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Electronic Supporting Information (ESI)

A tumor-targeting protein nanoparticle based on Tat peptide and enhanced green fluorescent protein

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Contents:

1. Materials

The pET28a-H6-EGFP plasmid was kindly gifted by Prof Xianen Zhang and Congqiang Cui of Wuhan Institute of Virology, Chinese Academy of Sciences. The pET28a-TatEGFP plasmid was kindly gifted by Catherine Tsilfidi of University of Ottawa, Canada.

2. Protein expression

The process of protein production was described in our previous study.[1] Briefly, E. coli BL21(DE3) pLysS cells including pET28a(+)/H6-EGFP or pET28a(+)/H6-Tat-EGFP plasmids were grown overnight in 1L Luria-Bertani (LB) medium at 37 °C and induced for 12 h at 22 °C by addition of IPTG (final concentration 1 mM). Then the bacterial cells were collected and resuspended in Binding/Wash Buffer with addition of PMSF before use. The cell was disrupted by sonication and the lysate was centrifuged at 15,000 rpm for 20 min at 4°C. The recombinant proteins were purified by Ni-IDA Sefinose Resin (Sangon, China). The elution solution was dialyzed against HBS Buffer (pH5.8) as described by Vazquez E. The purity of the

fusion protein was determined by 12% SDS- PAGE, the concentration was quantified using Bradford reagent (Solarbio, China).

3. Synthesis of rhodamine-B-labelled BSA

The rhodamine-B-labelled BSA (RhoB-BSA) were synthesized as below. rhodamine-B, (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were dissolved in deionized water, and the pH value of the solution was adjusted to 5.4. After 2 h of reaction, bovine serum albumin was added. After 12 h of reaction, the solution was dialyzed against distilled water for 12 h (MWCO=3500), the final suspension in dialysis bag was freeze-dried. The concentration of RhoB-BSA was determined by BCA method (Sangon, China).

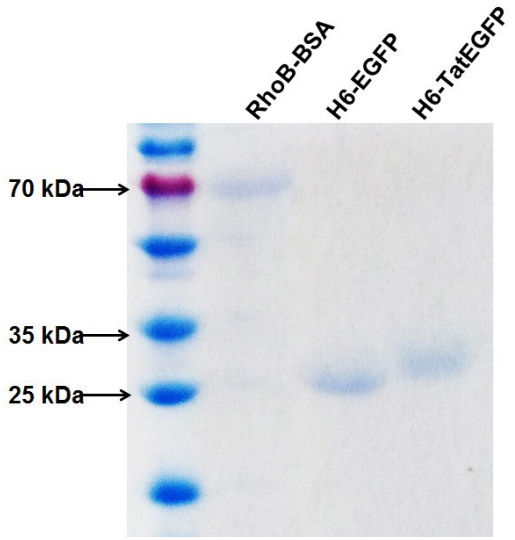

Fig. S1 SDS-PAGE analysis of RhoB-BSA, H6-EGFP and H6-TatEGFP proteins.

4. Characterization of protein nanoparticles

The sizes and structures of nanoparticles were examined by dynamic light scattering (DLS), transmission electron microscopy (TEM), and atomic force microscopy (AFM). The DLS and TEM analysis were performed according to the literature of Guan.[2] The AFM analysis was performed according to the Cespedes's report.[3]

5. Fluorescence emission spectroscopy

The fluorescence emission spectra of RhoB-BSA, H6-EGFP and H6-TatEGFP were determined by a Perkin-Elmer LS55 spectrofluorometer. The RhoB-BSA was excited at wavelength of 540 nm, while H6-EGFP and H6-TatEGFP proteins were excited at wavelength of 480 nm.

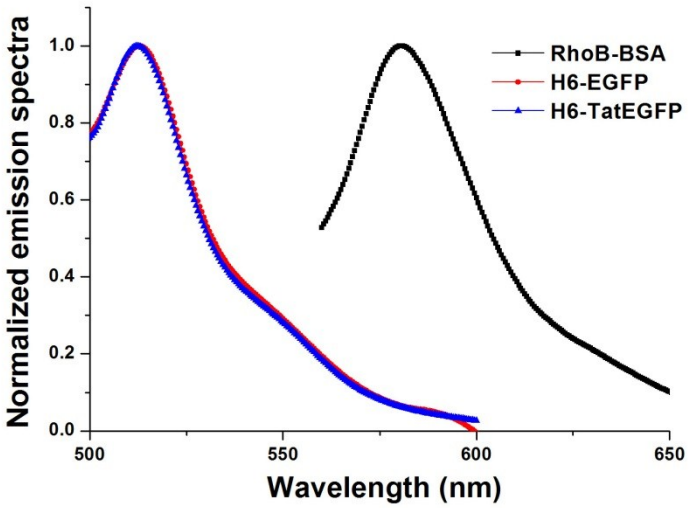

Fig. S2 The fluorescence emission spectra of RhoB-BSA, H6-EGFP and H6-TatEGFP proteins.

6. Stability analysis

The stability of H6-EGFP and H6-TatEGFP was analyzed by measuring fluorescence emission in mouse plasma and serum as described by Cespedes. [3] Mouse plasma was obtained by centrifugation of total blood at 600g for 10 min at 4 °C. The initial fluorescence signals (time 0) were designed as 100%, at certain time point the fluorescence were detected using an exitation wavelength of 480 nm.

7. In vivo bioimaging

All animal experiments were performed in compliance with the guidelines established by the Animal Care and Use Committee of Beihua University, and all procedures were approved by the Animal Care and Use Committee of Beihua University. Nine Kunming mice were utilized to implant xenograft H22 mouse carcinoma. To develop the tumor xenograft, 1 million H22 cells were injected to lateral aspect of anterior limb of the mice. After tumor volume reached 30~70 mm^3, xenograft-bearing mice were divided randomly into 3 groups: RhoB-BSA, H6-EGFP and H6-TatEGFP. 2 μg RhoB-BSA and 500 μg H6-EGFP and H6-TatEGFP were injected to xenograft-bearing mice via a tail vein, respectively. The fluorescence observations were carried out 2 h after vena tail injection by imaging system. The imaging system (CRI Maestro 500FL) used in this study consisted of a light-tight box equipped with a 150 W halogen lamp and an excitation filter system (575-605 nm) to excite NIR BODIPY. Fluorescence was detected by a CCD camera equipped with a C-mount lens and an emission filter (Longpass: 645

nm cut-in).

8. Reference

1. X. Guan, X. Hu, F. Cui, Y. Li, X. Jing and Z. Xie, *Macromol. Biosci.*, 2015, **15**, 1484-1489.
2. X. Guan, X. Hu, Z. Li, H. Zhang and Z. Xie, *RSC Advances*, 2015, **5**, 22957-22964.
3. M. V. Cespedes, U. Unzueta, W. Tatkiewicz, A. Sanchez-Chardi, O. Conchillo-Sole, P. Alamo, Z. Xu, I. Casanova, J. L. Corchero, M. Pesarrodona, J. Cedano, X. Daura, I. Ratera, J. Veciana, N. Ferrer-Miralles, E. Vazquez, A. Villaverde and R. Mangues, *ACS Nano*, 2014, **8**, 4166-4176.