# Supplementary Material

# Photosensitizer-driven nanoassemblies of homodimeric prodrug for self-enhancing activation and synergistic chemo-photodynamic therapy

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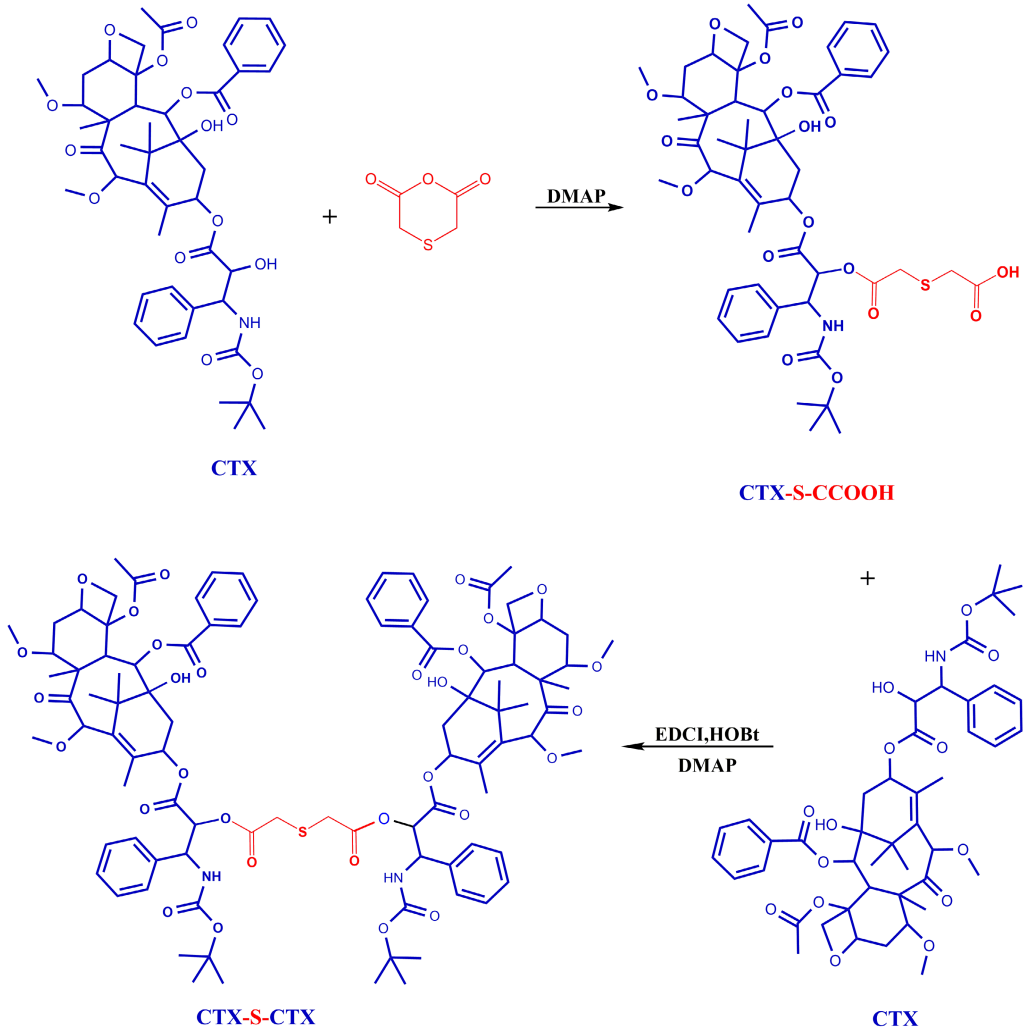


**Figure S1.** Synthetic route of CTX-S-CTX.

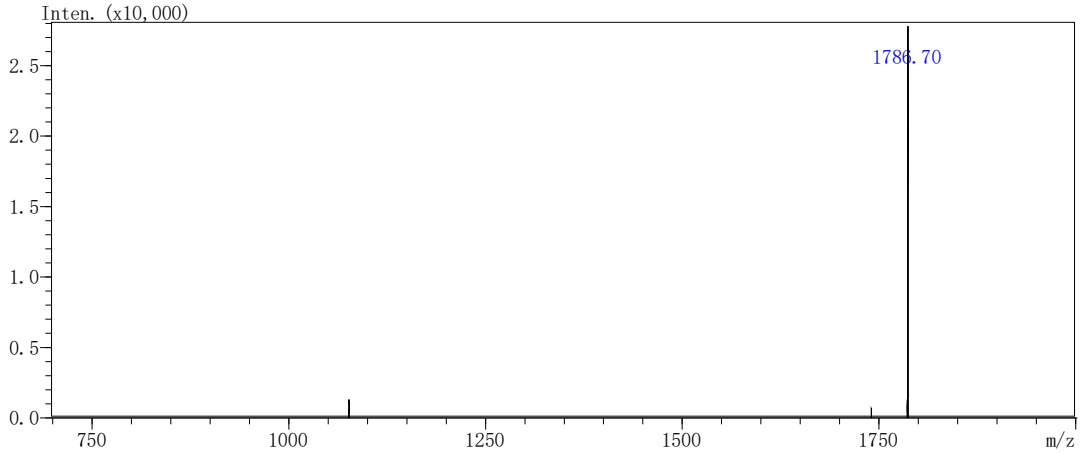


**Figure S2.** Mass spectrum of CTX-S-CTX.

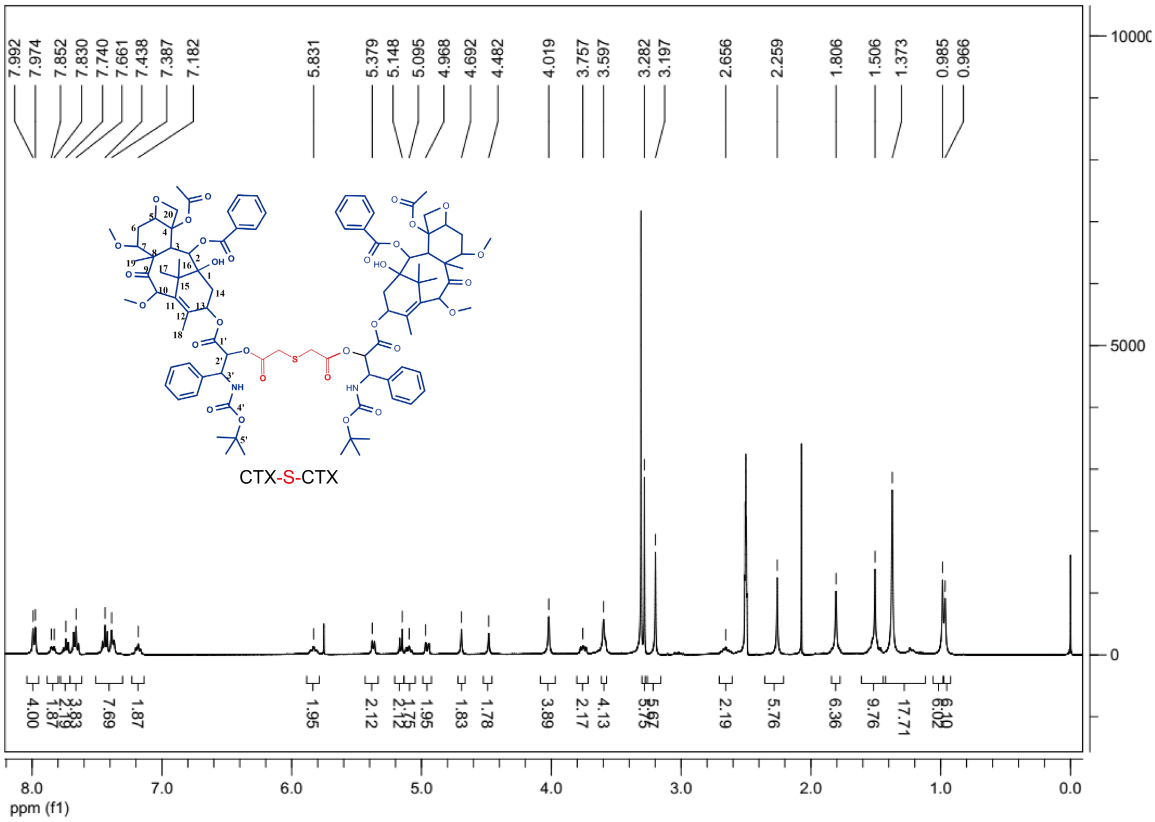


**Figure S3.** $^{1}$H-NMR spectrum of CTX-S-CTX

**$^{1}$H NMR results:** $^{1}$H NMR (400 MHz, DMSO-d6, ppm): δ 7.97-7.99 (d, 4H, Ar-H), 7.83-7.85 (d, 2H, Ar-H), 7.740 (t, 2H, Ar-H), 7.661 (t, 4H, Ar-H), 7.39-7.44 (d, 6H, Ar-H), 7.182 (t, 2H, –NH–), 5.831 (t, 2H, 13-CH), 5.379 (d, 2H, 2-CH), 5.148 (d, 2H, 3'-H), 5.095 (t, 2H, 10-CH),4.968 (s, 2H, 2'-H), 4.692 (d, 2H, 5-CH), 4.482 (d, 2H, 7-H), 4.019 (s, 4H, 20-$CH_2$), 3.757 (m, 2H, 7-CH), 3,597 (s, 4H, $CH_2SCH_2$), 3.282 (s, 6H, 10-$OCH_3$), 3.197 (s, 6H, 7-$OCH_3$), 2.656 (m, 2H, 3-CH), 2.259 (s, 6H, -OAc), 1.806 (s, 6H, 18-$CH_3$), 1.506 (10H, 14-$CH_2$, 19-$CH_3$), 1.668 (s, 6H, 19-$CH_3$), 1.373 (s, 18H, $C(CH_3)_3$), 0.985 (s, 6H, 16-$CH_3$), 0.966 (s, 6H, 17-$CH_3$).

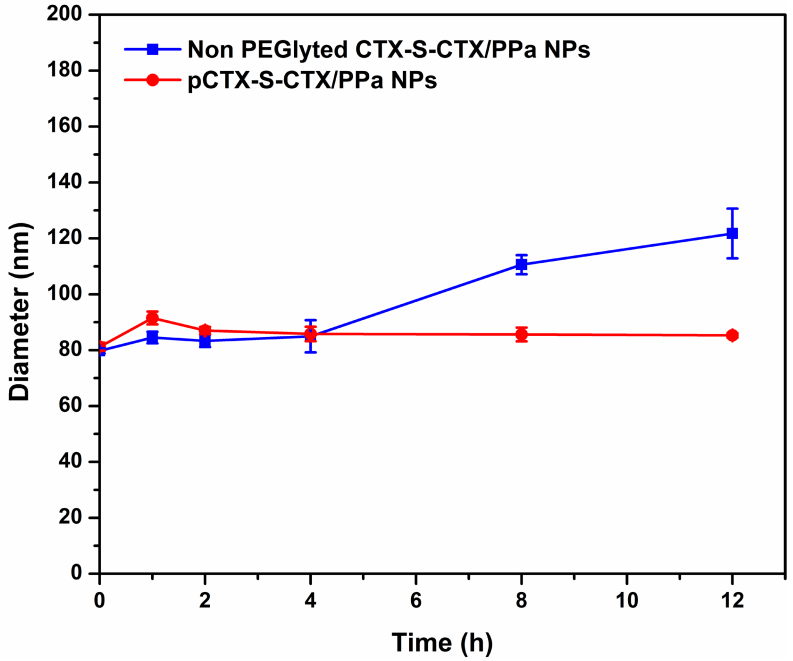


**Figure S4.** Changes in particle size of non-PEGylated CTX-S-CTX/PPa NPa and pCTX-S-CTX/PPa NPs in PBS containg 10% FBS.

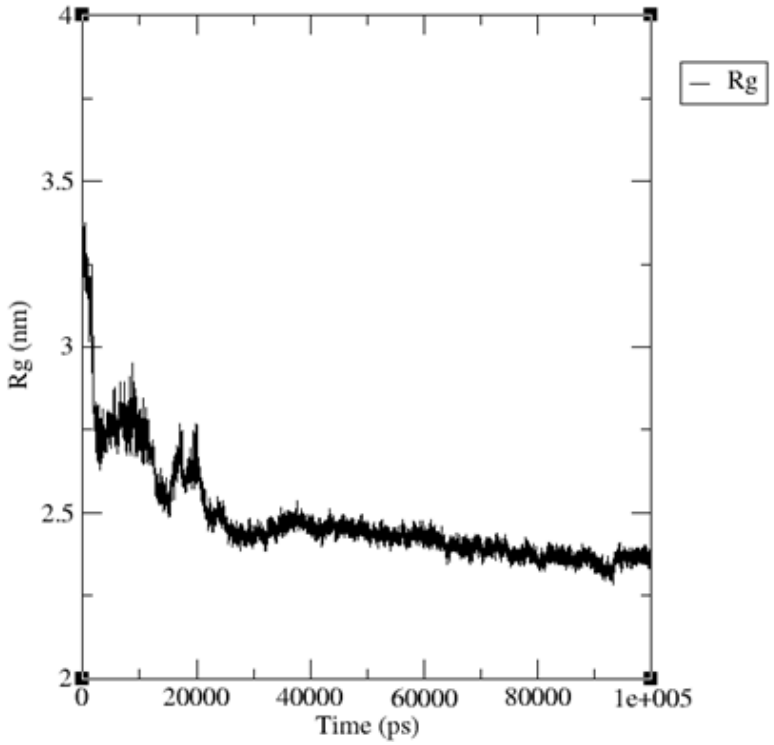


**Figure S5.** Gyration radius of the aggregated molecules during simulation.

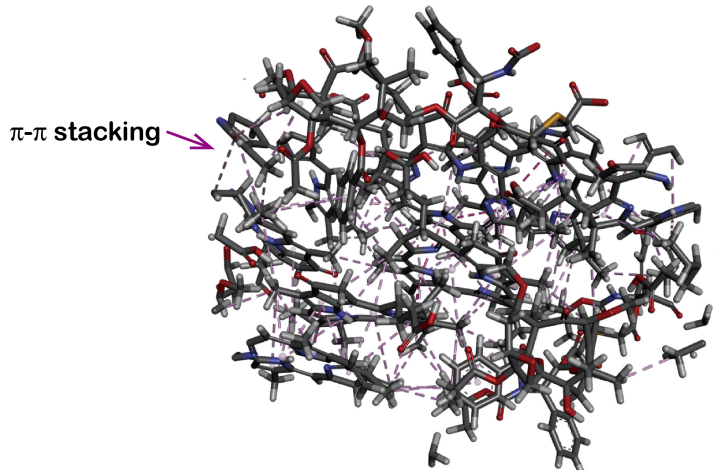


**Figure S6.** π-π stacking interaction between the selected PPa and other molecules within 1 nm after assembly.

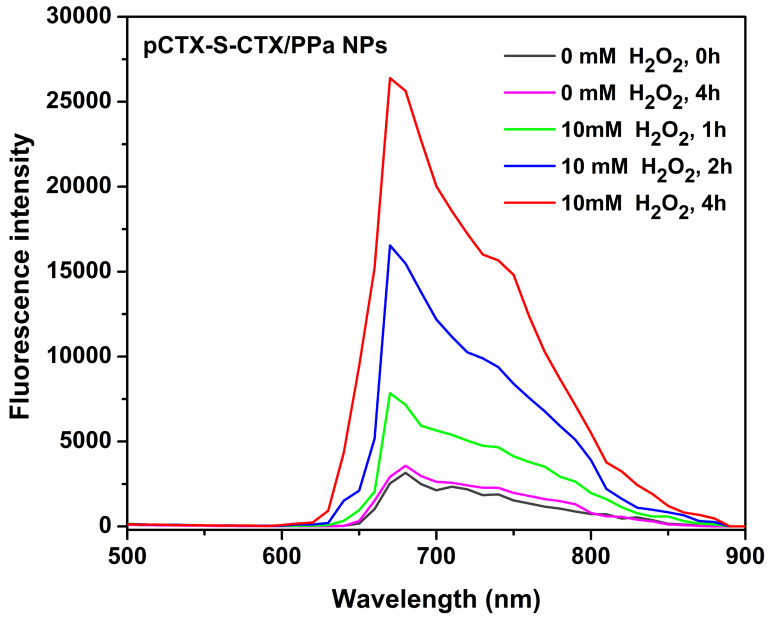


**Figure S7.** Fluorescence intensity of pCTX-S-CTX/PPa NPs in release medium containing $H_2O_2$ (10 mM) for 1, 2, or 4 h.

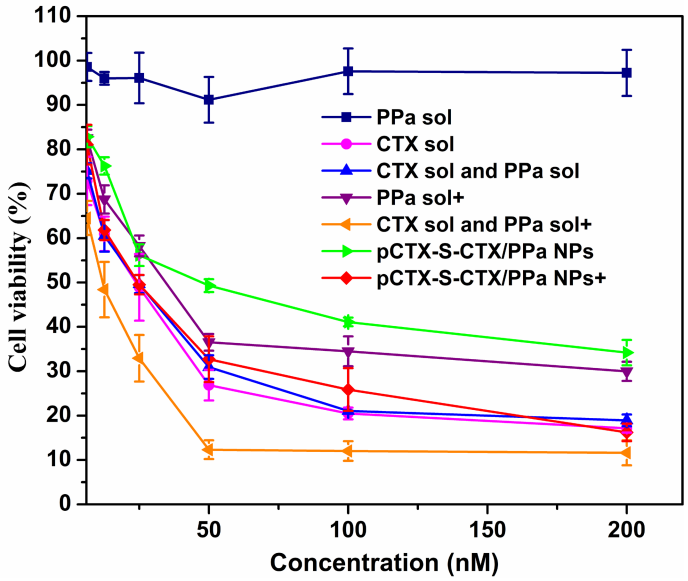


**Figure S8.** Cell viability of 4T1 cells treated with various formulations for 72 h. (+): with laser, (-): without laser.

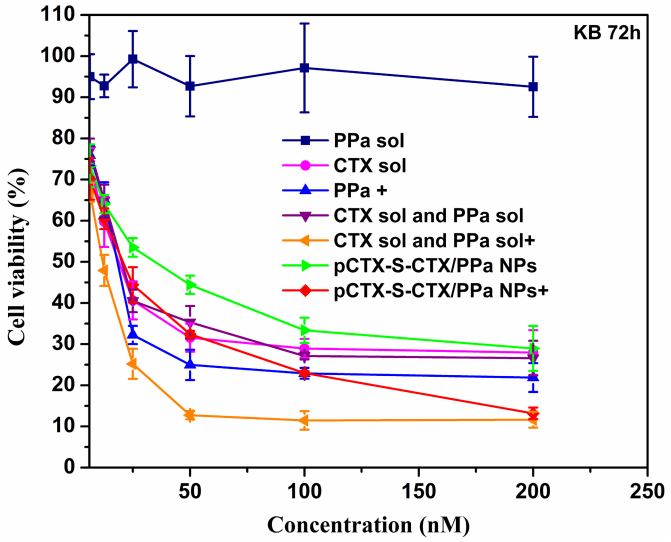


**Figure S9.** Cell viability of KB cells treated with various formulations for 72 h. (+): with laser, (-): without laser.

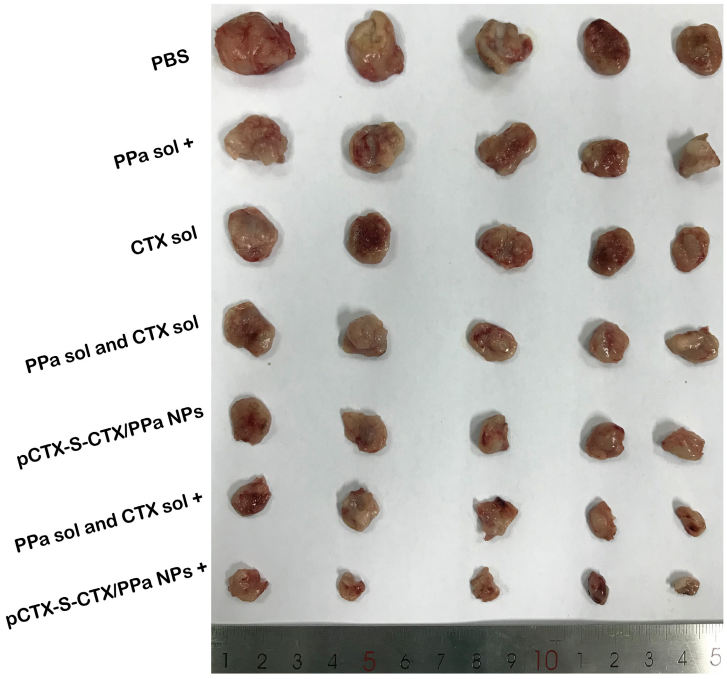


**Figure S10.** Photographs of excised tumor after various treatments. (+): with laser, (-): without laser.

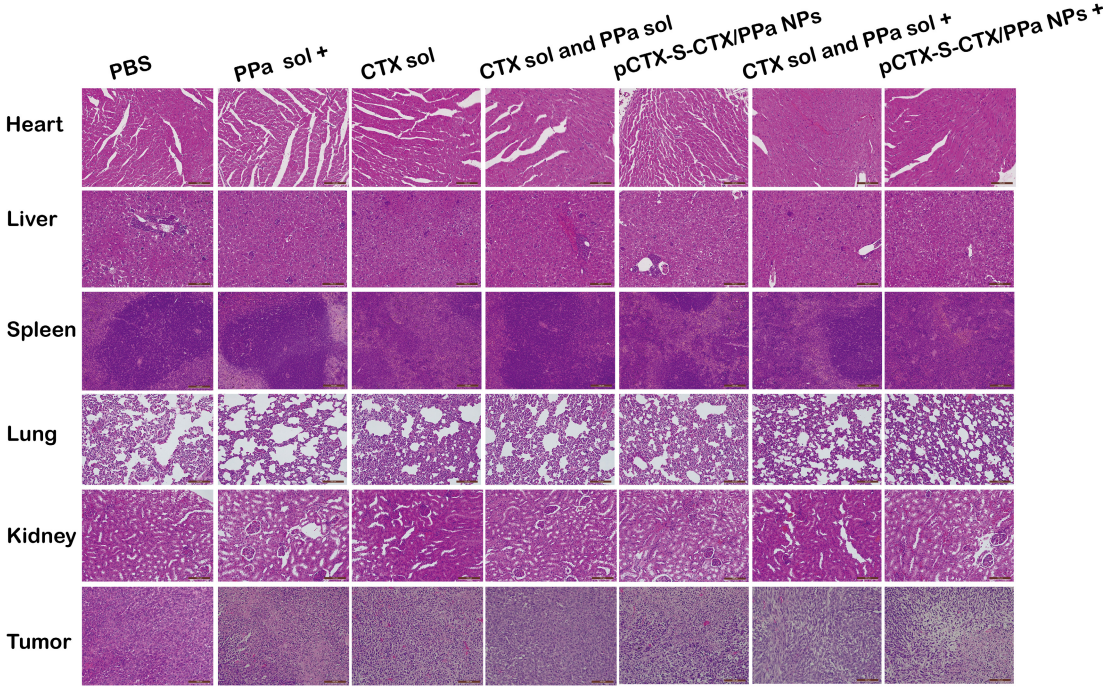


**Figure S11.** H&E staining of major organs and tumors. Scale bar = 100 μm. (+): with laser, (-): without laser.

**Table S1.** Characterization of non-PEGylated CTX-S-CTX/PPa NPs and combination index (CI) of CTX sol and PPa sol (+) at various ratios, (+): with laser (n=3).

| CTX-S-CTX/PPa | Diameter (nm) | PDI | CTX/PPa | CI (CTX and PPa) |
|---|---|---|---|---|
| 1:8 | 90.45±3.50 | 0.22±0.027 | 1:4 | 0.87 |
| 1:6 | 88.74±1.76 | 0.19±0.02 | 1:3 | 0.53 |
| 1:4 | 79.28±3.91 | 0.17±0.04 | 1:2 | 0.45 |
| 1:2 | 77.71±1.38 | 0.16±0.10 | 1:1 | 0.91 |
| 1:1 | 76.64±1.67 | 0.15±0.03 | 2:1 | 1.01 |
| 3:2 | 78.27±3.51 | 0.205±0.07 | 3:1 | 1.03 |
| 2:1 | 79.22±1.81 | 0.19±0.08 | 4:1 | 1.07 |

Notes: $CI = X \times AB_{combination}/A_{alone} + Y \times AB_{combination}/B_{alone}$. $X$ is the dose proportion of CTX in the mixture solution of PPa and CTX, $Y$ is the dose proportion of PPa in the mixture solution of PPa and CTX, $A_{alone}$ is the $IC_{50}$ of CTX solution, $B_{alone}$ is the $IC_{50}$ of PPa solution, and $AB_{combiantion}$ is the $IC_{50}$ of mixture solution.

**Table S2** Characterization of CTX-S-CTX/PPa NPs and the CIs of CTX solution and PPa solution with laser irradiation (+) at various ratios (n = 3).

| Formulations | Diameter (nm) | PDI | Zeta (mV) | Drug loading |
|---|---|---|---|---|
| Non-PEGylated CTX-S-CTX/PPa NPs | 79.28±3.91 | 0.17±0.04 | -14.6±0.56 | 97.1% |
| pCTX-S-CTX/PPa NPs | 88.70±3.08 | 0.19±0.03 | -19.9±1.40 | 80.9% |

**Table S3** $IC_{50}$ values of various formulations against 4T1 or KB cells at 48 or 72 h (n = 3). (+): with laser, (-): without laser.

| Formulations | 4T1 | | KB | |
|---|---|---|---|---|
| | 48 h | 72 h | 48 h | 48 h |
| PPa sol (-) | >1000 | >1000 | >1000 | >1000 |
| CTX sol (-) | 31.59±3.54 | 20.34±0.83 | 13.82±0.26 | 9.30±0.66 |
| PPa sol (+) | 57.35±3.24 | 41.10±1.14 | 23.59±3.06 | 18.95±1.02 |
| CTX sol and PPa sol (-) | 34.93±4.16 | 22.73±1.72 | 12.89±2.07 | 10.05±0.76 |
| CTX sol and PPa sol (+) | 14.56±1.40 | 10.30±0.67 | 6.97±0.34 | 4.93±0.71 |
| pCTX-S-CTX/PPa NPs (-) | 87.17±3.92 | 55.96±4.29 | 33.59±1.57 | 27.30±3.76 |
| pCTX-S-CTX/PPa NPs (+) | 39.77±1.59 | 26.54±1.27 | 16.86±1.99 | 10.70±0.14 |

**Table S4.** $IC_{50}$ values of PPa solution (-), CTX sol (-), and pCTX-S-CTX/PPa NPs (-) against 4T1 cells at 48 h (n = 3). (+): with laser, (-): without laser.

| Formulations | LO2 |
|---|---|
| PPa sol (-) | >1000 |
| CTX sol (-) | 111.18±3.51 |
| pCTX-S-CTX/PPa NPs (-) | >1000 |

**Table S5.** Pharmacokinetic parameters of PPa solution and pCTX-S-CTX/PPa NPs (n = 5).

| Formulations | $AUC_{(0-\infty)}$（μg/L*h） | $t_{1/2}$（h） | $C_{max}$（μg /L） |
|---|---|---|---|
| PPa sol | 9453.82±1437.54 | 3.67±0.41 | 3732.95±426.33 |
| pCTX-S-CTX/PPa NPs | 28761.59±1098.95 | 3.37±0.51 | 11425.56±578.82 |