Quenching of CdSe quantum dot emission, a new approach for biosensing†

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Received (in Cambridge, UK) 14th January 2005, Accepted 22nd April 2005
First published as an Advance Article on the web 20th May 2005
DOI: 10.1039/b500664c

The emission of CdSe quantum dots linked to the 5′-end of a DNA sequence is efficiently quenched by hybridisation with a complementary DNA strand with a gold nanoparticle attached at the 3′-end; contact of the quantum dot and gold nanoparticle occurs.

Semiconductor quantum dots (QDs), such as ZnS-capped CdSe nanocrystals, are extremely attractive fluorescent probes for biosensing applications possessing: very high fluorescence yields, extinction coefficients several times higher than conventional organic fluorophores, low photobleaching, but most importantly narrow, symmetric emission peaks (approx. 25–35 nm, full width at half maximum) which can be tuned as a function of the particle size.[1] Due to these properties QDs have been exploited for both cellular[2] and DNA[3] sensing strategies, where the QDs have found use as highly stable and emissive labels on biomolecules, enabling binding interactions to be imaged.

There are a number of other biosensing strategies that rely upon quenching of fluorescent dyes with organic quencher molecules. For instance, dimethylaminophenyl-azobenzoic acid has been applied in a number of DNA sensor approaches such as molecular beacons,[4] Scorpion primers[5] and TaqMan probes.[6] More recently the use of biosensor molecules containing gold nanoparticles as quenchers has been demonstrated. In this context molecular beacon probes containing a fluorescent dye and a gold nanoparticle have been reported by Dubertret *et al.*[7] Like fluorescent dyes, the emission of QDs is affected by their electromagnetic interaction with metallic films; Bawendi *et al.* have shown that the emission of QDs in contact with smooth gold surfaces is quenched and enhanced (by surface enhanced excition emission) when QDs are held away from the surface on rough gold surfaces.[8] Wargnier *et al.* more recently have shown that the emission of the QDs is quenched when associated with oppositely charged gold nanoparticles; a long range fluorescence resonance energy transfer (FRET) process was proposed.[9] Oh *et al.* reported a similar finding for assemblies of streptavidin coated QDs with avidin coated gold nanoparticles.[10] Our key goal is to exploit the excellent emission properties of QDs for the creation of novel DNA sensing approaches, whereby the fluorescence emission might be quenched by close contact with a gold nanoparticle; singly-labelled QDs and gold nanoparticles with complementary DNA oligonucleotides are used to form simple assemblies rather than multi-particle assemblies.

Effective close contact with gold nanoparticles could be achieved by hybridisation of an oligonucleotide with a 5′-end functionalised with a QD and a complementary oligonucleotide with a 3′-end functionalised with a gold nanoparticle (Fig. 1). The quenching of QDs with gold nanoparticles linked to DNA strands was suggested in some very recent studies by Gueroui *et al.*, where the emission was quenched by gold in a distance dependent manner.[11] Our approach is different; oligonucleotide conjugates of gold nanoparticles and QDs were synthesised to provide hybrids with close contact of the gold nanoparticle and the QD. The QDs used here were essentially tagged with an oligonucleotide chain with no coating, lipid,[11] or polymer.[1]

Single-stranded DNA, (5′-TGC AGA TAG ATA GCA G-3′), was linked at the 5′-end to CdSe–ZnS core-shell quantum dots. To achieve this, a 5′-aminohexyl substituted oligonucleotide was first synthesised by introduction of the 5′-MMT amino modifier C6 amidite (Link Technologies) in the synthesis by standard phosphoamidite methods on an ABI 394 synthesiser. The carboxylic acid functionalised QDs (Adirondack Green, Evident Technologies) were treated with 1-ethyl-3-(3-dimethylamino-propyl)carbodi-imide.HCl and *N*-hydroxylsulfo-succinimide (0.5 M sodium phosphate buffer, pH 6) for 30 min. Following ultracentrifugation (40 min, 32,000 *g*) the resulting pellet was washed with water. After dispersion of the functionalised QDs in a 0.1 M sodium borate buffer (pH 8.3) to a concentration of 0.8 μM, one equivalent of the 5′-aminohexyl-substituted oligonucleotide was added and mixed for 2 hours at room temperature and then stored overnight (4 °C). Purification was achieved by ultracentrifugation (80 min, 32,000 *g*) at 4 °C and subsequent washing of the pellet with water. The conjugate (QD–DNA) was re-dissolved to a concentration of 10 μM in water, under which conditions QD–DNA is stable when stored at 4 °C. The QD–DNA sample was found to be thermally unstable.

The complementary sequence was linked at the 3′-end to 1.4 nm dia. gold particles as follows. A 5′-CTG CTA TCT ATC

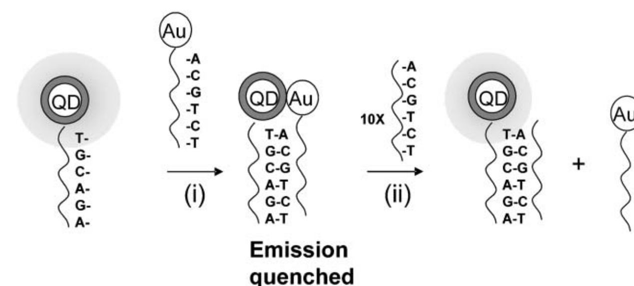

Fig. 1 Schematic diagram showing (i) addition of 1 equivalent of the Au–DNA to the QD–DNA to yield the hybrid, (ii) addition of ten equivalents of the unlabelled complementary oligonucleotide to the hybrid to displace the Au–DNA from the QD–DNA.

† Electronic supplementary information (ESI) available: further details of additional experiments performed using QD–DNA–biotin on streptavidin surfaces. See http://www.rsc.org/suppdata/cc/b5/b500664c/

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TGC-3′-aminomodifier C7 CpG was synthesized by utilisation of 3′-aminomodifier C7CPG 1000 (Link Technologies) as a solid support in the synthesis. The single *N*-hydroxysuccinimidyl ester functionalized 1.4 nm gold particles (Nanogold, Nanoprobes) at a concentration of 15 μM in 20 mM sodium phosphate buffer at pH 7.5 were mixed with 1.5 equivalents of the above 3′-amino-modified oligonucleotide and then purified by ultracentrifugation (32,000 *g*) and washing of the pellet with buffer (3 ×) to yield the 3′- gold nanoparticle end labeled DNA conjugate (Au–DNA) shown in Fig. 2.

The Au–DNA was mixed with QD–DNA in a ratio of 1 : 1 at a concentration of 0.4 μM in 0.3 M sodium chloride, 10 mM sodium phosphate buffer solution (pH 7.0). In order to avoid inner filter effects and light re-absorption the concentration of the QD–DNA was held at 0.4 μM, which corresponds to an optical density of 0.1 at the excitation wavelength of 322 nm. The emission spectrum for the QD–DNA is illustrated in Fig. 3, plot i. Addition of the Au–DNA to the QD–DNA resulted in significant quenching of the emission (85%), plot ii, which was complete within 1.5 h. Addition of ten equivalents (4 μM, final concentration) of pure unmodified oligonucleotide (5′-CTG CTA TCT ATC TGC-3′) results in recovery of the emission in one further hour to a level that is the same within experimental error to that obtained when the QD–DNA is mixed directly with the pure oligonucleotide, in the absence of DNA–Au, for 2.5 h (Fig. 3, plots iii and iv).

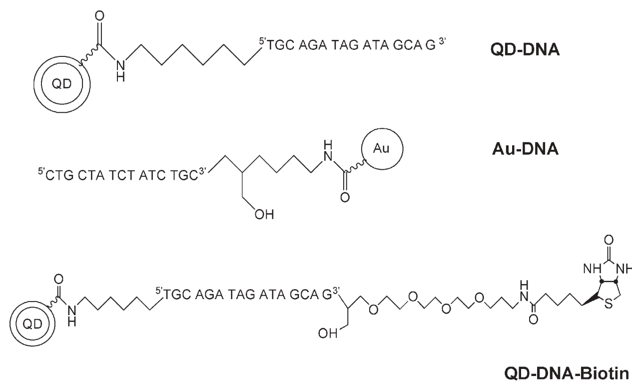

Fig. 2 Structures of QD and gold nanoparticle oligonucleotide conjugates showing chemical linkage groups.

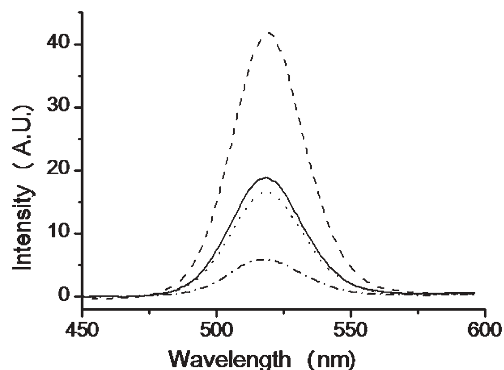

Fig. 3 Emission spectra of i) QD–DNA (—), ii) QD–DNA and Au–DNA after 1.5 h (–·–), iii) addition of 5′-CTG CTA TCT ATC TGC-3′ to sample ii) after 1 h (—), iv) mixture of QD–DNA and 5′-CTG CTA TCT ATC TGC-3′ after 2.5 h (····).

The rather slow hybridisation of the Au–DNA strand with the QD–DNA was considered to be due to association of the oligonucleotide strand onto the QD surface to which it is covalently attached; association of unlinked DNA onto isolated QDs is well established.[12] The reduced emission yield of the QD–DNA hybridised to the pure complementary oligonucleotide shown in plot iv after 2.5 h in the sodium chloride/phosphate buffer compared to that for the starting QD–DNA (plot i), is a result of the reduced emission yield of the QD–DNA, an observation which has been previously reported for QDs in ionic solutions[13] (see also technical data, Evident Technologies) and verified for single-stranded QD–DNA under our conditions (results not shown).

QD–DNA/Au–DNA hybrid assemblies were examined by transmission electron microscopy (FEI CM200 FEG microscope). QD–DNA was mixed 1 : 1 with Au–DNA (2 pM in 10 mM sodium phosphate buffer, pH 7.0) and then incubated ∼1.5 hours at room temperature, diluted 10-fold and deposited on a glow-discharged C film and negatively stained with methylamine vanadate (Nanoprobes Inc., USA). In the image (Fig. 4) the QDs can be observed as lighter particles (4 nm dia.) against the darker stain and the gold particles as smaller dark black dots (1.4 nm dia.) (Assemblies of QD–DNA/Au–DNA consist of single QDs and single gold nanoparticles in close contact. Some of the assemblies have been identified by arrows in Fig. 4).

In order to evaluate the QD quenching by hybridisation with a complementary Au–DNA on a surface, and the application of QD–DNA conjugates for single molecule studies, 5′-QD-labelled oligonucleotides were attached at the 3′-end to a glass slide in low densities (Fig. 5). The oligonucleotide (5′-TGC AGA TAG ATA GCA G-3′) was prepared with a 3′-end functionalised with a biotin group linked by tetraethylene glycol (TEG), to hold the QD–DNA sequence from the surface, and a 5′-aminohexyl group, which was used for attachment of the QDs (Fig. 2). The oligonucleotide was prepared using standard oligonucleotide synthesis methods by incorporation of 3′biotin TEG CPG and 5′ MMT amino modifier C6 amidite (Link Technologies).

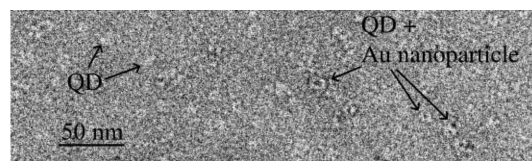

Fig. 4 Electron micrograph of negatively stained QD–DNA/Au–DNA assemblies (pairs).

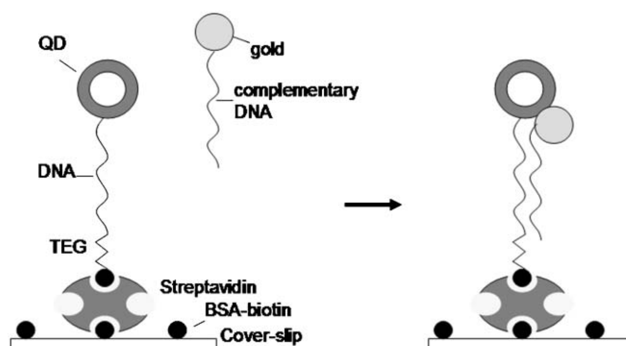

Fig. 5 Schematic figure of hybridisation of Au–DNA with QD–DNA biotin, associated to the streptavidin coated glass cover slip.

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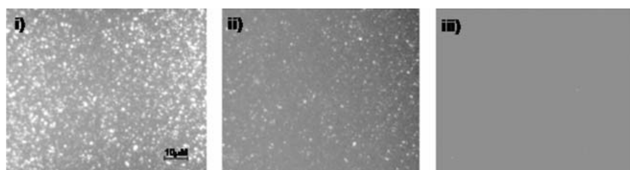

Fig. 6 Epifluorescence image of i) QD–DNA–biotin associated on the streptavidin, ii) after addition of Au–DNA (5 min), iii) after addition of Au–DNA (15 min).

Streptavidin was immobilised on glass slides according to the method of Tang *et al.*[14] QD–oligonucleotide–biotin was diluted with TNT buffer (0.1 M Tris, 0.15 M NaCl, 0.5% Tween 20, pH 7.5) to a final concentration of 0.156 μM and 10 μl was spread over an area of $\sim 1\ cm^2$ on the streptavidin-coated glass cover slip and immobilised by incubation at 37 °C for 15 min. The slides were washed in water and then observed by epi-fluorescence microscopy (Zeiss Axiovert 200 M microscope equipped 100× oil immersion objective (NA = 1.4) with Axio Cam HRm, HBO 50 W mercury lamp and N2 filterset (excitation G365, emission LP420, beamsplitter FT 395)). The emission from the single-stranded QD–DNA–biotin molecules associated on the glass surface is easily detected as bright spots (larger in dimension than the QDs) in the image due to the long exposure and accumulation of images (Fig. 6i).

Au–DNA (10 μl, 1.5 μM) in 0.3 M sodium chloride, 10 mM sodium phosphate (pH 7.0) was added and images captured (exposure 12.6 s. 100%, illumination light) (Fig. 6ii). After 5 minutes emission from the majority of the QD–DNA–biotin molecules was quenched. Within 15–20 minutes, the emission of the QDs could not be detected at all (Fig. 6iii). The sample was washed with buffer and then complementary DNA (5′-CTG CTA TCT ATC TGC-3′) (10 μl at a concentration of 15 μM) in 0.3 M sodium chloride, 10 mM sodium phosphate buffer solution (pH 7.0) was added and incubated for 1.5 hours at room temperature; the recovery of the QD emission is observed.

A further experiment was performed, except this time instead of using Au–DNA, with the sequence 5′-CTG CTA TCT ATC TGC-3′, similar 3′-gold nanoparticle labelled samples were prepared with the non-complementary sequence 5′-T-GCA-GAT-AGA-TAG-CAC-T-3′ aminomodifier C7 CpG by the same chemistry and purification methods as previously used to prepare the DNA–Au (Fig. 2). Addition of this non-complementary DNA–gold nanoparticle conjugate to the surface associated QD–DNA–biotin did not result in any loss of the QD emission (20 min).

Samples of QD–DNA biotin were incubated with i) unmodified oligonucleotide (5′-CTG CTA TCT ATC TGC-3′), ii) Au–DNA, and iii) non-complementary DNA gold nanoparticle conjugate in solution prior to association to the streptavidin coated slides (see ESI for details). The emission of the QDs was quenched where the Au–DNA/QD–DNA–biotin hybrid was formed.

The emission from QD–DNA–biotin molecules on the surface could be visualised using conventional epifluorescence microscopy. Addition of the complementary Au–DNA conjugate results in hybridisation and contact of the QD with the gold nanoparticle and quenching of the QD emission, whereas addition of the gold nanoparticle 3′-end labelled non-complementary sequence does not.

In summary, we have shown that the emission of QDs is effectively quenched by contact with gold nanoparticles as a result of a DNA hybridisation event using specially prepared DNA conjugates. QD–gold nanoparticle DNA conjugates have significant potential for use as DNA fluorogenic probes[15] suitable for the detection of single molecule (or low copy numbers) of DNA in the solution phase or in cellular systems.

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