Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Loading and Triggered Release of Cargo from Hollow Spherical Gold Nanoparticle Superstructures

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METHODS

Materials and Characterization. All solvents and chemicals were obtained from commercial sources and used without further purification. 0.1 M HEPES buffer (HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was made by directly diluting 1.0 M HEPES buffer (pH = 7.3 ± 0.1; Fisher Scientific) with water (NANOpure, Barnstead DiamondTM System.; 18.2 MΩ). TEAA buffer (TEAA = triethylammonium acetate; ~1.0 M in water; pH = 7.0; Sigma-Aldrich) was directly used. Peptide (AAAYSSGAPPMPPF or AA-PEP_{Au}) was synthesized and purified by New England Peptide with final purity of 99%. Reverse-phase high-pressure liquid chromatography (HPLC) was performed at ambient temperature with an Agilent 1200 liquid chromatographic system equipped with diode array and multiple wavelength detectors using a Grace Vydac protein C4 column (214TP1010, 1.0 cm × 25 cm). Transmission electron microscopy (TEM) samples were prepared by pipetting one drop of solution onto a 3-mm-diameter copper grid coated with carbon film. TEM was conducted on either a JEOL 200CX instrument operated at 200 kV and equipped with a Gatan CCD image system or FEI Morgagni 268 operated at 80kV and equipped with an AMT side mount CCD camera system. UV-Vis spectra were collected using an Agilent 8453 UV-Vis Spectrometer with a quartz cuvette (10 mm path length) at room temperature.

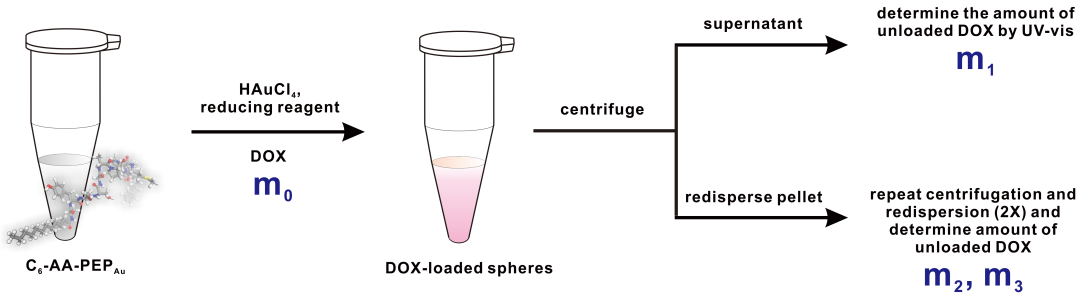
Preparation of large spheres or DOX-loaded large spheres using *in situ* loading method. C_6-AA-PEP_{Au} was synthesized according to the previously reported

method.[1] Lyophilized C_6-AA-PEP_{Au} (~1.87×10^{-8} mol) was completely dissolved in 110 μL 0.1 M HEPES buffer (pH = 7.4) and 15 μl 1.0 M TEAA buffer (pH = 7.0) in a plastic vial. (For DOX-loaded large spheres, 2 μL 1 mg/mL aqueous doxorubicin hydrochloride solution was added and vortexed at this time.) This solution was allowed to incubate for 30 min. Thereafter, 4 μl of aqueous 0.01 M chloroauric acid ($HAuCl_4$) solution was added to the above solution. The mixture was immediately vortexed for 1 min. and then left undisturbed at room temperature for one day. For DOX-loaded spheres, the excess drug molecules were removed by centrifugation (13200 rpm, 20 min, 3X). Thereafter, the spheres were redispersed in 125 μL of fresh buffer. Products from multiple syntheses were studied using TEM and UV-Vis spectroscopy.

Preparation of medium spheres or DOX-loaded medium spheres using *in situ* loading method. Lyophilized C_6-AA-PEP_{Au} (~1.87×10^{-8} mol) was completely dissolved in 125 μL 0.1 M HEPES buffer (pH = 7.4) in a plastic vial. (For DOX-loaded medium spheres, 2 μL 1 mg/mL aqueous doxorubicin hydrochloride solution was added and vortexed at this time.) This solution was allowed to incubate for 30 min. During the 30 min. incubation time, a fresh gold ion precursor solution was prepared: 0.1M chloroauric acid ($HAuCl_4$) in 1.0 M triethylammonium acetate (TEAA; pH = 7.0) buffer was incubated for 10 min. at room temperature. Thereafter, this mixture was centrifuged (10 min, 5K rpm). After the 30 min. peptide solution incubation period, 0.7 μL of the supernatant of the centrifuged gold ion precursor

solution was added to the peptide conjugate solution. The mixture was immediately vortexed for 1 min. and then left undisturbed at room temperature for one day. For DOX-loaded spheres, the excess drug molecules were removed by centrifugation (13200 rpm, 20 min, 3X). Thereafter, the spheres were redispersed in 125 μL of fresh buffer. Products from multiple syntheses were studied using TEM and UV-Vis spectroscopy.

Determination of DOX loading efficiency. See Scheme S1, where m_0 is the initial amount of DOX added, m_1, m_2, and m_3 are the amount of DOX washed away after centrifugation.



$$\text{Loading Efficiency} = \frac{m_0 - (m_1 + m_2 + m_3)}{m_0} \times 100\%$$

Scheme S1. Procedure used to determine DOX loading efficiency.

Leakage Studies. Leakage tests were performed in HEPES buffer (pH = 7.4), PBS buffer (pH = 7.4), and acetate buffer (pH = 5.0). At each time point, DOX-loaded spheres were centrifuged and the supernatant was analyzed for DOX using UV-Vis spectroscopy. All measurements were conducted in triplicate.

Proteinase K Stability Studies. To the sphere suspensions in either HEPES (pH = 7.4) or PBS buffer (pH = 7.4), 5 µl of 20 mg/ml Proteinase K was directly added. The mixture was incubated at 37 °C for up to 27 h. UV-Vis spectra were collected at different time points.

Enzyme treatment of DOX-loaded spheres. To DOX-loaded sphere suspensions, 5 µl of 20 mg/ml Proteinase K was directly added. The mixtures were incubated at 37 °C for up to 32 h. The release profiles of DOX were investigated in HEPES buffer (pH = 7.4) and PBS buffer (pH = 7.4). At each time point, the solution was centrifuged and the amount of DOX released in the supernatant was determined by comparing its absorbance at 485 nm to a DOX calibration curve. After each time point, the remaining solid was re-dispersed in fresh buffer solution containing 5 µl of 20 mg/ml Proteinase K (~1-2 seconds of sonication were employed for better dispersion).

Laser treatment of spheres or DOX-loaded spheres. To the spheres or DOX-loaded sphere suspensions, laser irradiation at 805 nm was applied for different lengths of time. The output from a commercial Ti:Sapphire laser system (Coherent Vitesse / Coherent Legend Elite, 805 nm, 100 fs, 5 kHz) was directed through a 1 cm diameter iris immediately before the sample. Average laser power was measured at the sample, using a thermopile detector (Coherent PowerMax). The 2.59 W average power is

0.660 mJ·cm-2 / pulse and the 1.25 W average power is 0.318 mJ·cm-2 / pulse. Samples were analyzed via TEM and UV-Vis spectroscopy after laser irradiation.

Supplementary Data

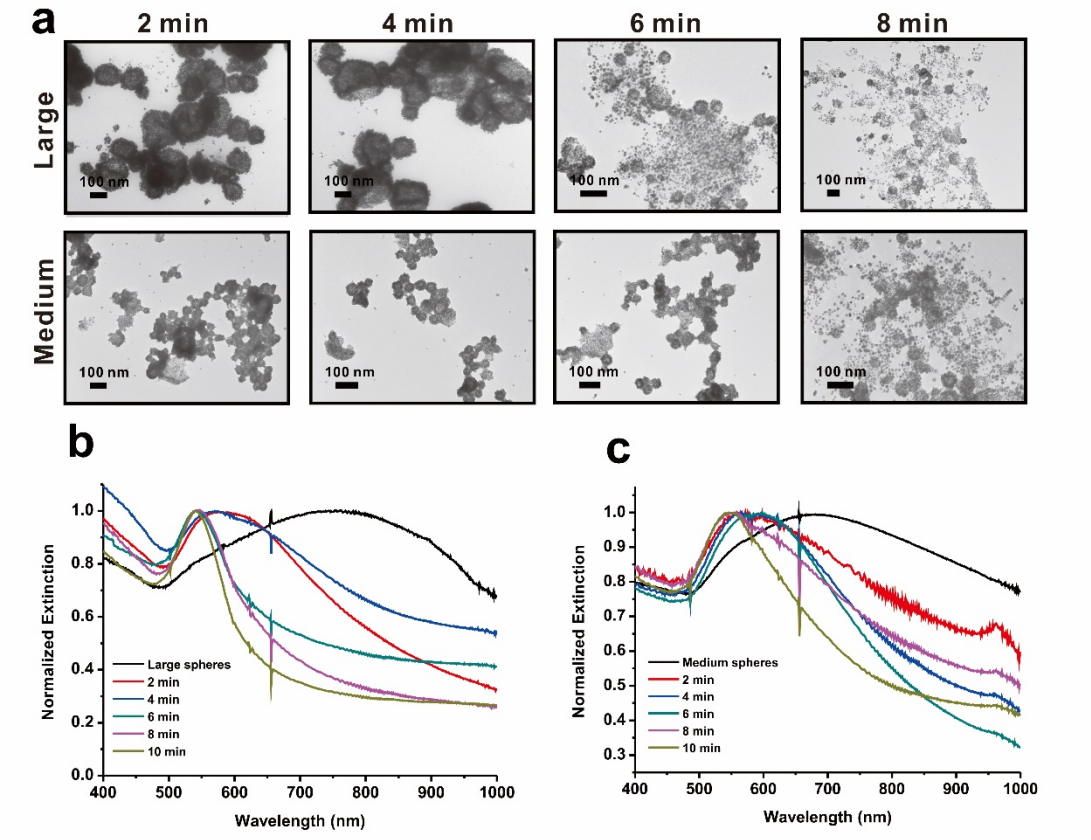


Figure S1. (a) TEM images of large spheres and medium spheres at different time points during irradiation with 805 nm laser at 2.59 W. (b) UV-Vis spectra of large spheres at different time points after irradiation with 805 nm laser at 2.59 W. (c) UV-Vis spectra of medium spheres at different time points after irradiation with 805 nm laser at 2.59 W.

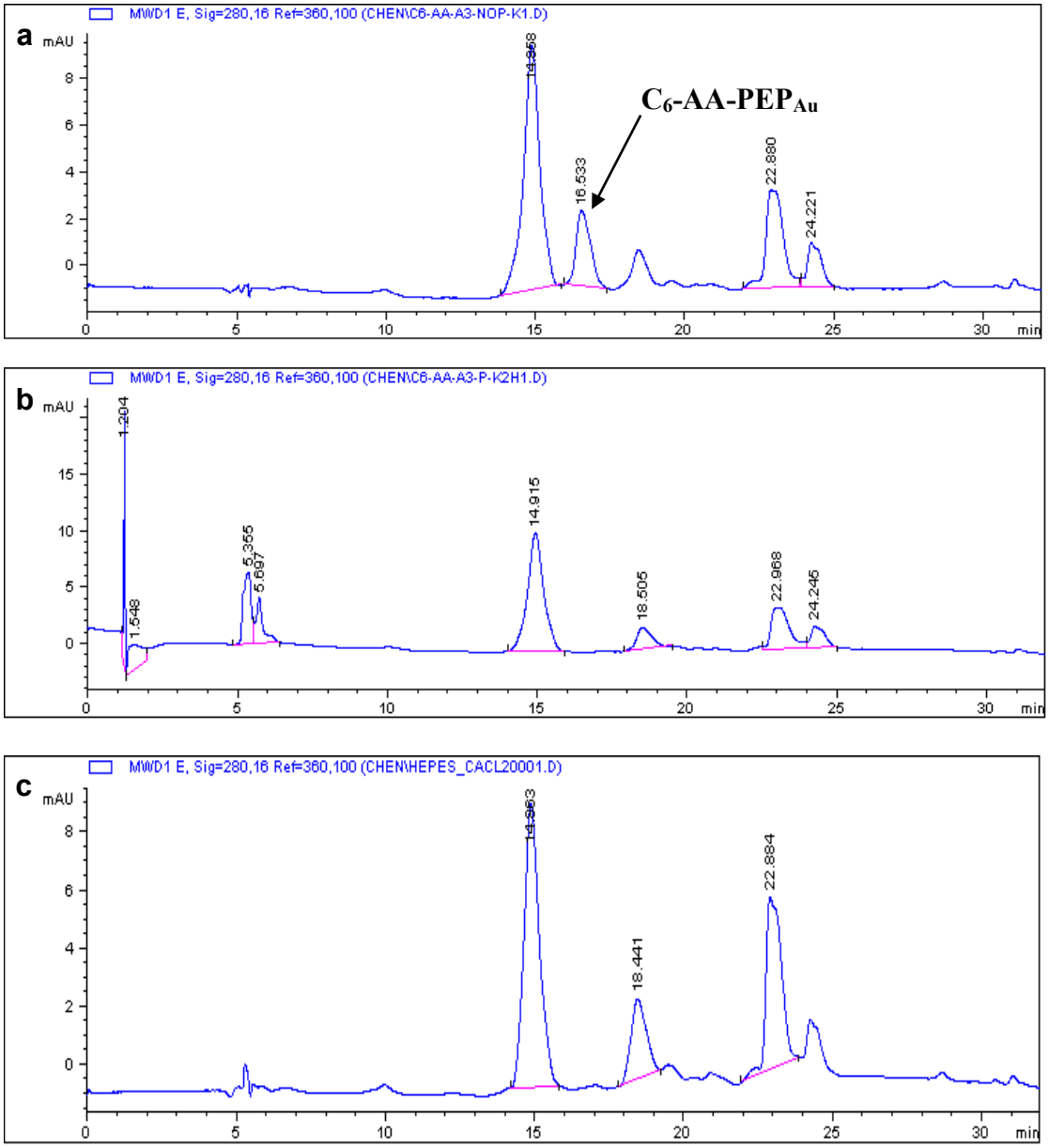


Figure S2. HPLC traces of: a) C_6-AA-PEP_{Au} in 0.1 M HEPES buffer (pH =7.4); b) C_6-AA-PEP_{Au} in 0.1 M HEPES buffer (pH = 7.4) after the addition of proteinase K for one day at 37 °C; c) 0.1 M HEPES buffer (pH = 7.4). The retention time for C_6-AA-PEP_{Au} is around 16.5 min.

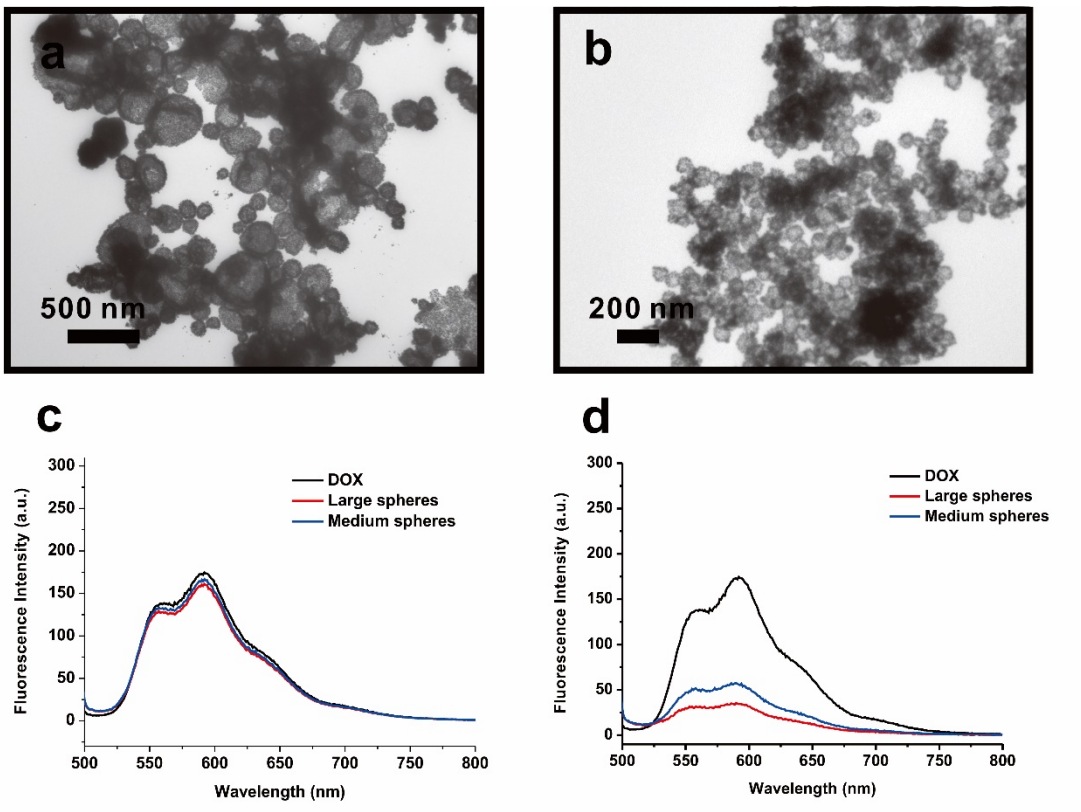


Figure S3. TEM images of DOX-loaded (a) large spheres and (b) medium spheres prepared using *in situ* loading method after removing excess drug molecules; (c) Fluorescence spectra of DOX-loaded large spheres and medium spheres using post-synthetic loading method without removing excess drug molecules; (d) Fluorescence spectra of DOX-loaded large spheres and medium spheres using *in situ* loading method without removing excess drug molecules. In both spectra, the black 'DOX' line is the spectrum for free DOX prior to loading into the spheres.

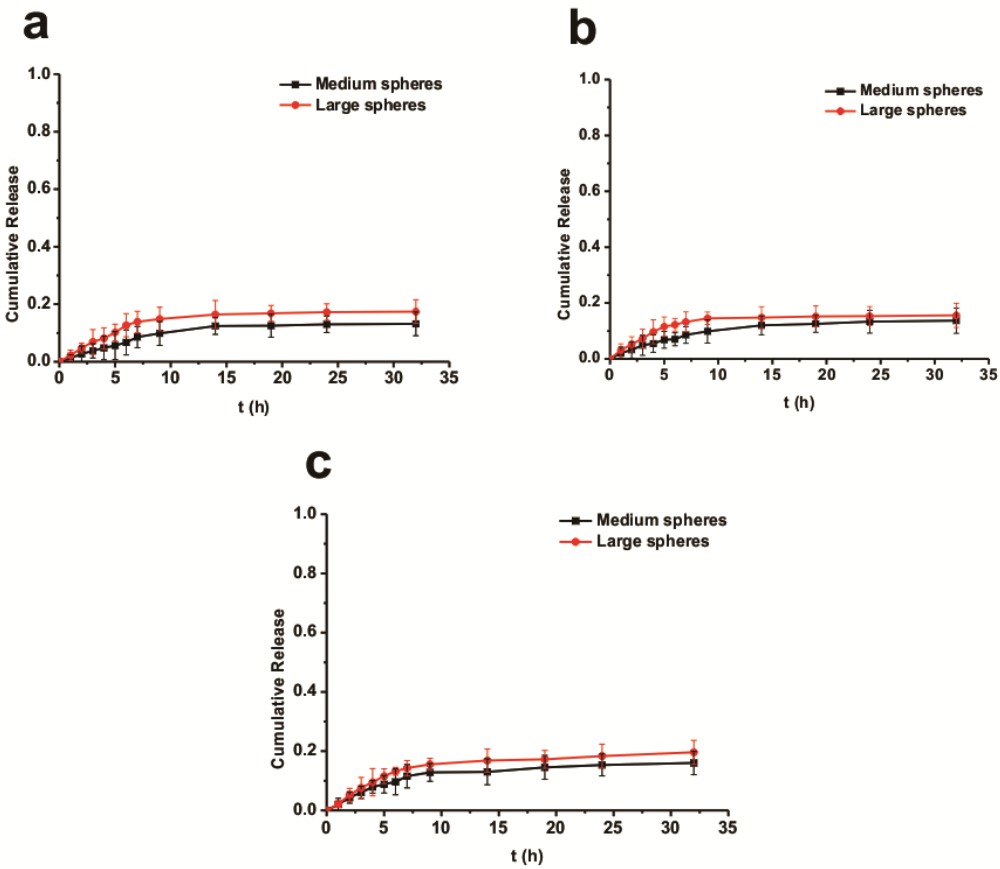


Figure S4. DOX leakage tests from large spheres and medium spheres in different buffers: (a) HEPES buffer (pH = 7.4); (b) PBS buffer (pH = 7.4); (c) Acetate buffer (pH = 5.0).

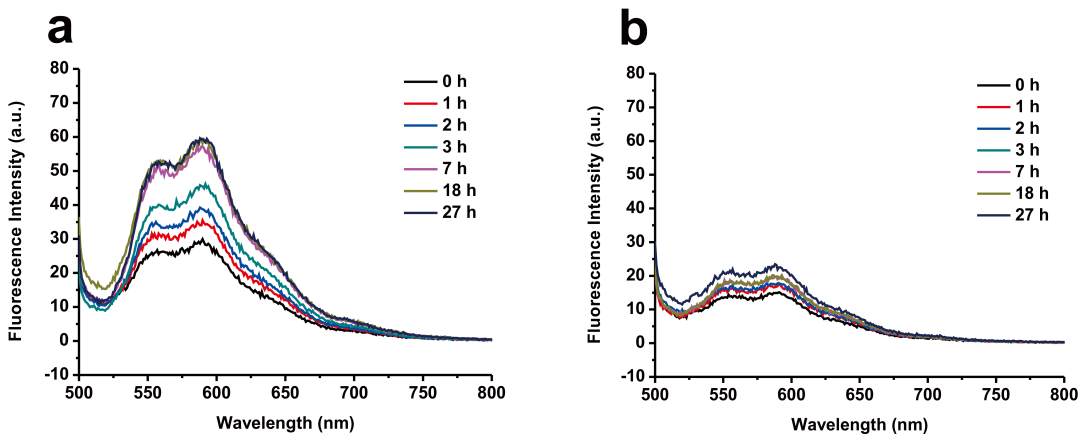


Figure S5. Time-dependent fluorescence spectra after the addition of proteinase K for DOX-loaded (a) large spheres and (b) medium spheres.

References:

1. C. Zhang, Y. Zhou, A. Merg, C. Song, G. C. Schatz and N. L. Rosi, *Nanoscale*, 2014, **6**, 12328-12332.