SUPPORTING INFORMATIONS for

Cetuximab functionalization strategy for combining active targeting and antimigration capacities of hybrid composite nanoplatform applied to deliver 5-fluorouracil: Toward colorectal cancer treatment

Ranran Chen[a, 1], Yuanjian Huang[b, 1], Lu Wang[a, 1], Jiahui Zhou[a, 1], Yuqian Tan[a], Chaofan Peng[a], Peng Yang[a], Wen Peng[b], Jie Li[a], Qiou Gu[a], Yuchen Sheng[c], Yan Wang[c], Guoqiang Shao[d], Qing Zhang[c,*] and Yueming Sun[b, *]

[a] Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China.

[b] Department of General Surgery, Colorectal Center, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China.

[c] Department of Pharmaceutics, School of Pharmacy, Nanjing Medical University, Nanjing 211166, China.

[d] Department of Nuclear Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China.

Corresponding Authors

[*]E-mail: sunyueming@njmu.edu.cn (Yueming Sun).
[*]E-mail: qzhang@njmu.edu.cn (Qing Zhang).

Author Contributions

[1] Ranran Chen, Yuanjian Huang, Lu Wang and Jiahui Zhou contributed equally to this work.

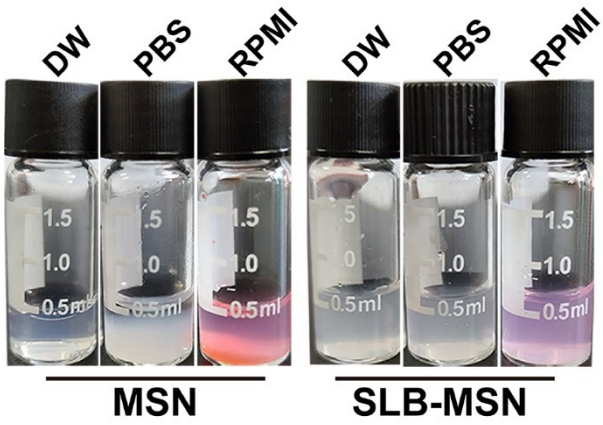

Figure S1. Dispersing stability of MSN and SLB-MSN in DW, PBS, RPMI media.

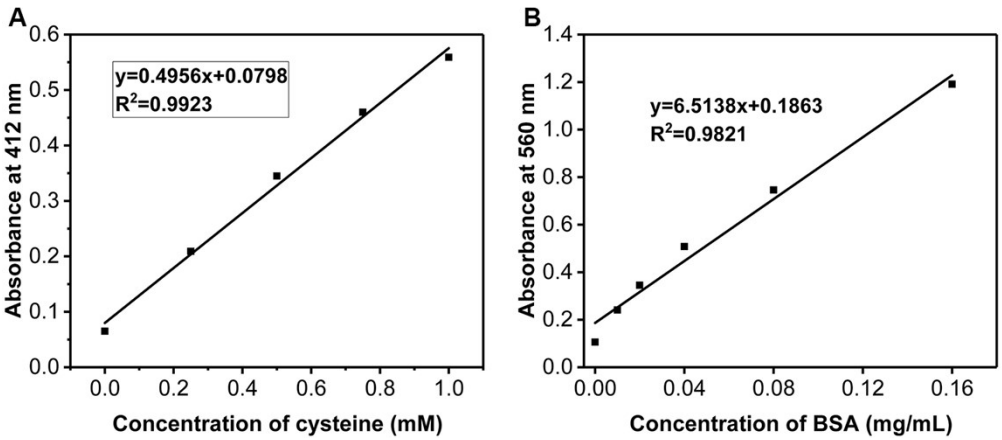

Figure S2. (A) The calibration curve of cysteine solution at serial concentrations after reaction with Ellman's reagent. (B) The calibration curve of BSA solution at serial concentrations.

Measurement of sulfhydryl groups on CTX using Ellman's reagent

The number of sulfhydryl groups on CTX was measured with Ellman's reagent (5,5'-Dithio-bis-2-nitrobenzoic acid). The cysteine solution (0.25 mM to 1 mM) was used to construct calibration curve for determination of sulfhydryl group (**Fig. S2 A**). Four mg of Ellman's reagent was dissolved in 1 mL of 0.1 M sodium phosphate buffer (PBS, pH 8.0, containing 1mM of EDTA). Then the obtained samples of CTX-SH were reacted with Ellman's reagent according to the following ratio: 50 μL of Ellman's reagent + 1 mL of the PBS + 250 μL of sample. After incubation for 15 minutes at room temperature, the absorbance of the resultant solution at 412 nm was detected to measure the concentration of sulfhydryl groups. According to the standard curve (**Fig. S2 A**), the concentration of sulfhydryl groups on CTX-SH was 0.067 mM. The concentration of CTX was calculated to be 2.21 mg/mL (which was ~0.0147 mM) by using the BCA assay (**Fig. S2 B**). Therefore, about 4.56 (=0.067/0.0147) sulfhydryl groups were conjugated with 1 CTX molecule after the reaction with Traut's reagent.

Determination of antibody density on the surface of nanoparticles

After modifying SLB-MSN with CTX, the concentration of CTX docked on nanoparticles was calculated to be 0.128 mg/mL according to the calibration curve of BSA solution (**Fig. S2 B**). Therefore, about 25.6 μg (0.128 mg/mL × 0.2 mL) of CTX was modified on 10 mg of SLB-MSN, which was equal to ~2.6 μg CTX per 1 mg of SLB-MSN. And the result indicated that the functionalization efficiency of antibody was ~0.26% (based on MSN). According to the previous study [1], we calculated the number of antibody molecules on one nanoparticle surface.

$$V_{MSN} = \frac{4}{3}\pi\left(d/2\right)^3$$

$$n_{MSN} = \frac{(m/\rho) + (m * Vpore)}{VMSN}$$

3 nm pore MSN, d = 57 nm, m = 0.01 g, ρ = 2.5 g/cm^3, V_{pore} =0.533 cm^3/g.

Thus, $n_{MSN} \approx 0.92 \times 10^{14}$. There were about 26 μg of antibody (170k Da) was modified on 10 mg of SLB-MSN (based on MSN). Averagely, there were approximately one antibody molecule were docked on one nanoparticle surface (based on MSN).

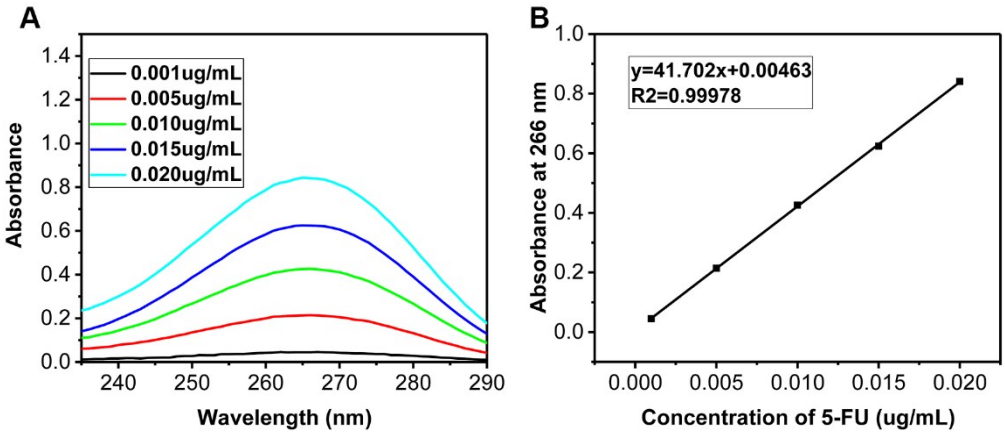

Figure S3. (A) The UV-Vis spectra of 5-FU solution at different concentrations. (B) The calibration curve for determination of 5-FU concentration.

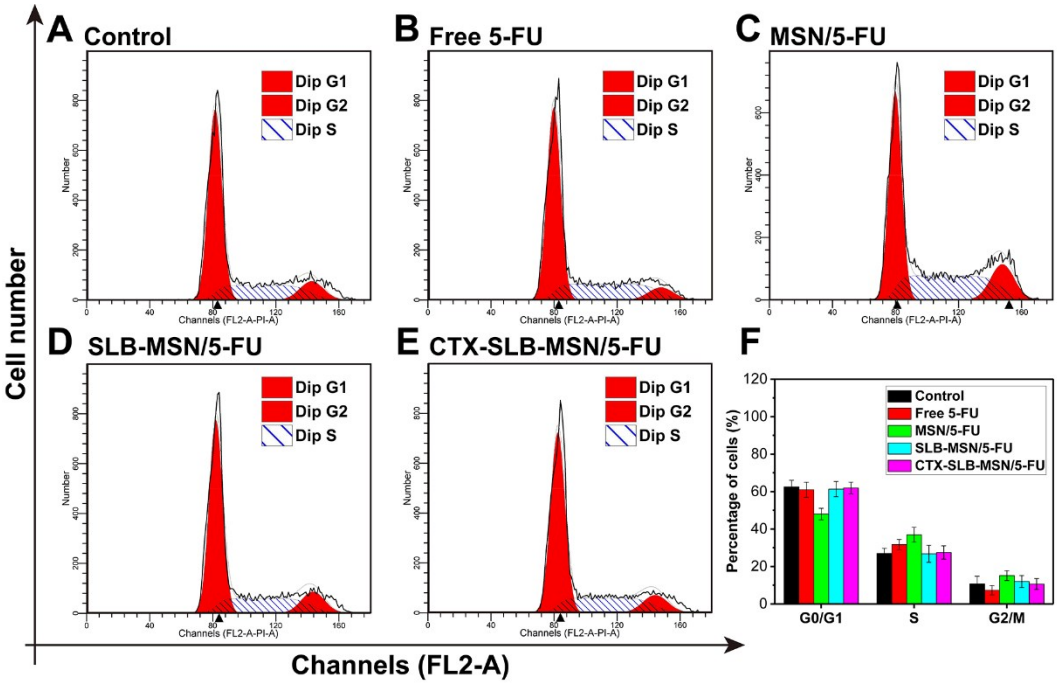

Figure S4. (A-E) The typical flow cytometry histograms of SW-620 cells treated with (A) complete medium (control), (B) free 5-FU, (C) MSN/5-FU, (D) SLB-MSN/5-FU, (E) CTX-SLB-MSN/5-FU. (F) The distribution percentage of SW-620 cells in each phase of cell cycle after different treatments. Mean ± SD, n =3.

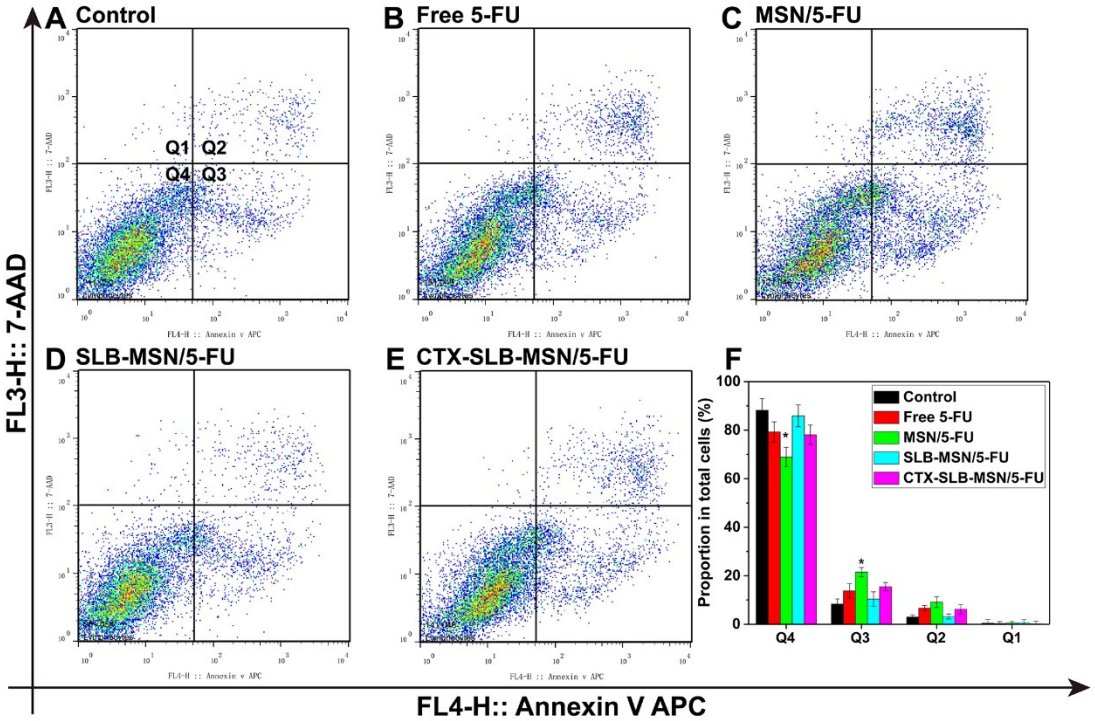

Figure S5. (A-E) The flow cytometric analysis of cell apoptosis for SW-620 cells treated with (A) complete medium (control), (B) free 5-FU, (C) MSN/5-FU, (D) SLB-MSN/5-FU, (E) CTX-SLB-MSN/5-FU, respectively. (F) The percentage of each cell population was presented after the corresponding treatments. Mean ± SD, n=3.

Table S1. The list of primary antibodies used in the study

Anti-EGFR antibody	Rabbit monoclonal	Abcam, #ab52894	WB: 1:5000
Anti-EGFR (phospho Y1068) antibody	Rabbit monoclonal	Abcam, #ab40815	WB: 1:2000
Anti-PI3K antibody	Rabbit monoclonal	Cell Signaling Technology (CST), #4249	WB: 1:1000
Anti-PI3K (phospho) antibody	Rabbit polyclonal	Abcam, #ab182651	WB: 1:500
Anti-AKT antibody	Rabbit monoclonal	CST, #4691	WB: 1:1000
Anti-AKT (phospho) antibody	Rabbit polyclonal	Abcam, #ab38449	WB: 1:500
Anti-GAPDH antibody	Mouse monoclonal	Abcam, #ab8245	WB: 1:5000

Table S2. IC_{50} (μg/mL) of drug loaded nanoparticles on colorectal cancer cells.

	Free 5-FU	MSN/5-FU	SLB-MSN/5-FU	CTX-SLB-MSN/5-FU
HCT-116	12.2 ± 1.2	8.2 ± 0.7	/	2.1 ± 0.4
SW-620	11.5 ± 0.8	6.7 ± 0.9	/	>20

[1] H. Meng, M. Wang, H. Liu, X. Liu, A. Situ, B. Wu, Z. Ji, C. H. Chang, and A. E. Nel, *ACS Nano*, 2015, **9**, 3540-3557.