

# Smart Nanomaterials Inspired by Biology: Dynamic Assembly of Error-Free Nanomaterials in Response to Multiple Chemical and Biological Stimuli

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## ABSTRACT

Three-dimensional functional nanoscale assembly requires not only self-assembly of individual nanomaterials responsive to external stimuli, such as temperature, light, and concentrations, but also directed assembly of many different nanomaterials in one-pot responsive to multiple internal stimuli signaling the needs for such materials at a specific location and a particular time. The use of functional DNA (DNAzymes, aptamers, and aptazymes) to meet these challenges is reviewed. In addition, a biology-inspired proof-reading and error correction method is introduced to cope with errors in nanomaterials assembly.

## 1. Promises and Challenges in 3D Nanomaterials Assembly

Assembly of nanoscale functional materials has long been a focus of research, because these materials may find promising applications such as in nanoelectronics, photonics, computing, environmental monitoring, medical imaging, and diagnostics. An ideal nanoscale photonic assembly is shown in Figure 1A. Toward making such a dream into reality, remarkable advances in synthetic techniques have already resulted in a diverse range of high-quality *individual* nanomaterials, such as nanoparticles, nanotubes, and nanowires,<sup>1</sup> which can serve as building blocks for assembly of more complex nanostructures such as that shown in Figure 1A.<sup>2–5</sup> Having these building blocks alone is not enough, just like having

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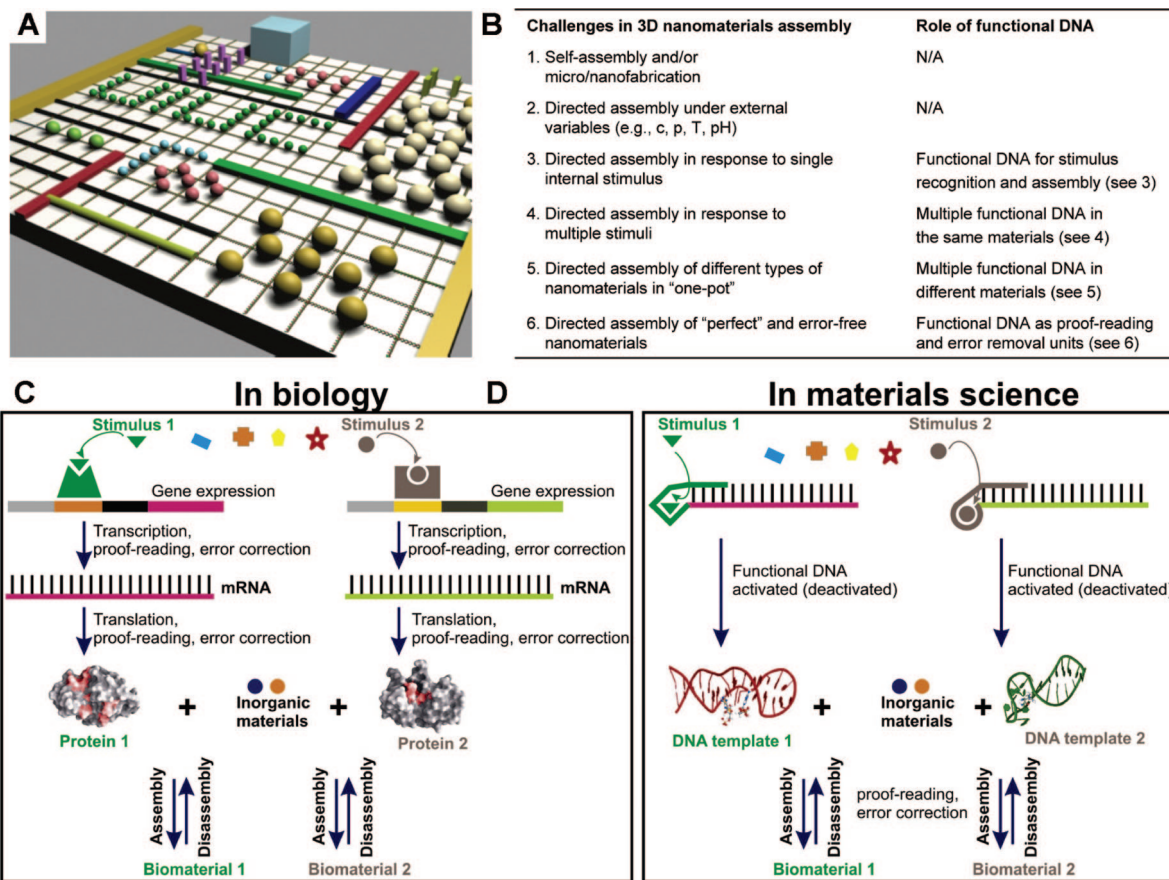
proteins, DNA, and carbohydrates alone is not enough to form a living and functional cell; dynamic control of the assembly and communication among these nanomaterials with high spatial and temporal resolution is required to make them into functional devices.

Biomaterials constantly provide inspiration to materials scientists and engineers. Most materials in biology are made of protein or protein scaffolds with inorganic minerals. Close examination of how these materials are made defines a number of challenges in a rough order of increasing difficulty (Figure 1B). For example, tremendous progress has been made not only in self-assembly of nanomaterials but also in directed assembly in response to external physical stimuli, such as temperature, light, ionic strength, or material concentration.<sup>6</sup> However, biomaterials are assembled under constant and ambient conditions, in response instead to internal chemical or biological stimuli that signal the need for initiation, growth, and termination of specific biomaterials at a specific location and at a particular time. Usually, many different materials grow in the same system, and in many cases, these materials are made in response to sophisticated multiple internal stimuli, often with cooperativity.<sup>7</sup> Finally, biology has developed a set of mechanisms to cope with errors in protein synthesis, which assures accuracy in the downstream materials assembly.

So then how does Nature achieve such an amazing feat? One way Nature accomplishes this is through genetic control (Figure 1C). In a biological system, such as a human body, many materials (teeth, bones, and soft tissues) are assembled at the same time. When the need for initiation of a particular material arises, related genes are turned on in response to corresponding stimuli to transcribe mRNA, which then translate into proteins to assemble biomaterials. Materials disassembly, such as release of iron in ferritin, can also be controlled in this way. Genetically controlled materials synthesis is “smart” in the sense that these materials are responsive to their chemical and biological environment and can, in turn, affect the environment. Furthermore, Nature makes defect-free proteins not because it does not make errors, but because it has developed elaborate systems of proof-reading and error correction.<sup>8,9</sup> Inspired by biology, stimuli-responsive peptide and protein-based synthetic materials assembly and disassembly have been reported.<sup>10–12</sup>

Nucleic acids are another important class of biopolymers. Recently, developments in biology have generated novel functional nucleic acids with binding and catalytic activities, just like proteins.<sup>13</sup> While most gene expressions are regulated through proteins, RNAs have recently been shown to be capable of fulfilling a similar role, and these are now called riboswitches.<sup>14</sup> These discoveries have thus remarkably expanded our understanding of nucleic acids from pure genetic materials to functional biopolymers

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**FIGURE 1.** Panel A shows an ideal 3D photonic assembly. In panel B, challenges in nanomaterials assembly and the role of functional DNA in meeting these challenges are listed along with the corresponding sections in the review. Panel C shows genetic control of stimuli-responsive assembly of materials in biology. In the same system, many different materials (dis)assembly occurs under constant ambient conditions with materials initiation, growth, and termination controlled by chemical stimuli with high spatial and temporal resolution, as well as error-correction mechanisms. Panel D present use of functional DNA to construct stimuli-responsive materials. Mimicking biological systems, functional DNA performs both functional (stimuli-responsive) and structural (materials assembly) roles, with no protein involvement required.

similar to proteins. On the materials science side, nucleic acids have been useful scaffolds and templates for assembly of inorganic nanoparticles to form many well-defined structures in the past decade.<sup>15–17</sup> We are interested in using functional nucleic acids to assemble and disassemble inorganic nanostructures in a way similar to Nature: to employ chemical and biological stimuli to activate or deactivate functional DNA for materials assembly; to use multiple stimuli to control different materials synthesis in one pot; and to grow materials at ambient conditions with proof-reading and error-correction mechanisms embedded (Figure 1D).

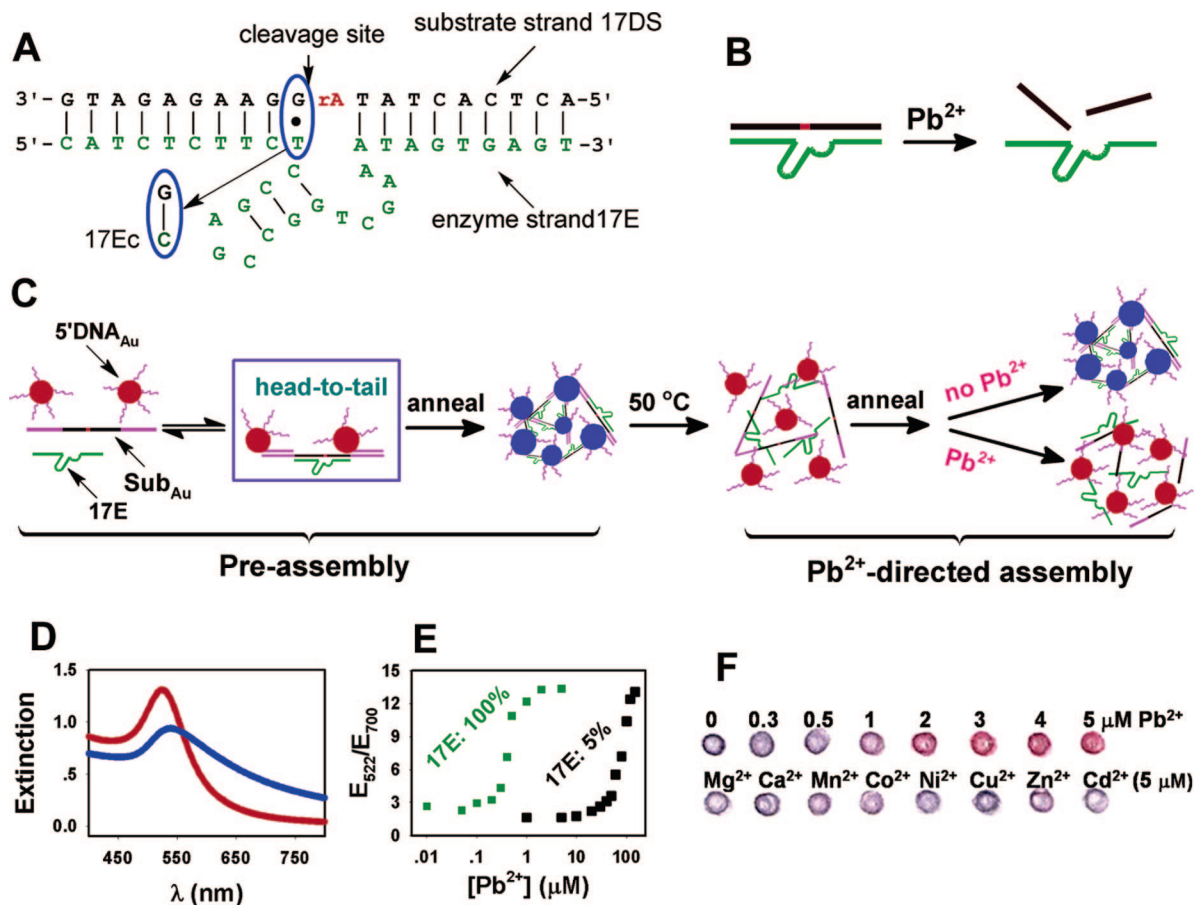
## 2. DNA and Functional DNA

To regulate DNA-linked nanomaterials by chemical stimuli, DNA and stimuli interactions need to be introduced first. The complementary strand of a DNA can be considered as a stimulus to the original DNA because the two strands can hybridize. Many organic molecules can interact with the major or minor grooves of DNA or act as DNA intercalators.<sup>18</sup> Some metal ions have been shown to interact with specific nucleotides. For example, it was recently reported that Hg<sup>2+</sup> can promote formation of thymine–thymine base pairs.<sup>19</sup> Since the early 1990s, many

short DNAs have been isolated with the ability to bind a broad range of molecules with high affinity and specificity, and such binding DNAs are known as DNA aptamers.<sup>20,21</sup> The species that can be recognized by aptamers range from small organic molecules to proteins and even intact viral particles.<sup>13</sup> In 1994, DNA was shown to be a catalyst for the first time,<sup>22</sup> and catalytic DNA molecules are called DNAzymes in this Account (also described as DNA enzymes, deoxyribozymes, or catalytic DNA elsewhere).<sup>23–27</sup> Importantly, most DNAzymes require a metal ion cofactor for activity and some DNAzymes show high metal specificity. These interactions between DNA and chemicals make it possible to design stimuli-responsive smart materials based solely on DNA.<sup>28</sup>

## 3. Directed Assembly in Response to a Single Internal Stimulus

**A. Head-to-Tail Alignment of Nanoparticles Assembled by DNAzymes.** In mimicking biology to construct stimuli-responsive materials, we employed a Pb<sup>2+</sup>-specific RNA-cleaving DNAzyme to direct the assembly state of gold nanoparticles in response to Pb<sup>2+</sup>.<sup>29</sup> The DNAzyme (Figure A) contains an enzyme strand (in green) and a substrate strand (in black).<sup>30,31</sup> The substrate contains a



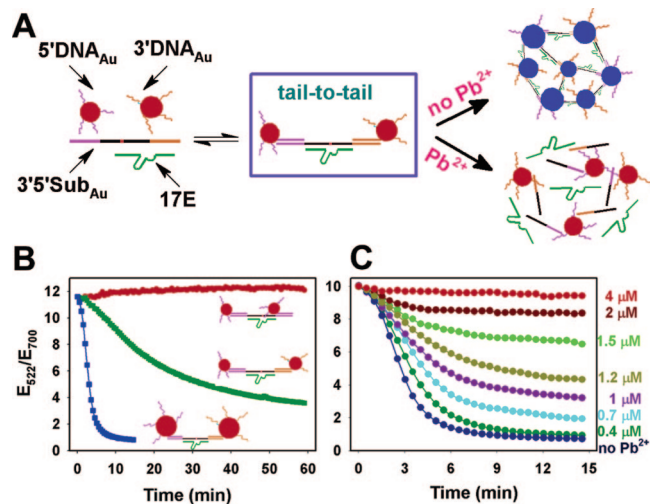
**FIGURE 2.** Panel A shows the secondary structure of the  $\text{Pb}^{2+}$ -specific DNAzyme. In panel B, in the presence of  $\text{Pb}^{2+}$ , the substrate is cleaved into two pieces. Panel C shows  $\text{Pb}^{2+}$ -directed assembly of DNAzyme-linked nanoparticles aligned in a head-to-tail manner. Panel D shows UV-vis spectra of disassembled (red) and assembled (blue) gold nanoparticles. Panels E and F show the assembly state or color of DNAzyme-linked nanoparticles in response to metal ions monitored by a spectrophotometer (E) or on a TLC plate (F).

single RNA linkage (rA) that serves as the cleavage site. In the presence of  $\text{Pb}^{2+}$ , the enzyme cleaves the substrate into two pieces (Figure 2B). The same DNAzyme motif (known as the 8-17 DNAzyme) has been independently selected under a variety of conditions by several research groups using different metal ions.<sup>24,32</sup> In a comprehensive study performed by Li and co-workers, the reason for the recurrence of this particular DNAzyme was attributed to its high catalytic rate, capability to cleave many dinucleotide junctions, small catalytic core, and high tolerance to nucleotide mutations.<sup>33</sup> To incorporate nanoparticle binding functions, the substrate was extended on both ends (Figure 2C). The nanoparticles were aligned in a head-to-tail manner, and only one set of nanoparticles was used. The nanoparticles were pre-assembled to ensure an optimal ratio between the DNAzyme and nanoparticles. After heating to 50 °C to fully disassemble the aggregates,  $\text{Pb}^{2+}$  can direct the outcome of assembly in the subsequent cooling process. If  $\text{Pb}^{2+}$  was present, the substrate was cleaved by the enzyme, and the assembly was inhibited. Otherwise, nanoparticles were re-assembled by the DNAzyme to form aggregates, accompanying a red-to-blue color change due to surface plasmon coupling. Upon assembly, the 522 nm plasmon peak decreased, while the extinction in the 700 nm region increased

(Figure 2D), and the extinction ratio at 522 nm over 700 nm was used to quantify the nanoparticle assembly state. A high ratio is associated with dispersed particles of red color, and a low ratio is associated with aggregated particles of blue color. With increasing concentration of  $\text{Pb}^{2+}$ , the extinction ratio increased, suggesting that nanoparticles were in a disassembled state (Figure 2E, green squares). The color change was also conveniently observed by spotting the nanoparticle solution on a TLC plate (Figure 2F). Only  $\text{Pb}^{2+}$  produced a red color, suggesting metal specificity.

**B. Tail-to-Tail Alignment of Nanoparticles Assembled by DNAzymes.** An annealing step (heating to 50 °C and subsequent cooling slowly to room temperature over 2 h) was needed to form head-to-tail aligned aggregates shown in Figure 2C. No assembly occurred by simple mixing of the DNAzyme and nanoparticles at room temperature (Figure 3B, red curve), which was attributed to the relatively large steric effects related to nanoparticle alignment.<sup>34,35</sup> Indeed, by changing the alignment to tail-to-tail, assembly at constant temperature was observed (Figure 3B, green curve). However, the rate of color change was relatively slow. It is known that the optical properties of a nanoparticle aggregate is governed by the size of the aggregate, instead of the number of nanoparticles in the



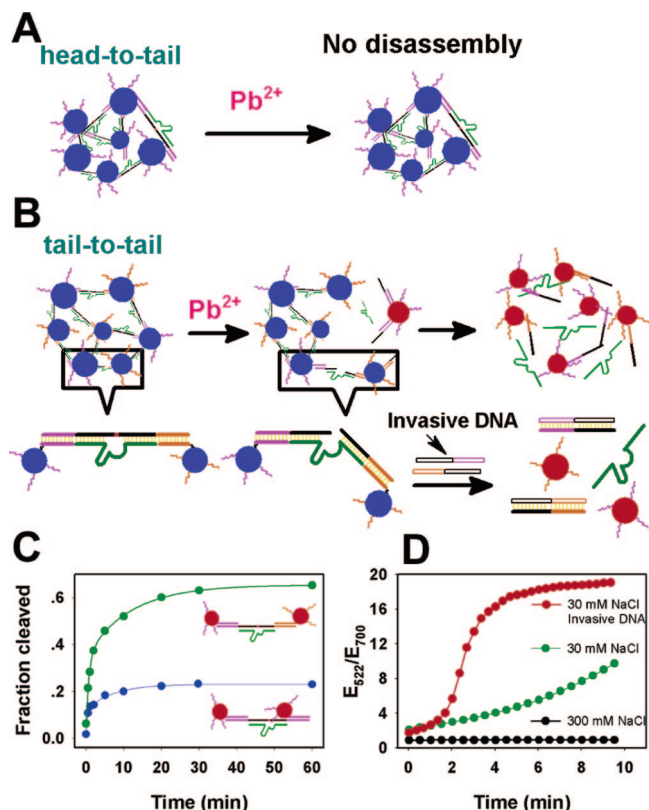


**FIGURE 3.** Panel A shows  $\text{Pb}^{2+}$ -directed assembly of DNAzyme-linked nanoparticle aligned in a tail-to-tail manner. Panel B shows the effect of nanoparticle alignment and size on the rate of color change. Panel C shows the kinetics of nanoparticle assembly in the presence of different  $\text{Pb}^{2+}$  concentrations.

aggregate.<sup>36</sup> Therefore, by using larger nanoparticles, the time needed to form an aggregate with a defined size should decrease, although the rate of assembly may not change. By changing nanoparticle diameter from 13 to 42 nm, a clear color change was observed in 5 min (Figure 3B, blue curve). Similarly,  $\text{Pb}^{2+}$  can direct the assembly state of the system (Figure 3A). The nanoparticles aggregated to different degrees at different  $\text{Pb}^{2+}$  concentrations (Figure 3C). Therefore, this system is also useful for colorimetric  $\text{Pb}^{2+}$  detection.<sup>35</sup>

**C. From “On/Off” Switches to Control of the Degree of Assembly.** The level of gene expression in a cell is often regulated by stimuli in a wide concentration range. In DNAzyme-linked nanostructures, it is also desirable to have materials assembly controlled by a wide stimuli concentration range. As a catalyst, the DNAzyme possesses multiple turnover properties, which allows tuning of the  $\text{Pb}^{2+}$  dynamic range. By changing the G·T wobble pair to a G·C Watson–Crick pair (Figure 2A, highlighted by blue circles), the DNAzyme activity is abolished. However, the mutated DNAzyme can still assemble nanoparticles. If only a small fraction of the active enzyme (17E) was used (i.e., 5%) with the rest being the inactive enzyme (17Ec), the  $\text{Pb}^{2+}$ -sensitive range shifted about 1 order of magnitude to higher  $\text{Pb}^{2+}$  concentrations (Figure 2E, black squares). This tuning property is unique and useful for sensing applications because it allows detection of  $\text{Pb}^{2+}$  in a wide concentration range without worrying about signal saturation.<sup>29</sup>

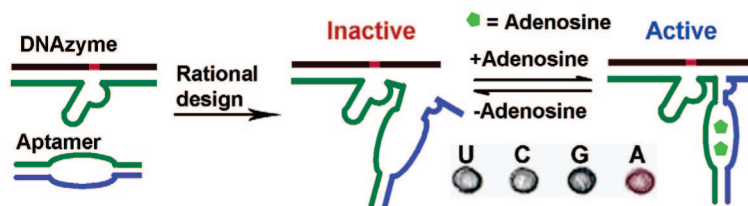
**D. From Directed Assembly to Directed Disassembly.** Although it may not be obvious, in biology materials disassembly is as important as assembly, such as the disassembly of ferritin in response to low iron levels and the degradation of biomaterials when they are no longer needed. Therefore, disassembly of nanoparticle aggregates was further investigated.<sup>37,38</sup> Most DNAzyme-linked gold nanoparticle aggregates contained hundreds to thousands of nanoparticles. Surprisingly, when  $\text{Pb}^{2+}$  was added to



**FIGURE 4.** In panel A, in head-to-tail aligned aggregates, the DNAzyme is inactive, and no  $\text{Pb}^{2+}$ -induced disassembly is observed. In panel B, in tail-to-tail aligned aggregates,  $\text{Pb}^{2+}$  can induce slow disassembly in low salt buffers. The linkages of nanoparticles before and after cleavage are zoomed in. By using invasive DNA, the rate of disassembly is accelerated. Panel C shows the kinetics of the DNAzyme cleavage reaction in nanoparticle aggregates. Panel D shows the kinetics of  $\text{Pb}^{2+}$ -induced disassembly under different conditions.

nanoparticle aggregates aligned in either configuration, no disassembly or color change was observed. To investigate the reason behind this, the 5'-ends of the substrates were labeled with  $^{32}\text{P}$ , and the kinetics of  $\text{Pb}^{2+}$ -induced substrate cleavage in nanoparticle aggregates was monitored. In the head-to-tail aligned aggregates, 22% of substrate was cleaved in 1 h, while in tail-to-tail aligned aggregates, 60% cleavage was observed (Figure 4C). From this study, it appeared that the DNAzyme was active in both aggregates, and we hypothesized that there should be inhibition of nanoparticle release after cleavage. To facilitate nanoparticle release, NaCl concentration was decreased from 300 to 30 mM for tail-to-tail aligned aggregates, and a slow color change was observed by addition of  $\text{Pb}^{2+}$  (Figure 4D, green curve). This color change was inhibited in 300 mM NaCl (black curve). However, even in low NaCl buffers,  $\text{Pb}^{2+}$  cannot accelerate the disassembly of head-to-tail aligned aggregates (Figure 4A), which suggested that the observed 22% cleavage was from the dangling DNAzyme with only one of its ends attached to nanoparticles, while the linking DNAzymes were not active.

To further accelerate disassembly in tail-to-tail aligned aggregates, two methods were developed. First, short DNAs complementary to the cleaved substrate fragments



**FIGURE 5.** An adenosine-activated aptazyme based on the  $\text{Pb}^{2+}$ -specific RNA-cleaving DNAzyme. A red colored spot was observed only in the presence of adenosine.

were added to invade the cleaved substrate (Figure 4B), which significantly accelerated the rate of disassembly (Figure 4D, red curve), and, as such, are called invasive DNA.<sup>37</sup> Alternatively, asymmetric DNAzymes were designed with one of the substrate binding arms elongated and the other one shortened to facilitate release of nanoparticles after cleavage.<sup>38</sup>

#### E. Application of DNAzymes to Other Nanomaterials.

In addition to gold nanoparticles, the DNAzyme has been used to functionalize other nanoscale building blocks such as carbon nanotubes.<sup>39</sup> It was demonstrated that the nanotube-conjugated DNAzyme maintained high activity, which opens the door for many applications ranging from chemically directed assembly of nanotubes to nanoscale cellular therapeutics. Recently, a  $\text{Cu}^{2+}$ -specific DNAzyme was incorporated in a two-dimensional DNA array, and  $\text{Cu}^{2+}$ -induced change of periodicity in the array was demonstrated.<sup>40</sup>

#### F. The Use of Aptazymes in Directed Assembly and Disassembly.

Most DNAzymes employ only metal ions for their reactions. Besides the  $\text{Pb}^{2+}$ -specific DNAzyme described above, there are also DNAzymes specific for  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{UO}_2^{2+}$ .<sup>41</sup> Therefore, many metal-responsive nanomaterials can be obtained. Aptamers are known to bind a broad range of molecules beyond metal ions. To expand the DNAzyme-based methodology to prepare materials responsive to other chemicals, aptamers were inserted into the DNAzyme, and such DNAzymes are known as allosteric DNAzymes or aptazymes.<sup>42</sup> An aptazyme designed by Sen and co-workers was chosen to assemble gold nanoparticles responsive to adenosine.<sup>43</sup> The aptazyme was built on the  $\text{Pb}^{2+}$ -specific DNAzyme (Figure 5). An adenosine aptamer was inserted into one of the substrate binding arms of the enzyme strand.<sup>44</sup> In the absence of adenosine, binding to the substrate was disrupted due to the bulging aptamer motif. As a result, aptazyme activity was inhibited. In the presence of adenosine, the interaction between the aptamer and adenosine strengthened the binding of the substrate and the enzyme, and cleavage of the substrate was allowed. As shown in the TLC plate in Figure 5, adenosine-directed assembly of nanoparticles was demonstrated. Only the sample with adenosine showed a red color, while other nucleosides produced blue colored spots.<sup>45</sup>

#### G. The Use of Aptamers in Directed Assembly and Disassembly.

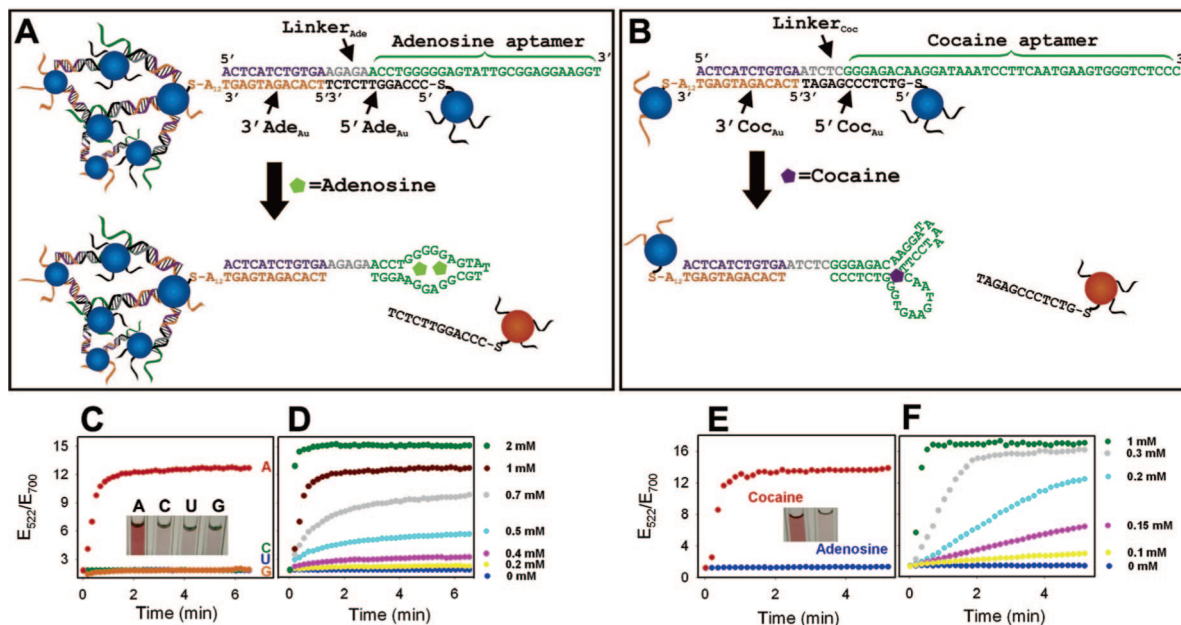
In previous DNAzyme and aptazyme-assembled materials, catalytic reactions were involved for stimuli recognition. The substrates were cleaved into two pieces, and therefore, the process was usually irreversible. In biological systems such as riboswitches,<sup>14</sup> binding-

based controls are the most frequently encountered. We further pursued the control of materials assembly states solely based on the binding function of aptamers. An aptamer can adopt two distinct binding states: binding to a complementary DNA and binding to its target molecule.<sup>46</sup> Taking advantage of this property, we prepared adenosine aptamer-linked nanoparticle aggregates as shown in Figure 6A.<sup>47–49</sup> Two kinds of DNA-functionalized gold nanoparticles were assembled by a linking DNA, which contained an adenosine aptamer (Figure 6A, in green) and an extension (in purple and gray). The purple part of the extension annealed to one nanoparticle ( $3'\text{Ade}_{\text{Au}}$ ). The gray part and a fraction of the aptamer sequence annealed to another ( $5'\text{Ade}_{\text{Au}}$ ). In the presence of adenosine, the aptamer switched its structure and bound adenosine.<sup>46</sup> As a result, the number of base pairs left to hybridize to  $5'\text{Ade}_{\text{Au}}$  decreased, leading to its dissociation and disassembly of the aggregate, changing the solution color from purple to red. The process was very specific for adenosine, and no other nucleosides could produce a similar color change (Figure 6C). Higher adenosine concentration induced faster disassembly (Figure 6D). Compared with nanoparticle assembly, which may take several minutes to hours, such disassembly can be finished in several seconds. The design is very general. We also constructed nanoparticle aggregates linked by a cocaine aptamer selected by Stojanovic et al. (Figure 6B).<sup>50</sup> In the presence of cocaine, a red color was produced; while in the presence of adenosine, no color change was observed (Figure 6E). The degree of disassembly also varied with cocaine concentration (Figure 6F). Similarly, materials responsive to potassium ions have also been obtained.<sup>51</sup>

Some proteins, such as thrombin and platelet-derived growth factors (PDGF), possess multiple aptamer binding sites. Therefore, these proteins can be used to cross-link aptamer-functionalized gold nanoparticles to form aggregates.<sup>52,53</sup> Such processes can also be considered as directed assembly with the stimulus being the proteins. Because of the requirement on the multivalency of aptamer targets, such assembly processes cannot be generalized to any chemical of choice.

## 4. Nanomaterials Responsive to Multiple Stimuli with Controllable Cooperativity

All the above materials were controlled by a single chemical stimulus. The next challenge would be to design materials whose assembly states are controlled by multiple stimuli simultaneously. Preferably, the cooperativity among



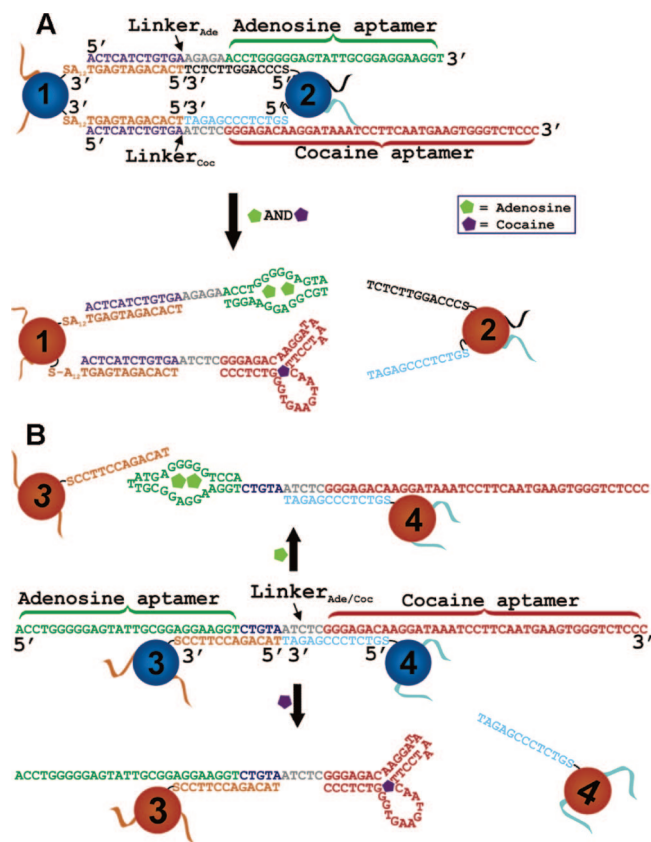
**FIGURE 6.** Chemically controlled disassembly of nanoparticle aggregates linked by an adenosine aptamer (A) or a cocaine aptamer (B). Specific disassembly of aggregates linked by an adenosine aptamer (C) and a cocaine aptamer (E), and kinetics of disassembly in the presence of different target concentrations in adenosine-aptamer-linked aggregates (D) and in cocaine-aptamer-linked aggregates (F).

the chemicals can be tuned so that more complex architectures with multifaceted properties can be fabricated. First, a system with high stimuli cooperativity was designed.<sup>51</sup> This system contained two kinds of nanoparticles: particles 1 and 2 (Figure A). Particle 1 was functionalized with one kind of DNA, and particle 2 was functionalized with two kinds of DNA. Both the adenosine aptamer and the cocaine aptamer were used to assemble the particles. To disassemble the aggregates, both adenosine and cocaine were needed. Neither molecule alone can induce significant disassembly. Therefore, the two molecules were highly cooperative in performing the disassembly task. In a separate system, two particles (particles 3 and 4) were assembled by a DNA containing both the adenosine aptamer and the cocaine aptamer (Figure 7B).

Therefore, either molecule can disassemble the aggregates, resulting in no cooperativity between the two stimuli.

## 5. Multiple Nanomaterials Responsive to Multiple Stimuli in One Pot

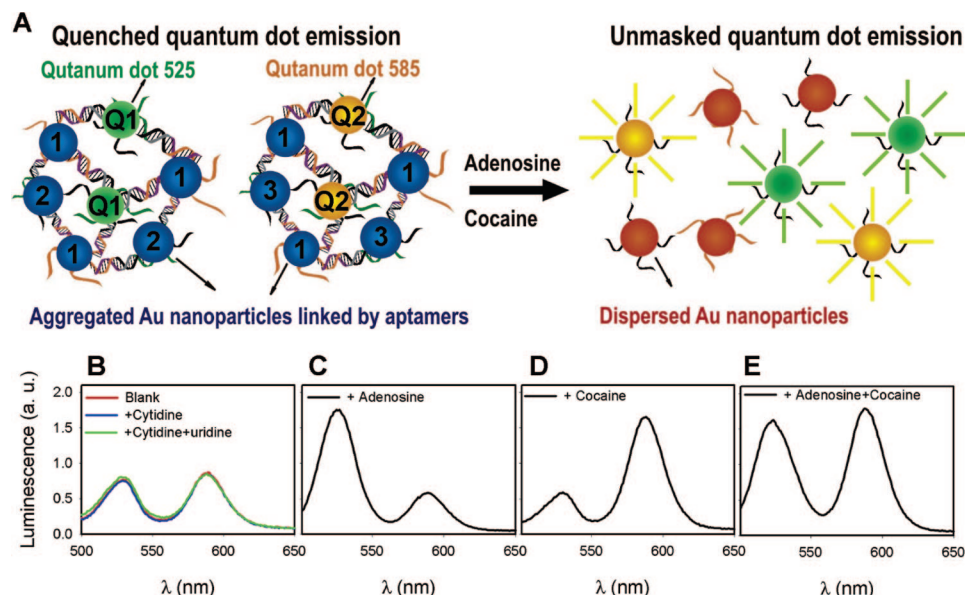
A further step toward meeting the challenges presented in Figure 1D is to assemble multiple different nanomaterials in one pot with the assembly state of each material controlled by a chemical stimulus. To achieve this goal, quantum dots (QDs) were introduced to encode different materials. The nanoparticle assemblies are shown in Figure A.<sup>54</sup> Using the adenosine responsive aggregates as an example, in addition to the gold nanoparticles 1 and 2, quantum dots Q1 (emitted at 525 nm) were also used. Q1 and gold nanoparticle 2 were functionalized with DNA of the same sequence, and therefore both particles can be linked to particle 1 by the adenosine aptamer linker. In the aggregated state, the emission of the QD was quenched because of energy transfer to the nearby gold



**FIGURE 7.** Chemically controlled disassembly of nanomaterials that requires both adenosine and cocaine (A) or either adenosine or cocaine (B).

nanoparticles.<sup>55</sup> Addition of adenosine disassembled the aggregates, resulting in increased emission intensity at 525 nm. Similarly, cocaine responsive aggregates were also prepared by incorporation of Q2 that emitted at 585 nm. The two kinds of aggregates were mixed, and two emission





**FIGURE 8.** Panel A shows a schematic of quantum dot encoded aptamer-linked nanostructures. Gold nanoparticles 1 and 2 and quantum dot Q1 were assembled by the adenosine aptamer DNA, while nanoparticles 1 and 3 and quantum dot Q2 were assembled by the cocaine aptamer. In both aggregates, quantum dot emissions were quenched. Addition of adenosine and cocaine disassembled the aggregates and increased emission intensity. Panels B–E show the steady-state emission spectra of mixed nanoparticle aggregates.

peaks at 525 and 585 nm can be observed (Figure 8B, red curve), corresponding to the adenosine and cocaine aggregates, respectively. Addition of cytidine (Figure 8B, blue curve) or cytidine and uridine (green curve) did not change the emission intensity of either peak. Addition of adenosine alone increased the 525 nm peak but not the 585 nm one (Figure 8C), while addition of cocaine alone increased the 585 nm peak but not the 525 nm one (Figure 8D). Addition of both molecules resulted in enhancement in both peaks (Figure 8E). This result demonstrated controlled disassembly of multiple different nanomaterials by different stimuli in one pot.

## 6. Proof-Reading and Error Correction in Nanomaterials Assemblies

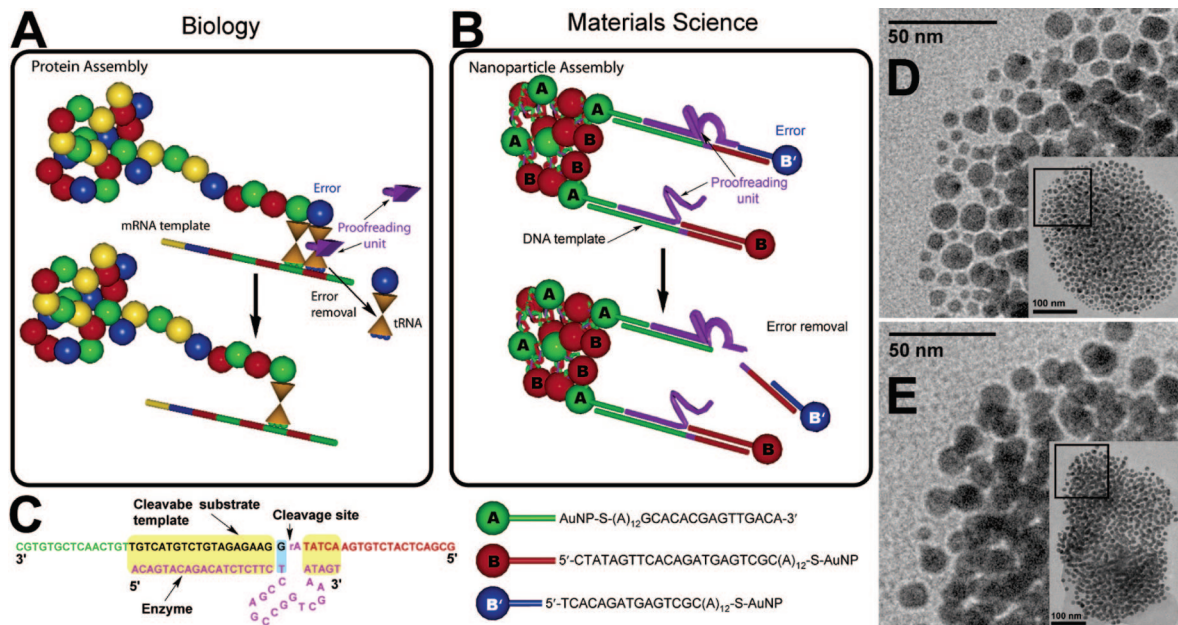
Errors occur in all nanoscale assembly processes, which is one of the major hurdles toward practical applications of assembled devices and structures. To reduce errors, most efforts have been focused on optimizing assembly processes to minimize errors or designing devices that can tolerate errors.<sup>56–58</sup> In biology, one can see that proof-reading and error-correction mechanisms have been used in many biological assembly processes, such as DNA transcription to RNA<sup>8</sup> and RNA translation to protein.<sup>9</sup> In the protein-assembly process, for example, a wrong amino acid could be incorporated. To overcome this problem, a protein enzyme is involved to proof-read the incorporated amino acid so that the wrong amino acid can be hydrolyzed, thus eliminating the error (Figure 9A).<sup>9</sup>

Inspired by biology, we want to introduce proof-reading and error correction in nanomaterials assembly.<sup>59</sup> As an initial step toward this effort, we used DNAzyme-directed disassembly of nanoparticles described in section 3D to demonstrate the concept. In the system, three kinds of nanoparticles encoded by three different DNA were

prepared. As shown in Figure 9B, two of the particles were defined as right particles (A and B), with the remaining one defined as a wrong particle (B'). The DNA attached to B was seven bases longer than that attached to B'. The substrate strand of the Pb<sup>2+</sup>-specific DNAzyme was used to template the assembly of nanoparticles (DNAzyme sequences shown in Figure 9C). One end of the substrate was attached with particle A, and the other end was designed to attach to B, even though B' could also be attached (Figure 9B). The enzyme strand (in purple) served as a proof-reading unit. When a wrong particle B' was incorporated, the enzyme can bind both ends to the substrate template. In the presence of Pb<sup>2+</sup>, which acted as a stimulus, the substrate was cleaved and the B' particle was removed. When a right particle B was incorporated, the enzyme can only bind one of its arms to the substrate and the active structure of the DNAzyme cannot form. As a result, the B particle was retained in the assembly. To differentiate error particles B' under TEM, B' was made smaller in size (5 nm diameter), while A and B were larger (13 nm, Figure 9D). After addition of Pb<sup>2+</sup>, most of the 5 nm particles were removed (Figure 9E), suggesting that the proof-reading DNAzyme can efficiently correct the errors in this system.

## 7. Summary and Outlook

By using the catalytic and binding functions of DNA, stimuli-responsive assembly and disassembly of nanomaterials have been demonstrated. These novel materials and processes are useful for colorimetric sensing of a broad range of analytes. In addition, by introduction of proof-reading and error-correction mechanisms into nanomaterials assembly, the assembled devices can be more robust and less vulnerable to defects.



**FIGURE 9.** Proof-reading and error removal in mRNA-templated protein assembly (A) or in DNAzyme-templated nanoparticle assembly (B). Panel C gives the DNAzyme sequence used for proof-reading and error correction. Panels D and E show TEM images of nanoparticle aggregates before (D) and after (E) error removal. Insets show the whole particle, and the zoomed parts are highlighted by black squares.

This Account only touches upon a tiny fraction of the powerful biological machinery available in making biomaterials and the benefits materials scientist can gain by mimicking biology. To expand dynamic assembly demonstrated for metallic nanoparticles, the methodology can be applied to the assembly of other nanomaterials, such as quantum dots and magnetic nanoparticles, resulting in new detection modes beyond simple color change.<sup>54</sup> Besides nanoparticles, functional DNA has also been shown to be active on other nanostructures, such as carbon nanotubes.<sup>39,60</sup> Incorporation of nanotube and nanowires in functional DNA-templated assembly may result in functional devices responsive to chemical stimuli. Due to the generality of the assembly process, it is possible to construct materials responsive to multiple analytes in one pot.<sup>54</sup> One can also envision the design of reversible assembly and disassembly processes in response to chemical stimuli, so that nanoswitches can be fabricated.

Further challenges in this emerging field include mimicking other aspects of biology in making biomaterials. For example, materials made in biology have highly ordered hierarchy structures. While DNA have been shown to be templates or scaffolds to form predictable one-, two-, and even three-dimensional structures, it remains difficult to functionalize these DNA templates or scaffolds with nanomaterials to form useful nanoscale devices such as such as photonic crystals. This challenge is likely to be met by designing rigid DNA/nanoparticle linkages. In addition, biological systems respond to the chemical stimuli not only passively but also progressively in such a way to change the environment. For example, an enzyme expression is turned on in response to the presence of a substrate to digest the substrate. It would be desirable to have synthetic materials made to possess similar properties such that the materials can be used not only for

sensing but also for autonomous repair and renewal. An even bigger challenge is to make materials that self-replicate and exponentially amplify. No matter what the methods are to meet these challenges, biology will always remain the biggest inspiration.

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