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Molecular catch and release: Controlled delivery using optical trapping with light-responsive liposomes

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Abstract

Gold-coated liposomes are maneuvered using an optical trap to achieve precise delivery of encapsulated molecular cargo. Movement and payload release from these plasmon resonant nanocapsules are independently controlled using a pulsed trapping beam. This technology enables *in vitro* delivery of a payload to a selected cell and may be applied to the interrogation of individual cells within their biological microenvironment.

Keywords

controlled release; nanoparticle; plasmon resonance; optical trapping; liposome

Nanoscale handling of molecular cargo offers the ability to examine binding interactions, chemical reactions, and signaling cascades, and may be applied to study cellular networks and disease development and progression.^[1–11] We previously introduced gold-coated liposomes, composite structures created via the *in situ* reduction of ionic gold onto the surface of 100 nm diameter thermosensitive liposomes.^[12] Their optical plasmon resonances, similar to that of solid gold shells, have unique tunability in the near-infrared range. Gold-coated liposomes are capable of light-induced release when illuminated with laser light of a wavelength corresponding to their plasmon resonance.^[13, 14] In this design, the thermosensitive liposome allows for the encapsulation of substances, and the plasmon resonant structure facilitates release of content through photothermal conversion upon on-resonant illumination.

High polarizability, characteristic of plasmon resonant structures, produces significant gradient forces acting upon gold nanoparticles in a focused beam of light,^[15] resulting in stable and efficient optical trapping of solid gold nanoparticles in a broad range of sizes, 18 to 254 nm.^[16–18] However, the dramatic heating of gold nanoparticles, as high as 266 K W^{−1} for particles 50 nm in diameter, has hampered the use of optical trapping in temperature sensitive assays, including various methods for the interrogation of biological systems.^[18–21] Here we report precise manipulation of gold-coated liposomes using the

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optical trapping technique. Tunability of materials properties in combination with a modulated trapping beam enables regulation of particle heating, and subsequently allows for unprecedented control of the movement and content release from these capsules.

Preparation of gold-coated liposomes (Experimental section provided in Supporting Information) yields plasmon resonance at 770 nm (Figure 1). In a previously introduced approximation,^[12] the gold coating is represented as an imaginary shell surrounding the liposome and partially filled with gold (Figure 1a). Extinction, scattering, and absorption efficiencies, Q_{ext} , Q_{sca} and Q_{abs} , respectively, can be calculated in the manner explained by the Mie theory combined with the Maxwell Garnet treatment of effective medium (Experimental), whereby varying the fill factor f in this shell-like structure produces optical resonances at different wavelengths. For example, Q_{abs} calculated for a liposome core of radius $R = 50$ nm and coated with a composite gold shell of thickness $d = 2$ nm (compare experimental data in Figures S1 and S2) and $f = 0.86$ reaches a maximum of $Q_{\text{abs}} = 2.628$ at a wavelength of 770 nm, corresponding to the maximum of the experimentally acquired extinction spectrum (Figure 1b). Similarly, polarizability of gold-coated liposomes depends on the dielectric constant of the composite shell and can be tuned by varying the amount of gold within this composite shell. Calculated polarizability of the described gold shell ($R = 50$ nm, $d = 2$ nm, $f = 0.86$) is plotted in Figure 1b (see Experimental for details). At 1064 nm, polarizability of the gold shell is $1.957 \times 10^{-17} \text{ cm}^3$, whereas polarizability of the PEG-coated liposome used as a template is approximately $1.769 \times 10^{-16} \text{ cm}^3$, indicating an order of magnitude enhancement in the gradient force acting upon the gold-coated liposome in comparison to uncoated liposome.

To establish conditions for selective trapping without release and for on-demand release from gold-coated liposomes, we introduced a pulsed trapping beam, where the frequency and duty cycle of modulation are controlled to maintain the trap or to initiate content release (Figure 1c and Experimental). Content release from these gold-coated liposomes is activated when the temperature of the liposome exceeds 41 °C, the main phase transition temperature of the major lipid component, DPPC.^[13] To analyze the temperature of a gold-coated liposome exposed to different laser modulation schemes, the heat equation was numerically solved for radial coordinate r (Experimental). This model comprised a central gold-coated liposome surrounded by PBS, with a system boundary set at $r = 10 \text{ }\mu\text{m}$. The thermal properties of gold, lipid, and water are listed in Table S1 and the absorption cross section of the gold coating was estimated as $4\pi \times (R+d)^2 \times Q_{\text{abs}}$, where $Q_{\text{abs}} = 1.272$ at 1064 nm. The temperature changes produced by 100 ns square pulses of laser light were analyzed at varying frequencies and power densities. As hydrophilic molecules require time spans on the order of milliseconds to transverse the liposomal membrane, transient temperature spikes associated with individual 100 ns pulses of light will not contribute to content release. Rather, by modulating the frequency of laser pulses, the cumulative baseline temperature of the gold-coated liposome can be controlled to affect content release. The baseline temperatures associated with different pulsing schemes were modeled over 2.25 second long durations using a computational model that employed continuous laser powers yielding baseline temperature trends equivalent to that of pulsed regimens (Experimental). As shown in Figure 2a, pulsing at 1 MHz yields a steady-state baseline temperature around 31 °C,

which may enable optical trapping of liposomes without content release; 2 MHz pulsing reaches a steady-state temperature of approximately 43 °C, thus enabling content release. Figure 2b shows the steady-state temperature distribution as a function of distance from the center of an optically trapped gold-coated liposome. Temperature changes are greatest over the volume of the liposome, and rapidly taper off to about half the peak change a distance of 58 nm away from the liposome surface.

Experimental realization of laser beam modulation requirements was achieved by placing an electro-optical modulator in the path of the trapping beam (Figure 1c). Beam pulsing controls the temperature of the particle, but it may be detrimental to stable trapping. This consideration is similar to that of beam sharing in multiple trap arrangements. The beam must be applied frequently enough so that the particle does not diffuse a significant distance from the trap. The diffusion coefficient of a 100 nm diameter liposome in water can be estimated as $D = 4 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ and the rms diffusion length l over time t is given by $l = (2Dt)^{1/2}$. Maintaining a liposome's diffusion length to within 10 nm between pulses, resulting in negligible changes to the trap stiffness and characteristic escape time, requires that the time between pulses not exceed 12.5 μs . Using DIC microscopy, we recorded particle movement and escape at decreasing frequencies, and demonstrated that gold-coated liposomes were stably trapped and moved using 100 ns laser pulses at a 1 MHz (Movie S1 in Supporting Information). An example of formation of user-defined motion patterns pattern using gold-coated liposomes is shown by DIC in Figure S3 and accompanying Movie S2. Fluorescence images of user-defined movement shown in Figure S4 and accompanying Movie S3 demonstrate stable encapsulation of the fluorescent dye over a 146 s duration.

To examine selective control of trapping and content release, gold-coated liposomes encapsulating Alexa Fluor 647 were prepared (Experimental). Individual liposome position and leakage of content were monitored via Alexa 647 fluorescence using a highly sensitive EMCCD. The dye was loaded at a relatively low concentration, approximately 12 dye molecules per liposome, to prevent fluorescence self-quenching. A single liposome was trapped using a train of 100 ns 1064 nm laser pulses at 1 MHz, with the laser operating at 12 mW average power. The localized fluorescence signal indicates that this laser pulsing scheme does not result in content release (Figures 2c1-2c3), which is in agreement with heat diffusion analysis (Figure 2a). As shown in Figure S5 the encapsulated content of gold-coated liposomes placed in the stationary trap is retained for durations of at least 30 seconds. In contrast, a 2 MHz train of 100 ns pulses with a 20 mW average power leads to rapid content release, with a concomitant loss of fluorescence signal, within 2 seconds (Figure 2c4-2c5). Uncoated liposomes maintain their fluorescent payload, even after 10 sec of exposure to the 2 MHz pulse regimen, illustrating that the gold-coating is required for this on-demand release functionality (Figure 2c6-2c8).

To demonstrate optical trapping and content release in a functional cellular system, we encapsulated TO-PRO-3 iodide, a cell impermeant nucleic acid stain, within liposomes. The liposomes were gold coated to produce plasmon resonance around 770 nm. We incubated these TO-PRO-3-loaded gold-coated liposomes with HEK293 cells held in a cell chamber set on the microscope stage, and activated the pulsed laser trap to propel liposomes into the

interior of a selected cell within the field of view (Figure 3a). The trap focus was set within the volume of the selected cell (highlighted in blue in Figure 3h and i) and the trapping laser was modulated to provide 500 ns pulses at a 500 kHz frequency, with an average power of 58.3 mW (Experimental). Within 1 minute of trap exposure, we observed intense fluorescence associated with nuclear binding of TO-PRO-3, indicating the selected cell was successfully loaded with the nuclear stain (Figures 3d and h). Gold deposition visualized by DIC indicates that the gold-coated liposomes, or their components, entered the cells (Figure S6). However, under the conditions of this cell assay, we could not temporally separate translocation and content release from liposomes. In contrast, the use of uncoated TO-PRO-3-loaded liposomes did not provide evidence for the release of the nucleic acid stain into the cell (Figures 3e and i). Calcein AM was introduced to test HEK293 cell viability after the optical loading procedure (Experimental). The strong and uniform calcein fluorescence co-localized with cells indicated the normal esterase activity of cells (Figure 3f and g).

We have demonstrated independent control of particle movement and content release using an optical trap by regulating the interactions of laser light with plasmon resonant gold-coated liposomes. Beam pulsing allows for precise control over the temperature of trapped gold-coated liposomes and subsequently payload release. Using both computational and experimental models, we have demonstrated that the pulsed optical trap producing 100 ns pulses at 1 MHz and 12 mW average power can maintain the trapping and movement of a gold-coated liposome without loss of content; rapid content release is then elicited by increasing pulse frequency to 2 MHz and 20 mW average power. This precise spatial and temporal control of gold-coated liposome function may enable localized release of selected agents with sub-cellular resolution. We further demonstrate the use of this adjustable optical trapping system for the delivery and release of a nuclear stain into the interior of a single cell, while maintaining cell viability. The ability to manipulate the location and timing of a content release may advance a wide variety of cellular studies requiring the precise release of select compounds, including examination of signal propagation in cellular networks, in a manner similar to the use of photouncaging of compounds in neurophysiology.^[22–24] The optical trapping and release method described here improves on photouncaging, as it does not require chemical modification of compounds, allows for release of many signaling molecules, and uses biologically safe NIR light. This delivery method is distinctly different from photoporation, which traditionally uses high-energy femtosecond laser pulses and relies on diffusion of content from the surrounding medium and into photoporated cells.^[25] In contrast to molecularly targeted agents,^[26–28] the performance of optical trap-based manipulation does not rely on the expression patterns of molecular markers or biological processes for delivery. Also, while microinjection techniques are capable of precise timing and dosage of a delivered material, microinjection requires disruption of the cellular membrane, which can lead to unviable cells, and is limited in the number of cells that may be simultaneously treated.^[29] Our interest in this platform technology stems from its potential to interrogate cellular activity within a biological environment, such as 3-dimensional models of communication in the cancer microenvironment. The spatial and temporal control of signaling events can be used to decipher the physiological events that

promote and prevent cancer development and progression in order to develop more effective diagnostic and treatment methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) The schematic representation of gold-coated liposomes used in the Mie Maxell Garnett calculations. In this model, gold reduced onto the surface of liposomes forms discrete spherical gold clusters of diameter d (shown in red) that partially fill an imaginary shell of radius R (grey). (b) Experimentally collected extinction spectra of uncoated (grey) and gold-coated (red) liposomes and modeled absorption efficiency (blue) and polarizability (green) of gold-coated liposomes. (c) Layout for optical trapping of gold-coated liposomes. The continuous wave 1064 nm beam is modulated by a Pockels cell (light modulator). That pulsed light is then expanded to slightly overfill the back aperture of the objective. After the beam expansion lenses, the beam passes through beam steering lenses, the first of which is controlled in the xyz directions via piezoelectric motorized actuators. The beam is then directed through a 60x 1.42 NA objective and to the sample by a VIS/IR dichroic mirror. The sample is monitored using a back-thinned EMCCD camera. Additional details available in Experimental Section of Supporting Information

Figure 2.

Selective trapping of and content release from gold-coated liposomes. (a) Computational analysis of the baseline temperature as a function of time at the center of a liposome-supported gold composite shell (distance = 0 in panel b) trapped using 100 ns laser pulses at a frequency of 1 MHz (long dash) or 2 MHz (short dash). (b) Computationally modeled steady-state baseline temperature as a function of distance from the center of the trapped gold-coated liposome using 100 ns laser pulses at a frequency of 1 MHz (dark grey) or 2 MHz (light grey). This data represents time = 2 s from data presented in panel a. In panels a and b, the transition temperature for DPPC liposomes is marked with a dotted line. (c) Gold-coated and uncoated liposomes trapped using 100 ns pulses at 1 MHz or 2 MHz frequencies, tracked via encapsulated fluorescent Alexa Fluor 647 dye. (c1-c3) Gold-coated liposomes trapped at a 1 MHz frequency retain their fluorescent content. (c4 and c5) Faster pulsing at 2 MHz leads to release of content within 2 seconds. (c6-c8) Uncoated liposomes retain their content, even at the faster 2 MHz pulsing frequency. Scale bar represents 1 μm in c1-c8.

Figure 3.

Optical insertion of gold-coated liposomes into cells. (a) Gold-coated liposomes encapsulating TO-PRO-3 diffuse into the path of the trap and are propelled to the interior of cells. (b-i) Cellular responses to optical manipulation of gold-coated (b,d,f,h) and uncoated (c,e,g,i) liposomes. Both uncoated and gold-coated liposomes were loaded with TO-PRO-3 iodide, a cell impermeant nucleic acid stain. Uncoated and gold-coated liposomes were incubated with HEK293 cells (shown in DIC, b and c) and exposed to optical trap radiation. The optical trap was operated to provide 500 ns pulses at a frequency of 500 kHz, with an

average power of 58.3 mW. Following 1 minute of trap exposure, TO-PRO-3 fluorescence was measured (d and e) and calcein AM was introduced to evaluate cell viability (f and g). Composite images of cells in DIC, TO-PRO-3 fluorescence, and calcein fluorescence for uncoated and gold-coated liposomes are shown in h and i, respectively. Cells exposed to the trap are marked with blue boxes. Fluorescence from TO-PRO-3 is indicated by red color and fluorescence from calcein is indicated by green color. Colocalization of TO-PRO-3 and calcein AM is marked by an orange/yellow color. Scale bar represents 20 μm in images b-i.