Electronic Supplementary Information

Evaluation of Red and Near Infrared Fluorescent Silver Nanoclusters as Potential in *vivo* Indicators of Tight Junction Opening

Xinyi Wang[a1], Na Wang[c1], Limei Li[a], Ruyue Xiao[b], Lan Yuan[b], Xiaoda Yang[✉b] , Na Li[d]

[a] College of Sciences, Shenyang Agricultural University, Shenyang 110161, China.

[b] State Key laboratory of Natural and Biomimetic Drugs and Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100083, China. Fax: +86-10-62015584; Tel: +86-10-82801539; E-mail: xyang@bjmu.edu.cn

[c] Department of Pharmacognosy, School of Pharmaceutical Sciences, Hebei Medical University, Shijiazhuang 050017, China

[d] Beijing National Laboratory for Molecular Sciences (BNLMS), The Key Laboratory of Bioorganic Chemistry and Molecular Engineering, Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

[1] Xinyi Wang and Na Wang contributed equally to this work.

✉Corresponding author

Characterization of Probes

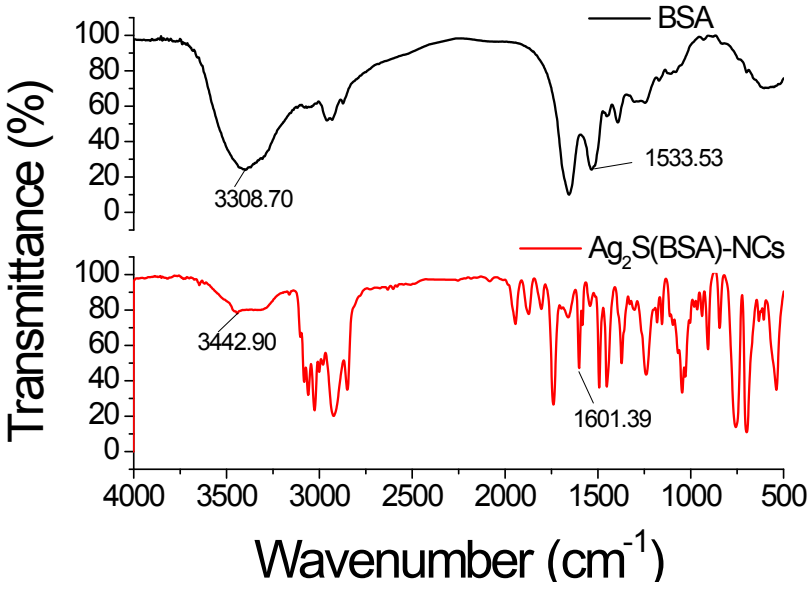

Fig. S1 FT-IR spectra of BSA and Ag_2S(BSA)-NCs. The peaks of 3308.70 cm^{-1} and 1533.53 cm^{-1} belong to the C-N stretching vibration and N-H bending vibration of BSA, respectively. The characteristic peakred-shift of BSA in Ag_2S(BSA)-NCs (increased by 134 cm^{-1} and 68 cm^{-1}) indicating the existence of interaction between the amino and Ag_2S nanoclusters.

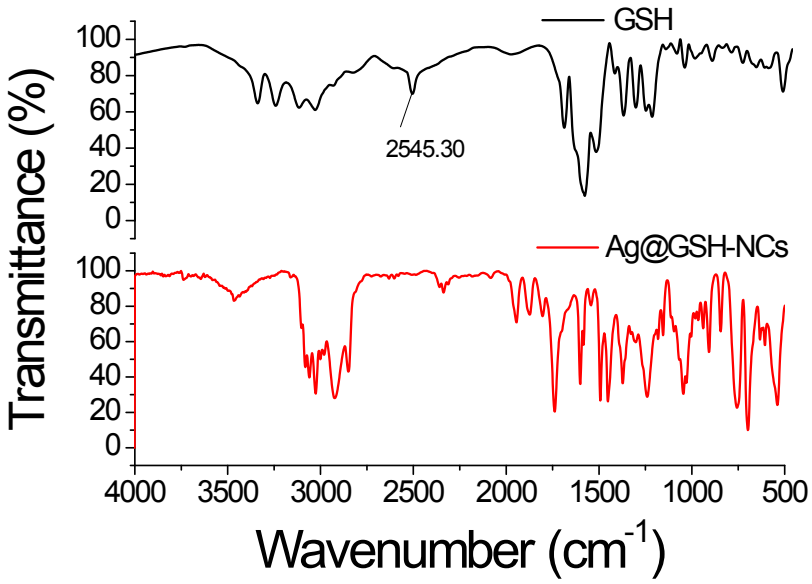

Fig.S2 FT-IR spectra of GSH and Ag(GSH)-NCs. The peaks of 2545.30 cm^{-1} belong to the SH bond stretching vibration peak of GSH. The disappearance of SH characteristic peak in Ag(GSH)-NCs indicates the interaction between GSH and Ag nanoclusters.

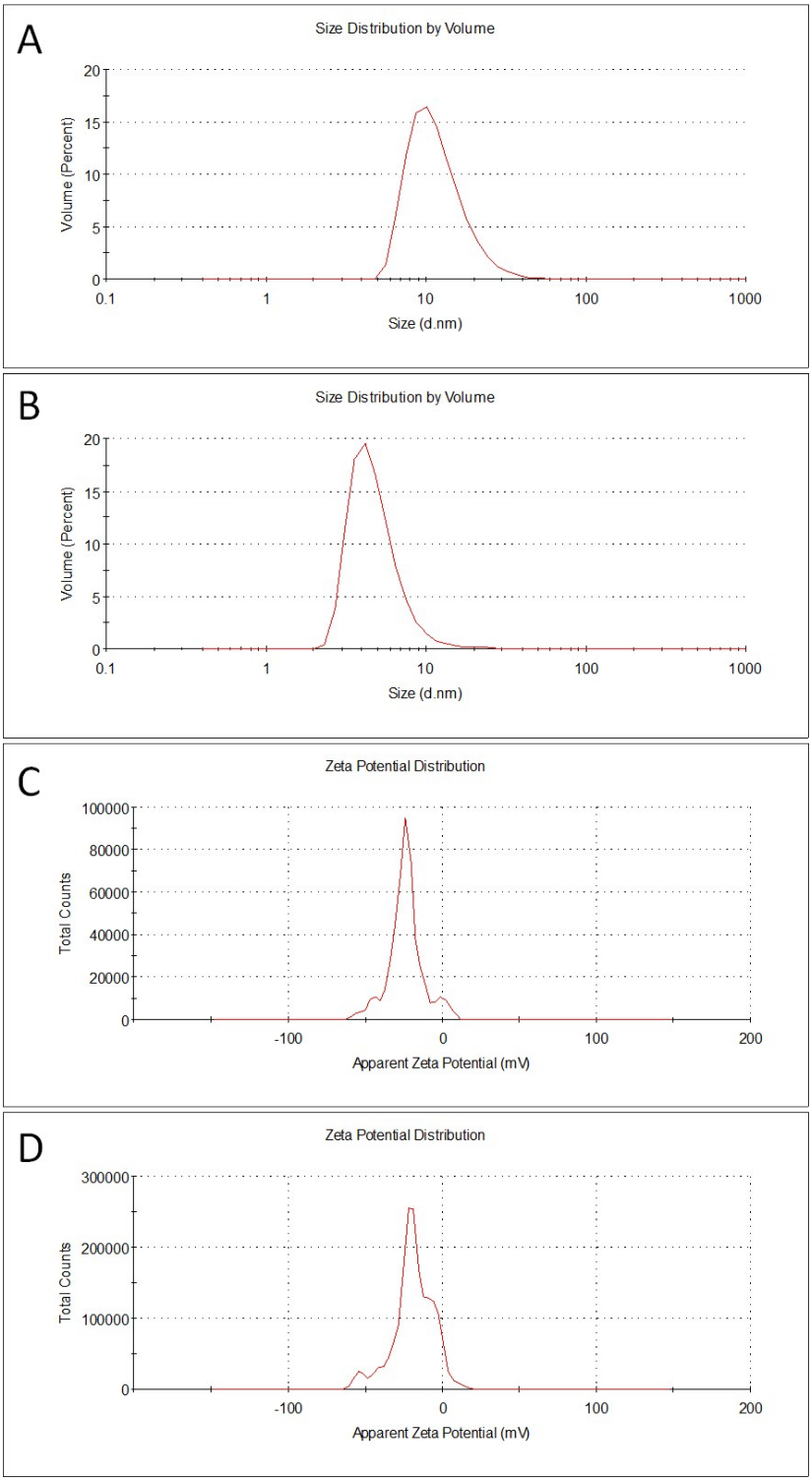

Fig. S3 Size and zeta potential distribution of AgNCs. (A) and (B) were the size distributions of Ag_2S(BSA)-NCs and Ag(GSH)-NCs dispersed in NF-DMEM culture media respectively. (C) and (D) were zeta potential distribution of Ag_2S(BSA)-NCs and Ag(GSH)-NCs dispersed in water respectively. Data were obtained by the dynamic light scattering (DLS) measurements.

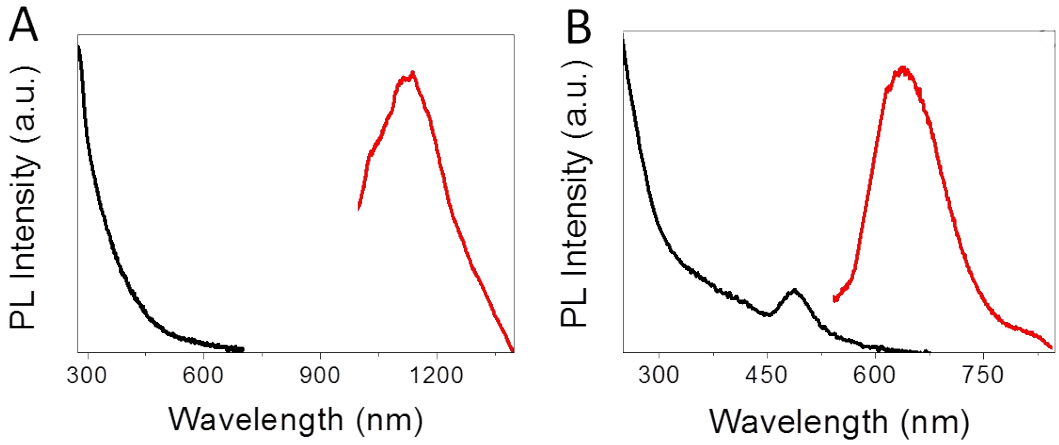

Fig. S4 The fluorescence excitation (black lines) and emission (red lines) spectra of (a) Ag_2S(BSA)-NCs (λem = 1050 nm for photoexcitation; λex = 500 nm for photoemission), (b) Ag(GSH)-NCs (λem = 640 nm for photoexcitation; λex = 488 nm for photoemission).

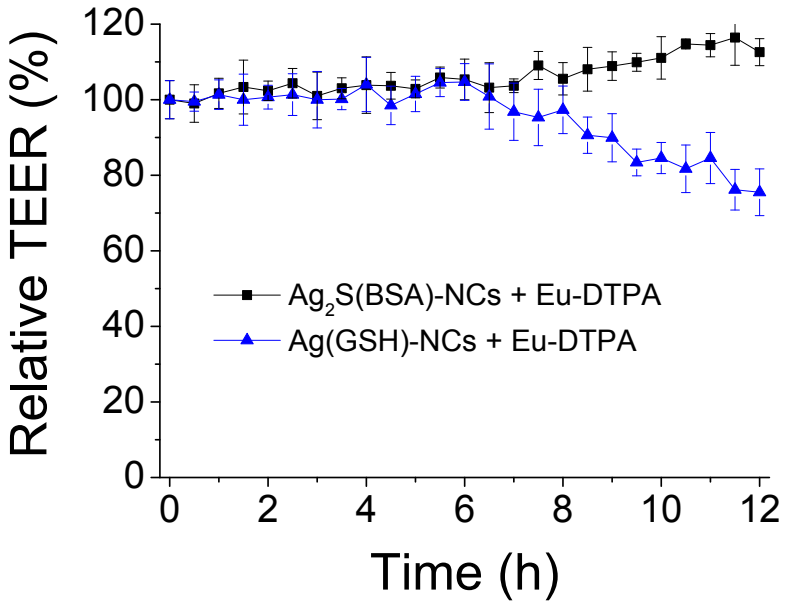

Fig. S5 TEER of MDCK cell monolayer after treated with AgNCs and Eu-DTPA combination. Ag_2S(BSA)-NCs, 75 mg/L; Ag(GSH)-NCs, 8 mg/L; Eu-DTPA, 20 μmol/L. All data were the mean ± SD of four replicates.

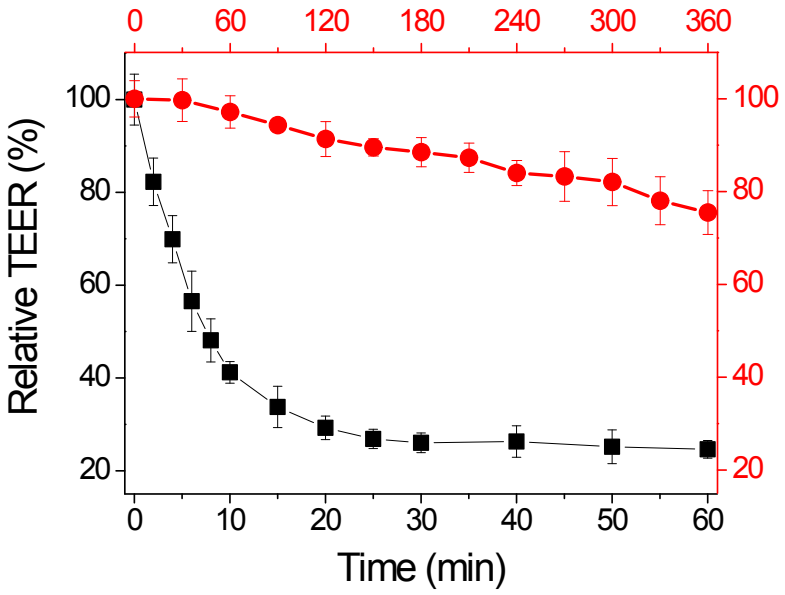

Fig. S6 TJ open treated by EDTA (0.5 mM, black) and $VO(acac)_2$ (80 μM, red).

Size exclusion Chromatography of Eu-DTPA and AgNCs

Eu-DTPA (2 μM) and Ag_2S(BSA)-NCs(150 mg/L) were dissolved in NF-DMEM medium and applied to a Sephadex G25 column (0.5 mL, GE Health Care). The column was eluted with NF-DMEM medium and monitored per 100 μL volume using a *Flexstation 3* microplate reader with a $\lambda_{ex/em}$of 340/616 nm and a measurementwindow from 600 to 1000 ms for Eu-DTPA, using Nanolog FL3-2iHR spectrometer with a $\lambda_{ex/em}$ of 500/1050 nm for Ag_2S(BSA)-NCs.

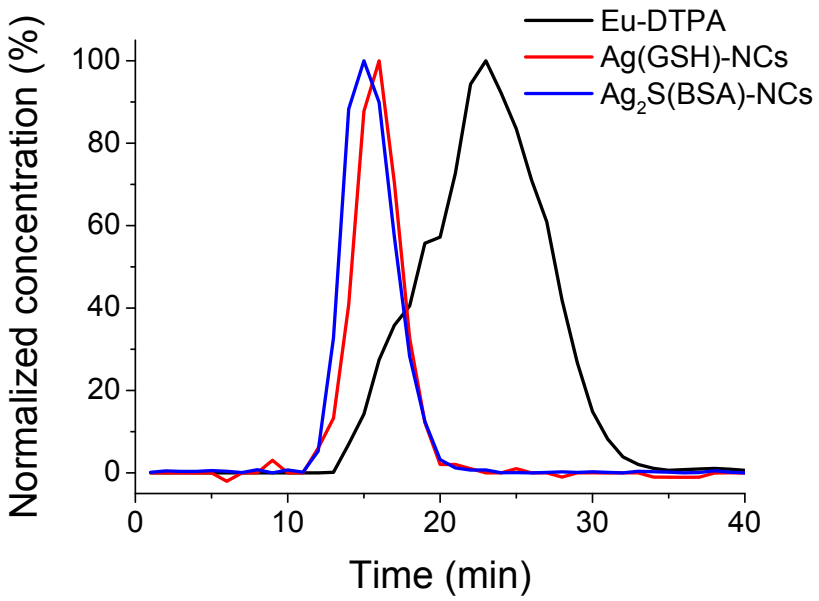

Fig. S7 Chromatograms of Eu-DTPA and AgNCs.