

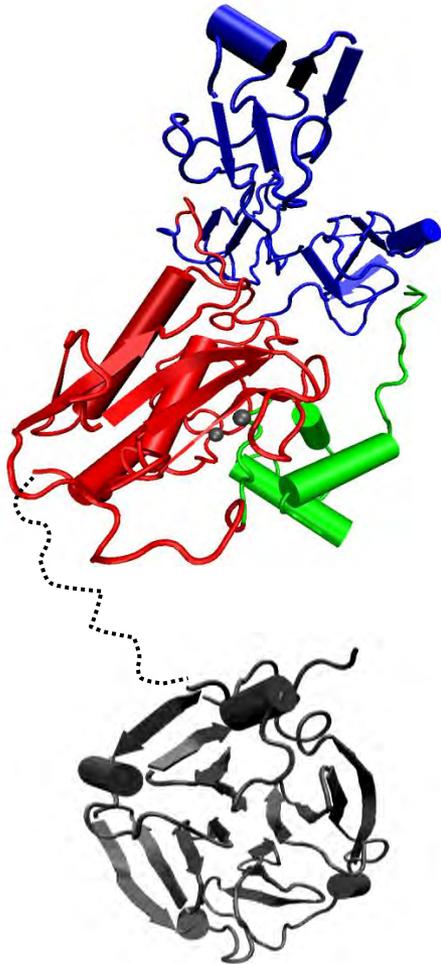
**Dynamics and morphology of dendritic  
spines driven by MMP-9  
Keeping tabs on methodology**

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Nencki Institute

Bioimage  
30 May 2011

## MMP-9

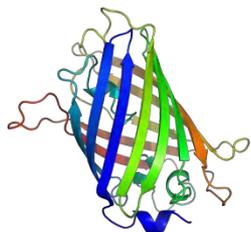
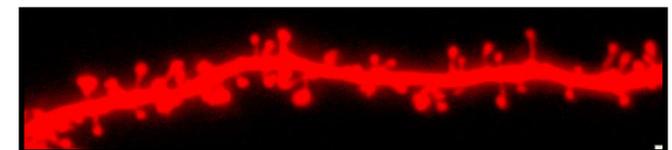
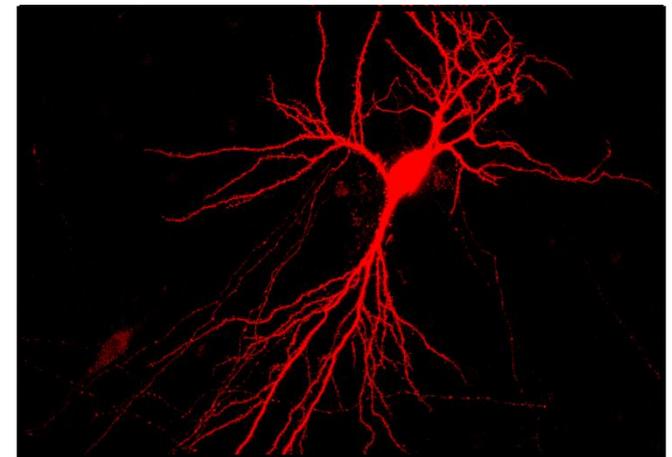
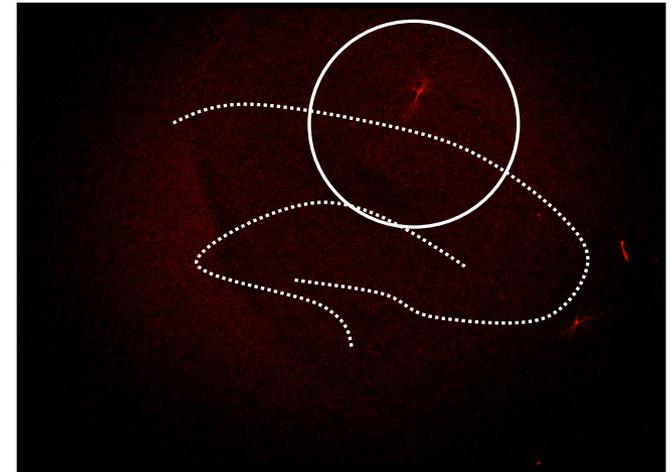
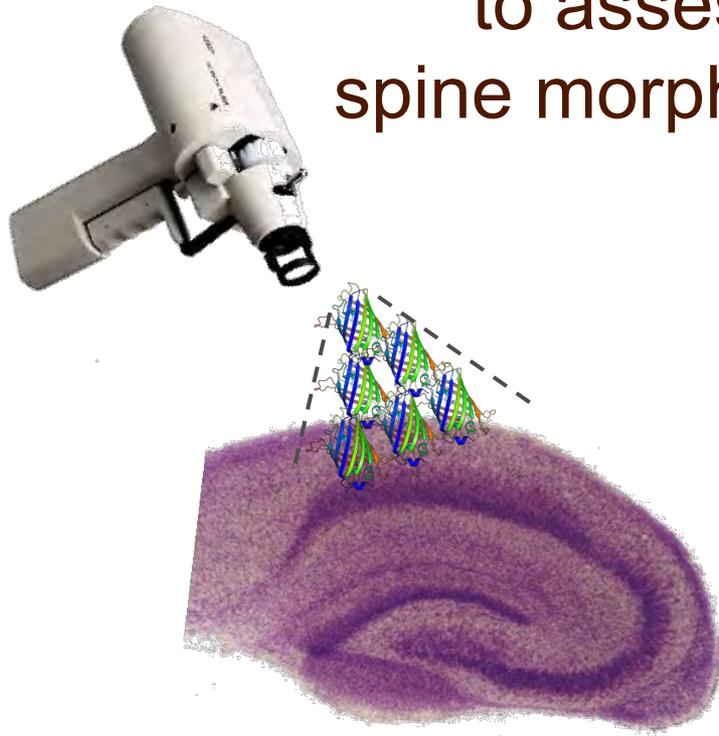


- extracellularly operating protease that is expressed by the neurons and released in response to enhanced neuronal activity (Michaluk et al., *J. Biol. Chem.*, 2007)
- plays a key role in synaptic plasticity associated with memory and learning processes; Nagy et al, *J. Neurosci.*, 2006; Okulski et al., *Biol. Psychiatry*, 2007
- was found to be present in a subset of dendritic spines bearing asymmetric synapses; Wilczynski et al., *J. Cell Biol.*, 2008.

## Dendritic spines

- membrane protrusions from neuron's dendrite that form component of synapses in the brain
- changes in dendritic spine morphology underlie learning processes (Xu et al., *Nature*, 2009; Yang et al., *Nature*, 2009)

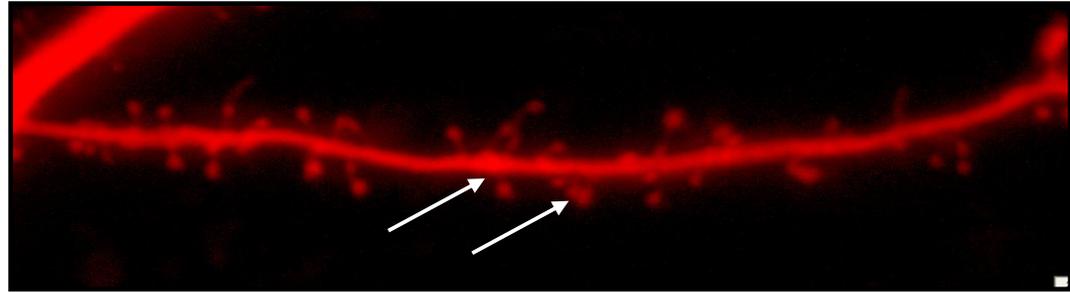
# Live Imaging to assess spine morphology



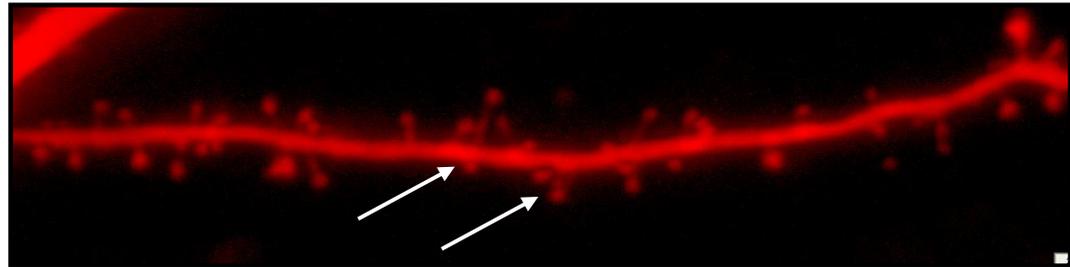
pCDNA3 – CMV **EGFP**  
pCX- beta actin **mRFP1**

Live imaging of organotypic  
cultures

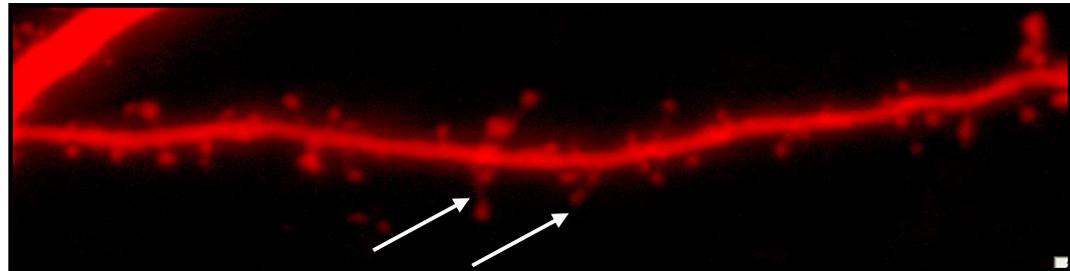
0 min.



30 min  
MMP-9



90 min  
MMP-9



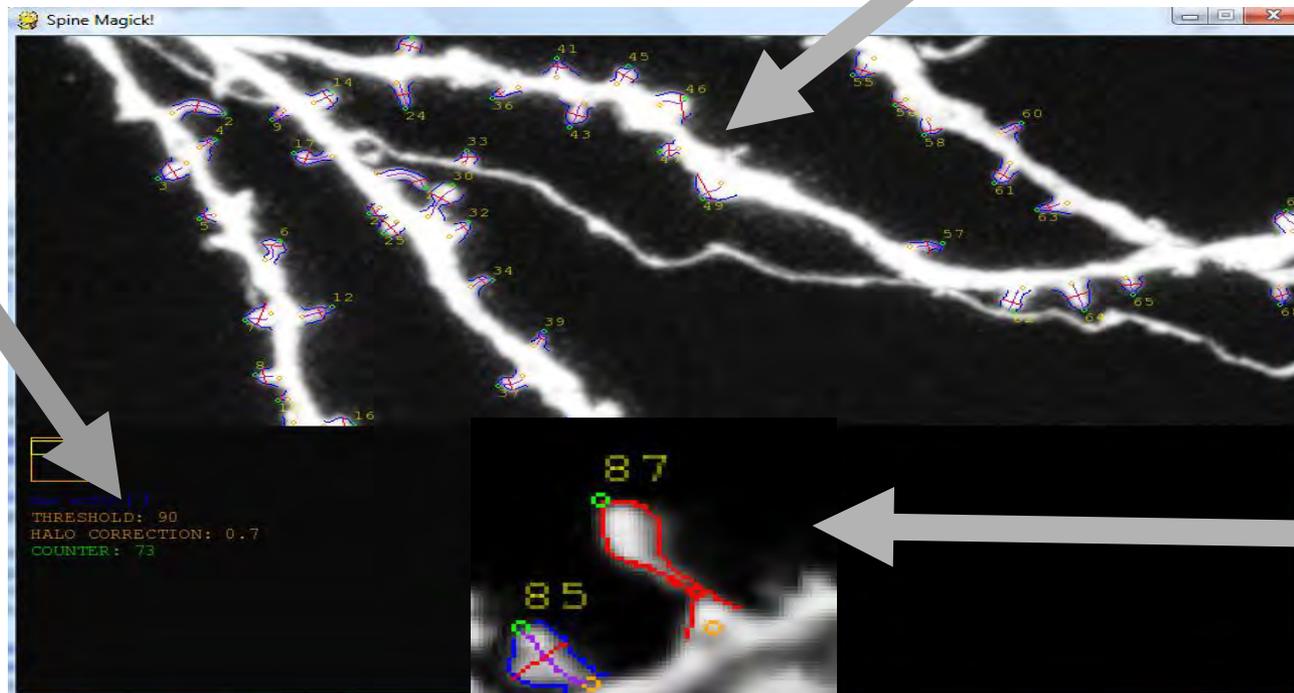
# Spine Magick!

Software for analysis of spine morphology

## Features:

- ✓ spine detection & surface recognition
- ✓ removal of artifacts
- ✓ morphometric analysis
- ✓ cataloguing the results
- ✓ linking to statistical tools

MAIN PROGRAM WINDOW



INTERACTIVE  
ZOOM  
WINDOW

# Automatic data management

Single experiment may involve hundreds of images and >10 000 spines

Dedicated data management system cataloging the results

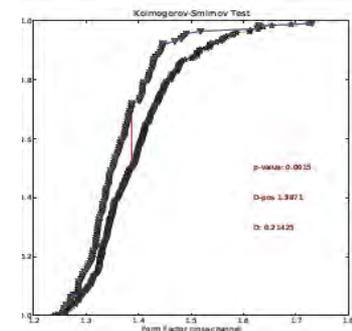
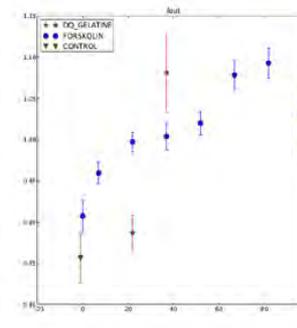
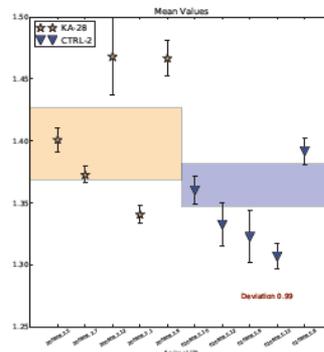
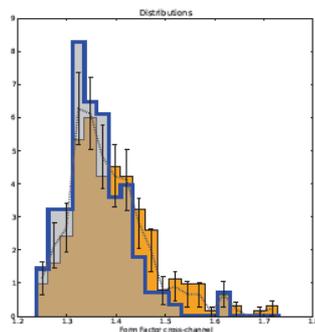
Checking against double-entries and improper/inconsistent records

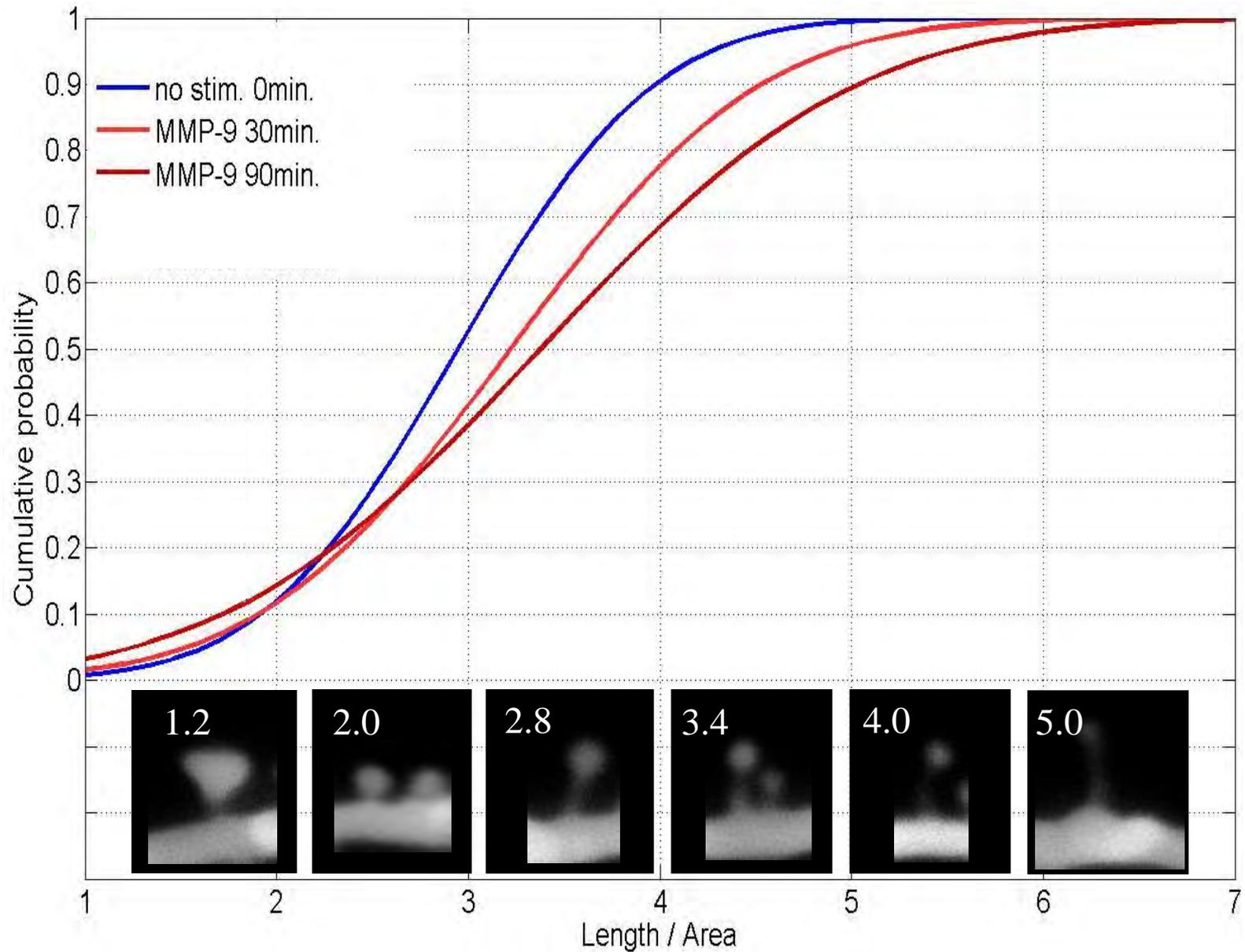
Linking to statistical tools facilitates data analysis

Life imaging requires identification of the same spines on different images

Assortment of statistical tests automatically performed

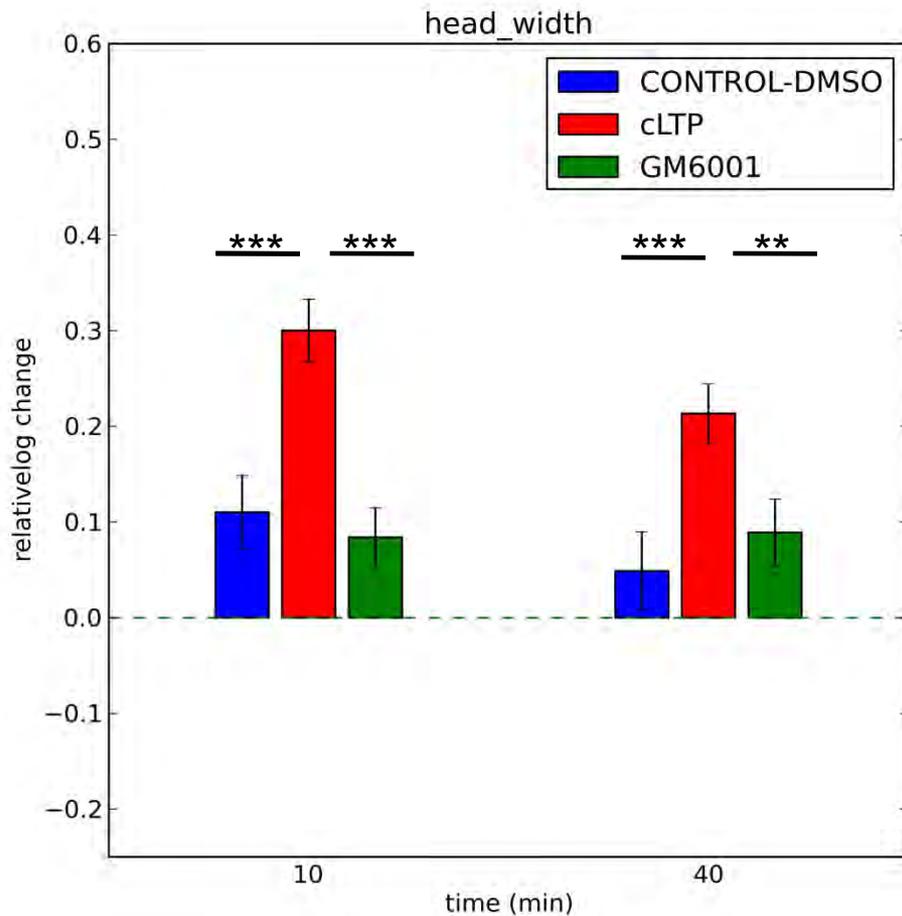
| Enter Scale:    |                    |
|-----------------|--------------------|
| Global Scale    | 0.07               |
| Experiment Name | FORSKOLIN_STIM     |
| Animal Type     | RAT_WISTAR         |
| Animal ID       | 1                  |
| Group ID        | FORSKOLINA         |
| Subgroup 1 ID   | 15                 |
| Subgroup 2 ID   | 17                 |
| Picture Nr      | 1                  |
| Brain Region    | HIPPOCAMPUSHODOWLA |
| Dendrite Rank   | APPICAL            |
| Notes           | 20.07.2010         |



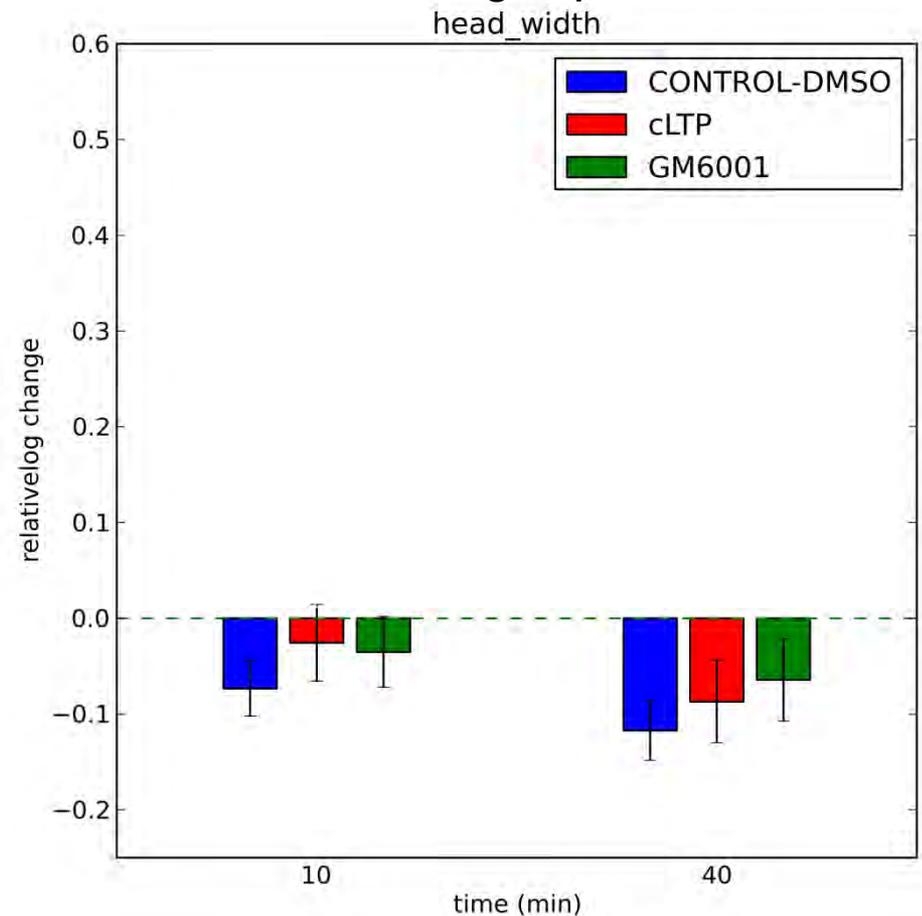


# Method Sensitivity (spines subpopulations analysis)

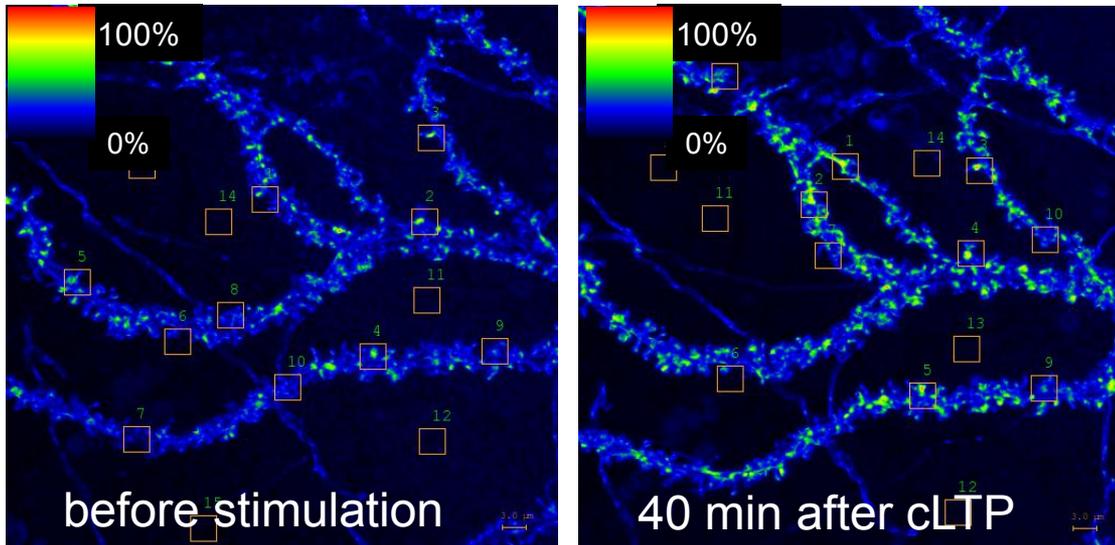
## Small spines



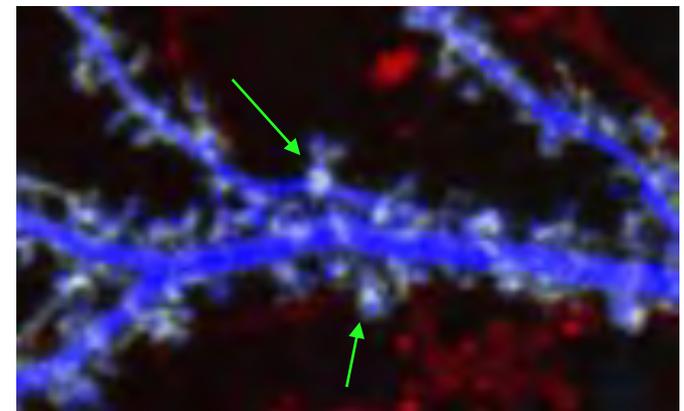
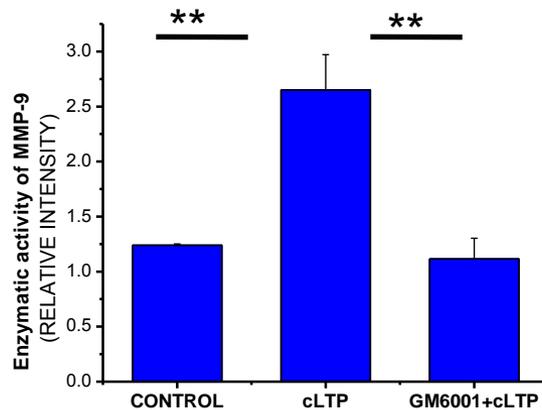
## Large spines



# Quantitative colocalization map to assess MMP-9 activity localization



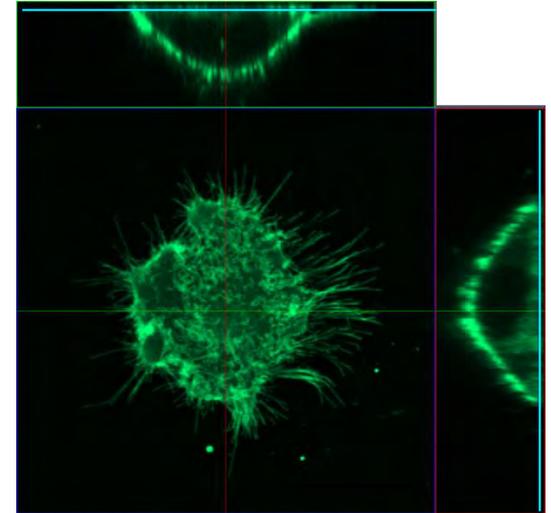
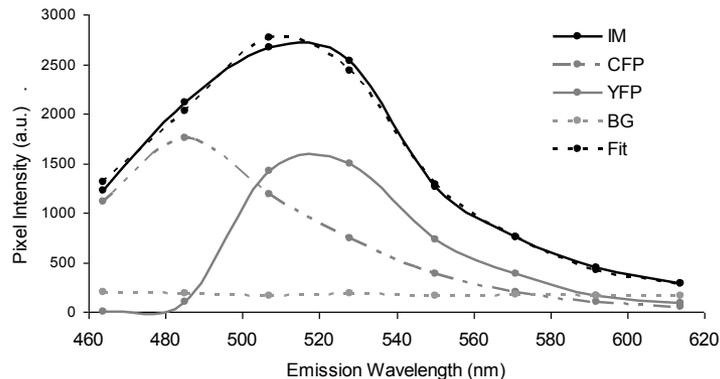
Enzymatic activity of MMP-9 on dendritic spines



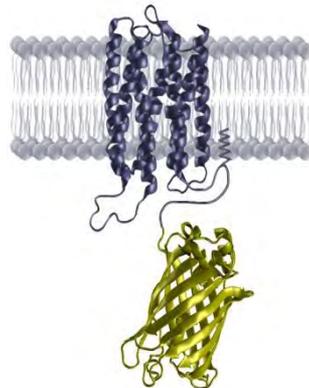
white color indicate colocalization

# Lux FRET to identify interacting partners

allows for the quantification of FRET efficiency and stoichiometry of interacting partners in living cells with high spatial and temporal resolution



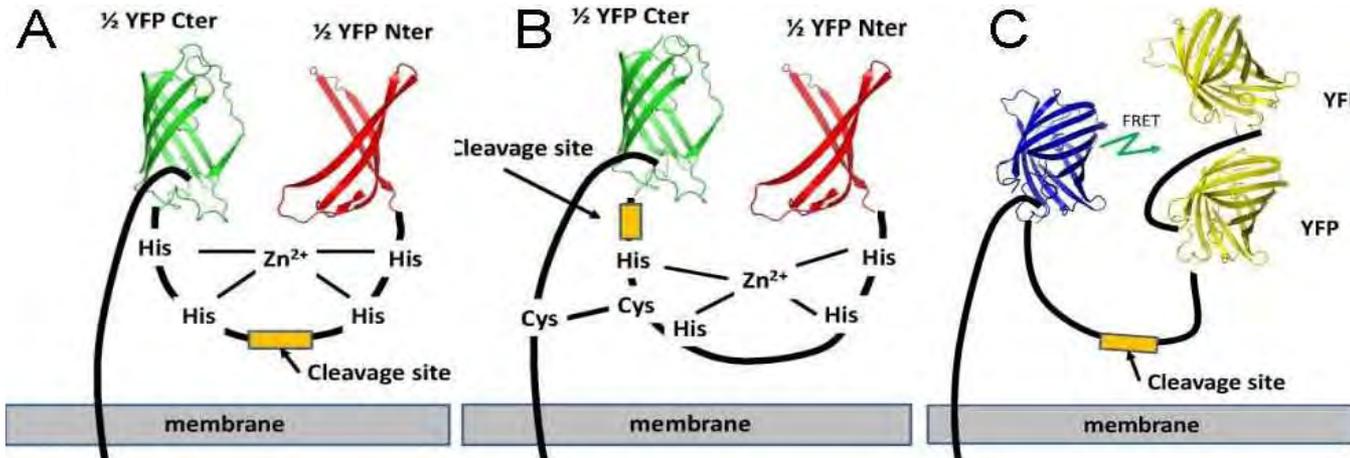
predict influence of  
incomplete labeling



Interactors  
(protein / peptide)

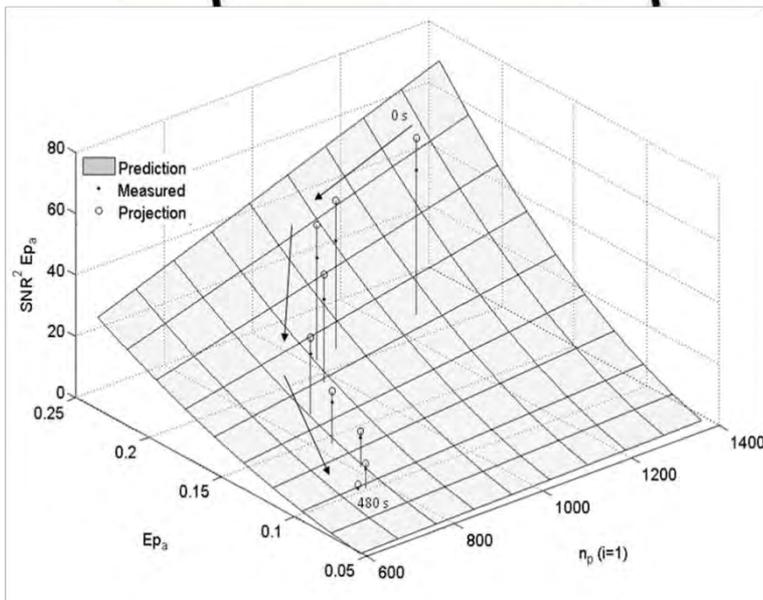
Labels  
(VFP, dye)

# Biosensor to detect MMP-9 activity



ERA-NET  
NEURON, 2009

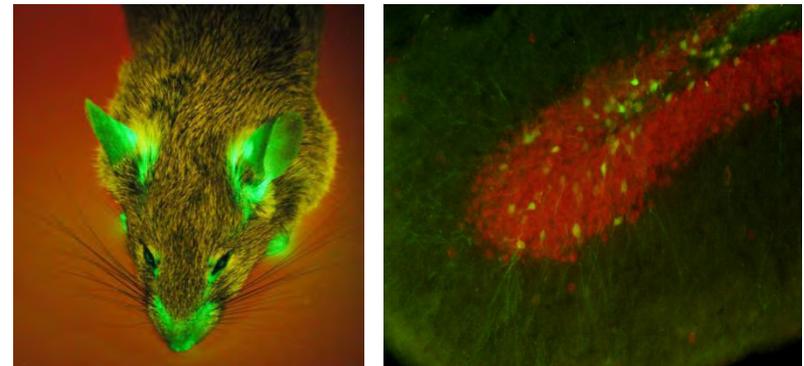
M. Stawarski



Wlodarczyk et al., *Biophys. J.*, 2008

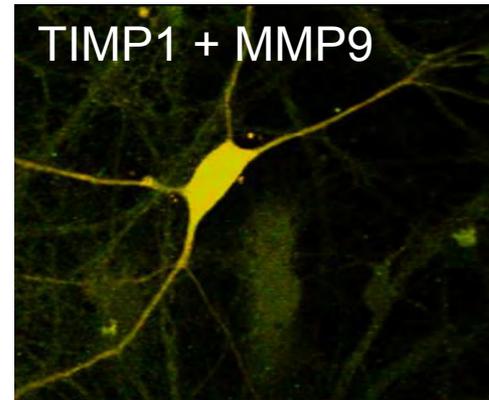
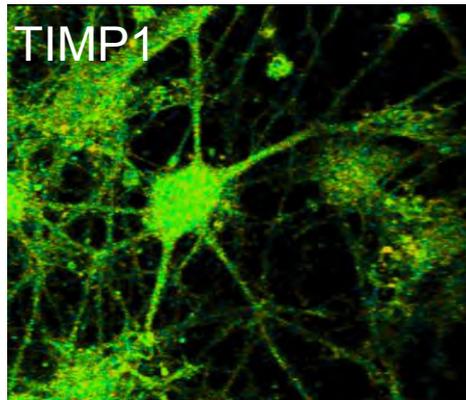
Woehler et al., *Biophys. J.*, 2010

## Stable transgene



Konopka et al., *Genesis*, 2009.

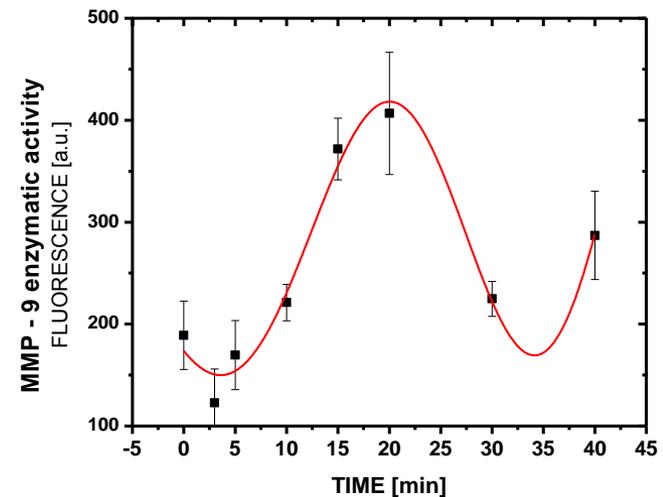
## FLIM to assess interaction kinetics



0ns FLUORESCENCE LIFETIME 5ns

Novel analysis of FLIM data, based on power-like decay model, to speed up refinement of the lifetime images and improve their contrast and quality

Włodarczyk, Kierdaszuk, *Biophys. J.*, 2003  
 Włodarczyk, Kierdaszuk, *Biophys. Chem.*, 2006  
 Włodarczyk, Kierdaszuk, *Eur. Biophys. J.*, 2007



# Summary

We employed

- Live Imaging on dissociated and organotypic cultures
- Detailed morphology analysis of dendritic spines
- Novel FRET based methods for protein-protein interaction study
- FRET based Biosensors

to get insight into effect of MMP-9 activity on synaptic plasticity

# Acknowledgement

■ Leszek Kaczmarek

■ Zsuzsanna Szepesi

■ Monika Bijata

■ Michal Stawarski

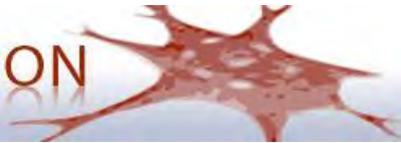
■ Blazej Ruszczycki



# Major international collaborators and support in the field of imaging



ERA-NET NEURON



A. Holtmaat; Geneva (live imaging; *in vivo* imaging)

A. Diaspro; Genua (high-resolution confocal microscopy)

E. Ponimaskin; Hannover (FRET sensors)

D. Choquet; Bordeaux (single receptor trafficking)

E. Gundelfinger; Magdeburg (ECM imaging)



**BIO-IMAGINE**

D. Choquet;

Polish-German  
(*application in progress*)

E. Neher; Goettingen (FRET based methods)

E. Ponimaskin; Hannover



**axregen**

axonal regeneration, plasticity and stem cells



INNOWACYJNA GOSPODARKA  
NARODOWA STRATEGIA SPÓJNOŚCI

INNOVATIVE ECONOMY  
NATIONAL COMMISSION STRATEGY