Supporting information

"Carrier-drug" layer-by-layer hybrid assembly of biocompatible polydopamine nanoparticles to amplify photo-chemotherapy

Lingyu Lv[a], Hao Cheng[a], Zhen Wang[a], Zhangyi Miao[a], Feng Zhang[a], Jie Chen[a], Gang Wang[a], Ling Tao[b], Jianping Zhou[a], Huaqing Zhang[a, *], Yang Ding[a, b, *]

[a]*State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing, 210009, China*

[b]*The High Efficacy Application of Natural Medicinal Resources Engineering Center of Guizhou Province, School of Pharmaceutical Sciences, Guizhou Medical University, Guiyang 550025, China*

* Corresponding authors.

E-mail: Yang Ding, dydszyzf@163.com; Huaqing Zhang, zhanghq0527@163.com.

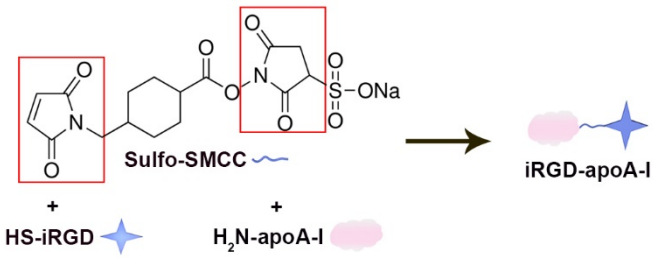

Fig. S1 Synthetic scheme for iRGD-apoA-I.

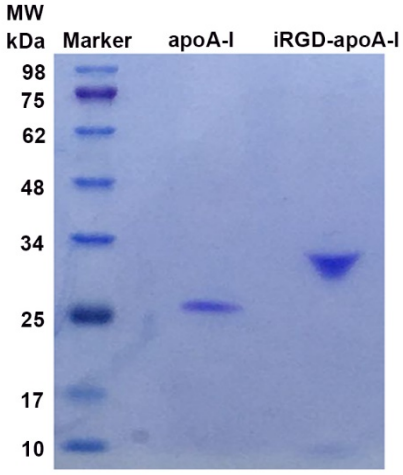

Fig. S2 SDS-PAGE analysis of apoA-I, iRGD-apoA-I, and marker, respectively.

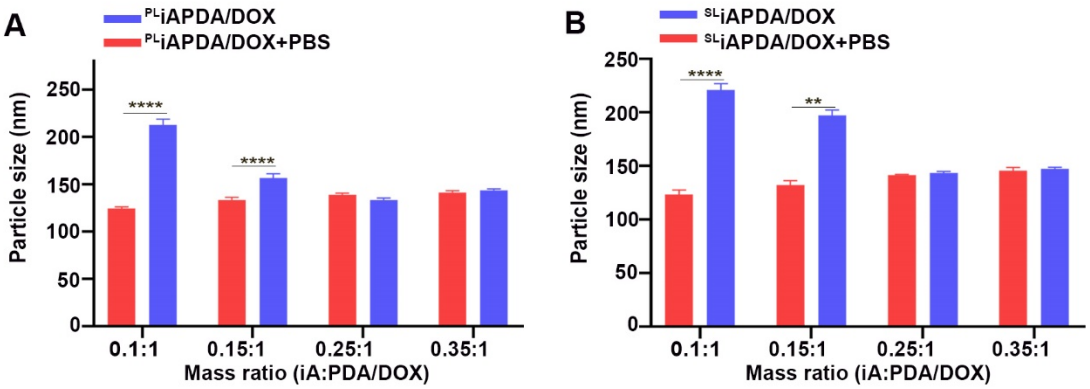

Fig. S3 The size distribution measured by dynamic light scattering of ^{PL}iAPDA/DOX (A) and ^{SL}iAPDA/DOX (B) prepared with different iRGD-apoA-I (iA): PDA/DOX mass ratio and incubation with PBS for 24 h. Data were presented as mean ± SD, n = 3. $^{**}P < 0.01$, $^{****}P < 0.0001$.

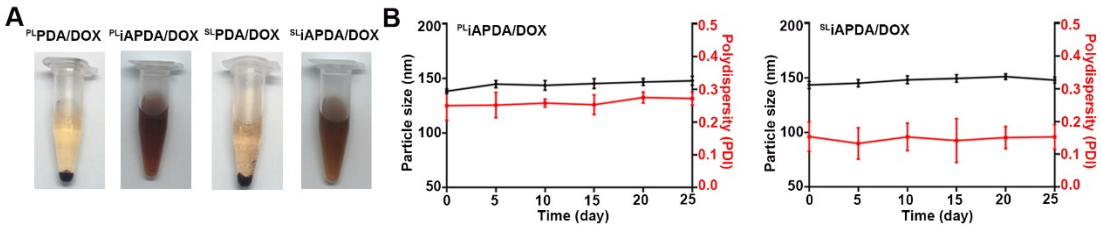

Fig. S4 (A) The stabilization of ^{PL}PDA/DOX, ^{SL}PDA/DOX, ^{PL}iAPDA/DOX, and ^{SL}iAPDA/DOX suspended in PBS after incubation for 24 h. (B) The change in the particle sizes and polydispersity index of ^{PL}iAPDA/DOX and ^{SL}iAPDA/DOX during incubation in PBS (pH = 7.4) at 4 °C for 25 days, respectively. Data were presented as mean ± SD, n = 3.

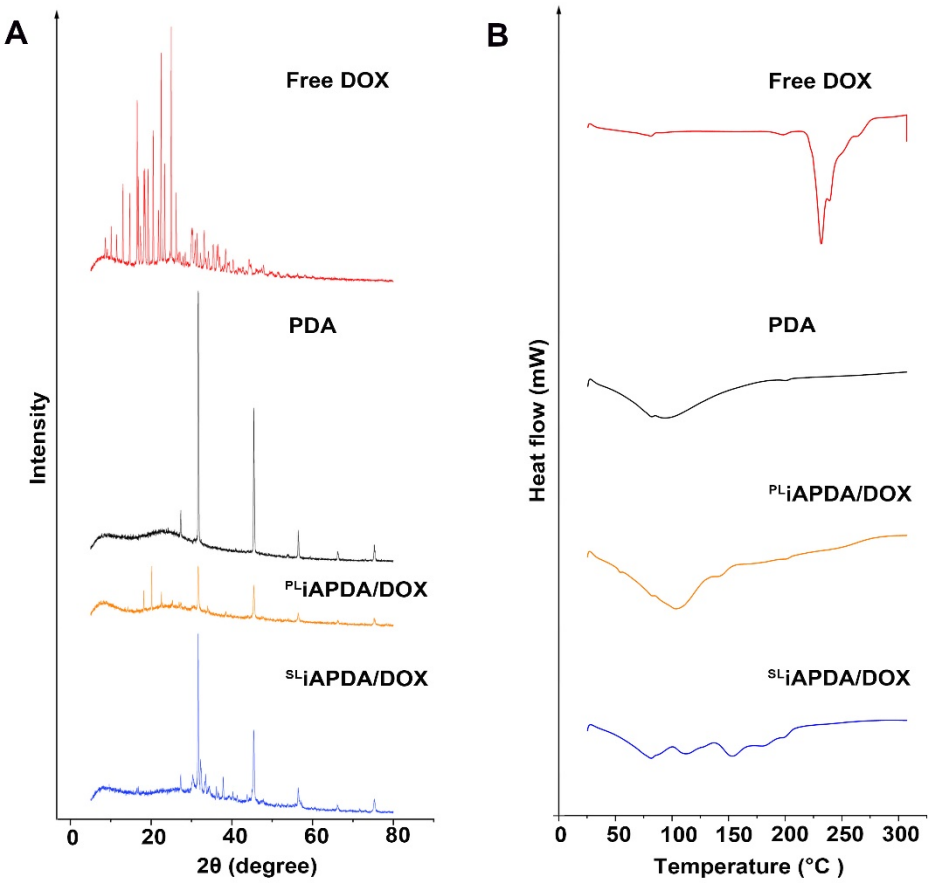

Fig. S5 XRD analysis (A) and DSC thermograms (B) of free DOX, PDA, ^{PL}iAPDA/DOX, and ^{SL}iAPDA/DOX.

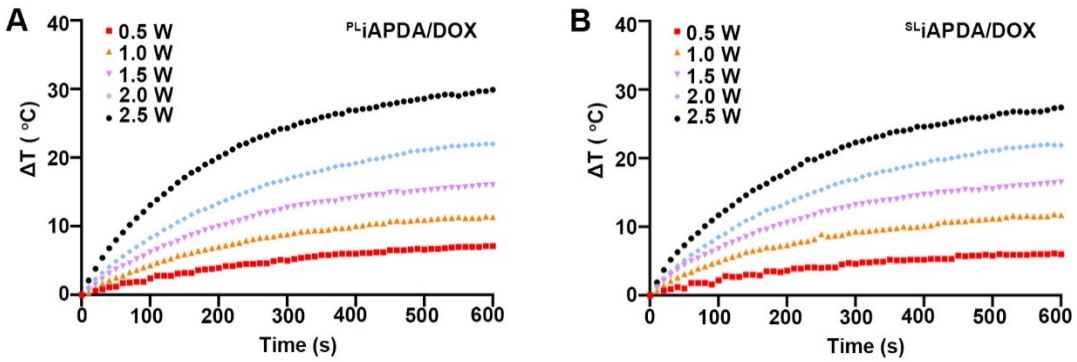

Fig. S6 The photothermal capability of PLiAPDA/DOX (A) and SLiAPDA/DOX (B) at various NIR laser power (200 μg/mL of PDA), respectively.

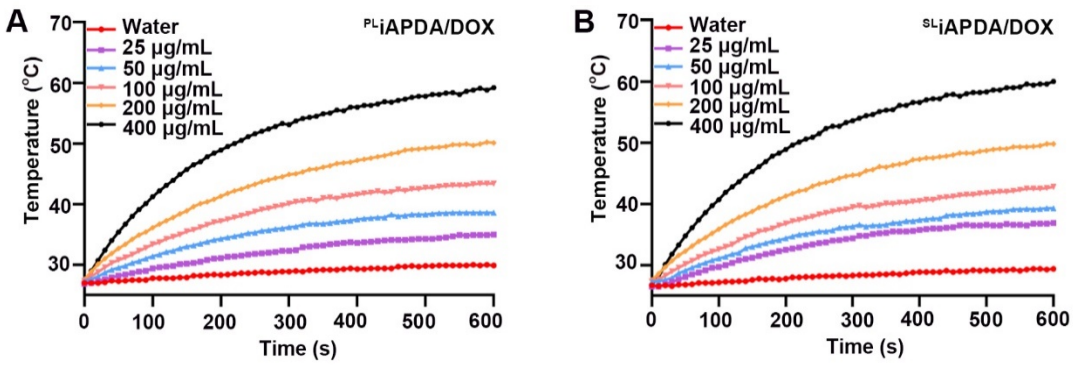

Fig. S7 The photothermal capability of PLiAPDA/DOX (A) and SLiAPDA/DOX (B) at different PDA concentrations (2 W/cm^2), respectively.

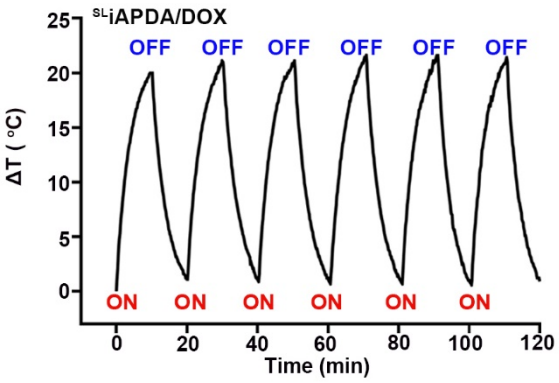

Fig. S8 Temperature changes of SLiAPDA/DOX under 808 nm NIR irradiation (2 W/cm^2) for six cycles (10 min of irradiation for each cycle).

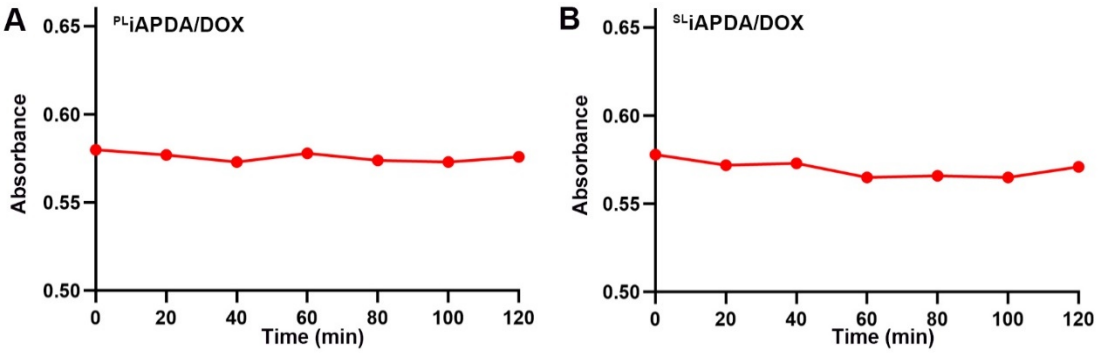

Fig. S9 Absorbance changes of ^{PL}iAPDA/DOX (A) and ^{SL}iAPDA/DOX (B) under 808 nm NIR irradiation (2 W/cm^2) for six laser on/off cycles, respectively.

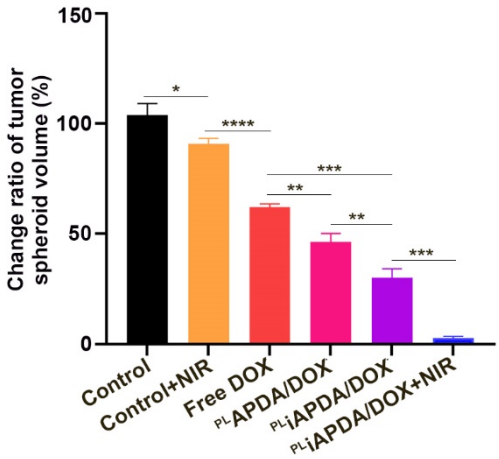

Fig. S10 Change ratios of 4T1 tumor spheroids volume (%) after applying various formulations and untreated control. Data were presented as mean ± SD, n = 5; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

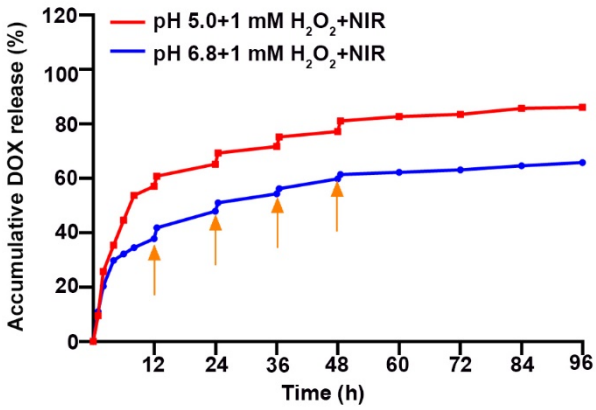

Fig. S11 Accumulative release of DOX from ^{PL}iAPDA/DOX triggered by NIR irradiation (2 W/cm^2, 10 min), ROS (1 mM H_2O_2), and different pH values (5.0, 6.8) to

simulate the tumor microenvironment, respectively. Data were presented as mean ± SD, $n = 3$.

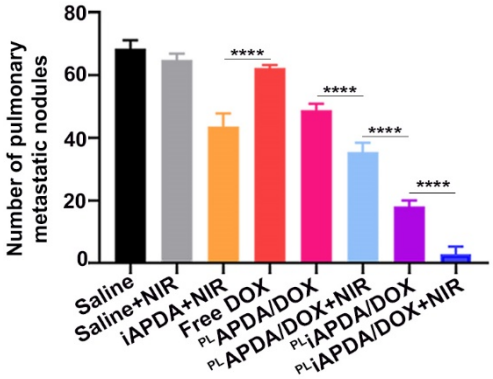

Fig. S12 Number of pulmonary metastatic nodules of 4T1 tumor-bearing mice after various treatments indicated. Data were presented as mean ± SD, $n = 5$; ****$P < 0.0001$.

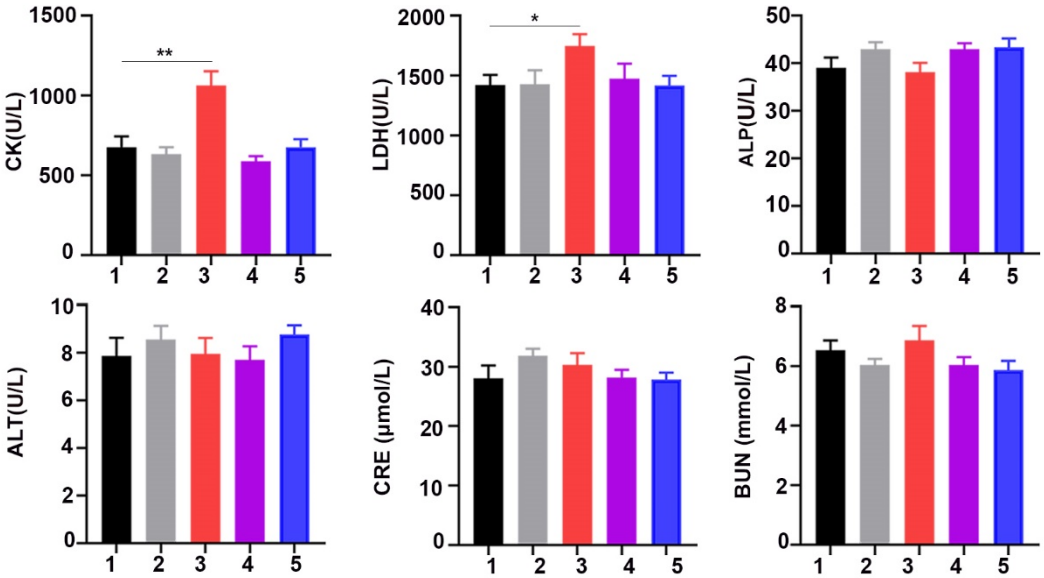

Fig. S13 Biochemical studies including heart functions (CK, LDH), liver functions (ALP, ALT), and renal functions (CRE, BUN) in healthy mice treated with various treatments (1, saline; 2, saline with irradiation; 3, free DOX; 4, ^{PL}iAPDA/DOX; 5, ^{PL}iAPDA/DOX with irradiation). Dose of DOX: 1.5 mg/kg; irradiation: 808 nm at 2 W/cm^2 for 10 min. Data were presented as mean ± SD, $n = 5$; *$P < 0.05$. **$P < 0.01$.

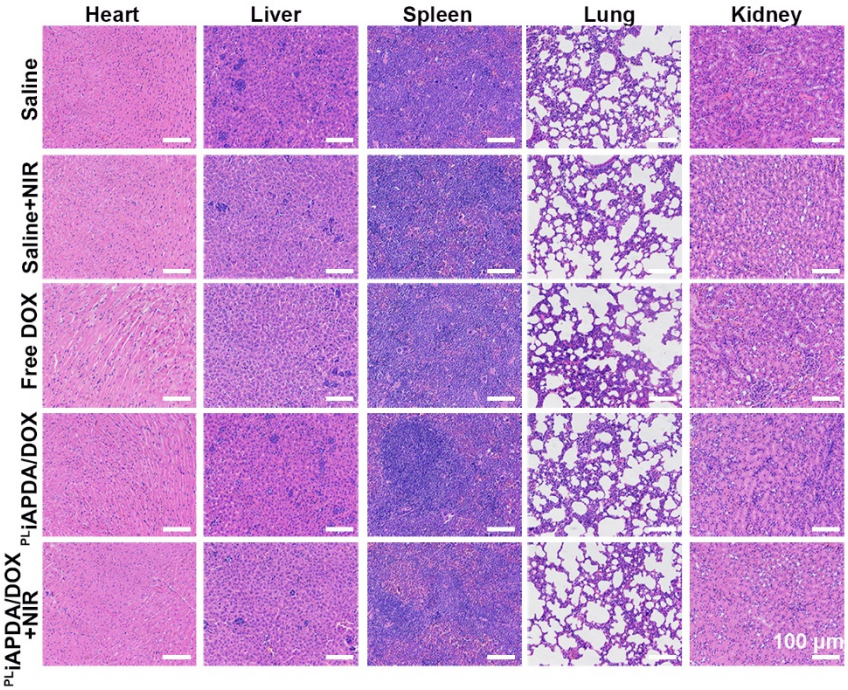

Fig. S14 H&E histopathological sections of tissues excised from mice in the study groups. Scale bar = 100 μm.

Table S1 Characterization of different formulations, including PDA, ^{PL}PDA/DOX, ^{SL}PDA/DOX, ^{PL}iAPDA/DOX, and ^{SL}iAPDA/DOX. Data were presented as mean ± SD, n = 3.

	Diameter (nm)	PDI	Zeta potential (mV)	EE (%)	DL (%)
PDA	84.54 ± 0.79	0.18 ± 0.03	-21.05 ± 0.60	---	---
^{PL}PDA/DOX	107.70 ± 2.73	0.25 ± 0.01	16.20 ± 1.08	79.71 ± 2.29	70.51 ± 0.59
^{SL}PDA/DOX	105.32 ± 2.14	0.15 ± 0.04	7.92 ± 1.01	24.39 ± 3.13	42.23 ± 1.11
^{PL}iAPDA/DOX	123.80 ± 1.87	0.19 ± 0.04	-29.31 ± 4.04	79.71 ± 2.29	57.79 ± 0.48
^{SL}iAPDA/DOX	119.10 ± 0.65	0.18 ± 0.03	-35.17 ± 6.02	24.39 ± 3.13	34.91 ± 0.91

Table S2 Grafting rate of iRGD-apoA-I to PDA nanoparticles in different formulations. Data were presented as mean ± SD, n = 3.

Mass ratio (iA:PDA/DOX)	^{PL}iAPDA/DOX	^{SL}iAPDA/DOX
0.1:1	(8.49 ± 0.27) %	(8.39 ± 0.30) %
0.15:1	(12.28 ± 0.15) %	(12.17 ± 0.19) %
0.25:1	(18.03 ± 0.27) %	(17.33 ± 0.29) %
0.35:1	(18.26 ± 0.31) %	(17.53 ± 0.72) %

Table S3 IC_{50} of iAPDA + NIR, Free DOX, ^{PL}APDA/DOX, ^{PL}APDA/DOX, ^{PL}iAPDA/DOX, and ^{PL}iAPDA/DOX + NIR.

	iAPDA +NIR	Free DOX	^{PL}APDA/ DOX	^{PL}iAPDA/ DOX	^{PL}iAPDA/DOX +NIR
IC_{50} (μg/mL)	46.68	1.53	1.28	0.95	0.61