

Nucleic Acid-Passivated Semiconductor Nanocrystals: Biomolecular Templating of Form and Function

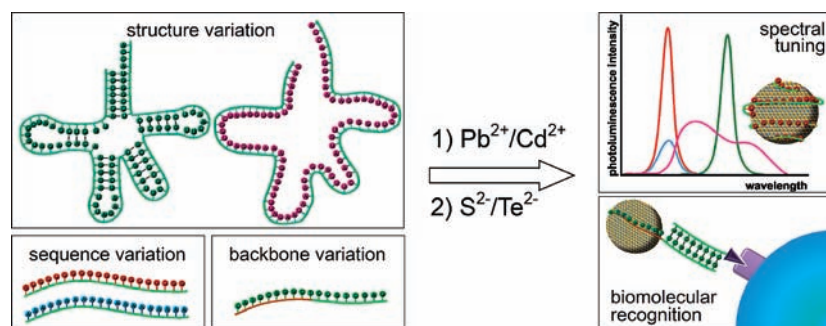
NAN MA,^{†,§} GRIGORY TIKHOMIROV,^{†,§} AND SHANA O. KELLEY^{*,†,‡}

[†]Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, and

[‡]Department of Biochemistry, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

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CON SPECTUS



Bright, photostable luminescent labels are powerful tools for the *in vitro* and *in vivo* imaging of biological events. Semiconductor nanocrystals have emerged as attractive alternatives to commonly used organic lumophores because of their high quantum yields and the spectral tunability that can be achieved through synthetic control. Although conventional synthetic methods generally yield high-quality nanocrystals with excellent optical properties for biological imaging, ligand exchange and biological conjugation are necessary to make nanocrystals biocompatible and biospecific. These steps can substantially deteriorate the optical characteristics of these nanocrystals. Moreover, the complexity of multistep nanocrystal synthesis, typically requiring inert and anhydrous conditions, prohibits many end users of these lumiphores from generating their own custom materials. We sought to streamline semiconductor nanocrystal synthesis and develop synthetic routes that would be accessible to scientists from all disciplines. In search of such an approach, we turned to nucleic acids as a programmable and versatile ligand set and found that these biomolecules are indeed appropriate for biocompatible semiconductor nanocrystals preparation. In this Account, we summarize our work on nucleic acids-programmed nanocrystal synthesis that has resulted in the successful development of a one-step synthesis of biofunctionalized nanocrystals in aqueous solution.

We first discuss results obtained with nucleotide-capped cadmium and lead chalcogenide-based nanocrystals that served to guide further investigation of polynucleotide-assisted synthesis. We investigated the roles of individual nucleobases and their structures in passivation of the surfaces of nanocrystals and modulating morphology and optical characteristics. The nucleic acid structures and sequences and the reaction conditions greatly influence the nanocrystals' optical properties and morphologies. Moreover, studies using live cells reveal low toxicity and rapid uptake of DNA-passivated CdS nanocrystals, demonstrating their suitability for bioimaging.

Finally, we describe a new approach that leads to the production of biofunctionalized, DNA-capped nanocrystals in a single step. Chimeric DNA molecules enable this strategy, providing both a domain for nanocrystal passivation and a domain for biomolecule recognition. Nanocrystals synthesized using this approach possess good spectral characteristics as well as high specificity to cognate DNA, protein, and cancer cell targets. Overall, this approach could make nanocrystal lumiphores more readily accessible to researchers working in the biological sciences.

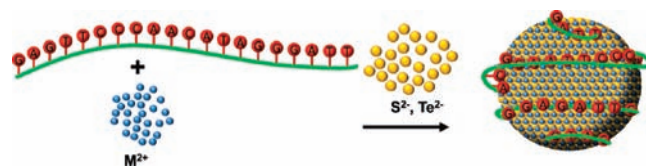
Introduction

Nanoscale semiconductor crystals exhibit size- and shape-dependent physicochemical properties that are distinct from those of the corresponding bulk solids.¹ These unique properties, arising from size-dependent bandgaps, discrete band structures, and confinement of charge carriers enable the diverse applications proposed for semiconductor nanocrystals in the area of physics,² biology,^{3,4} and medicine.^{5,6} The application of semiconductor nanocrystals as fluorescent labels for the *in situ* investigation of cellular processes in life sciences and medicine has in particular received much attention. Indeed, emission tunability, brightness, and superior photostability have made nanocrystals a promising alternative to conventionally used organic fluorophores for *in vitro* bioimaging.⁷

Great strides have been made in the preparation of monodisperse nanocrystals with controlled spectral properties.^{1,8} Most preparation methods involve synthesis in organic solvents and postsynthetic modification to render nanocrystals biocompatible and biospecific.^{9,10} We sought a “greener”,¹¹ simpler, safer, and efficient alternative to the organic routes for biocompatible nanocrystals synthesis and, in this search, turned to biological systems for inspiration. We designed a new approach that would employ a biological template, a nucleic acid molecule, to serve as a modulator of nanocrystal synthesis (Scheme 1).

The role of nucleic acids in living organisms is to store and transfer genetic information, a function seemingly unrelated to semiconductor nanocrystals synthesis. However, a closer examination of the nucleic acids reveals that they have many characteristics advantageous for nanocrystal synthesis: (1) The anionic oxygen of phosphate, the hydroxyl groups of sugar moieties, and nitrogen and oxygen atoms of nucleobases can interact with metal ions that are precursors for nanocrystals, for example, Cd^{2+} , Pb^{2+} , and Hg^{2+} .^{12–14} (2) The three-dimensional structures of naturally occurring and artificial nucleic acids are well-defined, and the sizes of many of these biological nanostructures are comparable to those of conventional QDs, which could serve to confine the nanocrystals' growth and control size. (3) Efficient chemical and biological techniques are currently available for polynucleotide synthesis^{15,16} and can be used to access a wide variety of nucleic acids with desired functionalities. (4) Nucleic acids are water-soluble and, if they could be used as ligands, could produce water-soluble nanocrystals that would not need ligand exchange to be compatible with cellular systems. This combination of proper-

SCHEME 1. Schematic Representation of Nucleic Acid-Templated Nanocrystal Synthesis



ties indicated to us that nucleic acids could be effective and functional ligands for nanocrystals.

Nucleic Acid Monomers As Semiconductor Nanocrystals Ligands

The large amount of data on the synthesis of nanoparticles accumulated in recent years has allowed the attributes of ligands capable of facilitating nanocrystal growth to be defined. Effective ligands for nanoparticle growth must have qualities that allow them to (i) form a precursor complex with metal ions to control ion release and nanoparticle growth rate as well as to facilitate heterogeneous nucleation, (ii) terminate the nanoparticle growth and prevent agglomeration once most precursors in the solution are consumed, (iii) passivate nanocrystal surface defects to promote maximal luminescence, and (iv) render nanoparticles soluble in the solvent in which the synthesis is conducted.¹⁷ To determine whether nucleic acids can indeed serve as ligands for semiconductor NP synthesis and to identify the DNA functional groups essential for this role, we initiated our work in this area with the four natural mononucleotides ATP, GTP, UTP, and CTP, and used these molecules as the sole ligand in the synthesis of PbS and CdS.

When PbS was synthesized using mononucleotide ligands in aqueous solution, only GTP was found to generate luminescent nanocrystals (Figure 1).¹⁸ ATP, CTP, and UTP produced mainly nonsoluble bulk materials with little luminescence. Therefore, functional groups unique to the base moiety of GTP appear to play an important role in nanocrystal formation. Previous studies suggest that either the exocyclic N2 or endocyclic N7 of GTP can interact with Pb^{2+} ions.¹⁹

To test the role of these moieties, the GTP analogues 7-methyl-GTP and inosine triphosphate (ITP, lacks N2) were investigated. When ITP was used instead of GTP, insoluble material was produced instead of nanocrystals. In contrast, methylation of the N7 resulted only in blue shift of the nanocrystals emission maxima relative to GTP (Figure 1), indicating that growth was slowed and smaller particles were formed. FTIR measurements suggested that the exocyclic N2 interacts directly with the semiconductor surface, therefore serving an essential role in nanocrystals surface passivation. Interaction

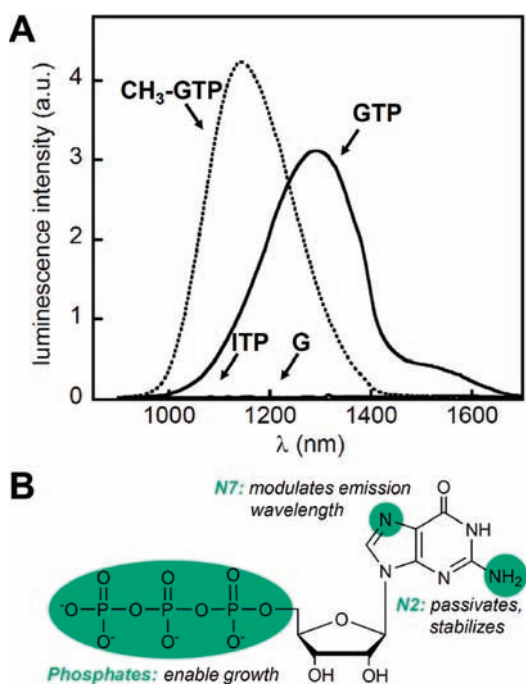


FIGURE 1. Effect of specific chemical functionalities present on GTP on PbS nanocrystal synthesis: (A) luminescence spectra obtained when GTP, G, ITP, and 7-CH₃-GTP were used for PbS synthesis; (B) proposed roles of phosphate and base functionalities on GTP in nanoparticle nucleation, growth, termination, stabilization, and passivation.¹⁸

with the N7 was not observed, indicating that this functional group is involved with the growth process but does not serve as a ligand.

The phosphate groups displayed on nucleotides were also shown to be essential for PbS nanocrystals formation. Non-soluble products were generated when G, the nucleoside lacking any phosphate, was used instead of GTP in nanocrystal synthesis, most likely because the negatively charged phosphate groups are necessary to prevent nanocrystal aggregation and to make nanocrystals soluble in water (Figure 1). Moreover, FTIR measurements indicate that the phosphate groups bind to Pb²⁺ ions initially; however, after the introduction of the S²⁻ source, the exocyclic N2 becomes the dominant binding site.¹⁸ Thus, the phosphate groups are important in the early stages of nanocrystal synthesis given that they serve to control the reaction by sequestering lead and feeding it into the reaction mixture with equilibrium control.

When nucleotide-mediated CdS nanocrystal formation was investigated, similar trends with some significant variations were observed.²⁰ The most luminescent CdS nanoparticles were again obtained in the presence of GTP. Materials synthesized with ATP, UTP, and CTP possessed luminescence at least an order of magnitude less than those produced by GTP. Interestingly, unlike in the case of PbS nanocrystals, using

7-methyl-GTP had a more substantial effect on CdS nanocrystals properties than the ITP analog. The luminescence intensity of 7-methyl-G-templated nanocrystals was reduced by about an order of magnitude compared with that of GTP-templated ones, which suggests that the endocyclic N7 plays a more important role in CdS nanocrystal synthesis than in the case of PbS. However, just as was observed for PbS, the use of GDP, GMP, and G in place of GTP yielded CdS materials with significantly reduced luminescence and solubility. This indicates that the negatively charged triphosphate groups of mononucleotides are critical for CdS synthesis just as they were for PbS. However, for CdS, the phosphate groups were found to participate directly in passivation, presumably together with the amino group of the base moiety.^{21,22}

In general, the studies performed with mononucleotide ligands indicate that the passivation of nanocrystals is dominated by the base moieties, while the phosphate groups prevent their aggregation by maintaining electrostatic repulsion between nanocrystals.

Polymeric Nucleic Acids as Nanocrystal Ligands: Effects of Sequence and Structure

Given the observations noted above linking the identities of nucleic acid monomers with nanocrystal properties, it can be envisioned that the virtually infinite number of sequence combinations in a DNA or an RNA strand could make these molecules highly tunable and versatile ligands for nanocrystals synthesis. Sequence determines the functionalities on each polynucleotide strand available for nanocrystal passivation, as well as the number of passivation sites and the spacing between them. Moreover, the secondary and tertiary structure of nucleic acids could affect the nanoparticle growth kinetics by interacting with the nanoparticle surface at different growth stages, leading to the formation of nanoparticles with different sizes or shapes.²³ This additional degree of freedom could be exploited to produce a greater diversity of nanocrystal products with unique properties.

To directly test these ideas and study how nanocrystals were affected by nucleic acid structure, nanocrystal size, morphology, and optical properties were monitored when a biologically active tRNA (*Escherichia coli* tRNA^{Leu}) was used as a ligand.²⁴ The wild type (WT) tRNA is a highly structured RNA molecule with a well-defined cloverleaf secondary structure and L-shape tertiary structure.²⁵ The overall size of a tRNA molecule is about 5 nm, which is comparable to the size of typical nanocrystals (Figure 2).

To vary the RNA structure and track its influence on nanocrystal formation, we generated an unstructured mutant (MT)

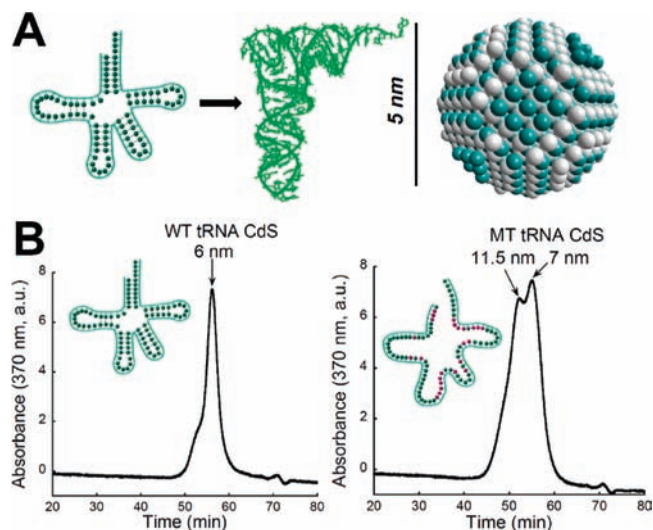


FIGURE 2. Transfer RNA as a nanocrystal template and ligand: (A) two-dimensional and three-dimensional WT tRNA structures and the same scale view of a 5.4 nm CdS spherical nanocrystal along the [111] axis; (B) gel filtration chromatography of WT and MT tRNA–CdS complexes.²⁴

tRNA by introducing several structure-perturbing mutations to disrupt the base pairing within all five stems of WT tRNA. Interestingly, the structural properties of CdS nanocrystals made with structured and unstructured RNA were quite distinct. Nanocrystals made with unstructured RNA were significantly larger and more polydisperse than those made with structured RNA. (Figure 2). TEM analysis revealed that WT tRNA produced monodisperse nanocrystals with spherical nanoparticles possessing 4.4 ± 0.4 nm diameters, while MT tRNA yielded larger and more irregular nanoparticles with diameters up to 7 nm. Gel filtration chromatography, which reported on the hydrodynamic diameters of the materials, supported this trend (Figure 2). Clearly, the structure of nucleic acids has a strong influence on CdS nanocrystals morphology. Since the unstructured MT RNA molecule is much more flexible compared with WT tRNA, it is likely to adopt various conformations when it binds to Cd^{2+} and CdS nanocrystal surfaces during the initiation and growth stages, which could result in coexistence of several growth regimes and, as a result, nanocrystal sizes.

With clear evidence that nucleic acids *structure* could modulate the properties of semiconductor nanocrystals, we desired to understand the roles of the *sequences* of extended unstructured oligomers. Four DNA homooligomers (A_{20} , G_{20} , C_{20} , T_{20}) were studied as ligands in CdS nanocrystals synthesis.²⁶ Unlike mononucleotides,²⁰ all four DNA oligomers produced water-soluble luminescent nanoparticles, demonstrating that these ligands have enhanced activity in nanocrystal growth. Moreover, increased stabilities were observed for nanocrystals synthesized with polynucleotides versus the correspond-

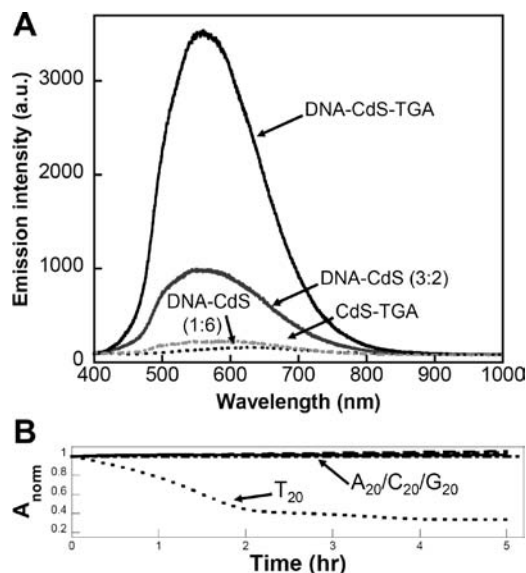


FIGURE 3. Luminescence and stability of DNA–CdS: (A) emission spectra for nanocrystals made with DNA and TGA as a coligand at different DNA/Cd/TGA ratios; (B) stability of DNA–CdS nanocrystals obtained with A_{20} , C_{20} , G_{20} , or T_{20} in a phosphate buffer monitored by absorbance at 430 nm.²⁶

ing mononucleotides most likely because the additive binding of adjacent nucleotides strengthens the overall binding through cooperativity. Interestingly, among the four homooligomer-passivated nanocrystals, those synthesized with T_{20} exhibit the lowest stabilities (Figure 3). Since thymine lacks an exocyclic amino group, it may be the poorest ligand, even when present in an oligomeric form.

Different quantum efficiencies were observed for CdS nanocrystals synthesized with the four 20-mer homooligomers.²⁶ Interestingly, despite lowered stabilities, the pyrimidine oligomers C and T produced CdS nanocrystals that exhibit higher luminescence intensity than those obtained with purine oligomers A and G. Given that the emission observed for these CdS nanocrystals represents that from “trapped states”, this trend may indicate that stronger ligands like A and G promote the formation of trapping sites, leading to higher luminescence intensities for materials with weaker ligands.

Clearly, nucleic acid sequence also influences nanocrystal properties. The effect of polynucleotide functional groups on nanocrystal passivation is enhanced when several of these functionalities act cooperatively in the same chain. The additive affinities of individual units in the oligomeric chains must be considered when extrapolating results obtained with mononucleotides to polynucleotides. This effect has been observed with nonbiological ligands,²⁷ and also appears to be operative with DNA ligands, as longer oligonucleotides yield more stable and emissive materials.²⁶ Moreover, the shape and rigidity of oligomeric chains, which is dependent on the

sequence, may also affect the binding of nucleic acids to nanocrystal surfaces.^{28,29} Others have investigated the dependence of CdS nanocrystal optical properties, size, and dispersity on oligonucleotide composition³⁰ and length.³¹ This work, in combination with ours, indicates that complicated DNA sequences could be designed to “encode” nanocrystal luminescence intensity and wavelength.

Optical Properties of DNA-Passivated Nanocrystals

For bioimaging applications, it is important to be able to prepare nanocrystals with luminescence maxima in a wide spectral range to provide versatility and compatibility with luminophores used for spectral multiplexing in multitarget assays and also to avoid overlap with biological tissues. In addition, individual populations of emitting nanocrystals should possess narrow spectral distributions as well as resistance to photobleaching.

The DNA-passivated CdS nanocrystals originally made exhibit broad luminescence spectra between 500 and 700 nm, which is a characteristic of trapped site emission rather than of band gap emission.^{28,32–34} The quantum yields of these first-generation materials were low because of the emitting state structure. However, the emission of these materials was improved with the addition of a low molecular weight thiol ligand, thioglycolic acid (TGA).²⁶ TGA molecules were proposed to bind to the exposed solvent trap sites on Cd²⁺-rich CdS nanocrystal surfaces through the strong Cd²⁺–thiol interaction, leading to a 3-fold increased luminescence intensity (Figure 3). The binding of the TGA ligand to the CdS nanocrystal surface may alter the energies of trap sites and reduce nonradiative decay probability.³⁴

Another parameter that significantly affected nanocrystal properties was the Cd²⁺ to S²⁻ ratio used for the synthesis. Several studies have reported that a slight excess of Cd²⁺ is necessary to generate highly luminescent CdS nanocrystals.^{35,36} This was also observed for DNA-passivated CdS nanocrystals where the nucleotide to Cd²⁺ to S²⁻ ratio that produced the nanocrystals with the highest luminescence was found to be 3:2:1.²⁶

Solution pH also affects nanocrystal optical properties. Postsynthetic activation of DNA-passivated CdS nanocrystals by the addition of NaOH and Cd(ClO₄)₂ resulted in a 2–3-fold increase in luminescence intensity without a change in emission maximum.³⁷ Nanocrystal luminescence was also increased by performing the synthesis at higher pH. This likely results from hydroxyl groups binding to the trap sites that are unpassivated at lower pHs.²⁰

Overall, limited progress was made with increasing the emission yields of DNA-passivated CdS. Very broad luminescence profiles were consistently obtained, and the quantum yields remained in the single digits despite extensive optimization. Dramatic increases in quantum yield, however, were realized by switching inorganic material. Indeed, the use of CdTe as a material system for DNA-passivated nanocrystals led to much more optimal luminescence characteristics, with photoluminescence quantum yield (PLQY) up to 15%.³⁸

One source of this improvement may be the slower kinetics of CdTe formation relative to CdS. CdS nanocrystals form instantly upon mixing of Cd²⁺–DNA complex with S²⁻ at 25 °C, while DNA-passivated CdTe nanocrystals grow over about an hour at 100 °C. It is believed that the growth of CdS nanocrystals at low temperature is under kinetic control,³⁹ which results in a rough surface that has various defects. The CdTe nanocrystals, in contrast, grow at a slower rate and elevated temperature closer to the equilibrium of growth and dissolution, which allows the CdTe nanocrystals to develop a more thermodynamically stable surface with fewer defects.⁴⁰ These materials have therefore constituted the focus of our most recent work,³⁸ which will be described in a subsequent section.

Cytotoxicity and Cellular Uptake of DNA-Passivated CdS Nanocrystals

Cytotoxicity has been a major concern associated with nanocrystals for biological applications. For cadmium-based nanocrystals, cytotoxicity can originate from the release of Cd²⁺ ions from the nanoparticle core, and the generation of reactive oxygen species upon irradiation of the nanocrystals.^{41–43} The stability of the ligand coating covering the nanocrystal surface is a major determinant of cytotoxicity. As mentioned above, DNA-passivated CdS nanocrystals are highly stable in high ionic strength phosphate buffers due to the cooperative binding of polymeric DNA molecules. To directly test their toxicity against living cells, we tested CdS nanocrystals synthesized with four different DNA homopolymers (A₂₀, C₂₀, G₂₀, T₂₀) by incubating HeLa cells with these nanocrystals. Little effect on cell viability was observed, suggesting that only a negligible amount of Cd²⁺ ions was released from DNA-passivated CdS nanocrystals.²⁶ Nanocrystals prepared by traditional organometallic procedures and solubilized via ligand exchange usually exhibit lower stabilities with increased surface decomposition and Cd²⁺ release.^{41,44} It should be noted, however that these materials were not directly compared with DNA-passivated nanocrystals and therefore solid evidence of their relative toxicities is lacking. A comprehensive analysis of

the toxicity profiles of the biotemplated materials will be warranted for further biological use.

The cellular uptake and localization of DNA-passivated CdS nanocrystals was also studied.²⁶ Rapid internalization and lysosomal sequestration was observed, which is consistent with previous studies of nonspecific cellular uptake of nanocrystals through endocytosis.⁴⁵ Interestingly, when HeLa cells were treated with TGA-coliganded CdS nanocrystals, less lysosomal trapping and more even distribution within cells were observed. Thus, a surface coating can alter the cellular trafficking of DNA–nanocrystals.

Generation of Biofunctionalized DNA–Nanocrystals Using a One-Pot Synthesis

The success achieved in using DNA molecules as templates for nanocrystal synthesis prompts an important question: can sequences be designed that will serve both as ligands and as recognition elements? Given that the most powerful applications of nanocrystals can only be realized if the materials can be made specific toward a biological target, this latter issue is of significant interest if the materials are ever to be widely used. Also, the studies performed to understand sequence and structure did not address whether sequences that were used as nanocrystal ligands remained active as binding partners for complementary sequences. The recognition ability of a DNA strand absorbed on the surface could be compromised since its phosphate and nucleobase functionalities are involved in the surface passivation. To circumvent this issue, we moved toward designing DNA-based ligands that would contain two chemically distinct domains with different roles for nanocrystal liganding and functionalization.

Bifunctional DNA templates with one domain responsible for nanocrystal passivation and the other domain for biorecognition were designed to test whether biofunctionalized nanocrystals could be generated with nucleic acid ligands and affinity domains.³⁸ Phosphorothioate DNA, where the negatively charged oxygen atom on the phosphate backbone is replaced by sulfur, was used as a nanocrystal passivating fragment. Phosphorothioate nucleotides have been shown to possess much higher affinities for Cd²⁺ ions than phosphate nucleotides.^{46–48} By combination of a phosphorothioate fragment with a phosphate fragment in one DNA strand, the phosphorothioate domain can be expected to bind to the nanocrystals, and the phosphate domain would then be free to interact with various biomolecules and to carry out recognition of a specific target (Figure 4). Thus, biofunctionalized nanocrystals for biotargeting and bioimaging could be obtained in a simple one-step aqueous synthesis.

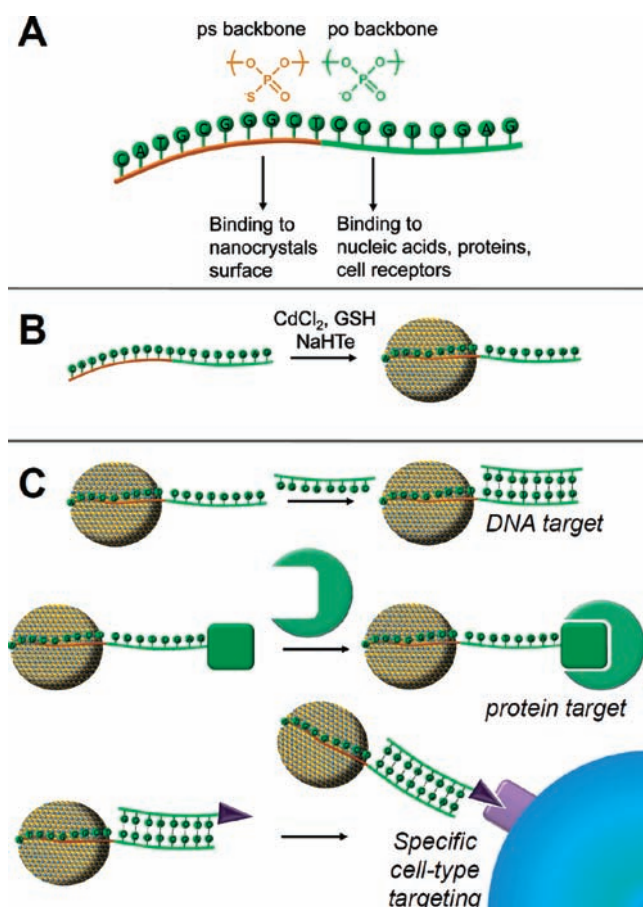


FIGURE 4. DNA-programmed and functionalized CdTe nanocrystals: (A) design of chimeric oligonucleotides with ligand (ps) and recognition (po) domains; (B) schematic representation of one-pot biofunctionalized nanocrystal synthesis; (C) binding to biological targets.

To test this concept, we designed a chimeric DNA template (ps–po) composed of a 10-nucleotide phosphorothioate DNA segment and a 10-nucleotide phosphate DNA segment that are joined together by a five-nucleotide poly-A linker.³⁸ The ps–po DNA produced monodisperse CdTe nanocrystals ranging from 6.0 to 6.5 nm in size with quantum yields of ~15%. To assess the availability of the DNA strands on nanocrystals to bind to biomolecule targets, we first evaluated the hybridization efficiency of each DNA domain with a DNA target. As expected, the po domain possessed much higher hybridization efficiency than the ps domain, which indicates that the ps domain is bound to CdTe nanocrystals more strongly than the po domain.

We then explored the binding of these nanocrystals to protein targets and cancer cells. The thrombin binding aptamer (TBA)^{49,50} was introduced as the po domain, and the binding of ps–po–TBA nanocrystals with thrombin was evaluated. A high binding efficiency was observed for the ps–po–TBA-passivated CdTe nanocrystals with thrombin, and the binding was

confirmed to be sequence-specific. PbS nanocrystals obtained in a similar one-pot procedure for thrombin detection have been reported recently⁵¹ indicating that other material systems could be used in such an approach.

We further evaluated the binding of nanocrystals with specific cancer cells by introducing a cancer cell-binding aptamer.⁵² Specific binding of aptamer-functionalized nanocrystals with CCRF-CEM cells, a leukemia-derived cell line, versus Ramos cells, a lymphoma-derived cell line, was observed. The discrimination of these two cell lines indicates that these biofunctionalized materials could be used for cellular imaging applications where tracking specific cell types is of interest.

These results are most significant because they demonstrate for the first time that functionalized semiconductor nanocrystals can be made in a single step using a ligand source that is available to any researcher who can access a source of synthetic DNA. This advance should be of value to those who desire to use nanocrystals for imaging and biomolecular detection but have difficulty using commercially available sources of nanocrystals and lack the infrastructure and equipment to use conventional multistep organometallic synthesis.

Summary

Semiconductor nanocrystals possess optical properties that are superior to traditional organic fluorescent dyes. However, wide adoption of nanocrystals for biological imaging has not yet been realized largely due to the absence of practical synthetic methods for the preparation of biocompatible and nontoxic nanocrystals appended with customized recognition domains. Our nucleic acid-directed synthesis of nanocrystals is conducted in water under mild conditions in the presence of mononucleotides, DNA or RNA, metal ions, and a chalcogenide source. The use of nucleic acids as ligands for nanocrystal synthesis provides a powerful tool for the rational design of nanocrystals via nucleic acid sequence variation. There are many unanswered questions about the structure of the complexes of nanocrystals with nucleic acids, for example: How many nucleic acid strands are there in the complex? What are their conformation and the mode of interaction with the nanocrystals surface? To answer these and other questions, nanocrystal structures and the growth mechanism will be studied in greater detail. Also, the use of *in vitro* evolution strategies may accelerate the search for optimal nucleotide sequences. The limited search through sequence space for nucleic acid ligands for semiconductor nanocrystals conducted so far has yielded sequences that are of great utility for making functional materials, and it is reasonable to expect

that a combinatorial approach could propel this research area forward significantly.

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BIOGRAPHICAL INFORMATION

Nan Ma received his B.S. degree in Chemistry from Peking University in 2004. He obtained a Ph.D. in Pharmaceutical Sciences at University of Toronto under the guidance of Professor Shana O. Kelley working on nucleic acids-templated semiconductor nanocrystals in 2009. He is currently a postdoctoral fellow at Stanford Medical School in the research group of Prof. Jianghong Rao.

Grigory Tikhomirov received a B.S. degree in Chemistry from Moscow State University in 2003. He earned his Ph.D. from the University of Alberta in 2008 working with Professor Hicham Feniri at the National Institute for Nanotechnology. He is currently a postdoctoral fellow in the laboratory of Professor Shana O. Kelley at the University of Toronto.

Shana Kelley is a Professor in the Departments of Biochemistry and Pharmaceutical Sciences at the University of Toronto. Kelley obtained her Ph.D. in 1999 in Chemistry from the California Institute of Technology in Chemistry and was a NIH Postdoctoral Fellow at the Scripps Research Institute from 1999 to 2000. Kelley has coauthored over 60 scientific publications and has developed new biosensing methods, nanomaterials for ultrasensitive biodetection, and novel bioconjugates for cellular studies. Since 2000, Kelley has received a Research Corporation Innovation Award, a Dreyfus New Faculty Award, a National Science Foundation CAREER Award, an Alfred P. Sloan Fellowship, a Camille Dreyfus Teacher-Scholar Award, and the Pittsburgh Conference Achievement Award and was named to *Technology Review's* list of Top 100 innovators and one of *Canada's Top 40 under 40*.

FOOTNOTES

*To whom correspondence should be addressed. E-mail: shana.kelley@utoronto.ca.

§These authors contributed equally to this work.

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