Journée de la Société Française de Radioprotection
Paris, 26 septembre 2017

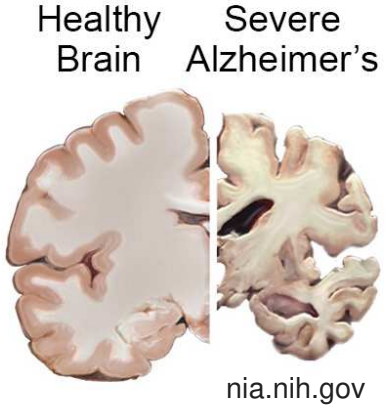
Nanoparticules fluorescentes pour l'étude du trafic membranaire et du transport intracellulaire dans les neurones.

François TREUSSART

ENS Paris-Saclay,
Laboratoire Aimé Cotton, Université Paris-Saclay

Biomedical context

Neuropsychiatric Disorders
Schizophrenia / Autism



Neurodegenerative Diseases
Parkinson / Alzheimer

1% World-wide frequency
Multiple risk factors
More than **100** genes

≠ Rare Brain Diseases (0.001% frequency & 1 impacted gene)

Need for
- Unbiased diagnosis
- Efficient treatments

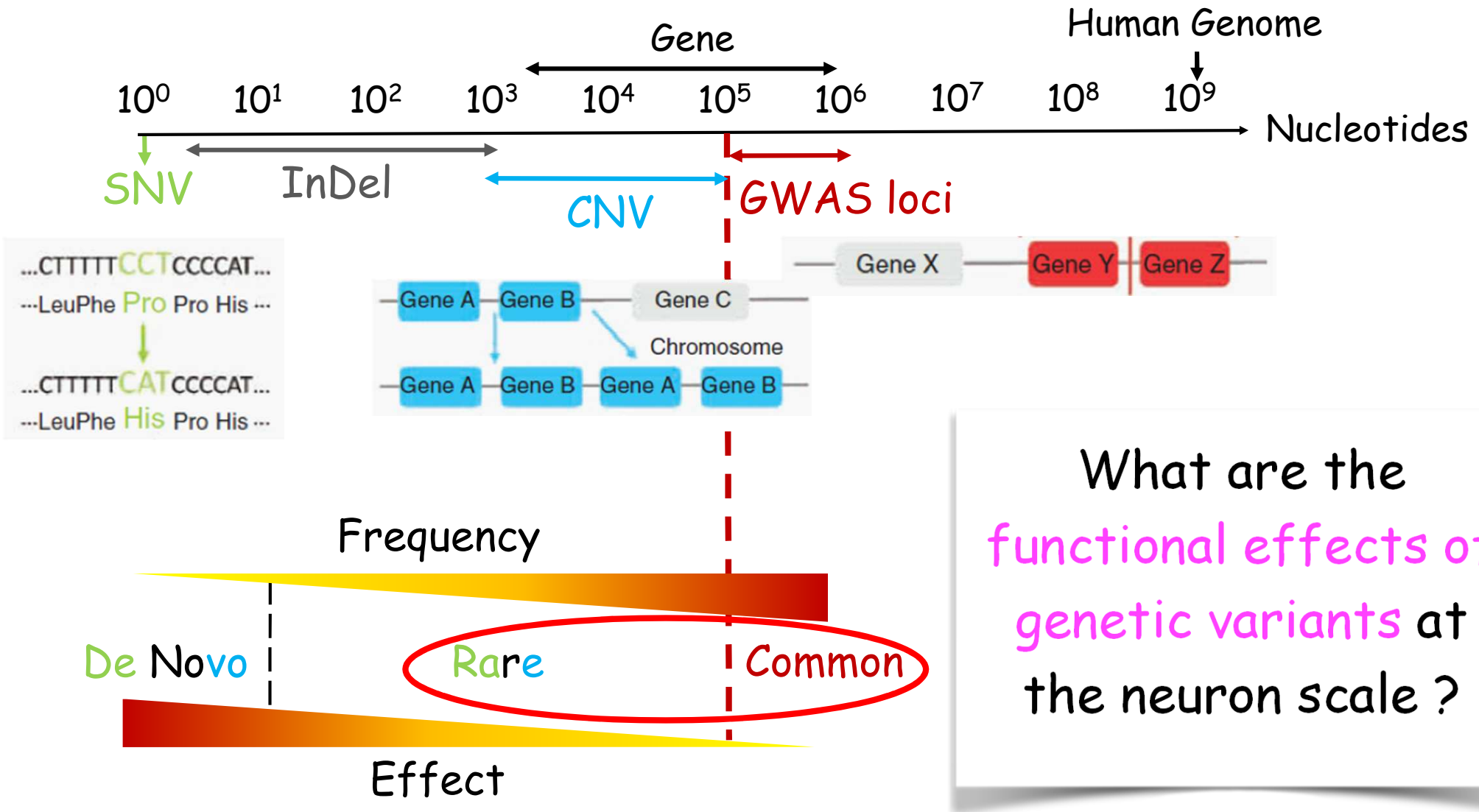
⇒ Dissect the underlying neurobiological process

No sample/biopsy of human brain (like for cancer)

⇒ Neurogenomic Data from Genome Sequencing

Polygenic Architecture of Common Brain Diseases

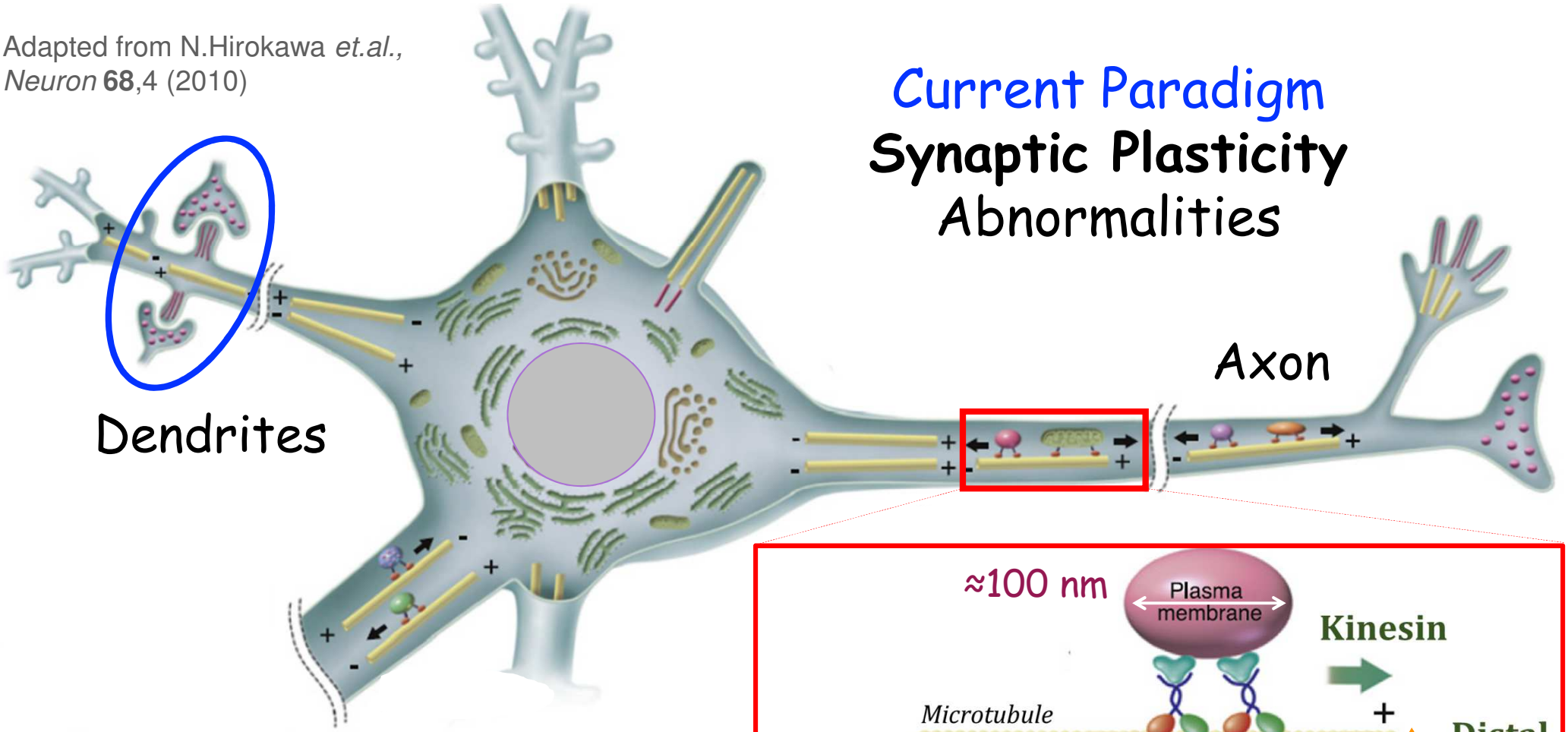
Genetic Risk Factors (change in gene **expression** or **function**)



What are the functional effects of genetic variants at the neuron scale ?

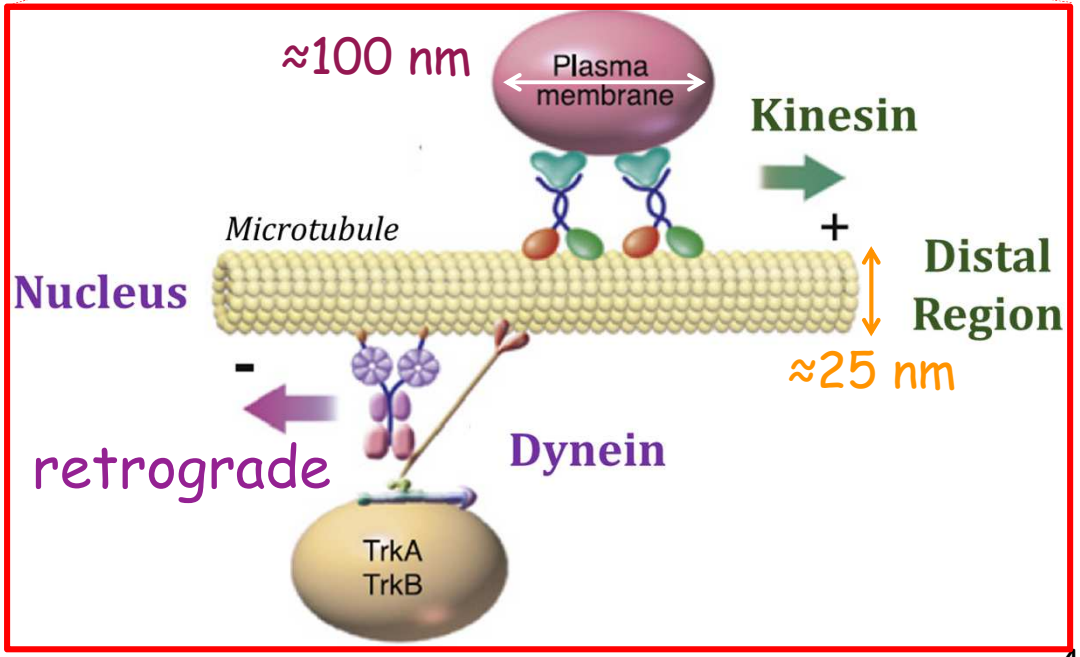
Measuring sub-neuronal functions

Adapted from N.Hirokawa *et.al.*, *Neuron* **68**,4 (2010)



Current Paradigm
Synaptic Plasticity
Abnormalities

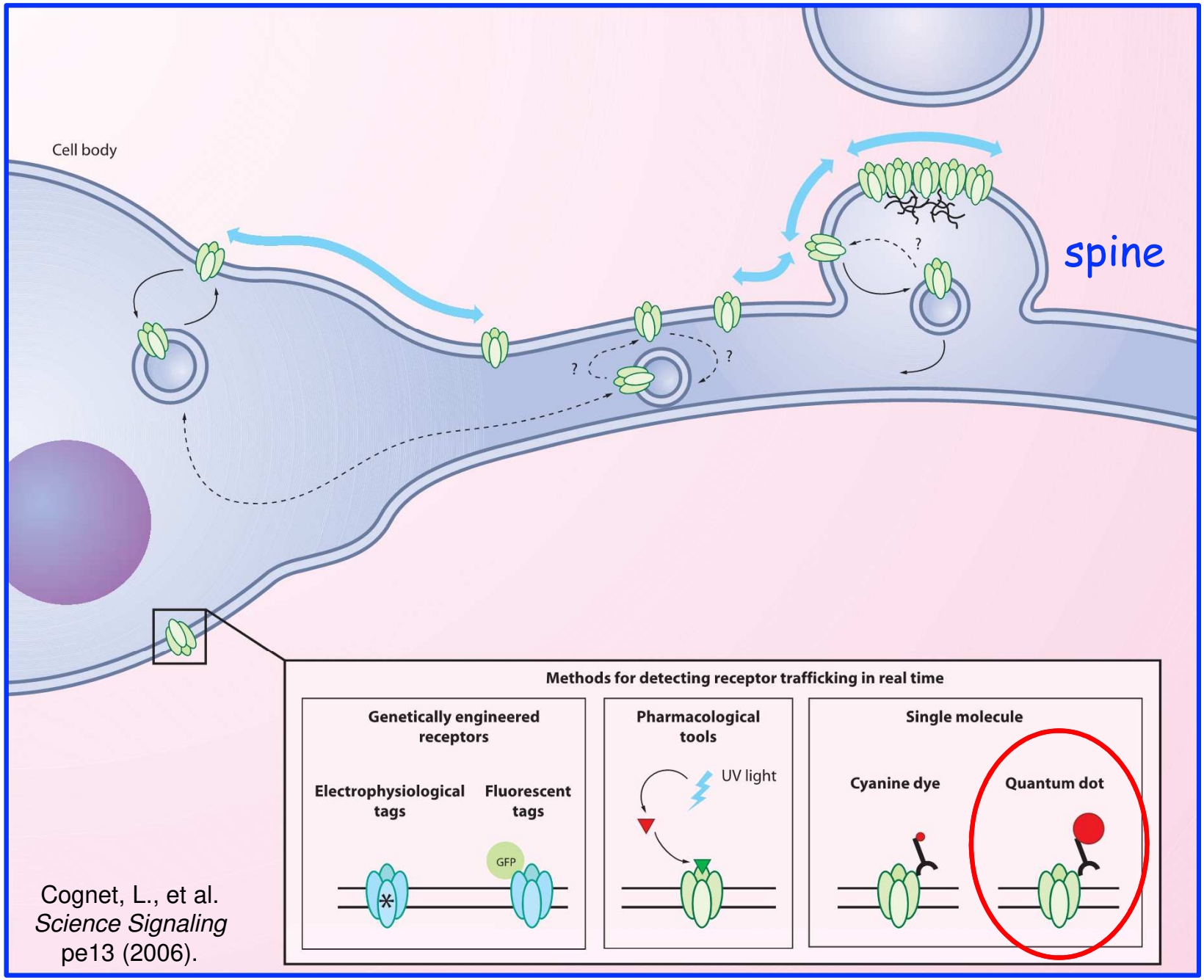
New Paradigm
Intraneuronal transport
Abnormalities



Traffic of synaptic receptors

Ionic channel-receptor AMPA & NMDA mediate **fast synaptic transmission**

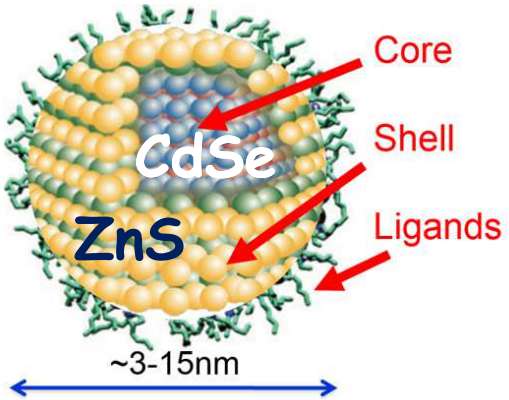
Modulation of their **concentration** at the spine ⇔ synaptic plasticity



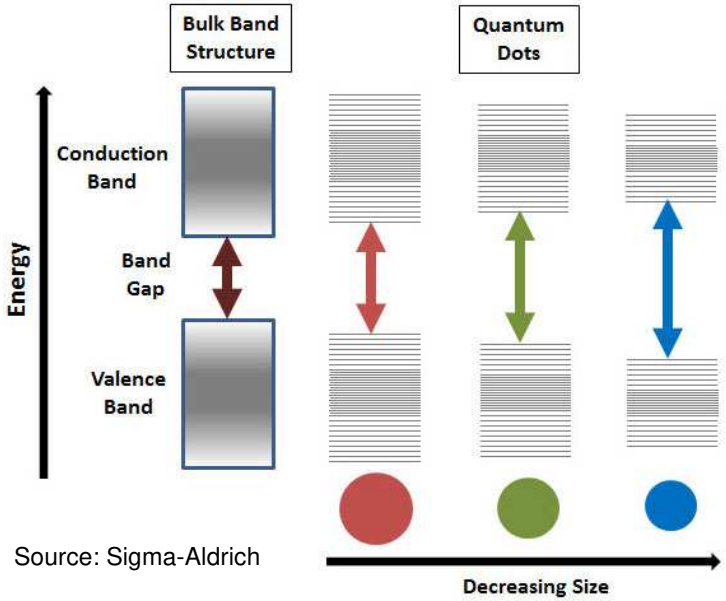
Cognet, L., et al. *Science Signaling* pe13 (2006).

Some fluorescent nanoparticles

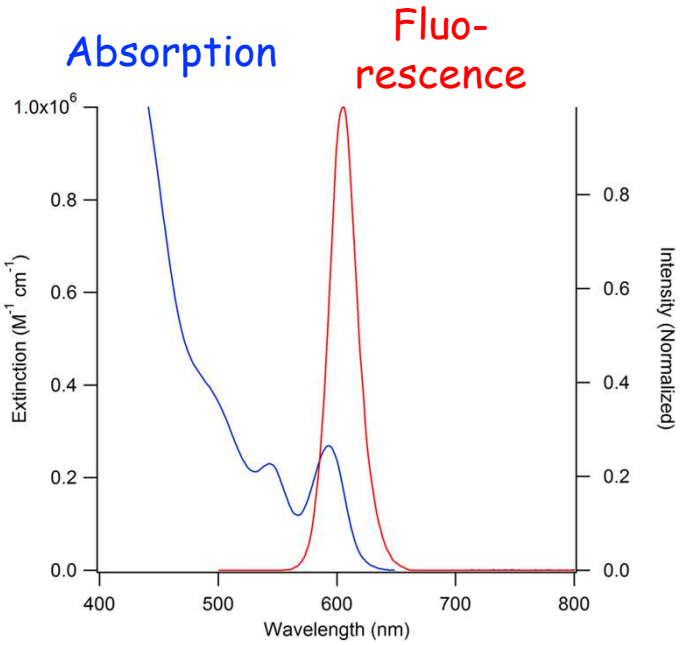
Quantum dots (QD)



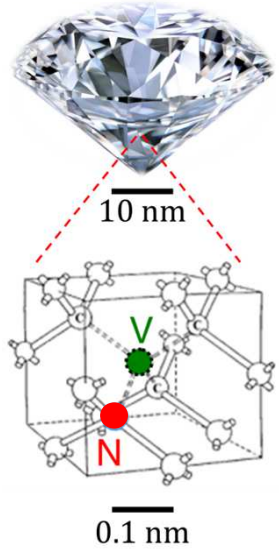
Core-shell
CdSe/ZnS

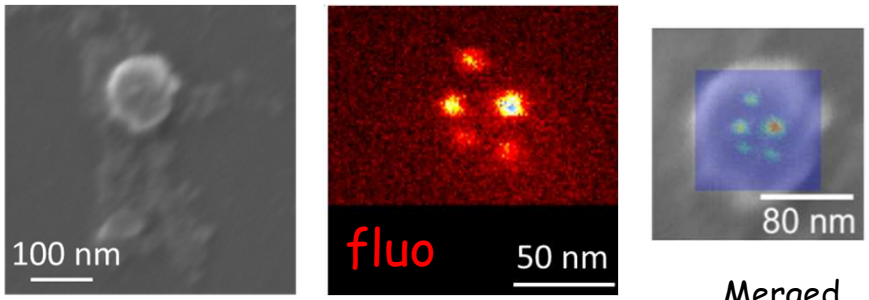


Source: Sigma-Aldrich



Fluorescent nanodiamonds (FND)

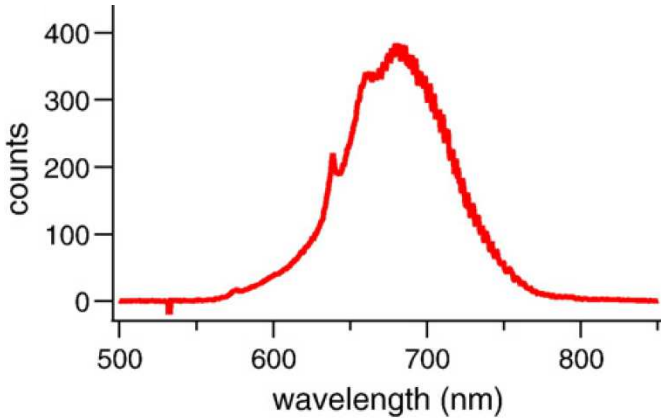




Merged

Arroyo, S. et al. *ACS Nano*, 2013

Faklaris et al. *Diam & Rel. Materials*, 2010



Perfectly photostable

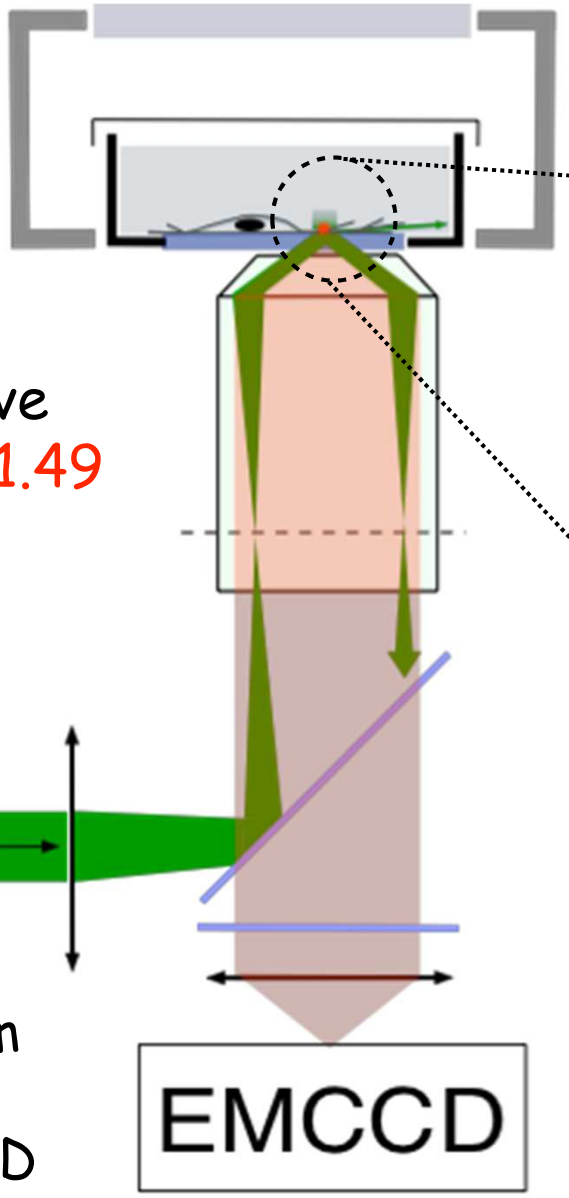
Live cell wide field fluorescence microscopy

incubating chamber
(37°C, 5% CO_2)

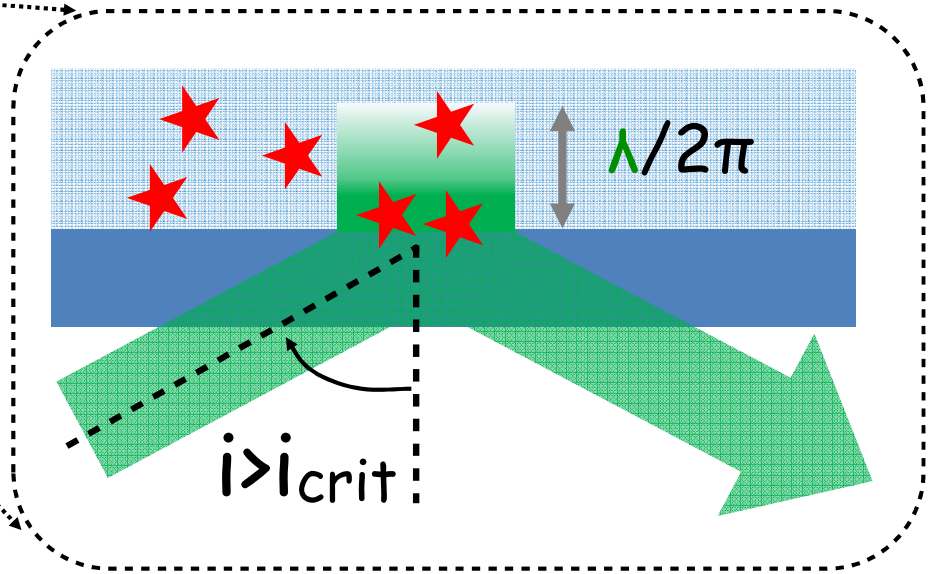
Objective
x100 NA=1.49

561 nm laser

high quantum efficiency cooled EMCCD detector



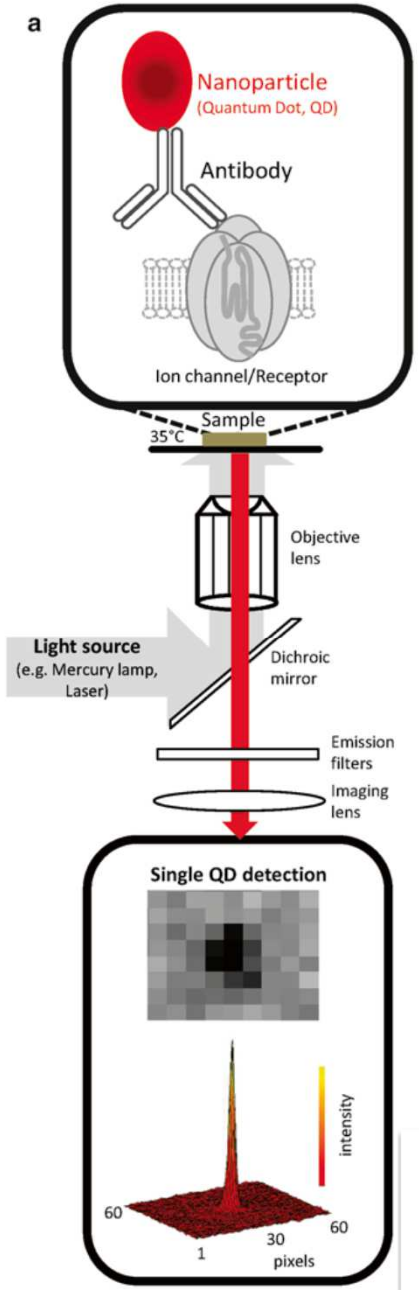
Total Internal Reflection Fluorescence Microscopy



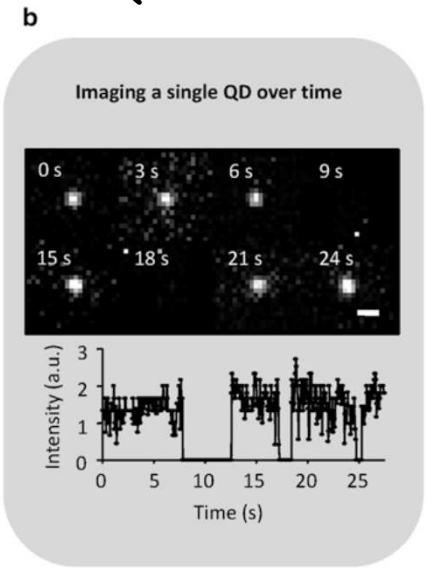
High signal/background
⇒ « fast » acquisition
(up to 50 ms/frame)

Measuring synaptic molecule trafficking

Single QD-receptor tracking

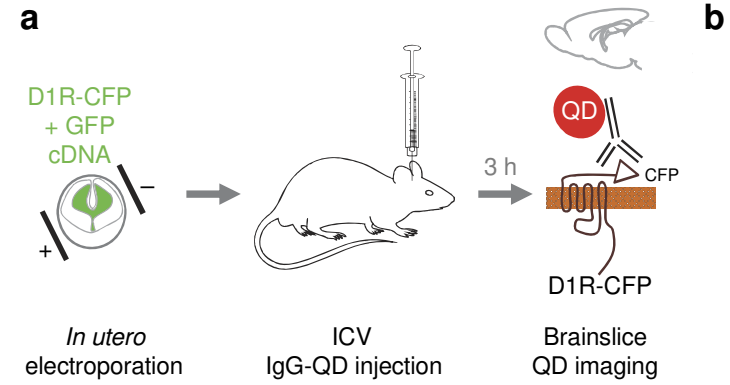


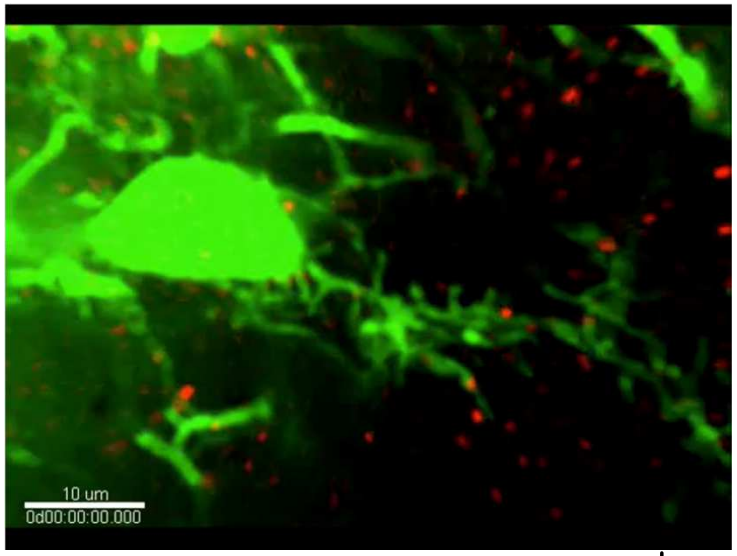
QDs blink



J. Varela *et al.*
Neuromethods **110** (2016)

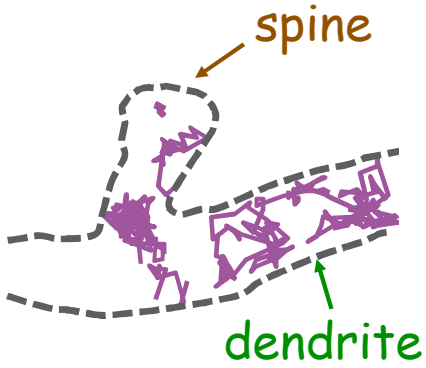
Localization with a
precision ≈10 nm

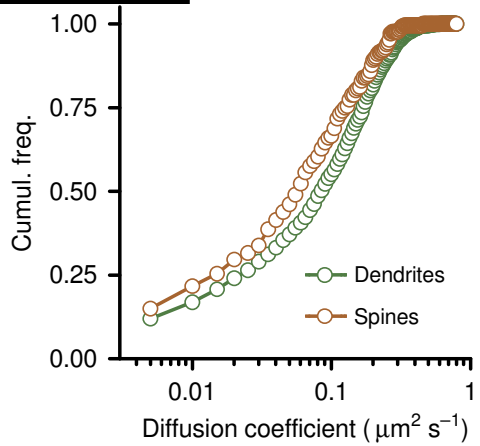




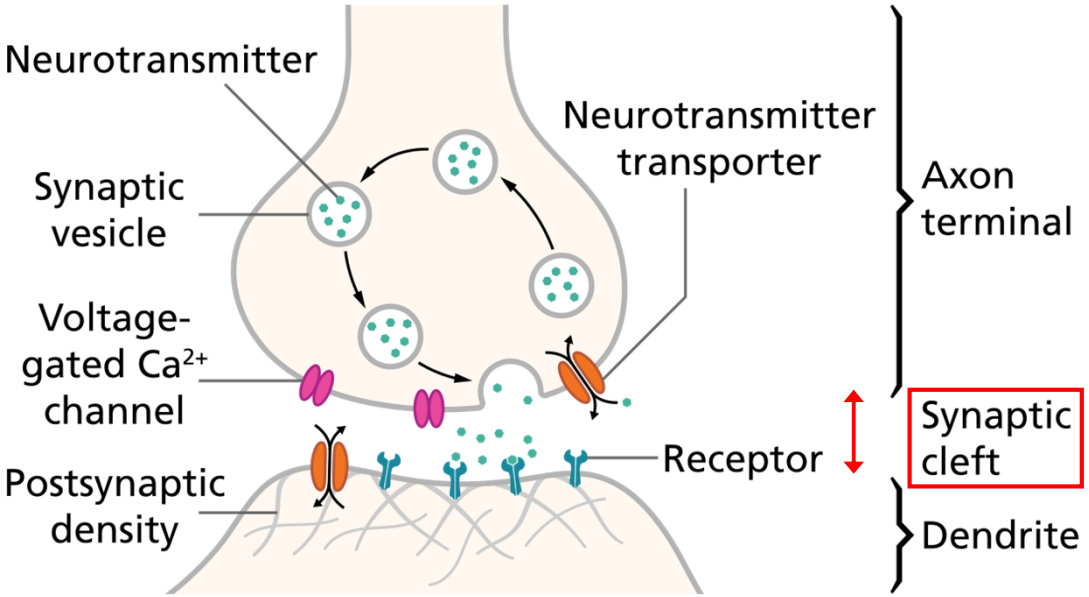
Varela, J.A., et al.
Nature Comm. **7**,
10947 (2016).

QDs
trajectories





Limitations and prospects



Source: Thomas Splettstoesser

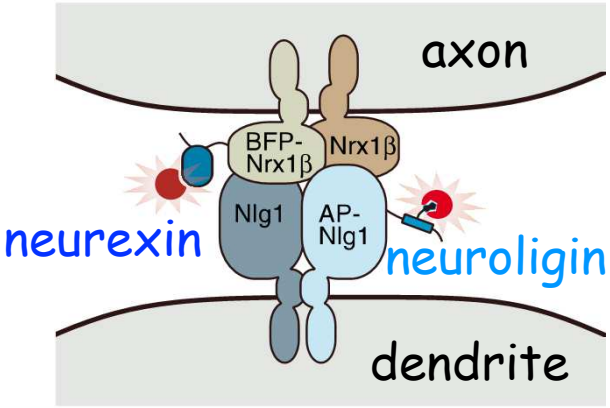
Gap ≈20 nm

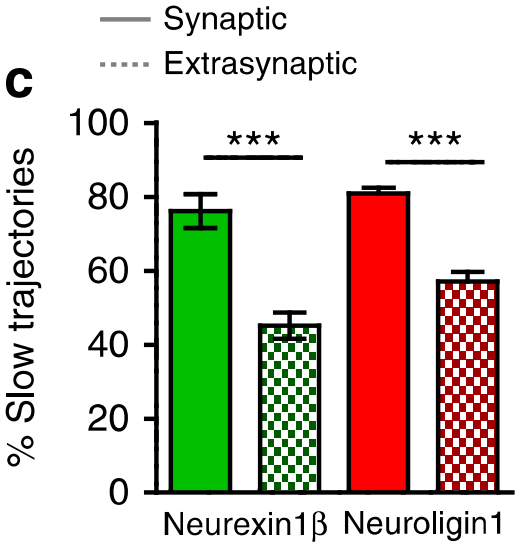
QD size ≈ 20 nm

➔ Limited accessibility to the cleft

Replace QD by dye

Study of adhesion molecule **dynamics** by Single molecule super-resolution imaging

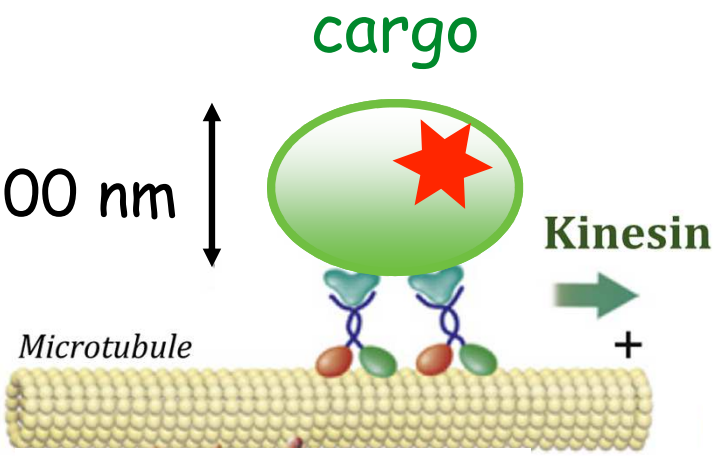




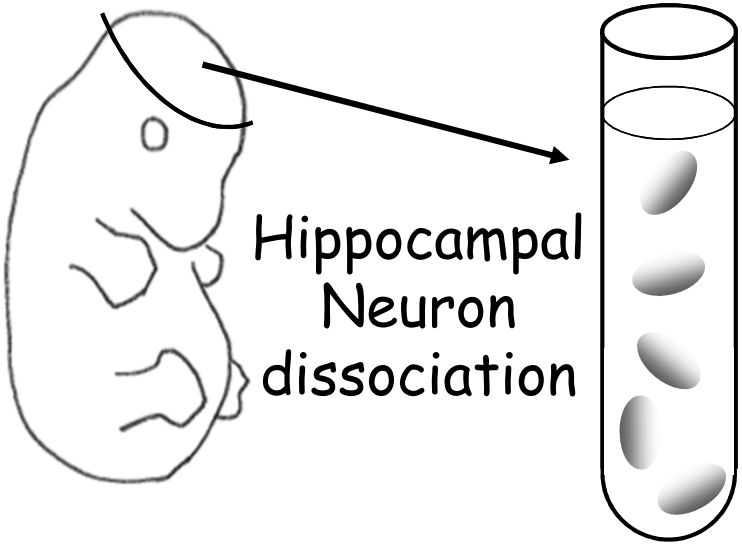
Chamma, I., *Nature Comm.* **7**, 10773 (2016).

Intraneuronal transport measurement

Labeling endosomal cargo with a single fluorescent nanoparticle spontaneously internalized



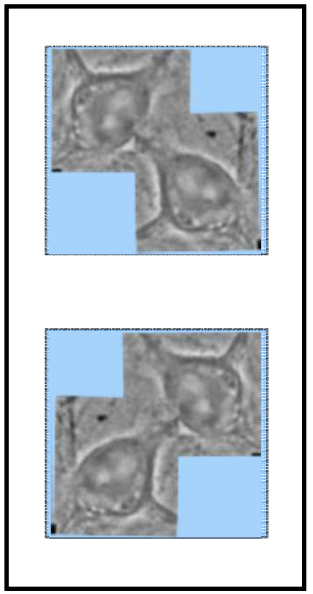
mouse embryo
(15.5 days after coïtum)



Hippocampal Neuron dissociation

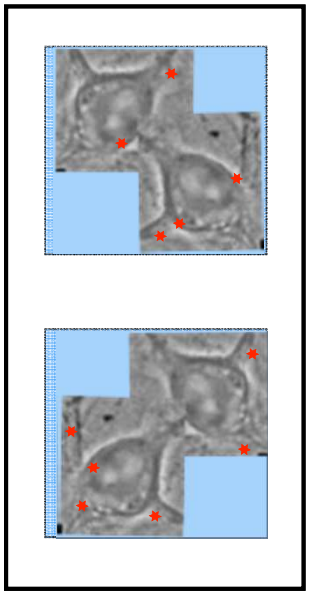
Cell culture on glass coverslip

wait 15-17 days



10 min **incubation** with QD or FND

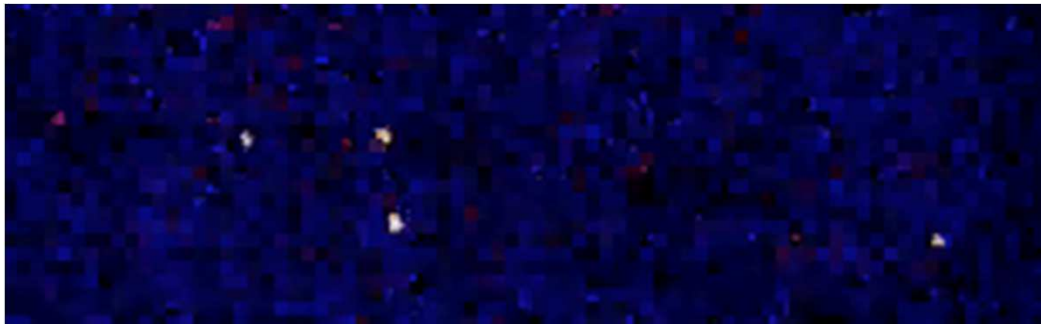
Day In Vitro 15



Measuring intraneuronal transport

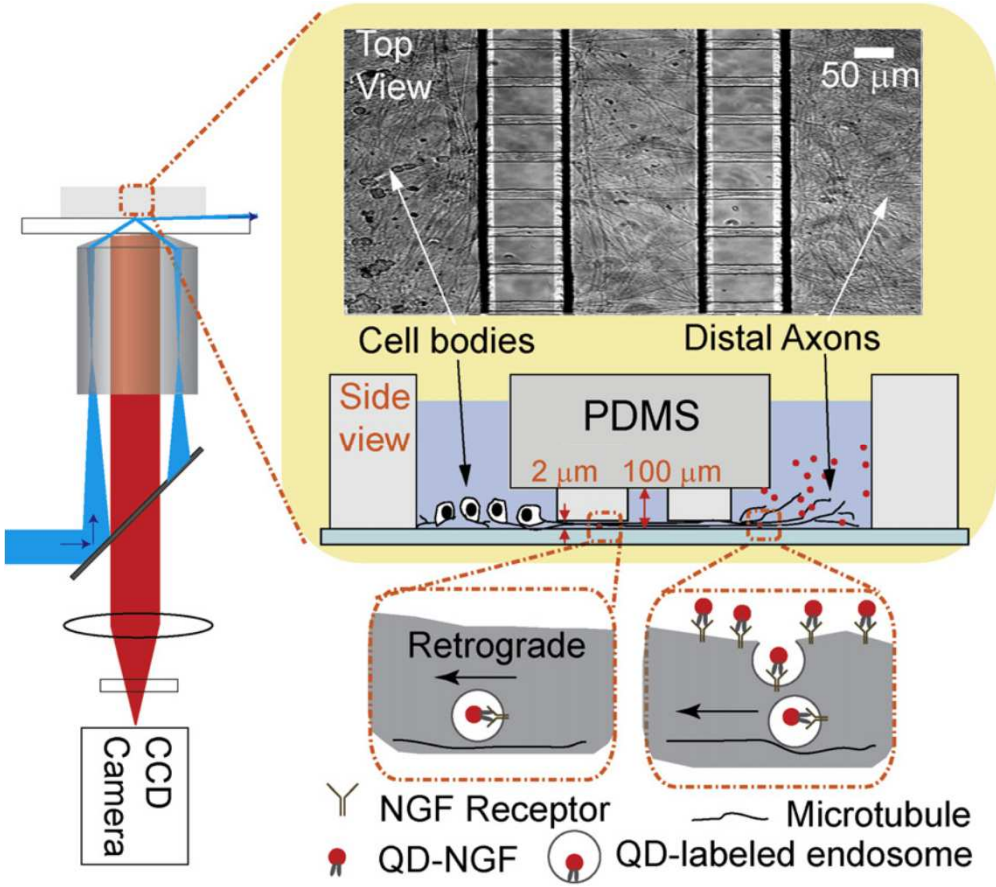
Intraneuronal transport measurement

Probing axonal retrograde transport by single QD tracking



Mudrakola, H. V., et al.
Structure **17**, 1433 (2009).

10 frames/s

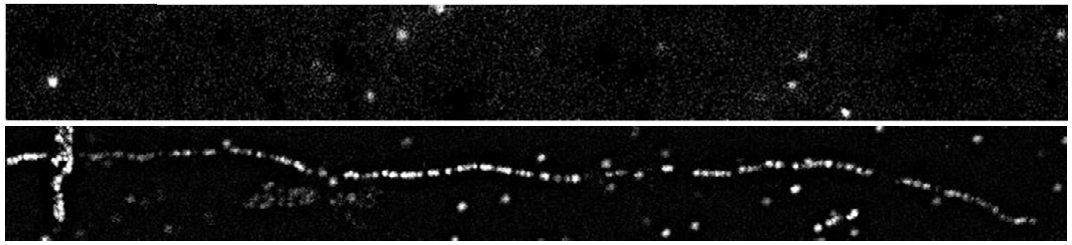


NGF: Nerve Growth Factor

QD blinking (1 QD per endosome)⇒ not precise tracking

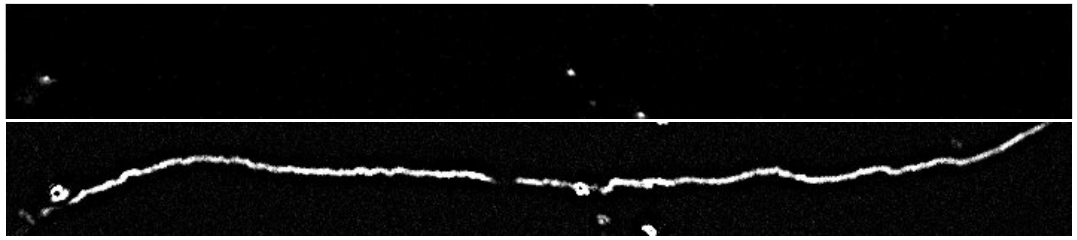
Quantum Dots vs FNDs for tracking

QDs



Strong **blinking**

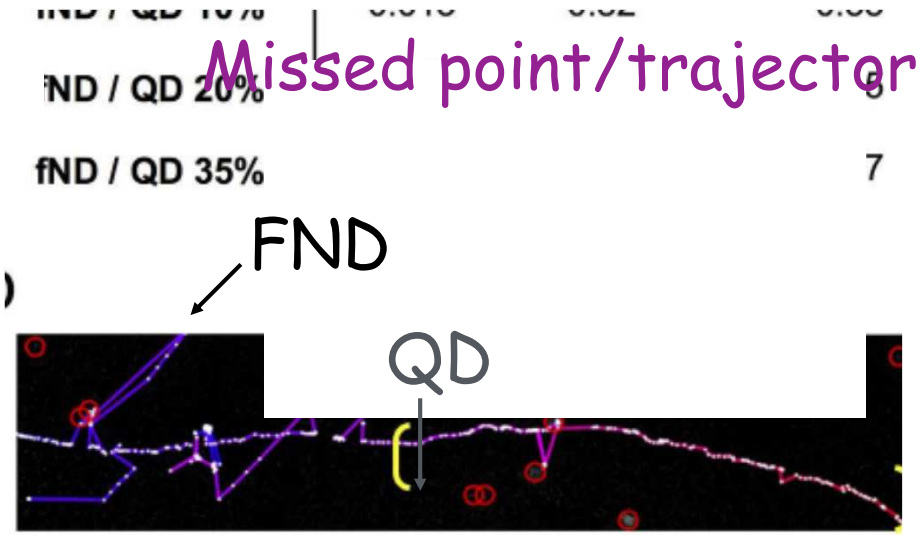
FNDs



Stable emission

Haziza et al.,
Nat. Nanotechnol. **12**, 322 (2017)

Missed point/trajectories



➔ artefacts in transport parameters measurements

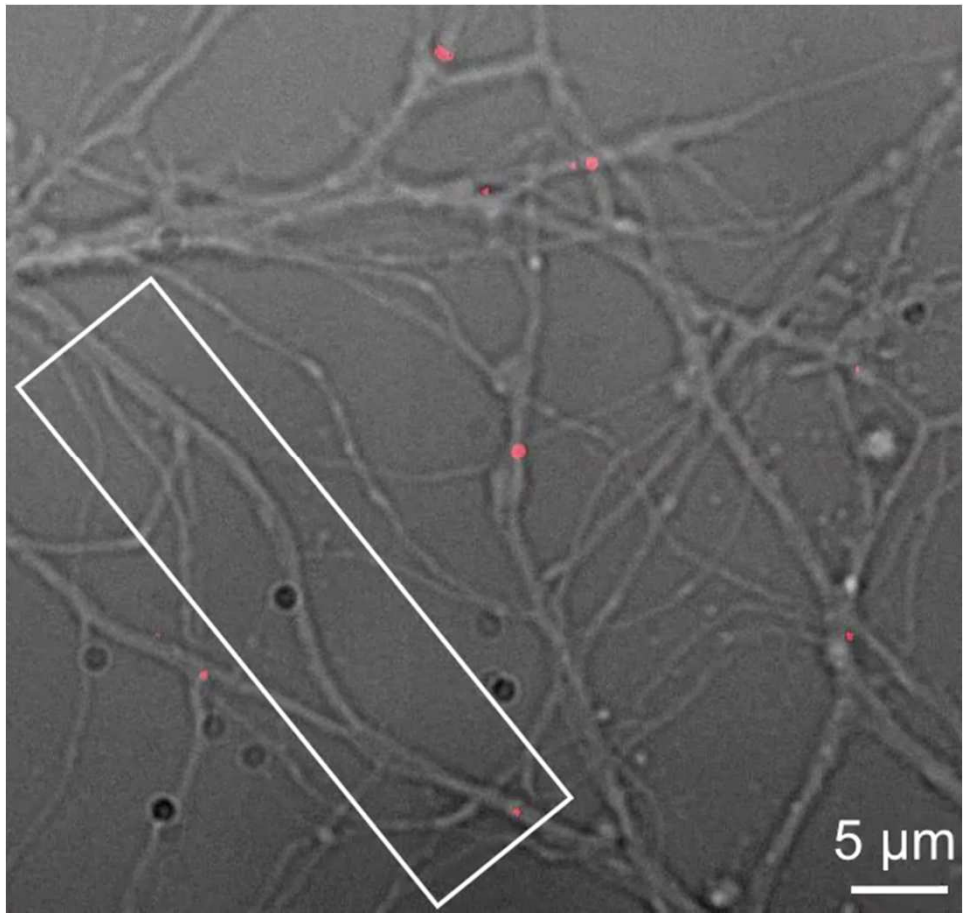
Nanodiamonds to track intra-dendritic transport

Hypocampal neurons + 30 nm sized FNDs

white light illumination (contrast) + laser (561 nm) illumination

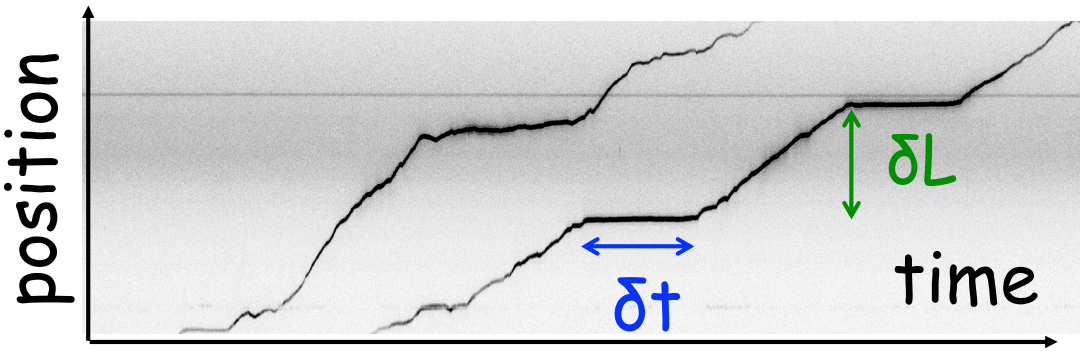
real time (20 frame/s)

soma



mostly **unidirectional** motion
with average speed ≈1.5 µm/s

Kymograph



GO & STOP motion

➔ parameters: velocity (slope),
pausing duration δt, run length δL

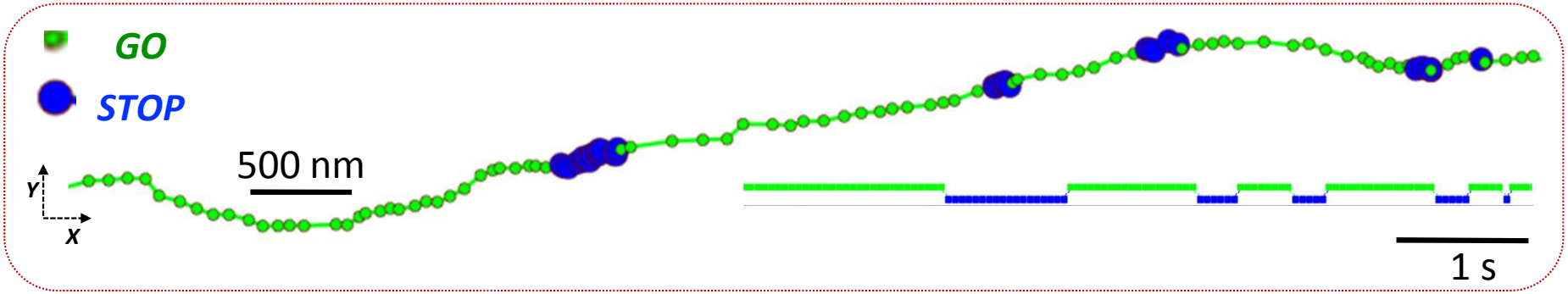
Haziza et al., *Nat. Nanotechnol.* **12**, 322 (2017)

Trajectories extraction

Tracking software: Icy (Pasteur Institute, Paris, France).
Probabilistic tracking (wavelet analysis): **fast** and **robust**

Open source
GPLv3

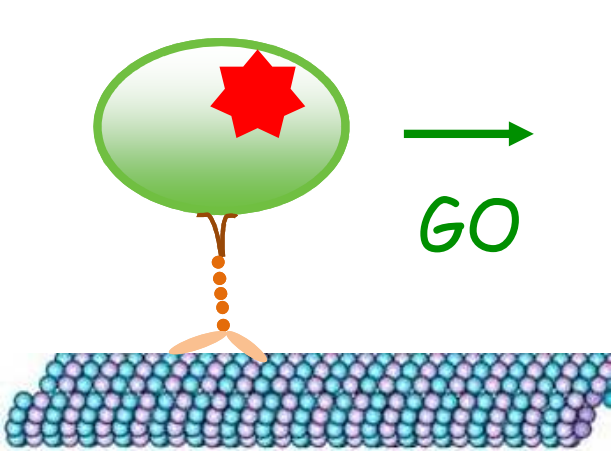
STOP (pausing) when frame-to-frame displacement <30 nm



⇒ Transport readouts

- velocity (in GO phase),
- processivity (duration between 2 stops)
- run length (between 2 stops)
- pausing duration
- pausing frequency

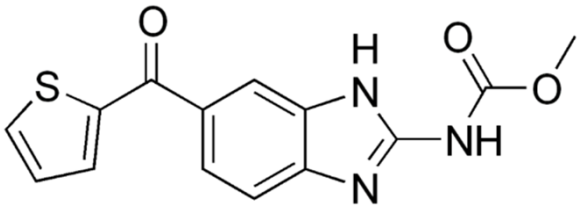
Transport monitored is Microtubule dependent

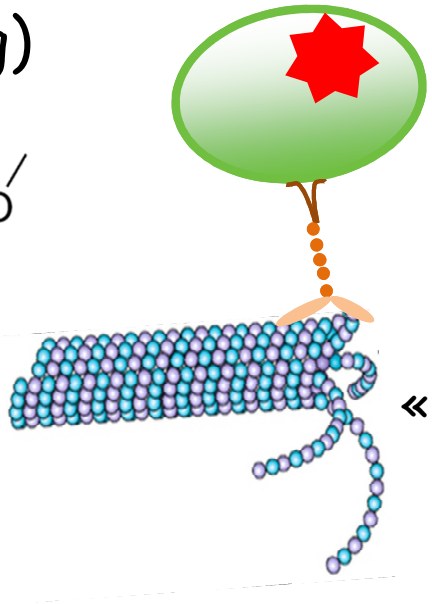


Microtubule
(polymer of tubulin)

GO

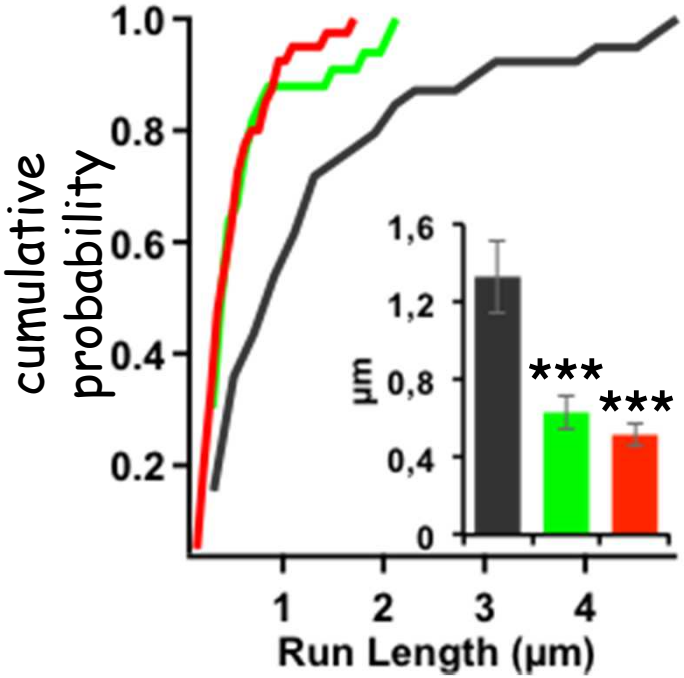
Nocodazole (drug)

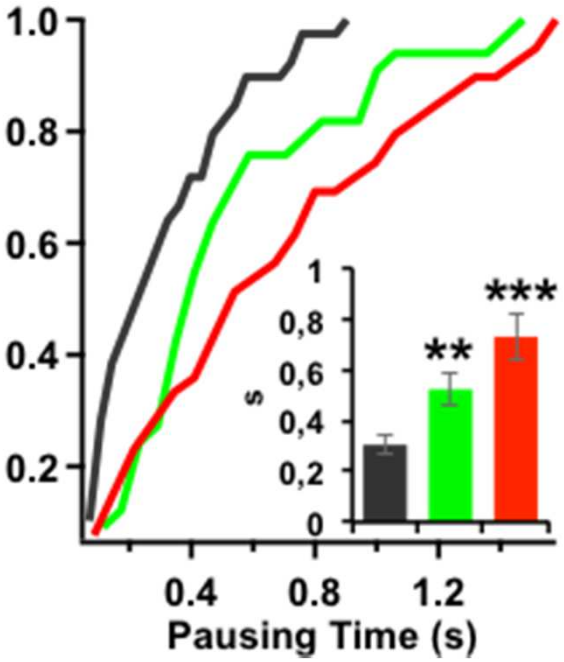




STOP

Depolymerization:
« catastrophe » event





Concentration

Control

2 nM

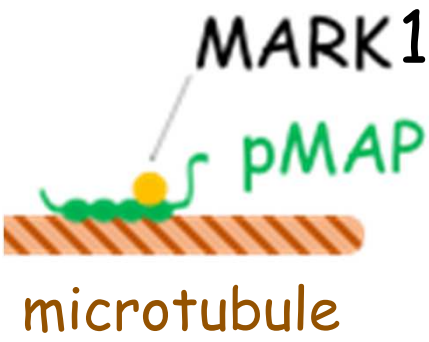
5 nM

⇒ nanomolar concentration sensitivity

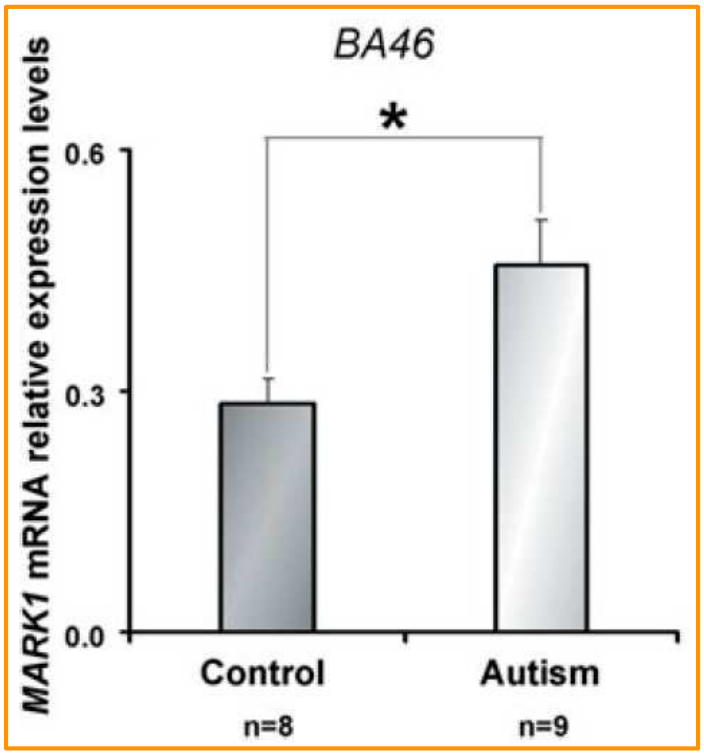
Haziza *et al.*, Nat. Nanotechnol. **12**, 322 (2017)

Application: functional impact of genetic risk factor

MARK1
Kinase of Microtubule Associated Proteins (MAP) —> phosphorylates the MAP (pMAP)



in brain of **Autism** patient (post-mortem)

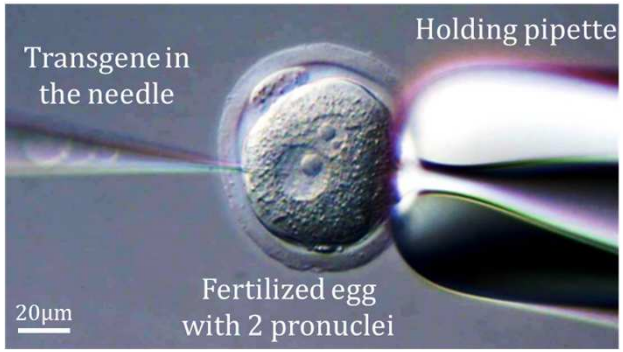


Over-Expression found : **+38%**

Maussion et al, *Hum. Mol. Genet.* **17**, 2541 (2008)

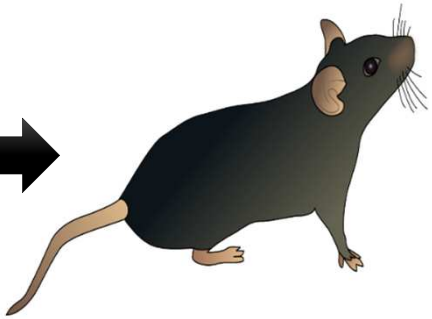
Subtle change in gene expression in brain of patients

Transgenic mouse to model Human Brain diseases



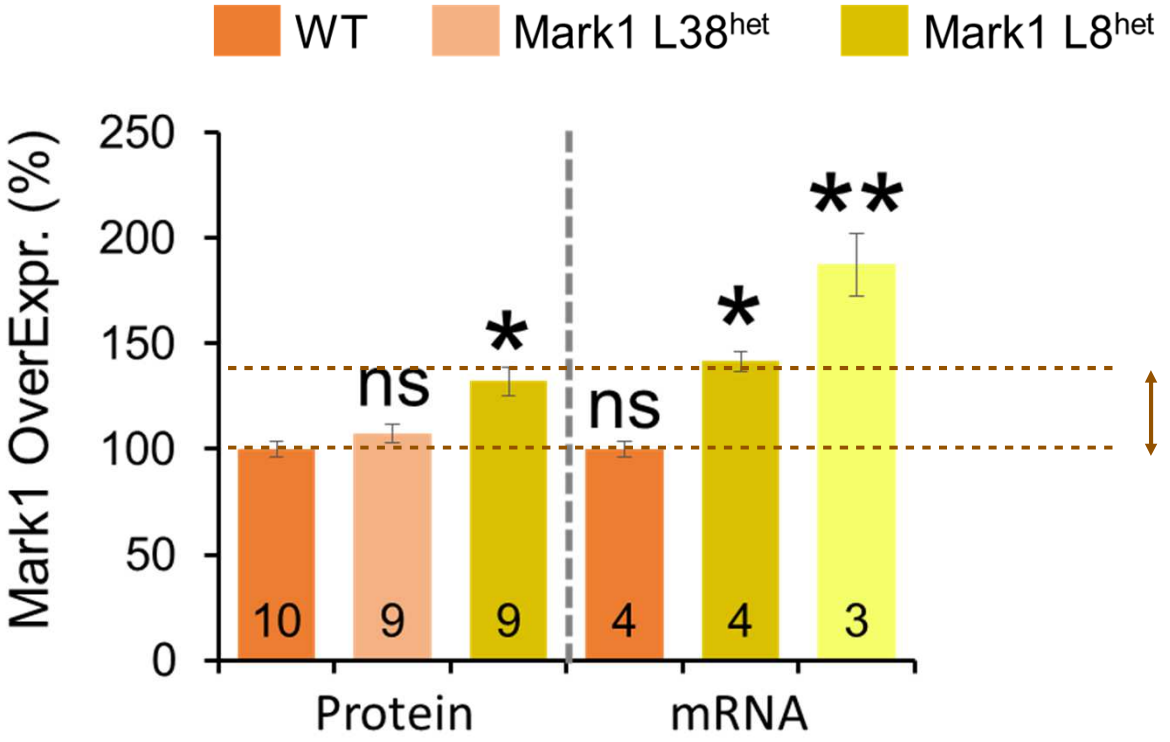
Collab. SEAT, CNRS UPS44, Villejuif

Mark1 mouse



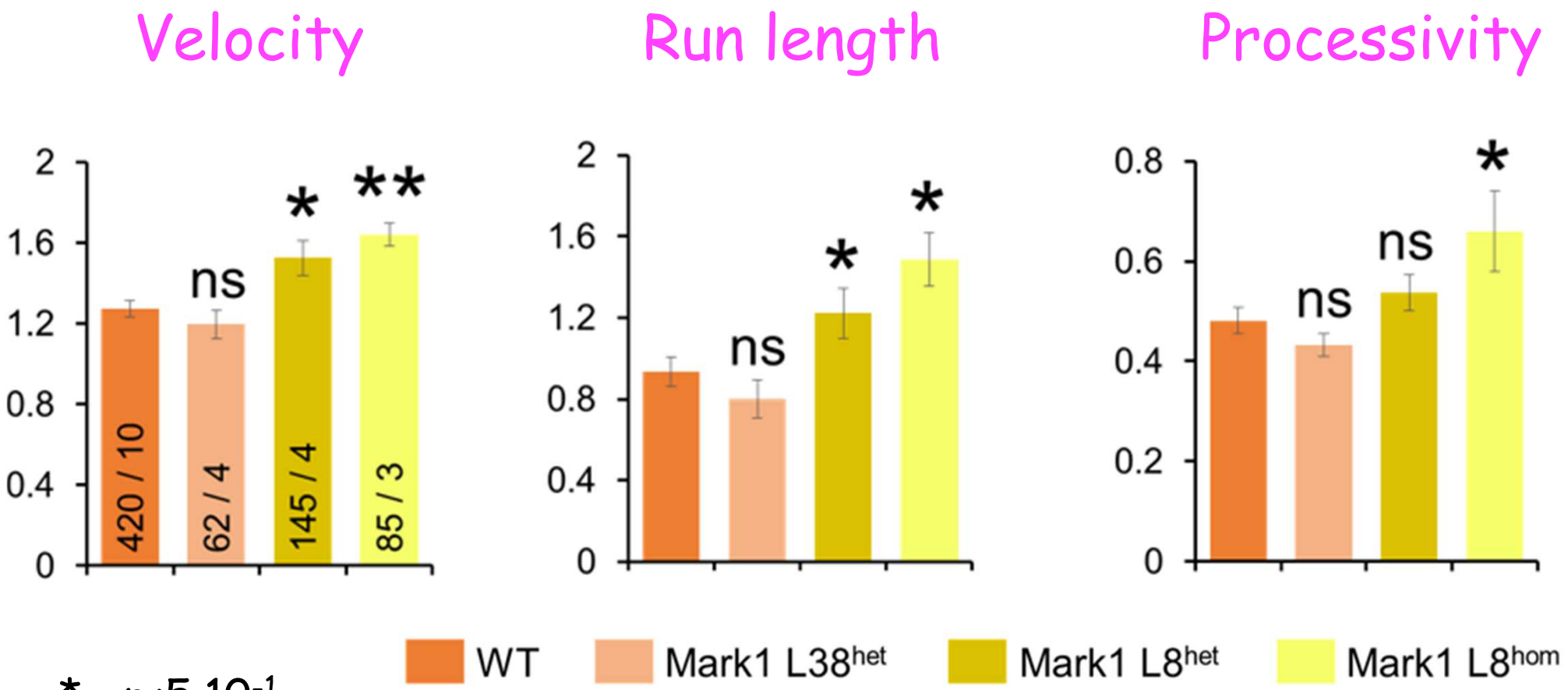
3 different lines

Wild Type (WT)



Mark1 Over-Production ≈30%

Common Variant *MARK1* / 1st order subtle perturbation



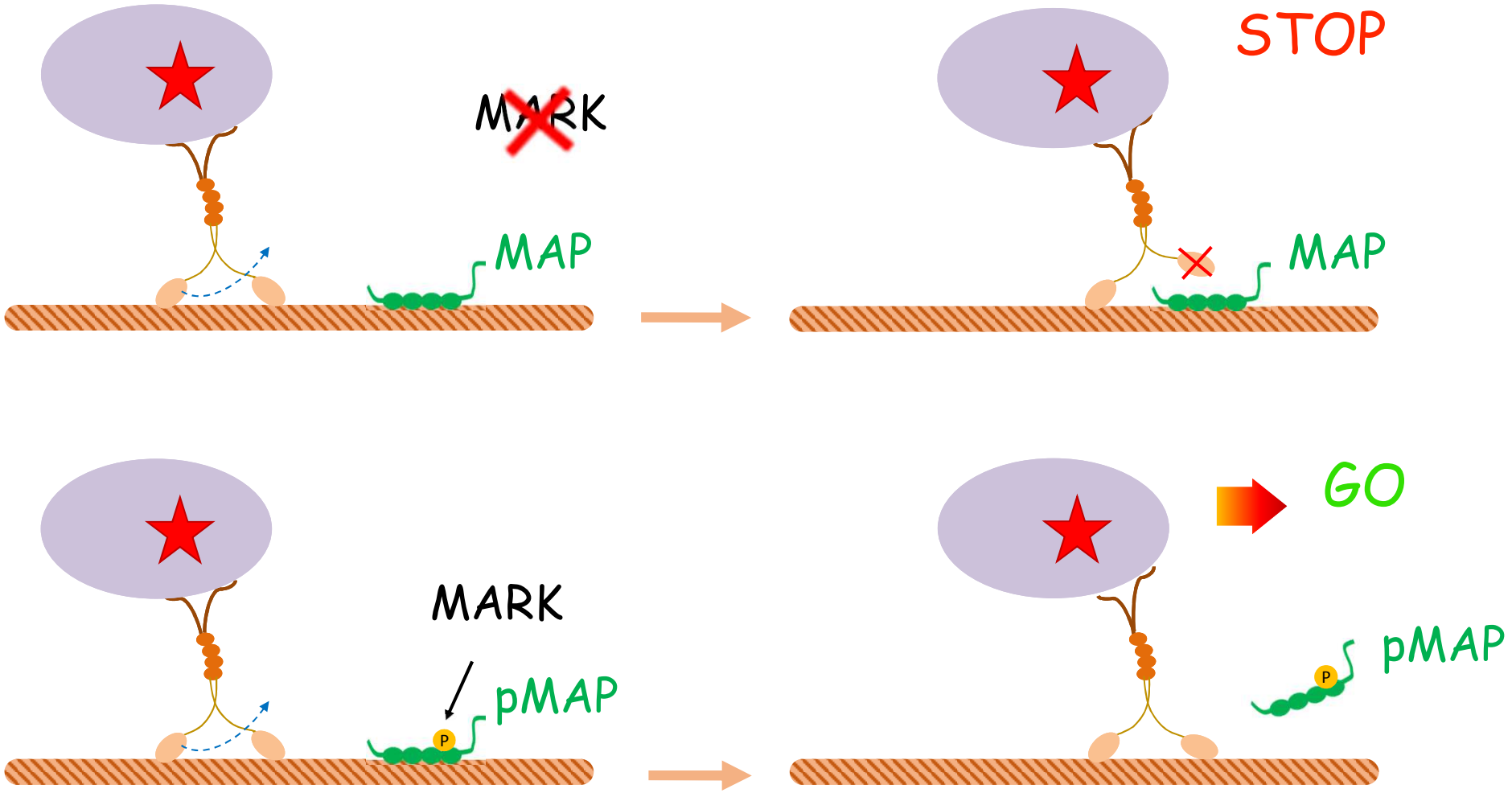
* $p<5.10^{-1}$
** $p<10^{-2}$
ns: non significant

⇒ significant changes of transport parameters in gene dosage manner

Haziza et al., *Nat. Nanotechnol.* **12**, 322 (2017)

« Mechanistic » interpretation

MAP = a **roadblock** of microtubule based transport



Inspired from Mandelkow et al, *JCB*, 2004

Prospects: probing neuronal network

Functional investigation of a neuronal network (brain slice)

(intact inter-neuron connections)

Complementary parameters

- calcium flux & membrane voltage sensing
- intraneuronal transport with FNDs (deep, 3D)

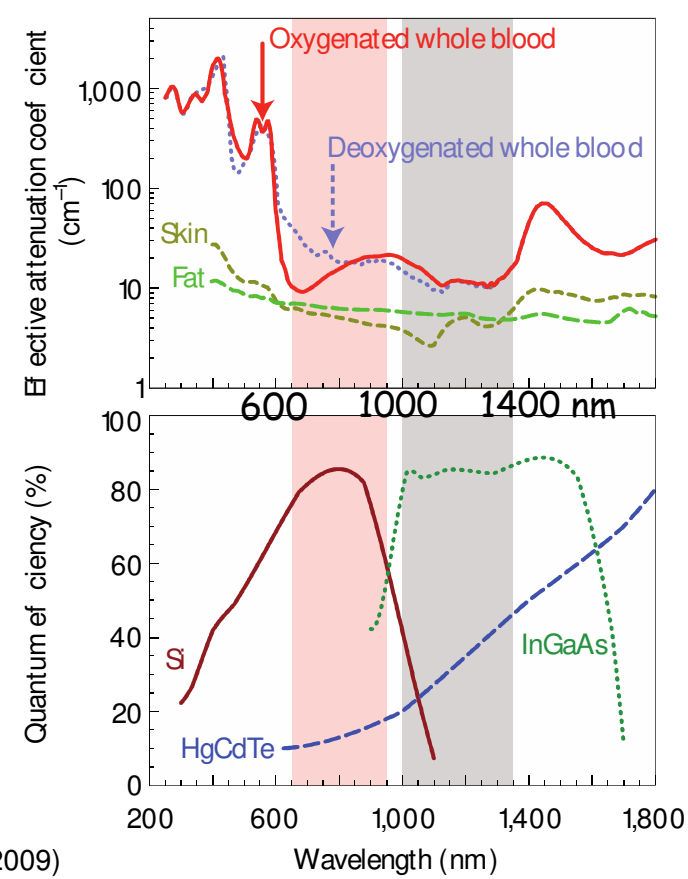
⇒ **fast** (100 frames/s) **background free** microscopy

Two-photon excitation microscopy

⇐

✓ Genetically encoded fluorescent probe (Ca^{2+}, V)

but NV has a **low 2-photons absorption** cross-section…



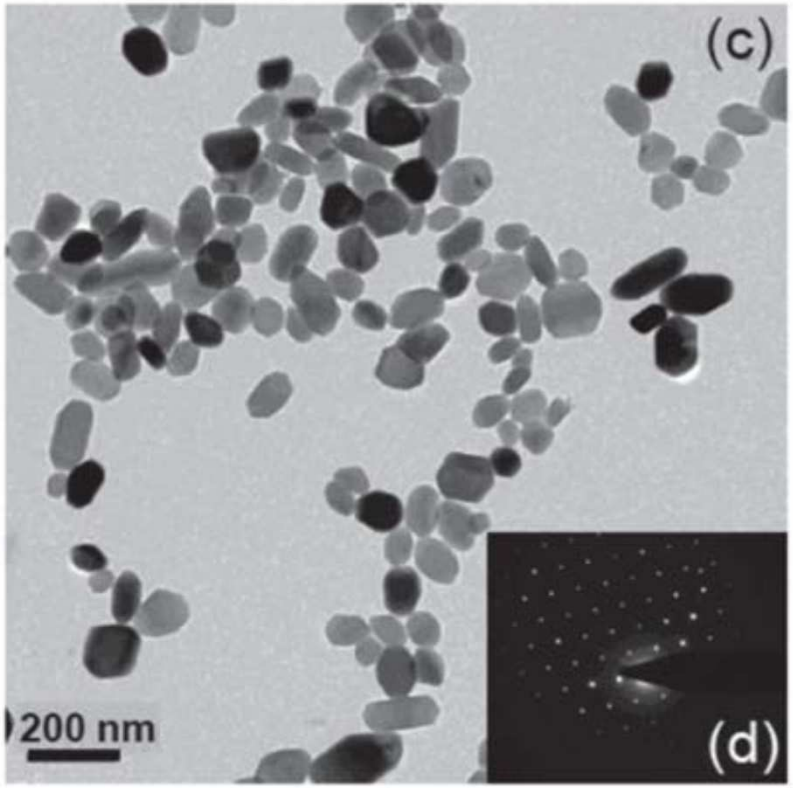
A. M. Smith et al. *Nat. Nanotechnol.* **4**, 710 (2009)

Background free imaging: alternative **nano labels**

nano-KTP ($KTiOPO_4$) ⇒ Second Harmonic Generation (ω+ω→2ω) [SHG]

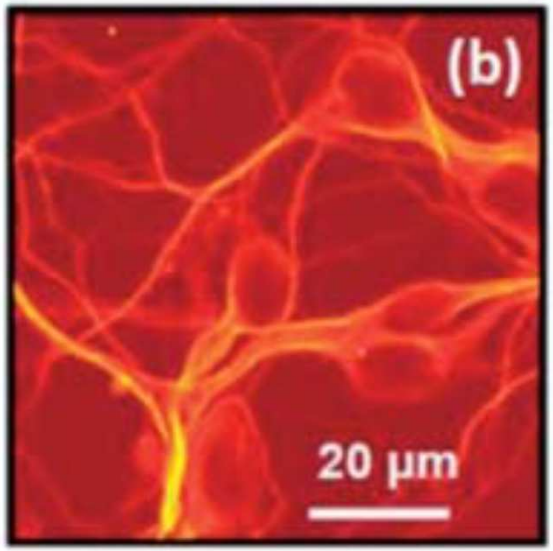
non linear labels

λ_{exc}=800-1300 nm / λ_{det}= 400-650 nm

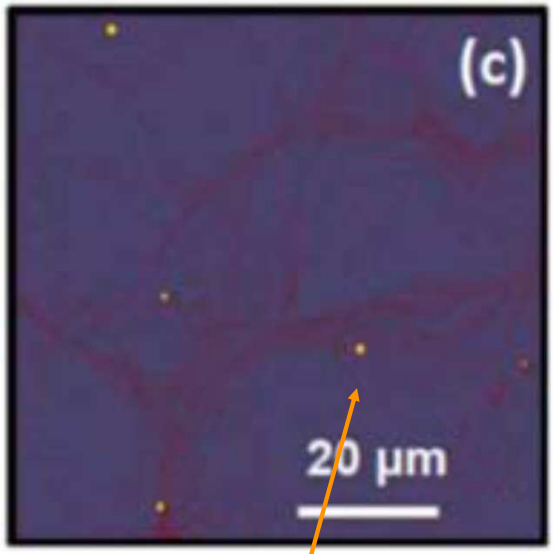


Pulsed infrared laser excitation (raster scan)

2-photon fluo



SHG



TRITC labeled neurons

nano-KTP

L. Mayer et al., *Nanoscale* **5**, 8466 (2013)

Toward a multimodal (fluo-SHG) nanoprobe: **nano-SiC**

3C-SiC

non centrosymetric

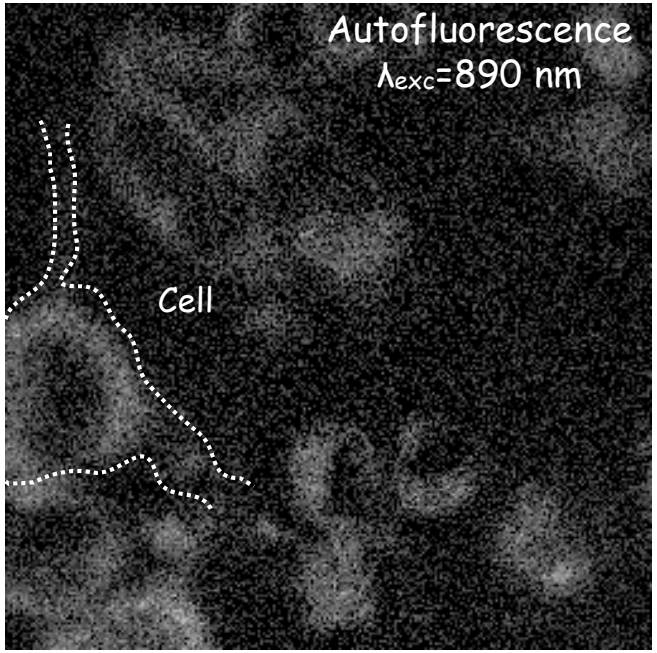
$$I_{2\omega} \propto \omega^2 d_{\text{eff}}^2 I_{\omega}^2$$

$d_{SiC} \approx 17$ pm/V

$d_{KTP} \approx 2$ pm/V

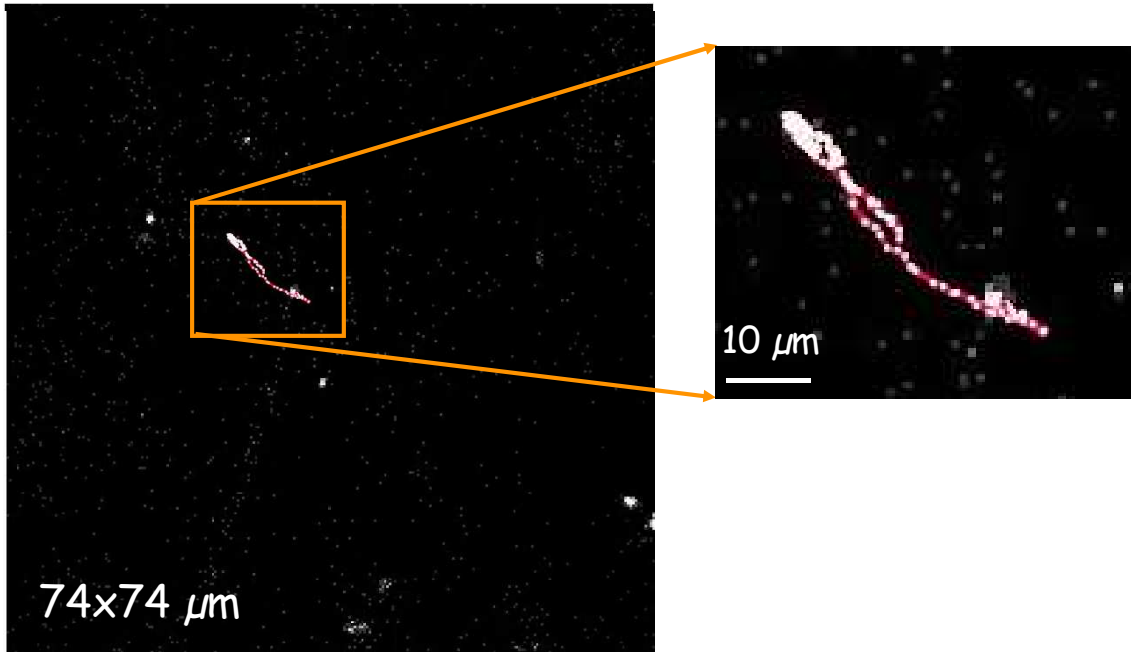
$$\Rightarrow \quad \boxed{I_{2\omega}^{\text{SiC}} / I_{2\omega}^{\text{KTP}} \approx 70}$$

size≈80 nm nano-SiC internalized in neuronal cell lines (SH5Y)



Tracking SHG response

(λexc=890 nm, detection filter 448±20 nm)



5 frames/s

NIR Fluorescence: embedded vacancy related defects V_{Si}^- ($\lambda_{em}^{max} \approx 900$ nm) ….and possibly $(V_C V_{Si})^0$ ($\lambda_{em}^{max} \approx 1150$ nm) and $N_C V_{Si}$ ($\lambda_{em} \approx 1250$ nm ?)

Summary

- **Single particle tracking** provide access to fast molecular dynamics in neurons
- QD are bright emitters but suffer from blinking
- FND **stable emission** allows a precise quantification of intraneuronal transport, revealing subtle genetic changes
- In thicker tissue (brain slices), **alternative nanolabels** compatible with NIR excitation are necessary:
  - SHG or/and fluorescence of **nano-SiC**
  - NIR emitting QD
  - Carbon nanotubes (λ_{em}≈1000 nm)
  - …

Acknowledgements

ENS Paris Saclay & INSERM U894

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- Xuan Loc LE

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- Aude-Marie LEPAGNOL-BESTEL
- Yan LOE-MIE
- **Michel SIMONNEAU**

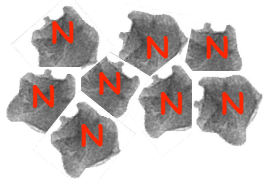
Academia Sinica (Taiwan)

- Feng-Jen HSIEH
- Huan-Cheng CHANG

Thanks for your attention

A perfectly photostable label: Fluo. Nanodiamond

Production of bright FND

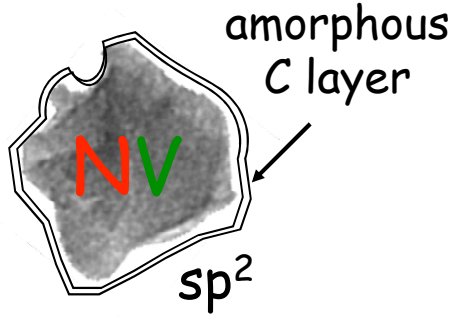


HPHT nanodiamonds (20-50 nm)
$[N_s^0] \approx 100$ ppm

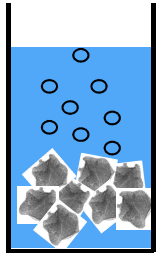
high energy (2-15 MeV) particle beam irradiation

pellet of NDs
(between Al or Co foils)

800°C vacuum annealing (2h)

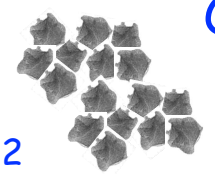


cleaning by strong **oxydation**



H_2SO_4 + HNO_3

+



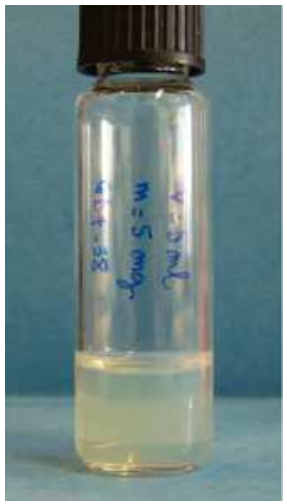
T≈500°C

water rinsing
+ water suspension

Mean value of
15 NV/FND

fND aqueous suspension

1 mg/mL
FND mean size≈30 nm



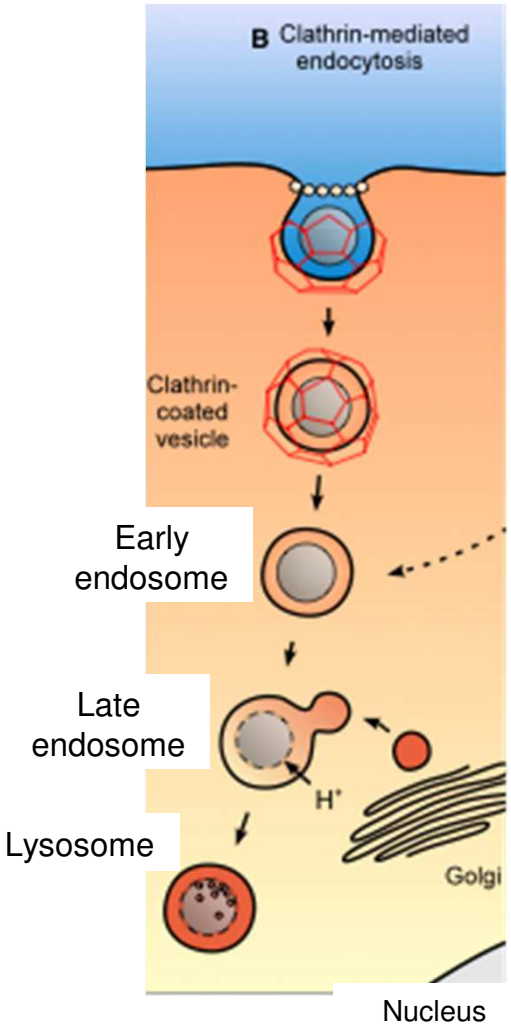
fNDs-labelled cargoes are **not Lysosomes**

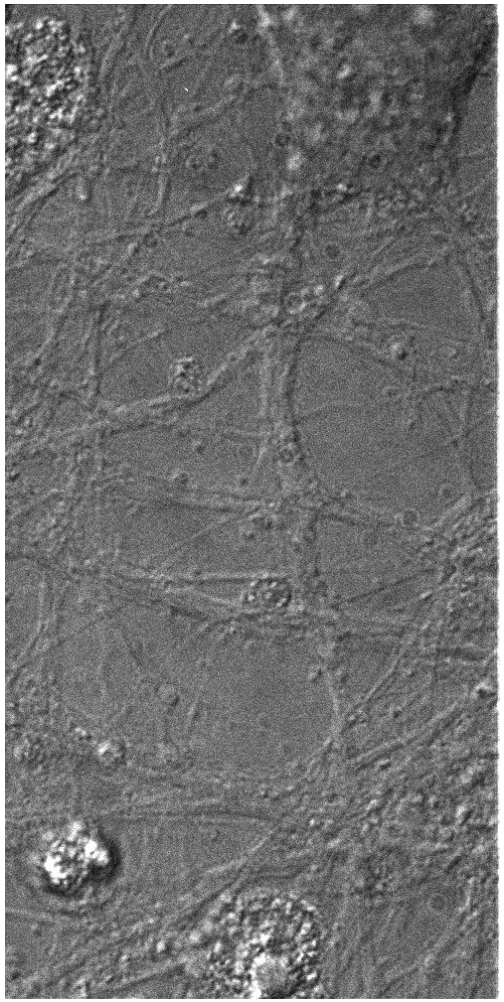
Simultaneous recording (DualView)

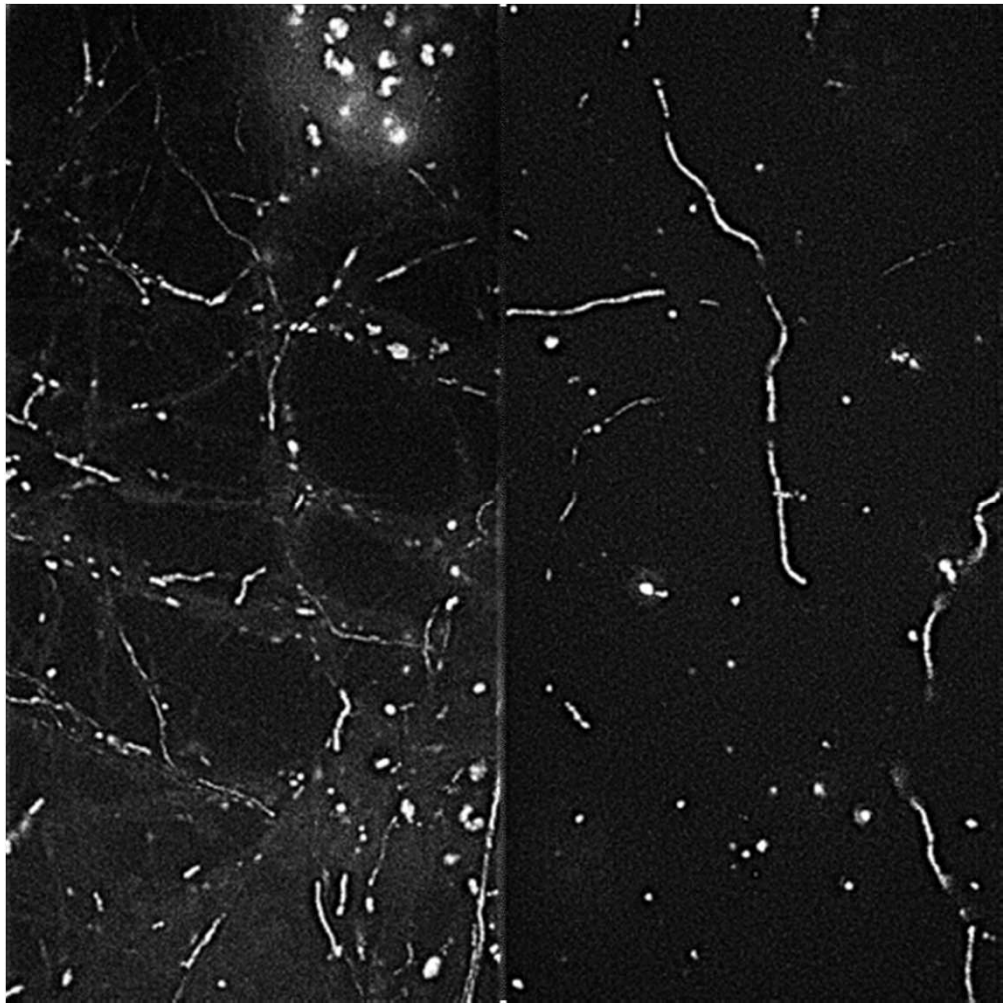
Lysosomes
LysoTracker

fNDs
(size≈30 nm)

DIC 100x





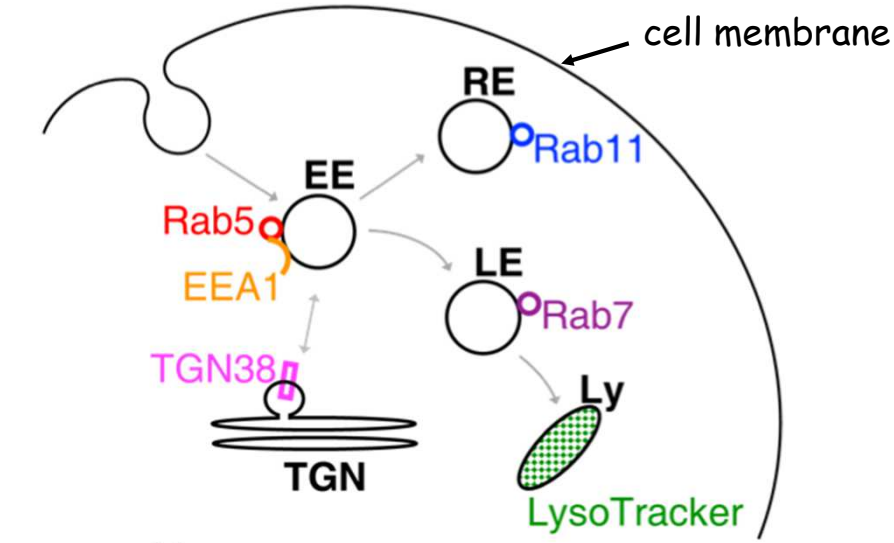


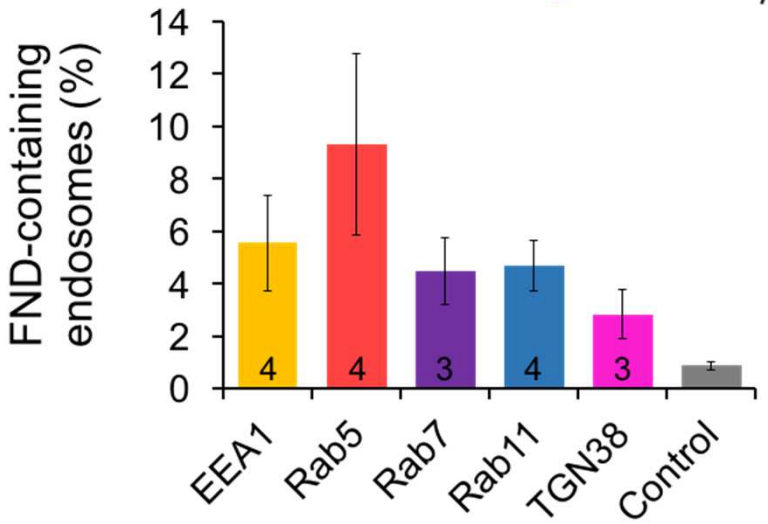
After 1h30 fNDs incubation **absence of colocalization**

Hillaireau, H. Couvreur, P. Cell. Mol. Life Sci. 2009.

Colocalization of fNDs with endosomal compartments

Endosomal markers: EEA1, Rab5, Rab7, Rab11
+ Trans Golgi Network protein (TGN38)

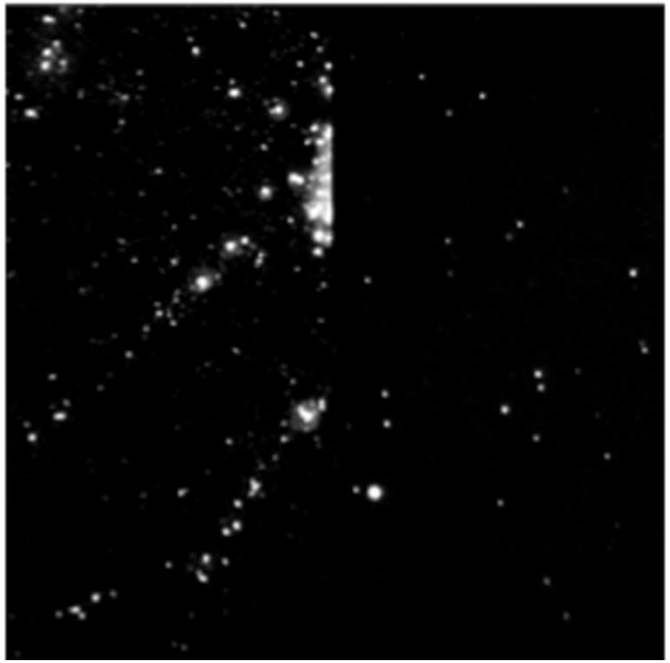




Immunofluorescence

Rab5

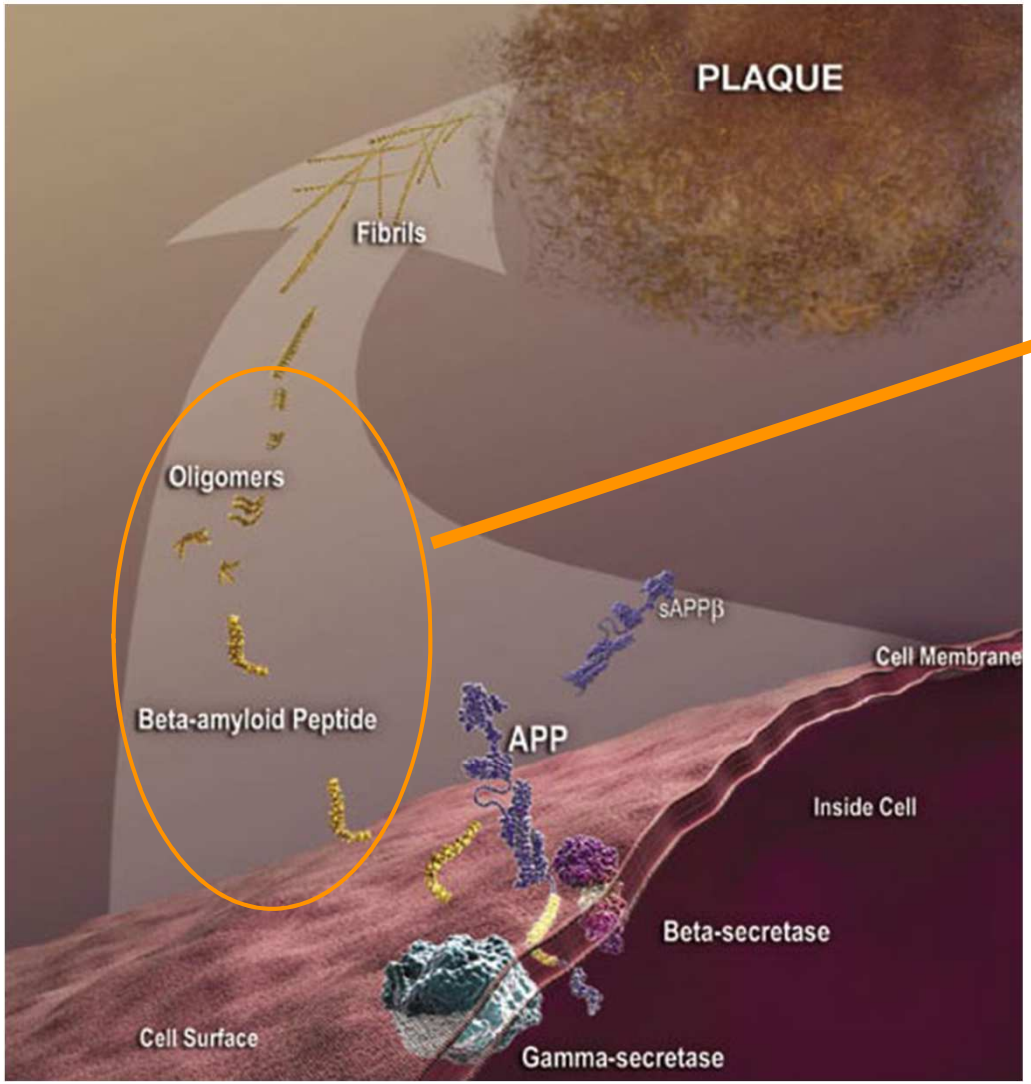
488nm Alexa488 561nm fND



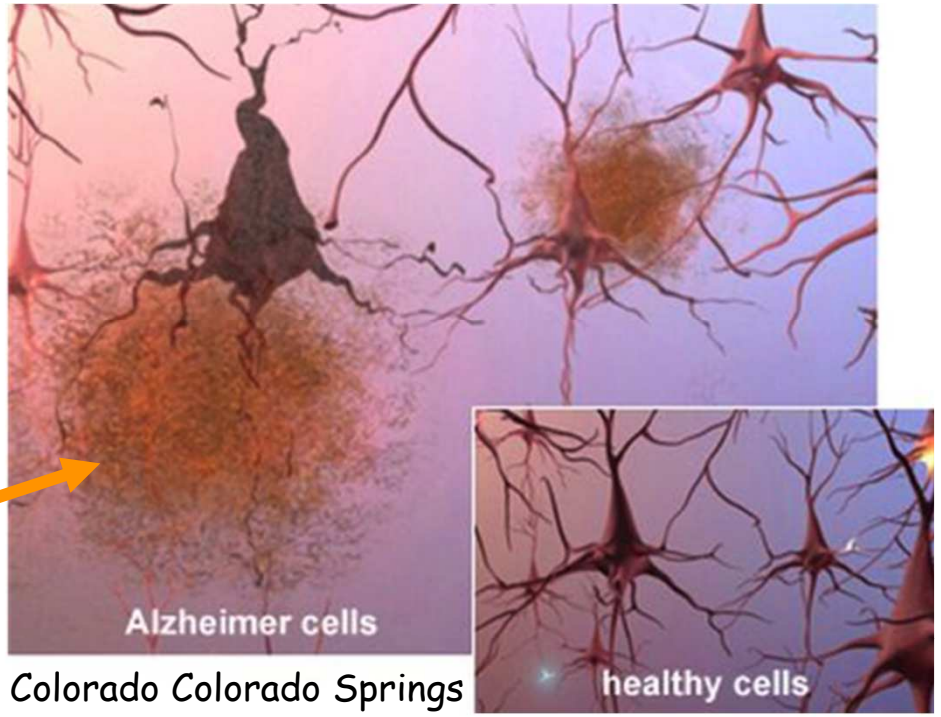
dualview

Pharmacological model of Alzheimer disease

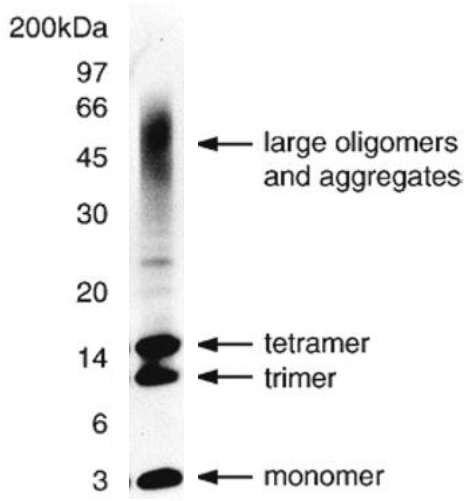
Beta amyloïd peptide Aβ42



National Inst. on Aging, NIH



Univ. Colorado Colorado Springs

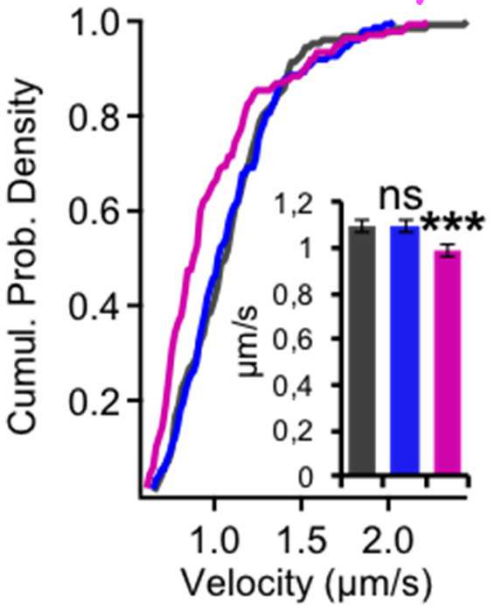


Adapted from Stine et al, *Methods Mol Biol* **670**, 13–32 (2011)

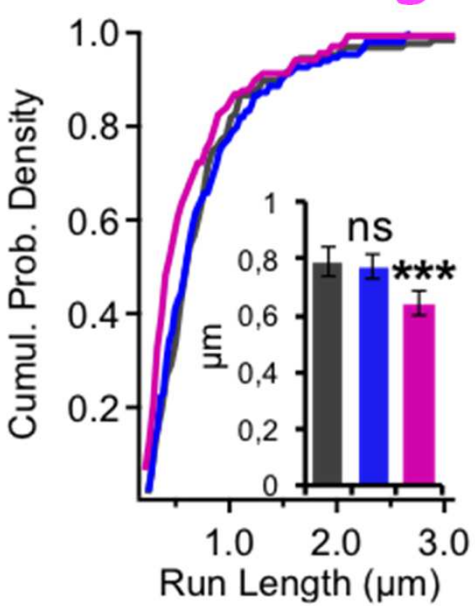
Purification
B. Allinquant (INSERM)

Impact of $A\beta$ on intraneuronal transport

Velocity



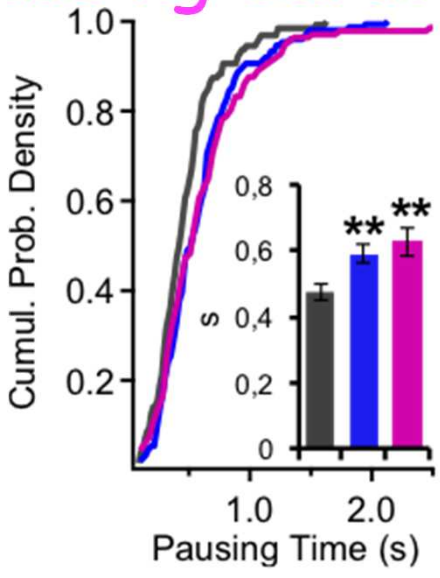
Run length



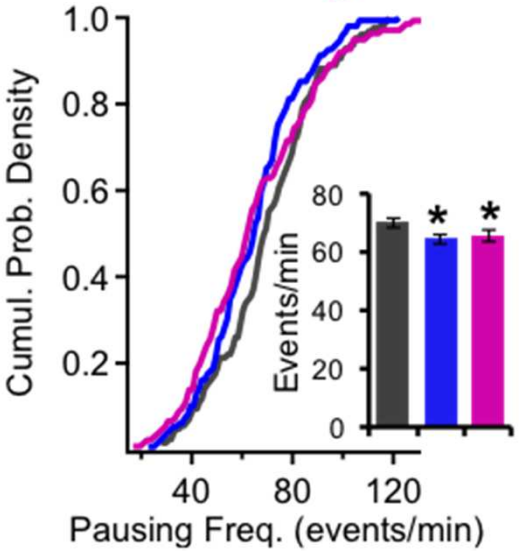
Beta amyloïd peptide [$A\beta 42$]

Ctrl
0.5µM
1µM

Pausing duration



Pausing freq.



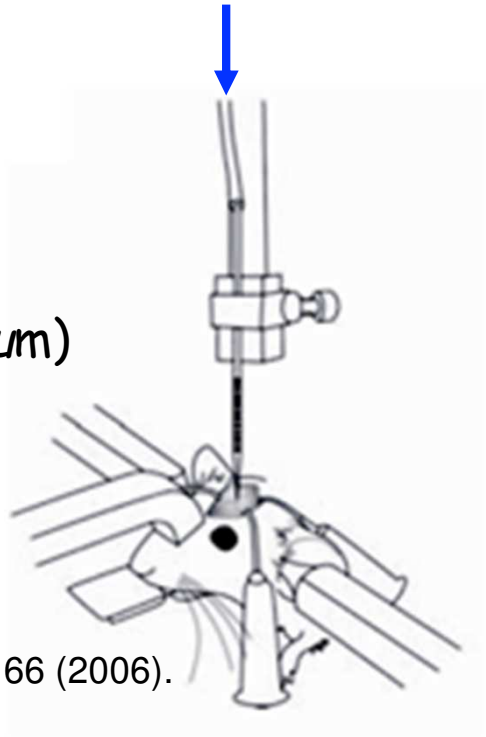
ns: non significant
***: $p<10^{-3}$

⇒ transport parameter impacted for [$A\beta 42$] =1 μM

Haziza *et al.*, Nat. Nanotechnol. **12**, 322 (2017)

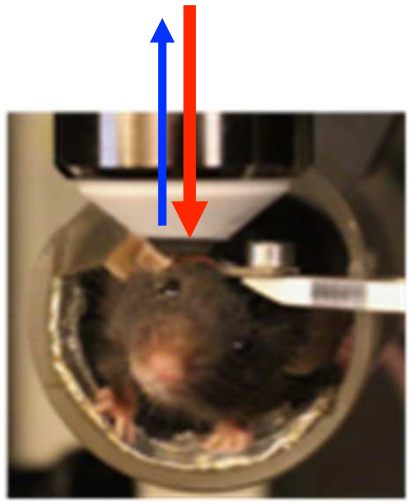
First try of nano-SiC tracking in brain of a living mouse

1. Injection of nano-SiC (80 nm) in the prefrontal **cortex** (depth≈200 μm)



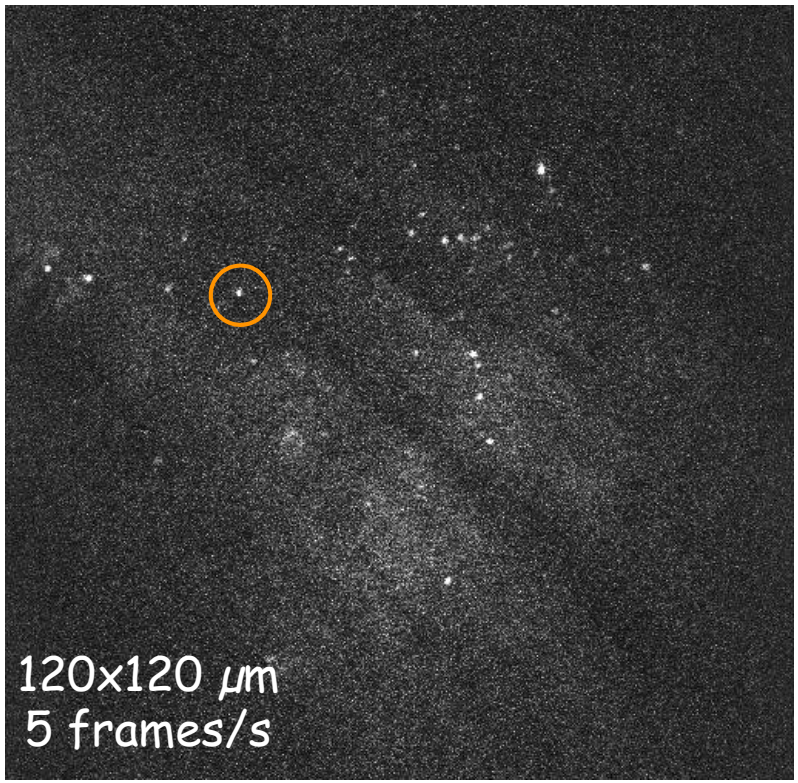
Cetin, A. et al.
Nat. Prot. **1**, 3166 (2006).

2. SHG imaging (λ_{exc}=1000 nm)



Collaboration:
Brice BATHELLIER, Neuro Paris-Saclay Institute

SHG imaging at 40 μm depth
(bandpass filter: 515±25 nm)



⇒ nano-SiC detectable