Supporting Information

Bioinspired peptosomes with programmed stimuli-responses for sequential drug release and high-performance anticancer therapy

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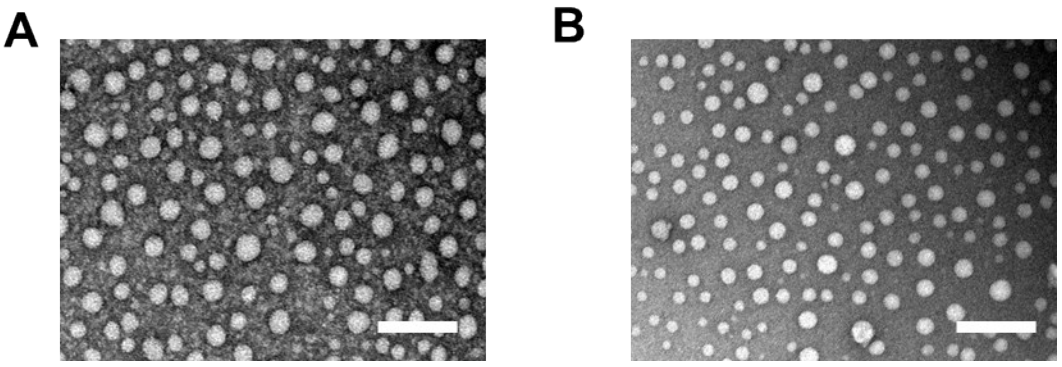

Figure S1. TEM images of peptosomes before (A) and after crosslinking (B) in PBS of pH 7.4. The morphology did not change after disulfide bonds cross-linking. Scale bar: 200 nm.

Table S1. Zeta potential, particle size and loading efficiency of different PSs formulation in PBS of pH 7.4.

Nanoparticle	Zeta potential (mV)	Size （nm）	Loading efficiency （%）
PS	-17.5±0.45	19.35±5.6	18.82
Mal-PS	+5.16±0.37	22.75±6.9	17.39
PePm/PS	+10.25±0.42	30.38±9.1	16.37

Upon surface decoration with Mal and PePm, the diameter and zeta potential of the obtained agents displayed gradual increase in sequence of PS, Mal-PS, and PePm/PS. The decoration process has no effect on the Cur loading efficiency.

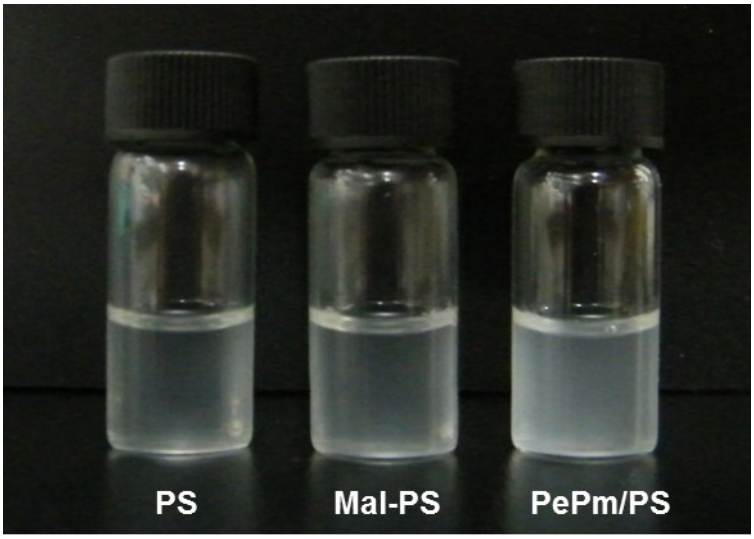

Figure S2. Photographs of the PS, Mal-PS, and PePm/PS aqueous solution. PS formulations possessed excellent dispersibility, which is favorable for intravenous injection.

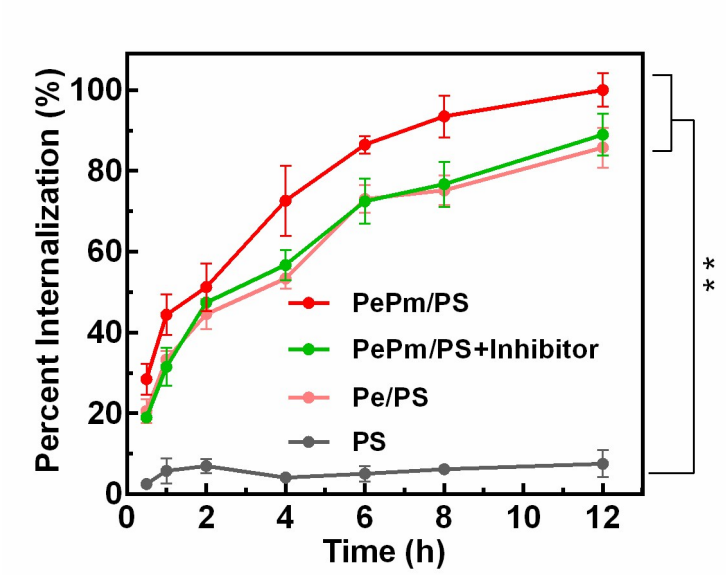

Figure S3. Cellular internalization kinetics of PePm/PS, PePm/PS + inhibitor, Pe/PS and PS in 4T1 cells. 4T1 cells were seeded in 24-well plate and incubated for 24 h to allow cell attachment. The cells were fixed with or without 10 nM MMP Inhibitor Ⅲ for 30 min, and then cultured with 0.1 mg/mL Cy-3 loaded PS formulations (red) for different time intervals until 12 h. Subsequently, cells were washed by PBS, and the uptake amount of Cy3-loaded PS was determined on a CyAn ADP 9 color flow cytometer (FCM). Data were obtained from 15000 cells for each sample.

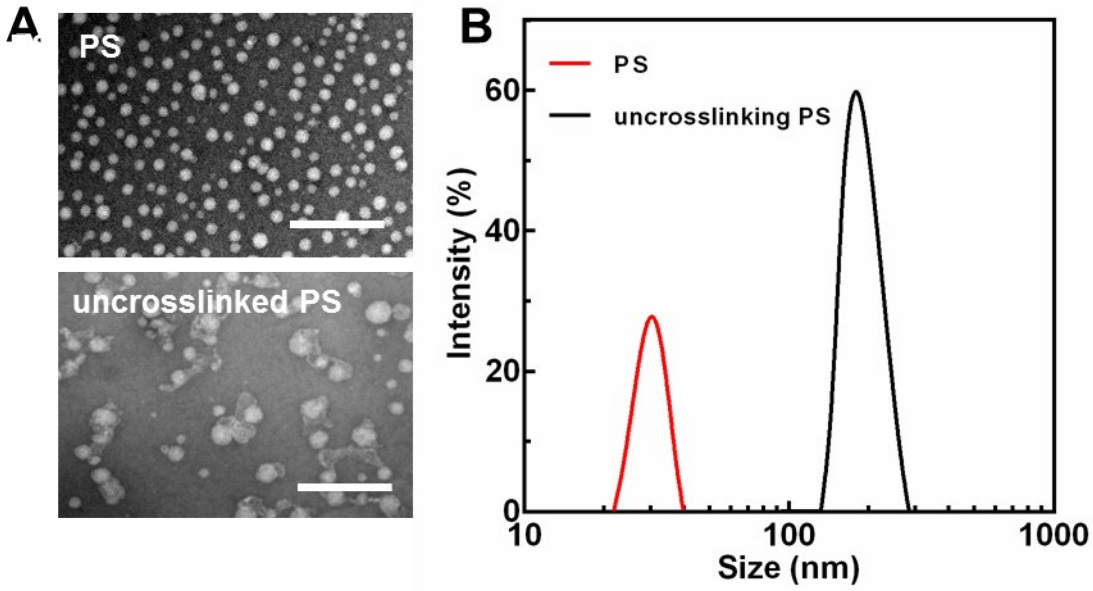

Figure S4. Peptosomes stability at pH 5 in PBS. (A) TEM images and (B) corresponding size varieties of PS and uncrosslinked PS at pH 5 in PBS; Scale bar: 200 nm. Usually PS without cross-linking is not stable at pH 5. After crosslink process, the stability of PS improves significantly in acidic environment. In this case, undesirable early release at lysosomes can be avoided.

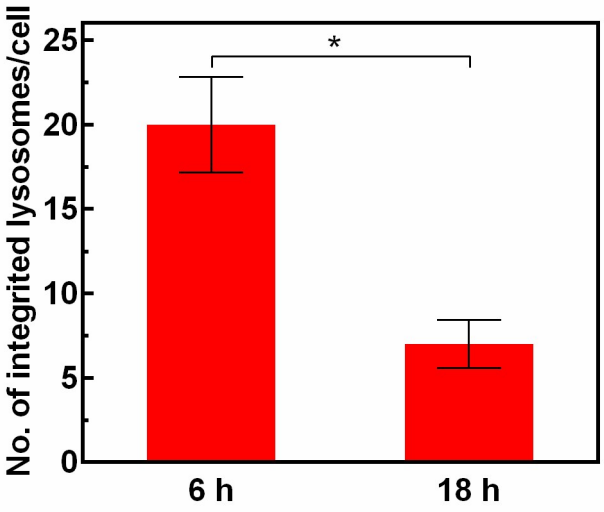

Figure S5. Quantitative analysis of integrated lysosomes in 4T1 after incubation with PePm/PS.

Cy3-labeled PePm/PS (0.1 mg/mL) were added to 4T1 cells and incubated at 37 °C. The cell nuclei were stained with Hoechst . Corresponding fluorescence images were taken using a

spinning-disk confocal system (UltraVIEW VoX, PerkinElmer) at 6 and 18 h. Number of integrated lysosomes/cell in the fluorescence images were analyzed by Columbus™ system.

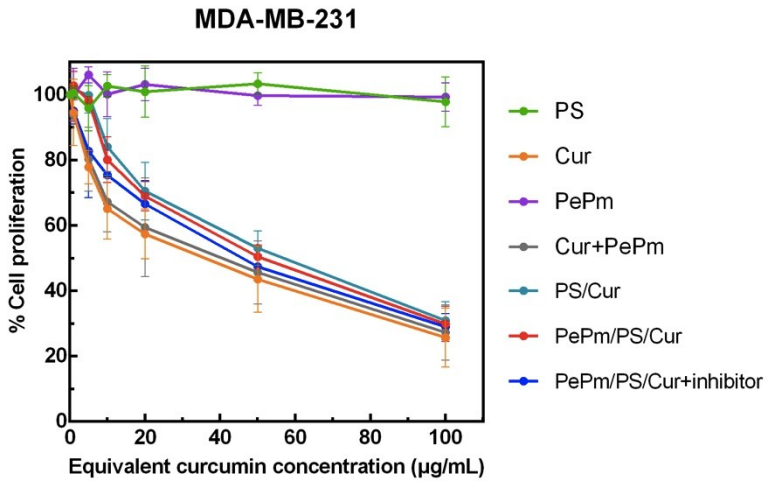

Figure S6. Cytotoxicity of different formulations on ErbB-2 negative human breast cancer cell line MDA-MB-231. As expected, little effect was observed on both free and conjugated PePm.

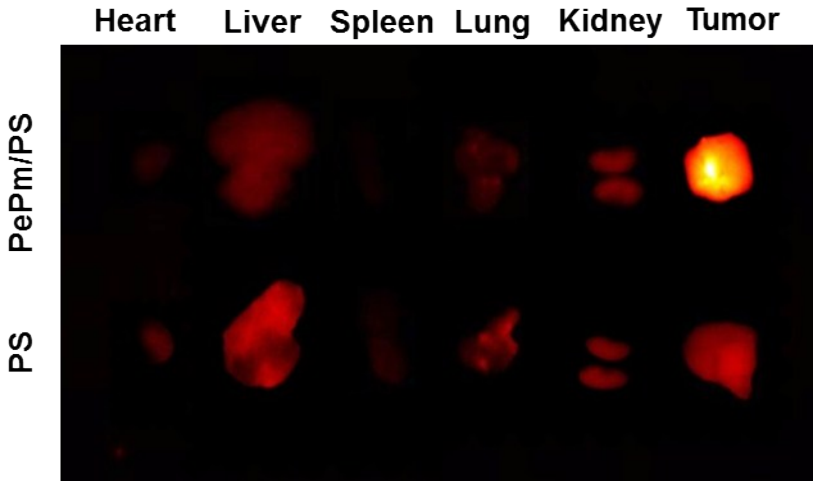

Figure S7. Analysis of drug distribution in visceral organs and tumor 24 h post intravenous injection. Representative NIRF images showing the PS and PePm/PS distribution in visceral organs and tumor.

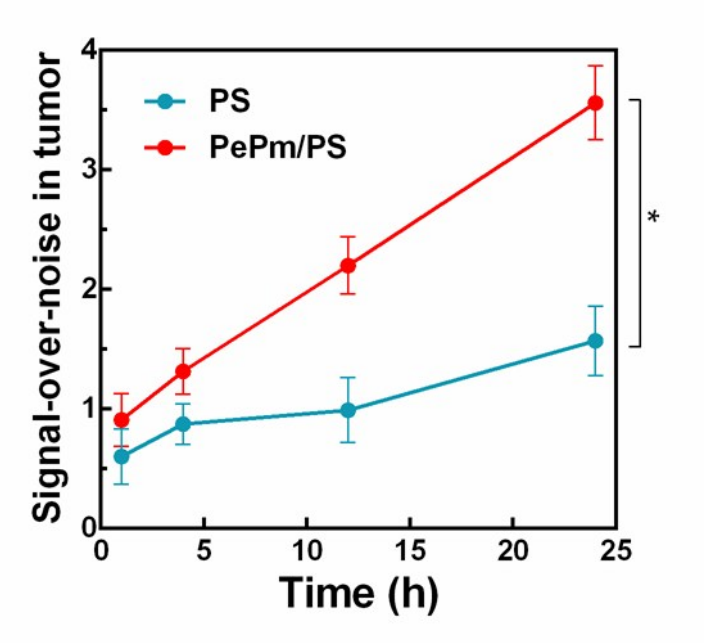

Figure S8. Signal-over-noise ratio (S/N) of fluorescence in tumor 24 h post injection. The S/N ratio was very small (~0.9) and only increased slightly at later time points after the injection of naked PS. In contrast, the ratio in the PePm/PS group reached 3.5 at 24 h after injection, demonstrating the desired guiding ability of Pe.

Table S2. The record of lung metastasis and bone metastasis during 40 days.

	Lung	Bone
PBS	6/6	5/6
Cur	6/6	5/6
PS/Cur	4/6	4/6
PePm/PS/Cur	0/6	0/6

Table S3. Determination of the serum biochemical parameters of tumor-bearing mice. Blood was sampled from six tumor-bearing mice each group (40 days after tumor cells injection). The aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) serum was analyzed by Toshiba Acute Biochemical Analyzer TBA-40FR. Compared to other groups, the serum chemistry values (AST, ALT, BUN, LDH, ALP) in PePm/PS/Cur group was within the normal range, manifesting the absence of hepatic, renal and cardiac toxicity.

	PBS	Cur	PS/Cur	PePm/PS/Cur	Normal range
AST	371±65	560±190	317±69	150±37	54~298
ALT	228±76	296±54	180±65	40±23	17~77
BUN	5.75±0.93	6.98±0.54	11.5±4.2	12.7±4.2	8~33
LDH	3822±672	3579±735	2564±553	660±198	215~1024
ALP	40±21	30±23	42±20	147±34	60~209

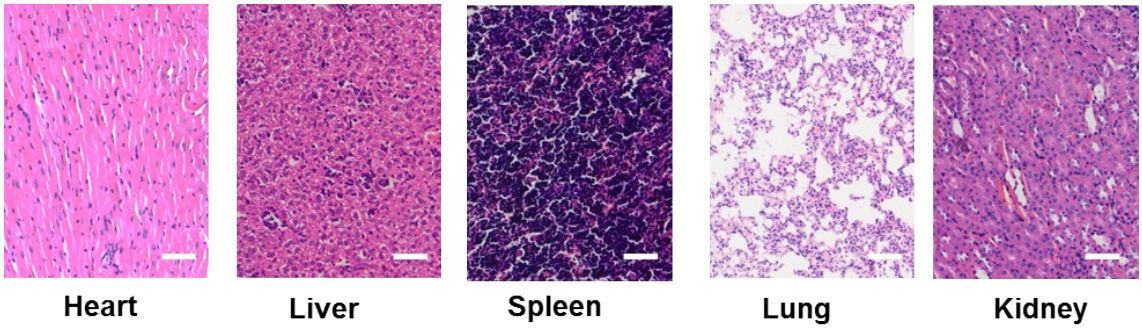

Figure S9. Histological examination using standard Hematoxylin and eosin (HE) staining of tumor-bearing mice organs after treatment with PePm PS/Cur (40 days after tumor cells injection). No organ damage was observed. Scale bar :100 μm.