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A General Approach for DNA Encapsulation in Degradable Polymer Microcapsules

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ABSTRACT We report a general and facile method for the encapsulation of DNA in nanoengineered, degradable polymer microcapsules. Single-stranded (ss), linear double-stranded (ds), and plasmid DNA were encapsulated into disulfide-cross-linked poly(methacrylic acid) (PMA) capsules. The encapsulation procedure involves four steps: adsorption of DNA onto amine-functionalized silica (SiO_2^+) particles; sequential deposition of thiolated PMA (PMA_{SH}) and poly(vinylpyrrolidone) to form multilayers; cross-linking of the thiol groups of the PMA_{SH} in the multilayers into disulfide linkages; and removal of the sacrificial SiO_2^+ particles. Multilayer growth was dependent on the surface coverage of DNA on the SiO_2^+ particles, with stable capsules formed from particles with up to 50% DNA surface coverage. The encapsulation strategy applies to nucleic acids with varied size and conformation and allows DNA to be concentrated over 100-fold from dilute solutions into monodisperse, uniformly loaded polymer capsules. The capsule loading can be controlled by the DNA:SiO_2^+ particle ratio, and for 1 μm diameter capsules, loadings of ~1000 chains of 800 bp dsDNA and more than 10 000 chains of 20-mer ssDNA can be achieved. The encapsulated DNA was released and successfully used in polymerase chain reactions as both templates (linear dsDNA and plasmid DNA) and primer sequences (ssDNA), confirming the functionality and structural integrity of the encapsulated DNA. These DNA-loaded polymer microcapsules hold promise as delivery vehicles for gene therapy and diagnostic applications.

KEYWORDS: layer-by-layer · DNA encapsulation · disulfide cross-linking · degradable capsules · polymer assembly

DNA is an important molecule with applications in gene therapy,[1] diagnostics,[2] nanorobotics,[3] and molecular evolution.[4–6] A common challenge for the effective and efficient use of DNA in these areas is to prevent its degradation, which can occur through either mechanical shearing forces[7] or chemical degradation by nucleases.[1] To prevent degradation, DNA can be condensed and/or protected by a physical barrier. There are a number of strategies employed in gene therapy to limit DNA degradation, including complexation of DNA with polycations,[1] block-copolymer micelles,[8] cationic lipids, or liposomes.[9] Alternatively, DNA can be confined within gel,[10] micellar,[11] and polymeric[12] microparticles. For applications requiring transcriptionally active nucleic acid, DNA may be encapsulated within liposomes,[13,14] water-in-oil emulsions,[4,5] and polyelectrolyte capsules.[15–17] The latter offer the potential of encapsulating DNA within tunable, semipermeable capsules that allow small solute molecules to diffuse through the capsule membrane,[18] while limiting the diffusion of large peptides such as proteins.

Polyelectrolyte capsules are typically prepared by using the layer-by-layer (LbL) technique, whereby the capsules are formed by the sequential deposition of polymers that interact through electrostatic, covalent, or hydrogen-bonding forces[19–21] onto sacrificial colloidal particles, followed by removal of the core particles. There have been several reports on the incorporation of DNA into multilayer thin films as one of the film components,[22,23] including multilayers on colloidal particles.[24,25] In contrast, there are only a few reports on encapsulation of uncomplexed DNA within the interior of polyelectrolyte capsules. Two examples include the "controlled precipitation" of DNA using spermidine onto the colloidal particle with subsequent assembly of the capsule[15] (preloading approach) and rehydration of preformed capsules in a DNA-containing solution[16] (postloading approach). Recently, we developed a polycation-free method for the encapsulation of DNA within polymer capsules, resulting in uncomplexed oligonucleotide DNA in the interior of capsules.[17] The encapsulation method involves the adsorption of DNA onto positively charged silica particles, followed by the LbL deposition of poly(methacrylic acid) (PMA) and poly(vinylpyrrolidone) (PVPON) multilayers, multilayer film cross-linking, and removal of the template silica particles. A signature feature of this hydrogen-bonded multilayer

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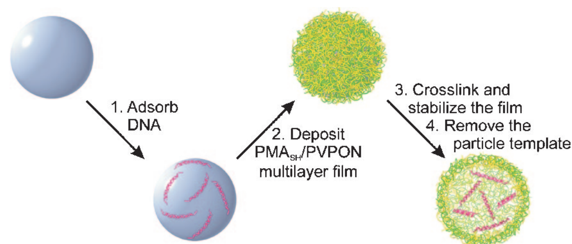

Figure 1. Encapsulation of DNA into degradable polymer capsules. Adsorption of DNA onto amine-functionalized silica particles (1) is followed by the assembly of a thin polymer film prepared *via* the alternating deposition of PMA_{SH} and PVPON (2). Oxidation of the PMA_{SH} thiol groups into bridging disulfide linkages (3) and removal of the core particles (4) result in stable polymer capsules. DNA chains are confined within the capsule interior and are released in a reducing environment.

film is that it is unstable above pH 7 when the PMA becomes ionized.[26] To impart stability at neutral pH, we used thiol-modified PMA (PMA_{SH}). When oxidized within the multilayer, disulfide bridges between PMA_{SH} chains provide stabilization to the film/capsules.[17,27] These capsules are stable at physiological pH but degrade and release their cargo once the disulfide linkages are destroyed in a reducing environment. This provides a mechanism for the release of products from microreactors[4,6] and for cellular delivery applications.[28,29]

The DNA sequence composition and length determine its conformation, which in turn affects the nature of the physical interactions with other biological and nonbiological molecules. Therefore, it is important that the encapsulation method is applicable to a wide variety of DNA forms. In this paper, we demonstrate that a broad range of nucleic acids can be encapsulated in a transcriptionally active form in degradable polymer capsules. We report the successful encapsulation of (i) short single-stranded (ss) oligonucleotides (~20 bases), which are representative of molecules used in antisense technologies; (ii) large supercoiled plasmid DNA (3 kbp), which has applications in gene therapy; and (iii) linear double-stranded (ds) DNA (800 bp), which may be used as a template for protein and DNA synthesis in microreactors. Amine-functionalized, positively charged silica particles (SiO_2^+) are used as sacrificial templates for the adsorption of DNA. Using the LbL technique, a thin film of alternating PMA_{SH} and PVPON layers is formed around the particles coated with DNA. The PMA_{SH} layers are then cross-linked, and the SiO_2^+ particles are dissolved (Figure 1). The nucleic acids are concentrated from dilute bulk solutions into the capsules, which are monodisperse in size and uniform in DNA loading, using only buffered aqueous solutions. We also provide evidence for the functional and structural integrity of both ss- and dsDNA that has been released from capsules by using them in polymerase chain reactions (PCRs).

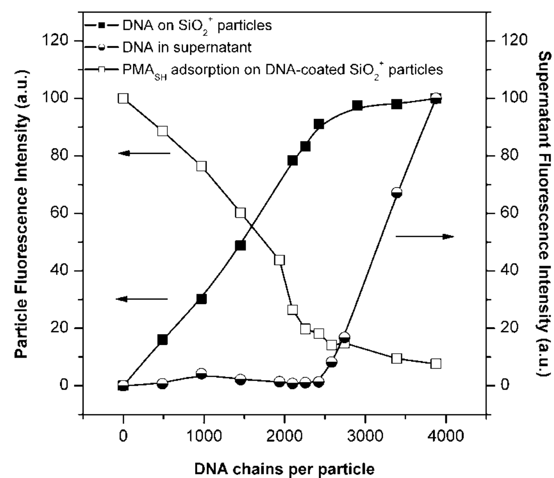

Figure 2. Adsorption isotherm for 800 bp dsDNA on amine-functionalized 1 μm diameter SiO_2^+ particles and PMA_{SH} adsorption onto DNA-coated SiO_2^+ particles. The data were obtained using fluorescently labeled DNA (Alexa Fluor 546) and PMA_{SH} (Alexa Fluor 488) and using flow cytometry and fluorescence spectroscopy to quantify the fluorescence of the particles and supernatant, respectively.

RESULTS AND DISCUSSION

DNA Adsorption onto SiO_2^+ Particles. Details of the adsorption of DNA onto amine-functionalized silica particles and encapsulation are given for the example of 800 bp dsDNA and 1 μm diameter SiO_2^+ particles; they were similar for all the DNA samples studied. Fluorescently labeled DNA of varying concentration (~1–20 nM DNA chains, corresponding to ~1–10 μM nucleic phosphor) was incubated with SiO_2^+ particles at pH 4 to effect the electrostatic adsorption of DNA. The fluorescence of the particles and that of the supernatant were independently analyzed by flow cytometry[30] and fluorescence spectroscopy, respectively (Figure 2). Below the surface saturation of the particles, the supernatant fluorescence remains at a background level, reflecting complete adsorption of nucleic acids from the solution onto the particle surface. Above the saturation coverage, the particle fluorescence plateaus, and the concentration of DNA in the supernatant increases (Figure 2). The surface saturation point was corroborated by zeta-potential measurements, which showed a progressive decrease in surface potential from +85 mV for the SiO_2^+ particles to −30 mV for the saturated dsDNA surface (Figure S1, Supporting Information). For the 800 bp dsDNA, saturation coverage occurs at ~2500 chains per 1 μm diameter SiO_2^+ particle, corresponding to 0.6 mg m^{-2}. The saturation coverage of the plasmid DNA was 0.7 mg m^{-2}, and it was 0.4 mg m^{-2} for the 22-mer DNA. As we previously reported, the surface saturation value for 30-mer ssDNA on 1 μm SiO_2^+ particles was 0.3 mg m^{-2}, corresponding to over 10 000 oligonucleotide chains per particle.[17]

Assembly of PMA_{SH}/PVPON Multilayers. Polymer multilayer formation was initiated by depositing fluorescently labeled PMA_{SH} onto the DNA-coated SiO_2^+ particles. The amount of PMA_{SH} adsorbed, monitored using flow cy-

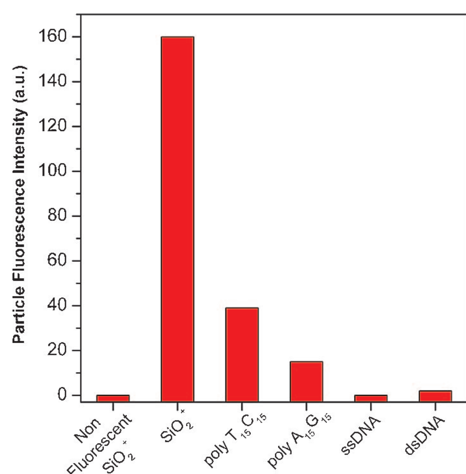

Figure 3. Fluorescence of the 1 μm diameter SiO_2^+ particles incubated with an excess of various ss- and dsDNA (resulting in saturation DNA coverage) and exposed to a solution of PMA_{SH} labeled with Alexa Fluor 488. Nonfluorescent SiO_2^+ refers to background fluorescence (control).

tometry, decreased progressively as the dsDNA surface coverage on the SiO_2^+ particles increased (Figure 2). Particles saturated with DNA showed minimal adsorption of PMA_{SH} for both the 800 bp dsDNA and 30-mer ssDNA (Figure 3). This is in contrast with the homopolymeric ssDNA sequences (poly$T_{15}C_{15}$ and poly$A_{15}G_{15}$) encapsulated previously,[17] which supported a higher level of PMA_{SH} adsorption on saturated surfaces. These data provide evidence that PMA_{SH} does not adsorb onto the randomly sequenced DNA immobilized on the surface of the template. The deposition of the first PMA_{SH} layer is therefore likely to occur on the vacant binding sites of the amine-functionalized silica particles.

The sequential adsorption of PMA_{SH} and PVPON[26] was performed on dsDNA-coated SiO_2^+ particles with various surface coverages (0, 50, 75, and 100% dsDNA). Polymer deposition was monitored *via* the fluorescence of Alexa Fluor 488-labeled PMA_{SH} on the SiO_2^+ particles using flow cytometry.[30] The amount of polymer adsorbed decreased as the DNA surface coverage increased (Figure 4), following the trend observed for the adsorption of the first PMA_{SH} layer. The DNA-saturated surface (100% dsDNA coverage) showed minimal multilayer growth. This observation also implies that the interaction between PVPON and DNA[31] is insufficient to promote significant PMA_{SH}/PVPON multilayer formation. For the SiO_2^+ particles with subsaturation DNA coverage, multilayer growth was linear, and the cumulative amount of PMA_{SH} adsorbed decreased as the surface area occupied by DNA increased (Figure 4).

To analyze the loss of dsDNA from the surface during film assembly, fluorescently labeled 800 bp dsDNA was used, and the fluorescence of the supernatant after deposition of each polymer was monitored (Figure S2, Supporting Information). The cumulative loss of DNA from the particle surface was determined to be less than 10%, regardless of the initial surface coverage.

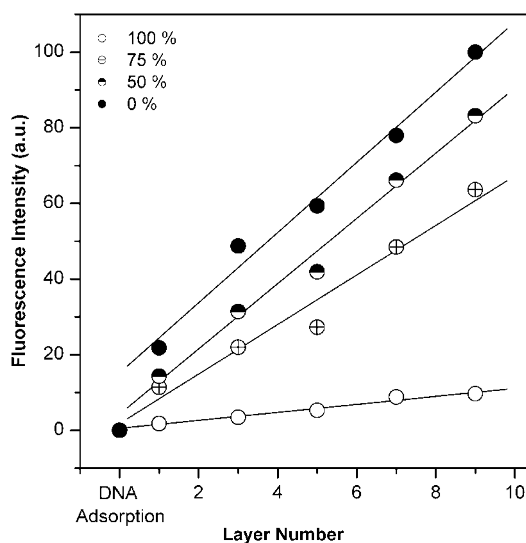

Figure 4. Flow cytometric analysis of the buildup of PMA_{SH}/PVPON multilayer films on 1 μm diameter SiO_2^+ particles with varying amounts of adsorbed 800 bp dsDNA (expressed as percent of saturation coverage), obtained by using a PMA_{SH} sample labeled with Alexa Fluor 488. Odd layer numbers indicate the deposition of PMA_{SH}; even layer numbers indicate the deposition of PVPON.

This relates to minimal ionization of PMA_{SH} at pH 4 (pK_a ~6.5) and correlates with the inability of PMA to displace DNA from its polyelectrolyte complexes in solution at pH 4.[32]

Aggregation of the particles during film assembly (defined here as formation of clusters of three or more particles) was ascertained using flow cytometry (Figure S3, Supporting Information).[30] The aggregation was more significant when PMA_{SH} was the outermost layer; however, it was reversible and decreased when PVPON, a low-fouling polymer, was deposited. For samples treated with an oxidizing reagent to cross-link the thiol groups of the PMA_{SH} chains, the level of aggregation was only marginally higher than that observed for uncoated SiO_2^+ particles.

Capsule Formation. To cross-link and stabilize the polymer multilayers (5 PMA_{SH}/PVPON bilayers), the films were oxidized with chloramine T at pH 6,[33,34] forming bridging disulfide linkages between the PMA_{SH} chains. Capsules were formed by removing the template SiO_2^+ particles with hydrogen fluoride (HF) buffered to pH 5 with ammonium fluoride.[35] The resulting capsules were suspended in pH 7 phosphate buffer and examined using fluorescence and confocal laser scanning microscopy (CLSM). As expected, no capsules were formed from the sample originating from the dsDNA-saturated silica particles, where multilayer growth was minimal. The sample originating from particles with 50% of the surface occupied with DNA produced stable capsules, identical in size to those obtained from SiO_2^+ without adsorbed DNA. The 75%-saturated particles gave rise to deformed, unstable capsules, perhaps due to an insufficient amount of PMA_{SH} within the multilayer film leading to decreased cross-linking efficiency. Thus, the opti-

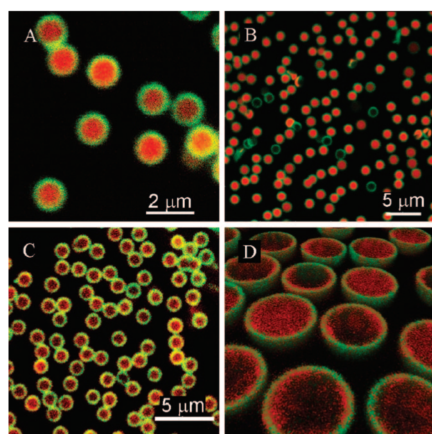

Figure 5. CLSM images of 1 µm diameter capsules filled with (A) 20 base ssDNA, (B) 30 base ssDNA, and (C) 800 bp dsDNA and (D) 3D reconstruction of the CLSM images of the 800 bp dsDNA-filled 3 µm PMA_{SH} capsules.

mal dsDNA content to maximize the nucleic acid loading without compromising the integrity of the capsule wall was established to be approximately 50% dsDNA coverage of the original template particle.

Using the procedure outlined above, we encapsulated DNA varying in composition and size, from 20-mer ssDNA to 800 bp linear dsDNA and 3 kbp plasmid dsDNA. The capsules filled with dsDNA and ssDNA were visualized by CLSM using fluorescently labeled PMA_{SH} (green) and DNA (red) (Figure 5). The capsules were monodisperse in size and uniform in loading, two features of utmost importance for reliable reagent/gene dosage. They could be stored at 4 °C for several weeks without signs of capsule degradation. For the samples prepared using 1 µm particles, each of the capsules contains ~1000 chains of 800 bp dsDNA. Using the measured DNA coverages (see earlier), the calculated loadings for the plasmid and 22-mer oligonucleotide are ~300 and >10 000 chains/capsule, respectively. As reported previously, the DNA loading for a 30-mer oligonucleotide was >10 000 chains/capsule.[17] For all the encapsulated DNA samples, the release of nucleic acids into the bulk solution was triggered when the capsules were placed in a reducing environment. This process is controlled by the thickness of the capsule wall and its cross-linking density, as well as the size of the cargo nucleic acid. A detailed study of the DNA release kinetics is the subject of ongoing research.

The amount of encapsulated ds- and ssDNA in the capsules is expected to be sufficient for most practical applications. Compartmentalized DNA replication is efficient using a single copy of a gene.[4] Successful gene transfer requires a few DNA chains to transfect the nucleus, and while gene silencing applications require far more copies of oligonucleotide, the amount of encapsulated ssDNA, 10 000 copies per 1 µm capsule,[17] should accommodate these applications as well.

DNA Functionality. The encapsulation procedure outlined here involves adsorption of DNA onto template particles and several other steps, including oxidation of the thiol groups with chloramine T and removal of the SiO_2^+ core *via* HF treatment. Each of these steps may potentially be harmful for the nucleic acid chains, leading to the loss of function. To confirm that the encapsulation process did not affect the function of the DNA, the DNA was released from the capsules and used in a PCR.

During a PCR, two template DNA chains are separated by heat treatment in the presence of a large excess of short oligonucleotide sequences (primers). Upon cooling, the primers hybridize with the complementary region on the DNA, forming a ds primer region. DNA polymerase binds to this complex and synthesizes new DNA chains. Nicking of the chain in the primer region would dramatically decrease or even stop the hybridization step. Chain scission at other bases along the template strand would result in the synthesis of shorter PCR products, and chemical modification of the bases at any site can also lead to a different amplified product. Thus, PCRs can be used as detailed and comprehensive tests to verify the structural and functional integrity of the released DNA.

The 800 bp dsDNA was released from PMA/PVPON capsules prepared using 1 or 3 µm amine-functionalized SiO_2^+ particles according to the method described above, including chloramine T treatment and removal of the SiO_2^+ particles with HF. Nonthiolated PMA was used to facilitate the analysis of the released DNA, as the reducing agent used to cleave the disulfide bonds *in vitro* interferes with the PCR (data not shown), and purification of DNA from the reducing agent could remove other components and artificially improve the reaction efficiency. These capsules were otherwise identical to those presented in Figure 5, with the exception that they were not stabilized with disulfide linkages, and the encapsulated DNA was released from the capsules by placing them into the PCR buffer, pH 8.4. The resulting solution, containing the capsule components (PMA and PVPON) and the released dsDNA, was supplemented with the remaining PCR reagents (primers, deoxyribonucleotides, Mg^{2+}, and Taq DNA polymerase) and underwent 25 reaction cycles.

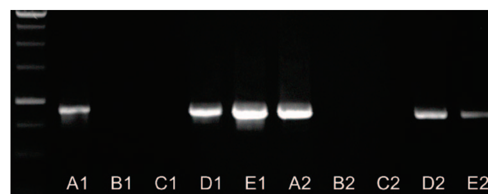

Figure 6. Gel electrophoresis analysis of the PCR products obtained using released 800 bp linear dsDNA as template: GeneRuler 1 kb DNA ladder, (A) nonencapsulated template control; (B,C) negative controls containing empty capsules (no template DNA) or no Taq DNA polymerase (with released DNA); (D,E) PCRs using released DNA at initial and 10-fold diluted concentration. Series A1–E1 corresponds to 1 µm diameter capsules, whereas A2–E2 corresponds to 3 µm diameter capsules.

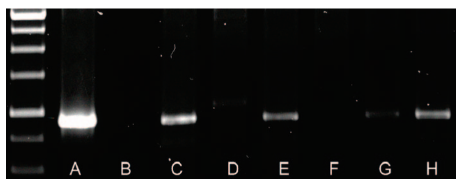

Figure 7. Gel electrophoresis analysis of the PCRs using released ssDNA as primers: GeneRuler 1 kb DNA ladder; (A) nonencapsulated primers control; (B) negative control containing empty capsules (no template DNA); (C) PCRs using released forward primer; (D) PCRs containing only nonencapsulated reverse primer; (E) PCRs using released reverse primer; (F) PCRs containing only nonencapsulated forward primer; (G) forward and reverse primer encapsulated together; and (H) forward and reverse primer encapsulated separately.

The amplified product was analyzed by gel electrophoresis (Figure 6) and had electrophoretic mobility identical to the mobility of the product amplified from the nonencapsulated template DNA. We performed this test using 3 kbp plasmid DNA and observed that the amplified product was identical to that amplified from the nonencapsulated plasmid (Figure S4, Supporting Information).

The procedure of encapsulation, release, and analysis was also applied to short oligonucleotides (22- and 27-mer) that act as primers in the PCR. When using the released dsDNA as the template for PCR, the success of amplification requires very few copies of dsDNA to remain intact; however, every DNA chain synthesized in a PCR must contain one of the primers. Encapsulated primers were used in PCRs, supplemented with all other reagents required for a successful PCR as described above. The PCR products obtained (Figure 7C,E,G,H) were identical to those generated using nonencapsulated primers (Figure 7A). This was valid for the cases when one of the two primers (lanes C and E) or both primers (lanes G and H) underwent encapsulation and release. When both primers were released, a product was generated when they were released from the same (G) and separate (H) capsules. This highlights the potential of coadsorption of multiple DNA sequences onto the same particle, similar to the coadsorption of blended polyelectrolytes.[36,37] While these results cannot rule out potential loss of function of some nucleic acid chains, the above data suggest that oligonucleotides, linear dsDNA, and plasmid DNA can be successfully encapsulated and remain functional in enzymatic reactions after the encapsulation procedure, including adsorption onto template particles, treatment with an oxidizing reagent, and removal of the core by aqueous HF. Investigation into the activity of released DNA within cellular systems is currently underway.

CONCLUSIONS

We have developed a novel method to encapsulate nucleic acids into degradable polymer capsules for applications as diverse as gene delivery, sensing, and compartmentalized reactions. The method accommodates ss- and dsDNA, from short oligonucleotides to linear or plasmid DNA in the kilo base pair size range. A distinguishing feature of the method is the ability to concentrate the DNA samples from a dilute solution (nanomolar chain concentration) onto the template particles to produce capsules with over a 100-fold increase in concentration compared to the initial solution. Multilayer growth decreased as the amount of DNA on the surface of the SiO_2^+ particles increased, with negligible growth observed on saturated DNA surfaces. Stable capsules were formed from particles with 50% DNA coverage, and the loading can be controlled by altering the DNA:SiO_2^+ ratio. The capsules formed are monodisperse in size and exhibit uniform DNA loading. The functional integrity of the encapsulated DNA, both ss- and dsDNA, is largely maintained, as confirmed *via* PCRs using encapsulated ss- and dsDNA as primers and templates, respectively.

METHODS

Materials. SiO_2 particles of 1 and 3 μm diameter were purchased from MicroParticles GmbH as a 5 wt % suspension and were used as received. Poly(methacrylic acid, sodium salt) (PMA), M_w 15 000, was purchased from Polysciences (USA), and poly(vinylpyrrolidone) (PVPON), M_w 55 000, dithiothreitol (DTT), *N*-chloro-*p*-toluenesulfonamide sodium salt (chloramine T), cystamine dihydrochloride, cysteamine hydrochloride, 5,5′-dithiobis-2-nitrobenzoic acid (Ellman's reagent), and 3-aminopropyltrimethoxysilane (APS) were purchased from Sigma-Aldrich and used as received. Dimethylsulfoxide, sodium hydrogen phosphate buffer, and sodium acetate buffer were purchased from Merck. Short oligonucleotides (20-, 22-, 27-, and 30-mer) were purchased from Geneworks, and pPCR-script Amp SK(+) plasmid was purchased from Stratagene. Taq DNA polymerase (native), PCR buffer, deoxynucleotide triphosphates (dNTPs), and the PureLink PCR purification kit were obtained from Invitrogen. Alexa Fluor 488 maleimide and Alexa Fluor 546 dUTP were purchased from Molecular Probes. High-purity water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore RiOs/Origin system (MilliQ water).

Methods. Flow cytometry was performed on a Becton Dickson FACS calibur flow cytometer using an excitation wavelength of 488 nm. Fluorescence measurements were conducted using a Fluorolog Horiba fluorescence spectrophotometer. Particles were imaged on an Olympus IX71 digital wide-field fluorescence microscope with a fluorescein filter cube and a Leica time-correlated single-photon-counting confocal fluorescence microscope. The images were processed and volume rendered using Imaris v4.2 software (Bitplane AG). Zeta (ζ)-potential measurements were taken on a Malvern zetasizer. PCR temperature cycling was carried out in an Applied Biosystems GeneAmp PCR System 9700.

Preparation of PMA_{SH}. A PMA sample with 12 mol % of thiol groups was synthesized from PMA and cystamine dihydrochloride *via* carbodiimide coupling, as described previously.[27] The thiol content in the resulting polymer was characterized using Ellman's reagent and a cysteamine standard curve.[38]

Fluorescence labeling of PMA_{SH} was carried out using a 1 g L^{-1} dimethylsulfoxide solution of Alexa Fluor 488 maleimide

(typically 10 μg) mixed with 1–10 mg of PMA_{SH} at a concentration of 10 g L^{-1} in pH 7.2 phosphate buffer. The reaction between the maleimide on the fluorescent dye and the thiol groups along the polymer was allowed to proceed overnight, after which time the polymer was purified *via* gel filtration and isolated *via* freeze-drying.

PMA_{SH} was incubated in a solution of 100 g L^{-1} DTT at pH 8 for at least 12 h and diluted with 10 mM sodium acetate buffer (pH 4) to the required concentration.

Synthesis of 800 bp DNA. The 800 bp dsDNA sample for adsorption and encapsulation experiments was synthesized by PCR using a mixture containing 0.5 mM dNTPs, 0.5 pM forward and reverse primers (5′-GTAATACGACTCATCATAGGGC, 5′-GGACTCAAGACGATAGTTACCGGATAAG), 2.5 U Taq DNA polymerase, and 100 ng of pPCR-Script Amp SK(+) plasmid in a total volume of 20 μL of the PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$). For the fluorescently labeled product, 0.01 mM AF546-dUTP was included. Cycling conditions were 5 min at 94 °C, 25 cycles of 55 °C for 30 s, 94 °C for 30 s, 72 °C for 3 min, followed by 72 °C for 7 min. Samples were purified using the PureLink PCR purification kit with the high cut-off binding buffer and eluting with MilliQ water at pH 7.1. A portion of the samples were separated on 1% agarose gel in TAE buffer (40 mM Tris-acetate, pH 8.3, 1 mM EDTA) and stained with ethidium bromide.

Preparation of SiO_2^+ Particles. A suspension of 1 μm diameter SiO_2 particles in 1 mL of ethanol was reacted with 250 μL of APS and 50 μL of 30% ammonia solution for 2 h. After this time, the particles were washed several times in ethanol and then three times in MilliQ water. The resulting particles had a ζ-potential of 83 ± 6 mV, as measured in 10 mM sodium acetate buffer (pH 4). SiO_2 particles of 3 μm diameter were functionalized in the same way, and the resulting particles had a ζ-potential of 72 ± 3 mV in 10 mM sodium acetate buffer (pH 4).

Adsorption. DNA adsorption was carried out in a 0.25 wt % suspension of 1 μm SiO_2^+ particles in 2–16 nM chain solutions of 800 bp DNA in 10 mM sodium acetate buffer (pH 4). After incubation for 15 min, the solutions were centrifuged for 2 min at 2000*g*, after which the fluorescence of the supernatant and that of the particles were quantified by fluorescence spectroscopy and flow cytometry, respectively. The ζ-potential of the particles was measured in 10 mM sodium acetate buffer (pH 4).

PMA_{SH} Adsorption and DNA Desorption. The DNA-coated SiO_2^+ particles (prepared as described above) were incubated in a 0.5 g L^{-1} solution of PMA_{SH} in 10 mM sodium acetate buffer (pH 4) for 15 min. The fluorescence of the supernatants and that of the particles were analyzed independently by fluorescence spectroscopy and flow cytometry, respectively.

Preparation of DNA-Containing Capsules. Portions 100 μL each of a 0.5 wt % suspension of 1 μm SiO_2^+ particles in 10 mM sodium acetate buffer (pH 4) were incubated with varying amounts of DNA corresponding to 100, 75, 50, and 0% of the surface saturation value. The DNA-coated particles were incubated in a 0.5 g L^{-1} solution of PMA_{SH} for 15 min. After being washed three times in 10 mM sodium acetate buffer (pH 4), the particles were suspended in a 0.5 g L^{-1} solution of PVPON for 15 min. Polymers were added sequentially until 10 layers had been deposited, after which time the particles were treated with 2 mM chloramine T in 10 mM 2-morpholinoethanesulfonic acid (MES) buffer (pH 6) for 2 min. The particles were washed with fresh buffer and dispersed in 50 μL of 10 mM sodium acetate buffer (pH 4), to which 200 μL of 2 M HF/8 M NH_4F (pH ~5) was added. The resulting capsules were washed by centrifugation (4500*g* for 5 min) and dispersion, which was repeated until the pH of the supernatant was equal to the pH of the fresh buffer solution.

PCR Analysis of the Encapsulated DNA. DNA-containing capsules were prepared as described above using 1 or 3 μm diameter SiO_2^+ particles and non-thiolated PMA and PVPON as capsule-constituting polymers. For analysis, the capsules were suspended in 20 μL of the PCR buffer, which resulted in immediate deconstruction of the capsules and release of the encapsulated DNA. PCRs were performed as described in the synthesis of the 800 bp sequence, using all the required components of the mix with the exception of the encapsulated DNA (3 kbp plasmid, 800 bp linear, or ssDNA as templates or primer sequences). The reaction product was analyzed *via* gel electrophoresis on a 1% agarose gel in TAE buffer with a GeneRuler 1 kb DNA ladder (Fermentas) and stained with ethidium bromide.

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Supporting Information Available: Zeta-potential of DNA-coated particles, fluorescence spectroscopy analysis of DNA desorption from particles, aggregation of particles monitored by flow cytometry, and gel electrophoresis analysis of capsule-released DNA. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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