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Nanoscale Superstructures Assembled by Polymerase Chain Reaction (PCR): Programmable Construction, Structural Diversity, and Emerging Applications

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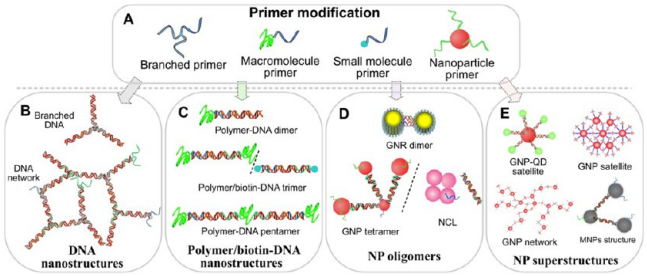

Polymerase chain reaction (PCR) is an essential tool in biotechnology laboratories and is becoming increasingly important in other areas of research. Extensive data obtained over the last 12 years has shown that the combination of PCR with nanoscale dispersions can resolve issues in the preparation DNA-based materials that include both inorganic and organic nanoscale components. Unlike conventional DNA hybridization and antibody–antigen complexes, PCR provides a new, effective assembly platform that both increases the yield of DNA-based nanomaterials and allows researchers to program and control assembly with predesigned parameters including those assisted and automated by computers. As a result, this method allows researchers to optimize to the combinatorial selection of the DNA strands for their nanoparticle conjugates.

We have developed a PCR approach for producing various nanoscale assemblies including organic motifs such as small molecules, macromolecules, and inorganic building blocks, such as nanorods (NRs), metal, semiconductor, and magnetic nanoparticles (NPs). We start with a nanoscale primer and then modify that building block using the automated steps of PCR-based assembly including initialization, denaturation, annealing, extension, final elongation, and final hold. The intermediate steps of denaturation, annealing, and extension are cyclic, and we use computer control so that the assembled superstructures reach their predetermined complexity. The structures assembled using a small number of PCR cycles show a lower polydispersity than similar discrete structures obtained by direct hybridization between the nanoscale building blocks. Using different building blocks, we assembled the following structural motifs by PCR: (1) discrete nanostructures (NP dimers, NP multimers including trimers, pyramids, tetramers or hexamers, etc.), (2) branched NP superstructures and heterochains, (3) NP satellite-like superstructures, (4) Y-shaped nanostructures and DNA networks, (5) protein–DNA co-assembly structures, and (6) DNA block copolymers including trimers and pentamers. These results affirm that this method can produce a variety of chemical structures and in yields that are tunable.

Using PCR-based preparation of DNA-bridged nanostructures, we can program the assembly of the nanoscale blocks through the adjustment of the primer intensity on the assembled units, the number of PCR cycles, or both. The resulting structures are highly complex and diverse and have interesting dynamics and collective properties. Potential applications of these materials include chirooptical materials, probe fabrication, and environmental and biomedical sensors.

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1. Introduction

DNA is well-suited for its own replication, the directed assembly of building blocks into various nanostructures for its physical and chemical stability, and ability to tolerate a variety of modifications.[1] Scaled-up production of highly organized nanomaterials with a predesigned blueprint is essential for sensing and optoelectronic materials and devices.[2,3] PCR as a classical basic tool in both research and analytical laboratories has been widely used and can amplify several orders of magnitude of a target DNA sequence with high accuracy and specificity. Typically, PCR consists of a series of 1−40 repeated temperature cycles, with each cycle commonly consisting of three discrete temperature steps, namely, denaturation (94−98 °C), annealing (50−65 °C), and extension (72−80 °C) (Figure 1). The polymerase binds to the primer−template hybrid and begins DNA synthesis in the 3′ to 5′ direction by adding deoxyribonucleoside triphosphates (dNTPs) to the template.[4] The primer-attached building blocks subjected to PCR amplification will be assembled. The important feature is that PCR can generate double stranded (ds) DNA with much higher molecular weights than chemical solid phase synthesis. The advantage of PCR assembly is programmability and controllability through design of primer and structure, cycling regime, DNA quantity, length, and sequence.

Various size-dependent properties can be enhanced and complemented by DNA, which provides the possibility to make unique structures, hybrid nanosystems with new functionalities. However, the challenge of efficient assembly of nanomaterials combining simplicity and variability remains. Combined with PCR amplification, various building blocks can be assembled: the organic motifs including small molecules (biotin, fluorescence labels, etc.)[5,6] and macromolecules (DNA, polymers, etc.).[7,8] Inorganic nanomaterials are used in a variety of fields by virtue of their unique electronic, magnetic, energy, optoelectronic, and catalytic properties.[9,10] Noble metal NPs and NRs,[11] semiconductor NPs,[12] and magnetic NPs[13] represent essential building blocks for PCR-based assemblies. The current status of applications for PCR assembly includes optical materials and sensing .[14−17] Geometrical chirality at different scales and polarization rotation in visible and infrared parts of the electromagnetic spectrum contribute to their unique set of properties.[14,15] PCR with high amplification efficiency can produce a large quantity of DNA in a short time, which is important for large scale probe fabrication and chemical signal amplification to develop ultrasensitive detection technology.[16,17] We review in this Account the current status of PCR assembly specifically as a tool for engineering of nanometer scale assemblies and superstructures, control of their organization, and applications.

2. Building Block Modification for PCR Amplification

2.1. NP Surface Chemistry and Primer Modification. Commonly used exchange ligand molecules with thiol groups and desired functional group (−COOH, $-NH_2$, etc.) can bind strongly to surfaces of inorganic NPs, for example, Au and Ag NPs, semiconductor NPs, or quantum dots (QDs), replacing the weaker bound ligands that the nanoparticles usually obtain originating from their synthesis. The functional group can be used with conjugation chemistry to modify the primer DNA.[12] DNA primer with a thiol group can be attached directly to Au NPs and CdSe/ZnS NPs by ligand exchange and aged to make the strand stretch. Magnetic NP−primer complexes can be prepared using 5′-end modified primer and thiol-functionalized magnetic NPs via S−S bonds.[18] The initial reaction ratio of DNA to NP can also help to have different quantities of DNA loading on the surface.[12]

2.2. Organic Molecules Modification. Conjugation chemistry was also mostly used to conjugate biomolecules to DNA. The *N*-hydroxysulfosuccinimide (NHS−biotin) was usually used as biotinylation reagent to react with primary amines and form stable amide bonds at pH 7−9.[5] For polymer modification with DNA, hydroxy-group-terminated polymers usually reacted with phosphoramidite chloride to yield phosphoramidite−polymer derivatives, which were then coupled to the 5′-end of the DNA on the solid support for primer modification.[19]

3. The Assembly of Functional Nanomaterials Based on PCR

The other enzyme catalytic approaches by enzymatic restriction, ligation, and rolling circle amplification (RCA) are alternative assembly methods to construct nanoarchitectures and have different characteristics compared with PCR.[20,21] This Account discusses characteristics of PCR assembly and applications with representative examples from our group with potential future perspectives. We also highlight the important accomplishments from others.

3.1. PCR-Assembled Bionanostructures. 3.1.1. PCR-Based DNA Assembly. DNA itself represents a nanoscale material that can be directly assembled into various important nanostructures including branched DNA.[22] PCR can construct three-dimensional DNA-based networks by using covalently connected bDNA Y-motifs as branched primers.[7] These PCR assembled DNA networks were made by branched

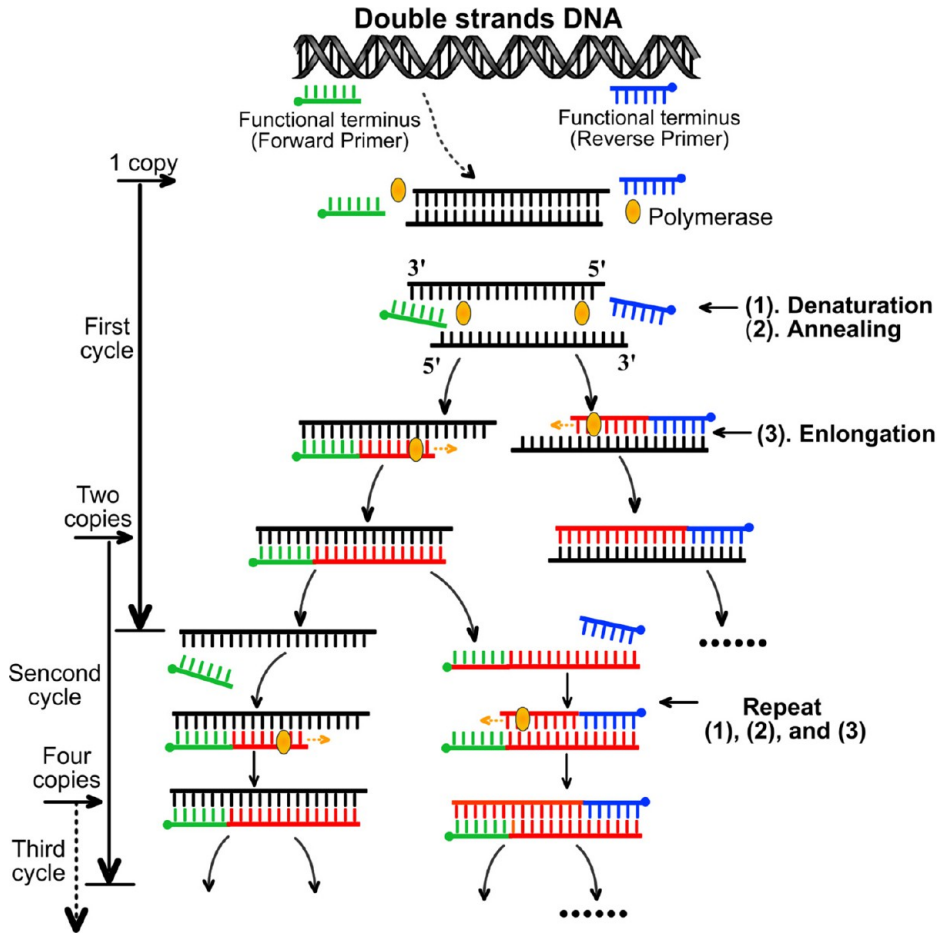

FIGURE 1. Schematic of PCR procedure. Typically PCR is performed with template DNA, forward and reverse primers, DNA polymerases, dNTPs, and cycled temperatures steps.

primer (I) and reverse primer (II) strands and suitable templates (Figure 2A,B). Extended branched primer strands were formed after the first cycle (Figure 2B, II), also forming DNA networks (Figure 2B, III). The approach is flexible, allowing tuning of the meshes of the network by variation of the template size. Further progress could allow access to useful DNA material. The DNAzyme dimer can be fabricated by PCR. The polymerization process resulted in the hairpin structure zipping off and generated the G-quadruplex DNAzyme that could yield colorimetric or chemiluminescent readout signals (Figure 2C).[23]

3.1.2. PCR-Based Copolymer Assembly. Block copolymers are attractive materials with variable and predictable morphologies.[24] The DNA block copolymer assembly by PCR has been described with DNA covalently attached to an organic polymer backbone.[8] Linear block copolymers have been introduced that contain DNA as a biological segment covalently linked to synthetic polymer units (Figure 2D,E).[25] For the preparation of DNA block copolymers (DBCs) with an extended double-stranded nucleic acid block, PCR in combination with ss-DBCs has been employed, yielding diblock, triblock, and pentablock architectures (Figure 2E). This synthetic route is comparable to the restriction and ligation method to yield well-defined biocompatible materials.[20,26] However, the use of PCR dynamic assembly of copolymers as an amplification tool for biosensing has not been exploited.

3.1.3. Functional Small Molecules Involved Assembly. Biotin-labeled oligonucleotides are routinely used in molecular biology.[27] Bis-biotinylated double-stranded (ds) DNA can be made by PCR and further assembly into a DNA–streptavidin linear structure,[28] which can be used for detection applications.[29] With forward and reverse primers labeled by biotin and a fluorescent dye, PCR can produce biotin–fluorescent chromophore hybrids.[6] PCR enzymatic incorporation of fluorophores into a growing DNA strand enables parallel, simultaneous observation of single-molecule reactions, which allows for real time sequencing.[17] The polymerization-assisted accumulation of small molecules could be adopted as amplified probes.

3.2. PCR-Based NP Assembly. Inorganic NP assembly is becoming more important for various applications.

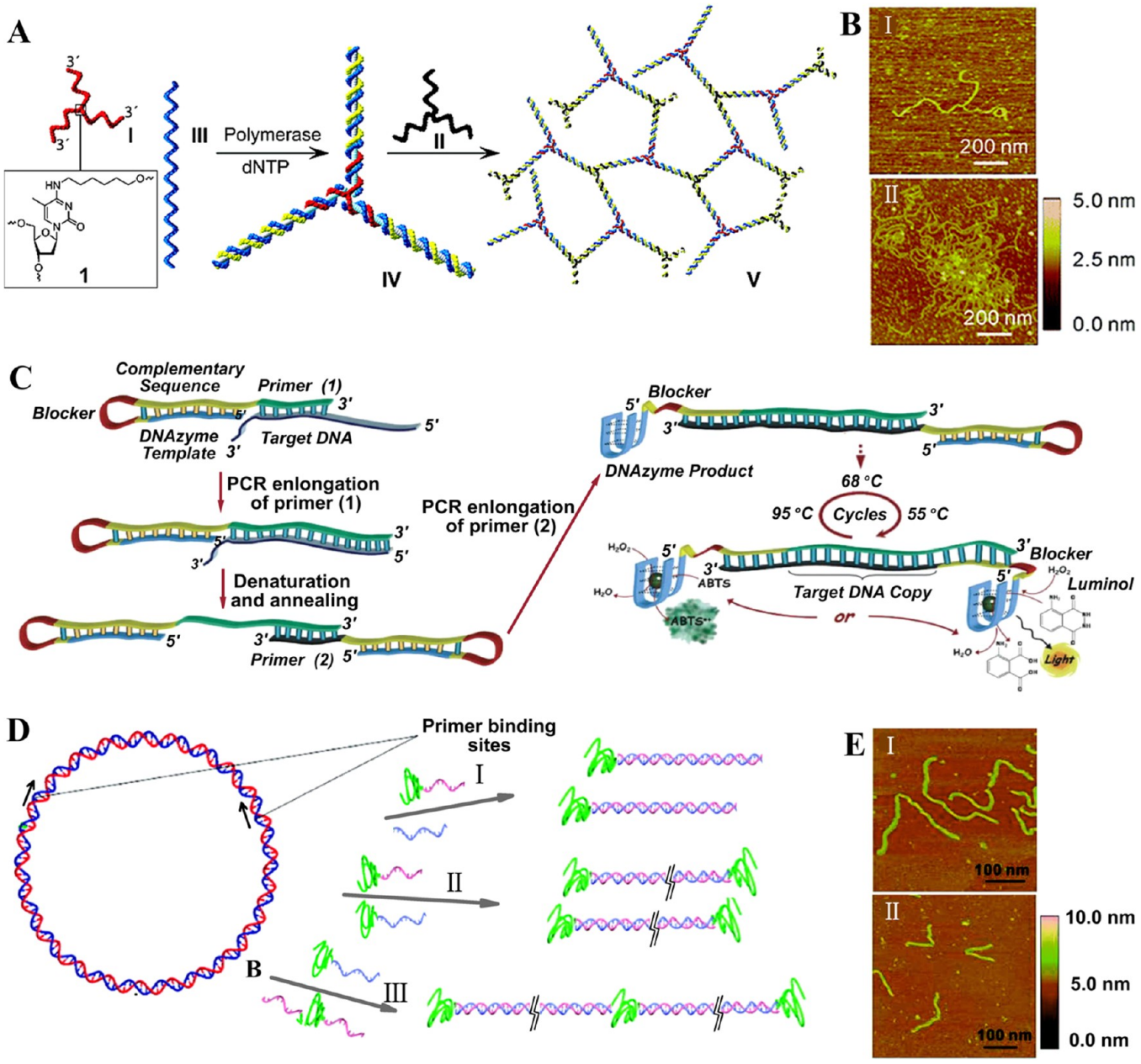

FIGURE 2. (A) Schematic of DNA network generation by PCR using branched primer strands.[7] (B) (I) first cycle reaction product using **I** and **II**; (II) DNA networks generated after 40 PCR cycles. (C) Scheme of replication of G-quadruplex DNAzyme-containing units by PCR.[23] (D) Schematic of the build-up of (I) DNA diblock, (II) DNA triblock,[8] and (III) DNA pentablock copolymers by PCR. (E) SFM images of DNA pentablock copolymers:[25] (I) DNA (500 bp)-*b*-PEG-*b*-DNA (500 bp); (II) DNA (225 bp)-*b*-PEG-*b*-DNA (225 bp).

Despite indirect Au NP assembly, two PCR procedures can be designated, namely, asymmetric PCR and symmetric PCR. For asymmetric PCR, only one primer is bound to the NPs and nanoparticles bearing double-strand DNA will be generated, including Au NPs,[30] magnetic NPs,[18,31] and copolymer NPs.[32] This asymmetric PCR can be an efficient tool for preparation of nanoparticle−DNA hybrids for further applications, for example, as nanoprobes. Symmetric PCR will be introduced in the following part.

3.2.1. Preparation of Nanomaterials with PCR Amplification. There are three parameters that can be modulated in the control of PCR assembly, including the primer, the number of cycles, and the template DNA, which have been investigated in Au NPs,[11,14,33,34] in Au NPs and QDs,[12] and in a magnetic NP assembly system.[13]

(1) The number of cycles, *N*. The *N* was an important factor for control over assembly. With increasing *N*, more NPs assembled in the course of the PCR process (Figure 3A).[14]

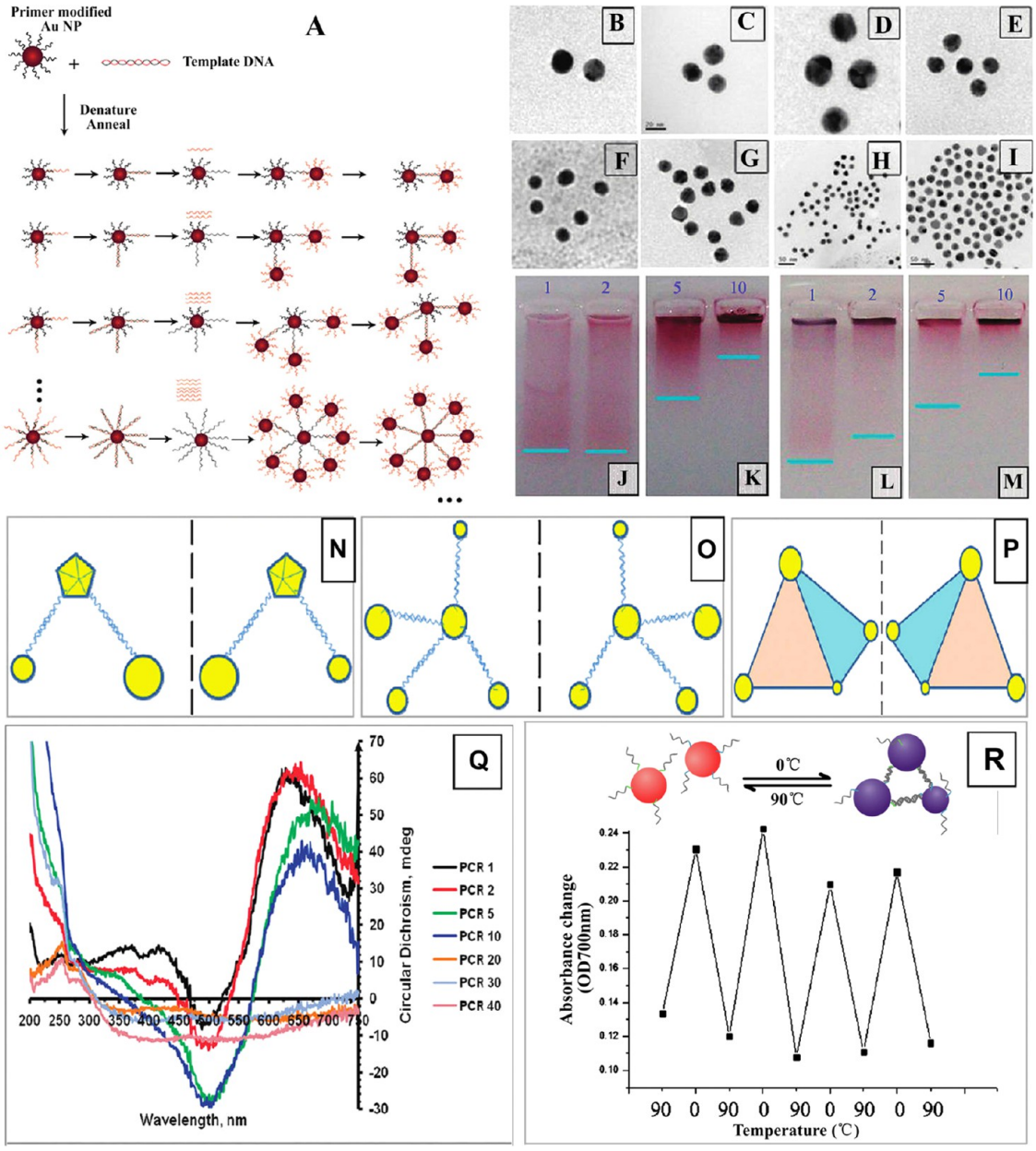

FIGURE 3. (A) Schematics of Au NP assembly by PCR.[14] (B–I) Characteristic TEM images with increase in the number of cycles. (J, K) Low and (L, M) high primer concentration with different cycles. (N–P) Schematics of chiral NP structures. (Q) Circular dichroism spectra of PCR products with different cycles. (R) The absorbance at 700 nm vs temperature/time profile.

From agarose gel electrophoresis results, the specific multimer geometry was not observed for $N = 1-5$, which means that the structure in the early stages is comparatively simple, and this is confirmed by TEM examination (Figure 3B–I): the increase of N results in the increase of the average number of NPs. Similarly reported by Cai et al,[11] the UV–vis spectral shift increased when N was increased from 5 to 20 in which the assembly degree was gradually increased.

(2) Primer. The primer density on the NP surface is another factor for PCR assembly.[14,33,34] The results for low and high densities of primer on Au NPs confirm this point (Figure 3J–M). For high primer concentrations, after five cycles the assembled structures include not only dimers but also some trimers and multimers. For a low primer concentration, the PCR products are dominatly monomers, dimers, and trimers, which was supported by gel electrophoresis (Figure 3J–M). High yield of gold core/shell NP dimer was achieved by controlling one primer on the NP surface (Figure 6). The geometry of assembled architectures can also be controlled by primer as reported for satellite-like assembly.[12] The Au NPs had a

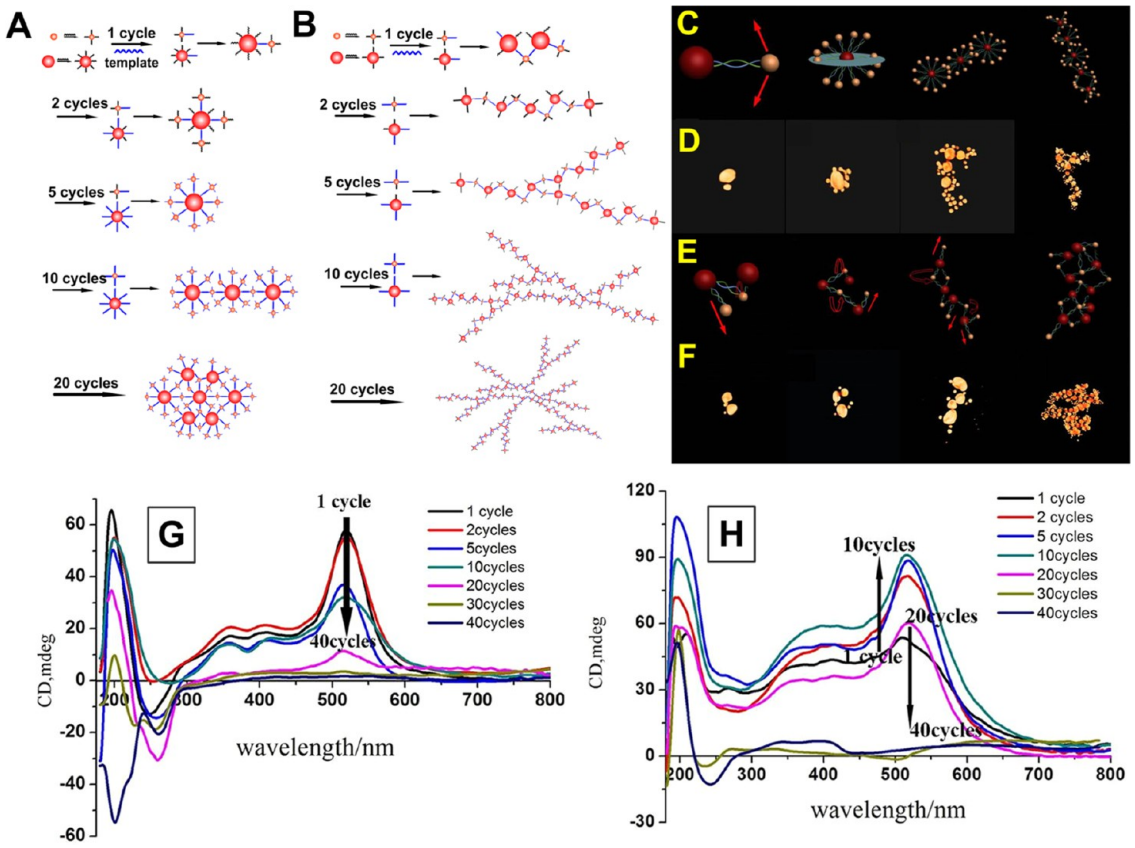

FIGURE 4. Schematics for self-assembly of Au NPs heterogeneously by PCR:[33] (A) asymmetric assembly PCR; (B) symmetric assembly PCR; (C−F) schematic models and corresponding tomography reconstruction from asymmetric PCR (C, D), and symmetric PCR (E, F); (G, H) circular dichroism spectra of superstructures created by asymmetric assembly PCR (G) and symmetric assembly PCR (H).

relatively large diameter of 18 nm, while the QDs mostly had diameters of less than 5 nm; hence the quantity of primer on Au NPs was relatively high while that anchored on the QDs was relatively limited.

(3) Template DNA. For a certain *N*, the number of particles involved in the assembly will increase with the starting template DNA concentration.[14] In fact, the DNA concentration increases with *N* according to an exponential function written as $X = X_0(1 + E_x)^N$, where X is the template DNA concentration, X_0 is the starting DNA template concentration, and E_x is the real amplification efficiency. As can be seen, the number of particles will increase with both the starting template DNA concentration and *N*. The parameter-dependent assembly can be particularly useful and important for developing biosensors.

3.2.2. PCR-Based Au NPs Assembly. There are two general strategies for PCR-based Au NP assembly including non-cross-linked (NCL) assembly and linked assembly.

(1) NCL Au NP assembly. Single-stranded DNA (ss-DNA) has strong attractive electrostatic interactions with citrate-coated Au NPs in which the adsorption of ss-DNA to the Au NPs enhances their repulsion and prevents aggregation.[35] When Au NPs are exposed to the mixture of probe and PCR-amplified DNA, the salt in the hybridization solution causes immediate Au NP assembly if the probes have hybridized to the amplified DNA target. This assembly method has been applied to single-base substitution detection.[16] PCR-coordinated "label-free" assembly can be a promising strategy for faster, simpler, and cheaper biosensors.

(2) PCR-based Au NP linked assembly. The most notable superstructures are from NPs modified with DNA.[36] This type of assembly demonstrates a new type of 3D superstructure from NPs (Figure 3). The complexity of the self-assembly (SA) material and the average number of nearest neighbors is controlled by *N*, primer, and template DNA. Our group also developed PCR to fabricate complex gold superstructures from heterogeneously sized gold NPs (Figure 4).[33] By control of *N* and primer density, two distinct superstructures were made by asymmetric and symmetric assembly PCR. This method allows assembly of small Au NPs around the large NPs (symmetric assembly PCR) and periodic branched NP structures consisting of small and large NPs (asymmetric

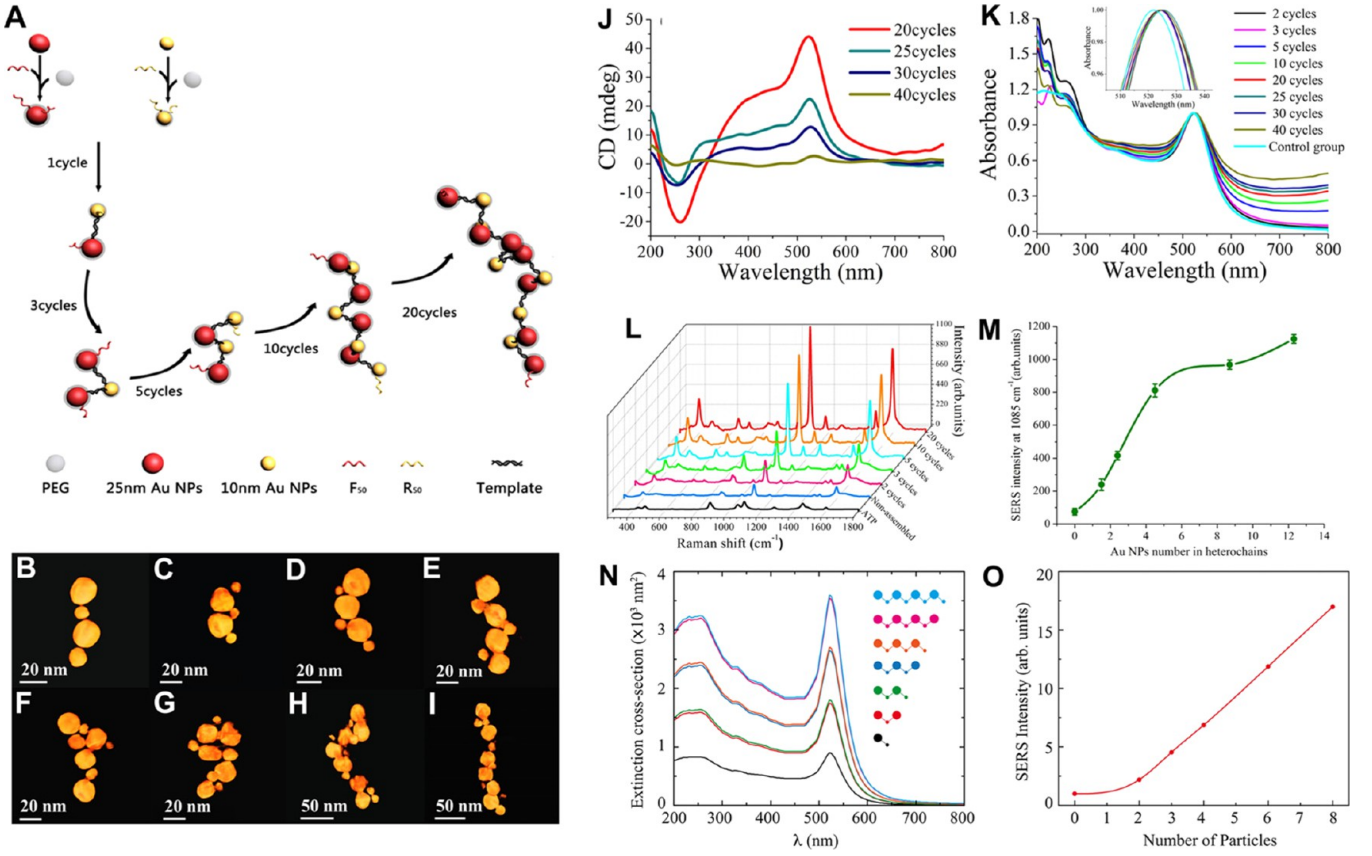

FIGURE 5. (A) Schematic illustration of heterochains assembled by PCR.[34] (B–I) Tomography images of heterochains. (J, K) The CD spectra (J) and UV–visible spectra (K) of heterochains. (L) The SERS spectra of heterochains. (M) Dependence of the Raman intensity at 1085 cm^{-1} on the number of NPs. (N) Calculated extinction cross-section of heterochains. (O) Simulated increases in SERS signal associated with the numbers of particles.

assembly PCR). Wang's group studied a case in which their majority was placed on gold aggregation for sensors.[12] Recently, PCR-based Au NP assembly was reported in which the amplified DNA further hybridized with two different gold nanoparticle–DNA probes to have selective assembly.[37]

Alternating 25 and 10 nm Au NP heterochains were successfully assembled by PCR (Figure 5). The length of each heterochain was also increased with PCR cycles. The heterochains displayed tunable plasmonic chirality and strong surface-enhanced Raman scattering (SERS) activity. These optical properties represent collective effects of NP superstructures and can be controlled by the number of PCR cycles.[34] The tunable strong optical response of the NP heterochains enables potential for biosensing applications.

Au NRs dimer with side-by-side (SBS) patterns based on PCR was fabricated by changing the surface chemistry of the nanorods.[38] The SBS dimer displayed characteristically strong bisignated CD signals corresponding to the plasmon bands of the nanorods, exhibiting good agreement with the simulations (Figure 6). The mechanism of chiral conformation comes from symmetrical breaking of the angled NR dimer conformation. The kinetic transitions of bands in CD corresponded well with the plasmon bands in absorbance. The chiral NR pairs and "ladders" enable the development of new liquid metamaterials and biosensors.

PCR-based Au NP assembly is the initial important part since they have strong surface plasmon resonance (SPR) properties with enhanced collective interaction. Other anisotropic gold nanomaterials (nanoprism, dodecahedra and octahedra, etc.) contributed even more important and fascinating assembly areas, which should benefit PCR assembly.[3]

3.2.3. Au NP–QD Complex Assembly and Magnetic NP Assembly. The investigation of the interactions between highly luminescent QDs and Au NPs embedded in controlled assemblies and how these interactions could affect the optical properties remains an interesting subject. Our group has also developed PCR-based complex assembly of Au NPs and QDs, whereby a satellite-like geometry structure was produced with QDs assembled around Au NPs (Figure 7).[12] The photoluminescence quenching in these architectures was closely related to the amplification length, which potentially indicates sensor applications. The assembly of magnetic NPs

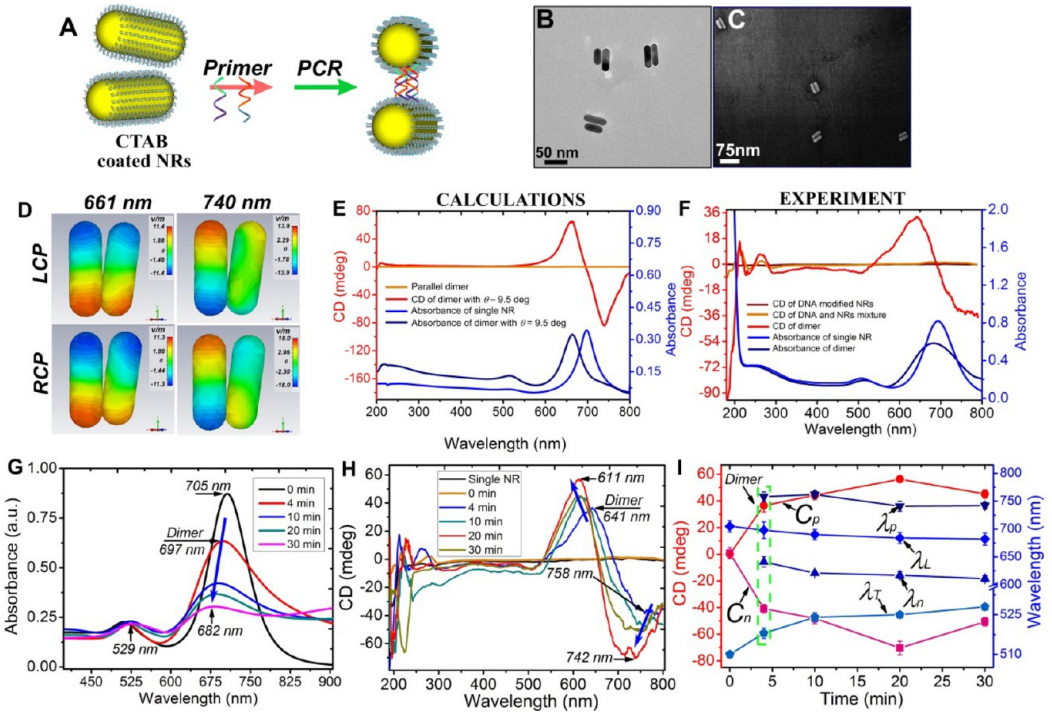

FIGURE 6. (A) Schematics of gold NRs dimers made by PCR.[38] (B, C) TEM and SEM images of NR dimer. (D) Surface E-field distribution of NR dimer. (E, F) Experimental data (E) and corresponding simulation results (F) for NR dimer. (G–I) UV–vis (G) and CD spectra (H) of NR assemblies and their profiles (I) at characteristic wavelengths.

represents another type of assembly with interesting collective magnetic interaction.[39] PCR-based assembly of γ-Fe_2O_3@SiO_2 NPs had been reported.[13] However, this work merely represents an initial step of magnetic NP assembly by PCR. Magnetic relaxation switch difference from dispersed and assembled states of magnetic NPs can be employed for sensors; if assembled by PCR, they should be sensitive considering the high amplification efficiency.

3.2.4. Performance of PCR Assembly Systems and Practical Issues. 3.2.4.1. Nanoparticle Effects on Amplification Efficiency and Specificity of PCR. Various nanomaterials have different impacts on PCR.[5] A high concentration of NPs slightly affected the PCR yield due to high adsorption. Typically, gold NPs can improve the efficiency of PCR, while gold NRs and QDs can increase the specificity by minimizing mispairing between the primers and templates. These beneficial effects may be attributed to the heat transfer property of NPs. However, the adsorption of primer-modified NPs could be minimized, and the concentration could be adjustable to achieve high PCR efficiency and specificity.

3.2.4.2. Stability, Yield, and Purification of PCR Assembled Products. The products from PCR assembly showed different stability against salt and heat, which was dependent on the stabilizer on NPs. The amplified DNA on the NP surface improved stability of the assembled superstructure; for example, PCR-based Au NP linked assembly had relative higher stability compared with NCL assembly, which was triggered fast under ionic strength.[16] The QDs and MNPs were less stable in PCR buffer or repeated temperature regimes. The DNA or other ligand-functionalized NP dispersion can reduce nonspecific aggregation.

The yield of products was variable for different PCR assembly systems and can be increased by purification technologies. The purification of PCR products was important to the specific structures, for example, the dimer, trimer, or multimer dominant products had been reported by gel electrophoresis,[14] and the yield of diblock copolymer was enhanced by centrifugation.[25] Additionally, the challenges to have high yield PCR products were also dependent on DNA engineering technologies. Higher yield structures can be achieved by controlling the number and region-specific modification of DNA on NPs.

4. Applications of PCR-Based Nanomaterial Assembly

PCR assembly combined diverse geometries and signaling modalities with specific molecular recognition of DNA. We further investigated the applications of optical materials based on chiral superstructures,[14,33,34] probe fabrication,[15,40,41] colorimetric sensors,[11,15,28,42] and fluorescence sensors.[12,17]

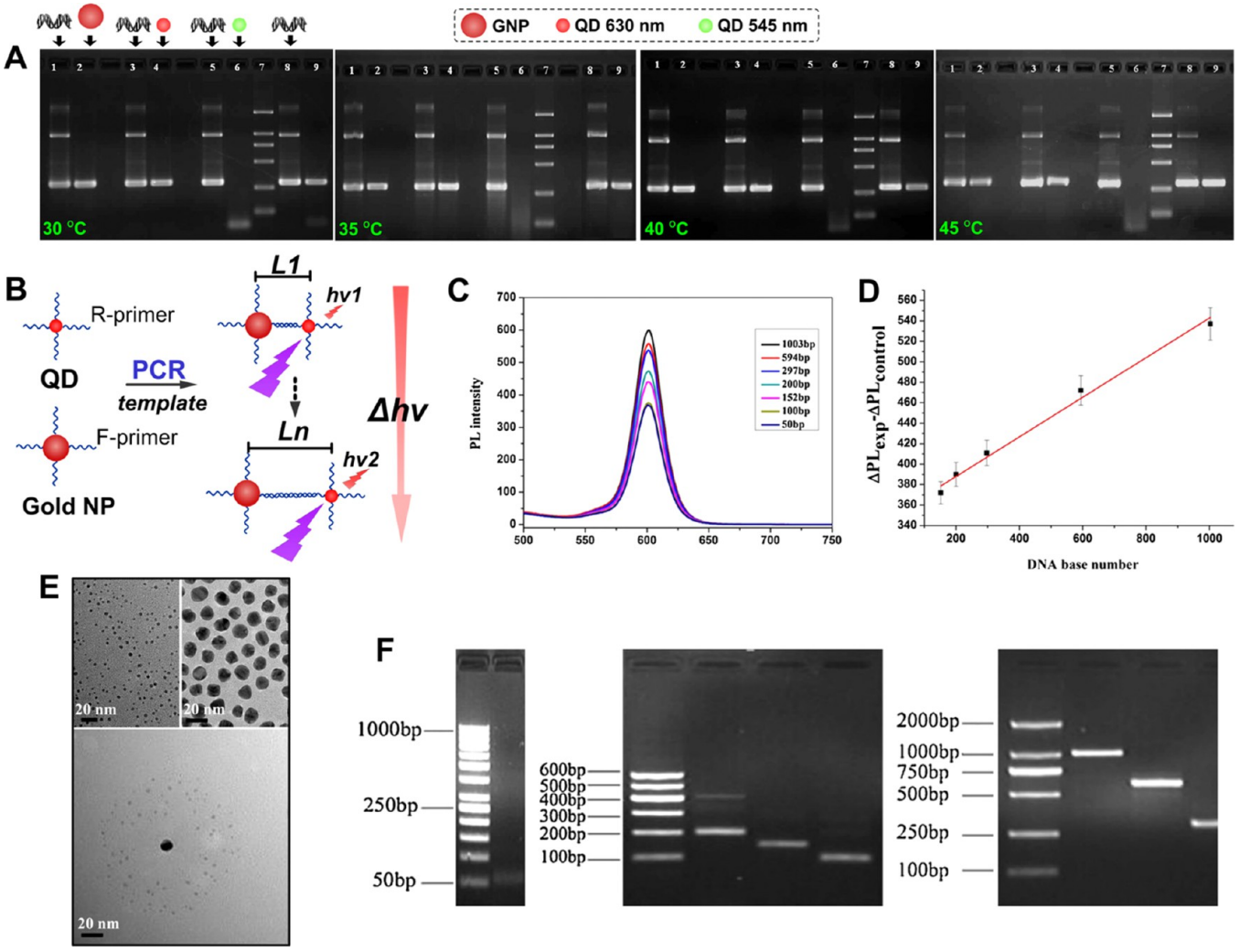

FIGURE 7. (A) Elimination effects of the QDs and gold NPs at different annealing temperatures.[4] (B) The complex assembly of Au NPs and QDs by PCR.[12] (C) The fluorescent spectrum of the PCR products. (D) The calibration curve of the DNA detection. (E) The TEM images for QDs, Au NPs, and the assembled satellite structure. (F) Agarose gel electrophoresis of PCR products with different amplification lengths.

4.1. Plasmonic Chirality and Optically Active Materials. Considering the relative rigidity of duplex DNA strands in PCR-assembled superstructures, the oligonucleotide bridges connecting the 3D structures can be chiral, as demonstrated in Figure 3. Some of the possible arrangements of NPs (Figure 3) have strong similarity with well-known chiral organic molecules in tetrahedral spatial organization, which has circular dichroism (CD) signal and changes with *N* (Figure 3).[14] The superstructures showed strong CD signal (65 mdeg) and typical "S" shaped CD signal at the plasmonic band of Au NPs around 550 nm. Quite different, the PCR-based heterogeneously sized gold NP assembly showed much stronger positive CD at the plasmonic band of Au NPs. The Cotton effect changed due to the size effect (Figure 3Q–R). The stronger CD should come from the symmetric breaking effect.[33,34]

Superchirality shall be important for novel sensors utilizing strong polarization rotation. DNA ultrasensitive sensors could be developed based on CD signal in our Au NP assembly system.[43,44]

4.2. Applications in Sensing. Prepared from biotin-labeled primers, DNA–streptavidin (STV) oligomers and antibody-modified DNA–STV oligomers were used as reagents in immuno-PCR (IPCR) for sensitive detection of proteins and other antigens and led to 100-fold enhanced sensitivity compared with conventional IPCR.[40] Biotin primer extended DNA can act as a magnetic capture hybridization probe for selective DNA purification, followed by PCR identification for rapid and sensitive detection of *Listeria monocytogenes* from milk.[5] Further probe applications in separation, sensors, and assembly can be practically useful.

4.3. Colorimetric Sensors. 4.3.1. Nanoswitch Thermometer. The most important feature of Au NPs is their optical

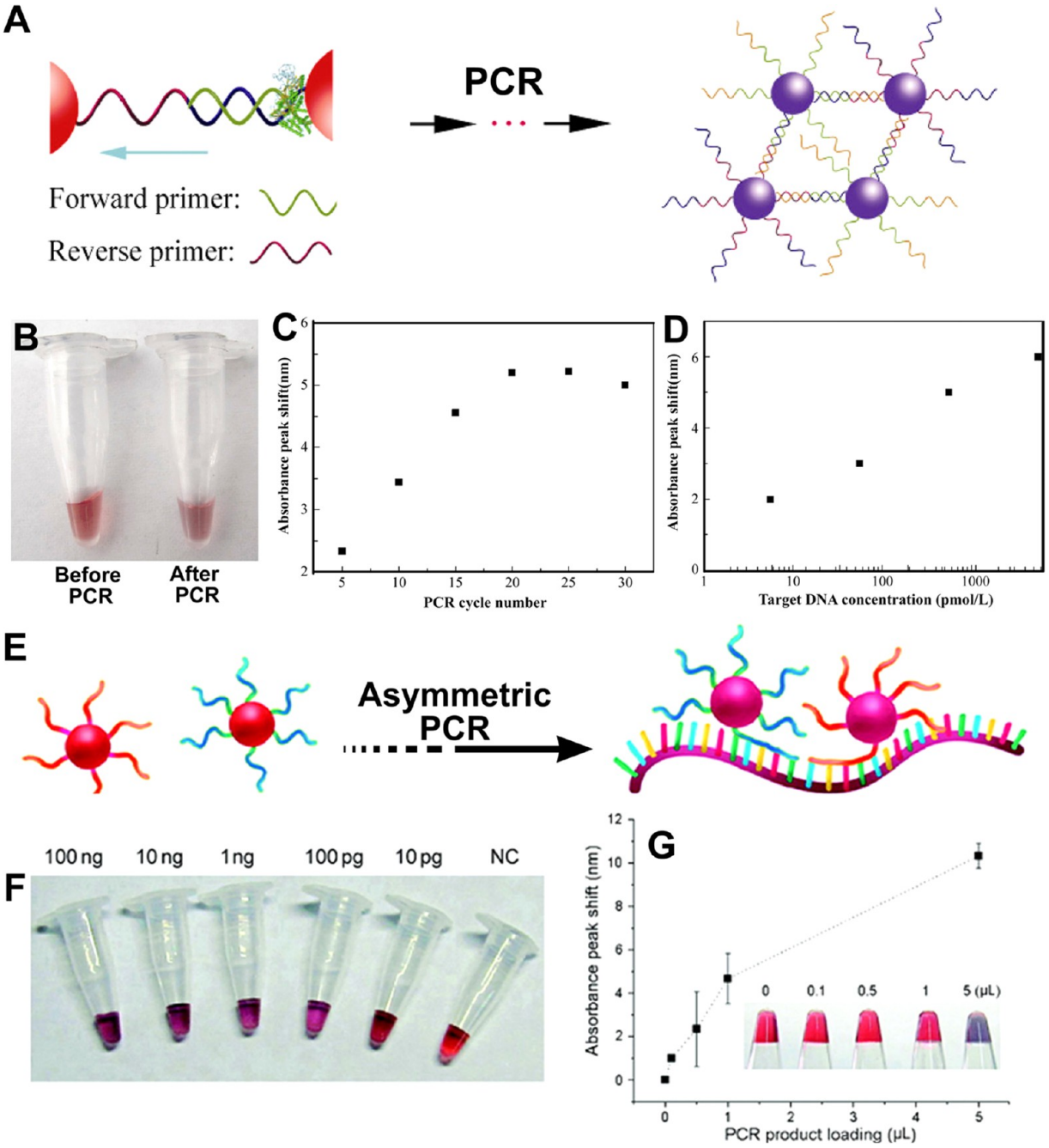

FIGURE 8. (A) PCR-based gold NP assembly for DNA detection. (B) Photographs of the PCR mixtures.[11] (C, D) Correlation of the absorbance peak shift with PCR cycles (C) and DNA concentrations (D). (E) Asymmetric PCR-based gold NP assembly for DNA detection.[37] (F) Photographs of PCR assembly mixtures. (G) Correlation of PCR loading volume and absorbance peak shift.

properties related to plasmon coupling interactions, which can be employed as a colorimetric readout in sensors where the color is closely related to the dynamic assembly. As-prepared PCR assembly products with integrated DNA can be thermally switchable between hybridization and dissociation (Figure 3R).[14] When the temperature was cycled between 0 and 90 °C, a dynamic increase and decrease in absorption at 700 nm was observed. The combination of this property with plasmon resonance interactions that display an optical output represents the foundation of a new family of sensing and optoelectronic devices.

4.3.2. DNA Target Detection. DNA biosensors with high sensitivity and specificity produced by PCR-based Au NP assembly have been reported (Figure 8A–D).[11] The assembly of Au NPs causes a significant shift in the extinction spectrum as the solution color changes from red to purple (Figure 8B). The

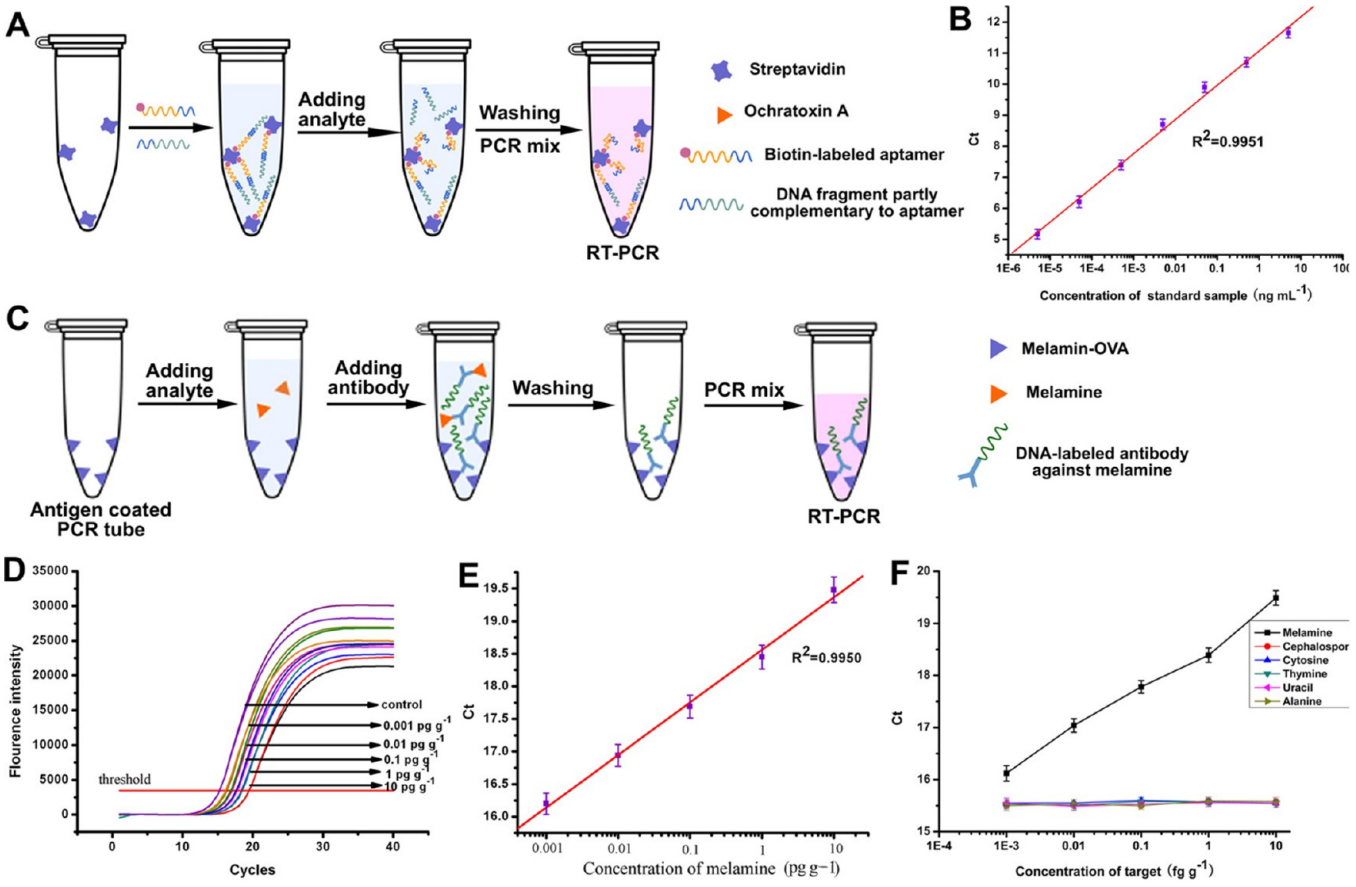

FIGURE 9. (A) Schematics for ochratoxin A detection by PCR.[45] (B) The calibration curve of OTA detection. (C) Schematics for MEL detection by PCR.[46] (D) The amplification curves at each concentration of MEL. (E) The calibration curve of MEL detection. (F) Ct value relationship between melamine and other targets.

detection sensitivity was 0.1 fmol by UV/vis spectroscopy (Figure 8C,D). Similarly, the asymmetric PCR coupled gold NP assembly method produced similar sensitivity (10 pg) for DNA (Figure 8G).[37] Linear amplification minimizes the potential risk of PCR product cross-contamination. The efficiency to detect *Bacillus anthracis* in clinical samples indicates the practical applicability. The quantitative analysis of M13 phage DNA by colorimetric signal from PCR-generated G-quadruplex DNAzymes was achieved with sensitivity of 90 molecules in 50 μL sample.[23]

4.3.3. Single Base-Pair Mismatch Detection. The NCL assembly by PCR became useful for fast single base-pair mismatch detection in less than 10 min. The anti-tag sequence labeled on the Au surface for NCL assembly has also been reported.[16] The NCL assembly exhibits extraordinary selectivity against terminal mismatches; single-base mismatches at the free ends of the duplexes make very stable dispersions.

4.4. Fluorescence Sensors. 4.4.1. Small Molecule Detection. A new type of biosensor based on PCR using an aptamer was developed to detect ocratoxin A (OTA). A complementary DNA fragment to the aptamer was used for the real-time quantitative PCR amplification (Figure 9A,B).[45] Similarly, by using a DNA-labeled antibody to recognize melamine (MEL) and amplified DNA as the signal reporter, a PCR based immunosensor was developed for the detection of MEL (Figure 9C−F).[46]

4.4.2. DNA Detection. Fluorescence-based assays take advantage of sensitive, rapid, and versatile methods for monitoring fluorescence signals.[47] We have presented Au NP and QD hybrid assemblies for DNA detection.[12] Generally, the fluorescence intensity was gradually quenched with decreasing length of the amplified sequence in a linear relationship in a range of 150 bp to 1003 bp. The lower limit of effective length of target DNA is ≥136 bp (3σ). This method could be a powerful tool for rapid DNA detection and potentially applied in single nucleotide polymorphism analysis.

4.4.3. Real-Time Detection. PCR enzymatic incorporation of fluorophores to the terminal phosphate moiety into a

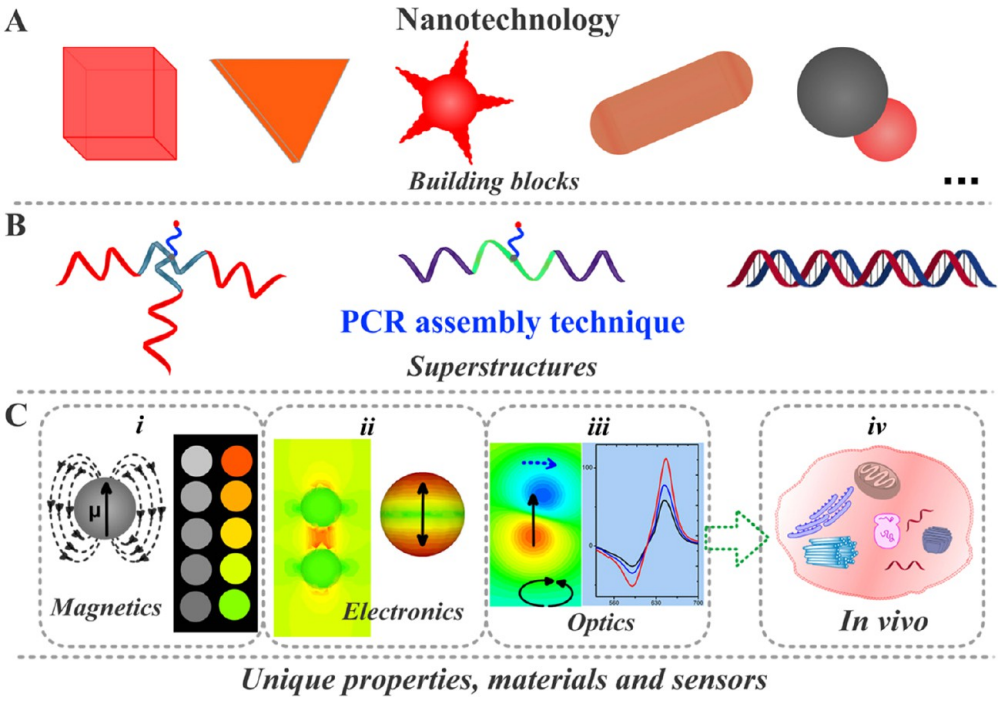

FIGURE 10. Perspectives of PCR-based nanoassembly. (A) Advanced nanotechnology provides promising complex and hybrid nanomaterials. (B) The well-developed PCR technique with sophisticated primer and templated DNA engineering endow efficient PCR assembly. (C) The unique properties and applications shall constitute the future aspects of PCR assembly fields.

growing DNA strand with nanostructure arrays provides a single-molecule, real-time sequencing method.[17] The sequence is determined by detecting fluorescence from binding of correctly base-paired phospholinked dNTPs in the active site of the polymerase. Combining the level of assembly with the high intrinsic speed and read length of single molecules, real-time DNA sequencing will enable low-cost rapid genome sequencing.

5. Summary and Outlook

The PCR assembly strategy alternatively provides an efficient and straightforward method for engineering nanoparticle assembly and sensor fabrication characteristics of controllability and high sensitivity. PCR's ability of patterning nanoscale building blocks with deliberate designs gives it tremendous potential for answering many outstanding challenges. For instance, (1) taking advantage of the easy manipulation and low-cost, primers should be designed for bioactive DNA nanostructures, such as DNA origami scaffolds with surprising stability in cell lysate; (2) thanks to the high efficiency and specificity of the amplification, large-scale assemblies with tunable yield with controlled spatial arrangements and site-specificity could connect the resulting nanoarchitectures to various applications;[48−51] (3) unique tunable properties of nanoscale assemblies should be exploited for sensors to meet the analytical goals of "3S+2A" (sensitivity, selectivity, speediness, accuracy, and automation).

Further chemical modifications, primer/DNA structure, and enzyme engineering endow PCR assembly with the capacity for linking of complex, hybrid nanoparticles, which can give assemblies with multiple functionalities and multisensing abilities and finally develop new diagnostic technologies (Figure 10). For example, preferential binding of surface ligands to Au NRs would allow for different assembly patterns by PCR, which would demonstrate interesting optical properties for new detection methods. The PCR assembly of nanostructures will need researchers to address some important challenges in biology, chemistry, physics, medicine, and engineering. Those future challenges include (1) construction of functional NPs with complex geometries and their primer attachment and (2) connection of the resulting nanoarchitectures to the macroscopic world for application.

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FOOTNOTES

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