Quantum dots in Molecular Imaging

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Introduction

Quantum dots (Qdots®) are nanoparticles exhibiting fluorescent properties that can be used for cell staining. In the early 1980s the first quantum dots were successfully synthesized in a research laboratory. About five years earlier Ascii et al. had demonstrated one dimensional confinement in quantum well structures. In less than a decade Ekimov et al. obtained the evidence for three dimensional confinement in quantum dots.

Qdots® are nanometer-scale (roughly protein-sized) atom clusters, containing from a few hundred to a few thousand atoms of a semiconductor material (cadmium mixed with selenium or tellurium), which has been coated with an additional semiconductor shell (zinc sulfide) to improve the optical properties of the material. These particles fluoresce in a completely different way than do traditional fluorophores, without the involvement of electronic transitions.

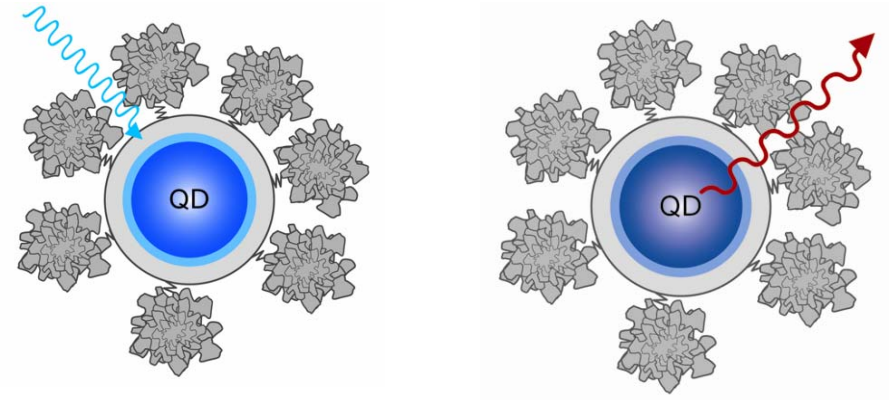

Figure 1: Structure of a Qdot® nanocrystal in excitation and emission state. The layers represent the distinct structural elements (core, shell and polymer coating) and are drawn roughly to scale. To the surface of the Qdot®s proteins are attached.

The two primary applications of Qdots® are optoelectronics and fluorescence tagging techniques. Both of these applications are thanks to the confinement of electron in the semiconductor material. Below, I will discuss the fluorescence applications.

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Fluorescence applications

Fluorescence tagging is the most common application of Qdots®, where they replace molecular dyes. In these applications the tagging substance is irradiated with light in the proper excitation wavelength, the light is absorbed and then re-emitted at a different wavelength (see figure 1). The presence of the tagging substance is proofed by detecting the right emission spectrum. For example, injecting a tagging substance into a particular biological cell makes it possible to identify the target cell amongst other cells by its fluorescence.

There are several reasons that make Qdots® more advantageous to molecular competition:

1) Qdots® can absorb a wide band of light for their excitation, but they emit in a very narrow band. In contrast, most molecular dyes can absorb only a very narrow band of wavelength, so most of the illuminating light is not used. Also, these molecules emit is a much wider band of wavelengths.

2) The Stoke´s shift of Qdots® is enormous compared to molecular dyes.

3) Their tagging property is controllable - with proper chemistry these objects can be attached to "molecules with a purpose". This is in contrast to traditionally used molecular tags having well defined binding characteristics. As a result a particular molecular tag may or may not bind with a given molecule or surface. Since Qdots® have a surface that could bind with a variety of molecules, they could be prepared (functionalized) so as to attach to well defined targets, even at the molecular level.

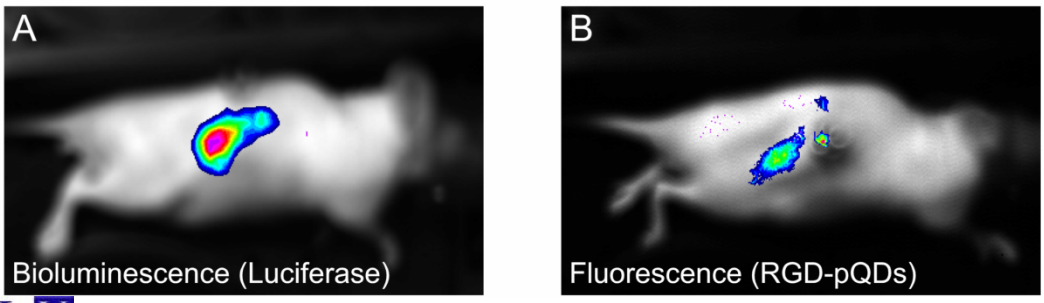
4) Qdots® are made with large non linear properties that have allowed researchers to employ them for deep noninvasive imaging. This is done by focusing long wavelength laser light that is not absorbed by tissue beneath the skin of a rat injected with Qdots® in the tail blood vessels. As the dots reach the focal point of the illuminating laser they frequency-double the laser radiation (absorb two of the long wavelength photons simultaneously) and emit light well into the visible. This "fluorescence" allows the imaging of the blood vessels/tumors without opening the tissue.

5) So et al. reported about self-illuminating Qdot® conjugates mimicking a natural BRET system. BRET is a phenomenon whereby a light emitting protein, which has

to be in a very close proximity (Förster radius of 2 – 10 nm) to a fluorescent protein, transfers its energy non-radiatively via dipole-dipole-interactions to this fluorescent protein. In this specific case So et al. coupled a Renilla mutant emitting light at a maximum of 480 nm to Qdot®655 and demonstrated that BRET emission can be imaged in cells and small animals. Again, the benefit is the sensitivity of bioluminescence combined with the enormous Stoke-s shift of Qdots®, because light in the near-infrared region penetrates easier animal tissues.

Whole-animal fluorescence imaging

1) Luciferase (luc) expressing human renal carcinoma cells (RC21-luc) were injected (100.000 cells per 10 µl PBS) under the renal capsule of Balb/c nu/nu mice (n=3) which led to the growth of luc-expressing renal carcinoma. Ten weeks after tumor inoculation, luciferase expression was assessed 5 minutes after intraperitoneal (i.p.) injection of luciferin (2 mg in 30 µl PBS). Total photon emission was acquired for 30 seconds. Fluorescence imaging of both RGD-pQDs and luciferase *in vivo* was accomplished using a peltier cooled CCD camera system NightOWL LB 981 (BERTHOLD Technologies, Germany). For fluorescent imaging, a 525±25 nm wavelength emission filter (Omega Optical, Glen Spectra Limited, England) was attached to the CCD camera. A 470±10 nm wavelength excitation filter (Omega Optical, Glen Spectra Limited, England) was inserted in front of the light source. Mice were anaesthetized with isoflurane/oxygen and placed in the imaging cabinet and images were acquired 10 minutes after intracardial injection of RGD-pQDs at a camera exposure time of 5 seconds.



Images courtesy of W.Mulder, I.Que, E.L.Kaijzel & C.W.G.M.Löwik. Submitted 2007.

Figure 2: Optical imaging of tumor angiogenesis: Luciferase expression (A) of a Balb/c nu/nu mouse with a luc-expressing renal carcinoma tumor after injection of luciferin (binning 7x7). The signal co-localizes with a strong fluorescence signal originating from intracardial administrated RGD-pQDs that are accumulated in the tumor, excitation 470 nm, emission 525 nm.

B) Balb/C nu/nu mice expressing prostate tumor (male) and B02F11RL-lucN11 mammary fat pads tumors (female) were anesthetized with isoflurane and followed by intravenously injection of 10nM Qtracker® 525 (Invitrogen). The mouse was placed in the LB 981 NightOWL under anesthesia and measured. The acquisition time was 5 second (Fig. 3).

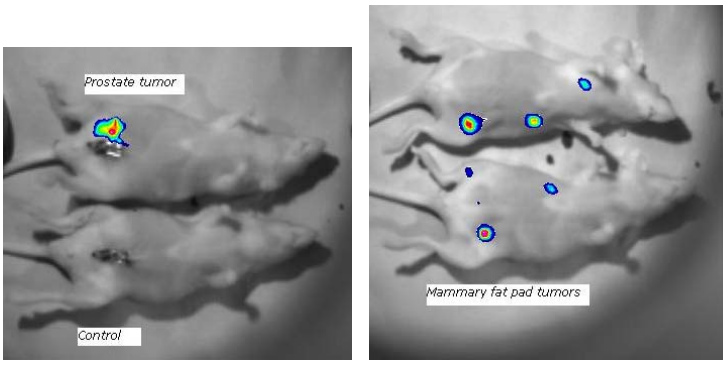


Image courtesy I.Que, N.V.Henriquez & G. van der Pluijm

Figure 3: Fluorescence signal of Qdots® in prostate tumor and B02F11 RL-luc N11cancer cells in mammary fat pads

Subcutaneously injections of $10E^6$, $10E^5$, $10E^4$, $10E^3$ and $10E^2$ breast cancer cells (MDA 231-Luc) labeled with Qtracker® 705 (Invitrogen).

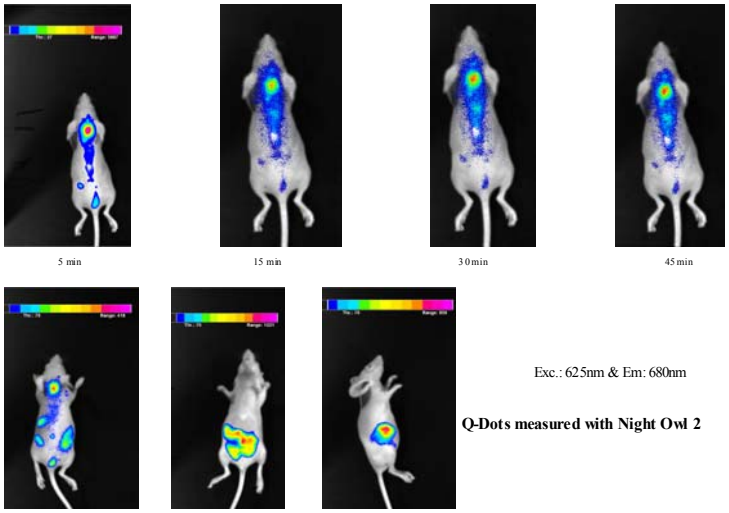


Image courtesy I.Que, N.V.Henriquez & G. van der Pluijm

Figure 4: Kinetics of subcutaneous injected breast cancer cells (MDA 231-Luc) labeled with Qtracker® 705 measured with LB 983 NightOWL II with excitation 625nm and emission 680nm.

Summary

Quantum dots (Qdots®), tiny light-emitting particles on the nanometer scale, are emerging as a new class of fluorescent probe for in vivo biomolecular and cellular imaging. In comparison with organic dyes and fluorescent proteins, Qdots® have unique optical and electronic properties: size-tunable light emission, improved signal brightness and simultaneous excitation of multiple fluorescence colors.

Qdots® molecular imaging is a new way of seeing biologic processes at work within cells and in small animals. Recent advances have led to the development of multifunctional nanoparticle probes that are very bright and stable under complex in vivo conditions. A new structural design involves encapsulating luminescent Qdots® with amphiphilic block copolymers and linking the polymer coating to tumor-targeting ligands and drug delivery functionalities. Polymer-encapsulated Qdots® are essentially nontoxic to cells and animals, but their long-term in vivo toxicity and degradation need more careful study. Bioconjugated Qdots® have raised new possibilities for ultrasensitive and multiplexed imaging of molecular targets in living cells, animal models and possibly in humans.

Material

- Balb C nude mouse (CBy/cby.CQ-fox1<nu>/J)
- NightOWL LB 981/ NightOWL II LB 983 (BERTHOLD Technologies)
- Qtracker® 525 / Qtracker® 705 (Invitrogen)

Literature

- *Parallel intravital microscopy, optical imaging, and MR imaging of tumor angiogenesis using avβ3-targeted paramagnetic quantum dots;* submitted. Willem J.M. Mulder, Karolien Castermans, Judy R. van Beijnum, Mirjam G.A. oude Egbrink, Patrick T.K. Chin, Clemes Lowik, Eric L. Kaijzel, Ivo Que, Gert Storm, Gustav J. Strijkers, Arjan W. Griffioen, Klaas Nicolay
- *Nanoparticles*; Invitrogen
- *Fluorescent nanocrystals for use in early cervical cancer detection*; Nida DL, Rahman MS, Carlson KD, Richards-Kortum R Follen M. Gynecol Oncol. 2005 Dec;99(3 Suppl 1):S89-94. Epub 2005 Sep 1.
- *Selection of quantum dot wavelengths for biomedical assays and imaging*; Lim YT, Kim S, Nakyama A, Stott NE, Bawendi MG, Frangioni JV. Mol Imaging. 2003 Jan;2(1):50-64.
- *Quantum dot nanocrystals for in vivo molecular and cellular imaging*; Smith AM, Gao X, Nie S. Photochem Photobiol. 2004 Nov-Dec;80(3):377-85.

- *Self-illuminating quantum dot conjugates for in vivo imaging*; So M-K, Xu C, Loening AM, Gambhir SS, Rao J Nature Biotechnology Letters, published online 26 February 2006
- Qdot® facts and hints:
  http://probes.invitrogen.com/products/qdot/hints.html
  http://www.evidenttech.com/quantum-dots-explained.html

Filters

Filters from BERTHOLD TECHNOLOGIES for the NightOWL imaging systems:

- Qdot®525: emission 520/10 (order number: 39805)
- Qdot®565: emission 550/20 (order number: 39806)
- Qdot®585: emission 600/20 (order number: 50477)
- Qdot®605: emission 600/20 (order number: 50477)
  emission 605/55 (order number: 46718)
- Qdot®655: emission 655/20 (order number: 51332)
- Qdot®705: emission 700/20 (order number: 50479)
- Qdot®800: emission 820/30 (order number: 50481)