Electronic Supplementary Information

Chelation Competition Induced Polymerization (CCIP):
Construction of Integrated Hollow Polydopamine Nanocontainers
with Tailorable Functionalities

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1. Experimental Section

1.1 Materials: Sodium borohydride (NaBH₄, ≥98.5%), cetyltrimethylammonium bromide (CTAB), gold (III) chloride trihydrate (HAuCl₄·3H₂O, 99.9+%), silver nitrate (AgNO₃, 99%), L-ascorbic acid, 2-Methylimidazole (99%), zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O), dopamine hydrochloride were purchased from Sigma-Aldrich.

1.2 Preparation of Gold Nanorods: Gold nanorods were synthesized using the seed-mediated method. Briefly, the seed solution was prepared by injecting 500 μL fresh NaBH₄ (0.01 M) into 2.5g CTAB solution (0.2 M) mixed with 1g H₂O and 120 μL HAuCl₄·3H₂O (0.015 M) at 25.5 °C under vigorous stirring (1000 rpm) for 120 s. Then the obtained seed solution was kept undisturbed at 25.5 °C for 1 h before use. To prepare growth solution, 10 mL of CTAB (0.1 M) mixed with 500 μL of HAuCl₄·3H₂O (0.015 M), 400 μL of AgNO₃ (0.004 M) and 124 μL of L-ascorbic acid (0.0788 M). Then the seed solution (100 μL) was injected into the growth solution. The resultant solution was kept in water bath at 27 °C overnight without disturbing for Au NRs growth.

1.3 Synthesis of Au NRs@ZIF-8: Before the preparation of Au NRs@ZIF-8 nanostructure, the Au NRs was firstly modified with surfactant PVP³. In brief, 1.0 ml of the obtained Au nanorods were centrifuged under 10000 rpm for 15 min and then dispersed in 1 ml PVP (Mn = 40000, 20 mg/ml) aqueous solution. The mixture was stirring at room temperature for 24 hours. 10 ml PVP-stabilized Au nanorods were collected by centrifugation at 10000 rpm for 15 minutes to remove excess
PVP and finally dispersed in 1 ml methanol.

In the encapsulation procedure, methanol was used as solvent. 10 ml solution of 2-methylimidazole (25 mM) and 10 ml 25 mM Zn(NO_3)_2·6H_2O (containing 1 ml PVP-stabilized Au nanorods methanol solution) were mixed and then allowed to react at room temperature for 1 hour without stirring. The product was collected by centrifugation at 5000 rpm for 10 min, washed several times with methanol, and final dispersed in 3.0 mL methanol.

1.4 Generation of Au NRs@PDA NCTs: 3.0 mL Au NRs@ZIF-8 methanol solution was added into 4.5 ml methanol, mixing with 1.5 ml dopamine solution (20 mM in methanol). The mixture was stirring and refluxing at 60 °C for 7 h, the color of which turned gradually from purple to black. The resultant was collected by centrifugation at 5000 rpm for 10 min, washed with methanol for three times, and freeze-drying overnight.

1.5 Characterization of Photothermal Effect: In order to assess the temperature increment by photothermal conversion, 2 mL of Au NRs@PDA NCTs solution with different concentrations (50, 100, 150, 200 μg/mL) were placed in the cuvette. Each solution was irradiated by an 808 nm NIR laser with the intensity of 2 W/cm² for 10 min, and the temperature increase was measured by a digital thermometer every 30 s.

1.6 Drug Loading and Release Performance of Au NRs@PDA NCTs: In a typical experiment, loading DOX into Au NRs@PDA NCTs was accomplished by mixing DOX hydrochloride together in phosphate buffer (PBS, pH 7.4) with a mass
ratio of 1:2 (DOX: Au NRs@PDA = 1: 2). The mixture was placed in the dark under ambient temperature overnight. After loading, the nanostructure was rinsed by centrifugation (4000 rpm, 10 min) and washed with PBS buffer for several times. All the supernatant was collected and measured with UV-vis spectrum to calculate the loading efficiency. Then the obtained Au NRs@PDA-DOX was stored at 4 °C in the dark for future use.

The release of DOX from Au NRs@PDA-DOX was studied by dialyzing Au NRs@PDA-DOX under room temperature in different buffers with pH of 3.0, 5.4, and 7.4 for 24 h. The released DOX was collected at different time and determined by fluorescence spectrum.

The laser-triggered drug release experiments were performed at different pH of 3.0, 5.4, and 7.4. An optical-fiber-coupled power-tunable diode laser (continuous wave) with wavelengths of 808 nm (maximal power = 10 W) was employed in this work. Au NRs@PDA-DOX nanostructure was dispersed in 10 mL of PBS buffer with different pH (3.0, 5.5, 7.4). The nanocontainers were irradiated by an 808 nm NIR laser (2 W/cm², 5 min for each pulse) with a time interval of 30 min. The released DOX before and after irradiation were collected. For each measurement, 1 mL of solution was centrifuged at 10000 rpm for 5 min. The amount of released DOX in the supernatant was determined using fluorescence spectrum.

1.7 Evaluation of Cytotoxicity and Cells Uptake: To access the cytotoxicity of Au NRs@PDA NCTs, the human oral epithelial carcinoma (KB) cells and Human
Embryonic Kidney 293 (293) cells were cultured in standard cell media, and then incubated with different concentrations of Au NRs@PDA nanostructures for 24 h. The cell viability was measured by using a standard methyl thiazolyltetrazolium (MTT, Sigma Aldrich) assay on 96-well plates. For the MTT assay, KB and 293 cells were seeded at 3000/well until adherent and then incubated with various concentrations of Au NRs@PDA nanostructures for 24 h. The standard MTT assay was carried out to determine the cell viabilities relative to the untreated cells.

Cell uptake and internalization experiments were conducted as following: Firstly, Au NRs@PDA NCTs were modified with Rhodamine B by EDC-NHS method. Then KB cells were incubated with Au NRs@PDA-Rhodamine B for 24 h, rinsed with PBS to remove the dead cells and fixed with 4% polyoxymethylene (PBS buffer) for 20 min. After that, 0.1% Triton X-100 was added to permeate the cytomembrane for 10 min and then the cells were incubated in a 3% bovine serum albumin (blocking agent) for 2 h at room temperature and washed with PBS buffer twice. The fixed KB cells were incubated by FITC-phalloidin (38 ng/mL) and DPI (2 μg/mL) to stain the cytoskeleton and nucleus, respectively⁴. The stained cells were washed by PBS buffer for several times and characterized by laser scanning confocal microscope.

1.8 Apoptosis performance in vitro: To evaluate the cancer cells eradication effect of the nanocontainers in vitro, KB cells were incubated with Au NRs PDA NCTs (100 μg/mL), Au NRs@PDA-DOX (150 μg/mL) and free DOX (50 μg/mL) for 4 h. Then free KB cells as control and incubated KB cells were irradiated by an
808 nm NIR laser at a power density of 2 W/cm² for 10 min, respectively. As for the control group, free KB cells and incubated KB cells without irradiation were presented. At last, MTT solution was added into the plates, and the cell viability was assumed by MTT assay. Each experiment was repeated for five times.

In order to provide a visual characterization of the apoptosis performance of the nanocontainers, ethidium bromide (EB) stain assay was performed by incubating KB cells (50000 cells per well on a 6-well plates) with Au NRs PDA NCTs (100 μg/mL), Au NRs@PDA-DOX (150 μg/mL) and free DOX (50 μg/mL) for 4 h. Then the free KB cells and incubated KB cells were irradiated by an 808 nm NIR laser at 2 W/cm² for 10 min. After that, the dead cells were stained by adding 2.0 μg/mL of EB in to the cell culture and incubated for 15 min and finally characterized by fluorescence microscope.

1.9 Characterization: Transmission electron microscopy (TEM) images were collected on a Hitachi H-800 electron microscope operated at 200 kV with a CCD camera. High-resolution TEM images and mapping images were recorded using a JEM-2100F electron microscope at an acceleration voltage of 200 kV with a CCD camera. SEM images were taken with a JEOL FESEM 6700F electron microscope with primary electron energy of 3 kV. The samples were sputtered with a thin layer of Pt prior to imaging. Thermogravimetric (TGA) curve was obtained on a STA 499 C Jupiter (NETZSCH) thermogravimetry analyzer. Elemental analysis was performed on an Vario EL cube elementar. Inductively Coupled Plasma (ICP) results were conducted by OPTIMA 3300DV spectrometer. Fourier transform
infrared (FT-IR) spectra were collected on Nicolet AVATAR 360 FT-IR spectrophotometer. UV-visible absorption spectra were obtained using a Shimadzu 3100 UV-vis spectrophotometer. X-Ray diffraction (XRD) analysis was performed by X-ray diffractometer, Model Rigaku Ru-200b, using a nickel-filtered Cu Kα radiation and the data were collected from 10° to 80°. To study the photothermal effect of our nanocontainers, an 808 nm diode laser (LEO photonics Co. Ltd.) was employed with the output power tunable from 0 to 10 W/cm². The temperature increment under laser irradiation was determined by an electric thermometer (Traceable Callbraion, 0.1 °C). Bright field and fluorescent images of KB cells were obtained by an Olympus IX71 inverted fluorescence microscope. The confocal fluorescent images of KB cells treated with Au NRs@PDA NCTs and Au NRs@PDA-DOX were taken by using a laser scanning confocal microscope OLYMPUS BX81 (FluoView FV1000).
2. Supplementary Figures

**Figure S1.** TEM images of A) Au NRs. B) SEM image of Au NRs@PDA NCTs. Inset: SEM image of a broken Au NRs@PDA NCTs with high magnification.
Figure S2. TGA curve of Au NRs@PDA NCTs. The weight loss is 62.3%
**Table S1.** Elemental analysis of Au NRs@PDA NCTs

<table>
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<th>Elements</th>
<th>The weight percentage /%</th>
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<tbody>
<tr>
<td>C</td>
<td>38.0</td>
</tr>
<tr>
<td>N</td>
<td>6.0</td>
</tr>
<tr>
<td>H</td>
<td>3.8</td>
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**Table S2.** ICP results of Au NRs@PDA NCTs

<table>
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<th>Elements</th>
<th>Concentration /ppm</th>
<th>The weight percentage /%</th>
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<tbody>
<tr>
<td>Zn</td>
<td>13.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Au</td>
<td>4.2</td>
<td>4.2</td>
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In our work, the 2-methylimidazole was firstly fixed in ZIF-8 crystals, chelating to Zn$^{2+}$. After adding dopamine, the stronger chelation of which grabbed Zn$^{2+}$ away, causing the release of 2-methylimidazole. The released 2-methylimidazole was dispersed into the solution, which could be proved by Figure S3. When pure colorless 2-methylimidazole mixed with colorless DA, the solution became black (Figure S3F, PDA is black), so we believed 2-methylimidazole could induce the polymerization of DA (Figure S3F), due to its weak alkaline in the solution, while only Zn$^{2+}$ could not induce the polymerization of DA (Figure S3D). After the formation of Au NRs@ZIF-8, the solution was centrifuged and the supernatant shows colorless (Figure S3A) and no change by mixing with DA (Figure S3C), suggesting no dissociative 2-methylimidazole could be left in the solution. After the formation of Au NRs@PDA NCTs, the solution was centrifuged and the supernatant was also colorless, as shown in Figure S3B. However, the color of solution changed to black by mixing with DA (Figure S3E), suggesting there was dissociative 2-methylimidazole in the solution during the decomposition of ZIF-8 and the formation of PDA NCTs. Therefore, it can be clearly proved that the 2-methylimidazole removed from the inside of the capsule, and released into the solution, which could induce the polymerization of DA.
Figure S4. TEM images of Au NRs@PDA NCTs prepared under different solvent ratio (H$_2$O: MeOH) at room temperature: (a) H$_2$O, (b) 2: 1, (c) 1: 1, (d) 1: 2, (e) 0.5: 2.5, (f) MeOH.
Figure S5. A, C, E) TEM images of Au NRs@ZIF-8, and B, D, F) TEM images of Au NRs@PDA NCTs with average sizes of 76 nm, 161 nm and 290 nm.
**Figure S6.** TEM images of Au NRs@PDA NCTs obtained with different concentration of DA after 48 hours: A) 0.5 mM, B) 1.0 mM, C) 2.5 mM, and D) 5.0 mM.
Figure S7. TEM images of A) ZIF-8, B) PDA NCTs intermediate, C) hollow PDA NCTs, D) Fe$_3$O$_4$, E) Fe$_3$O$_4$@ZIF-8 and F) Fe$_3$O$_4$@PDA NCTs. Inset in F): Fe$_3$O$_4$@PDA NCTs with large magnification.
Figure S8. Confocal fluorescence images of KB cells incubated with Au NRs@PDA-Rhodamine B for 24 h: blue-nucleus; green-cytoskeleton; red-Au NRs@PDA-Rhodamine B.
Figure S9. UV-vis spectrum of Au NRs (black), Au NRs@ZIF-8 (red), Au NRs@PDA (blue), ZIF-8 (green), PDA (pink).

The LSPR band of the Au NRs red-shifted from ca. 780 nm (black line) to about 810 nm (red line), suggesting the occurrence of encapsulation that alters the dielectric constant of the local environment of the Au NRs and the growth of hybrid spheres that scatter light in the UV-vis range because of their larger size than Au NRs, which corresponded to Lu’s paper3. After forming the Au NRs@PDA NCTs, the surface of Au NRs is encapsulated with a layer of PDA, which will maintain the changing of the dielectric constant, causing similar absorption in 810 nm (blue line).
Figure S10. A) Release of DOX from Au NRs@PDA-DOX NCTs under pH 3.0 (grey line), pH 5.5 (pink line), pH 7.4 (orange line). B) NIR-triggered release of DOX from Au NRs@PDA-DOX NCTs. The samples at different pH values were irradiated with an NIR laser (2 W·cm$^{-2}$) for 5 min at different time points.
**Figure S11.** TEM images of A) Au NRs@PDA NCTs. B) Au NRs@PDA NCTs dispersed in pH 5.5 buffer for 24 h. C) Au NRs@PDA NCTs dispersed in pH 3 buffer for 24 h.

Au NRs@PDA NCTs were partially broken after 24 h dispersing in pH 3 and 5.5, and the etching degree in pH 3 buffer is more serious than that in pH 5 buffer. Such broken can further enhance the drug release, which is shown in Figure S10. The reason of the structure broken under acid environment is under investigation. And the Au NRs remain the original morphology, suggesting their stability in faintly acid environment and no influence on their PTT performance.
Figure S12. Fluorescence microscope images of KB cells incubated with A) saline, B) 100 μg/mL Au NRs@PDA NCTs, C) 150 μg/mL Au NRs@PDA-DOX NCTs, and D) 50 μg/mL free DOX under irradiation of 2 W/cm² 808 nm laser for 10 mins. The insets in B) and C) show the borderline between the irradiation and non-irradiation regions and scale bar is 200 μm.

Reference