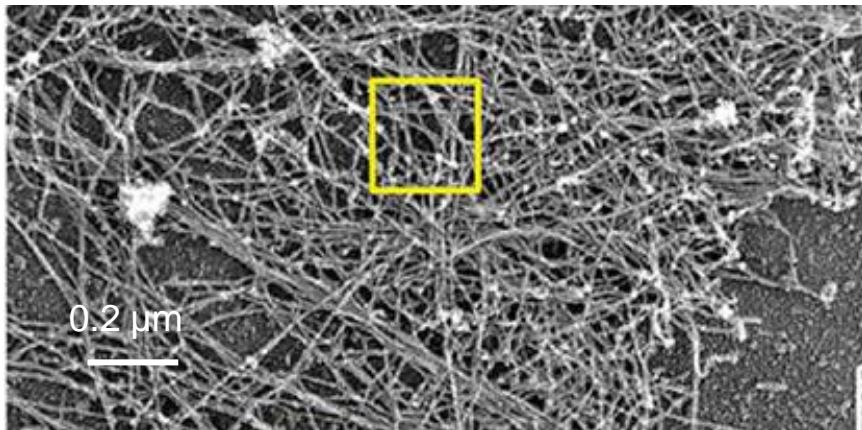
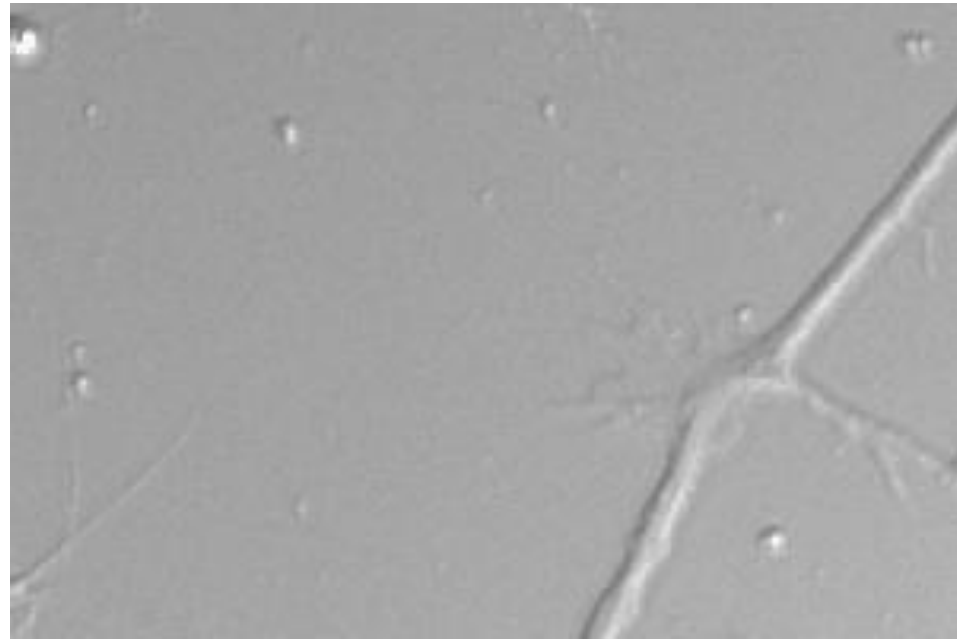
Outline

1. Growth cone migration
2. Post-synaptic morphing
3. Synapse formation

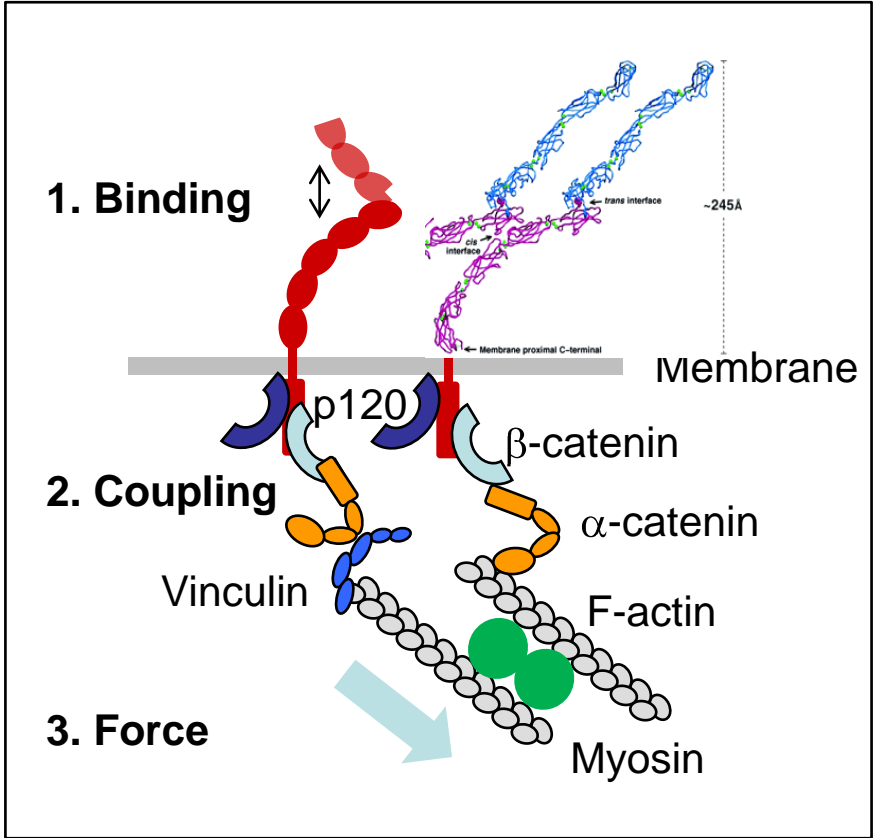
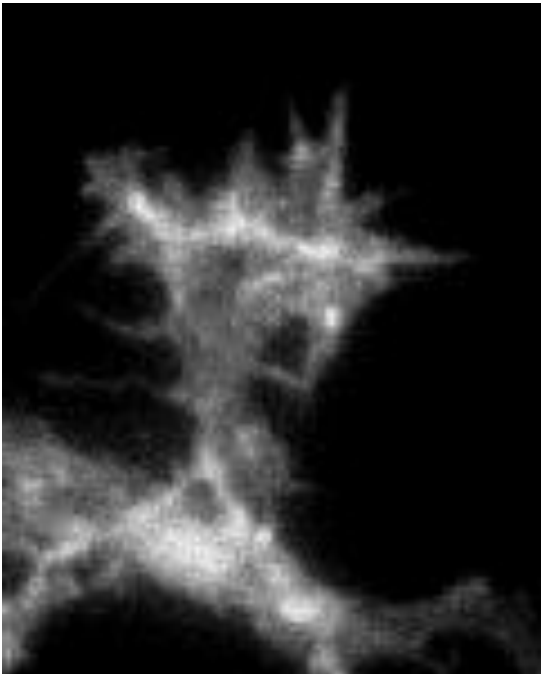
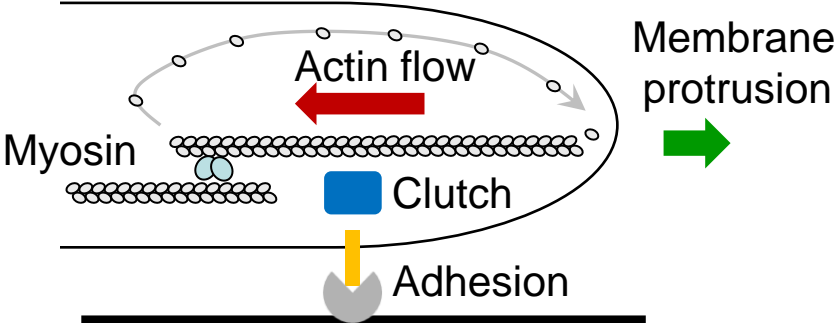
Axis 1: Growth cone motility



 F-actin  Microtubules  Myosin II  Actin flow



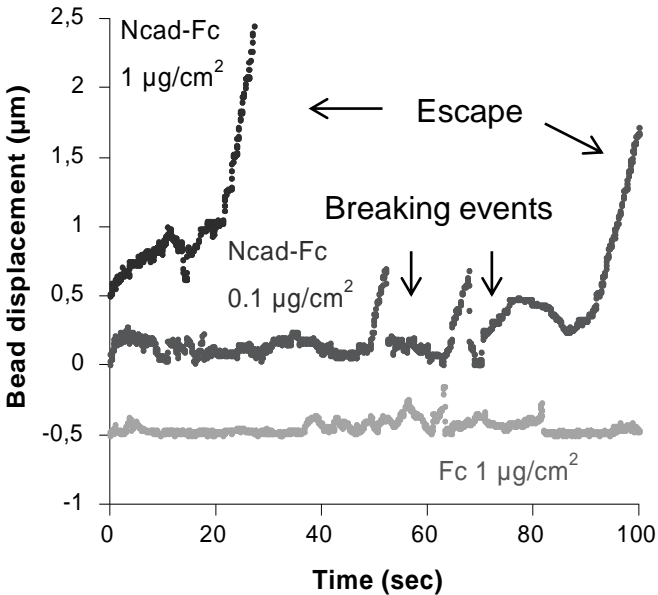
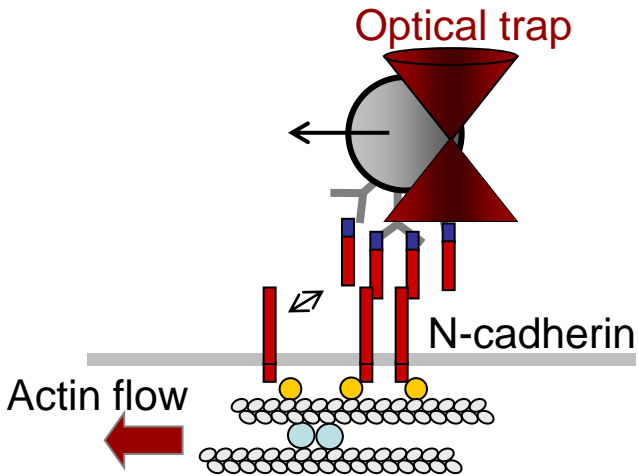
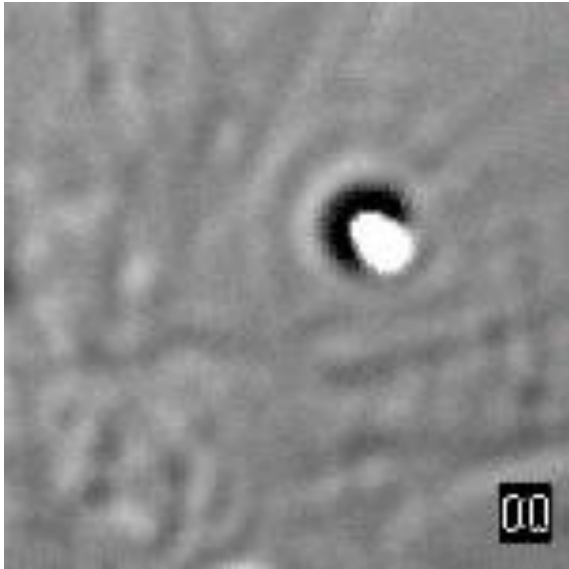
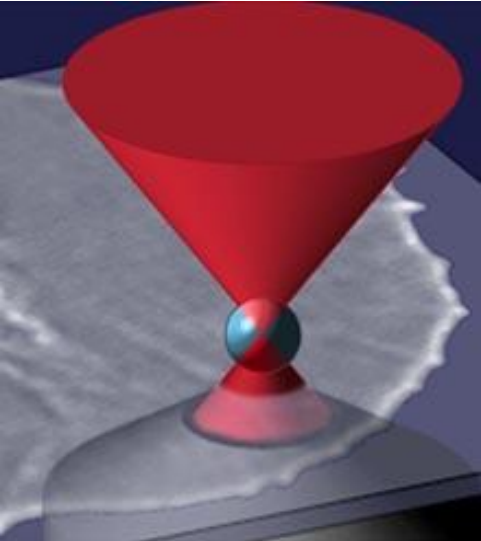
The molecular clutch mechanism: focus on N-cadherin



Boggon et al.
Science 2002

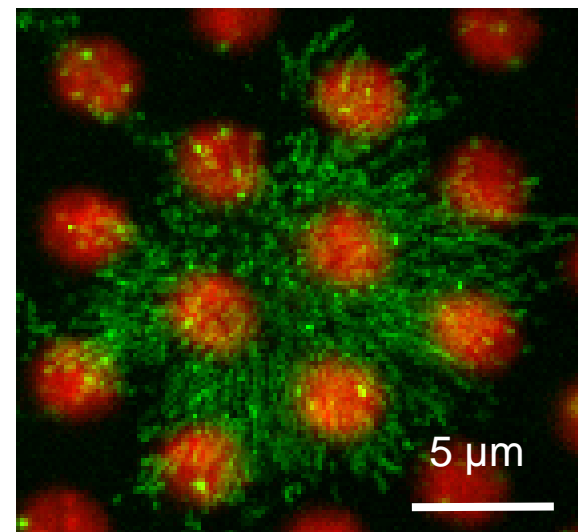
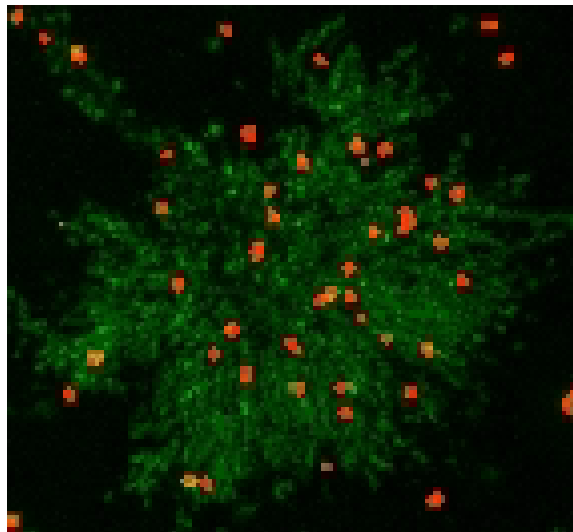
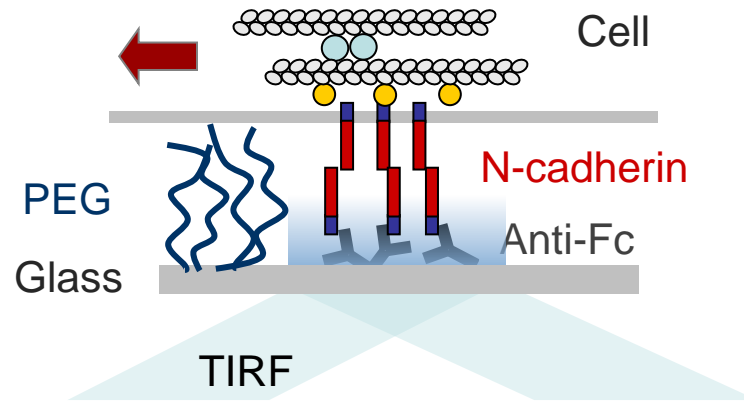
Giannone, Mège, Thoumine
Trends Cell Biol 2009

N-cadherin based molecular clutch in the growth cone



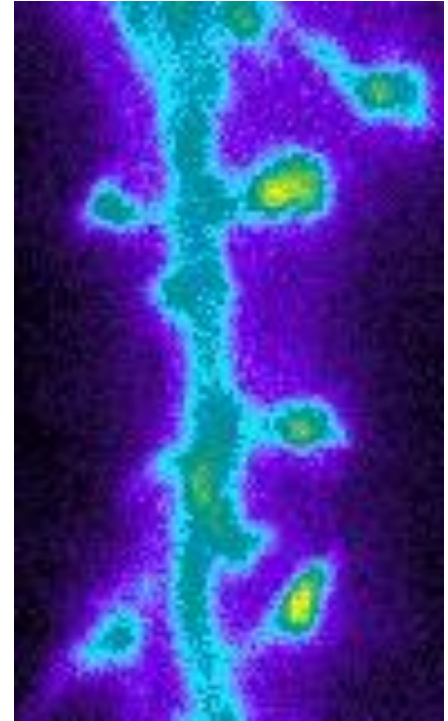
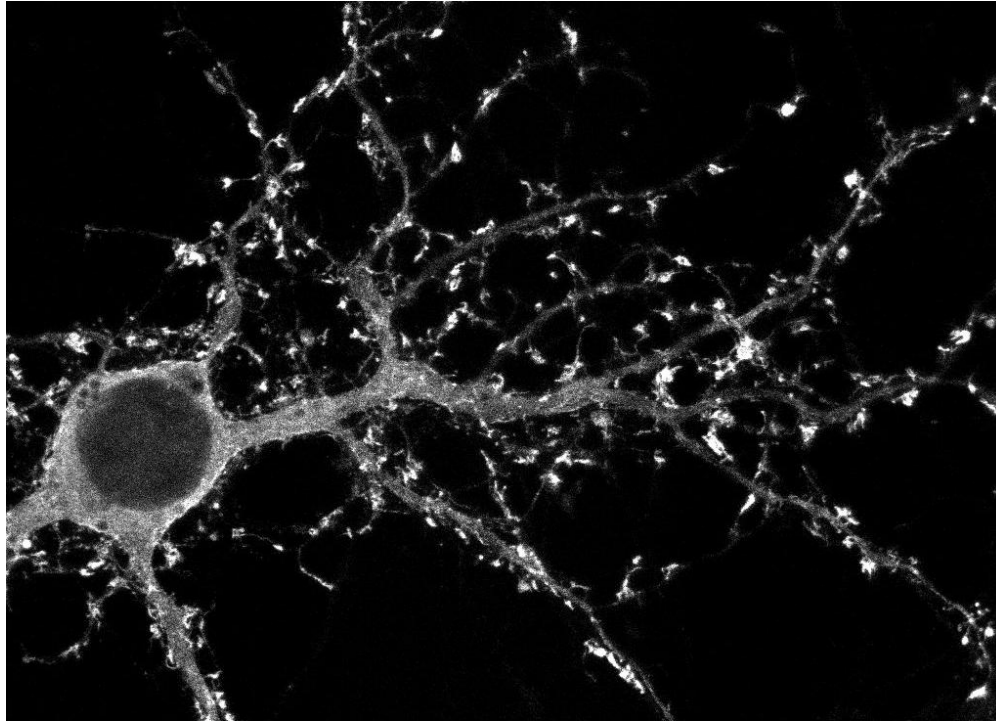
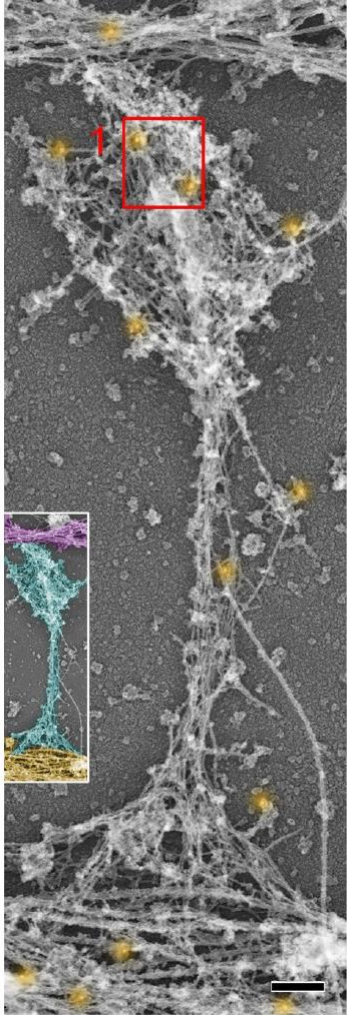
Bard, et al. *J. Neurosci* 2008

Mapping the actin flow in growth cones

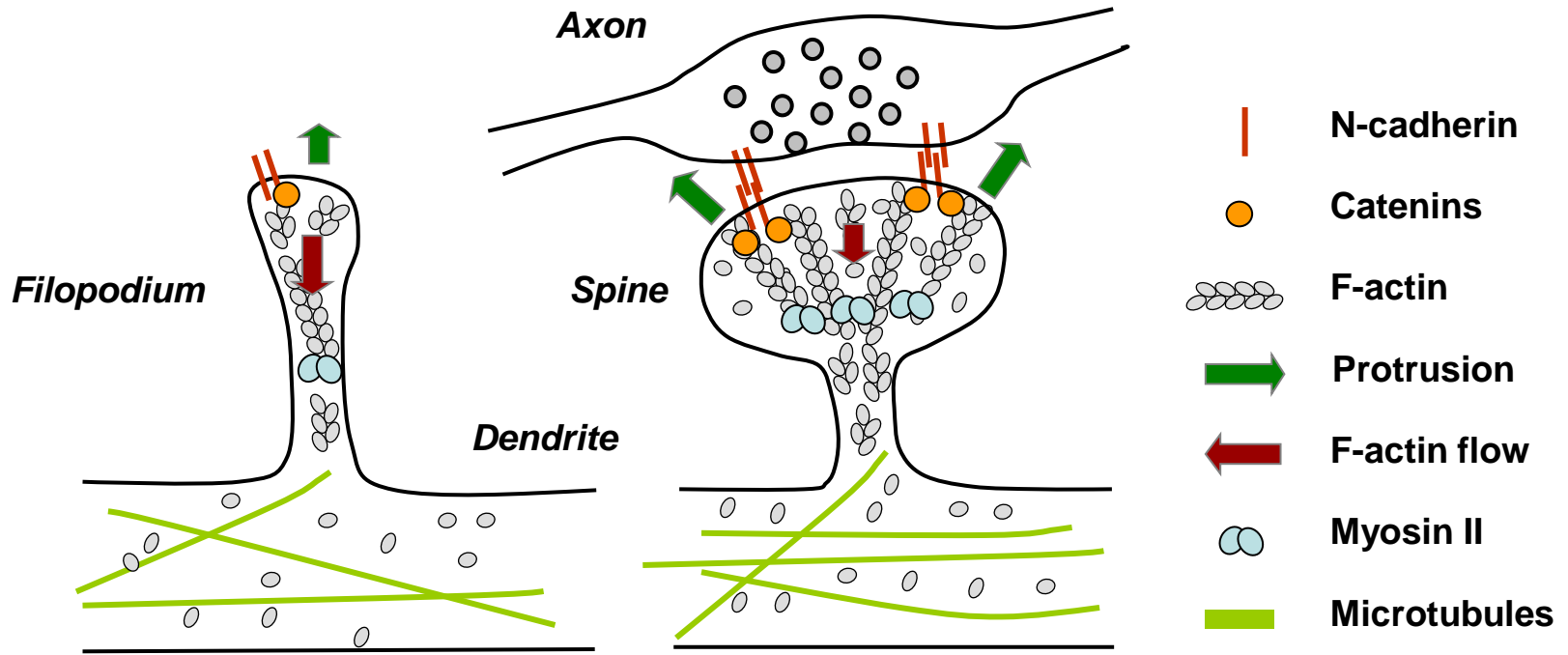


Garcia et al., *unpublished*

Axis 2: Dendritic spine morphing

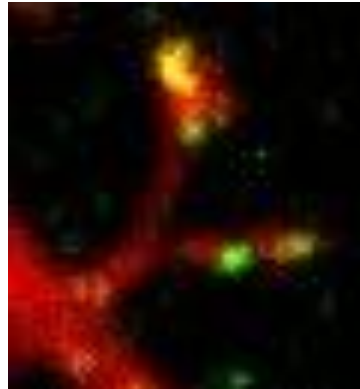


Rationale



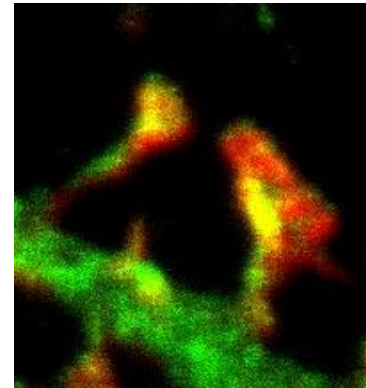
Subspine localization and dynamics

N-cadherin-GFP



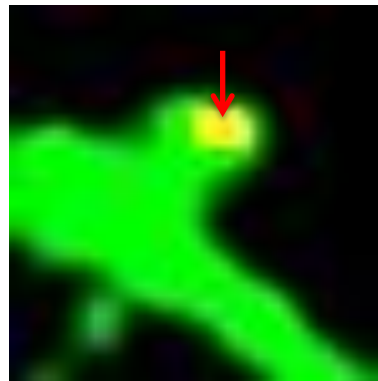
RFP

Myosin-GFP

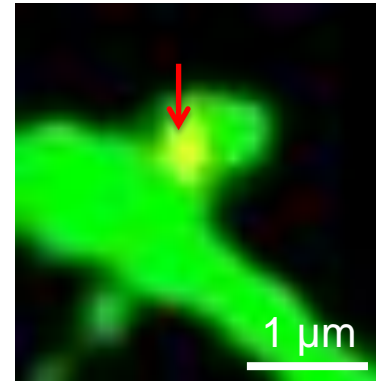


F-actin

Actin retraction upon myosin stimulation

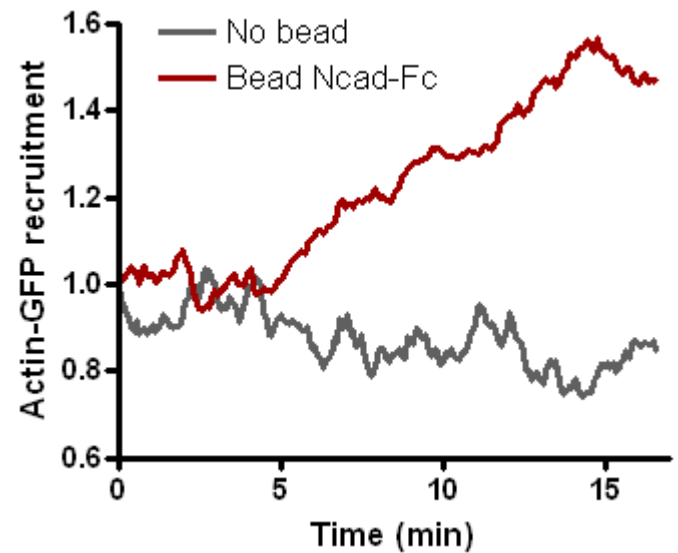
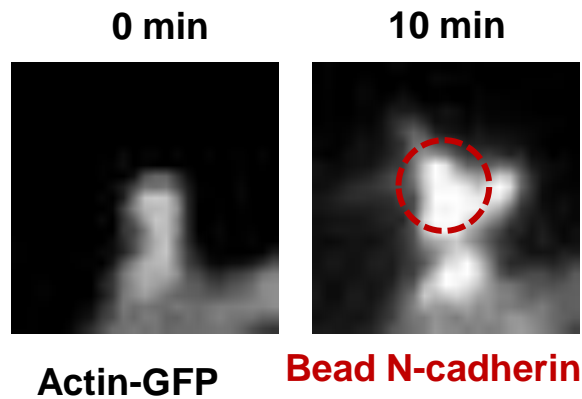
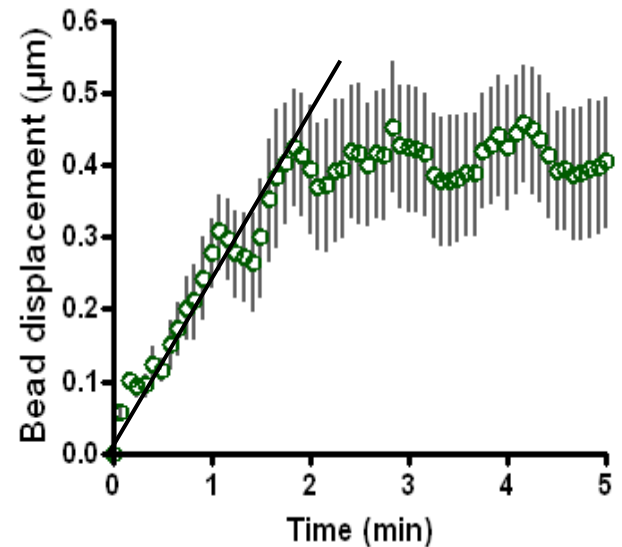
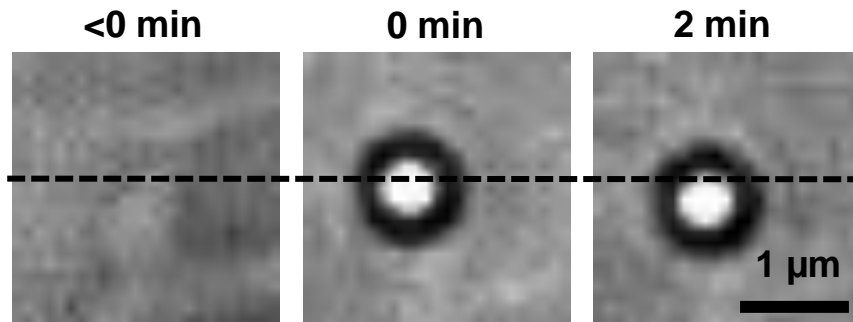


0 min

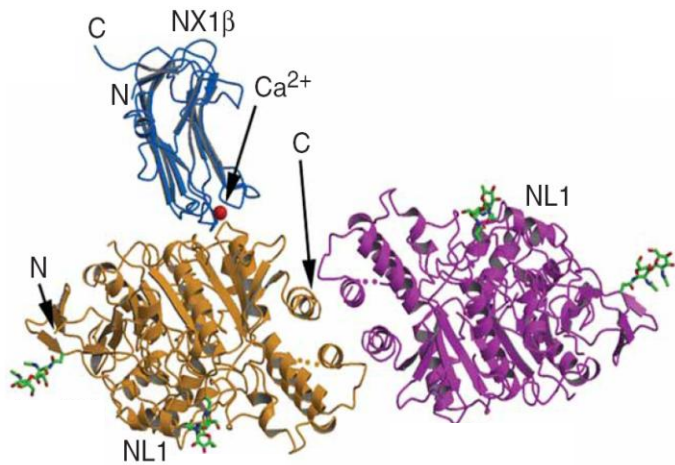


10 min

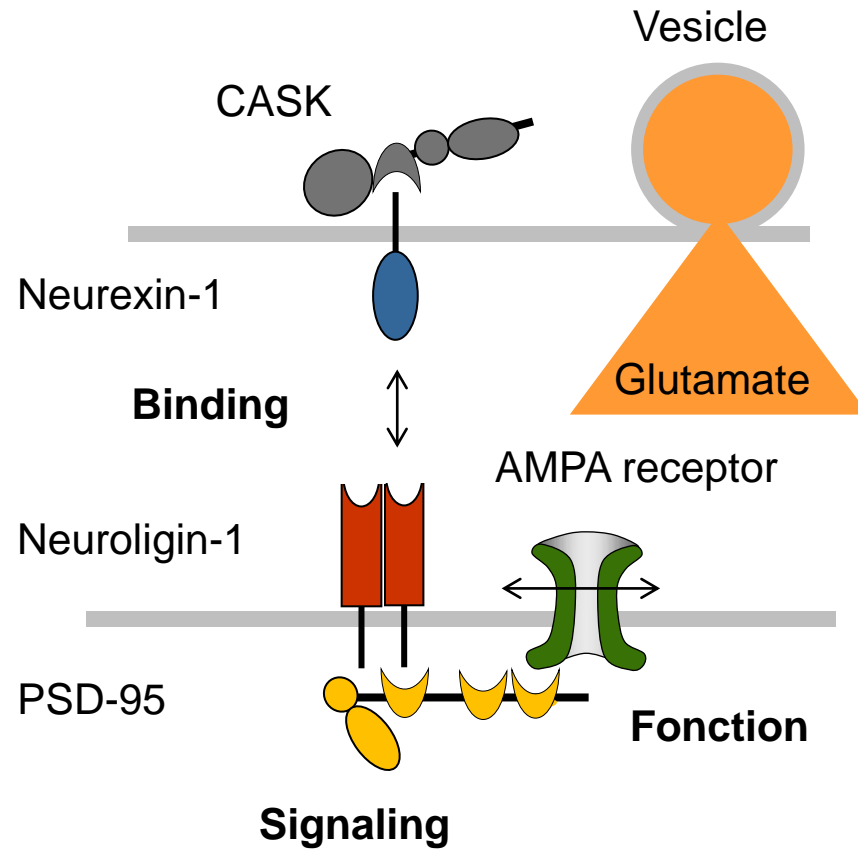
Optical tweezers experiments



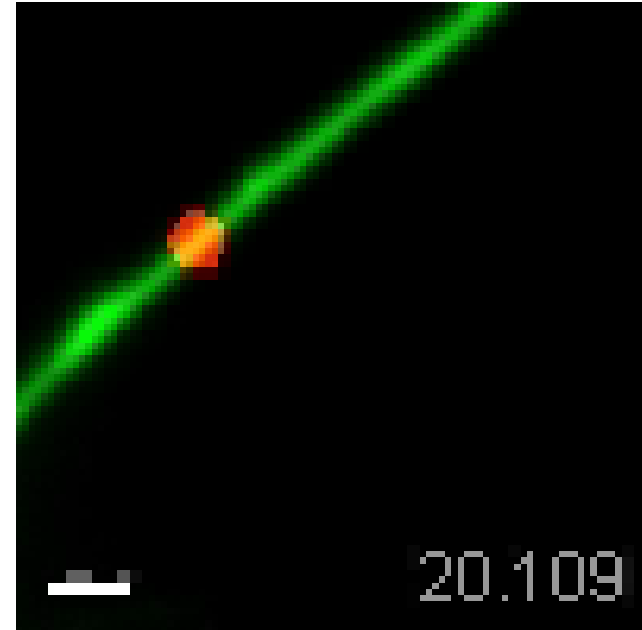
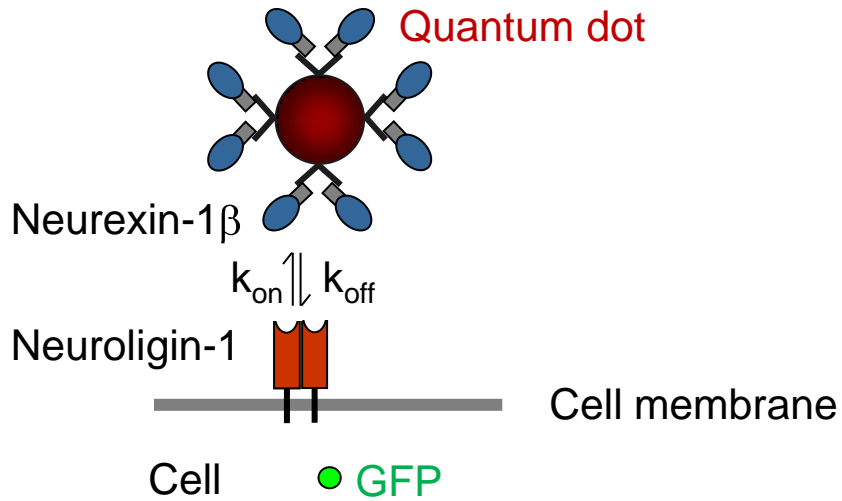
Axis 3: Synapse differentiation via neurexin/neuroligin adhesion



Sudhof, Nature 2008
Craig & Kang, Curr Opin Neurobiol 2007

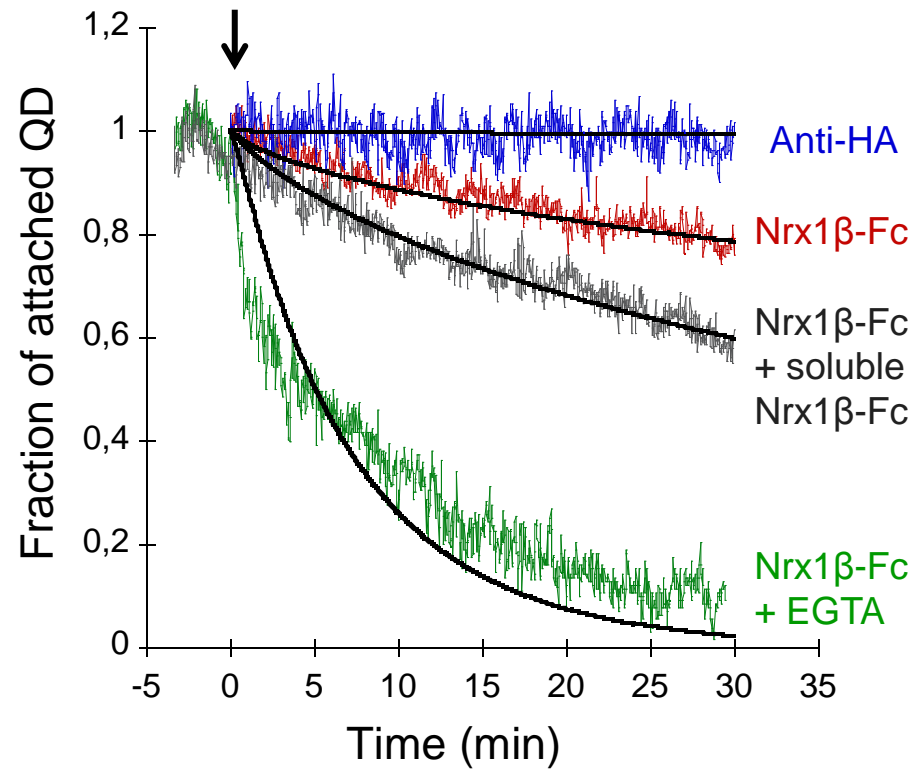
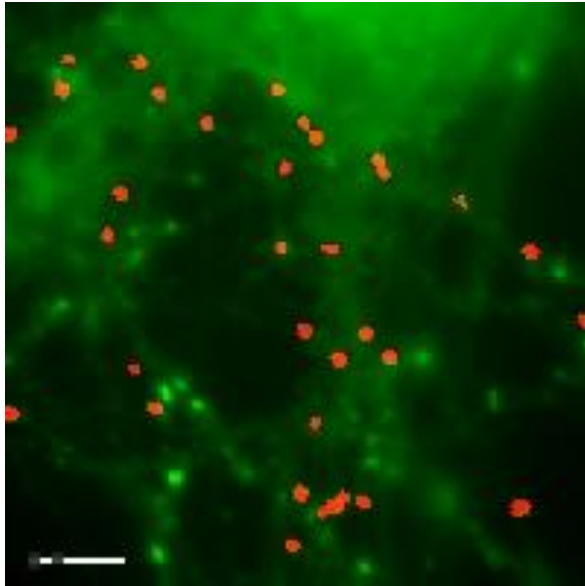


Detachment of neurexin-coated Quantum dots

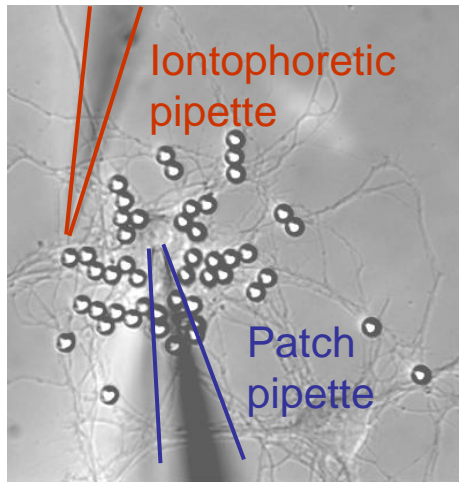


Individual detachment event

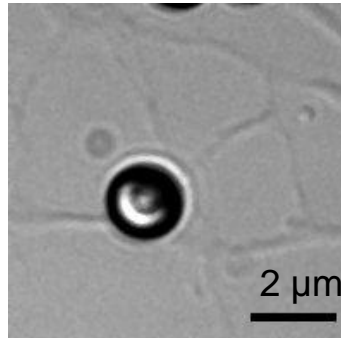
A sensitive method to probe protein interactions



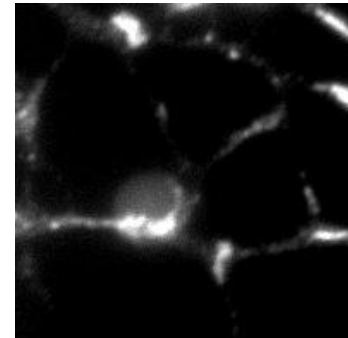
Assembly of functional post-synapses



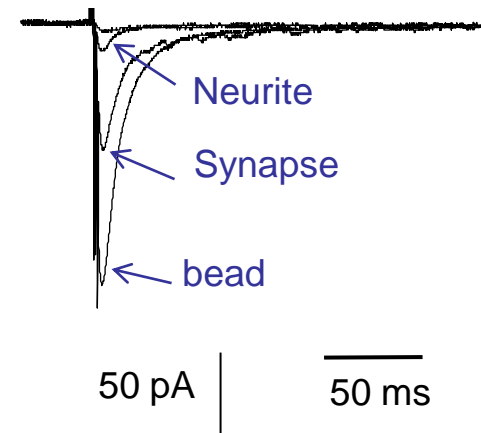
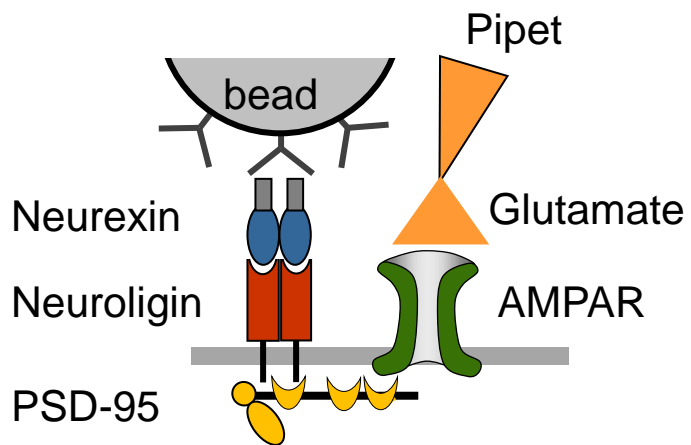
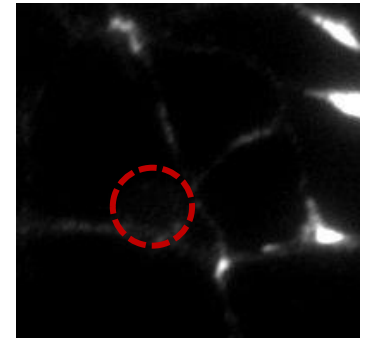
DIC



PSD95-mCherry



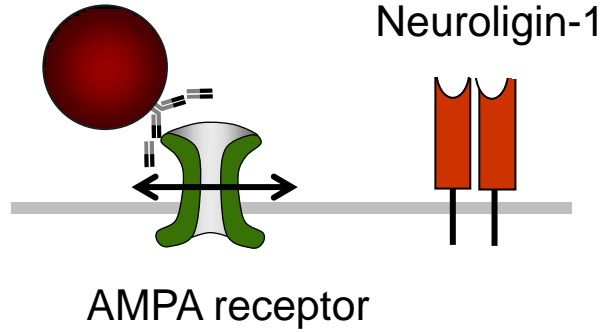
Synaptotagmin



Heine et al. *PNAS* 2008

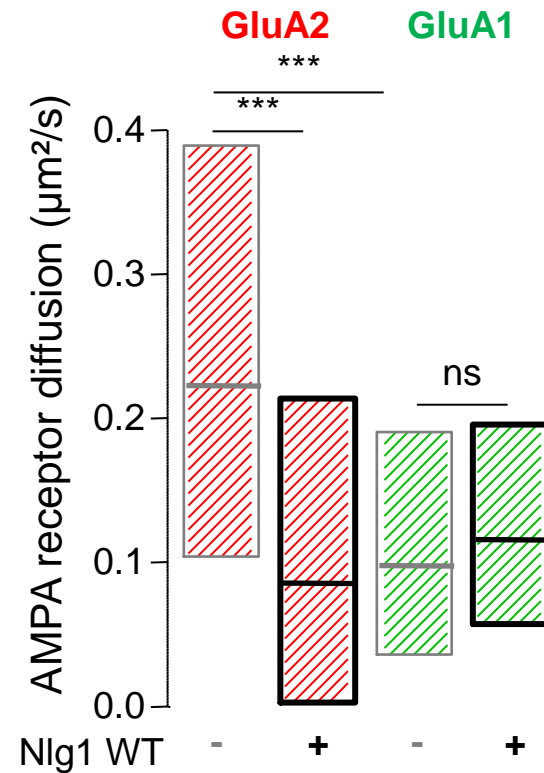
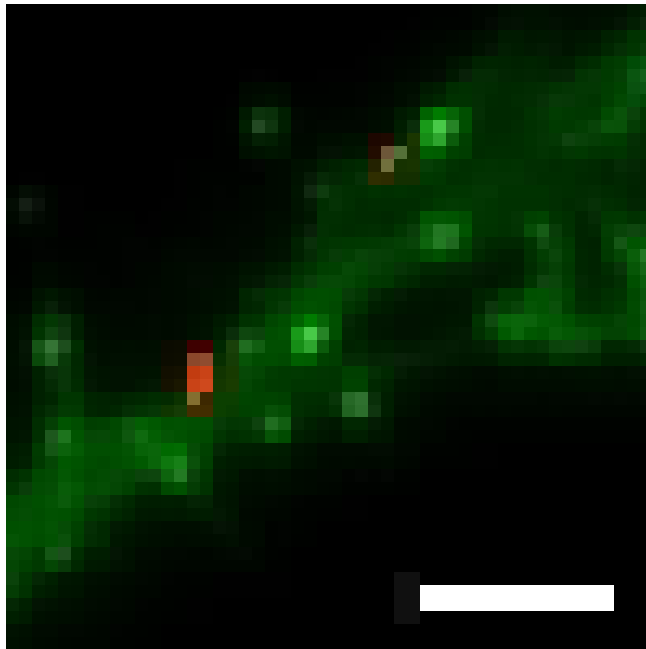
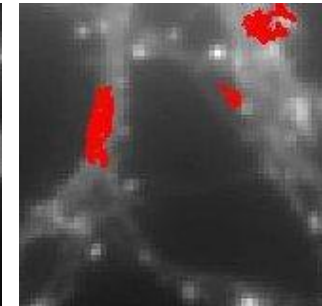
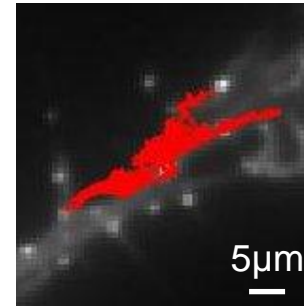
Neurotrophin-1 expression decreases AMPAR lateral diffusion

Quantum dot

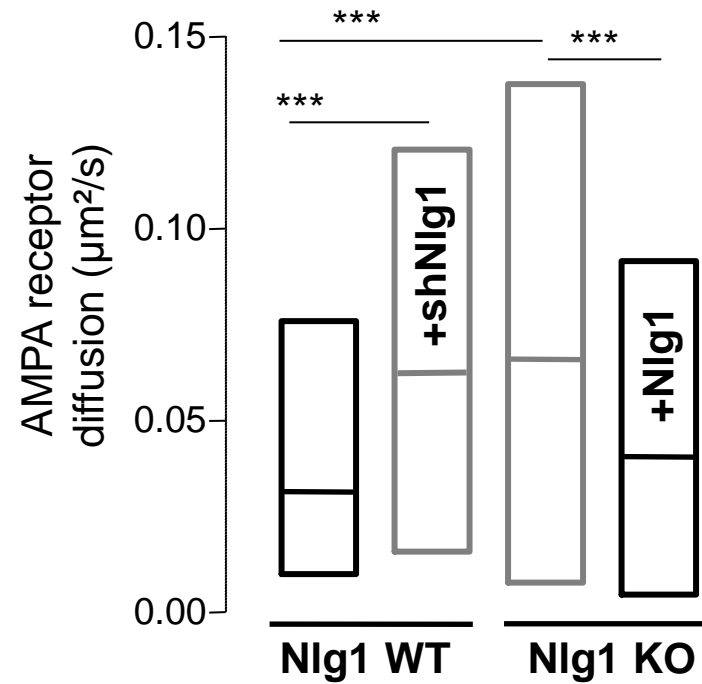
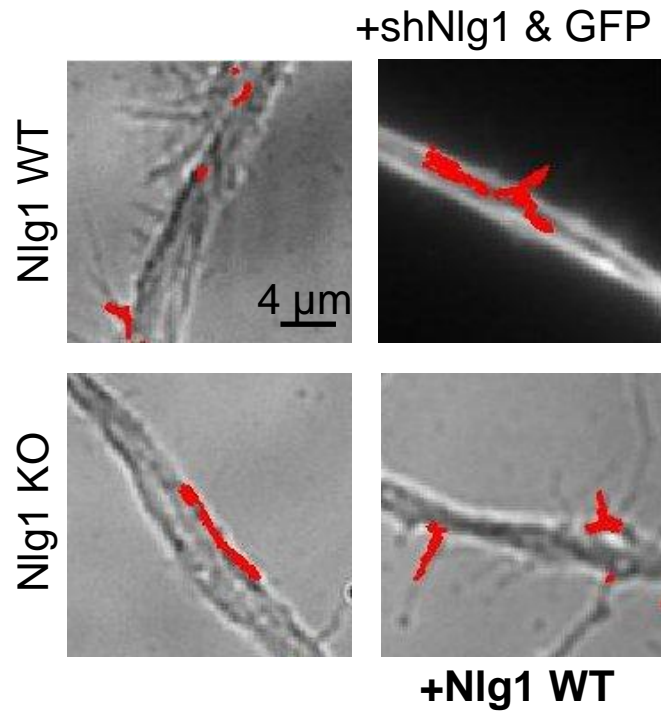


Control

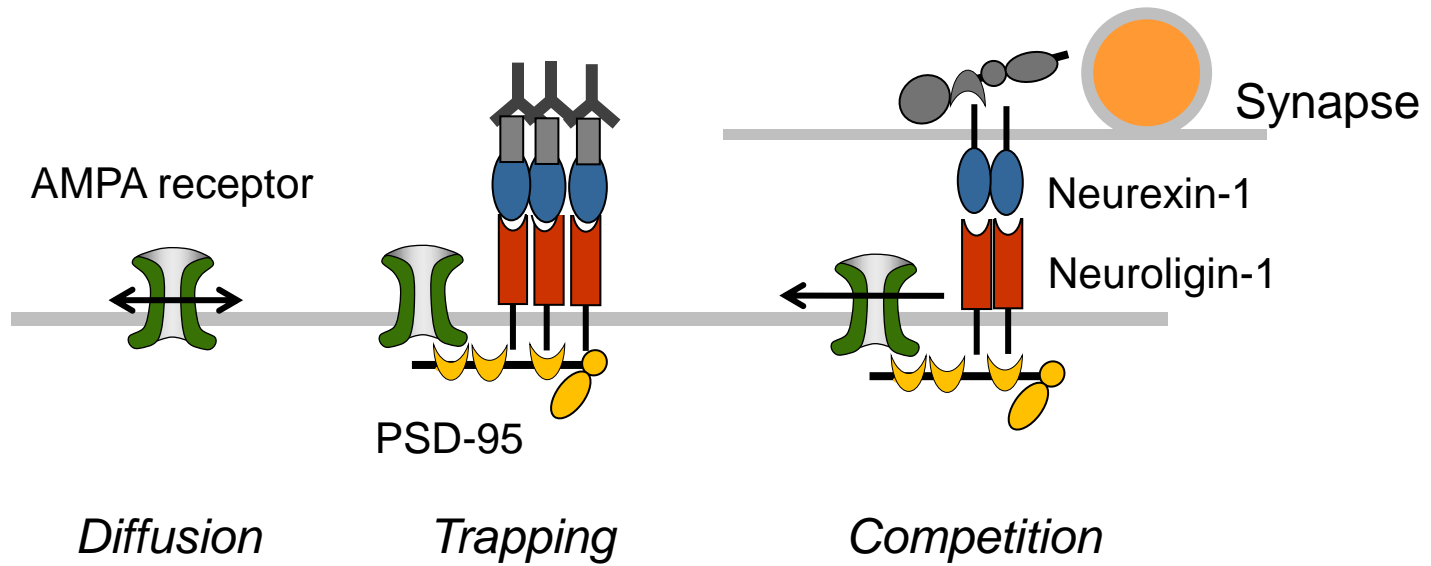
+ Neurotrophin-1



Neuroigin-1 bi-directionally modulates AMPAR lateral diffusion

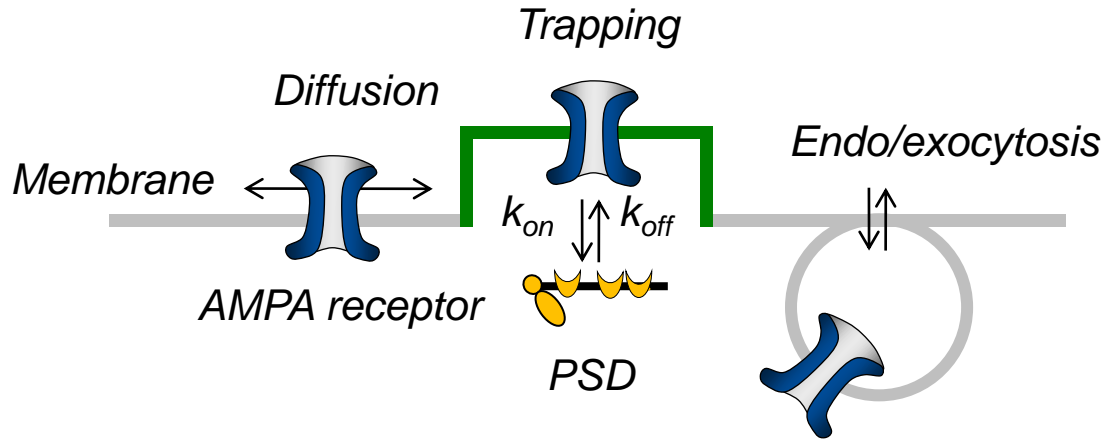


Summary

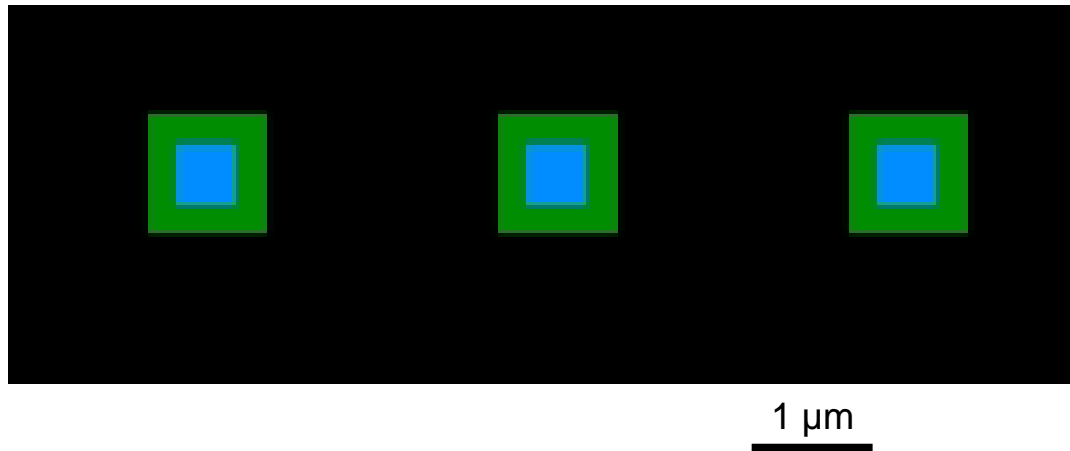


Model parameters

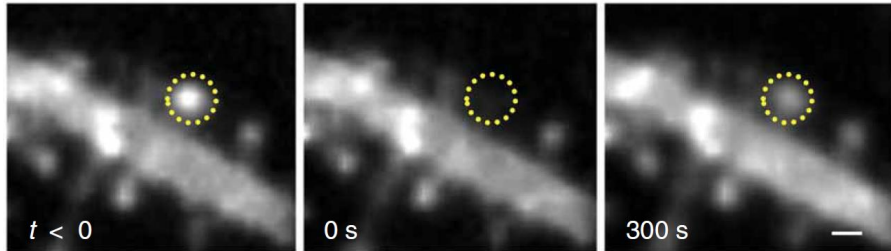
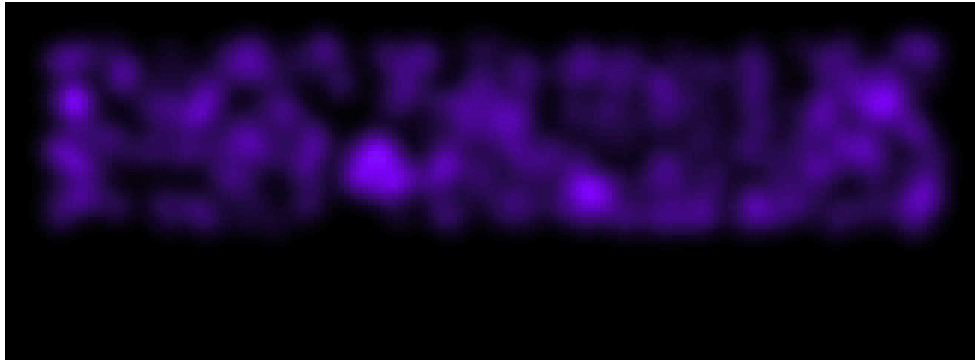
Model



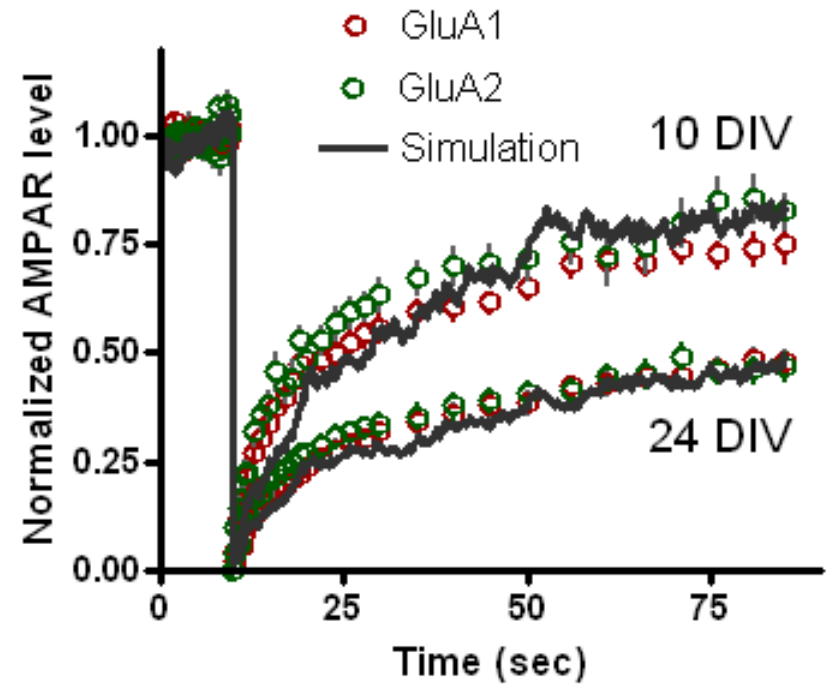
Simulation



Predicting FRAP experiments

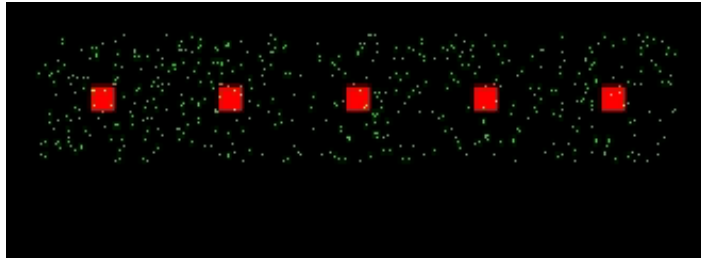


Ashby et al., J Neurosci 2006
Frischknecht et al., *Nat Neurosci* 2009

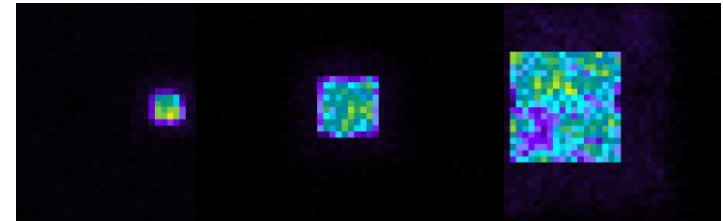


AMPA enrichment at synapses

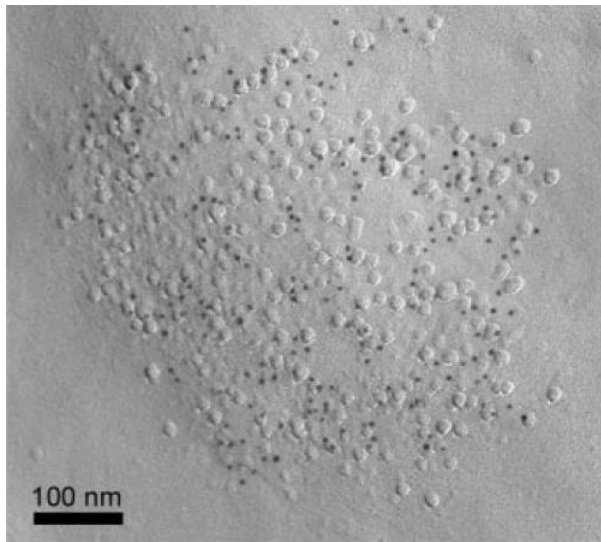
Projection



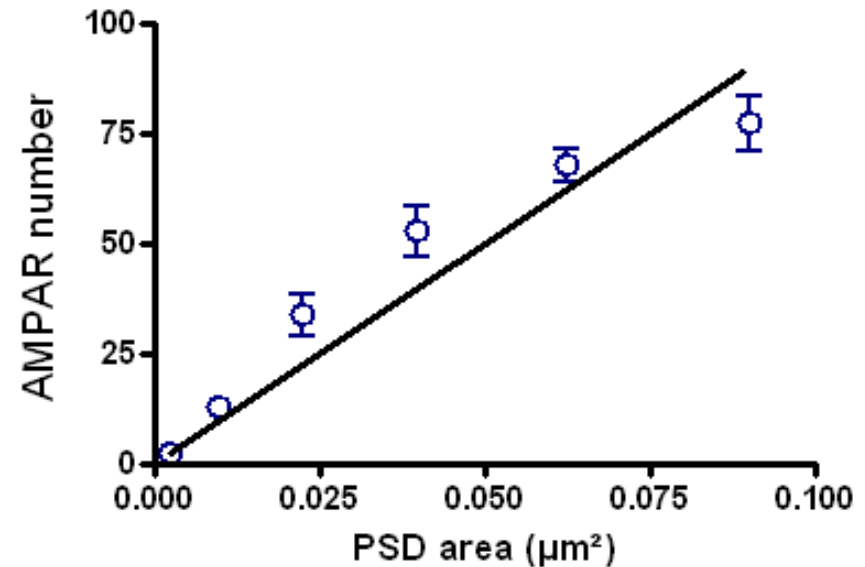
Synapse size



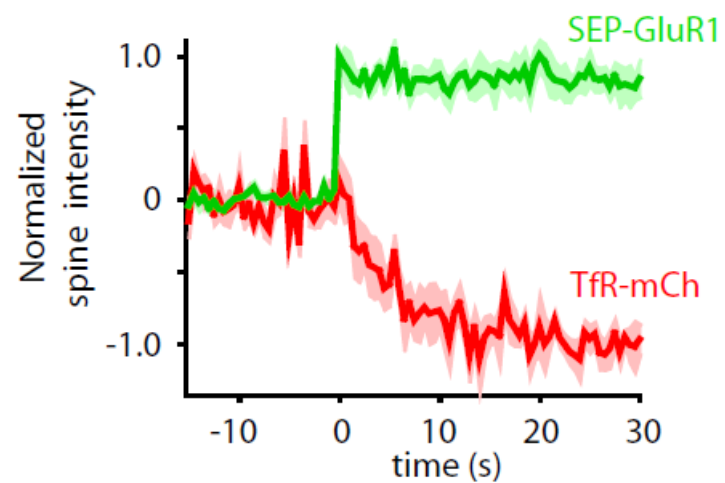
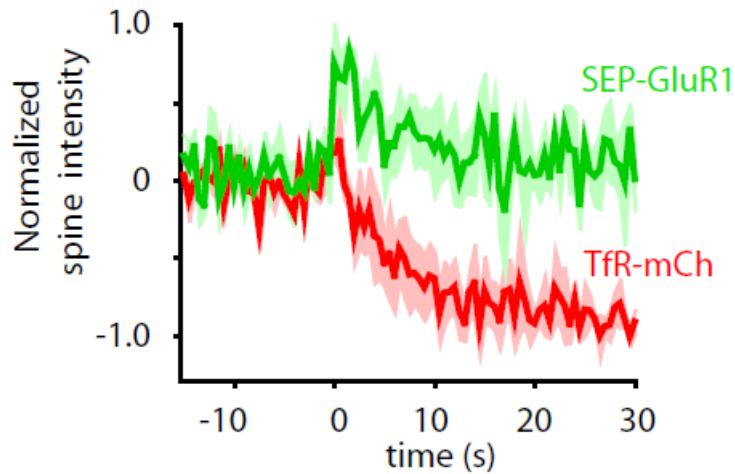
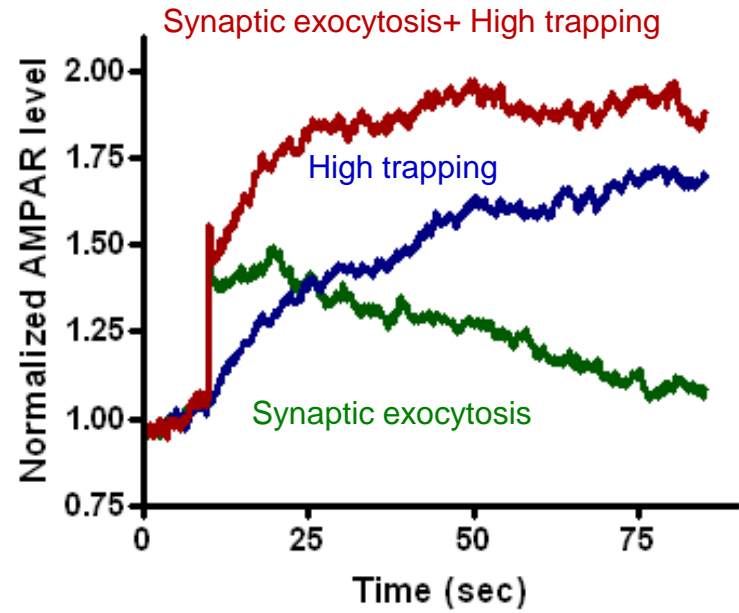
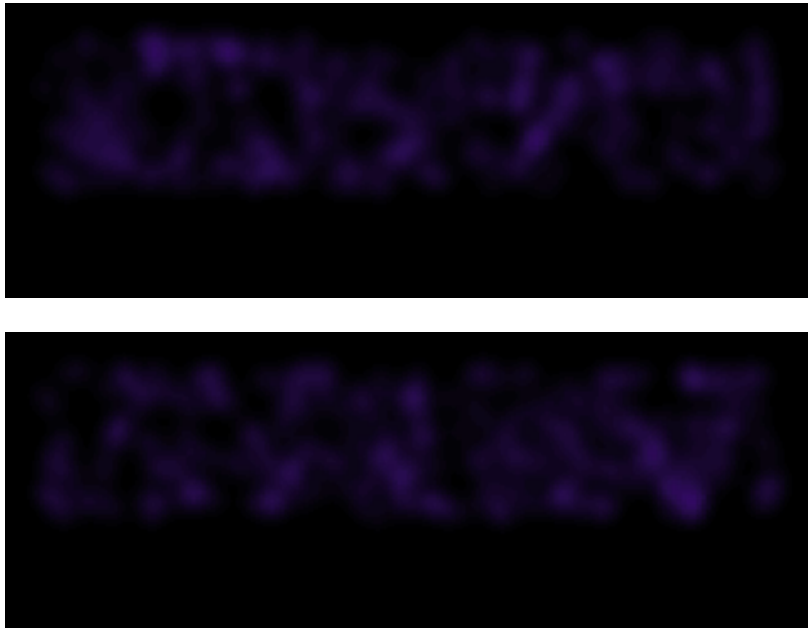
PSD = 0.1 μm 0.2 μm 0.4 μm



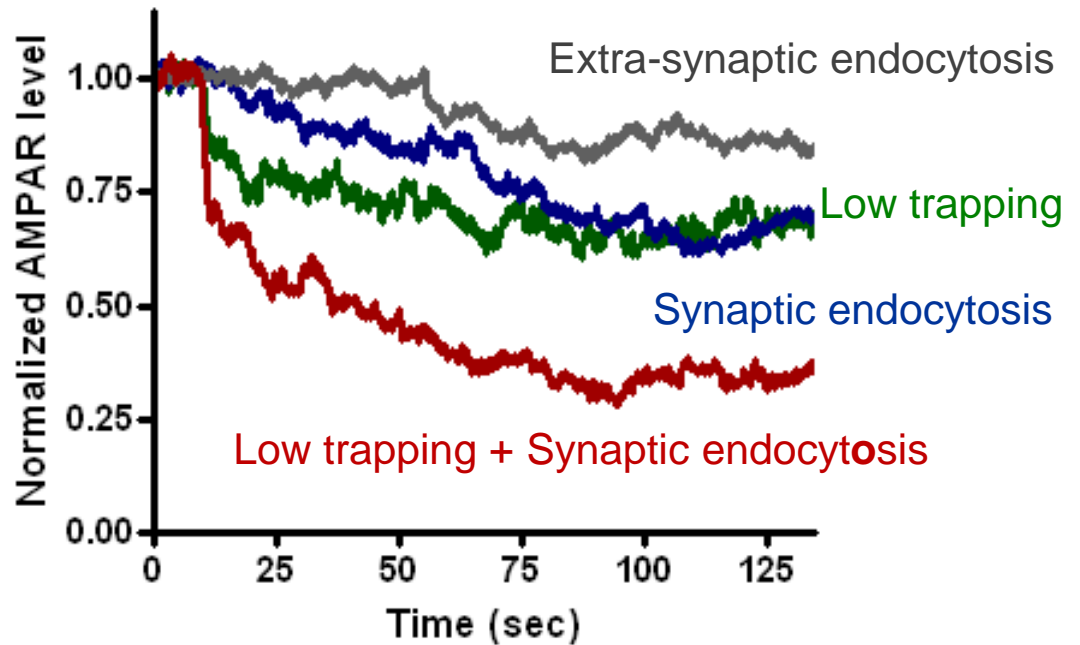
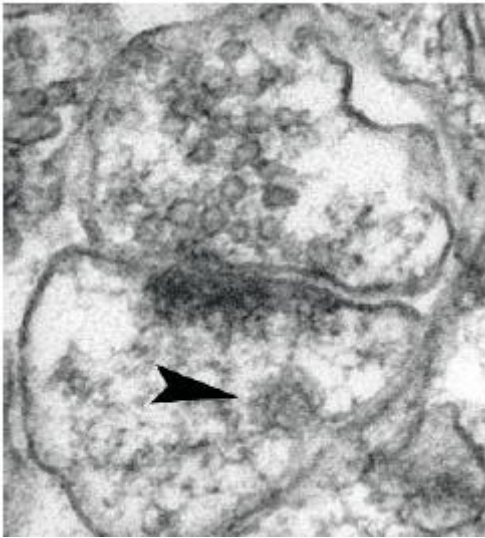
Tanaka et al., *J Neurosci* 2005



Synaptic AMPAR exocytosis



Introducing AMPAR endocytosis



Conclusions

A sequential scenario:

1. Growth cones migrate to find their targets
2. Synapses are assembled
3. Synapses undergo morphological and functional plasticity

Acknowledgments

Biophysics of Adhesion & Cytoskeleton

M. Mondin
K. Czöndör
M. Garcia
A. Chazeau
F. Néca
B. Tessier
O. Rossier
G. Giannone

CYTOO SA

A. Fuchs
A. Argento

Collaborators

M. Heine
R. Frischknecht
J.B.Sibarita
E. Hosy
D. Choquet
V. Labrousse
C. Mulle

Reagents

P. Scheiffele
N. Brose
F. Varoqueaux
O. Schlüter
M.Ehlers
R.M.-Mège

Bordeaux Imaging Center

C. Poujol
P. Legros
F. Levet
S. Marais

Animal care

A. Vimeney
A. Lacquement
N. Grosjean

Cell culture

C. Breillat
D. Bouchet
A. Frouin
R. Sterling

Funding

