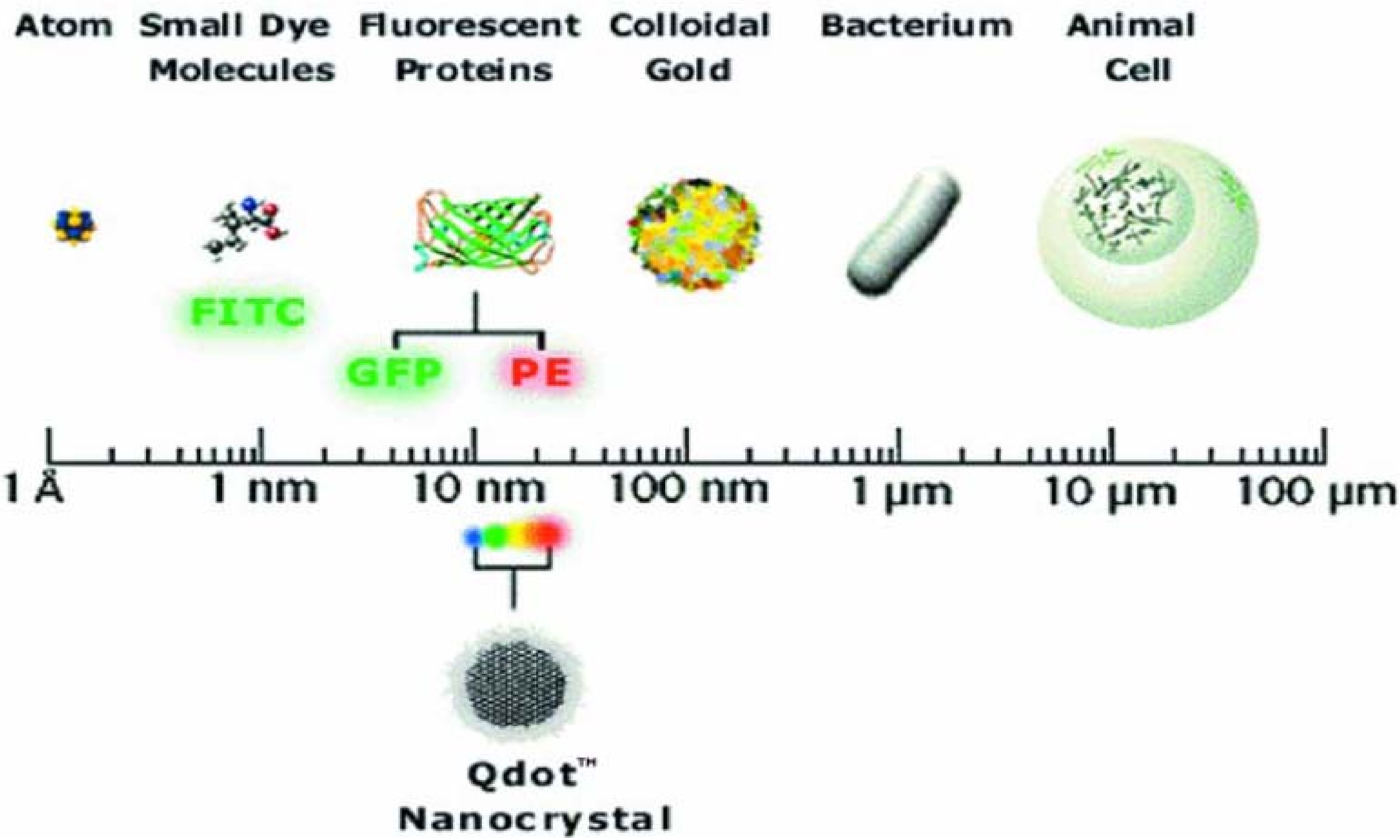
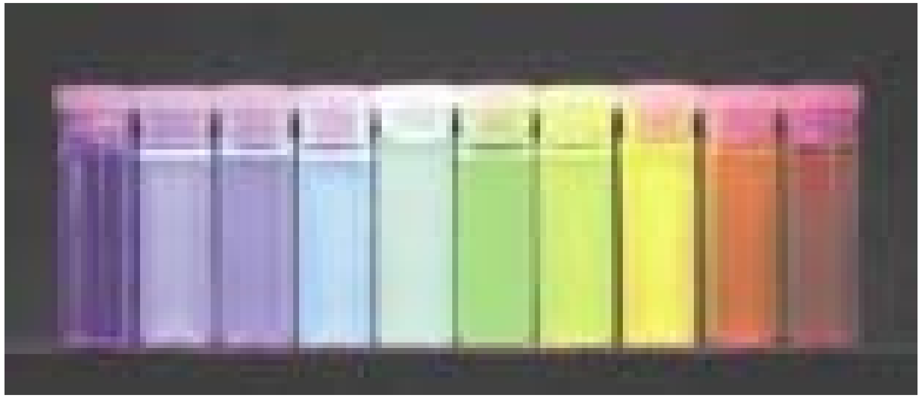
Applications of Quantum Dots in Biology



Atom
Small Dye Molecules
Fluorescent Proteins
Colloidal Gold
Bacterium
Animal Cell
FITC
GFP
PE
1 Å
1 nm
10 nm
100 nm
1 μm
10 μm
100 μm
Qdot™ Nanocrystal

Quantum Dots (QDs)

- Quantum dots (QDs) are semi conductor crystals
- Useful fluorescent markers for bio-imaging
- Emission wavelength is tuneable by QD ***size (nanometer range)***
- Narrow emission wavelength from common excitation wavelength
- Stable against photo bleaching
- They are robust, limited photo bleaching
- Some problems still be overcome, including aggregation, non-specific adsorption, toxicity.

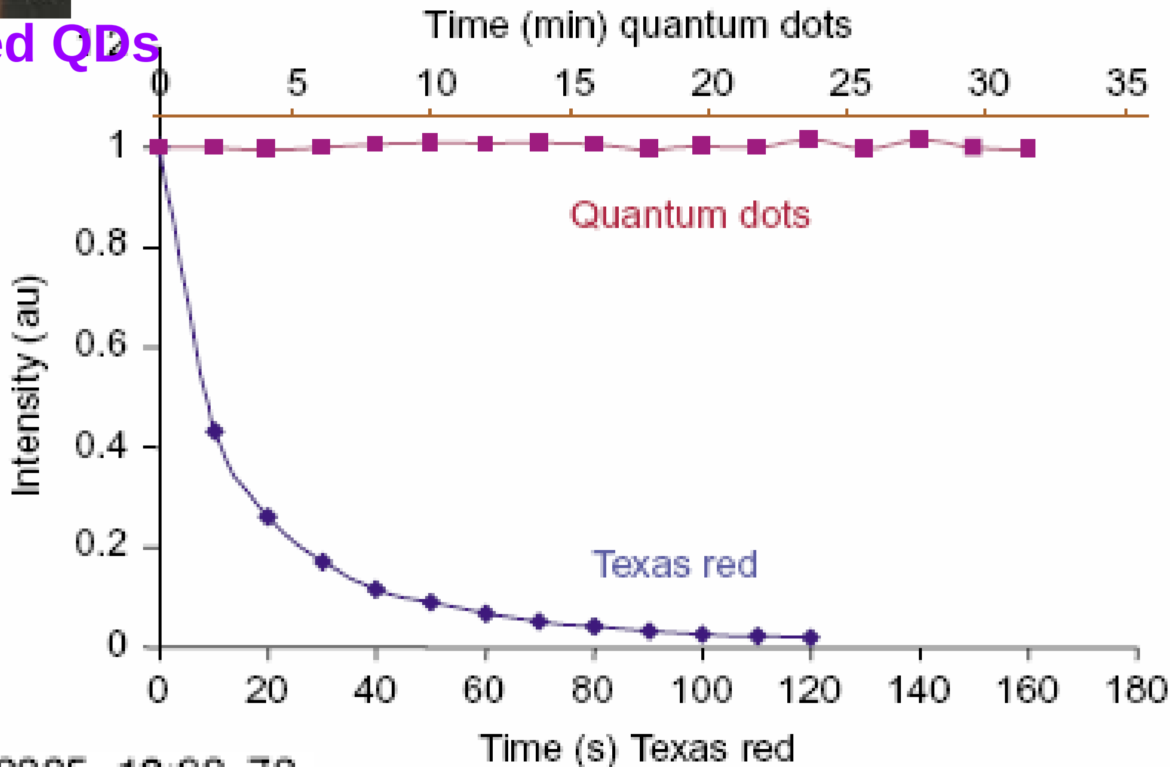


Comparison of QDs and organic dyes

Rhodamine dye

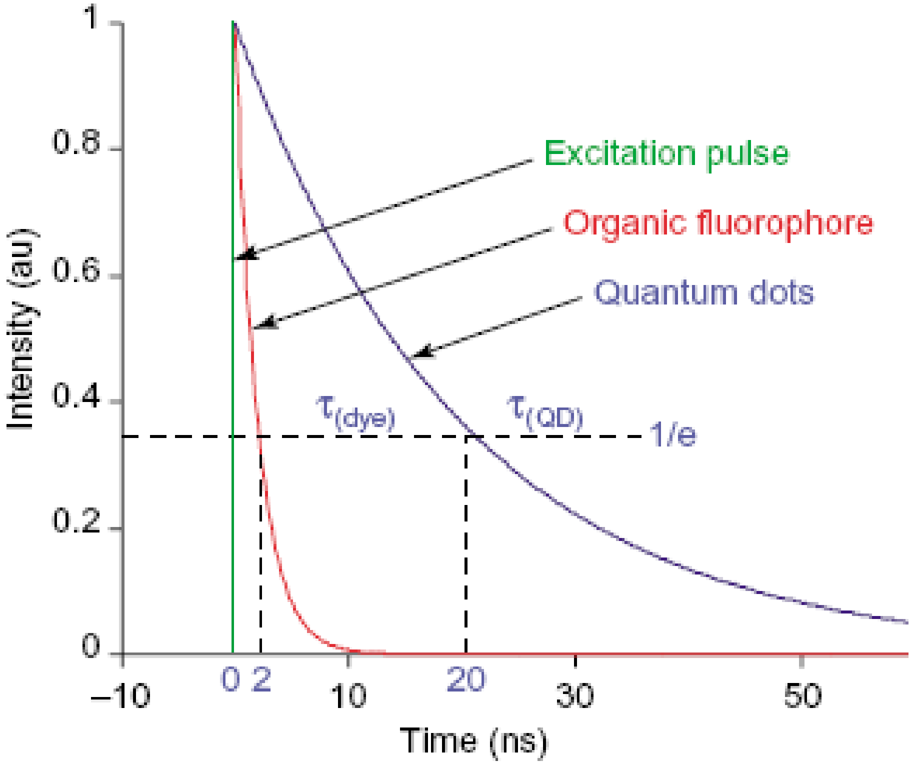
Green and red QDs

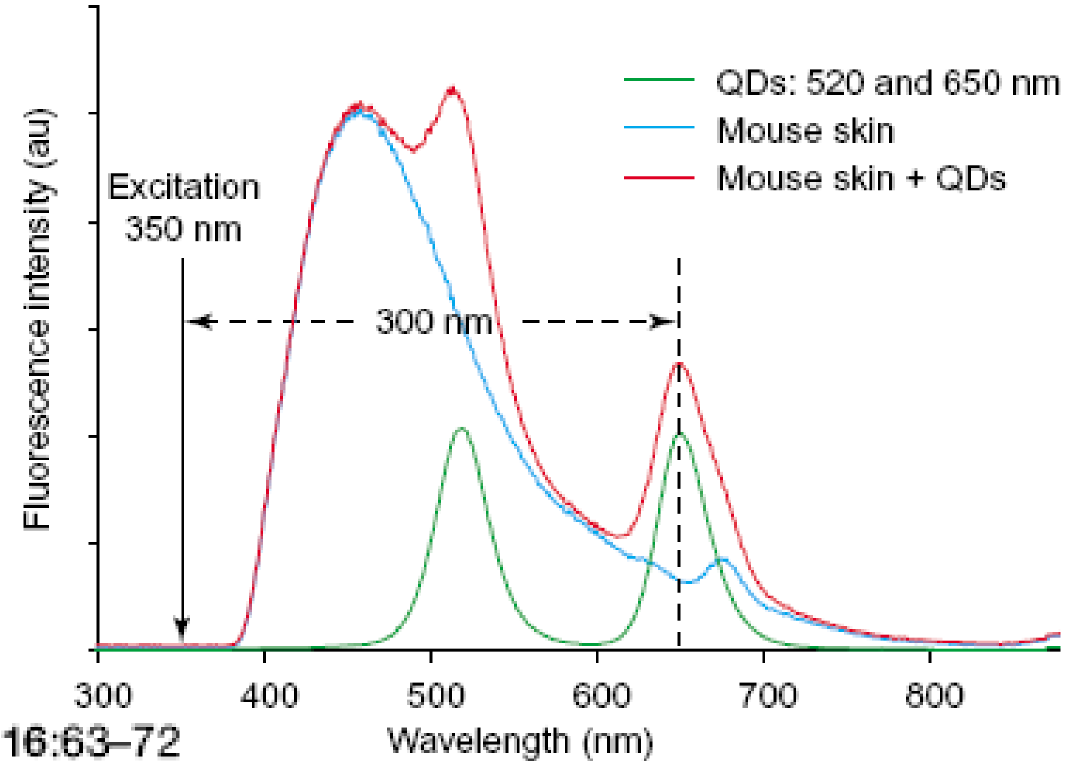
QDs are more photostable than organic dyes



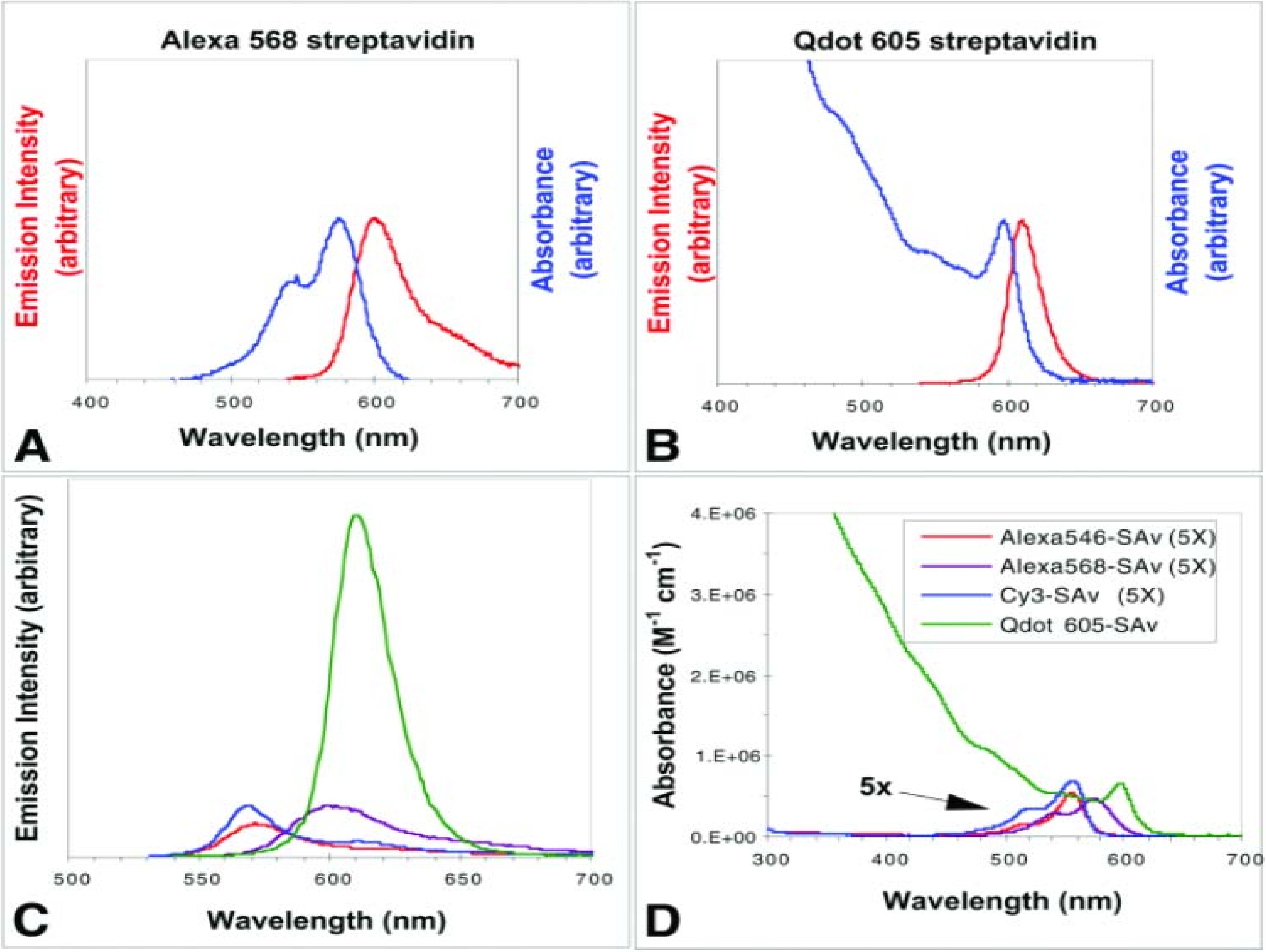
Current Opinion in Biotechnology 2005, 16:63–72

QDs have longer lifetimes than organic dyes



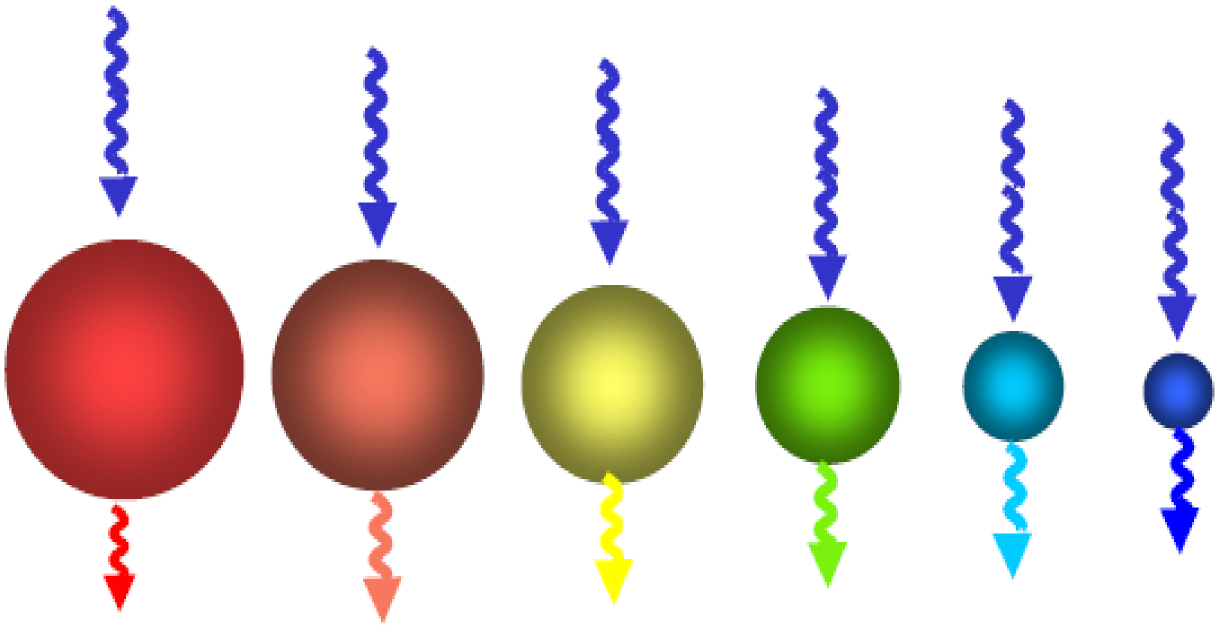


Current Opinion in Biotechnology 2005, 16:63–72



Alexa 568 streptavidin
Emission Intensity (arbitrary)
Absorbance (arbitrary)
400
500
600
700
Wavelength (nm)
A
Qdot 605 streptavidin
Emission Intensity (arbitrary)
Absorbance (arbitrary)
400
500
600
700
Wavelength (nm)
B
Emission Intensity (arbitrary)
500
550
600
650
700
Wavelength (nm)
C
4.E+06
3.E+06
2.E+06
1.E+06
0.E+00
Absorbance (M-1 cm-1)
Alexa546-SAv (5X)
Alexa568-SAv (5X)
Cy3-SAv (5X)
Qdot 605-SAv
5x
300
400
500
600
700
Wavelength (nm)
D

Optical Properties of Quantum Dots



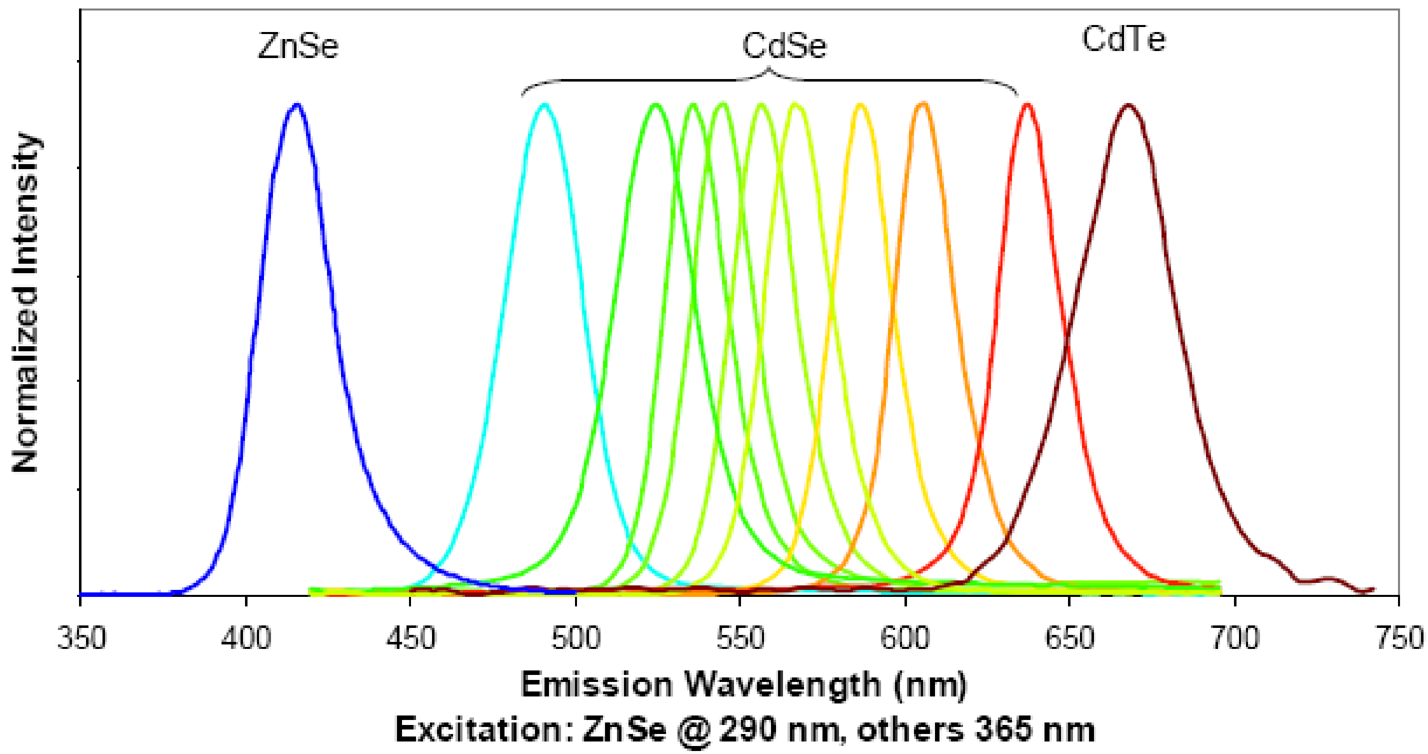
Red: bigger dots!

Blue: smaller dots!

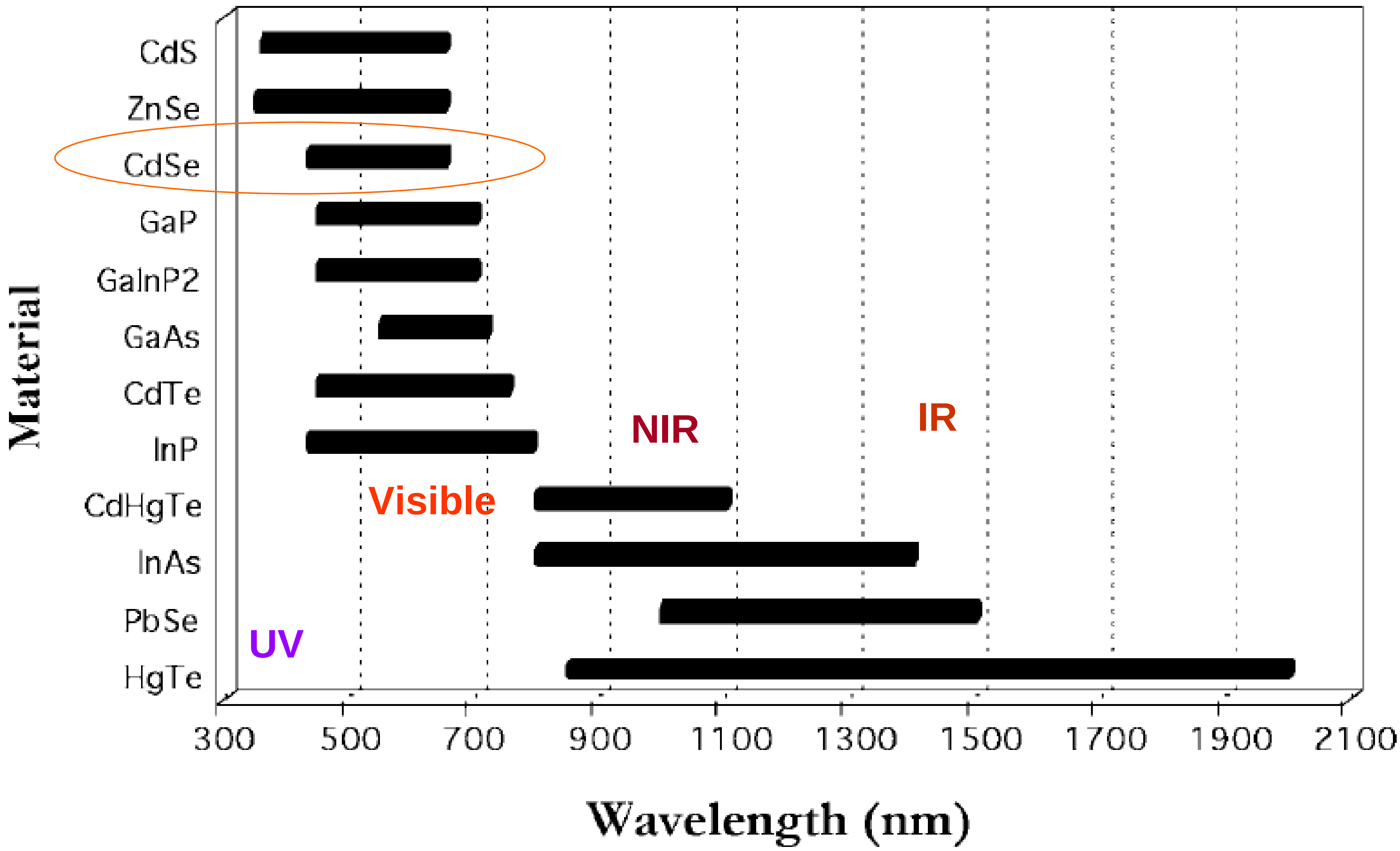
Nanocrystals absorb light then re-emit the light in a different color – the size of the nanocrystal (at the Angstrom scale) determines the color

Six different quantum dot solutions are shown excited with a long wave UV lamp

Many sharp, distinct colors



- **Extremely broad absorption spectra allow all colors to be excited with a single excitation wavelength**
- **Water-soluble nanocrystal quantum yields are as high as 90%**
- **Narrow, symmetric emission spectra minimize overlap of adjacent colors**



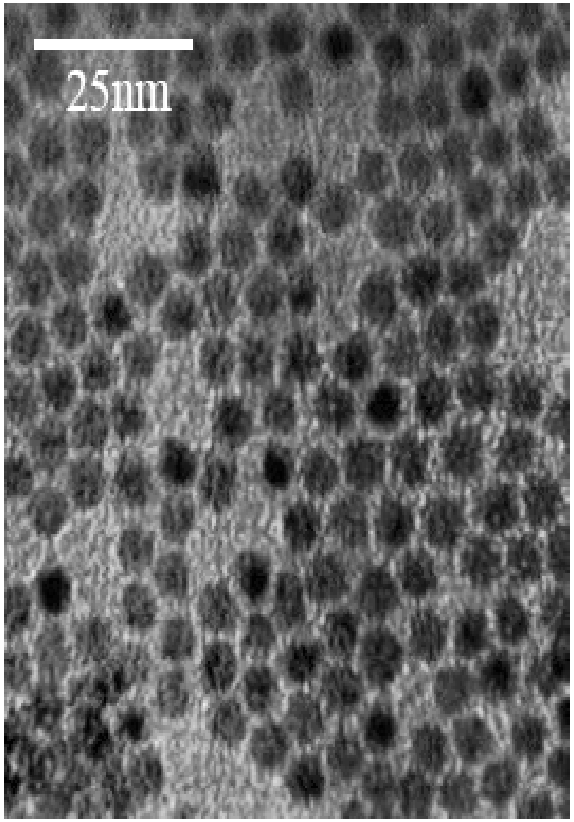
Material
CdS
ZnSe
CdSe
GaP
GaInP2
GaAs
CdTe
InP
CdHgTe
InAs
PbSe
HgTe
UV
Visible
NIR
IR
300
500
700
900
1100
1300
1500
1700
1900
2100
Wavelength (nm)

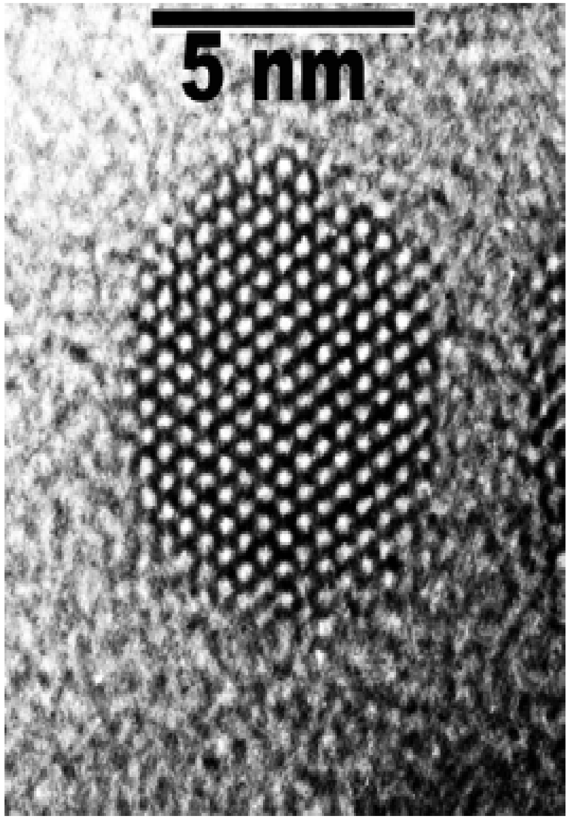
Table 1
Optical Properties of Quantum Dots Compared to Common Dyes[a]

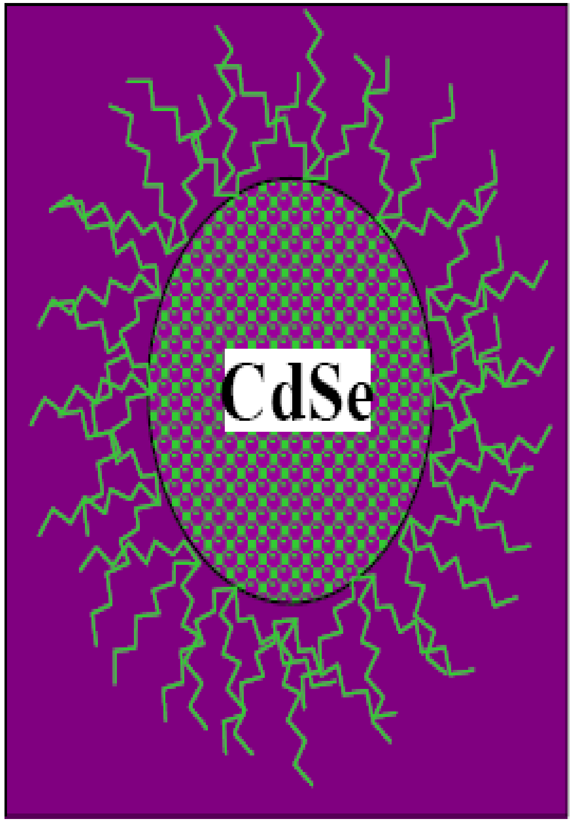
Fluorescent dye	$\lambda_{excitation}$ (nm)	$\lambda_{emission}$ (nm)	$\varepsilon(mol^{-1}\text{-}cm^{-1})$
Qdot 525	400	525	280,000
Alexa 488	495	519	78,000
Fluorescein	494	518	79,000
Qdot 565	400	565	960,000
Cy3	550	570	130,000
Alexa 555	555	565	112,000
Qdot 585	400	585	1,840,000
R-Phycoerythrin	565	578	1,960,000
TMR	555	580	90,000
Qdot 605	400	605	2,320,000
Alexa 568	578	603	88,000
Texas Red	595	615	96,000
Qdot 655	400	655	4,720,000
APC	650	660	700,000
Alexa 647	650	668	250,000
Cy5	649	670	200,000
Alexa 647-PE	565	668	1,960,000

[a]The extinction coefficients (ε) are generally much larger for quantum dots than for fluorescent dyes. Furthermore, the excitation wavelength ($\lambda_{excitation}$) can be much farther from the emission ($\lambda_{emission}$).

Qdot™ nanocrystals are highly fluorescent, molecular-sized semiconductor crystals



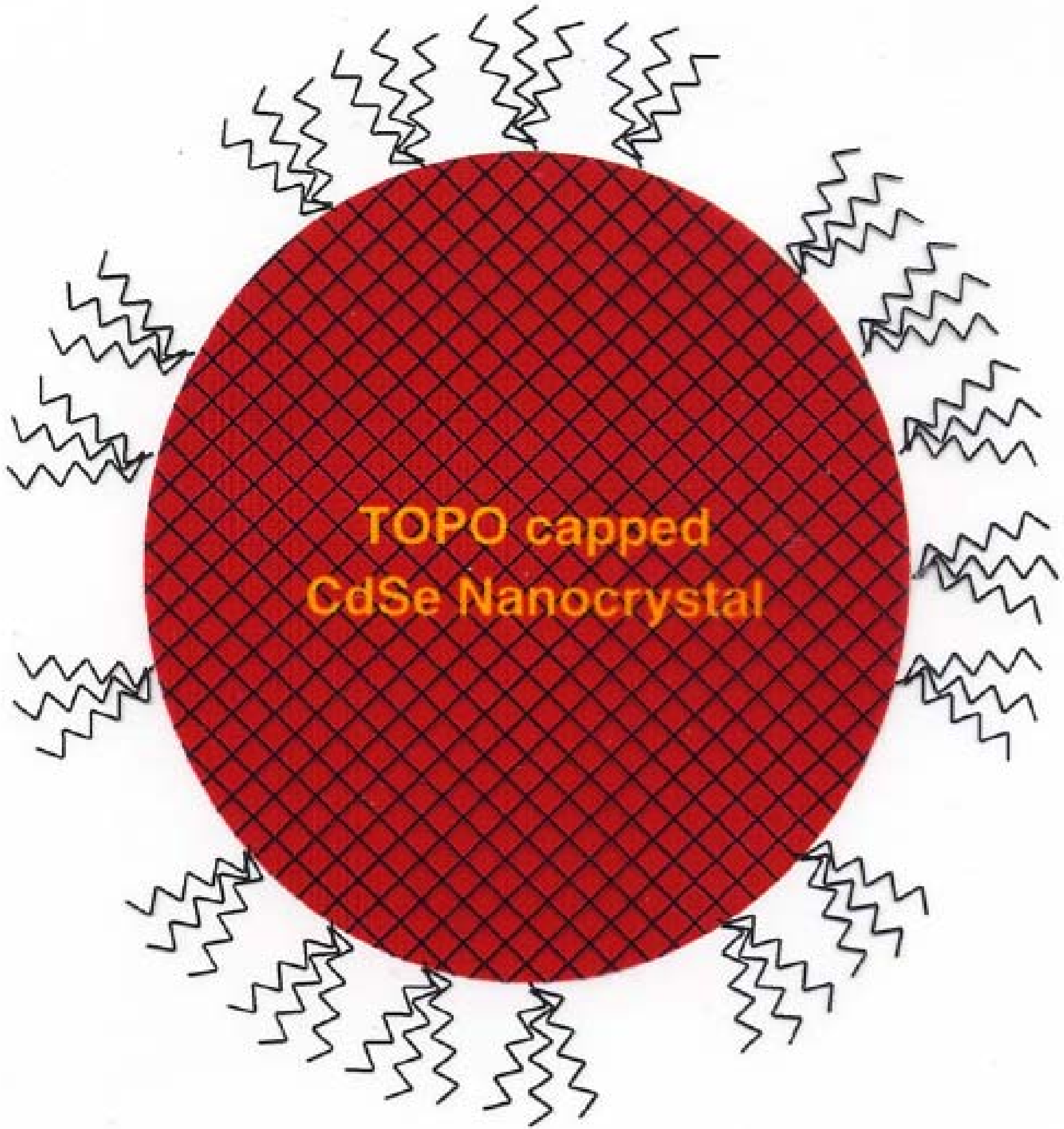




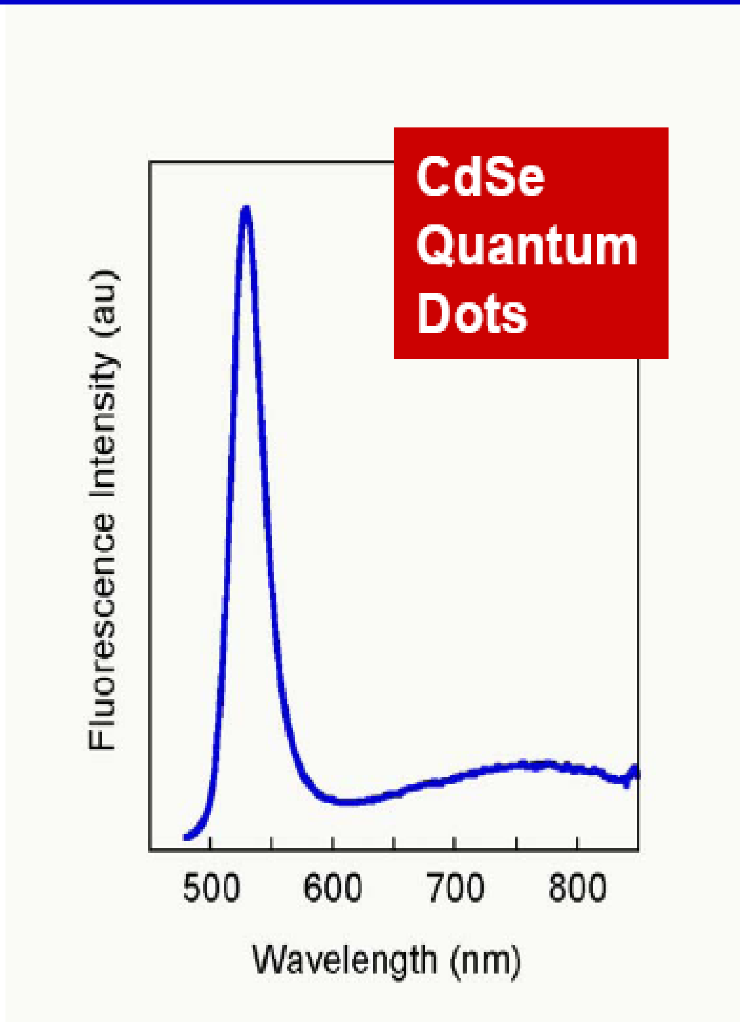
Size: Tunable from ~2-10 nm (±3%)

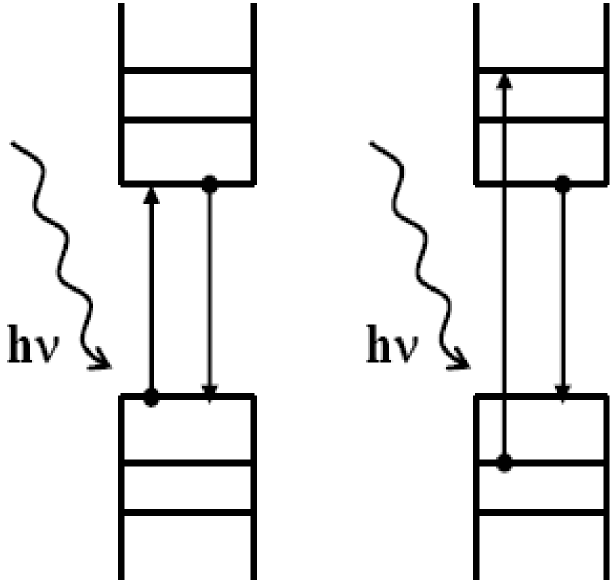
Structure: Highly crystalline

Quantum dots have imperfect surfaces

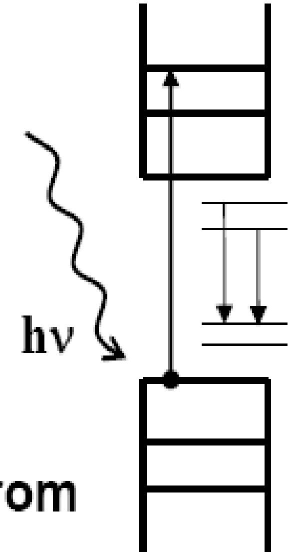


Low Quantum Yields

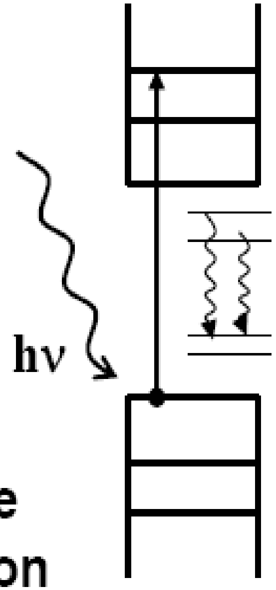




radiative recombination → fluorescence



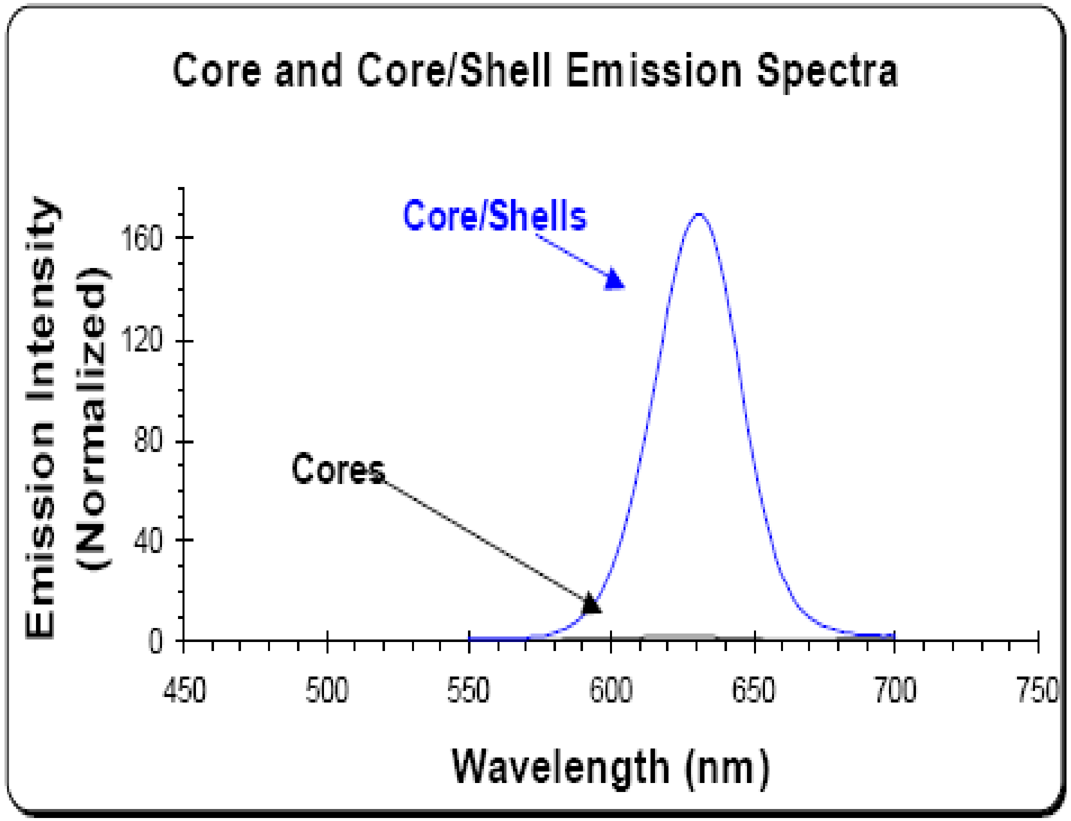
emission from trap states

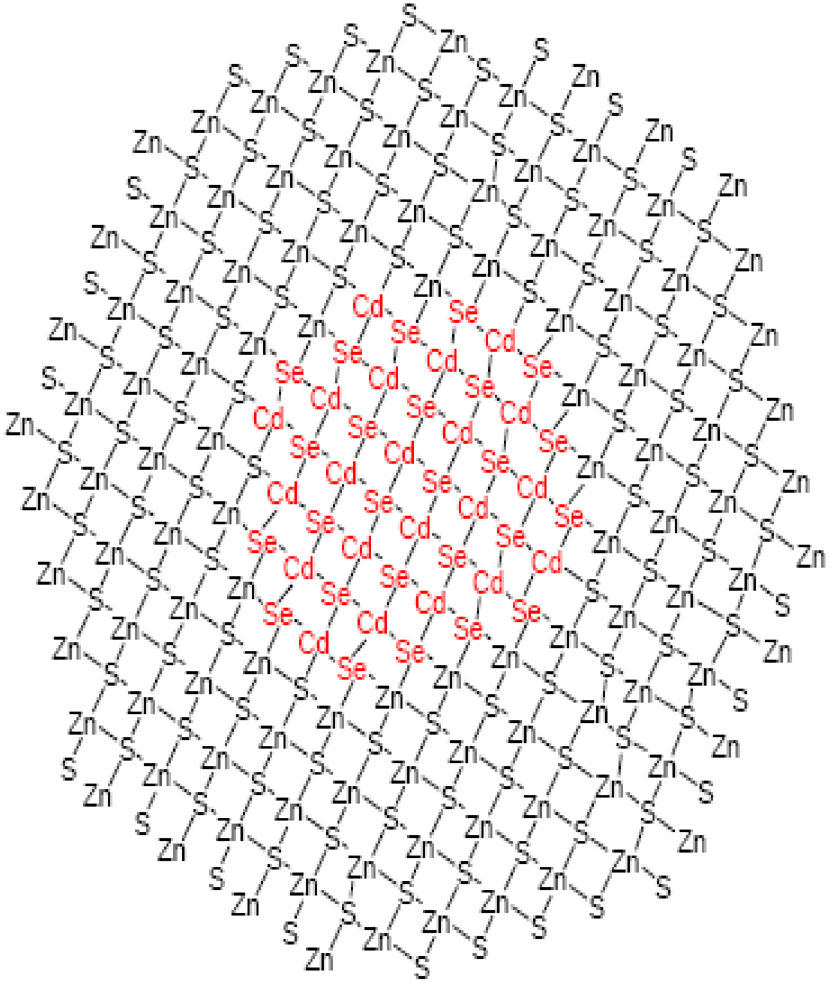


non-radiative recombination

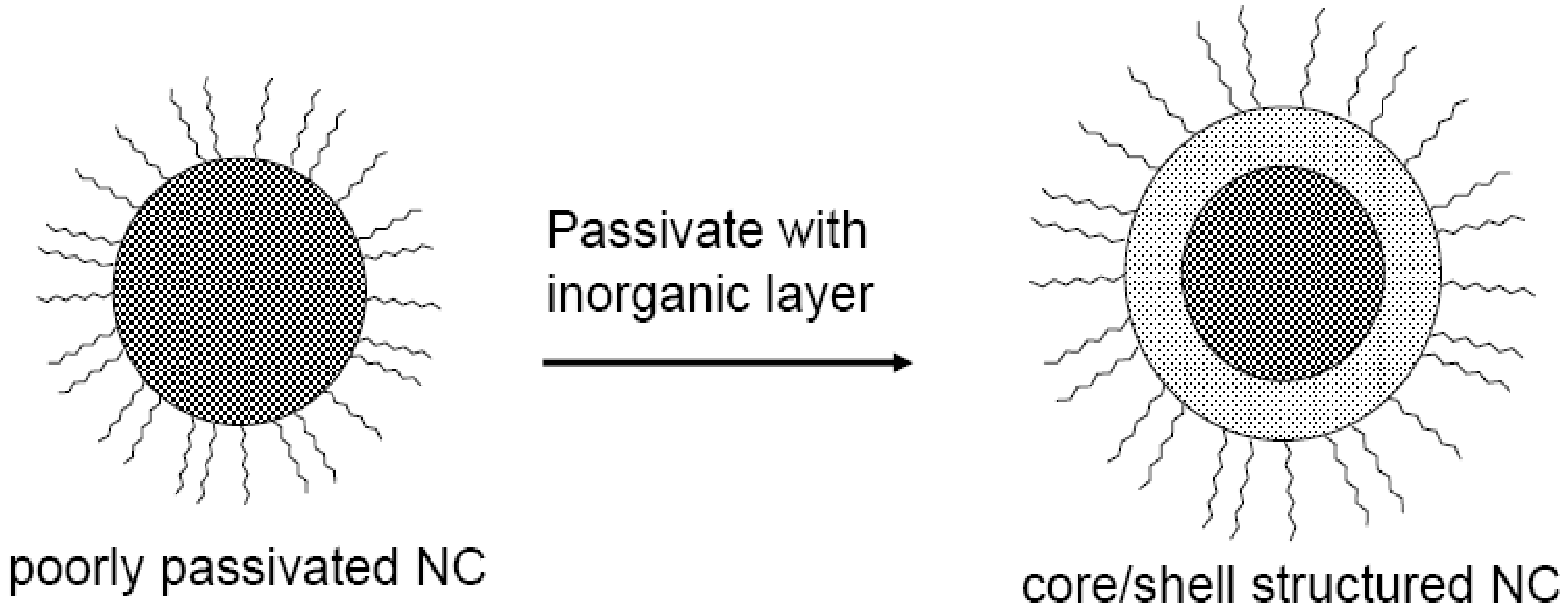
Basic Structure – Shells and Brightness

A shell of a higher band-gap material (ZnS), produces a more stable and brighter structure

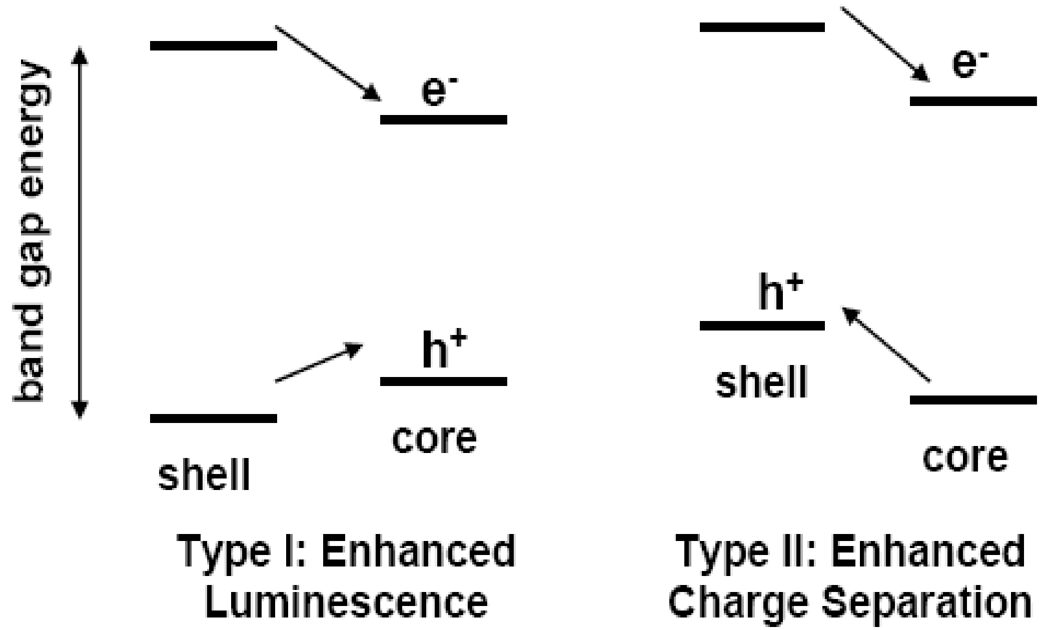




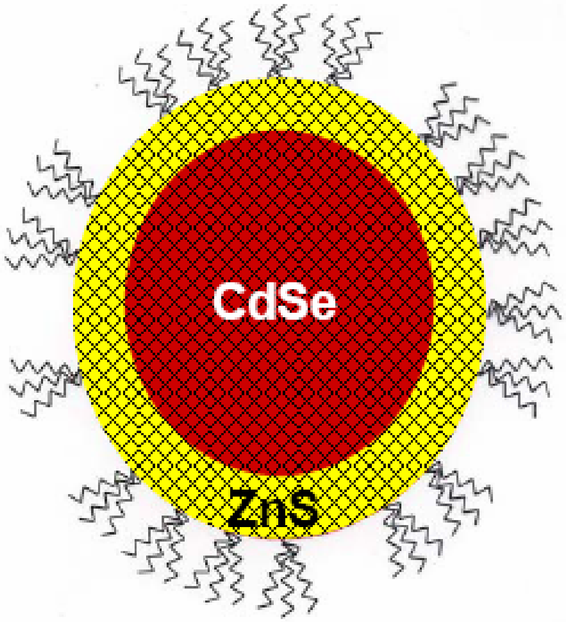
Surface Passivation

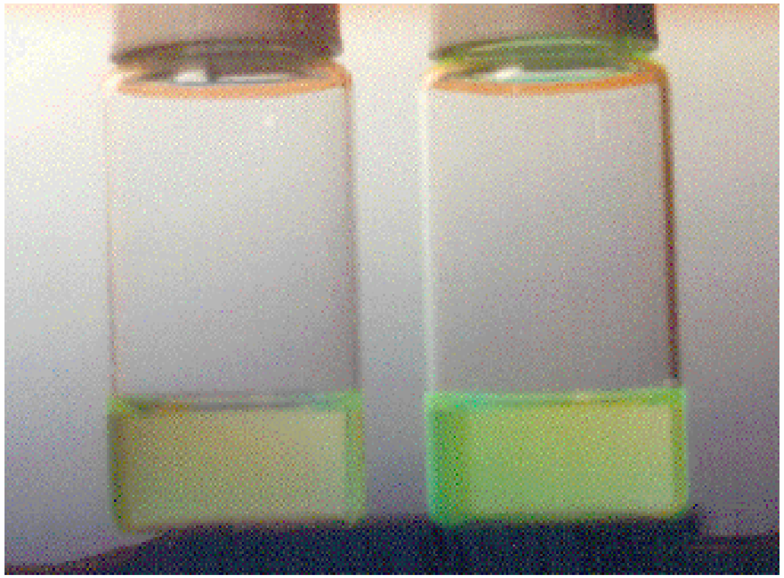


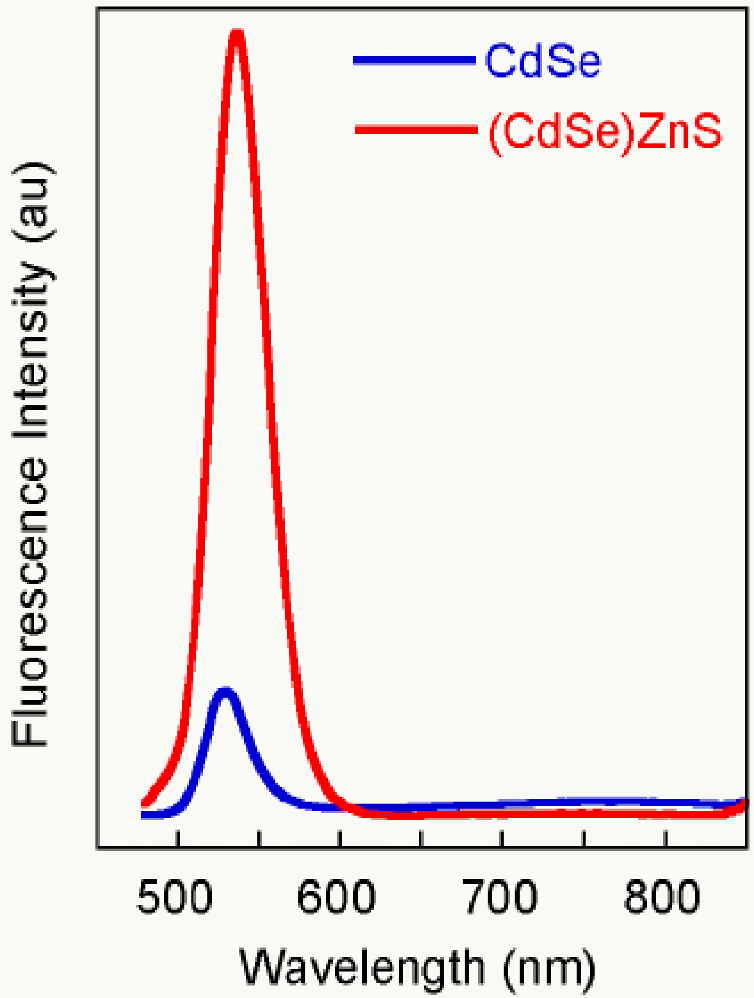
Layered heterostructures for enhanced optical or electronic properties



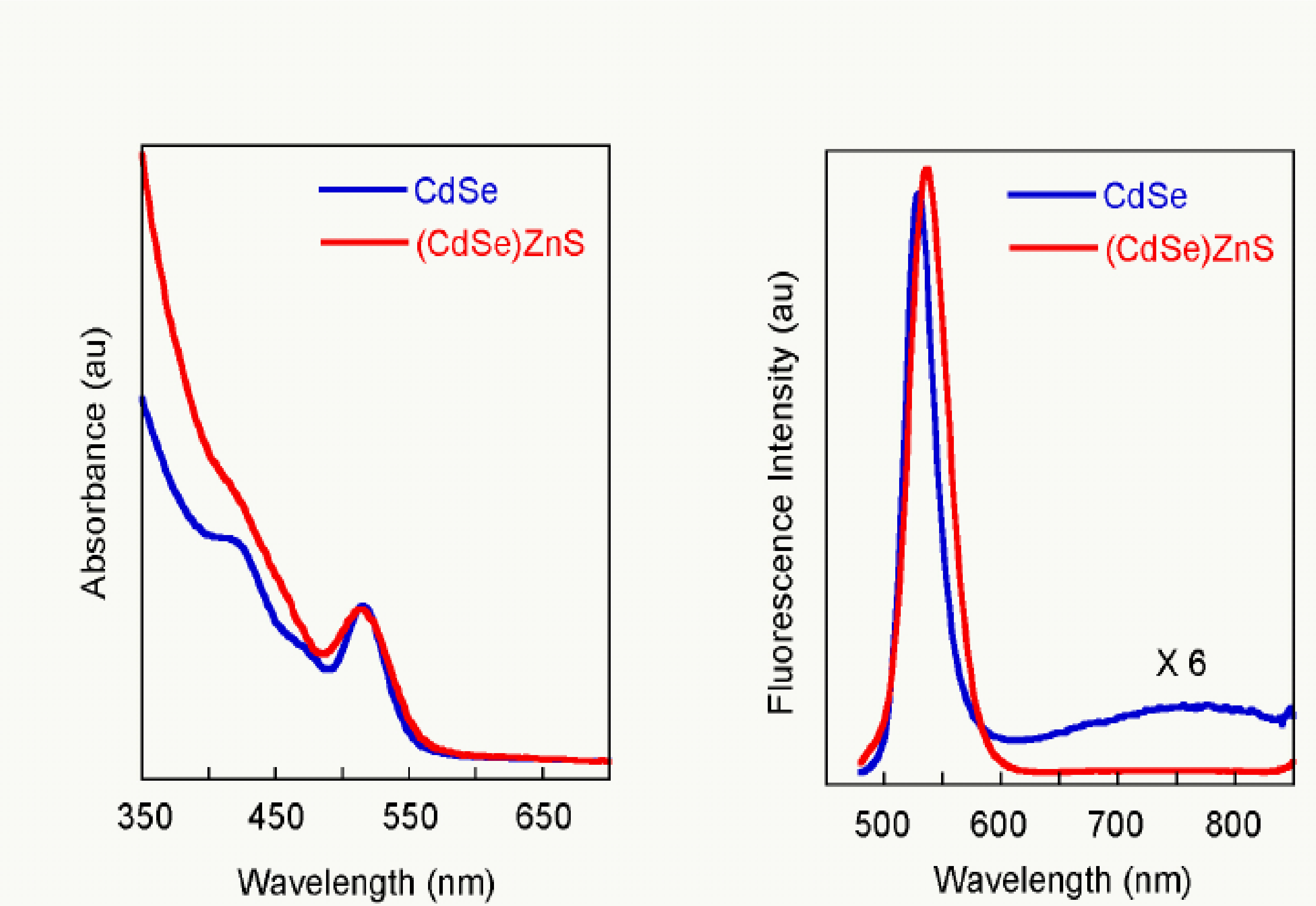
Enhanced Fluorescence





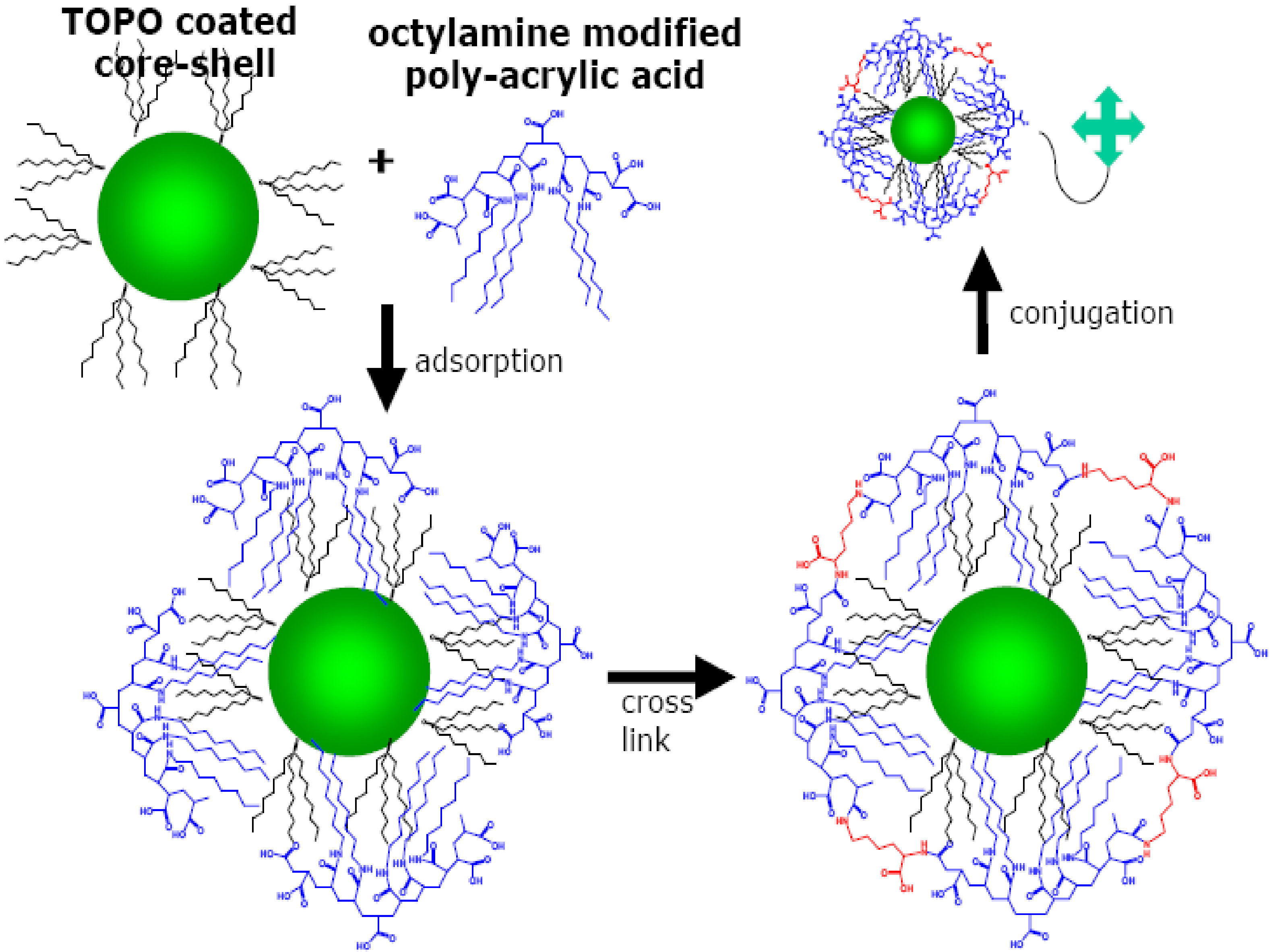


Maintain narrow size dispersions with added shell layer

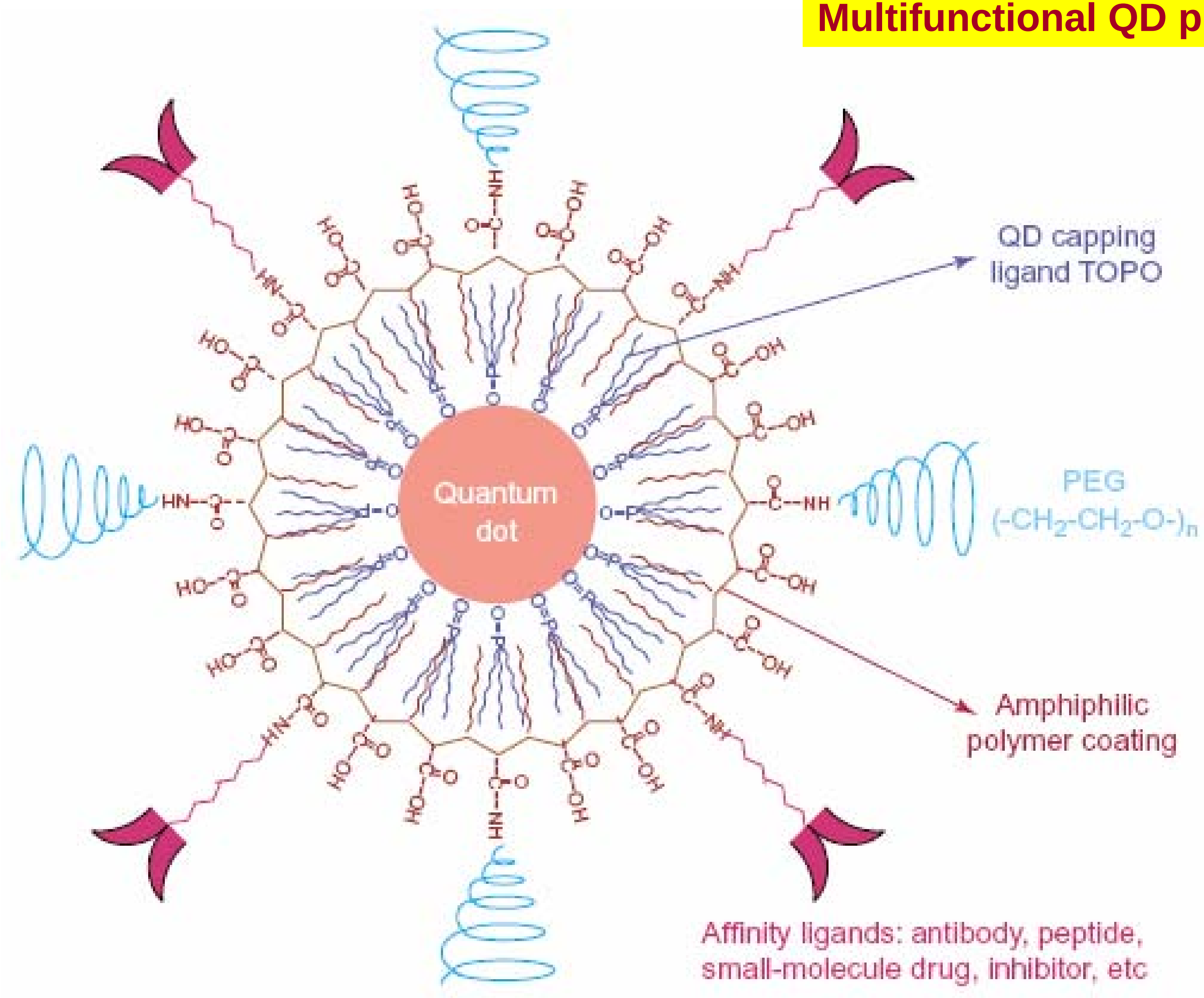


Three Approaches to Achieving Colloidal Stability in Aqueous Milieu

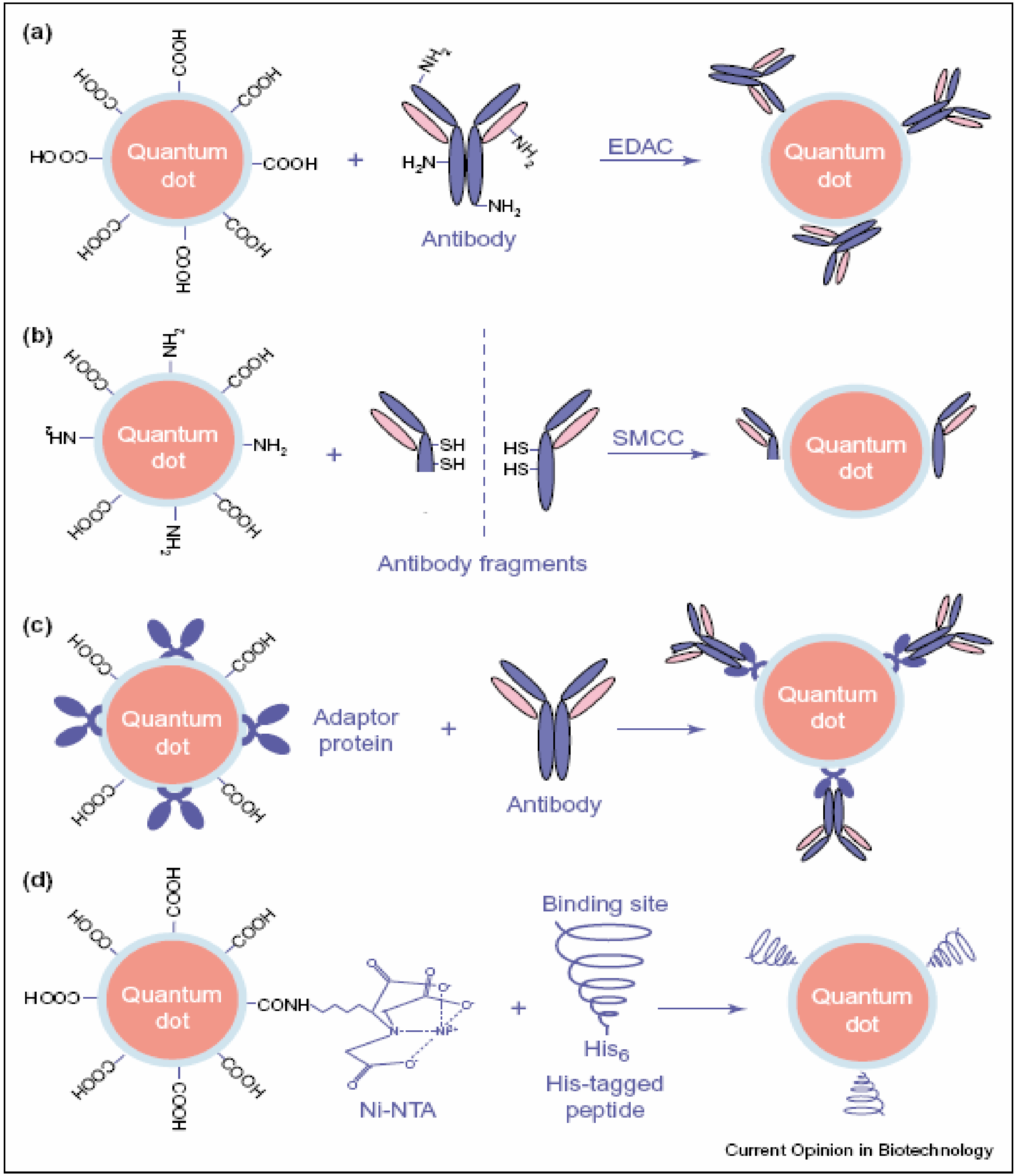
New Chemistry—AMP Strategy



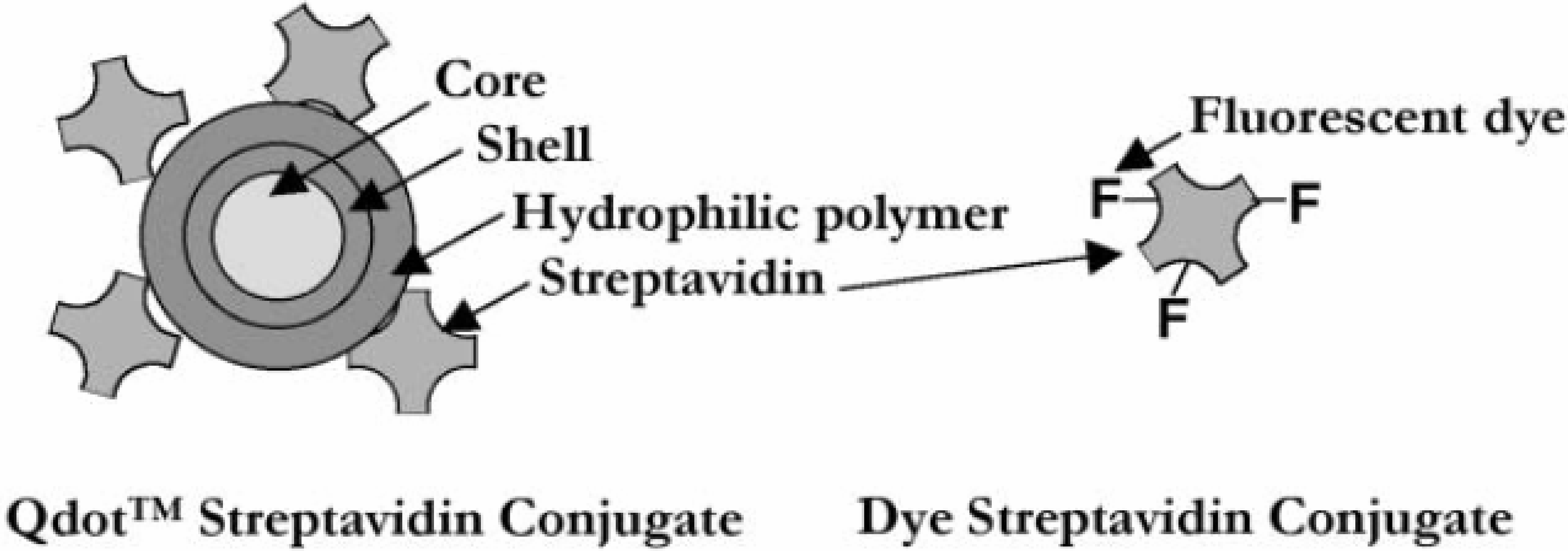
Multifunctional QD probe

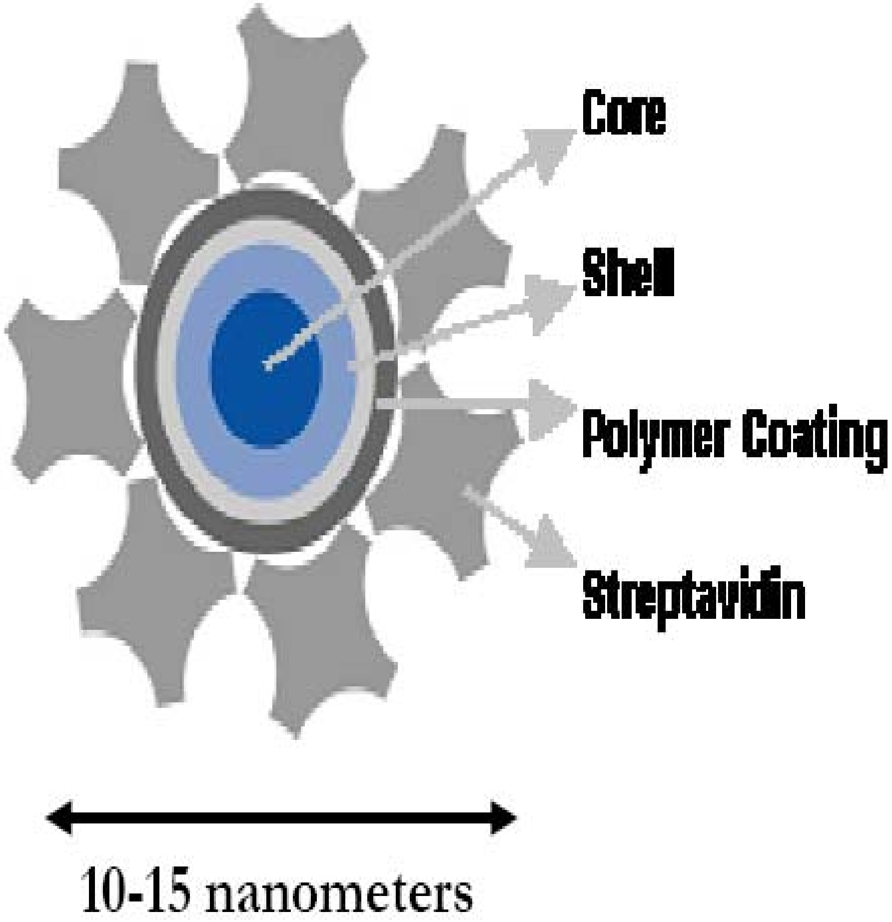


Current Opinion in Biotechnology 2005, 16:63–72

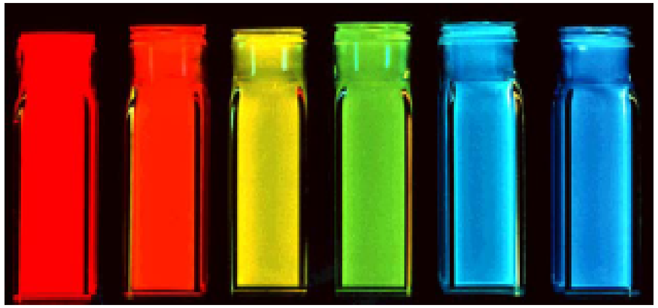


Different methods of protein conjugation with QDs

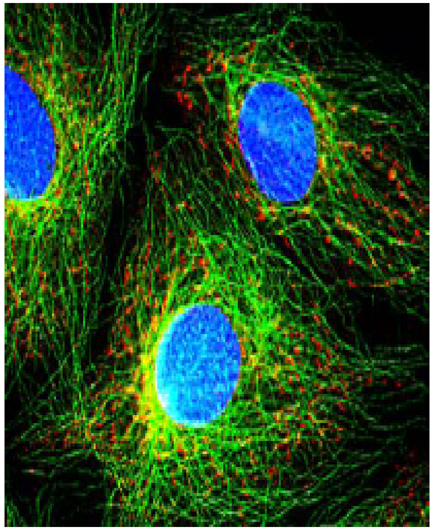

Core
Shell
Hydrophilic polymer
Streptavidin
Fluorescent dye
F
F
F
Qdot™ Streptavidin Conjugate
Dye Streptavidin Conjugate



- The core determines the nanocrystal color
- The shell dramatically enhances the brightness
- The polymer coating makes the nanocrystals stable and soluble in water and buffers
- Streptavidin, or other biomolecules are covalently attached to the polymer

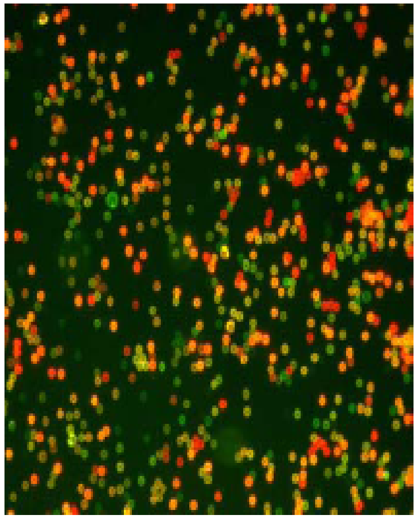


Quantum dots



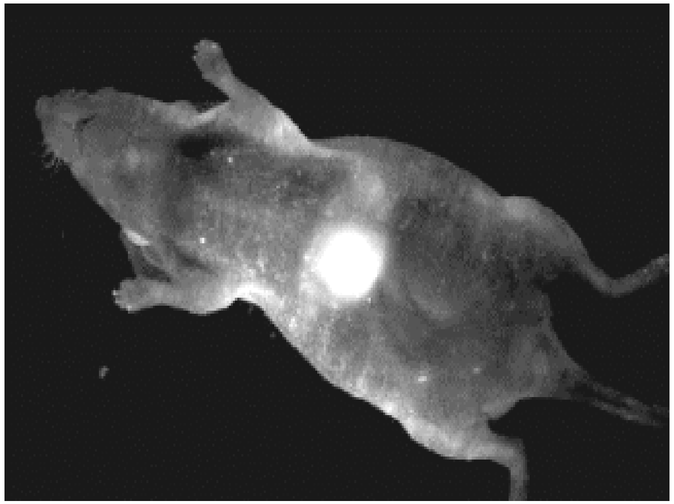
Bio-labeling:

Detection reagents for microscopy, DNA chips, flow cytometry, immunoassays, …



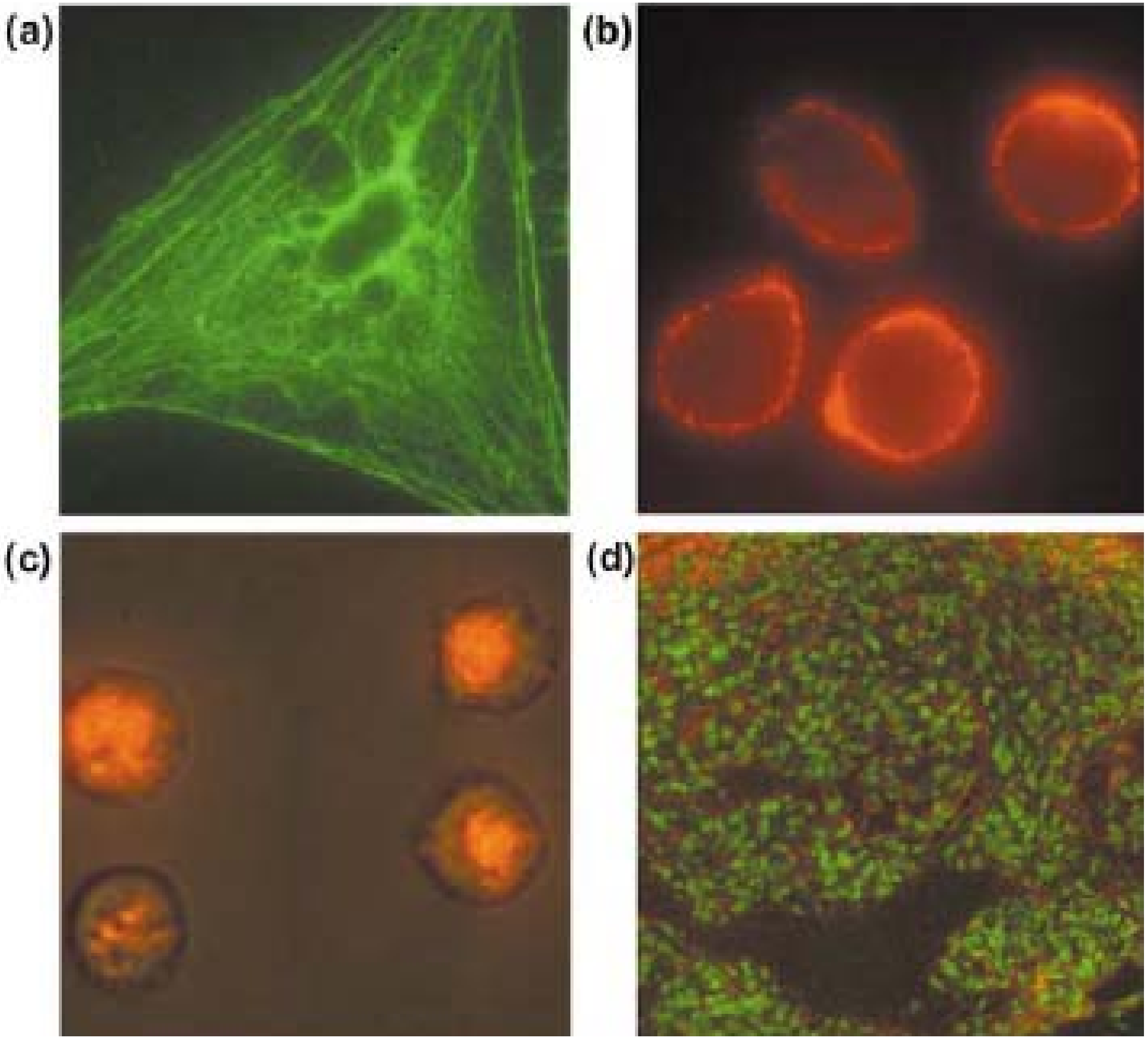
QBead™ encoded beads:

Platform for Multiplexed assays



Future applications

Live cell imaging
in vivo imaging,…



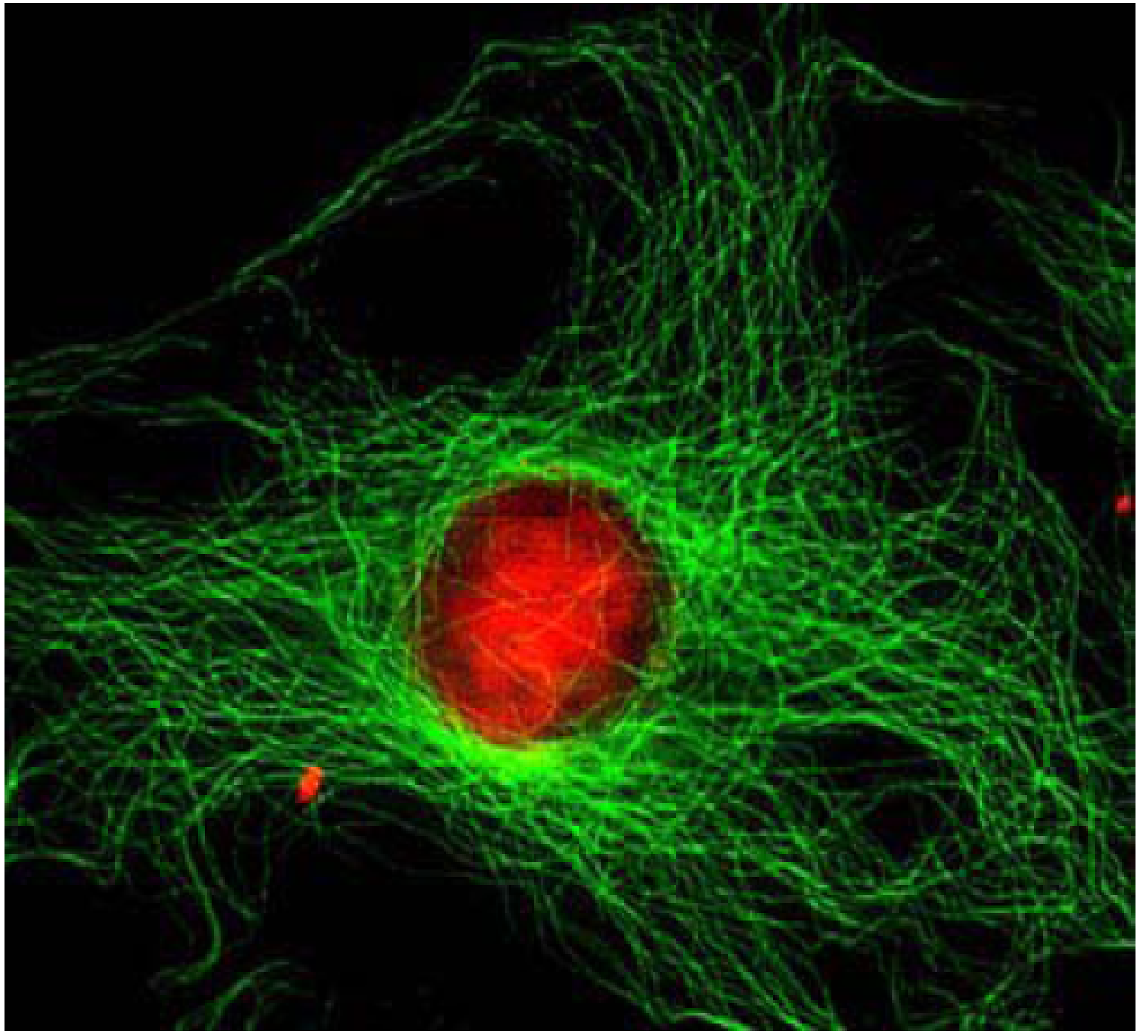
(a) Actin staining (green QDs) on fixed 3T3 fibroblast cells

(b) Live breast tumor cells with red QD antibody conjugates

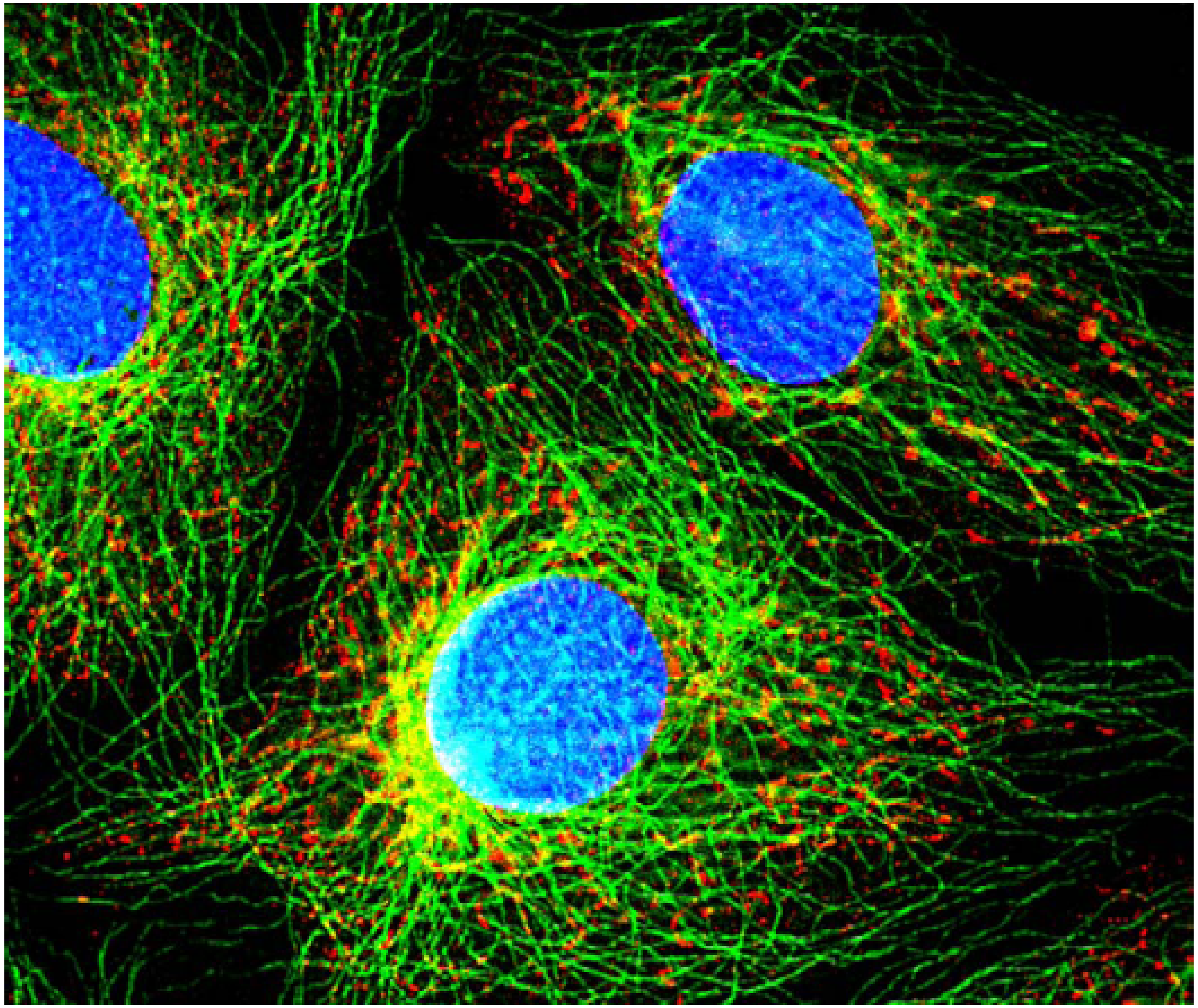
(c) Live mammalian cells with QD-Tat peptide conjugates

(d) Tissue specimens with red QDs and nuclear green dye

Current Opinion in Biotechnology 2005, 16:63–72

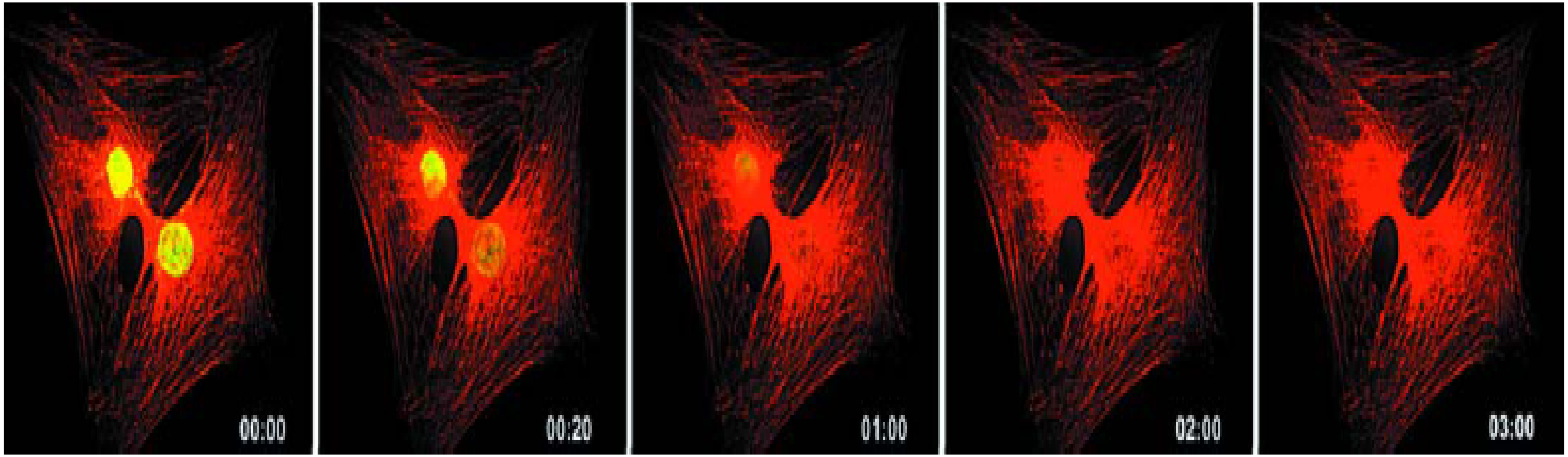


- Nuclear antigens (red) labeled with Qdot™ 630-streptavidin conjugates
- Microtubules (green) labeled with Alexa Fluor® 488 anti-mouse IgG
- Images captured every 10 seconds, over 180s for both colors



NIH 3T3 mouse fibroblast cells

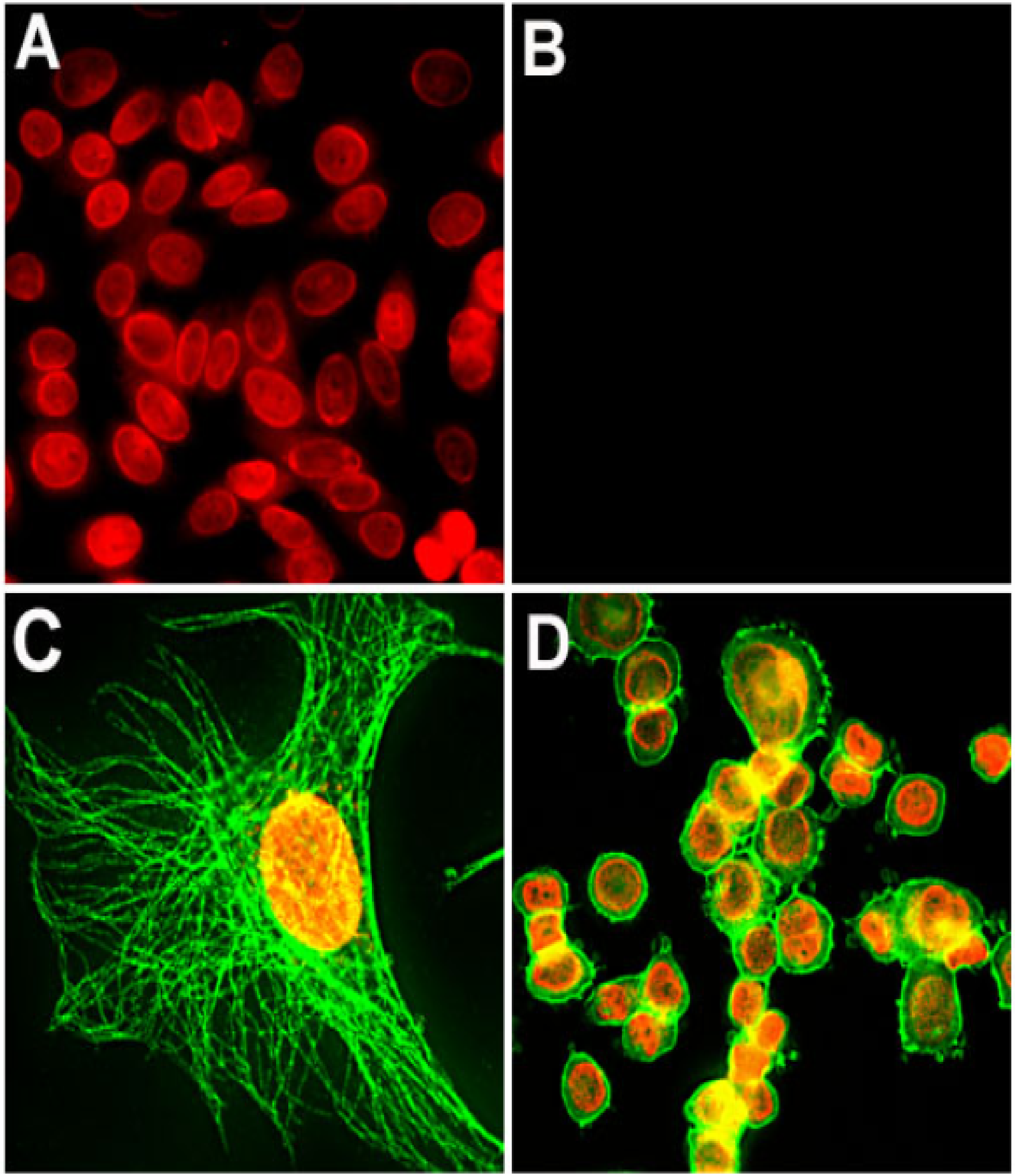
- Microtubules stained with Qdot™ 525-streptavidin conjugate
- Mitochondria stained with Qdot™ 605-streptavidin conjugate
- Nuclei were counterstained with Hoechst blue dye



QD streptavidin conjugate (red) labeled 3T3 fibroblast cells

Nuclei stained with Alexa Fluor dye

As a function of time, 0, 20, 60, 120, 180 seconds, dye emission completely faded with no change in QD emission



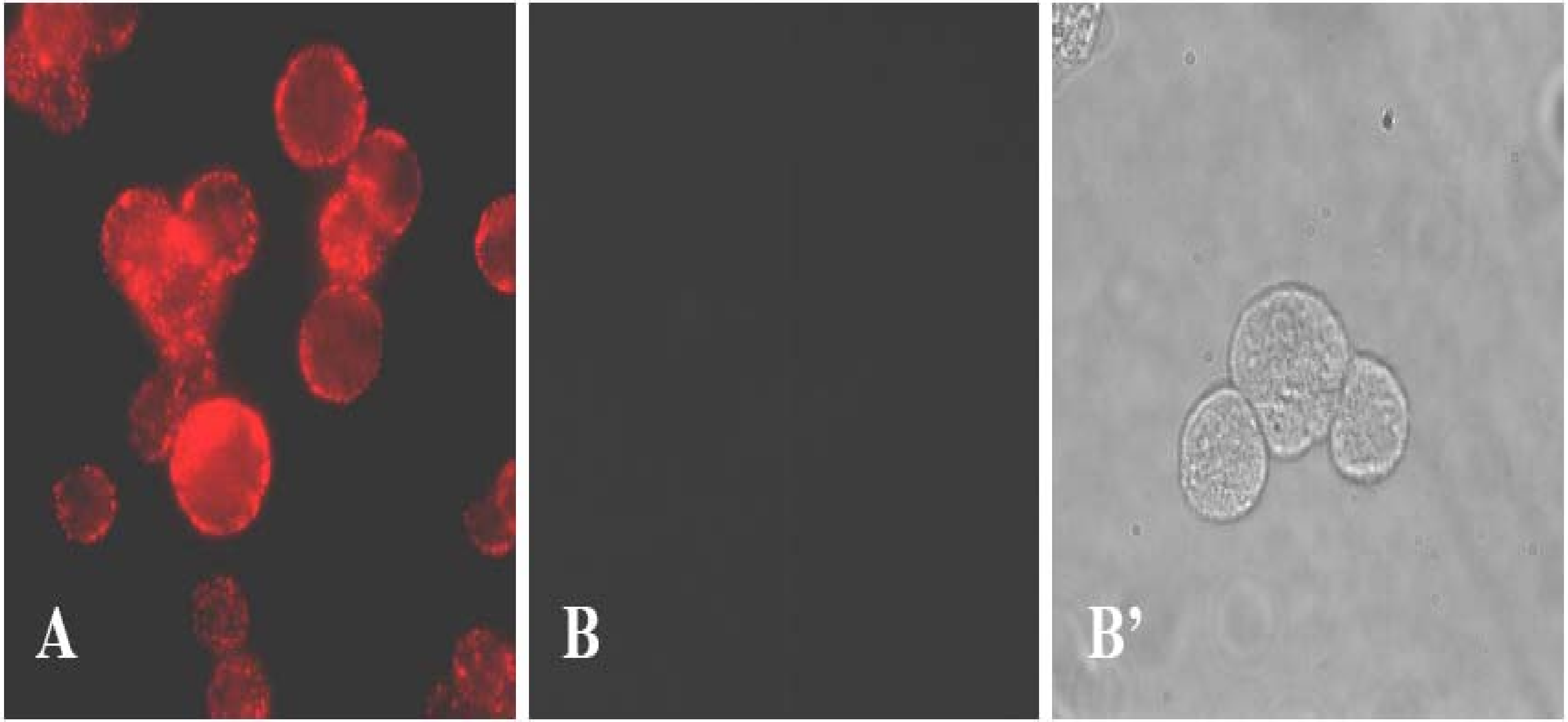
A.Nuclear antigens in the nuclei of human epithelial cells were stained with ANA, anti-human IgG-biotin and 630 nm QD-streptavidin.

B. is normal Human IgG control

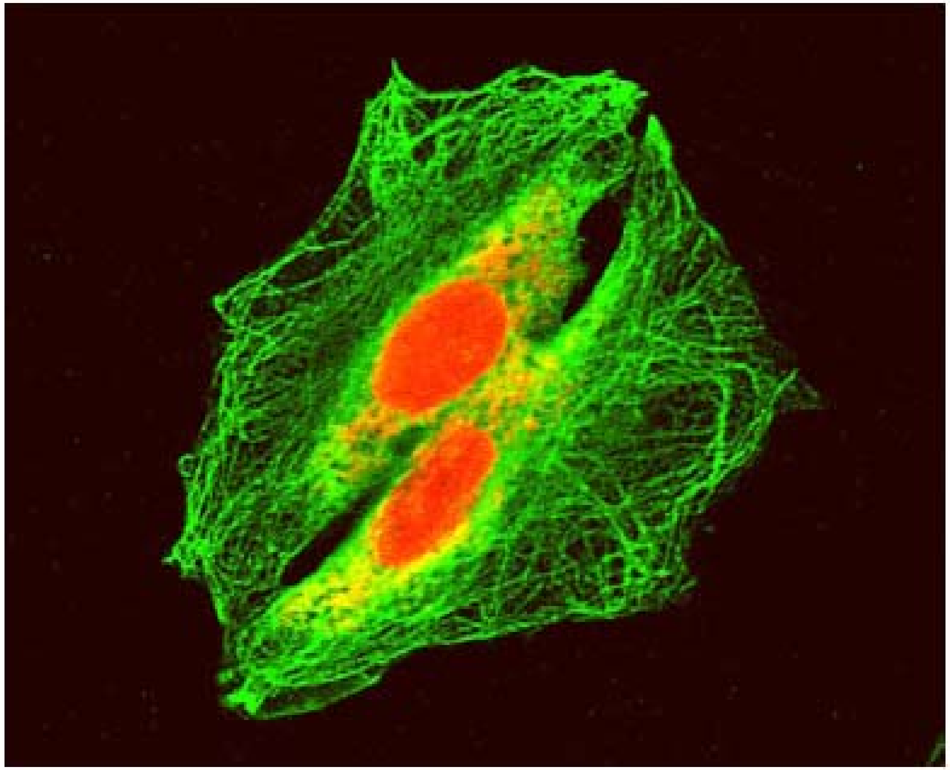
C.The nucleus of a 3T3 cell was stained with ANA, anti-human IgG-biotin and 630 nm QD-streptavidin (red). The microtubules were labeled with mouse anti-tubulin antibody, anti-mouse IgG-biotin and 535 nm QD-streptavidin (green).

D. Her2 on the surface of SK-BR3 cells were stained green with mouse anti-Her2 antibody and 535 nm QD linked to anti-mouse IgG. Nuclear antigens were labeled with ANA, anti-human IgG-biotin and 630 nm QD-streptavidin (red).

X.Wu et al. Nature Biotechnology



- Live SK-BR-3 cells sequentially incubated with humanized Anti-Her2 antibodies (1°), biotinylated goat anti-human IgG and Qdot™ 605-streptavidin
- (B) control without the primary antibody
- (B′) bright field image of (B)

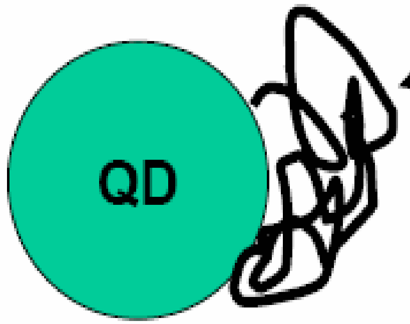


- Microtubules labeled with Qdot™ 535 conjugates
- Nucleus labeled with ethidium bromide

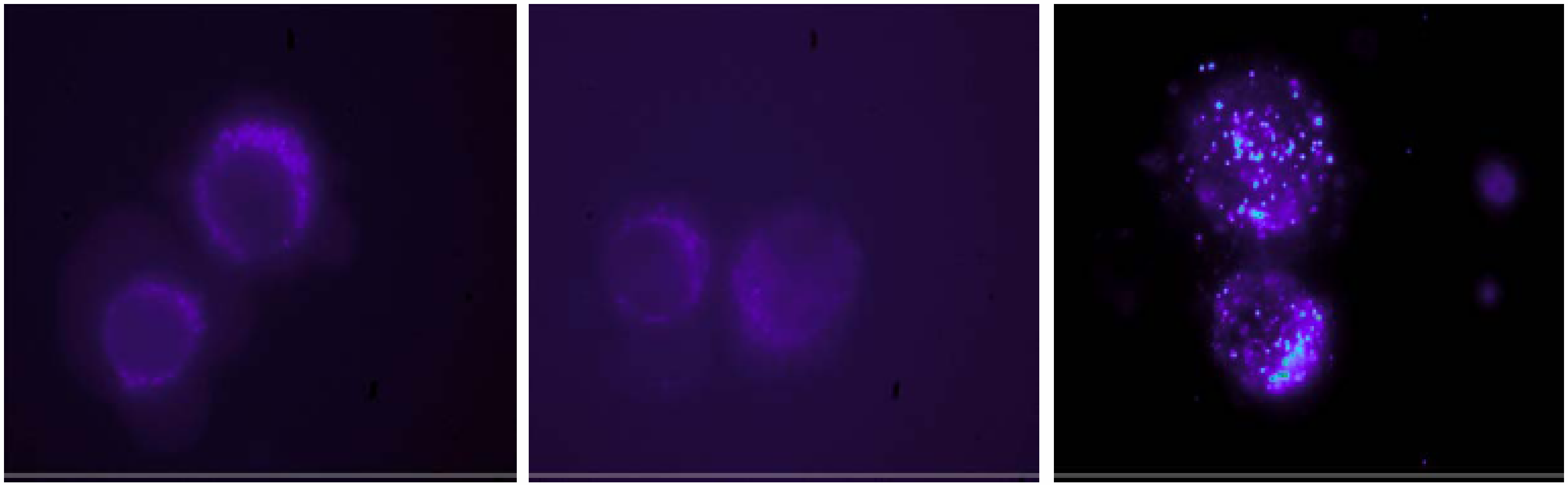
[Note: Photostability of Qdot™ conjugates so that material out of focal plane still accessible to subsequent interrogation]

Utilization for Cell Analysis

Emitting light



Protein directs Quantum Dots into cells.



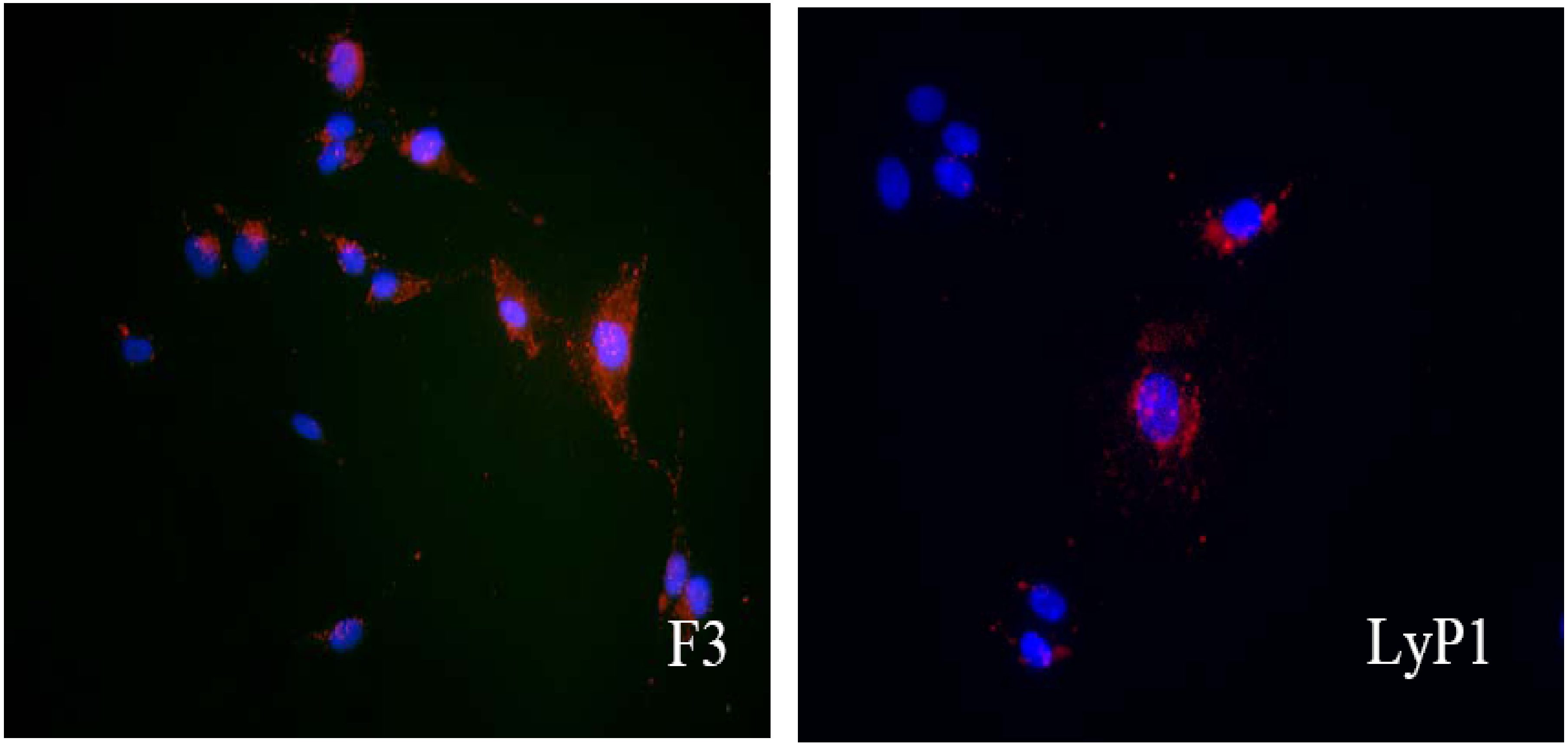
HeLa Cells

HeLa Cells
MAA Quantum Dots

HeLa Cells
Transferrin- Quantum Dots

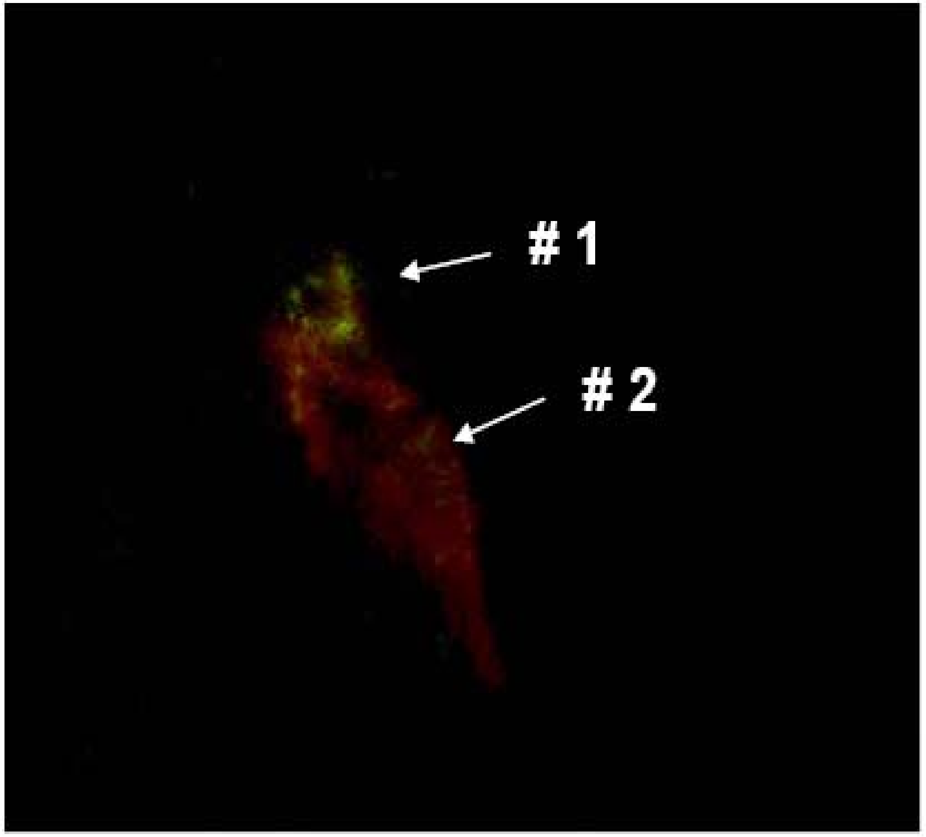
Chan & Nie (1998), Science, 281, 2016

More pictures of Quantum Dot-Cells



Akerman et al. (2002), PNAS, 99, 12617.

Diagnosing Cancer



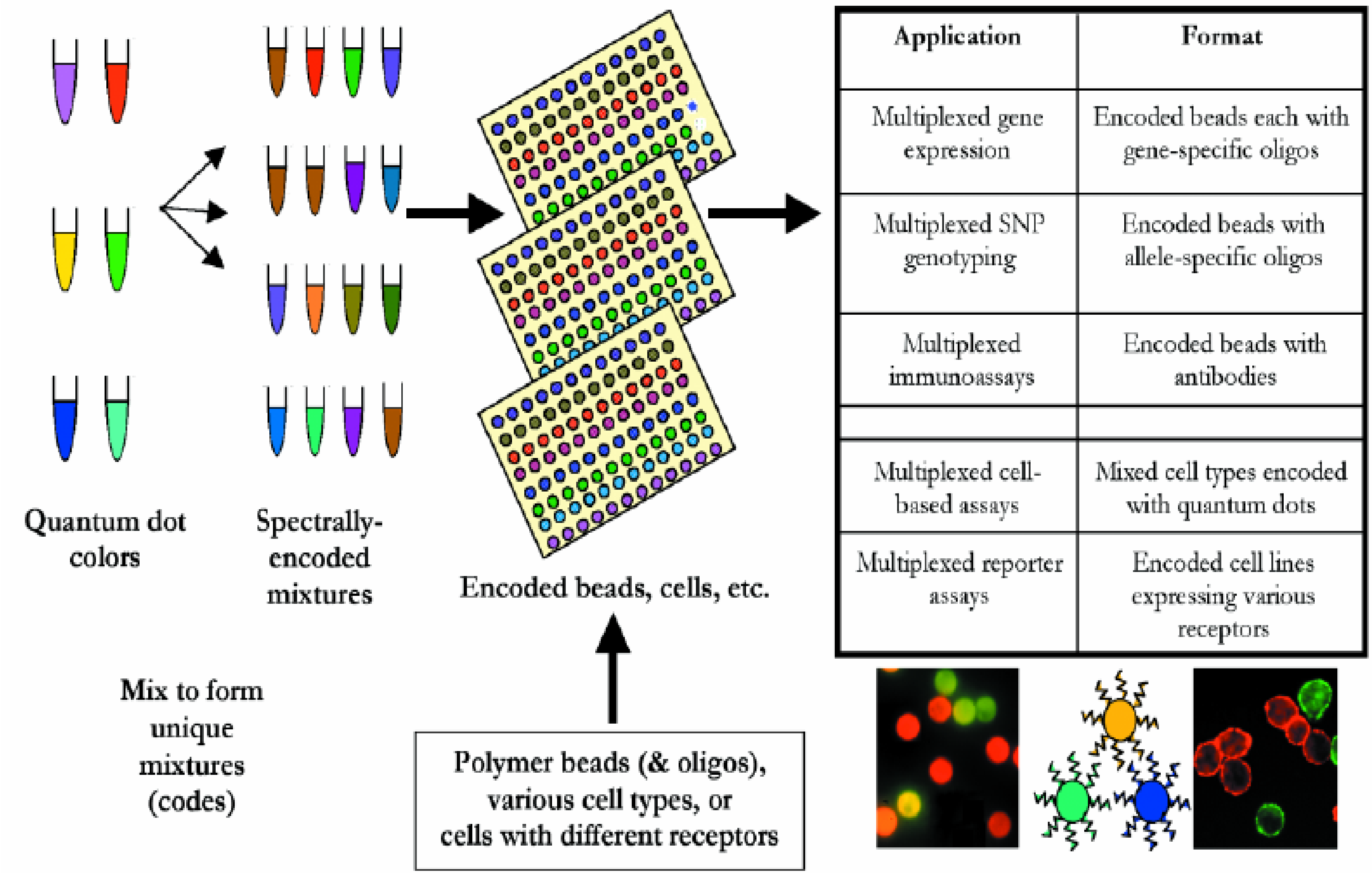
1: Quantum Dots with Homing Peptide 1
2: Quantum Dots with Homing Peptide 2

Akerman et al. (2002), PNAS, 99, 12617.

Glowing Mice!

Molecular targeting and in vivo imaging of prostate tumor using QD-antibody conjugates

Nie & Gao, Georgia Tech/Emory University



Application	Format
Multiplexed gene expression	Encoded beads each with gene-specific oligos
Multiplexed SNP genotyping	Encoded beads with allele-specific oligos
Multiplexed immunoassays	Encoded beads with antibodies
Multiplexed cell-based assays	Mixed cell types encoded with quantum dots
Multiplexed reporter assays	Encoded cell lines expressing various receptors

Fig. 6. Concept of encoding using quantum dots. Quantum dot colors can be mixed to produce spectral codes. These mixtures can be combined with polymer beads to produce encoded beads that can be subsequently coupled to distinct oligonucleotides or other affinity molecules. Alternatively, the quantum dot spectral codes can be used to label cells to differentiate cell lines, or cell lines bearing different receptors. SNP, single nucleotide polymorphism.