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Nanocrystal Molecules as Unique Systems for Structural, Mechanical, and Optoelectronic Studies

By

Charina L. Choi

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate Division of the University of California, Berkeley

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Abstract

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Doctor of Philosophy in Chemistry

University of California, Berkeley

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A spherical nanocrystal, also known as a quantum dot, is often referred to as an artificial atom due to its remarkable discrete and size-tunable physical properties. Just as bonding between natural atoms forms molecules, coupling between discrete groupings of artificial atoms creates artificial nanocrystal molecules. There are two primary mechanisms by which this coupling can occur: plasmonic coupling through organic linkers, and electronic coupling through inorganic interconnections. Analogous to natural molecules, artificial nanocrystal molecules with a myriad of physiochemical properties may be obtained from a discrete set of atomic building blocks. Enormous potential exists for exciting and useful behaviors to arise in these new materials. Thus, the design and characterization of nanocrystal molecules is a significant endeavor.

This dissertation explores the new physical properties that can arise in a nanocrystal molecule, and demonstrates the use of artificial molecules as probes in biological and materials systems. Chapter 1 provides an introduction to nanocrystal molecules, highlighting their synthetic preparation and physical properties.

Biomolecules can serve as the organic linker bonds between plasmonic nanocrystals, and additionally provide interesting systems to probe. Chapter 2 describes the use of gold nanocrystal molecules to elucidate a force-generating ultrastructure present in prokaryotic systems.

Inorganic interconnections enable nanocrystal molecules of different shapes and behaviors. Chapter 3 investigates the strain-dependent photoluminescence of CdSe/CdS nanocrystal molecules with three different topologies: the sphere, rod, and tetrapod. The effects of shape on strain-dependence are discussed. Importantly, tetrapod nanocrystals are observed to exhibit differential photoluminescence behavior under hydrostatic and non-hydrostatic environments, suggesting that the tetrapod may be useful as a gauge of local stress.
Chapter 4 presents the design and implementation of a luminescent nanocrystal stress gauge. The tetrapod stress gauge is calibrated in a simple uniaxial tensile geometry. Tetrapod luminescence is further used to reveal the local microscale stress profile of a polymer fiber. The microscale measurements support previous observations of local mobility and predictions of local stress behavior.

In addition to shape, the chemical composition of artificial atom components alters the optoelectronic behavior of the resultant nanocrystal molecule. Typically, an organic or nanocrystal fluorophore exhibits radiative emission from a single transition. Chapter 5 discusses the observation of multiple radiative emission peaks in fluorescence spectroscopy studies of single CdSe/CdS tetrapods. The dependence of multiple emission on external factors including CdS arm length, incident power, and excitation energy suggest that this photophysical phenomenon is due to spatially direct and indirect transitions within the nanocrystal molecule. Unique nanocrystal molecule designs will likely allow further control over the lifetime, oscillator strength, and energy of the indirect transition.

A significant advantage of colloidal nanocrystals is their ease of incorporation into many different systems. Chapter 6 highlights the tetrapod quantum dot as a dynamic nanocrystal probe that fluorescently reports cellular forces with spatial and temporal resolution. Rat-derived cardiomyocytes are cultured on a luminescent tetrapod array and induce clear time-dependent spectral shifts in the array emission. The small size and colloidal state of the tetrapod suggest that it may be further developed as a tool to measure cellular forces in vivo and with nanoscale spatial resolution. The tetrapod can additionally be utilized to convert biochemical inputs into optical signals, which may be useful for applications including synthetic biology designs.

Concluding remarks are presented in Chapter 7.
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Chapter 1.  
From Artificial Atoms to Nanocrystal Molecules


Quantum dots, which have found widespread use in fields such as biomedicine, photovoltaics, and electronics, are often called artificial atoms due to their size-dependent physical properties. Here this analogy is extended to consider artificial nanocrystal molecules, formed from well-defined groupings of plasmonically or electronically coupled single nanocrystals. Just as a hydrogen molecule has properties distinct from two uncoupled hydrogen atoms, a key feature of nanocrystal molecules is that they exhibit properties altered from those of the component nanoparticles due to coupling. The nature of the coupling between nanocrystal atoms and its response to vibrations and deformations of the nanocrystal molecule bonds are of particular interest. We discuss synthetic approaches, predicted and observed physical properties, and prospects and challenges toward this new class of materials.

1.1 Nanocrystal Molecules from Coupled Quantum Dots

Since their development more than 20 years ago, spherical semiconductor nanocrystals—known as quantum dots—have demonstrated remarkable size-dependent physical properties. Among the phenomena studied are the band gap, radiative rate, melting temperature, and solid-solid phase transition pressure, which can all be controlled solely by varying the size of the nanocrystal from 100 to up to 10,000 atoms. Because a quantum dot exhibits discrete and tunable structure in its optical and electrical characteristics, it is often referred to as an artificial atom. The ability to make such artificial atoms has developed significantly in the intervening years, and it becomes natural to consider whether artificial molecules may be created from well-defined groupings of single nanocrystals. Just as a hydrogen molecule has markedly different properties from individual hydrogen atoms, a key feature of an artificial molecule is that it should possess significantly altered properties from those of the component nanoparticles. This distinguishes the work described here from many current and important efforts in which, for instance, quantum dots are combined with magnetic nanoparticles to provide for multimodal capabilities. The distinct groups of nanoparticles considered here must have strong coupling between the particles. In this review we examine major efforts by the field to construct and characterize the properties of nanocrystal molecules. We consider nanocrystal molecules in which the individual nanocrystals are coupled via two main mechanisms: plasmon coupling between metal nanocrystals and direct electronic coupling arising from inorganically connected nanocrystals. In both cases we are interested in the nature of the interparticle coupling, and also in how this coupling changes as the nanocrystal molecule vibrates or deforms. Bond vibrations in organic or small molecules can be spectroscopically observed; we see that this is true for alterations or deformations of nanocrystal molecule bonds as well.
1.2 Plasmonically Coupled Nanocrystal Molecules

A plasmon is a collective oscillation of the valence electrons in a metallic particle. The resonance frequency of a metal plasmon depends critically on the material composition, especially the electron density in the metal. Most metals exhibit plasmon resonances in the far ultraviolet, but in the noble metals Cu, Ag, and Au, there is a d → s interband transition that mixes with the plasmon resonance and shifts it to the visible region of the electromagnetic spectrum. In nanoparticles, the plasmons couple strongly to optical fields, producing intense light-scattering spectra; this effect can be classically described using Mie theory\(^7\). The light scattering from the noble-metal nanoparticles depends strongly on size with intensity varying as the sixth power of the radius. The light-scattering spectrum from a single noble-metal nanoparticle of 20-nm diameter or larger can readily be observed in a dark-field microscope equipped with a standard tungsten lamp. As two metal nanoparticles are brought into proximity, the charge oscillations couple to each other, resulting in altered optical and electronic behavior that is highly distance dependent. This coupling effect is exploited in making metal nanocrystal molecules with new capabilities. Plasmon coupling occurs through a dielectric medium and readily extends over distances comparable to the nanocrystal diameter, making it particularly useful for situations in which metal nanoparticles are linked via biological macromolecules.

1.2.1 Directed Assembly of Metal Nanocrystals Using Biological Macromolecules

There are two reasons for using biological macromolecules to effect the metal nanocrystal assembly. First, biological macromolecules are capable of chemical recognition and assembly to a degree of sophistication that substantially exceeds what is otherwise possible. Thus a wide range of nanocrystal molecules, with a variety of symmetries and degrees of interconnection, can be readily achieved using oligonucleotides or peptides to arrange the nanoparticle assembly. Second, by using biological macromolecules as the glue that holds the nanoparticles together, biological sensing is automatically built into the resulting nanocrystal molecules. Any
conformational change in biomolecule linker directly results in a change in the distance between the nanoparticles, which should produce a detectable signal if the nanoparticles are coupled to each other strongly.

Alivisatos and Schultz demonstrated one of the first uses of DNA to organize nanocrystal molecules in 1996, with a subsequent experiment in 19998,9 (Figure 1.2). In this experiment, Au nanoparticles conjugated to complementary strands of DNA were brought together via Watson-Crick base pairing, resulting in distinct Au nanoparticle dimers and trimers. This work appeared simultaneously with work by Mirkin and Letsinger, who used DNA to assemble Au nanoparticle arrays10. Indeed, if the nanocrystal is thought of as an artificial atom, then the construction of artificial solids is an important and complementary activity to the construction of artificial molecules11,12,13.

The use of biological macromolecules to assemble metal nanocrystals has been further developed to design more complex structures. Nanocrystal assemblies consisting of a central nanocrystal with many satellite nanocrystals attached have been achieved by a variety of methods14,15. Chains of metal nanoparticles linked by single-stranded DNA have been synthesized through the use of DNA ligase, and subsequent pair bonding of complementary DNA strands yields chains of metal nanoparticle pairs16 (Figure 1.3a). Chiral Au nanoparticle assemblies, adapted from Seeman’s17 complex DNA nanostructures, have been demonstrated in the form of left- and right-handed four-nanoparticle pyramids18 (Figure 1.3b). Programmable dynamic behavior of Au nanoparticle assemblies has also been achieved19 (Figure 1.3c). Future combinations utilizing these demonstrated operations of central branch points, linear extensions, chirality, and dynamic capabilities can be used to create a wide variety of nanocrystal molecules with tunable collective properties.

Figure 1.2. DNA-directed assembly of 5- and 10-nm Au particles into dimer and trimer nanocrystal molecules. Figure adapted from Reference 9.

Figure 1.3a. Chiral Au nanoparticle assemblies, adapted from Seeman’s complex DNA nanostructures, have been demonstrated in the form of left- and right-handed four-nanoparticle pyramids. Figure 1.3b. Programmable dynamic behavior of Au nanoparticle assemblies has also been achieved. Figure 1.3c. Future combinations utilizing these demonstrated operations of central branch points, linear extensions, chirality, and dynamic capabilities can be used to create a wide variety of nanocrystal molecules with tunable collective properties.
1.2.2 Alternative Methods for Construction of Plasmonic Nanocrystal Molecules

Plasmon-coupled nanocrystal molecules may also be constructed without the aid of biomolecules. For example, spherical metal nanoparticles can assemble together via the polymerization of organic surface ligands. When two thiol ligands of different lengths arrange on the surface of an Au nanoparticle, they form alternating latitude ring-like domains. This phenomenon is not observed on flat surfaces and thus is likely driven by surface curvature. Topological considerations and molecular-dynamics simulations demonstrate that this surface...
arrangement is possible only if a single thiol molecule sticks out at each pole\textsuperscript{20}. Stellacci and coworkers\textsuperscript{21} demonstrated that the thiol molecules at either end of the particle can be replaced with longer-chain carboxy-terminated molecules, resulting in reactive sites at the particle poles. Diamines are then utilized to polymerize the divalent Au nanoparticle monomers, resulting in Au nanoparticle chains. Alternatively, Novak & Feldheim\textsuperscript{22} used stiff thiol-functionalized phenylacetylenes to assemble Ag and Au dimers, trimers, and tetratomers.

In addition to particle assembly, varying the nanocrystal shape is an alternative and powerful method for tuning plasmon resonances. This is accomplished mainly by altering synthetic conditions, without the aid of any nanoparticle linker molecules. For example, Au nanorods are grown via a seed-mediated synthesis, with the aspect ratio controlled by varying the amount of cationic surfactant present\textsuperscript{23}. Au and Ag nanoprisms are synthesized by photochemical or thermal methods\textsuperscript{24}. In comparison with simple spheres, nanorods and nanoprisms have optical properties much more enhanced and tunable by varying the particle dimensions\textsuperscript{25}. Au nanoshells are grown by the reduction of Au on the surface of a solid core that is either dissolved, in the case of an Ag core, or plasmon inactive, such as a silica core\textsuperscript{26}; the optical properties of an Au nanoshell exhibit greater sensitivity to particle dimensions in comparison with its solid counterpart. The use of dielectric cores as a structural synthetic tool has also led to the formation of nanoeggs, nanomatryushkas, and nanorice (Figure 1.3d), all with unique optical properties\textsuperscript{27}. The diverse plasmon resonance spectra of these shaped nanoparticles can be thought of as arising from the coupling together of simpler systems, so that these complex shapes, too, are nanocrystal molecules\textsuperscript{27,28}.

### 1.2.3 Theoretical Considerations of Plasmonic Nanocrystal Molecules

The ability to design and synthesize plasmon-coupled nanocrystal molecules is significant as new behaviors are predicted for these assemblies. The electromagnetic field–induced charge displacements within two noble-metal particles will couple to each other as the particles are brought together. In analogy with molecular bonding, when two metal nanoparticles are brought close together, the plasmon resonance splits into a lower and a higher frequency mode. Constructive interference will occur between the enhanced fields of each particle, leading to the red-shifting of the lowest-energy coupled plasmon resonance, and an enhanced electromagnetic field in the particle junction (Figure 1.4a)\textsuperscript{29}. Similarly, destructive interference produces a mode of higher frequency.

In consistence with the notion of individual nanocrystals as artificial atoms, Nordlander and Halas\textsuperscript{27} have contributed greatly to the field by using a method analogous to molecular orbital theory to model the interaction between two plasmonic particles (Figure 1.4b). The charge oscillations in two metal nanocrystals, with a separation less than their diameter, will strongly couple to each other. The single plasmon resonance of one particle will now split into two resonances: a longitudinal mode, in which the charge oscillations are aligned along the interparticle axis, and a transverse mode, in which the charge oscillations are perpendicular to the interparticle axis. As the two particles get closer together, the longitudinal mode shifts to lower energy, whereas as they move further apart, it shifts to higher energy. In addition, the light-scattering intensity from the longitudinal mode of a pair of strongly coupled particles can be up
to four times more intense than that of a single particle. Thus, the plasmon resonance energy and the light-scattering intensity from plasmon-coupled nanoparticles serve as reporters of interparticle separation and orientation\textsuperscript{30}. This technique has also been utilized to consider plasmon coupling in trimers and quadramers of metal nanoparticles\textsuperscript{31}, as well as hollow metal nanospheres\textsuperscript{32,33}.

The plasmon shift as a function of interparticle distance also depends greatly on the size, shape, and composition of the metal nanoparticle; the dielectric constant of the surrounding medium; and the direction of light polarization relative to the interparticle axis\textsuperscript{34,35}. Larger nanoparticles exhibit greater plasmon shifts for a given center-to-center distance. Ag nanoparticles generally have sharper plasmon resonances than Au due to a larger polarizability, making the effects of interparticle coupling easier to see\textsuperscript{35,36}. The surface plasmon light-scattering peak of a single particle will red shift as the dielectric constant of the surrounding medium is increased because the charge oscillations in the metal particle induce a polarization in the dielectric medium. Generally, the effect of local dielectric changes is smaller than effects that arise when another noble-metal nanoparticle is placed nearby, as the second nanoparticle is far more polarizable than the typical dielectric medium.

Care must be taken in calculating the magnitude of the interparticle coupling. It is not enough to include simple dipole-dipole terms to describe the polarizations in each nanoparticle, because the nanoparticles are within a diameter of each other and higher-order terms in a multipole expansion are needed. Pinchuk & Schatz\textsuperscript{7} developed a quantitative theory to describe these phenomena. El-Sayed and coworkers\textsuperscript{35} provided a scaling description that empirically describes the fractional plasmon shift for an Au nanodisc pair in solution:

\[
\frac{\lambda}{\lambda_0} \approx a \cdot \exp\left(\frac{-(R/D)}{\tau}\right), \quad (1)
\]

where \(\lambda\) is the plasmon resonance of the Au particle pair, \(\lambda_0\) is the plasmon resonance of the individual particles, \(R\) is the interparticle center-to-center distance, \(D\) is the particle diameter, \(\tau\) is the decay constant, and \(a\) is the amplitude of the fractional shift in the system. From the simulation, the authors find that \(\tau\) has a value of \(~0.2\); that is, the plasmon coupling decay length is roughly 0.2 times the particle diameter. This value is universal, regardless of particle size (Figure 1.4c), shape, metal type, and medium dielectric constant, all of which affect only the amplitude of the fractional shift. Although the expression is calculated for light polarization oriented parallel to the interparticle axis, experimental polarization effects only become significant for larger particles (\(~87\) nm)\textsuperscript{37}. 
1.2.4 Observed Properties of Plasmonic Molecules and Their Use in Microscopy and Spectroscopy

One feature of plasmon scattering cannot be overemphasized when considering the use of plasmons as reporters in microscopy and spectroscopy: The light scattering from a single noble metal nanoparticle does not change with time at all. It is just constant. In a noble-nanocrystal molecule, the light scattering will change only if the distance between the nanoparticles changes (small changes due to the variation of the dielectric constant of the surrounding medium can be detected but are an order of magnitude smaller than those due to particle proximity). Otherwise, the spectra are invariant. This is not true of any other type of single-molecule optical label. Organic dyes show intersystem crossing and bleach, lasting only a few tens of seconds under typical single-molecule conditions. Colloidal quantum dots luminesce much longer, but generally also show blinking, with a wide range of on and off times\textsuperscript{38,39}. Plasmon resonances do not suffer
from these drawbacks. Indeed, plasmonic nanocrystal molecules provide faithful trajectories for the analysis of the conformational changes of individual biological macromolecules.

A starting point for these studies is shown in Figure 1.5a. When a second particle binds to a single particle immobilized on a surface, the light-scattering intensity increases, and the plasmon peak red-shifts for both Ag and Au particles, as expected. This so-called plasmon ruler technique has been used to study the cutting of DNA by the EcoRV restriction enzyme (Figure 1.5b). In this experiment, Au nanoparticle dimers linked by oligonucleotides containing the EcoRV cutting site are deposited on a substrate and imaged in a dark-field microscope. When the enzyme is flowed into the chamber, the plasmon resonance blue shifts as expected—the second particle flows away as soon as it is cut. The EcoRV enzyme is known to first bend and then cut the DNA, and this can be seen in the light-scattering intensity, which increases slightly and then abruptly decreases as the DNA is cut. The plasmon ruler has been calibrated to correlate the distance between Au nanoparticle pairs with spectral shifts and has proved useful for further biological studies, including the observation of single caspase-3 enzyme cutting events in vivo.

Fluorescence resonance energy transfer techniques have been extremely useful in studying conformational changes in many systems. However, their use is limited by the lifetime and
distance dependency \( \sim \frac{1}{R^6} \) of the dyes: Typically tens of seconds and distances of 1–10 nm are accessible in a given experiment. Because of the invariant plasmon signal over time, and the size-dependent near-exponential distance decay \( e^{-R/D} \), Equation 1, plasmon rulers provide an additional tool capable of studying distances from 1 to 70 nm, for any length of time desired.

The electromagnetic field enhancement at the junction between two or more metal particles has been observed experimentally \(^{45,46,47}\). This has proved useful for surface-enhanced Raman spectroscopy studies down to the single-molecule level. Indeed, many unique metal nanostructures have been designed for this purpose \(^{48,49}\). As we focus here on plasmon spectral shift effects, we refer the reader to comprehensive reviews on surface-enhanced Raman spectroscopy \(^{50,51}\) and note that there have been important applications of metal nanocrystal molecules in this area. The enhanced electromagnetic field effect has also been utilized for electromagnetic energy transport and subwavelength optical waveguiding \(^{52,53}\).

### 1.3 Electronically Coupled Nanocrystal Molecules

Plasmon coupling occurs through space or a dielectric medium. Another way to produce coupling between two nanocrystals is through tunneling and exchange coupling of electrons. This is precisely analogous to molecular bonding. For this to be a significant effect, two nanocrystals need to be joined by a medium through which the quantum-confined electrons can tunnel. The effective masses of the electrons in a semiconductor quantum dot can vary from 1 to 0.01. The length scale over which two dots can then be coupled will depend critically on the potential of the electron in the medium that separates the two dots. Generally, it is necessary to try to lower the potential as much as possible to promote the coupling; for this reason, it is common to attempt to grow dots that are interconnected by an inorganic semiconductor medium, in which the electron potential is higher than that in the dots themselves, but lower than in vacuum or typical dielectric or insulating media. Thus, for such nanocrystal molecules to be studied, it becomes important to be able to grow complex nanocrystals in which regions of differing composition are connected together in well-defined ways. Although there are many versions of electronically coupled nanocrystal molecules, we highlight one case, that of semiconductor tetrapods, because of their beautiful symmetry (they are analogous to a methane molecule) and because the influence of mechanical deformations on their electronic properties can be investigated.

#### 1.3.1 Synthesis of Nanocrystal Molecules with Inorganic Interconnections

Semiconductor nanocrystals, typically consisting of II-VI or III-V semiconductor materials, have been synthesized via gas-phase, molecular-beam epitaxy, and colloidal methods. We focus here on colloidal synthesis, in which the resulting nanocrystal can be considered a chemical reagent, able to be dissolved in a fluid, spun into a polymer, attached to an electrical circuit, or reacted with other chemicals \(^{2,54}\). The colloidal synthesis of semiconductor nanocrystals has developed significantly from quantum dots to nanocrystal molecules of many different shapes, sizes, and chemical compositions (Figure 1.6) \(^{55,56,57}\). Dots (Figure 1.6a), rods (Figure 1.6b), discs, and
tetrapods (Figure 1.6c) allow for nanocrystal molecules extended in zero, one, two, and three dimensions, respectively.

![Image of tetrapods](image_url)

Shape control to achieve nanocrystal molecules is largely accomplished by controlling reaction temperature, which favors one crystal structure over another, and the organic surfactant molecules used, which bind to selective crystal faces and promote growth of other less-protected facets. For example, a tetrapod consists of a zinc-blende (cubic) core with four (111) facets at the tetrahedral angle, each projecting a wurtzite rod terminated with the (0001) facet. The temperature is controlled during the synthesis to favor nanocrystal nucleation in the zinc-blende structure, and subsequent wurtzite growth. Alkylphosphonate ligands such as methyl- and hexyl phosphonic acid and quaternary ammonium salt compounds preferentially bind to specific crystal facets to facilitate tetrapod synthesis.

Core/shell particles, comprising a shell grown epitaxially on a core particle of different material, add another layer of complexity and control to the nanocrystal molecule family. Typically, the core is synthesized first by traditional colloidal methods, and the shell is grown either by successive monolayer adsorption or quick nucleation followed by growth on the nanocrystal core. Type I band alignment core/shell heterostructures with the larger band-gap material as the shell result in extremely bright nanocrystals, as both electron and hole carriers are confined to the
core. These nanoheterostructures were first demonstrated by Bawendi and coworkers\textsuperscript{63}, who synthesized CdSe/ZnS quantum dots with 30\%–50\% quantum yields at room temperature. Such structures are now routinely used, particularly in biological fluorescence-tagging experiments. Type II core/shell heterostructures allow the electron and hole to be segregated to different materials, which is ideal for many photovoltaic and catalytic designs. Other arrangements of wave-function confinement and delocalization are possible via the careful choice of core and shell material.

Hollow particles and nested or yolk-shell nanocrystal molecules are synthesized by leveraging the Kirkendall effect: that ions diffuse through interfaces at different rates\textsuperscript{64}. The deliberate selection of core and shell materials followed by successive oxidation or sulfurization results in partially hollow, nested particles, leading to fully hollow spheres upon longer reaction times. These nanocrystals are used in surface and catalysis studies\textsuperscript{65}.

Of particular note are the Au-tipped semiconductor nanocrystal molecules demonstrated by Banin and coworkers\textsuperscript{66,67}, the first observed metal-semiconductor contact within a colloidal nanocrystal. Metal regions on semiconductor nanocrystals are an interesting class of more complex nanocrystal molecules that provide additional handles for electrical contacts\textsuperscript{68} or biological functionalization via thiol or amine linkers. Current methods provide nonepitaxial Au deposition on semiconductor structures; the Au tip size may be increased by further reduction of the metal in solution. Au-tipped tetrapods bear a striking resemblance to the Au-tipped pyramids constructed by DNA assembly mentioned in Section 1.2.1\textsuperscript{18}. These two structures provide a direct comparison between plasmonic through-space and electronic coupling of four tetrahedrally related Au particles.

1.3.2 Theoretical Consideration of Electronically Coupled Nanocrystal Molecules

Wang and coworkers have developed the semiempirical pseudopotential and \textit{ab initio} methods to model electron and hole wave functions in semiconductor nanocrystals. We direct the reader to other literature on this subject\textsuperscript{69,70,71}, noting that the optical and electronic properties of a nanocrystal are clearly dependent on nanocrystal shape from these calculations (Figure 1.7a–c)\textsuperscript{72}. These theoretical studies are consistent with the concept of rods and tetrapods (and other shapes) as nanocrystal molecules with properties distinct from a series of uncoupled dots in the same geometric arrangement. Furthermore, the mechanical properties of a nanocrystal, related to its shape, are inherently linked to its optoelectronic properties. Schrier et al.\textsuperscript{73} predicted that when the top arm of a tetrapod is mechanically compressed while its three supporting arms are flattened along the substrate, inducing arm bending, the electron and hole wave functions of a tetrapod are spatially altered; strain in the top arm reduces the probability of electron or hole localization in that arm (Fig. 1.7c–d). The resulting wave-function overlap results in a red shift of the energy gap. This calculation was recently experimentally confirmed\textsuperscript{74} and is discussed further in Section 1.3.3.
1.3.3 Observed Properties of Semiconductor Nanocrystal Molecules

Semiconductor nanocrystal molecules exhibit altered optical, electronic, and mechanical properties from their quantum-dot counterparts (Figure 1.8). As the aspect ratio of a wurtzite dot is increased along the $c$ axis to form a rod, the optoelectronic structure changes because of the inherent dipole in the rod structure (the two faces perpendicular to the $c$ axis are not equivalent). The particle begins to emit polarized light\textsuperscript{70,75} as well as exhibit electric-field-dependent photoluminescence\textsuperscript{76}. In the case of CdSe/CdS core/shell rods\textsuperscript{61,77,78}, in which the hole is localized in the CdSe core while the electron is more delocalized over the core and the CdS shell\textsuperscript{79,80,81}, the changes in optoelectronic properties are more pronounced (Figure 1.8a). As the length of the CdS rod shell is increased, the electron and hole wave-function overlap is decreased, leading to a decrease in the radiative decay rate. An external electric field applied to the CdSe/CdS rod additionally affects carrier wave-function localization and thus optoelectronic properties\textsuperscript{82}.
Figure 1.8. Semiconductor nanocrystal molecules exhibit altered optical, electronic, and mechanical properties. (a) The electron and hole wave-function overlap can be engineered in CdSe/CdS core/shell nanorods by tuning the CdS rod length (top) and the electric field (bottom). Figure adapted with permission from Reference 82. Copyright 2005 American Chemical Society. (b) Plots of the differential conductance as a function of $V$ and $V_g$ at $T = 5$ K of single nanocrystals. (i) A CdSe nanorod, (ii) a CdTe tetrapod showing coupled hopping behavior, and (iii) a CdTe tetrapod showing delocalized behavior. Insets show scanning electron micrographs of single nanocrystals contacted with electrodes. Figure adapted with permission from Reference 84. Copyright 2005 American Chemical Society. (c) Deviatoric stresses induce new radiative transitions in CdSe/CdS core/shell (i) dots, (ii) rods, and (iii) tetrapods. Schematics of core/shell nanocrystals are shown in insets. Figure adapted with permission from Reference 74. Copyright 2009 American Chemical Society.
Even more so than rods, coupled behavior is observed in the optoelectronic properties of tetrapods. In a single-electron transistor experiment, rod and tetrapod particles are immobilized on a 10-nm Si$_3$N$_4$ dielectric substrate with a metal back gate, and are contacted by electron beam lithography with Pd electrodes at both ends of the rod, or three arms of a tetrapod (with the fourth arm protruding upward). The electrical current as a function of voltage is measured for many gate voltages, and the differential conductance ($\partial I/\partial V$) is plotted as a function of $V$ and $V_g$ (Figure 1.8b). In the case of a single rod transistor, we observe a smooth set of Coulomb diamonds, indicating a single Coulomb-charging energy ladder (Figure 1.8b, panel i). This is similar to that observed for a single dot. In the case of a single tetrapod transistor, two types of behavior are observed. The first is a sawtooth-like Coulomb diamond structure (Figure 1.8b, panel ii), a signature akin to single-electron hopping in a system of weakly coupled quantum dots. This behavior results from the electron being transported through the arm-branch point arm in series: modeled, to the simplest approximation, as three Coulomb-charging energy ladders. The second tetrapod transistor behavior exhibits a large Coulomb diamond with two or three smaller diamonds alternating along the $V_g$ axis (Figure 1.8b, panel iii). This behavior is expected for a strongly coupled arm-branch point-arm system, with the charge carrier delocalized throughout the tetrapod, similar to lithographically patterned dots on a two-dimensional electron gas. Thus we see that a single tetrapod nanostructure behaves electronically as a collection of coupled quantum dots.

The optoelectronic coupling of tetrapods is sensitive to mechanical deformation (Figure 1.8c). That unique mechanical properties are observed for tetrapods as compared with dots and rods seems intuitive, as particle shape is clearly tied to its response to stress and strain. Nie and colleagues have observed strain-dependent optical properties of core/shell quantum dots with synthetically inherent internal stress. In these materials, a shell is epitaxially grown onto a core of softer material. As the shell thickness is increased, the softer core experiences increased strain, leading to an optical band-gap red shift. Ensemble measurements of CdSe/CdS core/shell dots, rods, and tetrapods all demonstrate a photoluminescence peak blue-shift with increasing pressure under hydrostatic pressure conditions (near-isotropic compression) in a diamond anvil cell (DAC). This results from increased atomic orbital overlap within the crystal, and is consistent with previous measurements on bulk and nanocrystal CdSe. However, upon application of pressure when solubilized in a nonhydrostatic medium (anisotropic compression), an additional photoluminescence peak at lower energy is observed for all three particle morphologies. This peak is attributed to a fraction of the particles within the DAC experiencing nonhydrostatic conditions. The difference between the two peaks reveals the net effect of deviatoric stress on the particles. Tetrapod nanocrystals are most greatly affected by net deviatoric stresses, with greater than 95% of the population affected by nonhydrostaticity; these particles exhibit a photoluminescence red shift with increasing anisotropic deformation. Because the strain state inside a DAC cannot be determined, future experiments are required to calibrate the fluorescence shift with force. With further development, these tetrapods may be effective as optical sensors of externally applied stress.
1.3.4 Chemical Transformations in Semiconductor Nanocrystals

Just as organic molecules may undergo chemical reactions, inorganic nanocrystal molecules may also undergo chemical transformations. Branching of nanocrystals, analogous to organic dendrimer formation, leads to tetrapod and multipod particles. Additional reactions in nanocrystals result in core/shell, nested, or hollow structures. Simple substitution reactions in particular highlight the ability of nanocrystals to transform as molecular entities. These reactions are demonstrated via a cation exchange mechanism. For example, CdSe quantum dots will quickly transform to Ag$_2$Se dots upon addition of excess Ag$^+$ in solution$^{86}$. The reaction is fully reversible, and CdSe dots are recovered upon addition of excess Cd$^{2+}$ (Figure 1.9a). Partial cation exchange has also been demonstrated, with the resultant nanocrystal morphology highly dependent on choice and concentration of cation$^{87,88}$. For example, when CdS nanorods are partially exchanged with Cu$^{2+}$, a binary nanorod structure forms with Cu$_2$S at one or both ends of the nanorod (Figure 1.9b). When CdS nanorods are partially exchanged with Ag$^+$, however, the CdS rod becomes partially striped with Ag$_2$S at regularly spaced intervals along the rod (Figure 1.6e). The interfacial strain between the two lattice structures and the chemical interaction dictates the resultant partially exchanged morphology. Full cation exchange of the CdS nanorod results in fully Cu$_2$S or Ag$_2$S nanorods.

That shape is highly preserved while the nanocrystal composition is thoroughly transformed into a different semiconductor material is remarkable. This observation suggests that cation exchange may be a new route toward the synthesis of nanocrystal molecules with complex compositions. Indeed, recent work has shown that semiconductor nanocrystals difficult to synthesize by established colloidal methods, e.g., PbS nanocrystals, can be achieved through a cation exchange mechanism$^{89}$.

Exchange, addition, and branching reactions result in a core set of transformation operations that can be performed sequentially in any order. The discovery of additional transformational tools and further development of multistep nanocrystal syntheses will result in a wide library of inorganic nanocrystals with a variety of properties.
1.4 In Situ Electron Microscopy for Observation of Nanocrystal Molecule Formation and Dynamics

As discussed above, many types of nanocrystal molecules can be synthesized, and unique properties distinct from those of the individual nanocrystal building blocks are obtained. The size, shape, structure, and composition of these particles can be characterized using transmission electron microscopy. Although these structures may be monitored roughly during formation by characterization of aliquots from synthesis, an ideal tool would enable visualization and characterization of nanocrystal molecule formation in situ. One such technique has recently been developed and used to observe the formation of Pt nanocrystals in real time. In this study, Pt nanoparticle precursors are injected into a liquid cell holder and placed into the transmission electron microscope, with growth of Pt nanocrystals catalyzed by the electron beam. Nanocrystals are observed to form both via monomer addition and particle coalescence. This observation is a first step toward understanding simple nanocrystal formation mechanisms and kinetics. Future work will investigate the formation of more complex chemical transformations of nanocrystal molecules. (a) Cation exchange reactions enable the reversible transformation of CdSe quantum dots to Ag2Se quantum dots. Figure taken from Reference 86. Reprinted with permission from AAAS. (b) Partial cation exchange enables the formation of CdS-CuS binary nanorod structures; the further addition of copper ions results in full cation exchange of the nanorod. Figure adapted with permission from Reference 88. Copyright 2009 American Chemical Society.
nanocrystals via reactions such as branching or cation exchange, as well as nanocrystal transformations during catalysis. These experiments are underway.

![Figure 1.10. Single colloidal Pt nanocrystal growth trajectories observed via transmission electron microscopy in situ.](image)

*Figure 1.10. Single colloidal Pt nanocrystal growth trajectories observed via transmission electron microscopy in situ. (a) Video images showing simple growth by means of monomer addition (left column) or growth by means of coalescence (right column). (b) Enlarged (1.5 times) color images of panel a. Arrows highlight distinct contrast changes indicating recrystallization from two separate particles in the case of coalescence. Figure taken from Reference 90. Reprinted with permission from AAAS.*

### 1.5 Future Prospects

Nanoscience has opened up the ability to create whole new classes of materials with designed properties. Early work in size control and characterization established that the artificial atom concept could be successfully applied to colloidal nanoparticles of inorganic solids, as if the periodic table itself acquired a third dimension corresponding to size. In the past decade, this analogy has developed further, with clear demonstrations that artificial atoms can be coupled together to create artificial nanocrystal molecules. Many of these effects have only been demonstrated at a proof-of-principle level, with limited numbers and types of nanoparticles connected to each other. As we learn to control the spatial arrangements of nanoparticles in new and more advanced ways, it should be possible to create ever more intricate and controlled nanocrystal molecules.
Chapter 2.
Protein-Nanocrystal Conjugates Support a Single Filament Polymerization Model in R1 Plasmid Segregation.


To ensure inheritance by daughter cells, many low-copy number bacterial plasmids, including the R1 drug-resistance plasmid, encode their own DNA segregation systems. The par operon of plasmid R1 directs construction of a simple spindle structure that converts free energy of polymerization of an actin-like protein, ParM, into work required to move sister plasmids to opposite poles of rod-shaped cells. The structures of individual components have been solved, but little is known about the ultrastructure of the R1 spindle. To determine the number of ParM filaments in a minimal R1 spindle, we used DNA-gold nanocrystal conjugates as mimics of the R1 plasmid. We found that each end of a single polar ParM filament binds to a single ParR/parC-gold complex, consistent with the idea that ParM filaments bind in the hollow core of the ParR/parC ring complex. Our results further suggest that multifilament spindles observed in vivo are associated with clusters of plasmids segregating as a unit.

2.1 The Eukaryotic R1 Segregation System

In eubacteria and archaea, many biologically important processes are carried out by genes encoded on large, low-copy number plasmids. These include genes conferring resistance to antibiotic and heavy metal toxicity, as well as genes involved in host cell invasion and pathogenicity. These large plasmids face a difficult challenge. To reduce the metabolic load they impose on the host cell, their copy numbers must be kept to a minimum (one to two per chromosome equivalent)\(^\text{91}\). At such low-copy numbers, they can no longer rely on chance for their maintenance in the bacterial population. To be stably maintained, these low-copy number plasmids must, like chromosomes, be actively segregated to daughter cells before division. Two classes of plasmid segregation systems (Types I and II) have been known and studied for years\(^\text{92}\), and recent work has uncovered several more\(^\text{93,94}\). Each system appears to be encoded on a single operon and to be composed of three pieces: 1) a centromeric DNA sequence, 2) a DNA-binding protein, and 3) a polymer-forming protein. The most well understood of these systems is the Type II segregation machinery encoded by the R1 drug-resistance plasmid. Segregation of R1 is driven by the par operon, which consists of a 150-bp centromeric sequence (parC), a repressor protein (ParR) that binds to the centromeric sequence, and a divergent actin-like protein (ParM) that polymerizes into dynamically unstable filaments in the presence of ATP\(^\text{95}\). Binding of the ParR/parC complex to ParM filaments stabilizes them against catastrophic disassembly and promotes their elongation via insertion polymerization at the interface with the ParR/parC complex. When both ends of a ParM filament are bound to ParR/parC complexes, elongation of the filament pushes the attached plasmids in opposite directions.
High-resolution structures of components of the R1 spindle are available\textsuperscript{96,97}, but provide little information about the ultrastructure of the R1 spindle itself. The atomic structure of ParM monomers reveals a basic similarity to conventional actin, whereas electron microscopy reveals that ParM and actin filaments differ significantly\textsuperscript{98}. Like actin, ParM filaments are structurally polarized, with distinct ends corresponding to the barbed and pointed ends of a conventional actin filament. Unlike actin, the long-pitch, two-start helix of ParM filaments is left-handed rather than right-handed, and the orientation of the ParM monomer with respect to the filament axis is significantly different from that of actin. Both free and ParR/parC-bound filaments elongate at equal rates from each end. This fact and the polar nature of ParM filaments place constraints on the mechanism of the interaction with the ParR/parC complex as follows. (a) ParR/parC contains two distinct ParM-binding sites, one for “pointed” ends and one for “barbed” ends; (b) spindles are composed of bundles of antiparallel filaments, and the complex recognizes an identical surface at each end of the bundle; or (c) ParR/parC interacts with a surface present at or near both filament ends.

X-ray crystallography of the ParR/parC complex reveals that multiple ParR dimers assemble into a helical ring with parC DNA running around the outside edge. This “segrosome” ring is hollow, with a 6-nm hole through the center. The size of the hole suggests that a single ParM filament (also 6 nm in diameter) might bind the inside of the ParR/parC ring complex. By forming a collar around the end of a filament, the ParR/parC complex would pose no barrier to elongation, and if the affinity of the complex for ParM depends on the nucleotide bound to the filament, hydrolysis of ATP within the filament could promote tracking of the complex with the elongating filament end. This is similar to the way in which the Dam1 complex is thought to interact with the dynamic ends of microtubules\textsuperscript{99}. In addition, a collar encircling a ParM filament might prevent monomer dissociation, providing an attractive explanation for how the ParR/parC complex stabilizes filaments against catastrophic disassembly. This model appears to be at odds with in vivo studies of R1 spindles that suggest plasmids are segregated by spindles composed of multiple filaments\textsuperscript{100}. However, R1 plasmids are also observed to cluster in vivo, and it is unclear whether multifilament spindles observed in vivo are assembled by pairs of individual plasmids or by pairs of plasmid clusters.

### 2.2 DNA-Au Nanocrystal Conjugates as Probes of R1 Structure

To better understand the coupling of the ParR/parC complex to ParM filaments, we determined the maximum number of filaments that can simultaneously associate with an individual ParR/parC complex. To do this, we attached defined numbers of parC-containing DNA molecules to gold nanoparticles and counted the number of filaments associated with each particle. Preparation of such discrete DNA-Au particles is well-established\textsuperscript{101,102}. We initiated ParM filament formation in vitro in the presence of DNA-Au nanoparticle conjugates and visualized associated ParM filaments by transmission electron microscopy. The high contrast of gold nanoparticles enabled us to identify the location of ParR/parC complexes relative to ParM filaments.

Garner et al.\textsuperscript{103} found that, in the presence of ParR, polystyrene particles conjugated to hundreds
of copies of parC DNA-stabilized bundles of up to 100 ParM filaments. To determine the number of ParM filaments that interact with a single ParR/parC complex, we prepared gold particles conjugated to single copies of parC-containing DNA, an established and well characterized procedure.\(^{101,102}\). Briefly, we incubated gold particles with thiolated parC-containing DNA to obtain particles conjugated to zero, one, two, or three parC sequences. We separated different sized conjugates by gel electrophoresis (Figure 2.1b), excised bands containing individual, conjugated species from the gel, and extracted and purified the monoconjugated particles.

![Figure 2.1](image)

**Figure 2.1.** Gold particles conjugated to defined numbers of parC-containing, double-stranded DNA molecules are prepared by covalent thiol-Au attachment and separated by gel electrophoresis. (a) The 150-bp parC construct consists of two regions (1 and 2), with a promoter (p) region in-between; c1, cp, and c2 denote the corresponding regions on the complementary strand. To incorporate a thiol group at one end of the construct, we used synthesized DNA. Each parC strand was divided into two parts (shown schematically as gray and black), for a total of four synthesizable oligonucleotides. The four oligonucleotides were hybridized together before conjugation to gold particles. (b) Conjugation of thiolated parC to gold particles resulted in a mixture of particles with different numbers of parC attached. The mixture was run in a 3% agarose gel to separate different types of conjugates. Particles conjugated to different numbers of parC sequences were then excised from the gel and extracted. Left lane, gold particles alone. Right lane, gold particles conjugated with DNA. Separation of gold particles with zero, one, two, and three DNA strands attached is clearly shown.

We mixed monoconjugated parC-Au (Figure 2.2a) with purified ParR and ParM, added ATP to induce filament formation, and observed the resulting structures by transmission electron microscopy. We then quantitated the specific association between ParM filaments and parC-Au monoconjugates by comparing and counting the types of structures observed in the absence and presence of the DNA-binding protein ParR (Figure 2.2i). In the absence of ParR, we rarely observed gold particles associated with ParM filaments (Figure 2.2h), whereas in the presence of ParR, we often observed gold particles closely associated with one or both ends of a ParM structure, with no observable gap between the filament and the gold particle. Addition of ParR increased the frequency of Au-M (Fig. 2, D and E) by >5-fold (Figure 2.2i). Likewise, in the presence of ParR, we observed Au-M-Au (Figure 2.2b-c), but never observed such Au-M-Au structures in the absence of ParR (Figure 2.2i). These data indicate that the majority of the Au-M and all of the Au-M-Au structures seen in the presence of ParR represent parC-Au nanoparticles.
bound specifically to ParM filaments via ParR.

We also observed gold particles in proximity to the end or side of ParM filaments but separated by an observable gap (Figure 2.2f-g). We scored all such cases in which gold particles were within 15 nm of a ParM filament and found that they occurred with equal frequency in the absence and presence of ParR, suggesting that they represent chance proximity between filaments and particles (Figure 2.2i). The majority of ParM filaments in the absence and presence of ParR were not colocalized with gold particles (Figure 2.2h). This is reasonable because we used 2.3 μM ParM, a concentration at which filaments can form spontaneously

Figure 2.2. Individual ParR/parC-Au complexes attach to one or both ends of a single ParM filament. (a) Monoconjugated parC-Au (schematic) was used as an R1 plasmid mimic to investigate the number of ParM filaments that would bind to a single parC. (b) and (c), Single Au M-Au filaments are shown. (d) and (e), Single Au-M filaments are shown. (f) ParM filaments with a parC-Au particle close to but separated from the filament end (M with gold close to end) were observed with near equal probability with and without the binding protein ParR. (g), ParM filaments with a parC-Au particle close to or touching the filament side (M with gold close to side) were observed with near equal probability with and without ParR. Scale bar is 50 nm. (h) Uncapped ParM filaments with no gold particles close by (M alone) were observed both with and without ParR. (i) Normalized histograms (occurrence divided by total number of filaments counted) indicate the numbers of observed structures when monoconjugated parC-Au, ParM, and ATP were combined with and without ParR.
To determine the number of filaments bound to an individual ParR/parC complex, we measured the width of the ParM filaments in Au-M and Au-M-Au structures. In both cases, we measured an average width of 6.0 ± 0.6 nm (Figure 2.3), in good agreement with the width of single ParM filaments\textsuperscript{104,105}. We never observed ParM structures >8 nm in width, indicating that none of the observed structures ($n = 80$) contained two filaments.

We observed singly capped filaments more frequently than doubly capped, consistent with a lower probability that a ParM filament will encounter two ParR/parC-Au complexes compared with one. The optimal ParM concentration for electron microscopy was \~2.5 µM. At higher concentrations (7–15 µM, near the ParM concentration estimated inside R1-containing bacterial cells), the filament density was too high to accurately distinguish capped from uncapped filaments. We also tried assembling spindles at high concentration and diluting them immediately prior to visualization, but found that the filaments were sheared and broken by pipetting. At lower concentrations (approaching 0.6 µM, the critical concentration for ATP ParM filaments\textsuperscript{103}), we observed capped filaments but with significantly reduced frequency.

Attempts to increase the encounter frequency by increasing the concentration of ParR/parC-Au particles in solution resulted in a density of gold particles too high to distinguish individual Au-M and Au-M-Au structures.

![Figure 2.3. Measurement of the widths of ParM structures in various relationships with ParR/parC-Au particles demonstrates that the binding capacity of the ParR/parC complex is a single ParM filament. Filament widths are shown for (a) Au-M structures, 53 total measured, and (b) Au-M-Au structures, 27 total measured. In both types of structures, an average width of 6 nm was observed, in agreement with the accepted width for single ParM filaments.](image-url)
To determine whether our failure to observe more than one ParM filament per gold particle reflects the binding capacity of the ParR/parC complex or an experimental artifact, we mixed ParM with gold nanoparticles conjugated to four or more parC sequences (Figure 2.4a). In the presence of ParR and ATP, the multiconjugated gold particles associated with the ends of bundles composed of up to six ParM filaments (Figure 2.4b-e). These data argue strongly that our results with monoconjugated gold particles reflect the binding capacity of the ParR/parC complex. The occurrence of multifilament spindles under these conditions is consistent with our previous observation of multifilament spindles formed by multiconjugated particles and suggests that, once a single filament spindle is formed, the probability of the tethered particles capturing additional filaments is relatively high.

![Figure 2.4. Multiconjugated ParR/parC-Au particles (four or more parC DNAs per gold particle) interact with bundles containing multiple ParM filaments. (a) Multiconjugated parC-Au (schematic) was used to investigate filament binding behavior to multiple parC. (b)–(e), ParM bundles with a ParR/parC-Au complex at one or both ends are shown. Scale bar is 50 nm.](image)

### 2.3 Structural Insights into R1 Plasmid Segregation

DNA-Au nanoparticle conjugates have been used for a variety of purposes, including nanoparticle self-assembly and as probes of biomolecular dynamics. For small gold nanoparticles (<15 nm in diameter) and DNA oligonucleotides longer than 100 bp, it is straightforward to tune the number of DNA molecules attached to each particle. Our in vitro results using DNA-Au monoconjugates to mimic the R1 plasmid demonstrate that a single ParR/parC complex binds either end of a single ParM filament. This implies that, in vivo, pairs of plasmids are segregated by polymerization of single ParM filaments. This is consistent with what is known about the physical properties of ParM filaments. Theoretical considerations and measurements of force produced by polymerizing actin filaments suggest that, in vivo, a growing ParM filament can generate forces on the order of 1 piconewton. Stiffness measurements indicate that ParM filaments have a persistence length (~10 µm) several times longer than the length of a bacterial cell (1–3 µm) and that, at bacterial length scales, ParM filaments require piconewton forces to buckle. These forces are several orders of magnitude greater than those required to push plasmids through bacterial cytoplasm.
Taken together with recent structural studies of the ParR/parC complex, our results suggest that a single 6-nm diameter ParM filament might fit within the ParR segrosome ring. This arrangement would explain three important experimental observations regarding ParM polymer dynamics. By forming a collar around the ParM filament rather than binding the end of the filament “face on,” the ParR/parC ring 1) could interact with identical surfaces at either end of a ParM filament, 2) would not be expected to affect the elongation rate of bound ParM filaments, and 3) could interact with no more than one filament at a time. One hypothesis for how the ParR/parC rings surf on the growing ends of ParM filaments would thus be that the ring has high affinity for ATP-bound portions of the filament and lower affinity for portions of the filament that have hydrolyzed bound ATP. An appealing analogy for this situation is the way in which Dam1 complexes are thought to encircle microtubules. Dam1 rings appear to prefer the plus-end of a microtubule, but by applying force to the complex, it can be moved away from the tip and slide along the entire length of the microtubule. Additional single molecule experiments will be required to determine whether this is also true for ParR/parC rings.

Finally, we previously found that, in vivo, R1 spindles are occasionally composed of at least two ParM filaments. Our results using monoconjugated gold particles suggest that these multifilament spindles contain at least two ParR/parC complexes at each end. This implies that clusters composed of multiple plasmids can segregate together as a single unit in vivo. This is also consistent with our previous observations of plasmid dynamics in vivo. Using plasmids labeled with fluorescently tagged DNA-binding proteins, we often observed segregation of plasmid foci with dramatically different fluorescence intensities, suggesting that the two foci contained different numbers of plasmids. Plasmid clustering is not well understood, but we suggest that it may play a previously unsuspected role in replication and segregation in vivo.
Chapter 3.
Strain-Dependent Photoluminescence Behavior of Nanocrystals with Spherical, Linear, and Branched Topologies


The photoluminescence of CdSe/CdS core/shell quantum dots, nanorods, and tetrapods is investigated as a function of applied hydrostatic and non-hydrostatic pressure. The optoelectronic properties of all three nanocrystal morphologies are affected by strain. Furthermore, it is demonstrated that the unique morphology of seeded tetrapods is highly sensitive to non-isotropic stress environments. Seeded tetrapods can thereby serve as an optical strain gauge, capable of measuring forces on the order of nanonewtons. We anticipate that a nanocrystal strain gauge with optical readout will be useful for applications including sensitive optomechanical devices and biological force investigations.

3.1 Strain-Induced Luminescence Effects in Electronically Coupled Nanocrystal Molecules

In recent years, a new generation of quantum confined colloidal semiconductor structures has emerged, with more complex shapes than simple quantum dots. These include nanorods and tetrapods. Beyond shape, it is also now possible to spatially vary the electron and hole potentials within these nanoparticles by varying the composition. Examples of these new structures include seeded dots, rods, and tetrapods, which contain a CdSe core embedded within a CdS shell. These structures may have many uses beyond those envisioned for simple quantum dots, which are frequently employed in luminescent applications. This paper is concerned with changes in the optoelectronic properties of tetrapods when the arms are bent. We demonstrate that seeded tetrapods can serve as an optical strain gauge, capable of measuring forces on the order of nanonewtons. We anticipate that a nanocrystal strain gauge with optical readout will be useful for applications including sensitive optomechanical devices and biological force investigations.

A tetrapod nanocrystal consists of a central core with four arms branching out at the tetrahedral angle. We have previously shown a few ways in which tetrapod arms can be bent. For instance, when a CdTe tetrapod (4 nm wide and 100 nm long arms) is deposited on a substrate through solvent evaporation, the fluid exerts a capillary force which pulls the tetrapod toward the substrate, in some cases permanently deforming the arms. Salmeron and co-workers used an atomic force microscope to press on the outward projecting arms of surface-immobilized CdTe tetrapods and have shown that for forces below 100 nN the tetrapod flexes elastically. Motivated by these observations, Wang and coworkers calculated the electronic level structure of a CdSe tetrapod with different degrees of arm bending, induced by nanonewton forces, and predicted a red shift of the energy gap with increasing strain.
Seeded tetrapods consisting of a CdSe core with CdS arms are highly luminescent\(^6\) and are very symmetric objects. When placed under an anisotropic stress, we expect a reduction in symmetry, which will influence the electronic level structure. To fully quantify such effects, we have examined a series of samples under diverse conditions of stress and strain. Specifically, we have examined the luminescence from seeded dots, rods, and tetrapods placed in a diamond anvil cell (DAC) as a function of applied pressure in a highly hydrostatic medium, which transmits pressure nearly isotropically, as well as a nonhydrostatic medium, which transmits pressure anisotropically. We compare the tetrapods to rods to separate out stress-induced strain effects in a single rod from effects specifically arising in a tetrapod consisting of connected rods. An additional important difference between seeded rods and tetrapods is that the seed in the rod case is of wurtzite (wz) or hexagonal symmetry, while in the tetrapod case it is of zinc blende (zb) or cubic symmetry. For this reason, we complete our studies by investigating simple wurtzite seeds under the same conditions of hydrostatic and nonhydrostatic stress. With this set of experiments, we can investigate the effects of deviatoric stresses on the luminescence of seeded tetrapods.

### 3.2 Nanocrystal Luminescence Inside a Diamond Anvil Cell

The particles under study were wz-CdSe/wz-CdS core/shell dots, wz-CdSe/wz-CdS core/shell rods, and zb-CdSe/wz-CdS core/shell tetrapods, with \(4.4 \pm 0.6, 4.0 \pm 0.4,\) and \(4.4 \pm 0.7\) nm cores, respectively. Dots were prepared following Li et al.\(^{113}\), and rods and tetrapods were prepared following Talapin et al.\(^6\) Structural and optical characterization demonstrate that the particles are of narrow size distribution. Transmission electron microscopy (TEM) images (Figure 3.1a-c) reveal dots of \(7.0 \pm 1.1\) nm diameter, rods of \(28.4 \pm 2.6\) nm length \(x 5.8 \pm 1.3\) nm width, and tetrapods of \(27.8 \pm 3.5\) nm arm length \(x 4.8 \pm 1.2\) nm arm width. Absorbance and fluorescence spectra (Figure 3.1d-f) show highly luminescent particles with broad absorption in the region below 500 nm, characteristic for CdS-containing particles, and a narrow photoluminescence (PL) peak of \(\sim 30\) nm full width at half-maximum, indicative of a monodisperse sample.

The samples were dispersed in a hydrostatic (1:1 (v/v) pentane/isopentane) or nonhydrostatic (toluene) pressure medium, and loaded into the DAC, with an initial pressure of 0.5-1 GPa. Toluene freezes at 1.7 GPa and shows high viscosity behavior above ambient pressures, indicative of a highly anisotropic, nonhydrostatic pressure transmitting medium\(^{118}\). The pressure in the DAC was increased to <6 GPa, and then decreased to ambient pressure. Fluorescence traces obtained during the experiment (Figure 3.2) demonstrate highly contrasting behavior between hydrostatic and nonhydrostatic pressure conditions and among particle geometries. The most obvious trend under hydrostatic pressure is a pressure induced blue shift of the PL peak. A slight asymmetric broadening of the peak at longer wavelengths is observed at higher pressures. The behavior under nonhydrostatic pressure is markedly different. For dots, the PL peak splits into a doublet at pressures above 0.7 GPa. For dots and rods, a shallower peak also typically arises at \(\sim 650\) nm. The PL peak in tetrapods instead slightly red shifts with increasing pressure, with the appearance of a small blue shoulder. The fluorescence behavior is reversible, with no apparent hysteresis, although the PL peaks are somewhat wider at the end of the experiment. The particles remained intact throughout the experiment as demonstrated by sample recovery post-compression (Figure 3.2 insets); we found that above 6 GPa, recovered nanoparticles were...
broken due to the high nonhydrostatic pressure environment within the cell. We did not observe the abrupt PL intensity decrease or peak broadening associated with a phase transition to rock salt\textsuperscript{119}; due to the mechanically stiffer CdS shell on the surface, these particles likely undergo this phase transition at higher pressures\textsuperscript{120}. The sharp spectral peak around 700 nm is from fluorescence of the ruby grains used as a pressure gauge within the DAC\textsuperscript{121}.

![TEM images and optical properties of CdSe/CdS core/shell nanocrystals with different geometries.](image)

**Figure 3.1.** Transmission electron microscopy (TEM) images and optical properties of CdSe/CdS core/shell nanocrystals with different geometries. TEM images for CdSe/CdS core/shell (a) dots (wz-CdSe core/wz-CdS shell), (b) rods (wz-CdSe core/wz CdS rod shell), and (c) tetrapods (zb-CdSe core/wz-CdS arms). Corresponding ensemble absorption (blue) and fluorescence (orange) spectra for CdSe/CdS (d) dots, (e), rods, and (f) tetrapods. The blue x40 and x5 traces are provided for clear visualization of the exciton absorption peak. All scale bars represent 20 nm.
Figure 3.2. Fluorescence spectra of CdSe/CdS nanocrystals in a diamond anvil cell (DAC) under hydrostatic and nonhydrostatic pressure. DAC fluorescence traces in hydrostatic 1:1 (v/v) pentane/isopentane medium for CdSe/CdS (a) dots, (b) rods, and (c) tetrapods, and in nonhydrostatic toluene medium for CdSe/CdS (d) dots, (e) rods, and (f) tetrapods. Subsequent spectra at different pressure points are shown in stacked offsets. Pressure (pressure gradient only if gradient ≥0.1) in GPa is labeled on the corresponding trace. Insets show TEM images of post-DAC recovered particles. All scale bars represent 20 nm. Fluorescence spike ∼694 nm is due to fluorescence from ruby grains, used to determine pressure inside the DAC.
3.3 Analysis of Strain-Induced Luminescence Shifts

To quantitatively evaluate the PL shifts of the particles, we fit the experimental data to a sum of Gaussian curves (see analysis methods in Supporting Information). PL peak energies as a function of pressure are shown in Figure 3.3a-c, and the percentage of particles in the red-most peak, calculated from the area under the fitted peak, is shown in Figure 3.3d. There are four notable observations from this analysis. First, under nonhydrostatic pressure for all three particle morphologies, two PL peaks are observed: a blue peak, whose energy as a function of pressure matches with that of the peak under hydrostatic pressure, and a red peak, which does not exist under hydrostatic pressure conditions. The two peaks are likely due to fluorescence from two populations within the cell, rather than two allowed transitions within individual particles, since the energy difference between the two peaks is much greater than the thermal energy available. However, future single particle fluorescence experiments are required to confirm this assessment.

That the blue peak and hydrostatic peak energies match so well suggests that the particles emitting at the blue peak experience a near-hydrostatic environment within the DAC, while those emitting at the red peak are affected by the nonhydrostaticity. Second, under nonhydrostatic pressure for dots, the blue peak further splits into at least two peaks, but possibly more, as evidenced by the greater spread in peak position (Figure 3.3a). The hydrostatic pressure peak for dots, with a broader red side, is also fit to two peaks for comparison. This blue-peak splitting phenomenon is not observed for rods or tetrapods. Third, for dots and rods under nonhydrostatic pressure, the energy of the red peak changes somewhat (-5.6 and 5.2 meV/GPa, respectively), while for tetrapods the red-peak emission red shifts at nearly two times this rate (-9.9 meV/GPa) (Figure 3.3a-c insets). Fourth, tetrapods are clearly the most affected by the nonhydrostatic pressure, with more than 95% of the population in this red-peak state (Figure 3.3d). This agrees intuitively considering the shape and larger size of tetrapods compared with dots and rods. This also demonstrates that tetrapods are the most suitable particle geometry for strain sensing as they are most likely to be strained. Under hydrostatic pressure, the particles are isotropically compressed, while under nonhydrostatic pressure, the particles additionally experience a net deviatoric stress which includes uniaxial and shear stresses. The difference between the two informs the net strain due only to nonhydrostatic stress. To investigate the optical effects of only nonhydrostatic stress on the tetrapods, which leads to arm bending, the energy difference between the nonhydrostatic peak and hydrostatic shoulder (Figure 3.2f) is plotted (Figure 3.3e). We observe a clear red shift with increasing stress. Although the strain state inside a DAC at nanometer size scales cannot be readily quantified, using the pressure gradient inside the cell and the particle size we calculate a force per particle on the order of nanonewtons. This is of the same magnitude as the forces previously observed to bend tetrapod arms\textsuperscript{73,117}. 


Figure 3.3. Deviatoric stresses induce new radiative transitions in semiconductor nanocrystals.

Fluorescence peak positions in CdSe/CdS (a) dots, (b) rods, and (c) tetrapods. Unfilled symbols denote the peak positions in 1:1 pentane/isopentane; filled symbols denote the peak positions in toluene. Under nonhydrostatic pressure, three radiative transitions are observed in dots and two radiative transitions are observed in rods and tetrapods. Insets in (a-c) provide a magnification of the emission points at the transition ~1.9 eV, with x-axis 0-6 GPa and y-axis 1.879-1.937 eV; a linear fit provides a guide to the eye. (d) Percentage of particles emitting in the transition ~1.9 eV under compression in toluene. Less than 50% of dots (circles) and rods (rod shapes) emit at this transition, while nearly 100% of tetrapods (crosses) do. (e) A difference plot of the fluorescence peak maxima for CdSe/CdS tetrapods compressed in hydrostatic and nonhydrostatic media provides a measure of the net deviatoric stress effect—which induces tetrapod arm bending—on tetrapod emission. A fluorescence peak red shift due to nonhydrostatic stress on the order of nanonewtons per particle is observed (see discussion). The linear fit shown provides a guide to the eye. Error bars show the standard error of peak position from the fit.
3.4 A Model of the Observed Photoluminescence Transitions

A model to explain the observed behavior is shown in Figure 3.4. In CdSe/CdS nanocrystals, the fluorescence recombination occurs in the CdSe core, with the electron relatively more delocalized throughout the nanocrystal while the hole is confined to the core.77,79,80,81,82,122 The conduction band is comprised mainly of Cd 5s orbitals. For dots and rods, with a wz-CdSe core, the valence band is comprised mainly of Se 4pₓ and 4pᵧ orbitals; the valence band of tetrapods, which have a zb-CdSe core, is comprised of Se 4pₓ, 4pᵧ, and 4pᶻ orbitals. Under hydrostatic pressure conditions the crystal bonds are compressed, and the greater wave function overlap leads to a blue shift of the energy gap, mainly due to an increase in the conduction band energy.123,124 Small deviatoric stresses lead to the observed fluorescence peak asymmetry particularly seen in dots. This effect is more obvious under nonhydrostatic pressure, where the blue peak for dots splits into at least two luminescent states due to stress. This effect has been previously observed for bulk CdSe stressed in different directions.125 For rods, blue-peak splitting is not as prominent due to the elongated CdS shell on the CdSe (100) faces; the mechanically stiffer CdS126 hinders compression along the CdSe c-axis. Therefore, fewer luminescent strain states are possible. Intermediate behavior for rods with the same size CdSe core but shorter CdS rod shells is observed (Figure A3.1). Under higher nonhydrostatic pressure conditions, experimentally achieved by particle dispersion in toluene, some bonds in the crystals are pulled apart by torque, leading to a red shift of the energy gap. Because the particles are randomly oriented with respect to the net uniaxial strain, the observed ensemble energy levels are broadened.

The distinction between tetrapods versus dot and rod particles is that the unique geometry of tetrapods makes them more physically sensitive to stress-induced effects. Once the medium has frozen (or become quite viscous) around the tetrapods, further application of anisotropic stress applies bending moments to each of the tetrapod arms, with each arm’s bending moment dependent on its orientation relative to the principal stress axes. Thus the arms of the tetrapod act essentially as lever arms, subjecting the zb-CdSe core to large shear stresses so that some bonds are stretched and others compressed relative to the hydrostatic configuration. The data suggest that the net result of arm bending is a red shifting of the energy gap. Consequently, forces which bend tetrapod arms may be optically detected.

While we considered other mechanisms for previously observed strain- and shape-dependent optical phenomena to explain our findings, they are ruled out given our experimental design. First, it has been hypothesized that the blue-PL peak split in CdSe quantum-dot solids arising at nonhydrostatic pressure conditions is due to an energy transfer mechanism between particles.127 This cannot be the central mechanism in our experiment, since the nanoparticle sample is dilute. Second, the appearance of the red peak under nonhydrostatic pressure conditions is not due to a type I-type II heterostructure transition, since this peak is also observed in our bare CdSe dot pressure studies (Figure A3.2). Finally, an aspect-ratio-induced crossover between the bands formed by the Se 4pₓ, 4pᵧ, and the Se 4pᶻ orbitals does not cause the red peak observed in the present study; the particle compression is too slight to increase the aspect ratio. However, under nonhydrostatic conditions the Se 4pₓ band may be at higher energy than the 4pᵧ and 4pᶻ bands for dots and tetrapods due to greater compression in the z-direction.123
3.5 Prospective Applications of Strain-Dependent Photoluminescence

In summary, our experiments demonstrate that the optical properties of highly luminescent CdSe/CdS nanocrystals are altered in response to external stresses. These particles, with the same long-lasting and narrow-emission advantages of shell-encapsulated quantum dots, provide a nanonewton-sensitive nanocrystal analogue of the mechanoresponsive polymeric materials recently described. Core/shell dots, because of their small size, isotropic shape, and lack of steric hindrance, may be employed to quantify nonhydrostaticity, for example within a DAC. Tetrapods, due to their unique geometry, are particularly suited for use as an optical strain gauge. Further development of tetrapods in this capacity requires precise calibration of the fluorescence emission shift with force by alternate methods, since the strain state inside a DAC at nanometer size scales cannot be readily determined. Synthetic tunability of size and shape may allow tetrapods sensitive to forces over a range of magnitudes. An optical strain gauge, sensing nanonewton forces, will be useful for optomechanical devices and investigations of biomechanical processes.
Chapter 4.  
A Luminescent Nanocrystal Stress Gauge


Microscale mechanical forces can determine important outcomes ranging from the site of material fracture to stem cell fate. However, local stresses in a vast majority of systems cannot be measured due to the limitations of current techniques. In this work, we present the design and implementation of the CdSe-CdS core-shell tetrapod nanocrystal, a local stress sensor with bright luminescence readout. We calibrate the tetrapod luminescence response to stress and use the luminescence signal to report the spatial distribution of local stresses in single polyester fibers under uniaxial strain. The bright stress-dependent emission of the tetrapod, its nanoscale size, and its colloidal nature provide a unique tool that may be incorporated into a variety of micromechanical systems including materials and biological samples to quantify local stresses with high spatial resolution.

4.1 The CdSe/CdS Tetrapod as a Luminescent Stress Sensor

Local microscale stresses play a crucial role in inhomogeneous mechanical processes from cell motility to material failure. Stress is a tensor representing force per unit area and is directly related to strain—a tensor that represents change in size and/or shape—via the stiffness constants of a material. Contact-probe techniques that measure stiffness such as atomic force microscopy\(^1\), indentation testing\(^2\), and optical coherence elastography\(^3\), and noncontact techniques that measure stress such as micro-Raman spectroscopy\(^4\), electron backscatter diffraction\(^5\), and polymeric post arrays\(^6\) have been used to quantify local mechanical behavior with high spatial resolution. However, these techniques remain limited to studies in specific material systems due to spectroscopic and geometric constraints. For example, although the mechanical behavior of cells can be indicative of significant aspects of their biology, including metastatic potential\(^7\), the stresses exerted by cells in physiologic three-dimensional culture systems cannot currently be quantified. A luminescent nanocrystal probe, with its small size, bright and narrow emission, and colloidal processability is ideally suited to measure local stresses in a variety of systems without spectroscopic requirements from or excessive perturbations to the material of interest.

We present here the design and implementation of a luminescent nanocrystal stress gauge, the CdSe-CdS core-shell tetrapod. The tetrapod, with a CdSe quantum dot at its core, has the same advantages as its widely used quantum dot predecessor, including tunable quantum confinement and high fluorescence quantum yields\(^8\). Four CdS arms protruding from the CdSe core confer a branched, three-dimensional structure on the tetrapod; these arms can act as dynamic levers to torque the CdSe core and alter its optical response. The tetrapod can be incorporated into many materials, yielding a local stress measurement through optical fluorescence spectroscopy of the electronically confined CdSe core states. In this report, we calibrate the stress response of the
tetrapod, and use this calibration to study spatially resolved mechanical behavior in single polymer fibers. We expect that tetrapods can be used to probe local stresses in many other mechanical systems.

The tetrapod nanocrystal is a tetrahedrally symmetric particle consisting of a zinc-blende core with four epitaxially attached wurtzite arms\textsuperscript{112} (Figure 4.1a). Atomic force microscopy studies on CdTe tetrapods demonstrated that nanonewton forces are capable of bending these lever arms\textsuperscript{117}. Furthermore, electronic level structure calculations on CdSe tetrapods predicted that force-induced arm bending, which causes a strain in the nanocrystal, results in a red shift of the energy gap\textsuperscript{73}. CdSe-CdS core-shell tetrapods, with a CdSe core and CdS arms, have high fluorescence quantum yields up to 60% due to quasi-type I band alignment of the heterostructure\textsuperscript{74}, and therefore present an optimal design for optical readout of local stresses. Compression of CdSe-CdS tetrapods in a diamond anvil cell revealed differential photoluminescence behavior under hydrostatic and nonhydrostatic pressure environments\textsuperscript{137}. These results suggested that the tetrapod might be useful as a gauge capable of sensing and optically reporting environmental stresses.

4.2 Calibration of the Luminescent Stress Gauge Using Single Polyester Fibers

To develop the tetrapods as a luminescent stress gauge, we first calibrated the fluorescence response of tetrapods to nonhydrostatic stress in a simple uniaxial geometry and then used this response to report local stresses in synthetic polymer fibers. Tetrapods were incorporated into single polyester fibers with known stress–strain properties and the tetrapod fluorescence was monitored under increasing tensile strain. CdSe-CdS tetrapods (4.8 ± 1.2 nm arm diameter and 27.8 ± 3.5 nm arm length) in low concentrations are easily incorporated into the fiber without perturbing the mechanical properties by dropping a few microliters of dilute solution in toluene onto the fiber (see Methods). The toluene quickly evaporates, and red fluorescence (Figure 4.1b)
shifted from that of tetrapods in solution (Figure 4.2a) is exhibited at all focal planes within the fiber, indicating that tetrapods are dispersed throughout. Diffusion of tetrapod fluorescence along the fiber following solvent evaporation was not observed, suggesting that tetrapods are relatively immobilized, along with their surface ligands. The chemistry of these surface ligands could be used to control tetrapod–matrix interactions; in this work, however, the tetrapods are simply dispersed in the matrix with no molecular scale anchoring. SEM images of a fiber cross-section confirm that tetrapods are embedded and well distributed within (Figure 4.1c).

Single polyester fibers with embedded tetrapods were affixed at both ends and tetrapod fluorescence spectra were monitored at a fixed spot with increasing tensile strain (see Methods). A clear spectral red shift is evident as a result of increasing fiber extension (Figure 4.2a). This shift is detectable with a spectral resolution of 0.01 nm in our system. The slight shoulder at higher energy in each trace (blue arrow), which matches the spectral peak of tetrapods in solution, is attributed to a fractional population of tetrapods on the surface of the fiber. Although the refractive indices of the fiber also change as the fiber is extended, this change is within ±0.02 and not large enough to induce the shifts we observe138,139,140. Similarly, the temperature change from tensile loading contributes a maximum fluorescence band-gap shift of about ± 0.02 nm141,142, a comparatively minor effect. The initial fluorescence wavelength maximum as well as the magnitude of the red shift varies both spatially along the fiber as well as among different fibers (Figure 4.2b), consistent with previous observations of mechanical variation in microstructure within and among single fibers74. In fact, the branched nature of the tetrapod may enable it to sense and report relevant length scales of spatial and dynamic heterogeneity down to the dimension of a tetrapod arm diameter. The initial fluorescence spectrum is recovered upon fiber failure and matrix relaxation, suggesting that under these experimental conditions the tetrapod deformation remains elastic, as expected from previous observations of fluorescence reversibility142.

Applied stresses to the tetrapod from fiber strain directly affect the energy gap, and therefore we studied the change in peak photoluminescence energy as a function of the fiber extension (Figure 4.2b). The linear elastic regime and onset of plasticity are readily identified in the photoluminescence behavior. The average of individual fibers is expected to reflect bulk behavior. The average fluorescence slope versus true strain in the elastic regime was correlated with the known average Young’s modulus of 8.3 ± 1.9 GPa72 in order to calibrate the stress gauge. We determined a fluorescence shift of −5.8 ± 1.2 meV/GPa (see Methods). This value compares reasonably to the theoretical prediction of −30.8 meV/GPa for single material CdSe tetrapods74, especially considering that differences in the strain dependence should exist due to altered wavefunction localizations within the hetero- and single material structures143,144, and that different sizes of tetrapods were considered in the two studies. Additionally, the measured response to uniaxial stress is about one-fourth the magnitude of the photoluminescence blue-shift response to hydrostatic pressure145. The calibration error is due to uncertainty from the linear fit; measurement error due to the spectral resolution and peak-fitting uncertainty corresponds to a stress resolution of 0.003 GPa.

Conventional measurement of the true stress in a fiber requires accurate knowledge of the diameter change, which is difficult to measure in fibers of microscale diameter. The tetrapod stress gauge avoids this difficulty by responding directly to the local true stress. As a proof-of
principle demonstration, we applied the polyester-derived calibration to find the Young’s moduli (E) of two high performance fibers of known E: Nomex® (10-µm diameter), a stiffer fiber than polyester, and nylon (120-µm diameter), a more compliant fiber. We found E = 18.9 ± 3.0 GPa and 4.3 ± 1.3 GPa for Nomex® and nylon, respectively, within the range of accepted values for these materials.\textsuperscript{146} (Fig. 2C). The reported error values represent the error in calculated modulus due to the linear fit (see Methods). The calculated E for Nomex® is slightly higher than the accepted value, likely because we typically focused on a kink band in these thin fibers to easily mark the location for repeated measurements. Two limiting behaviors of sensitivity to the Young’s modulus of the host medium exist. If the tetrapod probe is stiffer than the host medium and will not be easily deformed, then stress is transferred to the probe. If the tetrapod probe is less stiff than the host medium, it will move with the medium, thus adopting the strain of the material. As a result, the upper limit that the tetrapod can detect is the modulus of the tetrapod itself. Tetrapod luminescence in tensile experiments on single Spectra® fibers, E = 103 GPa, suggests a Young’s modulus of 50 GPa, which is roughly the Young’s modulus of bulk CdSe; this result suggests that the sensitivity of the tetrapod stress gauge is limited to materials with moduli less than 50 GPa. Although nanoparticles at higher concentrations can change the mechanical properties of a polymer matrix,\textsuperscript{146} the dilute amounts of tetrapods incorporated here did not significantly affect the mechanical behavior of these fibers as evidenced by an unchanged true strain to failure and unaffected site of failure (see Methods).

\textbf{Figure 4.2. Calibration of the tetrapod stress gauge.} (a) Fluorescence spectra of tetrapods embedded in a single polyester fiber under extension. A fluorescence red shift is clearly observed with increasing strain. A spectral shoulder matches that of the tetrapods in solution (blue arrow). (b) The change in energy of the tetrapod emission maximum as a function of extension. Traces of single polyester fibers (dotted lines) reveal varied mechanical behavior, consistent with variations in microstructure domains within and among single fibers. The average (solid line) in the elastic regime is correlated with the known Young’s modulus of polyester fiber to calibrate the tetrapod stress gauge. Error bars depict the standard deviation of fiber behavior, consistent with previous observations of mechanical variation within and among single fibers; fit error is within the data markers. (c) Emission maxima of tetrapods in Nomex® (cyan) and nylon (blue) single fibers as a function of fiber extension (dotted lines) and the corresponding average (solid lines) demonstrate an accurate measure of the Young’s modulus of these fibers (black, literature values). The error bars represent the error in calculated modulus due to linear fit.
4.3 Tetrapod Photoluminescence as a Probe of Local Microscale Stresses

The luminescent tetrapod stress gauge enables the imaging of local stresses within a complex medium. The ability to study local mechanical behavior is critical to an understanding of a material’s response to external perturbations; for example, polymer fibers do not respond homogeneously under tensile strain, and a local instability may ultimately become the site of fiber failure. Ediger and coworkers\(^\text{146}\) studied the local mobilities associated with plastic flow within a polymer glass below the glass transition temperature. Upon perturbation with a fixed stress above a low-stress threshold, they observed not only an increase in the polymer mobility, as predicted by the Eyring model, but also a subsequent increase in the homogeneity of local mobility rates. Further polymer creep and recovery, leading to a decrease in the polymer mobility, resulted in a respreading of the mobility rate distribution. They suggested that local stresses would mirror this behavior, with greater stresses in slower regions enabling higher mobility\(^\text{74}\). We used the tetrapods to spatially resolve the local stress profile of a semi-crystalline polyester fiber under increasing tensile strain (Figure 4.3). Tetrapods were incorporated along an 8-mm stretch of polyester fiber, and fluorescence spectra were collected at adjacent spots 400 µm in length, of similar spot size to that studied by Ediger and coworkers\(^\text{122}\), as the fiber was extended up to 22.5% (Figure 4.3a, see Methods). The ensemble red shift with increasing tensile strain is clearly seen in the data. In addition, we observe a stress distribution narrowing in the elastic regime, followed by a distribution widening (Figure 4.3b). This result indicates that the stress first becomes more homogeneous with strain and then increases in heterogeneity, correlating well with the previous observations of local mobility.

4.4 Stress Sensing in Tetrapods of Varying Dimensions

The CdSe-CdS tetrapods studied here present an optimal size and shape for sensing stress. Tensile stretching experiments on single polyester fibers embedded with tetrapods of similar diameter but longer (42.8 ± 2.8) or shorter (15.1 ± 1.7) arm lengths both exhibit reduced stress sensitivity (Figure 4.4a). We hypothesize that, although a longer tetrapod arm may increase the amount of torque on the CdSe core and thus the stress sensitivity, an arm that is too long should additionally buckle, reducing the stress sensitivity. The effects of torque sensitivity and buckling will be maximized and minimized, respectively, in an optimal tetrapod arm aspect ratio for stress sensing. In addition, the tetrapod morphology is unique to sensing anisotropic stresses. Similar polyester fiber tensile experiments using CdSe-CdS quantum dots and rods demonstrate that these nanoscale geometries cannot specifically detect anisotropic stresses (Figure 4.4b). Quantum dots (7.0 ± 1.1-nm diameter) exhibited a blue shift with increasing fiber strain, similar to their behavior under hydrostatic pressure\(^\text{147}\). Quantum rods, which from different viewing axes appear similar to either the dots or the tetrapods, exhibited either a slight red or blue shift with increasing tensile strain, suggesting that local rod orientation relative to the fiber affects the response to strain. No average net shift was observed for these particles.
Figure 4.3. Local stresses in a single polyester fiber. (a) A spatially resolved profile of the stresses along a fiber with increasing tensile strain. Fluorescence along the same section of fiber is monitored throughout; however, this length increases as the fiber is extended and thus fluorescence spectra are collected at a greater number of 400-µm spots. Twenty data points are collected at 0% extension up to 26 data points at 22.5% extension. (b) Area-normalized histograms of the tetrapod emission maximum along the fiber at increasing fiber extension using data from the stress profile in A, with 0.1-nm bin size. The stress distribution narrows with fiber extension and then widens, indicating that the stresses initially become more homogeneous upon tensile strain and become increasingly heterogeneous after plastic onset.
Due to its nanoscale size, three-dimensional branched geometry, bright luminescence signature, and colloidal processability, the tetrapod nanocrystal possesses the ability to report both spatially and dynamically resolved stresses in many mechanical systems. The high quantum yield of the tetrapod enables luminescence measurements down to the single particle scale. Utilizing dark-field or superresolution microscopies, the stress profile within a material may ultimately be mapped with spatial resolution of a single tetrapod. Tetrapods may also be biofunctionalized for studies including local stress measurements within biological fibers and three-dimensional cell culture, a method complementary to the use of collagen contrast, bead fluorescence, and gold nanorod scattering for measurement of local mechanical strains in a matrix. In both the mechanobiology and single tetrapod studies, the stress symmetry and its orientation relative to the tetrapods may be more complex than simple uniaxial or hydrostatic deformations on a tetrapod ensemble, and a deeper understanding of the optoelectronic response to other stress states is essential. Current efforts toward these goals are underway. Future synthetic work will allow creation of tetrapods of different sizes sensitive to stresses of a variety of magnitudes, and tetrapods of different compositions with alternate optical band gaps and particle stiffness, expanding the range of material systems and processes that may be probed.
Chapter 5.
Spatially Indirect Emission in a Luminescent Nanocrystal Molecule


Recent advances in the synthesis of multicomponent nanocrystals have enabled the design of nanocrystal molecules with unique photophysical behavior and functionality. Here we demonstrate a highly luminescent nanocrystal molecule, the CdSe/CdS core/shell tetrapod, which is designed to have weak vibronic coupling between excited states and thereby violates Kasha’s rule via emission from multiple excited levels. Using single particle photoluminescence spectroscopy, we show that in addition to the expected LUMO to HOMO radiative transition, a higher energy transition is allowed via spatially indirect recombination. The oscillator strength of this transition can be experimentally controlled, enabling control over carrier behavior and localization at the nanoscale.

5.1 Kasha’s Rule for Luminescent Molecules

Kasha’s rule is a principle governing luminescence behavior in organic molecules: radiative emission occurs only from the lowest energy excited state of the molecule. This rule follows from kinetic considerations: non-radiative internal conversion between excited states of a given multiplicity is typically fast relative to radiative emission, while internal conversion and radiative emission rates are competitive for relaxation from the lowest excited state to the ground state. Rare molecular exceptions to Kasha’s rule exist via decreased non-radiative rates caused by large excited state level spacings, or thermal population of low-lying excited states followed by radiative emission. Highly luminescent nanocrystal molecules, which consist of electronically coupled quantum dot atoms typically in a type-I core/shell configuration, also obey Kasha’s rule due to relatively fast non-radiative relaxation from higher energy excited states. However, utilizing recent advances in the synthesis of more complex nanocrystal heterostructures, it is conceivable that a nanocrystal molecule with bright photoluminescence may be designed to radiatively emit from multiple excited states to the ground state. Here we demonstrate a nanocrystal molecule, the CdSe/CdS core/shell tetrapod, which breaks Kasha’s rule by exhibiting radiative transitions from multiple excited states. Using single particle luminescence spectroscopy, we study the scaling of this violation with experimental parameters and quantify the distribution of energy differences between transitions. We show that in addition to the expected LUMO to HOMO radiative transition, a higher energy transition is allowed via spatially indirect recombination.
Figure 5.1. Emission from two discrete energy states is observed in single CdSe/CdS tetrapod nanocrystal molecules. (a) The CdSe/CdS core/shell tetrapod consists of a zincblende CdSe core (red) with wurtzite CdS arms (yellow). (b) TEM image of CdSe/CdS tetrapods with 30 nm arms. The tetrapods are oriented with three arms on the substrate and the fourth arm protruding out of the plane of the page. (c) Photoluminescence excitation (orange, collected at the emission peak) and emission (blue) spectra from an ensemble of CdSe/CdS tetrapods. (d) Luminescence spectra of a representative 30 nm-arm single tetrapod at different time frames (0.1 s integration time). Two emission peaks are clearly seen. (e) The single tetrapod time-integrated emission spectra (dotted curves) summed together (purple curve) reproduce the Gaussian-shaped ensemble fluorescence band; shown here is data for tetrapods with 30 nm arms. Sample traces from singly-emitting (black) or multiply-emitting (red) single tetrapods are shown for comparison. The amplitude of the ensemble curve is reduced on this plot for clarity.
5.2 Electronic Structure and Ensemble Behavior of CdSe/CdS Tetrapods

The CdSe/CdS core/shell tetrapod is a tetrahedrally symmetric nanocrystal molecule consisting of a zinc-blende CdSe quantum dot core and four epitaxially attached CdS arms (Figure 5.1a-b). In analogy to organic molecules, the energy levels of the tetrapod can be described using the language of molecular orbitals, although it is noted that nanocrystal molecule energy levels are not orthogonal. The HOMO of the CdSe/CdS tetrapod involves a hole that is dominantly localized within the central CdSe core, while the LUMO is centered within the core but has a substantial probability of presence in the four arms. The quasi-type I band alignment of this CdSe/CdS heterostructure results in high luminescence quantum yields of 30-60% \(^{156}\). The photoluminescence spectrum from an ensemble of CdSe/CdS tetrapods taken at low excitation fluence exhibits a single emission peak at \(\sim 1.9 \text{ eV}\) (Figure 5.1c), corresponding to emission of an electron into the HOMO level; the peak width of \(\sim 0.12 \text{ eV}\) at room temperature is typically considered to be a convolution of the temperature-dependent single particle intrinsic linewidth and sample polydispersity due to quantum size effects \(^{79,81}\). The ensemble excitation spectrum contains a second higher energy absorption threshold (\(\sim 2.4 \text{ eV}\)) above the lowest band gap due to absorption in the CdS arms (Figure 5.1c). At high excitation fluence, emission from the recombination of carriers in CdS may also occur \(^{155}\); this is analogous in effect to a molecule containing two uncoupled chromophores.

On the basis of the electronic structure alignment of a CdSe/CdS nanocrystal heterostructure \(^{157}\) and the single particle emission behavior of other semiconductor nanocrystals \(^{156,158}\), it is expected, at least for low excitation fluence, that a single CdSe/CdS tetrapod would emit from only one transition with energy corresponding to the HOMO-LUMO gap of the combined core-shell system. In this Letter, we use single particle luminescence spectroscopy to show that tetrapods can emit radiatively at multiple energies within the spectral range of the CdSe optical band gap (Figure 5.1d). This behavior is obscured in ensemble measurements due to sample polydispersity, and also because a majority of tetrapods exhibit a single radiative transition during the observation time (Figure 5.1e).

5.3 Factors Affecting the Propensity for Multiple Radiative Transitions

The propensity for radiative transitions from multiple states is affected by several factors, including tetrapod arm length, incident photon fluence, and excitation energy. We studied these effects by measuring the photoluminescence spectra and intensity traces from single tetrapods using a home-built single molecule confocal microscope \(^{159}\). Data was collected for tetrapods with 15, 30, or 45 nm arms using 2.33 eV or 2.54 eV laser excitation at intensities ranging from 0.3 µW to 18 µW (Methods). In total, 260,400 spectral frames from 651 tetrapods were analyzed.

Tetrapod arm length is an important factor governing the electron wavefunction delocalization since longer arm lengths increase the probability that an excited electron is located in the CdS arm \(^{158,160}\). We found that increasing the tetrapod arm length increased the occurrence of emission from multiple peaks at energies around the CdSe band gap (Figure 5.2a), suggesting that these multiple emissions were related to an increased occurrence of excited electrons located in the CdS arms. The incident photon flux affects the rate of exciton generation. If multiple peak emission were caused by multiple excitons, we would expect an increase in occurrence with
increasing laser intensity\textsuperscript{161,162}; instead we observed a decrease (Figure 5.2b). The photon energy used to excite the system can alter the spatial origin of carriers due to different band gaps of the heterostructure components; 2.33 eV photon excitation around the CdSe band gap predominantly excites carriers located in the core, whereas photons at 2.54 eV are likely to excite some carriers in the CdS arms as well (Figure 5.1c). We found that the propensity for multiple radiative transitions was similar regardless of photon energy (Figure 5.2c), demonstrating that the mechanism for multiple emissions was mostly independent of the spatial origin of excited electrons and holes. Recent studies of single tetrapods at cryogenic temperatures have not revealed multiple luminescence transitions at these energies, suggesting that thermal energy also plays a key role in this phenomenon.

![Image of Figure 5.2](image_url)

**Figure 5.2.** The multiple emission peaks are due to recombination of the CdSe-confined hole with an electron primarily located either in the CdSe or CdS. The fraction of single tetrapods exhibiting multiple peak emission as a function of (a) tetrapod arm length (at 2.54 eV excitation and 1 µW power), (b) incident laser power (for 30 nm arms and 2.54 eV excitation), (c) excitation wavelength (for 45 nm arms and 1 µW power). (d) Histogram of the energy difference between emission peaks for single tetrapods which emitted radiatively at multiple energies (45 nm arms and 1 µW incident power). This total histogram plot represents data from 18,000 spectra from 45 tetrapods. (e) Histogram of the on- and off-dwell times for the lower (P1) and higher (P2) energy peaks in single tetrapods (45 nm arms and 1 µW incident power).
5.4 Spatially Indirect Emission in the CdSe/CdS Tetrapod

All of the above observations support a model in which the additional radiative transition is caused by spatially indirect emission across the CdSe/CdS heterojunction. Similar behavior has been observed previously in type-II nanocrystal heterostructures in the form of charge-transfer emission\textsuperscript{161,162,163}. The probability of spatially indirect radiative recombination will increase with increasing CdS arm length. Because spatially indirect transitions are slower relative to the rate of direct transitions, increasing the incident photon flux may saturate the indirect channel before the direct channel, resulting in a reduction of the relative yield of indirect transitions\textsuperscript{164,165}. Temperature also plays a role in the propensity for the indirect transition: thermal energy at room temperature allows a non-negligible population of electrons in a CdS-dominant excited state, increasing the probability of a spatially indirect emission, regardless of the spatial origin of the carrier.

The energy difference between the multiple emission peaks provides additional insight into the nature of the radiative transitions. An intensity-weighted histogram of the energy of all observed transitions relative to the lowest energy transition shows three peaks (Figure 5.2d histogram for tetrapods with 45-nm CdS arms; Figure A5.3, Methods, Appendix 5). The first peak, labeled “P1” and centered around 0 eV, represents emission from the lowest energy transition, while “P2” labels the two remaining peaks on this total histogram: a broader peak around 60 meV and a much smaller, sharper peak around 110 meV. Only two of the 106 total multiply emitting particles exhibited emission at three peaks; most particles exhibited emission from only two states. The existence of two higher energy transitions further supports a model in which additional emissions are caused by spatially indirect transitions. Although a tetrapod is tetrahedrally symmetric, this symmetry is broken when it is immobilized on a substrate; tetrapods preferentially adsorb with three arms slightly bent on the substrate and the fourth arm upright\textsuperscript{166} (Figure 5.1b). Strain from arm bending increases the Cd-S bond lengths especially near the CdSe core, lowering the energy of the CdS conduction band\textsuperscript{85}. Thus, the energy of indirect emission from an electron in a strained arm would be lower relative to emission from the upright arm. It is important to note that arm bending induces strain in both the CdSe core and the three bent CdS arms, slightly altering the band structure of both components; only the fourth CdS arm remains unstrained relative to the equilibrium configuration. The lower and higher energy P2 emission peaks can be attributed to transitions involving electrons in the strained and unstrained arms, respectively.

Transition dwell times in and out of both P1 and P2 emission states follow the power law behavior characteristic of blinking from a single emitter\textsuperscript{39} (Figure 5.2e), demonstrating that the duration of luminescence for both radiative transitions is likely dictated by blinking phenomena and also suggesting that the transitions are independent.

The above analysis assumes that the lowest energy transition is the direct transition (Supplementary Information), which is likely considering the measured bulk and nanostructured band alignments\textsuperscript{79,81} and electronic structure calculations\textsuperscript{156}. However, further experiments combining single particle fluorescence spectroscopy with lifetime measurements will allow definitive assignment of emission peak energies to a spatially direct or indirect transition.
The existence of multiple allowed radiative emissions provides another means to probe carrier behavior across a nanoscale heterojunction. From the fluence-dependence data (Figure 5.2b) we can calculate a radiative lifetime for the spatially indirect emission (Supplementary Information). For a system with a single radiative channel,

\[ N_{em} = N_{abs} \times (1 - e^{-k/I}) \times \phi, \]

where \( N_{em} \) is the number of emitted photons, \( N_{abs} \) is the number of absorbed photons, \( k \) is the radiative rate, \( I \) is the rate of incident photons, and \( \phi \) is the intrinsic quantum yield of the transition; \( e^{-k/I} \) is the loss of quantum yield from saturation of the recombination channel. By extension of this expression to a two-channel system, using a literature value for \( 1/k_{direct, \text{CdSe}} \leq 10 \text{ ns}^{167} \), we calculated a lower limit for the radiative lifetime of \( 1/k_{indirect, \text{CdS} \to \text{CdSe}} \sim 200 \text{ ns} \) (Methods). This value is in good agreement with reported values of spatially indirect luminescence lifetimes in other systems\(^{168,169}\).

A schematic of the allowed transitions in the CdSe/CdS tetrapod nanocrystal molecule is shown in Figure 5.3. Electronic structure calculations of CdSe/CdS tetrapods\(^ {156}\) show that the LUMO is concentrated in the CdSe core with some probability extending to the CdS arms, while the LUMO+1 is located primarily within each of the four CdS arms. The first eleven hole excited states are confined within the CdSe core. Hence, the hole is expected to undergo relaxation to the HOMO level before radiative recombination can take place. The primary mode of emission is therefore expected to be due to radiative recombination of a CdSe-localized hole (HOMO) with an electron primarily in the CdSe core (LUMO), corresponding to a spatially direct recombination. However, reduced coupling between the LUMO and the LUMO+1 states due to their small spatial overlap can allow radiative recombination of a CdSe-localized hole in the HOMO with an electron largely located within the CdS arms (LUMO+1) before this electron can relax to the LUMO level. The latter corresponds to a spatially indirect recombination. The spatially separated CdSe and CdS components in the tetrapod and the band alignment of the resulting heterostructure create a partially coupled system in which radiative emission from multiple states is allowed.
5.5 Control Over Spatially Direct and Indirect Transitions

In this work, we demonstrate at the single particle level that the oscillator strength of the spatially indirect transition can be tuned by changing the arm length of the tetrapod; we predict that the lifetime and energy of this transition can also be altered by appropriate structural modifications. The excited state lifetime of a CdSe/CdS tetrapod increases with arm length\(^{61,82}\); the lifetime of the indirect transition should thereby increase as well. A multipod with many CdS arms protruding from a CdSe core might exhibit a spatially indirect transition with the same lifetime as a tetrapod with similar arm lengths, but with increased oscillator strength due to the greater number of arms. A CdSe/CdS tetrapod with extremely thick arms may reduce the quantum confinement in CdS and result in a type-II heterostructure\(^{163,170}\), changing the energy of the indirect transition. Mechanical strain, which can alter the electronic structure and optical behavior in nanocrystal systems\(^{74,85,171}\), may also provide a way to achieve a desired configuration of direct and indirect transitions in a nanocrystal system.

Control over spatially direct and indirect transitions is important for access to both novel fundamental nanoscale behavior and also the rational design of functional systems. Control over carrier localization and transport is extremely important in many applications including photovoltaics and solar-to-fuel. Additionally, multi-color emission within a single highly luminescent particle may provide a unique optical tool. Advances in synthetic techniques to increase the structural and behavioral homogeneity of these complex systems will be crucial efforts toward these goals.

![Figure 5.3. Schematic of the allowed spatially direct and indirect transitions in the CdSe/CdS tetrapod nanocrystal molecule. The LUMO-HOMO transition occurs primarily within the CdSe core, corresponding to a spatially direct recombination. Reduced coupling between the LUMO and the LUMO+1 states due to their small spatial overlap additionally allows radiative emission of an electron from the LUMO+1, located primarily within the CdS arms, to the HOMO, concentrated primarily in the CdSe core (see text). This transition corresponds to a spatially indirect recombination. The lifetime of this transition is calculated to be \(\sim 200\) ns using the fluence-dependent data (Fig. 2b and Supplementary Information).](image)
Chapter 6.
A Nanocrystal Sensor for Luminescence Detection of Cellular Forces

Quantum dots have been used as bright fluorescent tags with high photostability to probe numerous biological systems. In this work we present the tetrapod quantum dot as a dynamic, next-generation nanocrystal probe that fluorescently reports cellular forces with spatial and temporal resolution. Its small size and colloidal state suggest that the tetrapod may be further developed as a tool to measure cellular forces in vivo and with macromolecular spatial resolution.

Cell-generated forces on the surrounding extracellular matrix play a crucial role in important processes ranging from stem cell differentiation to cancer metastasis\textsuperscript{136,172,173}. These forces have been measured directly with spatial resolution\textsuperscript{174} by quantifying cell-induced displacements in microfabricated arrays of fluorescent particles\textsuperscript{175} and elastomeric vertical cantilevers\textsuperscript{135}. Such methods have yielded important insights into cell mechanical behavior and mechanotransduction\textsuperscript{174}. However, while cellular mechanics are ultimately generated by structures at the size scale of a single protein, current techniques cannot map forces with nanoscale spatial resolution. Furthermore, current techniques do not allow for in vivo measurements due to geometric restrictions. Here we demonstrate that the luminescence wavelength of a colloidal nanocrystal, the tetrapod quantum dot, shifts in response to cell-generated stresses. This result creates a path towards studies of cellular forces with nanoscale resolution and geometric flexibility.

The CdSe/CdS tetrapod nanocrystal consists of a zinc blende CdSe quantum dot core with four tetrahedrally protruding wurtzite CdS arms. The tetrapod has dimensions of 4 nm for the core and arm diameters and 15-50 nm for the arm length. Like its spherical quantum dot predecessor, the CdSe/CdS tetrapod exhibits bright and narrow photoluminescence, with a Gaussian-shaped ensemble emission spectrum. Previous work showed that the peak emission wavelength of the tetrapod shifts as a function of applied stress\textsuperscript{74,176} and is sensitive to perturbations on the nanonewton (nN) scale\textsuperscript{73}. Because cell-generated forces are also on the order of nanonewtons\textsuperscript{174}, we postulated that tetrapods might be able to sense and fluorescently report cellular mechanical stresses.

To investigate this idea, we designed a luminescent tetrapod array on which cells could be cultured (Figure 6.1a, Figure A6.1), using tetrapods of 25 nm arm length. An ensemble of these tetrapods exhibits luminescence peak emission at 1.94 eV with a peak full-width-half-maximum of 0.09 eV. As-synthesized tetrapods are not water soluble, so we first made them biocompatible using an amphiphilic polymer wrapping technique\textsuperscript{177}. The resultant carboxylate-functionalized tetrapods were covalently attached to an amine-functionalized transparent substrate via amide bond formation chemistry, achieving a tetrapod monolayer array (Figure A6.1). We chose to culture HL-1 rat cardiomyocytes\textsuperscript{178}, which exhibit spontaneous and periodic contractions in vitro, on top of a tetrapod monolayer to apply periodic stresses to the luminescent array (Figure 6.1b). The morphological and contractile phenotype of the cells was similar to those grown in culture without the tetrapod monolayer.
To determine whether cell contractions could switch the luminescence emitted by the tetrapod array, we built an acousto-optic tunable filter (AOTF) microscope to collect spatially resolved luminescence spectra of the array in real time (Figure A6.2). The AOTF microscope images the intensity of light emitted by the sample as a function of band pass. Images taken at multiple band pass frequencies are stacked together to create a map with spectral data at each image pixel. Luminescence spectra of the tetrapod array were collected by imaging the emission intensity of the array at 100 band pass frequencies ranging from 1.81-2.14 eV. We collected 324 spectra simultaneously in 350 ms over a total area of 18 x 18 pixels with a pixel resolution of 5.1 µm. Each spectrum was fit to a Gaussian curve, and the emission peak energy at each pixel was used to create a spectral map. Twenty successive spectral maps were imaged for a given spot to observe luminescence behavior over time.

We observed clear time-dependent color shifts in the tetrapod array emission due to cardiomyocyte contractions (Figure 6.1c, Figure A6.3). Fluorescence red shifts relative to a control array were observed, indicating a breaking of tetrahedral symmetry in the nanocrystals induced by cell beating. Utilizing results from electronic structure modeling of uniaxially compressed tetrapods, we calculate an average shift-inducing force of 0.7 ± 0.4 nN per tetrapod. This range of values is consistent with previous measurements of contractile forces from cardiomyocytes. Although the measured force behavior is likely altered relative to physiologic mechanics due to the stiff and two-dimensional culture substrate, the experimental geometry provides a simple system to probe nanocrystal fluorescence in response to cell-generated stresses. Importantly, the observed emission shifts demonstrate that tetrapods can sense cellular forces and respond with luminescent readout.

The small size and colloidal nature of the tetrapod allow its further development towards imaging cellular stresses with macromolecular spatial resolution and in a variety of biological geometries. Cellular forces are generated by cytoskeletal proteins such as microtubules and actin filaments, and are localized to subcellular structures such as focal adhesions. Nanoscale resolution will allow quantitative investigations of how stresses are sensed and relayed at single protein complexes consisting of integrin clusters to the cytoskeleton. Furthermore, colloidal particles can be treated as a chemical reagent, enabling use of the tetrapods to study processes that occur on flat surfaces, as shown in the present work, in three-dimensional matrices, or even within tissues or between cells. The key to measurements in more complex geometries will be careful anchoring of the nanocrystal through smart surface functionalization for maximal tetrapod deformation by the stress of interest.

Because the tetrapod responds optically to cell-generated stresses, it can also be used to convert biochemical inputs into optical signals, which may be useful in a synthetic biology circuit, for example. Future work will enable fully quantitative force measurements through a detailed characterization of the tetrapod response to biologically relevant stress states and more physiologic studies of cell mechanics by attachment of tetrapods within softer extracellular matrix-like materials. These studies will provide an important complement to measurements of in vitro cellular stress and tension, inter-macromolecular forces, and extracellular matrix strain.
Figure 6.1. Beating cardiomyocytes induce shifts in the fluorescence color of a luminescent tetrapod nanocrystal array. (a) Schematic of a cell grown on a fibronectin-coated monolayer of tetrapod nanocrystals. (b) HL-1 cells grow and beat on a tetrapod substrate. Synchronized contractions are exhibited over the entire area. Yellow lines highlight one area that contracts (right) and relaxes (left). (c) Spectral map snapshots of peak shifts from a cardiomyocyte-perturbed tetrapod array over time. Snapshots are taken from the area of tetrapods directly below the area shown in b. The change in peak energy at each pixel is plotted relative to a standard spectral map containing the highest peak energies observed; each frame represents 350 ms spectral integration time. Side length in b and c is 40.7 µm.
Chapter 7.  
Concluding Remarks

Nanocrystal molecules are a remarkable new class of materials. Analogous to the abundant physiochemical diversity created using only the set of periodic table building blocks, a range of exciting properties can be created through unique couplings of artificial atoms. The work presented in this dissertation demonstrated the use of nanocrystal molecules in studying the ultrastructure of mitotic machinery, optoelectronic behavior of nanostructures under pressure, microscale stresses in polymer fibers, nanoscale spatial radiative transitions, and cellular forces. This work represents only a small fraction of what is possible. The field of nanocrystal molecules is rich with opportunities for further designs, studies, and technologies.
References.


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Appendix.

A1. Summary Points for From Artificial Atoms to Nanocrystal Molecules

1. Well-defined groupings of single nanocrystals may couple to form a nanocrystal molecule. The physical properties of a nanocrystal molecule, including its optical, electronic, and mechanical properties, are significantly altered from those of the component nanoparticles due to the interparticle coupling.

2. Plasmonically coupled nanocrystal molecules are distinct groupings of coupled metal nanocrystals that may be assembled together via biological macromolecules, organic linkers, or synthesis-mediated shape control.

3. Electronically coupled nanocrystal molecules are distinct groupings of nanocrystals coupled through tunneling and exchange coupling of electrons, typically through an inorganic semiconductor medium.

4. Similar to organic molecules, inorganic nanocrystals may undergo chemical transformations, including exchange, addition, and branching reactions.

5. In situ transmission electron microscopy has been recently developed to observe Pt nanocrystal formation in real time. Further advancement of the technique may provide the capability to visualize the formation of more complex nanocrystal molecules.

6. Nanoscience has opened up the ability to create new classes of materials with designed properties. Future progress should allow the creation of ever more intricate and controlled nanocrystal molecules.
A2. Supporting Information for Protein-Nanocrystal Conjugates Support a Single Filament Polymerization Model in R1 Plasmid Segregation

*Preparation of Gold Colloid.* Samples of 5- and 10-nm gold colloid were prepared according to a literature procedure, described in detail by Claridge et al. The gold particles were used as an electron-dense tag for parC strands.

*Hybridization of Thiolated Double-stranded parC DNA.* The 150-bp parC was composed of four oligonucleotides (synthesized and purified by polyacrylamide gel electrophoresis, Integrated DNA Technologies, Coralville, IA) as shown in Fig. 1A. The following oligonucleotide sequences were used (cpc1 denotes the strand containing the cp and c1 regions; p2 denotes the strand containing the p and 2 regions): c2, 5'- (thiol) -TTT TTG GTG TTT TTT TGG TGT GTG TTT GGG TAT GTT TTT GGT TTT AAA TGG GTT TGT TTG-3'; cpc1, 5'-TCA AGT TTA CCC CAT TTC AAC CAT CAA TCA ATG ATT TGT CTT GTT TTG GTG TTT TAT TGG GTT GGG TAT GTG TTT TGT GTT TGT TAA AAA-3'; 1, 5'-TTT TTA AAC AAA ACC AAA CAA CCC ATA CCC AAC CCA ATA AAA CAC CAA AAG ACA-3'; and p2, 5'-AAT AAT CAT TGA TTT ATG GTT GAA ATG GGG TAA ACT TGA CAA ACA AAC CCA CTT AAA ACC CAA AAC ATA CCC AAA AAC ACA CCA AAA AAA CAC C-3'. A solution with 6 µM of each of the four oligonucleotides in 100 mM NaCl was heated at 80 °C for 2 min and then cooled to room temperature over 1 h to allow the strands to hybridize.

*Synthesis of parC-Au Conjugates.* DNA-Au conjugates were prepared and isolated according to Zanchet et al. and Loweth et al. A 3% agarose gel was used to separate different types of conjugates.

*Protein Purification.* Untagged ParM and ParR were purified following procedures described by Garner et al.

*Mixing of parC-Au Conjugates with Other Par Components and ATP*—ParM and ParR were diluted to 23 and 2 µM, respectively, in Buffer F (100 mM KCl, 30 mM Tris-HCl (pH 7.5), 1 mM MgCl2, and 1 mM Tris(2-carboxyethyl)phosphine). A 20-µl solution of 15–30 nM parC-Au, 350–700 nM ParR, and 2.3 µM ParM in Buffer F/FA (1% (w/v) 400-centipoise methylcellulose, 100 mM KCl, 30 mM Tris-HCl (pH 7.5), 1 mM MgCl2, and 500 µM Tris(2-carboxyethyl)phosphine HCl) was mixed and incubated on ice for 10–20 min while transmission electron microscopy grids were plasma-ionized. The sample was pipetted onto a strip of Parafile, and 1.5 µl of ATP (100 mM) was added for a total of 15–60 s to initiate ParM polymerization. Experiments without ParR were performed similarly, but with Buffer F in place of ParR to maintain similar sample volume and salt concentration. Control experiments of parC-Au particles alone, all components without ParM, and all components without ATP were performed, with no filaments observed in any of these cases (Figure A2.1). Occasionally gray wisps of ~50 nm in length attached to the gold particles were observed, all in control experiments, leading us to hypothesize that the wisps are parC-Au strands. These occasional observations were not surprising; uranyl acetate is a known stain for large DNA sequences, but DNA on the order of hundreds of base pairs (such as parC) is more difficult to visualize.

*Preparation and Visualization of Samples by Transmission Electron Microscopy.* Carbon-coated
copper transmission electron microscopy grids (Ted Pella) were ionized for 30 s in a Harrick Plasma PDC-32G oxygen plasma cleaner to increase surface hydrophilicity; the grids were then floated carbon-side down for 1–10 s on the 15-µl sample + 1.5 µl of ATP (10 mM) reaction. The sample was wicked off and allowed to partially air-dry. Once grids were nearly dry, 10 µl of 2% uranyl acetate was added to each grid and incubated for 1 min. The uranyl acetate stain was wicked off, and the grids were then immersed sequentially in two 50-ml tubes of deionized water for a total of 50 s. The water was wicked off, and grids were allowed to air-dry prior to analysis. Transmission electron microscopy was performed using a Phillips Tecnai G2 20.

**Determination of Normalized Structure Occurrence.** Wide-field images (magnification x19,500–38,000) for experiments with and without ParR were analyzed, and the observed structures on each image (ParM structures capped on both ends with a gold particle (Au-M-Au), gold particles associated with one end of a ParM filament structure (Au-M), ParM with gold close to end, ParM with gold close to side, ParM filaments alone) were counted manually. The normalized structure occurrence was obtained by dividing the number of that structure type by the total number of structures observed; normalized occurrences for experiments with and without ParR were tabulated separately.

**Measurement of ParM Filament Width in Au-M and Au-M-Au Structures.** Images (magnification x38,000–97,000) of Au-M structures (53 total) and Au-M-Au structures (27 total) were analyzed using NIH ImageJ image analysis software (rsb.info.nih.gov/ij/). A line scan perpendicular to the length of each ParM filament yielded a plot of pixel intensity *versus* distance; the distance between the darkest points of the scan was taken to be the filament width. Five line scans for each filament were taken at different points along the filament, and the mean of the resulting widths was taken to be the average width of the filament. The histograms for each type of structure were obtained by binning the widths: widths of $4.5 < x \leq 5.5$ nm were binned as 5 nm, and so on, up to 8 nm.

![Figure A2.1](image_url)

**Figure A2.1.** Control experiments demonstrate that the filaments observed are ParM filaments with ATP-initiated polymerization. No filaments are formed in any of these control cases, as expected: (a) parC-Au particles alone; (b) no ParM; (c) no ATP. Scale bar is 100 nm.
A3. Supporting Information for Strain-Dependent Photoluminescence Behavior of CdSe/CdS Nanocrystals with Spherical, Linear, and Branched Topologies

Synthesis of CdSe/CdS core-shell particles. CdSe/CdS dots were prepared by initial synthesis of wz-CdSe dots and subsequent growth of CdS shell layers, following Peng and colleagues\textsuperscript{113}. CdSe/CdS rods and CdSe/CdS tetrapods were prepared following Talapin and colleagues\textsuperscript{61}.

Nanocrystal characterization. TEM images were obtained with a Phillips Tecnai G2 20. Absorbance spectra were measured on a Shimadzu UV-3600 UV-Vis-NIR spectrophotometer with 1 nm slit widths. Ambient fluorescence spectra were measured with a Jobin Yvon Horiba Fluorolog fluorimeter with 400 nm wavelength lamp excitation and 2 nm slit widths; a photomultiplier tube was used as a detector for the spectral range 420-800 nm.

Diamond anvil cell studies. The diamond cell used was a Diacell (now Easylab, U.K) diamond anvil cell in a screw-driven mode. We used type Ia low-fluorescence diamonds with 350-\mu m culet size. The gasket hole was 150 \mu m, drilled with an electronic discharge machine, with a 69-100 \mu m pre-indentation. We used spring steel gaskets for 1:1 pentane:isopentane measurements and stainless steel gaskets for the toluene measurements. Annealed ruby grains were used for the measurement of pressure. Ruby fluorescence spectra were taken at 3-4 spots inside the DAC to quantify the pressure gradient across the cell. An argon ion laser with 488 nm excitation wavelength was used for all fluorescence studies.

Analysis methods. All spectral curvefits and statistical analyses were performed using Mathematica 7. Ruby peak spectra, taken at a high spectral resolution, were fit to two Lorentzian peaks. Nanocrystal fluorescence spectra, taken for a large spectral range, were fit to a sum of one to four Gaussian peaks and one Lorentzian peak. Gaussian peak 1 was used to fit the main PL blue-peak; Gaussian peak 2 was added to the sum for dot experiments in the case of blue-peak splitting. Gaussian peak 3 was used to fit the red peak arising under non-hydrostatic pressure conditions. Gaussian peak 4 was used to fit any residual broad surface state luminescence appearing \sim 800 nm. The Lorentzian peak was added to the sum if the ruby fluorescence peak in the nanocrystal spectrum was observed. Standard error in peak position from the fit with the experimental data was calculated and used as the value for the error bars in Figure 3.3a-c. Standard errors and the covariance of blue-peak and red-peak positions for tetrapods were used to calculate the error bars in Figure 3.3e. All spectra and graphs were plotted using Plot 0.997.
Figure A3.1. A longer CdS rod shell limits stress-able axes in CdSe dots. Fluorescence peak positions of CdSe/CdS core/shell rods with 4 nm wz-CdSe cores and total rod length of (a) 12 nm, (b) 21 nm, and (c) 46 nm. The second blue peak appears only in the 12 nm rods, and is induced at higher pressure than in the CdSe/CdS dots. In 21 and 46 nm CdSe/CdS rods, no second blue peak is observed, suggesting that the longer CdS rod inhibits certain CdSe compression modes.

Figure A3.2. The photoluminescence peak ~650 nm is also observed in bare CdSe dots. DAC fluorescence traces with pressure ± pressure gradient (GPa) in the non-hydrostatic pressure medium, toluene, for 4 nm zb-CdSe bare dots (0.7, 2.3 ± 0.1, 3.4 ± 0.1, 4.4 ± 0.1, 5.4 ± 0.2, 4.9, 4.3 ±0.1, 3.3 ± 0.1 GPa). The phase transition to rock salt, which is highly medium dependent\(^{118}\), is not observed under these experimental conditions.
A4. Supporting Information for A Luminescent Nanocrystal Stress Gauge

Synthesis of CdSe-CdS Dots, Rods, and Tetrapods. CdSe-CdS dots were synthesized following Li et al.\textsuperscript{113}, and CdSe-CdS rods and tetrapods were synthesized following Talapin et al.\textsuperscript{61}.

Polymer Fibers. Transparent polyester thread (Coats and Clark, 90-µm diameter), transparent nylon thread (Sew-Gude, 120-µm diameter), single fibers removed from spun Nomex\textsuperscript{®} (The Thread Exchange, 10-µm diameter), and Spectra\textsuperscript{®} thread (Gudebrod GX2, 130 denier, 20-µm diameter) were used for these experiments.

Incorporation of Tetrapods into Single Polymer Fibers. CdSe-CdS nanocrystals (1–5 µL, 9.1 x 10\textsuperscript{-10} M in toluene) were pipetted onto a 3-mm (single spot studies) or 1-cm (stress profile studies) length of fiber, with a total of about 10–15 mol nanocrystals added to the fiber spot. The toluene quickly evaporates, resulting in single polymer fibers with nanocrystals embedded within (Figure 4.1b-c). In most experiments, a kink band or a transverse band inherent to the fiber, or a black permanent mark on the fiber, was used as a reference mark to ensure that fluorescence spectra were collected at the same spot with subsequent fiber extension.

Tensile Fluorescence Experiments. Each end of a single polymer fiber was secured by winding under a washer and screw to a platform on a micrometer stage, with about 2-cm initial distance between the screws. One platform was fixed in position, whereas the other could be controlled using the micrometer stage screw. The distance between the two screws was calibrated with the micrometer screw reading using digital calipers. A micrometer stage controller was used to extend the fiber. The nanocrystal fluorescence was excited with a 488-nm Ar\textsuperscript{+} laser (Lexel Laser, Inc., 95) with 2-mW power and 400-µm spot size at the sample. Brightfield and fluorescence images were taken with a digital microscope camera (Paxcam 2+). The fluorescence spectra were monitored using a home-built inverted fluorescence microscope with a spectrometer (Acton Research Corporation, SpectraPro-3001) and CCD detector (Princeton Instruments, Model 7509-0001). Exposure times of about 0.1 s were used to collect spectra. For stress profile experiments, fluorescence was monitored similarly, with a reference mark on the polyester fiber used as the initial point for fluorescence measurement. Tetrapods were embedded over an 8-mm length of single polyester fiber. A fluorescence spectrum was taken at 20 adjacent spots of 400-µm spot size at 0% fiber extension. Subsequent spectra were taken at 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, and 22.5% extension, with an increasing number of spots up to 26 adjacent spots taken at 22.5% extension due to increased fiber length. The length of fiber exhibiting tetrapod fluorescence increased as the fiber was extended and spectra were collected over this entirety, with the first spectrum always taken at the reference position (Figure 4.3a).

Stress Gauge Calibration and Young’s Modulus Measurements. The tetrapod fluorescence emission maximum in polyester fiber was averaged from 17 individual experiments and plotted versus the true strain ($\epsilon_t = \ln |1 + (l - L)/L|$, where $L$ is the original length of the fiber and $l$ is the final length of the fiber). The slope in the elastic region was determined using a linear least-squares fit on the first five mean data points, representing data from $\epsilon_t$ 1/4 0 to 0.022 (0% to 2.2% fiber extension), with $r^2 1/4 0.99$. The error reported represents the 95% confidence interval for the fit in slope. The tetrapod fluorescence red shift as a function of stress was then calculated using the reported average Young’s modulus of 8.3 GPa for this polyester fiber\textsuperscript{142}.

To calculate the Young’s modulus in single Nomex\textsuperscript{®} and nylon fibers using the
polyester-derived calibration, we determined the slope of the average emission maximum versus strain for these fibers as described for polyester above. Four Nomex® experiments and five nylon experiments were used to obtain the average values. The slope in the elastic region for Nomex® was determined using the first 12 data points, representing data from $\epsilon_t = 0$ to 0.017 (0% to 1.7% fiber extension), with $r^2 = 0.95$. The slope in the elastic region for nylon was determined using the first 10 data points, representing data from $\epsilon_t = 0$ to 0.049 (0% to 4.9% fiber extension), with $r^2 = 0.88$. The error in measured Young’s modulus for both Nomex® and nylon represents the 95% confidence interval for the fit in slope. For all three fibers, a maximum $r^2$ and subsequent difference in slope were used to determine the elastic limit. The data points used to calculate Young’s modulus are within the range of extensions considered in typical mechanical Young’s modulus measurements for the respective material fibers.

**Mechanical Behavior of Single Fibers With and Without Embedded Tetrapods.** The percent extension to failure was measured for single fibers with and without tetrapods embedded, reported as [with tetrapods; without tetrapods]: polyester [21.2 ± 3.7; 23.4 ± 2.2], Nomex® [16.0 ± 12.1; 30.9 ± 10.4], and nylon: [15.4 ± 1.5; 16.9 ± 1.8]. The results with and without tetrapods are within error. The difference in percent extensions to failure and large standard deviations for Nomex® may be due to the thin diameter of Nomex®, which makes fiber manipulation difficult. The location of fiber failure for all three fibers was unaffected by the addition of tetrapods.
A5. Supporting Information for Spatially Indirect Emission in a Luminescent Nanocrystal Molecule

Tetrapod synthesis. CdSe/CdS tetrapods were synthesized and purified following Talapin et al.\textsuperscript{61}. Transmission electron microscopy to image the tetrapod samples was performed using a Phillips Tecnai G\textsuperscript{2} 20, and core and arm sizes were measured using ImageJ. For the studies in this work, three different tetrapod samples with average ± standard deviation arm dimensions [length, width] of [15.7 ± 2.3, 5.2 ±0.8], [32.4 ± 2.5, 5.1 ± 0.7], and [42.7 ± 3.8, 4.0 ± 0.5] nm are reported in the paper as samples with 15 nm, 30 nm, and 45 nm arms respectively for clarity.

Immobilization onto glass substrates. Transparent coverglass (no. 1.0) substrates were cleaned in ‘piranha’ solution (25% H\textsubscript{2}O\textsubscript{2}:75% H\textsubscript{2}SO\textsubscript{4}) at 60° C, then sonicated in deionized water. The glass surfaces were etched using a 1M KOH solution and then thoroughly rinsed with deionized water. The substrates were soaked in a (3-Aminopropyl)triethoxysilane/acetone solution (3% v/v) to produce a slightly hydrophobic surface. A drop of CdSe/CdS tetrapods (~70 pM) were then spin coated (2000 rpm for 1 min.) onto the substrate surface. Tetrapods on the surface were located on average >1 µm distance apart from each other, ensuring a near-unity probability of having a single tetrapod localized within a diffraction-limited laser spot size.

Microscope design. A home-built sample scanning confocal microscope was used for fluorescence spectroscopy of single tetrapods. Laser light (2.33 eV or 2.54 eV) was spatially filtered using a single mode optical fiber and focused on the sample by a 60x oil objective (NA = 1.43). The diameter of the diffraction-limited laser spot is 460 nm (2.33 eV) or 420 nm (2.54 eV). The sample was mounted on a piezoelectric stage with 100 µm x 100 µm travel. The sample was raster scanned in 100 nm steps to obtain a fluorescence image of the immobilized tetrapods. Individual tetrapods were then positioned at the waist of the excitation laser. Photoluminescence emission from the sample was collected by the same objective and split using a broadband beamsplitter to simultaneously measure intensity time traces and photoluminescence spectra. A single photon counting Avalanche Photodiode (MPD, Italy) measured intensity time traces (0.01 s integration time) from 10% of the emitted beam that was bandpass filtered (600-700 nm). The remaining 90% of emission was sent through a spectrometer (300 BLZ grating, centered at 640 nm) and imaged on a charge coupled device (CCD) camera (Andor iXon X3 897) to measure luminescence spectra (0.1 s integration time). Each particle was illuminated for 40 s.

Single particle measurements. To study the effects of arm length, CdSe/CdS tetrapods with arm lengths of 15 nm (78 particles), 30 nm (171 particles), or 45 nm (108 particles) were excited with a 2.54 eV laser at 1.0 µW laser power. To study effects of incident photon flux, tetrapods with 30 nm arms were excited at 2.54 eV with laser powers of 0.3 µW (55 particles), 1.0 µW (171 particles), 3.0 µW (62 particles), or 18.0 µW (47 particles). The effect of excitation energy was investigated by exciting 45 nm-arm tetrapods with 2.33 eV (130 particles) or 2.54 eV (108 particles) at 1.0 µW laser power. For the twice-compared conditions of (30 nm CdS arms, 2.54 eV excitation, 1.0 µW laser power) and (45 nm CdS arms, 2.54 eV excitation, 1.0 µW laser power), the same respective tetrapod cohorts were used. Emitters which did not exhibit blinking behavior were not included in the single particle analysis. Only particles that exhibited multiple peaks at energy differences greater than 40.0 meV were considered as multiple emitters, eliminating any contributions from exciton coupling to longitudinal optical phonons in CdSe or CdS. In total, 260,400 spectral frames from 651 single tetrapods were analyzed.
**Total histogram analysis.** We collected 400 spectral frames (0.1 s integration time) per particle. Data was confirmed to be from single particles by ensuring that the corresponding intensity profile exhibited complete on/off blinking (Figure A5.1). To quantify the energy difference between peaks (Figure 5.2d), the following analysis was performed: for each particle exhibiting multiple-peak emission, each spectral frame was fit to 0, 1, or 2 Lorentzian curves (depending on the number of emissions observed in that frame) (Figure A5.3a). The intensity-weighted peak energies from each frame were then plotted on a histogram (Figure A5.3), representing the frequency of emission at a given energy over time for a single particle. These peaks were fit to Gaussian curves, and the maximum of the lower-energy Gaussian peak was considered as the zero-energy difference reference point for that particle. The area-weighted peak positions for a given particle were then plotted versus the change in energy from the lowest excited state (pink histogram in Figure A5.3c), and this data was compared across multiple tetrapods. All peak position histograms for tetrapods under a given condition were summed to give an overall histogram of emission energies relative to the reference point (Figure 5.2d, black outlined plot in Figure A5.3c). Tetrapods which showed a blue-shift of the emission peak over time, commonly observed in quantum dots due to photooxidation\(^{182}\), were not included in this plot for clarity.

**Area and shape of peaks in the relative transition energy histogram.** The ratio of the lower and higher energy P2 peak areas for tetrapods with 45 nm arms is found to be 7.5:1 from our experimental data in Figure 5.2d. Considering three strained arms and a Boltzmann-weighted distribution based on the energy difference between the levels, a ratio of peak areas is estimated to be 22:1. The lower value of the ratio observed in practice is possibly due to a reduced indirect transition rate caused by interfacial strain. The shape of the peaks in the total histogram is expected to be Gaussian for an ergodic system. However, size and surface polydispersity in our sample results in heterogeneous, non-ergodic optoelectronic behavior: energy levels and fluorescence quantum yields differ among individual tetrapods. Some tetrapods emitted significantly more photons than others over their spectral collection time, biasing the total histogram towards the spectral features of the brighter tetrapods. Furthermore, differing amounts of strain in the tetrapod arms and core due to heterogeneity in immobilization positions or tetrapod dimensions may also cause dispersion in the energy of the CdS conduction band relative to the ground state.

**Energy of indirect transition.** The analysis in the paper assumes that the lowest energy transition is the direct transition, which is likely given that the bulk CdS conduction band is at a higher energy than that of the bulk CdSe, and also because the conduction band offset of CdS in a CdSe/CdS nanorod was measured to be +0.3 eV in STM studies\(^{81}\). Electronic structure calculations demonstrate that an electronic wavefunction primarily located in the CdS arms is at higher energy than one largely existing in the CdSe core\(^{156}\). However, recent experiments by Borys et al. suggest that some colloidal-synthesized tetrapods may have a type-II band alignment, an observation also made more likely due to the thicker, less quantum-confined arms used in that study\(^{160}\). It is possible that some of the tetrapods studied here had a lower-energy indirect emission relative to the direct transition, whether due to the intrinsic structure of the tetrapod or the imposed strain; this would change the peak positions observed in our total histogram analysis as well as the relative areas under each peak. A total histogram plotting only data from tetrapods in which the lowest emission energy observed was also the most frequent
emission shows similar behavior to that seen in Figure 5.2d, supporting the general conclusions from our analysis. Further experiments combining single particle fluorescence spectroscopy with lifetime measurements will allow definitive assignment of emission peak energies to a direct or indirect transition.

**Indirect lifetime measurement.** Following the expression in the main body of the paper, we used

\[
\gamma = \alpha * \left[ \left(1-e^{-k_{\text{direct}}(\alpha+1)/\lambda_{\text{direct}}} \right) * \phi_{\text{direct}} \right] / \left[ \left(1-e^{-k_{\text{indirect}}(\alpha+1)/\lambda_{\text{indirect}}} \right) * \phi_{\text{indirect}} \right],
\]

where \( \gamma = \frac{N_{\text{em,direct}}}{N_{\text{em,indirect}}} \), \( \alpha = \frac{e^{-E_0/kT}}{e^{-E_1/kT} + e^{-E_2/kT}} \) with \( E_0, E_1, \) and \( E_2 \) representing the energies of the lowest, middle, and highest energy transitions observed, \( I \) = the incident photons per second, \( \phi_{\text{direct}} \) and \( \phi_{\text{indirect}} \) the intrinsic (low intensity) quantum yields of the respective materials, and \( k_{\text{direct}} = 1/10 \text{ ns} \) for CdSe quantum dots similarly sized to our tetrapod core using previously reported values\(^{167}\).

\[\gamma = \frac{(1-\gamma_{P2}^2)\cdot\gamma_{\Omega2}}{(\gamma_{P2}^2)\cdot\gamma_{\Omega2}}, \]

where \( \gamma_{P2} \) is the fraction of emission under peak P2 in total histograms for each cohort such as the one shown in Figure 5.2d and \( \gamma_{\Omega2} \) is the fraction of tetrapods emitting multiple peaks (data shown in Fig. 2b). Using the data from each intensity cohort, we calculate the following values:

<table>
<thead>
<tr>
<th>Power (µW)</th>
<th>( \gamma_{P2} )</th>
<th>( \gamma_{\Omega2} )</th>
<th>( \gamma )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>0.5322</td>
<td>0.1455</td>
<td>11.9199</td>
</tr>
<tr>
<td>1.0</td>
<td>0.5051</td>
<td>0.1111</td>
<td>16.8253</td>
</tr>
<tr>
<td>3.0</td>
<td>0.3462</td>
<td>0.0968</td>
<td>28.8507</td>
</tr>
<tr>
<td>18.0</td>
<td>0.2841</td>
<td>0.0426</td>
<td>81.6446</td>
</tr>
</tbody>
</table>

To calculate \( \alpha \), we used the total histogram from the 0.3 µW data (low intensity regime) to calculate \( E_0, E_1, \) and \( E_2 \) by taking probability-weighted values of the energy peaks over the change in energy values \([-40.5 \text{ meV}, 40.5 \text{ meV}], [41.5 \text{ meV}, 89.5 \text{ meV}], \) and \([90.5 \text{ meV}, 150.5 \text{ meV}] \) respectively. We calculated \( E_0 = 0.0067 \text{ eV}, E_1 = 0.0634 \text{ eV}, \) and \( E_2 = 0.1146 \text{ eV} \). Then, \( \alpha = 7.9817 \).

To calculate \( \phi_{\text{direct}} \) and \( \phi_{\text{indirect}} \), we plotted \( \gamma \) as a function of the incident power to obtain a \( \gamma \) value at 0 µW incident power (representing the intrinsic quantum yield limit). This data fit to a quadratic plot of \( \gamma = -0.154x^2 + 6.75x + 10.04 \) with \( R^2 = 0.9999 \) with \( x \) = the incident power. Setting \( \gamma(x=0) = 10.04 \) into equation (1), we calculated an expression for \( \phi_{\text{indirect}} \) as a function of \( \phi_{\text{direct}} \). Using a value of \( \phi_{\text{direct}} = 0.3 \) (30% intrinsic quantum yield for a typical sample), \( \phi_{\text{indirect}} = 0.22 \). Taking an average of \( k_{\text{indirect}} \) from the 0.3 µW, 1.0 µW, and 3.0 µW data sets weighted by the number of particles observed in each cohort, we calculated a lifetime for the indirect transition of \( \tau_{\text{indirect}} = 204 \pm 30 \text{ ns} \). The 18.0 µW data was not included in the \( \tau_{\text{indirect}} \) calculation due to fast photobleaching and therefore many fewer fluorescence spectra observed compared to the other data sets.
Figure A5.1. Fluorescence intensity blinking verifies collection of emission from a single particle. A sample intensity trajectory of a single CdSe/CdS tetrapod with 45 nm arm length, excited with 2.33 eV photons at 0.60 kW/cm².

Figure A5.2. Multiple radiative transitions near the expected CdSe band gap are observed in tetrapods under all the conditions studied. Shown are spectra from tetrapods with [CdS arm size, excitation energy, excitation intensity] of (a) [15 nm, 2.54 eV, 1.0 µW], (b) [30 nm, 2.54 eV, 1.0 µW], (c) [45 nm, 2.54 eV, 1.0 µW], (d) [30 nm, 2.54 eV, 0.3 µW], (e) [30 nm, 2.54 eV, 3.0 µW], (f) [30 nm, 2.54 eV, 18.0 uW], (g) [45 nm, 2.33 eV, 1.0 µW].
Figure A5.3. Obtaining energy change histograms from raw emission spectra. (a) Each raw spectrum (black) from a single tetrapod (0.1 s integration time, 40 s total observation time) is fit to a Lorentzian function (red). The center of the Lorentzian peak(s) (orange lines) and the fitted peak areas are recorded. The peak width represents the intrinsic linewidth under experimental conditions. (b) The Lorentzian peak centers from each frame (orange) are weighted by their corresponding peak areas, and plotted as a weighted histogram representing the probability of emission for the single tetrapod under study. The peak widths represent the distribution of spectral diffusion for the particle. The peaks in this histogram are fit to Gaussian peaks (light blue), and the center of the lowest energy peak is set as the zero-point reference energy for this tetrapod. The difference between the energy of each data point and the zero-point (blue) are recorded. (c) The area-weighted peak centers are plotted on an energy change histogram (blue); data from all tetrapods within a sample cohort are plotted together to represent cohort statistical behavior.
A6. Supporting Information for A Nanocrystal Sensor for Luminescence Detection of Cellular Forces

**HL-1 cell culture on luminescent tetrapod substrates.** CdSe/CdS tetrapods were synthesized following previously reported methods. A polymer-wrapping procedure was used to water-solubilize the tetrapods and create carboxylic acid groups on the tetrapod surface. Tetrapod monolayer substrates (Figure A6.1) were fabricated by covalently linking the tetrapods to transparent coverglass slides (no. 1.0) functionalized with a dense amine surface (custom made SuperAmine slides, ArrayIt) via a 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC)/N-hydroxysulfosuccinimide (Sulfo-NHS) coupling reaction. CdSe/CdS tetrapods (4.5E-8 M in PBS buffer), EDC (0.24 mg, Sigma), and sulfo-NHS (0.66 mg, Pierce) were reacted overnight with the amine-coated coverglass to achieve monolayer coverage. Each slide was washed thoroughly with PBS buffer to ensure that only covalently bound tetrapods remained.

Tetrapod monolayer substrates were placed inside cell culture wells, and HL-1 cells were cultured on the tetrapod monolayer according to culture conditions previously described. Briefly, the nanocrystal substrate was first coated with a dilute solution of fibronectin (Sigma) for 1-2 hours. HL-1 cells were grown in Claycomb media (Sigma) supplemented with norepinephrine (100 µM), FBS (10%), L-glutamine (4 mM), and penicillin/streptomycin. The cells were plated onto the nanocrystal monolayer and allowed to adhere overnight. One to two days later, the medium was changed, supplemented with HEPES buffer (25 mM), and the cells were imaged. HL-1 cells cultured on fibronectin-coated tetrapod substrates exhibited qualitatively similar morphological and contractile phenotypes as those grown on normal tissue culture plates.

**Acousto-optic tunable filter (AOTF) microscope.** A schematic of the microscope is shown in Figure A6.2. Our design is based on previous work utilizing an AOTF for spatially resolved spectral imaging. Our samples were excited at 2.54 eV with an Ar+ laser (3 mW total power, 300 µm spot size). Luminescence from the sample is collected through an objective (Zeiss 40x LD Plan-Neofluar, NA = 0.6 with correction collar) and this light is collimated using achromatic doublet lenses and sent through the AOTF (97-02838-01, Crystal Technology, LLC). An iris is used to block the undeflected beam, and the deflected beam is focused using an additional achromatic doublet lens onto a CCD (Andor iXon 897).

The computer sets 1000 radio frequencies (RFs) to be applied by the direct digital synthesizer (DDS) onto the AOTF. We calibrated the AOTF and found that $x_{eV} = 0.0205*(x_{MHz} - 91.41) + 1.942$, where $x_{MHz} = the~applied~RF~(MHz)$ and $x_{eV} = the~light~frequency~allowed~to~deflect~through~the~crystal~(eV)$. Typically, we applied a sweep of 100 different frequencies ranging from 85-101 MHz, which corresponds to 1.81-2.14 eV. The CCD collects an intensity image at each RF frequency with a total time (including exposure time and transfer time) of 3.5 ms per frequency, for a total spectral collection time of 350 ms. We used a custom made printed circuit board (PCB, Impact Zone) to synchronize the AOTF and CCD; the time offset between the two was 10 ns.

**Spectral data collection and analysis.** Samples were taken from a 37°C incubator and imaged on our microscope at room temperature. HL-1 cells exhibited beating on the tetrapod substrate throughout data collection. At each spot imaged, a brightfield movie (25 s, 10 frames per s), brightfield image (9.2 ms exposure time), and fluorescence image (9.2 ms exposure time) were
collected using a camera (PaxCam 2+, MIS, Inc.) mounted on top of the microscope. Emitted light from the tetrapods was then sent through the AOTF to obtain spatially resolved photoluminescence spectra of the substrate (20 spectra at each pixel simultaneously, 350 ms total integration time per spectra). A brightfield movie was taken after spectra collection to determine whether any perturbations to cardiomyocyte contractions had been induced by laser excitation. In all spots studied, no changes in beating behavior following laser excitation were observed.

The time resolution for spectral collection was limited to 350 ms total integration time by the luminescence intensity reaching the CCD. Although the rate of cardiomyocyte beating was ~0.5-1 Hz, we were unable to consistently optimally capture in succession the stress changes within one beat cycle due to the rapid timescale of contraction (~100 ms) and variations in beating periodicity. Future work will increase spatial and temporal resolution via improvements to tetrapod quantum yield and microscope design. Use of electrical stimuli and addition of a microscope stage with temperature and CO$_2$ control may further optimize sub-cycle stress imaging through synchronization of beating with spectral measurements and a more consistent beat frequency.

Spectral maps of shifts in peak emission were plotted to highlight changes in array luminescence (Figure 6.1c and Figure A6.3). The change in energy at each pixel is calculated relative to a standard spectral map for a given area containing the highest peak energies observed at each pixel. A per pixel offset calculation is required since emission from the AOTF deflected beam was slightly spatially dispersed. The highest peak energies emitted by cardiomyocyte-perturbed array areas correspond to the average frequency emitted by an unperturbed control array, suggesting that the highest energies observed in a perturbed array represent a relatively unstressed state. This observation strongly advocates that cell-generated forces red shift tetrapod luminescence in the experimental geometry, in concordance with previous calculations and demonstrations that symmetry-breaking deformations induce red shifting of the tetrapod energy gap.

To calculate the average force, the average and standard deviation in offset value over all pixels in a given area (6480 total, from 324 pixels per frame and 20 frames) were calculated and converted from MHz to eV using our determined calibration and eV to nN following previous electronic structure calculations. The force values reported in the main text were calculated using data from the area depicted in Figure 6.1b-c and Figure S6.3a. An identical calculation on data from a control area (depicted in Figure S6.3c) determined an average force of 0.3 ± 0.2 nN, suggesting the minimum threshold of force detectable in our system. Work to thoroughly characterize the luminescence response of tetrapods to a set of all relevant stress states will provide an experimental eV to nN conversion, enabling more precise quantitative measurements of cell-generated forces.
Figure A6.1. Fabrication of luminescent tetrapod substrates. (a) Fluorescence image of CdSe/CdS tetrapod monolayer substrate emission upon excitation with a 2.54 eV Gaussian-shaped Ar$^+$ beam. Scale bar is 100 µm. (b) CdSe/CdS tetrapod nanocrystals as-synthesized are surface-functionalized with a phosphonic acid ligand (left). Following an amphiphilic polymer wrapping procedure the tetrapods are water soluble, with exposed carboxylic acid functional groups (center). The tetrapods are covalently linked to a transparent amine-functionalized coverslip substrate using ethyl(dimethylaminopropyl) carbodiimide (EDC)/sulfo-N-hydroxysulfo succinimide (sulfo-NHS) coupling chemistry. Incubation of the coupling reaction followed by thorough washing of the substrate results in a tetrapod nanocrystal monolayer covalently attached to the coverslip surface.

Figure A6.2. Diagram of the AOTF microscope used to collect photoluminescence spectra of a two-dimensional tetrapod array with spatial and temporal resolution.
Figure A6.3. Beating heart cells induce shifts in the luminescence color emitted by a tetrapod array. (a)-(b) Snapshots of the change in peak emission over time of a fibronectin-coated tetrapod array periodically stressed by cardiomyocyte contractions. a-b exhibit different areas on the luminescent array; data in a is from the same area shown in Fig. 1b-c. (c) Snapshots of the change in peak emission over time of a fibronectin-coated tetrapod array without cardiomyocytes. a-c show ten successively collected frames with a spectral integration time of 350 ms each. The change in peak energy at each pixel is plotted relative to a standard spectral map containing the highest peak energies observed. The side length of each frame is 40.7 µm.