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Proofreading and Error Removal in a Nanomaterial Assembly**

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Recent progress in the self-assembly of nanomaterials promises to revolutionalize a number of science and engineering fields such as molecular electronics, photonics, and computation.[1–7] A key hurdle to realizing such a potential is the presence of imperfect structures or errors in the assembly process. To overcome this problem, major efforts have been focused on optimizing the assembly process to minimize errors or to design devices that can tolerate errors.[8–11] We argue that it could be equally valuable to investigate proofreading and error removal during and after assembly, as demonstrated beautifully in a number of processes in biology, such as in mRNA-templated protein synthesis (Figure 1 a).[12] Herein, we provide the first experimental demonstration of proofreading and error removal in nanomaterial assembly by using DNAzymes to specifically locate and remove errors in DNA-templated gold nanoparticle assemblies (Figure 1 b). The concept demonstrated here can be applied to the assembly of other nanomaterials using other bio- or biomimetic molecules.

We chose to demonstrate the proofreading and error-removal process in a DNA-templated nanoparticle assembly because remarkable progress has been made in the field, which has resulted in both nanostructures with high resolution, controllability, and versatility[1–3,13–15] and nanomaterials with potential for practical applications such as colorimetric sensing.[16–19] For a design strategy, we searched biological processes for guiding principles. As shown in Figure 1 a, errors could occur in protein synthesis on an mRNA template where an incorrect amino acid might be incorporated. To prevent such a process, proofreading units (such as GTPase elongation factor-Tu) are employed to identify such an error and remove the wrong amino acid.[12] Similarly, errors could occur in a DNA-templated gold nanoparticle assembly, where each nanoparticle is coded by DNA molecules with a unique sequence, just as each amino acid is coded by a transfer RNA (tRNA) molecule. Among the three kinds of nanoparticles in the system, two (A and B) are defined as "correct" particles to be assembled on the template, while the remaining one (B′) is

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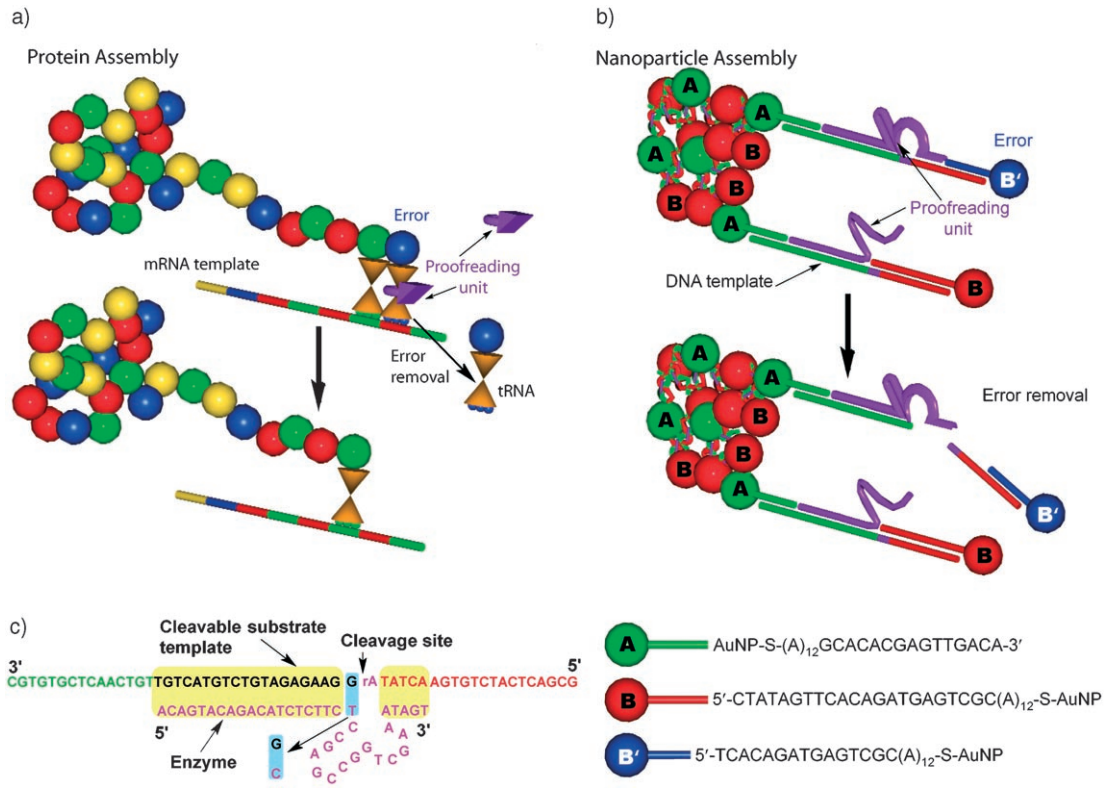

Figure 1. Proofreading and error removal in biology (a) and in materials science (b). a) The last tRNA moiety and its amino acid do not match the mRNA template (coded by colors) and are removed by the proofreading unit. b) The error-removal process is triggered by adding Pb^{2+} ions. c) Secondary structure of the DNAzyme used for proofreading and error removal in DNA-functionalized gold nanoparticle assemblies. The DNA sequences attached to gold nanoparticles (AuNP) are also shown. $(A)_{12}$ denotes a polyadenine spacer containing 12 A bases.

defined as an "error" particle (Figure 1b). Nanoparticle A can bind to the 3′ arm of the DNA template, while nanoparticle B binds to the 5′ arm of the template, both through sequence-specific hybridization. The error particle B′ can also bind to the 5′ arm of the template. However, the DNA attached to B′ is seven bases shorter than that attached to nanoparticle B. As a result, the affinity of B′ to the template is less than that of B, similar to the decreased affinity between an error tRNA and the mRNA template.

Instead of using protein enzymes for proofreading and error removal in protein synthesis, we chose to use a DNAzyme (DNA with catalytic activity), because it has the advantage of performing both structural roles to assemble nanoparticles and functional roles for proofreading and error removal.[20–22] The DNAzyme contains a substrate strand and an enzyme strand that form two duplex regions (Figure 1c, highlighted in yellow). In the presence of Pb^{2+} ions, the substrate is cleaved into two pieces at the rA position by the enzyme. The two duplex regions are designed to be asymmetrical, with one side containing 19 base pairs while the other side contains only five base pairs. Therefore, the cleaved fragment with the shorter binding arm can be easily released. To allow the DNAzyme substrate to be a template for nanoparticle assembly, the two overhangs on the substrate strand are complementary to the DNA attached to the nanoparticles. If a correct particle B is incorporated, binding of the longer arm of the enzyme to the DNA template is permitted while binding of the shorter arm to the DNA template is inhibited so that the active structure of the DNAzyme cannot form. As a result, the template is not cleaved and particle B is incorporated. When a B′ error particle is incorporated, the enzyme can bind both its arms to the substrate template and form an active structure to cleave the substrate and remove the B′ particle. This process is summarized in Figure 1b.

To demonstrate the specific removal of B′ particles from the assembly, a series of nanoparticle assemblies were prepared in such a way that the number of A particles equaled the sum of B and B′ particles. First, two extreme scenarios, 100% B (no B′) and 100% B′ (no B), were tested. The UV/Vis spectra of the two samples before and after error removal by Pb^{2+} addition are presented in Figure 2a and b, respectively. The sample with 100% B particles had only a slight increase of extinction at the 522-nm peak after error removal, which suggests that the assembly did not undergo significant changes (Figure 2a). However, for the sample with 100% B′ particles, the extinction at 522 nm increased whereas the extinction in the 700-nm region decreased significantly, thus resulting in a higher 522 to 700 nm extinction ratio. This finding suggests that nanoparticles in the A/B′ system were in the disassembled state (Figure 2b).[18,19] To test the effectiveness of proofreading and error removal in between the two extreme conditions, samples with various percentages of B′ particles were prepared and the 522 to 700 nm extinction

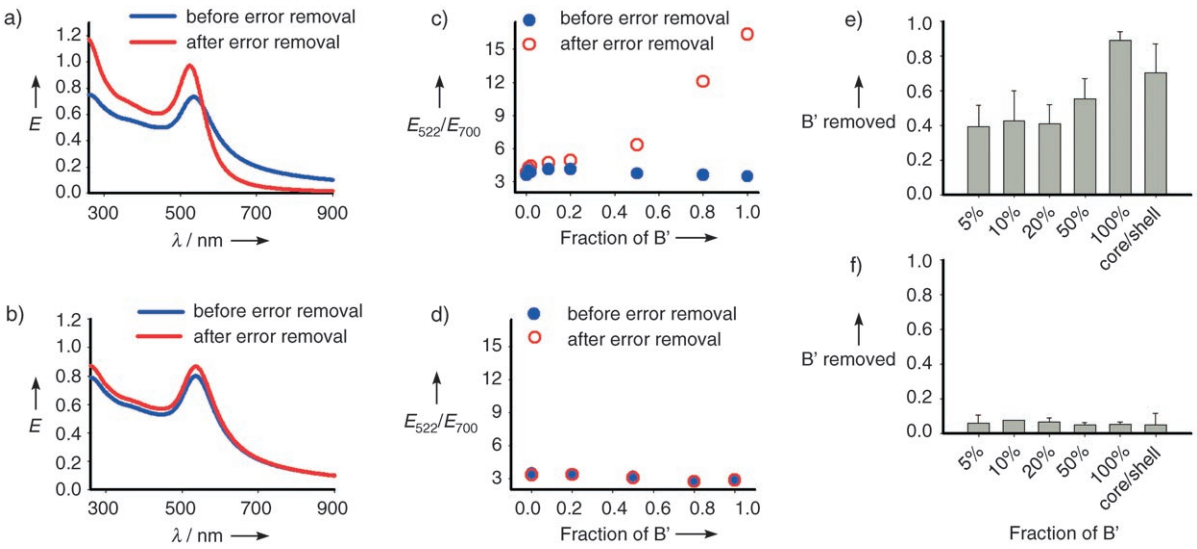

Figure 2. UV/Vis spectra of the nanoparticle assemblies made of particles A/B′ (a) or A/B (b) before and after proofreading and error removal. Extinction (*E*) ratios of the samples with various fractions of B′ particles before and after error removal with the native active enzyme (c) or mutated inactive enzyme (d). Fluorescence-based quantification of B′ particles removed from nanoparticle assemblies with the native active enzyme (e) or mutated inactive enzyme (f).

ratios before and after error removal were plotted against the fraction of B′ particles (Figure 2c). The extinction ratio increased with an increase of B′ particles, which suggests successful removal of the B′ particles from the assembly. To confirm that the change in the extinction ratio was a result of the error-removal mechanism, a control experiment was performed by replacing the enzyme strand with an inactive mutant. The mutated enzyme differs by only one base from the native enzyme (the T base highlighted in blue in Figure 1c was mutated to a C base.[23]) No change in the extinction ratio was observed by error removal with the mutated enzyme (Figure 2d). This control experiment strongly suggests that the DNAzyme is the key element in the proofreading and error-removal process.

To further investigate the extent of error removal, fluorescein-labeled DNA was used to functionalize the B′ particles in addition to the original fluorescein-free DNA. After addition of Pb^{2+} ions, the samples were centrifuged at 2000 rpm for 2 min. At such a speed, the nanoparticle assemblies were forced to the bottom, while dispersed particles remained in the supernatant. The isolated precipitate was redispersed and recentrifuged twice to completely separate the assembled nanoparticles from the dispersed nanoparticles. The fluorescein-labeled DNA was then displaced into solution by adding a high concentration of β-mercaptopropionic acid.[24] The percentage of B′ particles released from the assembled nanoparticles was calculated by comparing the fluorescence intensity from the assembled nanoparticles to that of the supernatant. As shown in Figure 2e, the percentage of B′ particles removed increased with the B′ particle percentage present in the assembled nanoparticles, from ca. 40% removal at a low percentage of B′ particles to almost complete removal at the 100% level. The incomplete removal of B′ particles at a low percentage in the assemblies is attributable to the fact that B′ particles embedded inside the assembled nanoparticles may not be released, even if the DNA template is cleaved. To support this hypothesis, assemblies of A and B particles were first prepared as a core, and then B′ particles were added as a shell to ensure that they were exposed on the surface of the assembly. Indeed, at a similarly low percentage of B′ particles, the percentage removal increased from ca. 40 to 70% for the core–shell structures. In addition, nonspecific release of nanoparticles in the process may contribute to the calculated percentage of error removal. This possibility was investigated by using the inactive enzyme mutant where the T base highlighted in Figure 1b was mutated to a C base (Figure 2f). Less than 10% of the B′ nanoparticles were released in the process of washing and handling. Therefore, nonspecific dissociations cannot account for the observed error removal.

The specific error-removal process was also confirmed by transmission electron microscopy (TEM). Instead of using 13-nm particles in all cases, 5-nm particles were used as the B′ error particles to differentiate them from the 13-nm correct particles (A and B). Typical TEM images of nanoparticle assemblies before and after error removal are presented in Figures 3a and b, respectively. The images indicate clearly that the number of 5-nm error particles in Figure 3a decreased significantly after error removal.

In summary, we have designed an experimental system for studying proofreading and error removal in a nanomaterial assembly. We showed that error particles can be specifically removed from the system. In addition, the error-correction process is controlled by a chemical stimulus (in this, case Pb^{2+} ions). By replacing the lead-specific DNAzyme with DNAzymes sensitive to other analytes, chemically controlled error removal can also be realized.[20,22,25,26] Finally, the concept presented here can be extended to other biomolecules, for example, to protein enzymes or biomimetic compounds such as chemical nucleases, for controlling the assembly not only of

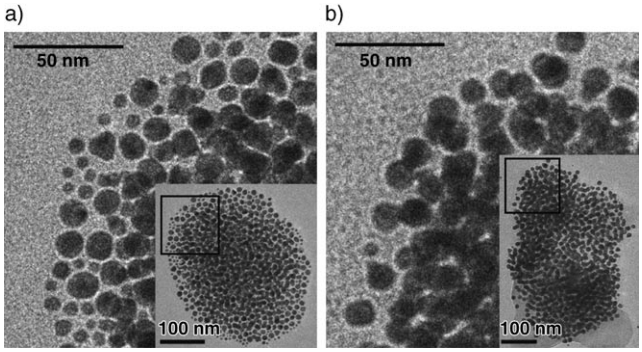

Figure 3. TEM images (acquired with a Philips CM200 TEM) of a nanoparticle assembly before (a) and after (b) proofreading and error removal. The complete nanoparticle assemblies are shown in the insets and the magnified regions are highlighted by squares. There are two kinds of nanoparticles in the images, with average diameters of 13 and 5 nm. The number of 5-nm error particles decreased significantly after the proofreading and error-removal process.

nanoparticles with defined size, shape, or composition, but also of other nanomaterials such as nanotubes or nanowires.

Experimental Section

Gold nanoparticles of 13- and 5-nm diameter were prepared by the citrate reduction[27] and $NaBH_4$ reduction[28] methods, respectively. Thiol-labeled DNA molecules were attached to gold nanoparticles by methods described in the literature.[27] The nanoparticles were purified twice by centrifugation, removal of the supernatant, and replacement with new buffer (100 mM NaCl, 25 mM Tris acetate, pH 8.2). To prepare fluorescein-labeled B′ particles, 5′-FAM-$(A)_{12}$-SH-3′ (20%) was also added in addition to the original DNA (80%).

Nanoparticles A, B, and/or B′ were mixed at designated ratios with a final extinction at 522 nm of ≈ 2. The system also contained substrate (100 nM), the enzyme strand (or the mutated enzyme, 200 nM), NaCl (300 mM), and Tris acetate buffer (25 mM, pH 8.2). The assembled nanoparticles were prepared by heating the sample at 65 °C for 1 min in a water bath (containing 60 mL water), and subsequently cooling to room temperature for 1 h. The sample was then centrifuged at 2000 rpm for 1 min. The supernatant was removed and the assembled nanoparticles were redispersed in NaCl (100 mM) and Tris acetate (25 mM, pH 8.2). To prepare samples for the TEM experiment, the 5-nm B′ particles were added later to form a core–shell (A/B–B′) structure, so that the B′ particles were mostly on the surface of the assembled nanoparticles, thus facilitating their release and observation.

Typically, two aliquots were taken from the prepared nanoparticle assemblies. Pb^{2+} ions (final concentration 20 μM) were added to one of the aliquots to initiate the error-correction process (reaction time 30 min), and the other aliquot was used for comparison.

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