Quantum Dots in Frogs

David J. Norris

Chemical Engineering & Materials Science
University of Minnesota

Fluorescent imaging with semiconductor nanocrystals
Quantum Dots via Gas-Phase Deposition:

- Semiconductor nano-particles
- Grown on a substrate
- e.g. InAs on GaAs
- Stranskii-Krastanow growth
- Pyramidal shape
- Extensive research...
- e.g. electrically pumped lasers

Not the focus of this talk
Colloidal Semiconductor Quantum Dots (Nanocrystals):

- Crystallites of semiconductor
- Chemically synthesized
- ~1000 atoms (3nm diameter)
- Coated with surfactants:
  - trioctylphosphine (TOP)
  - trioctylphosphine oxide (TOPO)

In addition to CdSe . . .
Many materials are now available

Size Control:

- Burst of nucleation controls size distribution
- Growth time controls mean size

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LaMer & Dinegar; *JACS* **72**, 4847 (1950).
Strong fluorescence

Quantum Yield ~10% at 300K


Quantum Yield ~80% at 300K
Size-dependent optical properties:

*CdSe Nanocrystals under uv light*

*photo by Felice Frankel; samples by Bawendi Group (MIT)*
Size-dependent optical properties of CdSe:

Size-dependent optical properties:


Why Study Nanocrystals?

Original Motivation:

- Fundamental science:
  - *What happens when a semiconductor becomes small?*
- Also of practical importance:
  - *Semiconductor devices are becoming small*
- Use optical properties in optoelectronic devices
  - *Lasers*
  - *Photovoltaics (solar cells)*
  - *Light-emitting diodes (LEDs)*

Progress:

- Tremendous progress over 20 years of research:
- Not only synthesis, but fundamental understanding . . .
Optical transitions understood in detail:

Semiconductor Nanocrystals:

Current status:

• Physics for many properties is understood
• Can be manipulated to make novel materials
  • artificial solids, etc..

• Applications?

• Traditional focus has been on opto-electronics
  • lasers
  • photovoltaics (solar cells)
  • light-emitting diodes (LEDs)

Biology?

• Can quantum dots be useful in biology?
Quantum dots finally come of age

Two reports demonstrate the specific labeling of cellular constituents with fluorescent quantum dot probes conjugated covalently or electrostatically to antibodies and streptavidin.

Thomas M. Jovin

Nature Biotechnology, January 2003

The phrase “less is more,” coined by Mies van der Rohe to describe mid-20th-century architecture, epitomizes the task facing researchers engaged in developing new types of probes for imaging applications. The challenge is to identify or devise the smallest probes possible that exhibit the highest selectivity, sensitivity, spectral versatility, stability, and capacity to penetrate cells and organelles. In recent years, quantum dots have come to the fore (for excellent and timely reviews, see refs. 1 and 2) as promising alternatives to chemical fluorophores3 and visible fluorescent proteins4. Now, four papers published in this issue5,6 and elsewhere7,8 demonstrate the successful application of bioconjugated quantum dots for labeling cells and macromolecular constituents of cells.

Despite the interest in quantum dots for use in imaging applications, it has been difficult to meet the requirements for reproducible fabrication and to overcome fundamental difficulties posed by the biological milieu. These include first, prevention of quenching of quantum dot emission in an aqueous environment; second, elaboration of strategies for the conjugation or adsorption of molecules of interest; third, suppression of nonspecific binding and aggregation; and fourth, provision for biological inertness (that is, absence of cellular toxicity). Of all these problems, the first has been particularly vexing; to use the vernacular associated with the whimsical banking institutions of contemporary Argentina, incompletely coated quantum dots tend to function as photon “corralitos” (photons in, no photons out).

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Dubertret, Skourides, Norris, Noireaux, Brivanlou & Libchaber; Science 298, 1759 (2002).
Fluorescent imaging in biology

Microscopy:
- Many biological experiments utilize fluorescent tags
- Organic dyes (fluoroscein, rhodamine, etc.)
- Fluorescent proteins (green fluorescent protein, GFP)

- Problem: even the best fluorescent tags have poor photostability
- Fluorescence fades quickly over time
- Severely limits experiments
Replace organic dyes with nanocrystals?


**Advantages of Nanocrystals:**

- Semiconductor nanocrystals exhibit high photostability
  - “Rock” that can be pounded with light
  - *Fluorescence can last days*
- Change size; get different colors in fluorescence
- Excite different size nanocrystals with a single laser

"Bio-object" **Nanocrystal**
Replace organic dyes with nanocrystals?

Problem

As synthesized nanocrystals are hydrophobic


Need to make the nanocrystals hydrophilic!

Simultaneously maintain fluorescence and colloidal stability!

Initial strategy: exchange surfactants on surface
Different Strategies

- **CdSe Core**
- **ZnS Shell**

**siloxane shell**

**monolayer of carboxylic acids**

**carboxylic acid + fusion protein**

**amine-modified polyacrylic acid**

**oligomeric phosphines**
Issue that we wanted to address:

NON-SPECIFIC ADSORPTION

Thus, lots of in vitro experiments
No experiments in vivo!
Approach: Use Micelles

amphiphilic molecule
(surfactant)

hydrophilic head group
hydrophobic tail

hydrophilic shell
hydrophobic core

micelle

H₂O
Our Route:

**Encapsulate hydrophobic quantum dot:**
- Keep initial hydrophobic ligand (TOP/TOPO)
- Thus, maintaining fluorescence
- Place inside a “bubble” to protect the dot
- And obtain water stability

**Use Micelles:**
- Form spontaneously
- Surfactant molecules used in nature for membranes
- Should be bio-compatible
The surfactant we chose:

poly(ethylene glycol) (PEG)

double hydrophobic chain

Block copolymer phospholipid:
- PEG block added to natural phospholipid
- PEG known as an extremely bio-compatible surface
- PEG will be on the outside of our micelle
- Can control the length of the PEG block

\[ N\text{-poly(ethylene glycol) phosphatidylethanolamine} \] [PEG-PE]
New Route:

Evaporate Solvent

Add water

Micelles self-assemble: simple 10 minute “synthesis”
Why did we choose this surfactant?

Previous studies on empty micelles

Johnsson M. et al.  

![Chemical structure of [PEG-PE]](image)

<table>
<thead>
<tr>
<th>PEG-X</th>
<th>$R_t$ (Å)</th>
<th>$R_c$ (Å)</th>
<th>L (Å)</th>
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<tbody>
<tr>
<td>750</td>
<td>51</td>
<td>34</td>
<td>17</td>
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<tr>
<td>2000</td>
<td>67</td>
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<td>35</td>
</tr>
<tr>
<td>5000</td>
<td>107</td>
<td>32</td>
<td>75</td>
</tr>
</tbody>
</table>
TEM of encapsulated quantum dots

Negative Staining
Basic Properties:

• Size = ~13nm (TEM)

• Colloidal Stability:
  • can boil in water
  • stable over a broad range of quantum dot concentrations
  • stable over a broad range of salt concentrations (up to 2M)
  • stable over a broad range of pH
Stability: pH

![Graph showing absolute fluorescence intensity over days for pH = 4, pH = 7, and pH = 10.](image)
Basic Properties:

• **Size** = ~13nm (TEM)

• **Colloidal Stability:**
  
  • can boil in water
  
  • stable over a broad range of quantum dot concentrations
  
  • stable over a broad range of salt concentrations (up to 2M)
  
  • stable over a broad range of pH

• **General Method:**
  
  • CdSe, PbSe, FePt . . .

  • quantum dots and quantum rods
Are QD-micelles bio-compatible?

**In vitro I: DNA Attachment**

biotin-modified ssDNA attached to streptavidin-modified 4% agarose beads
Are QD-micelles bio-compatible?

*In vitro II: directed self-assembly*
In Vivo Imaging?

Explore:

- Toxicity?
- Stability in a living organism?
- Non-specific adsorption?
- Aggregation?
Why Frogs?

Xenopus:
- Embryos are large
- Very sensitive to perturbations (good for toxicity tests)
- Quick: 5 days from fertilization to feeding tadpole
- Extremely well studied system
- Large number of embryos can be obtained
Injecting QD-micelles into frogs

In vivo imaging with quantum dots

Dubertret, Skourides, Norris, Noireaux, Brivanlou, & Libchaber; Science **298**, 1759 (2002).
Photostability

Dubertret, Skourides, Norris, Noireaux, Brivanlou, & Libchaber; Science 298, 1759 (2002).
Toxicity?

<table>
<thead>
<tr>
<th>Stage 19-20</th>
<th>n=55</th>
<th>n=39</th>
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<tbody>
<tr>
<td></td>
<td>1.5nl in 1 out of 8</td>
<td>Control</td>
</tr>
<tr>
<td>Normal</td>
<td>38 69%</td>
<td>27 70%</td>
</tr>
<tr>
<td>Defects</td>
<td>15 27%</td>
<td>12 30%</td>
</tr>
<tr>
<td>Dead</td>
<td>2  4%</td>
<td>0  0%</td>
</tr>
</tbody>
</table>

Dubertret, Skourides, Norris, Noireaux, Brivanlou, & Libchaber; Science 298, 1759 (2002).
Different colors . . .
Why bright green spots?
Also learned something new . . .

Quantum Dots become localized in nucleus:
- Occurs at specific stage of development
  *Mid-blastula transition*
- Embryo starts to make its own genetic material
- Pores in nuclear membrane open; dots go into nucleus

Surprise:
- Green dots go in, red dots get stuck!
- First evidence for size of the nuclear pores
What leads to stability?

- **Micelle**: ~ 1 week
- **Micelle + Quantum Dot**: indefinitely
Conclusions

Quantum Dot Micelles:
- New method to make nanocrystals bio-compatible
- Good stability *in vitro* and *in vivo*
- Low toxicity *in vivo*
- Low non-specific adsorption
- Easy coupling (bio-conjugation)
- *In vivo* imaging
Future Challenges:

Loss of Stability:

• bovine serum at 37°C: aggregation after 1 week

• pH 12: aggregation after days
Future Challenge:

• Whole Animal Imaging:
  • Injected mice with quantum dots
  • Image blood vessels through foot on confocal microscope
  • After a few days the quantum dot fluorescence could not be detected anymore
  • Organs harvested: quantum dots in kidney, not in lung or liver.
Detection of single quantum-dot-micelles:
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