PREPARATION, PHYSICO-CHEMICAL PROPERTIES AND APLLICATION OF QUANTUM DOTS IN BIOANALYSES

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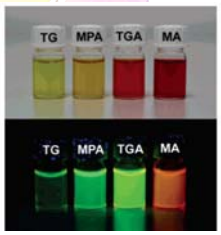
Semiconductor nanocrystals – quantum dots

- semiconductor nano-scale inorganic crystals (1-10 nm)
- core from elements of II. and VI. or III. and V. group
- sufrace modified by polar organic molecules to increase hydrophilicity

Quantum dots - modification

- different materials (ZnS; ZnSe; PbS; CdS; CdSe; CdTe)
- surface coated (CdSe/ZnS, polymery)
- silanization
- ligands (TOP/TOPO, oleic acid, dithiotreitol, thioglycol, 3-mercaptopropionic acid, 2-mercaptoethylamine)



J. AM. CHEM. SOC. 2006, 128, 10171-10180 9 **10171**
Hao Zhang, Dayang Wang, Bai Yang

Quantum dots - preparation

1st step: preparation of hydogen telluride

$4\ NaBH_4 + 2\ Te + 7\ H_20 \rightarrow 2\ NaHTe + Na_2B_4O_7 + 14\ H_2$

a) 6 hours 0 °C
b) 30 minute 80 °C
c) over night 2 - 8 °C

2nd step: quantum dots formation

$CdCl_2$ + NaHTe + MPA or MA + heating

MPA 3-mercaptopropionic acid: $HS\text{-}CH_2\text{-}CH_2\text{-}COOH$
MA 2-mercaptoethylamin: $HS\text{-}CH_2\text{-}CH_2\text{-}NH_2$

Quantum dots - coating

CdTe/CdS

CdTe + $CdCl_2$ + Na_2S + MPA or MA + heating

CdTe/CdS/ZnS

CdTe/CdS + $ZnCl_2$ + Na_2S + MPA or MA + heating

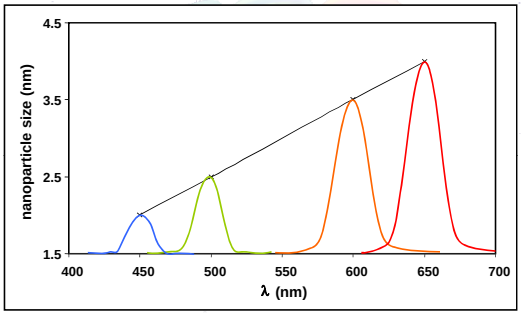
Quantum dots - preparation

Dependence of QDs size on refluction time

- different refluction times in second reaction step
- increase of emmision wavelength and particle diameter with refluction time

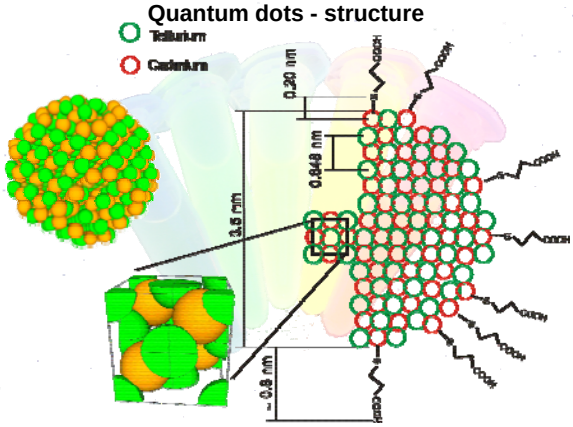
Refluxing time	10 min	1 h	2.5 h	13.5 h	16.5 h	27 h	41 h	44 h
Emission maximum (nm)	498	517	536	652	667	701	737	750
Particle size (nm)	1.99	2.67	2.86	4.02	4.17	4.80	4.87	5

Quantum dots - preparation

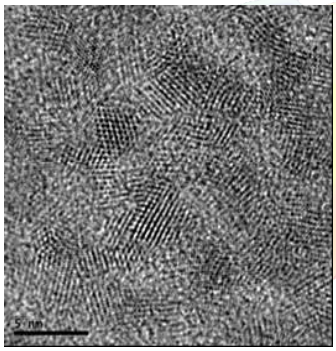


Eychmuller, A.; Rogach, A. L. Chemistry and photophysics of thiol-stabilized II-VI semiconductor nanocrystals*Pure and Applied Chemistry* **2000**, *72*, 179-188.

Quantum dots - structure



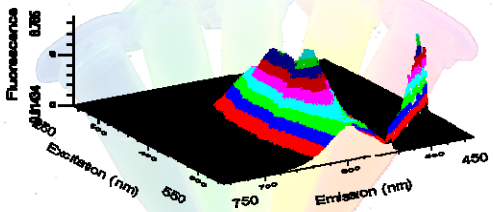
Quantum dots - structure



HRTEM image

Symmetry space group F - 43m
a = 6.48 Å

Quantum dots – fluorescence spectrum

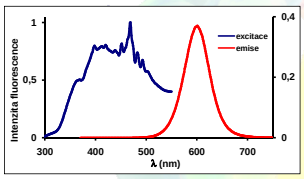


- wide excitation spectra with maximum at 469 nm

- narrow emission spectra with maximum at 600 nm
- bandwidth 58 nm at half height

Quantum dots – optical properties

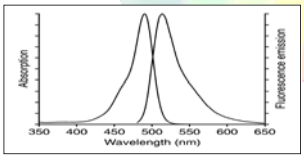
Excitation and emmision spectra of fluorescence



Quantum dots 600 nm

- excitation maximum 469 nm

-emmision maximum 600 nm

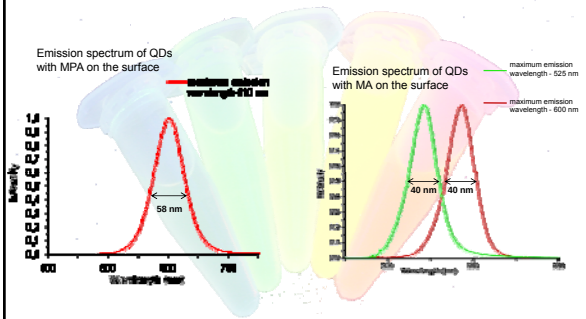


Fluorescein

- excitation maximum 494 nm

- emmision maximum 521 nm

Quantum dots – optical properties



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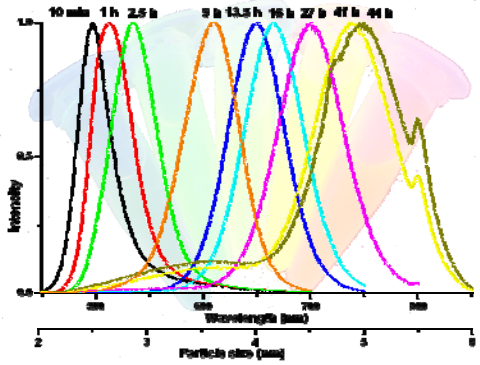
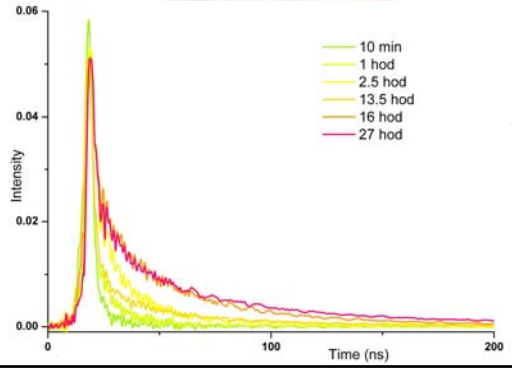
Quantum dots – optical properties



Quantum dots – optical properties

Fluorescence lifetimes



Quantum dots – optical properties

High chemical- and photostability

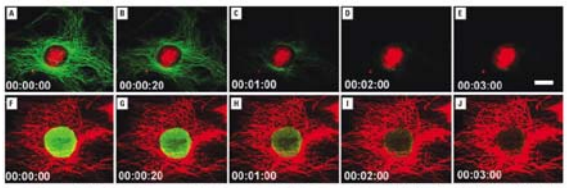


Figure 1. Fluorescent photostability and fluorescence intensity of quantum dots (QD 630) compared with organic dye Alexa 488. (A–E) Nuclei are labeled bright red with QD 630–streptavidin; actin fibers are stained green with Alexa 488. (F–I) Images of actin fibers are labeled red with QD 630–streptavidin; nuclei are labeled green with Alexa 488. Numbers in the bottom left corner indicate elapsed time. Scale bar, 10 μm. From Wu et al. (2003) and reproduced with permission from Quantum Dot Corp. (Hayward, CA).

Quantum dots – quantum yield

Freshly prepared QDs

Standard: fluorescein **91**

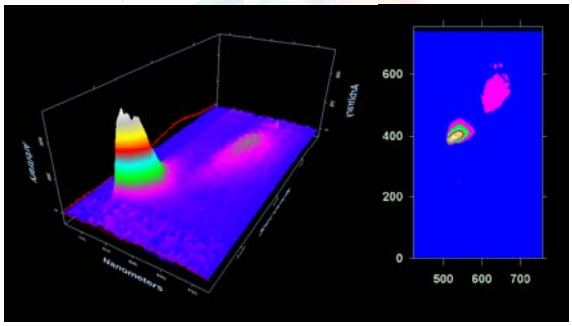
Quantum dots – quantum yield

QDs aging

Standard: fluorescein **91**

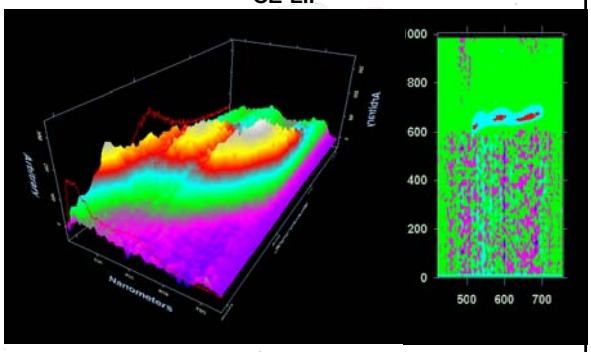
Quantum dots – separation

CE-LIF



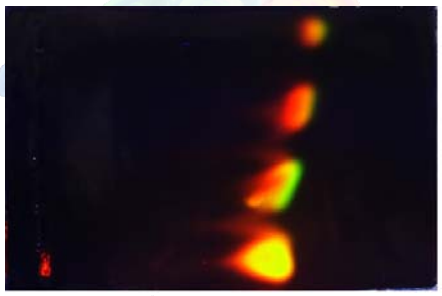
PVA coated capillary 20/30 cm, i.d. 75μm, gel 3% LPA 10MDa in 50mM TRIS/TAPS buffer, pH = 9
QD 2,8 + 3,7 nm (525 + 610 nm 1:1), injection 10s, separation volatge 3 kV

Quantum dots – separation
CE-LIF

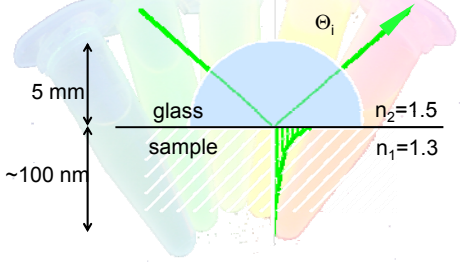


Coated capillary Dolnik 15/25 cm, i.d. 50μm, gel 3% LPA 10MDa in 50mM TRIS/TAPS buffer, pH = 9
QD 2,8 + 3,5 + 4,3 nm (525 + 595 + 675 nm 5:3:1), injection 10s, separation voltage 3 kV

Quantum dots – separation
Gel electrophoresis

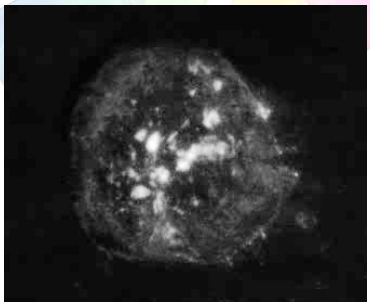


Quantum dots – non-selective labeling
TIRF (*Total Internal Reflection Fluorescence*)



TIRF instrumental, Nd:YAG laser (2x) 532 nm, inverted microscope Intraco Micro objective 100x/1,25GLYC

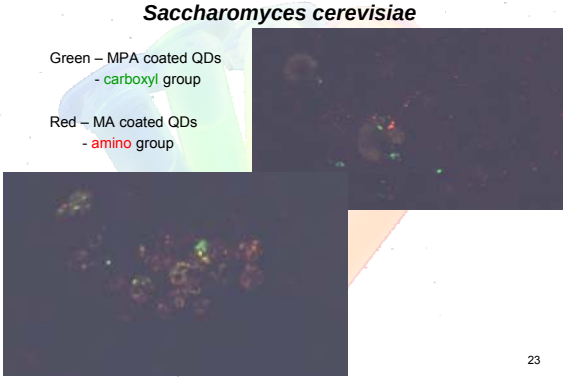
Quantum dots – non-selective labeling
TIRF image of human lymphocyte



Quantum dots – non-selective labeling
Saccharomyces cerevisiae

Green – MPA coated QDs
- carboxyl group

Red – MA coated QDs
- amino group



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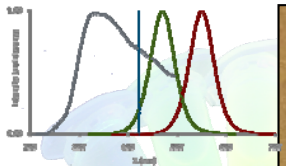
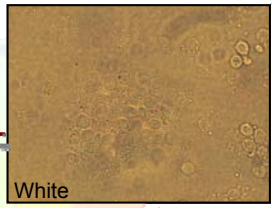
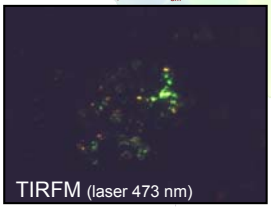
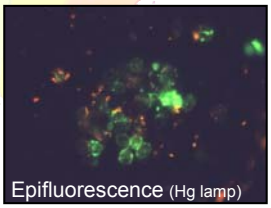


excitační spektrum, excitační laser 473 nm
emisní spektrum, λ_{em} = 522 nm
emisní spektrum, λ_{em} = 610 nm







Quantum dots – non-selective labeling

Human lymphocytes

Saccharomyces cerevisiae

Quantum dots - conjugation

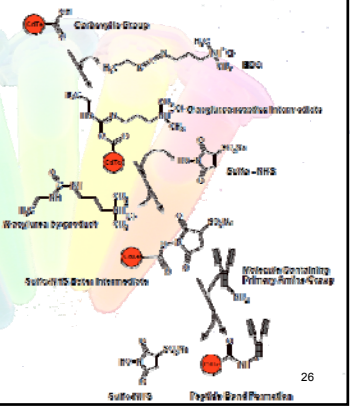
1-ethyl-3-(3-dimethyl -3-aminopropylcarbodiimide hydrochloride **(EDC)** and *N*-hydroxysulfosuccinimide **(Sulfo-NHS)**:

carboxylic group of MPA on the QDs surface

catalyzers: EDC and Sulfo-NHS

amino group e.g. from antibody

formation of peptide bond



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Quantum dots - conjugation

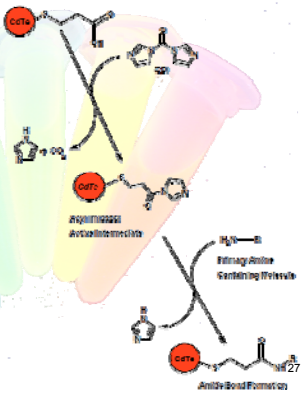
N, N´-carbonyldiimidazole **(CDI)**:

carboxylic group of MPA on the QDs surface

catalyzers: CDI

amino group from ovalbumin

formation of peptide bond



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Quantum dots - conjugation

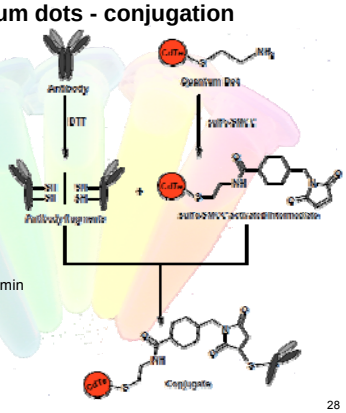
Sulfosuccinimidyl -4-(N-maleinimidomethyl) -cyklohexane-1-carboxylate **(Sulfo-SMCC)**:

amino group of MA on the QDs surface

catalyzers: Sulfo-SMCC

thiol group from anti-ovalbumin

formation of thioether bond



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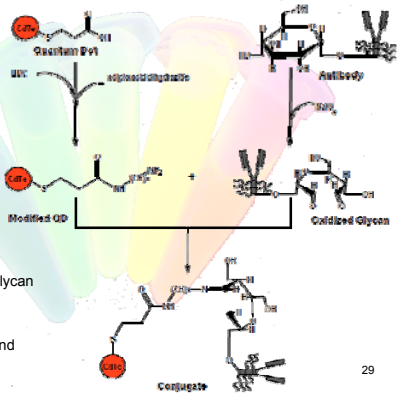
Quantum dots - conjugation

Oxidation of antibody glycan:

carboxylic group of MPA on the QDs surface modified by adipic acid dihydrazide

oxidation of antibody glycan by $NaIO_4$

formation of etheric bond



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Quantum dots - conjugation

Avidin-biotin interaction:

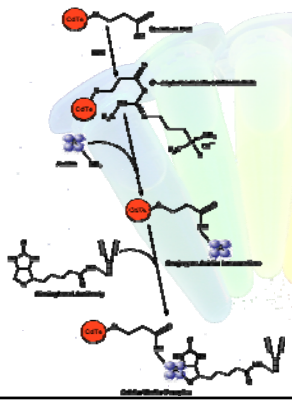
carboxylic group of MPA on the QDs surface

catalyzers: EDC (1-ethyl-3-(3-dimethylaminopropylbodiimide hydrochloride)

amino group from avidin

formation of peptide bond

afinity interaction avidin-biotinylated antibody



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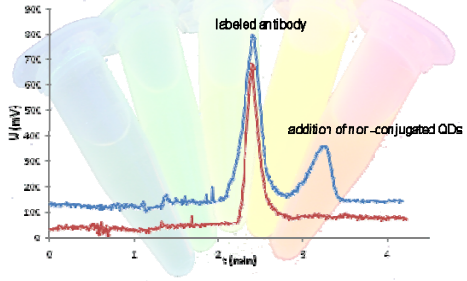
Quantum dots – conjugates separation

Checking of EDC/NHS conjugating process



Characterization of 3.5 nm CdTe QDs and their conjugates by CZE-LIF,
uncoated capillary 12/20 cm, voltage 6 kV, injection 1 s, 100 mM TRIS/TAPS buffer pH = 8.3

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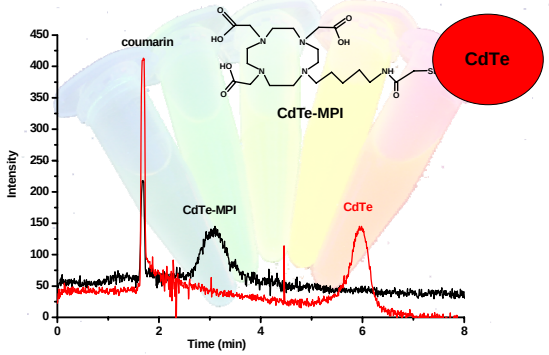
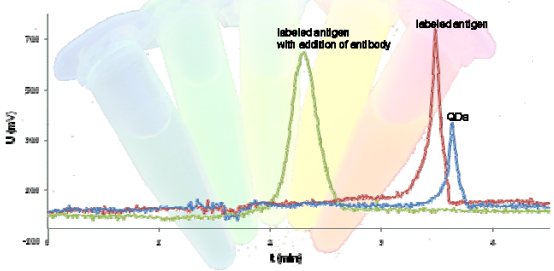
Quantum dots – conjugates separation



Quantum dots – conjugates separation

Conjugation of antigen via *N, N´*-carbonyldiimidazole (CDI)



Characterization of 3.5 nm CdTe QDs and their conjugates by CZE-LIF,
uncoated capillary 15/20 cm, voltage 6 kV, injection 5 s, 100 mM TRIS/TAPS buffer pH = 8.3

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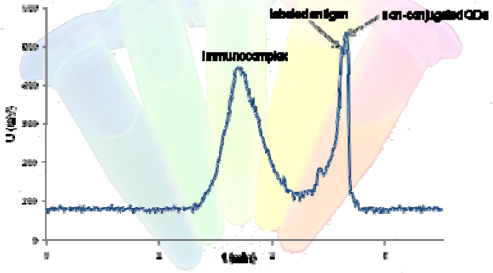
Quantum dots – conjugates separation

CE Competitive imunoassay



Characterization of 3.5 nm CdTe QDs and their conjugates by CZE-LIF,
uncoated capillary 15/20 cm, voltage 6 kV, injection 5 s, 100 mM TRIS/TAPS buffer pH = 8.3

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Quantum dots – conjugates separation

- Magnetic particles conjugated with antibody via oxidation of antibody glycan
- QDs or FITC conjugated with antigen via EDC/Sulfo-NHS

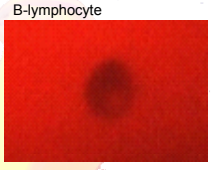
QDs conjugate **FITC conjugate**

control **control**

Quantum dots – selective cell labeling

Quantum dots were conjugated with antibody against CD3 protein, a membrane protein specific for T-lymphocytes while B-lymphocytes do not contain it. Antibody was also conjugated with FITC for control.

B-lymphocyte



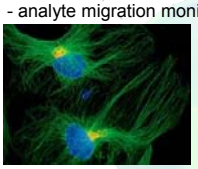
T-lymphocyte

T-lymphocyte

Quantum dots – application

Vizualization of organelles in cells

- analyte migration monitoring in cells, pathogenes detection



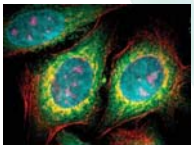
HeLa

HeLa

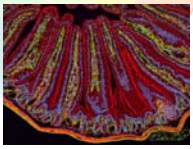
nucleus – 655 nm

Golgi apparatus – 585 nm

microtubuls – 525 nm



Hep-2 cells

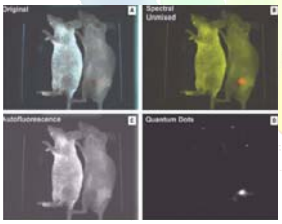


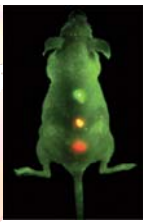
Mouse small intestine

Quantum dots – application

Cancer tissues labeling

- imaging and localization of cancer tissues
- using conjugated QDs with specific cancer antibodies





Quantum dots – application

Solar cells

- Solar cells from CdSe nanorods (Prof. A.Paul Alivisatos, Lawrence Berkeley National Laboratory)

- QDs absorbed broader spectrum of sun light

- QDs allows deposit photosensitive layer on wide range of materials

- price

Acknowledgment

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Fluorescence and fluorescence lifetimes measurement

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– Department of Chemistry, Faculty of Science, Masaryk University

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