

Supporting information

Title:

Self-engineered Binary Nanoassembly Enabling Closed-loop Glutathione Depletion-amplified Tumor Ferroptosis

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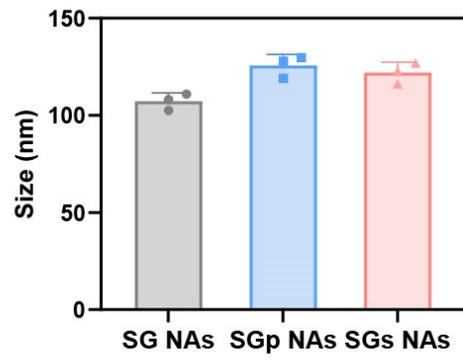


Figure S1. The DLS size of non-PEGylated and PEGylated NAs (n = 3).

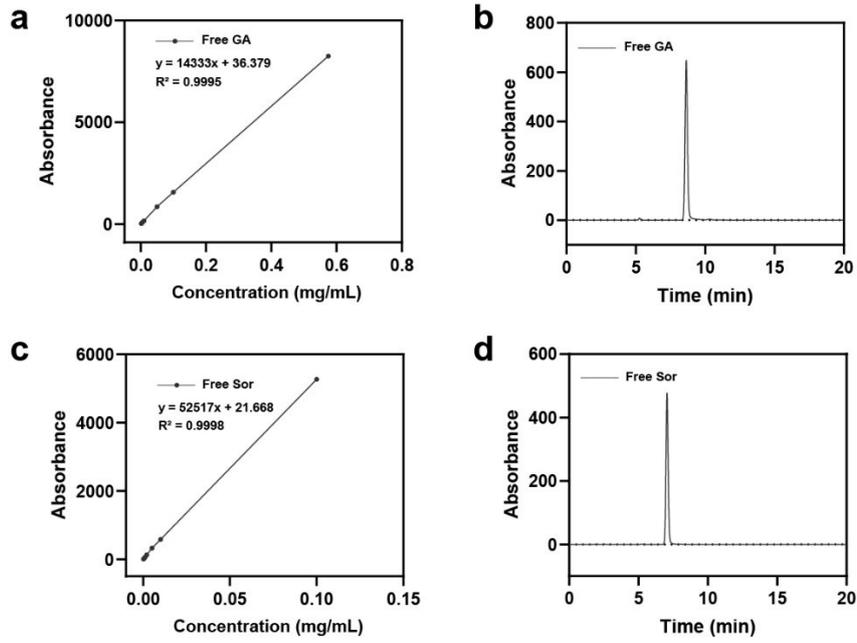


Figure S2. Standard curves and corresponding high performance liquid chromatography of free GA (a,b) and free Sor (c,d), respectively.

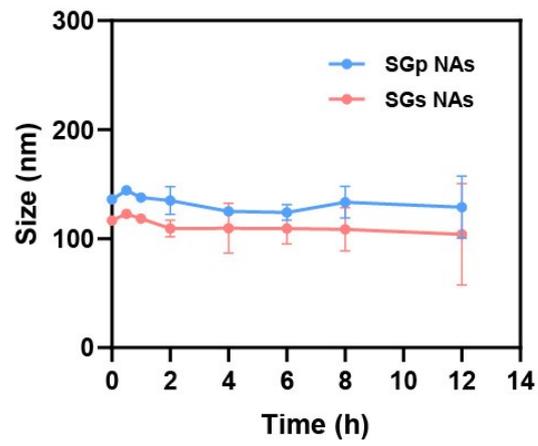


Figure S3. Colloidal stability of SGp NAs and SGs NAs incubated in PBS (pH 7.4) containing 10% serum (n = 3).

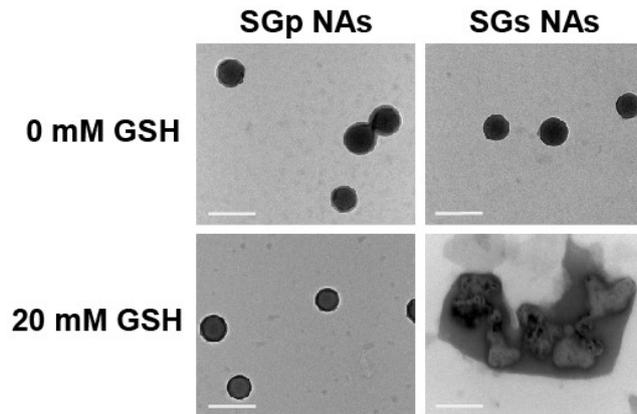


Figure S4. Particle size distribution of SGp NAs and SGs NAs in the presence of 0 mM and 20 mM GSH were obtained by TEM. Scale bar: 200 nm.

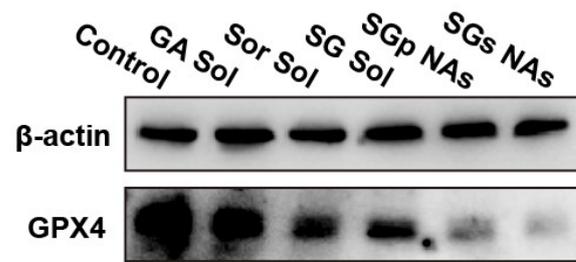


Figure S5. Western blotting analysis results of intracellular GPX4 protein in 4T1 cells after treatments.

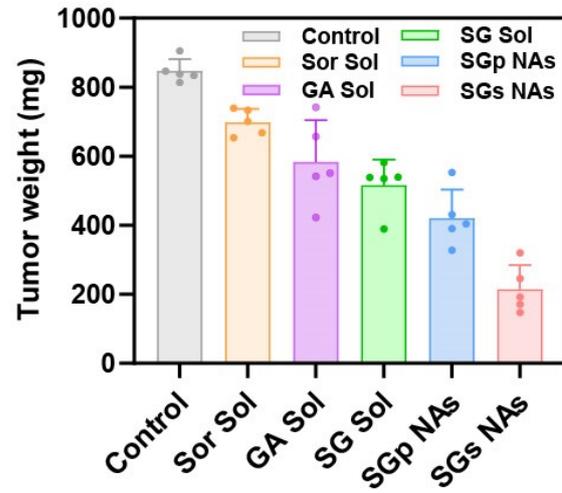


Figure S6. Tumor weight of the mice (n = 5)

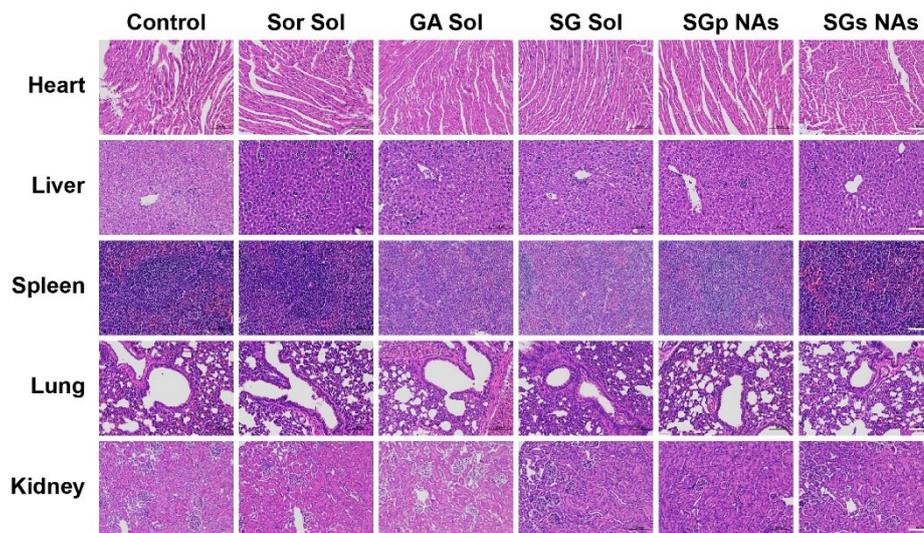


Figure S7. The H&E staining images of major organs after different treatments. Scale bar = 100 μm .

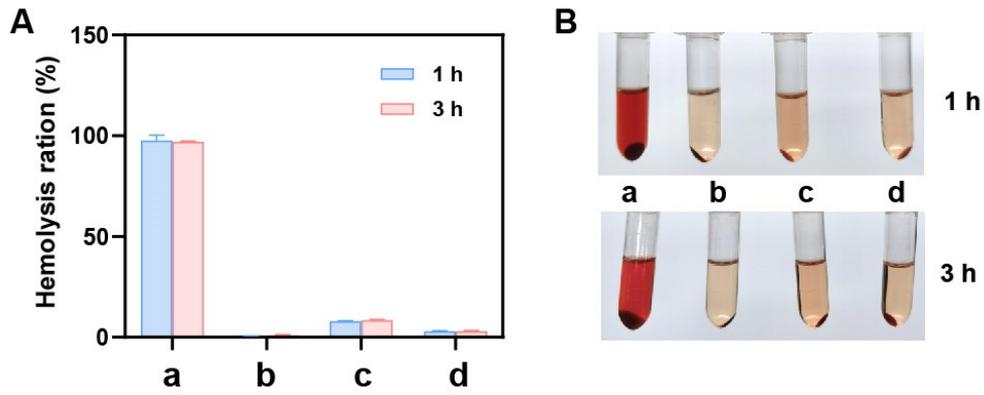


Figure S8. Hemolysis tests. (A) Hemolysis ratios of red blood cell solutions after incubating with a (water), b (saline solution), c (SGp NAs), and d (SGs NAs) for 1 h and 3 h, respectively. Saline solution and water were used as negative and positive controls, respectively (n = 3); (B) Photographs of centrifuged red blood cell solutions after various treatments for 1 h and 3 h.

Table S1. Particle sizes and PDI of Sor@GA NAs at various molar ratios (n=3).

Sor:GA	Size (nm)	PDI
1:5	118.7 ± 1.058	0.099 ± 0.052
1:4	116.2 ± 1.872	0.088 ± 0.063
1:3	115.4 ± 1.473	0.069 ± 0.025
1:2	119.6 ± 3.235	0.181 ± 0.029
1:1	109.8 ± 4.062	0.073 ± 0.052
2:1	131.3 ± 2.723	0.087 ± 0.012
3:1	129.4 ± 5.002	0.069 ± 0.079
4:1	122.8 ± 3.404	0.055 ± 0.055
5:1	129.9 ± 0.289	0.067 ± 0.053

Table S2. Synergistic cytotoxicity of Sor@GA NAs at various molar ratios (n=3).

Sor:GA	CI
1:5	0.45
1:4	0.33
1:3	0.35
1:2	0.35
1:1	0.23
2:1	0.27
3:1	0.46
4:1	0.47
5:1	0.49

Table S3. Mean size, polydispersity index, and Zeta potential of SGp NAs and SGs NAs.

Formulations	Size (nm)	PDI	Zeta potential (mV)
SGp NAs	130.3 ± 1.768	0.106 ± 0.021	-32.67 ± 0.478
SGs NAs	114.4 ± 2.207	0.073 ± 0.018	-32.43 ± 0.330

Table S4. IC₅₀ value of different formulations against 4T1 cells.

Formulations	IC ₅₀ (nM)
Sor Sol	161.7 ± 56.27
GA Sol	70.18 ± 22.70
SG Sol	31.67 ± 9.155
SGp NAs	20.50 ± 5.343
SGs NAs	13.03 ± 1.039

Table S5. Pharmacokinetic parameters after various treatments (n = 6).

Formulations	$t_{1/2}$ (h)	$AUC_{0-24\text{ h}}$ ($\mu\text{g}\cdot\text{h}/\text{mL}$)	C_{max} ($\mu\text{g}/\text{mL}$)	MRT (h)
Cy7 Sol	0.451 ± 0.256	535.212 ± 324.809	1094.17 ± 251.236	0.439 ± 0.164
SGp-Cy7 NAs	3.483 ± 0.669	2458.857 ± 1158.726	1484.176 ± 2255.113	2.895 ± 0.752
SGs-Cy7 NAs	2.738 ± 0.525	2650.222 ± 911.425	1399.499 ± 291.189	2.914 ± 0.500