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Plasmonically Controlled Nucleic Acid Dehybridization with Gold Nanoprisms

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The irradiation of anisotropic gold nanostructures at their surface plasmon resonance (SPR) is an efficient method for converting light into heat,^[1] particularly in the near-infrared region where absorbance by biological tissues is minimal.^[1–7] These techniques however, typically rely on high-power pulsed lasers that can cause irreversible damage to the local environment and have only recently been used to investigate a particle-selective response.^[4,5,8–10] Few reports to date focus on the use of SPR-mediated heating to release drugs or nucleic acids under mild conditions amenable to temperature-sensitive environments.^[11–13] Herein we show that organic–inorganic hybrid nanostructures consisting of a triangular gold nanoprism core modified with a dense shell of fluorophore-labeled oligonucleotide duplexes can be used for selective, SPR-mediated, and non-destructive release of nucleic acids. Our results introduce a class of tunable nanostructures that facilitate the rational control over the release of hybridized cargo using light-controlled modulation of the local temperature.

The ability to heat nanostructures and cause a local temperature change using external stimuli is of considerable interest for hyperthermic cancer therapies,^[1–3,7] drug delivery,^[5,14] DNA actuation,^[15] and microsecond DNA melting analysis.^[9] However, recent methods using radio-frequency electromagnetic fields to generate heating of iron oxide nanostructures are not particle-selective and require the collective interaction of many magnetic nanoparticles in order to generate local heat. In general, this approach requires high local concentrations (typically mg mL^{−1}) of particles and is unable to effect heating at a single-particle level.^[14] On the other hand, SPR-based heating methods using gold nanostructures under femtosecond-pulsed laser irradiation report temperature increases in excess of 900°C.^[16] Such high local temperature increases can cause acoustic cavitation and have been used to damage sensitive environments such as lipid membranes and ablate cancer cells.^[1–3,5,7] These conditions can even melt the nanoparticle core^[5,8] and dissociate nanoparticle capping ligands (e.g. thiols), which results in the destabilization and aggregation of the colloidal particles.^[4] These experiments demonstrate the promising

applications of nanoparticles in achieving local heating remotely. However, of the current methods, magnetic heating is less efficient with individual particles, while the SPR heating of metal nanostructures has been used primarily in destructive, largely irreversible processes. Therefore, it remains a challenge to develop materials that mediate non-destructive, reversible processes remotely.

One candidate for such a material is the oligonucleotide-functionalized gold nanoparticle.^[17] The discovery of these particles has led to the development of a number of important systems for antisense gene regulation,^[18] intracellular mRNA detection,^[19] and ultrasensitive biodiagnostics.^[20] Recently, procedures have been developed to expand oligonucleotide functionalization to anisotropic nanostructures such as gold nanorods and triangular nanoprisms which exhibit SPRs that can be tailored to lie in the biologically inert near-infrared region.^[21,22] Herein, we show that the optical properties of anisotropic nanostructures and the thermal properties of nucleic acids can be used to create hybrid nanomaterials capable of light-controlled oligonucleotide release. Further, we demonstrate that oligonucleotide-modified gold nanoprisms are chemically, morphologically and functionally stable under several hours of laser irradiation and thus represent a class of nanostructures capable of SPR-mediated heating to dehybridize oligonucleotides.

In a typical experiment, triangular gold nanoprisms (120 ± 12 nm edge length, 7.5 ± 0.5 nm thick),^[23] which have a strong dipole SPR in the near-infrared region (~ 1200 nm),^[24] were functionalized with a thiolated oligonucleotide containing a 15-base recognition sequence hybridized to a fluorescein-labeled complementary sequence.^[22] Using these particles, we hypothesized that the heat generated by SPR-specific irradiation would dissociate the attached duplex, and release the fluorophore-labeled oligonucleotide (Scheme 1). To test this hypothesis, we designed an apparatus for simultaneous SPR excitation of the nanoprisms and measurement of fluorescence intensity consisting of a near-infrared laser coupled to a fluorescence spectrometer (Figure 1). In this setup, the excitation and emission of the fluorophore-labeled complementary oligonucleotide can be monitored during the irradiation of the sample by a 500 mW continuous wave 1064 nm laser source. Because gold efficiently quenches fluorescence in a distance-dependent fashion,^[25,26] we take advantage of the quenching ability of the nanoprism–oligonucleotide conjugate to monitor the dehybridization of the fluorophore-labeled oligonucleotide in real time. This setup can be used to qualitatively assess the effect of laser irradiation on the hybridization state of the attached duplex.

Using this configuration, we proceeded to test the ability of SPR-mediated heating of oligonucleotide-modified nanoprisms to dehybridize the fluorophore-labeled complementary oligonucleotides. Without laser irradiation, the system exhibits a constant fluorescence signal. Under near-infrared laser irradiation (1064 nm), we observe an increase in fluorescence intensity, which we assign to photothermal generation of heat sufficient to denature the duplex (Figure 2b). Over the course of 3 h, the fluorescence intensity becomes constant, indicating that an equilibrium has been reached. In this experiment, only a small area of the cuvette is irradiated (spot size = 0.45 mm) where nanoprisms diffuse into the beam, undergo SPR-mediated local heating and release their oligonucleotide cargo via temperature-induced duplex dehybridization. Simultaneously, oligonucleotide-modified nanoprisms diffuse out of the beam, cool down and rehybridize to fluorophore-labeled complements. The dehybridization kinetics are slow because of several factors: 1) The experimental setup allows for irradiating only approximately 0.1% of the sample, and thus time must be allowed for prisms to diffuse into the beam. 2) Heating of the prisms is not instantaneous and requires time before the melting temperature of the duplex is reached. 3) Dehybridization is not expected to be immediate, even at the duplex melting temperature. Although this process is dependent on the geometry of the experimental setup, we estimate

that on average 10% of all fluorophore-labeled oligonucleotides are dehybridized (Supporting Information, Figure 1). Interestingly, 1064 nm laser irradiation of a free fluorophore-quencher-labeled duplex in the absence of Au nanoprisms results in no change in fluorescence, confirming the necessity of the inorganic nanoparticle to convert the incident light into heat (Supporting Information, Figure 2). It is important to note that the concentrations used in these experiments are low (45 pM) and result in nanoprisms separated by an average distance of 4 nm. These values are much greater than the longest observed SPR interactions,^[27] and suggest the origin of the local heating does not rely on interparticle interactions but is active at the single-particle level.

The local heating observed with these conjugates is dependent upon the resonance of the incident laser light with the SPR wavelength. Changes to the nanoparticle morphology will shift the SPR, and thus diminish the efficacy of the system. It is known that the high-energy vertices of anisotropic noble metal nanostructures are susceptible to surface reorganization effects under mild conditions.^[28,29] Because elevated temperatures have been implicated in these processes, we monitored the morphological evolution of oligonucleotide-modified nanoprisms before and after laser irradiation by transmission electron microscopy (TEM) and UV/Vis–NIR spectroscopy. Interestingly, representative TEM analysis did not identify any discernable change in the nanoprism shape, edge length, or tip sharpness (Figure 3a). Morphology was unchanged even after 9 h of laser irradiation—well beyond a typical experiment (3–4 h). Moreover, UV/Vis–NIR spectra taken before and after irradiation indicate that the SPR dipole peak at 1200 nm does not change significantly (Figure 3a). Because the nanoprism SPR dipole peak position is extremely sensitive to morphological features such as tip truncation,^[30,31] it is particularly crucial for SPR-mediated applications that the nanoprism morphology remains stable under experimental conditions. Indeed, the lack of spectral peak shift demonstrates the structural stability of these conjugates when subjected to laser irradiation. However, the UV/Vis–NIR spectra do indicate a slight drop in SPR peak intensity. This minimal drop in intensity, however, also occurs in a control experiment where the shutter on the laser head is closed and the sample does not receive any irradiation (Supporting Information, Figure 3). Thus, this change cannot be attributed to laser-induced morphology or concentration changes and is likely due to particles sticking to the walls of the sample cuvette.

The morphological invariance of oligonucleotide-modified nanoprisms under laser irradiation is important in maintaining the function of these conjugates. Morphological reorganization in the case of gold nanoprisms has been determined to significantly shift the plasmon resonance by hundreds of nanometers towards higher energy wavelengths.^[30] Indeed, inducing nanoprism surface reorganization by removing excess ligands from solution blue-shifts the dipole plasmon resonance to approximately 700 nm (Supporting Information, Figure 4). At this frequency the conjugates do not have appreciable extinction at the laser wavelength. If this were to take place, the photothermal efficiency of the nanoprism would decrease as the SPR peak absorbance blue-shifted away from the laser excitation until the effect ultimately became negligible. Because the plasmonic properties of the nanoprisms are unaffected, however, the nanostructures are expected to remain functional.

To probe the importance of plasmonic wavelength in these experiments, analogous duplex-functionalized 13 nm spherical Au nanoparticles were prepared and studied in the context of a similar experiment. These nanoparticles have an SPR absorbance at 532 nm and no appreciable extinction at the laser wavelength (Figure 2a). Using the spherical nanoparticles under identical experimental conditions and at an identical concentration (45 pM) to those used for nanoprisms, 1064 nm laser irradiation does not yield a significant change in fluorescence, demonstrating that the effect requires a plasmonic nanostructure that absorbs

at the laser irradiation wavelength (Figure 2b). The slight increase in fluorescence is attributed to heat generated by the nearby laser source. This contribution is diminutive when considering the change in fluorescence observed for oligonucleotide-modified nanoprisms. This experiment also accounts for any extrinsic heating of the solution, the cuvette, or the sample compartment that might otherwise explain the behavior observed in the case of the anisotropic gold nanoprism. Finally, this result confirms that light-induced heating can discriminate between nanoparticle types based on their specific SPR.

El-Sayed and co-workers have shown that femtosecond-pulsed laser irradiation ($\lambda = 400$ nm) is able to dissociate the Au–S bond for spherical gold nanoparticles functionalized with thiolated oligonucleotides.^[4] In order to rule out this process as the driving force behind the observed oligonucleotide release, the chemical stability of the Au–S bond on the nanoprism surface was investigated. Specifically, a cyanine 5.5 (Cy5.5) dye-labeled thiolated oligonucleotide was synthesized to determine the stability of the covalently bound oligonucleotide during excitation using the previously described experimental setup (Figure 1). Under 1064 nm laser irradiation, no change in fluorescence intensity is observed, which indicates that the thiolated oligonucleotide remains bound to the gold nanoprism surface (Figure 3b). Furthermore, after removal of the inorganic Au component of the oligonucleotide-nanoprism conjugate by oxidizing with potassium cyanide, the fluorescence intensity of the Cy5.5 labeled thiolated oligonucleotide increases approximately by a factor of four (Figure 3b). This represents the maximum fluorescence possible in the system and demonstrates that the Cy5.5 is being quenched by the Au nanoprism because it has been localized through the Au–S bond.

The chemical stability of the Au–S bond under continuous-wave 1064 nm laser irradiation illustrates an important finding for light-controlled nucleic acid release. While oligonucleotide release is possible using a Au–S bond dissociation mechanism,^[4] there are several reasons why Au–S dissociation is undesirable. First, ligands are required to maintain stability against particle aggregation. For the majority of applications under consideration, solution-phase stability both before and after irradiation are of crucial importance. Second, Au–S-mediated release does not allow one to denature an immobilized duplex that melts at a designed temperature for sequence tunable release. Finally, Au–S dissociation may be an irreversible process and thus does not allow release and subsequent rehybridization of a cargo.

To probe the advantages of Au–S bond stability, we next investigated the possibility of release followed by sequestration of fluorophore-labeled oligonucleotides. These experiments also address the concern of thermal damage to oligonucleotides during release from the particle surface due to potentially high local temperatures.^[16] We hypothesized that oligonucleotide damage would correlate strongly with an inability for the released nucleic acids to rehybridize back with the complementary oligonucleotides bound to the particle. Several hours of nanoprism irradiation immediately followed by several hours of no irradiation allows the system to reach equilibrium at each stage and results in a fluorescence signal which rises and falls back to a value equal to the original signal (Figure 4a). This observation is attributed to the ability of the released oligonucleotides to rehybridize with the nanoprism conjugates after irradiation has ceased. Because the system is able to recover its original fluorescence value, we conclude that the released nucleic acids are not degraded by local heating effects. Furthermore, the consistent response of the oligonucleotide-modified nanoprisms through four non-equilibrium cycles of irradiation and darkness implies an extraordinary stability against changes in electrostatic surface charge and photothermally-induced temperature gradients (Figure 4b). In addition, any cumulative damage to the photothermally dehybridized oligonucleotides is undetectable after these four cycles, since approximately the same fluorescence values are reached at every peak and

valley. The released oligonucleotides are therefore able to bind and participate in hybridization repeatedly, demonstrating significant functional persistence over time, and the ability to sequester a released nucleic acid in a reversible manner.

We report the first example of combining light-controlled, SPR-mediated, and fully reversible release of nucleic acids from triangular gold nanoprisms. Under continuous-wave laser irradiation, these conjugates are morphologically, chemically, and functionally stable. Not only can they be used to release a nucleic acid payload, the stability of these conjugates allows sequestration of a biochemical cargo all under spatiotemporal control. Furthermore, these light-controlled processes can be repeated four times in a single experiment without conjugate degradation. Oligonucleotide-modified gold nanoprisms can be manipulated in the near-infrared region making them relevant structures for study in biological systems as well as ideal tools for the analysis of photothermally generated temperature gradients, controllable gene regulation agents, intracellular drug delivery vectors, and photocatalytic nanostructures.

Experimental Section

Preparation of Oligonucleotide-Modified Au Nanoprism Conjugates: Synthesis and functionalization of Au nanoprism conjugates followed literature methods.^[22,23] Briefly, nanoprisms were synthesized using the seed-mediated growth method, and were allowed to separate from spherical nanoparticles that form concomitantly by settling in a conical centrifuge tube overnight. After purification of the Au nanoprisms, thiolated oligonucleotides (Sequence: 5'-TTATGACATTTCCTA A₁₀ SH-3', Cy5.5-labeled sequence: 5'-TTATGACATTTCCTAA₁₀-Cy5.5-SH-3') were treated with dithiothreitol (100 mM) in disulfide cleaving buffer (170 mM phosphate buffer, pH 8.0) for 1 hour and purified with a desalting column. Approximately 1.2 OD₂₆₀ (3.93 μ M) of the purified thiolated oligonucleotide and 1.2 OD₂₆₀ (6.55 μ M) of the fluorophore-labeled complementary oligonucleotide (Sequence: 5'-6-FAM-TAGGAAATGTCATAA-3') were added to Au nanoprisms that had been centrifuged (3 min at 8000 RPM) twice to remove excess cetyltrimethylammonium bromide.

After allowing this mixture to react for 1 hour, the solution was slowly brought to 150 mM NaCl and 10 mM phosphates in the presence of surfactant (0.01% sodium dodecyl sulfate) over the course of several hours.^[17,18] After allowing functionalization to occur overnight, oligonucleotide-modified Au nanoprism solutions were purified of excess oligonucleotides by centrifugation three times (3 min at 8000 RPM) and resuspension in physiological salt buffer (150 mM NaCl, 10 mM phosphates, and 0.01% sodium dodecyl sulfate). Oligonucleotide-modified nanoprisms were then diluted to 0.5 OD₁₂₀₀, which corresponds to a concentration of ~45 pM, and aliquoted for future experiments. All oligonucleotides were synthesized in-house with phosphoramidites purchased from Glen Research Corp. and all buffer reagents were purchased from Sigma-Aldrich Inc.

Simultaneous SPR Excitation and Measurement of Fluorescence Intensity: A 500 mW 1064 nm continuous-wave diode pumped solid-state Nd:YVO₄ laser (Newport Corp., Excelsior Scientific class) was coupled to a fluorescence spectrometer (Horiba Jobin Yvon Inc., Fluorolog-3). A pyrex dielectric mirror (Newport Corp., wavelength range: 1030–1090 nm) was used to reflect and align the beam through the sample chamber. Measurement of the laser power using a power detector (Newport Corp., 818P series) confirmed a high reflectivity from the mirror with a loss of at most 5% of the laser power.

In all fluorescence experiments, samples were allowed several hours inside the instrument to reach thermal equilibrium so as to ensure that changes in fluorescence were due to photothermally generated heat.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic illustration of the laser-coupled fluorescence spectrometer for the SPR excitation of oligonucleotide-modified Au nanoprisms and simultaneous fluorescence detection.

Figure 2.

a) Extinction spectra of oligonucleotide-modified Au nanoprisms and spherical nanoparticles. The vertical (-----) illustrates 1064 nm laser for comparison. b) Spectra of fluorescence intensity as a function of time for a solution of oligonucleotide-modified Au nanoprisms and spherical nanoparticles that have both been hybridized to fluorescein-labeled complements. The arrow denotes the onset of 1064 nm laser irradiation of the solution.

Figure 3.

a) Extinction spectra of oligonucleotide-modified Au nanoprisms before and after 9 h of 1064 nm laser irradiation. The inset shows representative TEM images before and after irradiation. Scale bars represent 50 nm. b) Fluorescence intensity of nanoprisms functionalized with a Cy5.5-labeled thiolated oligonucleotide during 1064 nm laser irradiation. The horizontal (-----) denotes the fluorescence value after oxidation of the nanoprisms using KCN.

Figure 4.

a) Equilibrium fluorescence intensity of oligonucleotide-modified Au nanoprisms with the irradiation of the 1064 nm laser shown (-----). b) Non-equilibrium fluorescence intensity of oligonucleotide-modified Au nanoprisms with the cyclic irradiation of the 1064 nm laser shown (-----).

Scheme 1.
SPR-mediated heating of gold nanostructures results in the release of oligonucleotides.