Quantum dot-nucleic acid/aptamer bioconjugate based fluorimetric biosensors

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Abbreviations used:

QD, quantum dot; FRET, Förster resonance energy transfer; BRET, bioluminescence resonance energy transfer; CRET, chemiluminescence resonance energy transfer; MPA: 3-mercaptopropionic acid; DHLA, dihydrolipoic acid; MAA, mercaptoacetic acid; NA, nucleic acid; GSH, glutathione; ssDNA, single-stranded DNA; EDC, *N*-(3-dimethyl-aminopropyl)-*N*-ethyl-carbodiimide; NHS, N-hydroxysuccinimide; TAMAR, tetramethylrhodamine; EG_3, 11-mercaptoundecyl tri(ethylene glycol); GNP, gold nanoparticle; SMF, single-molecule fluorescence; CDL, chelating dendritic ligand; FS, fluorescence spectroscopy; STV, streptavidin; HR, hydrodynamic radius; FWHM, full width at half maximum; FP, fluorescent protein.

Abstract

Over the past 10 years, fluorescent semiconductor quantum dot (QD)-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. This mini-review presents a brief account of the recent development on QD-nucleic acid (NA), particularly NA aptamer, conjugates based biosensors using the Förster resonance energy transfer (FRET) 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 concluded with challenges and outlook for the QD-NA/aptamer bioconjugate sensors.

NA Aptamer

NA Aptamers are short 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 SELEX (systematic Evlution of Ligand by Explontial Enrichment).[1,2] It is possible to select a 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 (nM to pM); production by totally *in vitro* protocols which combinated with chemical purification, can eliminate batch-to-batch variation; are more robust against thermal and chemical denaturation (they can sustain several rounds of denaturation/renaturation process without loosing ligand binding affinity), and can easily incorporate bespoke functional groups and/or fluorescent lables 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 DNAs, proteins, environmental pollutants, street drugs, viruses to whole cancer cells.[5,6]

QD-FRET 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 FWHMs of ~25-40 nm) are particularly attractive to FRET based sensing applications because such spectra 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 sourse, yet producing specific, narrow and synmetric emissions of different colours, which is very useful for multiplexing. Further, 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 which make direct excitation of QDs unavoidable. Furthermore, their relatively long lifetimes (*ca.* 10-100 ns *v.s.* < 5 ns for most organic fluorophores) also make dye to QD FRET inefficient. Therefore almost all most QD-FRET based sensors reported so far have exclusively used QD as the donor and dye as acceptor, but not vice versa.[9-11]

Because FRET efficiency (*E*, sensitivity) decreases dramatically 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 due to the signficant sizes of the QDs.[10] HRs for water-soluble QDs alone (core + surface coating, *ca*. ~5-25 nm depending on solubilisation strategies) can often be comparable or greater than the R_0 (*ca*. 4-7 nm) of most QD-dye FRET pairs prior to bioconjugation.[10,11] As a result, most QD-FRET sensors have relied on increasing the number of acceptor per QD (*n*) to improve the *E*, because $E = 1/[1 + r^6/(nR_0^6)]$ in a single-donor-multiple acceptors FRET system.[10] However, such systems are inefficient and often unsuitable for low *n* situations (*e.g.* 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] Desipte several strategies have been reported to make water-soluble QDs, so far none can produce QDs that are compact (HR < 5 nm), highly stable in biological buffers and resisting 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 solublised *via* capping with amphiliplic polymer[21] or PEGylated lipipids [22] are stable, resisting non-specific adsportion, but have large HRs (> 10 nm), limiting the sensitivity. **Table 1** summarises a few frequently used QD-bioconjugation chemistries for making compact QD-DNA conjugates and their *Es* at low *n* situations.

Table 1. FRET efficiencies (*E*) of some compact QD-DNA conjugates prepared *via* different bioconjugation chemistries at $n = 1$ except for those specifically indicated in the bracket. Abbreviations for "xxx-QD_{yyy}" are: xxx = QD surface capping ligand, $_{yyy}$ = QD emission wavelength (nm).

QD-dye FRET system	DNA conjugation chemistry	*E*	Readout	Ref
MPA-QD_{550}-Alexa 594	Thiolate self-assembly (ZnS shell)	81%	SMF	[14]
MPA-QD_{550}-Alexa 594	Thiolate self-assembly (ZnS shell)	~50%	FS	[14]
DHLA-QD_{550}-Atto 647N	Thiolate self-assembly (ZnS shell)	34%	FS	[11]
DHLA-QD_{510}-TAMAR	His_6-tag-metal affinity (ZnS shell)	60% (n=2)	FS	[16]
DHLA-QD_{590}-Cy5	His_6-tag-metal affinity (ZnS shell)	~21%	FS	[16]
MAA-QD_{520}-Cy3	Covalent coupling (surface ligand)	52%	Life time	[23]
MAA-QD_{600}-Alexa 647	Covalent coupling (surface ligand)	6.7%	Life time	[23]
EG_3-QD_{550}-Alexa 594	Covalent binding (surface ligand)	28%	FS	[17]
STV-QD_{585}-Cy5	STV-biotin interaction	~5.5%	SM	[12]

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 [16] used the His_6-tagged DNA and DHLA-QD self-assembly to produce compact QD-DNA conjugates and efficient FRET (E = 60% for n = 2). 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 MPA- or DHLA- QDs, producing compact QD-DNA conjugates and high E at low n situations.[11,14] Interestingly, the MPA (a monodentate ligand)-QD produced stronger E than the DHLA (a chelative ligand)-QD (80% *v.s.* 34% for n = 1), suggesting the QD surface capping ligands strongly affect the DNA conformation in the QD-DNA assemblies. For the MPA-QD, the DNA strands may wrap around the QD, yielding small r and strong E; while 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 hybridise to its complementary DNA, whereas DHLA-QD-ssDNA could.[11,14] Similar structural differences were also observed in a recent QD-FP (His_6-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_6-tag-Zn^{2+} coordination in the bioconjugation.[23]

Another approach to compact QD-DNA conjugate is to covalently couple the DNAs to 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 a MAA-capped QD. However, its specificity was rather low, with a FRET ratio of only ~2 between complementary and non-complementary DNA target, suggesting MAA cannot resist non-specific adsorption.[23] We found that EG_3 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 quantitation of low nM specific complementary DNA.[17] More recently, we have developed a novel CDL that can provide stable, compact and entangled capping to the QD, allowing highly specific detection of pM complementary DNA target.[11,18]

The third common approach to QD-DNA conjugate is *via* biospecific interactions, *e.g.* biotin-streptavidin interaction.[12,25,26,28-30] This process is very simple, by simply mixing commercially available STV-QD with biotinylated DNA usually lead 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.* $E \sim 5\%$ at $n = 1$).[12] These systems are generally suitable only for high *n* situations [26,28-30] or those using very efficient quenchers, e.g. GNPs.[25]

QD-DNA/Aptamer FRET sensors

Figure 1 summarises some common readout approaches in QD-NA/aptamer conjugate based biosensors. Schemes (**A-E**) are QD-DNA conjugate sensors for DNA detection and schemes (**F-J**) are QD-aptamer sensors for non-DNA target detection. Their specific sensing performance (*e.g.* detection limit and dynamic range) are summarised 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 the FRET process, allowing assays to be carried out in a convenient, separation-free format even with excess of unbound species.[10,11]

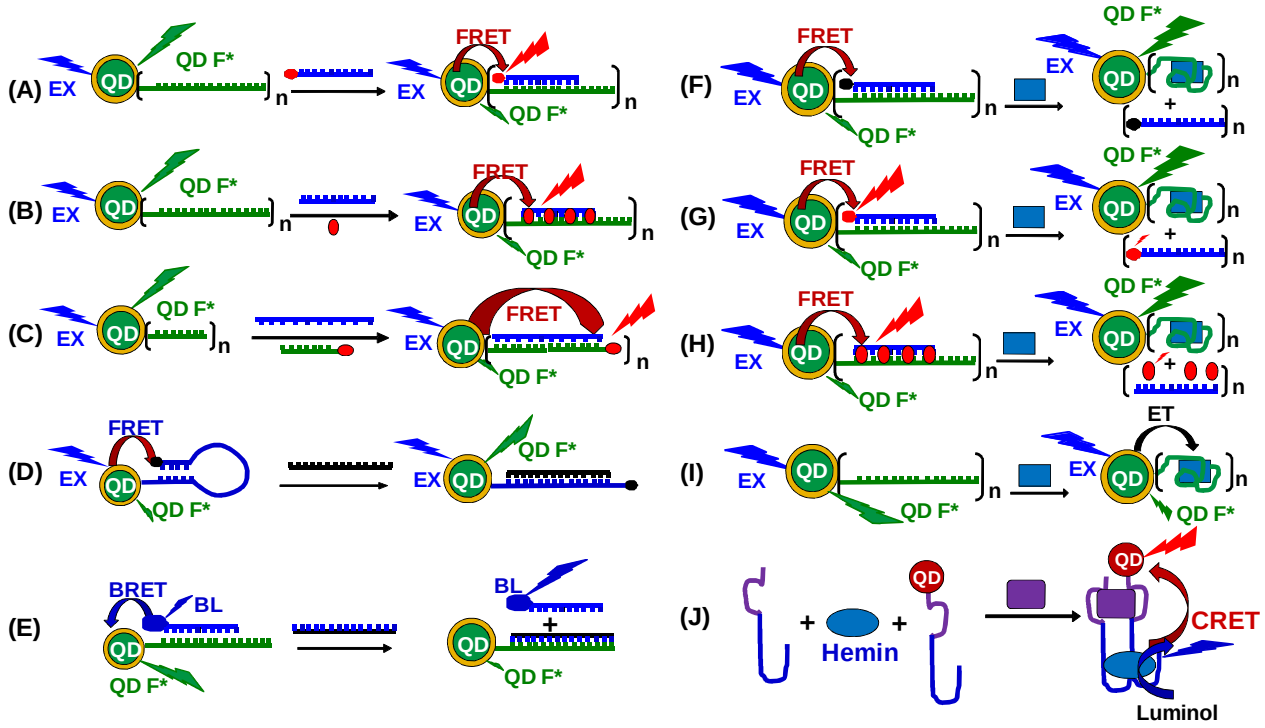

Figure 1. Schematics of commonly used sensing mechanisms in the QD-NA/aptamer conjugates based fluorimetric biosensors. Red dots: fluorescent or intercalating dyes; black dot: organic or GNP quencher; blue object: luciferace.

Schemes (**A-C**) are "target on" assays, where hybridisation of target DNA to the QD-DNA conjugate produces the readout FRET signal: (**A**) directly uses the hybridised DNA targets (dye-labelled) for FRET readout;[11,18,24] (**B**) is a label-free alternative to (**A**), which uses intercalated dye molecules within the hybridised dsDNA as acceptor for FRET readout;[17] and (**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] (**D**) uses the FRET change resulting from conformational change of a QD-conjugated molecular beacon upon target DNA hybridisation for signal readout. [16] (**E**) is a displacement assay, where target DNA binding displaces the luciferase-DNA from the QD-DNA conjugate, reducing luciferase to QD BRET signal as target readout.[27]

Schemes (**F**-**J**) show common sensing mechanisms used in QD-aptamer biosensors for non-DNA targets. (**F**-**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 [26,28] DNAs in (**F**); dye-labelled DNAs in (**G**);[11,29] and unlabelled DNAs with intercalated dyes in (**H**)),[30] leading to FRET changes as readout signal. (**I**) uses the QD fluorescence quenching by QD to bound target electron transfer as signal readout.[31,32] (**J**) is target induced assembly assay,[33] where the aptamer sequence is split into two halves, each connected to half of a hermin binding domain. The presence of target assembles the two-half aptamer units, leading to the formation of a complete hermin binding site acting as chemical luminescence centre and CRET signal for target readout.[34,35]

Table 2. Summary of the sensing performance of some QD-NA/aptamer biosensors

Sensing Mechanism	Target	Detection Method	Detection limit	Detectable range	Ref
QD-BRET	DNA (22 mer)	FS	20 nM	20-130 nM	[27]
QD-FRET	DNA (30 mer)	SMF	48 fM	0.048-48 pM	[13]
QD-FRET	DNA (30 mer)	FS	1 nM	1-200 nM	[17]
QD-FRET	DNA (30 mer)	FS	0.5 nM	0-12 nM	[18]
QD-FRET	DNA (24 mer)	FS	12 nM	2-50 nM	[23]
QD-FRET	DNA (30 mer)	FS	35 pM	0-2 nM	[11]
QD-FRET	Thrombin	FS	10 nM	10-1000 nM	[11]
QD-MB FRET	Thrombin	FS	1 nM	1-500 nM	[30]
ET quenching	Thrombin	FS	10 nM	10-210 nM	[32]
ET quenching	Cocaine	FS	1 μM	1-1000 μM	[32]
QD-FRET	Mucin-1	FS	250 nM	0.25-2 μM	[33]
QD-FRET	Cocaine	SMF	0.5 μM	1-8 μM	[29]
QD-GNP quenching	Cocaine	FS	120 μM	50-1000 μM	[25]
QD-GNP quenching	Adenosine	FS	50 μM	50-2000 μM	[25]
QD-ET quenching	Hg^{2+}	FS	10 nM	0.01-100 μM	[31]
QD-ET quenching	Ag^{+}	FS	1 μM	1-30 μM	[31]
QD-CRET	ATP	FS	0.1 μM	0.1-100μM	[34]

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 ~ 3 orders of magnitudes, than those for small-molecule targets (nM *v.s.* μM). This appears to be a positive reflection of the differences in binding affinities (*e.g.* nM v.s. μM) 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 readout over conventional FS based assays (48 fM *v.s.* nM), which can be attributed to the extremely high detection capability of the SMF approach, down to single-molecule (QD) level.[13] For most QD-NA/aptamer biosensors using conventional FS readout, their sensitivities are comparable 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 nM sensitivity for DNA/protein, and μM 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 so far 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, most likely due to limited stability and specificity of current QD-aptamer sensors.

Summary and Outlook

Significant progress in QD-NA/aptamer fluorimetric biosensors has been made over the past 10 years, where several assay formats capable of detection of different targets have been developed. Thanks for 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 both the numbers of publications and citations over the past 10 years (see Supporting Information, Figure 1). Despite these, most QD-NA/aptamer sensors reported so far 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, have yet to match those of current "gold-standard" clinical assays such as ELISA (typically with pM sensitivity). Future researches are likely to focus on improving the sensitivity, specificity and robustness of the QD-aptamer biosensors *via* optimisation of water-solubilisation [19] and QD-DNA conjugation [20] strategies, and extend their applications in real clinical samples.

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Reference

1 Tuerk, C. and Gold, L. (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **249**, 505-510.

2 Ellington, A. D. and Szostak, J. W. (1990) *In vitro* selection of RNA molecules that bind specific ligands. *Nature* **346,** 818-822.

3 Hermann, T. and Patel, D. J. (2000) Adaptive Recognition by Nucleic Acid Aptamers. *Science* **287**, 820-825.

4 Bunka, D. H. J. and Stockley, P. G. (2006) Aptamers come of age - at last. *Nat. Rev. Microbiol.* **4**, 588-596.

5 Song, S.P., Wang, L.H., Li, J., Zhao, J.L. and Fan, C.H. (2008) Aptamer-based biosensors. *Trends Anal. Chem*. **27**, 108-117.

6 Liu, J., Cao, Z. and Lu, Y. (2009) Functional nucleic acid sensors. *Chem. Rev.* **109**, 1948-1998.

7 Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., Sundaresan, G., Wu, A. M., Gambhir, S. S. and Weiss, S. (2005) Quantum Dots for Live Cells, in Vivo Imaging, and Diagnostics. *Science* **307**, 538–544.

8 Medintz, I. L.; Uyeda, H. T.; Goldman, E. R. and Mattoussi, H. (2005) Quantum Dot Bioconjugates for Imaging, Labelling and Sensing. *Nat. Mater*. **4**, 435–446.

9 Somers, R. C., Bawendi, M. G. and Nocera, D. G. (2007) CdSe nanocrystal based chem.-/bio-sensors. *Chem. Soc. Rev*. **36**, 579-591.
10 Medintz, I. L. and Mattoussi, H. (2009) Quantum dot-based resonance energy transfer and its growing application in biology. *Phys. Chem. Chem. Phys.* **11**, 17–45.
11 Zhang, H.Y., Stockley, P.G. and Zhou, D.J. (2011) Development of Smart Nanoparticle-Aptamer Sensing Technology. *Faraday Discuss.* **149**, 319-332.
12 Hohng, S. and Ha, T. (2005) Single-Molecule Quantum-Dot Fluorescence Resonance Energy Transfer *ChemPhysChem.* **6**, 956-960.
13 Zhang, C. Y., Yeh, H. C., Kuroki, M. T. and Wang, T. H. (2005) Single-quantum-dot-based DNA nanosensor. *Nat. Mater*. **4**, 826-831.
14 Zhou, D.J., Piper, J. D., Abell, C., Klenerman, D., Kang, D.-J. and Ying, L.M. (2005) Fluorescence Resonance Energy Transfer between a Quantum Dot Donor and a Dye Acceptor Attached to a DNA. *Chem. Commun.* 4807-4809.
15 Uyeda, H. T., Medintz, I. L., Jaiswal, J. K., Simon, S. M. and Mattoussi, H. (2005) Synthesis of Compact Multidentate Ligands to Prepare Stable Hydrophilic Quantum Dot Fluorophores. *J. Am. Chem. Soc.* **127**, 3870–3878.
16 Medintz, I. L., Berti, L., Pons, T., Grimes, A. F., English, D. S. Alessandrini, A., Facci, P. and Mattoussi, H. (2007) A Reactive Peptidic Linker for Self-Assembling Hybrid Quantum Dot−DNA Bioconjugates. *Nano Lett*. **7**, 1741-1748.
17 Zhou, D.J., Ying, L.M., Hong, X., Hall, E.A., Abell, C. and Klenerman, D. (2008) A Compact Functional Quantum Dot-DNA Conjugate: Preparation, Hybridization and Specific Label-free DNA Detection. *Langmuir* **24**, 1659-1664.
18 Zhou, D.J., Li, Y., Hall, E.A., Abell, C. and Klenerman, D. (2011) A chelating dendritic ligand capped quantum dot: preparation, surface passivation, bioconjugation and specific DNA detection. *Nanoscale* **3**, 201-211.
19 Liu, W., Greytak, A.B., Lee, J., Wong, C.R., Park, J., Marshall, L.F., Jiang, W., Curtin, P.N., Ting, A.Y., Nocera, D.G., Fukumura, D., Jain, R.K. and Bawendi, M. G. (2010) Compact Biocompatible Quantum Dots via RAFT-Mediated Synthesis of Imidazole-Based Random Copolymer Ligand. *J. Am. Chem. Soc.* **132**, 472-483.
20 Bernardin, A., Cazet, A., Guyon, L., Delannoy, P., Vinet, F., Bonnaffe, D. and Texier, I. (2010) Copper Free Click Chemistry for Highly Luminescent Quantum Dot Conjugates: Application to *in Vivo* Metabolic Imaging. *Bioconjugate Chem*. **21**, 583–588.
21 Yu, W.W., Chang, E., Falkner, J.C., Zhang, J.Y., Al-Somali, A.M., Sayes, C.M., Johns, J., Drezek, R. and Colvin, V.L. (2007) Forming Biocompatible and Nonaggregated Nanocrystals in Water Using Amphiphilic Polymers. *J. Am. Chem. Soc.* **129**, 2871-2879.
22 Dubertret, B., Skourides, P., Norris, D. J., Noireaux, V., Brivanlou, A. H. and Libchaber, A. (2002) In Vivo Imaging of Quantum Dots Encapsulated in Phospholipid Micelles. *Science* **298**, 1759-1762.
23 Zhang, Y., Zhang, H.Y., Hollins, J., Webb, M.E. and Zhou, D.J. (2011) Small-molecule ligands strongly affect the Förster resonance energy transfer between a quantum dot and a fluorescent protein. *Phys. Chem. Chem. Phys*. **13**, 19427–19436.
24 Algar, W. R. and Krull, U. J. (2007) Towards multi-colour strategies for the detection of oligo-nucleotide hybridization using quantum dots as energy donors in fluorescence resonance energy transfer (FRET). *Anal. Chim. Acta* **581**, 193-201.
25 Liu, J., Lee, J. H. and Lu, Y. (2007) Quantum Dot Encoding of Aptamer-Linked Nanostructures for One-Pot Simultaneous Detection of Multiple Analytes, *Anal. Chem*. **79**, 4120-4125.
26 Levy, M., Cater, S. F. and Ellington, A. D. (2005) Quantum-Dot Aptamer Beacons for the Detection of Proteins, *ChemBioChem*. **6**, 2163-2166.

27 Cissell, K. A., Campbell, S. and Deo, S. K. (2008) Rapid, single-step nucleic acid detection, *Anal. Bioanal. Chem*. **391**, 2577-2581.
28 Kim, G.I., Kim, K. W., Oh, M. K. and Sung, Y. M. (2009) The detection of platelet derived growth factor using decoupling of quencher oligonucleotide from aptamer/quantum dot bioconjugates. *Nanotechnology*, **20,** 175503.
29 Zhang, C.Y. and Johnson, L. W. (2009) Single quantum-dot-based aptameric nanosensor for cocaine, *Anal. Chem*. **81**, 3051-3055.
30 Chi, C. W., Lao, Y. H., Li, Y. S. and Chen, L. C. (2011) A quantum dot-aptamer beacon using a DNA intercalating dye as the FRET reporter: application to label-free thrombin detection, *Biosens. Bioelectron*. **26**, 3346-3352.
31 Freeman, R., Finder, T. and Willner, I. (2009) Multiplexed Analysis of Hg^{2+} and Ag^{+} Ions by Nucleic Acid Functionalized CdSe/ZnS Quantum Dots and Their Use for Logic Gate Operations. *Angew. Chem., Int. Ed*. **48**, 7818-7821.
32 Raichlin, S., Sharon, E., Freeman, R., Tzfati, Y. and Willner, I. (2011) Electron-transfer quenching of nucleic acid-functionalized CdSe/ZnS quantum dots by doxorubicin: a versatile system for the optical detection of DNA, aptamer-substrate complexes and telomerase activity. *Biosens. Bioelectron*. **26**, 4681-4689.
33 Cheng, A. K. H., Su, H., Wang, Y. A. and Yu, H.-Z. (2009) Aptamer-based detection of epithelial tumor marker mucin 1 with quantum dot-based fluorescence readout, *Anal. Chem.* **81**, 6130-6139.
34 Freeman, R.; Liu, X. Q. and Willner, I. (2011) Chemiluminescent and Chemiluminescence Resonance Energy Transfer (CRET) Detection of DNA, Metal Ions, and Aptamer-Substrate Complexes Using Hemin/G-Quadruplexes and CdSe/ZnS Quantum Dots. *J. Am. Chem. Soc*. **133**, 11597–11604.
35 Sharon, E.; Freeman, R. and Willner, I. (2010) CdSe/ZnS Quantum Dots-G-Quadruplex/Hemin Hybrids as Optical DNA Sensors and Aptasensors. *Anal. Chem*. **82**, 7073–7077.
36 Peng, H., Soeller, C., A. Vigar, N. A., Caprio, V. and Travas-Sejdic, J. (2007) Label-free detection of DNA hybridization based on a novel functionalized conducting polymeret al., *Biosens. Bioelectron*. **22**, 1868-1873.
37 He, L., Musick, M. D., Nicewarner, S. R., Salinas, F. G., Benkovic, S. J., Natan, M. J. and Keating, C. D. (2000) Colloidal Au-Enhanced Surface Plasmon Resonance for Ultrasensitive Detection of DNA Hybridization. *J. Am. Chem. Soc.* **122**, 9071–9077.
38 Deisingh, A. K. and Thompson, M. (2001) Sequences of E. coli 0157:H7 detected by a PCR-acoustic wave sensor combination. *Analyst*, **126**, 2153-2158.