Nanoparticle binding to urokinase receptor on cancer cell surface triggers nanoparticle disintegration and cargo release

Shijie Li¹,², Cai Yuan¹, Jincan Chen², Dan Chen¹, Zhuo Chen², Wenlie Chen³, Shufeng Yan², Ping Hu², Jiping Xue¹, Rui Li², Ke Zheng¹,*, Mingdong Huang¹,*

1. Fuzhou University, Fuzhou, Fujian 350116, China.

2. State Key Laboratory of Structure Chemistry, Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences, Fuzhou, Fujian 350002, China.

3. Fujian Academy of Integrative Medicine, Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian 350122, China.

*Corresponding author contact e-mails: HMD_lab@fzu.edu.cn or zhengke_qust@163.com
Figure S1. A) Study on the optimal ethanol concentration for the ATF-HSA:CPZ@RRNP or HSA:CPZ@NP preparation. The diameters of nanoparticles can be tuned by ethanol concentration. B) Effect of the starting protein/CPZ feed molar ratio on the amount of drug loaded per protein. The feed molar ratio of 1:10 was used for both nanoparticles.
Figure S2. A) UV-Vis spectra of ATF-HSA:CPZ@RRNP (red) and HSA:CPZ@NP (green) at pH 8.0 showed characteristic absorption of CPZ. B) Both ATF-HSA:CPZ@RRNP and HSA:CPZ@NP were quite stable for at least 14 days based on dynamic light scattering (DLS) in buffer solution (20 mM Tris-HCl, 50 mM NaCl, pH 8.0). C) Both ATF-HSA:CPZ@RRNP and HSA:CPZ@NP were stable in fetal bovine serum (FBS) for at least 2 days at 37 °C. 5 µM ATF-HSA:CPZ@RRNP or HSA:CPZ@NP was mixed with FBS at a volume ratio of 9:1 and incubated at 37 °C for 2 days. Then the size of ATF-HSA:CPZ@RRNP or HSA:CPZ@NP was measured by DLS. The result showed the size of both nanoparticles did not change after adding 10% FBS. D) Gel shift experiment showed that addition of recombinant soluble uPAR to monomer ATF-HSA:CPZ (lane 1) from disintegrated ATF-HSA:CPZ@RRNP or to ATF-HSA (lane 2, ATF-HSA expressed by Pichia pastoris, control) caused the ATF-HSA:CPZ or ATF-HSA band to shift, demonstrating the direct binding of ATF-HSA:CPZ to uPAR protein, the only ATF-HSA (lane 3) as a control. E) Native gel shift assay demonstrated ATF-HSA:CPZ@RRNP disintegrated into small aparts after incubation with uPAR receptor for 2.5 h (lane 1). Only expressed ATF-HSA from Pichia Pastoris as a negative control (lane 2) and another sample containing recombinant uPAR and ATF-HSA (1:1) from Pichia Pastoris as a positive control (lane 3).
Figure S3. Both ATF-HSA:CPZ@RRNP and HSA:CPZ@NP showed no phototoxicity on H1299 cells (A) and HELF cells (B) in the absence of light.
Figure S4: Representative images of laser scanning confocal microscope of the nanoparticles incubated with H1299 for 8 min. (A) targeting nanoparticle ATF-HSA:CPZ@RRNP, (B) non-targeting nanoparticle HSA:CPZ@NP.
Figure S5. Intracellular localization of nanoparticles by laser scanning confocal microscope. A) Both nanoparticles (ATF-HSA:CPZ@RRNP and HSA:CPZ@NP) were localized in cytoplasm of H1299 cell, not in nucleus (gray, red and blue colors represent bright field, nanoparticles and DAPI-stained nucleus, respectively). B) Both nanoparticles were co-localized with lysosome (gray, red, green colors represent bright field, nanoparticles, LysoTracker-stained lysosomes, respectively). C) Both nanoparticles were co-localized with mitochondria (gray, red, green colors represent bright field, nanoparticles and MitoTrack-stained mitochondria, respectively).
Figure S6. Representative fluorescence images of H22 tumor-bearing mice taken at different time points (1, 2, 4, 8, 12, 24, 48, 72, 96 h) post intravenous injection of ATF-HSA:CPZ@RRNP (top) and HSA:CPZ@NP (bottom).

Figure S7. Body weight of H22 tumor-bearing Kunming mice during the 7-day photodynamic therapy (10 mice per group).