Supporting Information

Bright Chemiluminescence Conjugated Polymer-Mesoporous Silica Nanoprobe for Imaging of Colonic Tumor in vivo

Yukun Li[a], Beibei Zhu[a], Wanyin Han[a], Wei Tang [a] Xinrui Duan[*a]

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, 620 Xi Chang'an Street, Xi'an, Shaanxi 710119, People's Republic of China.
E-mail: duanxr@snnu.edu.cn

Table of content

1. Calculation of the molar particle concentration of nanoparticles

All nanoparticles were diluted with water from 10 μg/mL to the appropriate concentration with a final volume of 1 mL. Afterward using a syringe to draw 1 mL of the above nanoparticle solution into the ZetaView nanoparticle-tracking analysis. Under suitable conditions, the process of determination of the particle concentration of

nanoparticles is conducted. The CPNs' molar particle concentrations (c) can be determined as follow:

$$c = \frac{N}{N_A V}$$

where c is the molar particle concentration, N is the number of particles, V is the average volume of the nanoparticles, N_A is Avogadro's constant.

2. Chemiluminescence quantum yield (QY) of nanoparticles

According to the standard luminol-H_2O_2 system with a known CL QY of 1.14×10^{-2} einsteins/mol at pH = 11.6 (0.1 M K_2CO_3),[1] we acquired the chemiluminescence quantum yield (QY) values. Briefly, A solution of 100 μL nanoparticles was placed in a quartz vial (10 μg/mL), 100 μL sodium hypochlorite was then added to the solution (50 mM), On top of that, a dynamic curve, referred from the mixture, is then determined by a CL analyze. Measure the data until chemiluminescence intensity was reduced to 1% of the highest value. CL detection with the voltage of PMT was -800 V, In the intensity mode, CL analyzer signal acquisition time was set to 0.1 s. The same operation was carried out with a mixture of luminol (200 nM, 100 μL, in 0.1 M K_2CO_3) and hydrogen peroxide (100 μL, 200 μM). The CLQY value is calculated according to the relative integral area under the chemiluminescence dynamic curve with the following formula：

$$\emptyset = \emptyset_{lum} \times \frac{I}{I_{lum}} \times \frac{n^2}{n_{lum}^2} \times \frac{[lum]}{[NPs]}$$

I is the total number of photons obtained by the dynamic curve from injection to 1% of the maximum CL intensity under the time integration, where ϕ is the quantum yield, n

is the refractive index of solvent. [Lum] refers to luminol concentrations. [NPs] refer to nanoparticles concentrations. Parameters with subscript luminol are values for the reference system.

3. LOD calculation

The LOD was calculated by following this equation: LOD= $\frac{3\sigma}{S}$. S represents the change amount of the analysis signal when the concentration of the measured substance changes by one unit, that is, the sensitivity of the method. σ is the standard deviation of CL signals from 14 blanks.

4. Preparation of Different ROS/RNS and other biological oxidants

70% tert-butyl hydroperoxide (TBHP), 30% Hydrogen peroxide (H_2O_2), and available chlorine 4.00-4.99% hypochlorite (ClO^-) aqueous solutions were used for further reactions. The concentration of ClO^- was calibrated in an aqueous solution according to its absorption value at λ=292 nm (ε = 350 $M^{-1}\cdot cm^{-1}$). The concentration of H_2O_2 was calibrated based on its absorption value at λ=240 nm (ε=43.6 $M^{-1}\cdot cm^{-1}$),[2] Two solutions with a concentration of 50 mM were prepared in turn Singlet oxygen solution can be got by mixing an aqueous solution of hypochlorous acid (50 mM) and an aqueous solution of hydrogen peroxide (100 mM). Hydroxyl radical (•OH) and tert-butoxy radical (•OtBu) were freshly prepared through Fenton reaction of 1 mM Fe^{2+} with 200 μM H_2O_2 and 200 μM TBHP,[3] respectively. Then sodium nitroferricyanide (149 mg) can be dissolved in deionized water (10 mL) to prepare an aqueous solution of nitric oxide. Sodium nitrite (35 mg) was dissolved in deionized water (10 mL) to prepare an aqueous solution of nitrite.

5. Cytotoxicity of nanoparticles by MTT assay

HCT116 cells were seeded in DMEM in a 5% CO_2 humidified incubator at 37 °C used for cytotoxicity analysis of PPV@MSN-CP1@FA. For MTT assay: The cells were digested with trypsin to ensure that there were 5000 cells in each well of 96 well plates. After attachment for 24 hours, the cells were treated with PPV@MSN-CP1@FA with a series of concentrations (0, 25, 50, 75, and 100 μg/mL) for 12 hours in triplicate. Then added 20 μL of 5 mg/mL MTT solution (in PBS) to each well and incubated for 4 hours. After that, the supernatant was removed, and 150 μL of DMSO was added. Then the 96 well plates were shaken for 10 minutes on a shaker to completely dissolve the crystals. Eventually, measuring the absorbance of each well using a microplate reader at 490 nm.

Table S1. Fluorescence quantum yield of CPs@MSN as the powder.

CPs	ϕ
PPV@MSN	60.60%
PFV@MSN	11.00%
PPV-PFV(1:9)@MSN	15.20%
PPV-PFV(1:1)@MSN	14.80%

Table S2. Molar Particle Concentrations and CLQY of CPs based nanoparticles

CPs	C (pmol/L)	ϕ (einsteins/mol)
MSN-CP1	3.34	6.97
PPV@MSN-CP1	6.1	9.36
PFV@MSNCP1	6.58	3.96
PPV-PFV(1:9)@MSN-CP1	5.94	5.37
PPV-PFV(1:1)@MSN-CP1	3.53	7.81

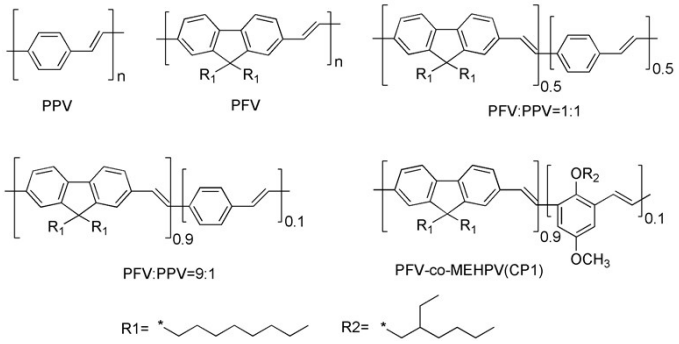

Figure S1 Chemical structures of polymers

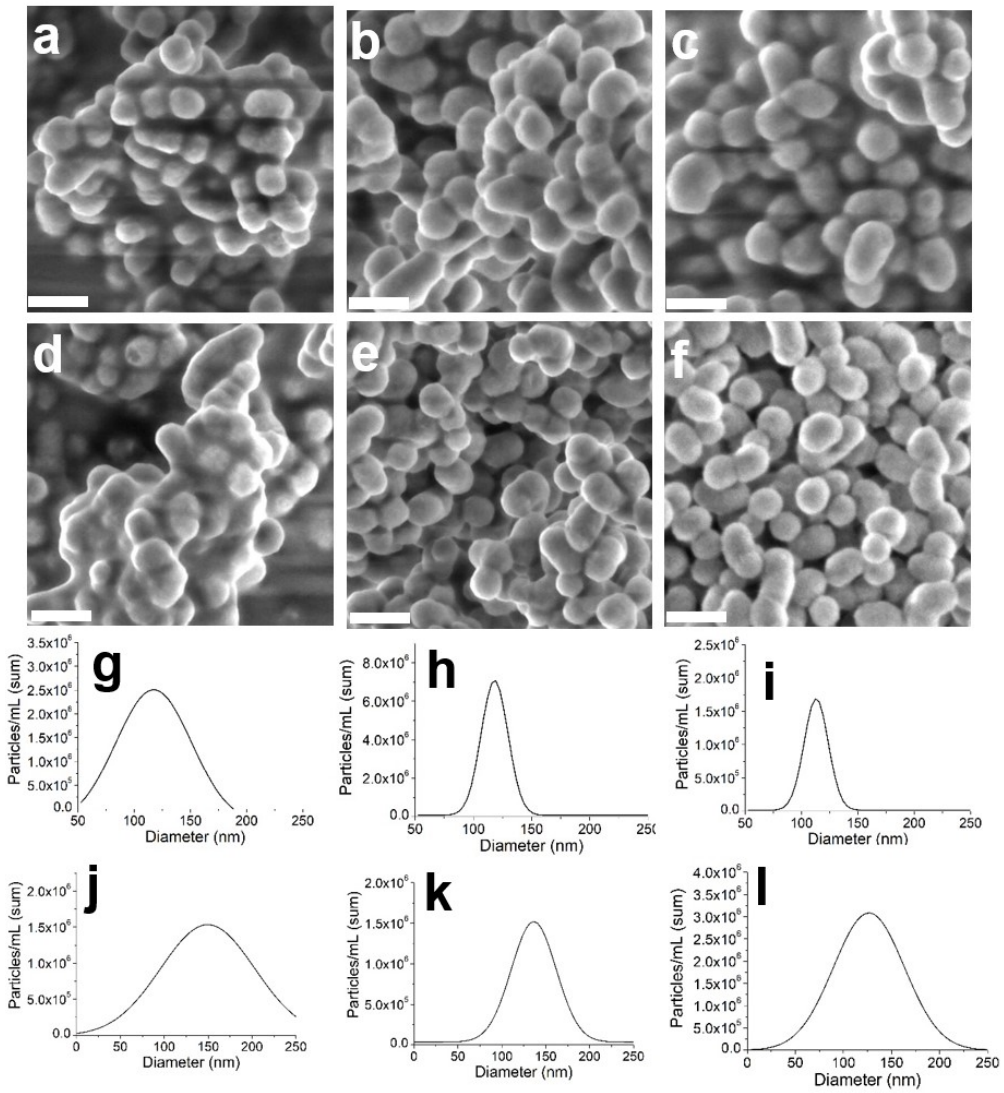

Figure S2 FE-SEM images of (a) PFV@MSN, (b) PPV@PFV(1:1)@MSN, (c) PPV-PFV(1:9)@MSN, (d) PFV@MSN-CP1, (e) PPV-PFV(1:1)@MSN-CP1, and (f) PPV-PFV(1:9)@MSN-CP1. Scale bar, 100 nm; NTA of (g) PFV@MSN, (h) PPV-PFV(1:1)@MSN, (i) PPV-PFV(1:9)@MSN, (j) PFV@MSN-CP1, (k) PPV-PFV(1:1)@MSN-CP1, and (l) PPV-PFV(1:9)@MSN-CP1.

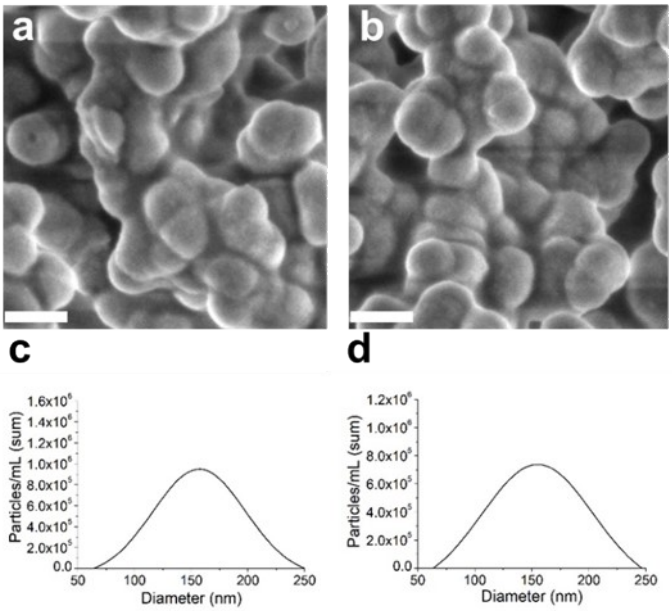

Figure S3 FE-SEM images of (a) PPV@MSN-CP1@SLB, and (b) PPV@MSN-CP1@FA. Scale bar, 100 nm; NTA of (c) PPV@MSN-CP1@SLB, and (d) PPV@MSN-CP1@FA.

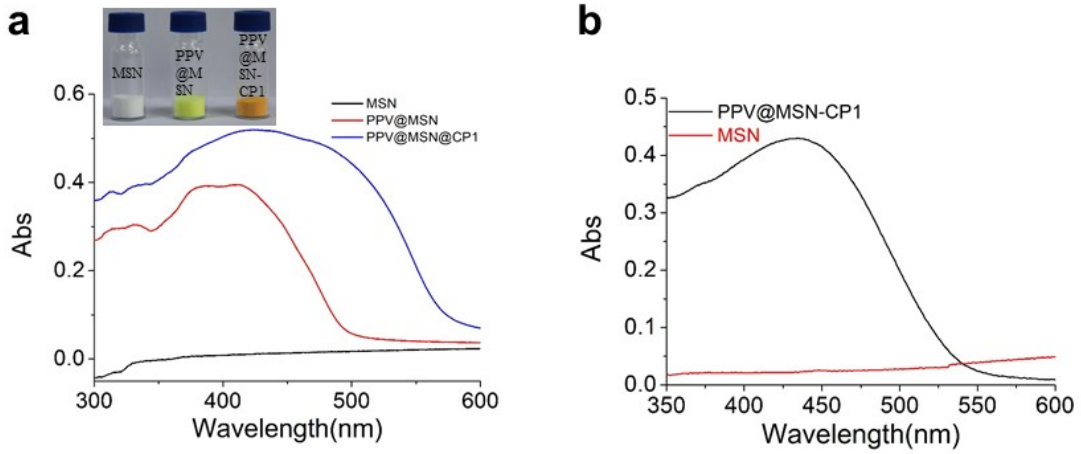

Figure. S4 UV-Vis absorption spectra of (a) MSN(black) PPV@MSN(red) PPV@MSN-CP1(blue) and (b) PPV@MSN-CP1(after etching)

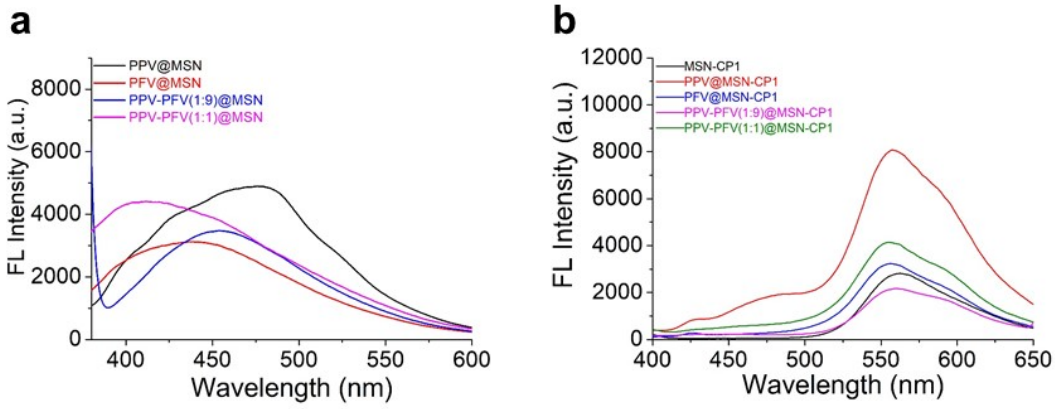

Figure S5. Fluorescence spectra of (a) 100 μg/mL PFV@MSN (λ_{em}=430 nm), PFV-PPV(1:1)@MSN (λ_{em}=420 nm), PFV-PPV(9:1)@MSN (λ_{em}=460 nm), and (b) 100 μg/mL PPV/PFV@MSN-CP1(λ_{em}=560 nm). The spectra were measured in ethanol.

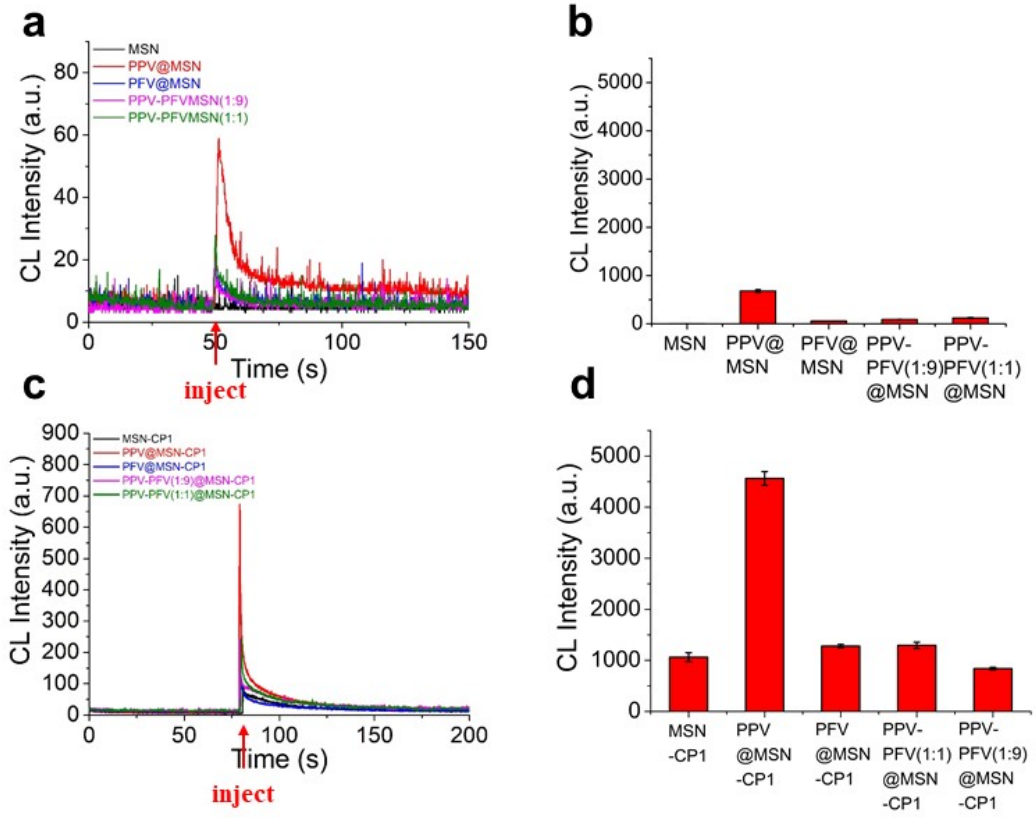

Figure S6. CL spectra of (a) 10 μg/mL PPV/PFV@MSN, and (c) 10 μg/mL PPV/PFV@MSN-CP1. Quantification of the CL of (b) 10 μg/mL PPV/PFV@MSN and (d) 10 μg/mL PPV/PFV@MSN-CP1. The CL was measured in ethanol. CL was induced by the addition of NaClO (50 mM). The error bars represent the standard deviations (n = 3). The spectra were measured in ethanol.

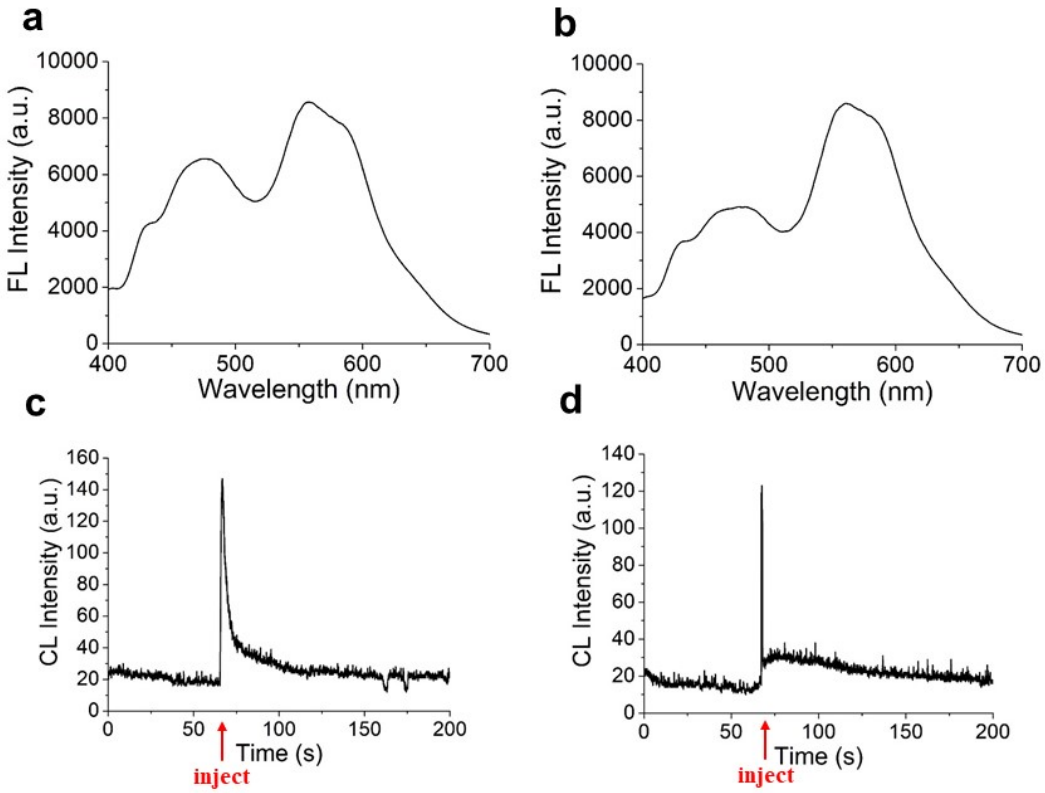

Figure S7. FL spectra of (a) 100 μg/mL PPV@MSN-CP1@SLB (λ_{ex}=490 nm) (b) 100 μg/mL PPV@MSN-CP1@SLB@FA (λ_{ex}=490 nm). CL of (c) 10 μg/mL PPV@MSN-CP1@SLB (d) 10 μg/mL PPV@MSN-CP1@SLB@FA. The FL spectra and the CL were measured in water. CL was induced by the addition of NaClO (50 mM).

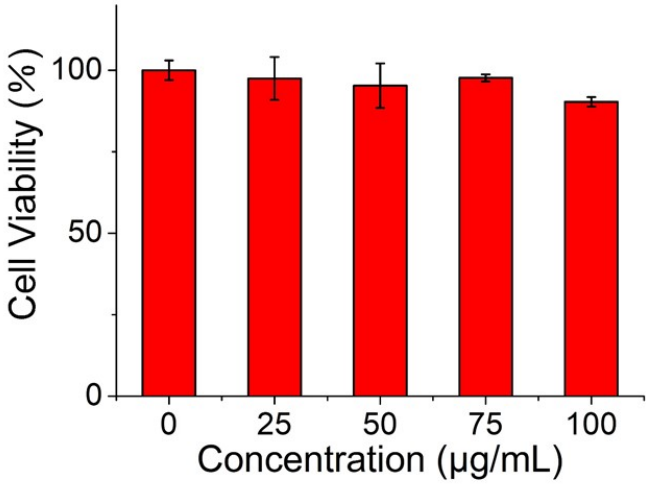

Figure S8. MTT assay for cytotoxicity of nanoparticles. The error bars represent the standard deviations (n = 3).

References

1 F.A. Augusto, G.A. de Souza, S.P. de Souza Junior, M. Khalid, W.J. Photochem Photobiol, 2013, 89, 1299-1317.
2 Y. Deng, S. Feng, Q. Xia, S. Gong, G. Feng, Talanta , 2020, 2015, 120901.
3 P. Zhao, K. Wang, X. Zhu, Y. Zhou, J. Wu, Dyes Pigm., 2018, 155,143-149.