Electronic Supplementary Information

Two-photon AgNPs/DNA-TPdye Nanosensing Conjugate for Biothiols Probing in Live Cells

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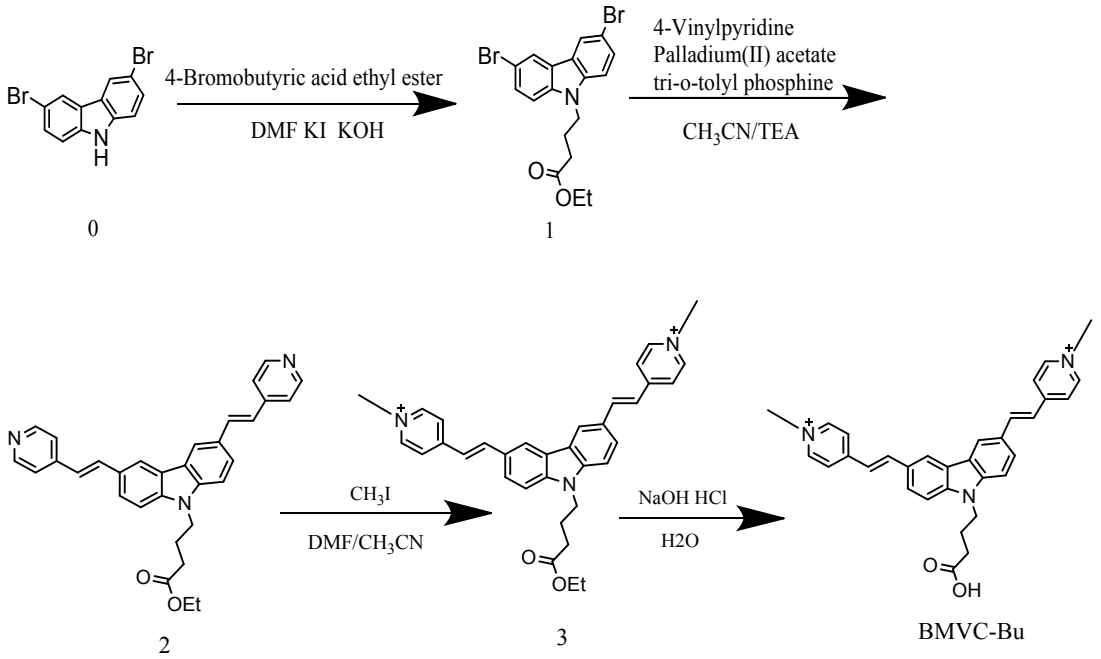
Preparation of the Two-photon Dye (TPdye: 4-[3,6-Bis(1-methyl-4 -vinylpyridium iodine)-9H-carbazol-9-yl)] butanoic acid, BMVC-Bu).



To a mixture of KOH (1.12 g, 20 mmol) and KI (80 mg, 0.48 mmol) dissolved in dry DMF (20mL) was added 3,6-dibromocarbazole (compound 0) (0.65g,2mmol), ethyl-4-bromobutanoate (1.15 mL, 8 mmol). The mixture was stirred at 60 ^{0}C under argon atmosphere overnight. After the addition of 100 mL H_2O to the final mixture, the mixture was extracted with ethyl acetate and then the organic layer was washed twice with water and once with brine, and dried over anhydrous Na_2SO4. After filtration, the solution was concentrated under reduced pressure to give crude product The final white powder Ethyl-4-(3,6-dibromo-9H-carbazol-9-yl) butanoate (compound 1) was obtained by chromatography using petroleum/ethyl acetate(5:1, V/V) as an eluent.[1]

4-[3,6-Bis(1-methyl-4-vinylpyridiumiodine)-9H-carbazol-9-yl)] butanoic acid was prepared as indicated in literature.[2] Ethyl-4-(3,6-dibromo-9H-carbazol-9-yl) butanoate (compound 1) (731.7 mg,1.7 mmol) was added into a mixture containing 4-vinylpyridine (667 mg), Palladium(II) acetate (5 mg) and tri-o-tolyl phosphine (50 mg) under the triethylamine (3 mL)/acetonitrile(9 mL) solvent pairs in a high pressure

bottle. The mixture was stayed at 105 ^{0}C for 48 h. After the reaction, the mixture was transferred to a flask and the solvent was removed under reduced pressure to give a yellow crude product, which was purified by chromatography on silica gel using CH_2Cl_2/CH_3OH (5:1,V/V) as an eluent to give Ethyl-4-[3,6-Bis(4-vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate (compound 2) as earth yellow solid. Excess CH_3I and Ethyl-4-[3,6-Bis(4-vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate (487.0 mg,1 mmol) in acetonitrile /DMF was refluxed for 4h,the orange red powder. Ethyl-4-[3,6-Bis(1-methyl-4-vinylpyridium iodine)-9H-carbazol-9-yl)] butanoate (compound 3) was obtained with a 90% yield after recrystallization twice using methanol. Then, Ethyl-4-[3,6-Bis(1-methyl-4-vinylpyridium iodine)-9H-carbazol -9-yl)] butanoate (compound 3) (193.0 mg, 0.25 mmol), sodium hydroxide (0.030 g, 0.75 mmol) were put into a 100mL flask containing the mixture solution of THF (4 mL) and water (1 mL) and the mixture was refluxed for 12 h. The diluted hydrochloric acid was added into the mixture to adjust pH=3 giving an orange red solid with a yield of 80%. ^{1}HNMR (d6-DMSO, 400 MHz, δ): 12.5 (s, 1H), 8.84 (d, 4H), 8.62 (s, 2H), 8.24 (d, 4H), 8.22 (d, 2H), 7.96 (d, 2H), 7,80 (d, 2H), 7.60 (d, 2H), 4.35 (t, 2H), 4.25 (s, 3H), 4.24 (s, 3H), 2.38 (t, 2H), 2.10 (m, 2H). MS (ESI) *m/z* for $C_{32}H_{31}N_3O_2$ 244.5 found, 244.5 (M^{2+}). Anal. calcd. for $C_{32}H_{31}I_2N_3O_2$: C, 52.19; H, 4.20; N, 5.65. Found: C, 52.19; H, 4.20; N, 5.64.

References

[1] X. B. Yan, S. P. Cheng and X. G. Chen, *Polymer.*, 2012, 53, 241-247.

[2] X. J. Feng, P. L. Wu and M. S. Wong, *Org. Lett.*, 2010, 12, 2194-2197.

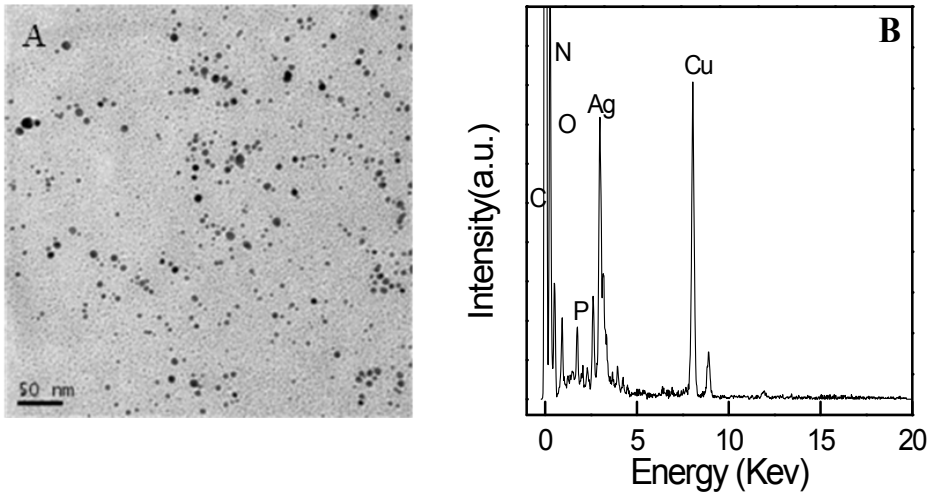


Figure S1. TEM image (A) and EDX spectroscopy analysis (B) of the AgNPs/DNA-TPdye conjugate.

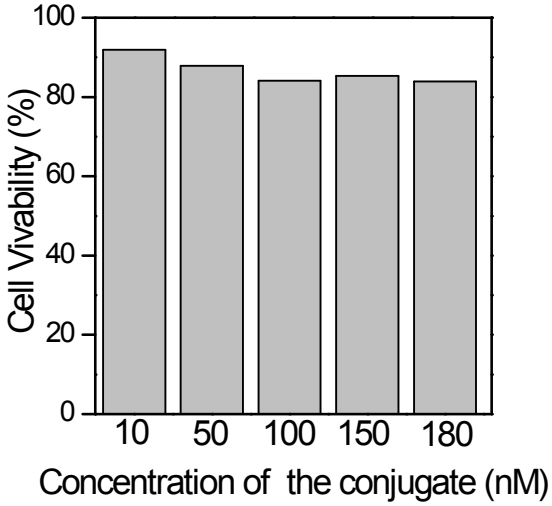


Figure S2. Cell viability of HeLa treated with different concentrations of AgNPs/DNA-TPdye conjugate for 24 h in fresh medium. The AgNPs/DNA-TPdye conjugate mixtures with different concentrations were prepared by mixing 946 μL of the fresh cell growth medium with 54 μL of 0.2, 0.9, 1.9, 2.8 and 3.3 μM AgNPs/DNA−TPdye conjugate prepared in the experimental section, respectively. All error bars were obtained through the detection of eight parallel samples. Note: The concentration of the conjugate refers to the concentration of DNA-TPdye.

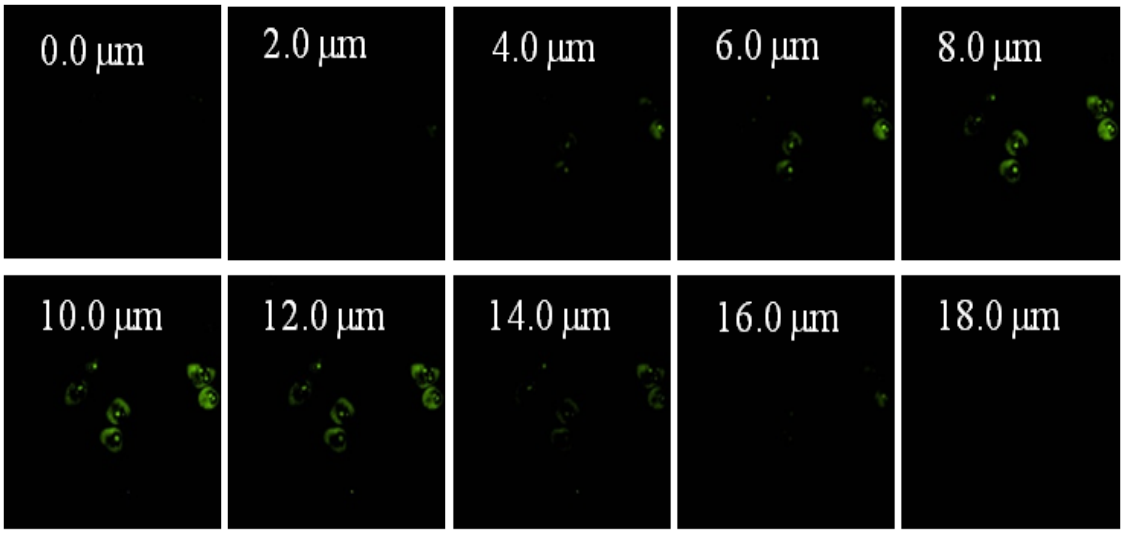


Figure S3. Z-scanning confocal fluorescence microscopy images of HeLa cells incubated with AgNPs/DNA-TPdye conjugate.