Optical Metrology of Nanomaterials and Nano-assemblies for Quantitative Biophotonics

The Fifth US-Korea Forum on Nanotechnology: Nano-Biotechnology

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0.2 μm

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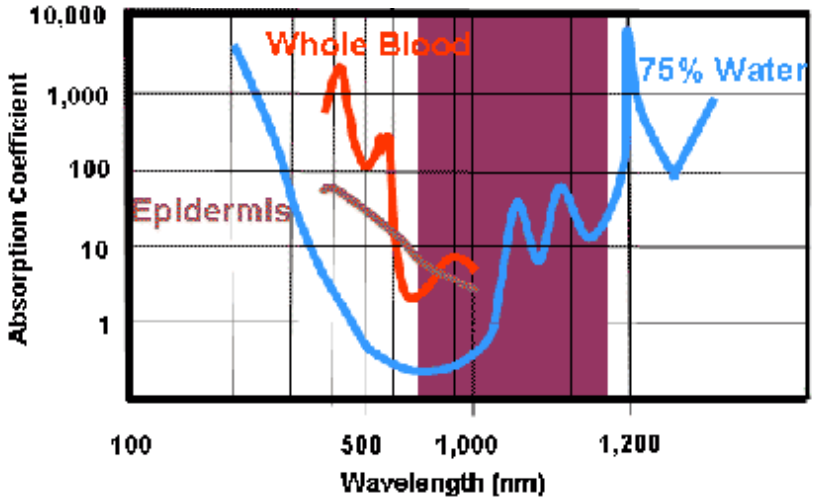
What is ‘Biophotonics?’

Biophotonics is **the study of the interaction of light with biological material**, where “light” includes all forms of radiant energy whose quantum unit is the photon.

Dennis Matthews
NSF Center for Biophotonics

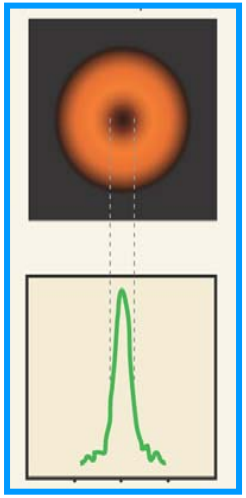
Absorption of photonic energy by a human body

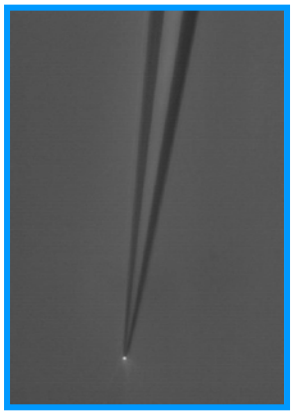


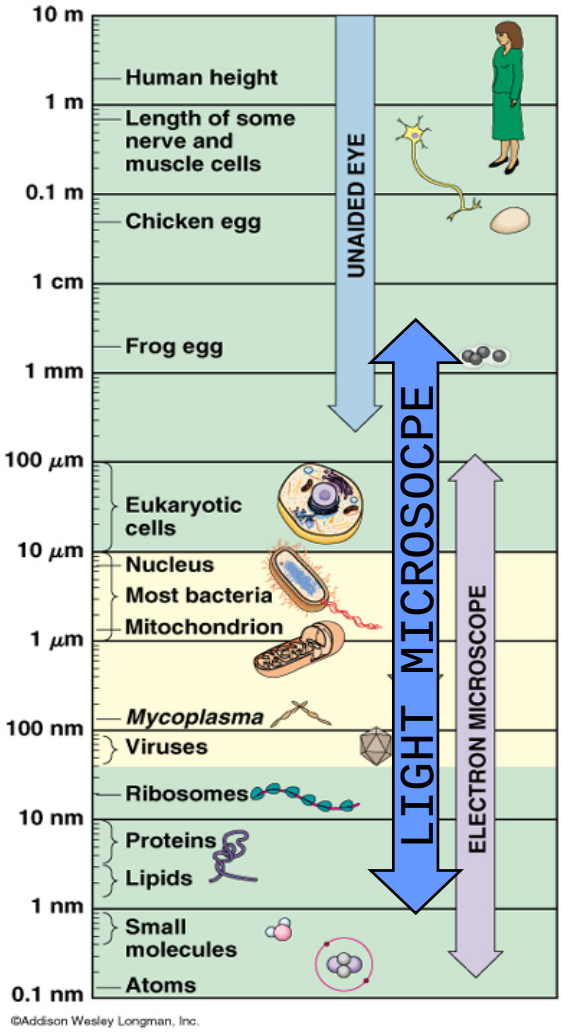
Photodynamic surgery
physics.upenn.edu

Ultimate goal of NANOBiophotonics for DYNAMICAL quantitative nanoscale imaging

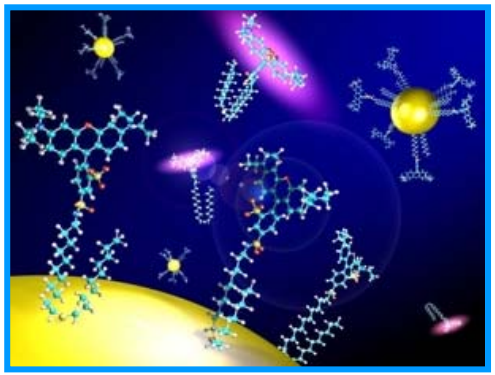
“Manipulated” photons

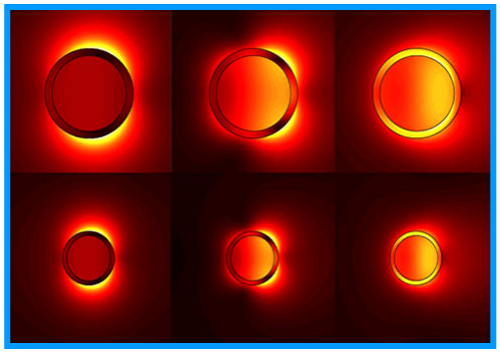






Nanomaterials:
Contrast agents or Manipulators





cohesiondev.rice.edu

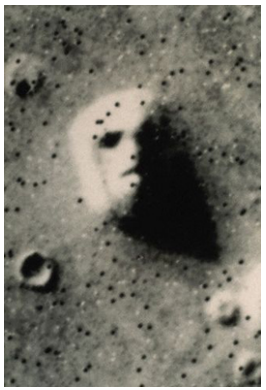
Measurement Strategy
Optical Metrology for Biophtotonics and Biophysics (OMB^2)

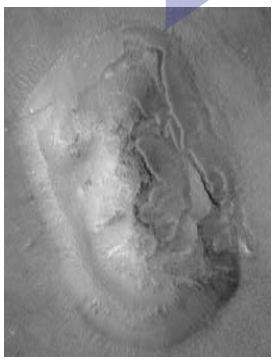
Integrated tools

Nanoprobes

Measurement confidence

Validated information

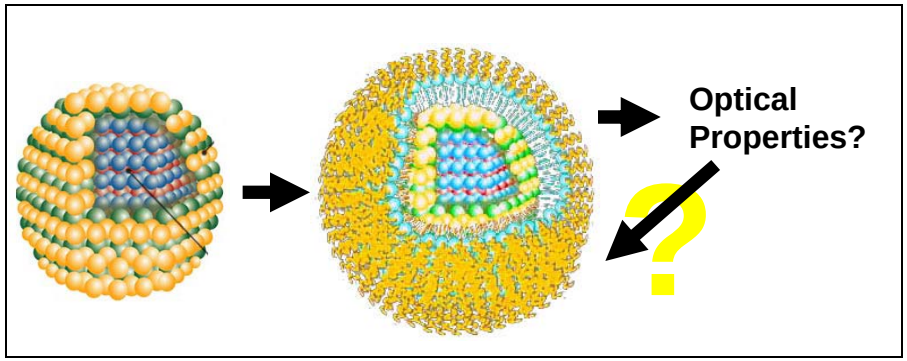




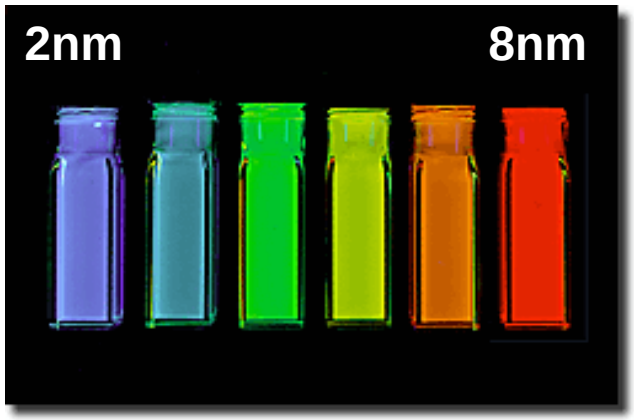
Diffraction-limited optical techniques with enhanced temporal resolutiion

Nanoscale details

Quantum Dot (QD)

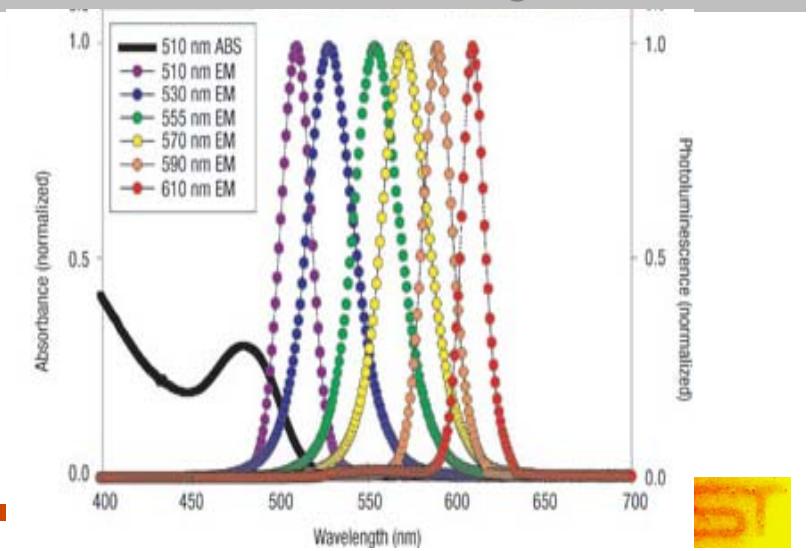


CdSe/ ZnS Functional Coating



A family of Qdot particles can be made to emit a full spectrum of colors when excited with a single excitation source.

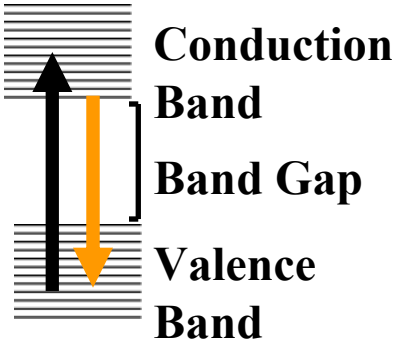
- **Attractive fluorophores for bio-imaging due to its broad absorption and narrow symmetric emission spectra**
- **Higher quantum yield and more photostable than conventional organic dye**
- **Size and composition dependent tunable absorption and emission pattern**
- **Bio-functional Coating**



Why single QD characterization?

"Sensor" for nanoscale environment.

• Probe electron-hole separation/recombination kinetics responsible for fluorescence intermittency



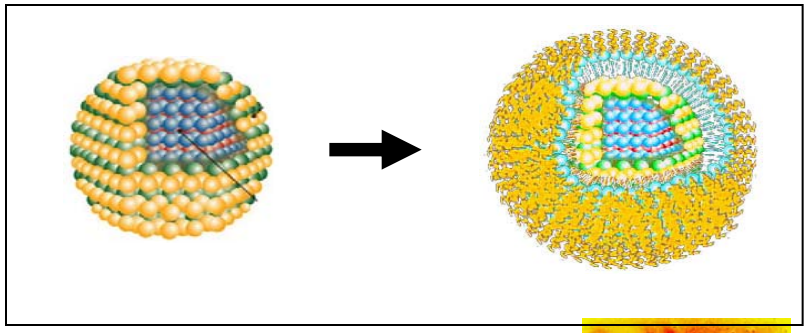
Local environment and fluorescence

• ZnS monolayers - *Nirmal et al, Nature, 383:802,* ***1996.***
  - addition of ZnS monolayers result in greater fluorescence
    - masks surface imperfections/defects
    - prevents air/solvent molecules from interacting with CdSe surface

• Oxygen/Argon atmosphere - *Koberling et al, Adv Mater, 13:672,* ***2001.***
  - fluorescence quenched in presence of oxygen
    - oxygen traps electrons at quantum dot surface

• β-Mercaptoethanol - *Hohng et al, JACS, 126:1324,* ***2004.***
  - near 100% blinking suppression
    - thiol moiety donates electrons

What is the effect of the BIOCONJUGATION, <u>functional coating</u> on the fluorescent properties of single quantum dots?



Coming up…

Measurable I: Surface hydrophilicity
→ Single particle tracking on QDs interacting with a lipid membrane

Measurable II: Distance and orientation
→ Fluorescence Energy Transfer using QDs as donors

Measurable III: electrostatic environment
→ Intermittency in fluorescence, "blinking" of single QDs

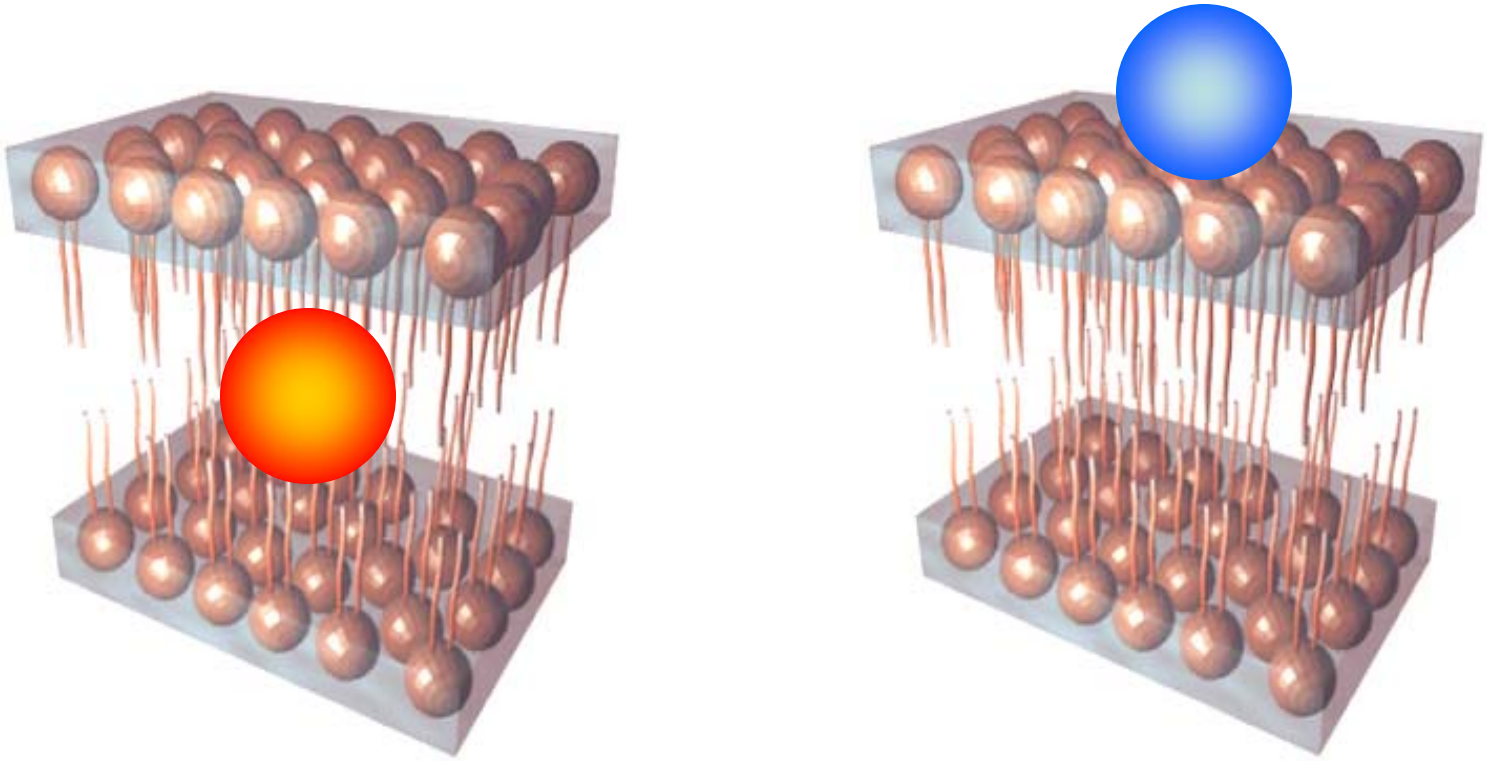
Measurable IV: Local concentration
→ Fluorescence from "clustered" QDs

An application
Nanosensor assembly and characterization of bacteriophage/QDs nanocomplexes

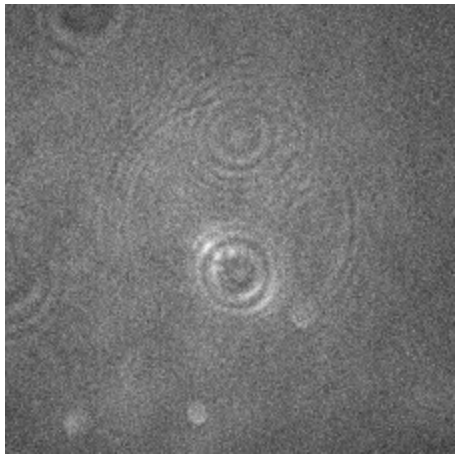
Measurable I: Surface hydrophilicity
Nanoparticles interacting with a lipid membrane

Nanoparticle vs membrane interactions

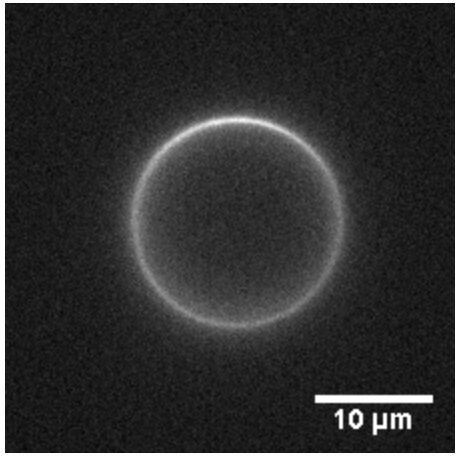
Hydrophobic vs Hydrophilic



single particle tracking of single nanocrystals

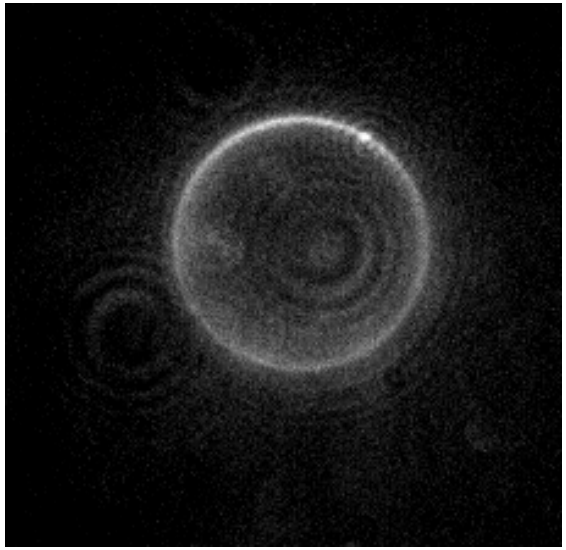


785 nm DIC



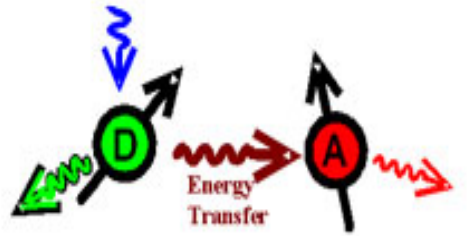
Fluorescence

Nanoshells trapped inside
a lipid vesicle

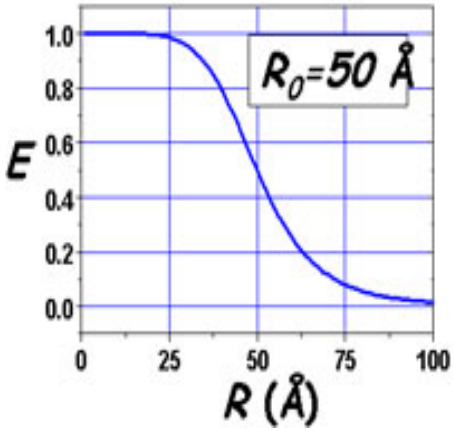


Combined 785 nm DIC
and Fluorescence

Measurable II: Distance and orientation
Fluorescence Energy Transfer using QD as donors



DONOR ACCEPTOR

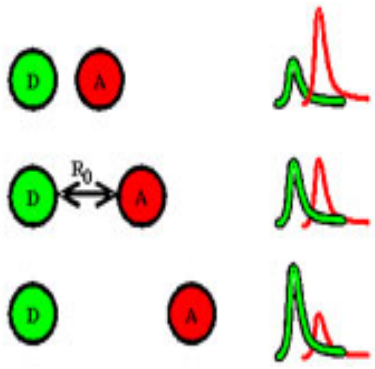


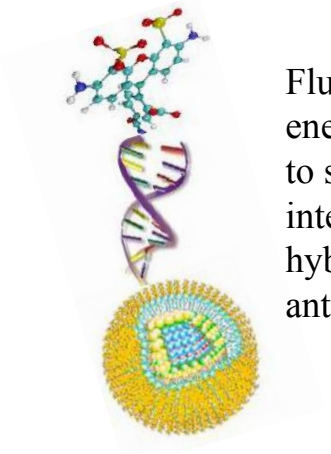
Energy Transfer Efficiency

$$E = \frac{1}{1+(R/R_0)^6} K(\theta)^2$$

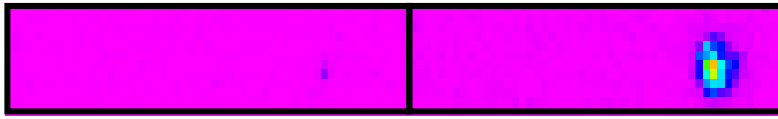
R_0=50% transfer efficiency distance
3nm~7nm

"Spectroscopic Ruler"





Fluorescence resonance energy transfer is measured to study biomolecular interactions such as DNA hybridization and antibody-antigen reaction.



Si NP

-----Biotin-CG ACG GTA TAG ATG
Cy5-GC TGC CAT ATC TAC----

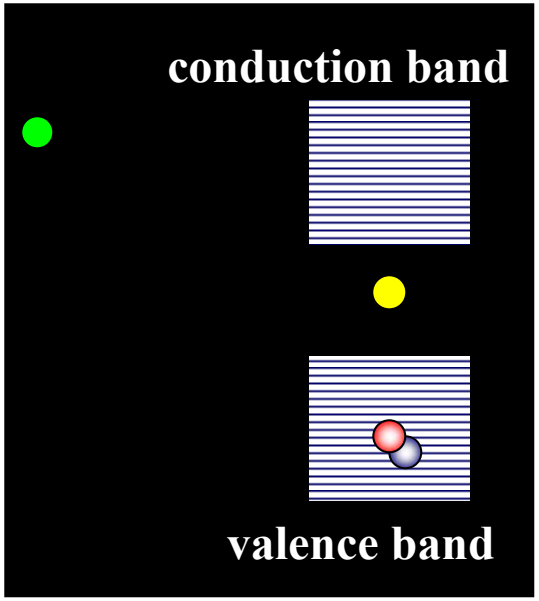
Human Papilloma Virus gene detection

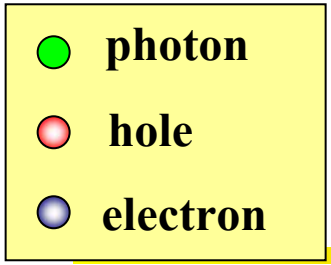
Measurable III: electrostatic environment

Intermittency in fluorescence, “blinking”

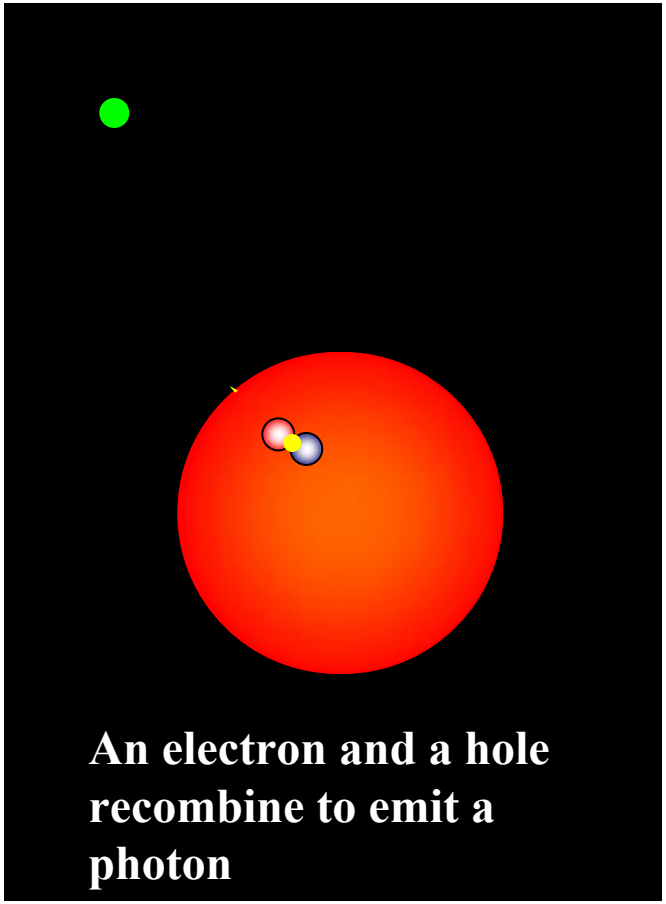
“a particle in a box”

Photoluminescence in Semiconductor

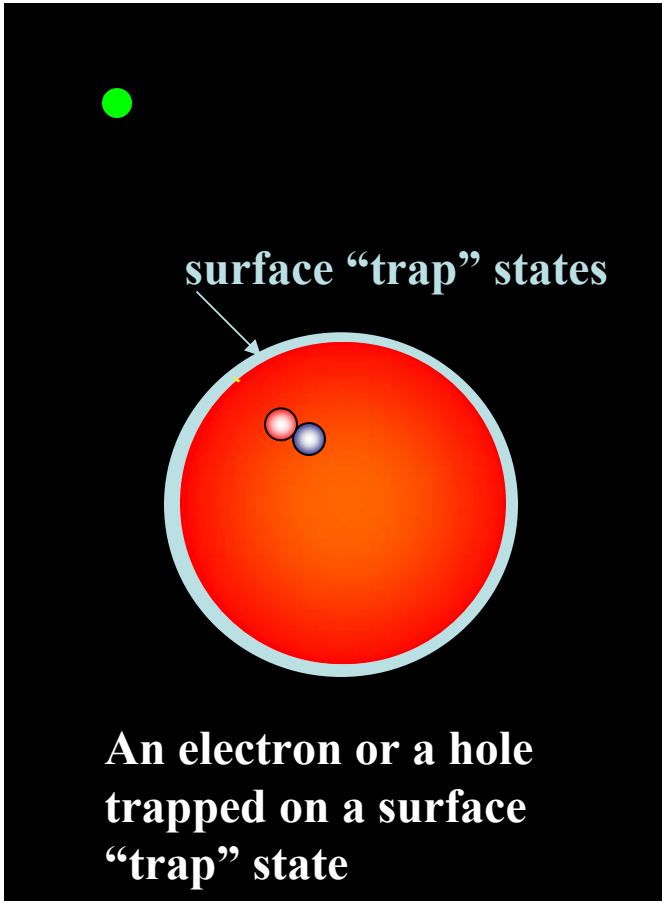




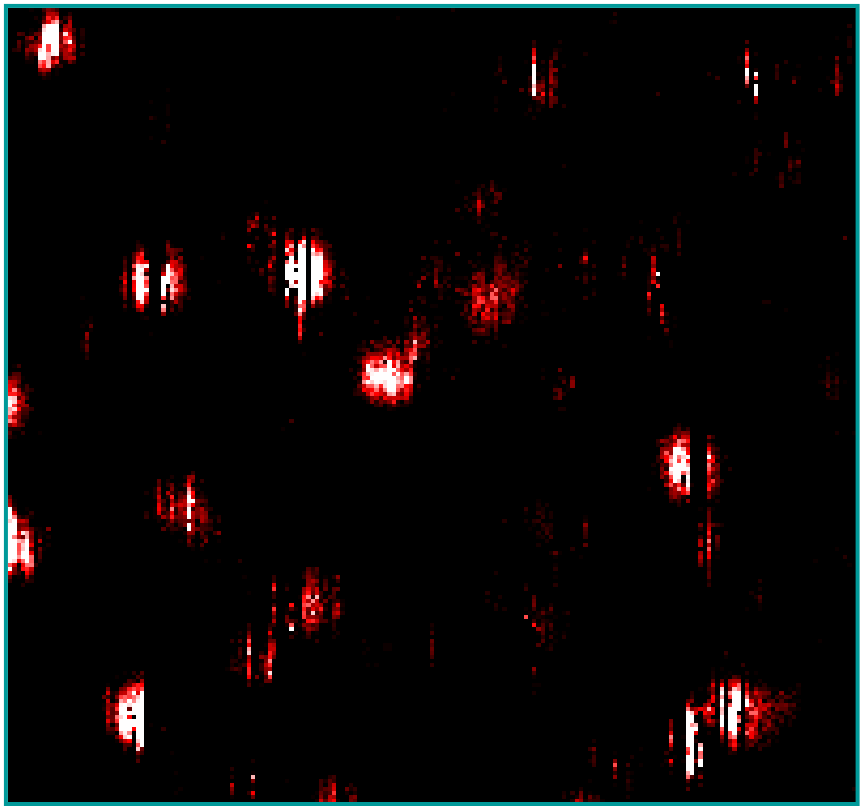
Fluorescent, “ON” state



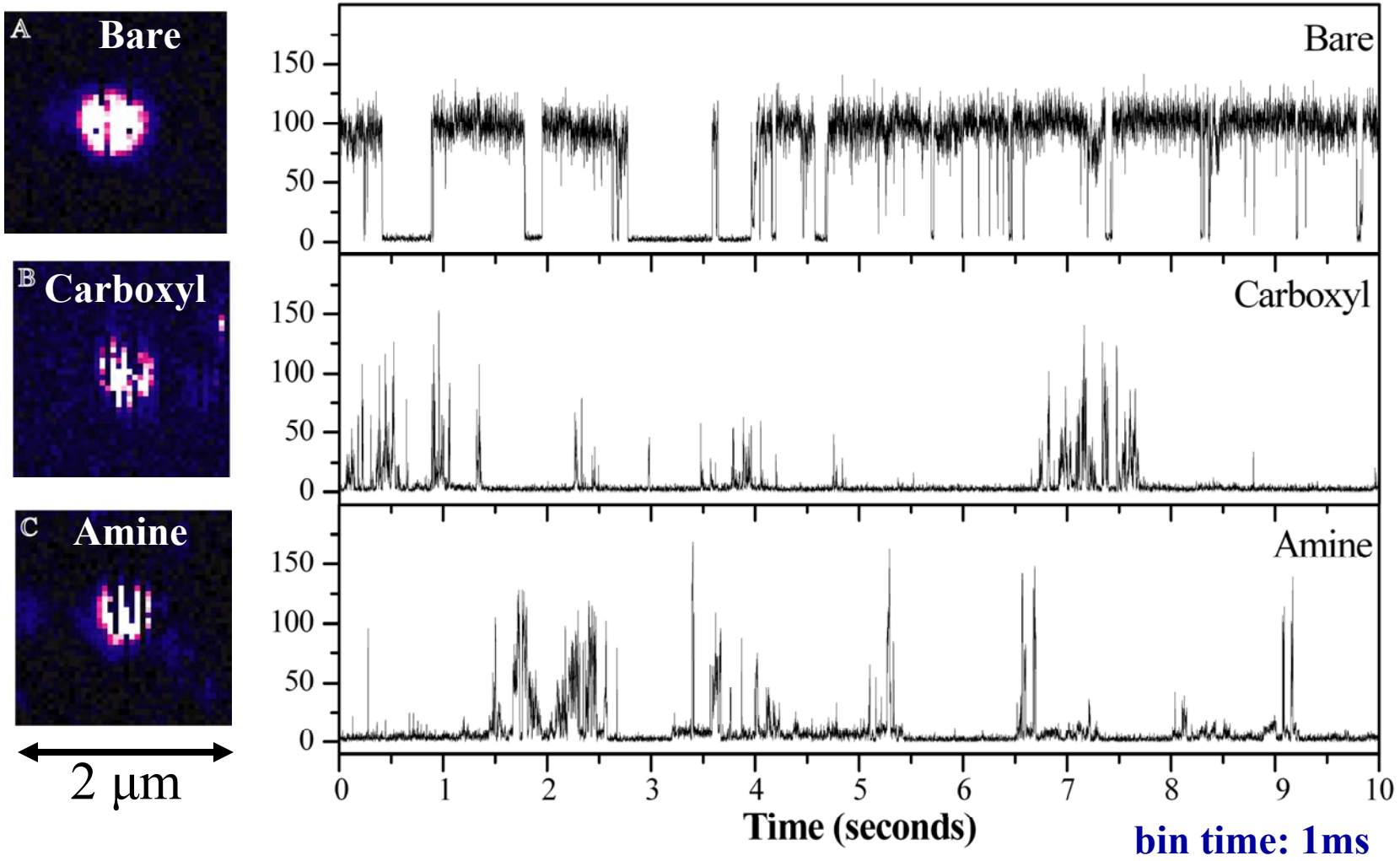
Trapped, “OFF” state



Confocal Fluorescence Microscopy of Single QDs

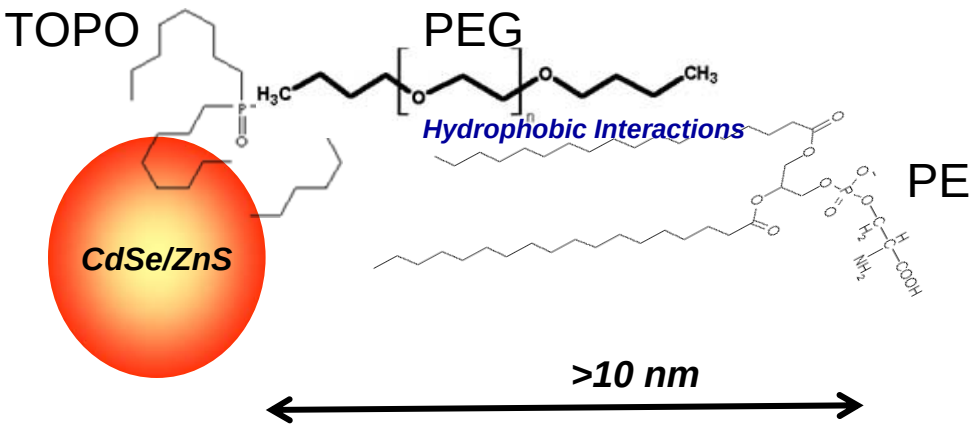


Surface functionalization results in shorter “on” periods of QD fluorescence due to increased surface traps

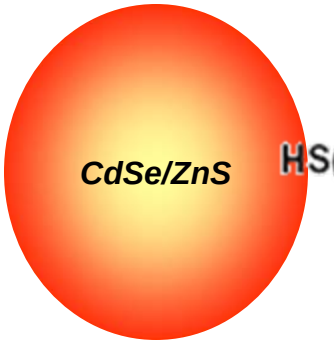


Surface conjugation chemistry of QDs

Non-covalent bonding

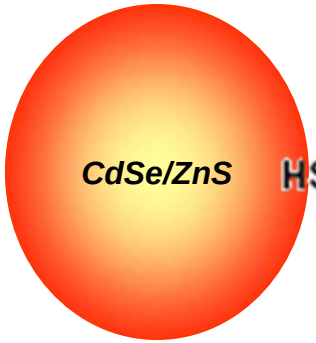


Thiol chemistry



$HS(CH_2)_{10}COOH$

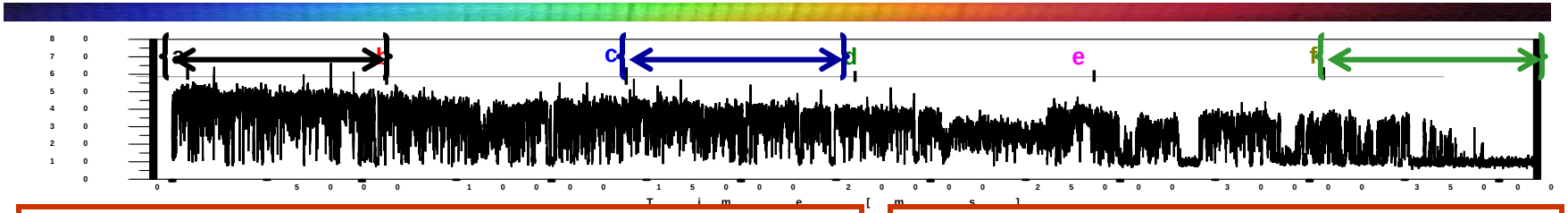
OR

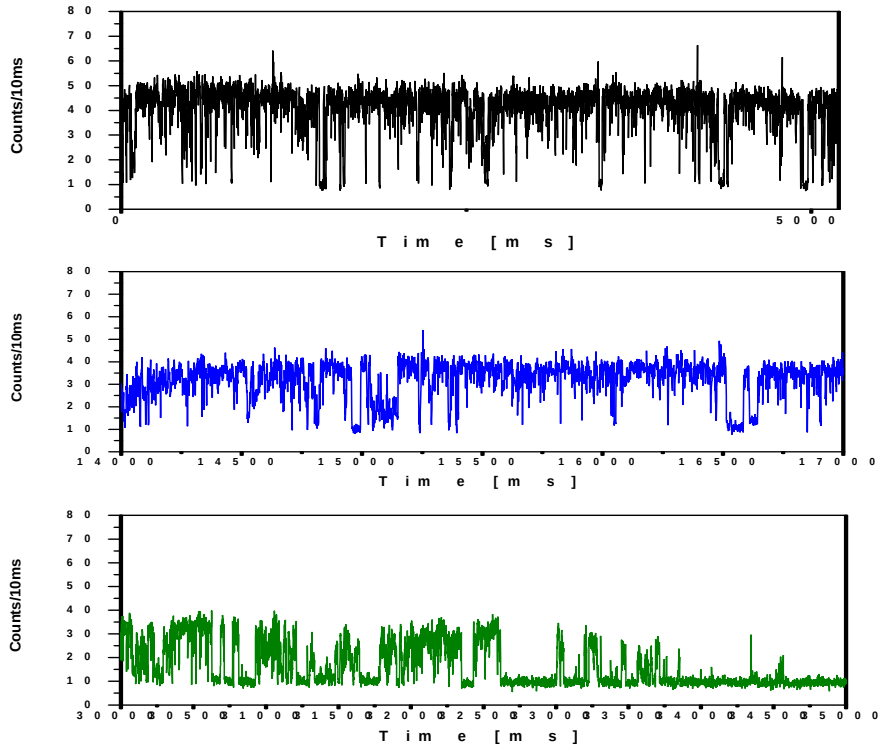


$HS(CH_2)_{10}COOH + H_2N\text{-DNA}$

mercaptoundecanoicacid (MDA) - QD

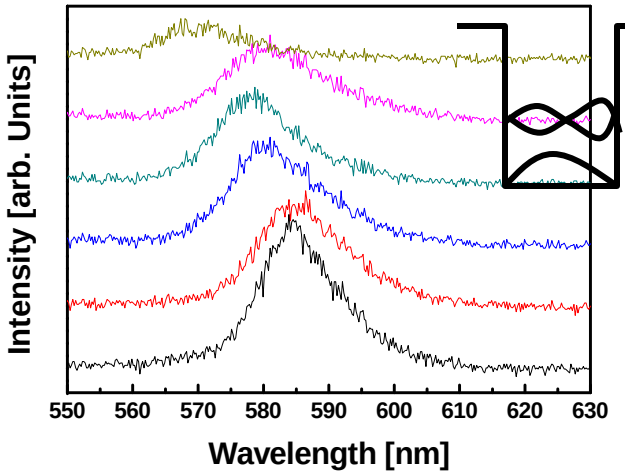
Dynamical fluorescence analysis of a single bio-conjugated QD



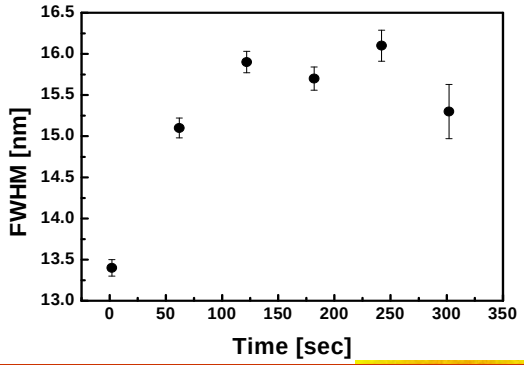


Increasing trap states
Decreasing quantum confinement size

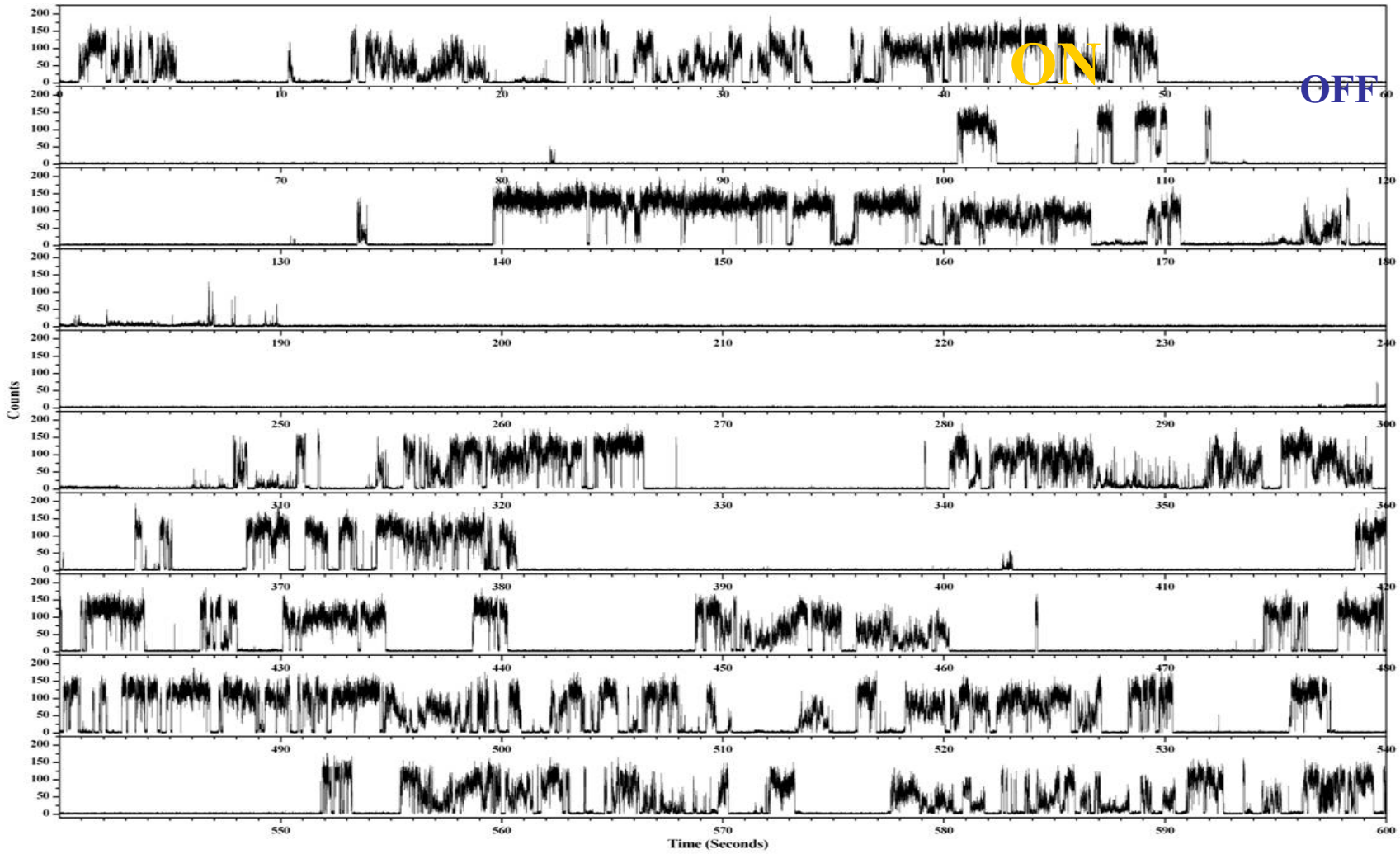
Spectral Shift



Spectral diffusion

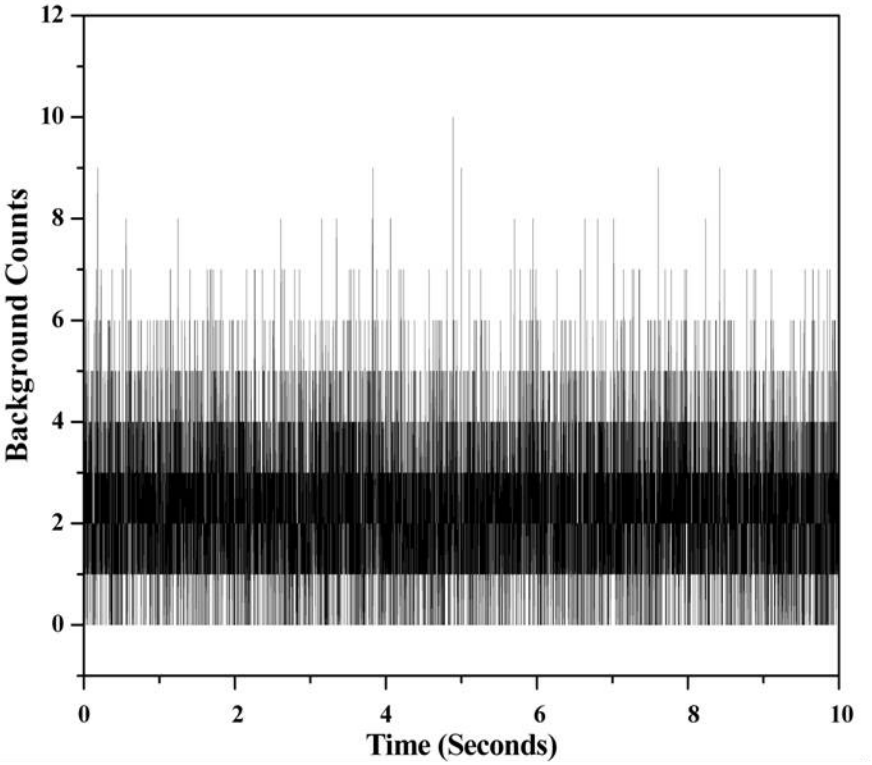


“blinking” of a single QD

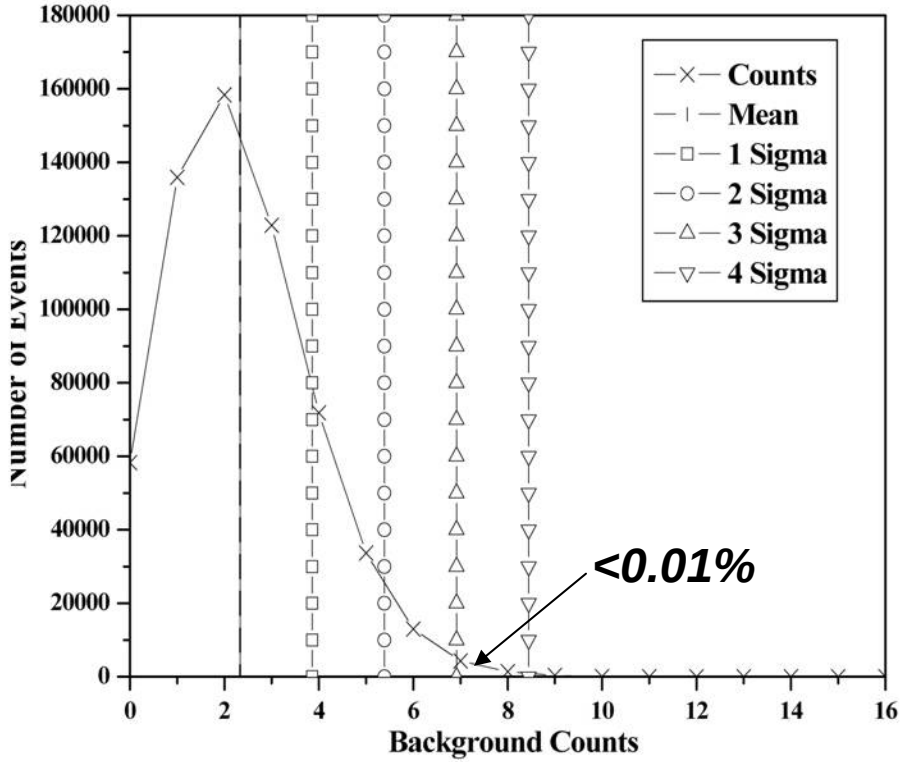


- **Quantized levels in blinking → “counter”**
- **No enhancement in the emission intensity → “measure cluster behavior”**

Quantum Dot - Blinking Analysis



Poisson Distribution

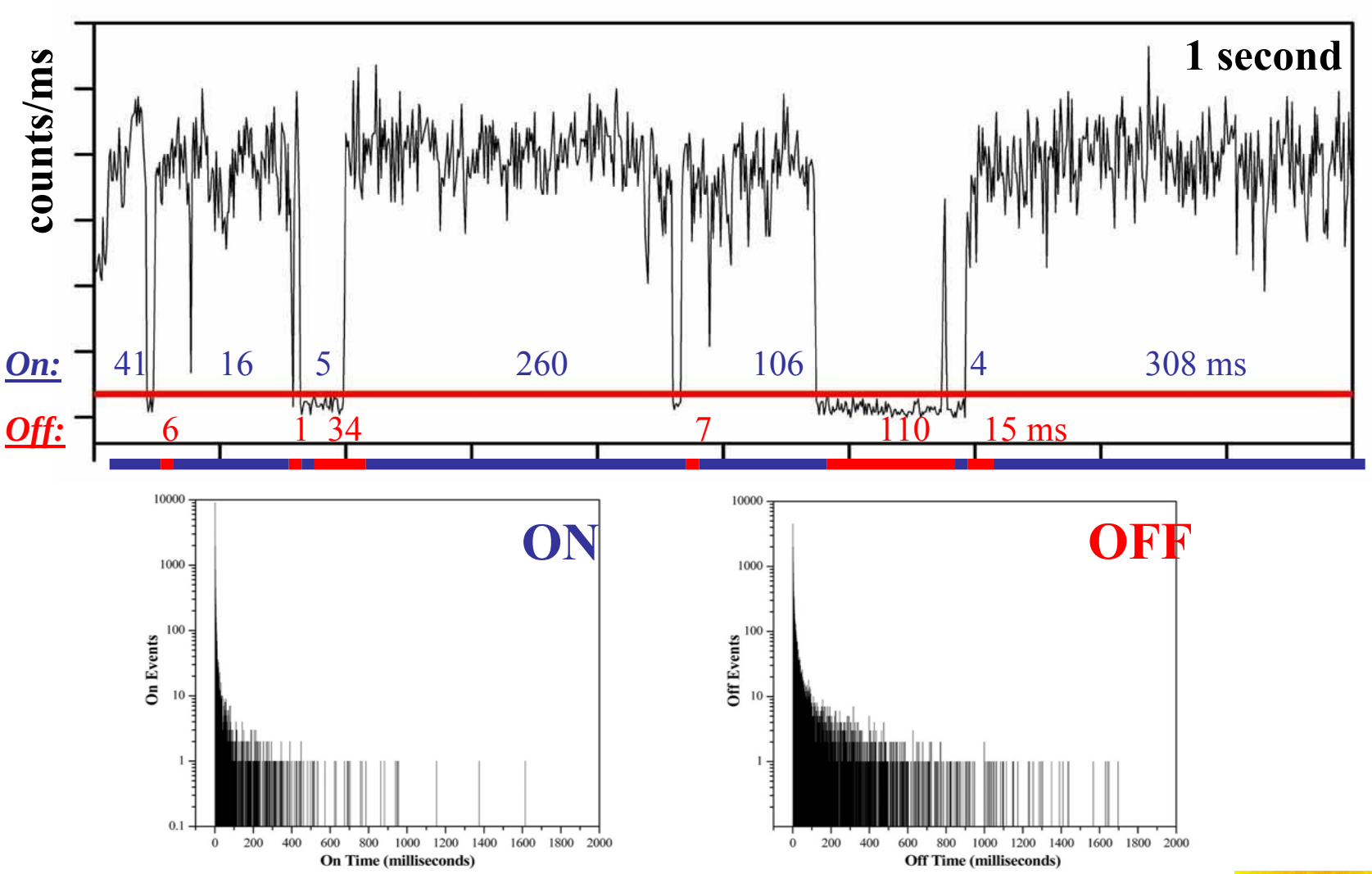


Background Counts: **Mean = 2.33 counts/ms, σ = 1.52**

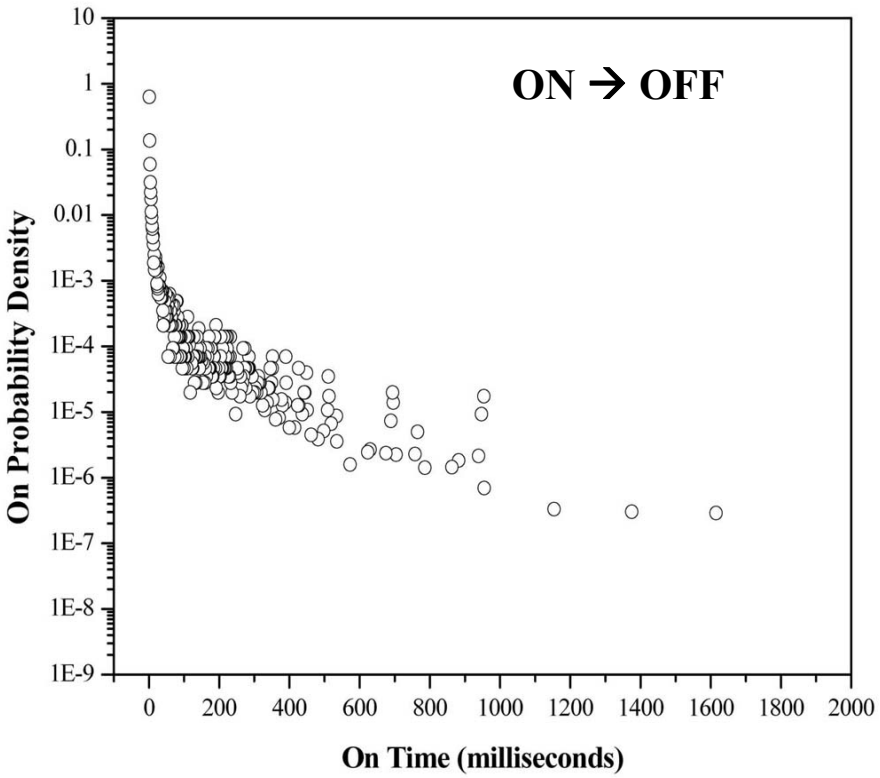
mean+σ = 3.86 +2σ = 5.40 +3σ = 6.92 +4σ = 8.44

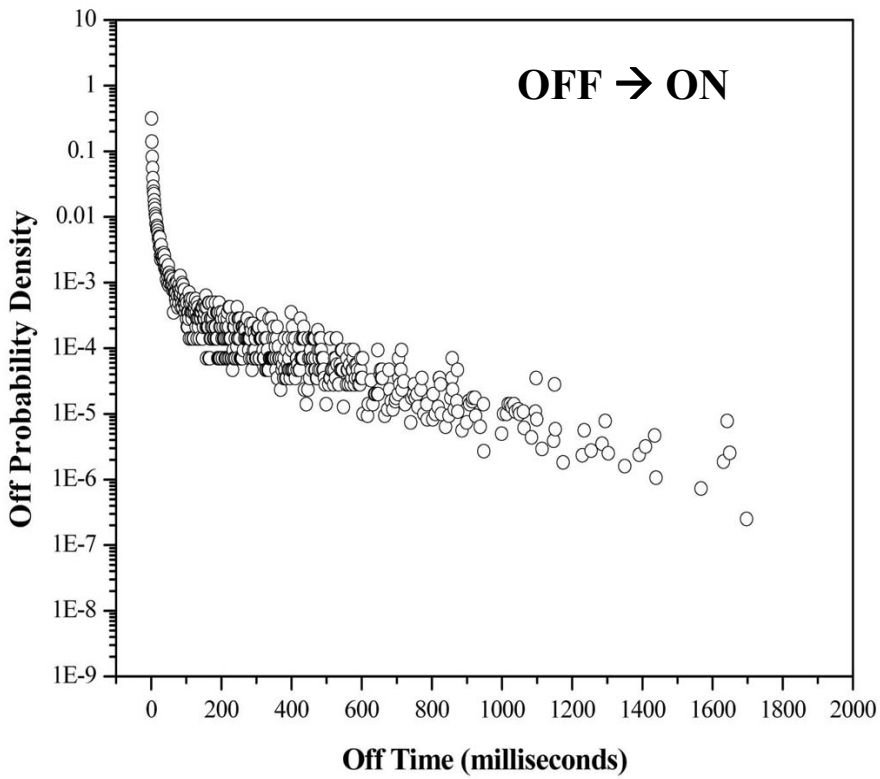
(Left) A 10 second background transient extracted from a 10 minute transient and collected from a dark region of the quantum dot sample. (Right) A histogram analysis of the background counts plotted relative to the mean and the standard deviation (σ) of the measurement. The mean+4σ value was found to be above **99.99%** of the background counts in the measurement and was employed as the threshold value. A threshold analysis procedure was performed following *Kuno et al. J. Chem. Phys. 115(2):1028, **2001**.*

Histogram Analysis (Log-Linear) of On and Off lengths



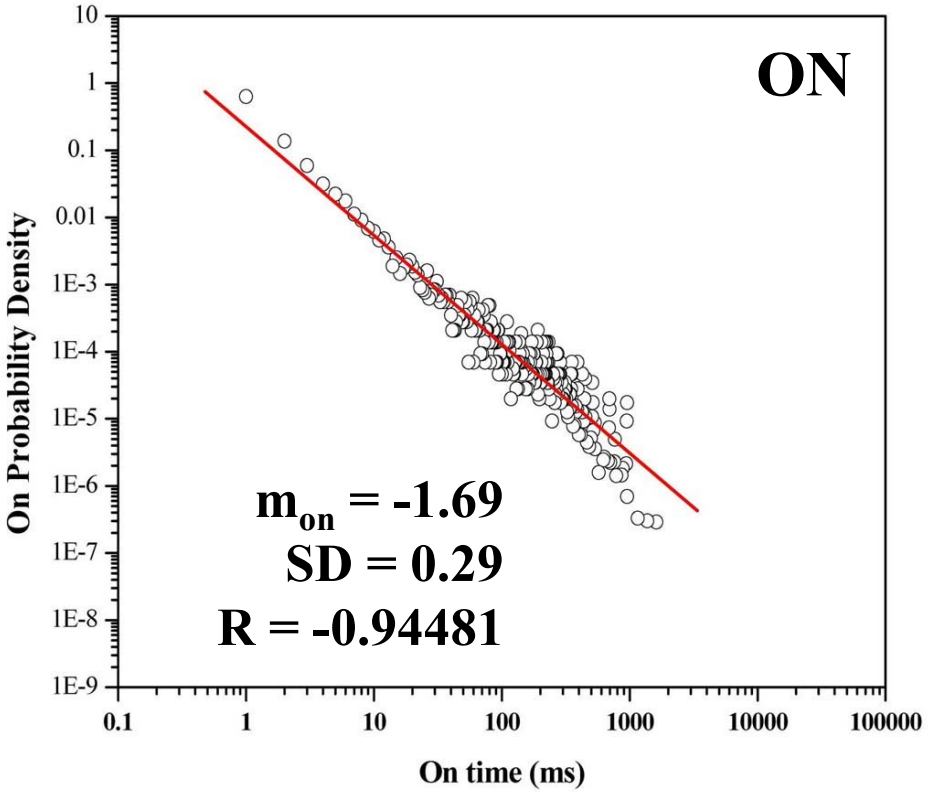
Probability Density (Log-Linear) of On and Off lengths

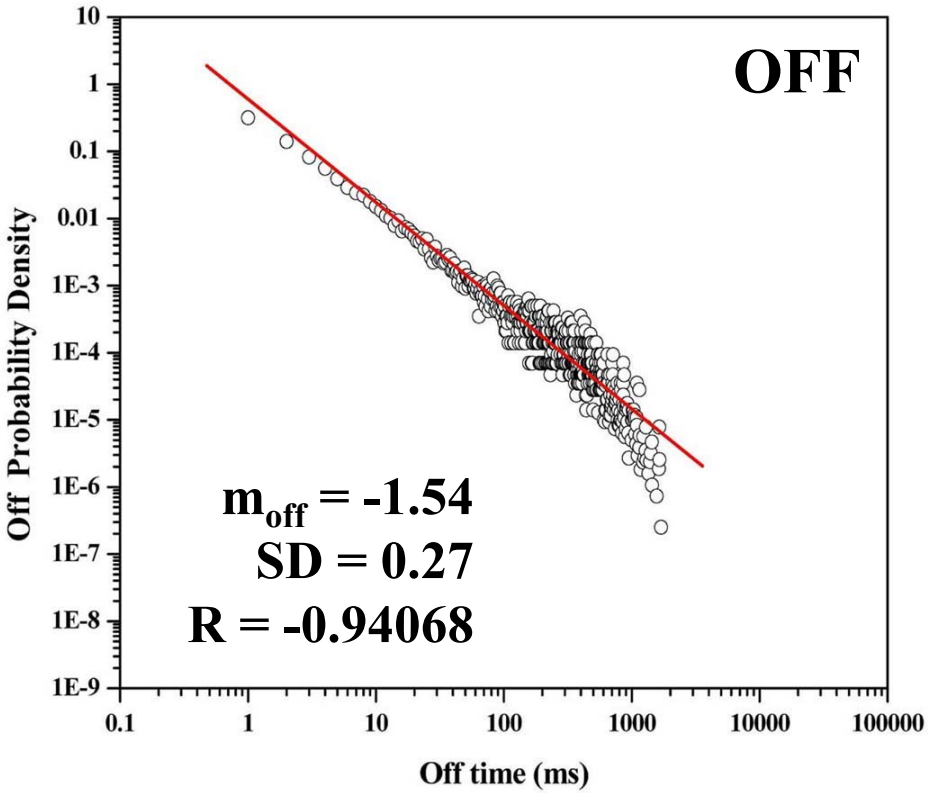




The on-time probability distribution (left) reflects the On → Off kinetics while the off-time probability distribution (rigt) reflects the Off → On kinetics. Curvature in the log-linear plot implies the blinking process is not exponential, therefore, a single recovery channel or single trap state is unlikely responsible for the blinking phenomenon.

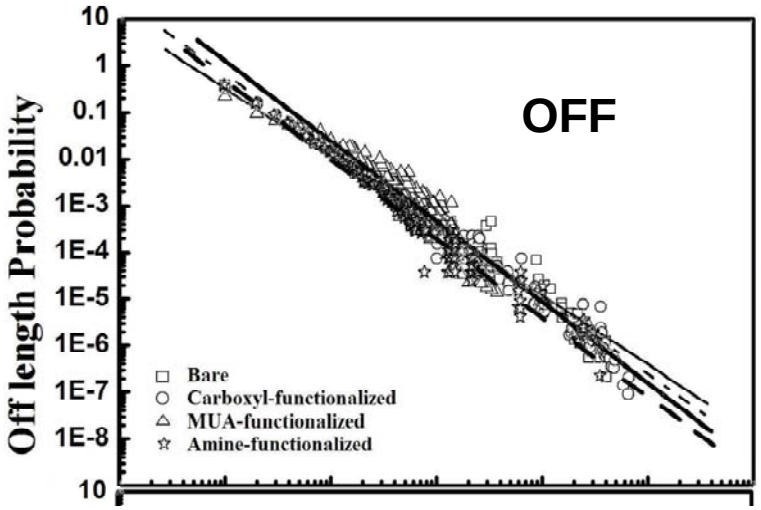
Probability Density (Log-Log) of On and Off lengths

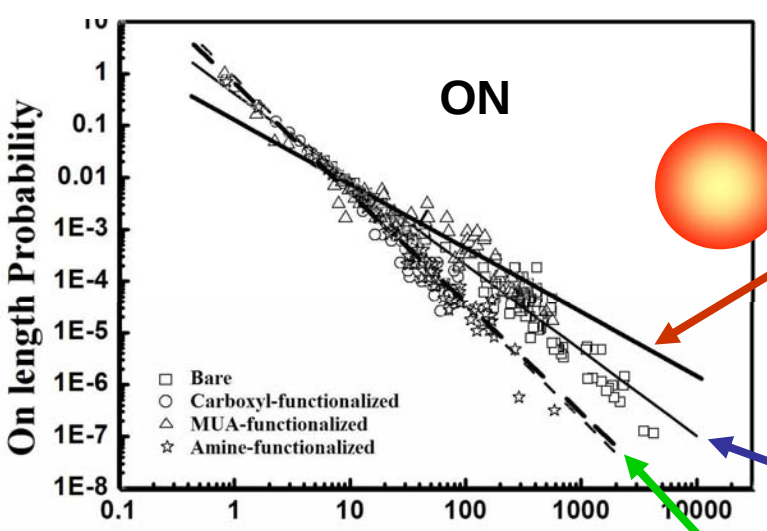




A linear log-log plot of the on-time (left) and off time (right) probability distribution implies that the blinking dynamics follow an inverse power law according to $P(\tau) \alpha \tau^{-m}$. *m* can be extracted from the graph using a least Chi-square fit to the data (red line) and allows the blinking dynamics of the bare, carboxyl, and amine quantum dots to be quantitatively compared.

Thiols (e-donating group) on the surface suppress blinking





$P(\tau) \propto (1/\tau)^m$

$S(CH_2)_{10}COOH$

Thiol (electron donating) group suppresses blinking.

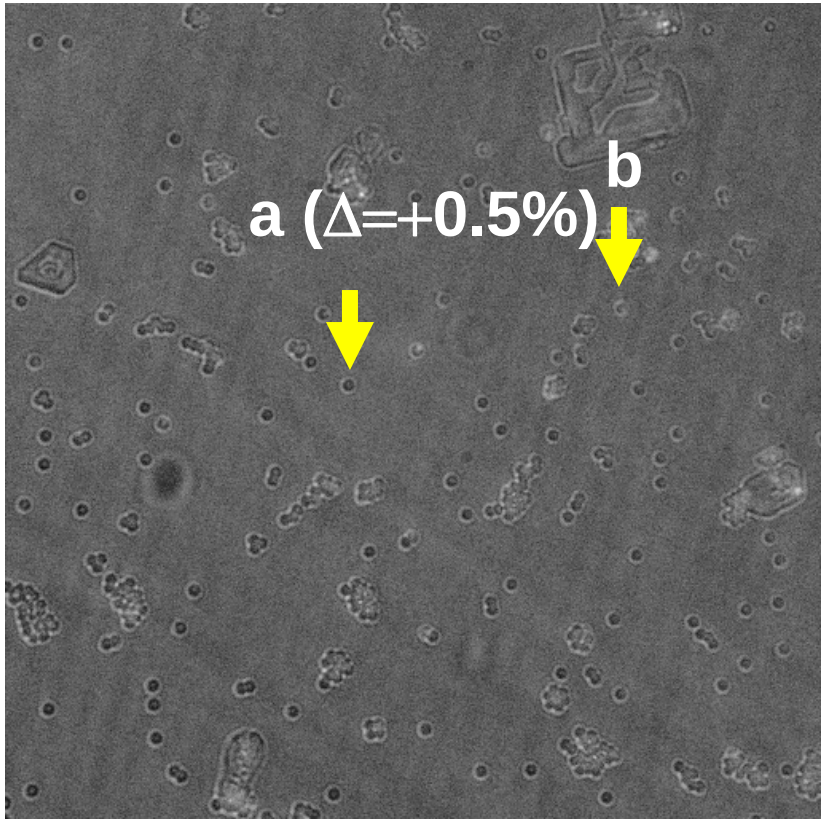
Bare QD

PEG-lipid carboxyl (amine)QD

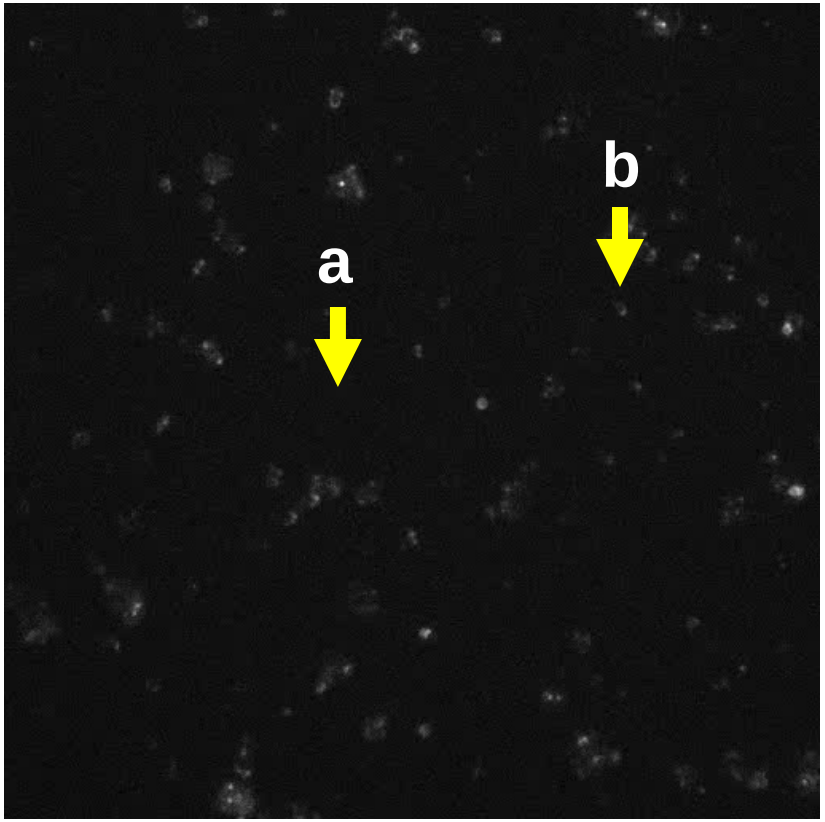
	Bare (N = 48)	Carboxyl (N =49)	Amine (N = 35)	MDA
m_{on}	-1.73	-2.29	-2.27	-1.48
R - m_{on}	-0.95	-0.97	-0.97	-0.93
SD - m_{on}	0.27	0.24	0.26	0.18
m_{off}	-1.38	-1.39	-1.64	-1.64
R - m_{off}	-0.94	-0.94	-0.95	-0.94
SD - m_{off}	0.30	0.29	0.28	0.26

Fluorescent Microscopy to count the # of QDs per bead

Bright Field

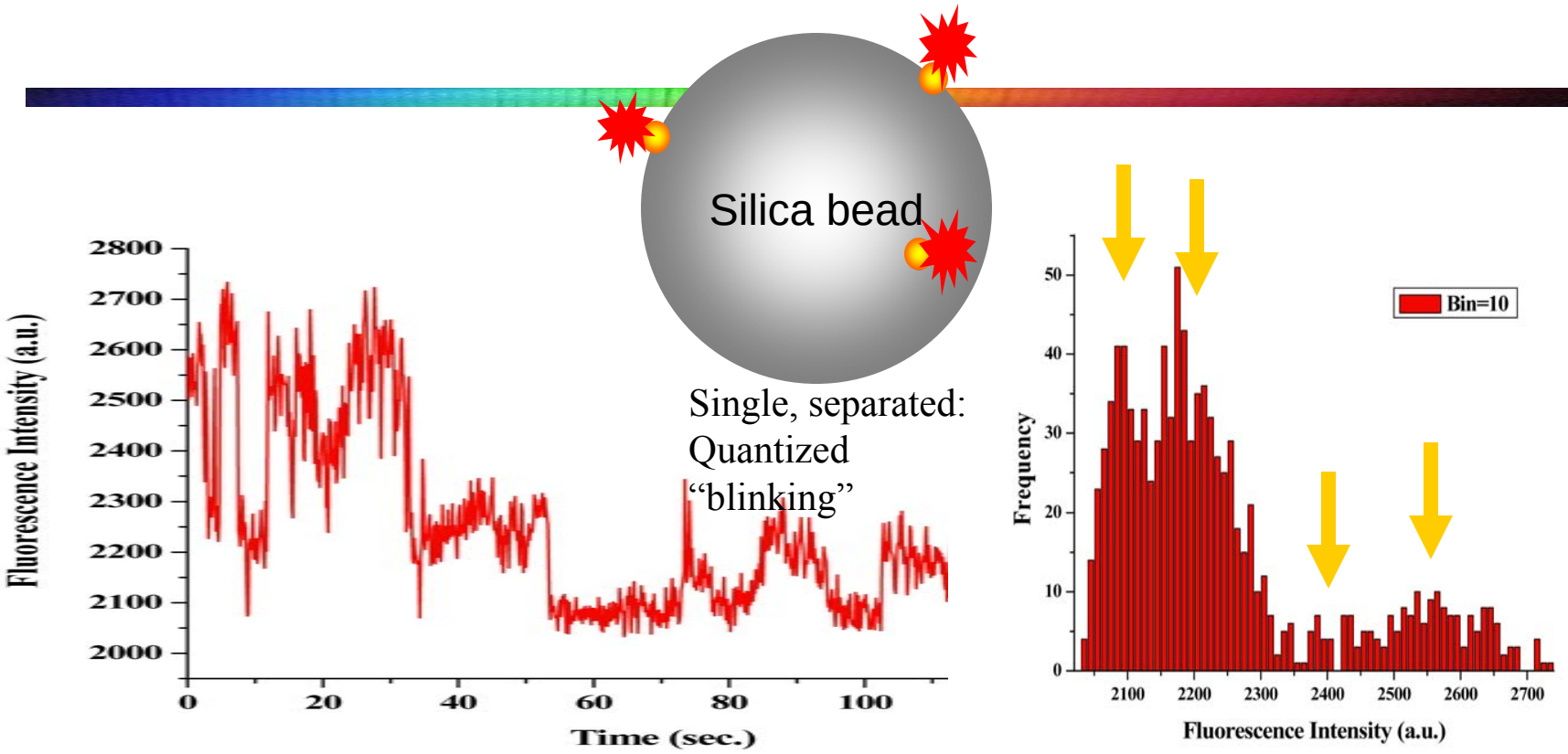


Fluorescent



Out of 256 ***single*** beads from **3 nM QD** sample that we looked at, 206 (≈ 80.5 %) beads showed fluorescence from attached QDs.

Blinking of single QDs conjugated onto Silica Bead



The estimated average intensity (≈ 150 counts) of each quantized step from a single QD allows for the detection limit determination of quantitative flow cytometry by estimating the number of QDs attached onto the beads detected above the threshold in the flow cytometry (as indicated with arrow 'a' in the micrographs in Figure 3). Maximum fluorescence signals of ≈ 3200 counts on average were observed from these bright beads corresponding to ≈ 8 QDs per bead.

Measurable IV: Local concentration
Fluorescence from “clustered” QDs

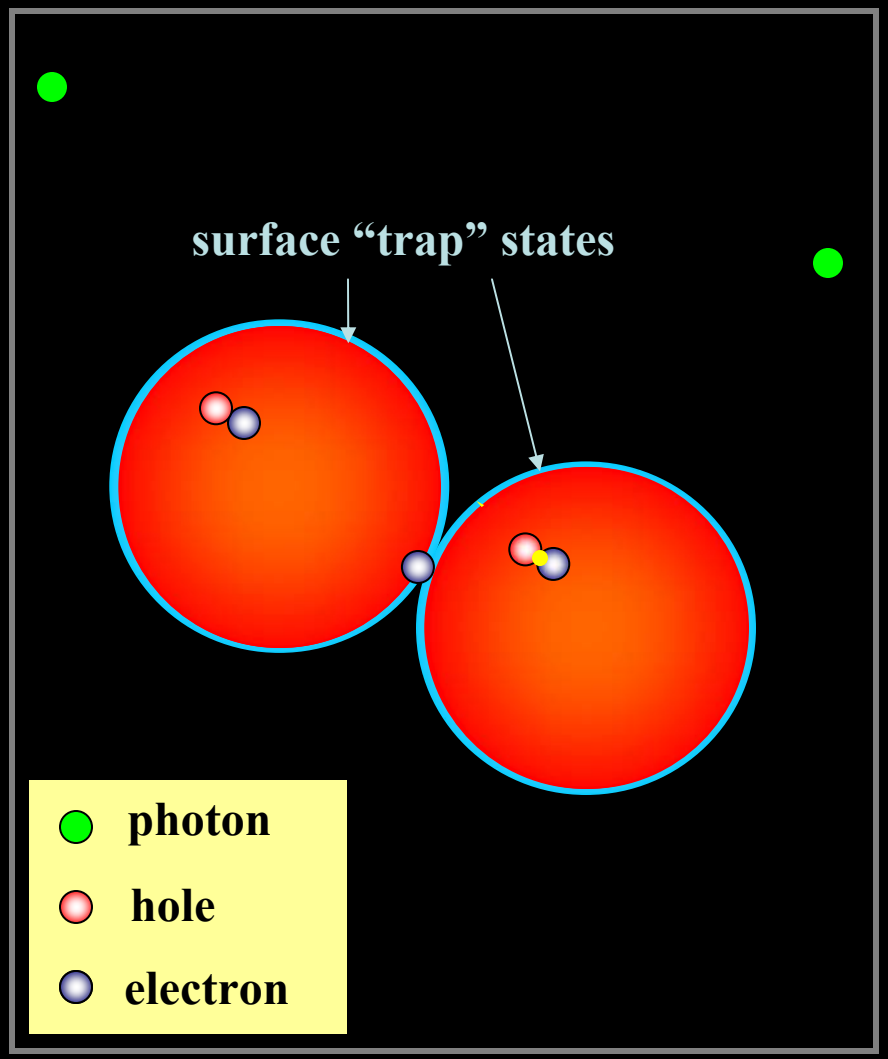
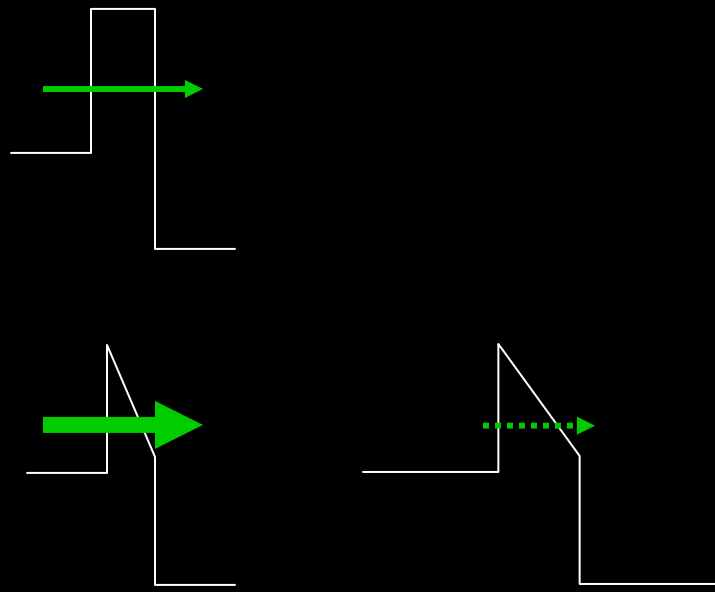
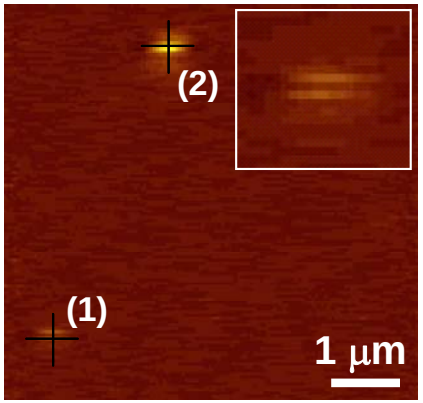
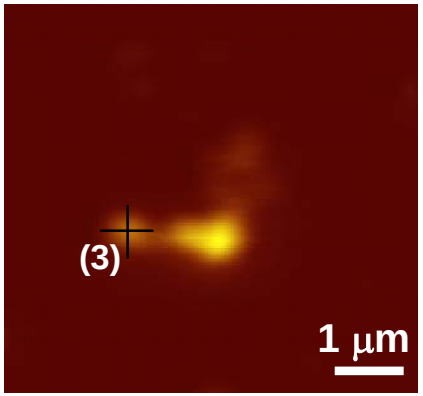


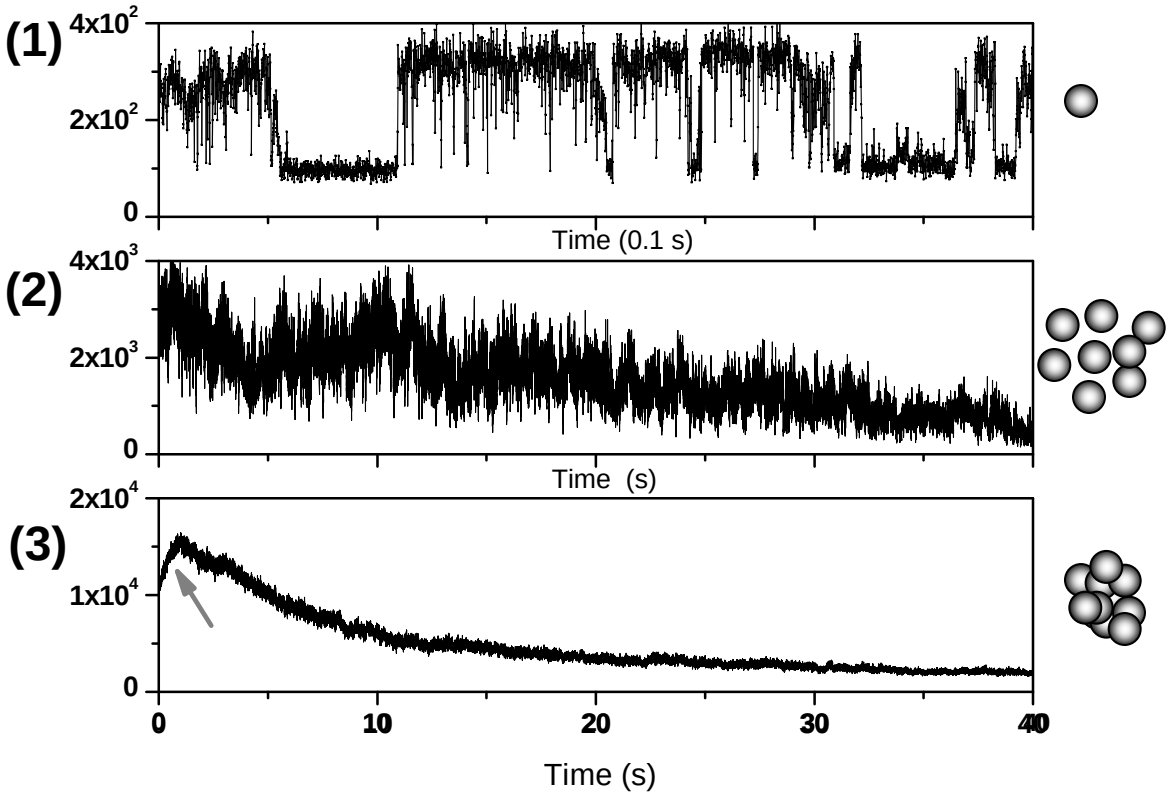
Photo-induced lowering of the tunneling barrier (i.e. oxide layer)



The difference between a group of *isolated* QDs and *clustered* QDs

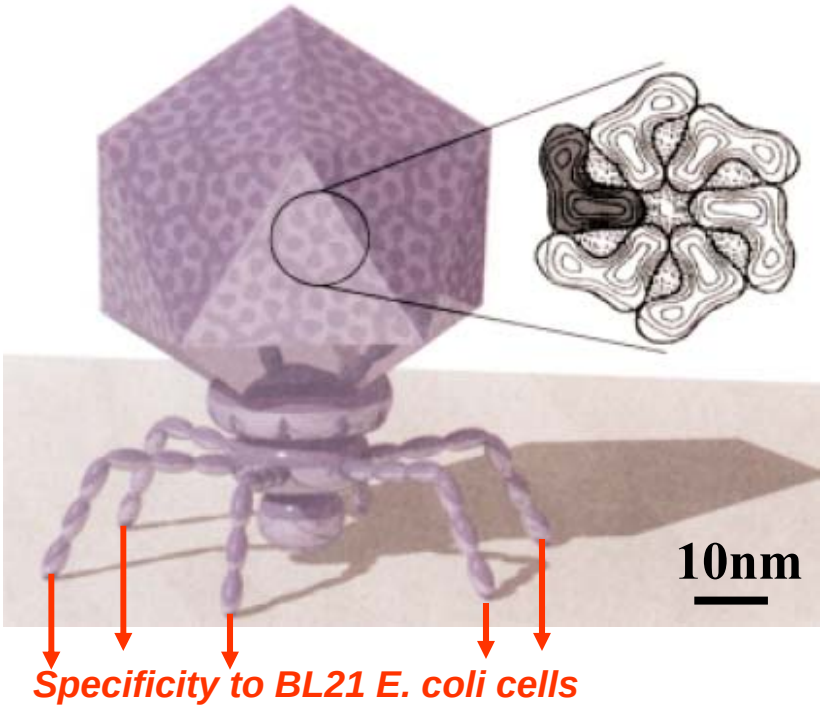






Confocal fluorescence images of QDs on glass substrates spun cast from a low concentration (a) and a high concentration (b) QD solution. Cross marked positions, (1), (2), and (3) in the images are the positions from which the time-trace of fluorescence intensities presented in (c), (d), and (e) are measured, respectively. Inset in (a) is a magnified view of the area over position (1) exhibiting the "blinking" behavior of a single QD.

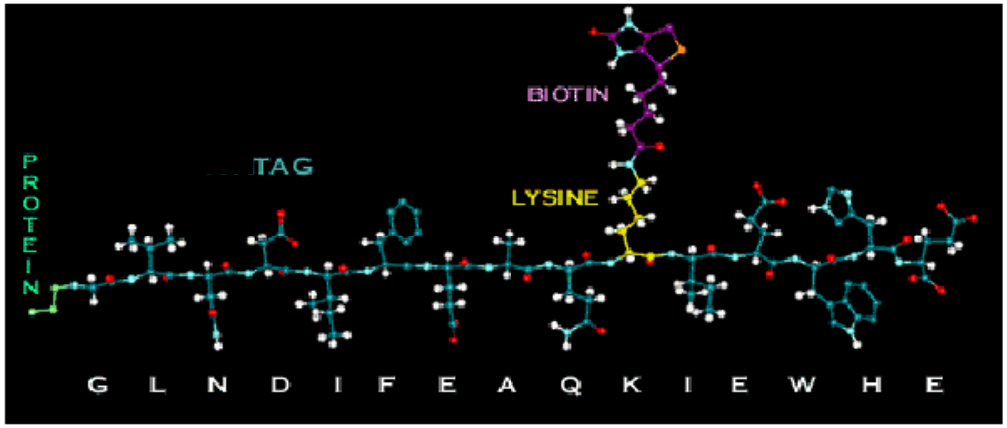
Nanosensor assembly of bacteriophage and QDs

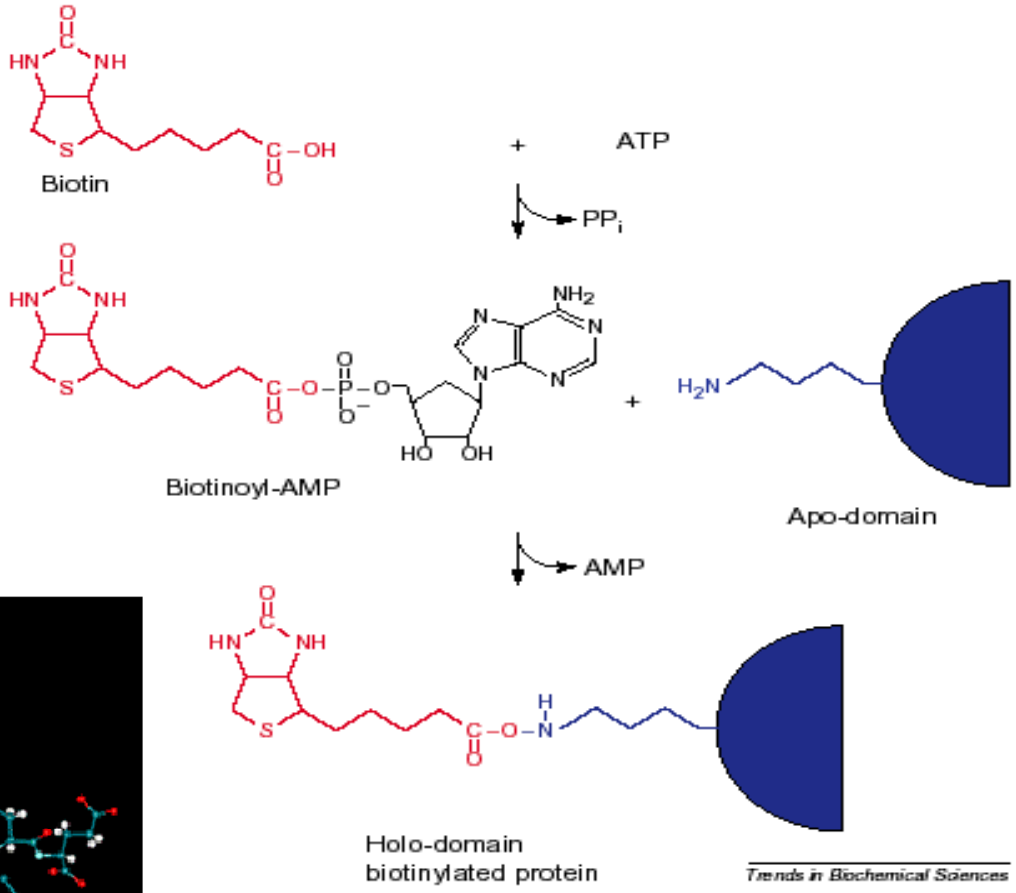


T7 phage: the capsid shell, head-tail connector, tail, and tail fibers are shown schematically. The diffraction pattern from polyheads (4) showing a hexamer capsid unit has been fit onto the surface of the icosahedral particle (diameter approx. 55 nm).
The monomer units are in gray. **(Newsletter of NOVAGEN Inc. Vol. No 6, October, 1996).**
Only a few GFP can be expressed to maintain the biological function of the phage.

Expressing Biotin protein ligase (BPL) on the capsid surface

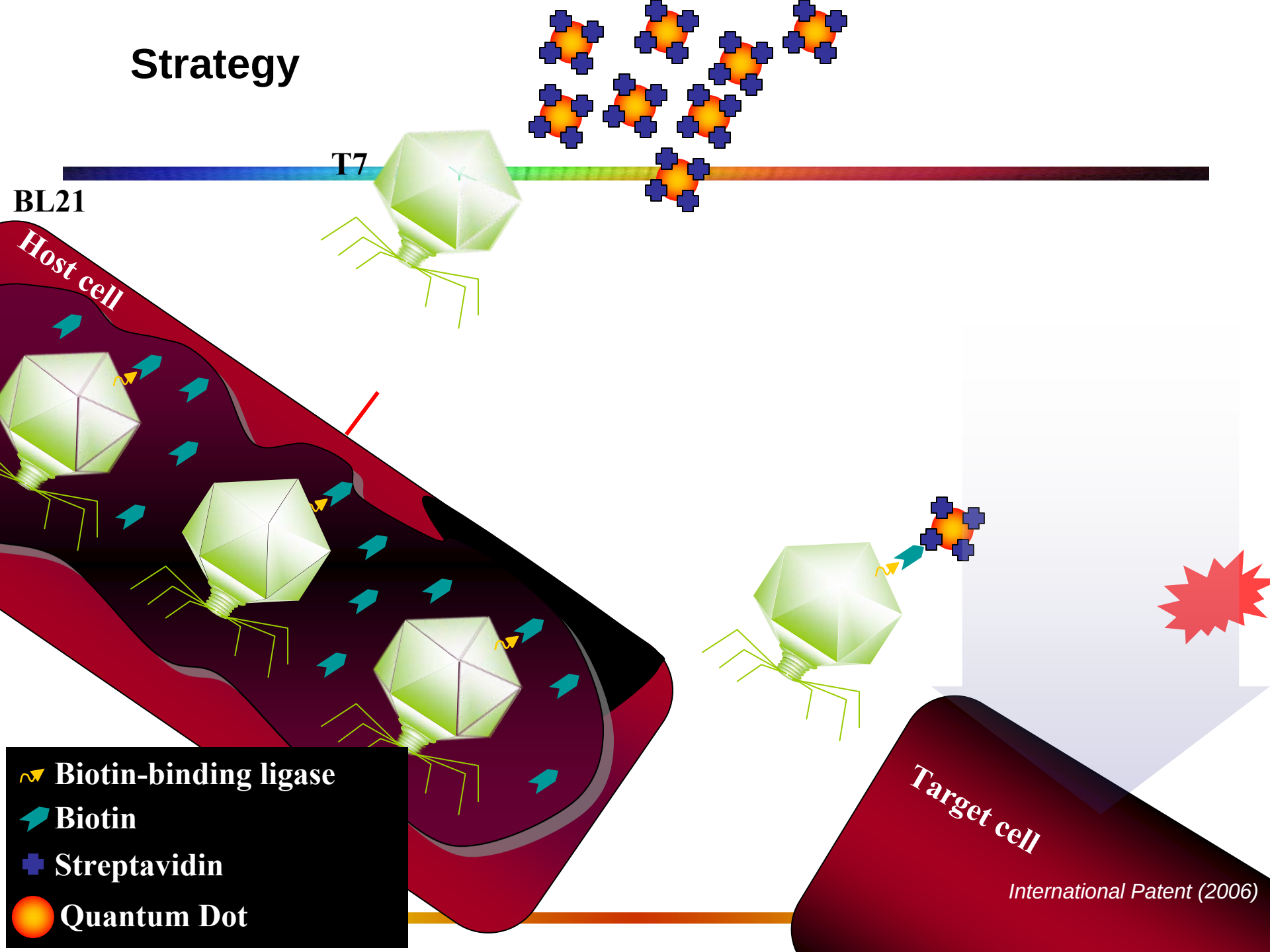
- **BirA (15aa):GLNDIFEAQKIEWHE the BPL of *E. coli* biotinylates only a single cellular protein, Biotin Carboxyl Carrier Protein (BCCP), a subunit of acetyl-CoA carboxylase (the enzyme catalyzing the first committed step of fatty acid synthesis)**





- **Negative control, myc (10aa): EQKLISEEDL**

Strategy


T7
BL21
Host cell
Target cell
Biotin-binding ligase
Biotin
Streptavidin
Quantum Dot
International Patent (2006)

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