DNA Functionalized Plasmonic Nanostructures for
Biosensing and Living Cell Imaging

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DNA Functionalized Plasmonic Nanostructures for Biosensing and Living Cell Imaging

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<tbody>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>AuNP</td>
<td>Gold nanoparticle</td>
</tr>
<tr>
<td>AuNR</td>
<td>Gold nanorod</td>
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<tr>
<td>AuNS</td>
<td>Gold nanosphere</td>
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<tr>
<td>AgNP</td>
<td>Silver nanoparticle</td>
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<tr>
<td>AgNS</td>
<td>Silver nanosphere</td>
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<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>LSPR</td>
<td>Localized surface plasmon resonance</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-carboxyethyl) phosphine</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface enhanced Raman scattering</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>MCH</td>
<td>Mercaptohexanol</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>FNA</td>
<td>Functional nucleic acid</td>
</tr>
<tr>
<td>SELEX</td>
<td>Systematic evolution of ligands by exponential enrichment</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Single-stranded DNA</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double-stranded DNA</td>
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<tr>
<td>MCF-7</td>
<td>Michigan cancer foundation-7</td>
</tr>
<tr>
<td>NIR</td>
<td>Near infrared</td>
</tr>
<tr>
<td>STM</td>
<td>Scanning tunneling microscopy</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>UPS</td>
<td>Ultraviolet photoemission spectroscopy</td>
</tr>
<tr>
<td>PDA</td>
<td>Polydopamine</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified eagle medium</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
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Summary

DNA Functionalized Plasmonic Nanostructures for Biosensing and Living Cell Imaging

Student: Wang Chenxu
Supervisor: Associate Professor Duan Hongwei

Research in this thesis focused on developing core-shell DNA-functionalized plasmonic nanostructures which exhibit integrated properties of the “hard” nanoparticle core and “soft” DNA shell. The grafted DNA strands that exhibit stimuli-responsive and/or hybridization-induced structural changes and specific recognition of protein targets provide a myriad of mechanisms to tailor the self-assembly of the plasmonic nanostructures and thus the interparticle plasmonic coupling. The distinctly different plasmonic properties of the assemblies and individual blocks offers the possibilities of developing sensors for the mechanisms inherent to the DNA strands. In addition, our work led to a new approach to preparing DNA-nanoparticle conjugates based on polydopamine coating to address the problems associated with the traditional method based on the labile metal-sulphur bond. We have demonstrated that it is possible to confine self-assembly of DNA-nanoparticle conjugates at certain stages to form discrete assemblies instead of macroscopic aggregates. A particular type of assemblies of plasmonic nanostructures we focused on is the “core-satellite” structures with a number of smaller nanoparticles surrounding the larger nanoparticles. Our results showed that the core-satellite assembly with defined sizes made it possible to sense particular
substances inside living cells and at single-particle level, which are not available for macroscopic assemblies.

First, we conjugated i-motif DNA and its complementary strand onto “satellite” and “core” nanoparticles respectively through Au-S bond and obtained core-satellite assemblies by carefully adjusting the hybridization time and ratio of two kinds of nanoparticle building blocks. Since i-motif DNA undergoes conformational change in acidic conditions (pH=5.0), we can modulate the assembly and disassembly of nanoparticles by pH changes. This process can be monitored through UV-vis spectroscopy and dark field imaging since the assemblies exhibit distinct plasmonic properties from individual building blocks. The pH-responsive assemblies were applied as nanoscale biosensors for intracellular pH analysis.

Second, a gold nanorod based plasmonic sensing approach towards virus genome sequence and genetic mutation was brought forward based on a sandwich DNA hybridization design. Binary nanoprobes were prepared by dividing complementary strand of target sequence into two individual parts and anchoring them on AuNRs and 14 nm AuNPs, respectively. Target-specific DNA hybridization would induce the assembly of nanoprobes and a dramatic red-shift of longitudinal SPR band was observed in response to different DNA concentrations. High sensitivity was achieved by this system and single nucleotide polymorphism (SNP) was detected with high specificity. Two hybridization strategies for DNA detection were applied and compared to display different hybridization dynamics, different sensitivity and respective advantages.

Third, we introduced polydopamine (PDA) shell to facilitate the DNA conjugation onto multiple colloidal nanoparticles. Dopamine can self-polymerize
into PDA in oxidizing conditions and subsequently adhere onto particles with metallic surface or polymeric surface. The thickness of PDA coating can be flexibly tailored to achieve excellent colloidal stability. On the other hand, the spontaneous reaction of nucleophilic groups such as thiol and amino groups with PDA through Michael addition and/or Schiff-base reaction enabled the ready coupling of DNA strands on the functional nanoparticles in high surface coverage. The resulting covalent linkage showed dramatically enhanced chemical and thermal stability in comparison with Au-S bond.

Finally, we conducted cell imaging experiment with AS1411 aptamer modified PDA-wrapped nanoparticles for targeted labeling of cancer cells. The unique adhesive properties of PDA allows for forming the PDA coating on a diverse range of nanoparticles of different functionalities and surface properties, which further enables the preparation of DNA-nanoparticle conjugates. We further showed that the DNA-nanoparticle conjugates can be assembled into discrete multifunctional core-satellite structures with magnetic nanoparticle core and plasmonic satellites, whose collective magnetoplasmonic properties hold great promise in bionanotechnology applications.
Chapter 1. Introduction

1.1 Backgrounds

1.1.1 Plasmonic Nanoparticles

When interacting with the oscillating electromagnetic wave of incident light, free conduction electrons in the metal would oscillate collectively in regard to its positively charged stationary metallic core.\textsuperscript{1,2} This phenomenon is called surface plasmon resonance (SPR) since it is usually resonant at a specific wavelength of the incident light. When it comes to structures in nanoscale, it is called localized surface plasmon resonance (LSPR). As shown in Figure 1.1a, the electric field of the light can cause the negative electron cloud in metal nanoparticles to polarize to one side, and a restoring force generated by the attraction of positive solid nanoparticle core will result in the oscillation of electron cloud in resonance with the incident light.\textsuperscript{3,4} The oscillation decays by light scattering and light absorption, which can be strongly enhanced at LSPR wavelength and result in characteristic peaks for different materials.\textsuperscript{5} The peaks usually locate in the visible region, and tunable properties of individual nanoparticles are utilized to adjust the peaks even to near infrared (NIR) region and enhance the oscillation strength so that the resulting plasmonic nanostructures can be suitable for more widespread applications such as in biological systems which requires NIR window for maximum penetration depth.\textsuperscript{2,3}

The oscillation frequency is dependent on electron density, effective electron mass and charge distribution, which are affected by the size, shape, the arrangement of nanostructures and dielectric environment.\textsuperscript{6-8} Plasmonic properties of metallic nanoparticles can be theoretically predicted by the following Mie equation (1):
\[ C_{\text{ext}}(\omega, R) = 12\pi \frac{\omega R^3 \varepsilon_{\text{m}}^{3/2}}{c} \frac{\varepsilon_2(\omega, R)}{[\varepsilon_1(\omega, R) + 2\varepsilon_{\text{m}}]^2 + \varepsilon_1(\omega, R)^2} \]  

(1)

Where \( C_{\text{ext}} \) is the extinction cross section, \( \omega \) is the frequency of exciting radiation, \( R \) is the radius of particles, \( c \) is the speed of light, \( \varepsilon_{\text{m}} \) is dielectric constant of surrounding solution, \( \varepsilon_1 \) and \( \varepsilon_2 \) represent the real and imaginary part of dielectric constant of particle material. Deducing from the equation, size and material of nanoparticles, along with the surrounding environment determine the plasmonic properties of metal nanoparticles.\(^7\)\(^9\) As shown in Figure 1.2a, the size increase of AuNSs lead to the continuous red shift of LSPR, and the color of colloidal solutions changes from burgundy red to purple and blue. AuNSs with a diameter of about 14 nm have a strong LSPR absorption peak at 520 nm when the peak of 40 nm AuNSs red-shifts to around 530 nm.\(^7\) Red shift of LSPR is resulted from electromagnetic retardation effect and color change of AuNS solutions is due to the increased absorption of red light and transmittance of blue light.

The influence of particle shape on LSPR can be found out in Figure 1.1b, as the isotropy of spherical AuNSs provides same electron oscillations along all directions which is in resonant with a particular frequency, while the anisotropy of AuNRs can realize electron oscillation along its transverse and longitudinal directions. Thus, typical AuNSs show one LSPR peak in the visible region and AuNRs have two LSPR band, one of which is generated by the plasmonic oscillation along the short axis of AuNRs and the other by the oscillation along the long axis.\(^10\) The position of these two peaks strongly depend on the aspect ratio, i.e. the length to width ratio, of AuNRs, whose increase would lead to the red shift of LSPR peaks even to the NIR region (Figure 1.2b).\(^2\)\(^,\)\(^11\) The easy size-tunability and NIR absorption property enable
AuNRs for widespread applications such as biodetection\textsuperscript{12}, cancer cell imaging\textsuperscript{13} and photothermal therapy\textsuperscript{14}.

\textbf{Figure 1.1.} Surface plasmon resonance that happens in the metal NSs (a) and metal NRs (b). (a). For spherical nanoparticles, one SPR band occurs in resonance with incident light.\textsuperscript{15} (b). metal NRs have two SPR frequencies corresponding to the transverse direction and longitudinal direction.\textsuperscript{15-17} Copy right 2011 RSC Publishing Groups and 2014 Nature Publishing Groups.
In general, the most important factors that influence plasmonic properties are size and shape of nanoparticles. With the increase of particle diameters and anisotropy, the resonance frequencies would red shift and resonance intensity would change as well. LSPR wavelength of plasmonic nanoparticles can be tuned by assembling nanoparticles in close distance, known as plasmon coupling.\textsuperscript{1,6} Plasmon coupling leads to a red shift of spectra which depends on the distance of neighboring nanoparticles.\textsuperscript{18} This phenomenon results in the construction of various target-responsive colorimetric sensors by functionalizing nanoparticles with responsive polymers\textsuperscript{19} and molecules\textsuperscript{20,21}. Among all the candidates that are utilized for surface functionalization of plasmonic nanoparticles, DNA is emerging as an excellent material for corona engineering\textsuperscript{9,22-24}. 

**Figure 1.2.** (a). UV-vis absorption spectra of AuNSs with various sizes. The inset shows different colors of colloidal gold nanoparticle solutions with different sizes. (b). UV-vis absorption spectra of AuNRs with a series of aspect ratios. The inset image shows the change of colors with the increase of aspect ratio.\textsuperscript{2} Copyright 2008 ACS Publishing Group.
AuNSs and AuNRs are widely used in bionanotechnology as model nanoparticles due to their easy synthesis method with large quantity, easy shape and size control, good bio-compatibility and outstanding optical properties for simple characterization. These great features made them good combination with DNA to function in biosensing and bioimaging.

1.1.2 Functional Nucleic Acids

DNA, widespread in organisms, is one kind of essential composing elements in life that plays crucial roles in storing, transmitting genetic information and directing cell behavior through replication and transcription. DNA is made of small, repeating units called nucleotides which are composed of three parts, deoxyribose, phosphate groups and nitrogen-containing bases. The four kinds of nitrogen-containing bases existing in DNA, namely, adenine, guanine, thymine and cytosine, form the structural foundation of molecular recognition property through hydrogen bonding of Watsons-Crick base paring. The length and double strand binding strength of DNA strands can be well controlled by changing the number of nucleotides. In a typical DNA molecule structure, sides of DNA strands are composed of alternating sugar and phosphate groups which form DNA backbone, and in the middle of DNA are nitrogen-containing bases which pair up by double (adenine and thymine) or triple (guanine and cytosine) hydrogen bond to hold together double stranded DNA. This unique sequence-specific recognition system, along with its structural versatility and precise manipulation of DNA strand length, allows DNA to be extensively explored for surface corona engineering of nanoparticles. The DNA strands, with careful design of sequences, can regulate the assembly of elementary nanoparticles into well-defined architectures. This
nanoparticle-DNA system is widely applied in diverse applications such as sensitive molecular detection\textsuperscript{32}, diagnostics\textsuperscript{33} and drug release\textsuperscript{34-36}.

Other than base pair recognition and hybridization of normal complementary DNA strands, functional nucleic acids (FNA), such as aptamer and DNAzyme, are also emerged as important agent for molecular recognition\textsuperscript{31,37} (biosensors) and cell targeting\textsuperscript{38} (bioimaging and drug delivery) \textit{via} interacting with specific proteins\textsuperscript{39}, metal ions\textsuperscript{40} or small molecules\textsuperscript{41} to induce structural transformation.\textsuperscript{31,37,42,43}

\subsection*{1.1.2.1 Aptamers}

Aptamers are single stranded oligonucleotides that can bind specific target molecules with high affinity and selectivity. In the presence of targets including adenine, proteins and metal ions, aptamers adaptively fold into specific structures to bind targets and the foundation of their recognition of targets is not based on base pairs.\textsuperscript{43,44} As reported by Takenaka and coworkers,\textsuperscript{45} the presence of potassium ions caused the common random coil DNA to fold into tetraplex structure, bringing the two ends of DNA in close proximity.

Aptamers are short oligonucleotide sequences that either naturally exist in organisms or are artificially screened from systematic evolution of ligands by exponential enrichment (SELEX). SELEX begins with the construction of oligonucleotide pool that contains a large number of randomly generated oligonucleotide sequences. Then, the target is incubated with this pool, followed by removing non-binding sequences using affinity chromatography. The binding sequence is eluted from the target and amplified by PCR to form the nucleic acid pool for subsequent selection cycle during which the strictness of elution conditions is improved to obtain oligonucleotide sequences with higher binding affinity.\textsuperscript{46} This
technique can select aptamers that can specifically bind numerous target ligands. For example, one aptamer was obtained by Missailidis group to specifically recognize MUC1, a transmembrane protein which is widely expressed in malignant epithelial cells.

Ions and small molecules such as $H^+$, $Ag^+$, $Hg^{2+}$, adenine and cocaine are common targets that aptamers can interact with apart from potassium ions we previously mentioned. The targets can induce the transformation of DNA strands from loose, single-stranded, random-coiled DNA to specific, rigid structure. The unique binding property enables aptamers to be involved in constructing various sensors with diverse targets. Ultrasensitive detection of heavy metal ions such as $Ag^+$ and $Hg^{2+}$ is realized by aptamer-based sensors with different sensing strategies.

$H^+$-sensitive aptamer is the DNA sequence with stretches of cytosine residues. When in acidic environment, the strand can form a four-stranded quadruplex structure based on cytosine base pairing, which is denoted as C quadruplex or i-motif. Cytosine rich oligonucleotides are widespread in telomeric DNA, which locates at the end of DNA and plays the key role in maintaining DNA integrity and cell fate control. Researches have shown that this C-rich DNA strand can form quadruplex structures via $C\cdot C^+$ base pairs in low pH environment. Typically, at pH around 5-6, nitrogen atom is protonated and the protonated cytosine forms a non-canonical base pair with a normal cytosine ($dC\cdot dC^+$) (Figure 1.3a), which could drive a DNA strand containing 4 stretches of CCC sequence to fold into a quadruplex structure (Figure 1.3b). This has been utilized in constructing optical probes and AuNP assembly. Fan and coworkers developed an optical probe for $H^+$ detection making use of the high affinity of single-stranded DNA (ssDNA) to AuNPs and poor
affinity of i-motif structure to AuNPs. After adsorption of ssDNA onto AuNPs, high stability against high salt concentration was displayed. However, acidic pH environment drove the formation of i-motif structure and detachment of DNA strand from AuNP surface, which would decrease the colloidal stability against salt and induce aggregation of AuNPs. In another work by Liu group\textsuperscript{57}, i-motif DNA was used to realize the reversible assembly of AuNPs (Figure 1.3c). At pH around 8, the DNA strand was in random coil status and hybridization took place between complementary DNA strands modified AuNPs. At acid pH around 5, cytosine-rich DNA was protonated to form C-quadruplex, leading to disassembly of AuNP aggregates and inducing a blue shift of LSPR peak (Figure 1.3d). This demonstrated that the introduction of aptamer for nanoparticle assemblies would broadens the applications of versatile plasmonic nanostructures.\textsuperscript{48}
Aptamers also display high binding affinity towards protein targets, making them widely applied as surface coating agents for modification of nanoparticles for protein detection\textsuperscript{59,60} and cancer cell targeting and therapy.\textsuperscript{61,62} Chang group demonstrated a SERS approach for detection of platelet derived growth factor (PDGF). The PDGF aptamer and 4-mercaptobenzoic acid were conjugated onto
AuNPs and Au necklace-like nanochain was prepared as SERS amplifier. PDGF can interact with DNA functionalized AuNPs and subsequently bind the nanochain to enhance SERS signals. Surface biomarkers, normally known as proteins on cellular membrane, are prevalent targets of aptamers for cancer cell imaging, targeted drug delivery and photothermal therapy. Nucleolin, an abundant RNA- and protein- binding phosphoprotein that locates widely in the nucleolus, cytoplasm and cell membrane, has been found to play essential roles in various cellular process such as DNA transcription, chromatin organization, protein transportation between nucleus and cytoplasm. Elevated expression level of nucleolin was discovered in cells with high proliferation rate, making it a potential biomarker of cancer cells such as breast and cervical cancer cells. A 26-mer guanine-rich oligonucleotide called AS1411 was known as nucleolin-binding aptamer that can form G-quadruplex structure when specifically interacting with nucleolin. Many recent researches demonstrated the application of AS1411 for cancer cell therapy and drug delivery, such as AS1411 modified micelles, liposomes for siRNA delivery and AS1411 functionalized AuNPs for cell imaging and photodynamic therapy.

1.1.2.2 DNAzymes

Another kind of FNA, DNAzymes, is oligonucleotides that exhibit catalytic properties. DNAzymes provide an effective catalytic label for bio-recognition events, and are widely applied in the analysis of metal ions and detection of DNA or other targets. Fluorescent and colorimetric sensors have been developed by functionalizing DNAzyme with specific molecules. In Lu group’s work, they demonstrated a DNAzyme and gold nanoparticles (AuNPs) based biosensor to detect Pb²⁺. A DNA substrate strand containing cleavage site for the DNAzyme was utilized to direct the assembly of DNA functionalized AuNPs. Massive AuNP
aggregates were formed in the presence of substrate strand and the color of AuNP solution changed from red to purple. DNazyme, possessing Pb$^{2+}$-dependent catalytic activities, were partly complementary to the DNA substrate strand and hybridized with this strand upon adding into reaction solution. When Pb$^{2+}$ existed in the environment, however, Pb$^{2+}$ could activate the DNazyme to perform its catalytic property and cleave DNA substrate strands, which resulted in the disassembly of aggregates and change of solution color back to red. A detection limit down to 100 nM Pb$^{2+}$ was reached.

1.1.3 DNA-Plasmonic Nanoparticle Conjugates

In order to utilize DNA for nanoparticle engineering, diverse modification methods were put forward for DNA functionalization of nanoparticles. Thiol groups are the most commonly employed functional moieties that are utilized to modify DNA strands to prepare DNA-nanoparticle conjugates since thiol group possesses strong affinity towards noble metal atoms and chalcogenide surfaces. Ever since Mirkin group and Alivisatos group utilized DNA to assemble AuNPs into macroscopic structures in 1996,22,23, many attempts have been made to modify DNA onto AuNPs. Thiolated DNA strands were modified onto AuNPs through the formation of gold-thiol bond. Since DNA has a negatively charged backbone, when utilizing DNA to functionalize negatively charged AuNPs such as citrate-stabilized AuNPs, electrostatic repulsion between DNA strands and AuNPs creates a barrier for loading DNA on the nanoparticles. One strategy is to use high concentration of salts to screen the charge, enabling DNA to attach onto AuNPs. Owing to the instability of AuNPs stabilized by weakly adsorbed citrate ions, a low concentration of NaCl (40 mM) could induce the aggregation of AuNPs. Researchers also showed that DNA loading amount is proportional to salt concentration. Therefore,
conventional method established by Mirkin group started with incubation of AuNPs and SH-DNA in low NaCl concentration with the aid of SDS to allow small quantity of DNA to load onto AuNPs and further stabilize AuNPs, followed by adding more NaCl into reaction solution to increase DNA loading. The gradual increase of NaCl concentration, accompanied with sonication at specific intervals, was called salt aging process. This method usually takes 1-2 days and requires great cautiousness during operation to prevent aggregation of AuNPs. Minor modification was made in other groups later by stabilizing AuNPs with BSPP or TCEP first to enhance their stability against salt. In 2012, Liu and coworkers introduced us new approaches to realizing efficient DNA functionalization. The first method was to use pH 3.0 citrate buffer to achieve the instantaneous and quantitative modification of AuNPs with thiol-DNA. The second one was to utilize depletion stabilization mechanism to stabilize nanoparticles of various sizes and kinds. The depletion agent was modification-free polyethylene glycol (PEG) with high molecular weight. The stability of nanoparticles was tested in the presence of PEG and results showed that PEG with high molecular weight can stabilize nanoparticles against high concentration of salt, various pH and organic solvents. 13 nm AuNPs can remain stable in the presence of 1 M NaCl in a 2% aqueous PEG 20000 solution. This new DNA modification method is fast (2-4 h), easy to control and of high loading efficiency.

In addition, several works demonstrated that modification-free ssDNA could adsorb onto AuNPs and the resulting DNA functionalized AuNPs are as stable as thiol-DNA stabilized AuNPs. Subsequent hybridization reaction of this DNA-AuNPs showed the retained molecular recognition property that could be used to
assemble plasmonic nanostructures. However, this method also required about 3 days by a salt aging process for successful functionalization.\textsuperscript{84}

Lu group\textsuperscript{85} and Xu group\textsuperscript{86} designed one-pot synthesis of DNA functionalized AuNPs. Lu group incubated seed AuNPs (20 nm AuNSs) with modification-free ssDNA first and then added NH\textsubscript{2}OH and HAuCl\textsubscript{4} solution to facilitate the reduction and growth. Xu group prepared the DNA-AuNPs by reducing HAuCl\textsubscript{4} with trisodium citrate in the presence of ssDNA at 37 °C for 12 hr. Both of two groups obtained DNA modified AuNPs with high colloidal stability and retained functionality of DNA strands on particle surface, as well as sequence-specific morphology and enhanced stability of DNA attached on AuNPs. However, evident disadvantages were observed that nanoparticle morphology was hard to control and DNA strands with unpredictable length were imbedded in particles. Furthermore, for Lu group’s method, seed AuNPs were coated with Au shell to carry DNA, which would greatly change the properties of seed nanoparticles. All these disadvantages, accompanying with the harsh requirements of particle materials, impeded the wider applications of the one-pot synthesis methods.

Apart from using thiol-DNA to interact with noble metal nanoparticles and chalcogenide surfaces to realize DNA conjugation, streptavidin-biotin interaction\textsuperscript{87}, amino-carboxyl condensation reaction\textsuperscript{88} and other methods were discovered to modify DNA strands onto colloidal nanoparticles (quantum dots, upconversion nanoparticles, magnetic nanoparticles, silver nanoparticles, etc).\textsuperscript{18} Moreover, Au shell was introduced onto nanoparticles as a substrate for DNA modification. Mirkin group grew Au shells onto silver nanoparticles (AgNPs) for the modification of DNA strands onto AgNPs since AgNPs were not stable to go through normal thiol-DNA modification process.\textsuperscript{89}
1.1.4 Current Challenges of DNA Applications

Owing to the extensive application of DNA-AuNP conjugates in sensitive colorimetric biosensors, cell imaging, intracellular therapeutic delivery and photothermal therapies, challenges were confronted for Au-S bond to remain stable in various environmental conditions such as high concentrations of salt, reductive thiol-containing small molecules and elevated temperatures. Previous researches had displayed that the dissociation of thiolated DNA strands from AuNP surface occurred at high temperature, in high salt concentrations or when exposed to various thiols. Liu group further demonstrated the breakage of Au-S bond in dimethylformamide (DMF). El-Sayed group confirmed that femtosecond laser heating resulted in photothermal instability of Au-S bond. It is reasoned that harsh environment may increase the susceptibility of Au-S bond to oxidation in air and water, leading to bond breakage. In addition, thiol-containing molecules can replace thiolated DNA from nanoparticle surface. Taton and coworkers’ work pointed out that high temperature may also lead to DNA degradation in the presence of gold surface which played a catalytic role. The instability of DNA-AuNPs in response to specific environment limited their potential applications. For instance, the conjugates cannot survive the experimental conditions of polymerase chain reaction (PCR) process which has high temperature and DTT as a stabilizer for polymerase enzyme.

Research efforts have been made in enhancing the thermal and chemical stability of DNA-AuNP conjugates by using multiple thiol-bearing DNA strands for nanoparticle modification. Mirkin and coworkers modified AuNPs with mono-hexylthiol, dithiane and tri-hexylthiol carried DNA strands, respectively and acquired substantially stable trithiol-DNA nanoparticle conjugates in comparison
with other nanoparticle probe (Figure 1.4). Le group prepared three kinds of DNA-AuNP probes through conjugation with monothiol, acyclic disulfide and cyclic disulfide, and proved that conjugation through acyclic disulfide showed highest thermal stability and cyclic disulfide bond realized highest chemical stability when treated by 50 µM MCH at room temperature. However, these multidentate-thiol-DNA strands are pretty expensive and complicated for synthesis and not commercially available, making monothiol DNA strands still widespread for nanoparticle engineering.

As for other modification methods, multiple functional groups of DNA are required and the methods are substrate-specific, making it necessary to develop a universal and facile approach for DNA functionalization.

Figure 1.4. Schemes of preparation of DNA-AuNP conjugates with different anchoring groups. DNA strands were conjugated onto AuNPs via monothiol, dithiols and trithiols, respectively. Copyright 2002 Oxford University Press.
1.2 Objectives

The first objective of this thesis is to construct well-defined plasmonic core satellite nanostructures from AuNPs modified with stimuli-responsive DNA aptamer and utilize them to monitor intracellular pH change process through plasmonic imaging under dark field microscopy. We also attempt to develop a simple and sensitive DNA detection system based on DNA functionalized anisotropic AuNR. Longitudinal plasmon band is recorded to determine the DNA concentration and discriminate single nucleotide polymorphism (SNP) sequence.

Furthermore, considering the disadvantages of traditional DNA modification methods, we seek to address these problems by introducing a new robust DNA conjugation method onto colloidal nanoparticles with the aid of PDA shell and explore the influence of PDA layer on nanoparticles. Investigation should be designed to prove the stability of DNA linkage. Finally, aptamers with specific recognition of biomarkers on cell surface are modified on PDA coated 50 nm AuNPs to interact with cancer cells and display the well-retained functions of aptamers on PDA shell. And we try to realize DNA hybridization based on three kinds of different PDA based platforms. The plasmonic properties are examined to confirm DNA hybridization mediated assemblies of core-satellite structures.
Chapter 2. Review of the Literatures

2.1 Plasmonics

2.1.1 Plasmon Coupling

LSPR band can be greatly changed through the control of distance between metallic nanoparticles. When two metallic nanoparticles are brought in close proximity within 2.5 times of nanoparticle diameter, the oscillation of one of the adjacent particles will induce the electron oscillation of the other one via near-field interaction, resulting in the coupling of plasmon resonance. The plasmon coupling will greatly enhance the electric field around the nanostructures and improve dipole-dipole energy, bringing about the red-shift of SPR band relative to individual nanoparticles, which, however, will decay quickly with the increase of distance between nanoparticles. In Figure 2.1, the presence of target DNA strands induced the assembly of DNA-AuNPs which significantly reduced inter-particle spacing, thus generating strong plasmon coupling and changing the color of reaction solution from red to purple. Since the electron cloud dipole interaction is greatly influenced by the inter-particle distance, different SPR bands can be achieved through the adjustment of the distance and the arrangement of nanoparticle assemblies such as the number, size and shape of individual nanoparticles.
Figure 2.1. In the presence of complementary DNA strands, DNA-AuNPs will hybridize together and aggregate when temperature is below melting temperature of DNA. The inset image shows the change of solution color from red to purple after aggregation. Copyright 2011 RSC Publishing Group.

2.1.2 Dark Field Imaging of Plasmonic Nanostructures

In addition to the easy tunability of plasmonic properties, plasmonic nanostructures with certain diameters can display distinct colors and signature scattering spectra corresponding to the specific LSPR frequency where light is strongly scattered. Even single plasmonic nanoparticles can be visualized because of the high scattering cross-section. Unlike normal fluorescent dyes or quantum dots, plasmonic nanoparticles are photostable and photoblinking-free even after continuous exposure to strong incident light, making them excellent agents for biosensing and bioimaging applications. To perform particle imaging, dark field microscopy is required which is an optical microscope assembled with a dark field condenser. After light enters the microscope from light source, a piece of disc with specific size will block light in the middle to generate an illumination ring, which will be focused on the samples after going through dark field condenser. Since the incoming light is incident at high angles, most of the light is transmitted, leaving
the light scattered by samples to enter objective lens, generating an image with black background and colored bright objects (Figure 2.2a). As shown in right panel of Figure 2.2b, scattered light displayed different colors relating to the size and materials of plasmonic nanoparticles, as 50 nm and 100 nm gold nanospheres (AuNSs) showed green and orange color, comparing with blue color of 50 nm silver nanospheres (AgNSs). The scattered light exhibits photostability against continuous excitation and can be influenced by plasmonic coupling, making plasmonic nanostructures good agents for protein sensing and cell imaging. Chen group demonstrated a nanoplasmonic ruler in which AuNPs were modified with a double-stranded DNA (dsDNA) carrying four endonuclease incision sites, and successive treatment of nuclease will result in a gradual change of scattering color and spectra due to the size change after DNA strand cleavage. El-Sayed group modified AuNRs with anti-epidermal growth factor receptor (EGFR) antibodies and acquired light scattering images to discriminate EGFR-overexpressed cancer cells from nonmalignant epithelial cell lines.
Figure 2.2. (a). Schematic illustration of light path that goes through microscope condenser and produces dark field images after interacting with the sample. (b). Distinct colors of scattered light by plasmonic nanoparticles under dark field microscopy. 50 nm AuNSs correspond to green light. 100 nm AuNSs to orange light and 50 nm AgNSs to blue light. Copyright wiki & 2005 ACS Publishing Group.

2.2 Self-Assemblies of DNA-Plasmonic Nanoparticles

Plasmonic nanostructures made from noble metals have been utilized in biosensing and bio-imaging due to their intense absorbing and scattering of light which strongly rely on their size, shape, composition and dielectric environment. Their properties can also be determined by the arrangement of individual metal nanoparticles in space, in which case neighboring nanoparticles are brought in close proximity to generate plasmon coupling and induce LSPR red-shift. The assembly
of metal nanoparticles can usually be realized through various surface modifications by small molecules, macromolecules and biomolecules.\textsuperscript{20,103} Of all the target-responsive smart coating molecules that are used in surface engineering, biomolecules, including DNA and proteins, are probably the most widespread items we used. DNA, with its programmable sequence, structural versatility, simple synthetic strategy and base pairing conducted molecular recognition, has attracted considerable research interest.\textsuperscript{9,22,23}

Since 1996 when Mirkin group and Alivisatos group did the pioneering work about introducing DNA into assembling plasmonic nanoparticle conjugates, various kinds of plasmonic nanostructures were constructed using DNA to connect individual nanoparticles and form previously designed patterns.\textsuperscript{104} Parameters such as distance between nanoparticles, number of nanoparticles in the system and structure patterns can be finely tuned through careful design of length and numbers of DNA strands. As displayed in Figure 2.3, DNA strands and DNA motifs hybridized from single strands, were designed to carry various functional groups (amino, thiol, biotin et al) and guide plasmonic nanoparticles to form nanoparticle superstructures, including macroscopic aggregates, one-dimensional (1D), 2D, 3D and discrete assemblies.\textsuperscript{9}
Figure 2.3. Schematic illustration of DNA mediated assembly of plasmonic nanostructures. Individual nanoparticles were assembled into various kinds of plasmonic assemblies from the guidance of single DNA strands and DNA motifs which can be rationally designed and created by means of DNA nanotechnology.\textsuperscript{9} Copyright 2011 Nature Publishing Group.

2.2.1 Macroscopic Aggregates.

In early researches, DNA mediated hybridization of AuNPs often formed macroscopic aggregates, with the color change of reaction solutions from red to purple due to strong plasmon coupling.\textsuperscript{23,105} These pioneer works shed light on DNA based biotechnology and revealed some basic properties of DNA mediated assemblies of AuNPs such as UV-vis spectra, melting temperature, color change and the reversibility of this process.\textsuperscript{106} Later, the color change and reversibility of DNA
mediated assemblies of nanoparticles into aggregates were utilized as colorimetric method in biosensors for DNA mismatch, methylation and metal ion detection.\textsuperscript{107} Recently, DNA linked nanoclusters found applications in diagnostics of cell surface marker in combination with SERS and LSPR detection at single cell resolution.\textsuperscript{108} Another interesting work is to modify DNA with azobenzene, a substance demonstrating wavelength dependent photo-isomerization property, to form photoswitchable conjugates, which can disassemble under UV light and conjugate again under blue light.\textsuperscript{109} However, further application was impeded by these irregular, uncontrollable macroscopic aggregates themselves for the reason that they cannot overcome size-limitation in biological barriers to be utilized in living system for cellular uptake and tumor targeting.

\textbf{2.2.2 One Dimensional (1D), 2D and 3D Assemblies.}

For nanoparticle assemblies with more complexity, one strategy is to guide nanostructure constitution with DNA template. The DNA template can be achieved through the self-assembly of well-designed building blocks after which individual nanoparticles will attach onto the template and form nanostructures with various geometries.\textsuperscript{110} 1D assemblies constructed by DNA and AuNPs rapidly emerged recently by using various construction methods. One common method was to construct DNA template while thiol groups were added onto the DNA blocks at specific position.\textsuperscript{111} The resulting DNA templates reacted with AuNPs to get 1D assemblies. Another method was to carry out AuNP modification to enable them to self-assemble with each other and form 1D assemblies.\textsuperscript{112} Presented in Kuzuya et al’s work\textsuperscript{113}, divalent DNA-AuNP conjugate was obtained by attaching DNA onto the polar region, that is, the “defect” region generated by 11-mercaptopundecanoic acid (MUA) and 4-mercaptophenylacetic acid (MPAA) on the surface of AuNPs,
which was necessarily formed in order to allow the alternation of concentric rings. These three works just involved the simple structure play of AuNP arrays and no practical applications or properties were discussed. Afterwards, Sleiman and coworkers displayed a remarkable work that they constructed 1D DNA-AuNP array through the capsulation of AuNPs by triangular DNA nanotubes which could realize precise positioning and selective loading of nanoparticles through the control of size of constructing triangular rungs. Specific DNA strands input would open the DNA nanotube and lead to selective cargo release of AuNPs.\textsuperscript{114}

For 2D assemblies, two works were displayed by Kiehl group that complicated DNA 2D arrays were constructed using basic DNA motifs and sticky ends of DNA in certain positions were reserved in these arrays for the hybridization with ssDNA modified AuNPs.\textsuperscript{115,116} Another special 2D nanoparticle arrays, free standing nanoparticle superlattice, were shown by Luo group. The specialty of this work concentrated on the role DNA played during the formation of nanosheets, that is, DNA played as a dry ligand here which are densely packed and can adjust the inter-particle distance through the change of DNA length. No Watson Crick base pairing occurred during the drying-mediated assembly.\textsuperscript{117}

Controllable 3D DNA-AuNP assemblies were displayed by several groups. Yan group\textsuperscript{118} used four kinds of DNA tiles, including one 5-nm AuNP carrying tile, to assemble and form various patterns of AuNPs, such as stacked rings, single spiral, double spirals and nested spirals (Figure 2.4). Mirkin group and Gang group realized nanoparticle crystallization and nanoparticle superlattices through careful design of DNA. Strand displacement and difference of melting temperature of different
strands were utilized to control the status of 3D assemblies and characterizations were conducted to investigate the properties.\textsuperscript{119,120}

\textbf{Figure 2.4.} Four possible patterns of formed DNA-NP nanostructures from the assembly of DNA tiles (left panel) and the corresponding TEM images of different patterns (right panel).\textsuperscript{118} Copy 2009 AACS Press.

2.2.3 Discrete Assemblies.

Most of the various works we previously discussed, relating to 1D, 2D and 3D nanoparticle assemblies, were focused on structure play and the construction of complicated assemblies. Few applications were involved in their works due to the size of nanostructures.

Obviously, discrete assemblies, comparing with other DNA-AuNP assemblies, play a more important role in practical applications such as bio-sensing and bio-
imaging due to their easy-to-control formation process and proper size to interact with cells. More importantly, easy synthesis strategy and proper size allow them to be clearly and thoroughly characterized before further use. Localized surface plasmon resonance (LSPR) and surface enhanced raman spectroscopy (SERS) are the most frequently related properties in practical applications.

Nanoparticle dimers assembled via ssDNA hybridization are the simplest assemblies. Alivisatos group firstly presented this structure formed by two ssDNA modified AuNPs and one long auxiliary DNA strand. Later, his group conjugated AgNPs with AuNPs via DNA hybridization and several kinds of heterodimers were obtained, followed by the careful investigation of plasmon coupling properties generated due to the close proximity of two nanoparticles. Bidault group also assembled AuNP dimers linked via dsDNA. The novelty of this work was the separation method of exact one DNA strand modified AuNPs and precise control of the distance between two individual nanoparticles via different orientation of two complementary strands, perpendicular and parallel, which could result in different intensity of plasmon coupling. The DNA-AuNP dimers can also be utilized in biosensing for enzyme activity detection and DNA sensing. Taking Ginger et al’s work as an example, the AuNP dimers were conjugated via dsDNA in which a DNA loop was introduced. Target DNA strand could recognize and hybridize with this loop and lead to the full extension of DNA double strands between AuNP dimers, which would bring about an increase of distance of AuNPs and a decrease of plasmon coupling, revealed by a blue shift of scattering spectra. In addition, Gang group introduced a high-throughput method to assemble AuNP dimers via stepwise surface modification of AuNPs.
Other than nanoparticle dimers, DNA based nanoparticle assemblies of increasing complexity were explored by many groups. Alivisatos group constructed various discrete QD-AuNP nanostructures. Another work was to make use of DNA scaffolds to direct the assembly of discrete AuNPs. Mirkin group constructed AuNP assemblies based on the asymmetric functionalization of AuNPs through precise temperature control and ligase mediated elongation of DNA strands. Another pattern of assemblies, triangular and rectangular assemblies were achieved using cyclic ssDNA as template to guide the construction. Nanoparticles of different size were applied and DNA strands which were partly complementary with cyclic ssDNA were utilized to realize write/erase and structural switching based on strand displacement. Moreover, DNA origami, created from the programmable folding of one long scaffold with other short staple strands, is predesigned to possess desired shapes. DNA functionalized nanoparticles can be incorporated into this rigid scaffold through the well preserved sticky ends of particular staple strands, resulting in multiple patterns of nanostructures such as linear chains and spiral chains. Based on strand displacement reaction, Seeman et al showed a quite novel work by constructing a nanoscale assembly line via a DNA origami and elaborately designed DNA-AuNPs. The DNA walker could move along the designed route and pick up cargos nearby. This nano-device provided a new method to assemble non-covalent blocks. Fine control of orientation, spacing and placement of nanoparticles make DNA origami an optimal miniature platform for applications such as DNA genotyping and drug delivery.

Another interesting DNA-AuNP assembly is core satellite superstructures. This sophisticated structure attracted considerable attention due to its strong plasmonic coupling and plasmonic gaps which can be associated with various detection
methods. Lazarides group and Kotov group presented quite remarkable works relating to core-satellite structure. Lazarides group used hairpin DNA sequences to link core and satellite nanoparticles and the distance between them could be increased when adding DNA strands that were complementary with hairpin DNA, resulting in the change of plasmonic scattering. Kotov and coworkers constructed several types of regiospecific assemblies with Side, End and Satellite conformation utilizing the difference of reactive activity between sides and ends of gold nanorods (Figure 2.5). The assemblies, with small size and good biocompatibility, were applied in monitoring intracellular components and metabolites in living cells making use of the greatly enhanced Raman scattering.

Figure 2.5. Schematic illustration of formation of various regiospecific AuNR-AuNP assemblies (left panel) and the corresponding TEM images of different nanostructures (side assemblies, end assemblies and core-satellite assemblies). Copyright 2012 ACS Publishing Group.
2.3 Plasmonic Nanostructures for Cell Imaging and Molecular Sensing Application

2.3.1 Living Cell Imaging and Cell Targeting

Plasmonic nanostructures exhibit strong light absorption and scattering at the LSPR frequency, enabling them to be visualized under dark field microscopy. Unlike normal fluorescent dyes, plasmonic nanoparticles scatter light several orders of magnitude larger than emission from dyes due to surface plasmon enhancement, and the nanoparticles do not undergo photoblinking or photobleaching which makes them suitable to be involved in experiments with long detecting time or high excitation energy, making plasmonic imaging and sensing extensively utilized for protein/DNA sensing, intracellular tracking and cancer cell detection.

Intracellular sensing of substances in cytoplasm is facilitated by plasmonic nanostructures due to their easy tunability of plasmonic properties by nanoparticle coupling and strong absorption. Proteins, with the properties of specific recognition binding and chemical reaction catalysis by enzymes, are widely applied in construction of optical plasmonic labels with bright colors and target response. Alivisatos group reported “crown nanoparticle plasmon rulers” which are composed of 40 nm AuNPs linked by peptides with a caspase-3 cleavage site. The strong plasmon coupling generated by neighboring AuNPs produced distinct orange color, and allowed the structure to be ideal optical probes for cell imaging. After successful uptake of plasmon rulers into cells, induced cell death promoted the activity of caspase-3 and a gradual drop of scattering intensity was observed due to the continuous loss of satellite nanoparticles which was cleaved by caspase-3. The
scattering color also changed from orange to green (Figure 2.6). This work provides new thoughts about probing specific intracellular enzyme activities. DNA is another good biomolecule for intracellular detection. Mirkin group\textsuperscript{138} constructed “nano-flares” by hybridizing Cy5-carrying DNA onto DNA functionalized AuNPs whose sequence is complementary with mRNA and would release Cy5-DNA in the presence of mRNA to exhibit red light of Cy5 (Figure 2.7). The nano-flares are utilized as intracellular probes for RNA detection, quantification and siRNA knockdown experiment. Kotov group\textsuperscript{122} demonstrated core satellite AuNR-AuNP assemblies for real-time intracellular detection of structural lipids of mitochondria and other local metabolic process with enhanced Raman scattering intensity.

Figure 2.6. (a). Release of satellite nanoparticles from crown plasmon rulers in the presence of caspase-3. (b-d). Gradual changes of scattering color with the treatment of caspase-3. (e-f). Scattering color of intracellular plasmon rulers changed from orange to dim orange or green after activation of caspase-3 for 100 min.$^{137}$ Copyright 2009 Highwire Press.
Figure 2.7. (a). Scheme of “Nano-flare”. AuNPs functionalized with recognition sequences are hybridized with reporter sequences which possess a Cy5 molecule. In the presence of mRNA in cells, reporter strands would be released and fluorescence will recover. (b-c). Nano-flare intracellular test. Targeting mRNA hybridized with reporter sequence and red fluorescence recovered, whereas non-complementary strands cannot hybridize with mRNA to exhibit red fluorescence.\(^{138}\) Copyright 2007 ACS Publishing Group.

Some proteins are responsible for cell communication,\(^{139}\) cell signalling\(^{140}\) and immune response,\(^{141}\) thus playing important roles in cell type-specific recognition and binding.\(^{142}\) Plasmonic nanoparticles are prepared with proteins to serve as intense optical labels for cell discrimination based on antibody-antigen specific interaction. El-Sayed group\(^{143}\) displayed the modification of AuNRs with anti-EGFR antibody to target EGFR which are overexpressed on cancer cell surface. Thus, cancer cells can be distinguished from normal cells through dark field imaging.
Moreover, AuNRs can generate heat once they are irradiated by lasers with a range of 650-900 nm, which would further induce cell death efficiently and selectively. Reinhard and coworkers\textsuperscript{144} achieved selective and efficient cancer cell labelling using anti-EGFR antibody modified 40 nm AuNPs which show bright green color under dark field imaging. The enrichment of EGFR on filopodia of cancer cells were observed through the interaction of neighbouring nanoparticles which yield plasmonnic coupling and display distinct yellow color (Figure 2.8). DNA strands with certain sequences are also found to have high affinity towards some cancer cell biomarkers, which enable them for cell targeting and subsequent drug delivery. These strands may naturally exist or artificially screened through SELEX.\textsuperscript{46} AS1411 is a nucleolin-binding aptamer under clinical trials. Another widely-used aptamer for cell targeting is MCT (mucin targeting aptamer) which shows high affinity to mucin 1 protein, a typical tumor marker overexpressed on the surface of bladder cancer, breast cancer and other types of cancer.\textsuperscript{145} MCT is selected by Missailidis group\textsuperscript{47} through SELEX. Duan group functionalized core-shell nanogap AuNPs with MCT aptamer to specifically label MCF-7 cells. The green-yellow scattering color was observed after incubation with MCF-7 cells, while scramble DNA showed no affinity to cell surface. Raman mapping and collected Raman spectra also indicated the detecting ability of MCT modified nanogapped nanoparticles.\textsuperscript{38}
Figure 2.8. (a). Dark field imaging of cancer cells interacting with antibody capped AuNPs. The filopodia was marked by white arrows. Inset image showed the scattering spectra of AuNPs on cell surface and filopodia region. (b). SEM images of AuNPs labelled filopodia of cancer cells. Copyright 2011 ACS Publishing Group.

The plasmonic labels can monitor intracellular process to reveal the influence of particle internalization. El-Sayed group\textsuperscript{146} conjugated nuclear localizing signal (NLS) and cancer cell penetrating (RGD) peptides onto AgNPs to incubate with cancer cells. By tuning the concentration of AgNPs, programmed cell death was realized due to the cytotoxicity of AgNPs when localized in cell nucleus. Moreover, behaviors of cell communities such as attracting, clustering and killing between neighboring cells can be monitored and visualized through the nuclear targeting
AgNPs. Another work\textsuperscript{147} was presented using RGD/NLS functionalized AuNPs to investigate the effects of particle localization which can cause cytokinesis arrest, DNA damage and apoptosis. The AuNPs@RGD/NLS conjugates were also utilized to carry out real time imaging of the whole cell cycle process including G1, S, G2 and different phases of mitotic phase. The combination of real time plasmonic-enhanced Rayleigh scattering and Raman spectroscopy provides corresponding information about changes of cell structures and intracellular contents during cell cycle.\textsuperscript{148,149}

Another intracellular process, acidification which occurs in cell endosome and liposome after endocytosis\textsuperscript{150,151}, is of particular interest for plasmonic imaging and pH induced cell therapy\textsuperscript{152}. In Kim and coworkers’ work, smart molecules modified 10 nm AuNPs were obtained whose surface charge can switch from negative to positive in mild acidic environment (pH=5.5). When incubating the “smart” AuNPs with cells, the decrease of pH in cell compartment induced charge change and caused serious aggregation among nanoparticles which can be monitored through dark field imaging, showing bright orange scattering color. The strong plasmon coupling resulted from AuNP aggregation caused absorption shift to NIR region and initiated photothermal therapy for cancer cells.\textsuperscript{153} In another work presented by Duan group, PEG and pH sensitive polymer functionalized AuNPs self-assembled to form plasmonic vesicles which could be destructed by external pH change from neutral to acidic environment (pH=5~6). When internalized into cells, plasmonic vesicles would collapse into single nanoparticles or small clusters with the change of scattering color observed with dark field microscopy. The cavity circled by AuNPs was perfect for drug delivery and their work demonstrated efficient delivery of doxorubicin into targeted cells and effective cell death.\textsuperscript{152}
2.3.2 Molecular Detection with Plasmonic Nanostructures

The unique properties of plasmonic nanoparticles also inspire researchers to construct sensing devices for molecular detection by functionalizing nanoparticles with responsive nature and synthetic polymers.\textsuperscript{19,154} Plasmon coupling of adjacent nanoparticles produce evident red shift of the spectra. When the number of nanoparticles is very high in the assembled system and the distance between them is close enough, the generated plasmon coupling can even lead to the visible color change of reaction solution\textsuperscript{107} (Figure 2.1). Mirkin group\textsuperscript{23} presented us a colorimetric detection system for polynucleotides which involved two detection probes that carried two DNA sequences respectively. The detection probes can be assembled and aligned in the presence of target strand which is complementary with DNA on the probes, resulting in the color change of particle solution from red to purple. The sharp melting temperature of AuNP structures provides high degree of discrimination to DNA strands with mismatch base. Lu group assembled nanoparticles \textit{via} adenosine aptamer-contained DNA targets and the presence of adenosine would induce the structural transformation to trigger dehybridization of nanoparticles. Quantitative analysis of color change was carried out by acquiring and comparing absorption spectra, and results showed a detection range of 0.3 mM to 2 mM\textsuperscript{155} (Figure 2.9).
Figure 2.9. Schematic representation of principles of colorimetric sensors for adenosine detection. The AuNPs are respectively modified with 3’ Adap and 5’ Adap which can hybridize with the linker DNA to form aggregates. The addition of adenosine into particle system will cause the disassembly and release hybridized AuNPs.\textsuperscript{155} Copyright 2006 Nature Publishing Group.

Further development for detection was made by introducing surface enhanced Raman spectroscopy (SERS) which is widely used to amplify discrimination signals to improve sensitivity.\textsuperscript{19} Mirkin group\textsuperscript{156} functionalized AuNPs with DNA strands carrying a Raman label and a sandwich assay was designed to capture the AuNP onto a substrate \textit{via} DNA hybridization. Then, the substrate was treated by silver enhancement solution to grow AgNPs around AuNPs and Raman labels. Largely enhanced Raman signals can be obtained through this system and multiplexed detection of virus DNA fragment was realized. Duan group\textsuperscript{19} designed a novel cadmium ion-chelating polymer that can specifically recognize Cd\textsuperscript{2+} through metal-ligand interaction. They grafted this polymer onto 40 nm AuNPs along with a Raman
tag and addition of Cd\(^{2+}\) induced aggregation of nanoparticles, giving rise to strong plasmon coupling and signal enhancement.

Recently, there is a clear trend that researchers try to use DNA strands to organize nanostructures into regular oligomers by pre-designed and controllable assembly.\(^{104}\) The oligomers such as dimers or trimers, are suitable for single molecule detection by analyzing the behavior of particle system. Yeo group\(^{133}\) immobilized DNA modified core AuNPs onto a glass substrate and satellite AuNPs were assembled with core in the presence of target miRNA. The resulting nanostructures were observed under dark field microscopy and changes were displayed in the scattering spectra and light color. Careful comparison of the ratio of peak intensity of scattering spectra between 680 nm and 550 nm showed a detection range from 1 pM to 10 µM. Nam group\(^{157}\) reported a high-yield method to construct SERS-encoded Au-Ag core shell nanostructures. They firstly built heterodimers between 20 nm and 30 nm AuNPs \textit{via} DNA modification, magnetic separation and target DNA induced assembly. Raman dye was arranged in the gap of dimers. Subsequent formation of silver shell on the surface of heterodimers was realized and the thickness of silver shells was controlled by adding different amount of AgNO\(_3\). Thus, the gap size is engineerable and a proper size is critical for molecule detection. The Raman signal of this structure was detected by a nano-Raman spectroscopy system correlated with atomic force microscope (AFM). Single dimer structure was examined under this system to obtain high sensitivity of single molecule detection and reproducible results were observed among dimers (Figure 2.10).
Figure 2.10. (a). Heterodimers of AuNPs were formed with the aid of target DNA strand. Raman dye was carried by one of the AuNP at the end of DNA so that it can be placed within the nanogap. (b). The single Au-Ag core shell structure was observed under Raman spectroscopy coupled with AFM. Copyright 2009 Nature Publishing Group.

Table 2.1. Summary of applications of plasmonic nanostructures.

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### 2.4 Polydopamine

#### 2.4.1 Adhesive Mechanisms of Catechol Groups

Catechol (1,2-dihydroxybenzene), an organic compound that carries two neighbouring hydroxyl groups on its benzene ring, is found possessing remarkable chemical versatility that enables it for complex oxidation driven cross-link, coordinating with different kinds of surface via neighbouring hydroxyl groups and chemical reactivity towards nucleophilic groups when exposed to oxidative environment. Such versatility allows catechols to be widely involved in adhesive process of marine organisms, and attracts great attentions from researchers.
to exploit catechols for surface modification and coating of various materials and explore the potential chemical reactivity for surface immobilization.\textsuperscript{161}

Catechol groups can attach onto diverse organic and inorganic surfaces such as metals, metal oxide, ceramics and polymers, and this remarkable adhesive property of catechols has been investigated by intensive scientific researches to propose different mechanisms of interaction modes between catechols and the adsorbed surfaces.\textsuperscript{162,163}

**Coordination bonding.** Catechol groups can react with silica containing compound and form organosilicon salts containing hexacoordinate silicate anion or penta-coordinate silicon atoms.\textsuperscript{164} Not only silicon atoms, but also metal atoms were found to interact with catechols through coordination bonding. Gratzel group realized the surface complexation of colloidal titania nanoparticles through catechol groups and proposed that stable complex structures were achieved by replacing the hydroxyl groups on hydroxylated TiO\textsubscript{2} surface and forming coordination bond through two oxygen atoms on the benzene ring.\textsuperscript{165} The coordination bonding was also shown when catechols were chemisorbed onto the surface of aluminium oxides.\textsuperscript{166}

**Bridge bidentate bonding.** In 1996, Regazzoni et al found that catechols adsorbed onto TiO\textsubscript{2}/aqueous solution interface undergoing protonation-deprotonation reaction and TiOH\textsuperscript{1/3} was formed to facilitate the chemisorption.\textsuperscript{167} Messersmith and coworkers adsorbed DOPA-containing mPEG onto TiO\textsubscript{2} surface and proposed that DOPA anchored the surface via bridge bidentate bonding to form charge transfer complex\textsuperscript{168} (Figure 2.11). Bidentate chelation was also suggested by FT-IR data to bring about the assembly of catechol groups onto Ti surface.\textsuperscript{169}
Furthermore, by immobilizing molecules onto Ti nanotubes and performing characterization via solid state NMR, Wu group proved that catechols interacted with TiO2 onto neighbouring surface Ti site through bridged bidentate bonding instead of bidentate chelation, which was further verified by quantum calculation.\textsuperscript{170}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{binding_mechanism.png}
\caption{Figure 2.11. Schematic illustration of binding mechanism of catechol groups onto TiO\textsubscript{2} surface via bridge bidentate bonding.\textsuperscript{162} Copyright 2011 RSC Publishing Group.}
\end{figure}

**Hydrogen bonding.** Further development of adsorption mechanisms of catechols onto materials’ surface was made in 2009 that Diebold et al used various experimental and theoretical methods such as UPS, STM and DFT to explore the bonding properties of catechol groups on the rutile TiO\textsubscript{2} surface. Results showed that catechols formed a densely packed monolayer and for a single catechol molecule, one of the two OH groups on benzene ring dissociated and O atom bound to a Ti atom whereas the other OH group interacted with the neighboring catechol via hydrogen bonding. This configuration can also transform into bidentate bonding with the breakage of hydrogen bond and oxygen atom bound to Ti surface.\textsuperscript{171} Moreover, Diebold group displayed the essential role of hydrogen bonding in the initial stage of self-assembly of catechols that catechols can release or recapture the
hydrogen atom in the OH group to modulate their facile diffusion ability across the surface.\textsuperscript{172}

Except for previously mentioned mechanisms, other physical-chemical adsorption mechanisms were also brought forward such as bidentate chelating bonding, $\pi-\pi$ stacking or free radical aryl-aryl coupling and covalent bonding after oxidization.\textsuperscript{161,162,173,174} Even though final conclusion for adsorption mechanisms of catechols may not be reached in current researches, we can deduce that catechol groups can assemble via a mixture of various adsorption modes onto a variety of organic or inorganic surfaces, which would be applied for surface modification purpose.\textsuperscript{161}

2.4.2 Self-Polymerization of Dopamine into Polydopamine

Dopamine (3,4-dihydroxyphenethylamine), a widespread neurotransmitter and hormone that exists in human brain and body, plays crucial roles in sending signals among nerve cells and in motivational control process which can help people learning and gaining good things.\textsuperscript{175} It is the most widely distributed catecholic derivatives that contains catechol and amino functional groups which makes it a universal coating agent on a wide spectrum of substrates such as metallic and polymeric surfaces.\textsuperscript{176}

Apart from universal adhesive property benefitted from its catechol group, dopamine can also undergo self-polymerization at slightly basic conditions.\textsuperscript{159} Typically, under oxidative environment, the dihydroxyl groups in dopamine deprotonate, and resulting dopaminequinone forms intramolecular cycles and undergoes another deprotonation to become dopaminechrome. Subsequent
intramolecular rearrangement will lead to 5,6-dihydroxyindole, which can be further oxidized and cross-link inter-molecularly to generate a polymer, that is, polydopamine (PDA) (Figure 2.12). Further research displayed the formation of (dopamine)/5,6-dihydroxyindole trimers through non-covalent physical assembly, attributing to PDA formation by their entrapment in PDA complex. The self-polymerization property, along with universal adhesive ability of catechol groups, renders dopamine the enormous potential as coating materials on surfaces made of virtually any materials via forming robust adhesive polymer PDA. Efforts have been made to coat substrates such as coins, Au stripe film, silicon wafer and super-hydrophobic anodic aluminium oxide surface, as well as realizing PDA-based surface engineering on colloidal nanoparticles ranging from inorganic surface (iron oxide, AuNPs and carbon nanotube) to organic surface (PLGA). Ji group synthesized PDA shell coated AuNPs and demonstrated the long-term stability and low cytotoxicity of PDA capped nanoparticles. Caruso and coworkers encapsulated PDA capsules on template particles (polystyrene, SiO₂ or CaCO₃) and obtained fluorescent-PDA capsules through removal of core particles and treatment with H₂O₂ in different pH conditions. Liu and coworkers fabricated iron oxide with PDA shell and adsorbed dye-labelled ssDNA probe onto PDA surface to detect its intracellular target mRNA, followed by initiating magnetic resonance imaging and photothermal therapy due to the strong NIR absorption and high conversion efficiency of PDA.

### 2.4.3 Chemical Reactivity and Applications of Polydopamine

Polydopamine coating also renders the coated substrates versatile reactivity with a variety of molecules for further functionalization. The most typical example is that
Catechol groups can react with amino or thiol groups via Schiff-base and/or Michael addition reactions under oxidative conditions. Schiff-base is formed with the occurrence of quinone whose C=O double bond can serve as a target of nucleophilic attack by amino nitrogen to form hemiaminal, followed by dehydration process to generate C=N bond (imine) at position 1 (Figure 2.12). For Michael addition reaction, thiol or amino groups can perform nucleophilic attack to the C=C double bond and subsequent radical coupling between polydopamine groups and thiol or nitrogen radicals produces the final products. Michael addition usually happens at position 2, 3 and 4 as shown in Figure 2.12. The reactivity of PDA with thiol and amino groups enables functionalization of substrates with various polymers or biomolecules, which would broaden the application of PDA-based materials ranging from colloidal nanoparticles to electrode or glass surface. Messersmith group functionalized PDA-coated surfaces with amino functionalized methoxyl-PEG to substantially reduce non-specific protein absorption after two day continuous exposure. They also modified the substrate with thiolated hyaluronic acid (HA) and obtained cell type-specific binding property related to the surface expression status of HA receptors, which further proved the biocompatibility of PDA layer.

Another work presented by them is to deposit PDA layer onto AuNRs and immobilize anti-EGFR antibody on the particle surface via amino groups to target OSCC15 cells and subsequently kill cancer cells through photothermal treatment. Yeo group coated poly(lactic-co-glycolic acid) (PLGA) nanoparticles with PDA, and further conjugated amino-functionalized folate, cRGD and polymers onto the PDA surface. The formed nanoparticles displayed no cytotoxicity and achieved expected interactions with targeting cells.
Figure 2.12. Possible structural transformation and polymerization mechanism of dopamine, and the conjugation mechanism of amino or thiol functionalized molecules with substrate coated with polydopamine layer. In alkaline pH environment, dopamine undergoes a series of structural changes and transforms to 5,6-dihydroxyindole for further polymerization into polydopamine. The PDA layer can react at position 1 with amino groups via Schiff-base reaction, or at position 2, 3, 4 with thiol and amino groups via Michael addition reactions.\textsuperscript{159} Copyright 2007 AACS Press.

Apart from PEG or proteins, another kind of biomolecules, DNA strands, can also be conjugated onto substrates via the reaction between PDA shell and thiol/amino groups. As previously mentioned, current DNA immobilization methods, including physisorption,\textsuperscript{84} biorecognition interaction (protein-specific binding),\textsuperscript{87} or covalent bonding (Au-S chemistry and amino-carboxyl condensation),\textsuperscript{76} have obvious disadvantages such as requirement of specific functionalization groups and relatively weak bonding which can be cleaved in high temperature, thiol-containing environment or long time storage.\textsuperscript{90} Improvement of DNA conjugation methods was
made by modifying DNA strands with dithiol- or trithiol- groups, which are not only difficult to process but also not commercially available. In addition, DNA immobilization onto non-metallic material substrate or polymer surface can be more challenging. For example, in order to develop a drug delivery system by conjugating ATP-aptamer on silica nanoparticles, Özalp and Schäfer firstly functionalized the nanoparticles with (3-mercaptopropyl) triethoxysilane and attached amino-modified DNA strands with the aid of sulfo-GMBS linker. Chen group realized DNA modification onto poly(methyl methacrylate) surface by spin coating alkyne-functional diblock copolymers on the substrate to subsequently react with azido-functionalized DNA via click chemistry. These substrate-specific DNA modification methods are not widely applicable and new approaches are needed for universal substrate coating of DNA.

Considering the self-polymerization and adhesive property of PDA shell and its reactive functional groups, PDA shell could solve these problems and act as reactive agents for substrate coating and further binding of DNA molecules. The C-S bond or C-N bond formed between catechol groups and thiol/amino groups may provide potential binding strength against harsh environment comparing with traditional conjugation methods which needs to be investigated. Messersmith group had demonstrated a simple and chemically facile method for one-step coating of silicon wafer with catecholamine polymers, followed by immobilizing amino-DNA strands to prepare a DNA microarray which can hybridize with target analytes and have low detection limit. Corn and coworkers displayed a controlled electrodeposition of PDA micropatterns onto gold thin films and then functionalized the film with amino-terminated ssDNA to fabricate DNA microarrays (Figure 2.13).
microarray was further applied for nucleic acid binding and protein interactions through SPR imaging\textsuperscript{195}.

Figure 2.13. Electrodeposition of PDA micropatterns on Au film and hybridization of fluorescent ssDNA with immobilized DNA strands. ssDNA was immobilized through amino groups.\textsuperscript{194} Copyright 2013 ACS Publishing Group.

Qu group utilized dopamine as reducing agent and coating materials for graphene oxide (GO). In this work, dopamine can reduce GO into rGO and directly stabilize rGO by self-polymerization into PDA shell to coat on rGO. Then, amino-ssDNA with pH response was immobilized onto the rGO@PDA and subsequently hybridized with its complementary strand which was incorporated with RGD peptide group for cell adhesion. This system can release adhered cells by dehybridizing dsDNA through pH decrease or temperature increase since graphene and AuNR have high photothermal efficiency (Figure 2.14).\textsuperscript{196}
Overall, dopamine has exhibited outstanding properties and it would largely broaden the range of materials for successful DNA modification. However, extensive investigations on the combination of DNA and dopamine are needed to thoroughly understand the process.

**Figure 2.14.** Preparation of cell adhesion substrate. (a). Step by step fabrication of substrates using rGO@PDA(1), AuNRs (2), amino-ended C rich DNA (3), G-rich DNA with RGD groups (4), tween 20 to reduce nonspecific binding (5) and cells (6); (b). Schematic illustration of NIR and pH induced DNA release. Copyright 2013 John Wiley and Sons Group.
Chapter 3. Stimuli-Responsive Plasmonic Core-Satellite Assembly: i-Motif DNA Linker Enabled Intracellular pH Sensing

3.1 Introduction

Localized surface plasmon resonance (LSPR), originating from the collective oscillation of free conduction electrons, plays a dominant role in shaping the optical properties of metal nanostructures.\(^2\) The dependence of LSPR on the intrinsic properties (size, shape, and chemical composition) of plasmonic nanomaterials and their immediate external environment forms the fundamental basis of metal nanostructure based sensing devices.\(^{197,198}\) In particular, the characteristic spectral red-shifts of LSPR, induced by plasmon coupling of neighboring nanoparticles in close proximity, have stimulated considerable research efforts in target-specific self-assembly of plasmonic nanostructures, leading to colorimetric sensors for a wide spectrum of biological and environmental targets.\(^{26}\) While early efforts mainly afford irregular macroscopic aggregates, recent development has shown a paradigm shift toward well-defined plasmonic assemblies with molecular-like structures.\(^{78}\) The resultant discrete assemblies of plasmonic nanoparticles not only show addressable optical signatures but also offer the possibility to overcome size-limitations in biological barriers such as cellular uptake and tumor targeting when used in living systems.\(^{152}\)

A layer of “soft corona” is often necessitated to direct the self-assembly of the “hard” inorganic cores by imparting specific recognition to the nanoparticle. Functional coatings that allow for biologically triggered transition between the well-defined assemblies and their building blocks are of particular interest because the
distinct change in plasmonic properties during the transition provides a new means
to monitor dynamic molecular and cellular processes, which is traditionally attained
by fluorescent resonance energy transfer (FRET). Unlike fluorescence probes
such as small molecular dyes, fluorescent proteins, and semiconductor quantum dots,
plasmonic nanostructures exhibit photobleaching-free and consistent scattering
signal at their LSPR wavelengths that are readily detectible by dark-field
microscopy at single-particle level. In combination with controlled self-assembly,
plasmonic imaging has emerged as a powerful technique for imaging and
spectroscopic detection in living cell.

Among the diverse collections of functional coatings for surface engineering of
plasmonic nanostructures, DNA has become the top candidate because of its
programmable sequence and base-pairing property. Significant progress has been
made in developing Au-DNA hybrid nanostructures, with DNA attached on the
nanoparticles through Au-S bond or non-covalent interactions. The base-pairing-
driven assembly of Au nanoparticles (AuNPs) has been explored to design sensors
for nucleic-acid-associated targets such as single-base-pair mismatch and DNA
methylation. Recently, the discovery of DNA structures such as aptamers and
quadruplexes has greatly diversified the potential targets of interest. Consequently,
plasmonic DNA nanostructures have found widespread use in diagnostics, food
safety, and environmental screening.

Here we present the development of stimuli-responsive core-satellite assembly of
binary AuNPs bridged by i-motif DNA linkers and its use for pH sensing in living
cells, as illustrated in Figure 3.1. Acidification is associated with a number of
physiological and pathological processes, and has raised considerable research
interest in developing molecular and nanoscale sensors for pH analysis at cellular
levels. The multiple stretches of cytosine in i-motif DNA are partially protonated at acidic conditions (pH 5.0), giving rise to C:C+ base pairing that drives the single-stranded DNA (ssDNA) to fold into quadruplex. This pH-triggered conformational change of i-motif has been used to modulate assembly of AuNPs by pH changes. However, existing studies mostly led to disordered macroscopic aggregates of AuNPs, which were not possible to be deployed in living systems as miniature sensors. One key finding of the current work is that well-defined core-satellite assembly of two different sized AuNPs can be constructed by tailoring the sequence and loading of complimentary ssDNA on the two sets of AuNPs and the ratio of core and satellite AuNPs.

Figure 3.1. Schematic illustration of the formation (a) and pH-triggered intracellular disassembly of the core-satellite nanostructure (CSNS) of DNA-functionalized 50 nm core and 14 nm satellite AuNPs.
3.2 Experimental Section

3.2.1 Materials and Characterization.

5'-thiol modified DNA strands were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). The DNA sequences are listed in Table 3.1. Hydroxylamine hydrochloride, sodium citrate tribasic dehydrate (99.0%), sodium acetate, potassium chloride, Poly (ethylene glycol) (Typical Mn 10,000), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), Dimethyl sulfoxide (99.9%), Sodium hydrogen carbonate, Lithium chloride and Poly-L-lysine (0.01% solution) were purchased from Sigma-Aldrich (Missouri, USA). 10×TAE buffer pH 8.0 and agarose were purchased from 1st base (Singapore). 10× TBE buffer pH 8.3 was purchased from Promega (Madison, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). Hydrogen tetrachloroauration (III) trihydrate (HAuCl₄·3H₂O, 99.99%) was purchased from Alfa Aesar (Ward Hill, Massachusetts, USA). Hoechst 33342, DMEM (Powder, High Glucose, no sodium pyruvate, no sodium bicarbonate) and 10× phosphate buffer saline (PBS) pH 7.2 were purchased from Life technologies (Carlsbad, USA).

Transmission Electron Microscopy (TEM) images were obtained by a Jeol JEM 2010 electron microscope operating at an acceleration voltage of 300 kV. UV-vis absorption spectra were acquired on a Thermo Evolution 500 UV-visible spectrophotometer and processed using OriginLab software. Dark field images of single particles and cells and, also, scattering spectra of single particles were conducted on an Olympus IX71 inverted microscope with an oil-immersion dark field condenser, combined with Photometrics CoolSNAP-cf cooled CCD camera and a PIXIS:100B spectroscopy CCD.
Table 3.1. Sequence Information of ssDNA Used in Chapter 3.

<table>
<thead>
<tr>
<th>ssDNA</th>
<th>Sequence</th>
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<tr>
<td>SH-A</td>
<td>HS-5' -TTTTTTTTTT-GGG TTA GGG TTA GGG TTA GGG-3'</td>
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<tr>
<td>SH-B</td>
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</tr>
<tr>
<td>SH-C</td>
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</tr>
<tr>
<td>SH-D</td>
<td>HS-5' -TTTTTTTTTTT-CAA CTT GCA ACT TGA ACT TGT-3'</td>
</tr>
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</table>

3.2.2 Synthesis of Gold Nanoparticles (AuNPs).

**14 nm AuNPs (Satellite NPs).** Uniform 14 nm AuNPs, were prepared using the previously published method. Briefly, add trisodium citrate water solution (205 mg in 4 mL DI-water) into a boiling aqueous HAuCl₄ solution (60 mg in 400 mL DI-water) under vigorous stirring. The color of reaction solution changed immediately from light yellow to colorless, and subsequent color change from colorless to burgundy red was observed in 5 min. The resulting solution was cooled to room temperature after another 30 min boiling. Store the 14 nm gold nanoparticle solution at 4 °C for further use.

**50 nm AuNPs (Core NPs).** 50 nm AuNPs, core structure, were prepared following the procedure mentioned in previous literature with minor modifications. Typically, seed AuNPs with a diameter of 20 nm were prepared firstly. An aqueous trisodium citrate solution (5 mL 38.8 mM trisodium citrate solution) was rapidly injected into a boiling HAuCl₄ DI-water solution (5 mg in 50 mL DI-water) under vigorous stirring. A color change from colorless to red was observed in 5 min. Heat the solution for another 30 min and then allows the 20 nm AuNP solution to cool to room temperature before further use.

Then, this 20 nm AuNP solution was used to synthesize 50 nm AuNPs. Briefly,
50 mL DI water was added into a 100 mL round-bottom flask. 2 mL of seed AuNP solution and 200 μL of 0.2 M NH₂OH·HCl were added into this flask consecutively. Afterwards, 3 mL of 0.1 % (wt) aqueous HAuCl₄ solution was dropwise added into the solution under vigorous stirring followed by 30 min reaction at room temperature. A gradual color change from light red to dark red was observed. Finally, adjust the concentration of trisodium citrate to 1 mM using 38.8 mM trisodium citrate solution. After another two hour incubation to allow citrate ligand to further modify the surface of nanoparticles, 50 nm AuNP solution was stored at 4 °C for further use.

3.2.3 DNA Functionalization of AuNPs.

The functionalization of AuNPs was realized using thiol-modified DNA strands following the methods described in the previously published literatures.

**Activation of Disulfide Bond-Modified DNA Strands.** We dissolved disulfide bond-modified DNA (powder) in 0.5×TAE buffer (containing 20 mM Tris-Acetate, 0.5 mM EDTA, pH 8.0) with 0.3 M sodium acetate. Aqueous TCEP solution, with a molar ratio of 20:1 to DNA strands, was added into DNA solution to cleave disulfide bond to obtain the thiol-modified DNA. The incubation continued for one hour at room temperature. After cleavage, thiol-modified DNA was stored at -20 °C for further use.

**Modification of Thiolated DNA onto 50 nm AuNPs.** 50 nm AuNP solution was placed into a beaker with vigorous stirring. Appropriate amount of DNA was added into AuNP solutions carefully, followed by adjusting the concentration of PEG 10,000 to 10 % (wt). Then, LiCl solution was added dropwise into DNA solution and the salt concentration was adjusted to 100 mM. After overnight incubation, AuNPs dispersions were centrifuged and the resulting DNA functionalized 50 nm
AuNPs were stored in 4 °C for further use.

**Modification of Thiolated DNA onto 14 nm AuNPs.** Generally, 3 mL 14 nm AuNPs solution was taken out from the fridge and let the temperature raise to room temperature. Then, certain amount of DNA strands were added into the solution with gentle shaking. Let the solution incubate in the drawer at room temperature overnight. Add 30 µL 500 mM Tris-Acetate 8.5 buffer into the solution with gentle stirring, after which 300 µL 1 M NaCl was added into the solution to raise the salt concentration up to 100 mM. Incubate overnight again to complete DNA modification. Centrifuge at 14000 rcf for 15 min to remove excess DNA strands. Store the Au\textsubscript{14}@DNA at 4 °C and use them as soon as possible since long time storage causes the breakage of Au-S bond and lowers the efficiency of DNA hybridization.

**3.2.4 Self-Assembly of DNA-Functionalized AuNPs.**

Two kinds of DNA functionalized AuNPs were mixed in a certain ratio after which the salt concentration was increased to 100 mM using 1 M NaCl. The reaction solution was maintained at room temperature for 1.5 h. After this hybridization procedure, the assembled core-satellite nanostructures (CSNSs) were collected by centrifugation and redispersed in 0.5×TBE buffer (44.5 mM Tris-borate, 44.5 mM boric acid, 1 mM EDTA, 100 mM NaCl, pH=8.3) at 4 °C for further use.

**3.2.5 Characterization of Samples.**

1% agarose gel was prepared by dispersing 0.5 g agarose powder in 50 mL 0.5×TBE buffer. Microwave the powder solution for 1-3 min to let it boiling and melt agarose. Let agarose solution cool for several minutes and pour into a casting tray with the well comb. Let gel sit at room temperature for 30 minutes until gel
solution completely solidified. Processed samples were loaded in 1% agarose gels using 0.5×TBE buffer as running buffer. Gel wells were sealed with 8% agarose gels. Gels ran at 100 V, 8 V/cm for 45min, after which images were taken by G-box.

3.2.6 Cellular Studies and Cytotoxicity Analysis

Culture of Macrophages. For the culture of macrophages, culture medium we use is DMEM powder with high glucose and no addition of sodium bicarbonate and sodium pyruvate. After dissolving DMEM powder in sterilized deionized water, extra amount of sodium bicarbonate (1.5 g/L) and fetal bovine serum (FBS, 10%) was added into DMEM solution. Antibiotics were not needed to culture macrophage.

Macrophages was cultured and grew in culture dish in the way of adherent growth. Cell passage should be done when a high percentage of macrophage coverage on the culture dish was reached. Macrophage could be re-dispersed into new culture medium using a scraper to scrape off cells from culture dish after the removal of old culture medium and PBS washing. A few drops of cell solution, which was obtained after cells being centrifuged and dispersed again, were added into new culture dish with culture medium. Cells would be incubated at 37 °C with a humidified environment containing 5% CO₂.

Microscope observation of macrophage, which included dark field imaging and fluorescence imaging, required cells to be cultured on coverslips that should be cleaned, sterilized and finally treated with poly-L-lysine for 5 minutes before being used. The procedures were pretty similar with cell passage except that cells would grow on coverslips.

Cellular Experiments. For cell imaging experiment, RAW 264.7 cells were
planted and grown on poly-L-lysine modified glass coverslips and incubated for one day. CSNSs or 50 nm AuNP@DNA in 1 mL PBS with the concentration of 10 pM was incubated with cells for 15 min. Then, cells were washed three times with PBS to remove free AuNP assemblies. A continuous incubation of cells with fresh medium was carried out. Cells were maintained in fresh medium before microscopy observation at certain time intervals. Hoechst 33342 was utilized to stain cell nuclei and localize nanoparticles. Control experiments at 4 °C were also conducted with cells on glass coverslips. The same concentration (10 pM) of CSNSs were used and the incubation of assemblies with cells was performed at 4 °C for 1 h. Hoechst 33342 is one kind of dye that carries positive charge and can enter cells through cell membrane to bind with double-strand DNA. It can emit blue fluorescence when excited by light at wavelength of 350 nm.

**Cytotoxicity Analysis.** A standard Cell Counting Kit-8 (CCK-8) was utilized to analyze the cytotoxicity of core-satellite assemblies following a general protocol. Briefly, RAW 264.7 cells were seeded in a 96-well plate with the concentration of 10000 cells/well. After a 24 h incubation in the cell incubator at 37 °C, CSNSs with final concentrations of 5 pM, 10 pM, 20 pM and 40 pM were incubated with cells for 30 min, 60 min, 120 min, 180 min and 240 min, respectively, after which 10 μl of CCK-8 solution was added to each well of the 96-well plate to incubate for another 4 h. The amount of an orange formazan dye, produced by the reduction of WST-8 (active gradient in CCK-8) by dehydrogenases in living cells, is directly proportional to quantity of living cells in the well. Therefore, by measuring the absorbance of cell solution in each well at 450 nm using a microplate reader, cell viability could be determined with the calculation of the ratio of absorbance of experimental well to that of the cell control well. All experiments were triplicated.
and results were averaged.

3.3 Results and Discussion

3.3.1 Self-Assembled pH-Responsive Plasmonic CSNSs

The “satellite” AuNPs of 14 nm with i-motif ssDNA attached through the Au-S bond were assembled with the 50 nm “core” AuNPs carrying the guanine (G)-rich ssDNA (the complementary strand of i-motif ssDNA) (Figure 3.1a) to form the core satellite nanostructures (CSNSs). Figure 3.2a shows that LSPR of the assembly formed at a feeding core-satellite ratio of 1:200 exhibited a red-shift of 14 nm relative to that of the core AuNPs (533 nm). While spectral properties of the CSNSs showed long-term stability at pH 8.0, the red-shift disappeared when the assembled structures were dispersed in pH 5.0 buffer, with a sharp blue-shift in the pH range of 5.0-6.0 (Figure 3.2a). Apparently, the complementary recognition of the ssDNAs and pH sensitive folding of i-motif DNA into closed quadruplex were retained when grafted onto AuNPs. The conformational change of i-motif detaches the “satellite” AuNPs from the “core” AuNPs, leading to reduced interparticle plasmonic coupling, corresponding to blue shift of LSPR peaks. In contrast, assembled structures directed by control DNA strands lack of i-motif sequence did not show the pH sensitivity (Figure 3.2b). TEM observation clearly displayed well defined CSNSs with an average number of ~40 satellites per 50 nm core nanoparticles (Figure 3.2c). We also used gel electrophoresis to investigate the assembly and disassembly of our CSNSs in buffer solution with different pH values (Figure 3.2d). The CSNSs of larger sizes migrate slower than the building blocks in the gel. And the i-motif CSNSs treated with pH 5.0 buffer clearly gave rise to two bands corresponding to the “core” and
“satellite” AuNPs. Consistent with the UV-vis results, the CSNSs built upon the control pH-insensitive ssDNA did not show changes at acidic pH.

Figure 3.2. (a) UV-vis spectra of DNA functionalized AuNPs and CSNSs: 14 nm satellite AuNPs (black line), 50 nm core AuNPs (red line), CSNSs at pH 8.3 (blue line) and disassembled CSNSs at pH 5.0 (green line). (b) UV-vis spectra of CSNSs formed through the hybridization of pH-insensitive control DNA strands: at pH 8.3 (black line) and at pH 5.0 (red line). (c) TEM image of the CSNSs. (d) Electrophoretic analysis of AuNPs and CSNSs. Lane 1: control sample at pH 5.0; Lane 2: control sample at pH 8.3. Lane 3: i-motif CSNSs at pH 5.0. Lane 4: i-motif CSNSs at pH 8.3. Lane 5: Au50@SH-A. Lane 6: Au14@SH-B.

We have found that the formation of well-defined CSNSs is highly dependent on the relative ratio of the core and satellite AuNPs. At a low ratio of 1:20, the LSPR red-shifted to 589 nm, suggesting a stronger plasmonic coupling. Performing
hybridization with a series of ratios up to 1:300 resulted in a gradual shift of the LSPR peak to shorter wavelengths, which became stable at a ratio of 1:200 (Figure 3.3). Obviously, at the low ratios, the limited number of “satellite” AuNPs has a higher possibility to interact with multiple cores to afford large aggregates. Correspondingly, higher ratios are favourable for the formation of well-defined CSNSs, because the crowding satellite particles would have little chances to be linked to the same “core” AuNPs. It should be noticed that hybridization time would also influence the fabrication of the CSNS nanostructures as well as ratios of two building blocks. Thus, the kinetic experiment was carried out to determine the optimal reaction time for hybridization. The optical signal response was recorded and the results showed that LSPR wavelength red shifted gradually with time went through, indicating successive linkage of “satellite” nanoparticles onto “core” nanoparticles (Figure 3.3b). The LSPR peak reached a plateau after 90-min hybridization, suggesting the completion of nanoparticle assembly. Therefore, 90 min would be sufficient for formation of CSNSs.
Figure 3.3. (a) UV-vis spectra of assemblies of different ratios of Au$_{50}$@SH-A to Au$_{14}$@SH-B. Inset: enlargement of the dashed region. (b) UV-vis spectra of time dependent hybridization of the Au$_{50}$@SH-A and Au$_{14}$@SH-B. A continuous red shift of hybridization solutions was observed within 90 min. Inset: peak wavelength of different time.

Since the G-rich ssDNA on the 50 nm “core” tends to form G-quadruplex in the presence of potassium ions (K$^+$), which are abundant in an intracellular environment, we also examined the stability of the i-motif CSNSs against K$^+$ ions. The LSPR of the i-motif CSNSs did not show any response to K$^+$ up to 110 mM (Figure 3.4). We reason that large ratio of i-motif DNA strand to the G-rich ssDNA resulting from the
core-satellite structures contributes to the stability observed here, and thermodynamics stability of fully complementary DNA duplex is higher than that of K\(^+\) ions induced G-quadruplex. This is supported by the fact that loading i-motif ssDNA on the core and G-rich ssDNA on the satellite nanoparticles gave rise to CSNSs sensitive to both pH and K\(^+\). The newly formed CSNSs exhibited a 4 nm blue shift when exposed to 20 mM K\(^+\) ions for 2 hours (Figure 3.5).

![UV-vis spectra](image)

**Figure 3.4.** UV-vis spectra of core-satellite nanostructures in the presence of different concentrations of K\(^+\). No obvious changes were observed except for dilution induced decrease of absorption intensity.
3.3.2 Single Particle Monitoring of pH-Responsive CSNSs.

The well-defined CSNSs offer the possibility to monitor their pH-triggered disassembly at the single-particle level. The 50 nm “core” AuNPs exhibit distinct green scattering light. And the 14 nm “satellite” AuNPs have negligible scattering and therefore are invisible under a dark-field microscope. As shown in Figure 3.6a, the attachment of multiple 14 nm “satellite” AuNPs on the 50 nm “core” AuNP led to CSNSs with bright yellow scattering light, resulting from the red-shift of scattering light (Figure 3.6c) due to interparticle coupling. Consistent with the bulk assay detected by UV-vis spectra, the color of the i-motif CSNSs turned into green and scattering spectra showed a blue-shift of 17 nm, confirming the possibility to use the CSNSs as miniature pH sensors. The CSNSs assembled by control DNA sequences showed similar yellow scattering color, but no color change of scattering light was observed when tuning pH of the solution to 5.0 (Figure 3.6b). Similarly, the scattering spectrum of representative control CSNSs showed hardly any
difference at pH 8.3 and pH 5.0 (Figure 3.6d).

**Figure 3.6.** (a-b). Dark field images of i-motif (a) and control CSNSs (b). Left: i-motif CSNSs. Right: control CSNSs. Upper: pH 8.3. Lower: pH 5.0. (c-d). Scattering spectral images of i-motif CSNSs (c) and control CSNSs (d). Left: i-motif CSNSs. Right: control sample. Au$_{50}$@SH-A and Au$_{50}$@SH-C (black line), CSNS (red line) and pH 5.0 buffer treated samples (blue line).

### 3.3.3 Intracellular Disassembly of Plasmonic CSNSs

We next investigated the use of i-motif CSNSs to detect the pH variation in living cells once they are uptaken through endocytosis process. The compact size of the CSNSs of 90-100 nm allows for efficient uptake by macrophage cells (RAW 264.7 cells) upon the incubation of 15 min (Figure 3.7a). Within 30 min, the yellow scattering signal from CSNSs in the cells became mostly green, suggesting the
disassembly of CSNSs into elementary units inside acidic intracellular compartments. It is well-known that pH in the endocytic pathway drops from 5.9-6.2 in early endosomes to 4.7-5.5 in late endosome/lysosomes, which agrees well with our imaging results. Plasmonic imaging also suggests that the DNA-functionalized AuNPs maintained excellent colloidal stability in the acid organelles. This is critical for the plasmonic imaging based on the CSNS assembly, because aggregation of AuNPs would lead to red-shifts of the scattering light and generate false imaging signals. In clear contrast, pH-insensitive control CSNSs showed no obvious color change in the same time scale (Figure 3.7c-3.7d), although they can also be efficiently uptaken by the cell. Cytotoxicity tests show that the cells treated with CSNSs are well-spread with viabilities of more than 90% (Figure 3.8). To confirm the mechanism of cellular entry of the CSNSs, the nanostructures were incubated with macrophages at 4 °C, under which the rigid cell membrane can block the non-specific endocytosis uptake of CSNSs by macrophage cells. Consistently, dark field image showed greatly reduced cellular uptake of CSNSs at 4 °C (Figure 3.9).
Figure 3.7. Dark field images of cells incubated with i-motif (a, b) and control (c, d) CSNSs after 15 min incubation (a, c) and 30 min post-incubation (b, d). Cell nuclei were stained with blue fluorescent Hoechst 33342.

Figure 3.8. Cytotoxicity test of nanoparticle assemblies with the concentration of 5 pM (black), 10 pM (red), 20 pM (blue) and 40 pM (magenta) in 30 min, 60 min, 120 min, 180 min and 240 min.
3.4 Conclusion

In summary, we reported a new class of well-defined core-satellite plasmonic assembly of binary AuNPs, with pH-responsive disassembly enabled by the i-motif DNA linker. Our results have shown that the i-motif CSNSs undergo endocytosis when cultured with macrophage cells and allow for plasmonic imaging of pH changes in the endocytic pathway in living cells. This concept of constructing CSNSs and the expanded library of stimuli-responsive DNA provides new opportunities to develop miniature plasmonic sensors for a diverse range of targets. The introduction of targeting ligands to recognize specific cell types, in combination with techniques for intracellular delivery of the plasmonic assembly, would further broaden their use for intracellular detection.
3.5 Declaration

Part of the work presented in this chapter has been published in the *Chemical Communications*. Reproduced by permission of The Royal Society of Chemistry.

Chapter 4. Simple and Label-Free Plasmonic Sensing of DNA and Single Nucleotide Polymorphism using Binary Nanoprobes

4.1 Introduction

Sequence-specific nucleic acid detection is of irreplaceable importance in disease diagnostics,\textsuperscript{156} food contamination control,\textsuperscript{206} human genome screening\textsuperscript{207} and intracellular mRNA analysis.\textsuperscript{138} In particular, single nucleotide polymorphism (SNP), genetic variation of a single nucleotide occurred in DNA sequence, has attracted considerable attentions since it is associated with genetic disease,\textsuperscript{208} pathogens and oncogene,\textsuperscript{209} thus making it necessary for DNA biosensors to possess high specificity for SNP discrimination.\textsuperscript{210} General methods for DNA and SNP detection are based on the signal response arising from hybridization or other recognition reaction with complementary strands and protein enzymes.\textsuperscript{210,211} The signals are traditionally transduced through fluorescence,\textsuperscript{212} electrochemical\textsuperscript{213} and colorimetric assays,\textsuperscript{214} which confront limitations such as photobleaching, complex operations and poor reproducibility.\textsuperscript{211}

To address these challenges, LSPR-based biosensors are designed due to the photostability of plasmonic nanoparticles and their intense interaction with incident light to serve as label-free optical labels.\textsuperscript{1,2,18} Through functionalization with target-responsive smart surface coatings molecules, LSPR-based sensors for various targets can be obtained.\textsuperscript{136} Mirkin and coworkers pioneered the development of colorimetric plasmonic sensors for SNP based on DNA-conjugated Au nanoparticles, which gave rise to considerable spectral red-shifts because of interparticle coupling upon sequence specific DNA hybridization.\textsuperscript{107} Among various kinds of plasmonic
nanoparticles, gold nanorods (AuNRs) have attracted plenty of interest due to the unique optical properties that the anisotropic shape of AuNRs affords two absorption peaks in response to the collective electron oscillation along transverse and longitudinal directions. Specifically, the longitudinal band of AuNRs shows particular sensitivity towards slight changes in surrounding environment resulted from surface functionalization or close proximity of other plasmonic nanoparticles, rendering them great potential for widespread applications such as the detection of environmental toxins, nucleic acid and proteins.

In this work, we present a simple AuNR-based optical sensing system for DNA and SNP detection based on a sandwich DNA hybridization platform as illustrated in Figure 4.1. The target sequence is chosen from gene fragment of human immunodeficiency virus (HIV) with 33 nucleotides. The complementary strand of the target DNA was split into two fragments which are further functionalized with one thiol group to be modified onto AuNRs and 14 nm AuNPs, respectively (Figure 4.1a). Assembly conditions should be optimized since hybridization time and ratios of two building blocks are key factors to complete the reaction and acquire optimal numbers of satellites for best sensing effects. Two hybridization strategies are utilized for DNA detection. For one-step method, DNA functionalized AuNRs and AuNPs are mixed together and the addition of target DNA would induce the assembly of two nanoprobes, leading to a characteristic red shift of longitudinal peak which can be continuously monitored and correlated with DNA concentration (Figure 4.1b). SNP discrimination was performed with target sequence which has a single-base mutation from G to C (Table 4.1). Two-step detection method includes consecutive hybridization of AuNR@DNA with target sequence and Au_{14}@DNA (Figure 4.1c-d). Different concentrations of target sequence would lead to distinct
amount of AuNPs to attach onto AuNR and UV-vis absorption information was obtained to analyze correlation relationships. One-step strategy displayed an exponential correlation between longitudinal band shift and DNA concentration while two-step strategy displayed a linear relationship. A lower limit of detection (LOD) of 16 pM and more precise variation at low concentrations can be reached by one-step strategy, whereas two-step method exhibits a broader detection range and behaves better at high concentration of target DNA. The DNA detection strategy demonstrated in this work should be an effective biosensor platform which shows comparable sensitivity and specificity for DNA detection and SNP analysis.

**Figure 4.1.** Schematic illustration of DNA detection through one-step hybridization and two step hybridization. (a). DNA functionalization of AuNRs; (b). One–step hybridization by incubating AuNR@DNA with Au_{14}@DNA in the presence of target DNA; (c-d). Two-step hybridization process by successive incubation of target DNA and Au_{14}@DNA with AuNR@DNA.
4.2 Experimental Section

4.2.1 Materials and Characterization.

5'-disulfide bond modified DNA strands were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). The DNA sequences are listed in Table 4.1. Silver nitrate, sodium citrate tribasic dehydrate (99.0%), Tris (2-Carboxyehtyl) phosphine (TCEP), L-ascorbic acid (LAA), polyvinylpyrrolidone (PVP) and sodium borohydride were purchased from Sigma-Aldrich (Missouri, USA). 10× TAE buffer (pH=8.0) and agarose were purchased from 1st base (Singapore). 10× TBE buffer (pH=8.3) was purchased from Promega (Madison, USA). All other chemicals were purchased from Sigma-Aldrich unless further noted.

TEM images were obtained by a Jeol JEM 2010 electron microscope operating at an acceleration voltage of 300 kV. UV-vis absorption spectra were obtained on a Shimadzu UV-1800 UV spectrophotometer and processed using OriginLab software. Fluorescence spectra was acquired by JOBIN YVON, Horriba Fluoro-Max-3 spectrometer. Zeta potential was obtained by Zeta potential ZetaPALS potential analyzer from Brookhaven Instruments Corporation.

Table 4.1. Sequence Information of ssDNA Used in Chapter 4.

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<th>Sequence</th>
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<tr>
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<td>DNA4</td>
<td>5’-AGAAGATATTTTGGGAATAAACATGACCTGGATGCA-3’</td>
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<tr>
<td>DNA5</td>
<td>5’-TTCGCTGGGCTAACTGGGCGCAGAGCTGGAGGGGC-3’</td>
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</tbody>
</table>

72
DNA1 and DNA2 are modified with thiol bond at 5' and 3' end, respectively. Their sequences are complementary with DNA3. DNA3 is a fragment of the genome of human immunodeficiency virus (HIV) selected from gene bank (AF405180.1). The 33-nucleotide sequence is statistically unique. DNA4 is the DNA strand with 1-bp mismatch (C-C) at the underlined position. DNA5 is the control strand which is not complementary with DNA1 and DNA2.

4.2.2 Synthesis of CTAB-Stabilized Gold Nanorods (AuNRs)

AuNRs were prepared based on a seed-mediated growth method described in a previous literature with small changes.\textsuperscript{218} Briefly, seed solution was prepared first. An aqueous solution of HAuCl\textsubscript{4} (0.2 mg/mL, 5 mL) was added into 5 mL CTAB DI-water solution (0.2 M) at 30 °C with vigorous stirring. The solution was dark yellow. Then, freshly prepared NaBH\textsubscript{4} solution (0.4 mg/mL, 0.6 mL) was injected into reaction solution. A color change from dark yellow to light brown was observed when the NaBH\textsubscript{4} solution was injected rapidly. Slowly stir the solution and age at 30 °C for 8 min before further use.

After seed preparation, the growth solution of AuNRs was prepared by consecutively adding HAuCl\textsubscript{4} (0.4 mg/mL, 120 mL) and AgNO\textsubscript{3} (0.68 mg/mL, 4.81 mL) into aqueous CTAB solution (0.2 M, 120 mL) at 30 °C. Afterwards, freshly prepared L-ascorbic acid (14 mg/mL, 1.643 mL) was added into the growth solution quickly with vigorous stirring. The color of the solution changed from dark yellow to colorless rapidly and previously-prepared seed solution (0.288 mL) was spontaneously added into the colorless solution. Stir the solution for another 2 hours at 30 °C to complete the growth of AuNRs. After synthesis, AuNRs solution was
centrifuged at 9500 rpm for 30 min to remove CTAB and other reactants. Redisperse the precipitate in DI-water. Repeat the centrifuge procedure one more time. UV-vis spectra data was obtained to determine the concentration of AuNR. The AuNR solution was stored at room temperature for further use.

4.2.3 Preparation of DNA Functionalized AuNRs.

Preparation of PVP and SDS Stabilized AuNRs. AuNR solution was diluted in a buffer solution which contains 10 mM phosphate buffer (PB) (pH=8.0) and 0.3% SDS. Centrifuge at 6000 rpm for 15 min. Remove the supernatant and disperse the precipitate in buffer solution to centrifuge again. The whole process was repeated three times. Finally, disperse the AuNRs precipitate in 5 mL buffer (10 mM PB 8.0 and 0.3% SDS) and further add 5 mL solution of 10% PVP (dissolved in ethanol) into the AuNR solution. Mix the solution and incubate at 40 °C with gentle stirring for 18 h. After incubation, AuNRs capped with PVP and SDS (denoted as AuNR@SDS-PVP) were centrifuged down at 6000 rpm for 15 min. Repeat the centrifuge process for three times and disperse AuNRs in buffer solution (PB 8.0+SDS) for further use.

DNA Functionalization of AuNR@SDS-PVP. In order to modify thiol-end DNA strands onto AuNR@SDS-PVP, salt aging buffer solution was prepared containing 10 mM PB 8.0, 300 mM NaCl, 4 mM MgCl₂ and 0.3% SDS.

Disperse AuNR@SDS-PVP in buffer solution (10 mM PB 8.0 and 0.3% SDS) to reach a concentration of 0.23 nM. Then, add certain amount of thiol-ended ssDNA into the 400 µL 0.23 nM AuNR solution. Sonicate for 5 seconds and incubate the solution in a drawer for 16 h. Then, treat the AuNR solution with a salt aging process. Generally, add 40 µL salting solution into AuNR solution and sonicate for 5 seconds
and let the solution sit for 30 min at room temperature. Repeat the salting process for 10 times to reach a final concentration of 10 mM PB 8.0, 150 mM NaCl, 2 mM MgCl$_2$ and 0.3% SDS of the whole solution. After salting, incubate AuNR and ssDNA solution in the drawer for another 16 h to finish DNA conjugation. Centrifuge for 3 times to remove excess ssDNA strands in the supernatant. The resulting AuNR@DNA was dispersed in DI-water and stored in 4 °C for further use.

**Optimization of Reaction Ratio.** 1% agarose gel was prepared by dispersing 0.5 g agarose powder in 50 mL 0.5×TBE 8.5 buffer. Dissolve agarose using microwave oven and pour the solution in the tray to make solidified gel. Samples (AuNR@CTAB, AuNR@SDS-PVP and AuNR@DNA with different reaction ratios of DNA) were loaded in the well to run at 100 V, 8 V/cm for 20 min. Take the image using G-box imaging system.

**Determination of DNA Amount on AuNRs.** Thiol-ended ssDNA with Cy5 molecule at the other end was conjugated onto AuNRs using the previous method and the same ssDNA was dispersed in buffer solution to go through all modification procedures as control samples. After reaction, centrifuge and compare the fluorescence spectra intensity to determine the number of DNA strands on AuNRs.

### 4.2.4 Hybridization of AuNR@DNA and Au@DNA

The concentration of AuNR@DNA and Au$_{14}$@DNA was obtained through calculation based on the readout of UV-vis spectra. Two hybridization strategies were applied.

**One Step Hybridization.** Mix AuNR@DNA with Au$_{14}$@DNA in a certain ratio, and add target DNA strands into the solution to initiate assembly. Increase NaCl concentration up to 100 mM to improve the hybridization efficiency. Hybridization
solution was placed at room temperature for two hours before we can characterize the assembly.

**Two Step Hybridization.** Mix AuNR@DNA with target DNA strands and increase salt concentration to 100 mM to let the solution stay at room temperature for 2 h. Centrifuge down AuNR@DNA-target DNA. Then, Au$_{14}$@DNA was added in a certain ratio. React completely and characterize the assembly. Keep the salt concentration at 100 mM at all times.

### 4.3 Results and Discussion

#### 4.3.1 DNA Functionalization of AuNRs.

Standard AuNRs, with the aspect ratio of 2.8, were obtained through seed-mediated growth method. TEM image shows the formation of 54 nm (length) × 19 nm (diameter) AuNRs (Figure 4.2a). After synthesis, CTAB capped AuNRs were functionalized with thiol-ended ssDNA with the aid of SDS and PVP, and the process was monitored via the change of LSPR peak and surface potential. While the LSPR of AuNR@CTAB showed two peaks at 512 nm and 709 nm corresponding to electron oscillation along transverse and longitudinal directions, the replacement of CTAB with SDS and PVP resulted in blue shifts of peaks to 510 nm and 684.5 nm (Figure 4.2b) since replacement of capping ligands decreased shell thickness and increased surface permeability as the CTAB bilayer around AuNRs was damaged. Subsequent modification of AuNR with ssDNA led to a red shift of LSPR to 511 nm and 690 nm since DNA layer increased the shell thickness and further changed the dielectric environment of nanoparticles. Considering the properties of different surfactants on AuNRs, zeta potential was performed to monitor surface modification
process. AuNR@CTAB possessed a positive charge of +26.8 mV, and the replacement of CTAB with SDS and PVP changed the surface potential of AuNRs to –12.4 mV, which further decreased to -23.2 mV because of the increase of charge density after DNA modification (Figure 4.2c). We also carried out gel electrophoresis to determine the optimal DNA amount to react with AuNRs. AuNR@CTAB stayed in the gel well (Figure 4.2d, lane 1), indicating the aggregation of colloidal particles in the presence of gel buffer solution. Functionalization with SDS/PVP and ssDNA endowed colloidal stability to the AuNRs and all the bands migrated into gels towards anode due to their negative charge. With the increase of ratios of DNA to AuNRs, the bands moved faster and maintained at the same position from the ratio of 2000:1 (Figure 4.2d), representing that the density of ssDNA stayed unchanged and ratio of 2000 is sufficient for successful conjugation of AuNRs. The quantity of ssDNA was measured using a Cy5 modified SH-DNA strand and a 13% fluorescence loss indicated that an average of ~260 strands were conjugated onto every AuNR.
Figure 4.2. Synthesis and characterization of AuNRs with DNA modification. (a). TEM images of AuNR@CTAB. (b). UV-vis absorption spectra of AuNR@CTAB (black), AuNR@SDS-PVP (red) and AuNR@DNA (blue). (c). Zeta potential of AuNR@CTAB, AuNR@SDS-PVP and AuNR@DNA. (d). Electrophoretic analysis of AuNR and AuNR@DNA. Red arrow denotes the flow direction. Lane 1: AuNR@CTAB; Lane 2: AuNR@SDS-PVP; Lane 3: AuNR with DNA 1:50; Lane 4: 1:100; Lane 5: 1:200; Lane 6: 1:400; Lane 7: 1:1000; Lane 8: 1:2000; Lane 9: 1:4000.

4.3.2 Target DNA Mediated Assemblies of AuNRs and AuNPs (NR-NP).

AuNRs, functionalized with DNA1 (denoted as AuNR@DNA1), was assembled with 14 nm AuNPs which was modified by DNA2 (Au14@DNA2) with the aid of target sequence DNA3. Prior to utilizing the two nanoprobes for DNA3 detection, the hybridization time and ratio of AuNRs to AuNPs need to be optimized in order
to control the detection process and allow sufficient time for reaction to acquire maximum signal discrimination. We firstly incubated AuNR@DNA1 with DNA3 in a ratio of 1:300 to achieve full coverage of target sequence to hybridize with Au14@DNA2. The samples were obtained after overnight reaction and centrifugation, and further dispersed in 100 mM NaCl for examination. Figure 4.3a displays UV spectra of the obtained assemblies from different ratios of core and satellite nanoparticles. When reacting at a high AuNR@DNA1-DNA3 to Au14@DNA2 ratio of 1:1, the spectra of NR-NP assemblies displayed that the longitudinal plasmon band red shifted from 690 nm to 752 nm and strong absorption occurred in the near infrared region of 750 nm to 1100 nm, indicating strong plasmon coupling between the building blocks. Owing to the small amount of Au14@DNA2, different AuNRs are brought in close proximity by cross-linking with AuNP, forming macroscopic aggregates. Thus, Au14@DNA2 with a large number is preferable to form controllable discrete assemblies. Consistently, gradual decreasing of reaction ratios by adding more Au14@DNA2 led to the continuous blue shift of peak wavelength which stabilized at 721 nm (Figure 4.3a). Therefore, we would choose 1:20 as an optimal ratio for subsequent applications.

It is also necessary to determine the ideal time for complete reaction since enough time is needed for the “building blocks” to hybridize together and redundant time can be deducted to accelerate DNA detection. A rapid red shift of 15 nm from 690 nm to 705 nm was observed after mixing the precursors together for 5 min, and the peak gradually red shifted to 720.5 nm after monitoring the reaction for three more hours (Figure 4.3b), indicating the successive hybridization of satellite particles with core NRs. Based on the results, two hours are sufficient for formation of complete superstructures. Interestingly, the rapid hybridization of two building blocks within
the initial five minutes offers the possibility for developing rapid response sensors.

Figure 4.3c showed the UV-vis spectra of hybridization samples guided by target sequence and control sequence under optimized conditions. A 30.5-nm red shift of the longitudinal band is observed from 690 nm to 720.5 nm, with a simultaneous red shift of transverse band from 511 nm to 523 nm, whereas the control samples with noncomplementary strands did not show any changes. TEM images clearly showed the formation of core-satellite NR-NP assemblies with uniform morphology (Figure 4.3d). A total of 12-14 satellite nanoparticles can be conjugated with single AuNR based on a crude counting. The successful realization of NR-NP assemblies and dramatic changes of optical properties suggests that we can utilize this binary system for DNA detection.

**Figure 4.3.** (a). UV-vis absorption spectra of nanoparticle assemblies formed by different ratios of AuNR@DNA1-target to Au_{14}@DNA2; (b). UV-vis spectra of core
satellite assemblies at different hybridization times. Longitudinal LSPR peak of assemblies showed a continuous red shift within two hours; (c). UV-vis spectra of AuNR@DNA1 (blue line), assemblies mediated by target sequence DNA3 (black line) and control sequence DNA5 (red line); (d). TEM images of AuNR-14Au assemblies. Inset image is a typical core-satellite assembly. Scale bar=20 nm.

4.3.3 One Step DNA Detection and SNP

To evaluate the performance of the binary nanoprobes, 10 µL core AuNR@DNA1 was incubated with 10 µL of Au14@DNA2 in a ratio of 1:20 in hybridization buffer (100 mM NaCl, 10 mM phosphate buffer pH 7.0). 0.5 µL of target DNA3 with different concentrations was added into the reaction solution to reach a final overall concentration of 0.016, 0.04, 0.08, 0.16, 0.4, 0.8, 1.6 and 4 nM. A subsequent 2-hour incubation was conducted to allow the assembly of nanoprobes, and the samples were examined using UV-vis spectra. The spectra were normalized for better comparison. The shift of longitudinal peaks of hybridization samples was analyzed to monitor the assembly status of AuNRs with AuNPs since longitudinal peak went through a total 30.5 nm red shift after hybridization due to its sensitive response to the changes of interparticle distance. Furthermore, longitudinal peak is only influenced by plasmon coupling which is associated with the number of 14 nm AuNPs assembled on the core and 14 nm AuNPs barely have UV-vis absorption in the range from 600 to 800 nm. As shown in Figure 4.4a, the increase of target DNA concentration led to a gradual red shift of longitudinal plasmon peak since more assembled satellites induced stronger plasmon coupling. The plot curve between wavelength shift of LSPR peak and DNA concentrations showed a good exponential
correlation with $R^2$ of 0.9902. Thus, the effective detection range is 16 pM to 4 nM. Notably, the limit of detection (LOD) is calculated to be 16 pM by inducing a distinguishable red shift of 1.1 nm (Figure 4.4b).

To explore the ability of this one-step hybridization system for SNP discrimination, LSPR shift of longitudinal peak was compared in the presence of 4 nM target strand DNA3, 4 nM mismatch DNA4 which carries a single base mutation from G to C and 4 nM control DNA5 which is not complementary with two probe sequences. The absorption spectra of three samples showed different peak shifts of 30 nm, 10 nm and 0.1 nm comparing with AuNR@DNA1 (Figure 4.5), demonstrating the high specificity of this detection method to identify single base mismatch DNA strand and random sequence from fully complementary strand.
Figure 4.4. (a). UV-vis absorption spectra of one step detection solutions with DNA concentration in the range of 0.016 nM to 4 nM. The figure displayed enlarged area of longitudinal peaks; (b). The relationship between the concentration of DNA and shift of peak wavelength. Inset image is enlarged region within the red frame.

![Figure 4.4](image)

Figure 4.5. (a). UV-vis spectra of hybridization solutions in the presence of 4 nM DNA5 (black line), 4 nM DNA4 (blue line) and 4 nm DNA3 (red line); (b). Bar chart of red shift of longitudinal LSPR peaks comparing with AuNR@DNA1.

![Figure 4.5](image)

4.3.4 Two Step DNA Detection and TEM Characterization

We also carried out two-step hybridization strategy for DNA detection initiated by incubating 10 µL of AuNR@DNA1 with 0.5 µL DNA3 in different concentrations from 48 pM to 24 nM. Perform centrifugation to obtain target DNA bearing AuNR and assemble with Au14@DNA2 with optimized conditions. The UV-vis spectra also showed a red shift of plasmon band with increased DNA concentration (Figure 4.6a) and a linear relationship with correlation R² of 0.989 between DNA concentration and LSPR shift (Figure 4.6b), which is not the same with one-step detection method. The different hybridization kinetics may result from
different behaviors of target strand that linker DNA in one-step system has a slightly higher possibility to link one satellite NPs with multiple NRs to give rise to stronger plasmon coupling at low concentration. This factor, along with the possible loss during centrifugation process, led to a higher LOD at 48 pM. Moreover, two-step detection method had worse discrimination ability at low DNA concentration with a red shift of 0.66 nm at 240 pM DNA3 (Inset image in Figure 4.6b).

However, two step method displayed controllable process to produce assemblies with different numbers of satellites with varying DNA concentration. We select several samples with different DNA concentrations to acquire TEM images. In the presence of 1.2 nM target DNA3, one to two satellite nanoparticles were attached onto AuNRs and the binding position showed no specific preference (Figure 4.7b). With the increase of DNA concentration, more Au14@DNA2 were assembled to the surface of AuNRs (Figure 4.7c), and at 24 nM DNA3, complete and uniform superstructures were formed (Figure 4.7d).
Figure 4.6. (a). UV-vis absorption spectra of two step-detection solutions with DNA concentration in the range of 0.048 nM to 24 nM. The figure displayed enlarged area of longitudinal peaks; (b). The linear relationship between the concentration of DNA and shift of peak wavelength. Inset image is enlarged region within the red frame.
Figure 4.7. (a). UV-vis spectra of assembled samples (b-d). TEM images of assemblies of AuNR@DNA1 and Au_{14}@DNA2 corresponding to different DNA concentrations 1.2 nM (b), 4.8 nM (c) and 24 nM (d).

4.4 Conclusion

In summary, we have reported a simple DNA detection and SNP identification approach based on target-induced assembly of binary probes. Our results showed that longitudinal plasmon band underwent dramatic red shift in the presence of target DNA, thus being utilized to analyze the hybridization status of nanoprobe. Two hybridization strategies were introduced, between which one-step hybridization was proved to be a simple and sensitive method to detect target HIV-sequence with a LOD of 16 pM. Also, high discrimination specificity towards SNP was displayed. Even though the two-step method did not show a comparable detection ability with
one-step method, it is an effective approach to generate uniform assemblies with
different geometry. The introduction of simple DNA detection methods depending
on variation of optical properties of plasmonic nanostructures provides new thought
to develop biosensors with high sensitivity and specificity. Further improvement can
be made by introducing SERS to enhance LOD and increasing hybridization
conditions such as higher temperature and lower salt concentration for SNP
detection.
Chapter 5. Robust Nanoparticle-DNA Conjugates Based on Mussel-Inspired Polydopamine Coating

5.1 Introduction

DNA, with its unique base pairing property, versatile programmable sequences, specific target recognition and simple synthesis of exact copies, has been utilized as popular candidate for surface engineering of nanoparticles.\(^{24,27,28,42}\) Ever since 1996 when DNA was firstly applied for gold nanoparticle (AuNP) assembly,\(^{22,23}\) numerous works have emerged as using DNA and its complex functional motifs to guide the assemblies of nanostructures,\(^{9,31,43}\) which can act as drug carriers,\(^{219}\) gene regulation materials\(^{220}\) and diverse biosensors to detect various targets such as metal ions, proteins, DNA and RNA strands.\(^{26,42}\) Ideal DNA conjugation method onto nanoparticles should possess facile reaction chemistry, produce high throughput of DNA nanoparticle conjugates and be broadly applicable on substrates of versatile materials. Currently, various strategies for the construction of DNA modified nanoparticles (AuNPs, silver nanoparticles, magnetic nanoparticles, upconversion nanoparticles, etc) have been put forward based on Au-S, Ag-S chemistry, streptavidin-biotin interaction, amino-carboxyl condensation reaction and polyA adsorption.\(^{76,87,88,221}\) However, evident disadvantages were found related to majority of these methods that time-consuming process and cautious operation are needed and resulting bond linkage is unstable when exposed to continuous heating, reductive environment and long term storage.\(^{90,92}\) Moreover, the various modification strategies are substrate-specific and require different functional groups of DNA. Therefore, a simple and universal DNA modification method with strong
bond stability is urgently required to broaden the application of DNA-nanoparticle conjugates.

In order to overcome the limitations of current DNA modification approaches on colloidal nanoparticles, a facile and universal strategy based on mussel-inspired polydopamine (PDA) was brought forward to construct robust nanoparticle-DNA conjugates. Polydopamine has become one of the important candidates as surface coating materials due to the self-polymerization of dopamine to form rigid PDA layer in slightly alkaline conditions, and subsequently undergo adhesive process onto a wide range of substrates through covalent or noncovalent interactions regardless of the surface properties.\textsuperscript{159,177} The resulting PDA layer, in possession of catechol and quinone groups, exhibits facile chemical reactivity with thiol and amino groups through Michael addition and/or Schiff base reaction.\textsuperscript{159,162,178} The obvious advantages of PDA shell including easy-formation, universal substrate coating and simple reactivity mechanism, has attracted considerable interests from researchers to utilize them for nanoparticle coating and further bioconjugation.\textsuperscript{176} Previous researches focused on PDA encapsulation onto electrode substrates and various nanoparticles such as metal nanoparticles, magnetic nanoparticles and polymeric nanoparticles, and carrying out surface modification with biomolecules for biosensing, photothermal therapy and drug delivery.\textsuperscript{163,181,182,185}

The combination of DNA and PDA was firstly brought forward by Messersmith et al., followed by Corn et al., who also used DNA to modify PDA-coated substrate for DNA and protein sensing.\textsuperscript{193,195} To further expand the applications, efforts were made to adsorb DNA probe onto PDA modified iron oxide for intracellular detection of mRNA targets and initiating photothermal therapy.\textsuperscript{185} Qu group immobilized amino-functionalized DNA strands on PDA capped graphene oxide as thermo-
responsive linkers. However, to the best of our knowledge, DNA conjugation onto PDA-coated colloidal nanoparticles was not systematically investigated. Here we present the synthesis of thin PDA layer encapsulated AuNPs (Au50@PDA) with different shell thickness and realize DNA modification onto colloidal Au50@PDA with thorough characterization. The PDA coating on 50 nm AuNPs was obtained by self-polymerization of dopamine in basic conditions (Figure 5.1a), and Au50@PDA we obtained displayed improved colloidal stability against high concentration of NaCl. Moreover, owing to the reactivity of PDA layer, 5’-thiol-ended and 3’-Cy5-tagged DNA strands were conjugated onto Au50@PDA (Figure 5.1b) to compare with traditional methods of 50AuNP-DNA conjugates, which displayed a great enhancement of bond stability against heat and thiol-containing molecules (Figure 5.1c). Our results suggested the successful preparation of nanoparticles with PDA layer of different thickness and great potential of DNA conjugation onto PDA to create multifunctional platforms that allows for applications under some harsh environment.
Figure 5.1. Schematic illustration of DNA functionalization of polydopamine (PDA) coated nanoparticles. (a). PDA encapsulation onto AuNPs; (b). DNA conjugation onto Au50@PDA and 50 nm AuNPs; (c). Heat treatment or thiol bearing small molecules (DTT or Cysteine) treatment to test and compare the bond stability. PDA structure is shown in the figure.

5.2 Experimental Section

5.2.1 Material and Characterization.

Disulfide bond or Cy5 molecule modified DNA strands were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). The DNA sequences are listed in Table 5.1. Hydroxylamine hydrochloride, sodium citrate tribasic dehydrate (99.0%), Poly (ethylene glycol) (Typical Mn 10,000), Tris (2-
Carboxyethyl) phosphine (TCEP), Dimethyl sulfoxide (DMSO) (99.9%), sodium dodecyl sulfate (SDS), Poly-L-lysine (0.01% solution) were purchased from Sigma-Aldrich (Missouri, USA). 10× TAE buffer (pH=8.0) and agarose were purchased from 1st base (Singapore). 10× TBE buffer (pH=8.3) was purchased from Promega (Madison, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). Hoechst 33342, DMEM medium (High Glucose, no sodium pyruvate, no sodium bicarbonate) and 10× PBS (pH=7.2) were purchased from Life technologies (Carlsbad, USA). All other chemicals were purchased from Sigma-Aldrich unless further noted.

TEM images were obtained by a Jeol JEM 2010 electron microscope operating at an acceleration voltage of 300 kV. UV-vis absorption spectra were obtained on a Shimadzu UV-1800 UV spectrophotometer and processed using OriginLab 8.0 software. Dark field images of single particles were collected by an Olympus IX71 inverted microscope with the aid of an oil-immersion dark field condenser and the photometrics CoolSNAP-ch cooled CCD camera. Scattering spectra of single particles were also conducted by this microscope with a PIXIS:100B spectroscopy CCD. Fluorescence spectra was acquired by JOBIN YVON, Horriba Fluoro-Max-3 spectrometer.

Table 5.1. Sequence Information of ssDNA Used in Chapter 5.

<table>
<thead>
<tr>
<th>ssDNA</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>DNA1</td>
<td>S-S-5’T-TTTTTTTTTTT-CTT CTC CAC AGG-3’-Cy5</td>
</tr>
<tr>
<td>DNA2</td>
<td>S-S-5’T-TTTTTTTTTTT-ATG TGG CTC GTA TG-3’</td>
</tr>
</tbody>
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5.2.2 Polydopamine (PDA) Encapsulation of Nanoparticles.

Four kinds of 50 nm AuNP@PDA (Au50@PDA) with different shell thickness
were prepared by adjusting the amount of dopamine in reaction solution. Typically, 50 mL of previously-synthesized 50 nm AuNPs were centrifuged at 1000 rcf for 12 min and redispersed in 2 mL DI-water. Take out 200 μL of the concentrated 50 nm AuNP solution and disperse them in 8 mL Tris-HCl buffer (pH=8.5), after which dopamine was added into reaction solution with vigorous stirring (final concentration is 0.01 mg/mL, 0.04 mg/mL, 0.1 mg/mL and 0.2 mg/mL, respectively). Keep stirring for another 6 hours to finish the reaction and centrifuge the product (800 rcf, 10 min) for three times. Re-disperse the precipitate in DI-water and keep at 4 °C for further use. MSN were encapsulated with PDA shell using a similar procedure as that of 50 nm AuNPs. And for polystyrene, the particles were first dispersed in 0.5% SDS aqueous solution, followed by adding Tris-HCl 8.5 buffer and dopamine to react and obtain PDA capped nanoparticles.

5.2.3 Colloidal Stability of Au@PDA Nanoparticles.

Place 1 mL aqueous nanoparticles solution (different kinds of samples with same concentration) into 1 cm path-length Poly(methyl methacrylate) (PMMA) cuvettes. Gradually increase the salt concentration of the particle solutions with 1 M sodium chloride (NaCl) solution. Collect the UV-vis absorption spectra of each nanoparticle solution from 400-900 nm.

5.2.4 Surface Functionalization of Au@PDA.

**NH2-PEG Functionalization of Au@PDA.** Au50@PDA were dispersed in 2 mL 10 mM Tris-HCl buffer (pH=8.5) and NH2-PEG solution with a concentration of 1 mM were added into the solution dropwise. After overnight reaction, Au50@PDA@PEG were centrifuged (800 rcf, 10 min) three times and dispersed in DI-water to store at 4 °C for further use.
**DNA Conjugation onto Au@PDA.** DNA strands with disulfide bond were activated with a certain ratio of TCEP using the method we previously mentioned. Au$_{50}$@PDA were dispersed in 10 mM Tris-HCl buffer (pH=8.5) and activated thiol-DNA with specific amount were added into the solution with vigorous stirring. Then, 1 M NaCl was added dropwise to adjust the salt concentration up to 150 mM and leave the reaction mixture for overnight reaction, after which the Au$_{50}$@PDA@DNA were collected by centrifuging for three times and the as-synthesized nanoparticles were stored at 4 °C for further use.

**5.2.5 Characterization Analysis of Nanoparticles.**

0.5% agarose gel was prepared by dispersing 0.25 g agarose powder in 50 mL 0.5×TBE 8.5 buffer. Microwave the powder solution for 2 min to let it boiling and melt agarose. Pour the agarose solution into a casting tray with well comb. Let gel solution sit at room temperature for 30 minutes until gel solution completely solidified. Processed samples (Au$_{50}$@PDA and Au$_{50}$@PDA@DNA with different DNA amounts) were loaded in the agarose gels using 0.5×TBE buffer as running buffer. Gel wells were sealed with 8% agarose gels. Gels ran at 100 V, 8 V/cm for 45 min, after which images were taken by G-box gel imaging system.

**5.2.6 Chemical and Thermal Stability of Au@DNA and Au@PDA@DNA.**

50 nm AuNPs and Au$_{50}$@PDA were functionalized with DNA1 strands which carried disulfide bond at 5’ end and a dye molecule Cy5 (DNA1) at 3’ end. Resulting Au$_{50}$@DNA1 and Au$_{50}$@PDA@DNA1 were dispersed in 10 mM Phosphate Buffer (PB) (pH=8.0) with the same concentration. The well-prepared DNA-nanoparticle conjugates were processed under two conditions. Heat-treated Condition: Au$_{50}$@DNA1 and Au$_{50}$@PDA@DNA1 with same concentration and same volume
were heated up to 75 °C for 24 hours. Then, the solutions were centrifuged (1000 rcf, 10 min) twice to remove all the precipitate and supernatant was kept for fluorescence measurement. Thiol molecule-treated condition: 1 M DTT and 1 M Cysteine were added into nanoparticle solutions to reach a concentration of 50 mM, respectively. The solutions were kept at room temperature for 4 hours for complete reaction. Centrifuge was carried out to remove the precipitate and supernatant was kept for fluorescence test. Cy5 molecule was excited with 610 nm laser light and emitted light at 660 nm.

5.3 Results and Discussion

5.3.1 Synthesis and Characterization of Au@PDA with Different Shell Thickness, PS@PDA and MSN@PDA.

50 nm AuNPs with highly uniform PDA shell can be easily acquired by dispersing AuNPs in a slightly alkaline Tris-HCl buffer, followed by adding dopamine solution and gentle stirring at room temperature for 6 h. Dopamine can self-polymerize into PDA in oxidative conditions and encapsulate AuNPs via covalent and non-covalent interaction to form a robust uniform PDA shell. Based on TEM observation, comparing with original 50 nm AuNPs, Au50@PDA with a PDA shell thickness of 3 nm (Figure 5.2a), 8 nm (Figure 5.2b), and 18 nm (Figure 5.2c) were obtained by adding 0.01 mg/mL, 0.1 mg/mL and 0.2 mg/mL dopamine solution respectively (the Au50@PDA with different shell thickness will be referred as Au50@PDA-3, Au50@PDA-8 and Au50@PDA-18 respectively). TEM images also showed that no self-polymerized PDA nanoparticles were formed during the reaction, indicating successful high throughput synthesis of monodisperse and uniform Au50@PDA. The
surface plasmon resonance of AuNPs is strongly affected by the change of surrounding environment, making it an effective parameter to monitor surface modification of AuNPs. After PDA coating, UV-vis spectra showed that LSPR band of Au50@PDA with different PDA shell thickness exhibited a red-shift of 2.5 nm, 22 nm and 32.5 nm in comparison with 50 nm AuNPs (533 nm) (Figure 5.2d), which was caused by the higher refractive index of PDA shell. As demonstrated in Figure 5.2d inset, the color of obtained Au50@PDA solution changed from bright red to purple since the increase of shell thickness leads to transmission of more blue light and absorption of more red light, which was in consistence with the red shift of LSPR peak.

In addition to metallic surface, we also tried to modify non-metallic and organic material surfaces with PDA shell. As shown in Figure 5.3, a 5 nm PDA shell was formed on the surface of MSN (Figure 5.3a, 5.3b) and PS (Figure 5.3c, 5.3d) after encapsulation process, and high throughput and uniform PDA-nanoparticle nanostructures were obtained. This further demonstrated the universal adhesive property of PDA and provided us facile and effective method to functionalize nanoparticles without regard to the nature of materials. In this work, we will use Au50@PDA as a general platform for further characterization of effects of PDA shell and its chemical reactivity since AuNPs are widely utilized and possess superior optical properties.
Figure 5.2. TEM images and UV–vis absorption spectra of Au$_{50}$@PDA. TEM images of (a) Au$_{50}$@PDA-3, (b) Au$_{50}$@PDA-8 and (c) Au$_{50}$@PDA-18. (d) UV–vis absorption spectra of Au$_{50}$@PDA with different shell thickness. The inset photograph showed the gradual color change of the corresponding samples.
We next performed single-particle imaging to monitor the formation of PDA layer on 50 nm AuNPs and its influence on scattering properties of AuNPs. 50 nm AuNPs displayed bright green scattering light under dark field microscopy, and gradually increased thickness of PDA shell caused the color change of Au50@PDA from distinct green to yellowish green, finally bright yellow scattering color (Figure 5.4a, 5.4b, 5.4c), which is consistent with the result of scattering spectra. Numerous single particles in the field of vision showed effective construction of homogeneous and uniform Au50@PDA core-shell nanostructures. Correspondingly, the collected scattering spectra of Au50@PDA went through a gradual red-shift (Figure 5.4d) up
to 35 nm when comparing Au50@PDA-18 with 50 nm AuNPs. This characterization process revealed the optical properties of PDA coated AuNPs which can be adjusted by PDA shell thickness and enabled Au50@PDA to be applied in broader optical imaging applications.

![Figure 5.4](image-url)

**Figure 5.4.** Single particle dark field imaging and scattering spectra of Au50@PDA. Dark field images of (a) Au50@PDA-3, (b) Au50@PDA-8 and (c) Au50@PDA-18. (d) Scattering spectra of 50 nm AuNPs and Au50@PDA with different shell thickness.

### 5.3.2 Colloidal Stability Test of Au@PDA and PEG Functionalized Au@PDA

PDA shell not only significantly changes the optical properties of AuNPs, but also endows AuNPs with enhanced colloidal stability and active reactivity with various
functional groups. PDA wrapped around AuNPs through the interaction between its catechol groups and AuNP surface to form a robust and dense PDA shell, which could greatly enhance colloidal stability of AuNPs against salt in the environment. In order to examine colloidal stability of the synthesized \( \text{Au}_{50}@\text{PDA} \), we gradually increased the concentration of NaCl in solution by adding specific volume of 1 M NaCl into 50 nm AuNPs and \( \text{Au}_{50}@\text{PDA} \) aqueous solutions. UV–vis spectra were collected to monitor the status of nanoparticles in salt solution since aggregation of AuNPs would result in a broader peak, baseline increase and even LSPR peak red shift. The results showed that 50 nm citrate capped AuNPs aggregated when exposed to 30 mM NaCl (Figure 5.5a). The 3-nm PDA shell of \( \text{Au}_{50}@\text{PDA}-3 \) can slightly improve particle stability by keeping nanoparticle stable at 30 mM NaCl solution (Figure 5.5b). Furthermore, \( \text{Au}_{50}@\text{PDA}-8 \) (Figure 5.5c) kept stable even when NaCl concentration was elevated up to 160 mM, which surpassed normal salt concentration in biological systems and made \( \text{Au}_{50}@\text{PDA} \) suitable agents for potential applications in cellular and \textit{in vivo} environment.

Moreover, PDA layer contains considerable amount of functional groups on the shell surface such as catechol groups, which can be further oxidized into quinone groups and allow for further functionalization of PDA encapsulated nanoparticles by conjugating with amino or thiol-terminated ligands \textit{via} Michael addition and/or Schiff base reactions. Therefore, we incubated amino-functionalized PEG (MW=5k Da) with \( \text{Au}_{50}@\text{PDA}-3 \) for overnight reaction in basic conditions. Salt stability test was performed for obtained \( \text{Au}_{50}@\text{PDA}-3@\text{PEG} \). It is found that colloidal stability of the nanoparticles increased significantly comparing with that of \( \text{Au}_{50}@\text{PDA}-3 \), for it can be stable when exposed to 160 mM NaCl (Figure 5.5d) which was 30 mM NaCl before functionalization. This process indicates the successful
functionalization of nanoparticles with thin PDA shell, proving that thin PDA shell can be widely used for nanoparticle coating and enable nanoparticles for multiple functionalization without greatly changing original properties of nanoparticles.

Figure 5.5. Salt stability test of 50 nm AuNPs and Au50@PDA. UV–vis absorption spectra of (a) 50 nm AuNP, (b) Au50@PDA-3, (c) Au50@PDA-8 and (d) Au50@PDA-3 conjugated with amino modified PEG (MW=5,000) in various concentrations of NaCl.

5.3.3 DNA Functionalization of Au@PDA.

Similar to amino groups, thiol-terminated ligand can also be conjugated onto PDA shell via Michael addition by reacting with C=C double bond in catechol groups or in the intra-molecular nitrogen containing ring. To determine the reaction between thiol-containing ligands and PDA-wrapped nanoparticles, we incubated
Au50@PDA-8 with thiol-terminated DNA strands which were further functionalized with a fluorophore Cy 5 (DNA1), and NaCl concentration was increased to 150 mM by adding 1 M NaCl solution, after which the mixture was kept at room temperature overnight. NaCl concentration was typically elevated during DNA modification process in order to screen the charge between negatively-charged DNA strands and nanoparticle surface to bring them in close proximity to improve the DNA modification efficiency. Certain amount of reaction solution was taken out at time point we previously set and centrifuged to remove the precipitate, and fluorescence intensity of supernatant was measured with an excitation light wavelength of 610 nm. The fluorescence spectra we collected showed a continuous decrease of fluorescence intensity for more than 90% (Figure 5.6a) in 8 hours after reaction started. This process was combination of both DNA adsorption and subsequent DNA conjugation. DNA adsorption was due to π–π stacking as well as electrostatic interaction between negatively-charged deprotonated catechol groups and positively-charged DNA’s nucleobases. DNA conjugation occurred after a covalent reaction between thiol groups and PDA shell. PDA and AuNPs together displayed a high quenching efficiency of fluorescence.

After finishing the reaction between thiol groups and PDA shell, we sought to optimize DNA ratio to react with Au50@PDA-8 by setting five ratios of DNA strands to nanoparticles and reacting under the same condition we previously used. UV-vis spectroscopy showed a red shift of 2 nm after DNA2 modification comparing with Au50@PDA-8 (Figure 5.6b). Based on these results, we found that nanoparticles were very stable during reaction process. Gel electrophoresis of Au50@PDA-8 and DNA2 modified Au50@PDA-8 displayed that bands of nanoparticles moved faster (Figure 5.6c) after reacting with DNA strands, and the moving distance of bands
stayed the same when the ratio of DNA2 to Au50@PDA-8 was 10000 and higher, indicating that 10000 was a preferable ratio for DNA conjugation on Au50@PDA-8. The increased surface charge density after DNA modification may lead to the enhanced electrophoretic mobility in gel.

Performing the DNA conjugation in the optimized ratio of 1:10000 and comparing the fluorescence intensity change, a 20.5% decrease was observed (Figure 5.7) after reaction, indicating that the DNA loading on a single Au50@PDA-8 nanoparticle is ~2050 strands. After calculation, the surface coverage of DNA on Au50@PDA-8 is 0.15 strand per nm², which is quite comparable with Au-S functionalization chemistry (for 50 nm AuNPs, 0.155 per nm², and for 80 nm AuNPs, 0.163 per nm²), proving that this PDA based DNA loading strategy is of high efficiency comparing with traditional methods.

Figure 5.6. Characterization of DNA conjugation process onto Au50@PDA. (a) Gradual fluorescence emission spectra change of Au50@PDA and DNA1 reaction solution after centrifuging and removing precipitates. Excitation: 610 nm, emission:
662 nm. (b) UV–vis spectra of Au₅₀@PDA conjugated with different ratio of DNA strands (2000, 4000, 10000, 20000). (c) Electrophoretic analysis of Au₅₀@PDA and Au₅₀@PDA@DNA₂ conjugates after reacting with different amount of DNA₂. Lane 1: Au₅₀@PDA; Lane 2: Au₅₀@PDA with DNA₂=1:1000; Lane 3: 1:2000; Lane 4: 1:4000; Lane 5: 1:10000; Lane 6: 1:20000.

**Figure 5.7.** Fluorescence emission spectra change of supernatant solution before and after Au₅₀@PDA and DNA₁ reaction.

### 5.3.4 Bond Stability of Au@PDA@DNA.

Based on the previous experiment, we have realized the conjugation of DNA onto Au₅₀@PDA-8 which is fast, facile and universal since a thin shell of PDA can virtually be coated onto various kinds of colloidal nanoparticles no matter the nanoparticles possess organic or inorganic surfaces.

Examining the reaction mechanism of conjugation between thiol or amino groups and catechol converted quinones on PDA shell, we found that both Michael addition
and Schiff base reaction can generate a covalent bond, either C-S or N-C bond. Considering the high stability of these covalent bonds and widely criticized instability of Au-S bond, we decide to compare the bond stability between Au50@PDA-8 and 50 nm AuNPs when linked with thiol-terminated DNA.

Au50@PDA-8 was conjugated with thiol-modified Cy5-tagged DNA strands using the method mentioned above and 50 nm AuNPs were modified with the same DNA through methods described in the experimental procedures. Same ratio of DNA strands to nanoparticles was applied for both two kinds of nanoparticles. Chemical stability was studied by treating two kinds of Au-DNA1 conjugates with both 50 mM cysteine and 50 mM DTT respectively at room temperature for 4 hours. Centrifugation was conducted and the supernatant solution was collected to measure the fluorescence signal. A significant increase of fluorescence intensity was observed for Au-S conjugated Au@DNA1 sample while control samples which was treated with same volume of water showed no fluorescence signal (Figure 5.8b), indicating the release of DNA1 strand from Au surface and recovery of Cy5 emission light. By contrast, C-S bond linked Au50@PDA-8@DNA1 showed no obvious fluorescent change before and after DTT and cysteine treatment (Figure 5.8a), demonstrating its superior chemical bond stability against small reducing agents, which would enable Au-DNA conjugates to be applied in intracellular redox environment which contains many kinds of anti-oxidizing reducing agents such as glutathione.

Thermal stability of Au-DNA conjugates was also examined by keeping two kinds of samples at 75 °C for 24 h. Fluorescence test showed that Au–S bond was cleaved after long-time continuous heating and Cy5 was released from nanoparticle surface to exhibit fluorescent signal (Figure 5.8d) while PDA mediated Au-DNA conjugates
remained stable after heat treatment (Figure 5.8c), displaying great resistance against high temperature.

![Figure 5.8](image)

**Figure 5.8.** Bond stability test of Au$_{50}$@DNA1 and Au$_{50}$@PDA@DNA1 treated under different conditions. Fluorescence spectra of supernatant solutions of (a, c) Au$_{50}$@PDA@DNA1 and (b, d) Au$_{50}$@DNA1 treated with (a, b) 50 mM cysteine, 50 mM DTT and (c, d) 75 °C heating, respectively. Excitation: 610 nm, emission: 662 nm.

### 5.4 Conclusion

In summary, we have reported a new robust DNA conjugation approach for colloidal nanoparticles by taking advantage of versatile PDA shells. The PDA shell, with tunable thickness, can be encapsulated on nanoparticles with organic or inorganic surfaces and largely enhance the colloidal stability of nanoparticles.
Plasmonic properties of 50 nm AuNPs can be changed corresponding to the different shell thickness. The highly packed PDA layer not only endows nanoparticles colloidal stability in high ionic strength, the facile reactivity also enables convenient loading of biomolecules. Our results demonstrated that thiol-ended DNA can be conjugated onto nanoparticles via PDA and the loading efficiency is comparable with traditional methods. More importantly, the DNA nanostructures was discovered to have distinct advantage that new and strong binding mode of DNA linkage showed dramatically improved thermal and chemical stability. This work sheds some light on PDA shell mediated DNA linkage onto multiplex colloidal nanostructures and expands the utility of DNA nanoconjugates for diagnostic applications in complex conditions.
Chapter 6. Aptamer-Targeted Cell Imaging and DNA-Mediated
Self-Assembly of Polydopamine Coated Nanoparticles

6.1 Introduction

Recently, multi-particle systems with certain controlled patterns, built from building blocks which are modified with natural and synthetic molecules, are of considerable research interest due to their wide fundamental and practical applications including plasmon modulation, intracellular real-time bioimaging, drug delivery, photothermal therapy and biosensors. Proteins, amphiphilic copolymers and even small molecules are involved for surface functionalization of nanoparticles to produce well defined nanostructures. Particularly, DNA, with its unique strand-specific recognition, selective targeting and structural diversity and controllability, offers novel approaches to directing the assembly of elementary nanoparticles with careful design. Since 1996, various nanostructures were constructed based on DNA corona engineering. However, problems need to be addressed that conventional DNA modification methods are surface-dependent and bond stability of DNA linkage is susceptible to surrounding environment.

Here we report the development of DNA functionalization of various polydopamine-coated particles and demonstrate the well-retained functions of conjugated DNA strands to conduct cancer cell imaging and assembly of diverse core-satellite nanostructures. Polydopamine has been developed as ideal materials for surface engineering since its catechol groups can be oxidized into quinone groups at basic conditions, followed by intramolecular cyclization, oligomerization and
intermolecular cross link to form polydopamine (PDA) which can strongly stick to substrates of virtually any materials via covalent or noncovalent bonding.159,177 Another advantage of rigid PDA shell as coating material is that its catechol and quinone groups exhibit universal chemical reactivity towards thiol and amino groups through Michael addition and/or Schiff base reactions, enabling facile surface functionalization with multiple molecules.176 The superior properties of PDA enables it to act as important auxiliary shell for DNA conjugation and construction of multi-particle system. In our previous research, PDA encapsulation and subsequent DNA conjugation onto colloidal nanoparticles were systematically explored and great enhancement of bond stability against continuous heating and reducing agent of high concentration was discovered.

As illustrated in Figure 6.1, we modified 5’-thiol-terminated DNA strands onto PDA-coated particles (Figure 6.1a) and displayed that DNA strands on PDA-nanoparticles still retain their functions for substance targeting and sequence specific hybridization. 50 nm AuNPs with PDA layer (Au50@PDA) were modified with cell targeting aptamer AS1411 to specifically bind with nucleolin on the membrane of MCF-7 cells, proving the well-performed function of PDA-linked aptamers (Figure 6.1b). Apart from realizing DNA modification onto inorganic surface via PDA, iron oxide nanoparticles embedded in polystyrene (IO@PS) were applied for DNA linkage. Well-defined core satellite nanoparticle assemblies of IO@PS@PDA, Au50@PDA and Au14@PDA can be built by tailoring one sets of complementary single-strand thiol-modified DNA onto the PDA capped particles (Figure 6.1c). Furthermore, micrometer-size magnetic nanochains (MNCs), assembled from IO@PS@PDA, were exploited for assembly with Au50@DNA to demonstrate the construction of superstructures from large size particles. Our results have shown that
optical properties of this superstructure is strongly affected by the number of “satellite” AuNPs conjugated on MNCs via DNA oligomer. All these findings suggested the fine establishment of DNA conjugation onto PDA-capped colloidal particles and the realization of DNA fundamental functions and aptamer based target-recognition properties. The construction of multi-particle systems indicates high potential for creating multifunctional platforms.

**Figure 6.1.** Schematic illustration of DNA modified PDA-capped nanoparticles for specific cell targeting and tailored assembly. (a). Thiol-ended DNA strands conjugate onto PDA layer. (b). Aptamer strand AS1411 functionalized particles can bind with specific proteins on cell membrane, thus enabling cell recognition. (c). DNA mediated assembly of PDA capped “core” particles with “satellite” nanoparticles. The particle represents gold nanoparticle (AuNP), iron oxide nanoparticles (IONP) and magnetic nanochain (MNC).
6.2 Experimental Section

6.2.1 Materials and Characterization.

5'-disulfide bond modified DNA strands were purchased from Shanghai Sangon Biotechnology Incorporation (Shanghai, China). The DNA sequences are listed in Table 6.1. Hydroxylamine hydrochloride, sodium citrate tribasic dehydrate (99.0%), sodium dodecyl sulfate (SDS), Tris (2-Carboxyethyl) phosphine (TCEP), dopamine, styrene, tetradecane, Poly-L-lysine (0.01% solution) were purchased from Sigma-Aldrich (Missouri, USA). 10× TAE buffer (pH=8.0) and agarose were purchased from 1st base (Singapore). 10× TBE buffer (pH=8.3) was purchased from Promega (Madison, USA). Sodium chloride was purchased from Merck (Darmstadt, Germany). Hoechst 33342, DMEM medium (High Glucose, no sodium pyruvate, no sodium bicarbonate) and 10× PBS (pH=7.2) were purchased from Life technologies (Carlsbad, USA). All other chemicals were purchased from Sigma-Aldrich unless further noted.

TEM images were obtained by a Jeol JEM 2010 electron microscope operating at an acceleration voltage of 300 kV. SEM images were obtained by a JSM-6700F FESEM. UV-vis absorption spectra were obtained on a Shimadzu UV-1800 UV spectrophotometer and processed using OriginLab software. Dark field images of single particles and cell imaging were collected by an Olympus IX71 inverted microscope with the aid of an oil-immersion dark field condenser and the photometrics CoolSNAP-cf cooled CCD camera. Scattering spectra of single particles were also conducted by this microscope with a PIXIS:100B spectroscopy CCD.
Table 6.1 Sequence Information of ssDNA Used in Chapter 6.

<table>
<thead>
<tr>
<th>ssDNA</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS1411</td>
<td>S-S-5’-TTTTTTTTTTT-GGT GGT GGT GGT TGT GGT GGT GG-3’</td>
</tr>
<tr>
<td>DNA1</td>
<td>S-S-5’-TTTTTTTTTTT-ACC TGG GGG AGT ATT GCG GAG GAA GTT-3’</td>
</tr>
<tr>
<td>DNA2</td>
<td>S-S-5’-TTTTTTTTTTT-CAT ACG AGC CAC AT-3’</td>
</tr>
<tr>
<td>DNA3</td>
<td>S-S-5’-TTTTTTTTTTT-ATG TGG CTC GTA TG-3’</td>
</tr>
<tr>
<td>DNA4</td>
<td>S-S-5’-TTTTTTTTTTT-GAT CCC TTG GAG CA-3’</td>
</tr>
</tbody>
</table>

6.2.2 PDA Encapsulation of Nanoparticles.

**PDA Encapsulation of 14 nm AuNPs.** 100 mL of freshly prepared 14 nm AuNPs were centrifuged down by 13000 rcf for 15 min and the precipitates were dispersed in 2 mL aqueous SDS solution (0.3%, m/v). 100 μL of concentrated nanoparticle solution was dispersed in 4 mL 10 mM Tris-HCl buffer to add 100 μL dopamine (4 mg/mL) into reaction solution dropwise. Keep stirring for 6 h and collect the product by centrifuge (10000 rcf, 15 min) for three times. Discard the supernatant and redisperse precipitate in DI-water and keep at 4 °C for further use.

**Synthesis of Polystyrene Wrapped Iron Oxide (IO@PS).** IO@PS and IO@PS@PDA were prepared followed the procedures we previously published.226 Magnetite nanoparticles (0.25 g) were dispersed in 6 mL aqueous SDS solution (1 mg/mL), and the reaction solution was treated with ultrasound in ice bath for 10 min to gain the miniemulsion of magnetite nanoparticles. This nanoparticle emulsion was mixed with 2.5 mg of potassium peroxydisulfate (KPS) and placed in a three-neck flask to stir for 30 min in nitrogen atmosphere. Subsequently, an as-prepared styrene emulsion including 1.25 mL styrene, 12.5 mg SDS, 10 mL DI-water and 8.25 μL tetradecane was added into the flask, followed by carrying out the reaction in an 80 °C oil bath for 20 h to obtain IO@PS nanoparticles. The newly-synthesized
IO@PS were collected with magnetic separation for three times and dispersed in 10 mL water to be applied in subsequent experiment.

**PDA Encapsulation of IO@PS.** Disperse 0.2 mL of IO@PS in 40 mL of Tris-HCl buffer (pH=8.5) and add dropwise 10 mg aqueous dopamine solution into IO@PS solution. The mixture was stirred for 4 h to complete the reaction and magnetic separation was conducted to collect dark brown product. The final product IO@PS@PDA was dispersed in water and stored at 4 °C for further use.

### 6.2.3 Synthesis of Magnetic Nanochains (MNCs).

The MNCs were synthesized based on the procedures we previously published. Disperse 50 µL IO@PS@PDA in 10 mL Tris-HCl buffer (pH=8.5) and add 0.5 mL aqueous dopamine solution (5 mg/mL). The reaction tube was placed in a magnetic field to react for 15 min, followed by incubating at room temperature for 4 h without disturbing to complete the reaction. The color solution changed from nearly colorless to dark brown, indicating the formation of magnetic nanochains. Magnetic separation was conducted twice to collect and purify MNCs, and the precipitate was dispersed in 1 mL DI-water for further use.

### 6.2.4 DNA Conjugation onto PDA Capped Particles

DNA strands with disulfide bond were activated with TCEP using the method we previously mentioned. 14 nm AuNP@PDA (Au_{14}@PDA) were dispersed in 3 mL Tris buffer to reach a concentration of 4 nM. Activated SH-DNA were added into reaction solution with vigorous stirring, and the salt concentration was slowly increased to 100 mM by adding 1 M NaCl. Leave for overnight incubation and Au_{14}@PDA@DNA was collected by centrifuge and dispersed in water for further
use. For 50 nm AuNP@PDA (Au50@PDA), the modification procedure was similar (nanoparticle concentration in the solution is about 0.05 nM).

For IO@PS@PDA and MNCs, 50 µL freshly prepared particles were dispersed in 3 mL Tris buffer and SH-DNA was added into the solution under vigorous stirring. 1M NaCl was added to reach a concentration of 100 mM. Overnight incubation was essential for completion of DNA conjugation. Magnetic separation was conducted to remove excess DNA strands and collect DNA conjugated products (IO@PS@PDA@DNA and MNC@DNA). Disperse in DI-water and store at 4 °C for further use.

6.2.5 Cellular Experiments.

MCF-7 cells were cultured in high glucose DMEM medium which was mixed with 10% FBS and 1× antibiotic-antimycotic solution. Cell incubator was set at 37 °C with 5% CO2 in a humidified environment. For imaging experiments including dark field and fluorescence imaging, cells were required to be cultured on glass coverslips which were completely rinsed and sterilized. To prepare this cell-grown coverslips, cells were disassociated from cell culture dishes using trypsin-EDTA for 1 min treatment and collected by centrifugation (800 rpm, 3 min). Then, cell precipitates were redispersed in fresh cell culture medium and planted on glass coverslips which were pre-treated with 1 mL poly-L-lysine for 5 min to promote attachment of cells. After one day incubation, Au50@PDA@DNA (AS1411 or scrambled DNA1) were dispersed in 1 mL 1× PBS solution with the concentration of 20 pM. The nanoparticle solutions were incubated with cells for 45 min in the cell incubator. After the nanoparticle-cell incubation, cell-carrying coverslips were washed three times with fresh PBS solution to remove free nanoparticles. Hoechst
33342 was applied for cell nucleus staining. Operating procedures were to follow the commercial protocols. To be brief, Hoechst 33342 was dispersed in 1 mL 1× PBS solution and the final concentration was 1 μg/mL. Then, prepared Hoechst 33342 solution was to interact with MCF-7 cells for 30 min in the cell incubator. After cell staining, cell coverslips were washed for another three times with fresh 1× PBS solution and observed under dark field and fluorescence microscopy.

### 6.2.6 Self-Assembly of DNA Functionalized PDA Nanocomposites.

DNA functionalized Au$_{14}$@PDA and Au$_{50}$@PDA were mixed together in a ratio of 1:200 (50Au:14Au) and salt concentration was adjusted to 80 mM. The solution was kept at room temperature for 1.5 h to complete hybridization. Then, the assemblies were collected by centrifugation and dispersed in 50 mM NaCl for further characterization. The assembly of IO@PS@PDA@DNA with Au$_{14}$@DNA and MNC@DNA with Au$_{50}$@DNA was performed with the same conditions. DNA2 was conjugated onto Au$_{50}$@PDA, IO@PS@PDA and MNCs. DNA3, which is complementary with DNA2 was modified onto Au$_{14}$@PDA, 14Au and 50Au.

### 6.3 Results and Discussion

#### 6.3.1 Cell Imaging of Au@PDA@Aptamer.

50 nm AuNPs were encapsulated with a uniform 8 nm PDA shell by dispersing the nanoparticles in dopamine-containing Tris-HCl buffer (pH=8.5) (Figure 6.2a). The PDA shell is formed due to the self-polymerization of dopamine at alkaline conditions. The PDA encapsulation led to a 22-nm red shift of LSPR band comparing with 50 nm AuNPs (Figure 6.2b) based on UV-vis spectra result due to
the increased refractive index after PDA modification. Successive functionalization of nanoparticles were enabled by this PDA shell since it possesses versatile chemical reactivity with thiol or amino groups via Michael addition and/or Schiff-base reaction. Oligonucleotides with amino or thiol groups can be attached onto nanoparticles through interacting with PDA shell. Our UV-vis results showed a red shift of 2 nm after DNA conjugation (Figure 6.2b) and the inset image of Figure 6.2b showed the dark field image of Au50@PDA@AS1411 which exhibited distinct and homogeneous greenish yellow color as Au50@PDA, indicating that nanoparticles remained stable during DNA modification process.

DNA conjugation with the aid of PDA has superior chemical and heat-resistance stability, inspiring us to carry out cellular experiments to explore the interaction between Au50@PDA@DNA and cells to check if PDA layer affects the biological functions of the linked DNA strands. The AS1411 strand we conjugated onto Au50@PDA is one kind of aptamer that can selectively bind nucleolin, a phosphoprotein which was found to be expressed on cell surface and plasma membrane of breast cancer cells.70,71 We conjugated thiol-ended AS1411 and scrambled DNA strands (DNA1) onto Au50@PDA respectively, followed by adding amino-PEG (MW=750) into reaction solution to further functionalize Au50@PDA@DNA and reduce its nonspecific adsorption of proteins. Generated nanostructures were incubated with MCF-7 cells, one kind of human breast cancer cells that overexpressed nucleolin on the cell membrane, for 45 min at 37 °C in a 5% CO2 containing incubator. After incubation, MCF-7 cell-bearing coverslips were rinsed with PBS three times to remove the unbinding nanoparticles, and placed on specimen stage to observe under dark field microscope. 45-min incubation allowed AS1411 to bind with nucleolin on cell surface and greenish yellow color was
observed (Figure 6.3a and 6.3b). Bright yellow color was observed on some parts of cells, which could be due to the plasmon coupling caused by close proximity of nanoparticles, indicating a nucleolin-rich region. Hoechst 33342 was applied to stain cell nuclei with blue color (Figure 6.3b and Figure 6.3d). In clear contrast, scramble DNA 1 sequence conjugated nanoparticles showed no interaction with cells and dark field image showed that no greenish yellow color dots were observed (Figure 6.3c and 6.3d). The clear dark background of coverslips further indicated the colloidal stability of nanostructures against high salt environment (PBS solution) and the resistance against non-specific adsorption of proteins.

Specific targeting effect of AS1411 towards breast cancer cells MCF-7 cells proved that PDA layer would not impede the function of aptamers and stability of nanostructures was retained after DNA modification to make a difference on cellular imaging. This provides the possibility of allowing the combination of aptamers and PDA capped nanoparticles to construct robust biosensors and bio-targeting drug carriers.
Figure 6.2. (a). TEM images of Au₅₀@PDA with an 8 nm PDA shell. (b). UV-vis absorption spectra of 50 nm AuNPs, Au₅₀@PDA and Au₅₀@PDA@AS1411. Inset image is dark field image of Au₅₀@PDA@AS1411.

Figure 6.3. Dark field images of cell imaging by Au₅₀@PDA modified with cell-targeting aptamer. (a,c). Dark field images and (b,d) overlaid dark field and fluorescent images of MCF-7 cells incubated with (a,b) Au₅₀@PDA@AS1411 and (c,d) Au₅₀@PDA@Scrambled DNA1. Blue color exhibited the position of cell nuclei which were stained with Hoechst 33342. Scale bar = 20 µm.
6.3.2 DNA Guided Core-Satellite Assemblies of Au@PDA

Other than functioning as aptamers, DNA strands are also applied as building blocks and DNA sensing sensors owing to its magnificent hybridization property based on rigid base-pairing among four nitrogen-containing bases. Some current researches related to PDA and DNA functions were only conducted on solid phase of electrodes, leaving it unknown in the solution phase of DNA-modified PDA-encapsulated colloidal nanoparticle structures.

In order to conduct DNA hybridization process after PDA conjugation, we modified Au$_{50}$@PDA with DNA2 as “core” AuNPs and Au$_{14}$@PDA with DNA3, which is complementary with DNA2, as “satellite” AuNPs to form core-satellite nanostructures. The nanoparticles were conjugated with thiol-containing DNA strands using procedures explained in experimental section, and hybridization process was conducted after DNA modification. After hybridization, the collected products were measured by UV-vis spectroscopy. An 8 nm red shift of LSPR of the assemblies were observed relative to that of the core Au$_{50}$@PDA@DNA2 (Figure 6.4c), whereas the control experiment, which was designed by conducting a hybridization process between Au$_{50}$@PDA@DNA2 and Au$_{14}$@PDA@DNA4 in which DNA4 was not complementary with DNA2, displayed no obvious peak shift (green line in Figure 6.4c) after the same process and sample centrifugation. This confirmed the selectivity and specificity of DNA hybridization property after conjugating with PDA. TEM observation clearly displayed the formation of core satellite nanostructures from the building blocks (Figure 6.4a and 6.4b). Consistently,
single-particle scattering spectra also showed a 9 nm red shift due to the plasmon coupling between $\text{Au}_{50}@\text{PDA}$ and $\text{Au}_{14}@\text{PDA}$ (Figure 6.4d).

Considering the red shift (14 nm) of core satellite nanostructures we previously reported\textsuperscript{227} which had no PDA shell on core and satellite nanoparticles, this 8 nm red shift was probably resulted from the weakened plasmon coupling since interparticle distance was longer because of the existence of 8 nm PDA shell on $\text{Au}_{50}@\text{PDA}$ and 5 nm on $\text{Au}_{14}@\text{PDA}$.

![Figure 6.4. Controlled assembly of $\text{Au}_{50}@\text{PDA}@\text{DNA}_{2}$ and $\text{Au}_{14}@\text{PDA}@\text{DNA}_{3}$. TEM images of $\text{Au}_{14}@\text{PDA}@\text{DNA}_{3}$ (a, 1), $\text{Au}_{50}@\text{PDA}@\text{DNA}_{2}$ (a, 2) and the core satellite assemblies (b). (c). UV-vis spectra image of nanoparticles and assemblies. (d). Scattering spectra of $\text{Au}_{50}@\text{PDA}@\text{DNA}_{2}$ and nanoparticle assemblies.](image)
6.3.3. Preparation and Characterization of Magnetoplasmonic Assemblies.

In order to further elucidate the versatility of this DNA modification method via PDA layer, we wrapped iron oxide nanoparticles (IO@PS), which has hydrophobic surface, with PDA shell (Figure 6.5a) and utilized the formed IO@PS@PDA for DNA conjugation and DNA directed assembly. IO@PS were prepared by embedding a bunch of magnetic nanocrystals in polystyrene (PS) matrix through miniemulsion polymerization. The same strategy with previous assembly experiment was applied to conjugate DNA2 onto IO@PS@PDA as “core” and DNA3 onto 14 nm AuNPs as “satellite” nanoparticles. After hybridization, magnetic separation was performed to remove excess 14 nm AuNPs and collect the precipitate. TEM and SEM images showed that 14 nm AuNPs were assembled onto IO@PS@PDA and uniform and large throughput core satellite nanostructures were obtained (Figure 6.5). UV-vis spectra of assemblies after magnetic separation displayed a peak at 539 nm comparing with the spectra of IO@PS@PDA@DNA2 (Figure 6.6). The results demonstrated that IO@PS@PDA and 14 nm AuNPs assembled together via DNA strands in consistent with TEM and SEM images. The 17 nm spectral red-shift was brought about by strong plasmon coupling resulted from close proximity of numerous 14 nm AuNPs. Meanwhile, control samples after hybridization showed that the LSPR band was at 522 nm where Au_{14}@DNA4’s peak locates, indicating no AuNPs were assembled onto core nanoparticles (Figure 6.6). The results confirmed that the DNA strands conjugated onto the PDA layer well retained their selectivity and specific recognition of DNA hybridization property.
Figure 6.5. Controlled assembly of IO@PS@PDA@DNA2 and Au_{14}@DNA3. TEM images of IO@PS@PDA (a) and nanoparticles assemblies (b). SEM images of IO@PS@PDA (c) and assemblies (d).

Figure 6.6. UV-vis absorption spectra of IO@PS@PDA@DNA2 (black), nanoparticle assemblies with a LSPR peak at 539 nm (red) and control samples with peak wavelength at 522 nm (blue).
DNA strands were conjugated onto PDA-coated nanoparticles to direct the formation of core-satellite assemblies and the proper size (50-200 nm) of resulting nanostructures are advantageous for a wide range of cellular applications such as bioimaging and intracellular sensing. However, assemblies with larger size are of equal importance since they are easy to recycle and serve as ideal colloidal platforms for multiplex sensing applications. Thus, further explorations were made to conjugate DNA strands onto micrometer-size structures and construct superstructure systems.

Magnetic nanochains (MNCs) were prepared by dispersing IO@PS@PDA in the dopamine solution and exposing this reaction mixture in a magnetic field which would help align IO@PS@PDA into 1D chain like structures. PDA shell was deposited to encapsulate and cross-link nanoparticles towards MNCs. As shown in SEM images (Figure 6.7a), MNCs with an average length of 15 µm and a diameter of 160 nm were obtained. The diameter is consistent with the diameter of IO@PS@PDA (130 nm) and external PDA shells, and the length of MNCs can be adjusted by sonication and shaking during chain formation. Then, thiol-ended DNA2 molecules were modified onto MNCs after overnight incubation in Tris-HCl buffer (pH=8.5) and 100 mM NaCl. 50 nm AuNPs were modified with DNA3 strands (denoted as Au50@DNA3) using previously mentioned methods as “satellite” nanoparticles to assemble with MNCs. 5 µL, 20 µL and 40 µL of Au50@DNA3 were mixed with MNC@DNA2 to yield superstructures (denoted as MNC-Au-1, MNC-Au-2 and MNC-Au-3, respectively). Following a 2-hour incubation of MNC@DNA2 and Au50@DNA3, hybridization of DNA2 and DNA3 strands took place and particle assemblies were obtained through magnetic separation. The
structures were examined by SEM and representative images clearly showed the formation of three types of MNC-50Au assemblies carrying with different amount of 50 nm AuNPs (Figure 6.7b, 6.7c and 6.7d). We further investigated MNC-Au-2 with TEM and the image clearly displayed the AuNPs on the surface of MNCs in clear contrast (Figure 6.7e). From an enlarged observation area, MNCs with an average diameter of 160 nm were assembled with uniform 50 nm AuNPs and the nanoparticles were dispersed in single layer (Figure 6.7f), indicating that no aggregation of AuNPs occurred and DNA hybridization is the only driving force for assembly.

To collect more information about the superstructures and understand their potential applications, UV-vis absorption spectroscopy was carried out to assess the plasmon coupling among the conjugated AuNPs. The spectrum of MNCs with PDA coating displayed a wide range absorption from 400 nm to 900 nm with no evident peaks (black line, Figure 6.8a), while the conjugation of Au50@DNA3 caused the emergence of a new peak at 552 nm (red line, Figure 6.8a). Since the LSPR peak of individual 50 nm AuNPs with DNA3 modification is around 533 nm, the assembly of AuNPs on MNCs led to a 19 nm red shift in LSPR. For MNC-Au-2 and MNC-Au-3, a continuous red shift of LSPR band to 616.5 nm and 678 nm (Figure 6.8a) was achieved. For MNC-Au-1, Au50@DNA3 with limited number was due to the close arrangement of Au50@DNA3 with increasing amount.

To further characterize the DNA driven core-satellite superstructures, scattering spectra and dark field color images were collected from MNC-Au-2 in buffer solution. Spectra from 10 MNCs and 10 MNC-Au-2 assemblies were collected and corrected by deducting the background scattering from the area with same size adjacent to particles. The typical curves were demonstrated in Figure 6.8b that
MNCs had a broad range scattering spectra from 450 nm to 700 nm with a peak at 565 nm and MNC-Au-2, with strong plasmon coupling from Au$_{50}$@DNA3, exhibited a stronger scattering centered at 640 nm. An over two-fold increase of scattering intensity and a red shift of 70 nm indicated the tight packing of AuNPs on the surface of MNCs. Color images acquired from dark field microscopy were consistent with the scattering spectra that MNCs displayed weak scattering light with no obvious color while strong and distinct orange and yellow light was presented by MNC-Au-2 (Figure 6.8c and 6.8d) corresponding to the scattering spectra around 640 nm. The evident changes of absorption and scattering properties of MNC-50Au assemblies make the structures desirable candidate for biodiagnostics.
Figure 6.7. SEM images (a-d) of magnetic nanochains (MNCs) (a), MNC-Au-1 (b), MNC-Au-2 (c) and MNC-Au-3 (d). TEM images (e-f) of MNC-Au-2 (e) and a magnified region of MNC-Au-2 (f).
Figure 6.8. (a). UV-vis spectra of MNC@DNA2 and assemblies of MNC@DNA2 with different ratios of Au$_{50}$@DNA3. (b). Scattering spectra of MNC@DNA2 and MNC-Au-2. Dark field images of MNC@DNA2 (c) and MNC-Au-2 (d). Scale bar=5 µm.

6.4 Conclusion

In summary, we have demonstrated the successful DNA conjugation onto PDA-coated nanoparticles without regard to inorganic metallic surface or organic polystyrene surface, as well as the well-retained DNA functions and properties. DNA aptamer with surface biomarker (protein) binding property was exploited to functionalize Au$_{50}$@PDA and brought about the specific recognition of targeted cancer cells. DNA hybridization property facilitated the preparation of plasmonic superstructures that assembled from DNA functionalized PDA-coated materials.
The three types of resultant nanoscale assemblies and micrometer-size assemblies are characterized with various experimental methods including TEM, SEM, UV-vis and dark field microscopy. The plasmonic properties of core-satellite assemblies undergo obvious variations which are induced by distance change of neighboring nanoparticles, which facilitates the construction of biosensors. The PDA shell enlarges the range of particles made from multiple materials to form assemblies via DNA strands. Particularly, the notable discrimination of optical properties of core-satellite assemblies and magnetoplasmonic assemblies, accompanied with intense optical labeling, easy magnetic recyclability and self-mixing property, enables these structures to find extensive applications in biosensing, biodiagnostics, magnetic separation and magnetothermal therapy.
Chapter 7. Conclusions and Future Research

7.1 Conclusions

In this thesis, we utilized DNA strands to construct plasmonic nanostructures and applied them in bioimaging and DNA sensing applications. Moreover, a new DNA modification method onto colloidal nanoparticles was put forward based on polydopamine based particle encapsulation, and DNA function on PDA shell was thoroughly investigated.

First, we prepared the well-controlled core-satellite plasmonic nanostructures using DNA modified AuNPs. The assemblies, when hybridized with the aid of i-motif DNA linker, can undergo pH decrease-stimulated disassembly. We demonstrated that the i-motif nanostructures can enter macrophages through endocytosis and subsequent maturation and acidification of intracellular organelles would induce the disassembly of the structure, enabling plasmonic imaging of intracellular pH changes.

Second, we demonstrated a simple and sensitive DNA detection method based on a sandwich DNA hybridization assay. AuNRs and 14 nm AuNPs are modified with DNA to serve as binary probes which can assemble in the presence of target sequence, enabling simple DNA sensing within a concentration range of 16 pM to 4 nM and evident discrimination signal towards SNP. The step-wise hybridization method, even with a higher LOD for DNA detection, can be efficient to produce nanoparticle assemblies with varying numbers of satellites.

Third, we have developed a new DNA conjugation method towards colloidal nanoparticles utilizing polydopamine shell which can strongly adhere onto the
inorganic or organic surfaces of particles and render them highly stable against high concentration of salt. PDA shell also provides versatile and facile chemical reactivity with thiol and amino groups, enabling DNA modification with high efficiency. The resulting DNA-PDA conjugates showed greatly enhanced chemical and thermal stability owing to the strong C-N and C-S covalent bonding.

Finally, we explored the function of DNA strands conjugated on the colloidal particles coated with PDA. AS1411, an aptamer that can bind with nucleolin, was modified onto Au50@PDA and mediated the cancer cell recognition. Self-assembly via DNA hybridization gave rise to two types of core satellite assemblies with DNA linked NP@PDA as “core” particles. Further investigation showed that micrometer-scale PDA-magnetic chains can also assemble with “satellite” nanoparticles. This micro-structure, with magnetic related characteristics and significant changes of plasmonic properties, can function as a new diagnostic platform.

7.2 Future Research

Since DNA strands exhibit great structural adjustability and responses to various targets, we could design more intracellular probes for bioimaging and biosensing applications, as well as apply them as an effective tool for constructing nanostructures and tuning the location and distance of building blocks. Also, considering the obvious advantages of PDA shell mediated DNA conjugation, we can use this method to design experiments in harsh environment. The detailed future researches are put forward in the following four aspects:

1) In previous works, we have developed the pH-responsive core satellite nanostructures to monitor the intracellular pH changes. Owing to the multiple
targeting functions and structural recognition property of DNA strands, in the next step, we will try to assemble plasmonic nanostructures with aptamers that are responsive to intracellular substances such as secreted proteins and RNA strands and realize real-time monitoring of the concentration gradients in living cells. Since many anticancer drugs can interact and bind with DNA, we can load the drugs with double-stranded DNA sequence and realize drug delivery in the presence of specific targets such as H⁺, ATP, Ag⁺ and mRNA.

2) In order to improve the detection limit and signal discrimination when the analytes are in low concentration, surface enhanced Raman spectroscopy (SERS) is introduced due to its dramatic signal amplification effect. AuNRs and their “satellite” nanoparticles could provide junctions between the neighboring nanoparticles, known as hot spot, where electromagnetic field and Raman scattering can be highly enhanced. Thus, we can introduce Raman dye into the junction by conjugating them to the end of DNA molecules. Moreover, since silver nanoparticles showed significant higher enhancement of Raman scattering than AuNPs, we can form silver shells on the core satellite nanostructures to obtain stronger signals.

3) Other than plasmonic nanoparticles, quantum dots, with unique optical properties such as high quantum yield, no photobleaching under long time excitation and narrow emission, are attracting more attentions recently. Its interaction with the electromagnetic field of plasmonic nanoparticles is quite interesting, which can lead to fluorescence quenching or fluorescence enhancement with the varying distance. Thus, DNA, which can precisely regulate the length through hybridization, is ideal agents to carry out the fluorescence interaction between QD and nanoparticles. We can introduce nanospheres and nanorods to assemble with QD. The resulting
nanostructures may be further used as biosensors for diagnostics and intracellular imaging.

4) The strong bond stability of PDA based DNA conjugation, along with the universal adhesive property of PDA layer, allow us to modify DNA onto various platforms and utilize them in complex conditions such as in PCR to produce nanostructures with high throughput or in intracellular drug delivery system. Also, we can design diagnostic probes for DNA, protein, mRNA or microRNA which requires reversible hybridization and multiple usages. Other properties of PDA shell such as strong NIR absorption and surface reducing properties, can be further exploited as drug delivery platforms and photothermal agent.
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