Quantum dot–nucleic acid/aptamer bioconjugate-based fluorimetric biosensors

Dejian Zhou1
School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

Abstract
Over the last 10 years, fluorescent semiconductor QD (quantum dot)–biomolecule conjugates have emerged as a powerful new sensing platform showing great potential in a wide range of applications in biosensing, environmental monitoring and disease diagnosis. The present mini-review is a brief account of the recent developments in QD–NA (nucleic acid), particularly NA aptamer, conjugate-based biosensors using the FRET (Förster resonance energy transfer) readout mechanism. It starts with a brief introduction to the NA aptamer and QD-FRET, followed by example approaches to compact QD–DNA conjugates, target readout strategies and sensing performance, and concludes with challenges and outlook for the QD–NA/aptamer bioconjugate sensors.

NA (nucleic acid) aptamer
NA aptamers are short ssDNA (single-stranded DNA) or RNA molecules selected from large random DNA or RNA molecule pools (e.g. 10^{14}–10^{15} molecules) by their ability to bind a specific target using an in vitro process termed as SELEX (systematic evolution of ligand by exponential enrichment) [1,2]. It is possible to select an NA aptamer against effectively any target of interest. Once an aptamer sequence is known, it can be produced by chemical synthesis and followed by stringent chemical purification to avoid batch-to-batch variations. Aptamers have several advantageous properties over the widely used antibodies as ligand-binding agents [3,4], including a wider target choice, higher ligand specificity with comparable binding affinity (nanomolar to picomolar), production by totally in vitro methods that combined with chemical purification can eliminate batch-to-batch variation, greater robustness against thermal and chemical denaturation (they can sustain several rounds of a denaturation/renaturation process without losing ligand-binding affinity), and ease of incorporating bespoke functional groups and/or fluorescent labels site-specifically for easy surface-/bio-conjugation and signal readout. As a result, NA aptamers have been used in developing sensors for a wide range of different targets: from DNA, proteins, environmental pollutants, street drugs and viruses to whole cancer cells [5,6].

QD (quantum dot)-FRET (Förster resonance energy transfer) and challenges
The unique size-dependent, bright and extremely photo-stable fluorescence of QDs make them well-suited for a range of bio-related applications, imaging, cell tracking/trafficking and multiplexed sensing, etc. [7–9]. Their broad absorption and narrow symmetric emission (with typical full-width at half-maximum of ~25–40 nm) are particularly attractive to FRET-based sensing applications because such spectral characteristics allow for wide selection of excitation wavelengths to minimize direct excitation of the acceptor, reducing background and improving sensitivity [10,11].

Besides, different-coloured QDs can be excited by a single light source, yet produce specific, narrow and symmetric emissions of different colours, which is very useful for multiplexing. Furthermore, multiple biomolecules can be arrayed to a single QD to create a multivalent/multifunctional QD bioconjugate for enhanced ligand-binding affinity and multiplexed sensing [8]. In general, QDs are excellent donors, but not acceptors, in FRET-based applications, because of their broad absorption spectra that make direct excitation of QDs unavoidable. Furthermore, their relatively long lifetimes (approximately 10–100 ns compared with <5 ns for most organic fluorophores) also make dye to QD FRET inefficient. Therefore almost all QD-FRET-based sensors reported so far have exclusively used QD as the donor and dye as acceptor, but not vice versa [9–11].

As FRET efficiency (E, sensitivity) decreases significantly with the increasing donor–acceptor distance (r) following the Förster dipole–dipole interaction equation: \[E = 1/[1 + (r/R_0)^6]\], where \(R_0\) is the Förster radius of a single donor–acceptor pair under which E = 50%, this has posed a considerable challenge to achieve high sensitivity for QD-FRET sensors owing to the significant sizes of the QDs [10]. HRs (hydrodynamic radius) for water-soluble QDs alone (core + surface coating, ~5–25 nm depending on solubilization strategies) can often be comparable with or greater than the \(R_0\) (~4–7 nm) of most QD–dye FRET pairs before bioconjugation [10,11]. As a result, most QD-FRET sensors have relied on increasing the number of acceptors per QD (n) to improve the E, because \[E = 1/[1 + n^6/(nR_0)^6]\] in a single-donor–multiple-acceptor FRET system [10].
However, such systems are inefficient and often unsuitable for low $n$ situations (e.g. $n = 1$) because of the small FRET change obtainable from a single target binding in such QD sensors [12,13]. Therefore considerable efforts have been focused on developing compact water-soluble QDs and effective bioconjugation chemistries to reduce $r$ values [14–20]. Despite several strategies having been reported to make water-soluble QDs, so far none could produce QDs that are compact (HR <5 nm), highly stable in biological buffers and resistant to non-specific adsorption, key requirements for robust, sensitive and specific clinical bioassays. In general, water-soluble QDs prepared via ligand exchange are compact, but often exhibit limited stability in biological buffers and cannot prevent non-specific adsorption, whereas QDs solubilized via capping with amphiphilic polymer [21] or PEGylated lipids [22] are stable, resisting non-specific adsorption, but have large HRs (>10 nm), limiting the sensitivity. Table 1 summarizes a few frequently used QD-bioconjugation chemistries for making compact QD–DNA conjugates and their $E$ values at low $n$ situations.

### Approaches to compact QD–DNA conjugates

The most straightforward approach to compact QD–DNA conjugates is to directly bind the DNAs to the QD surface (via Zn$^{2+}$ in CdSe/ZnS core/shell QDs). Here, two different approaches have been reported. Mattoussi and co-workers used the His$_8$-tagged DNA and DHLA (dihydrolipoic acid)–QD self-assembly to produce compact QD–DNA conjugates and efficient FRET ($E = 60\%$ for $n = 2$) [16]. They have also used such QD–DNA conjugate for label-free DNA detection by incorporating a DNA molecular beacon. We found that thiolated DNAs could self-assemble onto or PEGylated lipids [22] are stable, resisting non-specific adsorption, but have large HRs (>10 nm), limiting the sensitivity. Table 1 summarizes a few frequently used QD-bioconjugation chemistries for making compact QD–DNA conjugates and their $E$ values at low $n$ situations.

However, such systems are inefficient and often unsuitable for low $n$ situations (e.g. $n = 1$) because of the small FRET change obtainable from a single target binding in such QD sensors [12,13]. Therefore considerable efforts have been focused on developing compact water-soluble QDs and effective bioconjugation chemistries to reduce $r$ values [14–20]. Despite several strategies having been reported to make water-soluble QDs, so far none could produce QDs that are compact (HR <5 nm), highly stable in biological buffers and resistant to non-specific adsorption, key requirements for robust, sensitive and specific clinical bioassays. In general, water-soluble QDs prepared via ligand exchange are compact, but often exhibit limited stability in biological buffers and cannot prevent non-specific adsorption, whereas QDs solubilized via capping with amphiphilic polymer [21] or PEGylated lipids [22] are stable, resisting non-specific adsorption, but have large HRs (>10 nm), limiting the sensitivity. Table 1 summarizes a few frequently used QD-bioconjugation chemistries for making compact QD–DNA conjugates and their $E$ values at low $n$ situations.

### Approaches to compact QD–DNA conjugates

The most straightforward approach to compact QD–DNA conjugates is to directly bind the DNAs to the QD surface (via Zn$^{2+}$ in CdSe/ZnS core/shell QDs). Here, two different approaches have been reported. Mattoussi and co-workers used the His$_8$-tagged DNA and DHLA (dihydrolipoic acid)–QD self-assembly to produce compact QD–DNA conjugates and efficient FRET ($E = 60\%$ for $n = 2$) [16]. They have also used such QD–DNA conjugate for label-free DNA detection by incorporating a DNA molecular beacon. We found that thiolated DNAs could self-assemble onto or PEGylated lipids [22] are stable, resisting non-specific adsorption, but have large HRs (>10 nm), limiting the sensitivity. Table 1 summarizes a few frequently used QD-bioconjugation chemistries for making compact QD–DNA conjugates and their $E$ values at low $n$ situations.

## Table 1 | FRET efficiencies ($E$) of some compact QD–DNA conjugates prepared via different bioconjugation chemistries at $n = 1$ except where specifically indicated within parentheses

<table>
<thead>
<tr>
<th>QD-dye FRET system</th>
<th>DNA conjugation chemistry</th>
<th>$E$ (%)</th>
<th>Readout</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPA–QD$_{550}$–Alexa Fluor® 594</td>
<td>Thiolate self-assembly (ZnS shell)</td>
<td>81</td>
<td>SMF</td>
<td>[14]</td>
</tr>
<tr>
<td>MAA–QD$_{520}$–Alexa Fluor® 594</td>
<td>Thiolate self-assembly (ZnS shell)</td>
<td>~50</td>
<td>FS</td>
<td>[14]</td>
</tr>
<tr>
<td>DHLA–QD$_{650}$–Atto 647N</td>
<td>Thiolate self-assembly (ZnS shell)</td>
<td>34</td>
<td>FS</td>
<td>[11]</td>
</tr>
<tr>
<td>DHLA–QD$_{510}$–TAMAR</td>
<td>His$_8$-tag-metal affinity (ZnS shell)</td>
<td>60 ($r = 2$)</td>
<td>FS</td>
<td>[16]</td>
</tr>
<tr>
<td>DHLA–QD$_{590}$–Cy5</td>
<td>His$_8$-tag-metal affinity (ZnS shell)</td>
<td>~21</td>
<td>FS</td>
<td>[16]</td>
</tr>
<tr>
<td>MAA–QD$_{520}$–Cy3</td>
<td>Covalent coupling (surface ligand)</td>
<td>52</td>
<td>Lifetime</td>
<td>[23]</td>
</tr>
<tr>
<td>MAA–QD$_{560}$–Alexa Fluor® 647</td>
<td>Covalent coupling (surface ligand)</td>
<td>6.7</td>
<td>Lifetime</td>
<td>[23]</td>
</tr>
<tr>
<td>EG$<em>3$–QD$</em>{550}$–Alexa Fluor® 594</td>
<td>Covalent binding (surface ligand)</td>
<td>28</td>
<td>FS</td>
<td>[17]</td>
</tr>
<tr>
<td>STV–QD$_{555}$–Cy5</td>
<td>STV-biotin interaction</td>
<td>~5.5</td>
<td>SMF</td>
<td>[12]</td>
</tr>
</tbody>
</table>

Abbreviations for ‘xxx–QD$_{yy}$’ are: xxx=QD surface capping ligand, yyy=QD $\lambda_{em}$ (nm). Cy3, indocarbocyanine; Cy5, indodicarbocyanine; EG$_3$, 11-mercaptopoundecyl tri(ethylene glycol); TAMAR, tetramethylrhodamine.

conformation in the QD–DNA assemblies. For the MPA–QD, the DNA strands may wrap around the QD, yielding small $r$ and strong $E$, whereas for the DHLA–QD, the DNA strands are extended, leading to big $r$ and weak $E$. This was supported by the observation that self-assembled MPA–QD–ssDNA could not hybridize to its complementary DNA, whereas DHLA–QD–ssDNA could [11,14]. Similar structural differences were also observed in a recent QD–fluorescent protein (His$_8$-tagged) self-assembly study, where $r$ value for the MPA–QD was found to be ~2.5 nm shorter than that of the DHLA–QD despite using identical His$_8$-tag-Zn$^{2+}$ co-ordination in the bioconjugation [23].

Another approach to compact QD–DNA conjugate is to covalently couple the DNAs with the QD surface functional groups. The binding strength and chain length of the ligands are critical for structural compactness (sensitivity) and specificity of the resulting QD–DNA conjugate sensor. For example, Algar and Krull [24] prepared compact QD–DNA conjugates with efficient FRET ($E$ up to 52% at $n = 1$) by coupling amine-modified DNA to an MAA ( mercaptoacetic acid)-capped QD. However, its specificity was rather low, with a FRET ratio of only ~2 between complementary and non-complementary DNA targets, suggesting that MAA cannot resist non-specific adsorption [23]. We found that EG$_3$ [11-mercaptopoundecyl tri(ethylene glycol)]-capped QD could effectively resist non-specific adsorption while maintaining a relatively compact QD–DNA structure ($E = 28\%$ for $n = 1$); the resulting QD–DNA conjugate is suitable for quantification of low-nanomolar-specific complementary DNA [17]. More recently, we have developed a novel chelating dendritic ligand that can provide stable, compact and entangled capping to the QD, allowing highly specific detection of picomolar complementary DNA target [11,18].

A third common approach to QD–DNA conjugate is via biospecific interactions, e.g. biotin–STV (streptavidin) interaction [12,25,26–29]. This process is very simple, by simply mixing commercially available STV–QD with biotinylated DNA usually leading to reliable efficient QD–DNA conjugation. The drawback here is the large size of...
the STV–QD (HR >12 nm), leading to inefficient FRET at low n situations (e.g. E≈5% at n = 1) [12]. These systems are generally suitable only for high n situations [26–29] or those using very efficient quenchers, e.g. GNPs (gold nanoparticles) [25].

**Figure 1** | Schematic diagrams of commonly used sensing mechanisms in the QD–NA/aptamer conjugate-based fluorimetric biosensors

Red dots, fluorescent or intercalating dyes; black dot, organic or GNP quencher; blue object, luciferase. BRET, bioluminescence resonance energy transfer.

**QD–DNA/aptamer FRET sensors**

Figure 1 summarizes some common readout approaches in QD–NA/aptamer conjugate-based biosensors. Figures 1(A)–1(E) are QD–DNA conjugate sensors for DNA detection and Figures 1(F)–1(J) are QD–aptamer sensors for non-DNA target detection. Their specific sensing performances (e.g. detection limit and dynamic range) are summarized in Table 2. A unique advantage for FRET-based biosensor over other techniques is its short-range interaction: it only detects species within the FRET range (e.g. <10 nm), any unbound species are undetected because they cannot participate in the FRET process, allowing assays to be performed in a convenient separation-free format even with excess of unbound species [10,11].

Figures 1(A)–1(C) are ‘target on’ assays, where hybridization of target DNA to the QD–DNA conjugate produces the readout FRET signal: Figure 1(A) directly uses the hybridized DNA targets (dye-labelled) for FRET readout [11,18,24]; Figure 1(B) is a label-free alternative to Figure 1(A), which uses intercalated dye molecules within the hybridized dsDNA as acceptor for FRET readout [17]; and Figure 1(C) uses the target DNA to cross-link the QD-capture and dye-labelled reporter DNAs, forming a capture/target/reporter sandwich for FRET readout [13]. Figure 1(D) uses the FRET change resulting from conformational change of a QD-conjugated molecular beacon upon target DNA hybridization for signal readout [16]. Figure 1(E) is a displacement assay, where target DNA binding displaces the luciferase-DNA from the QD–DNA conjugate, reducing luciferase to QD-BRET (bioluminescence resonance energy transfer) signal as target readout [30].

Figures 1(F)–1(J) show common sensing mechanisms used in QD–aptamer biosensors for non-DNA targets. Figures 1(F)–1(H) are displacement assays where target-aptamer binding displaces the signal DNA strands from the QD–aptamer conjugate (e.g. GNP [25] or quencher-linked [26,27] DNAs in Figure 1(F); dye-labelled DNAs in Figure 1(G) [11,28]; and unlabelled DNAs with intercalated dyes in Figure 1(H) [29]), leading to FRET changes as readout signal. Figure 1(I) uses the QD fluorescence quenching by QD to bound target electron transfer as signal readout [31,32]. Figure 1(J) shows a target-induced assembly assay [33], where the aptamer sequence is split into two halves, each connected to half of a haemin-binding domain. The presence of target assembles the two-half aptamer units, leading to
the formation of a complete haemin-binding site acting as chemical luminescence centre and chemiluminescence resonance energy transfer signal for target readout [34,35].

A number of different targets, DNAs, proteins, metal ions and small molecules have been detected by the QD–NA/aptamer fluorimetric sensors (Table 2). The sensitivity and dynamic range vary significantly from target to target and assay types. In general, the detection limits achieved for macromolecular targets are significantly lower, by approximately three orders of magnitude, than those for small-molecule targets (nanomolar compared with micromolar). This appears to be a positive reflection of the differences in binding affinities (e.g. nanomolar compared with micromolar) and energies of these two types of aptamer–target binding interactions [4–6]. Another notable observation is the significantly higher sensitivity achieved for DNA detection by using the SMF (single-molecule fluorescence) readout over conventional FS (fluorescence spectroscopy)-based assays (48 fM compared with nanomolar), which can be attributed to the extremely high detection capability of the SMF approach, down to a single-molecule (QD) level [13]. For most QD–NA/aptamer biosensors using conventional FS readout, their sensitivities are comparable with or better than most other more established electrochemical [36], surface plasmon resonance [37] or quartz crystal microbalance [38] -based biosensors using direct detection without further target amplification (which typically have nanomolar sensitivity for DNA/protein, and micromolar for small-molecule/metal-ion targets). An advantage for the QD–NA/aptamer FRET biosensor is its ratiometric signal, which is effectively insensitive to instrument noise and/or signal fluctuations, allowing for accurate reliable detection even in the presence of excess of unbound species [10,11]. It should be noted that most of the QD–FRET biosensors reported to date have only demonstrated the ‘proof-of-principle’ application in clean buffers. Few have showed robust operation in complex media, such as serum or other biological fluids, which are more relevant to real clinical samples, probably due to limited stability and specificity of current QD–aptamer sensors.

<table>
<thead>
<tr>
<th>Sensing mechanism</th>
<th>Target</th>
<th>Detection method</th>
<th>Detection limit</th>
<th>Detectable range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>QD-BRET</td>
<td>DNA (22-mer)</td>
<td>FS</td>
<td>20 nM</td>
<td>20–130 nM</td>
<td>[30]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>DNA (30-mer)</td>
<td>SMF</td>
<td>48 fM</td>
<td>0.048–48 pM</td>
<td>[13]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>DNA (30-mer)</td>
<td>FS</td>
<td>1 nM</td>
<td>1–200 nM</td>
<td>[17]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>DNA (24-mer)</td>
<td>FS</td>
<td>0.5 nM</td>
<td>0–12 nM</td>
<td>[18]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>DNA (30-mer)</td>
<td>FS</td>
<td>12 nM</td>
<td>2–50 nM</td>
<td>[23]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>DNA (30-mer)</td>
<td>FS</td>
<td>35 pM</td>
<td>0–2 nM</td>
<td>[11]</td>
</tr>
<tr>
<td>QD-MB-FRET</td>
<td>Thrombin</td>
<td>FS</td>
<td>1 nM</td>
<td>1–500 nM</td>
<td>[29]</td>
</tr>
<tr>
<td>ET quenching</td>
<td>Thrombin</td>
<td>FS</td>
<td>10 nM</td>
<td>10–210 nM</td>
<td>[32]</td>
</tr>
<tr>
<td>ET quenching</td>
<td>Cocaine</td>
<td>FS</td>
<td>1 μM</td>
<td>1–1000 μM</td>
<td>[32]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>Mucin-1</td>
<td>FS</td>
<td>250 nM</td>
<td>0.25–2 μM</td>
<td>[33]</td>
</tr>
<tr>
<td>QD-FRET</td>
<td>Cocaine</td>
<td>SMF</td>
<td>0.5 μM</td>
<td>1–8 μM</td>
<td>[28]</td>
</tr>
<tr>
<td>QD-GNP quenching</td>
<td>Cocaine</td>
<td>FS</td>
<td>120 μM</td>
<td>50–1000 μM</td>
<td>[25]</td>
</tr>
<tr>
<td>QD-GNP quenching</td>
<td>Adenosine</td>
<td>FS</td>
<td>50 μM</td>
<td>50–2000 μM</td>
<td>[25]</td>
</tr>
<tr>
<td>QD ET quenching</td>
<td>Hg²⁺</td>
<td>FS</td>
<td>10 nM</td>
<td>0.01–100 μM</td>
<td>[31]</td>
</tr>
<tr>
<td>QD ET quenching</td>
<td>Ag⁺</td>
<td>FS</td>
<td>1 μM</td>
<td>1–30 μM</td>
<td>[31]</td>
</tr>
<tr>
<td>QD-CRET</td>
<td>ATP</td>
<td>FS</td>
<td>0.1 μM</td>
<td>0.1–100 μM</td>
<td>[34]</td>
</tr>
</tbody>
</table>

**Summary and outlook**

Significant progress in QD–NA/aptamer fluorimetric biosensors has been made over the last 10 years, where several assay formats capable of detection of different targets have been developed. Thanks to their unique short-range interaction readout mechanism and ratiometric signal [10,11], the QD–NA/aptamer FRET-based sensors can provide reliable multiplexed detection of different targets in a convenient separation-free manner. Since NA aptamers can be selected against virtually any targets of choice [1–4], the QD–NA/aptamer sensor thus appears to be a highly promising new sensing platform with broad biotechnological, environmental and clinical applications [8–11]. This is evident from the exponential growth in the number of both publications and citations over the last 10 years (see Supplementary Figure S1 at http://www.biochemsoctrans.org/bst/040/bst0400635add.htm). Despite these, most QD–NA/aptamer sensors reported to date have only demonstrated 'proof-of-principle' applications in clean buffers. Few have attempted in biological fluids or real clinical samples, limited mainly by the sensitivity, specificity and robustness of current QD–NA/aptamer sensors. Specifically, their sensitivity and specificity, especially assay robustness,
are yet to match those of current 'gold-standard' clinical assays such as ELISA (typically with picomolar sensitivity). Future research is likely to focus on improving the sensitivity, specificity and robustness of the QD-aptamer biosensors via optimization of water-solubilization [19] and QD-DNA conjugation [20] strategies, and extend their applications in real clinical samples.

**Funding**

This work was supported by the Leeds Biomedical Health Research Centre (BHRC) and the University of Leeds.

---

**References**


Received 2 March 2012
doi:10.1042/BSI201200599

©The Authors Journal compilation ©2012 Biochemical Society
SUPPLEMENTARY ONLINE DATA

Quantum dot–nucleic acid/aptamer bioconjugate-based fluorimetric biosensors

Dejian Zhou
School of Chemistry and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.

Figure S1 | The exponential growth in the number of publications (A) and citations (B) concerning QD–DNA fluorimetric biosensors over the last 10 years.

Numbers were obtained from the Web of Science database using topic search terms of 'QD' and 'DNA' and 'fluorescence'.

Received 2 March 2012
doi:10.1042/BST20120059

©The Authors Journal compilation ©2012 Biochemical Society