**Supplementary Material**

# Tumor-targeted hybrid protein oxygen carrier to simultaneously enhance hypoxia-dampened chemotherapy and photodynamic therapy at a single dose

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**Keywords:** hybrid protein nanoparticle, targeted oxygen carrier, tumor hypoxia, chemotherapy, photodynamic therapy

**Supplementary Figures**

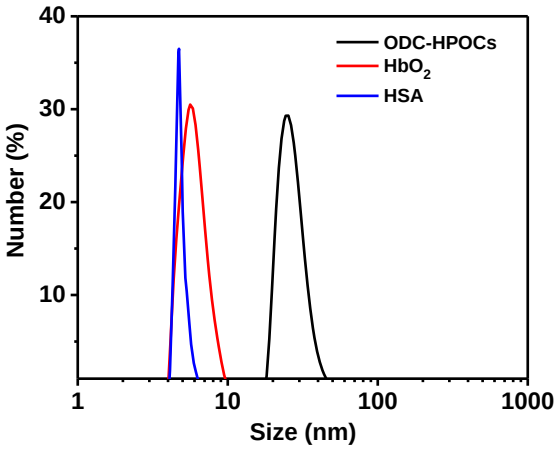


**Figure S1.** Size distribution of $HbO_2$, HSA and ODC-HPOCs.

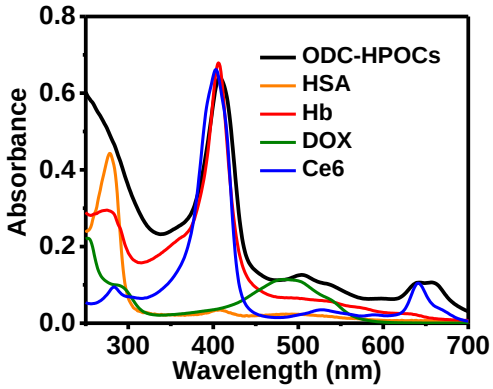


**Figure S2.** UV-Vis absorption spectra of ODC-HPOCs.

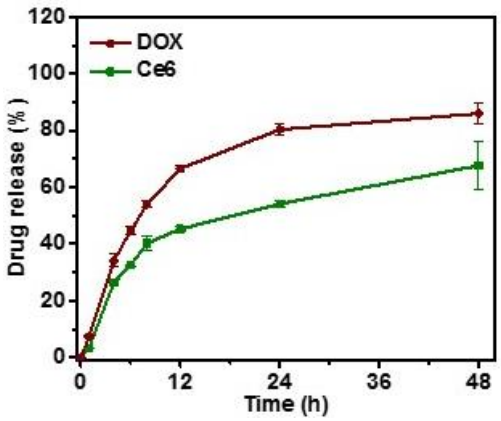


**Figure S3.** The releasing profiles of DOX and Ce6 in ODC-HPOCs.

**Table S1**. Fitting results of experimental DOX and Ce6 release data to different kinetic equations.

| Samples | Kinetic model | Equation | Correlation coefficient |
|---|---|---|---|
| **DOX** | First-order | $y=64.6025(1-e^{-0.1064x})$ | **0.9658** |
| | Higuchi | $y=13.9290x^{1/2}+4.9211$ | 0.8835 |
| | Ritger-Peppas | $y=20.8482x^{0.3950}$ | 0.9137 |
| **Ce6** | First-order | $y=85.7680(1-e^{-0.1233x})$ | **0.9987** |
| | Higuchi | $y=10.8034x^{1/2}+0.5513$ | 0.8942 |
| | Ritger-Peppas | $y=12.9517x^{0.4452}$ | 0.9034 |

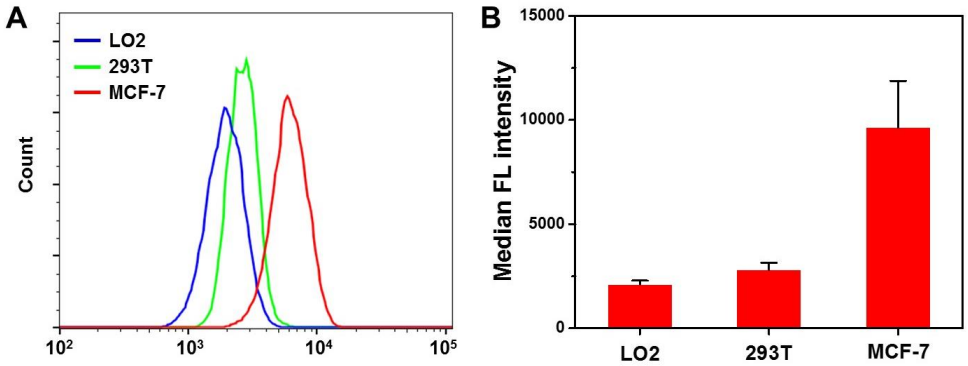


**Figure S4**. (A) Cellular uptake of Ce6 in cancerous (MCF-7) and normal (LO2 and 293T) cell lines after 2 h incubation with ODC-HPOCs. (B) Median fluorescent intensities of Ce6 uptake in different cells with flow cytometric analysis (Figure S4A).

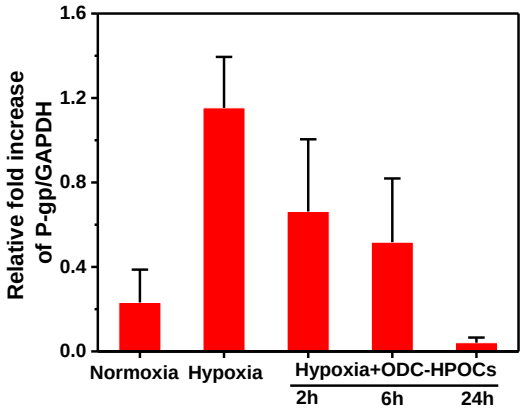


**Figure S5.** Semi-quantification of the P-gp expression (Figure 2D) *in vitro* (n=3).

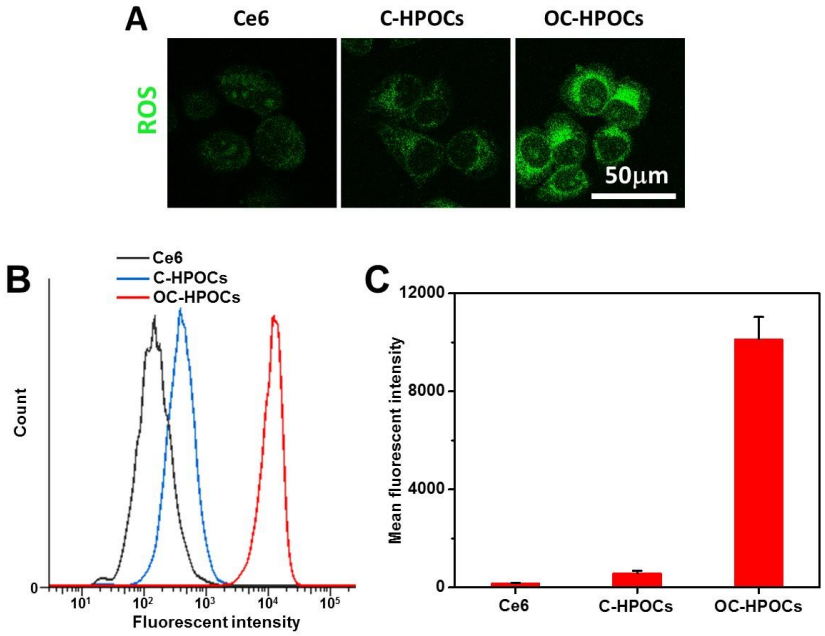


**Figure S6.** (A) Fluorescence imaging of ROS generation in MCF-7 cells treated with Ce6, C-HPOCs and OC-HPOCs (Ce6-encapsulated HPOCs) with laser irradiation. (B) Flow cytometric profiles of ROS generation in MCF-7 cells after PDT treatment with Ce6, C-HPOCs and OC-HPOCs. (C) Mean fluorescent intensities of ROS probe in the flow cytometric analysis (Figure S6B).

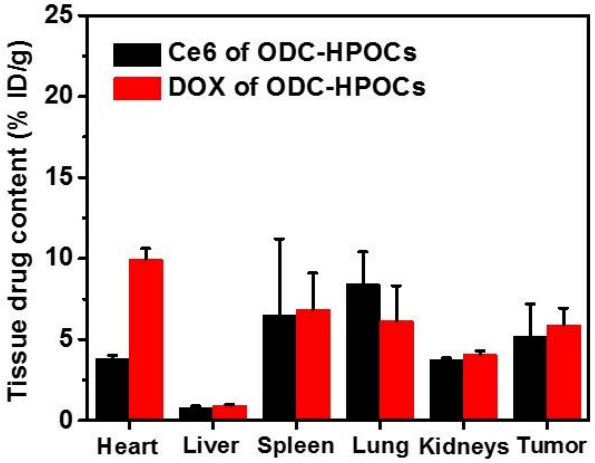


**Figure S7**. Quantitative biodistribution analysis of ODC-HPOCs by measuring fluorescence of Ce6 and DOX in tissue lysate (in the unit of % ID/g).

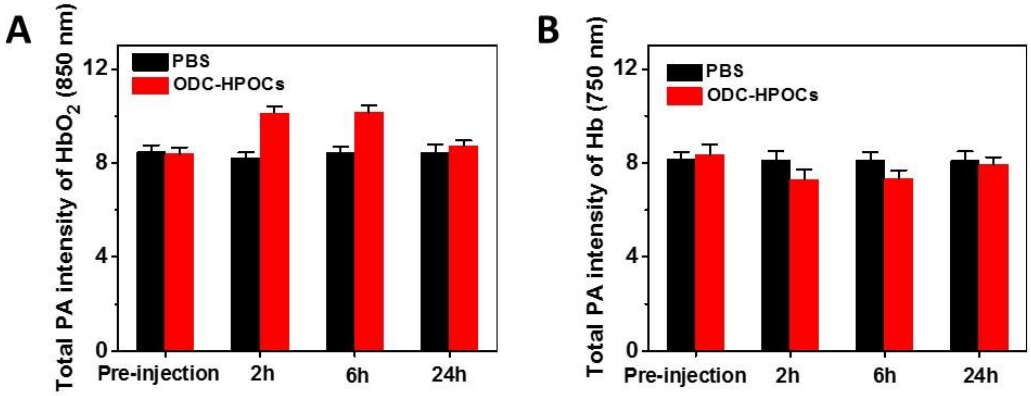


**Figure S8.** (A) Quantified PA intensities of $HbO_2$ (850 nm) around the tumor at indicated time points. (B) Quantified PA intensities of Hb (750 nm) around the tumor at indicated time points.

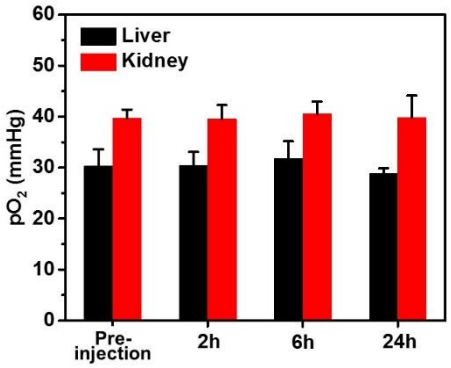


**Figure S9.** Measurement of $pO_2$ in liver and kidney by OxyLite device (Oxford Optronix, UK) after mice were intravenously injected with ODC-HPOCs.

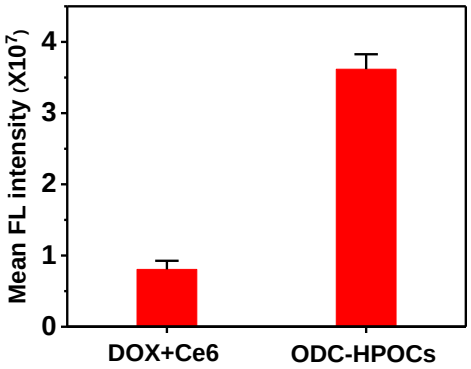


**Figure S10.** Quantified FL intensities of DOX around the tumor at 24h after intravenous administration of DOX+Ce6 and ODC-HPOCs, respectively.

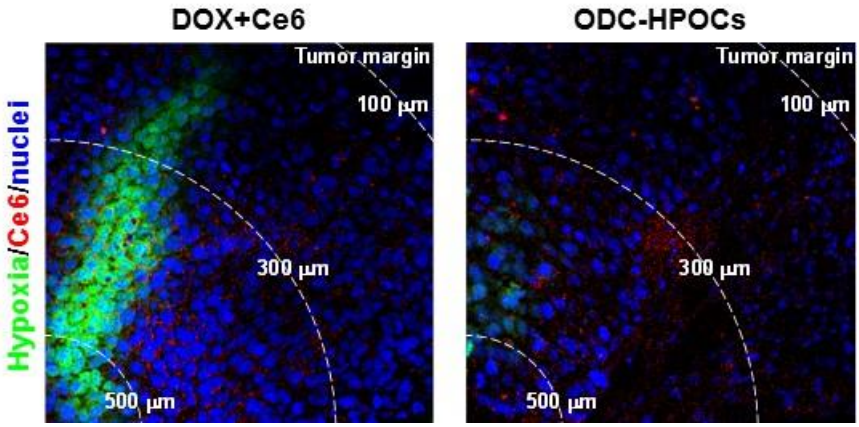


**Figure S11.** Distribution of hypoxia (green), Ce6 (red) and nuclei (blue) in tumor 24h after intravenous injection of DOX+Ce6 and ODC-HPOCs.

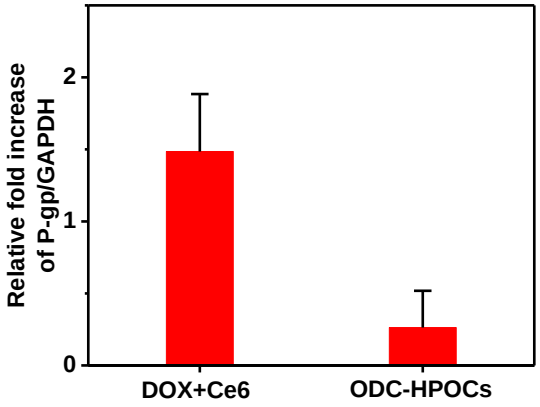


**Figure S12**. Semi-quantification of the P-gp expression *in vivo*.

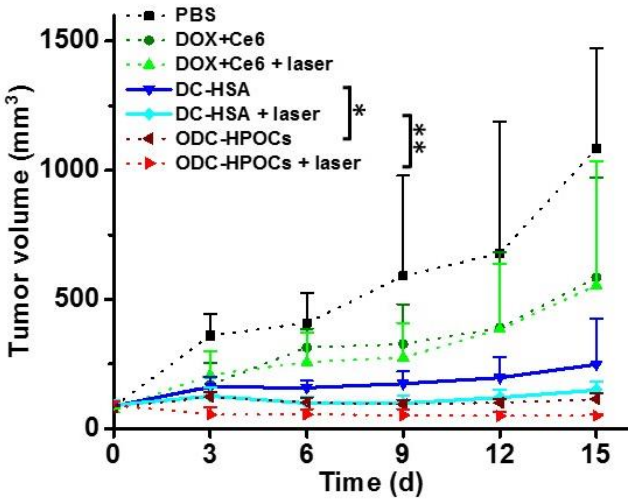


**Figure S13.** Tumor growth curves of DC-HSA and DC-HSA+laser. The groups of Figure 4I were added as dash lines for comparison. *P < 0.05, **P < 0.01.

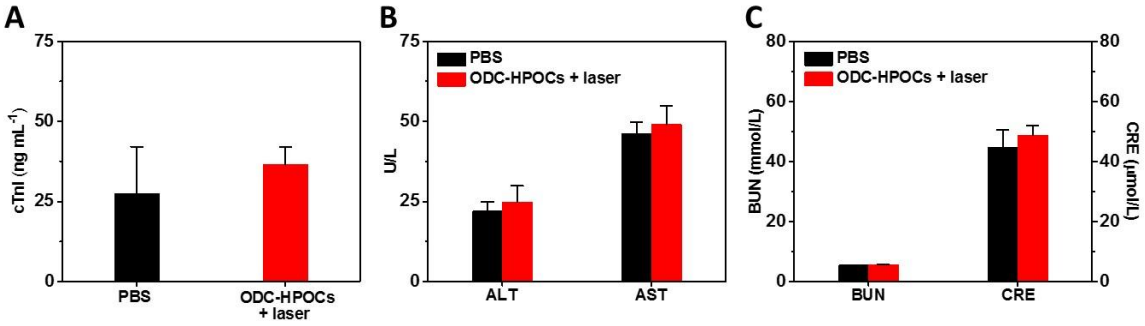


**Figure S14.** Serum levels of (A) cTnI (cardiac function marker), (B) ALT/AST (liver function marker) and (C) BUN/CRE (renal function marker) of mice on 15 day after different treatments (n=5).