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Reversible Loading of Nanoscale Elements on a Multicomponent Supramolecular Polymer System by Using DNA Strand Displacement

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Nucleic acids are excellent building blocks to enable switchable character in supramolecular polymer materials because of their inherent dynamic character and potential for orthogonal self-assembly. Herein, DNA-grafted squaramide bola-amphiphiles are used in a multicomponent supramolecular polymer system and it is shown that they can be addressed by DNA-labeled gold nanoparticles (5 and 15 nm) through sequence complementarity. These nanoparticles can be selectively erased or rewritten on-demand by means of DNA-strand displacement.

Since their inception three decades ago, interest in supramolecular polymer materials has continued to rise, with the field striving towards function. Supramolecular polymers consist of monomer units self-assembled through molecular recognition or stacking by using a combination of noncovalent interactions, such as hydrogen bonding, π stacking, charge, and solvophobicity, to provide networked structures with dynamic character over several length scales.[1–9] These features make supramolecular polymer materials excellent candidates for the construction of modular multicomponent systems, in which functional monomers can be introduced by simply matching the noncovalent interactions of the native and functional monomer units.[10–12]

Engineering of function within shape-persistent, one-dimensional supramolecular polymers consisting of amphiphiles can involve tethering[13–17] or embedding[18–21] of specific (bio)molecular or nanoscale components. For example, bioactive peptides[12,14,22] and drugs[16,23] have been incorporated into them through the use of various covalent chemistries to prepare monomers that self-assemble into supramolecular polymers with a multivalent presentation of a given component for applications in the biomedical area. Taking this one step further, if noncovalent and orthogonally addressable tethers are grafted onto supramolecular polymers consisting of amphiphiles, reversible, potentially on-demand displays of application-specific components can be envisioned.

A particularly attractive class of molecules for the dynamic display of functional units are nucleic acids. DNA has been widely exploited as a nanotechnological building block because of its precise dimensions, sequence programmability, and dynamic character.[24–28] The recent use of DNA on its own or in combination with covalent polymers, amphiphiles, or nanoparticles has resulted in stimuli-responsive scaffolds sensitive to specific nucleic acid inputs or to (bio)molecules through the introduction of aptamers.[29–34] It is thus highly appealing to introduce these types of features into supramolecular polymer materials based on amphiphiles to exploit their potential for orthogonal self-assembly to tune both the properties and function of materials. However, only a few groups have examined this powerful combination thus far.[35–38]

Previously, our group demonstrated the self-assembly of a squaramide (SQ)-based bola-amphiphile into supramolecular polymers in water.[39] The self-assembly of the monomer was driven by a combination of strong hydrogen-bonding interactions afforded by the ditopic SQ unit,[40] and the hydrophobic domain in which they were embedded relative to the peripheral oligo(ethylene glycol) chains. We herein report the synthesis of a SQ bola-amphiphile decorated with DNA oligonucleotides and evaluate its incorporation into a multicomponent supramolecular polymer for the reversible loading of single-stranded (ss) DNA-labeled gold nanoparticles (AuNPs) of distinct sizes with various presentations by orthogonal self-assembly (Scheme 1).

DNA-coupled SQ-based monomers were synthesized (see the Supporting Information) by treating SQ-Az with 5′-hexynyl oligonucleotides through copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC). The resulting monofunctionalized DNA–SQ bola-amphiphile conjugates (SQ-**D1**, SQ-**D2**) were purified by ultrafiltration, and quantified and characterized by UV/Vis spectroscopy and MALDI-TOF MS, respectively. Gel electrophoresis of the SQ-**D1/2** conjugates showed increased gel retention relative to the uncoupled DNA (Figure S1 in the Supporting Information). Spherical AuNPs with 5 kDa maleimide-functionalized oligo(ethylene glycol) capping groups were conjugated to 5′-thiol DNA oligonucleotides (Au15-**D3**, Au5-**D3**, Au5-**D4**). By UV/Vis spectroscopy, 24 or 360 DNA strands per 5 or 15 nm AuNP were estimated on average (Figure S2).

As a first approach, conventional TEM was pursued to image the reversible loading and exchange of AuNPs by orthogonal self-assembly on the DNA-grafted SQ supramolecular polymers. The morphology was retained when the SQ polymer (50 μM)

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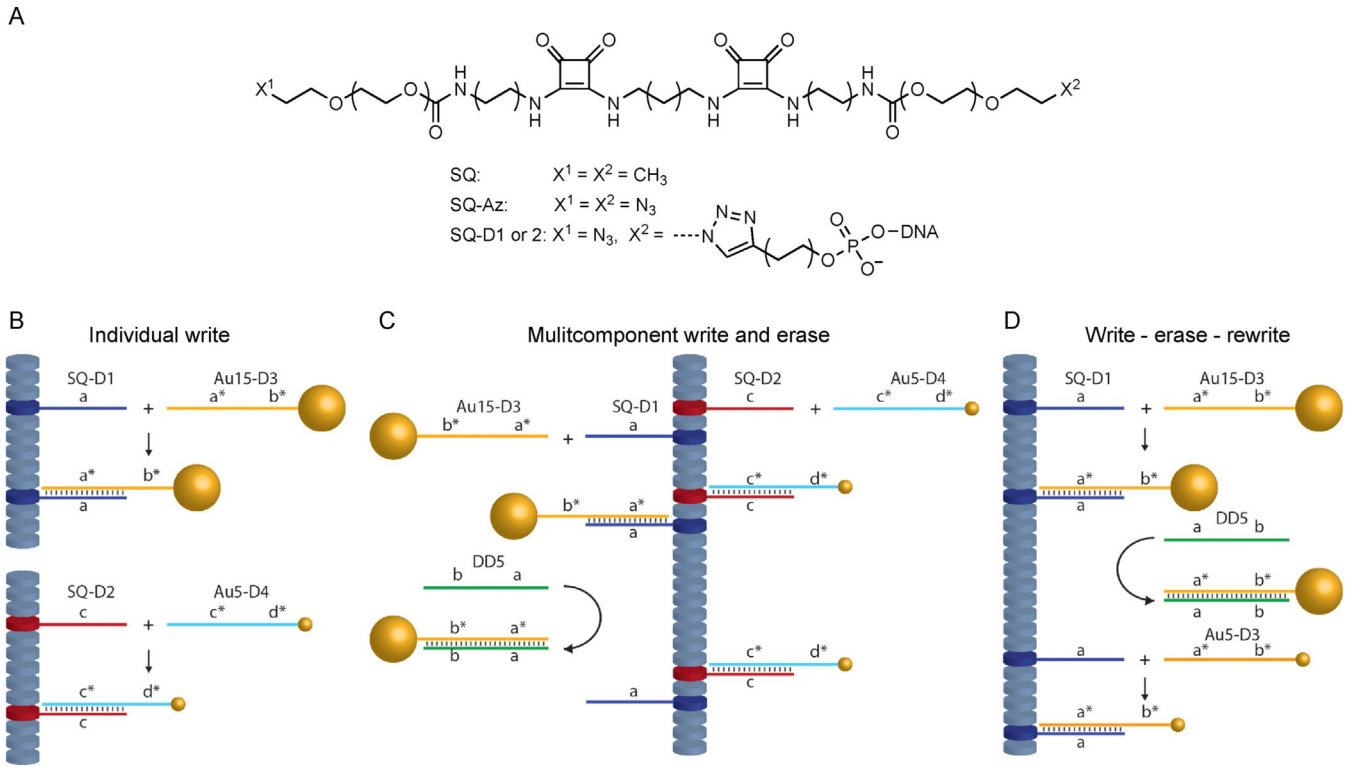

Scheme 1. A) Structures of bis-SQ bola-amphiphiles SQ, SQ-Az, SQ-**D1**, and SQ-**D2** used herein; these compounds contain methyl, azide, or DNA end groups. B)–D) Schematic illustration of the reversible loading of SQ supramolecular polymers with AuNPs by orthogonal self-assembly. B) The SQ supramolecular polymers are functionalized with a 16-mer DNA (SQ-**D1** or SQ-**D2**; 1 mol%), which can be written on with a complementary 24-mer DNA-functionalized with 15 (Au15-**D3**) or 5 nm (Au5-**D4**) AuNPs to form partial duplexes with a toehold. C) If DNA-containing monomers (SQ-**D1** and SQ-**D2**; both 0.5 mol%) are self-assembled with SQ, distinct DNA sequences can be labeled simultaneously by complementary AuNP–DNAs of 5 and 15 nm (Au15-**D3** and Au5-**D4**, respectively). By using a 24-mer DNA displacement strand (**DD5**) fully complementary to Au15-**D3**, an AuNP can be selectively erased from the supramolecular polymer. D) The single 16-mer DNA tether (SQ-**D1**) can also be used to write–erase–rewrite AuNPs of various sizes. First, 1 mol% SQ-**D1**, containing SQ supramolecular polymer, is written with 24-mer DNA-labeled Au15-**D3**, which afterwards can be displaced using **DD5**, resulting in the regeneration of the SQ-**D1** tether. Introduction of Au5-**D3** then allows for its rewriting on the supramolecular polymer.

was functionalized with 1 mol% SQ-**D1** (Scheme 1A) by mixing in a solution of DMSO/H_2O (9:1), lyophilization, and rehydration in phosphate-buffered saline (PBS, 1×). The mixing protocol was further supported by zeta-potential measurements, in which an increasingly negative value was obtained with increasing DNA concentration (0–5 mol%; Table S2 and Figure S3). To validate the orthogonality of the DNA self-assembly approach on supramolecular polymers, several experiments were performed: individual writing of 5 (Au5-**D4**) and 15 nm (Au15-**D3**) AuNPs (Scheme 1B); writing of 5 and 15 nm (Au5-**D4**, Au15-**D3**) AuNPs simultaneously and erasing of the 15 nm AuNP (Scheme 1C); and writing of a 15 nm particle (Au15-**D3**), erasing it, and rewriting with a 5 nm AuNP (Au5-**D3**; Scheme 1D).

The writing of 5 (Au5-**D4**, 250 nM) or 15 nm (Au15-**D3**, 25 nM) AuNPs individually on the SQ supramolecular polymer (50 μM) with either SQ-**D1** (0.5 μM) or SQ-**D2** (0.5 μM) resulted in their partial hybridization on the self-assembled aggregates (Scheme 1B and Figure 1A–C). Areas of low aggregate density showed clear labeling, but their width, in several cases, is roughly three times greater than that of the native SQ bola-amphiphile (average width ≈7.5 nm), and is suggestive of their clustering when dried on the carbon grid (Figure 1; representative TEM images of higher density can be found in Figure S4). Moreover, the addition of Au5-**D4** or Au15-**D3** to solely SQ supramolecular polymers without DNA tethers did not result in their writing on them (Figure S5). Further increasing the level of complexity, the potential for dual writing of both 5 (Au5-**D4** (540 nM)) and 15 nm AuNPs (Au15-**D3** (70 nM)) simultaneously on a SQ supramolecular polymer by using grafted DNA tethers of distinct sequences (SQ-**D1** and SQ-**D2**) was demonstrated (Scheme 1C and Figure 1D). To show the reversible labeling of the DNA-SQ supramolecular polymers, addition of an equimolar ratio of a fully complementary displacement DNA strand (**DD5**) to Au15-**D3** resulted in its selective toehold-mediated removal (Figure 1E). Finally, a more complex sequence involving the writing of a 15 nm AuNP (Au15-**D3**, 25 nM), erasing it by adding the fully complementary DNA strand **DD5**, and rewriting with a 5 nm AuNP (Au5-**D3**, 125 nM) was validated by the colocalization of the particles and supramolecular polymers in the first and last steps (Scheme 1D and Figure 1F–H). These experiments prove that DNA can be used as an orthogonal and reversible handle for self-assembly of nanoscale components on a multicomponent supramolecular polymer consisting of amphiphiles.

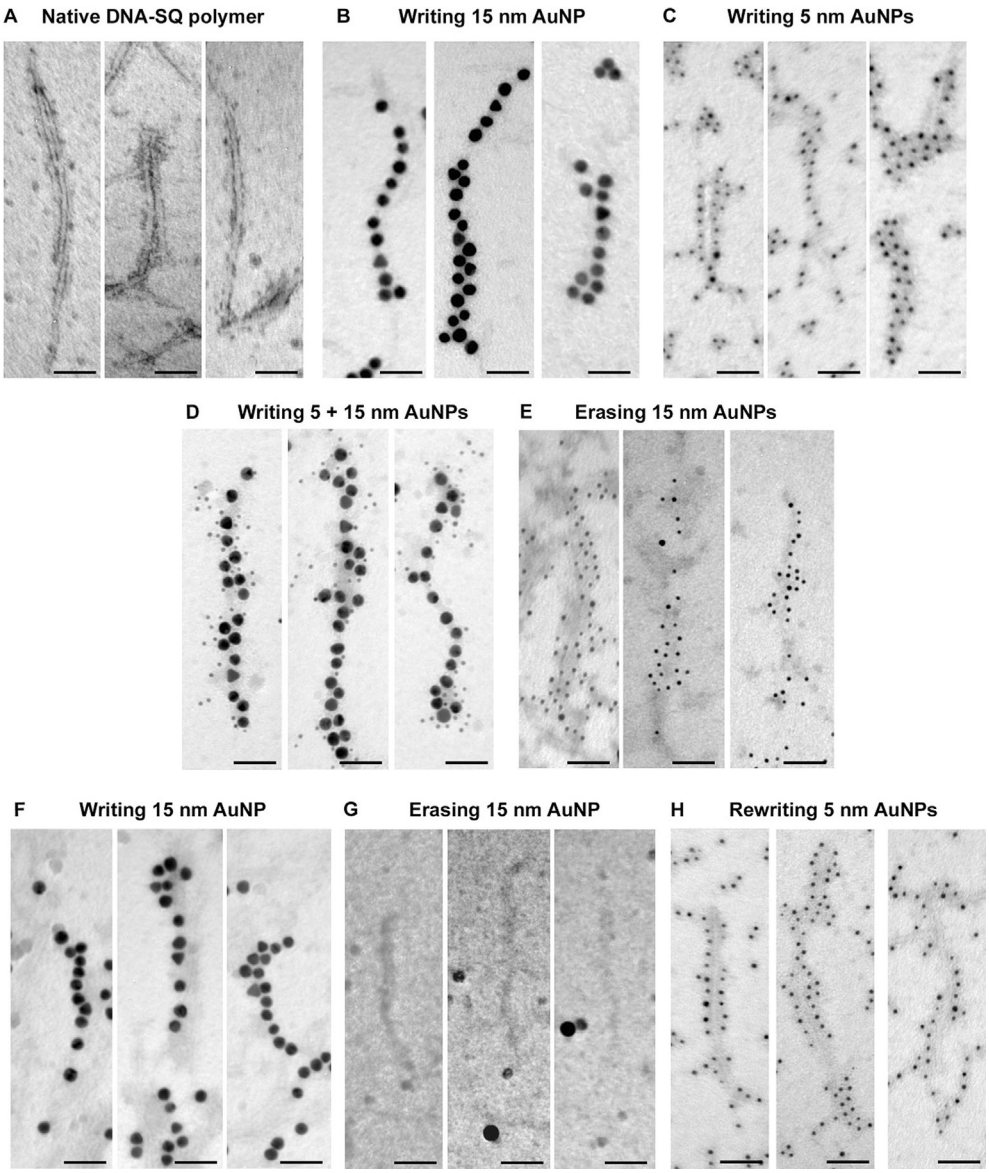

Figure 1. Conventional TEM images of SQ/SQ-**DX** multicomponent supramolecular polymers with and without AuNPs stained with uranyl acetate (0.8%): A) SQ supramolecular polymers (50 μM) bearing SQ-**D1** (0.5 μM) or SQ-**D2** (0.5 μM), B) SQ supramolecular polymer written on with 15 nm AuNPs (Au15-**D3**, 25 nM), C) and 5 nm AuNPs (Au5-**D4**, 250 nM). D) Dual writing of both 5 and 15 nm AuNPs (Au15-**D3**, 70 nM and Au5-**D4**, 540 nM) on independent DNA strands (SQ-**D1** and SQ-**D2**, both 0.25 μM), E) and selective erasing of 15 nm AuNPs using **DD5**. F) SQ supramolecular polymers written on with 15 nm AuNPs (Au15-**D3**, 25 nM), G) which are erased by using an equimolar amount of DNA displacement strand (**DD5**), H) and are rewritten on with 5 nm AuNPs (Au5-**D3**, 125 nM). Scale bars 50 nm.

To gain further insight into the orthogonal self-assembly process on SQ supramolecular polymers, namely, the writing and erasing of AuNPs in the solution phase, we used a combination of gel electrophoresis, fluorescence quenching, and thermal denaturation experiments. DNA hybridization (writing) and displacement (erasing) events on the DNA-grafted SQ supramolecular polymer were first probed by polyacrylamide gel electrophoresis (20%). Because of the small pore size of the acrylamide gel, an unconjugated 5′-disulfide DNA (disulfide-**D3**) was used instead of the corresponding AuNP–DNA to indirectly probe hybridization events. Thus, the SQ supramolecular polymer (50 μM) with SQ-**D1** (0.5 μM) was mixed with equimolar amounts of complementary unconjugated disulfide-**D3**; this resulted in the appearance of a major band of higher gel retention, which was consistent with the formation of a partially hybridized DNA duplex on the SQ supramolecular polymer (Figure 2A, lane 4). As expected, a control sample based on a combination of noncomplementary SQ-**D1** and **DD5** did not yield a band of decreased mobility indicative of the lack of duplex formation (Figure 2A, lane 5). Mixing of **DD5** and disulfide-**D3**, as performed in AuNP erasing experiments, resulted in the formation of a complete duplex with an even higher retention (Figure 2A, lane 6). Moreover, premixing the SQ supramolecular polymer containing 1 mol% SQ-**D1** and disulfide-**D3** with the subsequent addition of **DD5**, as performed in the write–erase cascade, displayed three bands correspond-

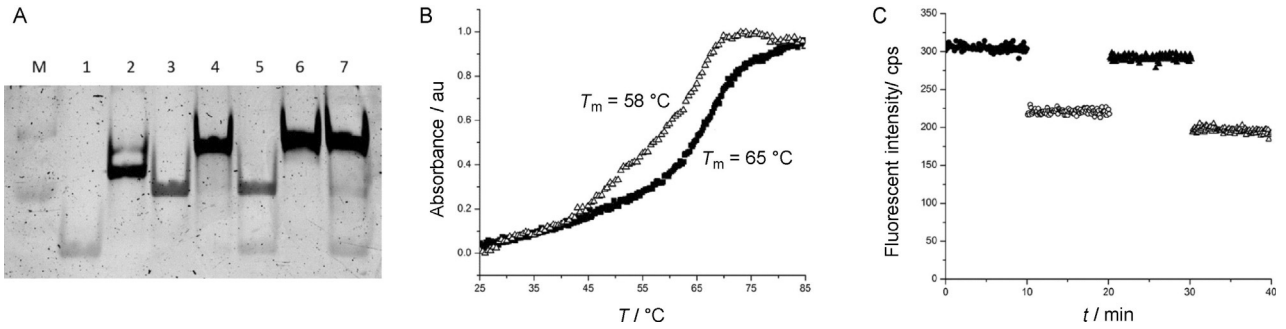

Figure 2. A) Polyacrylamide gel electrophoresis (20%) showing orthogonal self-assembly on SQ supramolecular polymers by using DNA: SQ-**D1** (lane 1), disulfide-**D3** (lane 2), and **DD5** (lane 3). The combination of SQ-**D1** and disulfide-**D3** forms a stable partial duplex (lane 4), whereas the combination of SQ-**D1** and **DD5** does not (lane 5). A mixture of **DD5** with complementary disulfide-**D3** forms a full duplex (lane 6). Premixed duplex of SQ-**D1** and disulfide-**D3** and subsequent addition of **DD5** shows the formation of the full duplex between **DD5** and disulfide-**D3**, along with excess **DD5** and SQ-**D1** (lane 7). B) Normalized thermal denaturation profiles of SQ (50 μM) with 1.0 mol% SQ-**D1** (0.5 μM) and complementary Au5-**D3** (125 nM; △) and unconjugated hexynyl-**D1** and disulfide-**D3** (■). C) Fluorescent quenching experiment with 2-AP-labeled DNAs on the SQ supramolecular polymer and a DNA-labeled 5 nm AuNP. The SQ supramolecular polymer (50 μM) with SQ-**D1**-2AP (0.5 μM) is fluorescent (●) until Au5-**D3** (125 nM) is added, resulting in quenching of the signal (○). Removal of Au5-**D3** from the supramolecular polymer by addition of equimolar amounts of **DD5**, fully complementary SQ-**D1** restores fluorescence signal (▲). Afterwards, the addition of new Au5-**D3** (125 nM) again results in fluorescence quenching (△).

ing to the toehold-mediated displaced full duplex between **DD5** and disulfide-**D3**, excess **DD5**, and SQ-**D1** of increasing electrophoretic mobility (Figure 2A, lane 7).

UV/Vis and fluorescence spectroscopy measurements were performed to further probe the coupling between AuNPs and the SQ supramolecular polymer through DNA. DNA-functionalized 5 nm AuNPs (Au5-**D3**) were combined in an equimolar quantity with complementary SQ supramolecular polymer with 1.0 mol% SQ-**D1**. By means of a thermal ramp measured by UV/Vis spectroscopy at $\lambda = 260$ nm from 25 to 85 °C, a lower melting temperature of 58 °C (Figure 2B), with a comparable profile to the unconjugated DNA strands (hexynyl-**D1** and disulfide-**D3**; $T_m = 65$ °C), was recorded due to the energetically less favorable hybridization of DNA on AuNP surfaces relative to the solution phase.[41] These results are indicative of DNA hybridization between the DNA-grafted SQ supramolecular polymer and AuNPs.

Fluorescent quenching experiments with a 2-AP labeled-DNA oligonucleotide were used to probe AuNP writing, erasing, and rewriting by strand displacement. The 2-AP oligonucleotides are fluorescent in their unhybridized state and become quenched upon duplex formation (Figure 2C). First, the fluorescence intensity of the SQ supramolecular polymer (50 μM) grafted with SQ-**D1**-2AP (2AP = 2-aminopurine; 0.5 μM) was monitored for 10 min (Figure 2C, ●). Next, Au5-**D3** (125 nM; Figure 2C, ○) was added to the fluorescently DNA-labeled supramolecular polymer. Mixing of the two solutions resulted in partial hybridization of DNA-conjugated AuNPs on the supramolecular polymer and quenching of the 2-AP fluorescence signal. The addition of **DD5** (Figure 2C, ▲) to this mixture resulted in the recovery of fluorescence due to toehold-mediated displacement of the Au5-**D3** DNA-conjugated 5 nm AuNP from the DNA-grafted SQ supramolecular polymer. Finally, the addition of a second round of Au5-**D3** (125 nM) resulted in a second decrease in fluorescence and suggested partial hybridization and reloading of the SQ supramolecular polymer (Figure 2C, △). Taken together, gel electrophoresis, UV/Vis thermal denaturation, and fluorescent quenching experiments show that ssDNA-labeled AuNPs can be orthogonally self-assembled on a multicomponent supramolecular polymer being written and erased from this scaffold.

In conclusion, we have shown that supramolecular polymers consisting of amphiphiles with DNA tethers can be addressed in a programmable and reversible way through the orthogonal self-assembly of DNA. Moreover, because of flexible azide–alkyne coupling chemistry and supramolecular mixing of the monomer components, multiple nanoscale elements can be tethered on the same DNA-labeled supramolecular polymer expeditiously, even simultaneously, through the introduction of several unique DNA sequences. This proof-of-concept study highlights the potential for the reversible labeling of these DNA-grafted supramolecular materials with several complex biological molecules (e.g., peptides, proteins); thus opening the door for the dynamic presentation of biochemical or biophysical signals.

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Conflict of Interest

The authors declare no conflict of interest.

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