To my wife and parents
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Selective molecular recognition through noncovalent interactions between specific molecules forms the basis of biological systems. Among all biomolecules, nucleic acids have drawn special attention due to the highly specific base-pairing. By molecular engineering, scientists have explored the potential of nucleic acids in functional materials and systems. Moreover, by mimicking naturally occurring systems, different types of artificial molecular recognition systems have also been developed, thus extending the variability and versatility of molecular recognition.

The first goal of this dissertation is the development of a novel method for bioanalysis with high sensitivity and selectivity based on enzymatic amplification. In this project, exonuclease III is used as a signal amplifier and graphene oxide as a fluorescence quencher. Exonuclease III can recognize and digest specific DNA hybridization structures to yield an amplified signal in the presence of targets. A similar strategy is also applied for the signal amplified analysis of telomerase activity, knowledge of which is essential not only for cancer diagnosis but also for the development of novel anti-cancer therapeutic agents.
The second part of this research focuses on the engineering of DNA-polymer hybrid hydrogels for the development of dynamic materials based on reversible DNA hybridization. The dynamic hydrogel takes advantage of the scaffold of the polymer network to enable molecular level changes to be collected and visualized through macroscopic volume change measurements. Therefore, the photoreversible DNA hydrogel can be used as a photon harvesting and conversion unit for a variety of applications, including actuators, solar absorbing units and sensors.

The last individual research project is the development of a reversible phase transfer system for nanoparticles and their catalytic applications. Using light as the stimulus, phase-transfer of nanoparticles is realized based on the photo-switchable molecular recognition between azobenzene ligands and alpha-cyclodextrin coated on nanoparticles. The photo-responsive phase transfer system is applied for the regulation of the AuNP-catalyzed reaction and catalyst recycling.

In summary, this research focuses on the engineering and applications of molecular recognition systems. Functional and smart materials and systems are constructed through rational molecular design and engineering. We envision that molecular recognition will be useful in various applications, including bioanalysis, dynamic materials development, and nanoparticle sciences.
CHAPTER 1
INTRODUCTION

Molecular Recognition

The term molecular recognition refers to the specific interaction between two or more molecules through noncovalent bonding, including hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, halogen bonding, electrostatic and/or electromagnetic effects. In addition to these direct interactions, solvent plays an indirect but significant role in the course of molecular recognition in solution. The molecular entities involved in molecular recognition are bridged by molecular complementarity.

In reality, few molecules are stand-alone entities, and therefore, many important properties of matter, including melting point, viscosity, and solubility are direct consequences of intermolecular interactions. Non-covalent interactions are also dominant in biological systems, as observed between receptor-ligand, antigen-antibody, DNA-protein, sugar-lectin, RNA-ribosome, etc, and these interactions are examples of molecular recognition in biological systems. The remarkable specificity, reaction regulation, and rate control of biological reactions are also the direct results of molecular recognition. All of these examples demonstrate the importance of molecular recognition to the properties of life and the understanding of biological processes.

More than 100 years ago, Emil Fischer described the receptor-guest interaction using the analogy of a lock and key. The modern field of molecular recognition began in 1967 when Pedersen synthesized macrocyclic receptors-crown ethers which were capable of binding alkali metals both effectively and selectively. Since then, various synthetic molecular recognition systems, such as crown ether-metal cation and
cyclodextrin-small molecule, have been developed for a wide range of applications, including drug delivery, molecular sensing and catalysis. The field has also accumulated a number of names including: host-guest chemistry and supramolecular chemistry. In 1987, Pedersen was rewarded for initiating this emerging multi-disciplinary field with a Nobel Prize.

**Biological Molecular Recognition Systems**

Life phenomena are a series and network of chemical reactions, which are regulated by genetic information inherited from generation to generation. The genetic information itself is generated and transmitted by a series of chemical processes. In each of those reactions, some characteristic process takes place, which distinguishes biochemical reactions from ordinary chemical reactions in solution. The process is commonly referred to as molecular recognition. For example, in order for an enzymatic reaction to occur, substrate molecules must first be accommodated in the enzyme’s reaction pocket to form a so-called “enzyme–substrate (ES) complex”. The molecular recognition process is extremely selective and specific at the atomic level, and that selectivity is the key for living systems to maintain life. Imagine what would happen if a calcium-binding protein erroneously binds another cation, for example, potassium ion. In that respect, the molecular recognition is an elementary process of life phenomena.

Deoxyribonucleic acid (DNA) is a biological molecule encoding the genetic instructions used in the development and functioning of all known living organisms and many viruses. Along with RNA and proteins, DNA is one of the three major macromolecules that are essential for all known forms of life. DNA hybridization, the molecular recognition interaction between complementary DNA strands, is one of the most fundamental processes in biology. In a DNA double helix, each type of nucleobase
on one strand forms hydrogen bonds with just one type of nucleobase on the other strand, a process called complementary base pairing. As a result of this complementarity, all the information in the double-stranded sequence of a DNA helix is duplicated on each strand, which is vital in DNA replication. Indeed, this reversible and specific interaction between complementary base pairs is critical for all the functions of DNA in living organisms.\textsuperscript{7} Through the manipulation of hybridization in living cells, DNA has become an essential tool for gene regulation.\textsuperscript{8-10} In addition to its critical functions in biological events, DNA hybridization has also been extensively employed in DNA-based bioanalysis in the fields of molecular diagnostics, environmental and food safety monitoring, pathogen identification, forensic sciences, etc.\textsuperscript{11-13}

**Artificial Molecular Recognition Systems**

Mimicking naturally occurring systems, chemists have developed artificial supramolecular systems exhibiting molecular recognition capability. Instead of studying the properties of single molecules, supramolecular chemistry focuses on the chemical systems made up of a discrete number of assembled molecular subunits or components and the weak and reversible intermolecular interactions involved. In supramolecular chemistry, host-guest chemistry refers to the domain of chemistry that describes the special binding of the guest molecules to the complementary host molecules.

Host-guest complexes are composed of two or more molecules or ions brought together by molecular recognition. The host component is a molecule or ion whose binding sites converge in the complex, while the guest component is any molecule or ion whose binding sites diverge in the complex.\textsuperscript{14} Common host molecules include cyclodextrins, cucurbiturils, porphyrins, crown ethers, zeolites, etc. Host-guest chemistry is observed in intercalation compounds, inclusion compounds, and molecular tweezers.
Review of Nucleic Acids

Nucleic acids are biological macromolecules to essential life. It has been revealed that nucleic acids are responsible not only for the storage and expression of genetic information but also for the regulation of a wide range of biological processes, including gene expression and silencing. As described below, depending on the chemical structures of the nucleotides, nucleic acids are divided into two groups: ribonucleic acids (RNA) and deoxyribonucleic acids (DNA). Over the last few decades, much effort has been devoted to research and applications of nucleic acids in diverse fields including biotechnology, biomedical and material science.

Composition and Structure of Nucleic Acids

Early degradation studies showed that nucleic acids were linear polymers composed of nucleotide units containing a phosphate group, a sugar ring, and a nucleotide base (Figure 1-1). The bases comprise five different planar aromatic heterocyclic molecules: adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U). As shown in Figure 1-2, individual nucleotide units are linked together through the 5’ to 3’ phosphodiester linkage between the phosphate group and the sugar ring.

The DNA anti-parallel duplex structure was first discovered by James Watson and Francis Crick. It was found that two linear nucleic acid polymers are bound to each other by hydrogen bonds between specific base pairs: A-T and C-G for DNA and A-U and C-G for RNA. The A-T and A-U base pairs each have two hydrogen bonds, while the G-C base pair has three hydrogen bonds. This specific molecular recognition interaction is generally called complementary base pairing. In the case of DNA, the two strands of nucleic acids align anti-parallel to each other to form the double helix structure (Figure 1-3) and the strength of the duplex structure is mainly determined by
the sequence-dependent stacking effects. Generally, the length of DNA strands and the ratio of G-C to A-T base pairs are the two main factors influencing the stability of duplex complexes. The nucleic acids are highly negative-charged due to the phosphate groups in the backbone. Although there are only four types of nucleotide bases in DNA or RNA, single nucleic acid molecules can have thousands or millions of base pairs. For example, a transfer RNA (tRNA) molecule may have around 80 nucleotides while a eukaryotic chromosome contains more than $10^8$ base pairs. With the four possible bases and a wide range of length, the storage capacity of genetic information of DNAs is virtually unlimited. Furthermore, the specific recognition of A-T and G-C pairs ensures high fidelity during the DNA transcription and replication processes. Each three-nucleotide segment codes for an amino acid and serves as the bridge between DNA and protein synthesis.

In summary, the sequence and structure determine the properties of a DNA duplex. The specific DNA hybridization and the underlying reversible hydrogen bonding form the basis of biological functions of DNAs as well as diverse artificial DNA-based systems with novel functions.

**Chemical Synthesis of Nucleic Acids**

In a biological environment, nucleic acids are synthesized through a series of enzymatic reactions. As research on nucleic acids based advanced, chemical synthesis of DNA and RNA became indispensable. After decades of development and optimization, we can not only synthesize short nucleic acids (up to 200 bases) with identical chemical structure to their natural counterparts, but also incorporate non-nucleotide groups for desired functions. The most widely used method for chemical
synthesis of nucleic acids is the solid-phase synthesis via phosphoramidite chemistry with an automated DNA synthesizer.\textsuperscript{17}

Briefly, individual phosphoramidite units bearing different bases are linked together in a DNA synthesizer to generate an oligonucleotide following a predesigned sequence. A phosphoramidite unit (Figure 1-4) is composed of three different functional groups: a nucleoside base, a sugar ring and protecting groups. To eliminate undesired side reactions, all nucleobases (except thymine) are converted to benzamide derivatives to protect the primary amines (Figure 1-4). The deoxyribose is protected by an acid-labile dimethoxytriyl (DMT) group coupled at the 5'-hydroxyl position. Capped with 2-cyanoethyl and diisopropylamino groups, the phosphate can be activated only under specific conditions. Other non-nucleotide phosphoramidites, such as fluorophores, quenchers and coupling groups, share the same design strategy.

Different from the 5' to 3' synthesis by enzymatic reactions, chemical synthesis of oligonucleotide starts from the 3' end on a solid support (controlled pore glass bead (CPG)) contained in a column. The addition of every nucleotide goes through a same cycle with four steps: detritylation, coupling, capping and oxidation (Figure 1-5). In the first step (detritylation), the trityl group protecting the 5'-hydroxyl of the starting nucleotide is removed by an acidic dichloromethane (DCM) solution of either 3% dichloroacetic acid (DCA) or trichloroacetic acid (TCA). The second step involves the coupling of the next nucleotide achieved by the addition of a phosphoramidite and tetrazole into the synthesis column. The protonation of diisopropylamine on the phosphoramidite leads to formation of an active intermediate which is coupled to the 5'-hydroxyl group generated in step one yielding an unstable phosphate triester linkage.
The reaction column is then washed to remove any extra tetrazole, unbound bases and byproducts. Since the coupling yield is not always 100%, some of the solid support-bound remains unreacted, which could react in later cycles resulting in an oligonucleotide with a deletion. Therefore, to block the residual unreacted 5’-hydroxyl sites of the starting nucleotides, capping of these sites is performed by acetylation using acetic anhydride and 1-methylimidazole in the third step. Finally, the fourth step of oxidation converts the unstable phosphite linkage between neighboring nucleotides to the highly stable phosphate linkage by treatment with iodine and water in presence of pyridine.

After the synthesis of the desired DNA sequences, the raw products with protection groups are cleaved from the solid support followed by deprotection treatment. Since the efficiency of chemical reactions throughout the DNA synthesis is not 100%, the products contain both desired and false sequences. Therefore, purification is needed to remove the impurities. In a typical method, reversed-phase high-performance liquid chromatography (HPLC) with a C18 column is employed using acetonitrile (ACN) and 0.1M TEAA water as the mobile phase. After purification, 80% acetic acid is added to the DNA sample to remove the DMT group and the concentration is determined by UV absorbance at 260nm.

**DNA Modifying Enzymes**

Multiple mechanisms have been discovered for repairing DNA and maintaining genomic integrity in cells, including base excision repair (BER) and nucleotide excision repair (NER). Many of the enzymes responsible for NER are also involved in transcription-coupled repair (TCR) processes. BER enzymes can correct DNA lesions and stop the propagation of mutations via specific and sequential enzyme activity. In
NER, nucleotides damaged by chemicals or ultraviolet radiation are removed by enzymes, creating a short-strand DNA gap which provides a template for DNA polymerase. DNA modifying enzymes can recognize and bind with specific DNA structures with subsequent implementation of their corresponding enzymatic functions. In this section, different types of DNA modifying enzymes are reviewed in regard to their functions.

Nucleases comprise a family of enzymes that cut DNA strands via the catalyzed hydrolysis of phosphodiester bonds. Nucleases are divided into two categories: exonucleases that digest DNA strands from the ends, and endonucleases that cut within strands. In molecular biology, the restriction endonucleases are the most frequently used nucleases due to their ability to cut DNA at specific sequences. For example, the EcoRI enzyme recognizes and cuts the sequence 5' - GAATT - 3'. These sequence-specific nucleases are essential tools for molecular cloning and DNA fingerprinting in biotechnology.

DNA ligase is a type of enzyme that catalyzes the formation of a phosphodiester bond to unite two DNA strands. Ligases are particularly important in the repair of double-strand breaks in living organisms. They are also extensively used in molecular biology experiments to form recombinant DNA.

Topoisomerases possess both nuclease and ligase activity. These enzymes regulate the supercoiling extent in DNA. They bind to either single-stranded or double-stranded DNA and generate intermediate break on the phosphate backbone which lead to a topological change. Afterwards, the topoisomerases seal the break and rejoin the
Topoisomerases are required in many of the DNA-involved processes including DNA replication and transcription.

Helicases are motor proteins that use energy derived from ATP hydrolysis to break hydrogen bonds between bases and separate two annealed strands of nucleic acid. These enzymes are essential for many cellular processes, such as DNA replication, transcription and DNA repair.

Polymerases are enzymes that synthesize polynucleotide chains from nucleoside triphosphates using the existing polynucleotide templates. The synthesis starts from the 5' hydroxyl group of the previous nucleotide and progresses in a 5' to 3' direction. Because of the templated synthesis based on accurate base-pairing, polymerases are able to produce nucleic acid strands with high fidelity. In DNA replication, a DNA-dependent DNA polymerase makes a copy of a DNA sequence.

DNA modifying enzymes form a class of DNA-binding enzymes with great biological significance. At the same time, they also provide us with tremendous tools for the manipulation of biological samples in molecular biology studies. In recent years, DNA-modifying enzymes have found new applications in the area of bioanalysis. Different strategies have been developed for enzyme-assisted signal amplification. One of the most widely used techniques is polymerase chain reaction (PCR), in which the DNA strands are exponentially amplified to achieve extreme sensitivity. Detection assays based on other types of DNA-modifying enzymes, such as nicking enzymes, exonucleases and helicases have also emerged.

**Graphene Oxide and Biosensing**

Graphene is a two-dimensional single layer of sp²-bonded carbon atoms. Graphene oxide (GO) shares a similar lattice structure, but is partially oxidized to
possess oxygen-containing functional groups. Both graphene and graphene oxide are attracting considerable attention due to their unique electrical, optical, thermal and mechanical properties.\textsuperscript{23, 24} Different from graphene, which is a zero-gap semiconductor, GO is intrinsically an insulator with defects caused by the oxygen bonded sp\textsuperscript{3}-hybridized carbon atoms. GO shows great potential as a biosensing platform because of its heterogeneous chemical and electronic structure, and it has successfully be applied in diverse areas, including electronics, functional hybrid materials, biosensing and biomedical applications.\textsuperscript{23-25}

In the field of biosensing properties, research has been focused on the optical properties of GO, including photoluminescence and quenching capabilities, as well as the interaction between GO and biomolecules. This has led to the development of novel biosensing mechanisms and methodologies. GO exhibits photoluminescence properties originating from the recombination of electron–hole pairs localized within a small sp\textsuperscript{2} carbon domain embedded in a sp\textsuperscript{3} matrix.\textsuperscript{26} The photoluminescence intensity depends on the preparation method and solvent conditions, and it covers a wide range of wavelengths including the ultraviolet, visible, and near-infrared regions.\textsuperscript{24, 27} For example, graphene quantum dots synthesized from oxidized carbon fibers emit light of different colors which can be tuned by different reaction temperatures.\textsuperscript{28} This tunable photoluminescence makes GO an invaluable probe for \textit{in vitro} and \textit{in vivo} bioimaging. It has been reported that nano-graphene oxide can be used for live-cell imaging in the near-infrared (NIR) with little background.\textsuperscript{29} Besides the interesting photoluminescence properties, GO offers excellent quenching capability for a broad range of fluorophores, such as organic dyes, quantum dots and conjugated polymers. With a high planar
surface, the quenching efficiency can be as high as 100% because of an increased number of FRET acceptors. Moreover, GO is also tested as a universal long-range quencher.

As mentioned above, GO has different oxygen-bearing functional groups decorated in the carbon network. Various molecules including biomolecules can be modified via covalent bonds. On the other hand, similar to single-walled carbon nanotube (SWNT), GO can also interact with molecules through noncovalent hydrophobic π-π stacking interactions. One of the notable examples is the adsorption of single-stranded DNA (ssDNA) on GO due to the interaction between the aromatic ring structures of the bases and the hexagonal cells of GO. However, experimental results indicate that double-stranded DNA (dsDNA) has minimal interaction with GO, because of the shielding effect of the rigid structure. The strong adsorption of ssDNA on GO and GO’s fluorescence quenching capabilities have led to the development of optical sensors for a variety of targets including DNA, RNA, proteins and other molecules.30, 31

Hydrogels and Their Applications

Hydrogels

Hydrogels are amazing materials which share properties with both liquids and solids. Composed of a cross-linked polymer network and fluid that fills the voids between polymer chains, hydrogels are ideal vehicles for chemical storage and transport. Over the last few decades, numerous studies have been carried out concerning the chemical and physical properties of hydrogels as well as different hydrogel materials. From our daily life to industry and academia, hydrogels have found application in a wide range of areas, including agriculture, cosmetics, food processing
and material engineering. Recently, hydrogels with high biocompatibility have emerged as a significant platform for pharmaceutical and biomedical research, because of the unique storage capability of biological fluids in their unique three-dimensional network structures.\textsuperscript{32-34}

The ability of hydrogels to absorb a large amount of water arises from the hydrophilic groups (-OH, -COOH, -CONH-, -NH\textsubscript{2}, etc) modified on the polymer backbone. On the other hand, hydrogels are not dissolved by water as a result of the crosslinks between the polymer chains. Figure 1-6 illustrates a typical hydrogel structure with a crosslinked network. With water molecules encapsulated inside the network, a hydrogel is a semi-penetrating material that allows molecules of relatively small size to diffuse within the network. However, large molecules or particles with incompatible sizes are blocked. Therefore, by tuning physical structure and chemical composition of the hydrogel, desired properties such as penetratibility, water content, size and stiffness can be achieved.\textsuperscript{35-37}

**Volume Transition of Hydrogels**

One of the most attractive features of hydrogels is triggered volume change in response to specific external stimuli, such as the pH, temperature, solvent composition and specific ions.\textsuperscript{38-40} Depending on the design and synthetic method, the volume change of hydrogels may occur continuously with the stimulus level, or undergo a discontinuous transition when the stimulus level reaches a critical point. The swelling equilibrium and the elastic modulus of hydrogels are dependent not only on the crosslinker and charge densities of the polymer network, but also on the concentration of polymers during the gel synthesis. It also should be noted that the swelling and
elastic properties under different conditions predicted by theories are only qualitatively related to the experimental observations.

The stimuli-responsive volume transition behaviors of hydrogels have attracted considerable attention over the last few decades, and numerous studies have been carried out to develop functional materials and devices by the manipulation of their volume change. Particularly, hydrogels that undergo volume changes in response to specific biomolecules are becoming increasingly important because of their potential applications in biomaterials and smart delivery systems. For example, glucose-sensitive hydrogels provide a platform for constructing self-regulated smart insulin delivery and release systems, in which the release of insulin can be regulated by the blood glucose concentration.\textsuperscript{41, 42} Another application area with great potential is microfluidics. Automatic microfluidic systems based on hydrogels with sensor-actuator properties offer extra functionalities over other systems or actuators. The working principles and the basic elements are demonstrated in devices like hydrodynamic transistors, micropumps and tunable microlenses.\textsuperscript{43-45}

**DNA Hydrogels**

A DNA hydrogel is a DNA-containing polymer hydrogel with broad applications in biomedicine, dynamic materials, biosensors, etc. Based on the chemical composition, DNA hydrogels can be generated by DNA strands solely via hybridization to form self-assembled structures, or by polymerization of DNA strands bearing polymerizable functional groups. In one example, branched X or Y-shaped DNA nanostructures with sticky termini were first produced by self-assembly of multiple DNA strands. The X or Y-shaped building blocks were then hybridized to and ligated with each other by T4 DNA ligase to form a hydrogel constructed only of DNA.\textsuperscript{46} This DNA hydrogel shows
excellent biocompatibility and can be fine-tuned by adjusting the initial concentrations and types of branched building blocks. This capability allows the hydrogel to be tailored for specific biomedical applications, such as controlled drug delivery, tissue engineering, and cell transplant therapy. However, these hydrogels have the disadvantage of requiring large quantity of DNA.

DNA polymer hybrid hydrogels are DNA hydrogels made of DNA and organic polymers. With an organic polymer backbone and DNA side chains, this type of hydrogel exhibits advantages combining the properties of both DNA and polymers. Thus, organic polymers can be readily modified with the multi-functionalities of DNA, such as target recognition and reversible hybridization. The most commonly used polymer backbone network is polyacrylamide (PAAm). In one example, Langrana at el. fabricated a DNA hydrogel using duplex DNAs as crosslinkers instead of bisacrylamide, the traditional cross-linking agent. Two DNA strands are grafted to the polymer backbone to form two DNA polymers via copolymerization of acrydite-modified DNA strands with acrylamide monomers. Followed by the addition of a third cross-linker DNA strand, the two DNA grafted polymers are crosslinked by DNA hybridization to form a 3D hydrogel network. This DNA crosslinked hydrogel possesses various advantages, including excellent biocompatible, sequence programmability and gel-sol reversibility originating from the reversible DNA hybridization. Moreover, the mechanical properties, pore sizes and melting behavior of the gel can be adjusted by the length and density of the DNA strands. In the last decade, functional DNA, such as aptamers and photo-responsive DNA structures, have also been incorporated into hydrogels to achieve target-specificity, stimuli-responsiveness, catalytic activity, etc.
Light-Responsive Nucleic Acids

Azobenzene

Azobenzene is a chemical compound composed of two phenyl rings linked by a N=N double bond. The term, azobenzene, is often used to refer to a wide class of molecules that share the core azobenzene structure with different chemical functional groups extending from the phenyl rings. One of the most intriguing properties of azobenzenes is trans-to-cis photoisomerization. (Figure1-7)

The two isomers of azobenzene can be switched with light of particular wavelengths. Generally, ultraviolet light triggers the trans-to-cis conversion, while the blue light facilitates cis-to-trans isomerization. The photoisomerization process is completely reversible under UV and visible irradiations. Additionally, the cis-to-trans isomerization of azobenzene is extremely rapid, occurring on a picosecond timescale, while the rate of the reverse thermal relaxation varies greatly depending on the derivatives: usually hours for azobenzene-type molecules, minutes for aminoazobenzenes, and seconds for the pseudo-stilbenes.51

The ability to regulate the isomerization of azobenzene opens many opportunities for the engineering and application of light-responsive molecular switches in photocontrolled processes or devices.52,53 For instance, azobenzene can be attached to ligands to modulate their binding affinity to acceptor proteins using light.54 Ion channels modified with azobenzene can be switched on or off by light to realize photo-control of electrical activity of neurons.55

Most of the azobenzene derivatives synthesized to date require the use of UV light for isomerization which limits the use of azobenzene-based molecular switches in biological and biomedical studies. For example, UV light has very limited capability of
penetrating biological samples. Moreover, UV light can interact with many biological molecules triggering cell apoptosis and other responses, thereby complicating study of cells and tissues. Therefore, azobenzene derivatives which can isomerize with the irradiation of visible or even NIR light are highly desirable for in vivo applications. With regard to need, a series of azobenzene derivatives has been synthesized to increase the wavelength range for photoisomerization.

**Azobenzene Incorporated Nucleic Acids**

In order to extend photo-response to oligonucleotides, Asanuma and coworkers first synthesized azobenzene-based phosphoramidite, which can be easily incorporated into the DNA chains via solid phase synthesis. The resulting azobenzene modified DNA strands can hybridize with their complementary strands and, more importantly, the hybridization can be reversibly regulated by irradiation with UV and visible light. The photo-responsive hybridization behavior is attributed to the changes in stacking interactions. Upon irradiation with visible light, the azobenzene moieties are in the planar trans configuration which stabilizes the hybridization by additional stacking interactions. However, when switched to UV light, the azobenzene moieties are converted to the cis configuration which introduces steric hindrance to destabilize the hybridization between DNA strands. (Figure 1-8)

The combination of molecular recognition and photocontrollability makes azobenzene-incorporated DNA an ideal building block for diverse applications. For example, DNA nanostructures and nanomotors have been constructed using light to manipulate molecular movements. Recently, a DNA nanomachine powered by light was reported using azobenzene incorporated DNAs to control the opening and closing movements of a DNA hairpin. In the design, azobenzene groups are incorporated into
the stem part of the DNA hairpin. Upon irradiation with UV and visible light, hairpin formation can be reversibly controlled. This simple system can be viewed as a single-DNA molecular motor, in which light energy is harvested and utilized to power the mechanical motions of DNA.

Besides the applications in light-powered molecular motors, these novel photo-responsive nanostructures can be further engineered to function as switches to control enzymatic reactions and binding affinities.64,65 In one example, the template-dependent DNA synthesis by T7 DNA polymerase can be regulated by light using an azobenzene bearing oligonucleotide as a modulator.66

**Cyclodextrin Based Host-Guest Complexes**

Cyclodextrins (CDs) are cyclic oligosaccharides composed of 5 or more α-(1, 4) linked glucopyranose subunits produced from starch by enzymatic conversion.(Figure 1-9) They share a similar cage-like supramolecular structure with a group of cyclic molecules such as cryptands, calixarenes, spherands and crown ethers. These compounds can interact with compatible molecules to form supramolecular complexes via noncovalent host-guest interactions. Compared to all other supramolecular hosts, cyclodextrins are the most important due to their ability to form host-guest complexes with hydrophobic molecules. The properties of the guest molecules can be modified significantly as a result of molecular complexation, which makes CDs indispensable components in a wide range of applications in many fields, such as the food industry and pharmaceutics. The biocompatible CDs with negligible cytotoxic effects have found important applications for drug delivery, separation processes, catalysis, environmental protection, fermentation, etc.
As mentioned above, the most notable feature of CDs is their ability to form inclusion complexes with a broad range of compounds by molecular recognition. In these inclusion complexes, a guest molecule resides within the cavity of the cyclodextrin molecule. The hydrophobic cavity of CDs provides a microenvironment for non-polar moieties of compatible sizes. There are no covalent bonds formed or broken during formation of the host-guest complex.\textsuperscript{67, 68} It has been reported that the enthalpy effect caused by the release of water molecules from the cavity is the main force driving complex formation. The displacement of water molecules by the hydrophobic guest molecules results in a more stable state where apolar–apolar host-guest association is obtained. The host-guest interaction is an equilibrium process, with the strength of local interactions as well as the size matching properties determine the binding strength. The typical solvent of the host-guest complexes is water, yet the complexation can occur either in solution or in the crystalline state. It is also feasible to form complexes in a co-solvent system or a non-aqueous solvent.

The ability of a cyclodextrin to include a guest molecule in the cavity arises from two key factors. The first is a steric effect, which depends on the relative size of the guest molecule compared to the size of the cyclodextrin cavity. For example, 1:1 (host:guest) complexes between pyrene and γ-CD cannot be formed due to the small size of pyrene. Instead, two pyrene moieties are included in the cavity of one γ-CD to provide the right fit. Another notable example is the interaction between α-CD and azobenzene, since azobenzene has two isomers, trans and cis, which possess different sizes. Although both are hydrophobic, only the trans isomer with the correct size can be recognized by α-CD via van der Waals interactions.\textsuperscript{69}
The second critical factor involves thermodynamic considerations, including contributions from cyclodextrin, guest, solvent, and other aspects. A favorable net free energy change is prerequisite for the formation of complexes. For instance, ferrocene can form a stable inclusion complex with β-CD in aqueous solution. However, after being oxidized to hydrophilic ferrocenium ion, the complexation behavior is strongly inhibited because of the unfavorable energy gain (Figure 1-11).
Figure 1-1. Chemical structure of the naturally occurring eight deoxyribonucleotides and ribonucleotides.
Figure 1-2. Structure of DNA and RNA oligonucleotides formed via phosphodiester bonds.
Figure 1-3. Double helix structure of DNA and complementary base-pairing bases via hydrogen bonds.
Figure 1-4. Structure of phosphoramidite and the four monomers of nucleic acid phosphoramidite.
Figure 1-5. Four-step cycle for successive addition of nucleotide to a growing DNA chain.
Figure 1-6. Structure of a typical hydrogel.

Figure 1-7. Photo-induced isomerization of an azobenzene molecule.
Figure 1-8. Scheme of the photoreversible DNA hybridization/dissociation.

Figure 1-9. Chemical structure of the three main types of cyclodextrins.
Figure 1-10. γ-CD/pyrene-pyrene complexation.

Figure 1-11. Redox-responsive β-CD/ferrocene complexation.
DNA Detection

Detection of nucleic acids has great importance for a wide range of applications, including gene therapy, forensic investigations and clinical diagnosis. Development of simple and sensitive DNA assays to detect unique target sequences has been a major goal of biosensor technology. Thus far, the polymerase chain reaction (PCR) has been the method of choice. Although it is extremely powerful in amplifying oligonucleotide targets, making even single-molecule detection possible, PCR is limited by its own complexity, as well as contamination and false-positive signals.\textsuperscript{71-73}

Recently, molecular beacons, which undergo a conformational change from a closed fluorescence-off state to an open fluorescence-on state in the presence of target nucleic acid, have emerged as an alternative by their simplicity and specificity.\textsuperscript{74-78} However, the sensitivity of detection is strictly limited because of the 1:1 hybridization ratio. In response, several signal amplification strategies have been developed to increase fluorescence. Particularly, enzymatic amplification involving DNA enzymes, such as nicking endonucleases\textsuperscript{79}, DNase I\textsuperscript{80} and exonuclease III\textsuperscript{81}, is of great interest. Meanwhile, novel materials, such as cationic polymers\textsuperscript{82}, quantum dots\textsuperscript{83} and gold nanoparticles\textsuperscript{84} are employed as detection platforms. Among all of these materials, GO has shown great promise in improving the detection sensitivity of nucleic acids.\textsuperscript{85-88} However, most of the GO based detection systems rely on the competition between the formation of target-ssDNA complex and the formation of GO-ssDNA complex. Few studies have focused on GO’s different affinity to long ssDNA (10 to 30 bases) and to single nucleotides.
In this paper, we present a rapid, sensitive and selective method to detect DNA using exonuclease III (ExoIII) as a signal amplifier and GO as a fluorescence quencher. This method takes advantage of the much higher binding affinity of GO to the ssDNA than to the single nucleotides. Exonuclease III catalyzes the digestion of duplex DNAs from blunt 3'-hydroxyl termini, but it has only limited activity on single-stranded DNA. GO is proven to strongly bind single-stranded DNA due to the hydrophobic and π-π stacking interactions with nucleobases. And due to the multivalent interaction between GO surface and each nucleotide of a ssDNA, a ssDNA generally exhibits higher binding affinity with GO than a single nucleotide. GO is also recognized as an effective quencher for a variety of fluorophores and greatly decreases the background noise and thus increase the signal-to-noise ratio in fluorescence measurements, in addition to functioning as a convenient and versatile platform for multicolor fluorescence analysis of DNAs.

Experimental Section

Materials and Instrumentation

The oligonucleotide sequences are listed in Table 1-1. DNA synthesis reagents were purchased from Glen Research (Sterling, VA). DNA probes were synthesized using standard phosphoramidite chemistry and purified using reversed phase HPLC. Fluorescein-dT and TAMRA-dT phosphoramidites (Glen Research, Sterling, VA) were used in the synthesis of signaling probes (S1 and S2). Exonuclease III and dNTPs were purchased from New England Biolabs (Ipswich, MA, USA). Graphene oxide (1mg/ml) was received as a gift from Professor Hongjie Dai, and 4-(1-pyrenyl) butyric acid (PBA) (Fisher Scientific) was used as a surface-blocking agent.
An ABI 3400 DNA/RNA synthesizer (Applied Biosystems) was used for DNA synthesis. Probe purification was performed with a ProStar HPLC (Varian) equipped with a C18 column (Econosil 5U, 250 × 4.6 mm) from Alltech Associates using acetonitrile and 0.1 M TEAA as mobile phases. A Cary Bio 100 UV/vis spectrometer (Varian) was used for probe quantitation. Steady-state fluorescence measurements were performed on a FluoroMax-4 spectrofluorometer (Jobin Yvon, Edison, NJ), using a 100μL quartz fluorescence cuvette.

**Preparation of Graphene Oxide**

Graphene oxide was synthesized from natural graphite powder by a modified Hummers method.(W. S. Hummers and R. E. Offeman, J. Am. Chem. Soc., 1958, 80, 1339.) Briefly, graphite powder (2 grams) was ground with NaCl to reduce the particle size. After removing the salt, the graphite was added to the concentrated H2SO4 (80 mL) and left stirring for 2 hours. Afterwards, KMnO4 (10 grams) was added gradually under stirring and the temperature of the mixture was kept to less than 20 °C. Successively, the mixture was stirred at 35 °C for 2 h. Keeping the temperature less than 50 °C, distilled water (180 mL) was added and then the mixture was stirred at room temperature for 3 hours. The reaction was ended by a final addition of distilled water (450 mL) and H2O2 (30%, 20 mL) solution. At the end, the mixture was repeated washed with 1:10 HCl aqueous solution, and then distilled water. Exfoliation was carried out by sonicating graphene oxide (2 mg mL-1) dispersion under ambient condition for 4 hours. At last, the resulted sample was centrifuged at 12 k rpm for 10 min, and the upper solution was taken for future experiments.
Fluorescence Measurement

Exonuclease III-aided target recycling was carried out in 120 μL of ExoIII reaction buffer (20mM Tris-HCl, 5mM MgCl2, 50mM NaCl, pH 8.0mM) in a test tube which contained 100 nM signaling probe, 10 μM PBA, 30 units of exonuclease III and varying concentrations of target at 37 °C for 30 minutes. Then, 6 μL graphene oxide (1mg/ml) was added to the reaction solution, followed by a 10 min incubation period for complete quenching of undigested signaling probes. Fluorescence was measured using 488 nm as the excitation wavelength for the FITC-labeled probe and 543 nm as the excitation wavelength for the TAMRA-labeled probe. Each experiment was repeated at least three times.

Detection of Telomerase in HeLa Cell Extracts

The telomerase was extracted by the CHAPS method. One million cell pellets were first suspended in 200 μL 1×CHAPS lysis buffer (0.5% CHAPS, 10 mM Tris−HC1, pH 7.5, 1 mM MgC12, 1 mM EGTA, 5 mM β-mercaptoethanol, 0.1 mM PMSF, 10% glycerol) and incubated on ice for 30 min. The mixture was centrifuged at 16000 rpm for 20 min at 4 °C, and the supernatant was collected. The resulting extract was stored at -80 °C.

In the detection, telomerase extract diluted in lysis buffer with the respective number of cells and TS primer (100 nM) were incubated in 20 μL of extension solution (50 mM Tris–HC1, pH 7.5, 1 mM MgC12, 1 mM EGTA, 50 mM KCl) at 30 °C for 0.5 h. Next, a mixture of dNTPs was added, and the final concentration was 0.2 mM. The mixture was incubated at 30 °C for 1 h, after which 20 μL of 10×ExoIII buffer and 160 μL of water were added. Then, signaling probe for telomere extension and ExoIII were added for signal generation. Finally, after incubation at 37 °C for 30 min, the
fluorescence signal was measured. For control experiments, telomerase extracts were heat treated (90 °C for 3 min).

**Results and Discussion**

**Principle of Amplified DNA Detection**

As shown in Figure 2-1, a single-stranded linear DNA with a fluorophore (red dot in Figure 2-1) -modified dT base (fluo-dT) at an internal position is employed as the signaling probe. It is designed to hybridize with the perfectly matched target DNA to form a duplex structure with a blunt 3’ terminus on the signaling probe, but with a protruding 3’ terminus on the target strand. After the formation of the duplex, exonuclease III digests the signal probe in a stepwise manner to generate single nucleotides, releasing fluo-dT and the target. In this manner, the released target can then bind to another signaling probe to initiate another round of digestion by exonuclease III and release another fluorophore from a signaling probe. This recycling of target can be repeated multiple times to accumulate free fluo-dTs. Finally, graphene oxide is added and strongly adsorbs single-stranded DNAs, but not the single nucleotides. As a result, undigested signaling probes bind to GO and are quenched, but fluo-dTs stay in solution to give a fluorescence signal.

**Fluorescence Quenching by GO**

The concentration of GO added to the test solution is very critical. GO in large excess can quench the signaling probes efficiently. However, it also increases the chance of absorption of the target DNA. As a result, the effective concentration of the target DNA that can hybridize with the signaling probes is reduced, causing a higher limit of detection. In this method, the concentration of GO required for best analytical performance was achieved by measuring the fluorescence intensity of probe solution
(100 nM) with the additional of GO. Different concentrations of GO, ranging from 0 to 70 ug/mL, was treated with the probes to get an optimal GO concentration. The kinetics data showed obvious quenching effect instantaneously within seconds. However, we waited another 5 minutes for every measurement until the quenching process was completed. In Figure 2-2, the fluorescence intensity with the increase of GO concentration was summarized. From the curve, 50ug/mL was chosen as the optimized GO concentration where the signal intensity reached a plateau and was only 0.5% of the original intensity.

**Effect of 4-(1-pyrenyl) Butyric Acid (PBA)**

PBA was added to the mixture solution as the surface-blocking agent of GO. The structure is shown in Figure 2-3, inset. It can be easily attached onto the surface of GO due to the hydrophobic π-π stacking interaction. In our scheme of signal amplification, signal is generated when ssDNA is digested into single nucleotides which escape from the GO surface. It is also known that single nucleotides can still have binding with GO although much weaker than ssDNA, which can potentially reduce the signal intensity. Therefore, a surface-blocking agent is needed to keep the fluorophore-labeled single nucleotides away from GO surface. At the same time, this blocking agent should not remove the ssDNA from GO surface in order to maintain a low background.

PBA is the chosen agent which keeps single nucleotides from the GO surface but does not compete with ssDNA. As shown in Figure 2-3, in the presence of PBA, the signal after ExoIII amplification is twice of that of amplification in the absence of PBA. On the other hand, the addition of PBA didn't show any significant effect on the background signal.
Premixing versus Postmixing Amplification

In this project, a postmixing amplification strategy was employed and GO was added for signal generation after the enzymatic amplification by quenching the remaining signaling probes. Another possible scheme involved a premixing amplification strategy, where the signaling probes were premixed with GO for complete fluorescence quenching. Afterwards, the target DNA and ExoIII were added to the solution. Due to the hybridization with the target DNA, the signaling probes were released from the GO surface to form duplex complexes which were digested to release fluorophore-labeled single nucleotides. However, compared with the postmixing method, the premixing strategy is much more time-consuming. The kinetic data showed that the signal was rising slowly and didn't reach a plateau during the time of the experiment. As a sharp comparison, the signal in the postmixing method under the same experimental condition reached the maximum within 40 mins. And more importantly, the amplification effect of signal was much larger than that of the premixing method. Therefore, the postmixing strategy was selected for more efficient signal amplification.

Verification of Amplified Detection Scheme

In this assay, exonuclease III-induced target recycling is conducted at 37°C for 30 min, followed by addition of graphene oxide. The amplification scheme is verified by comparison with the conventional “premixing” method (Figure2-4), using GO for DNA detection. Upon addition of 5 nM target (T), the conventional “premixing” method shows a small signal difference from the background signal (Figure 2-5). However, the signal is greatly enhanced by exonuclease III and GO aided amplification, which leads to a huge increase in fluorescence signal (Figure 2-5). Meanwhile, the background signal resulting from nonspecific digestion of signaling probes (S) by exonuclease III is
much lower compared with that of previously reported methods using exonuclease III. Such low background is achieved by the design of a signaling probe in which the internal fluo-dT is more than 10 bases from the 3’ end. In addition, graphene oxide shows excellent quenching ability for fluorescence dyes. When considering both factors, this assay allows a high signal-to-noise ratio, leading to a very low limit of detection. In addition to dramatic signal enhancement, rapid detection of DNA target is also achieved with an assay time of about 40 minutes.

**Optimization of Experimental Parameters**

To achieve the best performance of this assay, several parameters, including concentration of signaling probes, amount of exonuclease III and time of reaction, were optimized. As shown in Figure 2-6, when the signaling probe concentration and reaction time were maintain constant, the amount of exonuclease III greatly impacted the performance of the assay. At low concentrations, although the background was low, the signal generated by enzymatic amplification was also low. When the concentration of exonuclease III was increased up to 70 units, the background started to increase dramatically. Therefore, a concentration of 30 units for exonuclease III was chosen to obtain a high signal-to-background ratio. Similarly, optimization was performed for the signaling probe concentration and the enzymatic reaction time.(Figure 2-7 and Figure 2-8) Eventually, the following parameters were chosen for the assay: 100 nM of signaling probe, 30 units of exonuclease III and 30 min of reaction time at 37 °C.

**Calibration Curve and Sensitivity**

Figure 2-8 shows fluorescence spectra generated by this assay for target DNA of different concentrations. In the absence of target (T), a very low fluorescence signal is obtained as a consequence of the resistance of single-stranded DNA to exonuclease III.
digestion. However, upon the addition of target (T), an increase in the fluorescence signal is observed in response to target (T) of increasing concentrations from 2 pM to 5 nM (Figure 2-9). Within the range from 2 pM to 1 nM, the peak intensity increases linearly with target (T) concentration (Figure 2-10). Based on the 3σ rule, the limit of detection is estimated to be 0.5 pM, which is about 20-fold lower than that of previous exonuclease III-aided strategies.\textsuperscript{81, 91} Exhibiting good selectivity, our assay can distinguish single-base mismatched target. As shown in Figure 2-11, a single-base mismatched target produces a signal less than half that of perfectly matched target of equal concentration.

**Multiplexed Detection**

It has been recognized that simultaneous detection of multiple targets holds new promise in molecular diagnostics.\textsuperscript{74, 92, 93} The use of GO as a fluorescence quencher makes multicolor DNA analysis feasible.\textsuperscript{88} With this assay, sensitive multicolor DNA analysis can be realized easily and rapidly as shown in Figure 2-12. Two probes (S1 and S2) labeled with FITC and TAMRA, respectively, are employed to demonstrate amplified multicolor DNA analysis of target T1 and T2. In the presence of 100 pM T1 only, a strong fluorescence signal is detected in the channel of S1 (FITC, excitation λ=488 nm), while no signal is observed in the channel of S2 (TAMRA, excitation λ=543 nm) (Figure 2-13 A, red). Similarly, 100 pM of T2 leads only to a fluorescence signal for TAMRA (Figure S8a, black). However, the presence of both targets T1 and T2 leads to a fluorescence signal in both channels (Figure 2-13 B, black).

Furthermore, to verify the general applicability of this assay for real-sample analysis, we investigated multiplexed DNA detection in 50% serum. Following the same procedure for multiplexed DNA detection in buffer, we achieved the signal for targets
spiked in the 50% serum. As shown in Figure 2-14, both background and signal increased compared with that in buffer due to the complexity of serum. However, multiplexed DNA detection was successfully performed with results similar to that in buffer. Therefore, the graphene-ExoIII based DNA assay showed good potential for clinical applications.

**Amplified Analysis of Telomerase Activity**

Telomerase is a ribonucleoprotein reverse transcriptase that catalyzes the synthesis of specific DNA sequence repeats (“TTAGGG” in all vertebrates) to the 3’ ends of eukaryotic chromosomes to protect chromosome from shortening. High expression of telomerase has been observed in most human cancer cells, compared with that in most normal somatic cells. The cancer cells obtain immortal life due to the high telomerase activity. Therefore, telomerase has been identified as a potentially biomarker for early cancer diagnosis and a target for drug treatment.

**Principle of the assay for detection of telomerase**

The enzymatic amplification mentioned above was applied for analysis of telomerase activity. The principle of the signal amplified analysis of telomerase activity is shown in Figure 2-15. The detection system consisted of a TS primer for telomere elongation and a ssDNA signaling probe (S-tel) complementary to the telomere sequence. The telomerase activity was evaluated by detection of telomere elongation product. In the first step, the telomerization reaction catalyzed by telomerase was allowed to precede in the presence of the nucleotide mixture dNTPs. TTAGGG repeat units were continuously added to the 3’-end of the TS primer by telomerase to form a longer single strand DNA.
Following the first step, the signal probe (S-tel) with a fluorophore-modified dT (fluo-dT) base and exonuclease III were added to the solution containing the telomere elongation. After the formation of the duplex, exonuclease III digests the signal probe in a stepwise manner to generate single nucleotides, releasing fluo-dT and the target. In this manner, the released telomere strand can then bind to another signaling probe to initiate another round of digestion by exonuclease III and release another fluorophore from a signaling probe. This recycling of telomere elongation product can be repeated multiple times to accumulate free fluo-dTs. Finally, graphene oxide is added and strongly adsorbs single-stranded DNAs, but not the single nucleotides. As a result, undigested signaling probes bind to GO and are quenched, but fluo-dTs stay in solution to give a fluorescence signal. Since the telomere elongation product could be displaced and trigger the digestion reaction of signaling probes circularly, obvious fluorescence enhancement could be observed even in the presence of a trace amount of the telomerase. Therefore, by monitoring the increase of fluorescence intensities, the telomerase activity could be detected with high sensitivity.

**Detection of telomerase activity in HeLa cells**

To demonstrate the capability of the our assay for analysis of telomerase activity, fluorescence signal from live HeLa cells was compared with that from heat-treated HeLa cells. It is well-known that heat can denature the reverse transcriptase protein of telomerase and therefore inhibit telomerase activity. Curves b and c in Figure 2-16 showed that the fluorescence signal was similar to the background. This result clearly confirmed the fluorescence signals were generated because of the telomerase activity.

To evaluate the sensitivity of the exonuclease III and GO aided telomerase assay, cell extracts from $1.0 \times 10^6$ HeLa cells were systematically diluted with buffer and
used for telomere elongation on TS primer followed by signal amplified analysis. Under the optimized conditions, the fluorescence intensities from cell extracts of different numbers of HeLa cells were plotted in Figure 2-17. The results showed that the signal intensities increased with the increased number of cells. The telomerase activity of HeLa extracts equivalent to 250-10,000 cells could be detected with the proposed method. The method also exhibited good reproducibility. The relative standard deviation was calculated to be 4.8% in a series of seven repetitive measurements of 1000 HeLa cells.

**Telomerase activity in different cancer cells**

Moreover, different types of cancer cells have different degrees of telomerase activity. It is important to differentiate the telomerase activity among different cancer cells for diagnostic purposes. Therefore, we also applied our assay for analysis of telomerase activity in different cancer cells. The fluorescence signals generated by cell extracts from 5,000 cells were acquired for three different cell lines, including HeLa, MCF-7 and K562 cells. It was found that MCF-7 cells show a lower telomerase activity than HeLa cells, while K562 cells exhibit a higher telomerase activity (Figure 2-18). This is consistent with previous reports and proves that our ExoIII/GO-based platform is able to discriminate between telomerase activities of different cancer cells.

**Conclusion**

In conclusion, we have demonstrated an amplified method for rapid detection of DNA based on ExoIII-induced target recycling and graphene oxide quenching. Binding of target DNA with signaling probe generates DNA duplex which has an overhang on the 3'-end of target DNA strand, triggering the digestion of signaling probe by ExoIII to release the target and cycle the entire reaction. With this approach, a sub-picomolar
detection limit can be achieved within 40 minutes at 37 °C. The method was successfully applied to multicolor DNA detection and, furthermore, sensitive detection of DNA in real samples was also achieved.

Moreover, we applied the proposed enzymatic amplification for analysis of the telomerase activity in cell extract using. Telomerase activity in cell extracts from as few as 250 HeLa cells could be detected by this novel strategy. We also demonstrated the application of the assay in the analysis of telomerase activity in different cancer cells, which proved its wide applicability and potential of distinguishing different cancer cells.
### Table 2-1. DNA sequences used in this work.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>T/T1</td>
<td>5'‐TAG GGT TGT ATG TAC TGG CTA CAT AGC CGA T-3'</td>
</tr>
<tr>
<td>S/S1</td>
<td>5'‐TGT AGC CAG –T(FAM)-ACA TAC AAC CCTA-3'</td>
</tr>
<tr>
<td>T1m</td>
<td>5'‐TAG GGA TGT ATG TAC TGG CTA CAT AGC CGA T-3'</td>
</tr>
<tr>
<td>T2m</td>
<td>5'‐TAG GGA CGT ATG TAC TGG CTA CAT AGC CGA T-3'</td>
</tr>
<tr>
<td>T3m</td>
<td>5'‐TAG GGA CTT ATG TAC TGG CTA CAT AGC CGA T-3'</td>
</tr>
<tr>
<td>T2</td>
<td>5'‐TAG CAC ATC GAG AAT AGC ATA AGA TAC TGA T-3'</td>
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<tr>
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<td>5'‐CTT ATG CTA –T(TAMRA)- TCT CGA TGT GCT A-3'</td>
</tr>
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<td>TS primer</td>
<td>5'‐AAT CCG TCG AGC AGA GTT-3'</td>
</tr>
<tr>
<td>S-tel</td>
<td>5'‐CCC TAA CCC- T(FAM)-AA CCC TAA CCC TA-3'</td>
</tr>
</tbody>
</table>

*Underlining indicates targeting region.
*Bold letters represent the mismatched bases.
*T1m, T2m and T3m are one-, two- and three-base mismatched targets, respectively.
Figure 2-1. Scheme of amplified DNA detection. Step 1. ExoIII-induced target recycling. Step 2. Fluorescence quenching of undigested signaling probes by graphene oxide.
Figure 2-2. The fluorescence intensity of signaling probe solution versus different concentrations of GO.

Figure 2-3. Signal generated by 100 pM target using the exonuclease III and GO aided method in the presence and absence of PBA.
Figure 2-4. Conventional pre-mixing GO-based method for DNA detection.

Figure 2-5. Comparison of signal enhancement using the combined ExoIII/GO-based scheme with the conventional GO-based scheme.
Figure 2-6. Fluorescence signal for blank (black) and for detection of 100 pM target (red), using different amounts of ExoIII. Experimental conditions: 100 nM of signaling probe and 30 min of reaction time at 37 °C. 30 units ExoIII were used for subsequent experiments to achieve a high signal, as well as a low background.
Figure 2-7. Fluorescence signal for blank (black) and for detection of 100 pM target (red) using different concentrations of signaling probes. Experimental conditions: 30 units of exonuclease III and 30 min of reaction time at 37 °C. 100 nM signaling probe was used for subsequent experiments.
Figure 2-8. Fluorescence signal for blank (black) and for detection of 100 pM target (red) after different reaction times. Experimental conditions: 100 nM of signaling probe and 30 units of exonuclease III at 37 °C. A 30-min reaction time was used for subsequent experiments.
Figure 2-8. Fluorescence spectra for amplified detection of target with different concentrations.
Figure 2-9. Plot of fluorescence intensity at 516 nm versus target concentration.
Figure 2-10. Determination of the limit of detection.

Figure 2-11. Comparison of fluorescence signal between perfectly matched and mismatched target. T is the perfectly matched target. T1m, T2m and T3m are one-, two- and three-base mismatched targets, respectively. All targets are at a concentration of 100 pM.
Figure 2-12. Amplified multicolor analysis of DNA targets using different fluorescent signaling probes.

Figure 2-13. Multicolor detection of different targets (100 nM S1, 100 nM S2, ExoIII 30 units, 30 min, 37 °C): (a) Signal in the presence of either 100 pM T1 (red) or 100 pM T2 (black); (b) signal in the presence of both 100 pM T1 and 100 pM T2 (black) and in the absence of either T1 or T2 (red).
Figure 2-14. Multicolor detection of different targets (100 nM S1, 100 nM S2, ExoIII 30 units, 30 min, 37 °C) in 50% serum: (a) Signal in the presence of either 100 pM T1 (black) or 100 pM T2 (red); (b) signal in the presence of both 100 pM T1 and 100 pM T2 (black) and in the absence of either T1 or T2 (red).
Figure 2-15. Scheme of signal amplified analysis of telomerase activity.
Figure 2-16. Fluorescence signal originating from 5,000 HeLa cells (red) and heat-treated 20,000 HeLa cells as control (black).
Figure 2-17. Analysis of telomerase activity in cell extracts from HeLa cells: relationship between fluorescence intensities and different amounts of cell extracts from different numbers of cells.
Figure 2-18. Fluorescence signal originating from heat-treated 20,000 and untreated 5,000 cells of different cell lines: HeLa, MCF-7 and K562 cells.
CHAPTER 3
MACROSCOPIC VOLUME CHANGE OF DYNAMIC HYDROGELS INDUCED BY REVERSIBLE DNA HYBRIDIZATION

Stimulus-Responsive Hydrogels

Selective molecular recognition between specific molecules forms the basis of biological systems and is achieved through noncovalent interactions such as hydrogen bonding, hydrophobic forces, van der Waals forces, electrostatic effects, etc. Reversible manipulation of molecular recognition events is of crucial importance in the stimulus-responsive regulation of biological processes, for example the reversible enzyme and receptor recognition regulated by kinase and phosphatase through phosphorylation and dephosphorylation. Mimicking naturally occurring systems, different types of stimulus-responsive molecular recognition systems have been developed, such as the oxidation-responsive ferrocene/β-cyclodextrin and photo-responsive azobenzene/α-cyclodextrin interaction, and explored for their applications in construction of novel functional materials. One emerging area involves stimulus-responsive hydrogels, which show great potential in various applications, such as biosensing, drug delivery, microfluidics and self-healing materials. Based on the different designs, several classes of hydrogels have been developed with sensitivities towards temperature, pH, light, biomolecules, and other stimuli. 

There has been great interest in photo-responsive hydrogels for the development of “smart” systems. Among all different types of stimuli, light is one of the most attractive since it can be delivered remotely and instantly with high accuracy. Furthermore, light-responsive materials also remain a forefront topic because of their possible application in solar energy harvesting and utilization. For example, Yamaguchi et al. developed photo-switchable gel assembly based on the
photoresponsive azobenzene/α-cyclodextrin interaction. Matsubara et al. synthesized photochromic hydrogels based on the structural change of hydrogel network regulated by azobenzene isomerization. Our group recently engineered drug-releasing hydrogels based light-triggered sol-gel conversion. There are also reports of dynamic hydrogels with volume changes. However, they either focus on different properties such as self-assembly and photochromic properties, or take advantage of effects other than molecular recognition. To our best knowledge, there have not been reports about dynamic hydrogels with reversible volume changes based on photo-switchable molecular recognition.

With the aim of developing new photo-responsive dynamic materials, we have engineered a DNA-crosslinked hybrid acrylamide polymer hydrogel with light-induced reversible volume change. The basic functional modules of this photo-responsive hydrogel are the photo-switchable DNA duplex complexes regulated by azobenzene isomerization driven by light. It has been demonstrated that an azo-benzene bearing DNA duplex dissociates when azobenzene converts to the cis-isomer upon UV light irradiation. However, the DNA duplex recovers when azobenzene converts back to the trans-form when visible light is applied. This new type of light-responsive dynamic hydrogel has the potential to fabricate actuator devices which can convert light energy into a macroscopic volume change for different applications. Furthermore, the design of hydrogels can also be applied to other photo-responsive molecular recognition systems.

Experimental Section

Materials and Instrumentation

All acrydite and azobenzene modified DNA oligonucleotides were synthesized via phosphoramidite chemistry on an ABI 3400 DNA synthesizer (Applied Biosystems,
Inc., Foster City, CA). DNA sequences used in the experiments are shown in Table 3-1. After the synthesis, the DNA sequences were deprotected in concentrated AMA (1:1 mixture of ammonium hydroxide and aqueous methylamine) solution at 65 °C for 30 min, followed by further purification on a ProStar HPLC system (Varian, Palo Alto, CA) with a C-18 reversed-phase column (Alltech, 5μm, 250mm × 4.6 mm) using acetonitrile and 0.1 M triethylammonium acetate (TEAA) aqueous solution as mobile phases. The collected DNA products were dried and detritylated with acetic acid. The detritylated aptamers were precipitated with ethanol and dried using a vacuum drier. The purified aptamers were then dissolved in DNA grade water and quantified by determining the UV absorption at 260 nm using a UV-vis spectrometer (Cary Bio-300, Varian).

**Synthesis of Acrydite**

The acrydite used in DNA sequences was synthesized in the lab by two steps. (Figure 3-1) First, 6-amino-1-hexanol (9.32g, 0.08mol) and TEA (16.16g, 0.16mol) in 100mL dichloromethane was cooled to 0 °C. Methacryloyl chloride (10g, 0.0957mol) was added slowly, and the reaction was stirred at 0 °C for 2 hours, after which 100mL of water was added to quench the reaction. The organic layer was washed with 5% HCl and dried. After evaporation of all solvent, the crude 6-hydroxyhexyl methacrylamide was used for the next step without further purification. To a solution containing 6-hydroxyhexyl methacrylamide (2 g, 10.8 mmol) in anhydrous CH₃CN (40 mL) at 0 °C, N, N' Diisopropylethylamine (DIPEA) (3.9 g, 30.0 mmol) was added in 15 minutes. Then, 2-cyanoethyl diisopropyl chlorophosphoramidite (2.9 ml, 13 mmol) was added dropwise, and the reaction mixture was stirred at 0 °C for 5 h. After removing the solvent, the residue was dissolved in ethyl acetate, and the organic phase was washed with NaHCO₃ solution and NaCl solution and dried over anhydrous magnesium sulfate. The
solvent was evaporated, and the residue was purified by column chromatography (ethylacetate/hexane/triethylamine 40:60:3) and dried to afford the title compound (3.33 g, 8.64 mmol, 80%) as an colorless oil. $^1$H NMR (CDCl$_3$): δ 5.92 (br, 1H), 5.63 (m, 1H), 5.27 (m, 1H), 3.86-3.72 (m, 2H), 3.66-3.49 (m, 4H), 3.30-3.23 (m, 2H), 2.61 (t, 2H), 1.92 (m, 3H), 1.58-1.50 (m, 4H) 1.37-1.32 (m, 4H) 1.17-1.13 (m, 12H). 13C NMR (CDCl$_3$): δ 168.6, 140.4, 119.3, 118.0, 63.8, 63.6, 58.6, 58.3, 43.2, 43.1, 39.8, 31.3, 29.7, 26.8, 25.8, 24.9, 24.8, 24.7, 19.0. 31P (CDCl$_3$): δ 148.

**Synthesis of Azobenzene Phosphoramidite**

Azobenzene phosphoramidite was synthesized according to the protocol reported by Asanuma et al. $^1$ and is shown as Figure 3-2. **Compound 1.** $^1$H NMR (CDCl$_3$): δ 7.96-7.38 (m, 9H), δ 7.12 (d, 1H), δ 4.33 (m, 1H), δ 4.09 (m, 1H), δ 3.77 (s, 6H), δ 3.57 and 3.42 (dd, 2H), δ 1.29 (d, 3H). **Compound 2.** $^1$H NMR (CDCl$_3$): δ 8.00-6.78 (m, 23H), δ 4.25 (m, 1H), δ 4.17 (m, 1H), δ 3.77 (s, 6H), δ 3.60 and 3.42 (dd, 2H), δ 1.23 (d, 3H). **Compound 3.** $^1$H NMR (CDCl$_3$): δ 8.00-6.79 (m, 22H), δ 6.62 (d, 1H), δ 4.48 (m, 1H), δ 4.39 (m, 1H), δ 4.21-4.10 (m, 2H), δ 3.77 (s, 6H), δ 3.57-3.34 (m, 4H), δ 2.76-2.72 (m, 2H), δ 1.30-1.25 (m, 15H). $^{31}$P NMR (CDCl$_3$): δ 149.

**Synthesis of Light-Responsive Dynamic Hydrogels**

The photosensitive hydrogels were prepared by a two-step polymerization method in buffer solution (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl$_2$, pH 8.0) (Figure 3-3). In step I, the linear-backboned DNA hybrid polymer was synthesized by copolymerization of acrylamide (4%) and acrydite-DNA (A, 3 mM) using ammonium persulfate (APS) and tetramethylethylenediamine (TEMED). Afterwards, the resultant DNA hybrid linear polymer synthesized in step I was mixed with azobenzene-modified
acrydite-cDNA (A’) at a 1:1 ratio. The mixture was heated to 90 °C and slowly cooled to room temperature to allow complete hybridization between A and A’. Then, acrylamide and N,N’-Methylenebisacrylamide (MBAA) were added to the mixture, and step II of the polymerization process was carried out to form the dynamic hydrogel using APS and TEMED. After polymerization, the dynamic hydrogels were immersed in buffer to remove the unreacted monomers at 22 °C. Control hydrogels were also prepared by the same method, but with different acrydite-DNA monomers. Control hydrogel (C1) was synthesized using complementary DNA monomers (A and ctrl-A’) without azobenzene modification, while control hydrogel (C2) contained noncomplementary DNA monomers (B and A’) with azobenzene modification.

**Measurement of Light-Induced Volume Change**

A 60 W table lamp and a 6 W handheld UV lamp (365 nm) were used as visible and UV light sources, respectively. During the light irradiation, the hydrogel was immersed in buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl₂, pH 8.0) in a glass vial. The temperature was maintained at 22 °C using a water bath. The hydrogel mass was measured by taking out the hydrogel from the solution, removing excess solution from the gel surface, and weighing. The hydrogel was kept in darkness during the entire process. The volume of the hydrogels (V) can be viewed as the sum of the volumes of the polymer network (V_p) and the aqueous solution stored in the hydrogels (V_w).

Assuming that the mass of polymer and aqueous solution are M_p and M_w and the densities are ρ_p and ρ_w, respectively, then V = V_p + V_w = M_p / ρ_p + M_w / ρ_w. Since the change of volume and mass of the hydrogel is caused by absorption and release of solution, the change of hydrogel volume can be expressed by ΔV = ΔM_w / ρ_w = ΔM / ρ_w.
Δwhere M is the observed mass change. Therefore, the percentage volume change is equal to the percentage mass change of the hydrogel.

Results and Discussion

Design of Light-Responsive Dynamic Hydrogels

Inspired by light-controlled DNA hybridization based on azobenzene isomerization\textsuperscript{59, 112, 113} (Figure 3-4 A), \textsuperscript{113, 114} we engineered a hydrogel using DNA duplexes as photoreversible crosslinks. This photosensitive dynamic hydrogel was prepared by grafting azobenzene-tethered, single-stranded DNA and its complementary DNA to the hydrogel network, so that the degree of crosslinking could be regulated by light. With a two-step polymerization method\textsuperscript{104}, we synthesized an acrylamide-backboned hydrogel network, in which linear acrylamide polymers are trapped, using N,N'-methylenebisacrylamide (MBAA) as a permanent crosslinker (Figure 3-3). The azobenzene-modified DNAs are grafted onto the crosslinked network, while the complementary DNAs are modified on the linear polymer. When exposed to visible light, it is expected that the hybridization between the two complementary DNAs will serve as extra crosslinks in the network, so that the hydrogel will have a smaller volume (Figure 3-4 B). However, when the hydrogel is exposed to UV light, the disruption of DNA hybridization removes the extra crosslinks and triggers a volume increase of the hydrogel. By alternating irradiation of visible and UV light, a reversible volume transition is thus realized. Therefore, the reversible macroscopic volume change driven by UV and visible light can be viewed as a process of converting light energy to mechanical energy, meeting the initial goal of this work in converting photonic energy to usable forms of energy.
Light-Induced Volume Changes of Dynamic Hydrogels

The ability of the hydrogels to undergo a light-induced volume transition was tested by monitoring the swelling upon UV light irradiation and shrinking with visible light irradiation. The hydrogels were synthesized under visible light in a shrunken state, in which all DNAs were hybridized. Before analysis of the photo-induced volume change, the hydrogels were soaked for three days in a large volume of buffer solution (10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl2, pH 8.0) and allowed to swell to equilibrium. Using a water bath, the temperature was maintained at 22°C throughout this process and the subsequent experiments. The percentage volume changes of the hydrogels were calculated from mass measurements considering the mass and density of polymer, DNA and water included.115-117

The light-responsive swelling was first analyzed by exposing the hydrogels to UV light (365 nm) at 2 hours after the start of monitoring under visible light. As shown by the black curve in Figure 3-5 A, the volume of the hydrogel increased abruptly following the irradiation of UV light and reached equilibrium after 3 hrs. We attribute this volume increase to the photodissociation of DNA duplexes in the hydrogel network by UV light-induced trans-to-cis isomerization of azobenzene moieties. It is well known that azobenzene isomerizes from the trans- to cis- configuration upon UV light irradiation, and previous work has shown that the non-planar cis- azobenzene moieties can destabilize and dissociate the DNA duplex by steric hindrance.59,111,112 As a result of this isomerization, the DNA duplex crosslinkers are removed, thereby yielding a larger hydrogel volume.

After the hydrogel swelled to equilibrium upon UV light irradiation, the light source was switched to visible light which caused the cis- azobenzene moieties to
isomerize back to the trans form. Along with the cis- to trans- isomerization of azobenzene moieties, the two complementary ssDNA strands hybridized again to form the duplex, since the planar trans- azobenzene moieties could stabilize the duplex by stacking interactions.\textsuperscript{59, 111, 112} Therefore, the hydrogel shrank as the DNA duplex crosslinkers recovered (black curve, Figure 3-5 B).

To prove that the swelling and shrinking of the dynamic hydrogel is indeed regulated by photo-reversible DNA hybridization, we carried out control experiments using two control hydrogels (C1 and C2). Both control hydrogels shared the same structure with the dynamic hydrogel: all were synthesized through the two-step polymerization using two acrylate ssDNA monomers. However, no photoresponsive azobenzene moieties were modified in the DNA strands of control hydrogel C1. As expected, no volume change was observed upon either UV or visible light irradiation (blue curves in Figure 3-5 A and B). Several types of hydrogels were recently developed with volume changes based on an infrared (IR) light-induced photothermal effect which changed the local temperature of the hydrogels,\textsuperscript{110, 118} the results for control C1 clearly rule out thermal effects as the cause of the volume change.

Control hydrogel C2 had azobenzene moieties in one DNA strand, but the two DNA strands were not complementary to each other. Therefore, no photoreversible DNA duplex crosslinkers were possible, even though the azobenzene moieties could still isomerize upon UV and visible light irradiation. Results from volume change measurements shown by the red curves in Figure 3-5 A and B indicated that the isomerization of azobenzene moieties themselves could not induce the volume change.
We performed another control experiment, in which the dynamic hydrogel was left in the dark after swelling to equilibrium upon UV light irradiation, to determine if the shrinking process would be affected by thermal relaxation of the cis-azobenzene moieties. Previous work has shown that azobenzene can automatically isomerize from the cis- to trans-state by thermal relaxation at room temperature without any light irradiation, although this process can take up to days and is much slower than the visible light-activated isomerization, depending on the presence of different substituents.\textsuperscript{119, 120} To determine if thermal relaxation is important in the hydrogel volume changes, the volume was monitored after the UV light was removed at the 3-hour time point. As shown in Figure 3-5 B (green curve), the shrinkage induced by thermal relaxation was much slower than that by the visible light, and only a small overall shrinkage was observed. Therefore, based on these extensive control experiments, the volume change of the dynamic hydrogel was induced solely by UV and visible light-regulated dissociation and association of azobenzene-modified DNA duplexes.

**Reversible Volume Changes of Light-Responsive Dynamic Hydrogels**

In order to make the light-responsive dynamic hydrogel useful for continuous harvesting and conversion of light energy, swelling and shrinking must be reversible. Therefore, we investigated the reversibility in response to alternate irradiation with UV (3hrs) and visible light (2hrs) for multiple cycles, as shown in Figure 3-6. In each cycle, the dynamic hydrogel swelled when the light source was switched from visible to UV and contracted when the light source was switched back to visible. For the control hydrogels C1 and C2, no volume change was observed. The volume change for the dynamic hydrogel was very reproducible, except that the expanded gel did not return to
its original compact volume when irradiated with visible light. We attribute the decreased contraction to incomplete rehybridization of DNA upon visible light irradiation. The hydrogel is synthesized in the shrunken state with all ssDNA hybridize to form duplexes. In the first cycle, all the duplexes are dissociated by UV light, resulting in a large volume increase. However, not all of the dissociated ssDNAs are able to rehybridize as a result of the diffusion and rearrangement of the polymer networks and DNAs. Consequently, the reduced number of DNA duplex crosslinkers yields a larger volume in the compacted state, compared to the initial volume. However, after the first cycle, the degree of volume change becomes more reversible, because diffusion and rearrangement reach equilibrium, and the number of photoreversible DNA duplex crosslinkers stays the same.

Effects of Synthesis Parameters on Volume Changes

The percentage of reversible volume change was also determined to be dependent on conditions during hydrogel synthesis. We prepared dynamic hydrogels using varying concentrations of N, N'-methylenebisacrylamide (MBAA), acrylate DNA monomer, and acrylamide monomer (Figure 4-7 to 4-9). We first changed the concentrations of MBAA and acrylate DNA monomer at fixed acrylamide concentration. Figure 4-10 A shows that the percentage of reversible volume change decreased as the concentration of MBAA increased at fixed acrylate DNA monomer concentration. On the other hand, at fixed MBAA concentration, the reversible volume change increased as the DNA monomer concentration increased (Figure 4-10 B). According to the design of our dynamic hydrogel, the volume of hydrogel can be divided into two portions: the reversible portion and irreversible portion. The reversible portion of the hydrogel volume is determined by the photoregulated DNA duplex crosslinkers, while the irreversible
portion depends on the permanent MBAA crosslinkers. As a result, the increase of MBAA concentration tends to increase the irreversible portion of volume and reduce the percentage of light-induced volume change. However, an increase in the acrylate DNA monomer concentration increases the reversible portion of volume and therefore enhances the percentage volume change. However, if the concentrations of both MBAA and acrylate DNA monomer are fixed, the acrylamide concentration within the range tested does not influence the percentage volume change, as shown in Figure 4-10 C. We attribute this result to the constant ratio of DNA concentration to MBAA concentration, which results in similar percentage volume change.

**Conclusion**

We have successfully constructed dynamic hydrogels with reversible volume changes based on photo-switchable DNA hybridization. The dissociation of DNA duplex crosslinkers in the hydrogels leads to the expansion of the hydrogel volume while the formation of DNA duplex crosslinkers causes the shrinkage of the hydrogel. With this approach, we are able to visualize the reversible dissociation and formation of the DNA duplexes through the volume change of hydrogels. Furthermore, during the last decade, considerable effort has been devoted to the development of DNA-based nanomachines.\textsuperscript{113, 121-125} However, most reported nanomachines fall short of practical use, as a consequence of their nanometer sizes and inconvenient energy sources. Our dynamic hydrogel takes advantage of the scaffold of the polymer network to convert molecular-level effects into macroscopic changes. The nanometer-sized, photoreversible DNA duplex crosslinkers are assembled into the hydrogel to create harvesting and conversion units for light energy. Macroscopic volume change of hydrogels is achieved by alternate irradiation with UV and visible light. The volume
change could be potentially converted to mechanical or electrical energy by using proper device designs. There have been several examples where attempts were made to utilize azobenzenes to harvest solar energy, including an osmotic pressure-driven solar mechanical device\textsuperscript{126} and a solar thermal fuel system composed of azobenzene functionalized carbon nanotubes\textsuperscript{127}. We believe that the light-reversible dynamic DNA hydrid hydrogels add a new concept to the solar energy field and could find applications in many areas, such as light-controlled actuators, microlenses and microvalves. These devices will be highly useful with remote control using photons in energy conversion. In addition, the DNA based photo-dynamic hydrogel can be coupled with other similar systems, such as photo-switchable azobenzene/α-cyclodextrin complexation, protein/substrate binding and protein/protein interaction.\textsuperscript{128-130}
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<td>B</td>
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Figure 3-1. Synthesis of acrydite.

Figure 3-2. Synthesis of azobenzene phosphoramidite
Figure 3-3. Preparation of the dynamic hydrogel. (A) Synthesis of light responsive dynamic hydrogels. (B) Chemical structure of the dynamic hydrogel.
Figure 3-4. Scheme of reversible volume change of dynamic hydrogels. (A) Light-controlled formation of DNA duplex based on azobenzene isomerization in the hydrogel. (B) Reversible volume transition of the DNA-crosslinked hydrogel regulated by UV and visible light.
Figure 3-5. Light induced volume change of dynamic hydrogels. (A) UV light-induced volume increase of the dynamic hydrogel containing complementary DNAs with azobenzene modification (black); (B) Volume changes of the dynamic hydrogel upon visible light irradiation (black) and in dark with no light (green) following UV light irradiation. Blue and red curves are for control experiments: Blue curve: control hydrogel (C1) containing complementary DNAs without azobenzene modification; Red curve: control hydrogel (C2) containing noncomplementary DNAs with azobenzene modification.
Figure 3-6. Reversible swelling and contracting of light-responsive dynamic hydrogels in response to alternate ir-radiation by UV and visible light. Black curve: dynamic hydrogel containing complementary DNAs with azobenzene modification; Blue curve: control hydrogel (C1) containing complementary DNAs without azobenzene modification; Red curve: control hydrogel (C2) containing noncomplementary DNAs with azobenzene modification.
Figure 3-7. Light-induced reversible swelling and shrinking of the light-responsive dynamic hydrogels synthesized using different concentrations of MBAA (DNA monomer: 0.5 mM; acrylamide: 10%): 0.0033% (black), 0.0067% (red) and 0.0133% (blue).
Figure 3-8. Light-induced reversible swelling and shrinking of the light-responsive dynamic hydrogels synthesized using different concentrations of DNA monomers (MBAA: 0.0033 %; acrylamide: 10%): 0.1 mM (black), 0.25 mM (red), 0.5 mM (blue) and 0.75 mM (green).
Figure 3-9. Light-induced reversible swelling and shrinking of the light-responsive dynamic hydrogels synthesized using different concentrations of acrylamide (DNA monomer: 0.5 mM; MBAA: 0.0033%): 6% (black), 8% mM (red), 10% (blue), 15% (green) and 20% (pink).
Figure 3-10. Percentage reversible volume change of light-responsive dynamic hydrogels synthesized under different conditions: (A) percentage volume change as a function MBAA concentration; (B) percentage volume change as a function of DNA monomer concentration; (C) percentage volume change as a function of acrylamide concentration.
Chapter 4
Reversible Phase Transfer of Nanoparticles Based on Photoswitchable Host-Guest Chemistry

Phase Transfer of Nanoparticles

Nanoparticles (NPs), by their unique optical, electrical and chemical properties, have attracted considerable interest in diverse research areas, including catalysis, sensing, electronics, biomedicine, and optics. The rapid growth in applications of NPs has been accompanied by the development of synthetic methods resulting in NPs with well-defined properties, such as shape, size, composition and surface modification. However, many of these synthetic strategies are realized with the aid of specific hydrophobic or hydrophilic ligands in their respective organic or aqueous solvents, while synthesized NPs possess distinct solubility in media with different polarities. Therefore, additional steps for phase transfer are usually required, especially between water and organic solvents, for specific applications. To date, the strategies developed involve either changing the wettability of the surface ligands or changing the properties of the solvents. However, reversible phase transfer of NPs between the immiscible phases still remains challenging and complicated. Most of the previous approaches required sequential addition of different ligands or drastic physicochemical change of the solvent, such as temperature and pH. Moreover, none of the reported reversible phase transfer strategies has been used for practical applications.

The use of light as an external stimulus for stimuli-responsive systems is of particular interest since light can be delivered remotely, precisely in space and time, and quantitatively. Smart materials based on photoresponsive systems have emerged as a dynamic research area, where the properties of matters can be reversibly modulated.
For example, light-switchable α-cyclodextrin (α-CD) and azobenzene interaction has been employed to control the viscosity of hydrogels\textsuperscript{149, 150}, the release of cargos from carriers\textsuperscript{151, 152}, and the assembly of macroscopic/microscopic objects.\textsuperscript{153, 154}

In this report, the photoswitchable host-guest interaction between α-CD and azobenzene is used as a trigger to induce the reversible phase transfer of the AuNPs in water/toluene. Furthermore, the application of the reversible phase transfer system is demonstrated in the regulation of catalysis and the recycling of AuNP catalyst. The separation and reuse problems limit the applications of homogeneous catalysts.\textsuperscript{155} The design of recyclable nanoparticle-based catalysts has been a major area in green chemistry where strategies including magnetic separation and gravitational sedimentation are involved.\textsuperscript{156, 157} However, these methods suffer from different disadvantages. For example, the magnetic separation requires magnetic ingredients in the nanoparticles which may be hard to achieve or render a low catalytic activity. We envision that our system of light-responsive recycling of nanoparticles would help the development of recyclable catalysts with high efficacy.

**Experimental Section**

**Preparation of Per-6-Thiol-α-Cyclodextrin-Coated Gold Nanoparticles**

The scheme for preparation cyclodextrin coated gold nanoparticle is shown in Figure 4-1. The experimental details are described as below.

**Synthesis of per-6-iode-α-cyclodextrin (2)**

According to the procedure of Gadelle and Defaye\textsuperscript{158}, to a mixture of triphenylphosphane (21 g) and I\textsubscript{2} (20.2 g) in DMF (40 mL) was added α-CD (1) (4.3 g). The mixture was stirred at 80 °C under an atmosphere of N\textsubscript{2}. After 18 h, the solution was concentrated to half volume under reduced pressure. The pH was then adjusted to
9-10 by adding sodium methoxide in methanol (3 M, 30 mL) with cooling. The solution was stirred at room temperature for 1 h, and methanol was added to form a precipitate. The precipitate was collected by filtration and allowed to air dry to yield per-6-iodo-α-cyclodextrin as a white powder. 1H NMR (DMSO-d6): δ 3.30 (t, 6H), δ 3.8-3.55 (m, 12H), δ 3.55-3.71 (m, 12H), δ 3.83 (bd, 6H), δ 4.96 (d, 6H), δ 5.60 (d, 6H), δ 5.81 (d, 6H).

**Synthesis of per-6-thio-α-cyclodextrin (3)**

Per-6-iodo-α-cyclodextrin (0.965 g) was dissolved in DMF (10 mL), and thiourea (0.301 g) was added. The reaction mixture was heated to 70 °C under a nitrogen atmosphere. After 19 h, the DMF was removed under reduced pressure to give a yellow oil product, which was dissolved in water (50 mL). Sodium hydroxide (0.26 g) was added, and the reaction mixture was heated to a gentle reflux under a nitrogen atmosphere. After 1 h, the resulting suspension was acidified with aqueous KHSO4, and the precipitate was collected by filtration, washed thoroughly with water, and dried. 1H NMR (DMSO-d6): δ 2.16 (t, 6H), δ 2.79 (m, 6H), δ 3.14 (br d, 6H), δ 3.25-3.41 (m, 12H), δ 3.72-3.82 (m, 12H), δ 4.89 (t, 6H), δ 5.58 (s, 6H), δ 5.73 (d, 6H).

**Synthesis of α-cyclodextrin-capped gold nanoparticles**

The α-CD-modified gold nanoparticles were prepared following the method reported by Liu and coworkers \(^\text{159}\). A 50 mg sample of HAuCl₄ was dissolved in 20 mL DMSO. This solution was quickly mixed with another 20 mL of DMSO containing 75.5 mg NaBH₄ and 10.5 mg per-6-thio-α-cyclodextrin. The reaction mixture turned deep brown immediately, but the reaction was allowed to continue for 24 h. At this point, 40 mL CH₃CN was added to precipitate the colloid, which was collected by centrifugation,
washed with 60 mL of CH$_3$CN: DMSO (1: 1 v/v) and 60 mL of ethanol, isolated by centrifugation, and dried under vacuum (60 °C) for 24 h.

**Preparation of Azo-Ligand**

The scheme for preparation azobenzene ligand is shown in Figure 4-2. The experimental details are described as below.

**Synthesis of 4-methylazobenzene (4)**

Following the procedure of Liu et al. $^{160}$, 3 g (27.8 mmol) of p-toluidine and 3 g (27.8 mmol) of nitrosobenzene were dissolved in 50 mL of glacial acetic acid and stirred under a nitrogen atmosphere for 24 h at room temperature. After removal of acetic acid under reduced pressure, the resulting solid was recrystallized from ethanol-water, followed by purification by column chromatography. 1H NMR (CDCl$_3$): δ 2.42 (s, 3H), δ 7.39-7.44 (m, 2H), δ 7.52-7.63 (m, 3H), δ 7.79-7.87 (m, 2H), δ 7.87-7.92 (m, 2H).

**Synthesis of 4-(bromomethyl)azobenzene (5)**

We followed the process of Kumar et al. to obtain 4-(Bromomethyl)azobenzene. $^{161}$ 4-methylazobenzene (2.5 g, 0.013 mol) was dissolved in 54 mL of CCl$_4$ followed by the addition of 2.28 g of N-bromosuccinimide (NBS), and 0.043 g of benzoyl peroxide (BPO) The mixture was refluxed for 24 h under a nitrogen atmosphere. The resulting solution was filtrated while hot, followed by the removal of solvent. The solid product was purified by column chromatography. 1H NMR (CDCl$_3$): δ 4.58 (s, 2H), δ 7.41-7.65 (m, 5H), δ 7.79-7.98 (m, 4H).

**Synthesis of (dimethylaminomethyl)azobenzene (6)**

We followed the process of Sperotto et al. to obtain (dimethylaminomethyl)azobenzene. $^{162}$ Dimethylamine dissolved in THF (10 mL, 2 M) was added to a solution of 4-(Bromomethyl)azobenzene (2.23 g, 8 mmol) in dichloromethane (DCM) at 0 oC.
The mixture was allowed to reach room temperature and was stirred overnight. Solvents were removed, and NaOH (15 mL, 4 M aqueous) was added. The mixture was extracted with hexane (3×80 mL). The aqueous layer was made strongly basic by adding solid sodium hydroxide, and the resulting solution was extracted with diethyl ether (3×80 mL). The combined organic layer was dried over magnesium sulfate and filtered. Solvents were removed, yielding the product as yellow oil. 1H NMR (CDCl$_3$): δ 2.26 (s, 6H), δ 3.59 (s, 2H), δ 7.40-7.71 (m, 5H), δ 7.71-7.89 (m, 4H). ESI-MS: m/z 240.15 [M+H]$^+$ (calc. for [C$_{15}$H$_{17}$N$_3$+H]$^+$ 240.32).

**Synthesis of azo-ligand (7)**

(Dimethylaminomethyl)azobenzene (4 mmol) in dry ethanol (25 ml) was mixed with an equimolar amount of 1-bromohexadecane (5 mmol), and the mixture was refluxed for 24 hours. Solvent was evaporated, and the crude azo-ligand compound was purified by column chromatography. 1H NMR (CDCl$_3$): δ 0.86 (t, 3H), δ 1.24 (bs, 26H), δ 1.81 (m, 2H), δ 2.81 (s, 6H), δ 3.57 (m, 2H), δ 4.28 (s, 2H), δ 7.45-7.60 (m, 5H), δ 7.67-7.79 (m, 4H). ESI-MS: m/z 464.40 [M]$^+$ (calc. for [C$_{31}$H$_{50}$N$_3$]$^+$ 464.75).

**Results and Discussion**

**Design of Light-Responsive Phase Transfer of Nanoparticles**

The present photoreversible supramolecular system consists of an azobenzene-containing surfactant (azo-ligand) and a thiolated α-CD (Figure 4-3 A). The key innovation of this approach is the light-induced reversible modification of the azo-ligand on the AuNP surface coated with thiolated α-CD (Figure 4-3 B). Azobenzenes represent a class of photoresponsive compounds that undergo reversible trans-to-cis isomerization by the irradiation of UV and visible light. While the trans isomer can form stable inclusion complex with α-CD by matching size and hydrophobic interaction, the
cis isomer cannot. As shown in Figure 4-4, the α-CD-coated AuNPs are initially dispersed in water as a consequence of the secondary hydroxyl groups of α-CD$^{163}$, while the trans-azo-ligands are dissolved in toluene. After agitation, the trans-azo-ligands bind to the α-CDs on the AuNP surface, which brings the hydrophobic alkyl chains to the periphery of the AuNP to form a reverse micelle-like structure. Therefore, the AuNPs are converted from hydrophilic to hydrophobic and transferred from water to toluene. Upon UV irradiation, azo-ligand isomerizes from trans to cis state, and cis-azo-ligand dissociates from the AuNP surface. As a result, the AuNP surface recovers its hydrophilic property, and the AuNPs are transferred back into water from toluene. Importantly, the toluene-to-water transfer process can be reversed when the cis-azo-ligands are converted to the trans state by the irradiation of visible light. As illustrated in the proposed scheme, the excellent reversibility of azobenzene isomerization allows the phase transfer of AuNPs between water and toluene to be performed reversibly for multiple cycles by alternating UV/Vis irradiation.

**Properties of Synthesized CD capped NPs and Azo-Ligand**

In our work, the per-6-thio-α-cyclodextrin and α-CD-coated AuNPs were prepared following the methods reported previously.$^{159, 163}$ As described in Figure 4-5 A, the UV/Vis spectra of the resulting water-soluble AuNPs dispersed in aqueous solution show an absorption peak at 516 nm. The average particle size was estimated as 3.6±0.5 nm using TEM (Figure 4-5 B). The azobenzene-containing surfactant was synthesized using the method described in the Supporting Information. The design of the azo-ligand was inspired by a ferrocene derivative described by Liu et al. More specifically, the positively charged nitrogen atoms transfer water molecules and counterions to the vicinity of AuNPs which assists in the formation of the interfacial
azobenzene-CD inclusion complexes.\textsuperscript{163} As expected, the azobenzene part of the surfactant ligand dissolved in toluene can be photoisomerized by UV/Vis irradiation. As shown in Figure 4-6, the absorption spectra of the azo-ligand synthesized in the trans state show a characteristic peak at 326 nm. Upon UV light irradiation at 365 nm, the intensity of this peak decreases dramatically, which indicates photoisomerization of the azo-ligand from the trans to the cis state (Figure 4-6). When visible light is applied afterwards, the peak intensity at 326 nm recovers as the azo-ligand undergoes cis-to-trans isomerization (Figure 4-7). The photoisomerization of the azo-ligand is totally reversible by monitoring the peak intensity at 326 nm upon alternating UV and visible light irradiation for multiple cycles (Figure 4-8).

**Phase Transfer of NPs from Water to Toluene by Trans-Azo-Ligand**

It has been reported that the host-guest interaction at the nanoparticle-solution interface can be used for phase transfer of nanoparticles from aqueous to organic solution, and vice versa.\textsuperscript{144, 163} Therefore, we first demonstrated that our new azo-ligand (trans) could also function as a phase transfer agent for the α-CD-coated AuNPs. Initially, the α-CD-coated AuNPs were dispersed in water (deep brown), while the azo-ligands were dissolved in toluene (yellow). Upon mixing the two phases, the α-CD-coated AuNPs were transferred from water (bottom layer) to toluene (top layer), as indicated by the color change of the two phases. The deep brown aqueous AuNP solution turned colorless, while the light yellow organic phase became colored (Vial 1, inset of Figure 4-9 and 4-10). However, in the absence of azo-ligands in the toluene phase, all the AuNPs stayed in the aqueous phase (Vial 2, Figure 4-9 and 4-10). The phase transfer of AuNPs was also characterized by the absorption spectra of aqueous and toluene phases. Figure 4-9 shows the absorption spectra of AuNPs in water before
(blue curve) and after phase transfer (black curve). Figure 4-10 shows the absorption spectra of the toluene phase. Before phase transfer (red curve, Figure 4-10), only the absorption band of azo-ligands (trans) was observed with no absorption at wavelengths longer than 500 nm. After phase transfer (black curve, Figure 4-10), a spectrum showing the absorption of both the azo-ligands (trans) and the AuNPs was achieved. Subtraction of the spectrum of the toluene phase before phase transfer from the spectrum after phase transfer resulted in a characteristic spectrum of AuNPs in toluene with a peak at 533 nm (Figure 4-11).

We also studied the phase transfer of AuNPs as a function of the concentrations of azo-ligand (trans) by maintaining a constant initial concentration (0.1 mg/mL) of AuNPs in aqueous solution. It was found that the concentration of nanoparticles transferred to the toluene phase (as measured by their absorbance at 533 nm, Figure 4-12) increases with the initial concentration of the azo-ligand in the organic phase. When the concentration of azo-ligand reaches 0.4 mM, a complete phase transfer of AuNPs is achieved as illustrated by the plateau.

The trans azobenzene of the azo-ligand can form inclusion complex with the α-CD-coated AuNPs, resulting in the phase transfer of AuNPs. However, the azo-ligand in the cis state cannot transfer the AuNPs into the organic phase. The azo-ligand (trans) dissolved in toluene was irradiated by UV light at 365 nm for 1 h to reach isomerization equilibrium before being used for the phase transfer of AuNPs. As shown in Figure 4-13, the spectrum of AuNPs transferred to the toluene phase, which was achieved by subtraction in the same way as that in Figure 4-11, shows only a small peak at 533 nm.
We attributed the limited amount of transferred AuNPs to the residual trans-azo-ligands after UV light irradiation.

**Photo-Reversible Phase Transfer of NPs**

The remarkable difference between trans and cis azo-ligand in the phase transfer of α-CD-coated AuNPs suggests that the formation of interfacial inclusion complexes and the phase transfer of AuNPs can be manipulated by UV/Vis light irradiation via the photoisomerization of azobenzene moieties. As shown by the pictures in Figure 4-14, UV light irradiation caused the AuNPs to dissolve in the organic phase (upper layer) because host-guest interactions were transferred back to the aqueous phase (lower layer). UV light-responsive phase transfer was also demonstrated by the absorption measurements of the organic phase (Figure 4-14). Upon irradiation by UV light, the absorption of trans azo-ligand at 326 nm decreases dramatically, which is indicative of the trans-to-cis isomerization of the azo-ligand. Concomitantly, the absorption of AuNPs at 533 nm in toluene disappears. Phase transfer of AuNPs to the aqueous phase, upon UV irradiation, depends on its duration and completes after about 1 h (Figure 4-15).

After the phase transfer of AuNPs from toluene to water by UV light irradiation, visible light was applied in order to reverse the transfer. As shown in Figure 4-16, after the irradiation by visible light, the AuNPs are again transferred to the organic phase. Absorption at both 326 nm and 533 nm then increases as a result of the visible light-triggered cis-to-trans isomerization of the azo-ligand and the concomitant phase transfer of AuNPs, respectively. This process is also time-dependent, and all the AuNPs are transferred to toluene after 30 mins of visible light irradiation (Figure 4-17).

The azo-ligand-mediated phase transfer of the α-CD-coated AuNPs is light-responsive. Based on the reversible isomerization of azo-ligand, the phase transfer of
AuNPs is also reversible. In a typical experiment, the irradiation of UV and visible light was continued for 60 and 30 mins, respectively, in each cycle. By monitoring the absorbance at 533 nm, which is attributable to the AuNPs in the toluene phase, the reversible phase transfer of AuNPs was observed as a result of the alternating UV and visible light irradiation over multiple cycles (Figure 4-18). It was also found that the absorbance of AuNPs in the toluene phase decreased after the light-triggered reversible phase transfer. We attributed the decreased absorbance to the formation of aggregated structures of AuNPs. However, most of the AuNPs stayed dispersed in both aqueous and organic phases during the reversible phase transfer, as shown in TEM images (Figure 4-19).

**Applications of Photo-Reversible Phase Transfer of NPs in Catalysis**

Metallic nanoparticles, such as gold and platinum NPs, have shown great promise in the fabrication of novel catalysts with better performance and versatility. Phase transfer is one of the most important techniques in the applications of functional nanoparticles. To demonstrate the applications of the phase transfer system, we applied the host-guest chemistry-based transfer strategy for the control of catalytic reactions. It has been reported that AuNPs coated by CDs through noncovalent interaction can catalyze the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of sodium borohydride (NaBH4) in aqueous solution.\(^ {164} \) Using this reaction as a model system, we first examined the catalytic ability of the obtained thiolated α-CD-capped AuNPs. The reaction was monitored by absorption measurements. The addition of AuNPs to the solution containing 4-NP and NaBH4 caused the fading and ultimate disappearance of the 400 nm peak (Figure 4-20), revealing the conversion of 4-NP to 4-AP.
Since the azo-ligand (trans) can transfer the α-CD-capped AuNPs from water to toluene, it is feasible to use this strategy to quench the catalytic reaction at will via removal of the catalytic AuNPs. As shown in Figure 4-21, the reduction of 4-NP to 4-AP was interrupted immediately upon the addition of the azo-ligand (trans) dissolved in toluene. However, without the azo-ligand (trans), the reaction proceeded to completion.

Moreover, we applied the photoreversible phase transfer for the recovery and recycling of catalytic α-CD-capped AuNPs to enhance their lifetime with both economic and environmental benefits. In a typical experiment (Figure 4-22), after the completion of 4-NP reduction in the 1st cycle, the AuNPs are recovered and transferred to the toluene phase by trans-azo-ligand. The aqueous phase with product is replaced by fresh water. Upon irradiation of UV light, the AuNPs are transferred back to water by the trans-to-cis isomerization of the azo-ligand. The 2nd cycle of reaction is performed after the addition of 4-NP and NaBH4. The AuNPs are then recovered and transferred to toluene upon irradiation of visible light which triggers the cis-to-trans isomerization of the azo-ligand. By the alternating irradiation of UV and visible light, the same batch of AuNPs can be recycled to catalyze multiple rounds of 4-NP reduction. As shown in Figure 4-23, the absorption of 4-NP at 400 nm decreases dramatically in each cycle of the reaction, indicating the sustained catalytic activity of the AuNPs during the light-regulated recovery and recycling process.

**Conclusion**

In summary, we demonstrated photoreversible phase transfer of AuNPs by the photoswitchable host-guest interaction between α-CD and azobenzene. The basic principle relies on the reversible surface modification of the α-CD-capped hydrophilic AuNPs with an azobenzene-containing surfactant ligand. The reversible phase transfer
can be performed for multiple cycles which are monitored by absorption spectra. The α-CD- capped hydrophilic AuNPs process catalytic activity that reduces 4-NP. Using the phase transfer strategy based on host-guest interaction, we were able to quench the catalytic reaction by removal of the catalytic AuNPs. Furthermore, the recovery and recycling of catalytic AuNPs were also realized by alternating UV and visible irradiation. The method of reversible phase transfer based on photoswitchable molecular recognition could bring more insight into nanoparticle surface engineering, thus improving and augmenting applications in different research areas.
Figure 4-1. Preparation of cyclodextrin coated gold nanoparticles. A) Synthesis of per-6-iodo-α-cyclodextrin. B) Synthesis of α-cyclodextrin-capped gold nanoparticles.
Figure 4-2. Preparation azobenzene ligand.
Figure 4-3. Scheme of reversible modification of azo-ligand on AuNPs. A) Structures of host per-6-thio-α-CD and guest azobenzene-containing ligand (azo-ligand). B) Photoreversible inclusion of azo-ligand in α-CD-coated AuNPs.
Figure 4-4. Light-responsive phase transfer of α-CD-capped AuNPs by azo-ligands between water and toluene phase.
Figure 4-5. A) Absorption spectrum of thiolated α-CD-coated AuNPs (3.6 nm) in water. B) TEM image of thiolated α-CD-coated AuNPs.
Figure 4-6. Absorption spectra of azo-ligand (trans) upon irradiation with UV light at 0 min (red), 15 min (green), 30 min (blue), 45 min (pink) and 60 min (black).
Figure 4-7. Absorption spectra of azo-ligand (cis) upon irradiation with visible light at 0 min (black), 15 min (pink), 30 min (blue), 45 min (green) and 60 min (red).
Figure 4-8. Reversible photoisomerization of azo-ligand (absorbance at 326 nm) in toluene upon alternating irradiation with UV and visible light.
Figure 4-9. The absorption spectra show absorbance of aqueous in vial 1 (black), vial 2 (blue) and vial 3 (red). AuNPs are transferred from aqueous to organic phase by azo-ligands (trans) (vial 1). Vial 2 has no azo-ligands in the toluene phase, and vial 3 has no AuNPs in the aqueous phase.
Figure 4-10. The absorption spectra shows absorbance of toluene phase in vial 1 (black), vial 2 (blue) and vial 3 (red). AuNPs are transferred from aqueous to organic phase by azo-ligands (trans) (vial 1). Vial 2 has no azo-ligands in the toluene phase, and vial 3 has no AuNPs in the aqueous phase.
Figure 4-11. Absorption spectrum of AuNPs in the toluene phase is achieved by subtraction of the red line from the black line in Figure 4-10.

Figure 4-12. Absorbance (533 nm) of AuNPs transferred to toluene phase containing variable concentrations of azo-ligand after equilibrating with aqueous solution containing AuNPs of a fixed concentration (0.1 mg/mL).
Figure 4-13. Absorption spectrum of AuNPs transferred to the toluene phase by azo-ligand (cis).
Figure 4-14. Phase transfer of α-CD-coated AuNPs from toluene to water phase by irradiation with UV light.
Figure 4-15. AuNPs (absorbance at 533 nm of toluene phase) transferred to aqueous phase from toluene phase upon irradiation with UV light at different times.
Figure 4-16. Phase transfer of α-CD-coated AuNPs from water to toluene phase by irradiation with visible light.
Figure 4-17. AuNPs (absorbance at 533 nm of toluene phase) transferred to toluene phase from aqueous phase upon irradiation with visible light at different times.
Figure 4-18. Reversible phase transfer of AuNPs. A) Absorption spectra of toluene phase upon irradiation with UV (red) and visible (black) light during reversible phase transfer. B) Reversible phase transfer of AuNPs (absorbance at 533 nm in toluene phase) between water and toluene phase.
Figure 4-19. TEM images of AuNPs during reversible phase transfer. A) AuNPs in aqueous phase after synthesis. B) AuNPs transferred to toluene by azo-ligand (trans). C) AuNPs transferred to water by UV light irradiation. D) AuNPs transferred back to toluene by visible light irradiation.
Figure 4-20. Catalytic activity of the α-CD-capped AuNPs. A) Reduction of 4-NP in the presence of NaBH4 and α-CD-capped AuNPs. B) Conversion of 4-NP to 4-AP monitored by absorption measurement. (0.3 mM 4-NP, 15 mM NaBH4, 25 µg/mL α-CD-capped AuNPs).
Figure 4-21. Absorbance (400 nm in water) during the reduction of 4-NP (0.3 mM) in the presence of NaBH4 (15 mM) and α-CD-capped AuNPs (6.7 µg/mL): reaction not quenched (black) and quenched by phase transfer of AuNPs from the addition of toluene containing azo-ligands at 5 min (red), 10 min (blue) and 20 min (pink).

Figure 4-22. Scheme of recovery and recycling of α-CD-capped AuNPs for the reduction of 4-NP by photoreversible phase transfer.
Figure 4-23. Multicycle reduction of 4-NP (absorbance at 400 nm) catalyzed by AuNPs recovered and recycled through the photoreversible phase transfer.
CHAPTER 5
SUMMARY AND FUTURE WORK

Engineering and Application of Molecular Recognition

Selective molecular recognition is achieved through noncovalent interactions such as hydrogen bonding, hydrophobic forces, van der Waals forces, and electrostatic effects. Naturally occurring molecular recognition interactions, like DNA hybridization, DNA-protein binding and antigen-antibody association, form the basis for biological processes. Obviously, studies on the fundamentals of molecular recognition are essential to answer questions concerning the mechanisms of biological processes.

On the other hand, molecular recognition provides a tool for manipulation of materials. Engineering of molecular recognition interactions enables the development of functional artificial materials and systems with applications in a wide range of fields. For example, DNA hybridization is the core component of numerous biosensing platforms for analysis of biological or environmental substances. Nanostructures based on DNA self-assembly have emerged as a unique type of biomaterials with precise structures and broad applications in biomedicine, DNA topological structures, stimuli-responsive materials, etc. Mimicking biological systems, non-biological molecular recognition systems are also developed. One notable example is the host-guest complex formation. Due to their chemical properties, DNA nanostructures can be easily synthesized and modified with different functional groups at a much lower cost compared with biological molecular recognition systems. Moreover, DNA materials are robust and can function under conditions where biological molecular recognition is inhibited.

This research has focused on the design of molecular recognition interactions for bioanalysis and dynamic materials involving three key projects: 1) the development of
an exonuclease III and graphene oxide-aided assay for DNA detection and telomerase analysis; 2) the development of dynamic polymer-DNA hybrid hydrogels with photoresponsive volume changes for utilization of photon energy; and 3) the reversible phase transfer of nanoparticles based on photoswitchable host-guest chemistry with applications in catalysis.

**An Exonuclease III and Graphene Oxide-Aided Assay for DNA Detection**

DNA detection is significant due to its applications in diverse fields, such as disease diagnosis, bio-weapon detection and forensic science. However, the current PCR-based methods suffer from limitations including the requirement of sophisticated equipment, contamination and replication errors. In order to develop comparable methods addressing those limitations, we proposed an amplified method for DNA analysis using exonuclease III and graphene oxide, in which enzyme-induced target recycling was employed. This method showed great signal enhancement compared with conventional hybridization-based method such as the molecular beacon assays. It is well known that multiple DNA biomarkers are associated with specific genetic diseases and we were able to apply the amplification strategy for simultaneous detection of multiple DNA targets without separation. Moreover, we analyzed telomerase activity in cancer cells with high sensitivity using our method with modifications. The results revealed the different telomerase activity levels in a variety of cancer cells, thus demonstrating the potential of our method for biomarker detection.

**Macroscopic Volume Change of Dynamic Hydrogels Induced by Reversible DNA Hybridization**

Functional materials regulated by photo-responsive molecular recognition enable the fabrication of devices such as switches, biosensors and actuators. In this project, we
proposed a rational design and synthesized a dynamic hydrogel by grafting azobenzene-tethered ssDNA and its complementary DNA to the hydrogel network using a two-step polymerization method. This hydrogel exhibited reversible volume change due to the photo-reversible DNA hybridization. The dynamic hydrogel took advantage of the scaffold of the polymer network to enable molecular level changes to be collected and visualized through macroscopic volume change measurements. We also studied the effect of different synthetic parameters on the volume change behaviors to verify structure-related properties. The photo-reversible DNA hydrogel can be used as a photon harvesting and conversion unit for a variety of applications, including flow valves, microlenses, solar absorbing units and sensors.

**Reversible Phase Transfer of Nanoparticles Based on Photoswitchable Host-Guest Chemistry**

Nanoparticles play an important role in applications like biological imaging, energy conversion, and catalysis, due to their unique optical, electrical and chemical properties. However, the distinct solubility differences of nanoparticles in media with different polarities limit their broader applications. In this project, we focused on developing a strategy for reversible phase-transfer of nanoparticles between aqueous and organic phases using light as the stimulus. The phase-transfer of nanoparticles was based on the photo-switchable molecular recognition between azobenzene ligands and alpha-cyclodextrin coated on nanoparticles. The reversible host-guest interaction changed the surface hydrophilicity of the nanoparticles. Using the phase-transfer strategy, we were able to stop the AuNPs-catalyzed reduction of 4-nitrophenol by removing AuNPs from the aqueous phase. We were also able to recycle the AuNP catalyst after the catalytic reaction without loss of catalytic activity.
Future Direction: Exonuclease III Based Signal Amplification for Detection of Mercury (II) ion

Mercuric ion (Hg$^{2+}$) is a highly toxic environmental pollutant which can accumulate in human bodies and cause serious health problems, such as DNA damage, organic function failure, brain damage, and immune system homeostasis disruption. Therefore, it is important to develop a simple yet effective method to monitor mercury levels in water with high selectivity and sensitivity.

Various methods have been developed for detection of Hg$^{2+}$ based on organic fluorophores, semiconductors, cyclic voltammetry, proteins, etc. Recently, it was found that Hg$^{2+}$ can mediate the formation of T-Hg$^{2+}$-T in DNA duplexes. The structure of DNA duplexes are stabilized by N–Hg bonds and the T-Hg$^{2+}$-T pairing exhibits higher bonding strength than regular T-T pair. The high specificity and bonding strength make it suitable for detection of Hg$^{2+}$ in aqueous solution.

Currently, we are developing a method for Hg$^{2+}$ detection based on exonuclease III induced signal amplification. (Figure 5-1) This assay is based on duplex formation mediated by T-Hg$^{2+}$-T base pairing. The signaling fluorophores are released from the DNA duplexes formed via T-Hg$^{2+}$-T by exonuclease III digestion. The signal amplification is achieved by the recycling of Hg$^{2+}$. 
Figure 5-1. Signal amplified detection of Hg$^{2+}$ using DNA and exonuclease III.
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BIOGRAPHICAL SKETCH

Lu Peng was born in Tianmen, China in November of 1985. He was inspired to become a chemist during high school. After graduating from Xiangyang No.5 High School, he attended University of Science and Technology of China to pursue a degree in chemical physics. In 2008, he began his doctorate study under the supervision of Dr. Weihong Tan at the University of Florida. After five years, Lu Peng completed his Doctor of Philosophy in chemistry in August 2013.