DNA base pair stacking assembly of anisotropic nanoparticles for biosensing and ordered assembly

Zhiyu HE,† Guoqing WANG,*†‡§ Xingguo LIANG,†§Tohru TAKARADA*‡ and Mizuo MAEDA‡

†College of Food Science and Engineering, Ocean University of China, 5 Yushan Road, Qingdao 266003, China
‡Bioengineering Laboratory, RIKEN Cluster for Pioneering Research, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
§Laboratory for Marine Drugs and Bioproducts of Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

* To whom correspondence should be addressed.
E-mail: gqwang@ouc.edu.cn
E-mail: ttkrd@riken.jp
Abstract

Anisotropic gold nanoparticles have attracted great interest due to their unique physicochemical properties derived from the shape anisotropy. Manipulation of their interfacial interactions, and thereby the assembling behaviors are often requisite in their applications ranging from optical sensing and diagnosis to self-assembly. Recently, the control of interfacial force based on base pair stacking of DNA terminals have offered a new avenue to surface engineering of nanostructures. In this review, we focus on the DNA base stacking-induced assembly of anisotropic gold nanoparticles, such as nanorods and nanotriangles. The fundamental aspects of anisotropic gold nanoparticles are provided, including the mechanism of the anisotropic growth, the properties arising from the anisotropic shape, and the construction of DNA-grafted anisotropic gold nanoparticles. Then, the advanced applications of their functional assemblies in biosensing and ordered assembly are summarized, followed by a comparison with gold nanospheres. Finally, conclusions and outlooks are given with challenges and opportunities on the futuristic directions in this field.
I. Introduction

Gold nanoparticles (AuNPs) have been extensively studied in the field of nanoscience and technology owning to the ease-of-preparation, chemical inertness, unique optical properties, and biocompatibility.1-3 The past two decades have further witnessed the emergence of a large variety of anisotropic AuNPs that were driven by the remarkable progress in the solution-phase synthesis of colloidal nanoparticle.4 As two classical examples of anisotropic AuNPs, gold nanorods (AuNRs) and gold nanotriangles (AuNTs) have drawn much attention owning to their optoelectronic properties and surface chemistry that are derived from the high shape anisotropy.5,6 For example, they have characteristic LSPR bands, which can be synthetically tuned across the visible to near-infrared regions by tailoring their aspect ratios.6,7 Moreover, their well-defined facets also allow for controlled interactions directed by region-selective functionalization, which makes them ideal building blocks for many functional nanomaterials.8 The shape-dependent properties have enabled applications in various fields ranging from optical sensing to therapeutics.9-13

Manipulating the assembly and thereby the optical properties of anisotropic AuNPs are essential toward the various applications. DNA has emerged as a powerful tool in this regard owning to its programmable base pairing and rich chemical functionality.14,15 One of the well-established strategies for controlled assembly is to connect the single-stranded (ss) DNA-modified AuNPs with short DNA crosslinkers to hybridize into sandwich configurations.16 In addition to the crosslinking method,
regulating the interfacial force among double-stranded (ds) DNA-grafted AuNPs is also capable of directing their assembly.\textsuperscript{17} In 2003, Maeda and coworkers reported a mode of non-crosslinked assembly of AuNPs.\textsuperscript{18} At high ionic strength, the formation of full-matched dsDNA on the closely packed surface DNA monolayer leads to a spontaneous aggregation of nanoparticles. It has also been found that this mode of non-crosslinking assembly occurs irrespective of the composition,\textsuperscript{19-21} size,\textsuperscript{22,23} and shape\textsuperscript{24} of the core particles, and is independent of the sequences of the dsDNA monolayers.\textsuperscript{21,24-26} These results suggest that the attraction force among the nanoparticles is generated by the DNA terminals of the outermost surfaces. The measurement of the attraction force among DNA blunt ends on small angle X-ray scattering (SAXS)\textsuperscript{27} and colloid probe-atomic force microscopy (CP-AFM)\textsuperscript{26,28} reveals that the decreased colloidal stability of nanoparticles is derived from the base stacking among the DNA terminals. Although the stacking force is naturally a type of weak interaction, closely packed dsDNA monolayer can provide sufficiently strong stacking attraction among DNA terminal base pairs for assembling nanoparticles. By sharp contrast, once the outermost of dsDNA has a mismatched or an extended base, the stacking of the terminal base pairs is greatly inhibited, and entropic repulsion arising from the fraying motion of the unpaired bases prevents the nanoparticles from assembly.\textsuperscript{26} By integrating with the fascinating optical and surface properties of anisotropic AuNPs, this single base pairing-dominated interfacial force has been utilized for construction of biosensors and functional assemblies.
This review mainly focuses on the base pair stacking-induced assembly of DNA-grafted anisotropic AuNPs (Fig. 1). We will firstly discuss the fundamentals of DNA-grafted anisotropic AuNPs, including the mechanism of directional growth of anisotropic AuNPs, and the process of DNA functionalization on their surface. Then, advanced applications including biosensing and ordered assemblies using DNA-grafted anisotropic AuNPs will be summarized, with an emphasis on the control of interaction among the particle surfaces by forming DNA blunt ends. A comparison of the properties with isotropic AuNPs will also be made, highlighting the diverse performances of anisotropic AuNPs caused by shape anisotropy. Finally, conclusions and outlooks on the opening opportunities in this field are given. Extended applications of DNA base stacking assembly of anisotropic AuNPs in biosensing and optical devices with more functions and better performances are expected.

Fig. 1  Schematic illustration of the applications of DNA base pair stacking assembly of anisotropic AuNPs in biosensing and ordered assembly.
II. Fundamentals of DNA-grafted anisotropic AuNPs

The fundamentals of DNA-grafted anisotropic AuNPs are worth discussion prior to their applications. Their special optical and surface properties are derived from the anisotropic structures that can be obtained by controlled growth in solution-phase synthesis.4 The establishment of base pairing-dominated interfacial interaction among the anisotropic AuNPs is based on replacement of the surface ligand by thiol-tagged DNA.29 In this section, the construction of DNA-grafted anisotropic AuNPs will be elaborated, including the synthesis of colloidal anisotropic AuNPs and the process of DNA functionalization on their surfaces.

Synthesis and properties of anisotropic AuNPs

Among the many synthetic methods developed for anisotropic AuNPs, chemical reduction in solution phase is popular due to the advantage of simplicity and efficiency.30 The formation of anisotropic AuNPs involves preferential anisotropic growth, which depends on the crystal symmetry of the nuclei and the selective binding of ligands.31 The twinned planes formed at the nucleation stage facilitates the breaking of symmetry, while the adhesion of ligands on some specific facets directs the anisotropic growth by slowing down the growth rate of that facet.32 However, the optimal conditions for the formation of twinned nuclei and the preferential growth are not always the same. Therefore, the process of nucleation is usually separated from the
growth to form well-defined seeds, which allows for better control of specific facet deposition to ensure the high yield of desired shapes.\textsuperscript{33} For instance, in a classical protocol for synthesis of AuNRs pioneered by Murphy et al.,\textsuperscript{5} the nucleation was performed under harsh reductant conditions, followed by an aging step. Then, the seed solution was injected into an Au stock solution containing mild reductant and silver ions for directional growth. The silver ions worked as a shape-directing reagent by preferentially depositing onto the \{110\} facet to form metal monolayer, accelerating the growth of \{100\} facet. Similar to the mechanism of the rod-like growth, the formation of AuNTs relies on the protection of \{111\} facet of the nuclei, leaving the fast growth of side facets.\textsuperscript{5} The shape control of AuNTs not only depends on the ligand that can selectively bond on \{111\} facet, but also involves the selection of the twinned nuclei by oxidant, such as H\textsubscript{2}O\textsubscript{2},\textsuperscript{34} which can remove the non-twinned nuclei by oxidative etching.\textsuperscript{35} The two processes can proceed in the same vessel, namely, “seedless growth”. In the growth, iodide worked as a dual-functional reagent, both selectively binding to the \{111\} facet and removing the non-twinned seeds.\textsuperscript{36}

The shape anisotropy is the origin of the intriguing optical properties of anisotropic AuNPs, which also leads to their uneven surface reactivity.\textsuperscript{33} When interacting with incident light, the coherent oscillation of conductive band electrons occurs on the particle surface, exciting LSPR bands ranging from the visible to near-infrared regions of the spectrum.\textsuperscript{37} The position and intensity of LSPR bands are closely related to the charge density of the particle surface, which are affected by many factors such as the size, shape, structure, and dielectric environment.\textsuperscript{38} For example, the electron
oscillation on AuNRs occurs in the two different axes, generating a relatively weak transverse band at ~520 nm and a strong longitudinal band in longer wavelength regions. While the transverse band is not sensitive to the change of the particle shape, the longitudinal band can be easily tuned by tailoring the width and length. As for AuNTs, the condition of the SPR band is determined by more factors, such as the edge length, thickness, and tip morphologies. Beside the characterized LSPR mode, the anisotropic shape also leads to the directional interactions that have the tendency to interact with the large surface, which inspires the separation of anisotropic nanoparticles from the isotropic AuNPs. In addition, the site-specific surface reactivity makes them typical subjects for face-selective functionalization. The surfaces with large curvature, such as the end faces of AuNRs and the tips and edges of AuNTs, have lower density of surfactant molecules, thereby promising faster ligand exchange. Therefore, the interfacial interaction of different faces can be controlled by the face-selective modification of functional ligands.

DNA functionalization of anisotropic AuNPs

The process of DNA functionalization of anisotropic AuNPs is essentially an exchange reaction between the native ligand and the thiol-tagged DNA, thereby forming Au-S covalent bonds to immobilize the DNA onto the particle surfaces. Salt aging step can be followed to maximize the DNA loading. For anisotropic AuNPs stabilized with cetyltrimethylammonium (CTA) halide, once DNA is introduced, the positively-charged CTA can lead to the compaction of DNA. Therefore, DNA
functionalization for CTA-capped AuNPs is usually carried out in the presence of sodium dodecyl sulfate (SDS) to disrupt the CTA-DNA combination, enabling efficient conjugation of thiol-tagged DNA to the particle surfaces.\textsuperscript{42,43} In addition, the sites with large curvature have lower steric hindrance for ligand exchange,\textsuperscript{40} leading to the preferentially adsorption of DNA added in low concentrations. After these regions have been mostly occupied, another DNA can be introduced to adsorb onto the low-activity regions. Therefore, the region-selective modification of anisotropic AuNPs with different DNA can be achieved by the ordered addition of different DNA sequences with predetermined ratio (Fig. 2a).\textsuperscript{41} It can be confirmed by the duplex formation with small gold nanospheres functionalized with the complementary DNA, as shown in transmission electron microscopy (TEM) images (Fig. 2b).\textsuperscript{41}

![Diagram](image.png)

**Fig. 2** Region-selective modification of DNA on anisotropic AuNPs. (a) Schematic illustration of selectively modification of two types of DNA sequences on different regions of AuNRs. (b) Schemes and TEM images of the confirmation of region-selective modification by site-selective assembly of AuNR with gold nanospheres (From Fig. 2 in Ref. 41, reproduced with permission).
III. Applications of DNA-grafted anisotropic AuNPs

The formation of fully matched DNA generates stacking force at the outermost DNA layers of anisotropic AuNPs. High density modification of DNA can lead to rapid assembly of the nanoparticles.\textsuperscript{44} The shortened interparticle distance caused by assembly induces coupled electron oscillation in the nanoparticles, and leads to a red shift of the LSPR band.\textsuperscript{37} This underlies a widely used principle of signal transduction in AuNP-based colorimetric biosensors.\textsuperscript{45} Moreover, the deposition of DNA species on different facet can be controlled, thereby promising directed organization of anisotropic AuNPs.\textsuperscript{41} In this part, we will discuss the applications of base pair stacking-induced assembly of DNA-grafted anisotropic AuNPs in detail, including biosensing and ordered assembly.

Biosensing

The key to biosensor design based on base pair stacking-induced assembly is the formation of DNA blunt ends on AuNPs.\textsuperscript{46} There are mainly two strategies to achieve this goal. One is to form fully matched dsDNA on the particle surface by hybridizing with the complementary sequence, and the other is to directly control the pairing status of DNA terminal bases.

When the target is a given DNA sequence in abundance or readily amplified, colorimetric detection based on base pair stacking assembly proceeds more rapidly than
that based on crosslinking assembly. This inspired research efforts on application of base pair stacking assembly of anisotropic AuNPs in food traceability and single-nucleotide polymorphism (SNP) genotyping. Wang et al. reported that the process of base pair stacking assembly would be accelerated in low polar solvent, as a result of enhanced interaction among the DNA blunt ends. Based on this finding, a method for quickly identification of DNA anti-counterfeiting code in alcohol has been established. In this work, the alcohol sample containing DNA anti-counterfeiting code leads to the color change of the DNA-AuNP probes, as a result of hybridization with the full-match ssDNA grafted on the particle surface.

By coupling with single-base primer extension, DNA base stacking-induced assembly of AuNPs has been successfully applied for colorimetric detection of single-base substitution. The applicability of AuNRs and AuNTs in SNP detection of human cytochrome P450 2C19 monooxygenase gene (CYP2C19) model (Fig. 3a) was also demonstrated. The workflow is shown in Fig. 3b. Firstly, single-base primer extension was performed on CYP2C19 gene model, in which chemically synthesized dsDNA termed CYP(G) worked as a template. In this step, four reaction samples containing each of ddATP, ddTTP, ddGTP, and ddCTP, respectively, together with typing primer and DNA polymerase, were mixed with the template. Then, AuNRs or AuNTs functionalized with the ssDNA that was complementary to the un-extended typing primer was added into each sample to implement the colorimetric assay. Upon mixing with the DNA-AuNR or DNA-AuNT probes, the resultant samples of ddATP, ddGTP and ddTTP, which generated un-extended typing primer, underwent a blue-to-
grey color change within 5 min, indicating the formation of fully matched DNA on particle surface (Fig. 3c). No color change can be observed in the ddCTP sample because it produced an extended typing primer and formed a dangling end on the probe surfaces (Fig. 3d). By taking advantage of the allele-specific single base primer extension, convenient yet rapid discrimination of extended and un-extended typing primers could be achieved with this approach, which is more user-friendly than the traditional SNP detection methods, such as invader assay and TaqMan assay.\textsuperscript{50}

Fig. 3  Schematic illustration of SNP detection based on the combination of single base extension and the base pair stacking-induced assembly of AuNRs. (a) The DNA sequence of the CYP2C19 gene model. (b) The workflow of colorimetric SNP typing of the CYP2C19 gene model with guanine (G) at the SNP site. (c) The assembly of anisotropic AuNPs caused by the hybridization of un-extended typing primer in the ddATP, ddTTP, and ddGTP samples. (d) The dispersion of anisotropic AuNPs caused by the hybridization of extended typing primer in the ddCTP sample (From Fig. 4 in Ref. 24, reproduced with permission).
The biosensors based on DNA base pair stacking assembly can also be designed through target-induced breathing of the DNA terminals. Small targets such as Hg$^{2+}$ and Ag$^+$ can easily penetrate to the AuNP surfaces and bind with terminal bases. This ensures effective control of terminal base pairing status, and thereby switching of interactions among the DNA terminal bases. By using cysteine (Cys) to regulate the formation of T–Hg$^{2+}$–T complex on DNA terminals, Zhang et al. developed a DNA-AuNR plasmon switch to rapidly detect Hg$^{2+}$ and Cys (Fig. 4). In this work, AuNRs were functionalized by the dsDNA with T–T mismatch at the penultimate position of the outermost terminals, making the DNA-AuNRs dispersed at the initial state. When Hg$^{2+}$ was introduced into the solution, the formation of T–Hg$^{2+}$–T complex on DNA terminals triggered the assembly of AuNRs, leading to solution color change from green to colorless. Then, Cys was added to remove the Hg$^{2+}$ from the T–Hg$^{2+}$–T complex, reproducing T–T mismatch at the DNA terminals, which resulted in the redispersion of AuNRs. This plasmon switch fueled by Hg$^{2+}$ and Cys achieved a rapid and repeated cycle at room temperature, with the detection limit of 10 nM and 1 μM for Hg$^{2+}$ and Cys, respectively. The generality of this method has also been demonstrated in a similar plasmon switch, where the reversible assembly of AuNRs modified with Ag$^+$-binding DNA sequence was fueled by Ag$^+$ and Cys.52
Fig. 4  Schematic illustration of the switching of dsDNA-AuNR assembly state regulated by Hg$^{2+}$/Cys-controlled DNA terminal base pairing. The binding of Hg$^{2+}$ in the penultimate base pair leads to the formation of blunt end and induces the aggregation of AuNRs (From dispersion to assembly). The formation of Cys–Hg$^{2+}$–Cys complex causes the recovery of mismatched end and induces the dispersion of AuNRs (From assembly to dispersion). The corresponding photographs for the color change of the solutions are inserted on the bottom (From Fig. 1 in Ref. 52, reproduced with permission).

**Ordered assembly**

Even though base pair stacking-induced random assembly of AuNRs and AuNTs are applicable to colorimetric biosensing, as described above, the full use of their anisotropic feature should be made. The structural anisotropy affords multiple forms of assembly, while the uneven surface activity implies region-selective functionalization, which promise more precise regulation of their shape-dependent optical properties. For example, the directed end-to-end or side-by-side assembly of AuNRs can cause red or blue shift of the SPR bands. 53

Since the interfacial force among the dsDNA-functionalized AuNPs is controlled
by the pairing of outermost DNA terminals at high ionic strength, the ordered assembly of AuNRs and AuNTs can also be achieved in this fashion.\textsuperscript{41,54} Wang et al. reported the region-selectively functionalized AuNR with DNA by adjusting the ratio of two different DNA sequences and the order of addition. Both complementary DNA and single-base-substituted DNA were simultaneously added, forming fully matched and terminal-mismatched dsDNAs on the side and the ends, respectively, allowing for the ordered assembly of AuNRs (Fig. 5a).\textsuperscript{41} Then, the assemblies were observed under TEM after evaporation on a substrate, where the capillary force facilitated the process of assembly. When the fully matched dsDNAs were on the ends of the AuNR, while the terminal mismatched dsDNAs were on the side, end-to-end assemblies were obtained. When the positions of the two types of dsDNAs were exchanged, the AuNRs were directed into side-by-side assemblies. These two forms of AuNR assemblies were also in-situ characterized by UV-Vis spectroscopy. By adjusting the parameters of the solvent environment such as temperature and ionic strength, dramatic LSPR shifts for the AuNRs occurred depending on assembly configuration. For the side-by-side assemblies, a red shift of the transverse band occurred accompanied with a blue shift of the longitudinal band (Fig. 5b). For the end-to-end assemblies, the longitudinal band underwent a red shift (Fig. 5c). These observations reveal the successfully manipulation of optical properties of AuNRs by the ordered assembly. Similar ordered assemblies have also implemented with AuNTs. When the top/bottom faces and the edges of AuNTs were modified with full-matched and terminal-mismatched dsDNA, respectively, the face-to-face assemblies of AuNTs were obtained (Fig. 5d).\textsuperscript{54} It is worth
noting that even though the selectivity of the DNA adsorption to different regions was not perfect, the base pair stacking assembly of AuNRs were highly ordered and selective. This can be attributed to the deterministic effect of the overall interaction, including stacking attraction or entropic repulsion, arising from the whole dsDNA monolayer. By comparison, the production of assemblies through crosslinking strategy is less selective due to the crosslinking behavior of the few mis-located DNA.⁴¹

Fig. 5 Schematic illustration of ordered assembly of anisotropic AuNPs directed by
the region-selectively modified dsDNA with different terminals. (a) Side-by-side assembly (top) and end-to-end assembly (bottom) of AuNRs with terminal-mismatched dsDNA and full-matched DNA modified on the end and side. (b) UV-Vis spectra of the side-by-side assemblies. (c) UV-Vis spectra of the end-to-end assemblies (From Fig.1 and Fig. 3 in Ref. 41, reproduced from with permission). (d) Face-to-face assembly (top) and edge-to-edge assembly (bottom) of AuNTs with terminal-mismatched dsDNA and full-matched DNA modified on the face and edge (From Fig. 1 and Fig. 4 in Ref. 54, reproduced with permission). The corresponding TEM images for each assembly are inserted on the right.

The region-selective functionalization of DNA with well-designed terminals could realize the simultaneous transformation of the interfacial properties on different surfaces, which made the forms of ordered AuNR assemblies convertible.\textsuperscript{41} The reagent that can control the pairing of DNA bases plays the key role in the transformation of assembly form. For example, Hg\textsuperscript{2+} can mediate the pairing of the thymine bases by forming stable T–Hg\textsuperscript{2+}–T complexes. Based on this property, the DNA sequences of bonding module and antibonding module were designed.\textsuperscript{55} In the bonding module, the T–T mismatch was set at the penultimate position, perturbing the stability of adjacent G–C base pair at the end. In the presence of Hg\textsuperscript{2+}, the formation of T–Hg\textsuperscript{2+}–T complex recovered the stability of the G–C base pair, thereby realizing the transition of the DNA terminal from unpairing to pairing (Fig. 6a). Analogously, the terminal of antibonding module was set as A–T base pair with a T-bulge moiety. After the addition of Hg\textsuperscript{2+}, the
adenine base was overhang on the terminal owning to the higher thermodynamic stability of the T–Hg$^{2+}$–T than the A–T base pair. In this way, the terminal of antibonding module was transformed from pairing to single-base overhang (Fig. 6b). By anchoring these two types of DNA on the different regions of AuNRs, the assembly behavior of the AuNRs can be altered with the addition of Hg$^{2+}$. When the ends and the side of the AuNR were modified with bonding and antibonding modules respectively, the repulsion and the attraction of the end and side induced the AuNRs to be assembled in a side-by-side manner. Upon the formation of T–Hg$^{2+}$–T, the interactions among the DNA terminals on different regions would shift into reverse, and the AuNR assemblies was transformed from side-by-side to end-to-end (Fig. 6c). Inversely, when the regions of the bonding and antibonding modules were exchanged, the transformation of end-to-end AuNR assemblies to side-by-side can also be triggered by the addition of Hg$^{2+}$ (Fig. 6d). The establishment of this external stimuli-responsive AuNR assemblies could provide useful ideas for the creation of optical switching devices.

![Fig. 6](image_url)

Fig. 6  Schematic diagrams for the Hg$^{2+}$-directed assembly of AuNRs achieved by regioselective modification of DNA with fine designed terminals. (a,b) The structural
change of (a) bonding module and (b) antibonding module induced by Hg$^{2+}$. (c) The transformation of side-by-side to end-to-end assembly of AuNRs with bonding module on the ends and antibonding module on the side. (d) The transformation of end-to-end to side-by-side assembly of AuNRs with antibonding module on the ends and bonding module on the side (From Fig. 5 in Ref. 41, reproduced with permission).

IV. Comparison to gold nanospheres (AuNSs)

In comparison to AuNSs, anisotropic AuNPs have unique shapes and characteristic LSPR, which are accompanied with distinct surface chemistry. The dramatic difference between the isotropic and anisotropic shapes can contribute to different performance in biosensing and ordered assembly.

Concerning biosensing applications, the important point is that anisotropic AuNPs can in principle form larger assemblies more rapidly by virtue of the large contact area. For example, isotropic AuNSs usually start to assemble in a point-to-point fashion due to the high curvature, in which attraction by only a few outermost DNA bases are involved. In sharp contrast, AuNTs can easily form face-to-face assembly because of the large plane area of the top/bottom faces. This is also the case for the spontaneous side-by-side assembly of AuNRs. The above inference should rationalize the much lower detection limit of Hg$^{2+}$ by DNA-AuNRs than DNA-AuNSs.$^{51,52}$ Besides, the higher sensitivity of AuNRs’ LSPR to the changes in their morphology and dielectric environment also contributes to the better performance of the sensor.$^{52}$
As for the ordered assembly applications, it should be pointed out that the region-selective functionalization can be easily achieved on anisotropic AuNPs compared to isotropic AuNSs, owning to the uneven surface activity caused by shape anisotropy. By site-selective control of DNA terminal base pairing, many directed assemblies of anisotropic AuNPs can be formed. For AuNSs that exhibit uniform surface activity, however, it would be much more difficult, even though a multi-step method has been used to prepare bifunctional monomer to construct assemblies with ordered spatial arrangement.\textsuperscript{56-60}

V. Conclusions and outlooks

Since the discovery of the terminal base pairing-dominated assembly of dsDNA-grafted nanoparticles, DNA base pair stacking has emerged as a highly potential strategy for surface engineering. The overall interactions generated by the whole surface dsDNA monolayer ensure rapidity and accuracy of the assembly behaviors of nanoparticles. Heavy metal ions, amino acids, and short DNA sequences can efficiently transform the interfacial attractive and repulsive forces, due to the high accessibility of DNA-grafted AuNPs for the small chemical signals. Based on the special surface and optical properties of anisotropic AuNPs, many plasmonic biosensors with good detection performances have been developed through the manipulation of the base pair stacking on particle surface by the targets. Region-selective functionalization of well-designed dsDNA enables precise control of the directional assembly of anisotropic AuNPs.
Despite the range of success in the ingenious design of biosensors and ordered assemblies, there are still numerous opportunities in the development of DNA-grafted anisotropic AuNPs. First, due to the easy tuning of their LSPR bands throughout a wide wavelength range, anisotropic AuNPs serve as ideal indicator candidates for colorimetric assays, a comparable role to isotropic AuNPs. They have the potential to be applied for simultaneous detection of multiplexed targets.⁶¹,⁶² Given the improved sensitivity of dark field microscopy,⁶³-⁶⁵ the assay based on assembly of anisotropic nanoparticles may also afford better detection performance through light scattering analysis, which is susceptible to the formation of a few small nano-clusters that is difficult to be observed with UV-Vis spectral analysis and the naked eye. Second, other anisotropic nanoparticles with structural complementary such as cubes and polyhedrons are also good candidates for the particle core, in addition to rods and triangles. As a result, more possible functional assemblies with distinct morphologies can be expected. Different composition of nanoparticle core, such as silver, may be more cost-effective and display stronger LSPR properties and wider tunability at the nanoscale.⁶⁶ This optical feature promises better performance in sensing. Third, since the base pair stacking assembly can be manipulated by the single base pairing at the DNA terminals, the control of assembly can be achieved more conveniently by many remote external stimuli other than the addition of chemicals.⁴⁴ For example, DNA base pair stacking assembly is indeed thermo-responsive. It possesses high thermal sensitivity because it strongly depends on the pairing status of terminal DNA base pair(s), other than the melting of whole DNA duplex that is required for disassembling crosslinking
assemblies. Therefore, the base pair stacking assembly exhibits more sensitive response to the change in temperature.\textsuperscript{44} Furthermore, by modifying light- or pH-response groups onto the DNA terminals, the assembly can also be controlled by light and pH, respectively. This may lead to the applications in external stimuli-response switches and devices for remote control, such as plasmonic thermometers and pH-meters. Finally, by taking advantage of the barcode capability of the programmable DNA sequence, the base pair stacking assembly of anisotropic AuNPs have the promising applications in barcoding, food traceability, and information storage.

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References

1. S. Eustis and M. A. El-Sayed, Chem. Soc. Rev., 2006, 35, 209.

2. P. Ghosh, G. Han, M. De, C. K. Kim, V. M. Rotello, Adv. Drug. Deliver. Rev., 2008, 60, 1307.

3. H. Jans and Q. Huo, Chem. Soc. Rev., 2012, 41, 2849.
4. M. Grzelczak, J. Pérez-Juste, P. Mulvaney and L. M. Liz-Marzán, *Chem. Soc. Rev.*, **2008**, 37, 1783.

5. X. Huang, S. Neretina and M. A. El-Sayed, *Adv. Mater.*, **2009**, 21, 4880.

6. J. E. Millstone, S. J. Hurst, G. S. Métraux, J. I. Cutler and C. A. Mirkin, *Small*, **2009**, 5, 646.

7. H. Chen, L. Shao, Q. Li and J. Wang, *Chem. Soc. Rev.*, **2013**, 42, 2679.

8. J. E. Millstone, D. G. Georganopoulou, X. Xu, W. Wei, S. Li and C. A. Mirkin, *Small*, **2008**, 4, 2176.

9. G. Paramasivam, N. Kayambu, A. M. Rabel, A. K. Sundramoorthy and A. Sundaramurthy, *Acta Biomater.*, **2017**, 49, 45.

10. P. R. Sajanlal, T. S. Sreeprasad, A. K. Samal and T. Pradeep, *Nano Rev.*, **2011**, 2, 5883.

11. N. Li, S. Han, C. Zhang, S. Lin, X.-y. Sha and W. Hasi, *Anal. Sci.*, **2020**, 36, 935.

12. K. Thorkelsson, P. Bai and T. Xu, *Nano Today*, **2015**, 10, 48.

13. A. J. Mieszawska, W. J. Mulder, Z. A. Fayad and D. P. Cormode, *Mol. Pharm.*, **2013**, 10, 831.

14. N. Liu and T. Liedl, *Chem. Rev.*, **2018**, 118, 3032.

15. L. Wang, J. Li, S. Song, D. Li and C. Fan, *J. Phys. D: Appl. Phys.*, **2009**, 42, 203001.

16. C. R. Laramy, M. N. O’Brien, C. A. Mirkin, *Nat. Rev. Mater.*, **2019**, 4, 201.

17. G. Wang, Y. Akiyama, N. Kanayama, T. Takarada and M. Maeda, “Targeted Nanosystems for Therapeutic Applications: New Concepts, Dynamic Properties,”
Efficiency, and Toxicity”, ed. K. Sakurai and M. A. Ilies, 2019, American Chemical Society, Vol. 1309, Chap. 6, 119.

18. K. Sato, K. Hosokawa and M. Maeda, *J. Am. Chem. Soc.*, 2003, 125, 8102.

19. T. Mori and M. Maeda, *Polym. J.*, 2002, 34, 624.

20. Z. Tang, T. Takarada and M. Maeda, *Langmuir*, 2018, 34, 14899.

21. K. Sato, M. Sawayanagi, K. Hosokawa and M. Maeda, *Anal. Sci.*, 2004, 20, 893.

22. K. Sato, K. Hosokawa and M. Maeda, *Analyst*, 2019, 144, 5580.

23. K. Sato, M. Onoguchi, Y. Sato, K. Hosokawa and M. Maeda, *Anal. Biochem.*, 2006, 1, 162.

24. G. Wang, Y. Akiyama, T. Takarada and M. Maeda, *Chem-Eur. J.*, 2016, 22, 258.

25. Y. Akiyama, H. Shikagawa, N. Kanayama, T. Takarada and M. Maeda, *Chem- Eur. J.*, 2014, 20, 17420.

26. N. Kanayama, T. Sekine, K. Ozasa, S. Kishi, T. Nyu, T. Hayashi and M. Maeda, *Langmuir*, 2016, 32, 13296.

27. X. Qiu, K. Andresen, L. W. Kwok, J. S. Lamb, H. Y. Park and L. Pollack, *Phys. Rev. Lett.*, 2007, 99, 038104.

28. T. Sekine, N. Kanayama, K. Ozasa, T. Nyu, T. Hayashi and M. Maeda, *Langmuir*, 2018, 34, 15078.

29. M. R. Jones, R. J. Macfarlane, B. Lee, J. Zhang, K. L. Young, A. J. Senesi and C. A. Mirkin, *Nat. Mater.*, 2010, 9, 913.

30. J. L. Elechiguerra, J. Reyes-Gasga and M. J. Yacaman. *J. Mater. Chem.*, 2006, 16, 3906.
31. Q. Zhang, Y. Hu, S. Guo, J. Goebl and Y. Yin, *Nano Lett.*, **2010**, *10*, 5037.

32. Q. Zhang, N. Li, J. Goebl, Z. Lu and Y. Yin, *J. Am. Chem. Soc.*, **2011**, *133*, 18931.

33. N. D. Burrows, A. M. Vartanian, N. S. Abadeer, E. M. Grzincic, L. M. Jacob, W. Lin, J. Li, J. M. Dennison, J. G. Hinman and C. J. Murphy, *J. Phys. Chem. Lett.*, **2016**, *7*, 632.

34. G. Wang, S. Tao, Y. Liu, L. Guo, G. Qin, K. Ijiro, M. Maeda and Y. Yin, *Chem. Commun.*, **2016**, *52*, 398.

35. Y. Ni, C. Kan, J. Xu and Y. Liu, *Superlattices Microstruct.*, **2018**, *114*, 124.

36. L. Chen, F. Ji, Y. Xu, L. He, Y. Mi, F. Bao, B. Sun, X. Zhang and Q. Zhang, *Nano Lett.*, **2014**, *14*, 7201.

37. X. Huang and M. A. El-Sayed, *J. Adv. Res.*, **2010**, *1*, 13.

38. C. L. Nehl and J. H. Hafner, *J. Mater. Chem.*, **2008**, *18*, 2415.

39. C. Zhao, G. Wang, T. Takarada, X. Liang, M. Komiyama and M. Maeda, *Colloids Surf. A Physicochem. Eng. Asp.*, **2019**, *568*, 216.

40. K. K. Caswell, J. N. Wilson, U. H. F. Bunz and C. J. Murphy, *J. Am. Chem. Soc.*, **2003**, *125*, 13914.

41. G. Wang, Y. Akiyama, N. Kanayama, T. Takarada and M. Maeda, *Small*, **2017**, *13*, 1702137.

42. X. Li, D. Sun, Y. Chen, K. Wang, Q. He, and G. Wang, *Biochem. Biophys. Res. Commun.*, **2018**, *495*, 2559.

43. B. A. Grzybowski, K. J. M. Bishop, C. J. Campbell, M. Fialkowski and S. K. Smoukov, *Soft Matter*, **2005**, *1*, 114.
44. G. Wang, Y. Akiyama, S. Shiraishi, N. Kanayama, T. Takarada and M. Maeda, 

*Bioconjugate Chem.*, **2017**, *28*, 270.

45. P. Valentini and P. P. Pompa, *RSC Adv.*, **2013**, *3*, 19181.

46. K. Sato, K. Hosokawa and M. Maeda, *Anal. Sci.*, **2007**, *23*, 17.

47. L. Wang, G. Wang, Y. Shi, L. Zhang, A. Ran, T. Takarada, M. Maeda and X. Liang, 

*Analyst*, **2020**, *145*, 3229.

48. K. Sato, K. Hosokawa and M. Maeda. *Nucleic Acids Res.*, **2005**, *33*, e4.

49. Y. Akiyama, G. Wang, S. Shiraishi, N. Kanayama, T. Takarada and M. Maeda, 

*ChemistryOpen*, **2016**, *5*, 508.

50. A.-C. Syvänen, *Nat. Rev. Genet.*, **2001**, *2*, 930.

51. N. Kanayama; T. Takarada and M. Maeda. *Chem. Commun.*, **2011**, *47*, 2077.

52. L. Zhang, C. Zhao, Y. Zhang, L. Wang, G. Wang, N. Kanayama, T. Takarada, M. Maeda and X. Liang, *Langmuir*, **2019**, *35*, 11710.

53. L. Vigderman, B. P. Khanal and E. R. Zubarev, *Adv. Mater.*, **2012**, *24*, 4811.

54. G. Wang, Y. Zhang, X. Liang, T. Takarada and M. Maeda, *Nanomaterials*, **2019**, *9*, 581.

55. N. Kanayama, T. Takarada, M. Fujita and M. Maeda, *Chem-Eur. J.*, **2013**, *19*, 10794.

56. Y. Akiyama, H. Shikagawa, N. Kanayama, T. Takarada and M. Maeda, *Small*, **2015**, *11*, 3153.

57. S. Shiraishi, L. Yu, Y. Akiyama, G. Wang, T. Kikitsu, K. Miyamura, T. Takarada and M. Maeda, *Adv. Mater. Interfaces*, **2018**, *5*, 1800189.

58. L. Yu, S. Shiraishi, G. Wang, Y. Akiyama, T. Takarada and M. Maeda, *J. Phys.*
Chem. C, 2019, 123, 15293.

59. G. Wang, L. Yu, Y. Akiyama, T. Takarada and M. Maeda, Biotechnol. J., 2018, 13, 1800090.

60. T.-Y. Yang, L. Yu, Y. Akiyama, T. Takarada and M. Maeda, Langmuir, 2020, 36, 5588.

61. C.-C. Chang, C.-P. Chen, T.-H. Wu, C.-H. Yang, C.-W. Lin and C.-Y. Chen, Nanomaterials, 2019, 9, 861.

62. J. Reguera, J. Langer, D. Jiménez de Aberasturi, L. M. Liz-Marzán, Chem. Soc. Rev., 2017, 46, 3866.

63. T. Bu, T. Zako, M. Fujita and M. Maeda, Chem. Commun., 2013, 49, 7531.

64. G. Wang, T. Bu, T. Zako, R. Watanabe-Tamaki, T. Tanaka and M. Maeda, Chem. Phys. Lett., 2017, 684, 310.

65. Y. Yano, M. Nisougi, Y. Yano-Ozawa, T. Ohguni, A. Ogawa, M. Maeda, T. Asahi and T. Zako, Anal. Sci., 2019, 35, 685.

66. C. M. Cobley, S. E. Skrabalak, D. J. Campbell and Y. Xia, Plasmonics, 2009, 4, 171.