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Selective Release of Multiple DNA Oligonucleotides from Gold Nanorods

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ABSTRACT Combination therapy, or the use of multiple drugs, has been proven to be effective for complex diseases, but the differences in chemical properties and pharmacokinetics can be challenging in terms of the loading, delivering, and releasing multiple drugs. Here we demonstrate that we can load and selectively release two different DNA oligonucleotides from two different gold nanorods. DNA was loaded on the nanorods *via* thiol conjugation. Selective releases were induced by selective melting of gold nanorods *via* ultrafast laser irradiation at the nanorods' longitudinal surface plasmon resonance peaks. Excitation at one wavelength could selectively melt one type of gold nanorods and selectively release one type of DNA strand. Releases were efficient (50—80%) and externally tunable by laser fluence. Released oligonucleotides were still functional. This proof of concept is potentially a powerful method for multiple-drug delivery strategies.

KEYWORDS: gold nanorods · DNA oligonucleotide · drug delivery · controlled release · selective · independent control · combination therapy

Increasingly, the use of multiple drugs, known as combination therapy, has been sought for improving treatment efficacy of diseases such as malaria,[1] cancer,[2] and HIV.[3] Though proven to be effective, the differences in the chemical properties (such as molecular weights, solubilities) and pharmacokinetics of the components of a drug mixture can create challenges for loading, delivery, and release of multiple drugs.[4] Even if a predetermined synergistic ratio is encapsulated in a carrier, this ratio may not be maintained at a target upon delivery or during release. Typically, the timing of the release of each species is crucial for drug efficacy, as has been observed for tumor treatment.[2] Therefore, for effective combination therapy, release rates of each drug must be controlled independently. Current solutions involve complex systems such as polymer multilayer carriers[5] or sophisticated bioMEMS implants.[6,7] Nanoscale carriers have gained attraction, but achieving different release windows for each drug in a mixture requires engineering intricate architectures.[2] Extending all of these strategies beyond two species or even changing the order of release is problematical. Clearly, an effective method to externally control release of each species independently and actively would ultimately lead to optimization of combination therapies for treatment.

Recently, gold,[8,9] magnetic,[10,11] and composite[12] nanoparticles have been exploited for both passive and active targeted delivery.[13,14] In the case of gold nanoparticles, the surface chemistry has been proven to be chemically versatile for loading biomolecules and optimizing physicochemical parameters.[9,15] Gold nanorods (NRs) have also become attractive for biological applications due to their optical properties.[16,17] Pulsed laser excitation in resonance with their longitudinal surface plasmon resonance (SPR_{long}) can heat NRs locally to high temperatures,[18] inducing melting. This triggered melting is exploitable for controlling the release of biomolecules conjugated to the NRs.[19] Since SPR_{long} is tunable by changing NR aspect ratio (AR), NRs with different ARs can be excited independently at different wavelengths. If different NRs are conjugated to different molecules, this strategy could be utilized for orthogonal triggered release of multiple species. Others have utilized a similar concept to independently control microfluidic valves using two different nanoparticles, gold colloids and gold nanoshells.[20]

Here we demonstrate selective release of two distinct DNA strands from two different NRs by matching laser excitation wavelength to the NRs' SPR_{long} (Scheme 1). We first demonstrate selective melting of two different NRs. Utilizing this concept, two different DNA oligonucleotides conjugated to each of the NRs were released selectively by irradiation at specific wavelengths, and released DNA was functional. The releases

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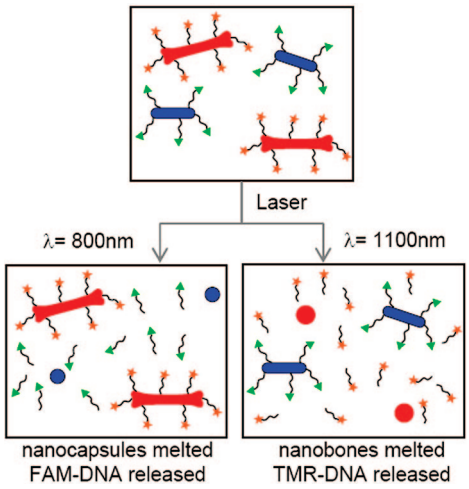

Scheme 1. Overview of selective release. Laser irradiation of DNA-conjugated nanocapsules (blue ovals) and nanobones (red bones) are exposed to λ_{800} irradiation (left), which melts the nanocapsules and selectively releases the conjugated DNA (labeled by FAM (green triangles)). Exposure to λ_{1100} irradiation (right) melts the nanobones, selectively releasing the conjugated DNA (labeled by TMR (orange stars)).

were also efficient (~50−80%) and externally controllable by tuning the laser fluence.

RESULTS AND DISCUSSION

NRs were synthesized[21−23] to have distinct ARs and morphologies, with SPR_{long} that overlapped with each of the laser excitation wavelengths. Short NRs, "nanocapsules", were ~11 nm × 44 nm, with <AR> = 4.0 (Figure 1a, inset) and SPR_{long} at 800 nm (Figure 1b, black), coinciding with the short wavelength excitation at ~800 nm (λ_{800}). Long NRs were bone-shaped (Figure 2a, inset), presumably due to preferential deposition at the nanorod ends from the excess reducing agent.[21] "Nanobones" were ~17 × 89 nm with <AR> = 5.4 and SPR_{long} at ~1100 nm (Figure 2b, black), coinciding with the long wavelength excitation at 1100 nm (λ_{1100}). Size analysis was done utilizing ImageJ.[24] Mixtures allowed distinction between their populations by both the AR and morphology (Figure 3a). TEM sizing of a mixture exhibited broader AR distribution due to overlapping peaks at 4.0 and 5.4. The 1.5−2.0 AR peaks were from the small presence of synthesis byproducts (spheres, cubes, and stars). Spectral overlap of nanocapsules and nanobones at both excitation wavelengths was minimal (Figure 3b, black).

Laser irradiation of gold NRs at SPR_{long} caused melting,[18,19] accompanied by a shape transformation to spheres. We studied the fluence dependence of nanocapsule and nanobone melting by monitoring absorption. The λ_{800} irradiation of nanocapsules caused the SPR_{long} to decrease in intensity and blue-shift with increasing fluence, while the ~520 nm peak increased (Figure 1b). This suggested that melting transformed nanocapsules into shorter rods and spheres. TEM images of nanocapsules after λ_{800} irradiation (inset) and size analysis showed the AR distribution shift to lower values (Figure 1a, blue),

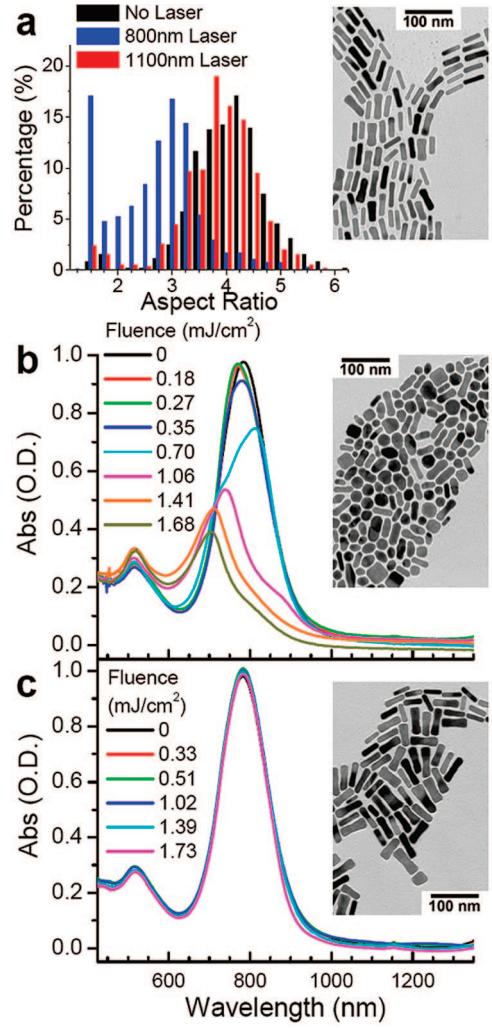

Figure 1. Melting nanocapsules. (a) AR histogram of nanocapsules exposed to irradiation at 800 nm at 1.68 mJ/cm² (blue) and 1100 nm at 1.73 mJ/cm² (red), and unexposed (black). TEM image of unexposed nanocapsule sample (inset). (b) Optical absorption spectra of nanocapsules upon exposure to λ_{800} irradiation. Fluence (mJ/cm²): 0 (black), 0.18 (red), 0.27 (green), 0.35 (blue), 0.70 (cyan), 1.06 (pink), 1.41 (orange), 1.68 (olive). Inset: TEM image of λ_{800} irradiated sample with a fluence of 1.68 mJ/cm². (c) Optical absorption of nanocapsules upon exposure to λ_{1100} irradiation. Fluence (mJ/cm²): 0 (black), 0.33 (red), 0.51 (green), 1.02 (blue), 1.39 (cyan), pink (1.73). Inset: TEM image of λ_{1100} irradiated sample with a fluence of 1.73 mJ/cm².

supporting shape transformation to spheres. To show that melting of nanocapsules requires matching irradiation wavelength to the SPR_{long}, we irradiated nanocapsules at 1100 nm. The absorption spectrum was unchanged (Figure 1c), demonstrating no significant effect. TEMs of nanocapsules after λ_{1100} irradiation (inset) were also unchanged, and size analysis showed little effect on the AR distribution (Figure 1a, red).

The melting of the nanobones also required the matching of the irradiation wavelength to their SPR_{long}. When nanobones were exposed to λ_{1100} irradiation, the 1100 nm peak decreased and blue-shifted with increasing fluence, while the ~520 nm peak increased (Figure 2c). TEM imaging after λ_{1100} irradiation confirmed a shape

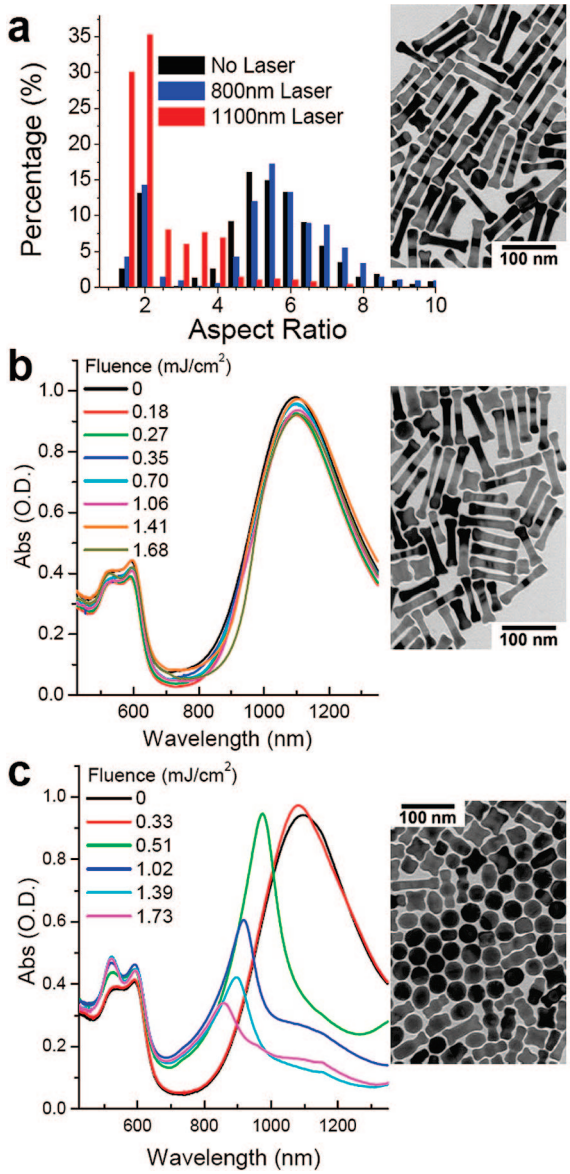

Figure 2. Melting nanobones. (a) AR histogram of nanobones exposed to irradiation at 800 nm at 1.68 mJ/cm^2 (blue) and 1100 nm at 1.73 mJ/cm^2 (red), and unexposed (black). TEM image of unexposed nanobones sample (inset). (b) Optical absorption spectra of nanobones upon exposure to λ_{800} irradiation. Fluence (mJ/cm^2): 0 (black), 0.18 (red), 0.27 (green), 0.35 (blue), 0.70 (cyan), 1.06 (pink), 1.41 (orange), 1.68 (olive). Inset: TEM image of λ_{800} irradiated sample with a fluence of 1.68 mJ/cm^2. (c) Optical absorption of nanobones upon exposure to λ_{1100} irradiation. Fluence (mJ/cm^2): 0 (black), 0.33 (red), 0.51 (green), 1.02 (blue), 1.39 (cyan), pink (1.73). Inset: TEM image of λ_{1100} irradiated sample with a fluence of 1.73 mJ/cm^2.

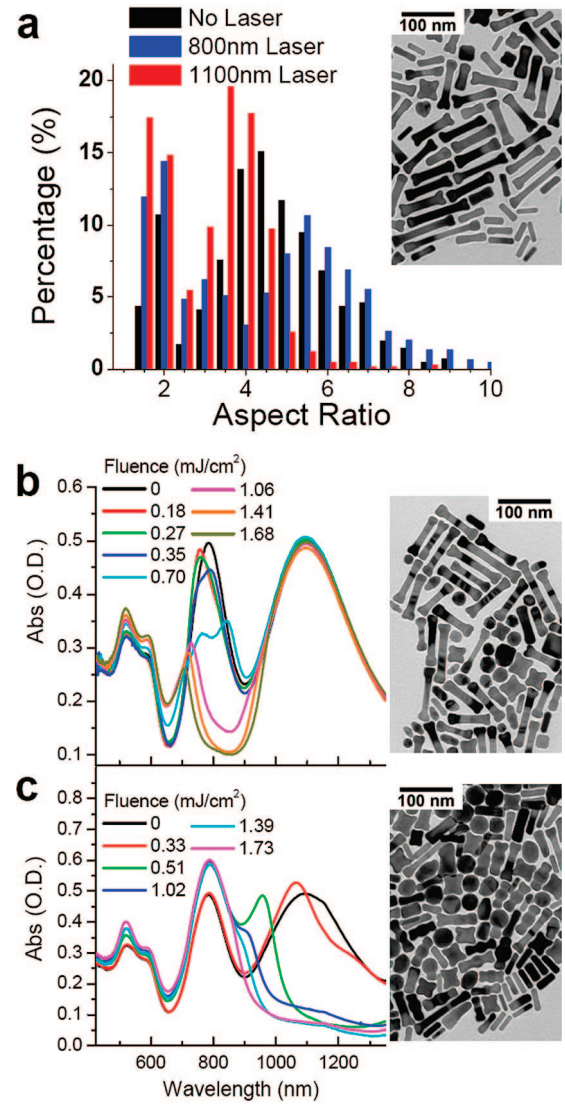

Figure 3. Selective melting of nanocapsule/nanobone mixtures. (a) AR histogram of nanocapsules−nanobones mixture exposed to irradiation at 800 nm at 1.68 mJ/cm^2 (blue) and 1100 nm at 1.73 mJ/cm^2 (red), and unexposed (black). Inset: TEM image of unexposed mixture. (b) Optical absorption spectrum of mixture after λ_{800} irradiation. Fluence (mJ/cm^2): 0 (black), 0.18 (red), 0.27 (green), 0.35 (blue), 0.70 (cyan), 1.06 (pink), 1.41 (orange), 1.68 (olive). Inset: TEM image λ_{800} irradiated mixture with a fluence of 1.68 mJ/cm^2. (c) Optical absorption spectrum of mixture after λ_{1100} irradiation. Fluence (mJ/cm^2): 0 (black), 0.33 (red), 0.51 (green), 1.02 (blue), 1.39 (cyan), pink (1.73). Inset: TEM image λ_{1100} irradiated mixture with a fluence of 1.73 mJ/cm^2.

transformation into shorter "candy-wrap" or ϕ-shaped particles and spheres (inset).[25] Size analysis confirmed an AR shift to lower values (Figure 2a, red). The λ_{800} irradiation had essentially no effect, as evidenced by no significant change in absorption scans (Figure 2b). TEM after λ_{800} irradiation (inset) and size analysis showed no significant changes in the AR distribution (Figure 2a, blue), indicating no shape transformation occurred.

We also selectively melted either the nanocapsules or nanobones when both were present in a mixture. The absorption scan had peaks at 800 and 1100 nm due to the presence of both species, and TEMs and AR histograms showed both populations (Figure 3a, black and inset). The λ_{800} irradiation caused the 800 nm peak to decrease, leaving the 1100 nm peak relatively unaffected (Figure 3b). After λ_{800} irradiation, fewer nanocapsules were present relative to nanobones. Spheres appeared, resulting from the nanocapsule shape transformation (inset). The AR peak at 4.0 decreased in intensity, and peaks at 3.0 or less increased, suggesting melting to form spheres and shorter NRs (Figure 3a,

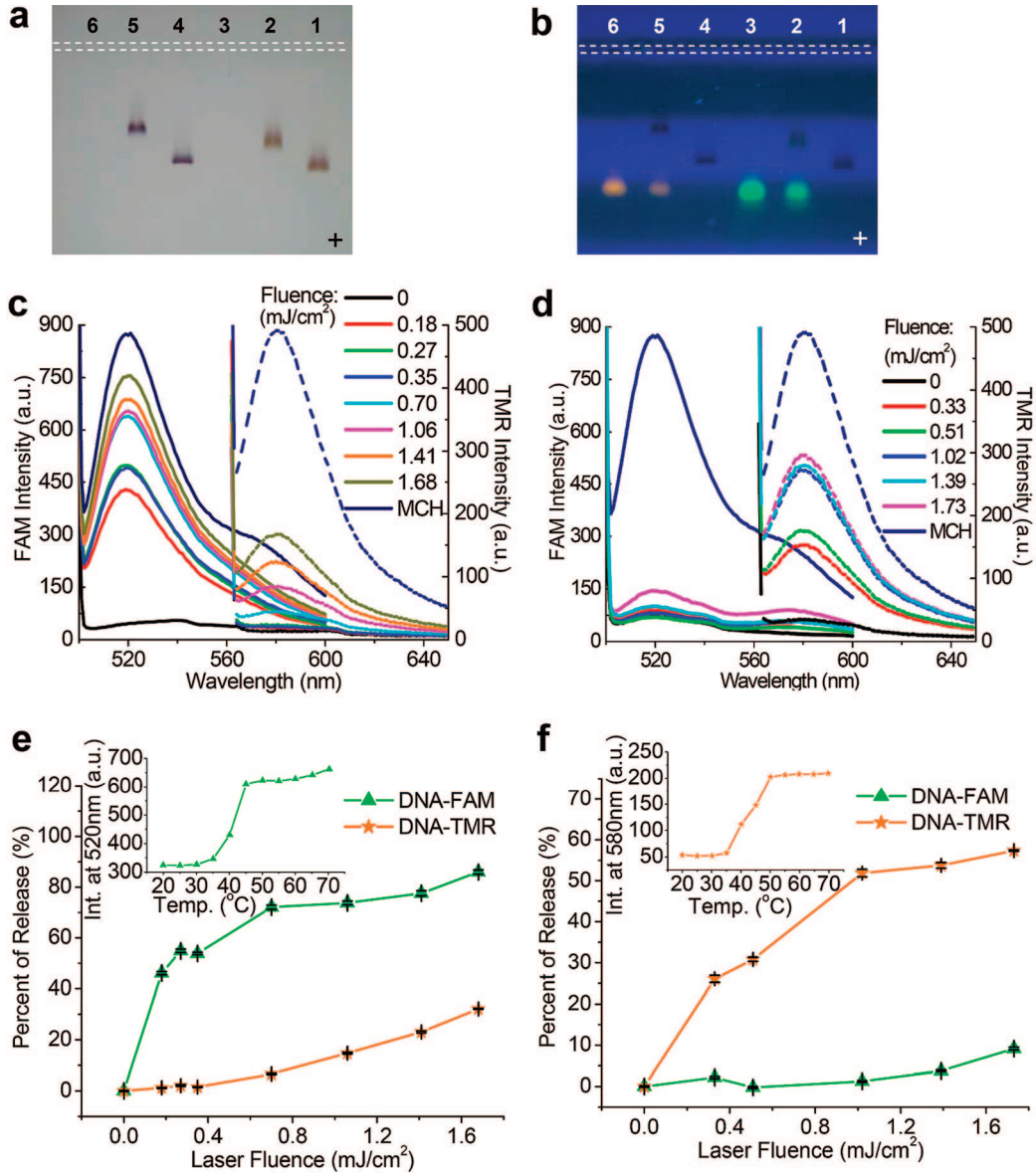

Figure 4. DNA functionalization of NRs and selective release: (a) white light and (b) UV images of gel electrophoresis. Lane 1: nanocapsules. Lane 2: FAM-DNA-SH + nanocapsules. Lane 3: FAM-DNA-SH. Lane 4: nanobones. Lane 5: TMR-DNA-SH + nanobones. Lane 6: TMR-DNA-SH. Dashed lines indicate positions of wells. Positive direction indicated. (c) Fluorescence spectra of supernatant after λ_{800} irradiation, FAM-DNA peaks (solid lines) and TMR-DNA peaks (dashed lines). Fluence (mJ/cm^2): 0 (black), 0.18 (red), 0.27 (green), 0.35 (blue), 0.70 (cyan), 1.06 (pink), 1.41 (orange), 1.68 (olive). (d) Fluorescence spectra of supernatant after λ_{1100} irradiation. FAM-DNA peaks (solid lines) and TMR-DNA peaks (dashed lines). Fluence (mJ/cm^2): 0 (black), 0.33 (red), 0.51 (green), 1.02 (blue), 1.39 (cyan), pink (1.73). (e) Percent released of FAM-DNA (green triangles) and TMR-DNA (orange stars) as a function of λ_{800} laser fluence. Inset: melting curve of released DNA (after λ_{800} irradiation of 1.68 mJ/cm^2) hybridized with a DABCYL-complement; monitoring fluorescence at 520 nm. (f) Percent released of FAM-DNA (green triangles) and TMR-DNA (orange stars) as a function of λ_{1100} laser fluence. Inset: melting curve of released DNA (after λ_{1100} irradiation of 1.73 mJ/cm^2) hybridized with a DABCYL-complement; monitoring fluorescence at 580 nm. Error bars represent standard deviation from 10 measurements.

blue). However, the peak at 5.4 was relatively unchanged. These results support that λ_{800} irradiation melted only the nanocapsules but not the nanobones. When the mixture was exposed to λ_{1100} irradiation, the 1100 nm peak decreased with increasing fluence (Figure 3c) while the 800 nm peak increased, presumably due to shape transformation of nanobones into ϕ-shaped NRs, which are expected to absorb at wavelengths lower than 1100 nm.[25] TEM imaging after λ_{1100} irradiation (inset) showed that the nanobones disappeared, with primarily nanocapsules, spheres, and ϕ-shaped NRs remaining. The AR peak at 5.4 decreased, while peaks at 4.0 or less increased, indicating nanobone shape transformation (Figure 3a, red). Evidently, λ_{1100} irradiation affected only the nanobones and not the nanocapsules. Thus, laser irradiation could selectively melt each species in a mixture, corroborating single-type NR melting studies.

NRs were conjugated to thiolated DNA 40mers, which were each labeled with different fluorophores

and thus distinguishable. Ligand exchange was necessary to avoid aggregation during DNA conjugation and was performed prior to conjugation to replace the positively charged CTAB surfactant with negatively charged mercaptohexanoic acid.[26] The conjugations were done *via* charge screening methods.[26−28] Gel electrophoresis assayed DNA conjugation (Figure 4a,b). Nanocapsules ran toward the positive electrode, indicating a negative charge from the mercaptohexanoic acid ligand coating the surface (Lane 1). Nanocapsules incubated with thiolated 6-carboxyfluorescein-labeled DNA 40mers (FAM-DNA-SH) were retarded, indicating a larger hydrodynamic radius due to conjugation (Lane 2). The UV image of the gel (Figure 4b) showed that the free FAM-DNA-SH band after conjugation (Lane 2) was dimmer than the equal-concentration free FAM-DNA-SH alone (Lane 3), also supporting conjugation to nanocapsules, which quench fluorescence. Nanobones showed similar results for thiolated tetramethylrhodamine- labeled DNA 40mers (TMR-DNA-SH). The TMR-DNA-nanobones band (Lane 5) was retarded compared to nanobones alone (Lane 4), and the free TMR-DNA-SH band (Lane 5) was dimmer than the equal-concentration free TMR-DNA-SH alone (Lane 6). These results confirm DNA conjugation to both species. DNA loading on the NR surface was quantified by two methods. The fluorescence change of the supernatant with and without NRs measured the loss of free DNA that was due to NR conjugation. In addition, purified NRs conjugated to DNA were treated with high concentrations of mercaptohexanol (MCH), which displaced conjugated DNA from the NR surface. This displaced DNA was quantified by fluorescence. Both methods gave similar estimates of the DNA loadings, which was approximately 114 DNA/nanocapsule and 284 DNA/nanobone.[26,29−31]

Finally, the mixture of purified FAM-DNA-nanocapsules and TMR-DNA-nanobones was laser irradiated for selective release (Scheme 1). Exposure to laser irradiation was followed immediately by centrifugation, and released DNA in the supernatant was quantified by fluorescence spectroscopy. After λ_{800} irradiation, the supernatant fluorescence at 520 nm increased with fluence (Figure 4c, solid lines), illustrating FAM-DNA release. However, increased fluorescence at 580 nm was much lower, indicating insignificant TMR-DNA release (dashed lines). Therefore, λ_{800} irradiation could selectively release FAM-DNA from nanocapsules, while leaving TMR-DNA-nanobones undisturbed. At fluences <1.00 mJ/cm^2, FAM-DNA release was selective, reaching ~70%, while TMR-DNA release was ≤10%, where 100% was the amount released by MCH treatment (Figure 4e). For fluences >1.00 mJ/cm^2, release of TMR-DNA from nanobones did increase, while FAM-DNA release was saturated. When the mixture was exposed to λ_{1100} irradiation, supernatant fluorescence at 580 nm increased (Figure 4d, dashed lines), while intensity at 520 nm was negligible (solid lines), illustrating TMR-DNA release with no significant FAM-DNA release. TMR-DNA release was selective, reaching 50−60% while FAM-DNA release was <10% (Figure 4f). Therefore, λ_{1100} irradiation could selectively release TMR-DNA from nanobones, while leaving FAM-DNA-nanocapsules undisturbed. Evidently, the NRs undergo a shape transformation at these fluences (Figures 1−3), which probably induces release due to gold−thiol bond dissociation.[32,33] We observed that re-adsorption of the released DNA back onto the melted gold nanorods after a long period of time (3 months) was minimal (Figure S1, Supporting Information). We also observed that the laser irradiation had no effect on the fluorescence of the FAM-DNA and TMR-DNA (Figure S2, Supporting Information).

We confirmed that released DNA was still functional and could hybridize to a complement. Released DNA was incubated with DABCYL-functionalized DNA complements and hybrid formation confirmed by melting curves. Dequenching of FAM at 520 nm (Figure 4e, inset) and TMR at 585 nm (Figure f, inset) was monitored with increasing temperature. Both curves were characteristic of functional hybrids, with T_m's coinciding with that of the plain DNA (T_m = 42 °C).

In conclusion, we demonstrate selective release of two distinct DNA oligonucleotides from two different NRs *via* selective laser-induced melting of NRs. Because laser fluence governs the degree of NR melting, yield, and specificity of DNA release, the controlled releases are externally tunable. Tuning NR synthesis parameters could extend this approach beyond two species. Since conjugation requires only standard thiol conjugation, it is potentially applicable to a wide range of molecules. NRs have relatively large surface area and the capacity to load hundreds of molecules, and ~80% of the payload can be released. NRs are chemically versatile, with customizable coatings, and others have demonstrated active targeting by decorating the NRs with moieties such as antibodies and cell receptor ligands. Others have also utilized laser-induced melting of a single type of NR for controlled release inside cells, while maintaining cell viability by tuning the laser parameters appropriately.[19] Therefore, this proof of concept of selective triggered release from NRs is potentially a powerful technique for improving drug delivery strategies.

METHODS

Gold Nanorod Synthesis. Both nanocapsules and nanobones were synthesized using the seed-mediated growth method. The preparation of the seed solution was the same for both. Typical protocol: 7.5 mL of 0.2 M CTAB solution was mixed with 0.25 mL of 0.01 M $HAuCl_4$ (for nanocapsules) or 2.5 mL of 0.001 M $HAuCl_4$ (for nanobones) in a beaker. While the solution was vigorously stirred, 0.6 mL of ice-cold 0.01 M of $NaBH_4$ was added and the solution turned brownish yellow. Vigorous stirring continued for another 2 min and then it was kept undisturbed at room temperature.

Nanocapsules were synthesized by single-surfactant seed-mediated growth method.[23] Typical protocol for growing nanocapsules: 10 mL of 0.01 M of $HAuCl_4$ was added into 237.5 mL of 0.1 M CTAB in a glass bottle, and the solution turned orange; 1.5 mL of 0.01 M $AgNO_3$ was added to the solution, followed by gentle mixing; 1.6 mL of 0.1 M ascorbic acid was added into the solution, followed by gentle inversion until the solution turned colorless. Two milliliters of seed solution was gently added to the growth solution. The solution sat on the bench undisturbed overnight, during which time it turned reddish brown.

Nanobones were synthesized by binary surfactant seed-mediated growth method.[21,22] Typical protocol: 125 mL of 0.001 M of $HAuCl_4$ was added into a mixture of 50 mL of 0.3 M CTAB and 75 mL of 0.3 M of BDAC (benzyldimethylhexadecylammonium chloride) in a glass bottle, and the solution turned orange. Five milliliters of 0.004 M $AgNO_3$ was added to the solution, followed by gentle mixing; 1.5 mL of 0.1 M ascorbic acid was then added into the solution, followed by gentle inversion until the solution turned colorless; 0.25 mL of seed solution was gently added to the growth solution. The solution sat on the bench undisturbed overnight, during which time it turned reddish purple. The resulting product was high aspect ratio gold nanorods. In order to turn these nanorods to nanobones, 4.63 mL of 0.1 M ascorbic acid was added into 250 mL of the nanorod solution, followed by gentle mixing. Reactions were left undisturbed at room temperature. After ~3 h, the solution turned blue, indicating formation of nanobones.

Nanorod Characterization. Nanorod concentrations were quantified by optical absorption using estimated extinction coefficients.[30] Cary 500 UV−vis−NIR spectrophotometer (Varian Inc.) was used to scan the absorption profiles of NRs solutions. On the basis of their SPR_{long}, we estimated the extinction coefficients to be 4.6×10^9 and 8.6×10^9 M^{-1} cm^{-1} for nanocapsules and nanobones, respectively. TEM imaging was done on a JEOL 2010 using holey carbon grids. Size analysis was done utilizing ImageJ.[24]

Ligand Exchange of Nanorods. The CTAB surfactant on the NR surface was replaced with mercaptohexanoic acid (MHA) by round-trip phase transfer ligand exchange as described in detail in previous work.[26] First we performed aqueous-to-organic phase transfer. Concentrated NR with CTAB surfactants (NR-CTAB) in water were put into contact with dodecanethiol (DDT). After addition of acetone, NRs were extracted into DDT by swirling the solution for a few seconds, upon which the aqueous phase became clear, indicating that no NRs remained. Next, organic-to-aqueous phase transfer was performed. To remove the excess DDT, the DDT-coated NRs (NR-DDT) were diluted in toluene. Centrifugation was performed to collect the NR-DDT. Methanol may be needed to precipitate the NR-DDT prior to centrifugation. The collected NR-DDT was resuspended in 1 mL of toluene by brief sonication. The NR-DDT in toluene was then added to 9 mL of 0.01 M mercaptohexanoic acid (MHA) in toluene at 95 °C and vigorously stirred. Reflux and stirring continued until visible aggregation was observed (within ~15 min), and then the solution was allowed to settle and cool to room temperature. Aggregation indicated that NRs were successfully coated by MHA, which are insoluble in toluene. The aggregates were washed twice with toluene *via* decantation and then once with isopropanol to deprotonate the carboxylic acid. The aggregates spontaneously re-dispersed in 1× tris-borate-EDTA buffer (TBE) and were no longer soluble in toluene. This ligand exchange protocol was performed for both types of NR (nanocapsules and nanobones).

DNA Functionalization of Nanorods. The 40mer DNA oligonucleotides with sequences 5′ HS-TTTTT TTTTT TTTTT TTTTT TTTTT TCGGC CCGTA TAATT 3′, fluorescently labeled at the 3′ ends with either 6-carboxyfluorescein (FAM-DNA-SH) or tetramethylrhodamine (TMR-DNA-SH), were purchased from Sigma Aldrich. DNA conjugation was achieved following charge screening protocols.[26−28] Charge screening (salt-aging) was necessary to compensate for electrostatic repulsion between the negatively charged ligand exchanged nanorods and DNA. Sodium dodecyl sulfate (SDS) surfactant was used to increase the stability of nanorods during the salt-aging process. First, FAM-DNA-SH and TMR-DNA-SH were reduced by tris[2-carboxyethyl]phosphine (TCEP) with TCEP/DNA ratio of 100:1. Then nanocapsules or nanobones in the concentration range of 5−10 nM were incubated with the reduced FAM-DNA-SH or TMR-DNA-SH, respectively, in 10 mM phosphate buffer with 0.3% SDS concentration. DNA to NR ratios were 200 FAM-DNA-SH/nanocapsule and 400 TMR-DNA-SH/nanobone. After 3 h of incubation, charge screening was performed with salting buffer of 0.6 M of NaCl, 0.3% SDS in 10 mM phosphate buffer. Eight microliters of the salting buffer was added to the 200 μL of the conjugation solution every 30 min followed by 10 s sonication. This step was repeated for a total of five times, which was then followed by overnight incubation.

Two methods were used to confirm NR-DNA conjugation. First, gel electrophoresis was used to observe mobility changes to assay any change in the hydrodynamic radius of DNA-conjugated NRs. Gel electrophoresis was performed with 0.5% agarose gels in 0.5× TBE. Glycerol was used for loading the samples to ensure the nanorods stayed in the wells prior to traveling in the gel matrix.

The second method to confirm the conjugation was quantification of the DNA loading onto the NR surface. This was accomplished by two methods. First, we quantified the free unconjugated DNA *via* fluorescence spectroscopy of the fluorophore labels (FAM for nanocapsules and TMR for nanobones) with or without nanorods present. This was done by collecting the supernatants after centrifugation of the conjugation solution. The second method was accomplished by chemical displacement of the conjugated DNA using literature methods.[26,31] Briefly, purified NR-DNA conjugates were incubated in 1 mM mercaptohexanol (MCH) overnight, displacing the DNA from the NRs. Free displaced DNA was separated from the NRs by centrifugation and quantified by fluorescence spectroscopy. Both methods gave similar estimates of the DNA loadings of approximately 114 DNA/nanocapsule and 284 DNA/nanobone.[30]

Laser Irradiation. Laser irradiation was achieved using pulsed femtosecond lasers. For the 800 nm irradiation, the 82 MHz output of a Ti:sapphire oscillator (Tsunami, Spectra-Physics) is amplified at 1 kHz by a Ti:sapphire regenerative amplifier (Spitfire, Spectra-Physics) pumped by the doubled output of a Q-switched Nd:YLF laser (Empower, Spectra-Physics). The system produces 50−475 μJ, with a duration of 100 fs centered at ~800 nm at a 1 kHz repetition rate. Spot size was 6 mm. Two filter lenses (900 nm short-pass and 700 nm long-pass) were placed prior to the sample holder. In a typical experiment, 50 μL of sample in 3 × 3 mm quartz cuvette was exposed to the laser for 60 s.

The 1100 nm was generated *via* a home-built two-stage BBO/$KNbO_3$ optical parametric amplifier pumped with the output of a Ti:sapphire multipass amplifier (Femtolasers: 30 fs, 1 kHz, 800 nm). Although the OPA is optimized for the production of 3 μm light, ~2.6−13.6 μJ pulses of 1100 nm light were generated, and the difference frequency between 800 nm and 3 μm is generated by the OPA and used for the experiments. The generated ~1100 nm has a duration of 45 fs/pulse with a repetition rate of 1 kHz. Spot size was 1 mm. The 900 nm long-pass filter lens was placed prior to the sample holder. In typical experiments, 50 μL of a sample in a 3 × 3 mm quartz cuvette was continuously mixed with pipet tip while being exposed to the laser for 60 s.

In the NR melting study, we had three samples: nanocapsules, nanobones, and a mixture of both. They were all suspended in a 10 mM CTAB solution. The concentration of nanocapsules sample was ~0.8 nM. The concentration of the nanobone sample was ~0.4 nM. The concentrations of the mixture were ~0.4 and ~0.2 nM for the nanocapsules and nanobones, respectively. After 800 or 1100 nm laser exposure, samples were diluted with 150 μL of a 10 mM CTAB solution. UV−vis−NIR absorption scans were performed for monitoring their shape transformations.

In the DNA release study, we used a mixture of FAM-DNA-nanocapsules and TMR-DNA-nanobones with concentrations of ~0.4 and ~0.2 nM, respectively, in 1× TBE. Samples were washed ≥three times with 1× TBE to remove free DNA prior to mixing. After 800 or 1100 nm laser exposure, samples were immediately diluted with 150 μL of 1× TBE and followed by centrifugation at 16100*g* for 5 min to remove the NRs and collect the

supernatants. Fluorescence spectroscopy was used to quantify the released DNA in supernatants. We quantified the fluorescence intensity of FAM due to FAM-DNA released from nanocapsule and TMR due to TMR-DNA released from nanobones.

To ensure that the released DNA from both nanocapsules and nanobones were still functional, we hybridized them with their complement. The complement was functionalized with a 5′ DABCYL (5′ DABCYL-AATTATACGGGCCG 3′) to quench the FAM and TMR in the hybridized state. Melting curves were obtained in a temperature-controlled Peltier module of the fluorescence spectrometer, where the increase of fluorescence of either FAM or TMR was monitored as a function of increasing temperature.

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Supporting Information Available: Additional supporting figures of the DNA release study. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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