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RNA-Templated Semiconductor Nanocrystals

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Biomolecules, possessing dimensions in the nanoscale regime, represent tools that could facilitate the construction of nanomaterials with high precision and programmability.[1] Colloidal semiconductor nanocrystals—exhibiting tunable optical properties that are closely tied to their sizes—are of particular interest as targets for precise engineering.[2−4] While quantum dots and other types of nanoclusters have been generated using the components of proteins[1,5] and nucleic acids[6−9] as ligands, the three-dimensional structures of discrete biomolecules have not been exploited to systematically control nanocrystal products. Our recent work demonstrating that nucleotides can be used as ligands in semiconductor nanocrystal synthesis represents an essential first step toward using biomolecular templates in nanomaterials engineering.[6] However, the use of nucleic acids' three-dimensional structures to modulate quantum dot properties has not yet been explored.

This report describes the use of RNA polynucleotides as nanocrystal ligands and templates. RNA exhibits highly versatile structural properties and conformations that can change dramatically with small changes in sequence.[10] Transfer RNAs (tRNAs), which are essential for protein biosynthesis, exhibit very well-defined and well-characterized structures.[11] tRNAs have highly conserved cloverleaf secondary structures and L-shaped tertiary structures[12] that are necessary for its biological function (Figure 1). Given that the size of tRNA molecules (5 nm in diameter) is quite comparable to that of conventional quantum dots, we imagined that this structure could serve as an appropriate template for nanocrystal formation.

To explore the use of tRNA as a structured ligand system for semiconductor nanocrystals, we monitored the effects of including the *E. coli* $tRNA^{Leu}$ (WT tRNA) in an aqueous synthesis of CdS.[13] Given the prior observations that the phosphate and base moieties of mononucleotides can support the production of luminescent nanocrystals in water-based syntheses,[6] these reactions were run in solutions containing only Cd^{2+}, S^{2-}, and WT tRNA, with no additional ligand added.[13] In the absence of tRNA, material was produced that displayed poor solubility—this material could be removed from solution easily via centrifugation. In the presence of tRNA, however, stably dispersed material was formed that displayed robust aqueous solubility.

The products of tRNA-templated CdS were characterized by gel electrophoresis and gel filtration chromatography.[13] The mobility of tRNA−CdS on an agarose gel was visualized by monitoring the CdS luminescence directly (Figure 2a). While material isolated from a synthesis performed in the absence of tRNA could not be electrophoresed because it would not enter the gel matrix (data not shown), the products of the synthesis containing tRNA exhibited high mobility, indicating that the negatively charged WT tRNA remains attached after the completion of the synthesis. For the tRNA−CdS sample, two discrete bands are visible by electrophoresis. The band with higher mobility corresponds to that observed for unbound WT tRNA visualized by ethidium staining (Figure 2b). Thus, it appears that one of the species produced represents a species similar in size and net charge to the native WT tRNA. The additional band observed, exhibiting lower mobility within the gel, could represent tRNA−CdS with altered mobility due to differences in CdS size, tRNA loading or conformation, or overall surface charge. Gel filtration, which can accurately measure hydrodynamic size without sensitivity to charge effects, indicated that the tRNA-templated material was somewhat monodisperse, with a hydrodynamic diameter of ∼6 nm. This observation suggests that the species visualized by gel electrophoresis differ in charge rather than size. It is noteworthy that a shoulder on the left side of the main peak was consistently observed, indicating that a small population of larger material may also be present.

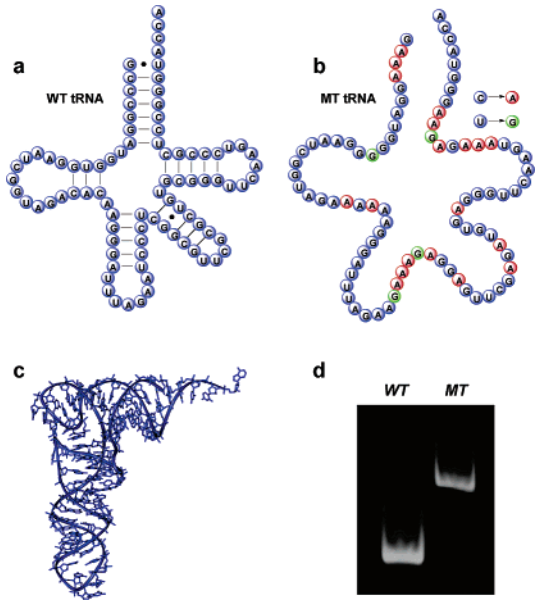

Figure 1. Two-dimensional and three-dimensional tRNA structures. (a) Primary and secondary structure diagrams of wild type (WT) *E. coli* $tRNA^{Leu(CUN)}$ depicted in a cloverleaf representation. (b) Primary structure of the mutated (MT) tRNA with disrupted base pairs in all five stems that would cause the tRNA to adopt a linear structure. These two RNA transcripts were prepared as described.[13] (c) L-shaped tertiary structure representative of that found in native tRNAs. (d) Native 8% polyacrylamide gel electrophoresis of WT tRNA and MT tRNA.

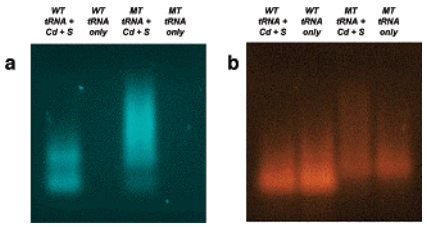

Figure 2. Gel electrophoresis analysis of tRNA−CdS. (a) CdS emission observed with products of syntheses performed with the WT and MT templates. (b) Ethidium bromide emission indicating the position of WT and MT tRNA not bound to CdS (ethidium is quenched by CdS, and therefore the tRNA−CdS complex is not visible in this image).

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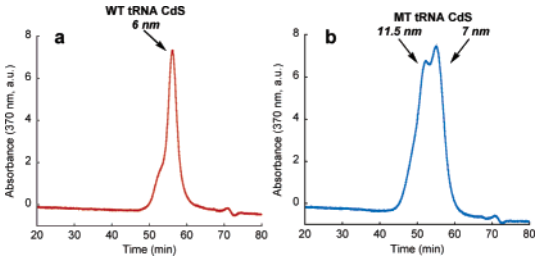

Figure 3. Gel filtration chromatography: (a) WT tRNA−CdS ($\lambda_{abs} = 370$ nm); (b) MT *E. coli* $tRNA^{Leu}$−CdS ($\lambda_{abs} = 370$ nm). See Supporting Information for standards used for sizing.

To deconvolute the influence of tRNA structure on CdS formation and properties, a destabilized tRNA mutant (MT tRNA) was designed by introducing 23 mutations into the WT tRNA sequence to disrupt the five stems existing in its cloverleaf secondary structure (Figure 1c). The design of the MT tRNA ablated base pairs that are critical for stable secondary structure, and hence we anticipated an unstructured conformation for the mutant. This expectation was confirmed by native gel electrophoresis (Figure 1d). The relative mobilities of WT and MT tRNA are strikingly different, suggesting a dramatic structural difference between these two RNAs. WT tRNA is a highly structured molecule with a compact conformation, and thus should bear a smaller drag force in the gel and exhibit higher mobility than the unstructured MT tRNA.

Gel electrophoresis and gel filtration chromatography (Figures 2 and 3) were again employed to compare the sizes of CdS particles synthesized with the MT versus WT tRNA templates.[13] While the WT tRNA produced two discrete bands when examined by agarose gel electrophoresis, the MT tRNA produced a material with less defined mobility and a seemingly large product distribution. Gel filtration analysis was consistent with this observation, as two main peaks were observed that indicated a much broader product distribution ranging from 7 to 11.5 nm in size. Clearly, larger structures are formed with the unstructured MT tRNA, while the folded and structured WT tRNA yields a single product type with a compact structure.

Transmission electron microscopy (TEM) was also used to compare the CdS products synthesized in the presence of the WT tRNA or MT tRNA. The products obtained with WT tRNA were predominantly <5 nm in diameter, with a mean diameter of 4.4 ± 0.4 nm, and spherical particles were easily visualized and numerous. Lattice fringes were apparent in the images, consistent with the formation of nanocrystalline products. The MT tRNA products, however, were significantly larger and had greater size dispersity. These particles often approached ~7 nm and had a mean diameter of 5.5 ± 1.0 nm, and spherical particles were difficult to identify. The differences visualized here are consistent with the gel electrophoresis and gel filtration results described above, indicating that greater dispersity and larger overall sizes were produced by the MT tRNA nucleotide-based ligand system that did not possess three-dimensional organization. The WT tRNA, however, supported the growth of smaller and more regular structures. It should be noted that there is a difference in the sequence composition of the two tRNAs used here; however, given the complete disruption of the tertiary and secondary structure in MT tRNA, it seems likely that the effects observed are most strongly influenced by the structural differences between MT and WT tRNA.

Nucleic acids-directed semiconductor nanocrystal growth likely involves the nucleotides playing roles in nucleation, growth, and passivation of the crystal, with the anionic oxygen atoms of the phosphate backbone and nitrogen atoms on the purine and pyrimidine bases acting as ligands. Organizing nucleotides within the context of a three-dimensional RNA structure facilitates the synthesis of products distinct from those made in the presence of an unstructured RNA, indicating that biomolecular structure could potentially be used to modulate and control nanomaterials' structure. The results reported here provide promise that designer materials with properties tailored by biomolecular scaffolds can be generated and engineered.

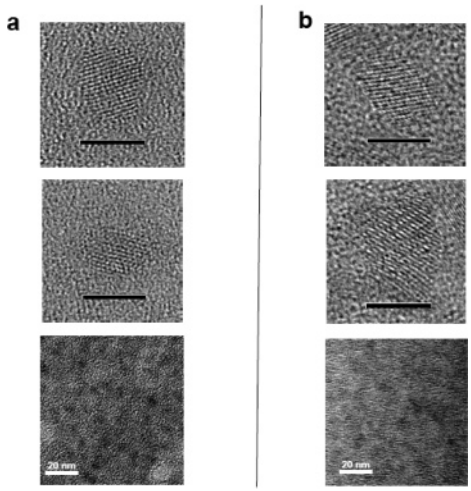

Figure 4. Transmission electron microscopy images of CdS synthesized with the (a) WT tRNA template or the (b) MT tRNA template. Scale bars are 5 nm for the upper images and 20 nm for the lower images in (a) and (b).

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Supporting Information Available: Information on tRNA synthesis, CdS quantum dots synthesis, and gel filtration chromatography. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) See Supporting Information for experimental details.

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