Supporting information

Photothermal-chemotherapy nanohybrid preparation by complexation of gold nanorods with polyamidoamine dendrimers having poly(ethylene glycol) and hydrophobic chains

Takuya Hashimoto, Eiji Yuba*, Atsushi Harada*, Kenji Kono

Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

***Corresponding authors: Eiji Yuba and Atsushi Harada**

Department of Applied Chemistry, Graduate School of Engineering,

Osaka Prefecture University, 1-1 Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

Tel: +81-72-254-9330; Fax: +81-72-254-9330; yuba@chem.osakafu-u.ac.jp,

harada@chem.osakafu-u.ac.jp

Synthesis of partially PEG-attached PAMAM dendrimer.

Partially PEG-attached dendrimer was synthesized according to Scheme S1[S1]. PAMAM-dendrimer (0.2437 g, 17.14 μmol, 1097 μmol amino groups) in methanol was evaporated and dried in vacuum to remove methanol and re-dissolved in 2 mL of distilled DMSO and PEG-NPC (1.696 g, 329.1 μmol, 0.3 equivalent to amino groups of PAMAM) in 6 mL of distilled DMSO was added, and this solution was stirred for 2 days at room temperature. The solution was dialyzed (molecular weight cut off: 12000-14000) against distilled water for 5 days. The compound was recovered by lyophilization and then further purified by a LH-20 column (GE Healthcare Japan, Tokyo, Japan) using methanol as the eluent. The yield was 1.06 g (65.6%). PEG content (Introduction ratio of PEG groups for amino groups of PAMAM-dendrimer molecule): 25.9%.

^{1}H NMR (400 MHz, D_2O): δ (ppm) 2.31 (br, $NHCH_2C\mathit{H_2}CO$), 2.58 (br, $NHCH_2C\mathit{H_2}NH$), 2.72-2.80 (br, $NHC\mathit{H_2}CH_2CO$, $NHCH_2C\mathit{H_2}NH_2$), 3.05-3.30 (br, $NHCH_2C\mathit{H_2}NHCO$, $NHC\mathit{H_2}CH_2NHCO$, $NHC\mathit{H_2}CH_2NH_2$, $NHC\mathit{H_2}CH_2NH$), 3.35 (s, $OC\mathit{H_3}$), 3.40-3.82 (br, $OC\mathit{H_2}C\mathit{H_2}$), 4.15 (s, $NHCOOC\mathit{H_2}$).

Preparation of CTAB−AuNR.

CTAB−AuNR was prepared by using seedless method[S2]. $HAuCl_4$ aqueous solution (20 mM, 7.5 mL) were mixed with CTAB aqueous solution (0.2 M, 300 mL) and kept stirred at 27 °C. Next, $AgNO_3$ (6 mM, 7.5 mL), ascorbic acid (6 mM, 2.666 mL) and ice-cold $NaBH_4$ solution (1 mM, 300 μL) were added into the above solution. The reaction solution was condensed by centrifugation (17,000 rpm, 30 min) to remove the supernatant containing excess of unreacted reagents. Then two cycles of wash-centrifugation (15,000 rpm, 10 min) using distilled water was performed for the purification of CTAB−AuNR.

Preparation of MHA−AuNR.

MHA−AuNR was prepared for the introduction of carboxylates to the surface of AuNR through the exchange reaction between CTAB and MHA. MHA solution in ethanol (5 mL, 10 mg) was added to CTAB-coated AuNRs dispersion (1.0 mg/mL, 30.9 mL) in distilled water and three times cycle of wash-centrifugation (14,000 rpm, 15 min) was performed for the removal of exchanged CTAB and excess MHA. and dispersed in pH 11.5 distilled water by adding 0.1 M NaOH aqueous solution for dispersion of nanoparticles.

Calculation of the number of PEG-dendrimers and oleoyl groups in Oleoyl−Dendrimer−AuNR.

From the result of weight loss during TGA measurements, the weight percent of PEG-dendrimer and AuNR in Dendrimer−AuNR were 2.88% and 91.09% respectively (Fig. 2B). To determine the composition of Oleoyl−Dendrimer−AuNR, following calculation based on structure of prepared AuNRs was conducted. Assume the shape of gold nanorod as a cylinder, surface area and volume of AuNR are follows:

Volume (V) = $\pi R(r/2)^2$

Surface Area (A) = $2\pi R(r/2)^2 + \pi Rr$

where R is height, r is diameter of AuNR. According to TEM image, R= 40 nm, r = 10 nm (Fig. S1), so that

$A = 2\pi \times 5^2 + 10\pi \times 40 = 1.41 \times 10^3$ nm^2,

$V = \pi \times 5^2 \times 40 = 3.14 \times 10^3$ nm^3.

Therefore, the molecular weight of AuNR is

$M_{AuNR} = 3.14 \times 10^3$ nm^3 $\times$ 59 nm^{-3} $\times$ 197 g/mol $= 3.65 \times 10^7$ g/mol.

The molecular weight of as-synthesized PEG-dendrimer is

$M_{PEG\text{-}dendrimer} = 14214 + 5000 \times 64 \times 0.259 = 9.71 \times 10^4$ g/mol.

The number of PEG-dendrimer molecules per AuNR is

$N_{PEG\text{-}dendrimer} = (2.88/ M_{PEG\text{-}dendrimer})/(91.09/ M_{AuNR}) =$ **12**.

In Oleoyl−Dendrimer−AuNR, weight percent of PEG-dendrimer and oleoyl group were 2.87% and 0.33% respectively according to Fig. 2B. From this value, oleoyl groups per PEG-dendrimer is

$N_{oleoyl\ groups} = (0.33/265.46)/(2.87/ M_{PEG\text{-}dendrimer}) =$ **42**.

To calculate the maximum volume *v* occupied by PEG-dendrimer surrounding AuNR, a nanohybrid is assumed as a cylinder with 46 (= 18 × 2 + 10) nm width and 40 nm length and two spherical caps at the edge of the cylinder with the diameter of 18 nm.

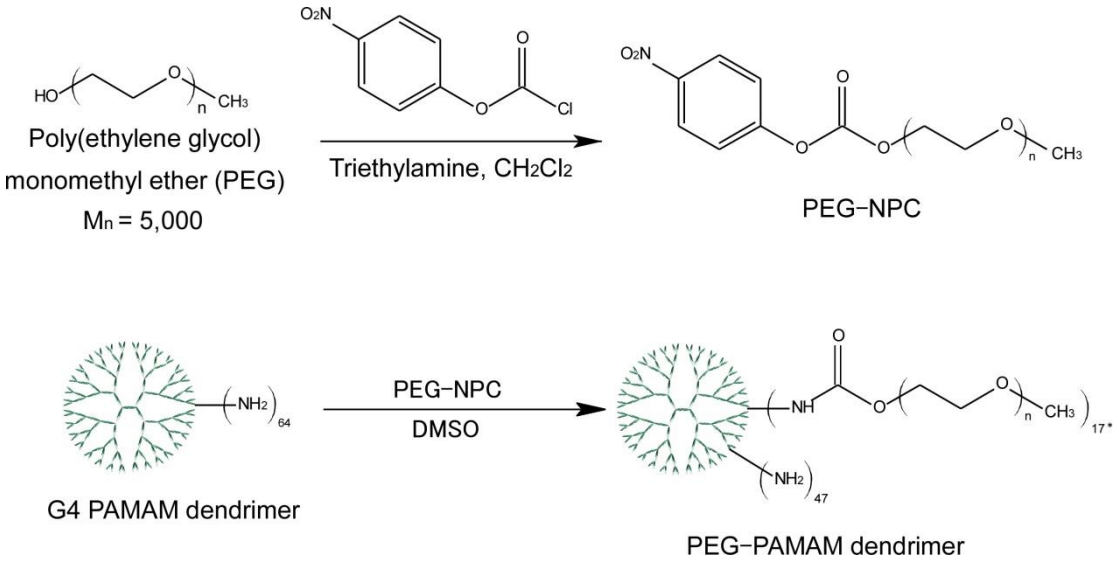
$$v = 2 \times \pi \int_0^{18} (23 - x)^2 dx + \pi \times (23^2 - 5^2) \times 40 = 8.86 \times 10^4 \text{ nm}^3$$

The maximum number of PEG-dendrimer conjugated (regarded as a sphere with 9 nm diameter) on the surface of AuNR can be calculated as:

$$\frac{v}{\frac{4}{3}\pi \times 9^3} = \mathbf{29}$$

This value is much higher than experimental value (11.9) from TGA probably because above calculation excludes the interdigitation between PEG-dendrimer spheres and these spheres contact to AuNR via PEG-terminus. In an actual nanohybrid, PEG-dendrimers are conjugated via amino groups in dendrimer and interdigitation

between PEG chains would take place, resulting in small dendrimer number compared with theoretical value.



Scheme S1. Synthetic route of PEG–PAMAM dendrimer. Average PEG content per dendrimer was determined as 17 from the integral of PAMAM dendrimer-derived proton signals compared with methoxy proton signal of PEG in ^{1}H NMR spectrum.

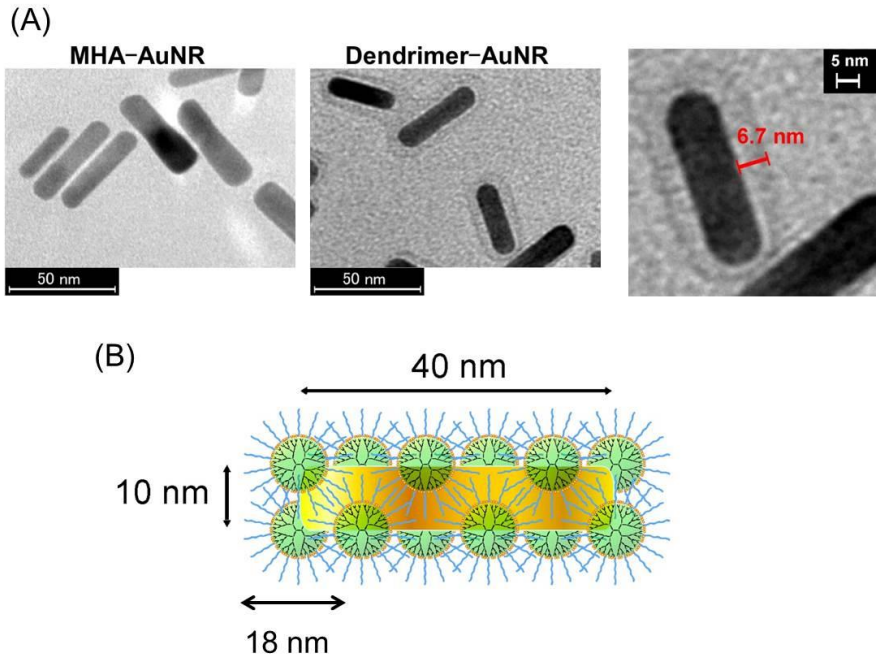


Figure S1. (A) TEM-images of MHA–AuNR and Dendrimer–AuNR stained with 2% aqueous sodium phosphotungstate. MHA–AuNRs had a dimension of about 40.2 ± 6.9 nm in length and 10.1 ± 2.0 nm in width. In the image of dendrimer-modified AuNR,

the shape of AuNR was kept after modification of dendrimer. And the layer about 6.7 nm in thickness was observed surrounding AuNRs (magnified image). Considering that the hydrodynamic size of G4-dendrimer was 4 nm[S3], PEG-dendrimer might cover on the surface of AuNR, which was observed as the layer on the AuNRs. (B) Plausible graphical morphology of nanohybrids composed of PEG-dendrimer and gold nanorod. The size of PEG-dendrimer was measured by DLS.

Table S1. Characterization of nanohybrids

	Experimental values	Theoretical values
Surface area of AuNR (nm^2)	-	1.41×10^3***
Cress section area of PEG-dendrimer (nm^2)	254*	-
Dendrimer / AuNR	11.9**	29*, ***
Oleoyl / Dendrimer	42**	46****

*Determined by DLS, **Determined by TGA, ***Determined by TEM, ****Determined by ^{1}H NMR.

Calculation of the photothermal conversion efficiency.

According to the literature (*J. Phys. Chem. C*, 2007, **111**, 3636–3641), the photothermal conversion efficiency η of nanohybrid was calculated as follows:

$$\boldsymbol{\eta = \frac{hA(T_{max} - T_{amb}) - Q_0}{I(1 - 10^{-A_{808}})} \times 100} \qquad (\mathbf{1})$$

Where, h is heat transfer coefficient, A is the surface area of container, T_{max} is maximum system temperature, T_{amb} is ambient temperature, and A_{808} is absorbance of nanohybrid dispersion at 808 nm.

Q_0 can be measured independently using a quartz cell containing DMEM without nanohybrid as follows:

$$Q_0 = 0.01085 \text{ W}$$

hA is determined by measuring the rate of temperature drop after NIR irradiation is turned off. To calculate hA, dimensionless driving force temperature θ and system time constant τ_s are introduced:

$$\boldsymbol{\theta = \frac{T_{amb} - T}{T_{amb} - T_{max}}} \qquad (\mathbf{2})$$

$$\boldsymbol{\tau_s = \frac{\sum_i m_i C_{p,i}}{hA}} \qquad (\mathbf{3})$$

Where, m and C_p are the mass and heat capacity, respectively.

Here, total energy balance for the system can be expressed by following equation:

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = Q_I + Q_0 - Q_{ext} \qquad (4)$$

Where, Q_I is the photothermal energy input by nanohybrids.

From eq. 2 and 3, eq. 4 can be rearranged as following eq. 5.

$$\frac{d\theta}{dt} = \frac{1}{\tau_s} \left[\frac{Q_I + Q_0}{hA(T_{max} - T_{amb})} - \theta \right] \qquad (5)$$

When laser irradiation is off, $Q_I + Q_0 = 0$. Therefore, eq. 5 is rearranged as following eq. 6.

$$\frac{d\theta}{dt} = -\frac{\theta}{\tau_s} \qquad (6)$$

Eq. 6 can be rearranged as following eq. 7 at the initial condition of $\theta = 0$ and $t = 0$.

$$t = -\tau_s \ln \theta \qquad (7)$$

By applying the linear approximation from the relationship between t and $\ln\theta$ (Figure S2), τ_s was calculated as follows:

$$\tau_s = 374.9 \text{ s}$$

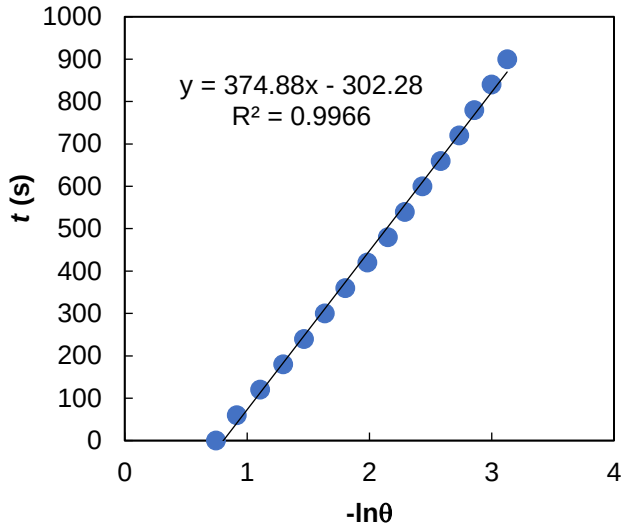


Figure S2. Time constant for heat transfer from the system using linear regression of

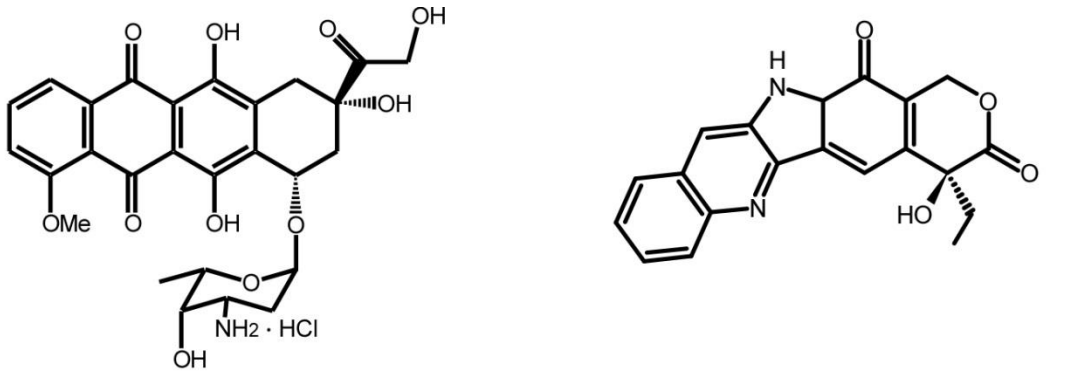
the cooling profile shown in Figure 2D (Profile at 600 - 1500 s was used.).

By introducing each value m_{H20} = 1 g, T - T_{max} = 22.1, $C_{p,H20}$ = 4.2 J/(g °C), $m_{quartz\ cell}$ = 1.53 g, $C_{p,\ quartz}$ = 0.772 J/(g °C) , A_{808} = 0.100, I = 6 W/cm^2, hA can be calculated as:

$$hA = 0.014 \text{ W/°C}$$

From eq.1, η of nanohybrid was calculated as:

$$\eta = \underline{24.8\%}$$



Doxorubicin hydrochloride (DOX·HCl)

(S)-Camptothecin (CPT)

Figure S3. Chemical structures of doxorubicin (DOX) and camptothecin (CPT).

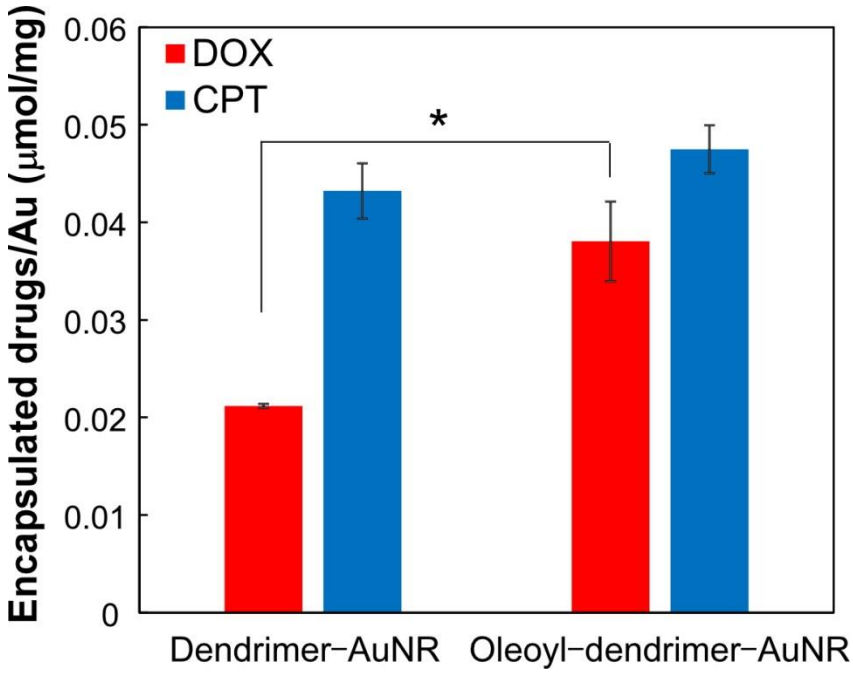


Figure S4. Amounts of DOX or CPT loaded in PEG−Dendrimer−AuNR nanohybrids

with or without oleoyl chains (Drugs/Au in feed is 0.061 mg/mg). *$p < 0.01$.

Table S2. LC and LE of DOX or CPT loaded in PEG−Dendrimer−AuNR nanohybrids with or without oleoyl chains (Drugs/Au in feed is 0.061 mg/mg).

	DOX		CPT	
	LC (wt%)	LE (%)	LC (wt%)	LE (%)
Dendrimer−AuNR	1.2±0.1	20.0±0.2	1.5±0.1	24.7±1.6
Oleoyl−Dendrimer−AuNR	2.2±0.2	36.0±3.9	1.6±0.1	27.1±1.4

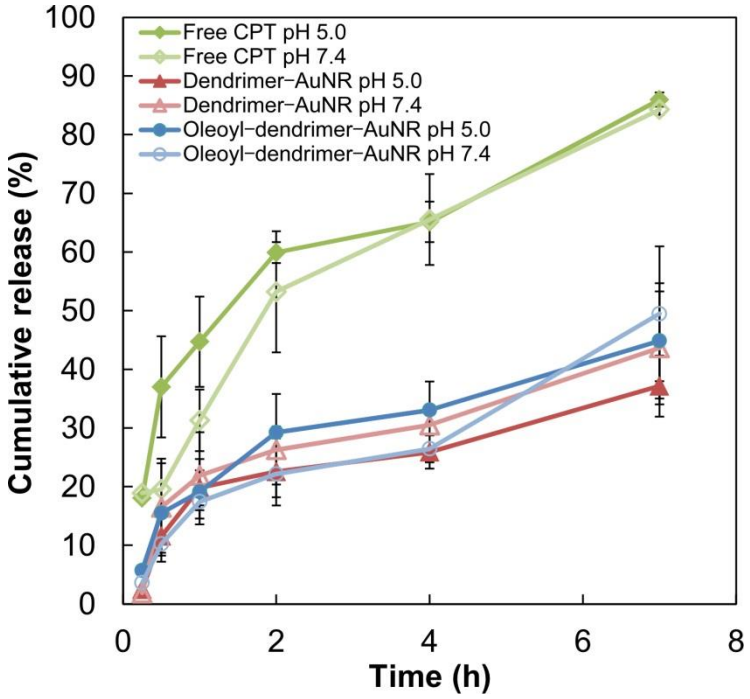


Figure S5. Release profiles of CPT from Dendrimer−AuNR or Oleoyl−Dendrimer−AuNR in PBS of pH 7.4 or pH 5.0 supplemented with 10% FBS.

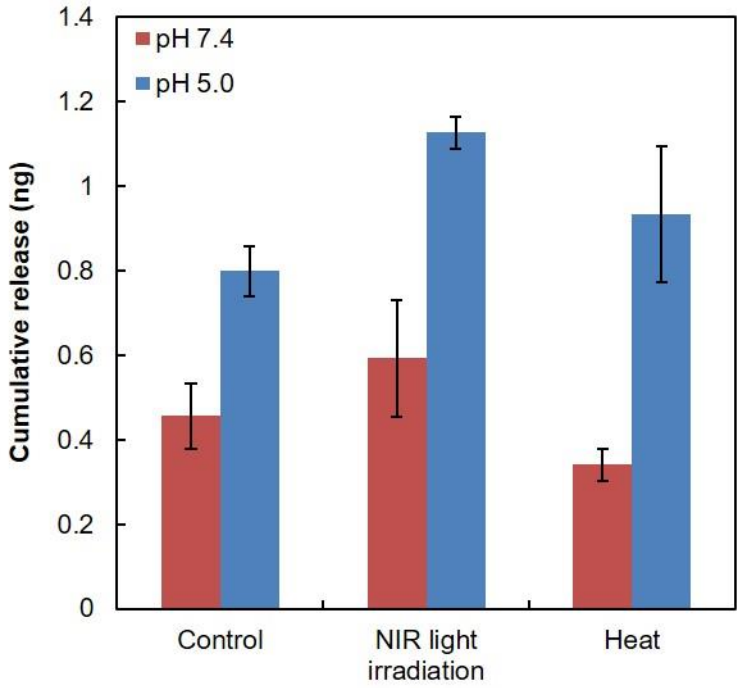


Figure S6. The influence of NIR light irradiation (808 nm, 6 W/cm^2, 15 min) or external heating (from room temperature to 45 ℃) on DOX release from DOX/Oleoyl-dendrimer-AuNR.

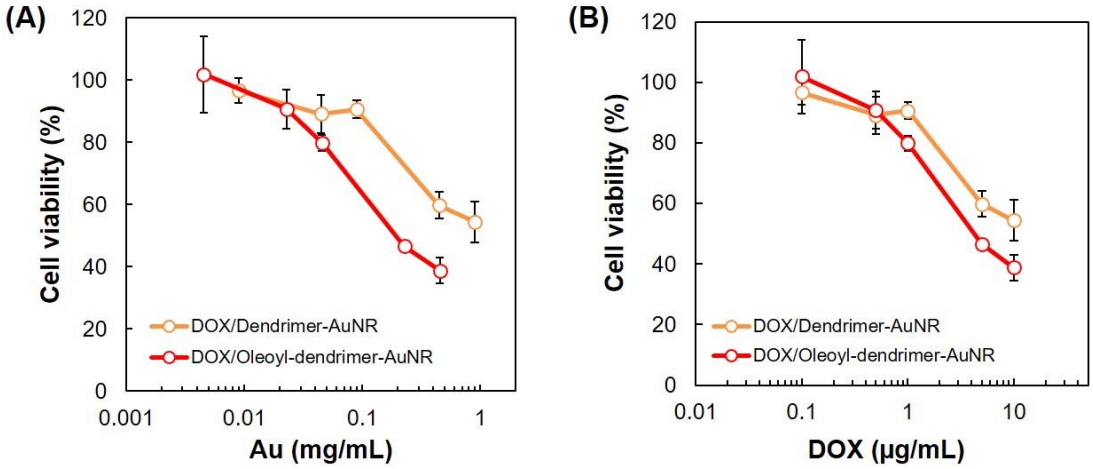


Figure S7. Viability of HeLa cells treated with various amounts of DOX-loaded nanohybrids for 3 h without NIR light irradiation. X-axis for same data was adjusted as Au concentration (A) or DOX concentration (B).

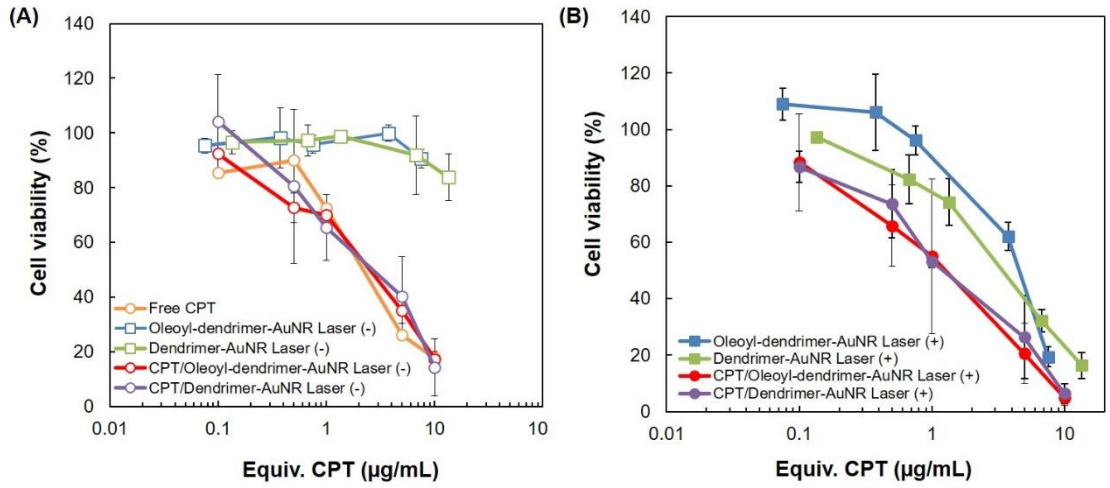


Figure S8. Dependence of cell viabilities on CPT concentrations for HeLa cells treated with free CPT, CPT/Oleoyl−Dendrimer−AuNR, CPT/Dendrimer−AuNR, Oleoyl−Dendrimer−AuNR, and Dendrimer−AuNR for 3 h without (A) or with (B) NIR irradiation (808 nm, 6 W /cm^2).

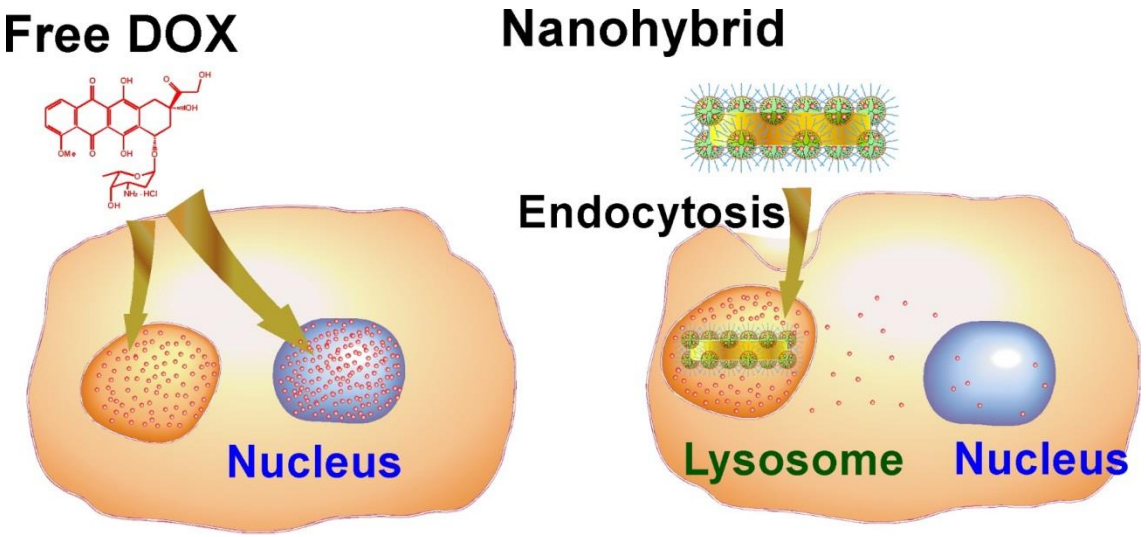


Figure S9. Graphical mechanism of cellular uptake of DOX or nanohybrids. Free DOX penetrates the cell membrane into nucleus. On the other hand, nanohybrids were taken up by HeLa cells through endocytosis and /or lysosomes.

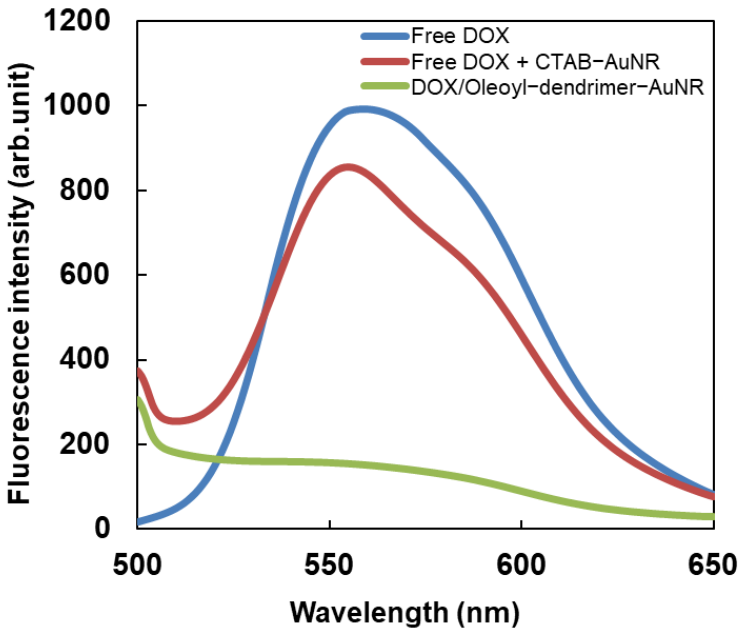


Figure S10. Fluorescence spectra of Free DOX, Free DOX + CTAB−AuNR, DOX/Oleoyl−Dendrimer−AuNR ((Ex: 468 nm, Em: 500-650 nm, [Au] = 4.2 μg/mL, [DOX] = 0.12 μg/mL).

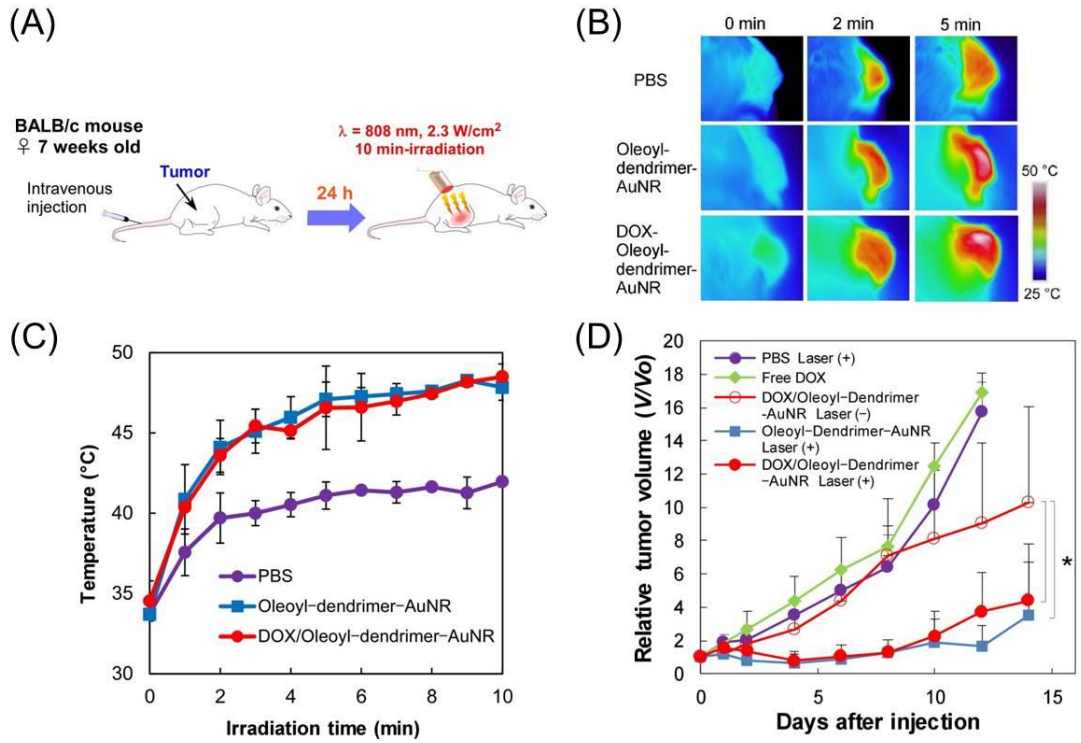


Figure S11. (A) Experimental representation of nanohybrid treatment via intravenous injection with NIR light irradiation for colon 26-bearing mice. (B) Thermographs of

DOX-unloaded nanohybrid, DOX-loaded nanohybrid or PBS intravenously injected mouse receiving photothermal treatment for different periods of time. (C) Time courses of temperature variation in the right tumor region under NIR laser irradiation (λ = 808 nm, 2.3 W/cm^2, 10 min) 24 h after intravenous injection of DOX-unloaded nanohybrid, DOX-loaded nanohybrid or PBS. (D) Tumor growth profiles of tumor-bearing mice after intravenous injection of DOX-unloaded nanohybrid, DOX-loaded nanohybrid, DOX or PBS (0.45 mg Au and 10 μg DOX) with/without NIR laser irradiation. Tumor volumes were normalized to their initial sizes. *p < 0.01.

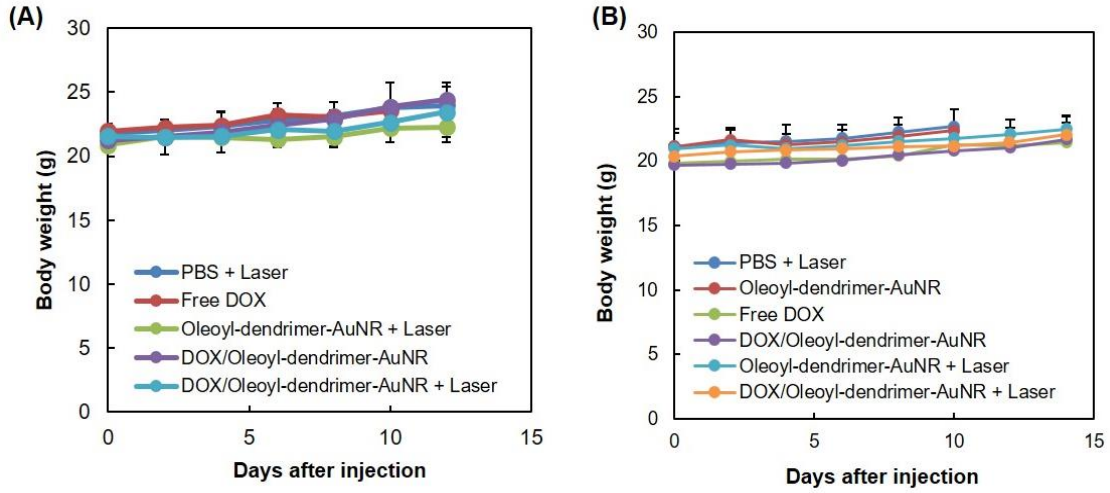


Figure S12. Body weight changes in BALB/c mice as a function of time after different treatments through (A) intravenous injection and (B) intratumoral injection.

References

S1) C. Kojima, K. Kono, K. Maruyama and T. Takagishi, *Bioconjug. Chem.*, 2000, **11**, 910–917.

S2) N. R. Jana, *Small*, 2005, **1**, 875–882.

S3) R. Esfand and D. A. Tomalia, *Drug Discov. Today*, 2001, **6**, 427–436.