成果報告

發展三合一癌症治療平台：結合 siRNA 與裝載抗癌藥物之中孔洞二氧化矽/金奈米棒之開發

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Mesoporous silica structure has shown its feasibility and been widely used as versatile solid support to construct hybrid materials for drug delivery. The construction of stimuli-responsive controlled release carriers is of crucial importance owing to its applicability in the area of 'on-demand' drug delivery. The stimuli-responsive drug reservoirs can effectively respond to external triggers by changing their physical or chemical properties in order to regulate the movement of the pharmaceutical cargos. To date, different kinds of capping agents, such as nanoparticle, cyclodextrin, dendrimer, collagen, and supramolecular assembly, have been designed to prevent guest cargos from leaving pores of mesoporous silica structure. The release stimuli can be associated with the changes in the light, temperature, pH, redox activation, enzymatic activity, and electrostatic interaction.

Light is an external stimulus and provides a great benefit to release a drug at a specific time and desired area at will. However, the reported photo-responsive controlled release motifs in mesoporous silica structure were mostly designed based on UV excitation. A few cases have been developed to use visible light-driven (~450 nm) gated ensembles. In order to toward practical drug delivery systems, a benign near-infrared (NIR) light, in which both blood and soft tissues transmission is optimal due to low energy absorption, is preferred to give maximum penetration. Recently, a two-photon NIR photo-activated mesoporous silica structure was created to initiate light-regulated drug release using a femtosecond Ti:sapphire laser (800 nm) to photocleave an o-nitrobenzyl ester moiety.

Additionally, the utility of the biomolecules as bio-gate remains in burgeoning stage. As compared to inorganic materials and organic molecules as gatekeepers, biomolecules provide a basis giving biocompatibility with minima toxicity to surrounding normal tissue, better cellular uptake to efficient intracellular drug release, and possible additional therapeutic approach. Based on heating to denature duplex DNA leading to uncapping of pores to release guest molecules, DNA-capped mesoporous silica structures were recently proposed to serve as controlled-release platforms. However, they remained at proof of principle level. It requires to further design a nanocontainer to adopt oligonucleotides as a nano-gate that can respond at cell level.

Herein, we have taken advantage of Au nanorods (NRs) character to absorb NIR light to buildup a NIR-responsive oligonucleotide-gated Au NRs@mSiO₂ (mesoporous silica shell) for intracellular drug delivery. Contrary to use of femtosecond laser, a continuous-wave diode laser (808 nm), with advantages of less expense and easy to handle, was employed to irradiate Au NR@mSiO₂ hybrid nanocontainers with double-stranded oligonucleotides tethered to the openings of silica mesopores. The remotely NIR-triggered intracellular drug release was achieved by means of the photothermal conversion of the Au NR@mSiO₂ under NIR diode laser exposure. The dehybridization of the DNA duplexes allowed anti-cancer drugs escape from mesopores into cancer cells. Different from other gating agents, such as inorganic nanoparticles, organic and polymeric molecules, oligonucleotide bio-gate provides additional important advantage for gene delivery. In this regard, we have also demonstrated using siRNAs attaching to the pore openings. Upon NIR irradiation, the released GFP-interfered siRNAs effectively silenced GFP expression in GFP expressing human epithelial carcinoma HeLa cells. Both Dox drug and GFP-interfered siRNA can be released in response to a sequential laser ON-OFF manner.
中文摘要:
本研究主要是發展一種可以近紅外光調控的金奈米棒複合藥劑進行癌症病灶治療，此金奈米藥劑的表面包覆一層具有孔洞的二氧化矽，產生的孔洞可裝填化療藥物，但為了確保藥物分子不會在運送過程中溢漏(為避免副作用產生)，故在孔洞表面修飾雙股 DNA 將洞口封住，此舉可以確保材料在未到達目的地的期間，藥物分子不會一直洩漏至外部。當利用近紅光波長照射此金奈米棒複合藥劑時，金奈米棒會吸收近紅外光並將光轉換成熱而將雙股 DNA 蓋子打開進而將藥物分子釋放出去並發揮其治療作用。使用 DNA 當蓋子目的是我們也可以將 DNA 蓋子改為「小干擾 RNA (small interfering RNA ; siRNA) 蓋子」使此治療藥劑也具有基因治療效用。以近紅外光來控制藥物釋放的優點是因為近紅外光是所謂的「生物之窗(biological window)」，可輕易穿透皮膚進行深層組織的治療，故可以針對較深層的組織進行治療。
前言及研究目的：

The stimuli-responsive controlled-release carriers are highly desirable in the clinical medicine when concerning the therapeutic efficacy and minimized adverse effect of cytotoxic drug. The stimuli-responsive drug reservoirs can effectively respond to external triggers by changing their physical or chemical properties in order to regulate the movement of the pharmaceutical cargos. Among the drug delivery systems, mesoporous silica nanoparticles (MSNs) have successfully shown their promising feasibility in the area of drug delivery because of the porous structure with high surface area, tunable size and biocompatibility. In particular, the readily functionalized surface has allowed researchers to design various gatekeepers, such as nanoparticle,\(^1\) cyclodextrin,\(^2\) dendrimer,\(^3\) collagen,\(^4\) and supramolecular assembly\(^5\) capping the pores in association with different stimuli using light,\(^1b,2c,3,5b-c,6\) temperature,\(^7\) pH,\(^1c-d,2a,5a\) redox activation,\(^1a,4\) enzymatic activity,\(^2b\) and electrostatic interaction\(^8\) to uncap pores.

Light is an external stimulus and provides a great benefit to release a drug at desired time and area, which is considered crucial to boosting drug efficacy in cancer treatment while minimizing side effects. The reported photo-responsive controlled release motifs in MSNs were mostly designed based on UV excitation involving an elegant cis-trans structural transformation in azobenzene-based derivatives,\(^6\) in cooperation with a photolabile linker \(\alpha\)-nitrobenzyl ester moiety,\(^1c,2c\) or supramolecular assembly in a pseudorotaxane system.\(^5b\) A few supramolecular ensembles have been designed to use visible light-driven (~450 nm) release of the entrapped guest molecules.\(^3,5c\) However, further step toward practical drug delivery systems prefer a benign near-infrared (NIR) light, in which both blood and soft tissues transmission is optimal due to low energy absorption, providing maximum penetration. Recently, Bao and co-workers constructed a two-photon NIR photo-activated MSNs consisting of a coumarin chromophore with large two-photon absorption cross section and a photocleavable \(\alpha\)-nitrobenzyl ester moiety. This enabled a femtosecond Ti:sapphire (800 nm) laser to initiate light-regulated drug release.\(^9\) Considering the importance of NIR-stimulus in biological applications, construction of NIR-driven gated nanocontainers is still in incipient area and strongly demanded.

Although the capping agents have been constructed as nanogates, the utility of the biomolecules as valves is still in burgeoning stage. As compared to inorganic materials and organic molecules as gatekeepers, biomolecules provide biocompatibility to surrounding normal tissue, better cellular uptake that leads to efficient intracellular drug release, and possible additional therapeutic approach. Recently, three groups Qu et al.,\(^10a\) Bein et al.,\(^10b\) and Martinez-Máñez et al.\(^10c\) proposed oligonucleotide-capped MSNs as the controlled-release platforms at proof of principle level. The double-stranded DNA duplexes were anchored to the pore mouths of MSNs to trap cargos inside the mesopores. Upon heating (\(\geqslant 45\) °C) MSNs colloids, the duplex DNA unwind, eventually uncapping of pores to release guest molecules.\(^10a-b\) However, practically still it is a challenge to adopt oligonucleotides as a nano-gate that can respond at cell level.

Au nanorods (NRs) with strong surface plasmon resonance are highly absorbent of light in the NIR region and can act as nanoheater by absorbing the NIR laser.\(^11\) Herein, we started with Au NRs as a template to buildup mesoporous silica (mSiO\(_2\)) shell. The double-stranded oligonucleotides (dsDNA) were tethered to mesopore pore openings to encapsulate anticancer Doxorubicin (Dox) drug. Contrary to use of femtosecond laser, a continuous-wave diode laser (808 nm), with advantages of less expense and easy to handle, was employed to irradiate Au NR@mSiO\(_2\) (AuMS) hybrid nanocontainers. The remotely NIR-triggered intracellular drug release was achieved by means of the photothermal conversion of the Au NRs, which induced dehybridization of the DNA duplexes. Different from other gating agents, such as inorganic
nanoparticles, organic and polymeric molecules, the oligonucleotide bio-gate can be replaced with functional nucleic acids to provide additional gene delivery. In this regard, we have demonstrated using siRNAs attaching to the openings of pores. Upon NIR irradiation, the released GFP-interfered siRNAs silenced GFP expression in GFP expressing HeLa cells (human epithelial carcinoma cell line, HeLa-GFP). Both Dox drug and GFP-interfered siRNA can be released in response to a sequential laser ON-OFF manner. Scheme 1 illustrates the design of the Au NRs with oligonucleotides-capped silica shell and the experimental performance.

**Scheme 1.** Schematic illustration of Au NRs with oligonucleotides-capped silica shell and the corresponding NIR light-controlled intracellular drug and siRNA release.
In a typical preparation, Au NRs (Figure 1a) were prepared using seedless growth method, developed by Murphy et al., with slight modification. The silica shell was built up in a reaction containing Au NRs, cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS) and NaOH solution. Subsequently, ethanol was used to wash resulting products to remove CTAB surfactant (Figure 1b). In TEM, the dark contrast Au nanorods were clearly encapsulated by the silica shell showing light contrast. In order to covalently immobilize oligonucleotide strands onto the silica surface, (3-aminopropyl)triethoxysilane (APTES) was added to react with the silanol groups of the mesoporous silica and tethered to the silica surface (Figure 1c). It should be mentioned that APTES molecules were able to access the interior surface of the pore channels as well. The 4-maleimidobutyric acid N-succinimidyl ester (GMBS) was then applied as linker to conjugate amine group of APTES and thiol group of thiolated duplex oligonucleotides.

Figure 1. TEM images of (a) Au NRs, (b) AuMS, (c) APTES treated AuMS, (d) AuMS-dsDNA, and (e) UV-vis spectrum of AuMS-dsDNA. All scale bars are 40 nm.

Prior to grafting duplex oligonucleotides to silica surface, single strand oligonucleotides of (SH-5’-(CH2)6-TTTTTCCCGC CGGG) were pre-hybridized with complementary oligonucleotides (5’-TTTTTCCGCGCGGG) in 4 °C PBS buffer. The quantification of the immobilized duplex oligonucleotides was performed with fluorescence-based method using FAM-labeled oligonucleotides (FAM: 6-carboxy-fluorescence). The self-complementary duplex oligonucleotides ((SH-5’-(CH2)6-TTTTTCCCGC CGGG and FAM-5’-TTTTTCCGCGCGGG) were anchored on silica surface. The amount of immobilization was measured from the decrease of fluorescence intensity of FAM-labeled oligonucleotides left in supernatants and calculated based on a calibration curve according to FAM-labeled oligonucleotide concentration. It was estimated that ~955 dsDNA on single AuMS. The final double-stranded oligonucleotide-modified AuMS (AuMS-dsDNA) showed the NRs with ~41 nm in length and ~11 nm in width covered by ~16 nm thick SiO2 shell (Figure 1d) dispersed in PBS buffer solution (pH 7.4). Figure 1e shows the the UV-vis absorption of AuMS-dsDNA colloids exhibiting two typical Au plasmon
resonances in transverse band (~520 nm) and longitudinal band (~800 nm) as well as an additional peak at 260 nm attributed from oligonucleotides absorbance. This provides an evidence for the presence of oligonucleotides on the AuMS surface.

The silica shell was characterized using N₂-sorption isotherm and BJH analysis to determine surface area and pore size distribution for a series of AuMS products (Figure S1). Initially, the as-prepared AuMS showed the specific surface area of ~70.3 m² g⁻¹ in the absence of clear porosity. Once the CTAB surfactants were removed following ethanol wash, the surface area significantly increased to ~422 m² g⁻¹ and a peak appeared at ~2.4 nm average pore diameter. Following modification with APTES, the surface area decreased to ~143 m² g⁻¹ with the similar pore diameter distribution. Although some surface was lost due to APTES conjugation, appreciable porosity was still available for drug loading. The characteristic synchrotron small angle X-ray diffraction signals were collected for the mesoporous evidence from AuMS and DOX-loaded AuMS (DOX-AuMS) samples (Figure S2), where AuMS has been treated with ethanol to remove CTAB. The ordering of mesoporous of AuMS appeared at 20 near 1.2 degree (corresponding to 2.2 nm using Cu kα source), d-spacing ~5.38 nm (X-ray wavelength: 1.1271Å). The signal decreased after DOX loaded into AuMS giving less ordering of the mesoporous structure. FT-IR analysis provided evidence for APTES and GMBS modification (Figure S3). Surface charge measurements presented an additional evidence for a series of Au NRs in different stages, i.e. 24.5 mV (Au NRs), -19.6 mV (AuMS), 29.9 mV (APTES treatment), 0.1 mV (GMBS treatment), and -8.3 mV (dsDNA treatment).

![Figure S1](image-url)

**Figure S1.** a) N₂ adsorption-desorption and b) pore-size distributions of AuMS (before ethanol wash), AuMS (after ethanol wash) and APTES-modified AuMS.
Figure S2. Small-angle X-ray diffraction (SAXD) pattern of AuMS and Dox-AuMS. The arrow indicates the diffraction signal from ordering mesoporous silica. The insert shows wide-angle X-ray diffraction (WAXD) data with Au NRs diffraction peaks indexed.

Figure S3. FT-IR spectra of AuMS (after ethanol wash), APTES-modified AuMS, and GMBS-modified AuMS colloids. As evidenced by IR measurements, a band at 1660 cm\(^{-1}\) was assigned to O-H species of silica. After treatment with APTES, a band associated with N-H species of amine appeared at 1565 cm\(^{-1}\). When conjugation with GMBS, a new band exhibited at 1705 cm\(^{-1}\) which is assigned to C=O species from maleimide of GMBS.

To perform NIR light-controlled intracellular drug release, we have selected anti-cancer Doxorubicin (Dox) as a guest drug. The Dox was mixed with GMBS-modified AuMS, followed by conjugation of thiolated duplex oligonucleotides in PBS buffer solution, as described in Supporting Information. The excess Dox was removed by centrifugation and repeated wash for at least three times. The encapsulated amount of Dox was determined to be \(\sim 2.2 \times 10^4\) molecules per Au NR, which was conducted from the difference in
fluorescence intensity of Dox between the initial amount and residue in supernatants and calculated based on a calibration curve according to Dox concentration. Both AuMS-dsDNA and Dox-loaded AuMS-dsDNA (Dox-AuMS-dsDNA) colloids were investigated for stability in dark under PBS buffer at 37 °C (Figure S4). The FAM-labeled oligonucleotides were used to monitor the stability and showed a stable hybridization with only ~2.7% liberated oligonucleotides after 72h. The capping efficiency was scaled with the Dox release profile. 10 % Dox leached in the initial 10 hours, thereafter no more release was noticed. The initial loss can be attributed to some loosening net structure originated from end-capping dsDNA. In comparison, encapsulated Dox was nearly (~99%) washed out after repeated wash four times for the Dox-loaded samples in the absence of end-capping dsDNA. The present AuMS-dsDNA still provides effective retention of guest molecule.

Figure S4. (a) Time-dependent stability of oligonucleotides in AuMS-dsDNA-FAM colloids. A 50 ppm (Au ion concentration) of AuMS-dsDNA-FAM colloids were distributed in PBS (10 mM, pH 7.4) at 37°C and the cumulative release of FAM-labeled oligonucleotides was monitored over time. (b) Time-dependent stability of Dox in Dox-AuMS-dsDNA colloids. A 50 ppm (Au ion concentration) of Dox-AuMS-dsDNA colloids was distributed in PBS (10 mM, pH 7.4) at 37°C and the cumulative release of Dox was monitored over time. All data were repeated triplicate.

Prior to examining NIR light stimulus to trigger drug release, the efficiency of photo-induced temperature increase of the solution from the Dox-AuMS-dsDNA colloids was investigated under different laser power as a function of irradiation time using a continuous-wave (CW) NIR diode laser (808 nm) (Figure S5). A thermocouple was used to measured the bulk solution temperature. The solution temperature was gradually raised as irradiation prolonged. Au NR has been known a highly absorbent of NIR light for photothermolysis in cancer cells. Herein, our aim is to evaluate therapeutic efficiency of intracellular drug release from a NIR light-responsive Au NRs. Hence to minimize the chance of apoptosis by hyperthermia effect at temperature

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45 °C, a laser power of 1.5 W/cm² was chosen, that will increase temperature not more than 42 °C. The denaturing temperature (Tm) of the present duplex oligonucleotides is approximately 47 °C. The heat distribution upon NIR laser irradiation is expected to be highly localized around the Au NRs, which enables to denature duplex oligonucleotides, and decayed exponentially within a few nanometers.\(^\text{13}\) The release profiles of FAM-labeled oligonucleotides and Dox were conducted in AuMS-dsDNA and Dox-AuMS-dsDNA colloids, respectively (Figure S6). The colloidal samples were all freshly prepared, and adjusted to 50 ppm of Au ion concentration and subjected to NIR laser (808 nm) irradiation. A pulsatile release mode was obtained, which demonstrated precise control of cargo release under light ON. A control experiment was also performed showing no response to CW 633 nm diode laser. The colloids dispersed in PBS solutions were placed in a well of 96-well. The samples were exposed to NIR light for 10 min, and then unilluminated for 5 min for each consecutive ON-OFF process. The irradiation denatured duplex oligonucleotides, opeing the pores, while cargos delivery was inhibited when NIR light was OFF. Approximately 32% Dox was released after three cycles of ON-OFF. It should be noted that laser beam has a limited spot area of 0.15 cm², which covered only 40% area of a well of a 96-well plate. The release percentage was estimated relative to the total DOX amount of a 50 ppm of Dox-loaded colloids. Not all of colloids were exposed to NIR irradiation, that influenced evaluation in Dox release, the same proportionally applied to oligonucleotide release in Figure S5a. Although NIR beam cannot completely cover a well of a 96-well plate, the therapeutic efficacy in cancer cells in response to NIR light-triggered drug release was not restricted (\textit{vide infra}).

**Figure S5.** The elevation profiles of the bulk solution temperatures for 50 ppm (Au ion concentration) of Dox-AuMS-dsDNA colloids in PBS (10 mM, pH 7.4) under NIR diode laser irradiation (808 nm) with powers of 1.0 W/cm², 1.2 W/cm², 1.5 W/cm², and 1.9 W/cm². The laser beam spot area was ~0.15 cm².
Figure S6. (a) Oligonucleotides release profile in AuMS-dsDNA-FAM colloids upon NIR diode laser irradiation (808 nm, power: 1.5 W/cm²) and diode laser (633 nm, power: 0.2 W/cm²). In comparison, using a lower 0.2 W/cm² irradiation by a 808 nm of diode laser resulted in oligonucleotides release as well. (b) Dox release profile in Dox-AuMS-dsDNA colloids upon NIR diode laser irradiation (808 nm, power: 1.5 W/cm²). An ON-OFF switching sequence was operated, where the samples were exposed to NIR diode laser for 10 min, and then un-illuminated for 5 min. The samples with 50 ppm (Au ion concentration) were distributed in PBS (10 mM, pH 7.4) in a well of a 96-well plate to experience laser exposure.

Using transmission mode dark field CytoViva microscope, light scattered and fluorescent signals were collected to evaluate negatively charged Dox-AuMS-dsDNA (-8.3 mV) uptake and NIR triggered intracellular drug release in A549 lung cancer cell line (Figure 2). 50 ppm (Au ion concentration) Dox-loaded colloids were incubated with lung cancer cells for 24h at 37 °C. After removal of uninternalized colloids by PBS wash, A549 cells were subjected to NIR light irradiation based on laser ON-OFF sequence, followed by additional 2h incubation. The dark field scattering images display cellular morphology and the cell-uptake of particles exhibits as bright spots (white arrow). The intact cells appeared in the absence of laser exposure. More cells destruction was observed when the ON-OFF steps increased, in particular after the third irradiation. Fluorescence images show Dox release upon NIR light trigger. Larger red area (emitted from Dox) was observed in proportion to the exposure cycle, while no apparent red fluorescence was seen in cells that experienced no laser exposure. Note that the fluorescence of Dox was significantly quenched when Dox was trapped inside AuMS-dsDNA. MTT assay was used to evaluate cell viability. Following the same procedure as those in cell-uptake studies, a 50 ppm of Dox-loaded colloids was incubated with A549 cells in 96-well plates for 24h in the dark, and then were exposed to laser following ON-OFF steps. With or without laser exposure, all cells were cultured for another 24 hours and subjected to MTT measurements. Cell-uptake efficiency quantified by atomic absorption spectrometer, gave ~45% Au NRs, corresponding to 22.5 ppm of Au ion concentration, internalized into cells. As seen in Figure 3, no significant drop in cell viability (> 90%)
for AuMS and Dox-AuMS-dsDNA treatments in the absence of laser irradiation. The free dsDNA have no influence in toxicity. The apparent cell death was observed upon laser irradiation. The cell viability (53%) reached nearly IC$_{50}$ after cells taken 3rd irradiation. Although we have controlled laser power to avoid hyperthermia effect from surrounding, still highly localized temperature generated around Au NRs can induce phothermal damage in cells. To compare this the AuMS colloids (no Dox loaded) were irradiated by laser, which showed cell viability above 90%. These results indicate that duplex oligonucleotides can be dehybridized to allow Dox escape from mesopores to the cells under phoactivation. Based on 45% of Au NRs taken up by cancer cells, we have estimated approximately 2.6 $\mu$M Dox entrapped into AuMS. Assuming complete release, which may not be the case experimentally, 2.6 $\mu$M Dox dropped viability to 80% which is still higher than that (53%) of cells taken 3rd irradiation.

![Figure 2](image-url)  
**Figure 2.** Dark field scattering and fluorescence images of A549 cell groups experienced either nil or NIR diode laser irradiation (1.5 W/cm$^2$). A549 cells were incubated with Dox-AuMS-dsDNA colloids (50 ppm in Au ion concentration) for 24 h in the dark, and then were exposed to laser following an ON-OFF sequence. After further 2h incubation, the images were examined using a 10× objective lens (CytoViva microscope). The insets show the enlarged dark field scattering images of A549 cells and the white arrows indicate the presence of nanoparticles.

![Figure 3](image-url)  
**Figure 3.** Cell viability studies of A549 cells in the presence of free Dox, free dsDNA, AuMS, and Dox-AuMS-dsDNA. A 50 ppm (Au ion concentration) in colloids was used to incubate with A549 cells. Irradiation performance was operated in an ON-OFF sequence using NIR diode 808 nm laser with 1.5 W/cm$^2$. Free dsDNA amount was determined based on the calculation of cell-uptake efficiency in 45%.
Using oligonucleotide bio-gate provides additional advantage for genomic therapy. We used a GFP expressing cell line (human epithelial carcinoma cell line, HeLa-GFP) to report silencing efficiency. The thiol-modified double-strand siRNA with sense strand SH-5’-(CH\textsubscript{2})\textsubscript{6}-GCAGCAGACUUUCUUCAAGTT-3’ and anti-sense strand 5’-CUUGAAGAAGUCGUGCUGCTT-3’ (MDBio, Inc, Taiwan) was conjugated with GMBS modified AuMS. In this regard, we did not encapsulate Dox drug and purely demonstrated GFP knockdown performance. With the identical experimental procedure and parameters in MTT assay, Fluorescence microscope was used to monitor GFP expressing HeLa cells seeded with siRNA-modified AuMS as a function of incubation time after laser irradiation. Figure 4 clearly shows laser-induced knockdown in HeLa-GFP cells. Upon laser irradiation, the liberated siRNA exhibited silencing activity. Quantification of GFP level indicates rapid and effective silencing when using more number of laser ON-OFF steps (Figure 4b). As incubation period prolonged, GFP expression was consistently reduced in all of experimental groups. Note that cells remained alive at 72h. Cells in the absence of laser irradiation exhibited no reduction in GFP expression.

**Figure 4.** (a) GFP expression in HeLa-GFP cells as a function of time. (b) Quantification of the integrated fluorescence intensity corresponding to Fig.4a showing silencing efficiency. A 50 ppm (Au ion concentration) of duplex siRNA-capped AuMS colloids was incubated with HeLa-GFP cells for 24h in the dark, and then was exposed to NIR diode laser (808 nm, 1.5 W/cm\textsuperscript{2}) following an ON-OFF sequence. The images were captured by fluorescence microscope.

In summary, we have prepared NIR light-responsive oligonucleotide-gated ensemble for intracellular drug delivery. Taking advantage of Au NRs character to absorb NIR light, CW NIR diode laser was employed to control cargo release through thermal dehybridization of duplex oligonucleotide. In view of therapeutic potential, this design is capable of siRNA delivery into cells for translational regulation as well as for cytotoxicity through anti-cancer drug delivery. For further downstream in vivo study, we will conduct the non-small cell lung cancer (NSCLC) targeting, using wild-type EGFR A549 and EGFR-mutation PC9 cells with EGFR overexpression in SCID mice. The conjugation of antibody with oligonucleotide will be achieved by anti-EGFR antibody bound with one of single strand oligonucleotides with terminal NH\textsubscript{2} group (5’-NH\textsubscript{2}-TTTTTCCGGCGCGGG), which is hybridized with SH-5’-(CH\textsubscript{2})\textsubscript{6}-TTTTTCCGGCGCGGG) attaching on Au nanoensembles.


