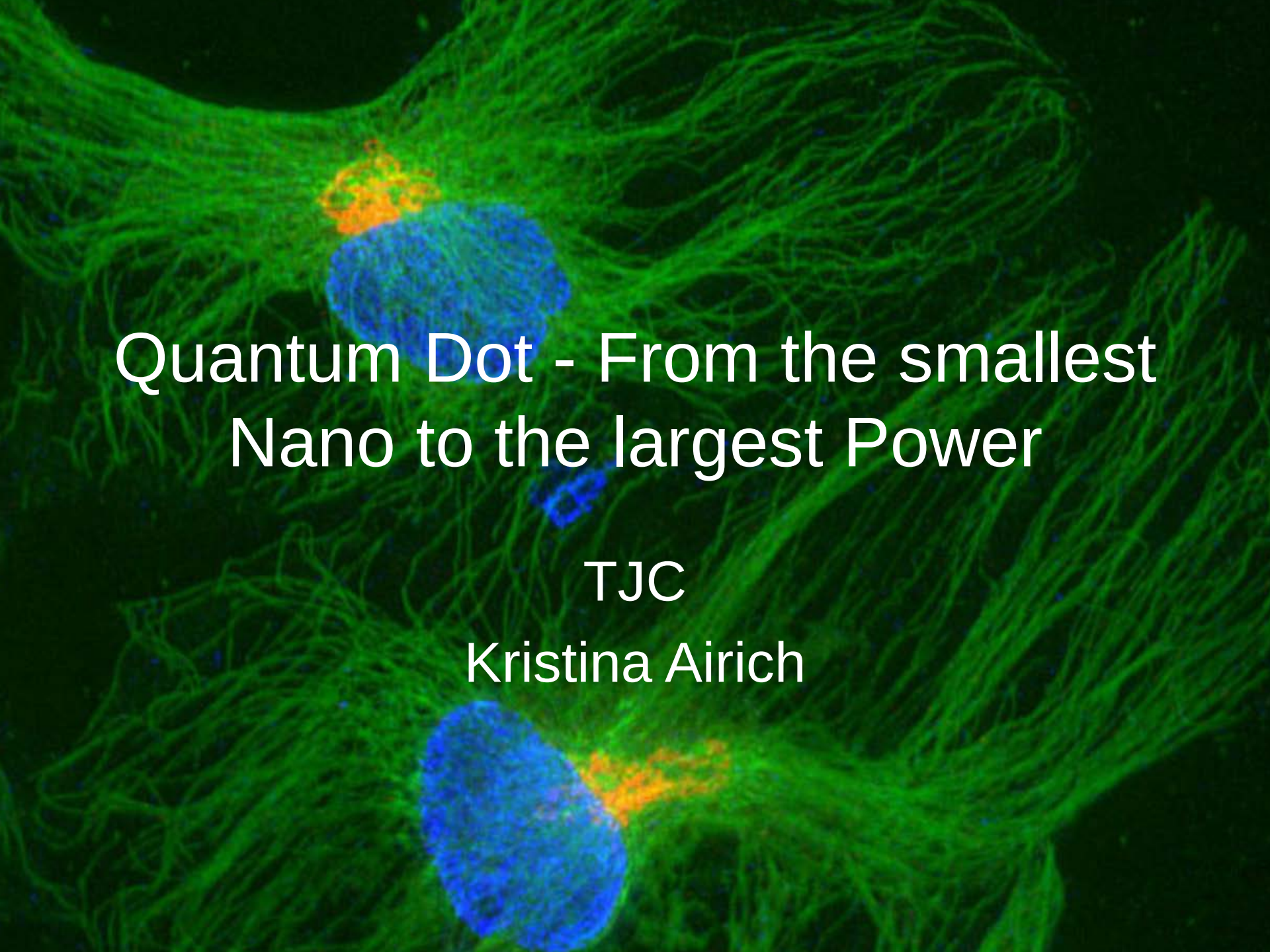
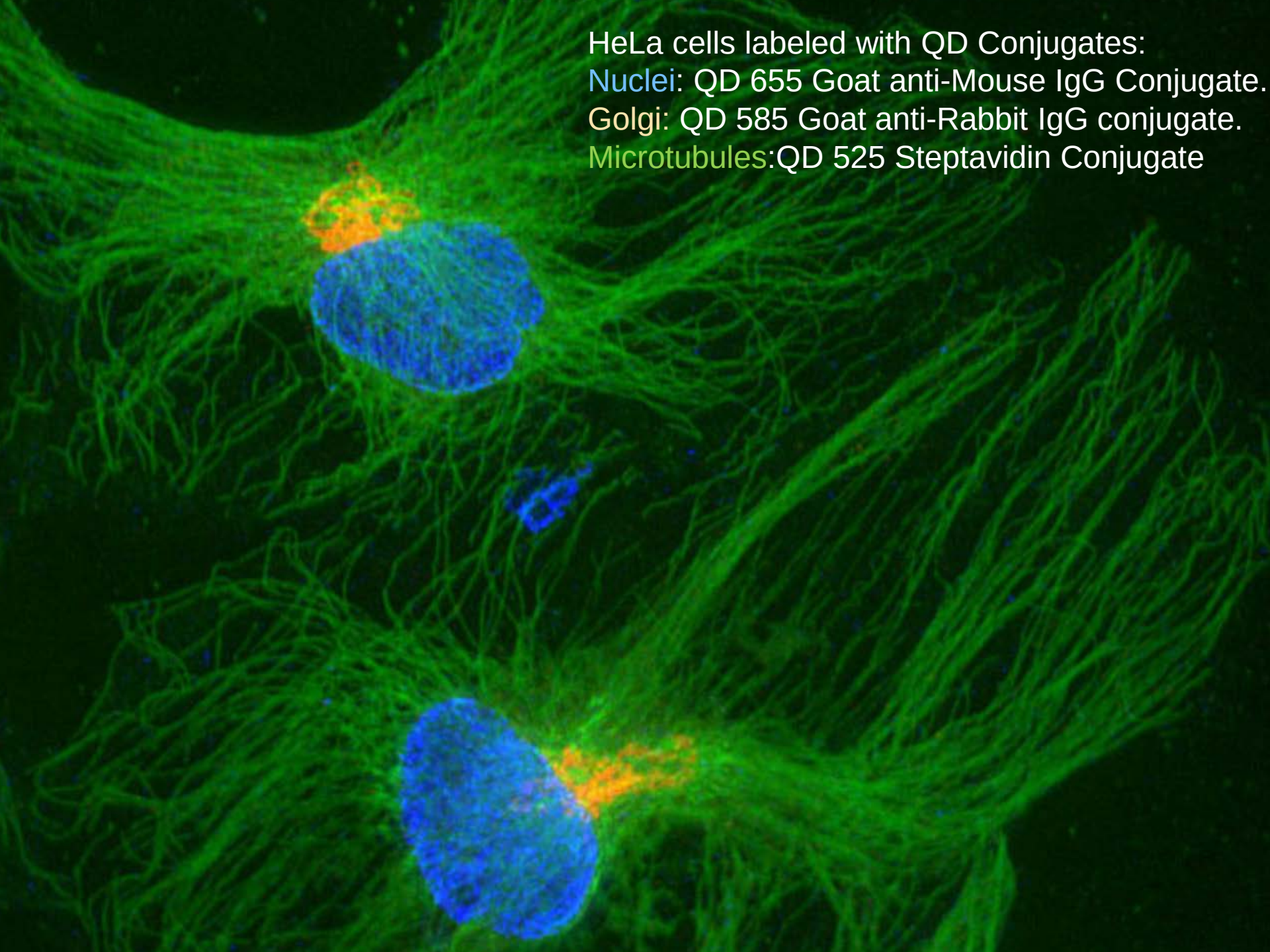


Quantum Dot - From the smallest Nano to the largest Power

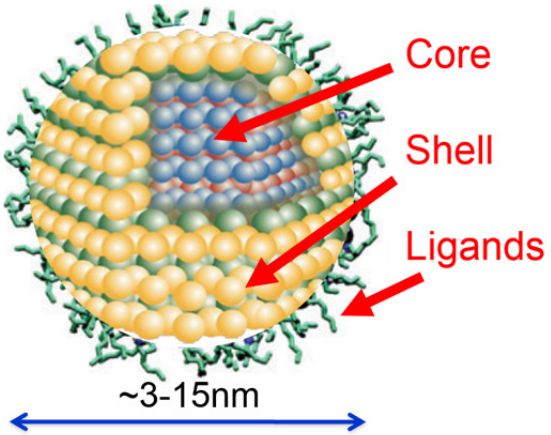
TJC

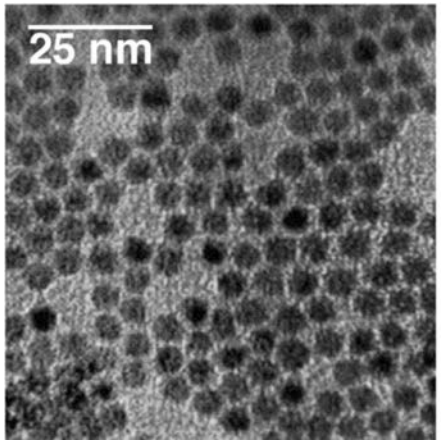
Kristina Airich

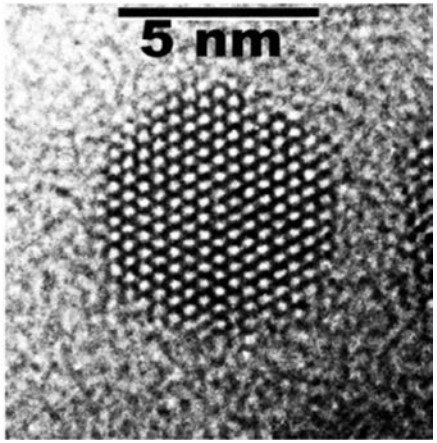

HeLa cells labeled with QD Conjugates:
Nuclei: QD 655 Goat anti-Mouse IgG Conjugate.
Golgi: QD 585 Goat anti-Rabbit IgG conjugate.
Microtubules:QD 525 Steptavidin Conjugate

Quantum Dots

- Quantum Dots are nanocrystals made of semiconductor particles
  Single electron trapped inside a cage of atoms
- have the ability to glow by emitting light after light excitation
- very bright, 50-fold excitation coefficients
- exceptional photostability

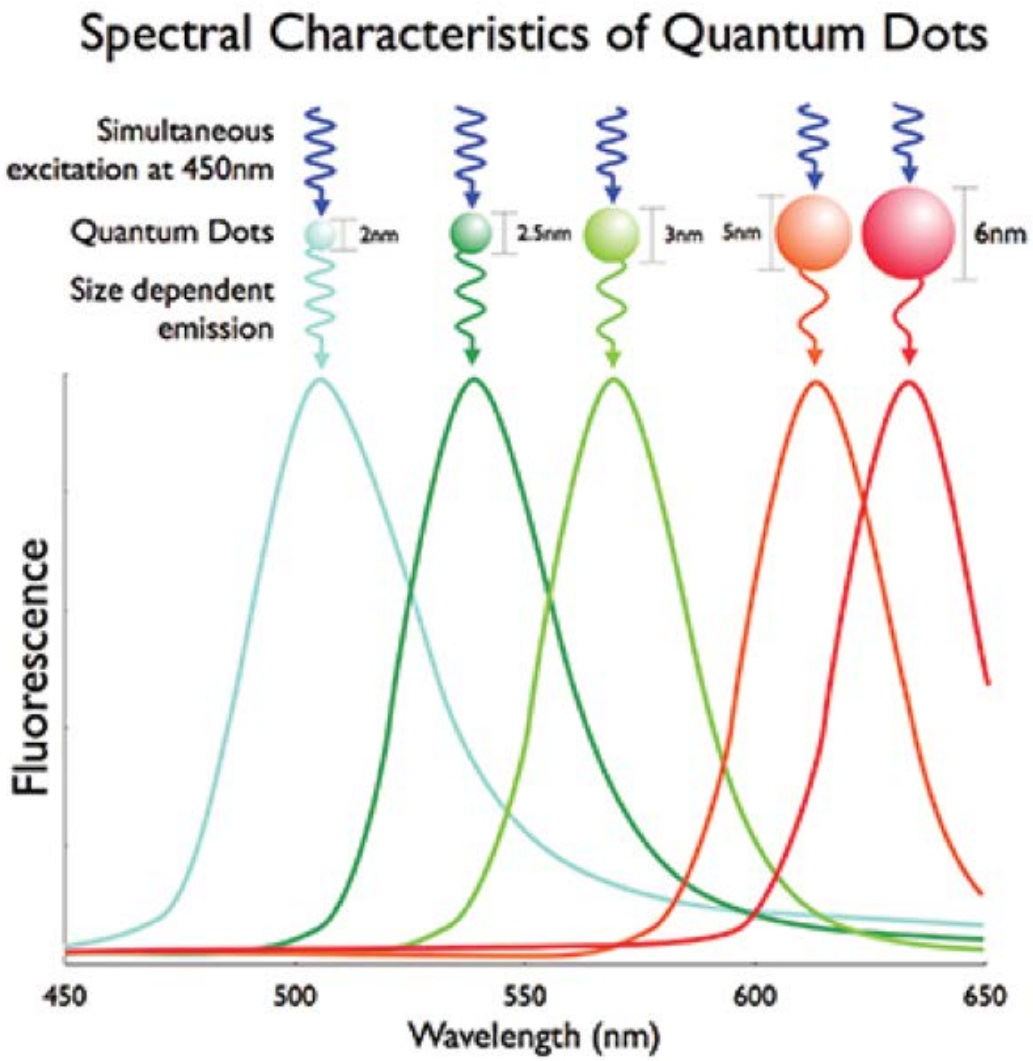






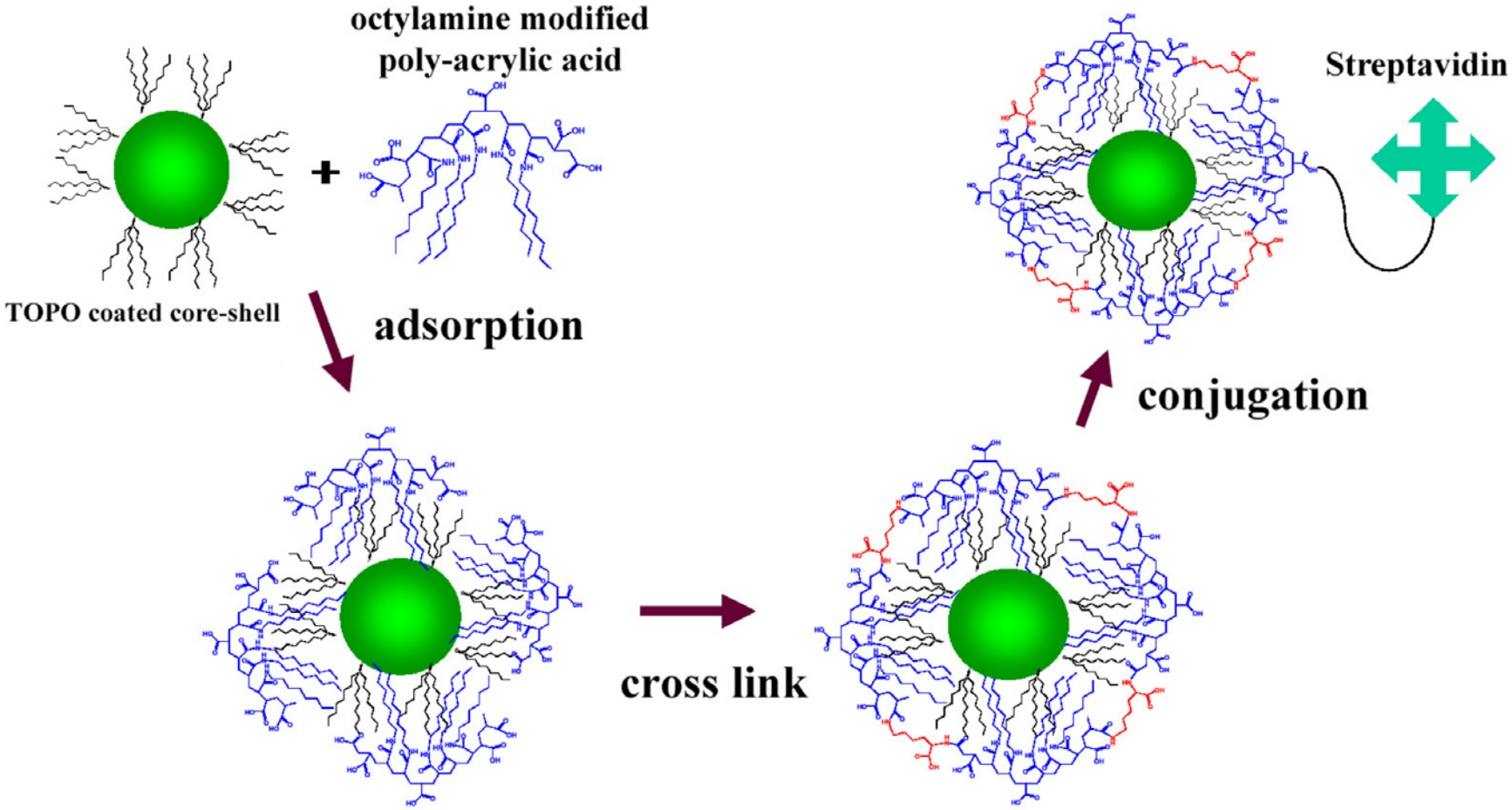
One dot showing
close packed atoms

Optics



- The color of emitted light depends on QD size
- Nanocrystal probes in aqueous buffer, all illuminated simultaneously with a handheld ultraviolet lamp

Coupling chemistry



chemical improvements have focused on reducing the size and increasing the chemical stability of the watersoluble QD

https://www.youtube.com/watch?v=ow6R08Q5Haw&feature=youtu.be

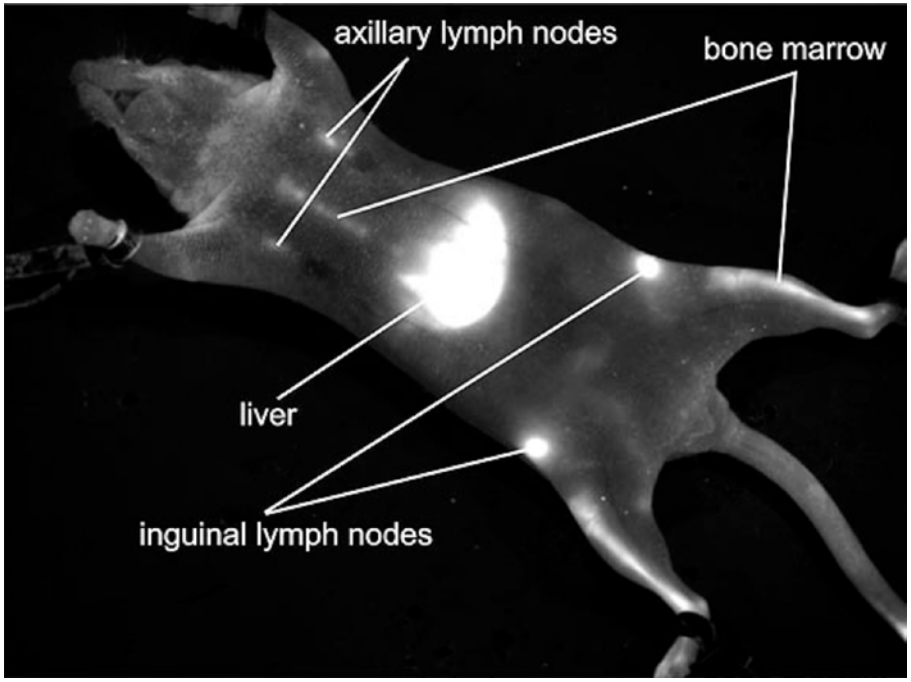
QD Application

- Simultaneous detection and analysis of multiple targets
- QD photostability ➔robust image acquisition and accurate quantitative analysis of staining intensity

Tools:
- flow cytometry
- Immunofluorescence
- in vivo imaging
- energy-transfer-based reporter systems

Application

Long-term experiments : QD remain fluorescent after at least four months in vivo.



Application

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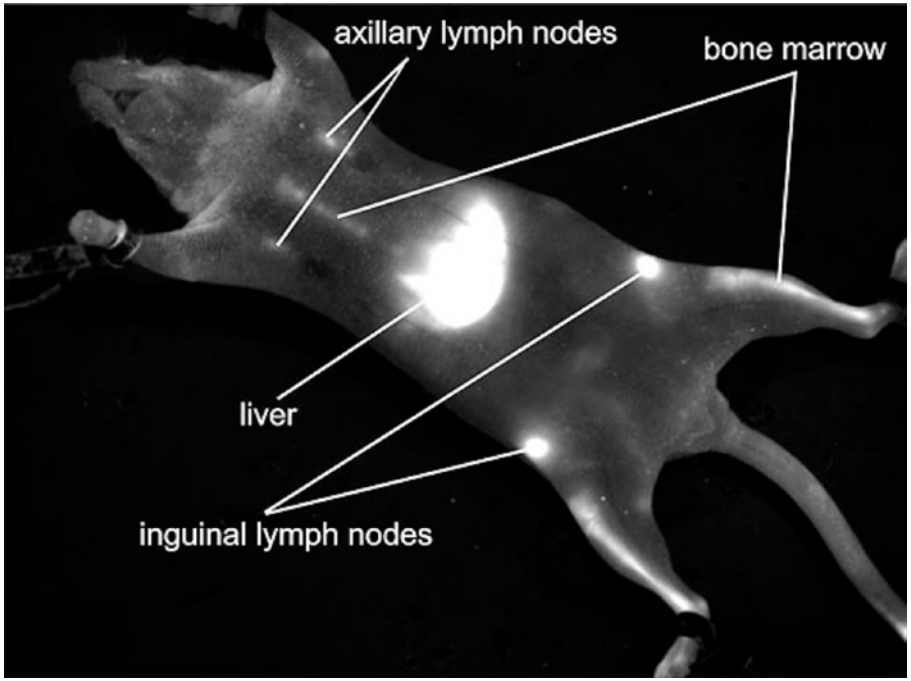
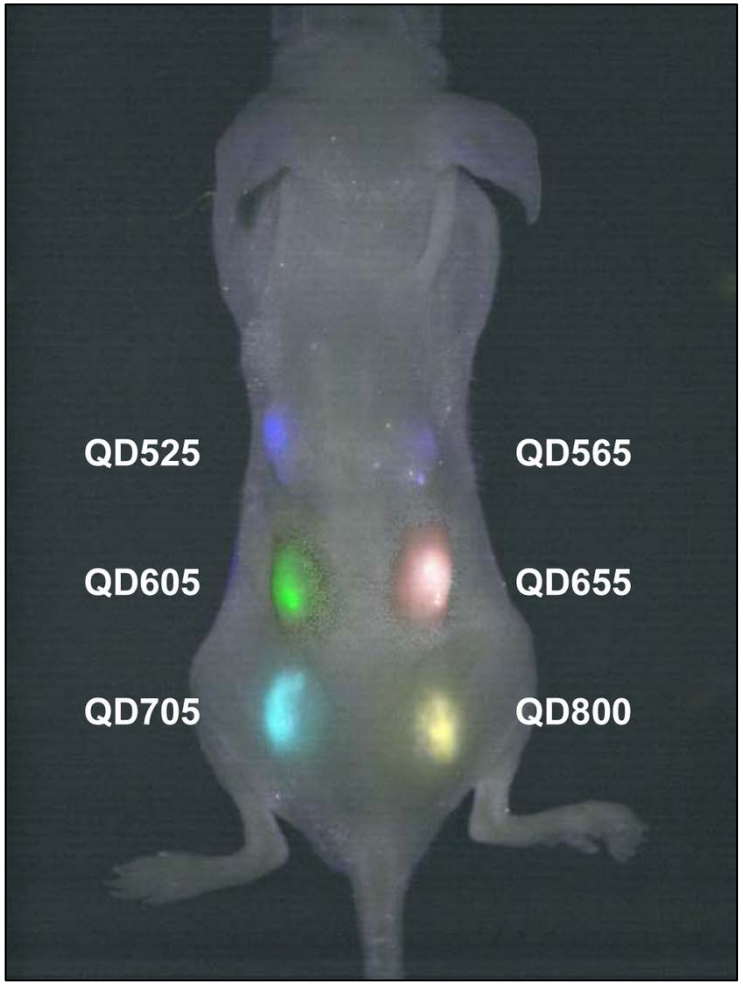


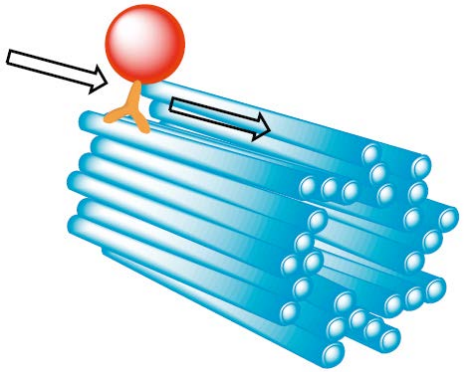


Multiplex imaging capability of QD in live animals: mouse embryonic stem cells labeled with different QD & image acquisition with single excitation light source right after injection.

Single Particle Tracking: living cells

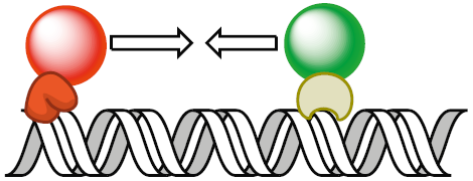
motor proteinslabeled with QD :
Myosin V on actin stepping

- Motion (trajectory of QD along the cytoskeletal track)



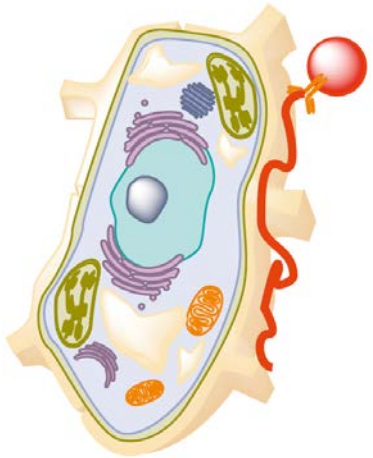
Multiple proteins labeled with QD:
DNA in process

- Mobilization
- Interaction (DNA-protein)
- Disruption (repair or replication)



Surface receptor labeled with QD:

- Receptor dynamics
- Diffusion



Single Particle Tracking: Receptors

biological targets:

- GPIanchored proteins
- AMPA-receptors
- Voltage gated ion channels
- IgE receptor
- receptor tyrosine kinases (insulin receptor)
- Dopamine receptors

Single Particle Tracking: Receptors

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- Dopamine receptors

Single-molecule imaging of the functional crosstalk between surface NMDA and dopamine D1 receptors

Laurent Ladepeche[a,b], Julien P. Dupuis[a,b], Delphine Bouchet[a,b], Evelyne Doudnikoff[c], Luting Yang[a,b], Yohan Campagne[a,b], Erwan Bézard[c], Eric Hosy[a,b], and Laurent Groc[a,b,1]

ARTICLE

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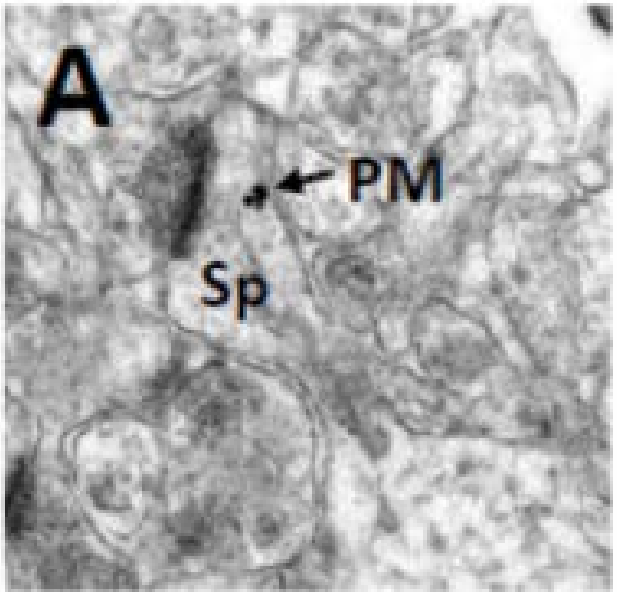
Targeting neurotransmitter receptors with nanoparticles *in vivo* allows single-molecule tracking in acute brain slices

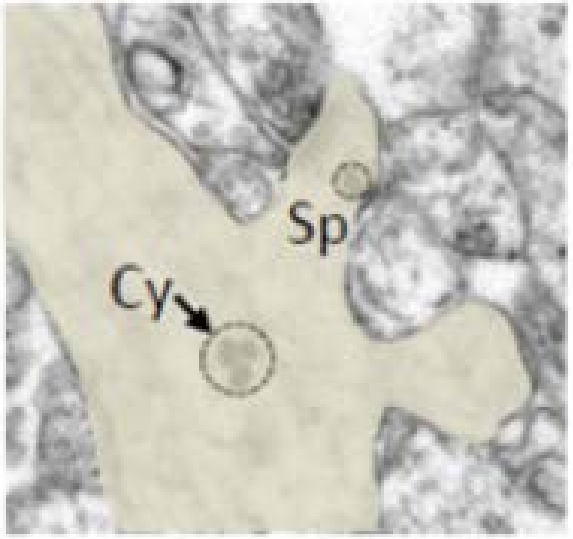
Juan A. Varela[1,2], Julien P. Dupuis[1,2], Laetitia Etchepare[1,2], Agnès Espana[1,2], Laurent Cognet[3,4] & Laurent Groc[1,2]

QD as Trackingsystem for Mobility & Diffusion: HPC culture

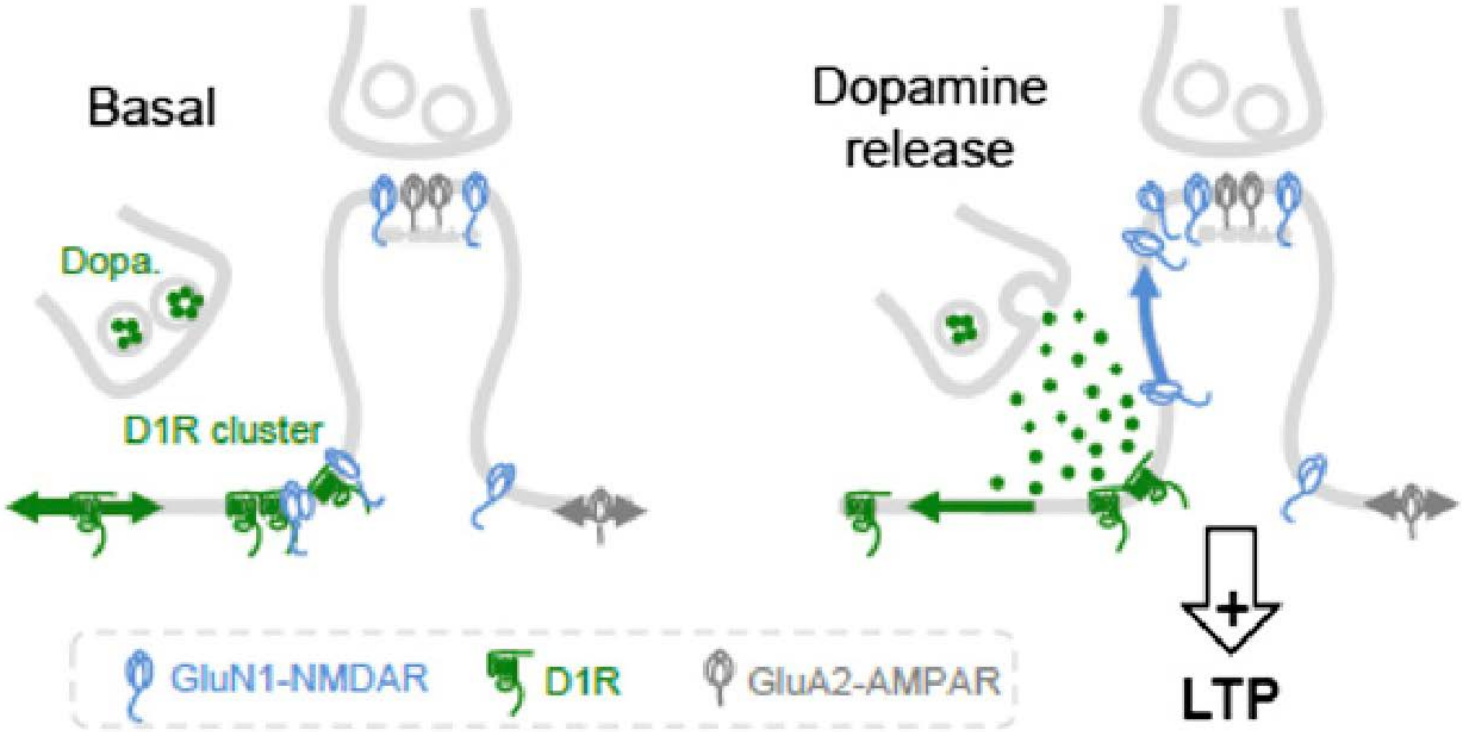


Electron micrographs: immunogold-labeled endogenous D1R protein close to the plasma membrane (PM) in a dendritic spine (Sp)…

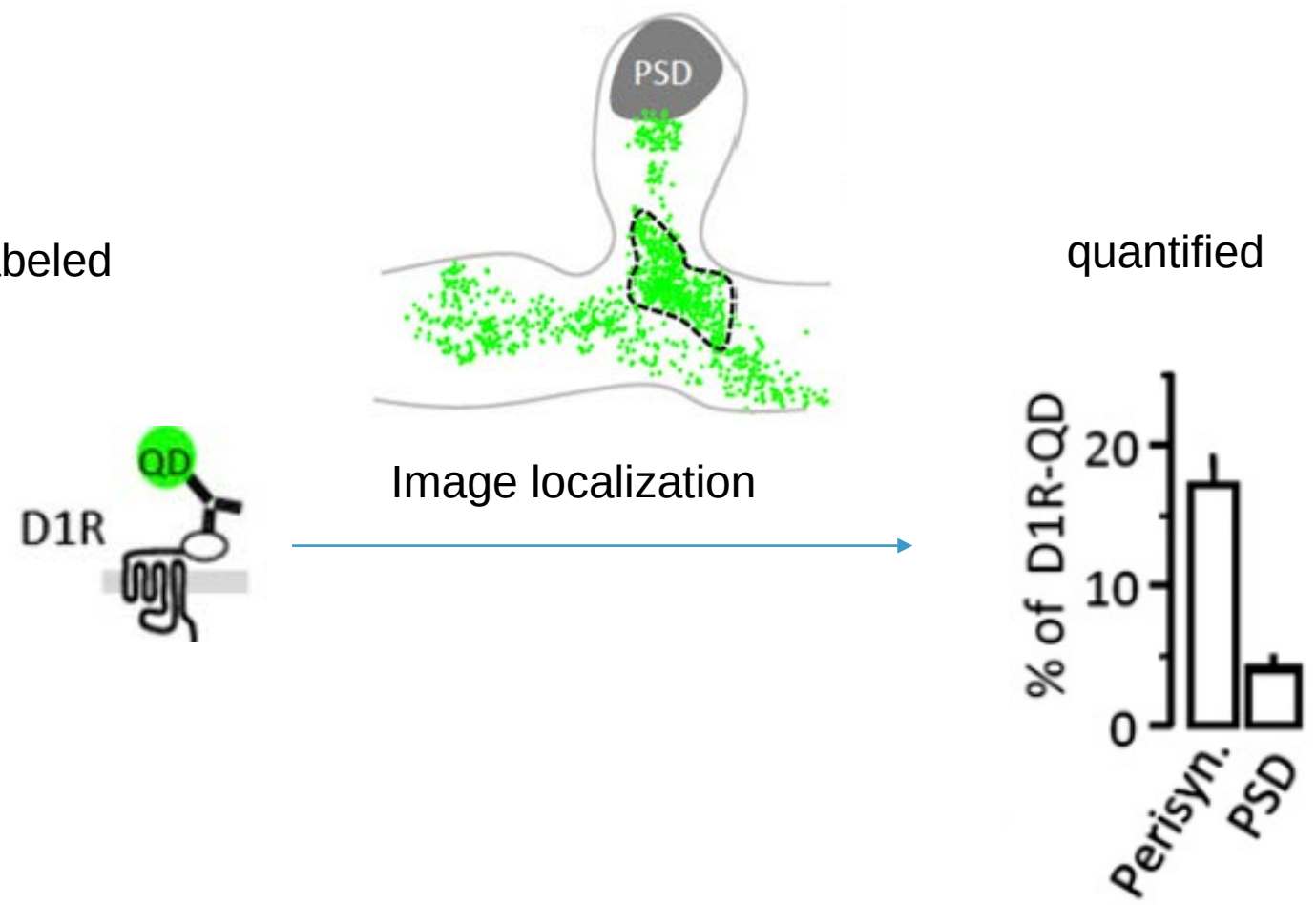
…and in the cytoplasm (Cy)
Circle: D1R



D1Rs dynamics



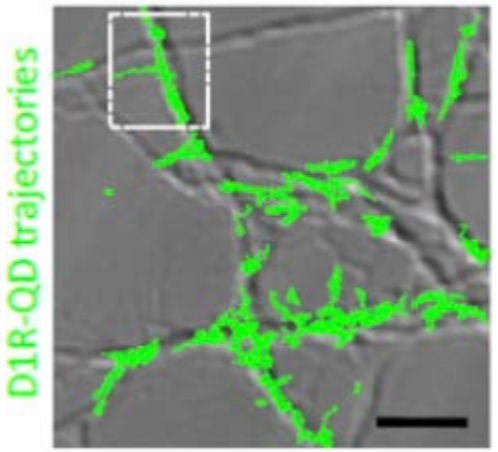
D1Rs dynamics



Percentage of single D1R–QD particles detected in the synaptic core (PSD) or the perisynaptic area

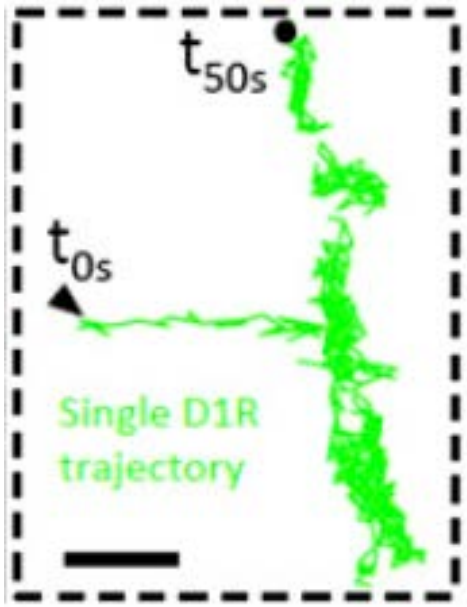
D1Rs dynamics

trajectories (1,000 frames, 20-Hz acquisition rate) of surface D1R in dendrites

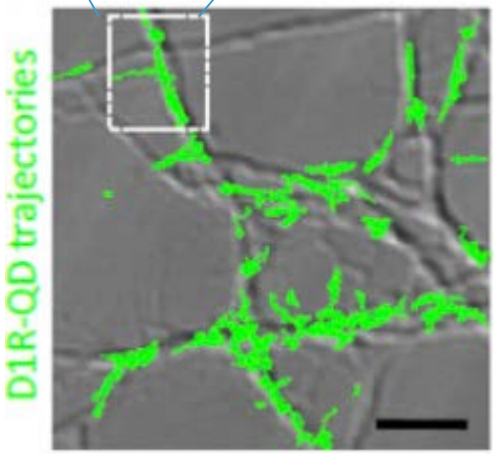


D1Rs dynamics

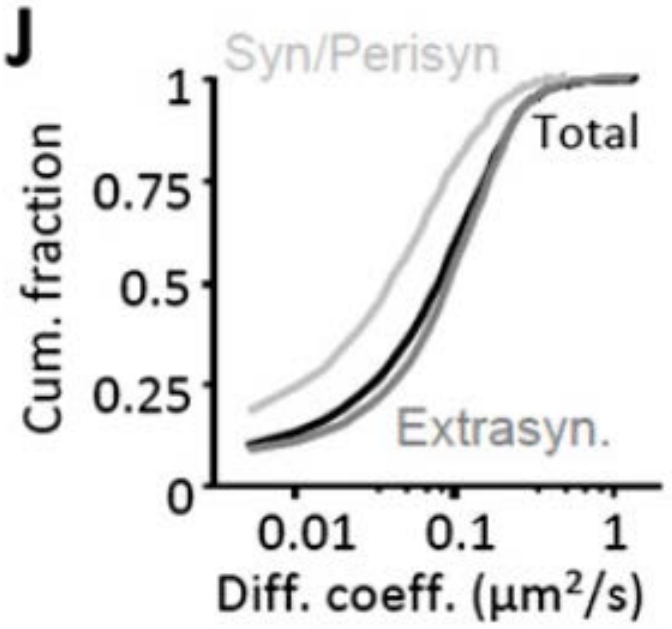
single D1R–QD trajectory from the dendritic field
Arrowhead: beginning
Black dot: (t50s).



trajectories (1,000 frames, 20-Hz acquisition rate) of surface D1R in dendrites



D1Rs dynamics



➔ surface diffusion of D1R–QD inside synaptic areas was significantly lower than in extrasynaptic compartments

QD as Thermodetectors

biological targets:

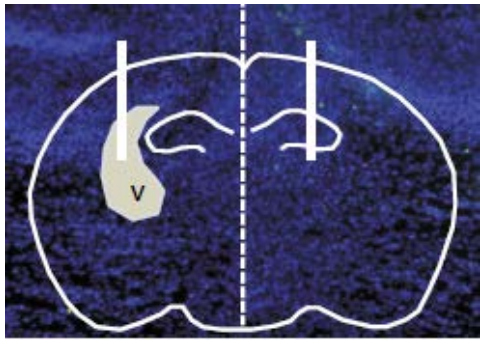
- GPIanchored proteins
- AMPA-receptors
- Voltage gated ion channels
- IgE receptor
- receptor tyrosine kinases (insulin receptor)
- Dopamine receptors

BUT: QD delivery issues currently limited the transfer of singleparticle tracking techniques to more integrated and preserved preparations, limiting investigations of receptor diffusion in the context of native tissue

QD as Trackingsystem for Mobility & Diffusion: Acute Brain Slices

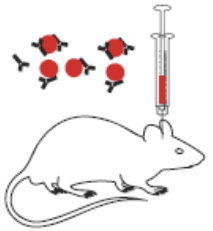
via the cerebrospinal fluid, intra-ventricular injections of QD in newborn rats (1–4 days old)

sacrificed: 3 h later

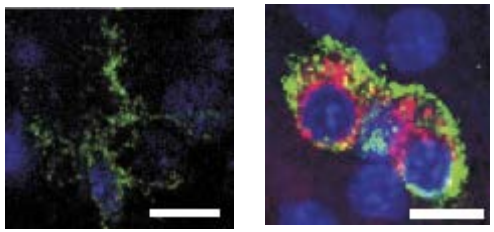


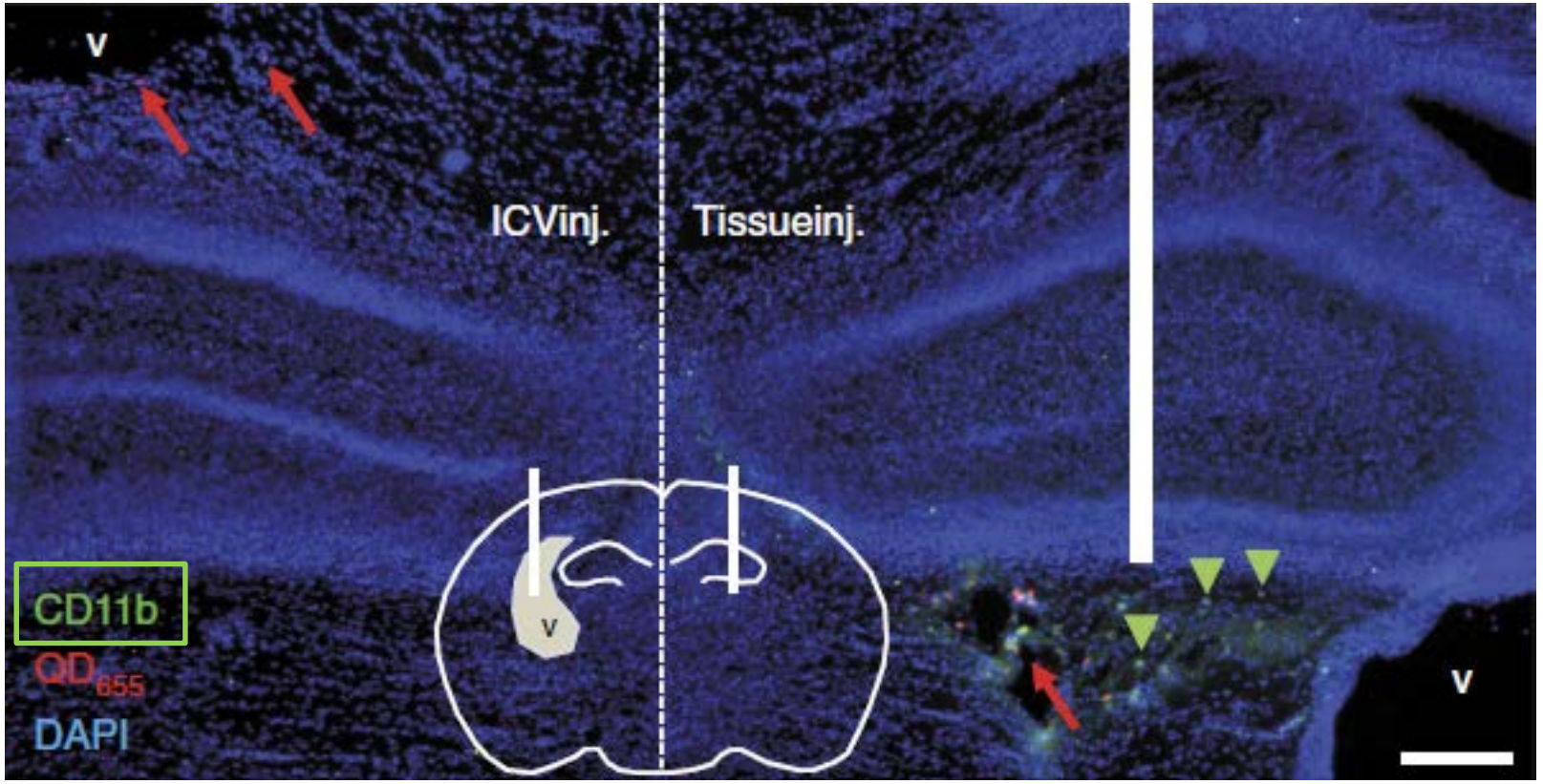
Dissection, Staining for Microglia (Inflammation) & Imaging

Brain distribution of untargeted QD after injection (3h post injection)



microglia with classical non-activated state

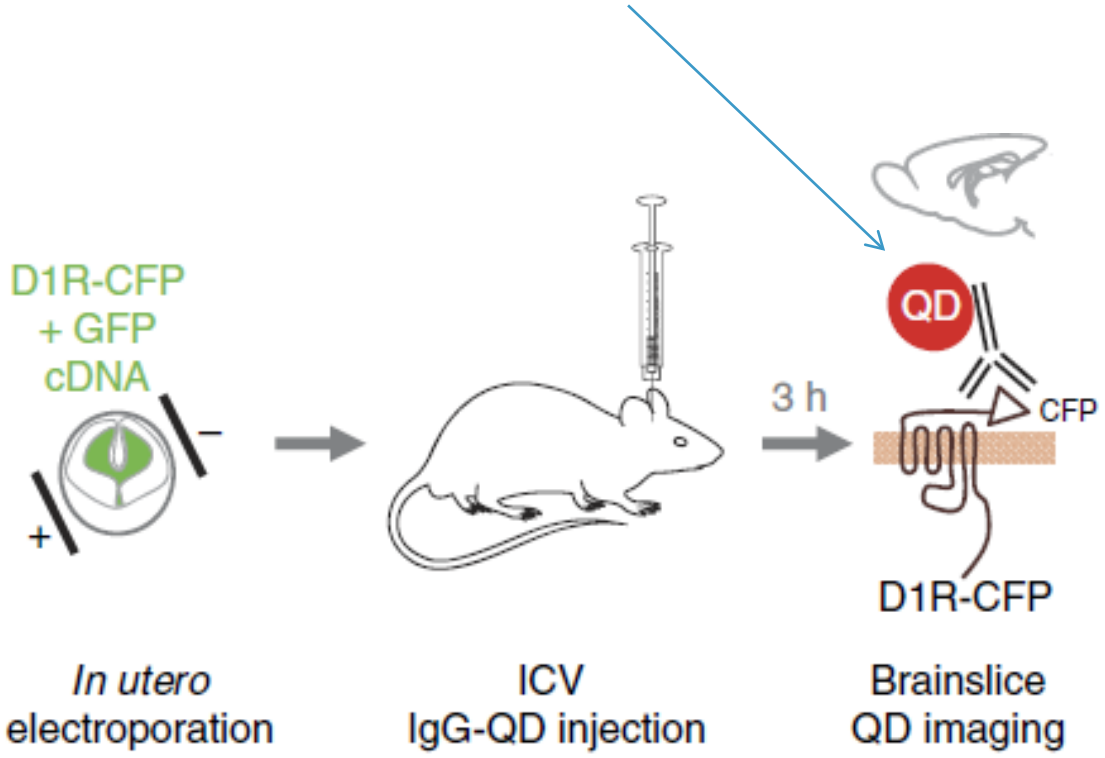




microglia with activated state (Inflammation)

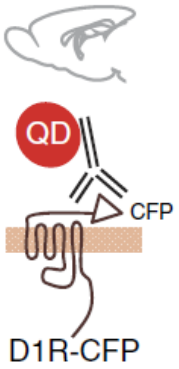
QD as Trackingsystem for Mobility & Diffusion: Acute Brain Slices

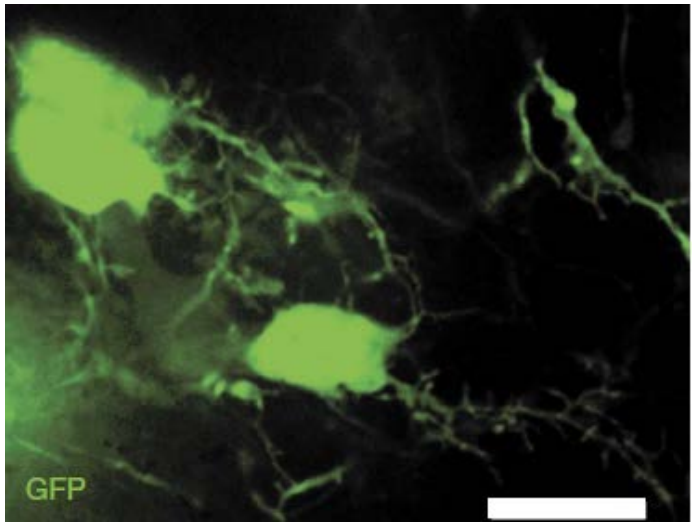
Labeled QD-antibody complex, that recognizes CFP motiv of D1R



HC neurons transfected with D1R (dopamine receptor) containing cyan fluorescent protein at N-terminus (D1R-CFP)

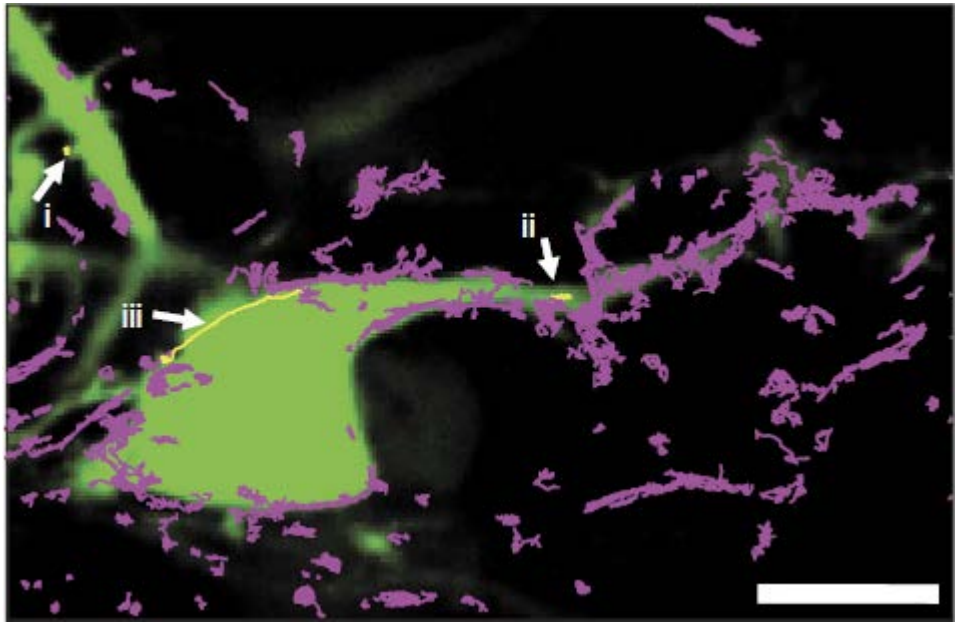
Single QD-D1 receptor detection in acute brain slices.

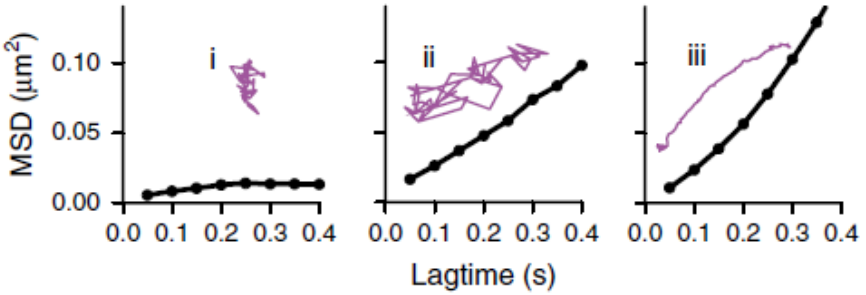




D1R labeld with QD (D1R-QD) can be tracked on the surface of HC neurons.

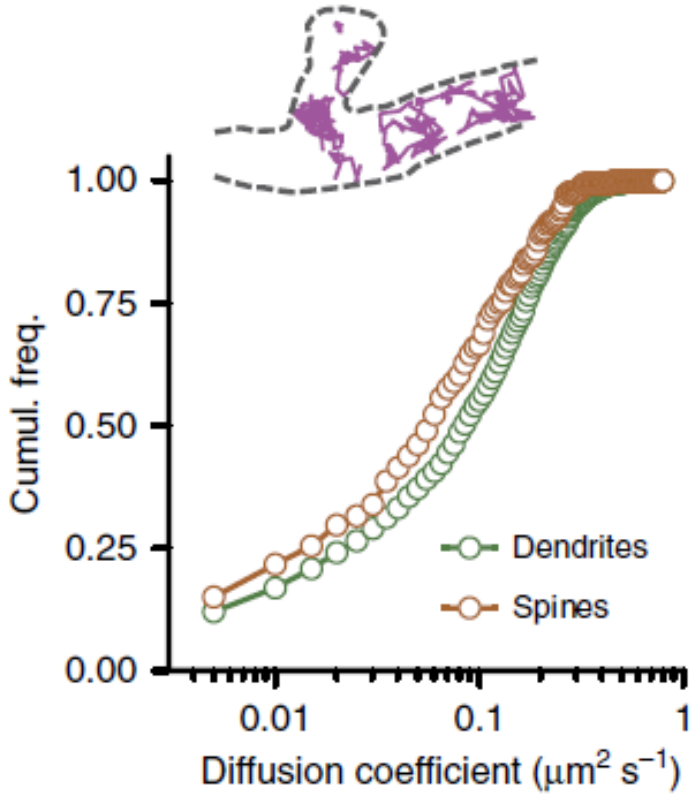
Imaged: 500 frames to retrieve individual QD trajectories



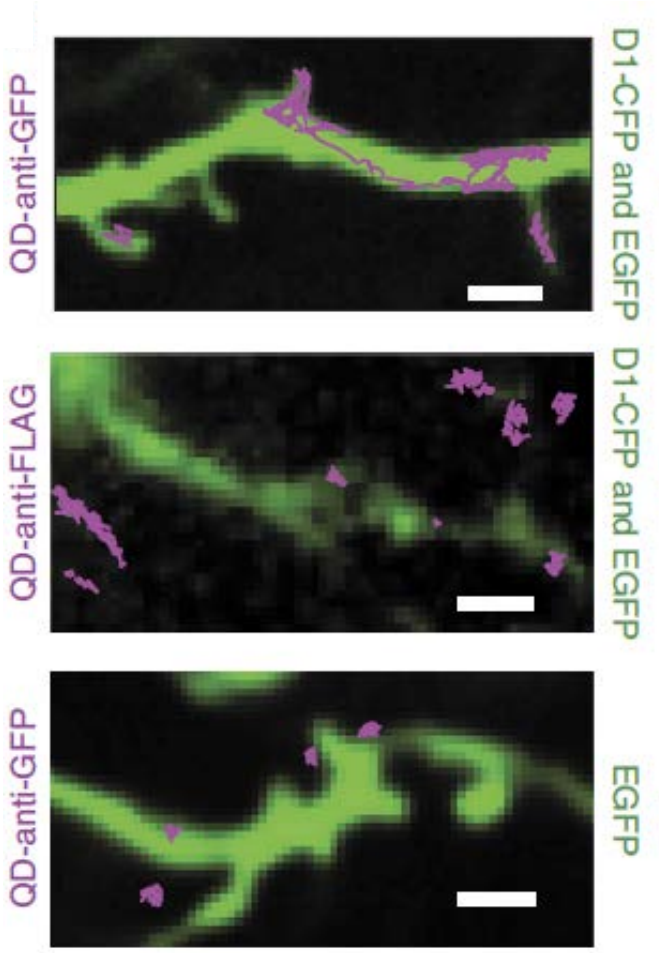


Analysis: QD trajectories diffusing along GFP-positive dendrites, calculating the mean squared displacement (MSD)

Diffusion coefficients of QD in spines lower than those on dendrites



Measuring diffusion (single-molecule tracking)



anti-GFP antibody binds CFP-D1R
➔trajectory difussing along mGFP-pos neurons

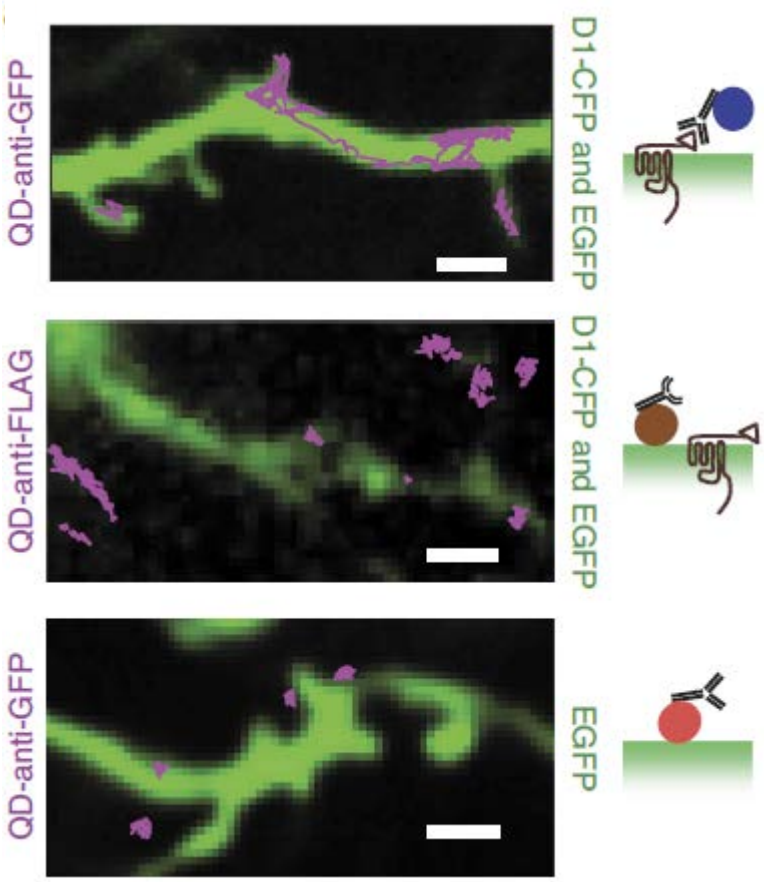
anti-FLAG antibody ➔did not show trajectories diffusing along mGFP-positive neurons

only with EGFP (no D1R-CFP) ➔ no trajectory

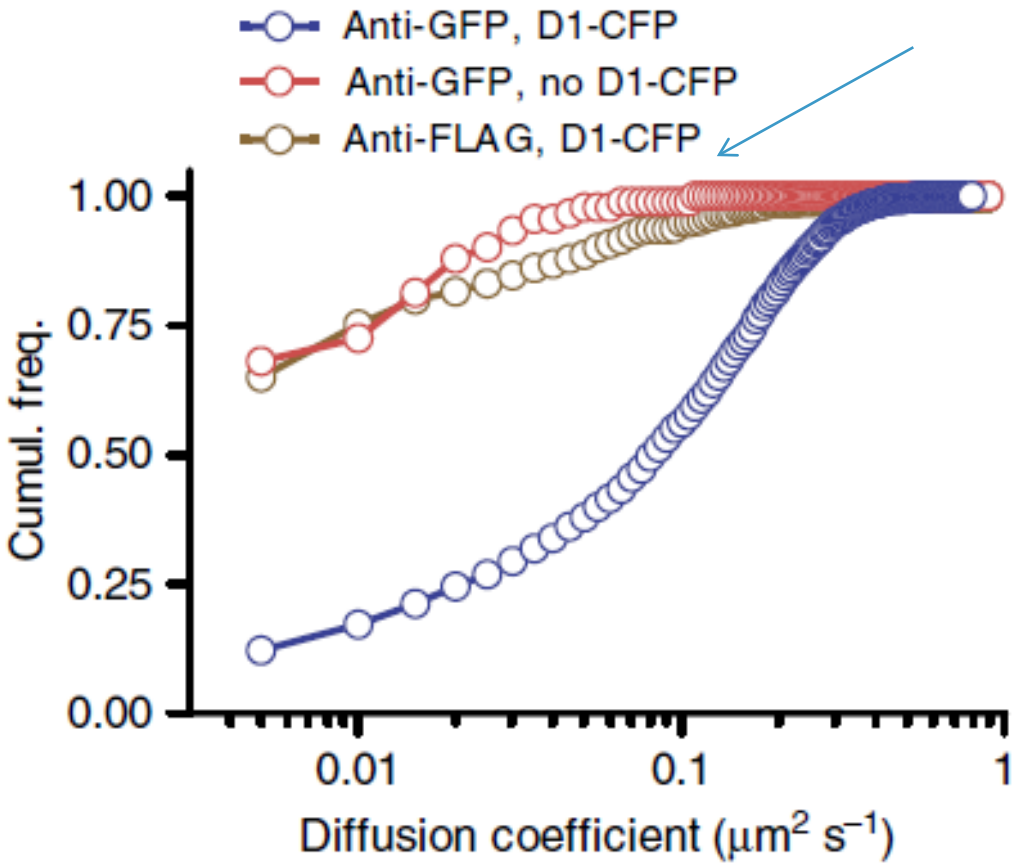
FPD1R (CFP located at the extracellular N-terminus of the receptor) and EGFP plasmids.

QD/anti-GFP antibody complex that recognizes the CFP motif of CFP-D1R

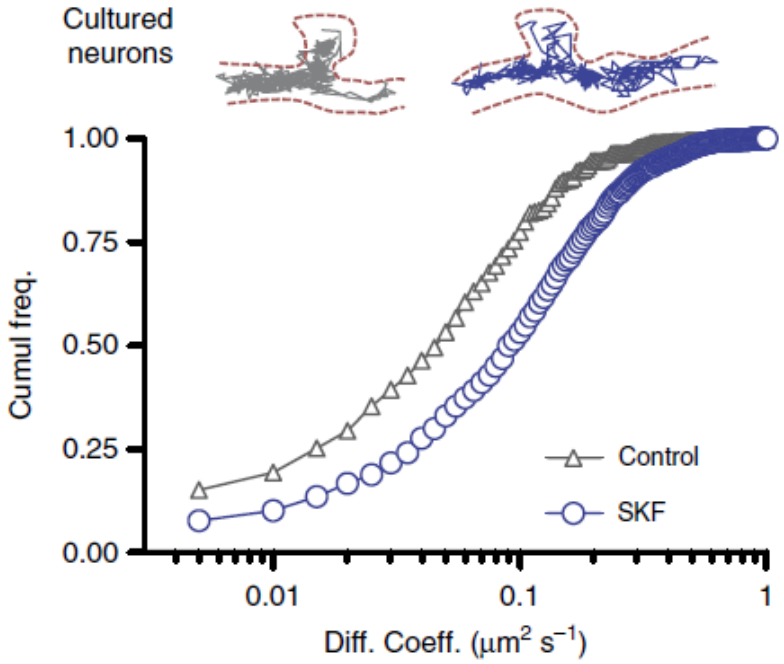
Measuring diffusion (single-molecule tracking)

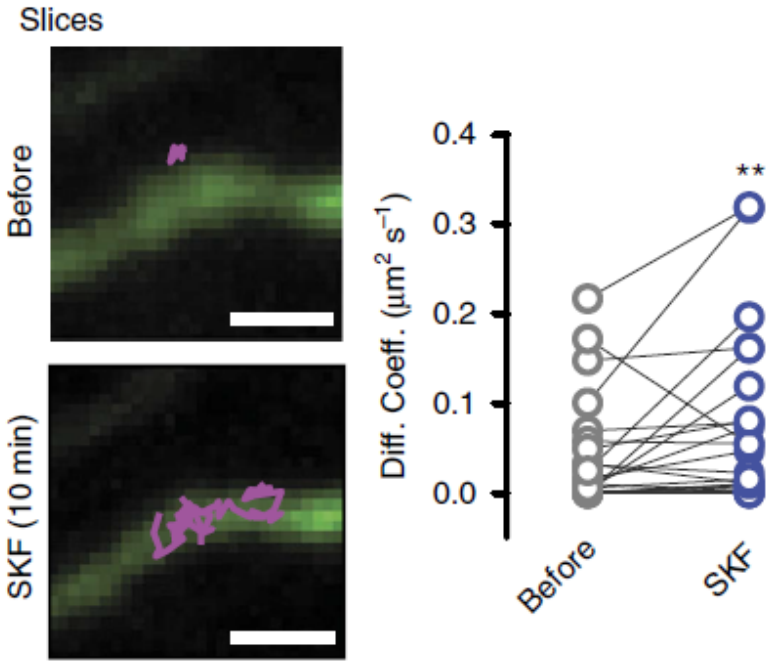


Immobile?



Effect of the dopamine D1R and D5R agonist SKF-38393 on D1R-QD mobility in intact brain tissue.





Conclusion

QD can be applied in:

- Cell cultures
- intact brain slice

Novel Findings: Biological relevance of the technique:

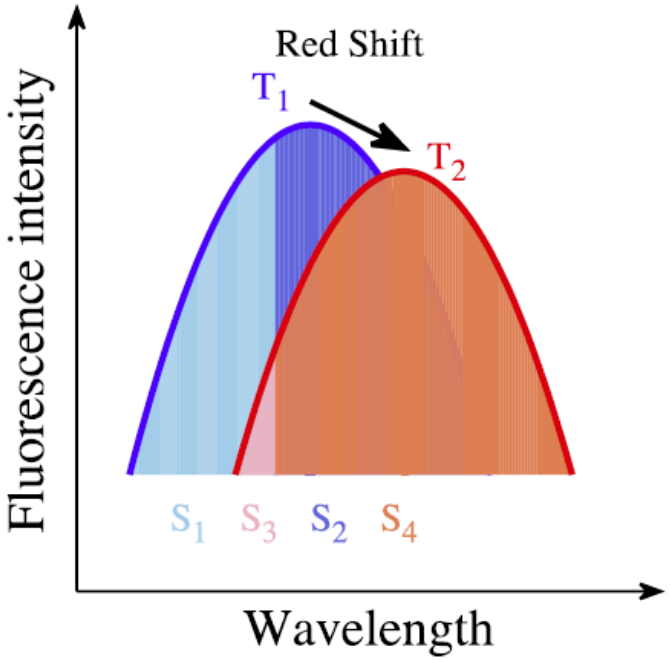
- surface diffusion of D1R–QD inside synaptic areas was significantly lower than in extrasynaptic compartments
- activation of modulates receptor surface dynamics (recycling)
- activation of D1R by its agonist increased the receptor mobility

Detection of Temperature Difference in Neuronal Cells

Ryuichi Tanimoto, Takumi Hiraiwa, Yuichiro Nakai, Yutaka Shindo, Kotaro Oka, Noriko Hiroi & Akira Funahashi

QD as Thermodetectors:

fluorescence spectrum of quantum dots exhibiting red shift to a longer wavelength dependent on the increase in temperature from T1 to T2



RESEARCH QUESTION: whether or not a ratio of fluorescence intensity lower an wavelength (λ) and that higher λ has dependency on temperature

Cell based study: QD as thermodetectors

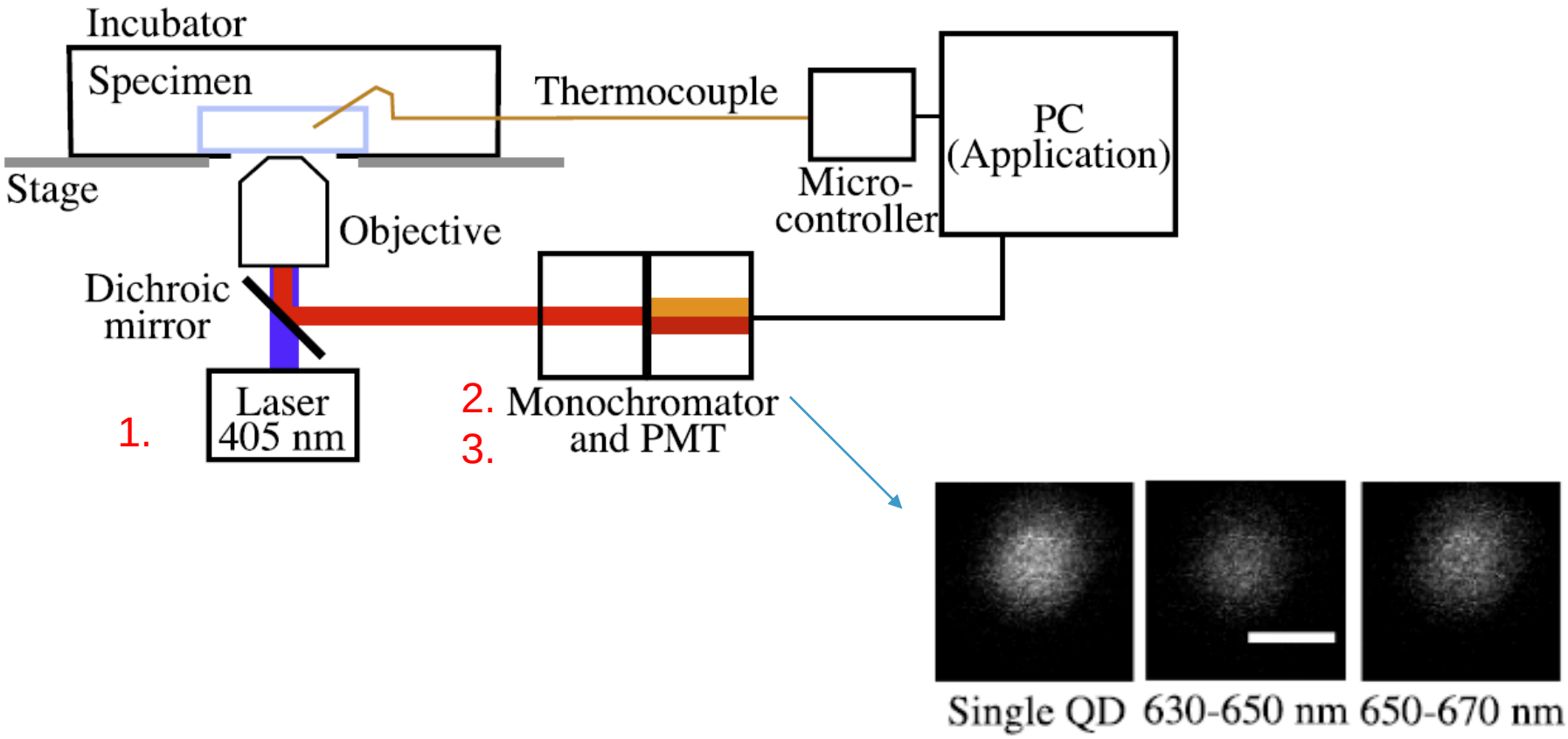
- Quantum dots have broader excitation wavelengths and brighter fluorescence than other thermodetectors ➔ easy to detect

- QD are endocytosed after neuronal differentiation of the human derived neuronal cell line, SH-SY5Y ➔ QD localized in cytoplsam

- sensing intracellular time-lapse temperature change, and spatial temperature difference

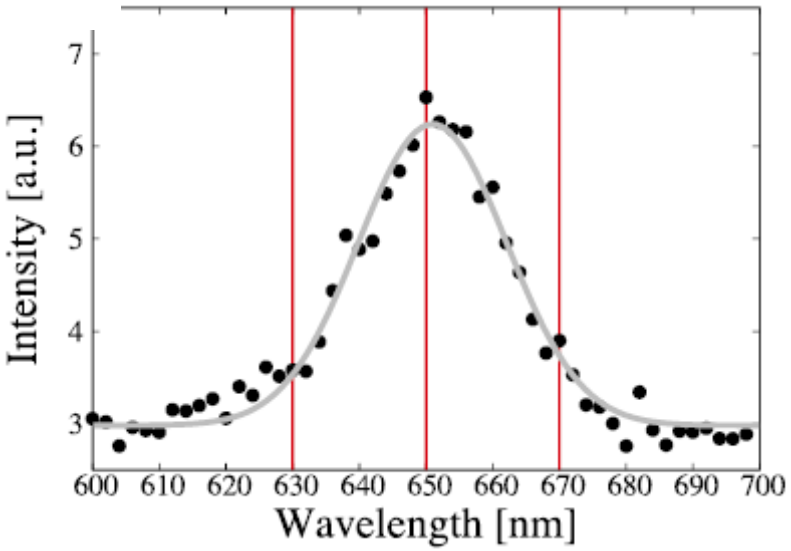
- detecting their temperature dependent shift of emission wavelength at maximum intensity requires a spectrograph

QD as thermodetectors



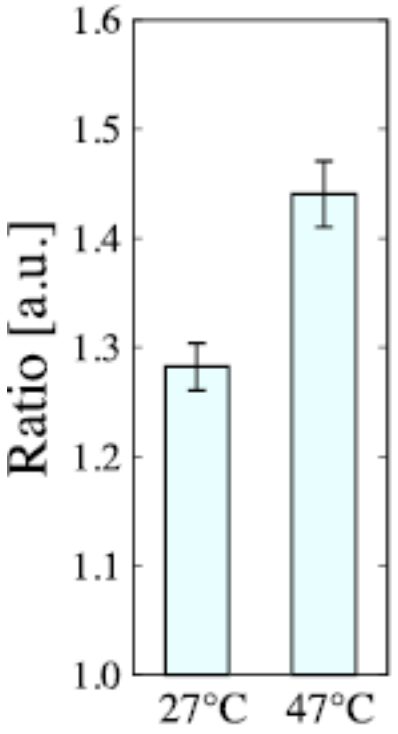
1. Laser: excited QD by 405 nm
2. Monochromator: emission spectrum splitted: range 630–650 nm & 650–670 nm
3. PMT: collected two emission spectra
4. ratiometry: confocal laser scanning microscope (FV1000, Olympus)

Parameter definition for ratiometric themometry



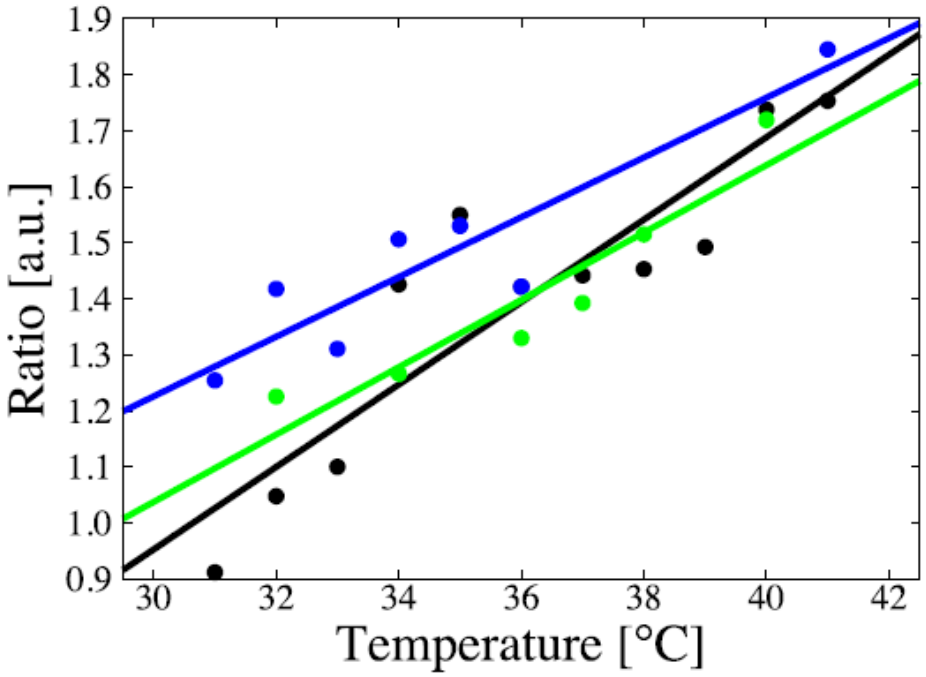
50 nM medium solution of QD crystals
➔measured its fluorescence spectrum with 2 nm resolution of wavelength

- Mean value of Fitted fluor. spectrum by Gaussian function: 651.0 nm.
- λ is set to 650 nm
- Fluorescence intensity ratio =fluorescence intensity in 650–670 nm divided by that in 630–650 nm
- exhibited significant difference between 27 °C and 47 °C



➔ ratio (λ = 650 nm) defined as thermosensitive parameter.

Fluorescence intensity ratio of single quantum dot as a thermosensitive parameter for time course analyses

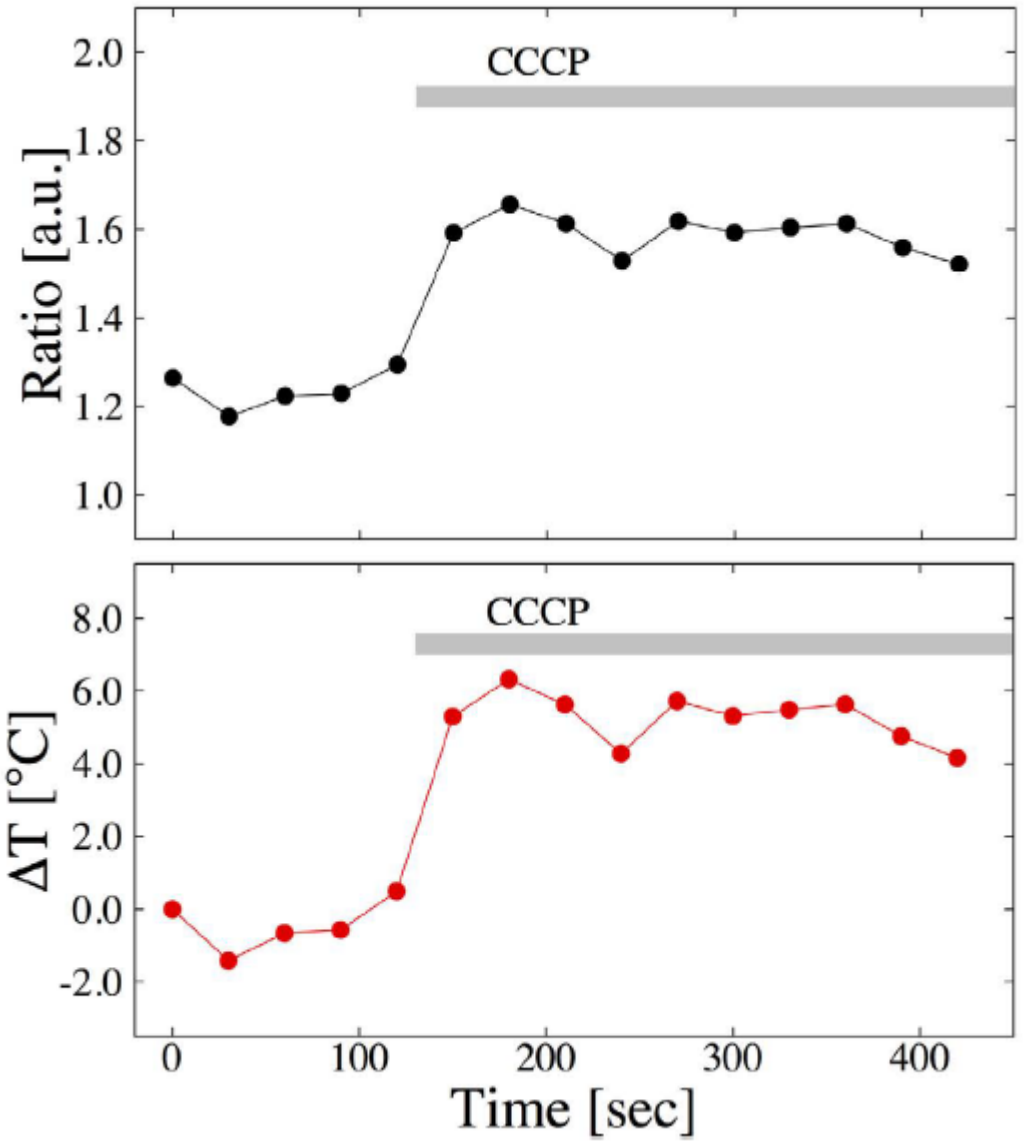


fluorescence intensity ratio of a single quantum dot at 31-41°C which was controlled by a stage-top incubator

Mean slope is 0.062/°C and this is the temperature sensitivity of the fluorescence intensity ratio for the thermometry.

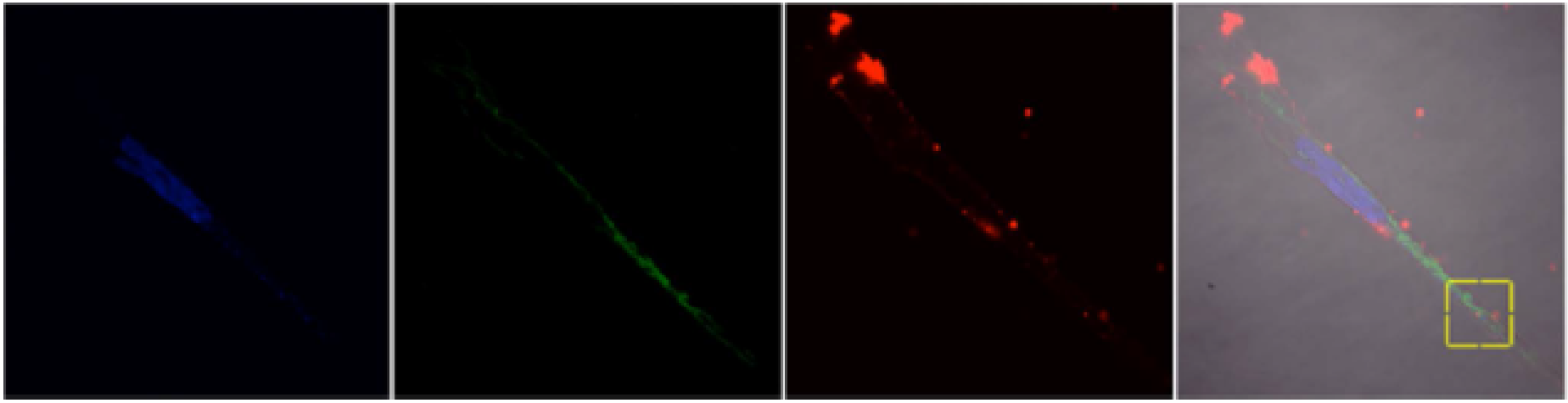
Measurement of thermogenesis in mitochondria

CCCP a **protonophore** and an uncoupler of oxidative phosphorylation, is known to accelerate the thermogenesis in mitochondria

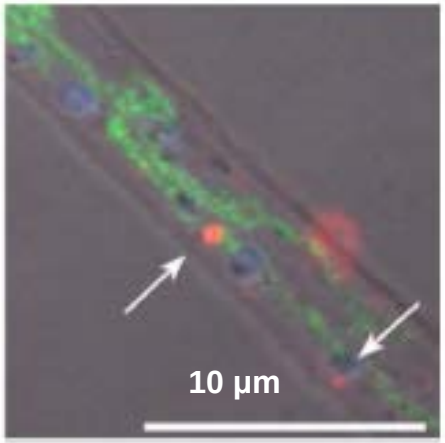


➔ fluorescence intensity ratio has ability to detect the temperature change in living cells.

Temperature difference in different compartments of neuronal cells

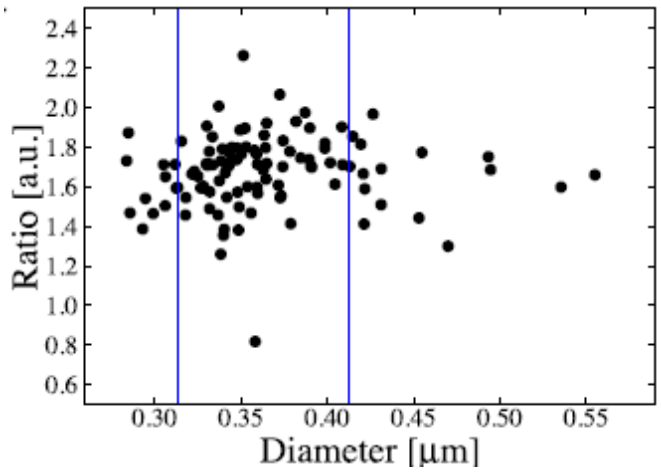


SH-SY5Y cells:
nuclei (blue), mitochondria (green), quantum dots (red), and the merge of fluorescence and DIC images

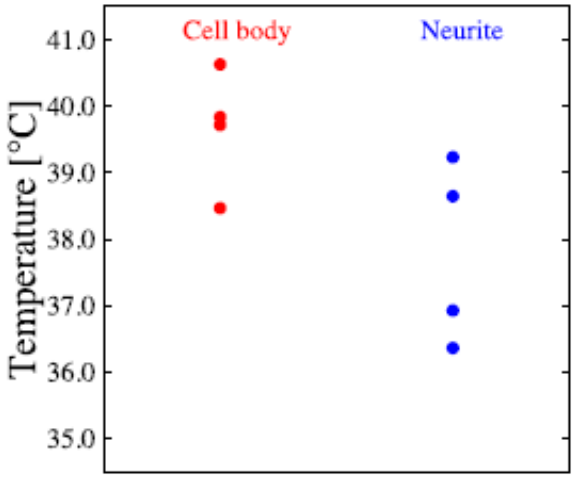


single quantum dot

Thermometry detected the temperature difference in a neuronal cell



estimated diameter of quantum dots and the fluorescence intensity ratio



The mean *R* value of the sample from the cell body was 1.74 and that from neurites
was 1.64

$$R = \Delta T / \dot{Q}_A$$

ratio of the temperature difference across an insulator and the heat flux through it
R varies with Temperature.

➔Temperature in the cell body is 1.6 °C higher than that in neurites.

Conclusion

QD advantages:

- Photostability
- Strong fluorescence
- broader excitation wavelengths
- Unique properties for various studies

➔ robust image acquisition and accurate quantitative analysis of staining intensity intact brain slice

Biological relevance of the technique:

- surface diffusion of D1R–QD inside synaptic areas was significantly lower than in extrasynaptic compartments
- activation of modulates receptor surface dynamics (recycling)
- activation of D1R by its agonist increased the receptor mobility
- temperatures in the cell body and neurites are different, suggesting inhomogeneous heat production in a cell

Future: high-throughput scale; Tumorbiology; Lab on Chip

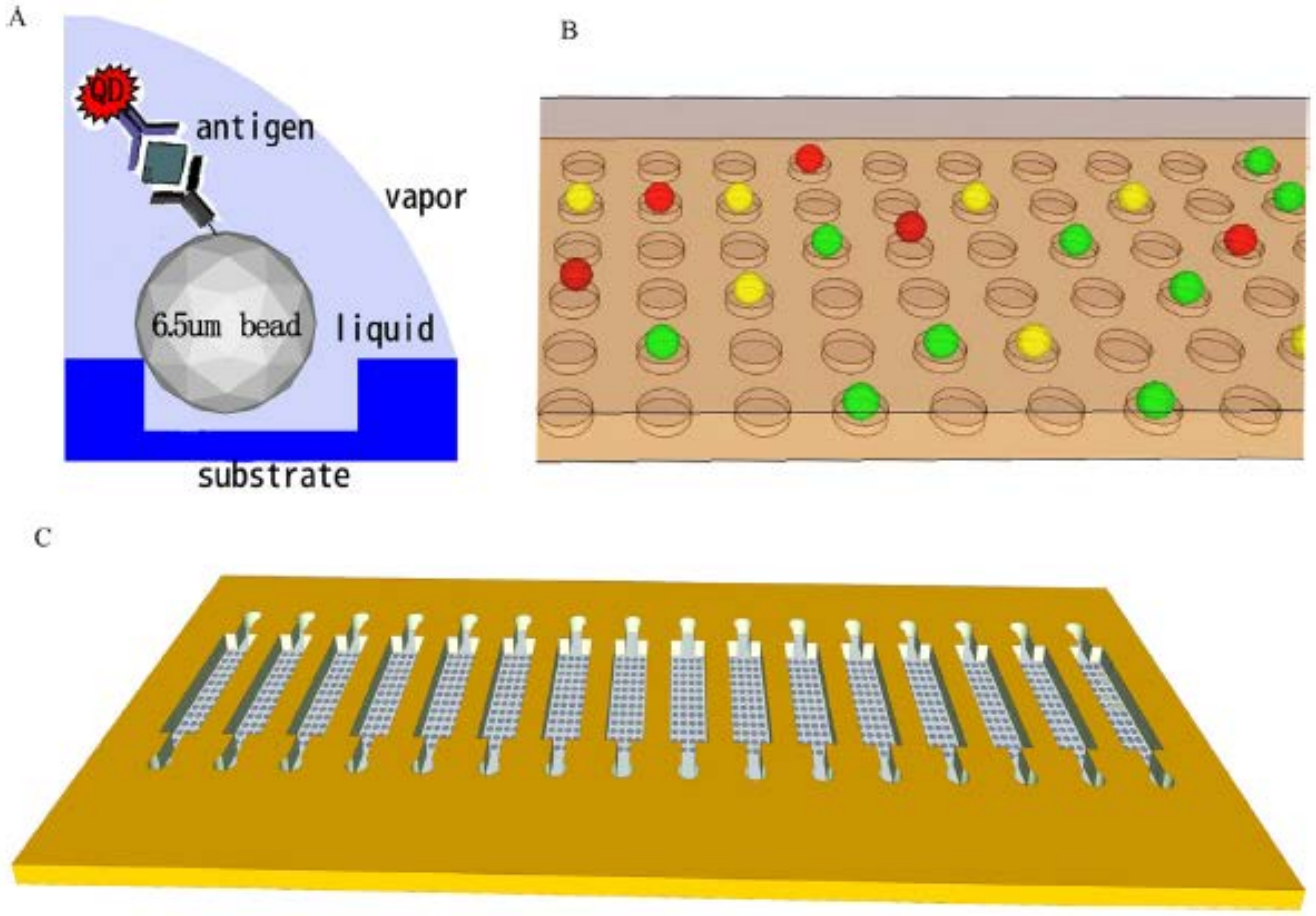


Fig. 1. Schematic illustrations of bead-based assay system for sensitive detection of three biomarkers using QDs. (A) Reaction principle for the bead-based sandwich assay; (B) Three colors of beads after reaction in the chip; (C) The structure of the chip.

Thanks for your attention!