Supporting Information

Materials

BSA (bovine serum albumin, ≥99%), fluorescein isothiocyanate (FITC, labeling efficiency with bovine serum albumin fragments>90%), rhodamin B isothiocyanate (RBITC, labeling efficiency with bovine serum albumin fragments>70%), and cysteine (97%) were purchased from Sigma-Aldrich. Trypsin was purchased from Hualan Chemical. Paraformaldehyde (99%) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. RPMI-1640 medium, fetal calf serum and antibiotics (penicillin 10,000units/mL, streptomycin 10,000μg/mL) were purchased from Hyclone Laboratories Inc. All other reagents were analytical grade and were purchased from Nanjing Chemical Reagent Co., Ltd.

NP characterization

The particle size and zeta potential of the NPs were determined using laser light scattering and a Zetaplus zeta potential analyzer (Brookhaven Instrument, USA), respectively. To determine the crosslinking degrees of the NPs, the primary amino groups of the NPs before and after the crosslinking reaction were detected using the OPA (o-phthaldialdehyde) method described by Violeta G[1], for the crosslink was carried out by the reaction between aldehyde and the primary amino groups of the protein fragments, and the crosslinking degrees of the NPs were determined using Eq.(1).

$$Crosslinking\ Degree = \frac{C_{NH2}^{'}}{C_{NH2}} \times 100\%$$

Eq(1)

Where C_{NH2} and $C_{NH2}^{'}$ were the amounts of primary amino groups before and after the crosslinking reaction, respectively.

To determine the primary amino groups using the OPA method, two reagents were prepared freshly as described below:

Reagent R_1 was made by combining 300μL of OPA (0.25M in ethanol) and 300μL of β-mercaptoethanol (4% in 0.1M sodium borate, pH9.5) and diluting to a final volume of 50mL with water.

Reagent R_2 was prepared by combining 500μL of 0.06M glycine and 500μL of β-mercaptoethanol (0.5% in 0.1M sodium borate, pH9.5) and diluting to a final volume of 30mL with water.

To determine the primary amino groups, the NPs suspension were diluted to 0.5mg/mL with water. Firstly, 200μL of the NPs was incubated with 2 mL of reagent

R_1 for 5min. Then the mixture was centrifuged at 20,000 rpm for 1h, and 2mL 0.1M sodium borate and 100μL of reagent R_2 were added to 50μL of the supernatant. The solution was incubated for 2min, and the absorbance at 340nm was measured. The amounts of primary amino groups could calculated according to a standard curve, which was made using glycine to provide certain amounts of amino groups.

Cell culture

Human breast cancer MCF-7 cells were cultured in RPMI 1640 medium containing 10% fetal calf serum and 1% antibiotics (penicillin 10,000 units/mL, streptomycin 10,000μg/mL) at 37 ℃ and 5% CO_2.

Confocal laser microscope observation

The fluorescence signals of the cells in the channel D and channel A could be recorded using the confocal laser scanning microscope (Leica TCS SP5, Leica, Germany) after fixing the cells with 4% paraformaldehyde. The signals in the channel D and A were excited at 488 nm and 543 nm, respectively.

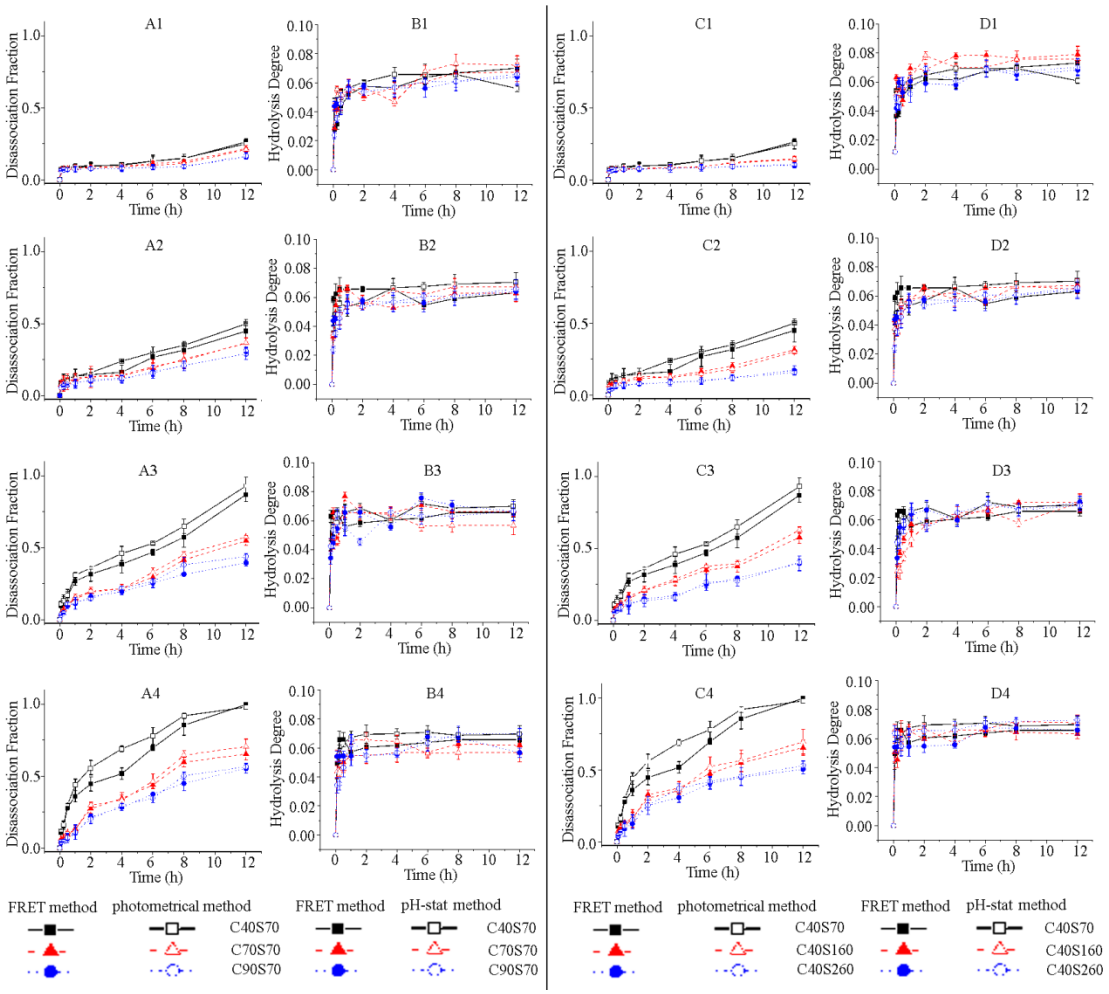

Fig.S1 Validation of the FRET-based method by detecting the degradation of albumin NPs with various crosslinking degrees (A1-A4, B1-B4) and diameters (C1-C4, D1-D4)

in trypsin buffers. The trypsin concentrations were set at 6.6 (A1, B1, C1 and D1), 33 (A2, B2, C2 and D2), 66 (A3, B3, C3 and D3) and 100μg/mL (A4, B4, C4 and D4). (n=3)

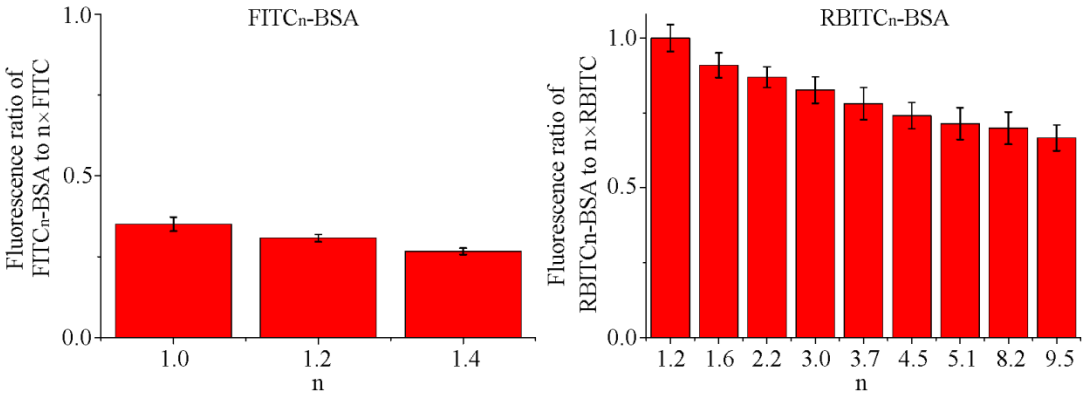

Fig. S2 The self quenching of FITCn-BSA and RBITCn-BSA, which was calculated as the fluorescence intensity ratio of FITCn-BSA (RBITCn-BSA) to FITC (RBITC) with the same dye concentration. The quenching intensity was dependent on the labeling content of FITC and RBITC, and the fluorescence of FITC-BSA and $RBITC_{9.5}$-BSA was quenched by 71% and 27%, respectively.. (n=3)

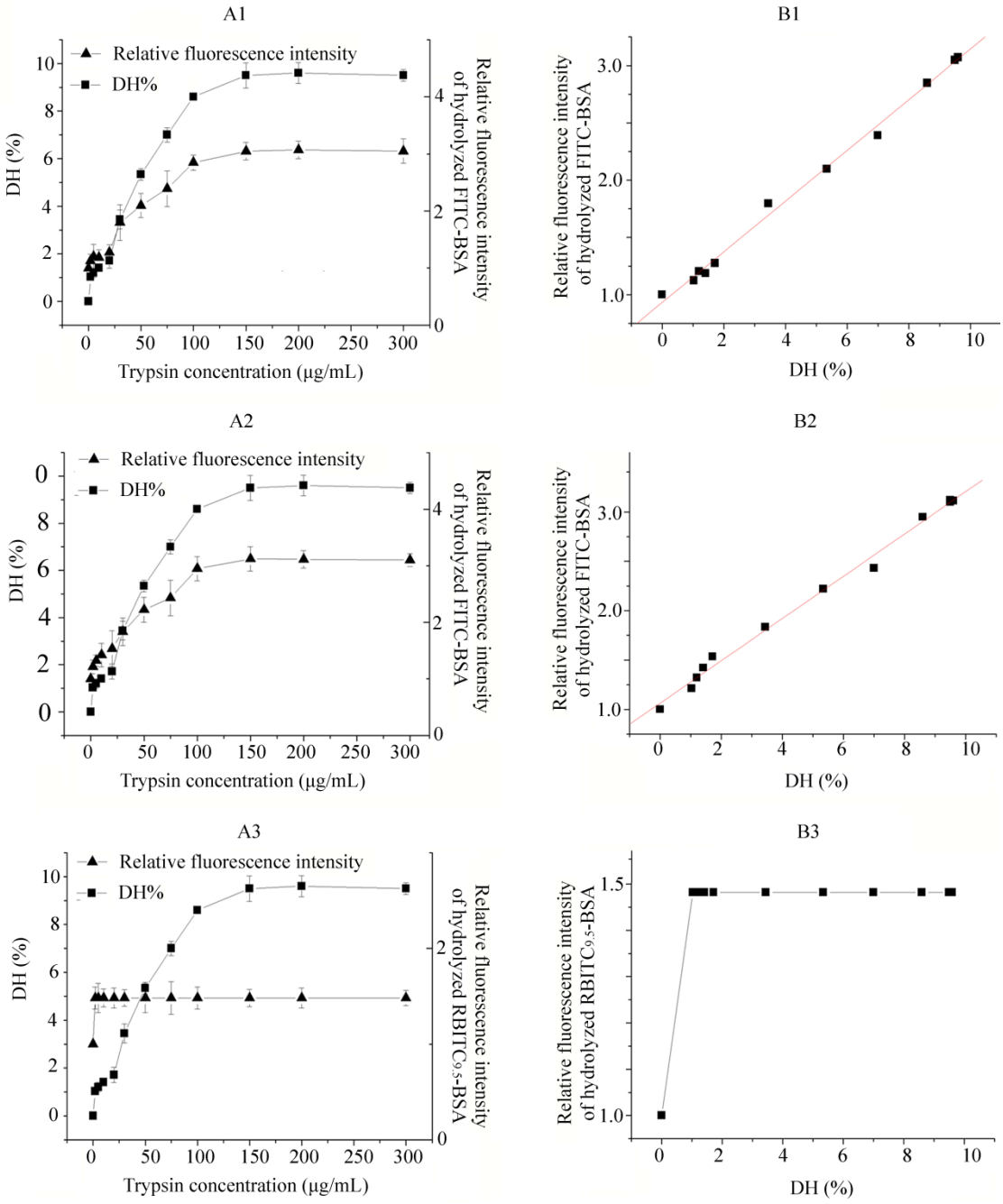

Fig.S3 The fluorescence increasing of FITC-BSA in channel D and F (A1, A2) and $RBITC_{9.5}$-BSA in channel A (A3) during the trypsin hydrolysis process, and the relationships between DH% and fluorescence increasing of FITC-BSA in channel D and F (B1, B2) and $RBITC_{9.5}$-BSA in channel A (B3). The fluorescence increasing was regarded as the relative fluorescence intensity of the hydrolyzed protein, which was calculated by deviding the fluorescence intensity of hydrolyzed protein by the initial intensity. The DH% was detected using the pH-stat method. The difference of the fluorescence increasing behaviors between FITC-BSA and $RBITC_{9.5}$-BSA might be due to the quenching intensity of FITC-BSA was highly larger than that of

$RBITC_{9.5}$-BSA. (n=3)

References

1. V. Janolino and H. Swaisgood, *Applied Biochemistry and Biotechnology*, 1992, **36**, 81-85.