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Functional DNA Nanostructures for Theranostic Applications

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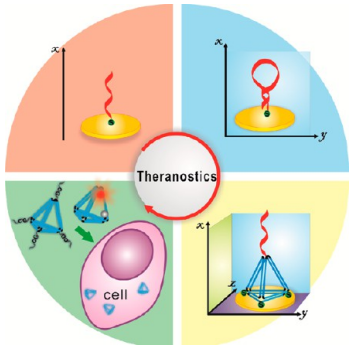
CONSPECTUS

There has been tremendous interest in constructing nanostructures by exploiting the unparalleled ability of DNA molecules in self-assembly. We have seen the appearance of many fantastic, "art-like" DNA nanostructures in one, two, or three dimensions during the last two decades. More recently, much attention has been directed to the use of these elegant nanoobjects for applications in a wide range of areas. Among them, diagnosis and therapy (i.e., theranostics) are of particular interest given the biological nature of DNA.



One of the major barricades for the biosensor design lies in the restricted target accessibility at the solid−water interface. DNA nanotechnology provides a convenient approach to well control the biomolecule-confined surface to increase the ability of molecular recognition at the biosensing interface. For example, tetrahedral DNA nanostructures with thiol modifications can be self-assembled at the gold surface with high reproducibility. Since DNA tetrahedra are highly rigid and well-defined structures with atomic precision and versatile functionality, they provide scaffolds for anchoring of a variety of biomolecular probes (DNA, aptamers, peptides, and proteins) for biosensing. Significantly, this DNA nanostructure-based biosensing platform greatly increases target accessibility and improves the sensitivity for various types of molecular targets (DNA, RNA, proteins, and small molecules) by several orders of magnitude.

In an alternative approach, DNA nanostructures provide a framework for the development of dynamic nanosensors that can function inside the cell. DNA tetrahedra are found to be facilely cell permeable and can sense and image specific molecules in cells. More importantly, these DNA nanostructures can be efficient drug delivery nanocarriers. Since they are DNA molecules by themselves, they have shown excellent cellular biocompatibility with minimal cytotoxicity. As an example, DNA tetrahedra tailored with CpG oligonucleotide drugs have shown greatly improved immunostimulatory effects that makes them a highly promising nanomedicine. By taking them together, we believe these functionalized DNA nanostructures can be a type of intelligent theranostic nanodevice for simultaneous sensing, diagnosis, and therapy inside the cell.

1. Introduction

DNA nanostructures are artificial self-assembled nanoscale objects that are rationally designed by exploiting the precise base-pairing ability of nucleic acids.[1] Since the pioneering work of Seeman in the early 1980s,[2] there has been tremendous interest in creating DNA nanostructures with controlled geometries and topologies.[3−5] DNA has a simple and precise self-recognition rule of A−T and G−C pairings; therefore it is easy to hold together multiple double-helical domains via the interchange of DNA backbones. The structural features of nucleic acids form the basis of constructing DNA nanoarchitectures with well-defined sizes and shapes, with flexible regions of single-stranded DNA (ssDNA) and rigid regions of double-stranded DNA (dsDNA). In addition, targeted insertions and deletions of nucleic bases paves the way to control of flexibility and stress of DNA nanostructures to engineer complex shapes with twists and curves.[6] More recently, the rapidly emerging DNA origami technology offers new opportunities for the construction of complex DNA structures.[7] DNA origami nanostructures could be further used as modular elements to form periodic and aperiodic supernanostructures, making it possible to design

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complex nanostructures in one, two, and three dimensions of virtually any shape.

DNA nanostructures can be readily functionalized with a variety of molecules and nanoparticles with nanometer-scale addressability.[8] Hence, it is possible to construct artificial functional systems by using DNA nanostructured scaffold with various decorations. For example, several groups developed nanoscale bioreactors by anchoring a pair of enzymes in predefined positions on DNA nanostructures, which showed improved cascade efficiency of the enzymes.[9,10] Likewise, gold nanoparticles (Au NPs) were arranged into plasmonic structures via DNA-mediated self-assembly.[11] Since DNA nanostructures are essentially biomolecules, it is intuitive to explore their biological applications in diagnostics and therapeutics. In this Account, we summarize recent progress in this direction, which is largely based on our own work in order to reflect our own perspective.

2. DNA Hybridization on the Gold Surface

Surface-based hybridization of nucleic acids has been popularly exploited in biosensing and microarray technologies for pathogen detection, mutation studies, and genotyping, the sensitivity of which is nevertheless hampered by crowding effects at the surface. For example, typical surface coverage of linear DNA probes on the Au surface is on the order of $10^{11} \pm 10^{13}$ molecules/cm^2, while the efficiency of hybridization is critically dependent on the surface density of DNA.[12]

As a classical approach to control of DNA immobilization at Au, ssDNA with end-tethered thiol is self-assembled onto the gold surface via the well-known thiol−gold chemistry.[13] Ideally, DNA probes should be well packed into an ordered monolayer with an upright orientation that is favorable for target hybridization (Figure 1). However, the assembly of DNA is influenced by several factors including electrostatic repulsion between strands, interactions between nitrogen atoms of bases and the Au surface, and strong interstrand entanglement.[14] Hence, the DNA layer has a rather complicated structure that largely restricts the accessibility of target DNA molecules (Figure 1). Tarlov and co-workers developed a two-step assembly strategy, which involves alkanethiol passivation that backfills the unoccupied space and removes nonspecifically adsorbed DNA to some extent.[13] While this protocol improves the surface assembly of DNA and increases the biosensing ability, it remains challenging to avoid interstrand interactions due to the flexible nature of ssDNA. Indeed, Ye's group recently identified the existence of many defects of DNA monolayers at Au and aggregation patches of DNA, by using electrochemical atomic force microscopy (AFM).[15]

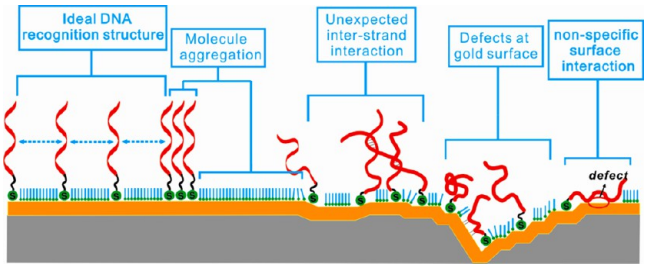

FIGURE 1. Schematic illustration of different states of bound ssDNA probes on the gold surface.

3. Biosensors with DNA Nanostructures

3.1. Structured DNA Probes: From One-Dimensional to Two-Dimensional. Structured DNA probes are single-stranded DNA with secondary structures. While these structures adopt conformations in three dimensions in reality, they can be regarded as pseudo-2D probes as a close approximation in most situations due to their simple structures. These probes with increased rigidity can in principle prevent interstrand entanglement or improve the orderliness of DNA layers on Au. In 2003, Fan et al. exploited the structural switching of a 2D DNA stem−loop structure immobilized at the Au electrode to develop a new type of electrochemical DNA (E-DNA) sensor.[16] Binding of the target DNA that is complementary to the loop sequence changes the 2D hairpin into a linear duplex (Figure 2a). This structural switching alters the electron transfer (ET) distance between the 5′-termini-modified ferroence and Au, producing measurable change in electrochemical signals with a detection limit of 10 pM. Ho's group and ours later increased the E-DNA sensitivity to the femtomolar range by using enzyme-based signal amplification (Figure 2b), which could sensitively detect mRNA in salivary samples.[17,20] Besides the use of hairpin probes, other 2D structures have also been explored to develop E-DNA-type sensors, for example, double- and triple-stem DNA probes.[21]

In a different line of studies, functional nucleic acids with pseudo-2D structures, for example, DNA or RNA aptamers and DNAzymes, are employed to develop electrochemical sensors for non-nucleic acids targets. For example, we designed an electrochemical sensor for adenosine triphosphate (ATP) by adapting the structural switching of an anti-ATP aptamer to the Au electrode setup.[18] ATP was found to induce the formation of the aptamer structure at the surface and responsively denature the initial duplex (aptamer and its complementary sequence) (Figure 2c). In another example, Plaxco and co-workers employed a Pb^{2+}-specific "8−17" DNAzyme to electrochemically detect lead

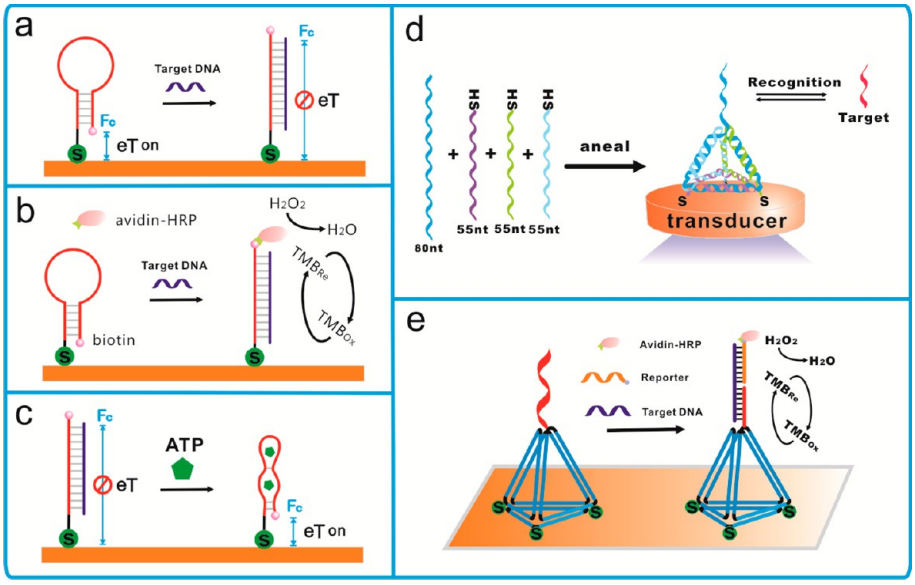

FIGURE 2. Schematic illustration of structured DNA probes. (a) Stem–loop structured DNA probe with Fc as signal molecule (adapted from Fan et al.[16]). (b) Stem–loop structured DNA probe with enzyme-based signal amplification (adapted from Liu et al.[17]). (c) ATP aptamer DNA probe (adapted from Zuo et al.[18]). (d, e) DNA tetrahedron-based structured probe (adapted from Pei et al.[19]).

ions.[22] The presence of Pb^{2+} selectively cleaved the methylene blue (MB) modified DNAzyme structure, resulting in conformation-specific electrochemical signals.

3.2. Three-Dimensional Probes with DNA Nanostructures. The introduction of 2D structures in DNA sensors has greatly increased the flexibility in the sensor design and improved the sensitivity and specificity. However, since 2D probes are attached to Au in the same single-point-attachment way as 1D ones, a passivation step with MCH is still a necessity that increases the heterogeneity of the sensor surface. In addition, 2D structures are usually not robust enough to survive in the crowding environment on the surface. Hence, it is critically important to precisely control the surface density to obtain high reproducibility of sensors. We reasoned that 3D DNA nanostructures might provide a new route to the solution of these challenging problems.

In 2010, we developed the first 3D DNA nanostructure-based E-DNA sensor by using an exquisite, rigid DNA tetrahedron.[19] This tetrahedron-structured probe (TSP) was synthesized from four designed oligonucleotides with self-complementary sequences (Figure 2d). The three vertices of the tetrahedron were modified with thiol to anchor the structure to the Au surface. The fourth vertex at the top of the bound tetrahedron was appended with a pendant ssDNA probe. Owing to the presence of three of thiol legs, TSP could be rapidly and firmly assembled onto the gold surface, which showed approximately 5000-times greater affinity compared with monothiolated DNA. More importantly, the high mechanical rigidity of TSP allowed it to stay on the Au surface with highly ordered upright orientation, even in the absence of the "helper" molecule MCH. AFM studies of Leitner et al. confirmed this directed surface attachment.[23] The surface density was measured to be $\sim 4.8 \times 10^{12}$ TSP cm^{-2}, corresponding to an interstrand spacing of $\sim$4 nm.[19]

Based on this DNA nanostructure-based TSP platform, we constructed an electrochemical DNA sensor with a conventional sandwich hybridization strategy[19] (Figure 2e). The capture probe appended on the TSP captured its target DNA as well as a biotinylated reporter DNA. The formation of the sandwich pair further brought a conjugate of avidin and horseradish peroxidase (HRP) to the proximity of the surface, producing intense electrochemical signal via substrate catalysis. This unoptimized sensor has a picomolar sensitivity. Moreover, this TSP-based DNA sensor possessed very high selectivity for single-base mismatches, exceeding that of ssDNA probe-based sensors by 25–100-fold increase in the discrimination factor.

We showed that sophisticated optimization of the sensor could increase the sensitivity that pushed the detection limit down to 10 fM.[24] More significantly, when poly-HRP, a polymerized avidin–HRP conjugate with up to 400 HRP molecules, was employed to further amply the electrochemical signal, we obtained an ultralow detection limit of 10 aM. This suggests that we can detect $\sim$60 molecules in 10 μL samples.

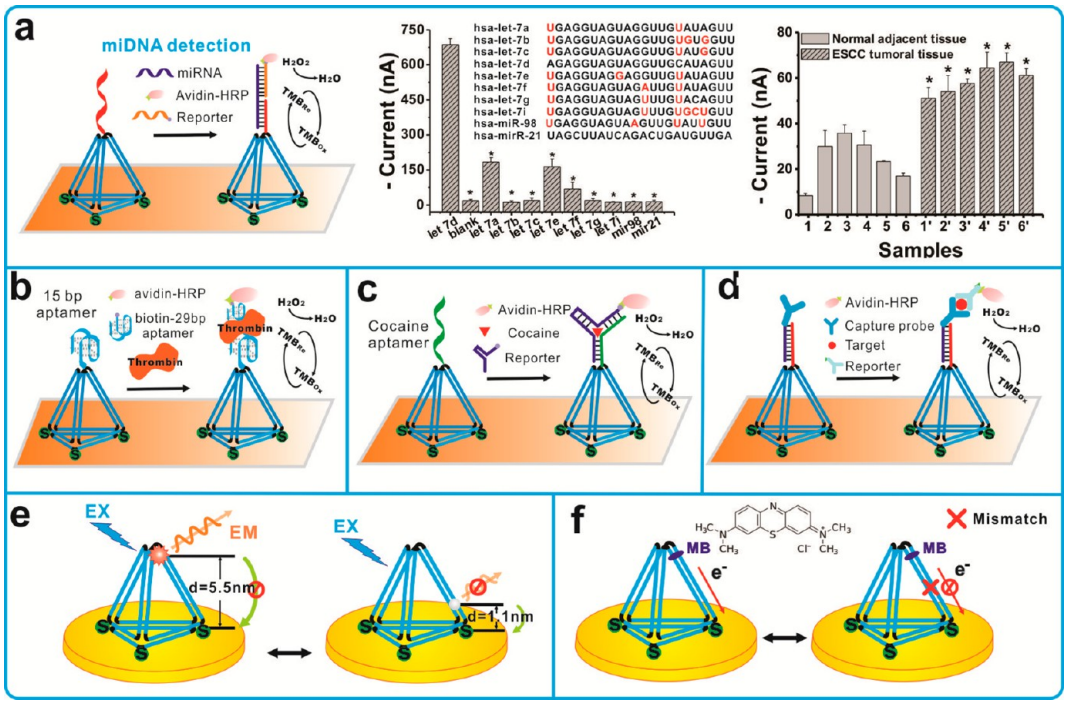

FIGURE 3. Applications of DNA tetrahedron-based structure probe. (a) miRNA sensor (adapted from Wen et al.[24]). (b) Thrombin sensor (adapted from Pei et al.[19]). (c) Cocaine sensor (adapted from Wen et al.[27]). (d) Electrochemical immunosensor (adapted from Pei et al.[28]). (e, f) Three-dimensional DNA nanostructures for studies of surface energy and charge-transfer mechanism associated DNA (adapted from Pei et al.[19] and Lu et al.[29]).

The extraordinarily high sensitivity of TSP-based E-DNA sensor arises from several factors. First, the TSP surface with 3D DNA nanostructures greatly increases the molecular recognition on the Au surface. On the one hand, the relatively thick TSP layer (~6 nm) places the recognition probe far from the surface, which reduces the surface effects and places the probes in a solution-phase-like environment. On the other hand, diffusion and convection at nanostructured interfaces are expected to be higher than those at macroscopic ones.[25,26] Second, the increased interstrand spacing due to the presence of the bulky DNA nanostructure offers the room for using multiple-enzyme amplification (e.g., poly-HRP), which greatly increases the E-DNA signal. Third, the use of rigid DNA tetrahedron leads to a highly stable and reproducible surface with minimal nonspecific adsorption. Hence, this type of E-DNA sensor has inherently low background. Given these unprecedented advantages, the use of DNA tetrahedra holds great promise to develop ultrasensitive biosensors for a variety of molecular targets.

3.3. Electrochemical Sensors with Three-Dimensional DNA Nanostructures. MicroRNA (miRNA) is a type of important biomarker for early phase cancer diagnosis. However, the sensitivity of conventional electrochemical sensors for miRNAs is typically in the range of picomolar to femtomolar, which does not support direct detection of low-abundance miRNAs without prior amplification. By employing the TSP-based platform, we developed an ultrasensitive electrochemical miRNA sensor (EMRS) for reliable quantitative detection of attomolar (<1000 copies) miRNAs with high sequence specificity[24] (Figure 3a). EMRS also showed high single-base mismatch discrimination ability, which could effectively distinguish closely related sequences in the family of human let-7 sequences. More importantly, EMRS exhibited excellent performance for analyzing expression levels of miR-21 in clinical samples of esophageal squamous cell carcinoma (ESCC).

Replacement of the DNA probe at the top vertex with an antithrombin aptamer sequence turns the E-DNA sensor into an immunological sensor for proteins,[19] that is, thrombin, with a low detection limit of 100 pM that excels that of the conventional aptamer-based sensor by 3 orders of magnitude (Figure 3b). Similarly, by incorporating a split aptamer of cocaine (Figure 3c), we developed an electrochemical sensor for small molecules.[27] This sensor exhibited excellent sensitivity with a detection limit of 33 nM in the complex media. Other examples include single nucleotide polymorphism (SNPs) genotyping with a three-way DNA junction, mercury ion detection with a mercury-specific oligonucleotide, and ATP detection with an anti-ATP aptamer.[30−32]

It is worthwhile to note that this platform provides a promising approach to anchor antibodies with the well-controlled

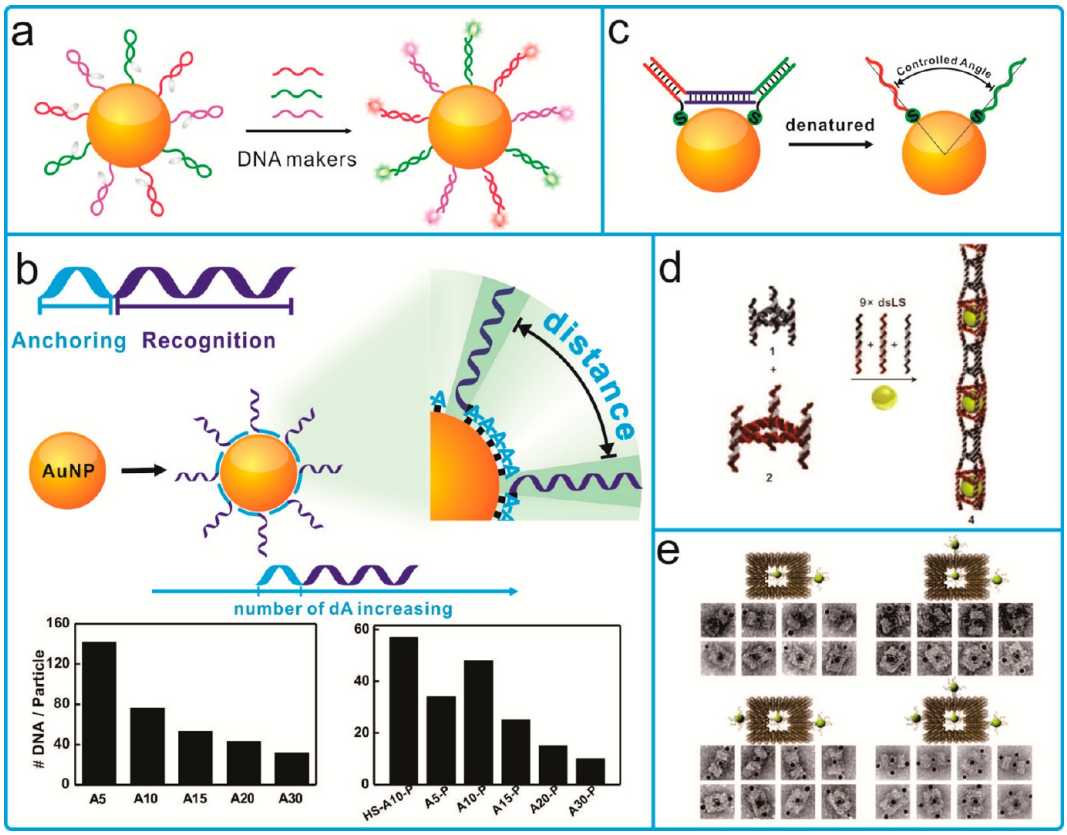

FIGURE 4. DNA nanostructures with AuNPs. (a) Multicolor nanobeacons (adapted from Song et al.[34]). (b) Polyadenine (polyA) blocks for adjusting the DNA probe conformations on nanoparticles (adapted from Pei et al.[35]). (c) dsDNA geometrical templates for controlling DNA numbers and positions on nanoparticles (adapted from Suzuki et al.[36]). (d) DNA nanotubes to encapsulate AuNPs (adapted from Lo et al.[37]). (e) DNA origami nanocage to encapsulate AuNPs (adapted from Zhao et al.[38]).

orientation to improve the performance of immunological sensing. For example, an antibody for tumor-necrosis-factor alpha (TNF-α) was first conjugated with a piece of oligonucleotide that is complementary to the DNA probe sequence on top of the tetrahedron[28] (Figure 3d). The antibody was then well placed on each tetrahedron with a 1:1 ratio. Thus-formed electrochemical immunosensors showed superior sensitivity and selectivity over conventional ones. In addition, given that the DNA hybridization is reversible, this sensor could be easily regenerated via dehybridization of the DNA linker.[28]

Surface-confined tetrahedral nanostructures also provide a platform for studies of energy and charge-transfer mechanism associated with DNA. Small molecules can be placed at different bases at the edge of the DNA tetrahedron. Because of the high rigidity of this scaffold, it is possible to measure the distance-dependent energy or charge-transfer mechanisms. In one of our studies, we attached a rhodamine dye to either the top vertex or one of the bottom ones of the tetrahedron[19] (Figure 3e). We found that the fluorescence was nearly quenched for the latter due to the strong Au-induced fluorescence quenching. However, the former exhibited strong fluorescence due to the attenuation of the Au-induced quenching at a long distance. By using this 3D DNA nanostructural framework, we also investigated the kinetics of DNA-mediated charge transport (CT) and different through-duplex and through-space CT mediated by DNA[29] (Figure 3f). When a DNA-intercalative redox molecule, methylene blue, was attached to different places on the tetrahedron, the CT was almost independent of the distance, suggesting the occurrence of highly efficient through-duplex CT. In contrast, a nonintercalative redox molecule, ferrocence, exhibited strong distance-dependent through-space CT. Our demonstration of distance-sensitive energy and charge transfer at this nanostructured interface sheds new light on the development of new target-responsive structural switching sensors and the design of DNA-based molecular electronic devices.

3.4. DNA Structures for Nanoparticle-Based Biosensors. It is very interesting to create effective DNA recognition layers on the nanoparticle surface. An important class of such systems is DNA functionalized gold nanoparticles

(DNA−AuNPs), which have found a wide spectrum of applications in self-assembled nanomaterials, biomolecular detection, and gene regulation.[33] The preparation of DNA−AuNPs typically relies on the use of thiolated oligonucleotides, which form a dense layer of DNA molecules on the Au surface.

Analogous to studies on the macroscopic Au surface, we employed hairpin-structured 2D probes to modify 15 nm AuNPs for the development of fluorescent DNA sensors.[34] By coimmobilizing three different probes onto nanoparticles, we constructed multicolor nanobeacons (nanoMBs) that could simultaneously sense three tumor DNA markers (Figure 4a). Because of the use of hairpin structures that were inherently sensitive to base mismatches, this nanoMB sensor showed negligible crosstalk. Recently, Li et al. employed the nanoMB-based multicolor probe to simultaneously detect three types of tumor-related mRNAs in live cells.[39] The fluorescence intensity correlated well with the concentration of the mRNAs and drug-induced changes in gene expression levels within cancer cells can be detected.

The structure of DNA layers on the surface of AuNPs plays an important role in their properties, such as hybridization kinetics and thermodynamics, nuclease resistance, and cellular uptake. However, it is much more difficult to tailor the structure of DNA layers on AuNPs than on the surface of bulk Au films, partially due to the instability of AuNPs in solution. In addition, it is relatively difficult to couple 3D DNA nanostructures with AuNPs with the highly curved surface. Hence, it remains a challenge to properly control the spatial arrangement and conformation of DNA probes on AuNPs.

Very recently, we reported a new strategy to circumvent this problem by exploiting nonspecific interactions between DNA bases and Au nanoparticles.[35] Instead of using thiolated DNA probes, we employed pure oligonucleotides with unmodified sequences containing a polyadenine (polyA) block (Figure 4b). Previous studies have shown that DNA bases interact differentially with Au primarily via their nitrogen atoms.[40] Interestingly, it was found that multiple consecutive adenines served as a strong anchoring block to Au due to the collective binding affinity of polyA to Au. Because of its multipoint attachment nature, nonspecific interactions between other bases and Au were minimized. By simply adjusting the length of polyA block, we could systematically modulate the lateral spacing and surface density of DNA probes on AuNPs. Significantly, the DNA−AuNPs conjugates prepared with this polyA strategy exhibited much faster hybridization kinetics than thiolated DNA−AuNPs, which further confirms that the conformation of DNA probes at AuNPs is critically important for their recognition ability.[35]

There have been great efforts to arrange DNA probes on the surface of AuNPs with complex DNA nanostructures. Suzuki et al. developed a method to immobilize DNA on AuNPs with precise control of their number and positions by using a dsDNA geometrical template[36] (Figure 4c). In another study, Sleiman and co-workers constructed a DNA nanotube with alternating larger and smaller capsules for the size-specific encapsulation of AuNPs, with selective release of the particles in response to externally supplied DNA[37] (Figure 4d). Yan's group demonstrated the ability to use a DNA origami nanocage to encapsulate AuNPs of various sizes[38] (Figure 4e). The spatial addressability of the DNA origami offered an opportunity for tuning the optical properties of AuNPs and further functionalization.

3.5. DNA Nanostructures for Biosensing in Homogeneous Solution. Self-assembled DNA nanostructures provide spatially addressable scaffolds for the development of biomolecular probe carrier platform for biosensing at the single-molecule level. For example, Yan and co-workers employed a rectangular-shaped DNA origami structure as a nanoscale gene chip for label-free detection of RNA[41] (Figure 5a). In their design, ssDNA probes were appended to predefined positions on the origami. Upon hybridization with target RNA, the increase in stiffness and height could be readily identified with AFM. Since the size of the DNA origami chip fits well with that of single cells, it holds great promise for gene expression analysis at the single-cell level.[41] We reported the design of an index-free nanochip by using an asymmetric DNA origami substrate with the shape of a Chinese map without using built-in index oligonucleotides[47] (Figure 5b). The asymmetric nature of the map ensures spatial addressability under AFM imaging.[42] As a further step, a toehold strand-displacement reaction was introduced on the nanochip to effectively differentiate single-base mismatches at ambient temperature[43] (Figure 5c). Seeman and co-workers later invented a visual chip for single-nucleotide polymorphisms (SNP) genotyping with the symbolic display[44] (Figure 5d,e). Each mutation in the genome could be visually displayed on this DNA origami under AFM.

In addition to the use of static 2D structures, dynamic DNA devices have also been designed with 3D DNA nanostructures. Turberfield et al. reported a reconfigurable DNA tetrahedron embedded with a hairpin structure.[48] The presence of a specific DNA strand that binds to the loop region of the hairpin responsively changed the conformation of the tetrahedron. We incorporated several functional DNA to different edges of the tetrahedron, which included the pH-responsive i-motif, anti-ATP aptamer, and T-rich MSO[45] (Figure 5f).

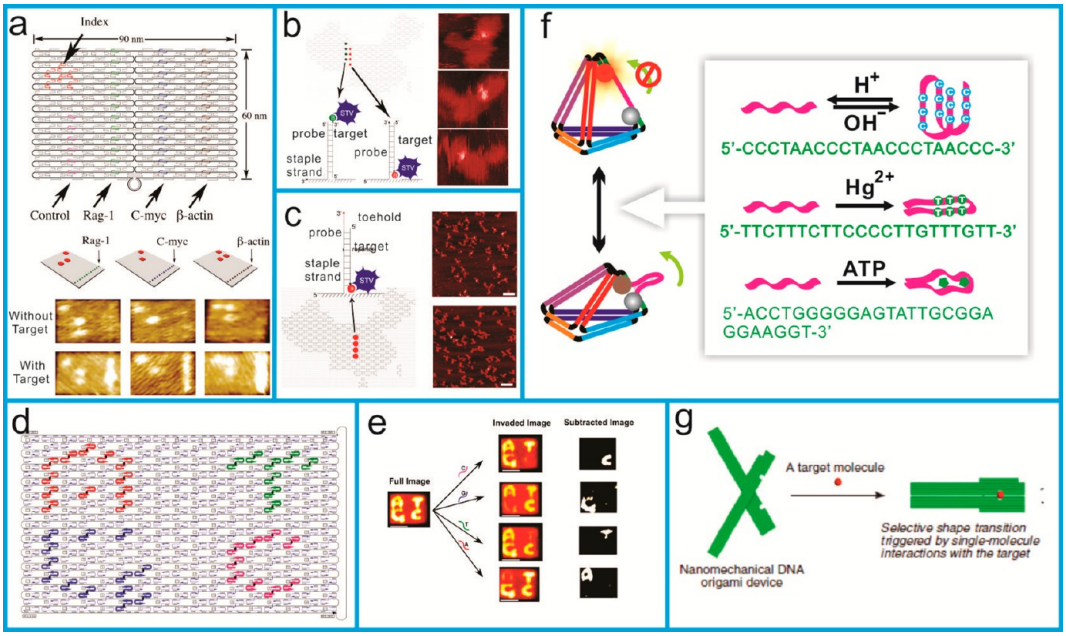

FIGURE 5. DNA nanostructures for biosensing in homogeneous solution. (a) Rectangular-shaped DNA origami nanochip (adapted from Ke et al.[41]). (b, c) Asymmetric DNA origami nanochip (adapted from Zhang et al.[42,43]). (d, e) Visual SNP genotyping on DNA origami chip (adapted from Subramanian et al.[44]). (f) Reconfigurable DNA tetrahedron embedded with pH-responsive i-motif, anti-ATP aptamer, and T-rich MSO (adapted from Pei et al.[45]). (g) Scissor-shaped DNA origami nanomechanical sensor (adapted from Kuzuya et al.[46]).

This DNA tetrahedron showed logical responses to one of the three targets, that is, proton, ATP, and Hg^{2+}. By combining individual tetrahedron-based molecular logic gates (AND, OR, XOR, INH), we further realized the complex operation, half-adder, with this DNA nanostructure. Kuzuya et al. developed a scissor-shaped nanomechanical DNA origami device, or "single-molecule beacons"[46] (Figure 5g), which could detect a variety of chemical and biological targets at the single-molecule level with AFM.

4. Theranostic DNA Nanostructures in Cells

Yan and co-workers showed that DNA origami nanostructures with different shapes, sizes, and probes were stable in cell lysate, which could be easily separated from the lysate[49] (Figure 6a). In 2010, Turberfield's group and our group independently found that DNA tetrahedra could cross the cellular membrane even in the absence of transfection reagents.[51,54] We further found that these small-sized tetrahedra were nontoxic and more stable than linear DNA inside the cell.[51] As such, we expect these 3D DNA nanostructures can serve as unique theranostic agents for imaging and therapy inside the cell.

By using the reconfigurable DNA tetrahedral nanodevice, we constructed an intracelluar ATP sensor, which could be effectively used for ATP imaging and mapping in the cell due to the high cell permeability and robust stability of the tetrahedra[45] (Figure 6b). Modi et al. reported the design of a proton-triggered DNA nanomachine for pH mapping in live cells, which could monitor endosome maturation that was associated pH variations[50] (Figure 6c). Also, they demonstrated that the synthetic DNA nanodevice could function autonomously in a multicellular living organism.[55]

By exploiting this excellent property of DNA tetrahedra, we developed a new type of noninvasive delivery system for CpG oligonucleotides, an immunostimulating agent. CpG motifs were appended to the DNA tetrahedron to form a functional nanostructure[51] (Figure 6d). These DNA nanostructures were readily internalized by macrophage RAW264.7 cells and remained substantially intact for several hours. By engaging the Toll-like receptor 9 (TLR-9), the CpG tetrahedron stimulated the production of cytokines appromixately 100-fold higher than single-stranded CpG. In a different study, Lee et al. constructed a DNA tetrahedral nanostructure with precisely controlled spatial orientation and density of siRNAs[52] (Figure 6e). They demonstrated that siRNAs were efficiently delivered to tumor cells of mice and silenced target genes in tumors. In addition to these pure DNA nanostructures, several groups also found that DNA-bound AuNPs could also be efficiently internalized.[56,57] Such a combination of inorganic and biomolecular nanostructures offers high flexibility in the design and versatility in functions.

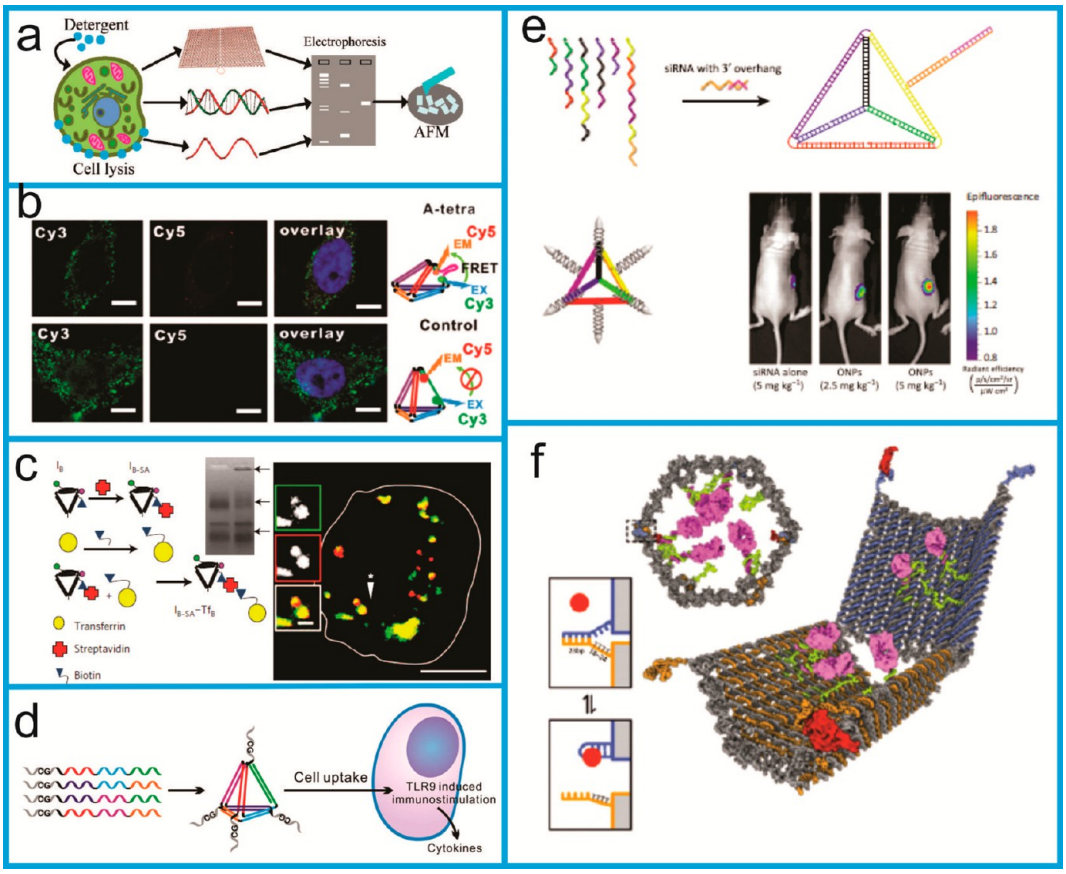

FIGURE 6. (a) Stability of DNA nanostructures in cell lysate (adapted from Mei et al.[49]). (b) Detection of intracellular ATP in living cells using reconfigurable DNA tetrahedron (adapted from Pei et al.[45]). (c) Mapping pH changes inside living cells using DNA machines (adapted from Modi et al.[50]). (d) CpG bearing DNA tetrahedron and its immunostimulatory effect (adapted from Li et al.[51]). (e) DNA tetrahedron delivery of siRNAs into cells and silencing of target genes in tumors (adapted from Lee et al.[52]). (f) Robotic DNA devices sense cell surface as inputs and induce cell signaling pathways (adapted from Douglas et al.[53]).

Active DNA nanostructures with logical control are particularly useful for theranostic applications. Douglas et al. described a robotic DNA device that senses receptor on the cell surface, which subsequently (and logically) induces cell signaling pathways[53] (Figure 6f). In their design, a barrel-shaped DNA–origami container was locked by two distinct DNA aptamers. When both apamers bound to the specific receptors, an AND logic gate was triggered to open the barrel and releases the cargo molecules. This prototype nanodevice represents an important step in development of a smart drug-delivery system with intelligent sensing and control.

As a type of novel material for drug delivery, self-assembled DNA nanostructures have shown unprecedented advantages including flexibility in design, ease of bioconjugation and inherent biocompatibility. Nevertheless, further investigations should be performed to obtain systematic information on extracellular and intracellular behavior of DNA nanostructures for their practical applications. These include mechanisms of endocytosis, pharmacokinetics, and geometric and multivalence effects of DNA nanostructures, which could be attractive topics in the future.

5. Conclusions and Outlook

Self-assembled DNA nanostructures have demonstrated great potential in a wide range of applications, including biosensing and drug delivery as described in this Account. Rationally designed DNA nanostructures can greatly increase the biomolecular recognition ability on the sensing interface with better controlled probe density and orientation, as well as surface passivation, providing a versatile and efficient platform to develop a wide range of electrochemical and optical biosensors for various proteins, nucleic acids, and small molecules. On the other hand, dynamic, cell-permeable DNA nanostructures have shown well controlled logical responses to environmental stimuli. Hence, it is possible to develop DNA nanostructure-based nanocarrier systems with embedded intelligent sensors for in vivo diagnosis and controlled released of drugs.

Given the convenience in designing DNA nanostructures with different size, geometry, and functional groups, we envisage that self-assembled DNA nanostructures will become a promising tool in theranostics. The state-of-the-art biosensors have not been able to challenge the most sensitive laboratory technologies, for example, polymerase chain reactions (PCR), in sensitivity. Precise engineering of the biosensing interface with highly tailorable DNA nanostructures is expected to improve the biomolecular recognition in both thermodynamics and kinetics, which should support ultrasensitive detection of biomarkers for early phase diagnosis of cancers and rapid screening of infectious diseases. Along a different line, self-assembled DNA nanostructures offer unprecedented opportunities to develop smart nanodevices that can work inside the cell or animals. Current nonviral drug delivery agents still lack the intelligence that a virus has by nature. Since DNA nanostructures have many similarities to viruses, it is possible to mimic viruses by using DNA nanostructures with well-defined functional structures. With the rapid progress in this area, we are very optimistic that these hopes, along with many others, will be realized in the near future.

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BIOGRAPHICAL INFORMATION

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Xiaolei Zuo obtained his B.S. degree from Central South University in 2002 and his Ph.D. at SINAP in 2008. After his postdoctoral research at the University of California at Santa Barbara and the Los Alamos National Laboratory, he became a professor at SINAP in 2012. He has published over 20 papers in peer-reviewed journals.

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FOOTNOTES

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The authors declare no competing financial interest.

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