ACCOUNTS of chemical research

Article

pubs.acs.org/accounts

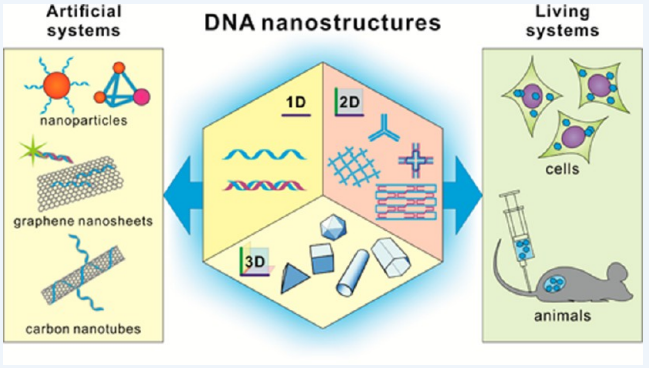
Physical and Biochemical Insights on DNA Structures in Artificial and Living Systems

Nan Chen,[†] Jiang Li,[†] Haiyun Song,[‡] Jie Chao,[†] Qing Huang,*[,†] and Chunhai Fan*[,†]

[†]Division of Physical Biology & Bioimaging Center, Shanghai Sychrotron Radiation Facility (SSRF), CAS Key Laboratory of Microscale Physics and Technology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China

[‡]Key Laboratory of Food Safety Research, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

CONSPECTUS: Highly specific DNA base-pairing is the basis for both fulfilling its genetic role and constructing novel nanostructures and hybrid conjugates with inorganic nanomaterials (NMs). There exist many remarkable differences in the physical properties of single-stranded (ss) and double-stranded (ds) DNA, which play important roles in regulation of biological processes in nature. Rapid advances in nanoscience and nanotechnology pose new questions on how DNA and DNA structures interact with inorganic nanomaterials or cells and animals, which should be important for their biological and biomedical applications. In this Account, we intend to provide an overview on many facets of DNA and DNA structures in artificial and living systems, with the focus on their properties and functions at the interfaces of inorganic nanomaterials and biological systems.



ssDNA, dsDNA, and DNA nanostructures interact with NMs in different ways. In particular, gold nanoparticles and graphene oxide exhibit strikingly different affinity toward ssDNA and dsDNA. Such binding differences can be coupled with optical properties of NMs. For example, DNA hybridization can effectively modulate the plasmonic and catalytic properties of gold nanoparticles. By exploitation of these interactions, there have been many ways for sensitive transduction of biomolecular recognition for various sensing applications. Alternatively, modulation of the properties of DNA and DNA structures with NMs has led to new tools for genetic analysis including genotyping and haplotyping.

Self-assembled DNA nanostructures have emerged as a new type of NMs with pure biomolecules. These nanostructures can be designed in one, two, or three dimensions with various sizes, shapes, and geometries. They also have characteristics of uniform size, precise addressability, excellent water solubility, and biocompatibility. These nanostructures provide a new toolbox for biophysical studies with unparalleled advantages, for example, NMR-based protein structure determination and single-molecule studies. Also importantly, DNA nanostructures have proven highly useful in various applications including biological detection, bioreactors, and nanomedicine. In particular, DNA nanostructures exhibit high cellular permeability, a property that is not available for ssDNA and dsDNA, which is required for their drug delivery applications.

DNA and DNA structures can also form hybrids with inorganic NMs. Notably, DNA anchored at the interface of inorganic NMs behaves differently from that at the macroscopic interface. Several types of DNA−NM conjugates have exerted beneficial effects for bioassays and in vitro translation of proteins. Even more interestingly, hybrid nanoconjugates demonstrate distinct properties under the context of biological systems such as cultured cells or animal models. These unprecedented properties not only arouse great interest in studying such interfaces but also open new opportunities for numerous applications in artificial and living systems.

1. INTRODUCTION

DNA is the carrier of genetic information inside a cell and plays pivotal roles in the survival and functions of an organism. For example, replication and transcription are two fundamental biological processes that rely on the highly specific base-pairing properties of DNA. Meanwhile, there also exist various forms of DNA secondary structures in nature, including hairpins, quadruplexes, and ribozymes, providing high versatility for exquisite modulation of functions of cells and organisms. These naturally existing 2D structured DNAs, as well as other functional nucleic acids (e.g., DNAzymes and aptamers), have become popular tools in many areas.

In a continuous effort to exploit DNA and DNA structures for materials applications, Nadrian Seeman pioneered the area

Special Issue: Nucleic Acid Nanotechnology

Received: December 31, 2013
Published: March 3, 2014

   dx.doi.org/10.1021/ar400324n | Acc. Chem. Res. 2014, 47, 1720−1730

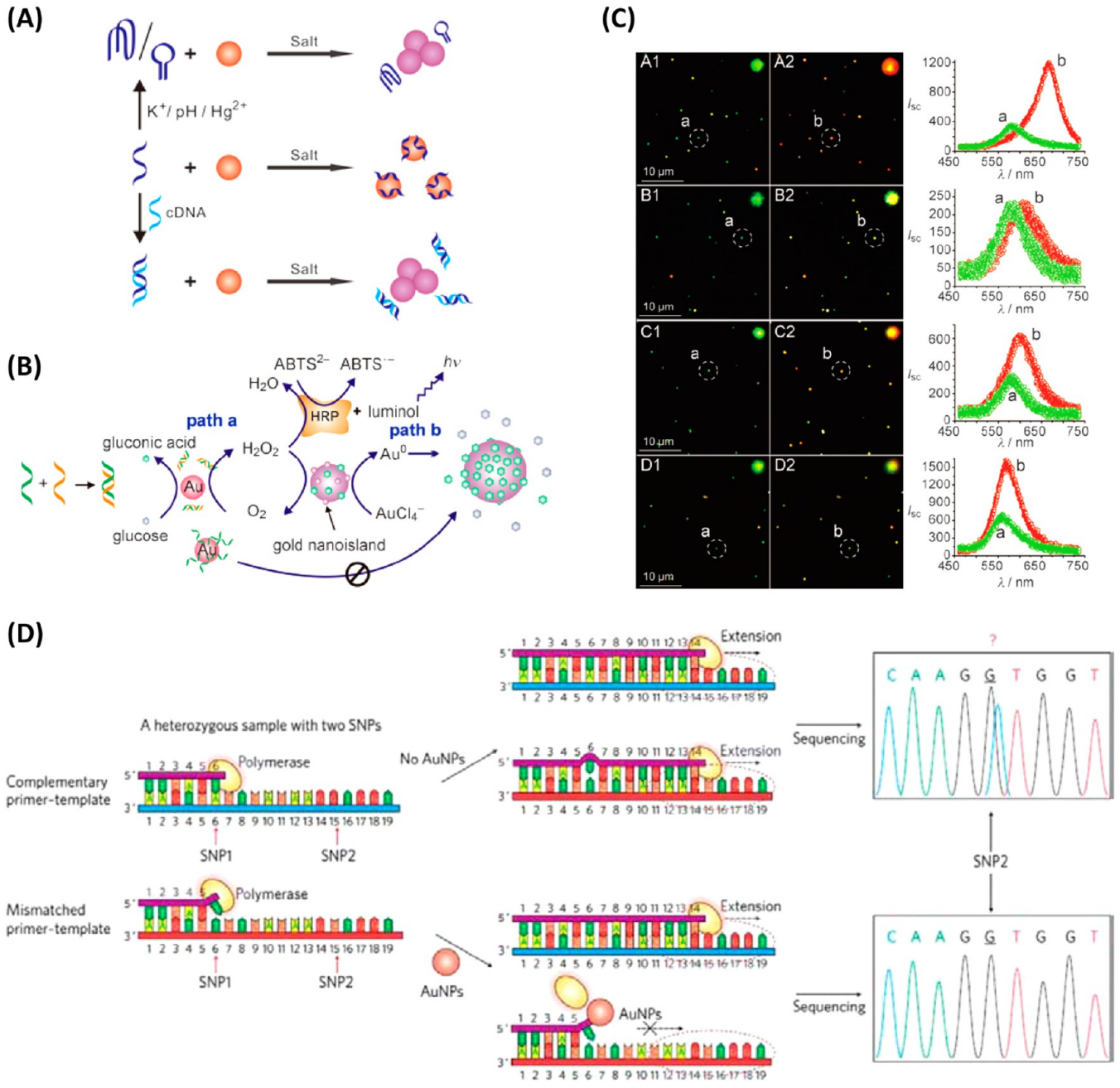


Figure 1. Interactions between DNA and unmodified AuNPs. (A) Unmodified AuNPs differentiate flexible ssDNA and rigid DNA structures. (B) DNA hybridization-regulated catalytic activity of AuNPs.[12] (C) DFM images of DNA hybridization-regulated seeded growth of AuNPs.[12] Reproduced from ref 12 with permission. Copyright 2011 Wiley-VCH. (D) AuNPs-enhanced allele-specific sequencing and haplotyping. Reproduced from ref 21. Copyright 2011 Nature Publishing Group.

of DNA nanotechnology since the 1980s, which has become a rapidly growing and prosperous area.[1] Compared with peptides or proteins, DNA has a much simpler composition of four nucleobases (versus 20 amino acids in the former), which are chemically stable, easy to design, and inexpensive to synthesize. More importantly, DNA hybridization processes can be precisely controlled following the simple and predictable A−T and G−C pairing rules and finely tuned with rational design of the sequence. Because of these unique properties of DNA, we have witnessed dramatic increase in the number and complexity of designed DNA nanostructures in the past 2 decades.[1,2] These DNA nanostructures with different sizes, shapes, and geometries have characteristic uniform size, precise addressability, excellent water solubility, and biocompatibility. Hence, they offer new opportunities for bottom-up construction of nanodevices and plasmonic nanostructures, and biomedical applications.[2−4]

As a new type of nanoscale materials, DNA and DNA structures have been extensively exploited in both artificial and living systems. In particular, it is highly interesting to explore biomedical applications of DNA and DNA structures given the biological nature of DNA.[3,5] Along these lines, there have been great efforts to interrogate unique physical and biochemical properties of DNA and DNA structures at various interfaces

dx.doi.org/10.1021/ar400324n | Acc. Chem. Res. 2014, 47, 1720−1730

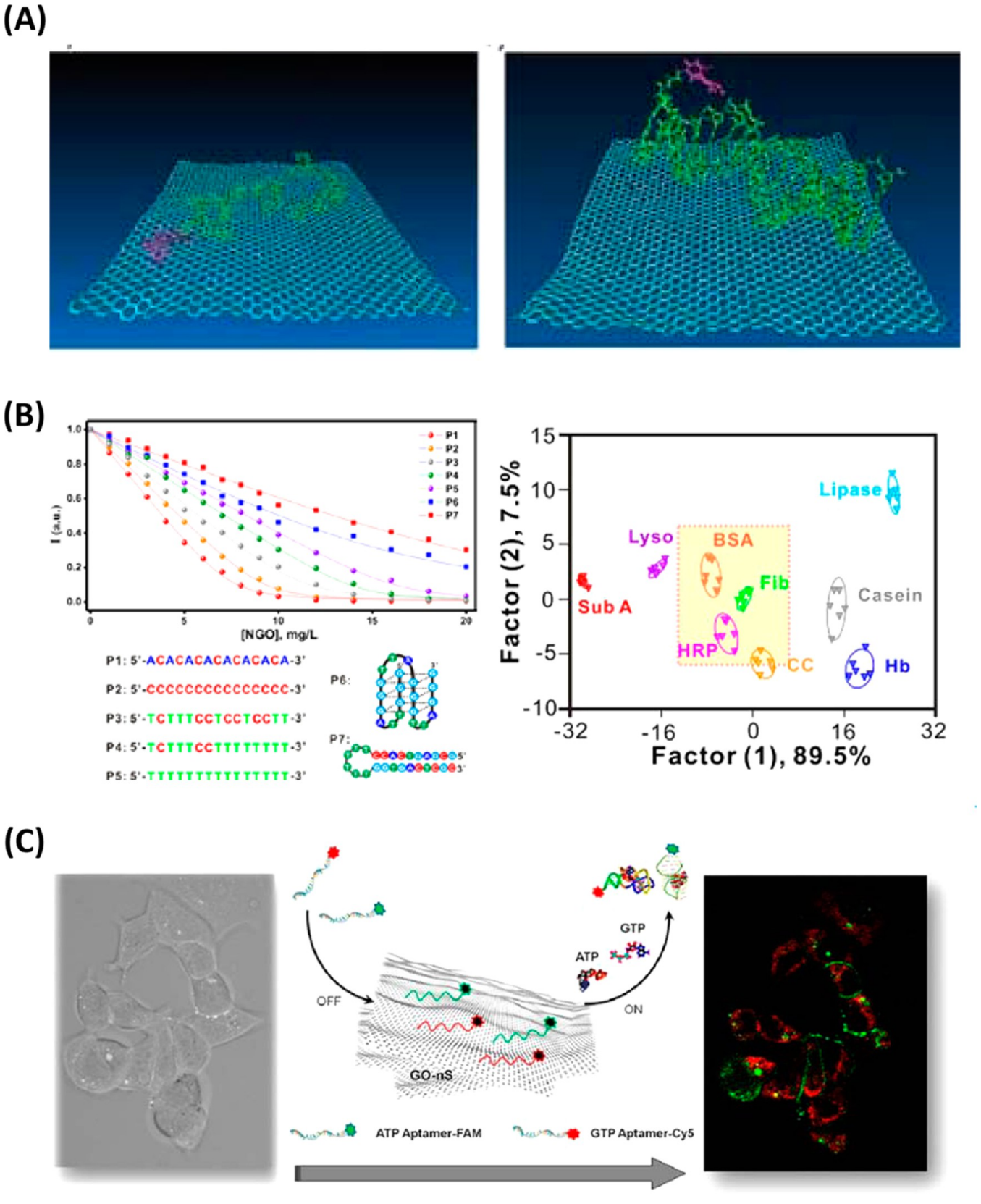


Figure 2. GO-based detection of biomolecules. (A) MD simulation of interactions between GO and ssDNA or dsDNA. Reproduced from ref 13. Copyright 2009 Wiley-VCH. (B) Protein identification with a GO-based sensing platform. Reproduced from ref 16. Copyright 2012 American Chemical Society. (C) In situ simultaneous imaging of ATP and GTP in living cells. Reproduced from ref 58. Copyright 2013 American Chemical Society.

dx.doi.org/10.1021/ar400324n | *Acc. Chem. Res.* 2014, 47, 1720−1730

ranging from artificial inorganic nanomaterials (NMs) to live cells and animals. Notably, hybrid DNA−metal nanoconjugates or pure DNA nanostructures demonstrate unique properties under the context of biological systems. In this Account, we intend to provide an overview on many facets of DNA and DNA structures in artificial and living systems, which are largely based on the research activities ongoing in our laboratory.

2. NATURALLY EXISTING DNA STRUCTURES

DNA and DNA structures can interact with inorganic NMs in various ways via electrostatic or hydrophobic interactions, hydrogen bonds, and coordination. NMs have been widely utilized in biological sensing applications, with AuNPs as a prominent example. Li et al. first found that single-stranded DNA (ssDNA) possessed greater adsorption affinity to AuNPs than double-stranded DNA (dsDNA).[6] This strikingly large difference should come from a combination of factors associated with the structural differences of ssDNA and dsDNA. First, ssDNA is a soft polymer, while dsDNA is a largely rigid rod-like helix, which can be reflected from their persistence lengths (ssDNA < 1 nm; dsDNA ≈ 50 nm). Hence, ssDNA has the flexibility to wrap the surface of AuNPs, while the greater rigidity of dsDNA prevents such binding. Second, nitrogen atoms of DNA bases are known to coordinate with gold, with adsorption energy larger than 100 kJ/mol. Given that, the exposed bases in ssDNA can strongly bind to the AuNP surface via Au−N coordination, which are shielded by the phosphate backbone in dsDNA. Third, citrate-stabilized AuNPs are negatively charged. DNA is also a highly negatively charged polyelectrolyte, so the surface charge of dsDNA doubles that of ssDNA. Thereby, the electrostatic repulsion between AuNPs and dsDNA is larger than that for ssDNA due to the doubling of surface negative charges in dsDNA. Importantly, the binding of ssDNA to the AuNP surface significantly increases the net negative charges of the latter and greatly stabilizes AuNPs in solutions of high ionic strength.

This discovery has led to the development of a large number of simple colorimetric bioassay methods.[6−9] These assays typically rely on the red-to-blue color change of AuNPs in their dispersed and aggregated forms, which provides a convenient means to visually detect target DNA with unmodified AuNPs.[6] We extended this strategy to the detection of a variety of non-nucleic acid targets with the introduction of aptamers, which are in vitro selected DNA or RNA sequences with high target-binding specificity (Figure 1A).[10] This finding paves the way to develop a generic strategy for rapid detection of a variety of metal ions, small molecules, and proteins.[7−9]

AuNPs have also proven to be catalysts for many chemical/biochemical reactions, a property that is not present in bulk gold. For example, we explored the glucose oxidase (GOx)-like activity of AuNPs to catalyze the oxidation of glucose, which was extremely sensitive to the surface properties of AuNPs.[11] Based on the differential ability of ssDNA and dsDNA in modulating the activity of AuNPs, we utilized catalytic AuNPs as a nanoplasmonic probe for studying biomolecular interactions. As a visual readout, the coupled seeded growth of AuNPs can be monitored in real time with dark-field microscopy (DFM) (Figure 1B,C).[12]

In addition to AuNPs, several other inorganic NMs, including graphene oxide (GO) and carbon nanotubes (CNTs), have been explored for their interactions with DNA.[13,14] We are especially interested in GO, a type of recently discovered one-atom-thick 2D nanomaterial with excitingly new properties.[15] Unlike AuNPs, GO interacts with ssDNA primarily via $\pi-\pi$ stacking interactions between aromatic bases and graphene sheets. Molecular dynamic (MD) simulation revealed that nearly all bases in ssDNA stably lie flat at the surface of GO (Figure 2A).[13] Interestingly, dsDNA has a low affinity for GO, possibly due to the shielding effect of negatively charged phosphate backbones.

Since GO is flat sheets with relatively homogeneous surfaces, it provides a reproducible platform for studying the adsorption of ssDNA with various sequences. Interestingly, we found that ssDNA of different sequences resulted in similar binding energies of ∼52.7 kJ/mol with less than 2% in variations.[16] This similarity reflects the homogeneity in the adsorption between ssDNA and GO via $\pi-\pi$ stacking, which differs remarkably with that in AuNPs that involve multiple distinct interactions (Figure 2B).

The difference in individual DNA sequences could be reflected by another parameter, the effective DNA footprint, β, which is defined as the average area of GO occupied by ssDNA.[16] The differentiation ability of GO toward ssDNA and dsDNA was exploited to develop fluorescent DNA sensors. By exploiting the superquenching ability of GO, we developed multicolor fluorescent DNA nanosensors for rapid detection of tumor-suppression genes[13] and other molecular targets[17,18] in homogeneous solution. In an alternative approach, we developed a GO-based platform for high-precision identification of a wide range of molecular or cellular targets. A small set of nonspecific ssDNAs, rather than specific receptors, were employed to interact differentially with GO, providing olfactory or gustatory-like pattern-recognition ability.[16]

The unique interactions between DNA and AuNPs/GO were also exploited to boost certain biological reactions. We previously found that the addition of AuNPs to the system of polymerase chain reaction (PCR) greatly increased the specificity.[19] Wen and co-workers found similar effects with GO in their studies.[20] In the machinery of DNA replication, single-stranded DNA binding (SSB) protein selectively binds to the ssDNA template, which forms the basis of the natural mechanism to avoid the occurrence of mutations. Apparently, AuNPs and GO resemble SSB in the in vitro PCR system, serving as chaperones to decrease the error rate. This nanomaterials-assisted PCR (nanoPCR) has proven highly useful in genetic analysis. We demonstrated that AuNP-based nanoPCR increased the ability for single-nucleotide-polymorphism (SNP) genotyping via inhibiting the amplification of mismatched primer/template pairs. This improvement in SNP genotyping is critically important for highly specific molecular haplotyping of long genome sequences.[21] A haplotype is a set of statistically associated SNPs on a single chromatid, which provides valuable genetic information for disease studies. By exploiting simple DNA−AuNP interactions, our nanoPCR provides the first inexpensive and scalable method for high-throughput analysis of long-range haplotypes (Figure 1D).[21]

3. DESIGNED DNA NANOSTRUCTURES

DNA can be used as engineering materials to construct artificial nanostructures via rational design and bottom-up molecular self-assembly. Since the 1980s, tile-based assembly and DNA origami technology have generated numerous DNA nanostructures including 2D and 3D crystal lattices, nanotubes, polyhedra, and even curved shapes.[22] Designed DNA nanostructures have exhibited unprecedented advantages

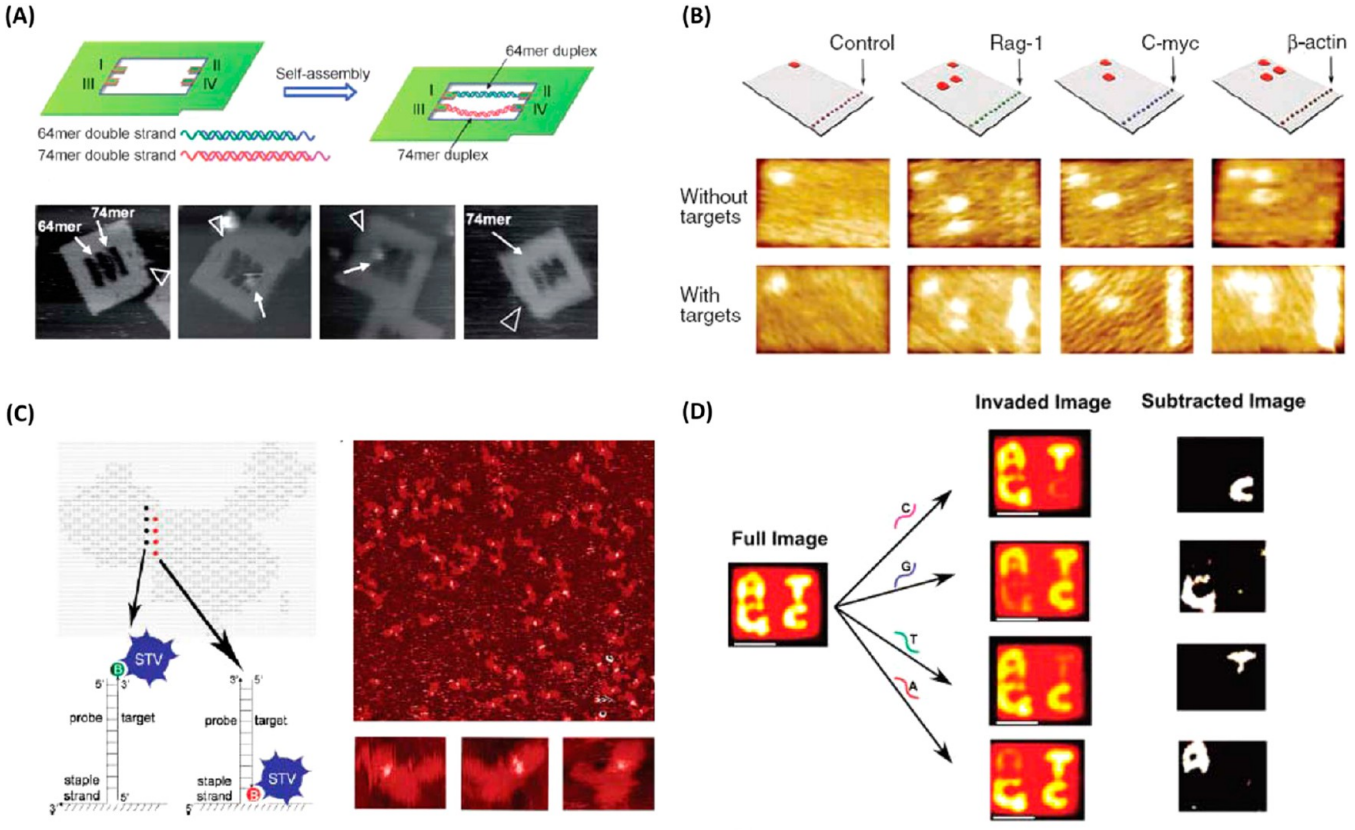


Figure 3. DNA nanostructures for biophysical and biosensing applications. (A) DNA frames for visualizing DNA methylation-induced conformational switch. Reproduced from ref 25. Copyright 2012 Wiley-VCH. (B) A rectangular DNA origami chip for label-free detection of microRNAs. Reproduced from ref 30. Copyright 2008 AAAS. (C) A map-shaped asymmetric DNA origami chip for SNP genotyping. Reproduced from ref 31. Copyright 2010 Wiley-VCH. (D) Visual SNP genotyping on DNA origami with a symbolic display. Reproduced from ref 33. Copyright 2011 American Chemical Society.

including predictable size and shape, capacity of multiple functionalization with high site-specificity, and excellent biocompatibility.

Since the early days of DNA nanotechnology, Seeman and co-workers have attempted to create periodic three-dimensional DNA lattices that can site-specifically anchor proteins in a confined space, with the hope to crystallize proteins that were otherwise impossible.[23] While this goal has not been realized, DNA nanotechnology has shown great power in protein structure determination with NMR. Rigid DNA origami rods were utilized in residual dipolar coupling (RDC) experiments to align detergent-reconstituted membrane proteins, which greatly facilitates NMR measurement and structural determination.[24] Likewise, DNA origami nanostructures provide a unique way to physically locate a single molecule on any predefined position of a nanostructure up to ∼100 nm. This unprecedented ability promoted single-molecule biophysical studies for DNA−DNA, DNA−protein, and other ligand binding processes at the single-molecule level (Figure 3A).[25−27]

In addition to their active roles in biophysical studies, designed DNA nanostructures have also proven of high utility in various applications including biological detection, bioreactors, and nanomedicine. We recently designed a rigid and structurally well-defined DNA tetrahedral scaffold to effectively modulate the lateral interaction between anchored DNA probes and greatly facilitate target accessibility at the biosensing interface.[28] Consequently, a DNA tetrahedral-based electrochemical sensor for microRNAs was developed with an extremely low detection limit of 10 aM, exceeding conventional electrochemical DNA/RNA sensors by 2−5 orders of magnitude.[29] We also demonstrated that this tetrahedral scaffold provides a versatile platform for the detection of a broad range of targets, including proteins, metal ions, and other small molecules, by simply replacing the pedant probe with various functional nucleic acids.[28]

Since DNA nanostructures are completely water-soluble, anchored molecular probes can in fact interact with their targets in homogeneous solution rather than at the heterogeneous interface. Ke et al. first reported the use a DNA origami-based rectangular structure to site-specifically anchor DNA probes and develop a solution-phase DNA array (Figure 3B).[30] As a step further, we developed a fully addressable DNA array by using an asymmetric DNA origami substrate with the shape of a Chinese map, which obviated the use of an internal index.[31] By introduction of a toehold strand-displacement reaction that could effectively differentiate single-base mismatches, this DNA origami chip demonstrated the ability to perform SNP genotyping (Figure 3C).[32] Seeman and co-workers later reported an ingenious design that converted the SNP genotyping results to visual graphical representations of four nucleotides (Figure 3D).[33]

Although these DNA origami arrays are essentially in the solution phase, there still exist significant steric and crowding effects at the interface of the DNA substrate. Pinheiro et al. investigated several steric parameters that could affect the hybridization kinetics of ssDNA probes anchored on a

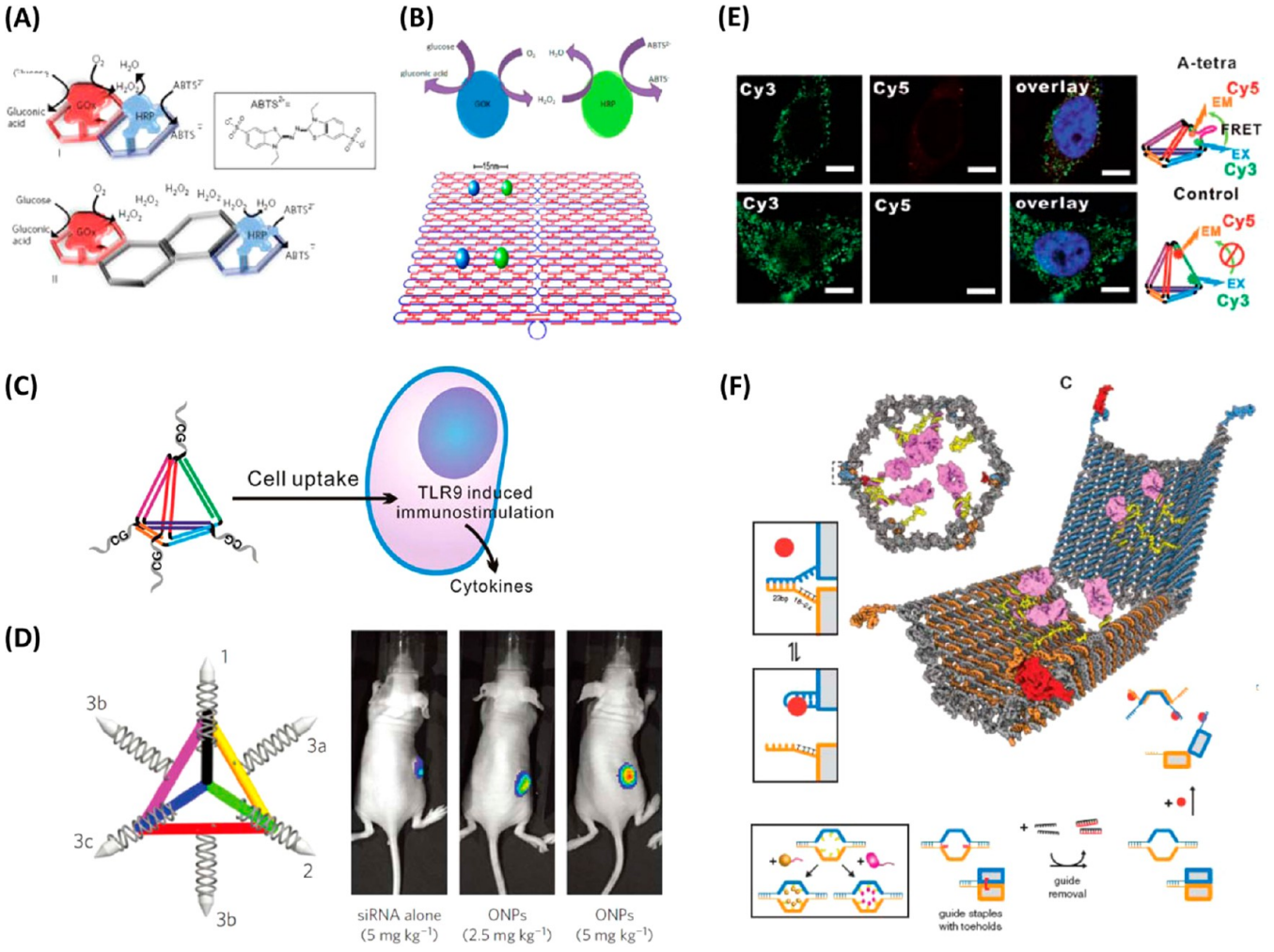


Figure 4. DNA nanostructures for biomedical applications. (A) Organization of GOx/HRP cascades on a two-dimensional DNA strip. Reproduced from ref 36. Copyright 2009 Nature Publishing Group. (B) Bienzyme cascade of GOx and HRP in short DNA nanotubes. Reproduced from ref 38. Copyright 2013 American Chemical Society. (C) CpG-bearing DNA tetrahedron with immunostimulatory effects. Reproduced from ref 5. Copyright 2011 American Chemical Society. (D) DNA tetrahedron-based delivery of siRNA in mice. Reproduced from ref 4. Copyright 2012 Nature Publishing Group. (E) Reconfigurable DNA tetrahedra for logic detection of intracellular ATP. Reproduced from ref 44. Copyright 2012 Wiley-VCH. (F) Targeted delivery of antibodies with nanorobots. Reproduced from ref 3. Copyright 2012 AAAS.

rectangular six-helix tile.[34] More recently, single-particle fluorescence resonance energy transfer (FRET) studies revealed that both the hybridization kinetics and thermodynamics were significantly altered on dense probe arrays while they were altered to a much less extent on sparse ones.[35] Clearly, these studies highlight the significance of systematic investigations of biomolecular interactions at the interface.

In addition to the anchoring of DNA probes, Willner's group and Yan's group attached an enzyme pair of glucose oxidase (GOx) and horseradish peroxidase (HRP) on either DNA strips or DNA origami (Figure 4A).[36,37] The precise spatial control of the bienzyme pair greatly reduced diffusion distances and increased the efficiency of enzyme cascade. Very recently, we constructed a high-efficiency nanoreactor by confining GOx and HRP in the internal side of short DNA origami nanotubes. The activity enhancement is a synergetic effect arising from both the stabilizing effect of the DNA nanostructure and the confining effect within the nanotube that resembles the crowded environments within cells (Figure 4B).[38]

Naked DNA oligonucleotides have a very short half-life in serum and are usually unable to penetrate cell membranes. However, recent studies have revealed that DNA nanostructures possess the ability to enter various cell lines with high resistance to enzymatic degradation.[5,39] We designed a DNA tetrahedral nanostructure appended with multivalent CpG oligonucleotides (ODNs). Unmethylated CpG ODNs can activate the mammalian immune system for both innate and adaptive immunity, which is a promising therapeutic tool to treat diseases including infection, cancer, and allergies (Figure 4C). We found that these nanostructures could be readily internalized by macrophage-like RAW264.7 cells and remained substantially intact in the cytoplasm for many hours. These CpG-pendant tetrahedral nanostructures are highly active as immunostimulatory agents, inducing high levels of cytokine secretion (TNF-α, IL-6, and IL-12).[5] More importantly, the ability to precisely control the valence of payloads offers the opportunity to tune the dosage of drugs. Liedl's group and our group later designed large DNA nanostructures that carried high loading of CpG ODNs, which further increase the efficacy as immunostimulatory drugs.[40,41]

A DNA tetrahedron was also utilized for siRNA delivery in a mouse model. Lee et al. designed a DNA tetrahedron modified

dx.doi.org/10.1021/ar400324n | Acc. Chem. Res. 2014, 47, 1720−1730

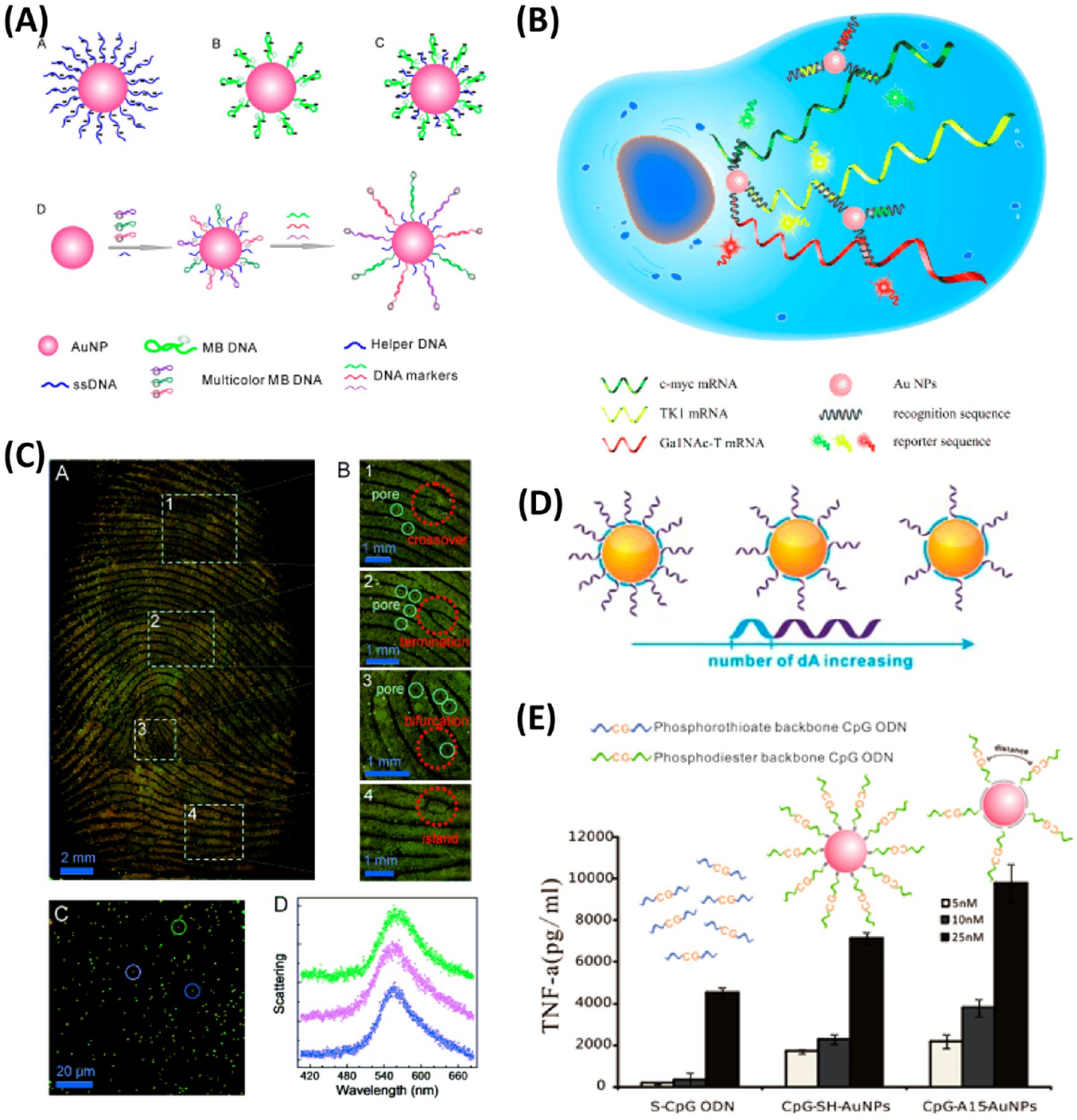


Figure 5. DNA–NM hybrids for biological applications. (A) AuNP-based multicolor nanobeacons. Reproduced from ref 45. Copyright 2009 Wiley-VCH. (B) AuNP-based multicolor nanobeacons for imaging of multiple mRNAs in living cells. Reproduced from ref 46. Copyright 2012 Wiley-VCH. (C) Dark-field images of sebaceous latent fingerprint. Reproduced from ref 48. Copyright 2013 Wiley-VCH. (D) PolyA-based spatial control of DNA assembly on AuNPs. Reproduced from ref 51. Copyright 2012 American Chemical Society. (E) The density of CpG on AuNPs regulates cytokine production. Reproduced from ref 56. Copyright 2014 Wiley-VCH.

with six RNA strands, and a tumor-targeting molecule, folate acid, which decreased both mRNA and protein levels of luciferase to ~50% in xenographic tumors (Figure 4D).[4] In a different study, Jiang et al. developed a DNA origami-based drug delivery system for doxorubicin (Dox), a widely used antitumor drug. Their system showed high levels of Dox loading and efficient cellular uptake, which could be used to reverse multiple drug resistance (MDR) of drug-resistant MCF-7/ADM human breast cancer cells.[42] In addition to these therapeutic systems, Delebecque et al. demonstrated an interesting example that RNA nanostructures could act as a scaffold to carry well-organized enzymes into bacteria, which greatly increase the hydrogen-producing cascade. Significantly, this nanoscale bioreactor could improve the hydrogen output of living bacteria.[43]

DNA nanostructures can be embedded with stimuli-responsive functional nucleic acids, for example, aptamers, i-motif structures, or DNAzymes, which bring dynamics to the

dx.doi.org/10.1021/ar400324n | Acc. Chem. Res. 2014, 47, 1720–1730

above-mentioned static structures. We designed a dynamic DNA tetrahedral nanostructure with anti-ATP aptamers embedded in one or more of edges. Binding of the target ATP induced significant conformational change, which in turn altered the FRET efficiency of the labeled fluorophore pair (Cy3 and Cy5). This nanostructure could enter cells and function as an intracellular sensor for monitoring ATP levels (Figure 4E).[44] Douglas et al. developed a container-like DNA nanostructure that was locked with two aptamers specific to two different receptors on the cell membrane. They successfully applied these DNA containers for targeted, logical release of proteins upon binding to cancer cells, which effectively induced cell death or apoptosis (Figure 4F).[3]

4. HYBRIDS OF DNA AND INORGANIC NANOMATERIALS

DNA can be anchored on the surface of inorganic nanomaterials via covalent or noncovalent attachment. Such nano−bio hybrids combine the highly specific recognition ability of DNA and optoelectronic properties of nanomaterials, providing new tools for both in vitro and in vivo studies.

A classic approach to prepare AuNP−DNA nanoconjugates is to modify AuNPs with thiolated DNA. These AuNP−DNA conjugates are extremely stable in solutions of high ionic strength. Because of the large surface area of AuNPs, many DNA strands can be loaded on a single nanoparticle, leading to collective effects that make surface-confined DNA molecules behave distinctly differently from their counterparts in solution. Our group developed multicolor nanobeacons by coassembling multiple hairpin-structured DNA probes labeled with different fluorophores on the surface of AuNPs, which could simultaneously detect three different DNA targets in solution (Figure 5A).[45] Recently, Tang and co-workers employed such nanobeacons to image multiple tumor-related intracellular mRNA in live cells (Figure 5B).[46] Zhang et al. replaced hairpin DNA probes with several aptamer sequences, which resulted in multicolor nanoprobes that could simultaneously detect adenosine, potassium ion, and cocaine with high specificity.[47] Very recently, aptamer-bound AuNPs were employed to serve as plasmonic nanoprobes for identifying latent fingerprints with fine structures under DFM. In addition to the conventional fingerprint identification, residual cocaine in the fingerprints could be detected semiquantitatively (Figure 5C).[48]

AuNP−DNA conjugates have also proven useful for the facilitation of biochemical reactions. Plaxco and co-workers employed DNA primer-modified AuNPs to improve the sensitivity and specificity of a PCR-based telomeric repeat amplification protocol (TRAP) for the analysis of expression levels of an important marker of tumor progression, telomerase.[49] In a different study, Park et al. demonstrated that AuNP−DNA conjugates could significantly improve the efficiency of in vitro translation of a protein. They found that both the nonspecific binding of translation-related factors to AuNPs and specific binding of target mRNA mediated by conjugated DNA were critical for the observed 2-fold enhancement.[50]

An alternative approach for preparing AuNP−DNA conjugates was recently developed in our laboratory. Instead of using expensive thiolated DNA to modify AuNPs, designed diblock ssDNA sequences with polyadenine (polyA) blocks could irreversibly bind to gold (Figure 5D).[51,52] The stability is comparable to that of thiolated DNA.[53] In addition to the reduced cost and simplified procedures, the use of this new polyA-based strategy greatly increases the ability to control DNA assembly on the AuNP surface. The lateral spacing and surface density of DNA strands can be effectively controlled by simply changing the length of the polyA.[51] By using such polyA-based AuNP probes, we could construct various DNA sensors with higher specificity and faster hybridization kinetics than those with thiolated DNA.

In 2006, Mirkin and co-workers found that AuNP−DNA conjugates could penetrate the cell membrane with high efficiency.[54] To test its ability to deliver a therapeutic ODN, we self-assembled thiolated CpG ODNs on the surface of AuNPs and evaluated their cellular effects. We found that CpG ODNs loaded on AuNPs were not only resistant to nuclease degradation but could easily enter RAW264.7 cells. After entering endosomes, CpG−AuNP conjugates bind to toll-like receptor 9 (TLR9) and activate the TLR9 signal pathway, which induces the secretion of proinflammatory cytokines with high efficiency.[55]

Our studies also revealed that the conformation of CpG on the surface of AuNPs is critical for its function.[55] To effectively modulate the surface density of CpG and its conformation, we adopted the polyA strategy to assemble CpG−AuNP conjugates with controllable and tunable density.[56] We found that polyA-based nanoconjugates showed higher immunostimulatory activity than their thiolated counterpart, by ∼20%. As a step further, we examined that whether CpG−AuNP conjugates could stimulate immune responses in vivo.[56] Administration of both CpG−SH−AuNPs and CpG−polyA−AuNPs via vein injection in mice showed effective stimulation of cytokine secretion, suggesting that these nanoconjugates survive in the complicated physiological environment (Figure 5E).[56]

GO can also be stably assembled with ssDNA. However, since their binding energy is only half of that of AuNPs modified with thiolated DNA (∼120 kJ/mol), target binding can disrupt the adsorption and release ssDNA from GO. For example, Lu et al. found that either DNA hybridization or aptamer-target binding released fluorophore-tagged ssDNA from GO and resulted in fluorescence emission.[57] Wang et al. designed an aptamer−GO nanocomplex for cell imaging, which could efficiently enter live cells due to the cell permeability of GO. By using ATP and GTP aptamers, they have successfully monitored intracellular ATP and GTP levels in real time (Figure 2C).[58]

5. CONCLUSIONS AND PERSPECTIVE

In this Account, we have summarized multiple ways DNA and DNA structures interact and function at the interface with inorganic materials and cells. Because of the inherent differences in their physical properties, ssDNA possesses remarkably higher affinity to many inorganic materials than dsDNA or DNA structures, which forms the physical basis for developing various biosensing strategies and opens new opportunities to regulate biochemical processes in vitro and in vivo.

The interface between DNA structures and cells is even more interesting. Naked DNA oligonucleotides are usually unable to penetrate cell membranes. However, DNA nanostructures and hybrid DNA−NM conjugates have shown potent ability to enter a variety of cell lines, which poses a scientifically interesting question that is also potentially useful in biomedical applications. While mechanistic studies on these interesting processes remain to be carried out, preliminary evidence shows

dx.doi.org/10.1021/ar400324n | Acc. Chem. Res. 2014, 47, 1720−1730

that these are multifactor processes involving various physicochemical properties of nanostructures, for example, size, shape, and charge, and the types of cells.[59] Their cellular fates are also important for the realization of cellular functions, which are dependent on specific endocytotic pathways. Cellular uptake usually involves several potential pathways, including phagocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, clathrin- and caveolin-independent pathways, and actin-dependent macropinocytosis. In a specific case, internalization via caveolin-mediated endocytosis does not merge with lysosomes, which provides a route to escape lysosomal enzymatic degradation. Given these considerations, it is important to elaborate the design of nanostructures for realizing their intracellular and in vivo applications.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: huangqing@sinap.ac.cn.
*E-mail: fchh@sinap.ac.cn.

Funding

This work was financially supported by the Ministry of Science and Technology of China (Grants 2012CB825805, 2013CB933802, and 2013CB932803) and the National Natural Science Foundation of China (Grants 21390414, 21329501, 31100716, 31371015, and 91313302).

Notes

The authors declare no competing financial interest.

Biographies

Nan Chen received her B.S. from Nanjing University and Ph.D. from Swiss Federal Institute of Technology in Zurich (ETHZ). She joined Shanghai Institute of Applied Physics (SINAP), Chinese Academy of Sciences, in 2010 as an associate professor. Her research interests focus on the cellular mechanisms of nanomaterials-related biological effects.

Jiang Li received his Ph.D. degree from Sichuan University in 2009. After that, he joined SINAP and was promoted to associate professor in 2013. His research interests are in self-assembly of multifunctional nanostructures based on DNA and their applications in biomedical diagnosis and therapies.

Haiyun Song received his Bachelor and Master degrees from Nanjing University in 1996 and 1999, respectively. In 2005, he received his Ph.D. degree from University of California, Los Angeles. After his postdoctoral research at University of Zurich, Dr. Song joined Shanghai Institute of Biological Science, CAS, as a Principal Investigator in 2010. He is now Professor and Head of the Laboratory of Metabolic Signaling Pathways in Institute for Nutritional Sciences, SIBS, CAS.

Jie Chao received her Ph.D. from Nanjing University in 2008. In 2006−2007, she was a visiting student in Prof. Seeman's laboratory at New York University. After her postdoctoral research at Nanjing University, she joined SINAP in 2011 as an associate professor. Her research interests focus on DNA nanostructures and their applications.

Qing Huang obtained his B.S. degree from Nanjing University in 1996 and Ph.D. from Sichuang University in 2003. After his postdoctoral research at Louisiana State University, he joined the faculty of SINAP in 2003. He is now Professor and Deputy Chief of the Division of Physical Biology at SINAP.

Chunhai Fan obtained his B.S. and Ph.D. from Nanjing University in 1996 and 2000. After his postdoctoral research at the University of California, Santa Barbara, he joined the faculty of SINAP in 2004. He is now Professor and Chief of the Division of Physical Biology and the Bioimaging Center at SINAP. He is also an associate editor of *ACS Applied Materials and Interfaces*. Dr. Fan has published over 200 papers in peer-reviewed journals.

REFERENCES

(1) Linko, V.; Dietz, H. The enabled state of DNA nanotechnology. *Curr. Opin. Biotechnol.* **2013**, *24*, 555−561.
(2) Li, J.; Fan, C.; Pei, H.; Shi, J.; Huang, Q. Smart drug delivery nanocarriers with self-assembled DNA nanostructures. *Adv. Mater.* **2013**, *25*, 4386−4396.
(3) Douglas, S. M.; Bachelet, I.; Church, G. M. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **2012**, *335*, 831−834.
(4) Lee, H.; Lytton-Jean, A. K.; Chen, Y.; Love, K. T.; Park, A. I.; Karagiannis, E. D.; Sehgal, A.; Querbes, W.; Zurenko, C. S.; Jayaraman, M.; Peng, C. G.; Charisse, K.; Borodovsky, A.; Manoharan, M.; Donahoe, J. S.; Truelove, J.; Nahrendorf, M.; Langer, R.; Anderson, D. G. Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery. *Nat. Nanotechnol.* **2012**, *7*, 389−393.
(5) Li, J.; Pei, H.; Zhu, B.; Liang, L.; Wei, M.; He, Y.; Chen, N.; Li, D.; Huang, Q.; Fan, C. Self-assembled multivalent DNA nanostructures for noninvasive intracellular delivery of immunostimulatory CpG oligonucleotides. *ACS Nano* **2011**, *5*, 8783−8789.
(6) Li, H.; Rothberg, L. Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 14036−14039.
(7) Wang, L.; Liu, X.; Hu, X.; Song, S.; Fan, C. Unmodified gold nanoparticles as a colorimetric probe for potassium DNA aptamers. *Chem. Commun.* **2006**, 3780−3782.
(8) Wang, J.; Wang, L.; Liu, X.; Liang, Z.; Song, S.; Li, W.; Li, G.; Fan, C. A gold nanoparticle-based aptamer target binding readout for ATP assay. *Adv. Mater.* **2007**, *19*, 3943−3946.
(9) Zhang, J.; Wang, L.; Pan, D.; Song, S.; Boey, F. Y.; Zhang, H.; Fan, C. Visual cocaine detection with gold nanoparticles and rationally engineered aptamer structures. *Small* **2008**, *4*, 1196−1200.
(10) Wang, L. H.; Li, J.; Song, S. P.; Li, D.; Fan, C. Biomolecular sensing via coupling DNA-based recognition with gold nanoparticles. *J. Phys. D: Appl. Phys.* **2009**, *42*, No. 203001.
(11) Luo, W.; Zhu, C.; Su, S.; Li, D.; He, Y.; Huang, Q.; Fan, C. Self-catalyzed, self-limiting growth of glucose oxidase-mimicking gold nanoparticles. *ACS Nano* **2010**, *4*, 7451−7458.
(12) Zheng, X.; Liu, Q.; Jing, C.; Li, Y.; Li, D.; Luo, W.; Wen, Y.; He, Y.; Huang, Q.; Long, Y. T.; Fan, C. Catalytic gold nanoparticles for nanoplasmonic detection of DNA hybridization. *Angew. Chem., Int. Ed.* **2011**, *50*, 11994−11998.
(13) He, S.; Song, B.; Li, D.; Zhu, C.; Qi, W.; Wen, Y.; Wang, L.; Song, S.; Fang, H.; Fan, C. A graphene nanoprobe for rapid, sensitive and multicolor fluorescent DNA analysis. *Adv. Funct. Mater.* **2010**, *20*, 453−459.
(14) Yang, R.; Jin, J.; Chen, Y.; Shao, N.; Kang, H.; Xiao, Z.; Tang, Z.; Wu, Y.; Zhu, Z.; Tan, W. Carbon nanotube-quenched fluorescent oligonucleotides: probes that fluoresce upon hybridization. *J. Am. Chem. Soc.* **2008**, *130*, 8351−8358.
(15) Geim, A. K.; Novoselov, K. S. The rise of graphene. *Nat. Mater.* **2007**, *6*, 183−191.
(16) Pei, H.; Li, J.; Lv, M.; Wang, J.; Gao, J.; Lu, J.; Li, Y.; Huang, Q.; Hu, J.; Fan, C. A graphene-based sensor array for high-precision and adaptive target identification with ensemble aptamers. *J. Am. Chem. Soc.* **2012**, *134*, 13843−13849.
(17) Li, F.; Huang, Y.; Yang, Q.; Zhong, Z.; Li, D.; Wang, L.; Song, S.; Fan, C. A graphene-enhanced molecular beacon for homogeneous DNA detection. *Nanoscale* **2010**, *2*, 1021−1026.
(18) Xu, H.; Wang, D.; He, S.; Li, J.; Feng, B.; Ma, P.; Xu, P.; Gao, S.; Zhang, S.; Liu, Q.; Lu, J.; Song, S.; Fan, C. Graphene-based nanoprobes and a prototype optical biosensing platform. *Biosens. Bioelectron.* **2013**, *50*, 251−255.

(19) Li, H.; Huang, J.; Lv, J.; An, H.; Zhang, X.; Zhang, Z.; Fan, C.; Hu, J. Nanoparticle PCR: nanogold-assisted PCR with enhanced specificity. *Angew. Chem., Int. Ed.* **2005**, *44*, 5100−5103.
(20) Jia, J.; Sun, L.; Hu, N.; Huang, G.; Weng, J. Graphene enhances the specificity of the polymerase chain reaction. *Small* **2012**, *8*, 2011−2015.
(21) Chen, P.; Pan, D.; Fan, C.; Chen, J.; Huang, K.; Wang, D.; Zhang, H.; Li, Y.; Feng, G.; Liang, P.; He, L.; Shi, Y. Gold nanoparticles for high-throughput genotyping of long-range haplotypes. *Nat. Nanotechnol.* **2011**, *6*, 639−644.
(22) Qian, L.; Wang, Y.; Zhang, Z.; Zhao, J.; Pan, D.; Zhang, Y.; Liu, Q.; Fan, C.; Hu, J.; He, L. Analogic China map constructed by DNA. *Chin. Sci. Bull.* **2006**, *51*, 2973−2976.
(23) Zheng, J.; Birktoft, J. J.; Chen, Y.; Wang, T.; Sha, R.; Constantinou, P. E.; Ginell, S. L.; Mao, C.; Seeman, N. C. From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature* **2009**, *461*, 74−77.
(24) Douglas, S. M.; Chou, J. J.; Shih, W. M. DNA-nanotube-induced alignment of membrane proteins for NMR structure determination. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 6644−6648.
(25) Rajendran, A.; Endo, M.; Sugiyama, H. Single-molecule analysis using DNA origami. *Angew. Chem., Int. Ed.* **2012**, *51*, 874−890.
(26) Wu, N.; Czajkowsky, D. M.; Zhang, J.; Qu, J.; Ye, M.; Zeng, D.; Zhou, X.; Hu, J.; Shao, Z.; Li, B.; Fan, C. Molecular threading and tunable molecular recognition on DNA origami nanostructures. *J. Am. Chem. Soc.* **2013**, *135*, 12172−12175.
(27) Langecker, M.; Arnaut, V.; Martin, T. G.; List, J.; Renner, S.; Mayer, M.; Dietz, H.; Simmel, F. C. Synthetic lipid membrane channels formed by designed DNA nanostructures. *Science* **2012**, *338*, 932−936.
(28) Pei, H.; Lu, N.; Wen, Y.; Song, S.; Liu, Y.; Yan, H.; Fan, C. A DNA nanostructure-based biomolecular probe carrier platform for electrochemical biosensing. *Adv. Mater.* **2010**, *22*, 4754−4758.
(29) Wen, Y.; Pei, H.; Shen, Y.; Xi, J.; Lin, M.; Lu, N.; Shen, X.; Li, J.; Fan, C. DNA nanostructure-based interfacial engineering for PCR-free ultrasensitive electrochemical analysis of microRNA. *Sci. Rep.* **2012**, *2*, 867.
(30) Ke, Y.; Lindsay, S.; Chang, Y.; Liu, Y.; Yan, H. Self-assembled water-soluble nucleic acid probe tiles for label-free RNA hybridization assays. *Science* **2008**, *319*, 180−183.
(31) Zhang, Z.; Wang, Y.; Fan, C.; Li, C.; Li, Y.; Qian, L.; Fu, Y.; Shi, Y.; Hu, J.; He, L. Asymmetric DNA origami for spatially addressable and index-free solution-phase DNA chips. *Adv. Mater.* **2010**, *22*, 2672−2675.
(32) Zhang, Z.; Zeng, D. D.; Ma, H. W.; Feng, G. Y.; Hu, J.; He, L.; Li, C.; Fan, C. A DNA-Origami Chip Platform for Label-Free SNP Genotyping Using Toehold-Mediated Strand Displacement. *Small* **2010**, *6*, 1854−1858.
(33) Subramanian, H. K.; Chakraborty, B.; Sha, R.; Seeman, N. C. The label-free unambiguous detection and symbolic display of single nucleotide polymorphisms on DNA origami. *Nano Lett.* **2011**, *11*, 910−913.
(34) Pinheiro, A. V.; Nangreave, J.; Jiang, S.; Yan, H.; Liu, Y. Steric crowding and the kinetics of DNA hybridization within a DNA nanostructure system. *ACS Nano* **2012**, *6*, 5521−5530.
(35) Johnson-Buck, A.; Nangreave, J.; Jiang, S.; Yan, H.; Walter, N. G. Multifactorial modulation of binding and dissociation kinetics on two-dimensional DNA nanostructures. *Nano Lett.* **2013**, *13*, 2754−2759.
(36) Wilner, O. I.; Weizmann, Y.; Gill, R.; Lioubashevski, O.; Freeman, R.; Willner, I. Enzyme cascades activated on topologically programmed DNA scaffolds. *Nat. Nanotechnol.* **2009**, *4*, 249−254.
(37) Fu, J.; Liu, M.; Liu, Y.; Woodbury, N. W.; Yan, H. Interenzyme substrate diffusion for an enzyme cascade organized on spatially addressable DNA nanostructures. *J. Am. Chem. Soc.* **2012**, *134*, 5516−5519.
(38) Fu, Y.; Zeng, D.; Chao, J.; Jin, Y.; Zhang, Z.; Liu, H.; Li, D.; Ma, H.; Huang, Q.; Gothelf, K. V.; Fan, C. Single-step rapid assembly of DNA origami nanostructures for addressable nanoscale bioreactors. *J. Am. Chem. Soc.* **2013**, *135*, 696−702.
(39) Walsh, A. S.; Yin, H.; Erben, C. M.; Wood, M. J.; Turberfield, A. J. DNA cage delivery to mammalian cells. *ACS Nano* **2011**, *5*, 5427−5432.
(40) Schuller, V. J.; Heidegger, S.; Sandholzer, N.; Nickels, P. C.; Suhartha, N. A.; Endres, S.; Bourquin, C.; Liedl, T. Cellular immunostimulation by CpG-sequence-coated DNA origami structures. *ACS Nano* **2011**, *5*, 9696−9702.
(41) Ouyang, X.; Li, J.; Liu, H.; Zhao, B.; Yan, J.; Ma, Y.; Xiao, S.; Song, S.; Huang, Q.; Chao, J.; Fan, C. Rolling circle amplification-based DNA origami nanostructures for intracellular delivery of immunostimulatory drugs. *Small* **2013**, *9*, 3082−3087.
(42) Jiang, Q.; S, C.; Nangreave, J.; Liu, X.; Lin, L.; Qiu, D.; Wang, Z. G.; Zou, G.; Liang, X.; Yan, H.; Ding, B. DNA origami as a carrier for circumvention of drug resistance. *J. Am. Chem. Soc.* **2012**, *134*, 13396−13403.
(43) Delebecque, C. J.; Lindner, A. B.; Silver, P. A.; Aldaye, F. A. Organization of intracellular reactions with rationally designed RNA assemblies. *Science* **2011**, *333*, 470−474.
(44) Pei, H.; Liang, L.; Yao, G.; Li, J.; Huang, Q.; Fan, C. Reconfigurable three-dimensional DNA nanostructures for the construction of intracellular logic sensors. *Angew. Chem., Int. Ed.* **2012**, *51*, 9020−9024.
(45) Song, S.; Liang, Z.; Zhang, J.; Wang, L.; Li, G.; Fan, C. Gold-nanoparticle-based multicolor nanobeacons for sequence-specific DNA analysis. *Angew. Chem., Int. Ed.* **2009**, *48*, 8670−8674.
(46) Li, N.; Chang, C.; Pan, W.; Tang, B. A multicolor nanoprobe for detection and imaging of tumor-related mRNAs in living cells. *Angew. Chem., Int. Ed.* **2012**, *51*, 7426−7430.
(47) Zhang, J.; Wang, L.; Zhang, H.; Boey, F.; Song, S.; Fan, C. Aptamer-based multicolor fluorescent gold nanoprobes for multiplex detection in homogeneous solution. *Small* **2010**, *6*, 201−204.
(48) Li, K.; Qin, W.; Li, F.; Zhao, X.; Jiang, B.; Wang, K.; Deng, S.; Fan, C.; Li, D. Nanoplasmonic imaging of latent fingerprints and identification of cocaine. *Angew. Chem., Int. Ed.* **2013**, *52*, 11542−11545.
(49) Xiao, Y.; Dane, K. Y.; Uzawa, T.; Csordas, A.; Qian, J. R.; Soh, H. T.; Daugherty, P. S.; Lagally, E. T.; Heeger, A. J.; Plaxco, K. W. Detection of telomerase activity in high concentration of cell lysates using primer-modified gold nanoparticles. *J. Am. Chem. Soc.* **2010**, *132*, 15299−15307.
(50) Park, S.; Hamad-Schifferli, K. Enhancement of in vitro translation by gold nanoparticle−DNA conjugates. *ACS Nano* **2010**, *4*, 2555−2560.
(51) Pei, H.; Li, F.; Wan, Y.; Wei, M.; Liu, H.; Su, Y.; Chen, N.; Huang, Q.; Fan, C. Designed diblock oligonucleotide for the synthesis of spatially isolated and highly hybridizable functionalization of DNA−gold nanoparticle nanoconjugates. *J. Am. Chem. Soc.* **2012**, *134*, 11876−11879.
(52) Opdahl, A.; Petrovykh, D. Y.; Kimura-Suda, H.; Tarlov, M. J.; Whitman, L. J. Independent control of grafting density and conformation of single-stranded DNA brushes. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 9−14.
(53) Zhang, X.; Liu, B.; Dave, N.; Servos, M. R.; Liu, J. Instantaneous attachment of an ultrahigh density of nonthiolated DNA to gold nanoparticles and its applications. *Langmuir* **2012**, *28*, 17053−17060.
(54) Rosi, N. L.; Giljohann, D. A.; Thaxton, C. S.; Lytton-Jean, A. K.; Han, M. S.; Mirkin, C. A. Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science* **2006**, *312*, 1027−1030.
(55) Wei, M.; Chen, N.; Li, J.; Yin, M.; Liang, L.; He, Y.; Song, H.; Fan, C.; Huang, Q. Polyvalent immunostimulatory nanoagents with self-assembled CpG oligonucleotide-conjugated gold nanoparticles. *Angew. Chem., Int. Ed.* **2012**, *51*, 1202−1206.
(56) Chen, N.; Wei, M.; Sun, Y.; Li, F.; Pei, H.; Li, X.; Su, S.; He, Y.; Wang, L.; Shi, J.; Fan, C.; Huang, Q. Self-assembly of poly-adenine-tailed CpG oligonucleotide-gold nanoparticle nanoconjugates with immunostimulatory activity. *Small* **2014**, *10*, 368−375.

(57) Lu, C. H.; Yang, H. H.; Zhu, C. L.; Chen, X.; Chen, G. N. A graphene platform for sensing biomolecules. *Angew. Chem., Int. Ed.* **2009**, *48*, 4785−4787.
(58) Wang, Y.; Li, Z.; Weber, T. J.; Hu, D.; Lin, C. T.; Li, J.; Lin, Y. In situ live cell sensing of multiple nucleotides exploiting DNA/RNA aptamers and graphene oxide nanosheets. *Anal. Chem.* **2013**, *85*, 6775−6782.
(59) Giljohann, D. A.; Seferos, D. S.; Patel, P. C.; Millstone, J. E.; Rosi, N. L.; Mirkin, C. A. Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. *Nano Lett.* **2007**, *7*, 3818−3821.