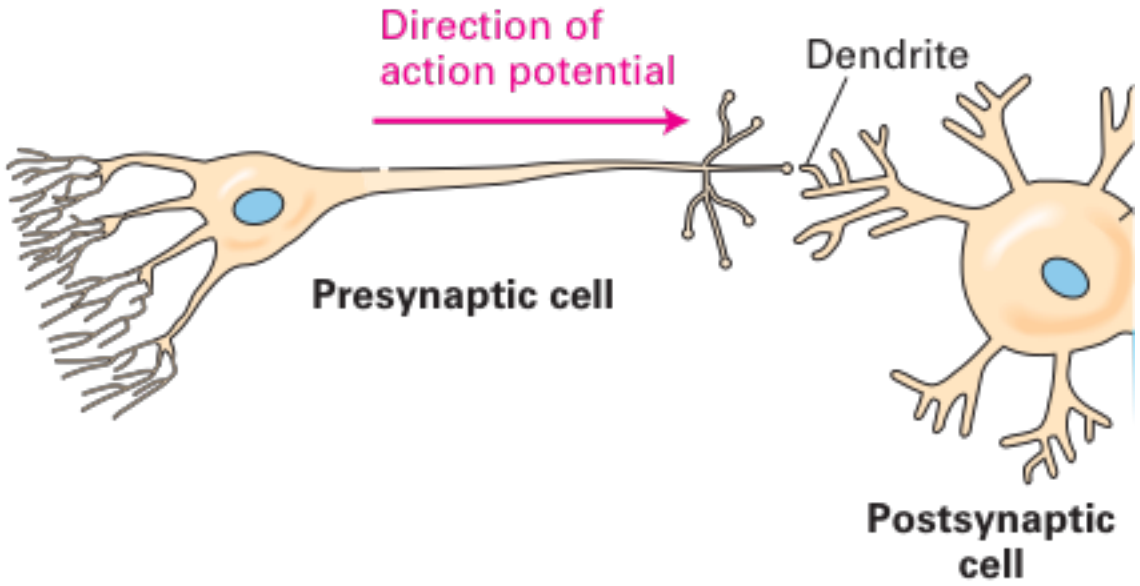
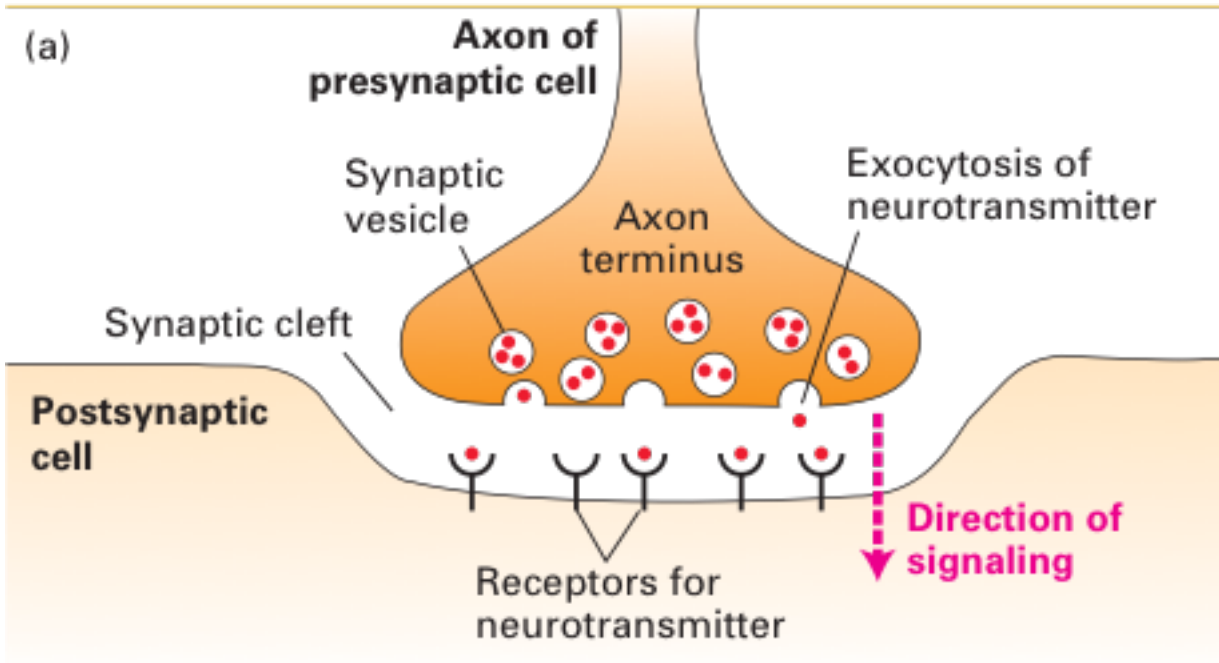
# Imaging Synaptic Transmission

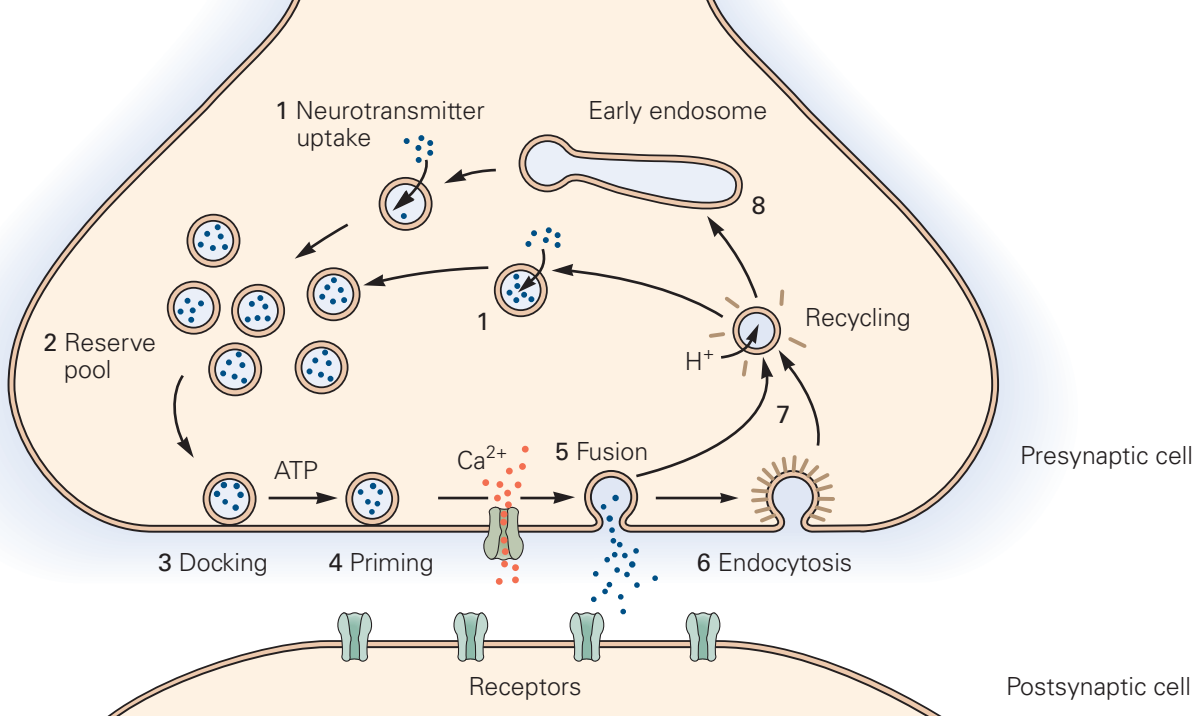
Emina Lemes

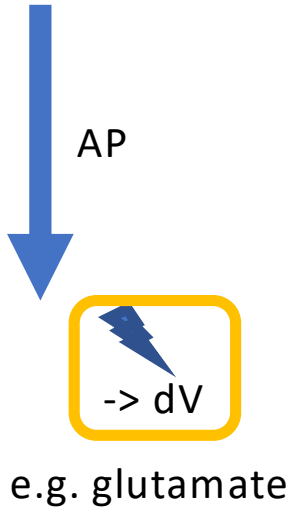


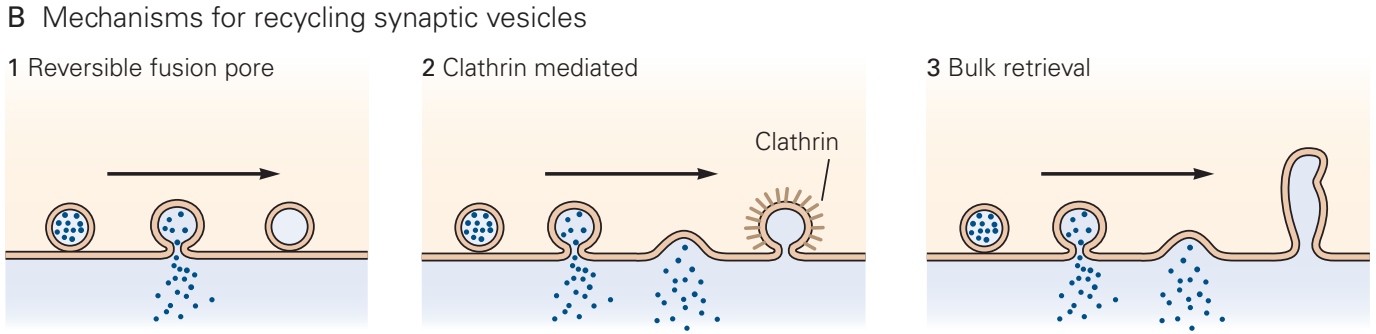

# Intro - Synaptic transmission

Lodish, 8th Edition, Fig 22-31 +Fig 22-3




e.g. glutamate




# Intro - Vesicle fusion

# Relevance

# Presynaptic alterations - Neurodegenerative diseases (ND)

**Table 1.** Summary of presynaptic phenotype by AD genetic factors

| Alzheimer's disease | | |
|---|---|---|
| Factor | Phenotype at presynaptic terminal | Ref |
| Amyloid Precursor Protein (APP) | • Negative regulator of synapse formation | 3 |
| | • Negative regulator for readily releasable pool of synaptic vesicle | 3 |
| | • Molecular Hub in presynaptic active zone (PAZ) | 4 |
| Amyloid beta (Aβ) | • Downregulation of presynaptic protein expression | 5 |
| | • Increase release probability (soluble Aß- normal condition) | 7 |
| | • Disruption of vesicle fusion ability by inhibiting VAMP2 function (pathologic Aβ) | 9 |
| BACE1 | • Negative regulator for excitatory synaptic transmission (homeostatic synaptic plasticity) | 15 |
| γ-secretase/Presenilin | • Presynaptic short-term plasticity, synaptic facilitation | 17 |
| | • Homeostatic synaptic scaling of excitatory synapses | 18 |
| Tau | • Synaptic stability (presynaptic proteins, synaptic vesicle) | 19, 20 |
| ApoE4 | • Downregulation of amount glutamate | 27 |
| | • Modulation of spontaneous vesicle release | 28 |

Bae JR, Kim SH. Synapses in neurodegenerative diseases. *BMB Reports*. 2017.

# ND – Alzheimer's disease

**Table 2.** Summary of presynaptic phenotype by PD genetic factors

| Parkinson's disease | | |
|---|---|---|
| Factor | Phenotype at presynaptic terminal | Ref |
| α-synuclein | • Impairment of dopamine release in SNpc | 38 |
| | • Impairment of synaptic vesicle endocytosis and reclustering | 39,40 |
| | • Reduction of synaptic vesicle recycling pool | 41 |
| LRRK2 | • Impairment of release and decreased DA uptake in SNpc | 48 |
| | • Impairment of synaptic endocytosis in presynaptic terminals | 45 |
| Parkin | • Reduction of dopamine release | 55 |
| | • Impairment of synaptic plasticity in striatal cells | 53 |
| PINK1 | • Impairment of synaptic plasticity and release of dopaminergic neuron | 62 |
| DJ-1 | • Defect of LTD through inhibitory effects of D2 receptor | 68 |
| Synaptojanin1 | • Slowed endocytosis rate for small stimulation by defect of phosphatase activity | 74,75 |
| Endophilin | • Regulation of Parkin expression | 76 |

Bae JR, Kim SH. Synapses in neurodegenerative diseases. *BMB Reports*. 2017.

# ND – Parkinsons's disease

**Table 3.** Summary of presynaptic phenotype by ALS and HD genetic factors

| ALS and Huntington's disease | | | |
|---|---|---|---|
| Factor | | Phenotype at presynaptic terminal | Ref |
| ALS | SOD-1 | • Axonal transport | 83 |
| | | • Synaptic degeneration | 84,85 |
| | TDP-43 | • Expression regulation of presynaptic protein | 88 |
| | | • Attenuation of synaptic transmission | 89 |
| | FUS | • Active zone formation, synaptic transmission | 93 |
| HD | HTT | • Synaptic transmission, release probability | 94 |
| | | • Synaptic vesicle dynamics | 95,96 |

Bae JR, Kim SH. Synapses in neurodegenerative diseases. *BMB Reports*. 2017.

# ND – Huntingtons's disease and ALS

- Glutamate release ->
  - high degree of heterogeneity among synapses
  - Study neuronal circuits of different brain regions

- To probe presynaptic function:
  - record presyn $Ca^{2+}$ dynamics
  - presyn release of glutamate

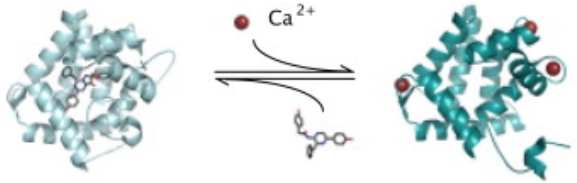
# Relevance in physiology - $Ca^{2+}$ entry and glutamate release

# Advances - Tools

- **Important factors**:
  - timeresolution
  - localization
  - non-confunded readout
  - specificity
  - invasiveness
  - timerange (months/weeks/days?)

# Advances– Calcium/Glutamate indicators

**Existing tools**

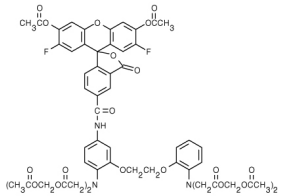



1. **Aequorin:** bioluminescent ca binding photoprotein

2. **Arsenazo III:** absorbance dye that changes its absorption spectrum as a function of bound calcium

implementation and use often tedious, mostly because of problems with dye delivery

3. **Fluorescent calcium indicators**, hybridization of EGTA/BAPTA (Ca-chelators) with fluorophores (Roger Tsien and colleagues: )
   - Quin-2, Fura-2, Indo-1, Fluo-3
   - Oregon Green BAPTA (OGB)
   - fluo-4 dye families (Paredes et al., 2008).



relatively easy to implement and provide large signal-to-noise ratios

4. **GECI**s, protein-based genetically encoded calcium indicators (Lab of Roger Tsien (Miyawaki et al., 1997)
   1. GCaMP-s

# Advances– Calcium indicators

**Existing tools for quantitative measurement** :

1. in situ microdyalisis
2. Microelectrode
3. glutamate dehydrogenase/oxidase coupled to NADH fluorescence readout

- Lack cellular resolution
- are confunded
- Have large responsetimes

4. **Biosensors composed of glutamate-binding proteins coupled to fluorescence readout**
    1. genetically encoded (like the Ca indicators (GECI))

    2. Glutamate (E) optical sensor: EOS (hybrid sensor made from AMPA R glutamate binding core and a small-molecule dye)

    3. PBP (bacterial periplasmic binding proteins)
        - FRET (FLIP-E , SuperGluSnFR)
        - Single wavelenghth indicators

# Advances – Glutamate indicators

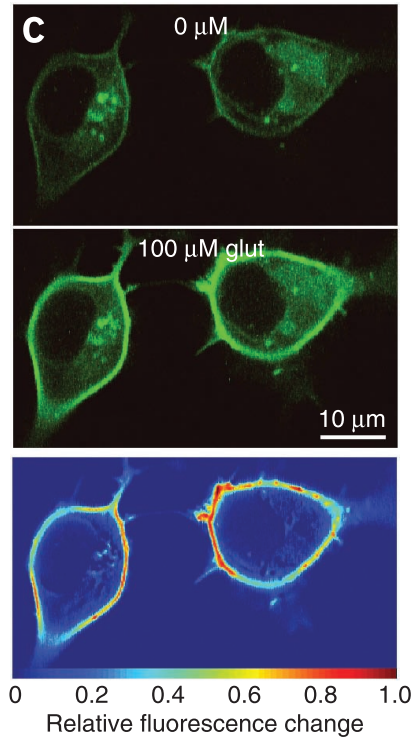
# Entry: 1st paper

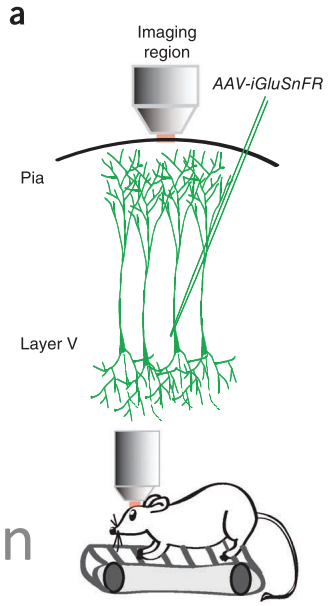
# An optimized fluorescent probe for visualizing glutamate neurotransmission

Jonathan S Marvin[1], Bart G Borghuis[1,2], Lin Tian[1,7], Joseph Cichon[3], Mark T Harnett[1], Jasper Akerboom[1], Andrew Gordus[4], Sabine L Renninger[5], Tsai-Wen Chen[1], Cornelia I Bargmann[4], Michael B Orger[5], Eric R Schreiter[1], Jonathan B Demb[2,6], Wen-Biao Gan[3], S Andrew Hires[1] & Loren L Looger[1]

**Engineered:**
an intensity-based, single-wavelength, glutamate-sensing fluorescent reporter (iGluSnFR)

- **highly sensitive**
- **localized**
- **High signal-to-noise ratio** and kinetics appropriate for *in situ* and *in vivo* imaging.

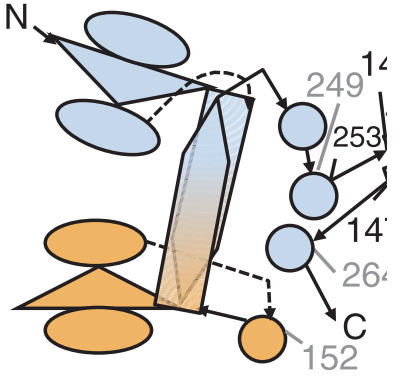


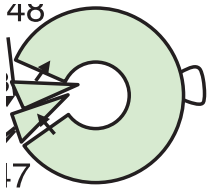

# What did they do and why is this amazing?

An optimized fluorescent probe for visualizing glutamate neurotransmission

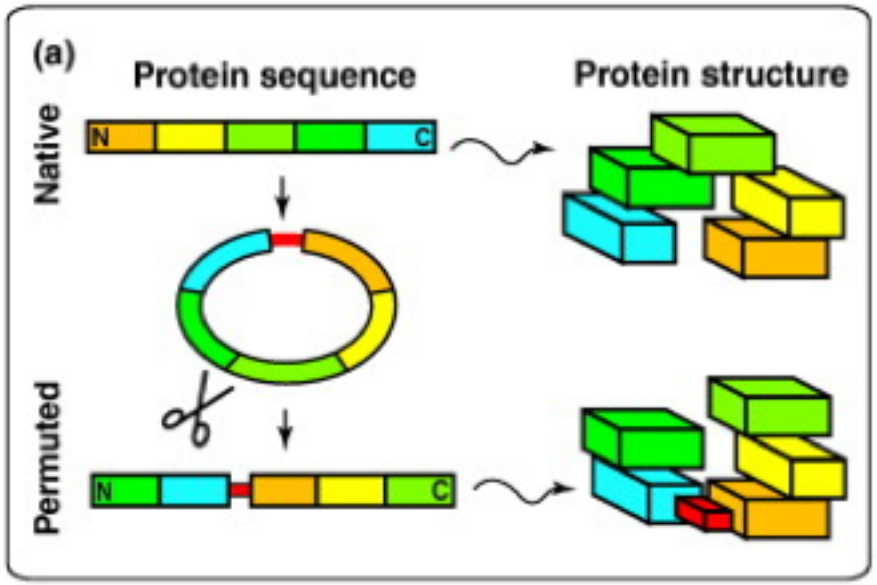
E.Coli
Glutamate/aspartate
Import solute-binding
Protein




cpGFP



Circular permutation:



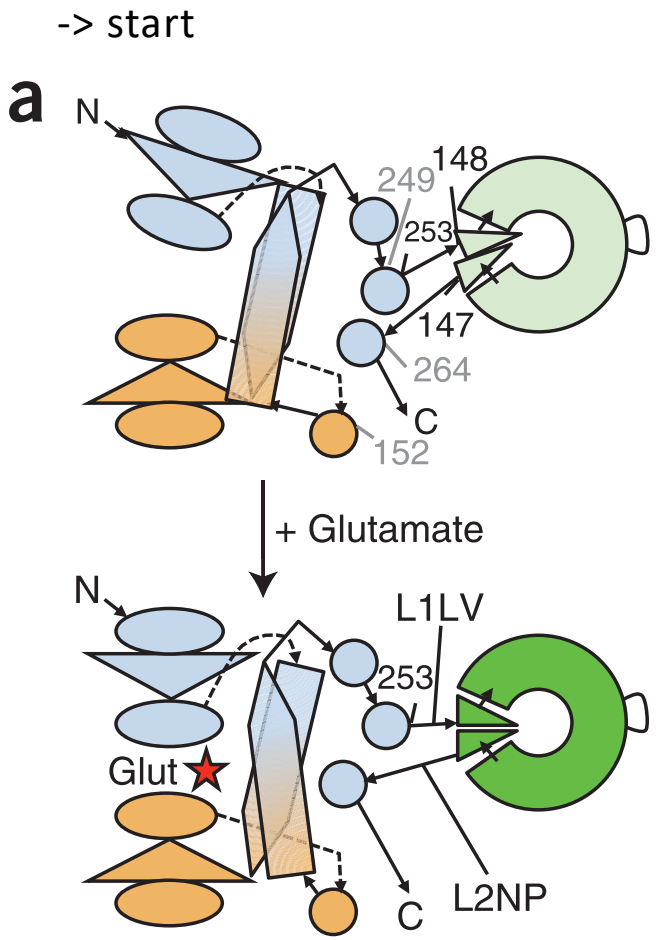

Yu Y, Lutz S. 2011.

- Glt1: Part of the ABC transporter complex involved in glutamate and aspartate uptake

# Sensor development – main players

An optimized fluorescent probe for visualizing glutamate neurotransmission

E.Coli
Glutamate/aspartate
Import solute-binding
Protein




cpGFP

ΔF

Exploit conformational change for detection of difference in Fluorescence upon ligand binding

expressed as $\Delta F/F = (F - F_0)/F_0$

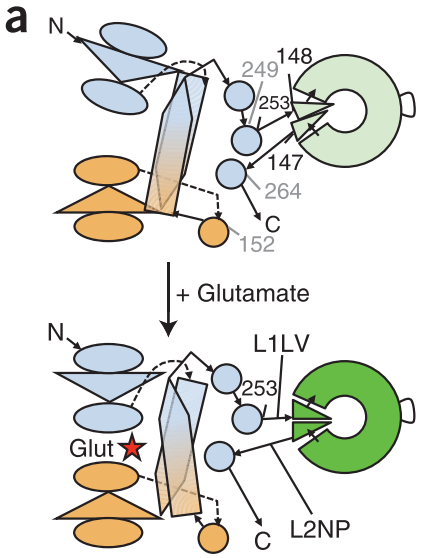
# Sensor development – principle

High throughput screening

**Optimization of two parameters**:

1. the cpGFP insertion site in the binding protein
2. the composition of the residues (‘linkers’) adjoining the two proteins.

Variant:
**GltI253.L1LV/L2NP**




# Sensor development - principle

An optimized fluorescent probe for visualizing glutamate neurotransmission

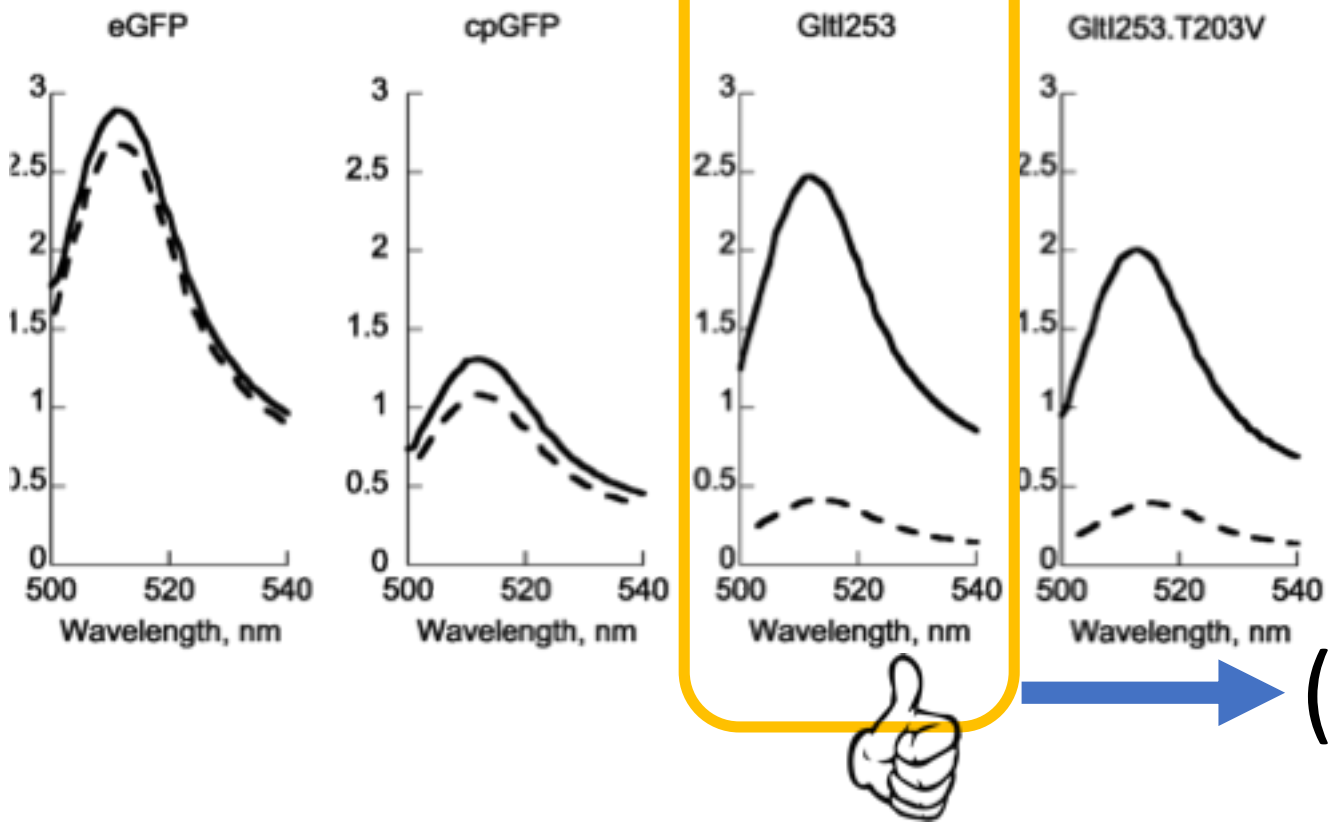
Variant:

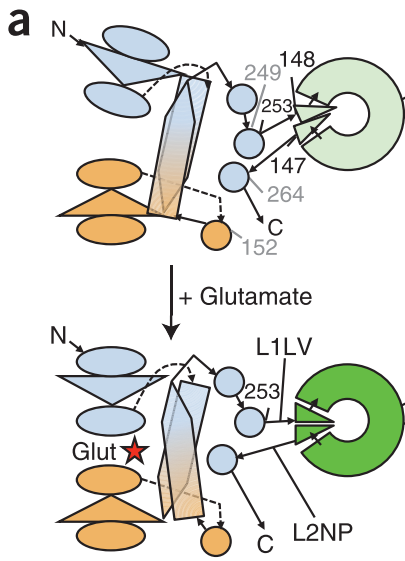
**GltI253.L1LV/L2NP**

Glutamate/aspartate Import solute-binding Protein

cpGFP

Emission spectra for 1 µM:



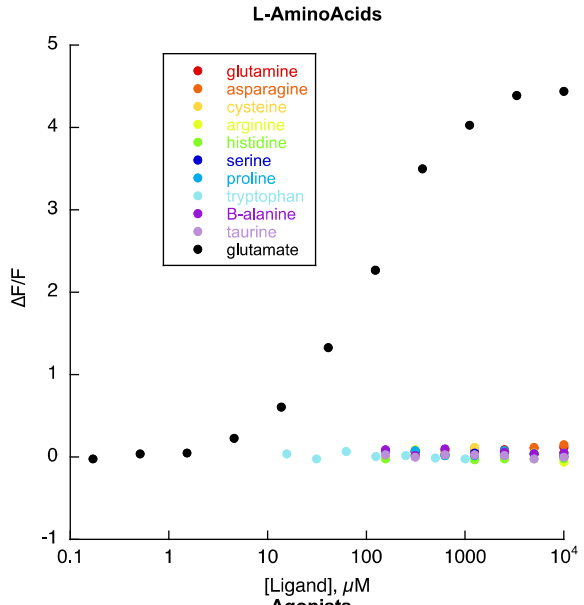



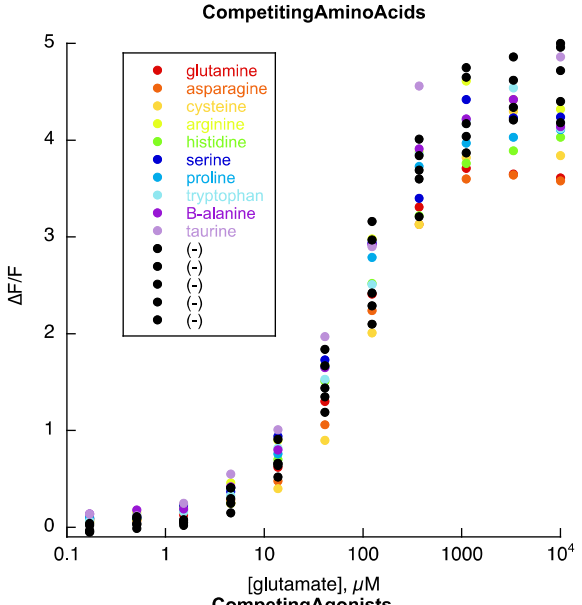

_____ PBS + 10mM glutamate

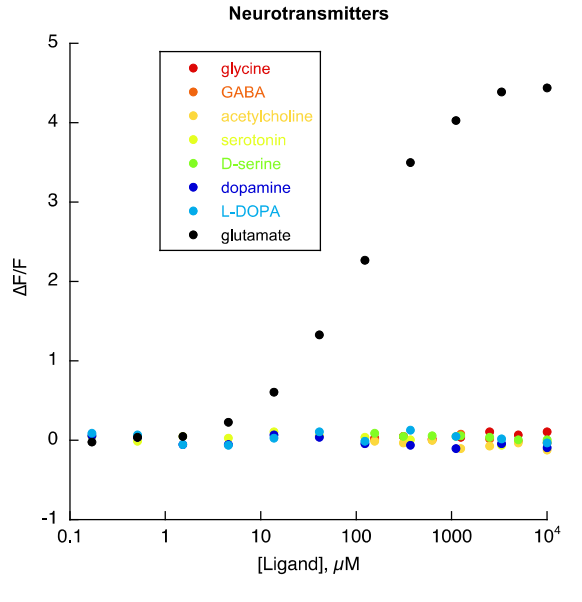
------- PBS

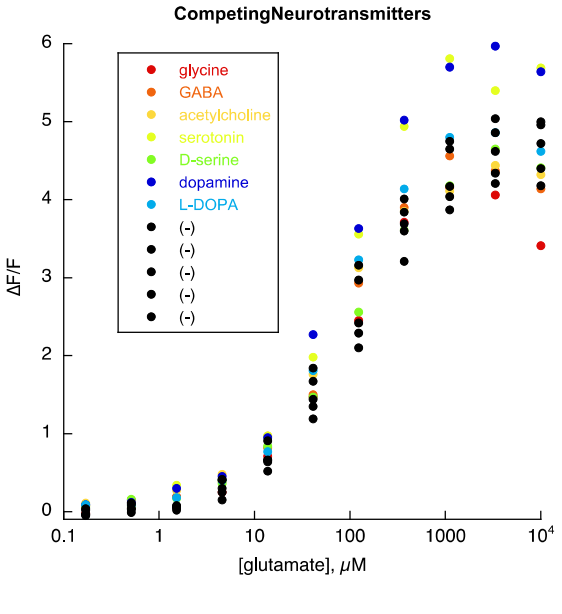
$(\Delta F/F)_{max}$= 4.5 +/- 0.1

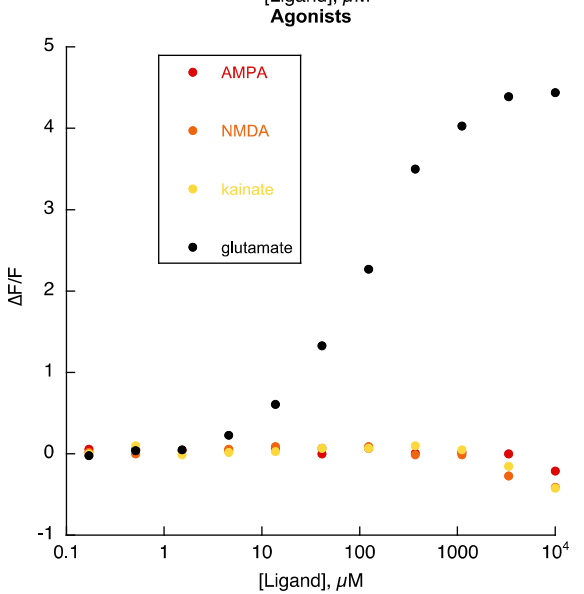
$(\Delta F/F)$max – glut / PBS

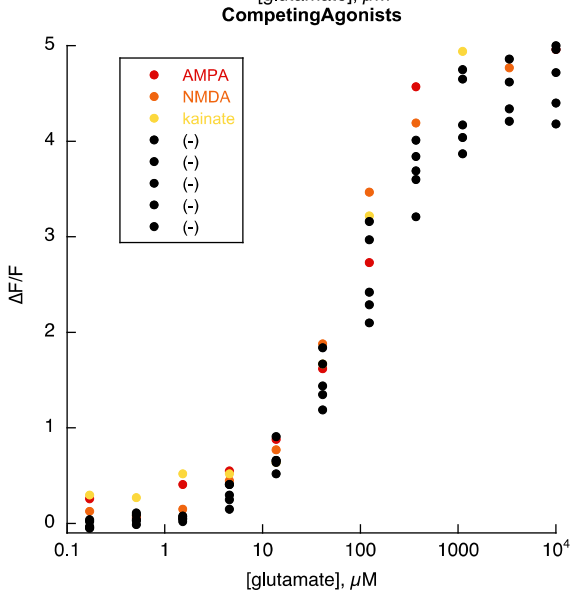


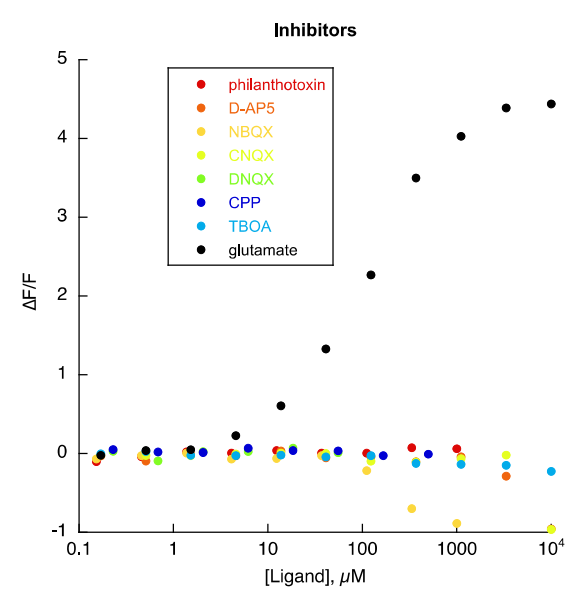



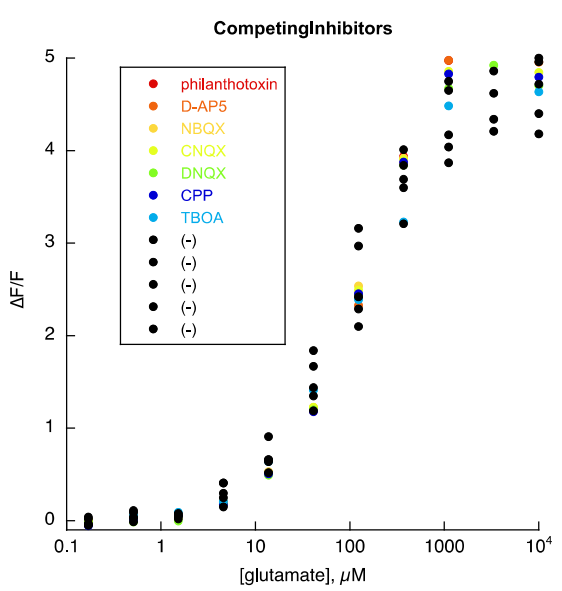



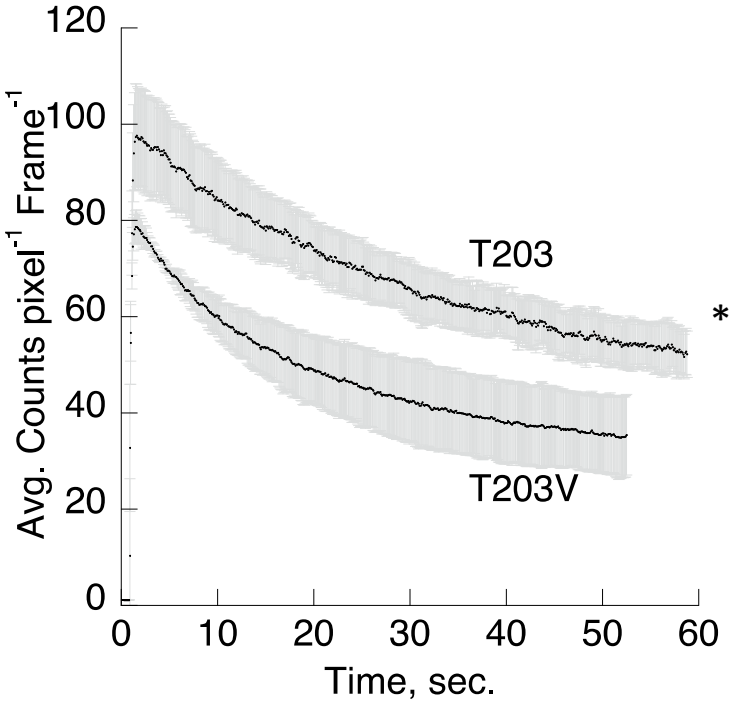




# GltI253.L1LV/L2NP – specificity

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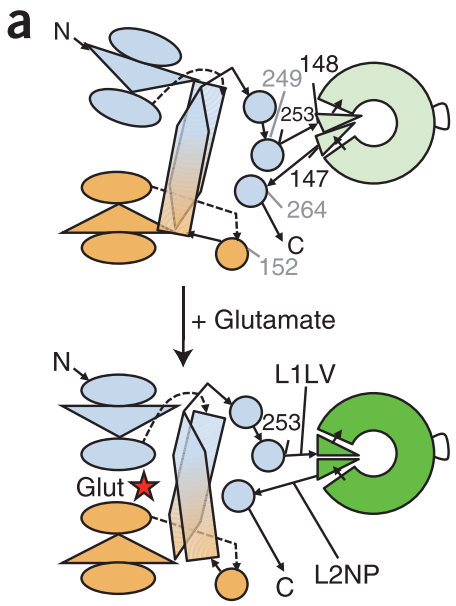
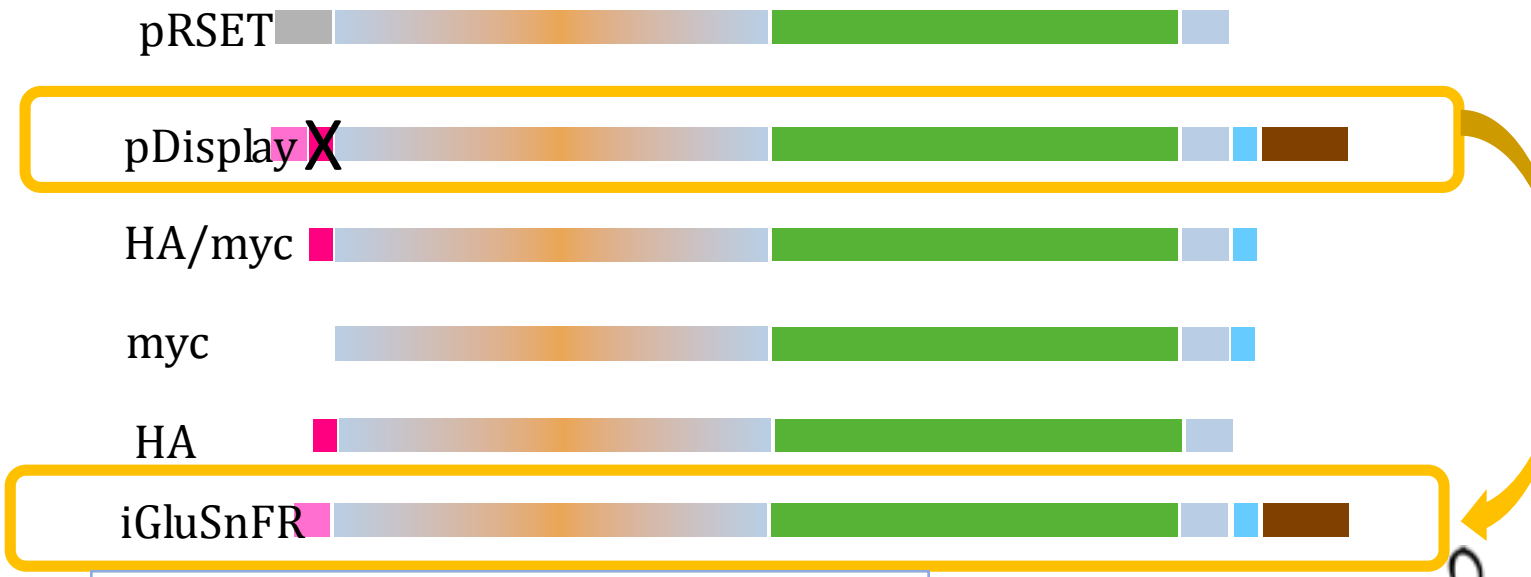
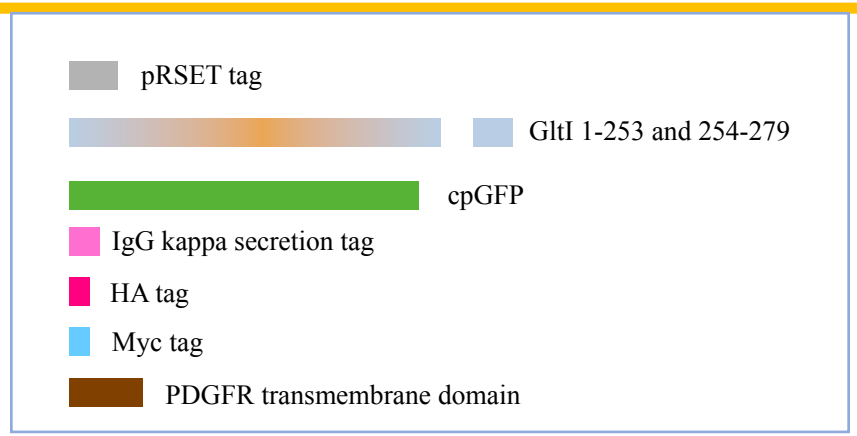
# GltI253.L1LV/L2NP – Photostability


Colors correspond

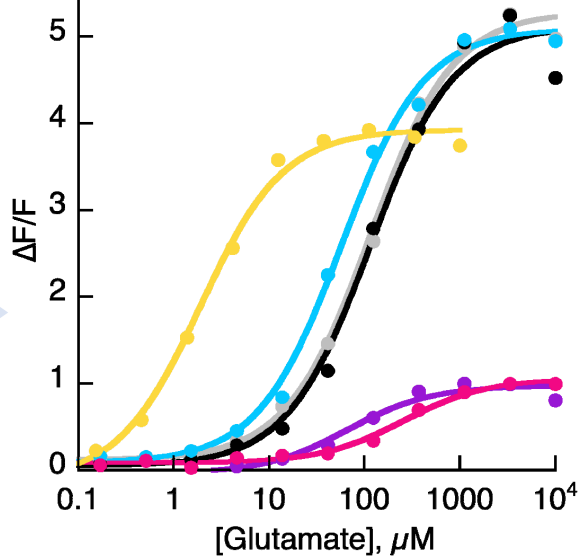


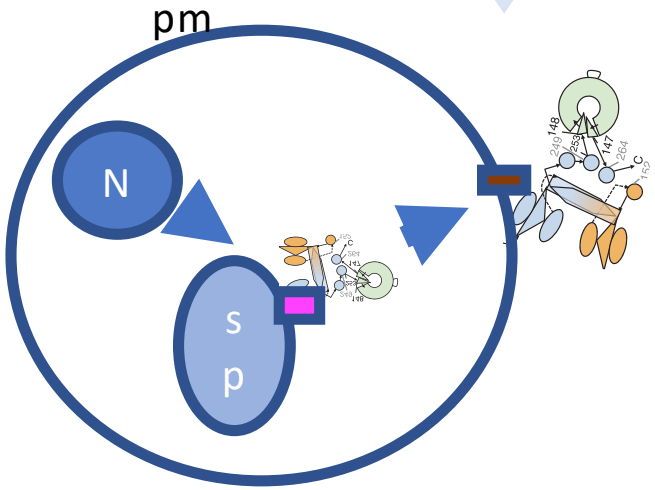

# iGluSnFR – Vectordesign



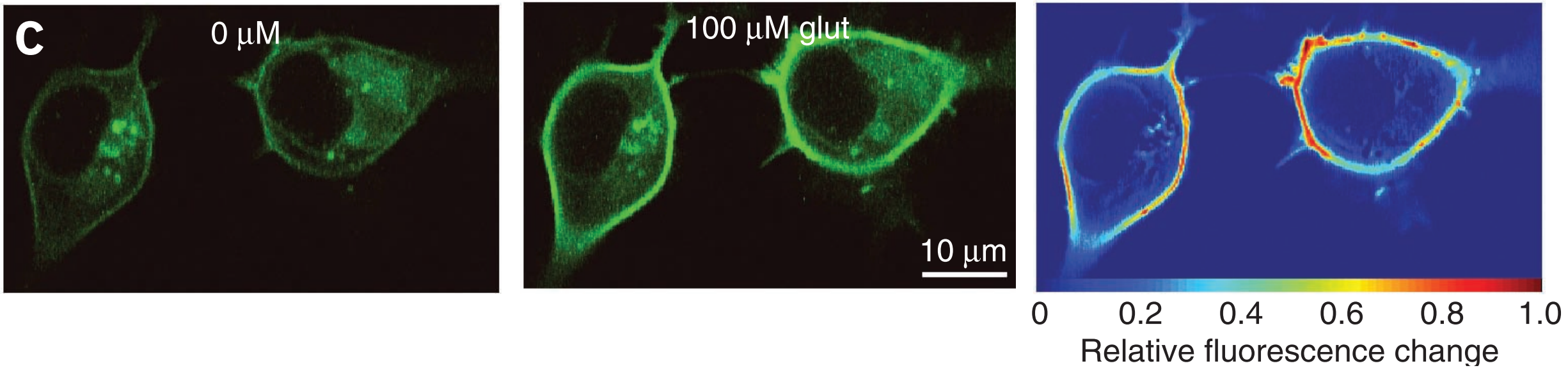




- bacterially expressed L1LV/L2NP



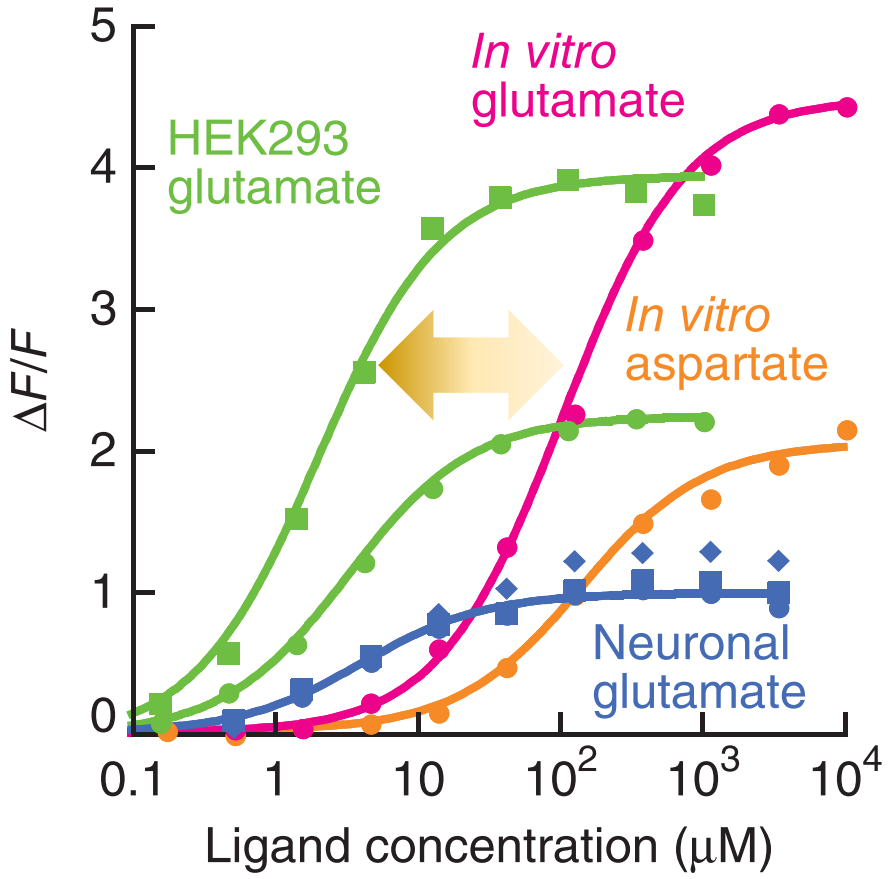




# iGluSnFR – Vectordesign




# iGluSnFR – in action

# iGluSnFR – soluble vs. bound

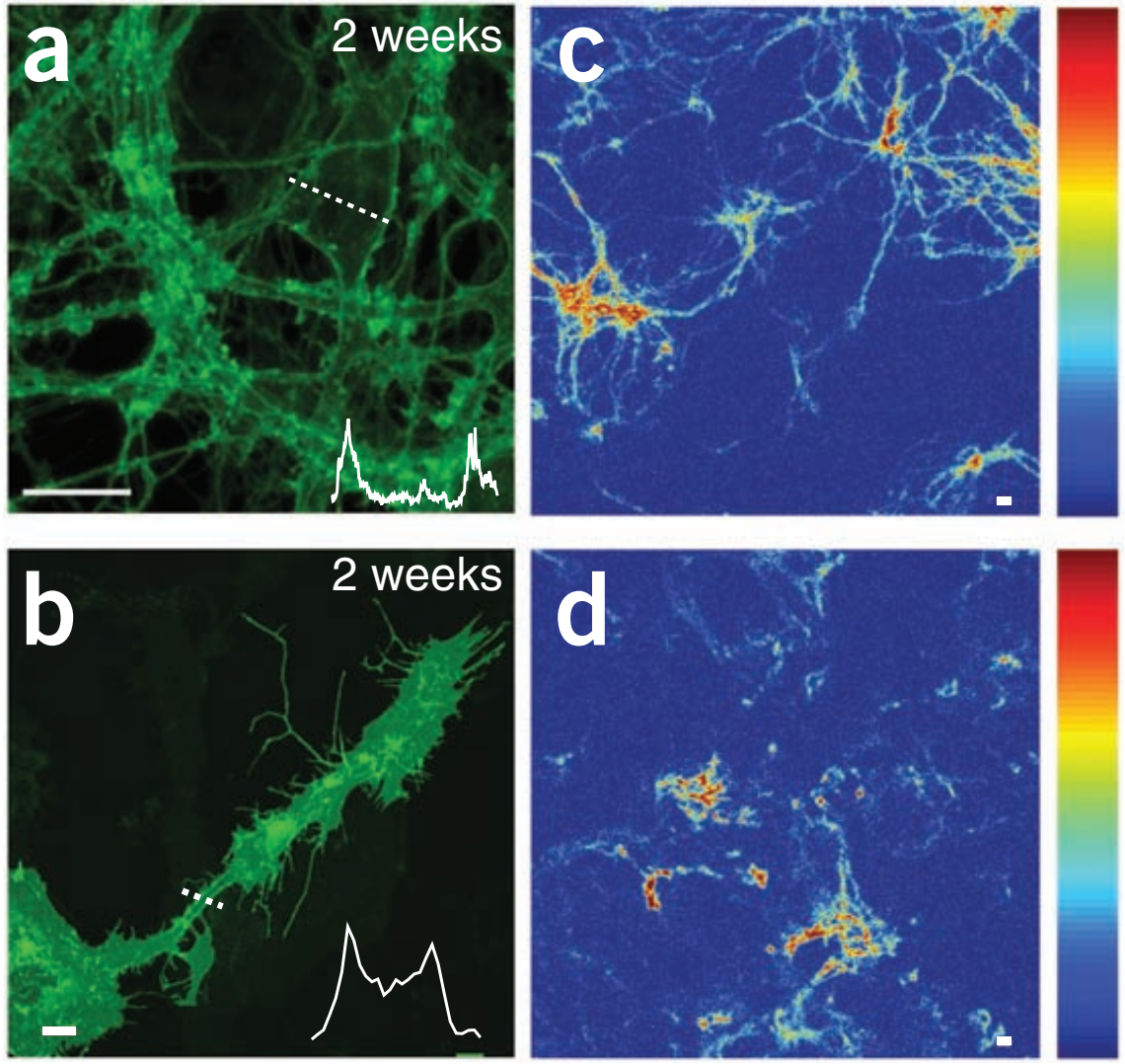


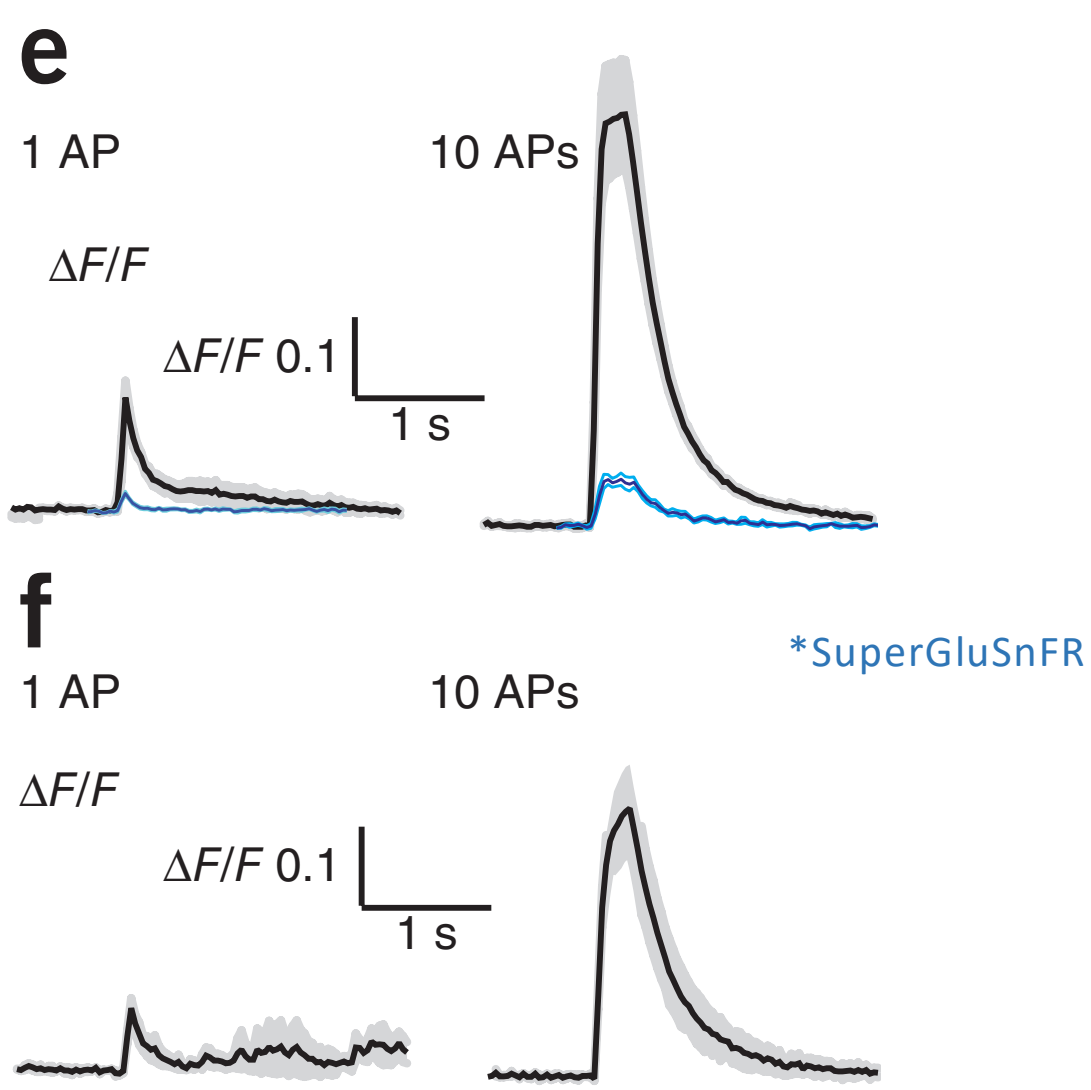

*In vitro* titration of L1LV/L2NP with **glutamate** and **aspartate** (orange) and
*in situ* titration of **iGluSnFR on HEK293** cells and **cultured neurons**

-> **25-fold** increase in affinity from that of the soluble protein

neuron

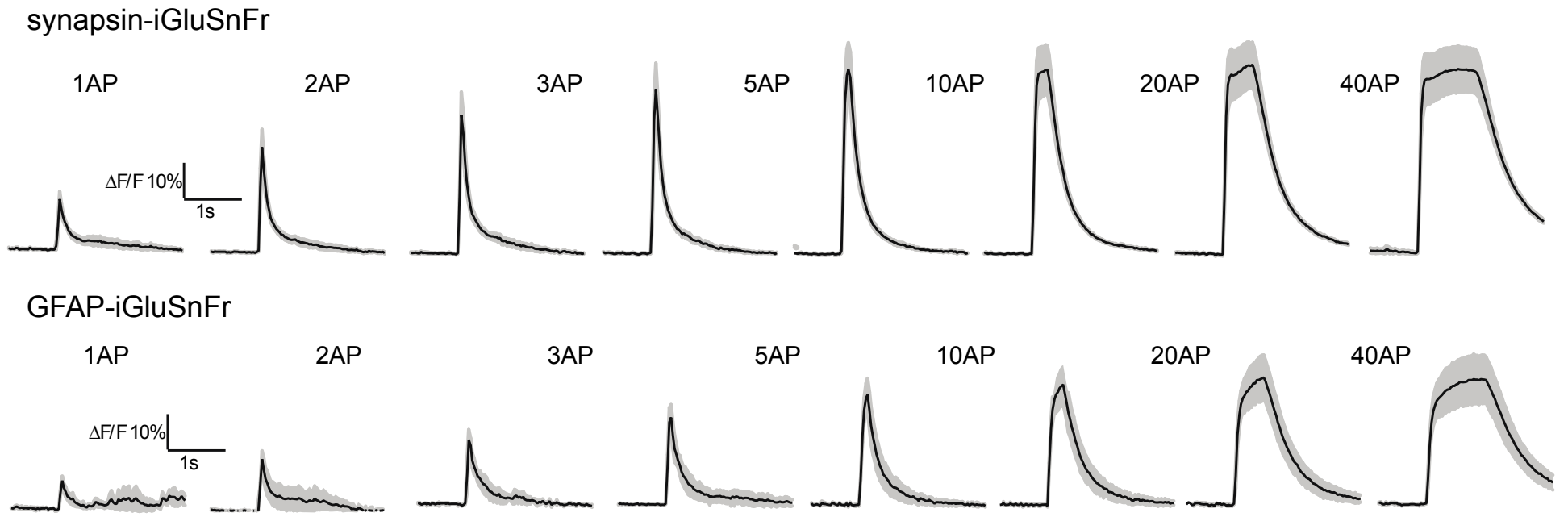
astrocyte



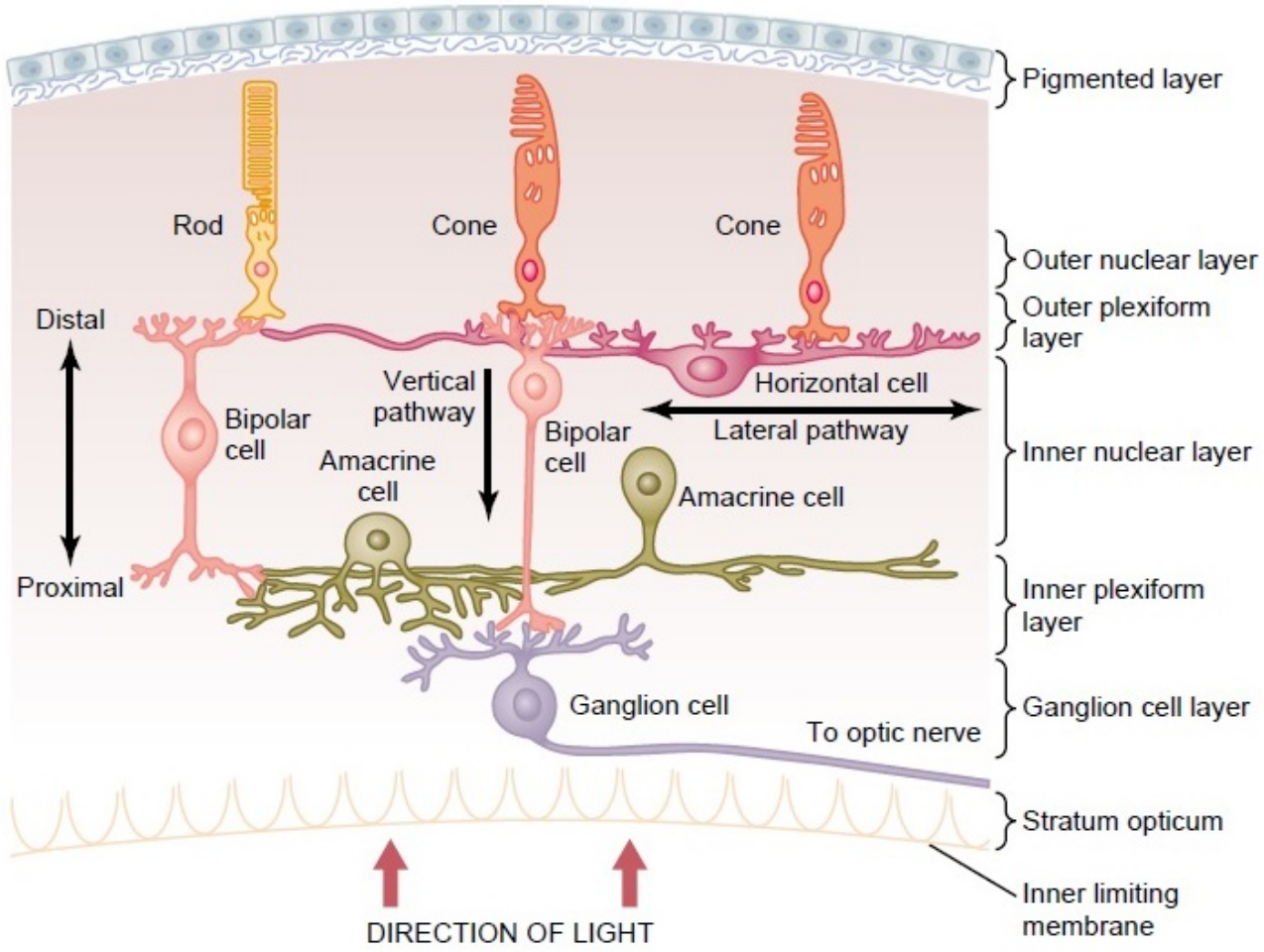




*SuperGluSnFR

# iGluSnFR – in neurons and astrocytes



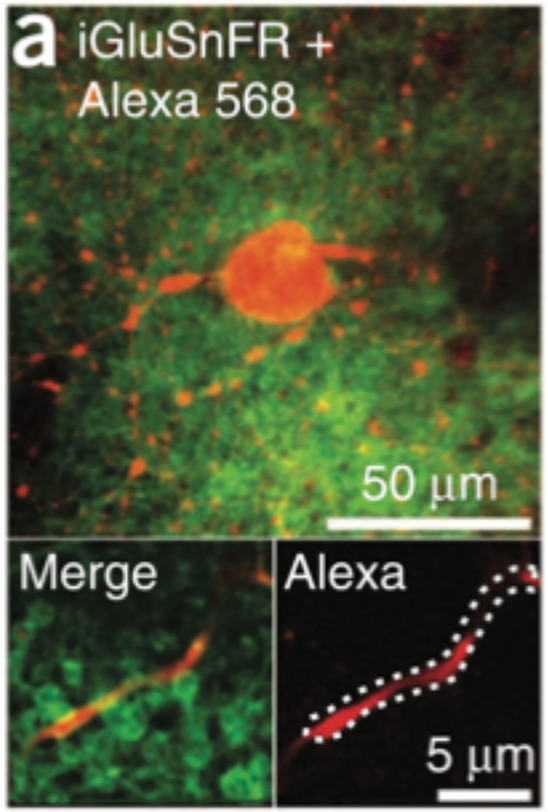

# iGluSnFR – in neurons and astrocytes

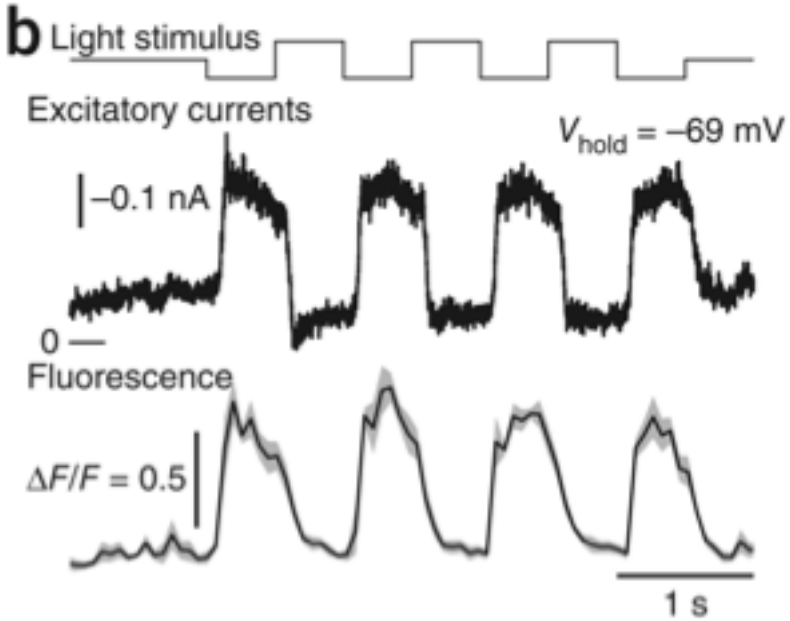


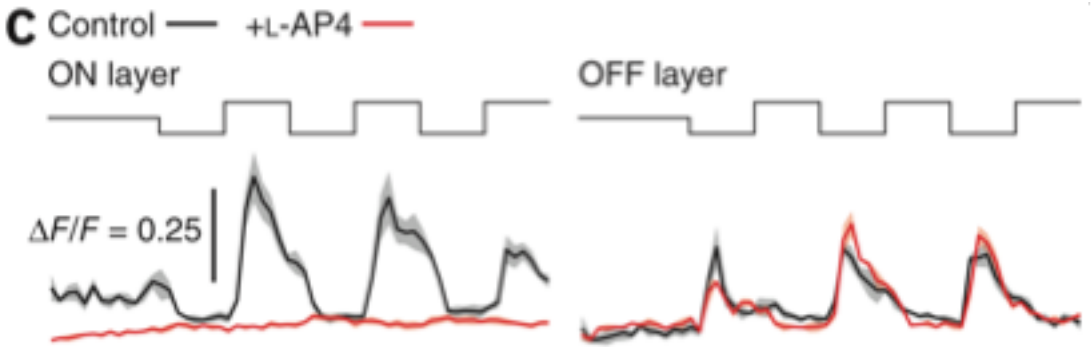

# Mouse retina – light evoked gluamate release

- iGluSnFR -> expressed in ganglion cells ,
- 14–21 d post-injection, targeted for whole-cell recording (OFF sustained type)

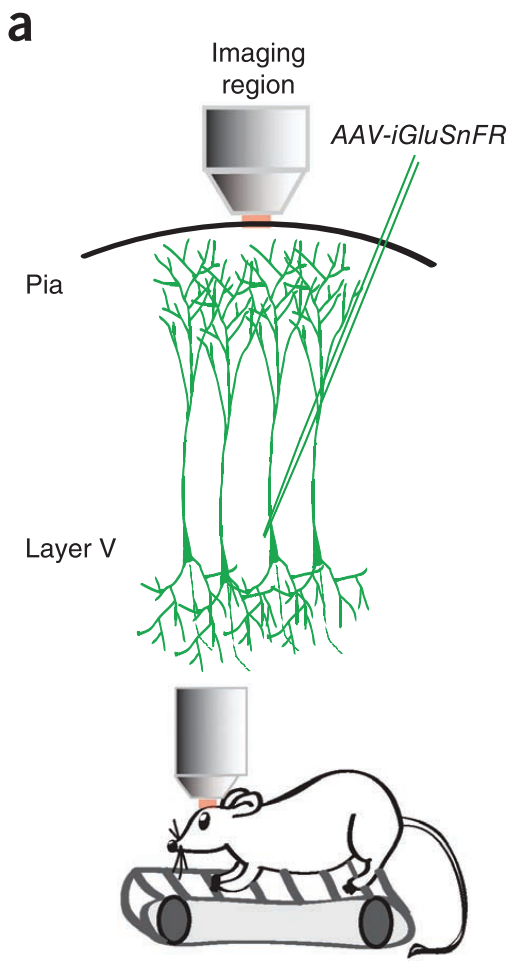
- iGluSnFR responses in ON and OFF layers
- L-AP4, selectively hyperpolarizes ON bipolar cells, blocking their synaptic release, but does not block release from OFF bipolar cells



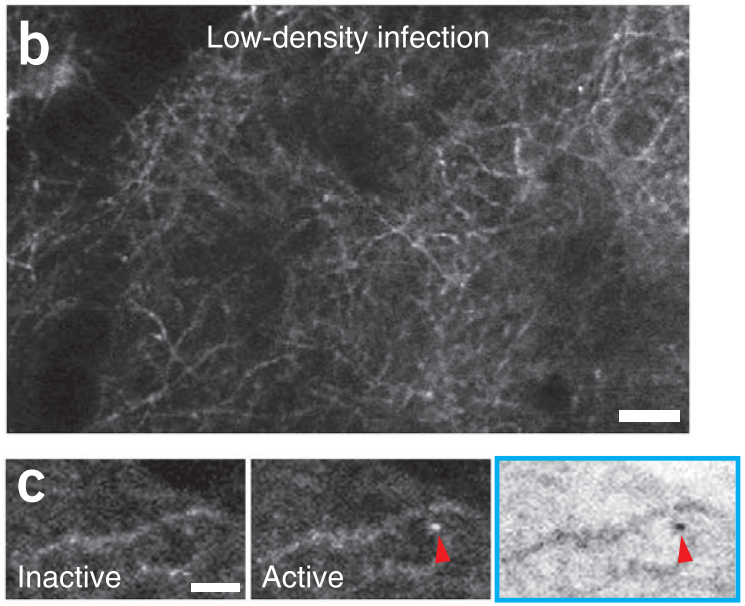



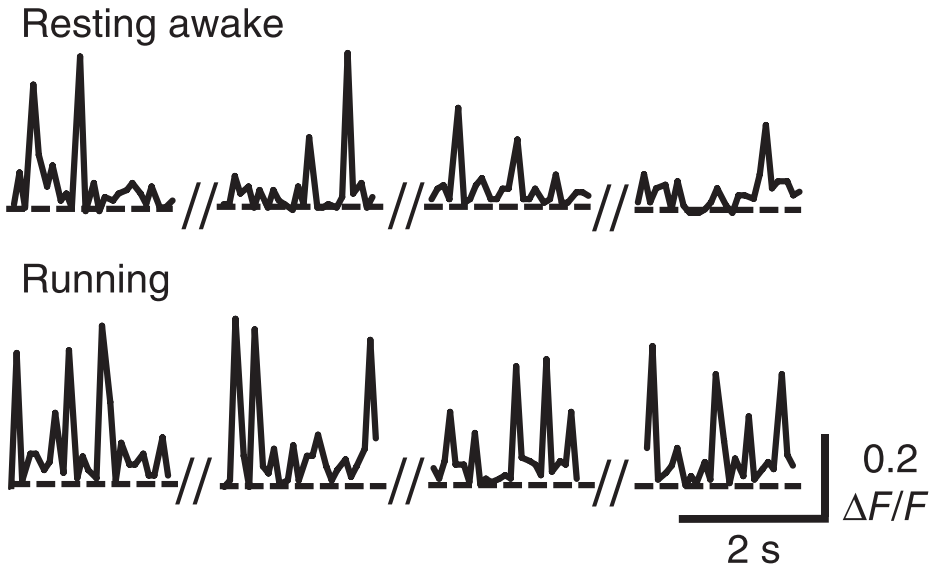

# Mouse retina – light evoked gluamate release




- Deliverey of iGluSnFR to layer V of the motor cortex
- Mice imaged while resting-awake or running
- Measured iGluSnFR fluorescence in response to motor tasks




iGluSnFR showing apparent dendritic spines (arrowheads)

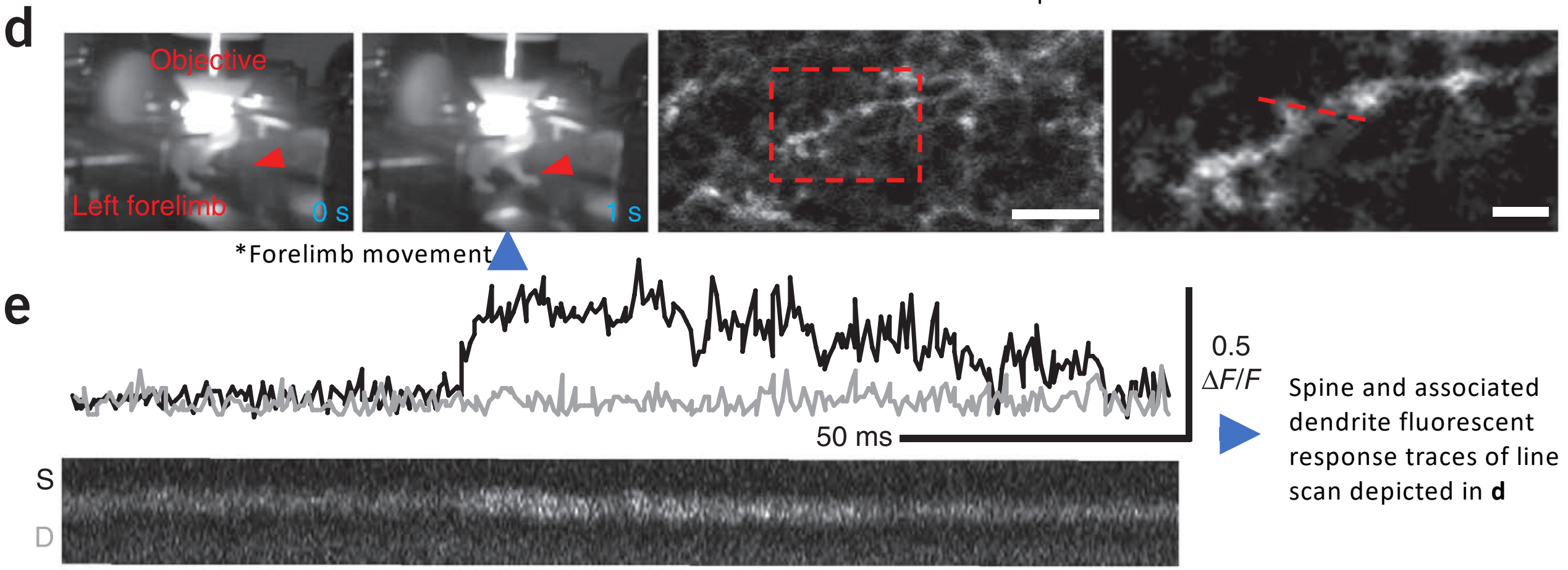



Glutamate transients during awake and fw running

# Mice – iGluSnFR functionality *in vivo*

- iGluSnFR fluorescence in response to motor tasks

two-photon images of a dendritic segment from the apical tuft of motor cortex




# Mice – iGluSnFR functionality *in vivo*

# iGluSnFR

## +

- Direct and **specific detection of glut release**
  - ***in situ*** and ***in vivo***
- Specific localization possible
- High SNR
- Could be directed towards postsynaptic membrane to monitor timing and localization of excitatory postsynaptic input from different afferent neurons along a dendritic arbour
- Better than Calcium sensors for reporting neuronal activity

**+** Since then : development new brighter variants for in vivo imaging

## -

- **For investigating full complement of glut synapse types -> lower affinity variants needed**
  - ~4 µM affinity of iGluSnFR pre- cludes quantitation of the millimolar glutamate concentrations

# Conclusion

# Entry: 2nd paper

"A preprint,
aka an invitation to be critical"
(Quote - Referee #3)

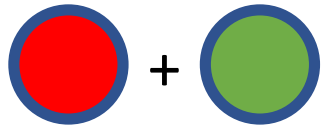
# Multiplex imaging of quantal glutamate release and presynaptic $Ca^{2+}$ at multiple synapses *in situ*

Thomas P. Jensen[1], Kaiyu Zheng[1], Nicholas Cole[1], Jonathan S. Marvin[2], Loren L. Looger[2], Dmitri A. Rusakov[1]

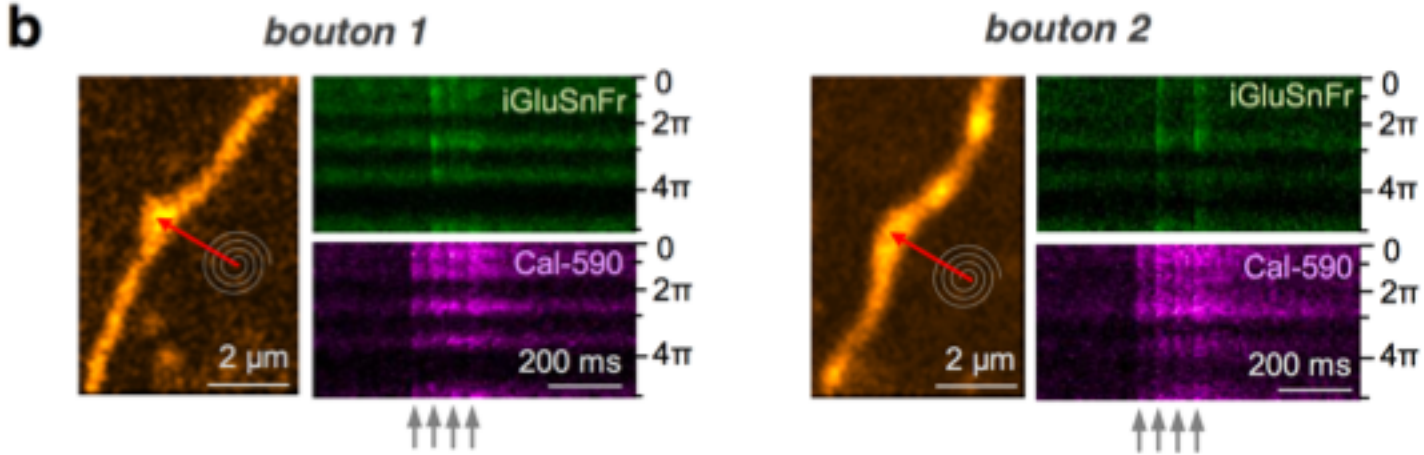
[1]UCL Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK

[2]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia, USA

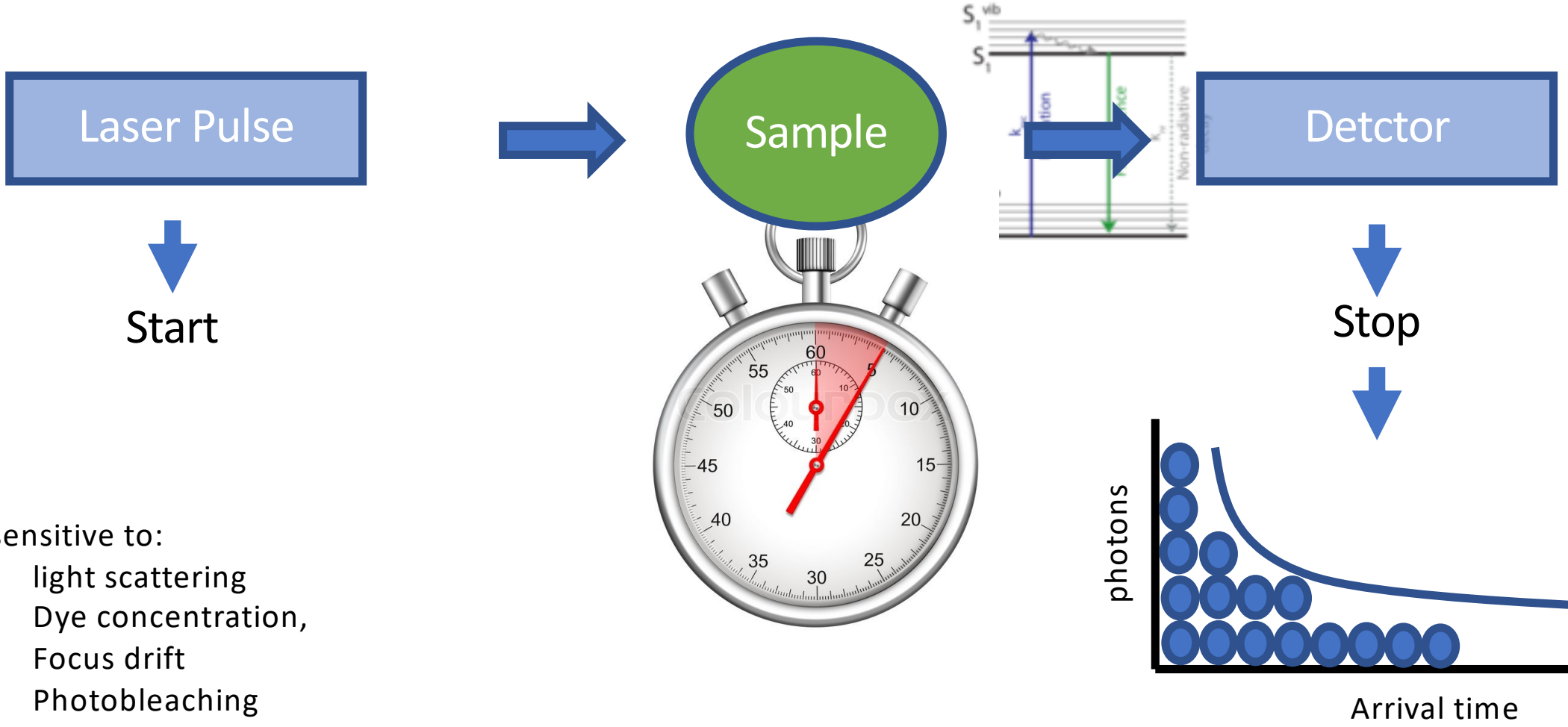
- Combined imaging of red-shifted fluorescent Ca2+ indicator (Cal-590) with iGluSnFR glutamate imaging at single synaptic boutons



- Makes it possible to conduct studies looking at the connection between presyn calcium signalling and glutamate response



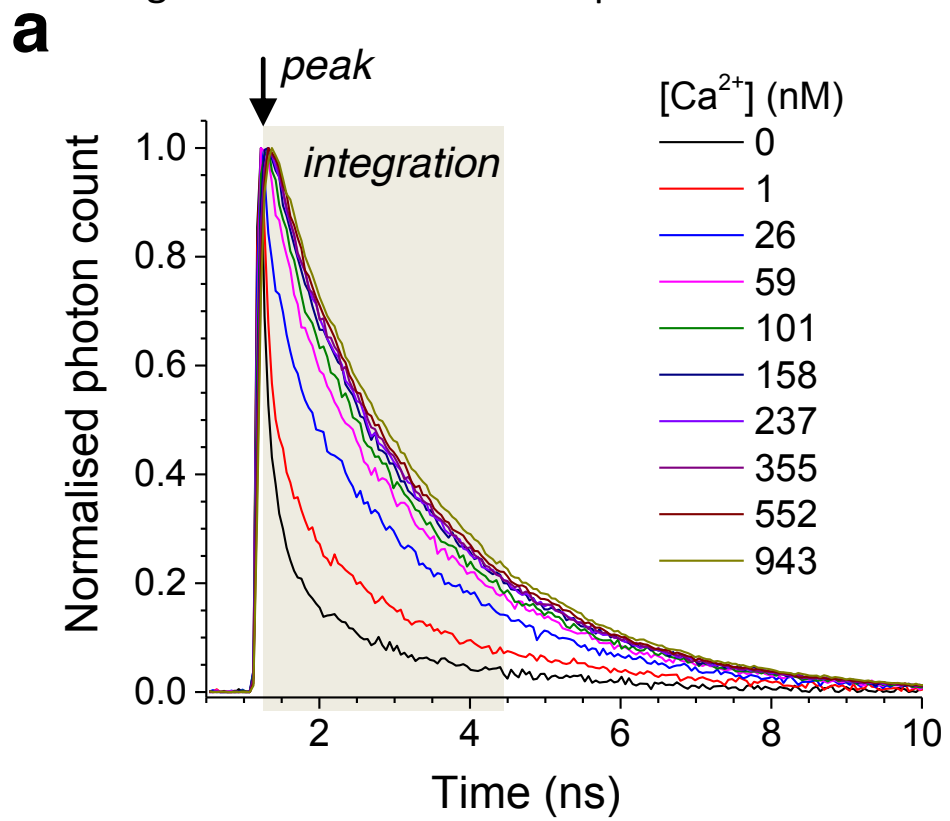

# What did they do and why is it amazing?




- Insensitive to:
  - light scattering
  - Dye concentration,
  - Focus drift
  - Photobleaching

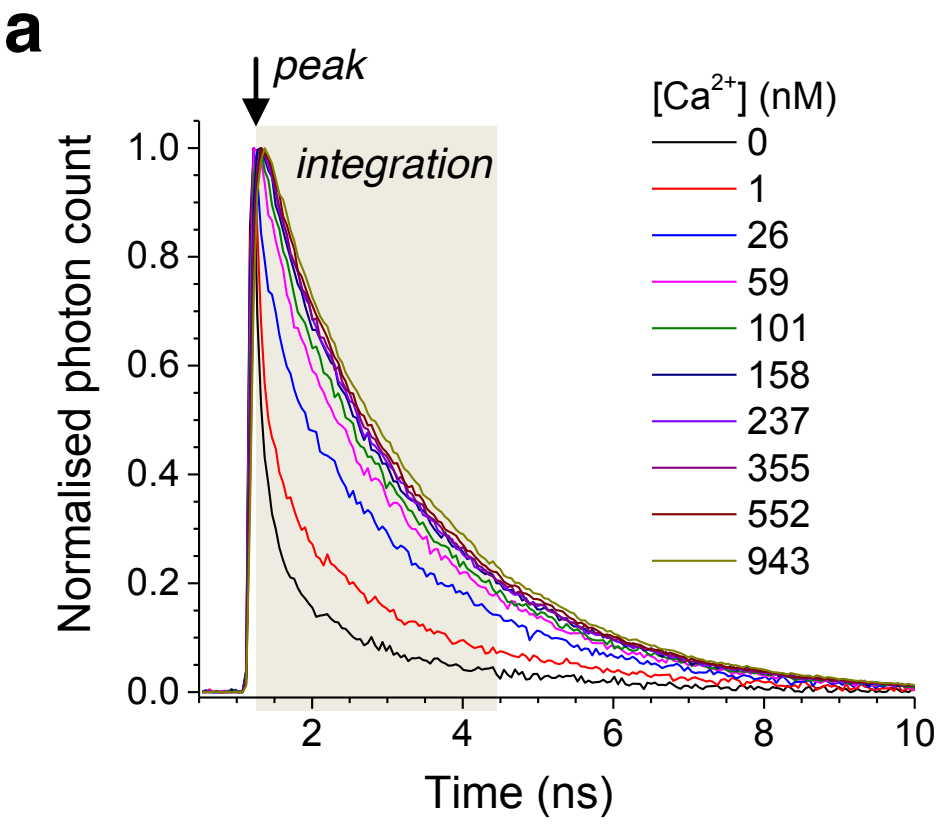
# FLIM - Fluorescence-lif etime imaging microscopy

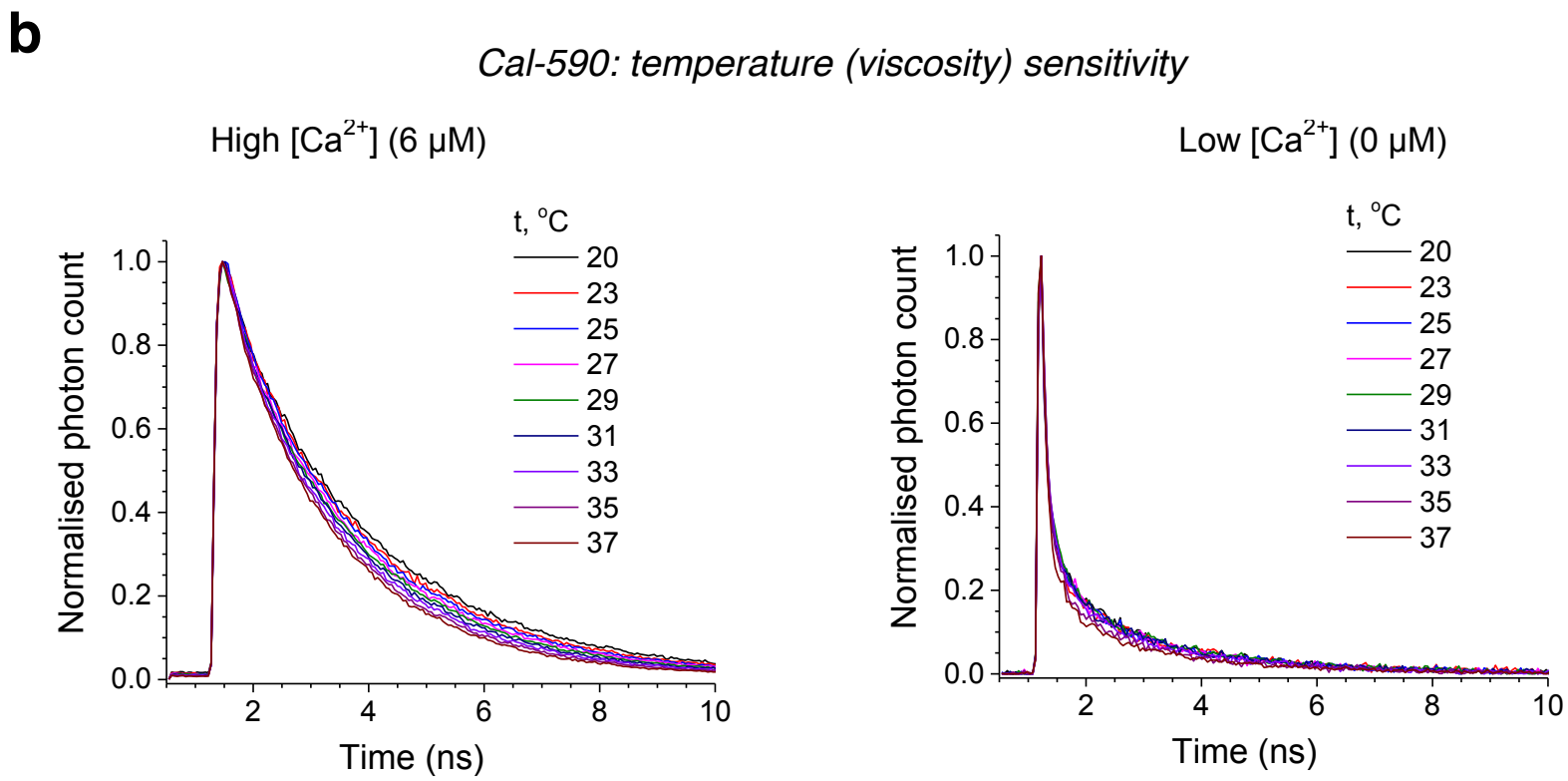
- Is Cal-590 FLIM readout correlated with [$Ca^{2+}$] ?
- using a series of Ca2+-clamped solutions




***Fluorescence-lifetime imaging microscopy**

# FLIM readout of red-shifted Cal-590

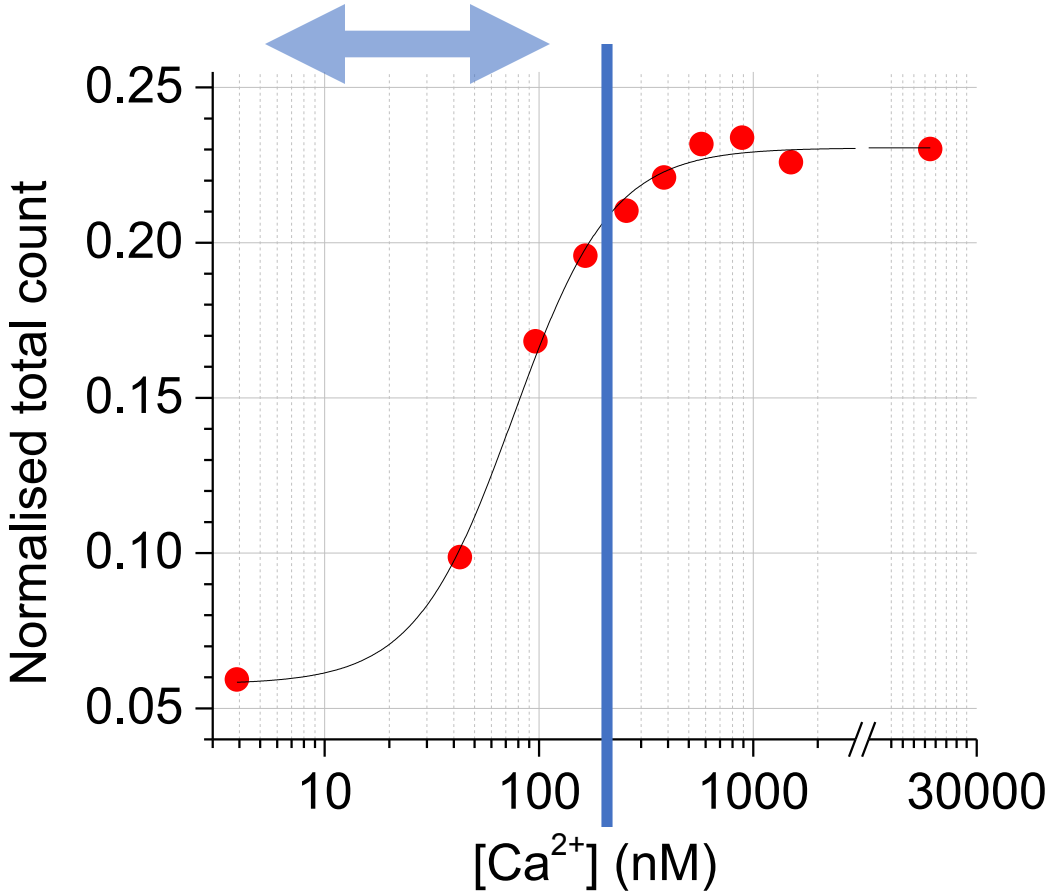



***Fluorescence-lifetime imaging microscopy**

# FLIM readout of red-shifted Cal-590

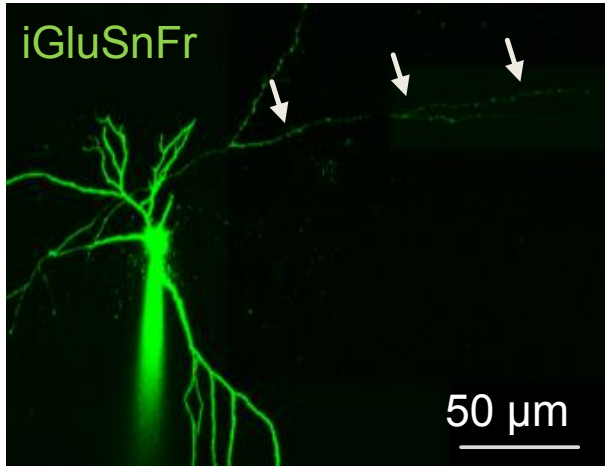
b

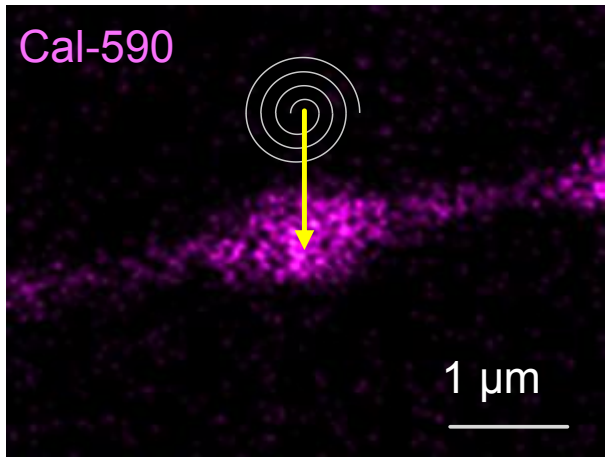



- **Quantitative calibration (Cal-590)**
- Normalized total count method (photon counts integrated along Ca sensitive components)
  - **-> greatest sensitivity in the 0- 200 nM range**

# Cal-590 peak sensitivity

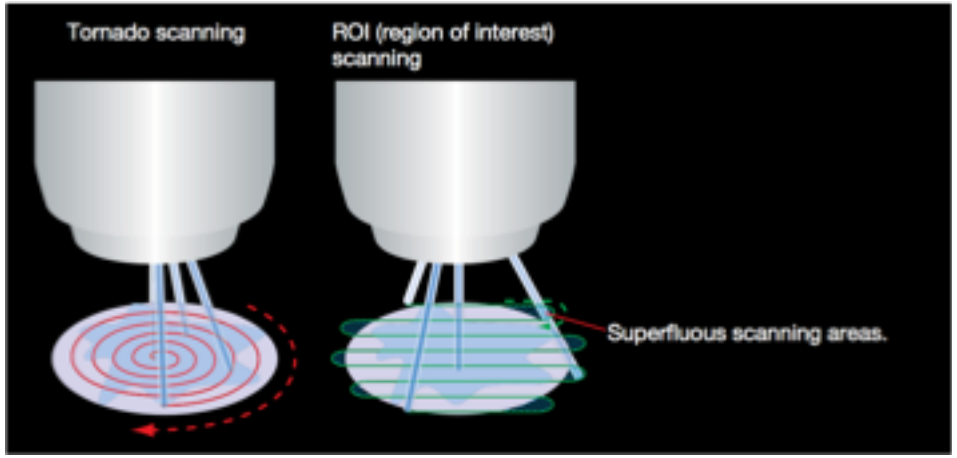
c



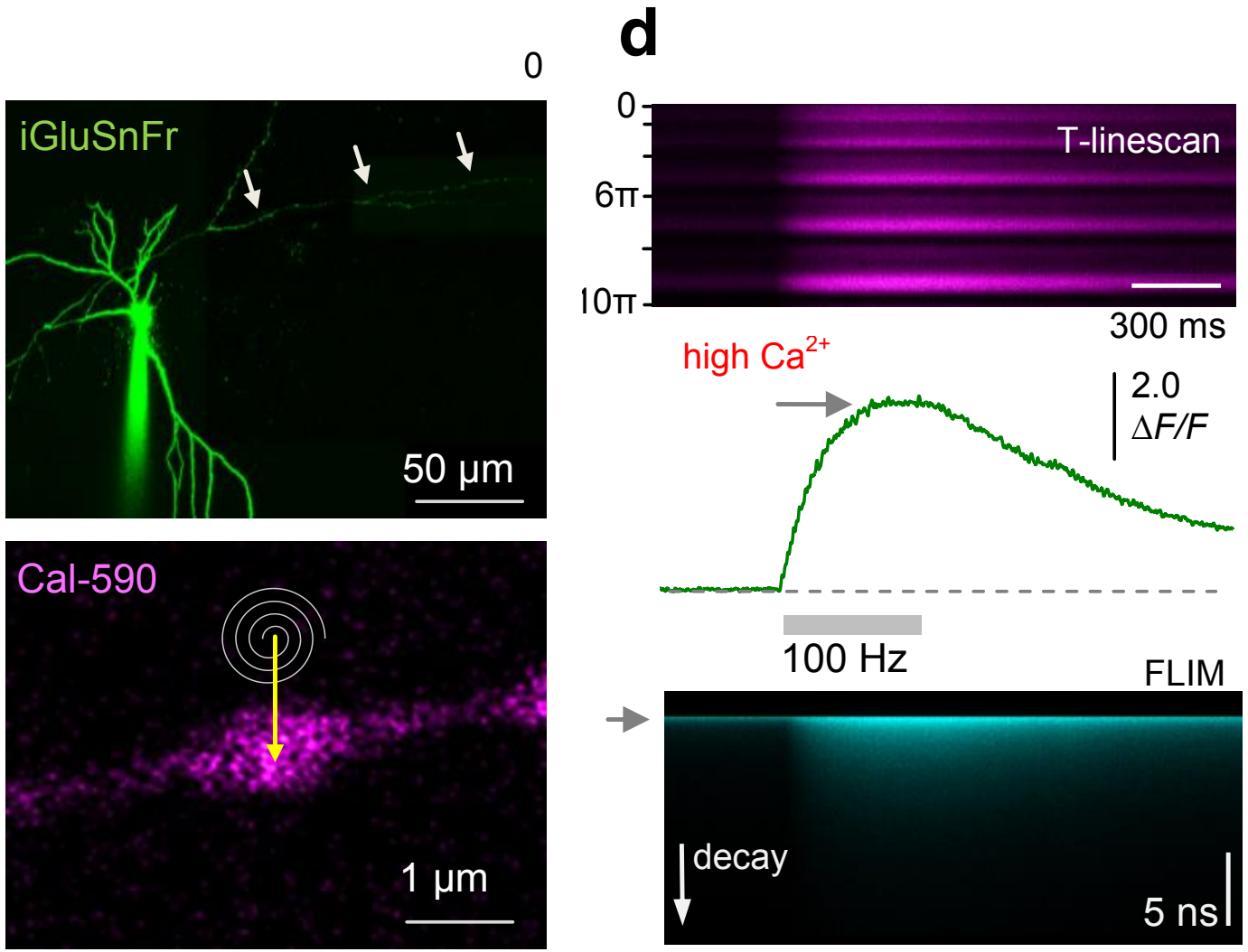




- organotypic **hippocampal brain slices**
- **Cell labeling through biolistic tranfection of iGluSnFR**
- **Patch loaded iGluSnFR expressing cell with Cal-590**
- **traced their axons for at least 150-200 microns from the cell soma, towards area CA1**
- **Tornado scanning of axonal bouton**

Spiral line-scanning mode

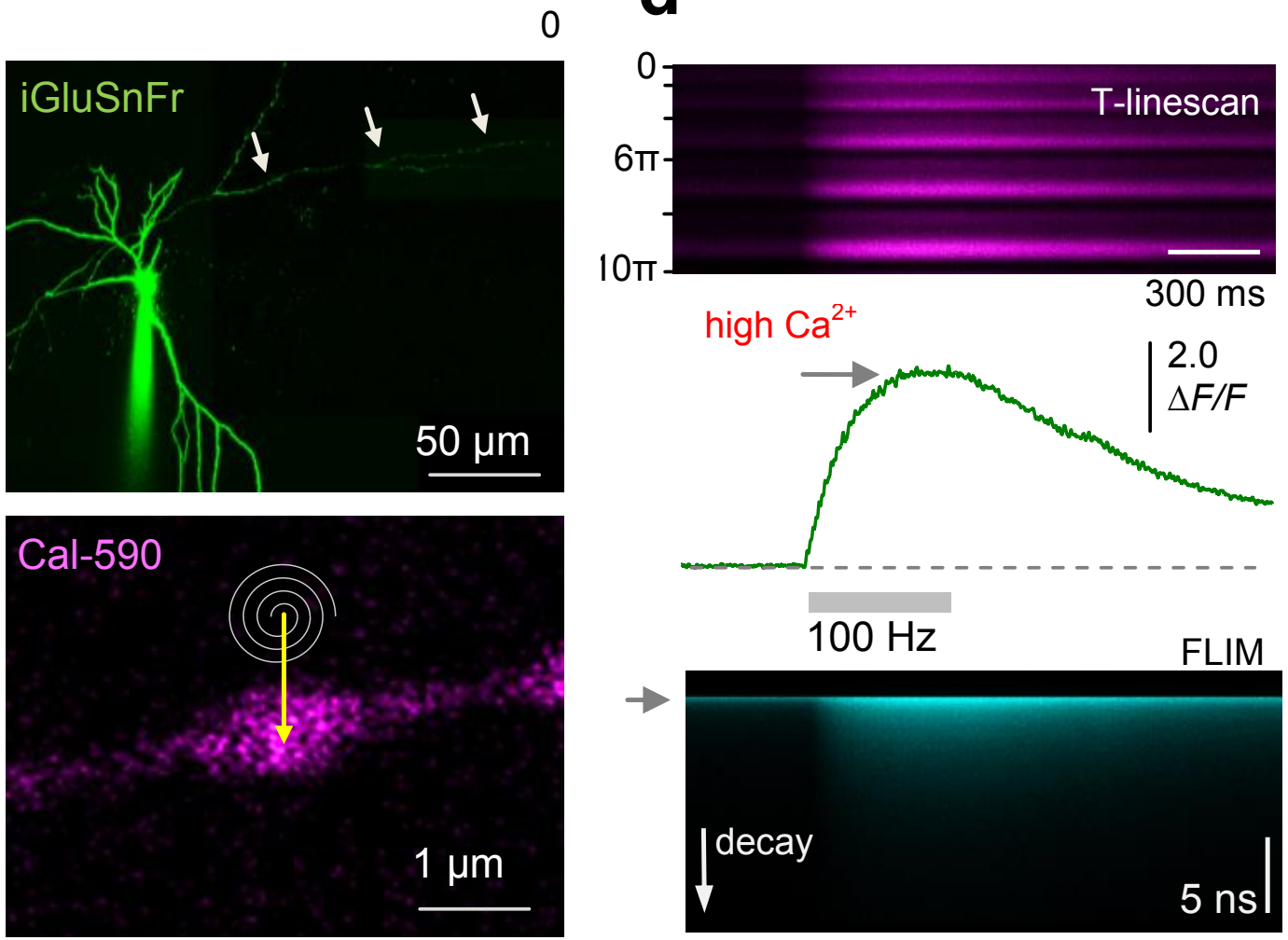


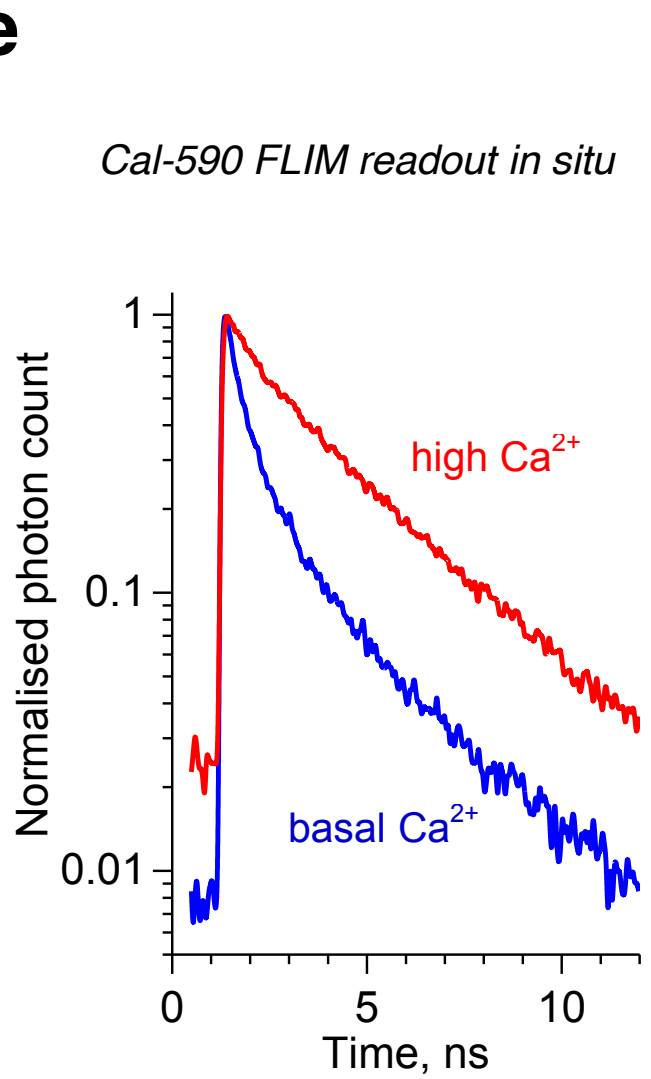

# *In situ* - Cal-590 in action




- **Example of a single-bouton Cal-590 signal**
- Represents 5 concenttric spiral circles
- 500 ms 100 Hz burst of spike-inducing somatic 1 ms current pulses
- fluorescence intensity (integrated over the 10π spiral scan) time course
- FLIM

# *In situ* - Cal-590 in action

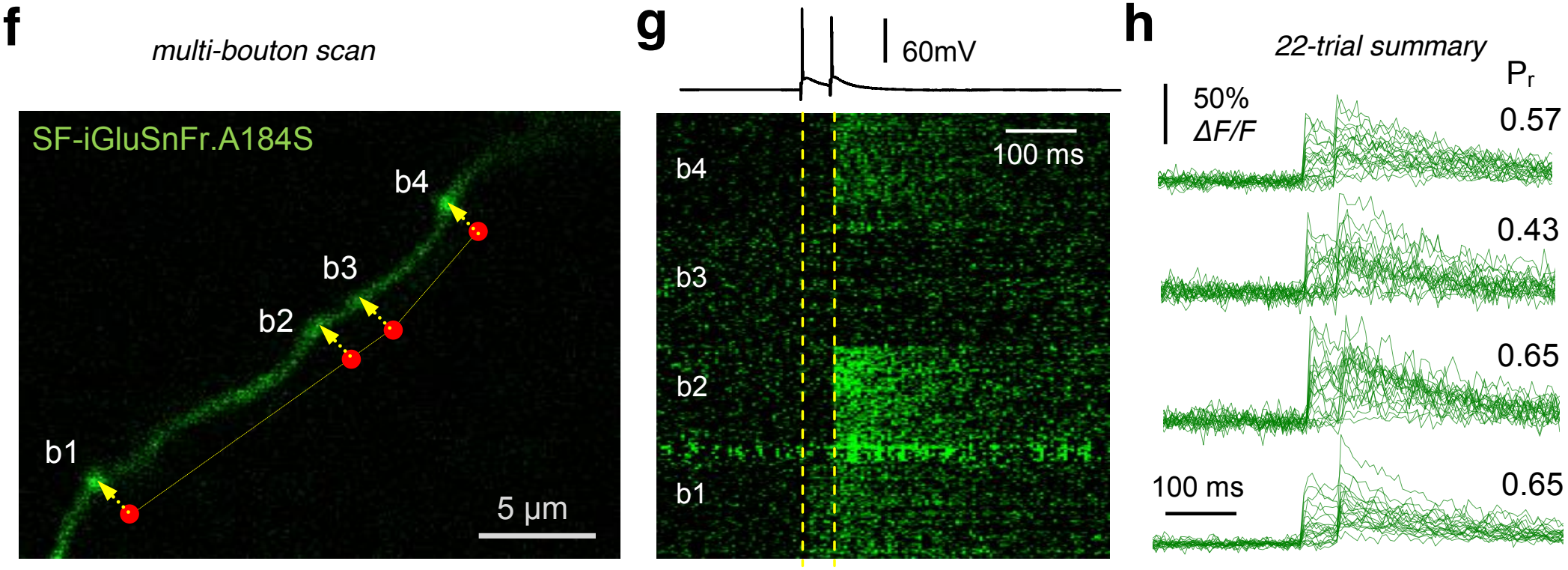



# *In situ* - Cal-590 in action

- Representation of FLIM readout at basal and high Ca from d

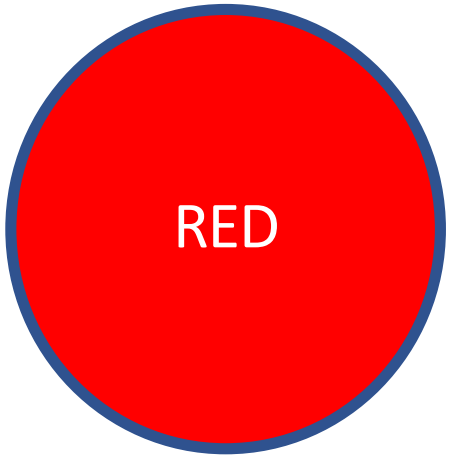
- CA3 pyramidal cell axon fragment showing four presynaptic boutons
- Linescan od iGluSnFR at 4 boutons recorded simultaneously
- During somatic generation of spikes
- x22



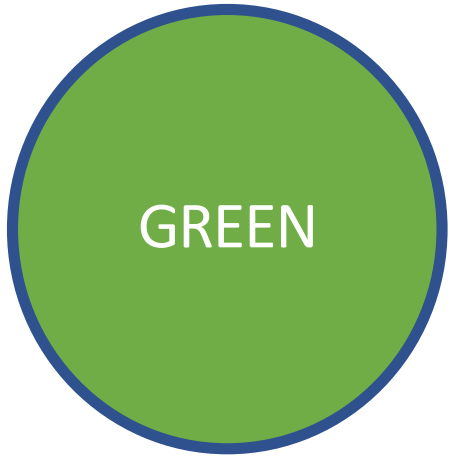

# iGluSnFR - readout

- no detectable photobleaching, over multiple trials
  - enables direct readout of release probability at multiple synapses from same circuitry

**Presynaptic**:
Cal-590
Emmission



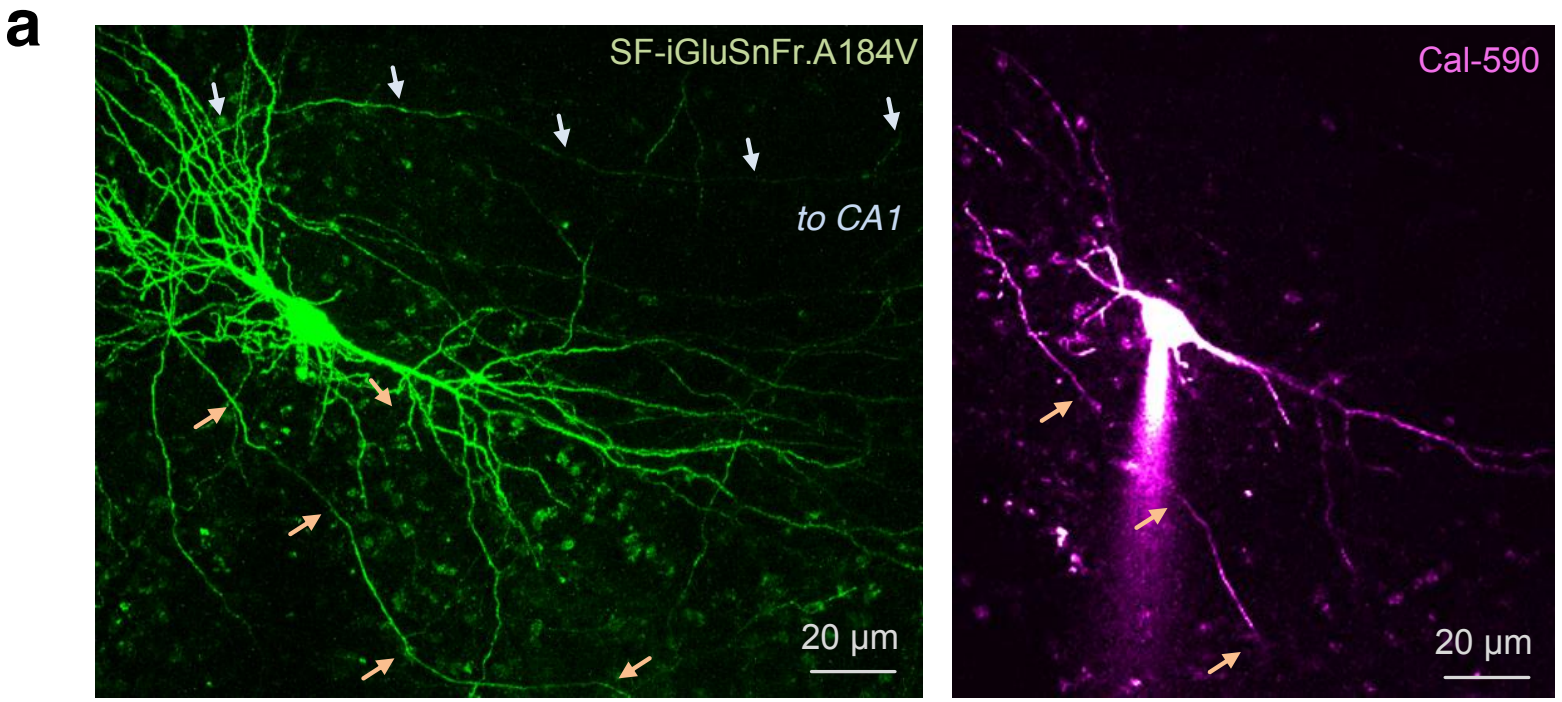

**Synaptic cleft**:
iGluSnFR.A184S
Emmission




- 2 dyes with 2 different emmission maxima
- Simultaneous imaging of Ca and glu possible?
  - -> in situ

# Cal-590 + iGluSnFR.A184S

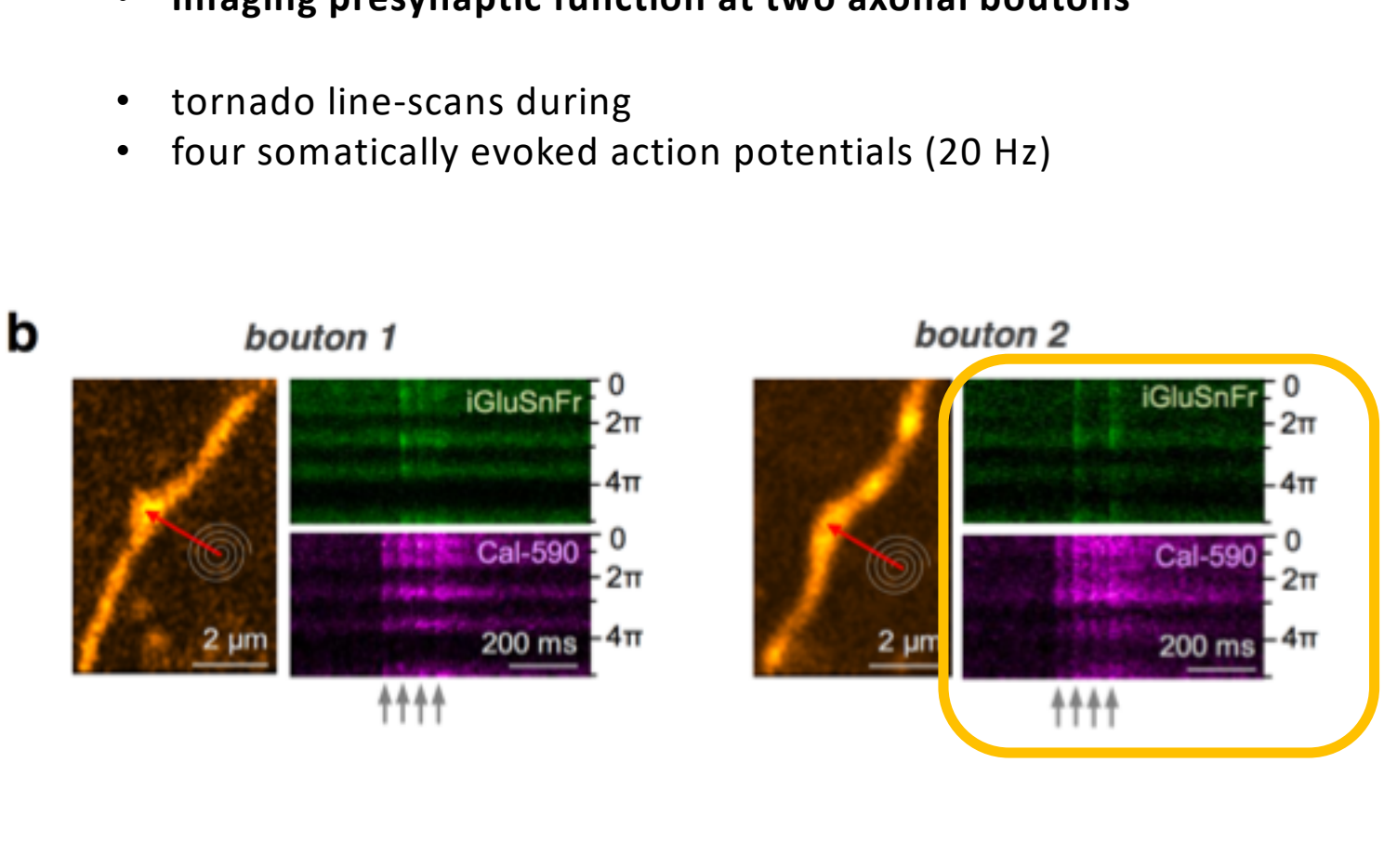
individually traced axonal boutons of CA3 pyramidal cells

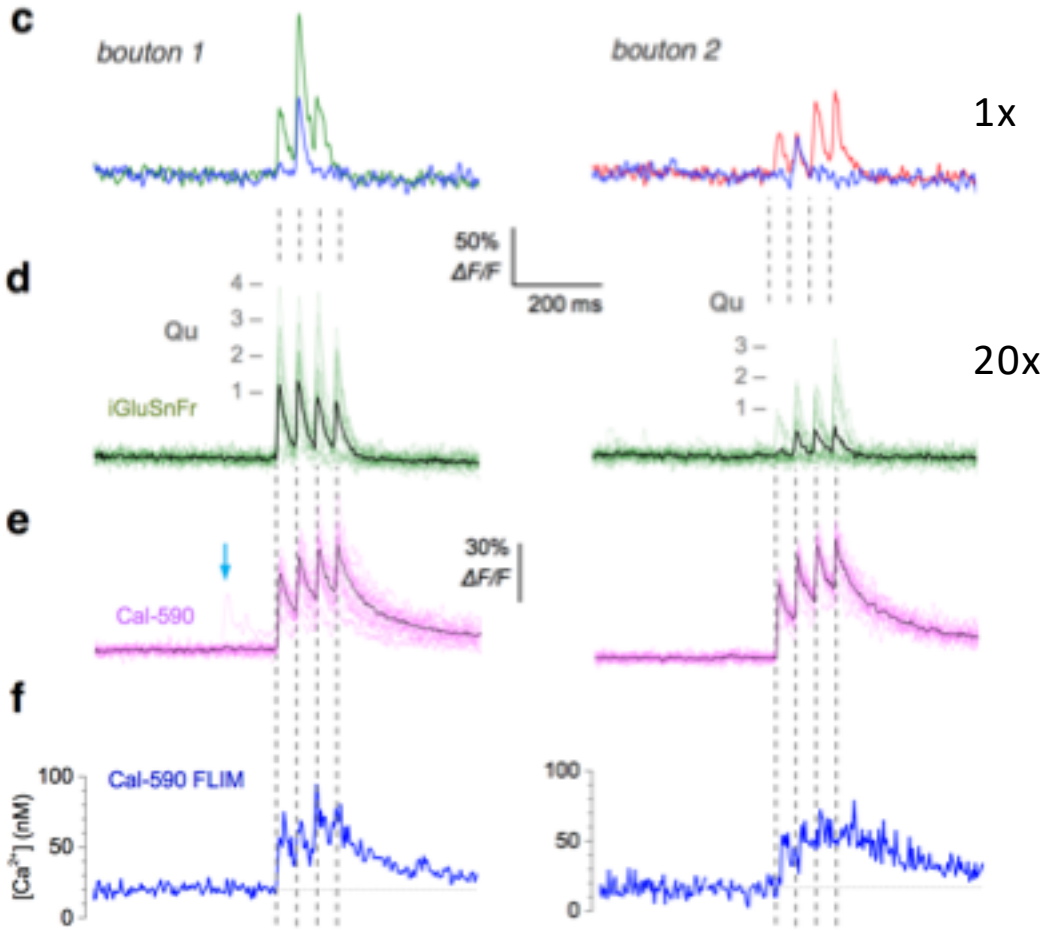



# Simultaneous monitoring of quantal glutamate release and presynaptic $Ca^{2+}$ dynamics

- **Imaging presynaptic function at two axonal boutons**

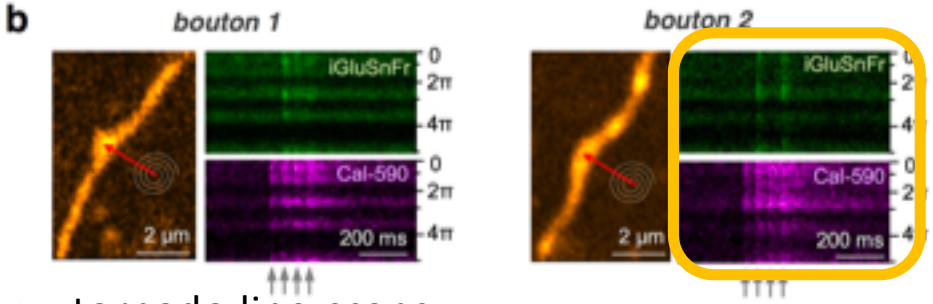
- tornado line-scans during
- four somatically evoked action potentials (20 Hz)




# Simultaneous monitoring of quantal glut release and presyn $Ca^{2+}$ dynamics

- Two characteristic single-trial, one-sweep recordings ->

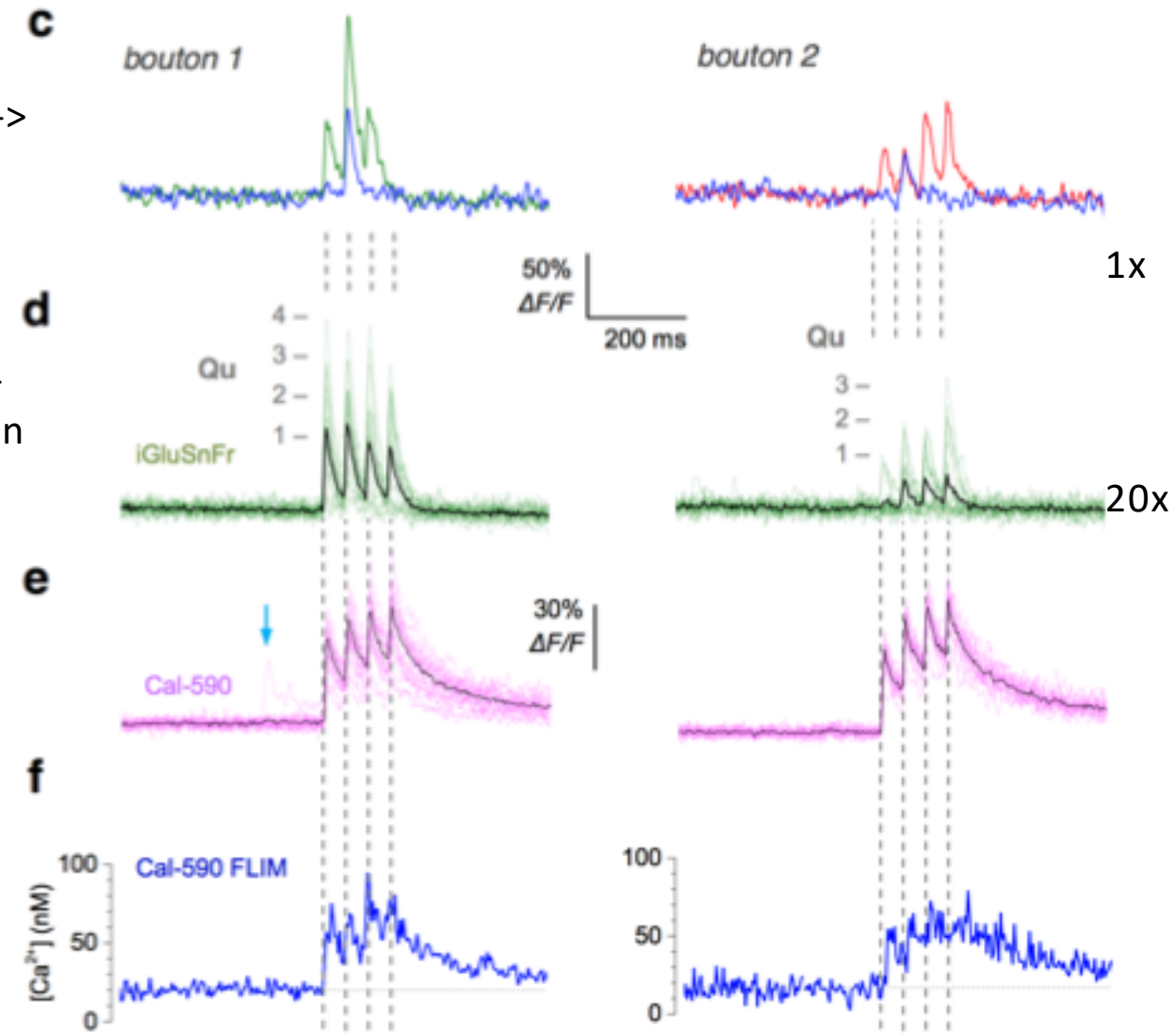



- tornado line-scans during four somatically evoked action potentials (20 Hz)

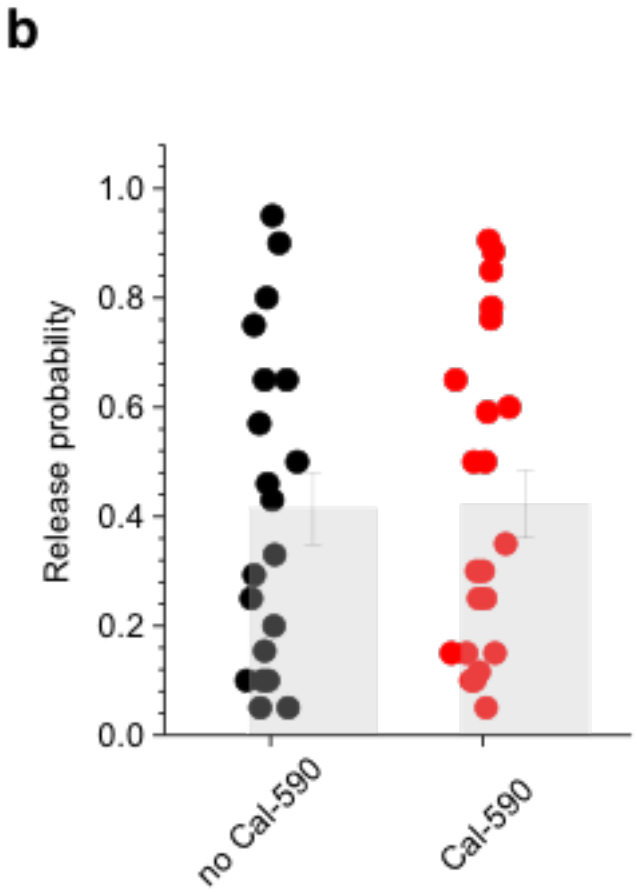
*relative short-term depression and facilitation

~= **ΔF/F**

d[Ca2+]

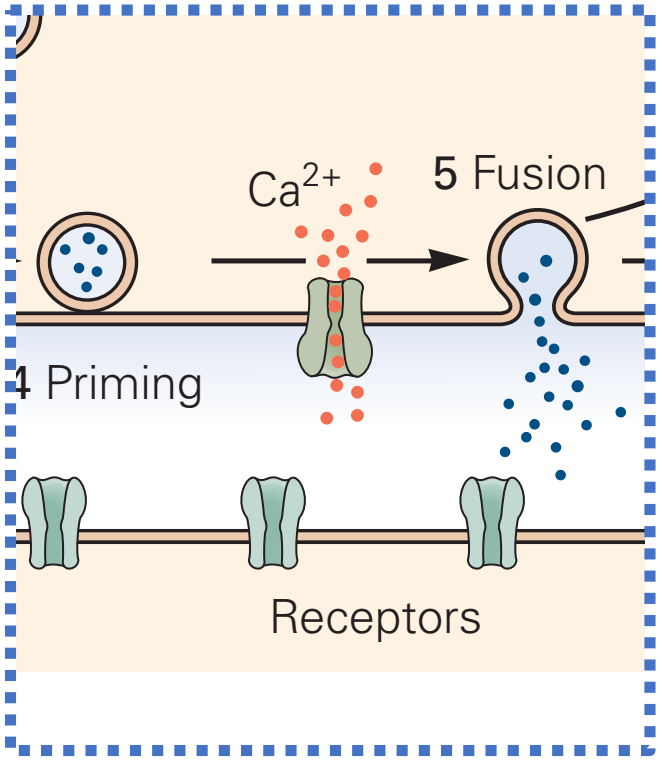



# Simultaneous monitoring of quantal glut release and presyn $Ca^{2+}$ dynamics

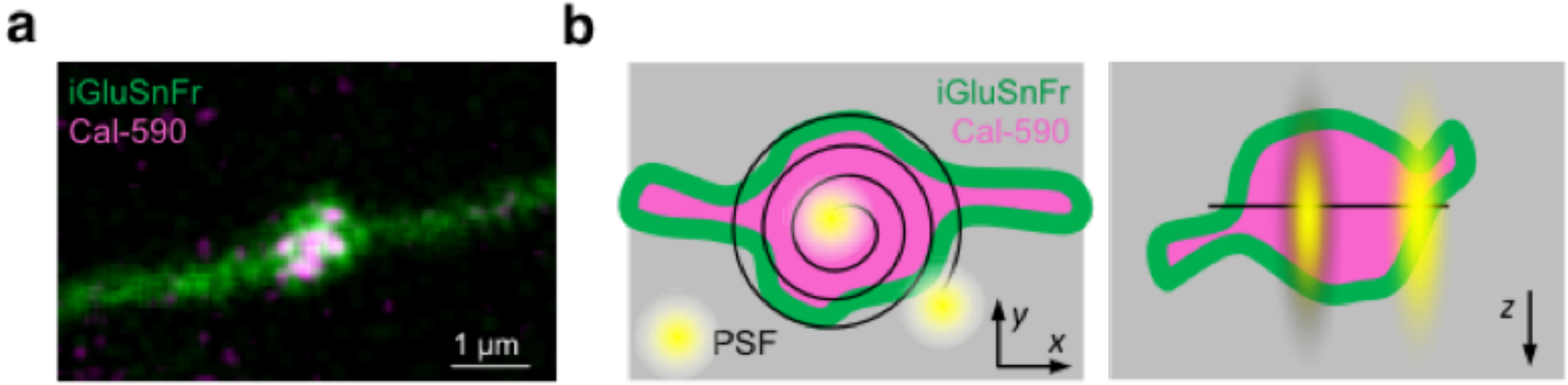



- Comparing glut release of Cal-590 loaded and not loaded
- average detected **release probabilities were indistinguishable**

# Testing $Ca^{2+}$ buffering interference

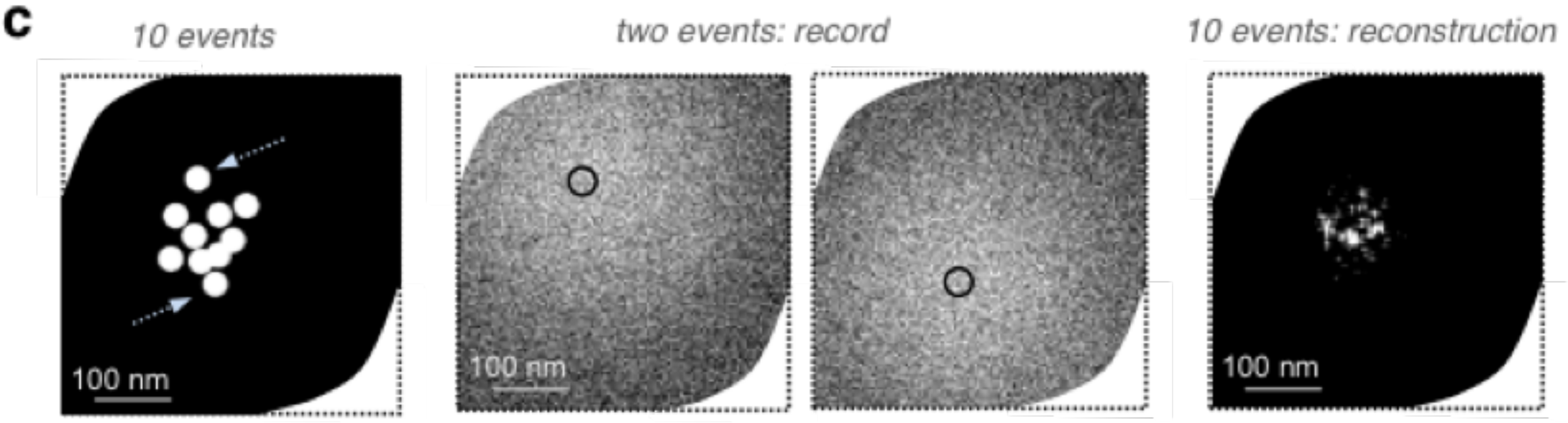



# Nanoscopic co-localisation of $Ca^{2+}$ entry and presynaptic glutamate release



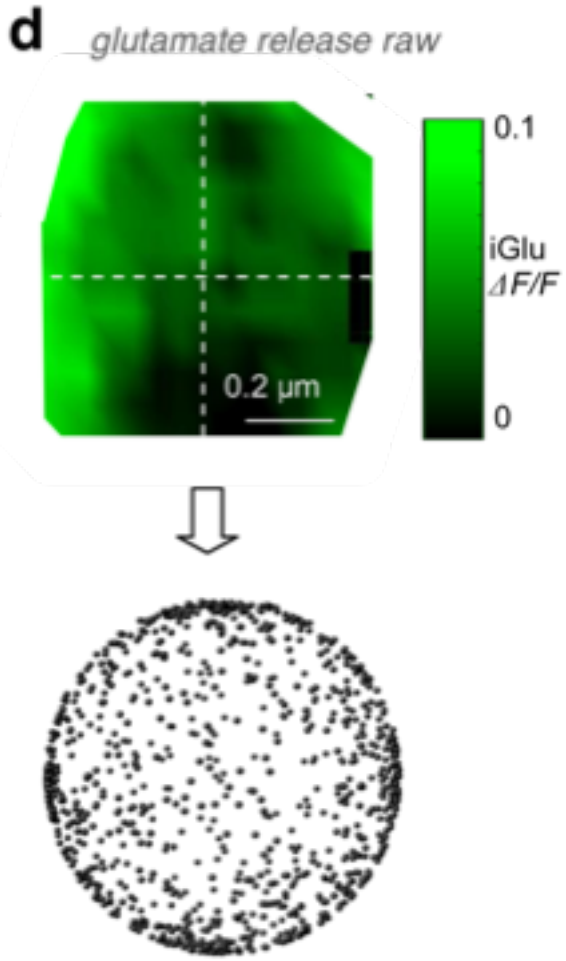

- 2-photon diffraction limit:
  - 0.2-0.3 µm (focal, x-y) ✸ Synaptic cleft: 2 - 4 nm
  - 0.8-1.2 µm in z
    - Note: when imaging xy -> z fluorometric signal is integrated

# Nanoscopic co-localisation of $Ca^{2+}$ entry and presynaptic glutamate release

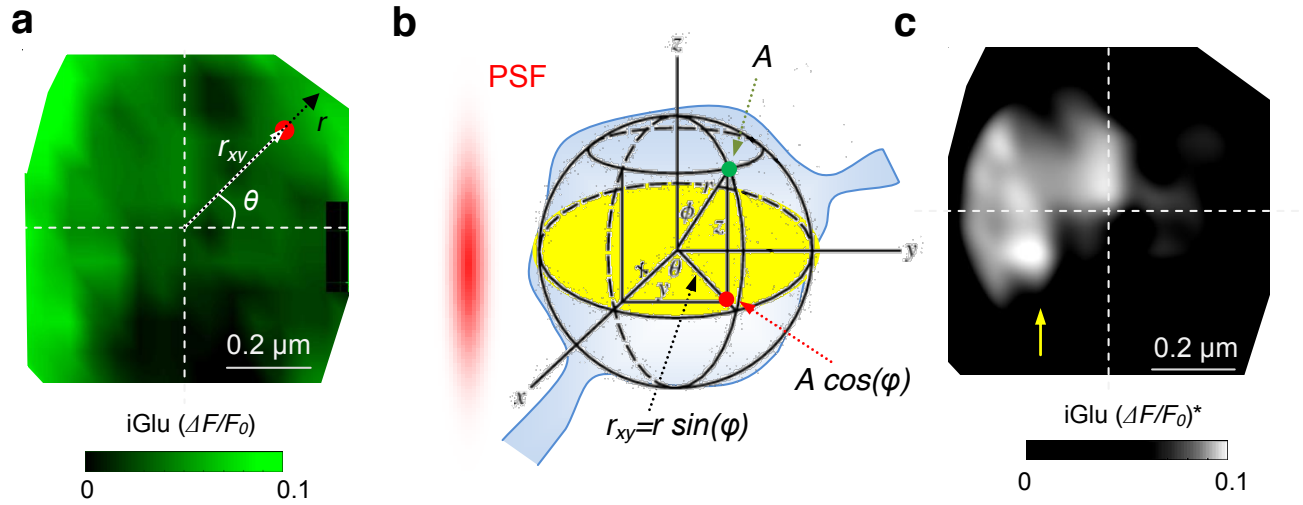



- Steep diffusion rates of both Ca2+ and glut -> causing concentrations to fall rapidly from site of action

# Sub- diffraction localization through multiple-exposure noise reduction

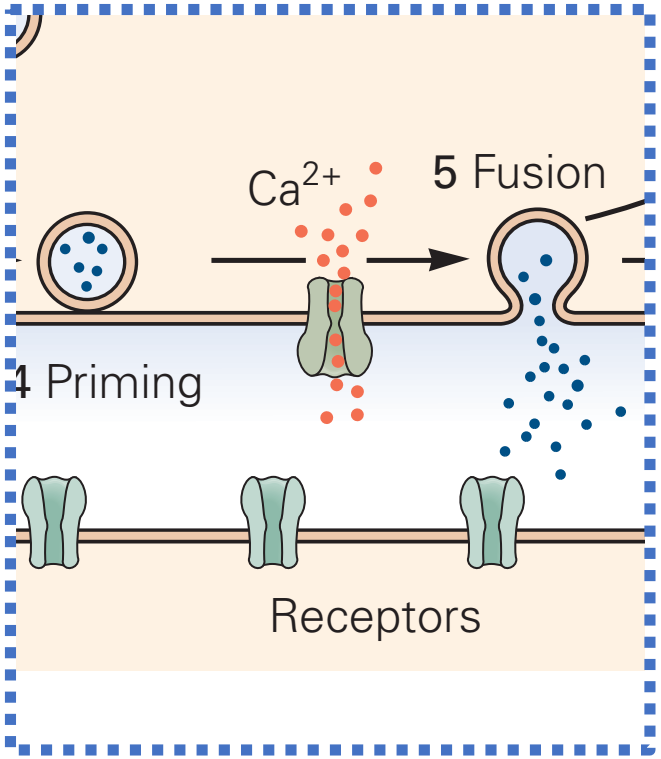


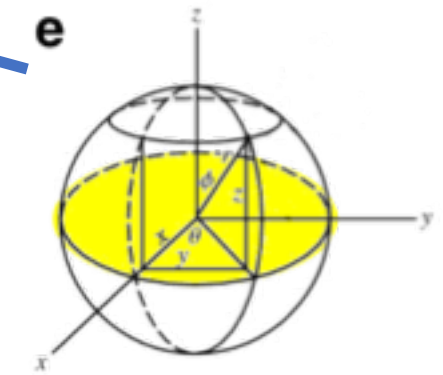

- Correction:

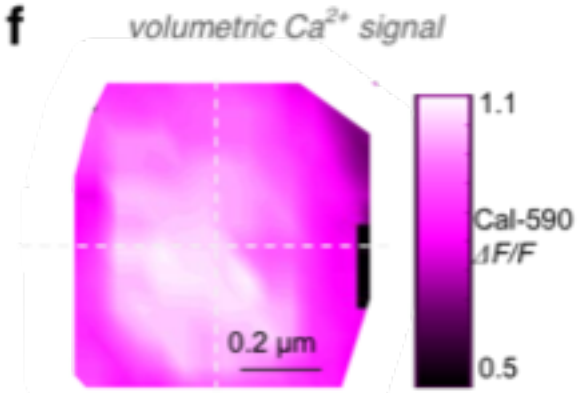


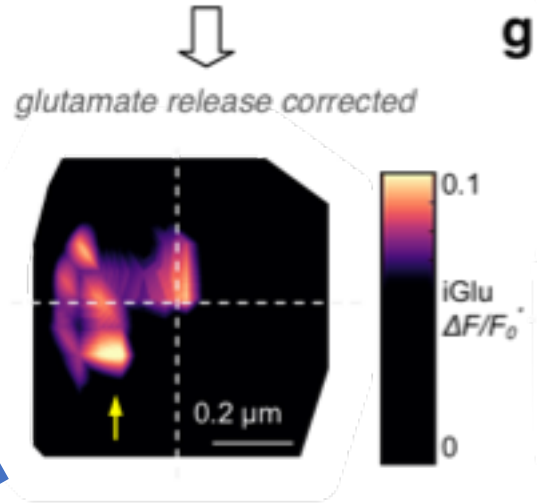

- overestimation corrected using trigonometric relationships for planar projection of a sphere

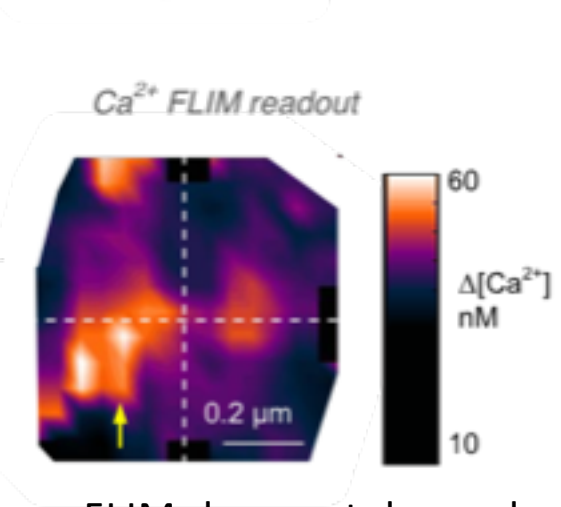
# Sub- diffraction localization - Correction



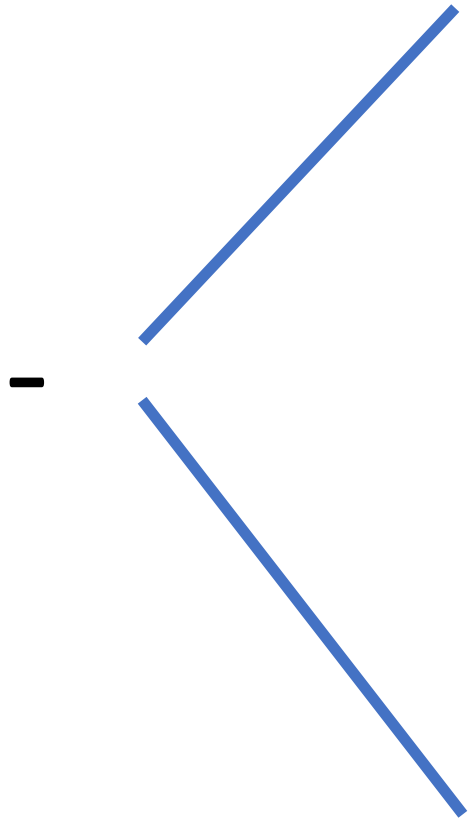




- More accurate localization of glut signal
- FLIM does not depend on indicator vol or signal intensity

# Nanoscopic co-localisation of $Ca^{2+}$ entry and presynaptic glutamate release

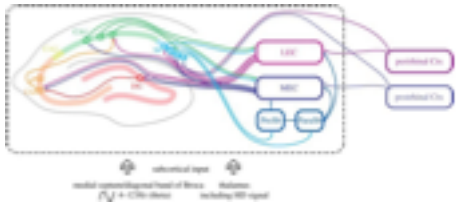


- Possible **Ca- buffering** by Ca-binding dye
  - effect of Ca2+ indicators on rapid presynaptic Ca2+ dynamics and release probability could be significant
  - **Reqcuires** seperate study
  - **Systematic comparison of release probabilities**

- Cal-590 has a relatively low affinity
- iGluSnFR fluorescence signal (for tracing) **– interference**?
- sub-diffraction localisation with current settings is feasible only in the x-y plane whereas in **the z plane** the signal location is more uncertain
- Correction bears possibility of error

# Conclusion – the questionable

+



- **Co-localization studies** through imaging and correction possible – (**2 dyes with separate emmission maxima**)
- **Possibility of further studying** :
  - Presyn spike detection
  - **Mechanistic relationship Calcium entry and glutamate release**
  - presynaptic Ca entry and glut release dependance **in diverse neural circuits, in different brain regions**

- Cal-590 low affinity -> less buffering than OGB-1
- No significant effect of axon loading with Cal-590 on average release probability
- Prev: OGB-1 /GECIs used to study neural network activity in freely-moving animals without apparent behavioural implications
- **Decrease Problems of live imaging *in situ* or *in vivo:***
  - **focus drift, photobleaching, or physiological changes in tissue light scattering or absorption**

# Conclusion – the good

Done : )