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Colloids

Exploiting the Directionality of DNA: Controlled Shrinkage of Engineered Oligonucleotide Capsules**

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Hollow micro- and nanocapsules are increasingly finding application in areas as diverse as drug delivery, diagnostics and catalysis.[1,2] This development has led to a growing need for capsules with nanoengineered properties, including composition, structure, and permeability. A versatile and widely used method for preparing hollow capsules is based on layer-by-layer (LbL) assembly.[3] This approach involves the sequential assembly of multilayer films on sacrificial colloidal templates, and subsequent removal of the core template, resulting in hollow capsules.[4,5] The LbL approach has predominantly exploited electrostatic interactions between oppositely charged polymers (polyelectrolytes) to facilitate multilayer buildup. Other interactions used for film assembly include hydrogen-bonding[6,7] and hydrophobic interactions,[8] as well as more recently, covalent bonding (through "click chemistry")[9] and biomolecule recognition.[10,11] An example of biomolecule recognition which is based on DNA hybridization introduces a new level of control over the multilayer assembly. The multilayer structure (and hence properties) can be tailored through engineering of the specific oligonucleotide sequences that hybridize through site-specific, programmable DNA base pairing. Furthermore, the use of common polymers in LbL assembly leads to each polymer layer adsorbed in a random orientation. In contrast, DNA is a directional molecule, as a result of the oligonucleotides in the double helix hybridizing in an antiparallel orientation (the 3′ end of one DNA strand hybridizes with the 5′ end of the second DNA strand and vice-versa). This feature allows control over the orientation of the oligonucleotide sequences within the film by engineering the oligonucleotide sequences. We have previously shown that the composition and sequences of the oligonucleotides play an important role in the structure and assembly of DNA multilayer films.[10,11]

Herein, we report the influence of oligonucleotide sequence composition on the formation of DNA capsules and examine the controlled shrinkage of the hollow capsules. These hollow capsules synthesized entirely from DNA are of interest because they have designed structural properties while also being biodegradable and biocompatible. Additionally, controlling the shrinkage through specific oligonucleotide sequences used in the assembly opens a new and versatile avenue to prepare DNA capsules in the nanometer-size regime, which is of importance for gene-therapy applications. Controlling the shrinkage of capsules may also offer advantages in modulating their permeability properties and in entrapping drugs. For example, upon capsule shrinkage, the thickness of the capsule wall would increase, thereby decreasing the permeability of the capsule wall and enabling entrapment of drugs or biomolecules inside the capsule. Further, if a drug or biomolecule is pre-encapsulated inside the capsule, and the capsule is stimulated to shrink, the concentration of material inside the capsule will increase. In our examples, shrinkage (up to 90% of the original volume) occurs as a consequence of removal of the core template supporting the DNA multilayers. This result is in contrast to previous studies, which have required post-treatment of the capsules to induce shrinkage. For example, heating capsules made from alternating layers of poly(diallyldimethylammonium chloride) (PDADMAC) and poly(styrenesulfonate) (PSS) resulted in shrinkage of up to 66% of the original capsule diameter.[12,13] Similarly, changes in pH[14] and salt concentration[15] have been used to induce shrinkage of capsules synthesized from various polymers, including the commonly used PSS and poly(allylamine hydrochloride) (PAH) pair. UV radiation has also been shown to induce shrinkage in capsules that contain UV-absorbing aromatic groups, such as PSS.[16] While these methods successfully induce shrinkage of capsules, some of the conditions are quite harsh (high temperatures or extremes of pH), limiting the usefulness of these methods, particularly for capsules comprising biomolecules such as DNA or proteins.

We have recently shown that if an oligonucleotide sequence is deposited on a surface and a completely complementary oligonucleotide is hybridized to the adsorbed layer, subsequent layer buildup is limited,[10] because the nucleotides available for hybridization are hybridized between the first two layers. However, if the second layer is designed so it contains two regions (or blocks), only one of which is complementary to the adsorbed layer, then multilayers can be formed by alternately depositing diblock oligonucleotides. Throughout this process one block is complementary to the single-stranded (ss) block in the film and the second block is free for hybridization in the subsequent layer (Scheme 1). The oligonucleotide blocks can either be homopolymeric (e.g. $A_{15}G_{15}$, $T_{15}C_{15}$), where each block contains a repeating sequence of the same nucleotide, or contain a specific oligonucleotide sequence (e.g. $X_{15}Y_{15}$, $X'_{15}Y'_{15}$, where X'_{15} and Y'_{15} are complementary to the X_{15} and

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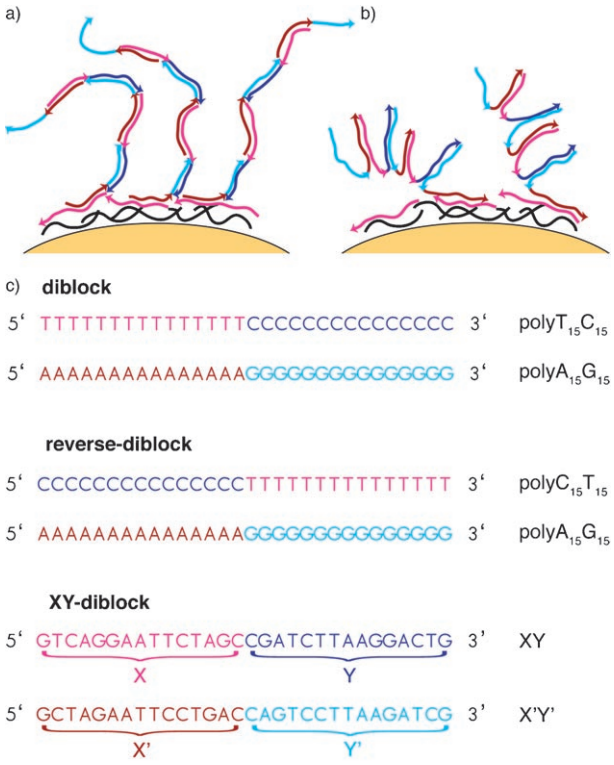

Scheme 1. Schematic representation of an idealized orientation of oligonucleotides assembled with different oligonucleotides (colors as in (c)) on particles: a) a diblock film, b) a reverse-diblock film. The arrows on the oligonucleotides correspond to the direction of the sequence (from the 5′ to the 3′ end). c) Oligonucleotide sequences used to assemble the capsules. The scheme does not indicate the hybridization or orientation of the sequences.

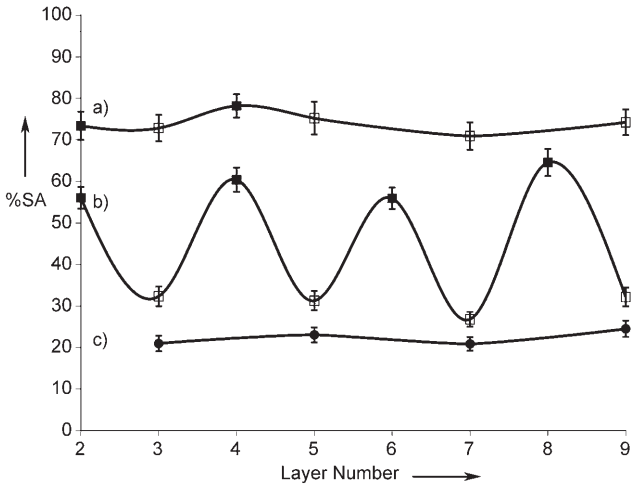

Figure 1. Dependence of capsule shrinkage (% of original surface area (%SA)) on layer number after silica core removal for a) homopolymeric diblock capsules, b) reverse-diblock capsules, and c) XY-diblock capsules. ■: deposition of a $T_{15}C_{15}$ or $C_{15}T_{15}$ layer, □: deposition of an $A_{15}G_{15}$ layer, ●: deposition of XY-diblock layer. Data points are the average for 20 capsules.

Y_{15} blocks respectively; Scheme 1c). The structure of the multilayer film can also be varied by changing the orientation of one of the diblock layers. In the simplest case, the oligonucleotides can be designed so that each layer adds to a continually growing helix (Scheme 1a). If the order of the blocks in one of the layers is altered (e.g. a reverse-diblock oligonucleotide is used), the film buildup is hindered, as the direction of the helix must change as each layer is deposited (Scheme 1b).

Capsule shrinkage occurred upon removal of the silica-particle template (using a pH 5 buffered hydrofluoric acid solution, which does not affect the activity of DNA[17]) for all of the oligonucleotide sequences examined (Figure 1). In all cases, stable capsules are formed only when two or more oligonucleotide layers are hybridized—this corresponds to a total of three layers, as the initial layer is electrostatically adsorbed to amine-functionalized silica particles. When only one layer is hybridized to the template particle, the DNA film immediately disassembles upon dissolution of the silica templates. Both of the diblock films (homopolymeric and XY) show no dependency on the final capsule size with layer number (Figure 1a and Figure 1c). Homopolymeric diblock films (assembled from alternating layers of $A_{15}G_{15}$ and $T_{15}C_{15}$) shrink to approximately 75% of the original surface area (from a diameter of 6 μm to 5.2 μm, corresponding to a decrease in volume of around 35%), and the morphology of the capsules is maintained after core dissolution (Figure 1a and Figure 2b). There is no discernable difference between capsules formed with two or nine layers of DNA. The capsules formed from XY-diblock oligonucleotides exhibit significantly greater shrinkage (Figure 1c and Figure 2c), shrinking to about 20% of the original capsule surface area once the core is removed (diameter of ca. 2.6 μm and a decrease in volume of ca. 90%). Again, the capsules formed with two or nine layers of XY-diblock oligonucleotides are indistinguishable. The morphology of the XY-diblock capsules is distinguishable from the homopolymeric diblock capsules, as the XY-diblock capsules are slightly distorted and are no longer perfectly spherical. This distortion is probably due to the shrinkage that occurs when the core was removed. The greater shrinkage of the XY-diblock capsules is unlikely to be due to the thickness of the film. The influence of capsule wall thickness on shrinkage would be expected to diminish as the number of oligonucleotide layers increases. This correlation was not observed for either of the diblock capsules. The above data shows that altering the sequences used to assemble the films results in different shrinkage behavior of the homopolymeric and XY-diblock capsules.

The shrinkage of the homopolymeric reverse-diblock system (assembled from alternating layers of $A_{15}G_{15}$ and $C_{15}T_{15}$) shows completely different behavior to the diblock capsules (Figure 1b and Figure 2d,e). After deposition of the first $C_{15}T_{15}$ layer and core removal, the capsules shrink to about 55% of the original surface area (corresponding to a diameter of 4.4 μm and a decrease in volume of ca. 60%), as compared to 75% of the original surface area for the homopolymeric diblock capsules. When a second layer of $A_{15}G_{15}$ is deposited on the core–shell particles, and the silica particles are subsequently dissolved, the capsules shrink to about 30% of the original surface area (a diameter of 3.3 μm and a decrease in volume of ca. 80%). This trend continues

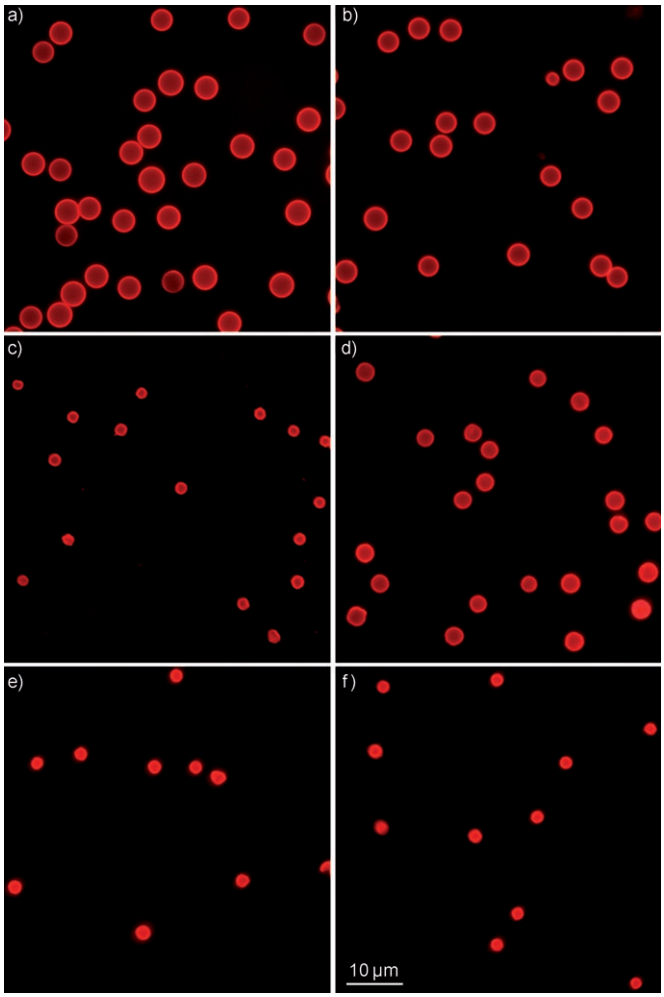

Figure 2. Fluorescence microscopy images of silica-core DNA-shell particles and DNA capsules. a) Core–shell silica–DNA particles, with the shell comprising nine layers of the homopolymeric diblock oligonucleotides. DNA capsules assembled from b) homopolymeric diblock oligonucleotides (nine layers), c) XY-diblock oligonucleotides (nine layers), d) reverse-diblock oligonucleotides terminated with $C_{15}T_{15}$ (eight layers), e) reverse-diblock oligonucleotides terminated with $A_{15}G_{15}$ (nine layers), and f) reverse-diblock oligonucleotides terminated with $C_{15}T_{15}$ and then hybridized with $A_{15}G_{15}$ (total of nine layers). The poly$A_{15}G_{15}$ oligonucleotide was modified with fluorescently labeled TAMRA. The scale bar corresponds to all of the images shown.

for all subsequent layers: when $C_{15}T_{15}$ was the outermost layer, the capsules shrank to between 55 and 65% of the original surface area, while when $A_{15}G_{15}$ was the outermost layer, the capsules shrank to approximately 30% of the original surface area (Figure 1b).

When the homopolymeric reverse-diblock hollow capsules with a terminal layer of $C_{15}T_{15}$ are incubated with $A_{15}G_{15}$, the capsules shrink to approximately the same size as the capsules prepared from $A_{15}G_{15}$-terminated core–shell particles (Figure 2f). The shrinkage occurs within 30 s, indicating that fast rearrangement occurs. In contrast, when hollow capsules terminated with $A_{15}G_{15}$ were incubated with $C_{15}T_{15}$, the capsules remained the same size, and they did not swell to the size of the capsules formed from $C_{15}T_{15}$-terminated core–shell particles (data not shown). The shrinking process may be considered analogous to the deflation of a balloon. Large capsules terminated with the $C_{15}T_{15}$ layer are able to shrink when the $A_{15}G_{15}$ sequence is hybridized to the film. However, when the $C_{15}T_{15}$ hybridizes to the $A_{15}G_{15}$-terminated capsules, there is no driving force for the capsules to swell, and the capsules remain the same size, although we would anticipate that the same rearrangement within the film would occur.

The deposition of the $C_{15}T_{15}$ layer in the homopolymeric reverse-diblock capsules corresponds to the hybridization of G with C, while the deposition of the $A_{15}G_{15}$ layer corresponds to the hybridization of T with A. Hybridization between G and C leads to the formation of three hydrogen bonds, while hybridization between T and A leads to the formation of only two hydrogen bonds. It is possible that shrinking of the capsules may be due to the number of hydrogen bonds formed as the layer is deposited, that is, the capsules shrink less when $C_{15}T_{15}$ is the outer layer because there are more hydrogen bonds with the G–C hybridization and a more rigid film is formed. However, as the whole capsule shrinks, it is likely that the entire film rearranges. To gain some insight into this, we conducted real-time quartz-crystal microgravimetry (QCM) measurements. QCM experiments reveal that deposition of the $A_{15}G_{15}$ layer in the reverse-diblock film induces an unusual increase in the QCM frequency after the initial decrease in frequency resulting from hybridization of the oligonucleotide to the film (Figure 3, layers 3, 5, and 7). Such an increase in the frequency would normally be attributed to material being

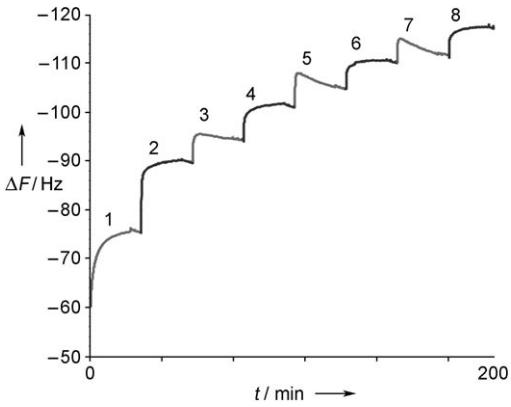

Figure 3. Real-time QCM buildup (frequency change vs. time) of the reverse-diblock DNA film. The numbers 1–8 correspond to the number of layers hybridized. The odd layer numbers correspond to the deposition of $A_{15}G_{15}$, and the even layer numbers to the deposition of $C_{15}T_{15}$.

lost from the film; however, as the subsequent layers of $C_{15}T_{15}$ continue to hybridize to the film, $A_{15}G_{15}$ must be deposited on the film. Additionally, flow cytometry experiments,[18] where each oligonucleotide was fluorescently labeled, showed regular layer buildup, and importantly no loss of fluorescence from either the $A_{15}G_{15}$ or the $C_{15}T_{15}$ layers was observed when $A_{15}G_{15}$ was hybridized to the film (data not shown). This result further demonstrates that the change in frequency

observed by QCM is not the result of DNA being lost from the film. The increase in frequency is likely to be due to water being expelled from the film when the rearrangement is induced. The diblock film exhibited no such increase in frequency, as observed using real-time QCM, with the frequency decreasing for each layer deposited (data not shown). We are currently further investigating the rearrangement mechanism.

We have demonstrated that the shrinkage of DNA capsules can be controlled by the composition of the DNA sequences used to assemble the multilayer films. Capsules formed from diblock oligonucleotides showed no dependence of the final capsule size on the number of layers, or on the sequences of the outer layer. Capsules prepared from homopolymeric sequences were more than twice the size of capsules formed from XY-blocks. In terms of volume, capsules assembled from XY-diblock oligonucleotides shrank to about 10% of the original volume of the core-shell particles; thus, in principle, being able to effectively increase the concentration of any entrapped molecules by an order of magnitude. If the orientation of the helix was reversed after each layer was deposited (reverse-diblock system), the capsule size was dependent on the sequence of the outermost layer. Capsules terminated with a $C_{15}T_{15}$ layer could be shrunk to approximately 50% of the original capsule size by hybridizing the capsules with an $A_{15}G_{15}$ layer. This process was not reversible, as $A_{15}G_{15}$-terminated capsules did not change size when a $C_{15}T_{15}$ oligonucleotide was hybridized with the capsules. We anticipate that these capsules will find use in drug delivery and microreactor applications, and the shrinking of the capsules can be used for both control of the final size of the capsules, as well as stimulating morphological changes in the capsules.

Experimental Section

Materials: Unless otherwise noted, all chemicals were obtained from Sigma-Aldrich (see Supporting Information for more details). Unlabeled oligonucleotides (Scheme 1c) were custom synthesized by Geneworks (Australia). The oligonucleotide poly$A_{15}G_{15}$ (AAAAAAAAAAAAAAGGGGGGGGGG-GGGGG) was modified with the fluorescent label 5′-carboxytetramethylrhodamine (TAMRA; SigmaGenosys). Approximately 6-μm-diameter silica particles were purchased from MicroParticles (Germany).

Methods: Quartz-crystal microgravimetry (QCM) measurements were performed using a Q-Sense D300 instrument (see Supporting Information for details). After initially depositing a layer of poly(ethylenimine) (PEI; 1 mg mL^{-1} for 5 min), DNA (500 μL of 4 μM in SSC buffer (500 mM NaCl and 50 mM sodium citrate, pH 6.5)) was adsorbed/hybridized to the film for 20 min. After each adsorption step, the film was washed with SSC buffer (2 ml).

Hollow DNA capsules: The DNA multilayers were deposited on positively charged, amine-functionalized silica particles (see the Supporting Information). The particles (10 μL) were suspended in a DNA solution (10 μM; 50 μL in SSC buffer), and the oligomers were allowed to hybridize to the surface for 20 min. After hybridization, the particles were washed three times in SSC buffer before addition of the next layer. To form hollow capsules, the silica core was dissolved by mixing the particle suspension (1 μL) with ammonium fluoride (8M; 1 μL) buffered HF (2M) at pH 5, and the capsules were visualized in situ. (*Caution! HF is highly toxic and great care must be taken when handling.*) Dissolution of the silica core occurred within 1 min.

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