Electronic Supplementary Information

A Nanogel Sensor for Colorimetric Fluorescent Measurement of Ionizing Radiation Doses

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Experimental

Materials:

Acrylamide (AAm, ≥98%), *N,N'*-methylenebis(acrylamide) (BIS, ≥99%), ammonium persulphate (APS, ≥98%), hexane (≥99.8%) and ethanol (EtOH, ≥99.7%) were obtained from Sinopharm Chemical (China). *N*-(3-aminopropyl)methacrylamide hydrochloride (APMA, ≥98%), bis(2-ethylhexyl)sulfosuccinate (AOT, ≥96%), polyoxyethylene-4-laurylether (Brij 30), *N,N,N',N'*-tetramethylethylenediamine

(TEMED, ≥99%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, ≥98%) and N-hydroxysuccinimide (NHS, ≥98%) were purchased from Aladdin (China). 5(6)-carboxytetramethylrhodamine succinimidyl ester (TAMRA-SE) and coumarin-3-carboxylic acid (CCA, ≥99%) were purchased from Sigma-Aldrich (China). 2-(*N*-morpholino)ethanesulfonic acid (MES, 0.1 M, pH=5.5) and phosphate buffered saline (PBS, 0.1 M, pH=7.4) were obtained from Yuanye Bio (China). Sodium borate buffer (0.5 M, pH=9.5) was purchased from Tianenze Bio (China). Deionized (DI) water was filtered to have a resistivity of 18.2 MΩ cm by a Milli-Q Plus system (Millipore Co.). Whatman 1# filter paper was purchased from GE Healthcare Life Sciences (U.S.).

Preparation of poly(AAm-co-APMA-co-TAMRA) (PAAT) nanogels:

First, TAMRA-SE (1 mg) was dissolved in a sodium borate buffer (250 μL) in the presence of APMA (0.5 mg) and stirred for 24 h in the dark at room temperature to obtain APMA-modified TAMRA (APMA-TAMRA) solution. The PAAT nanogels were prepared by inverse microemulsion polymerization that was modified from a previously published procedure.[1] Briefly, AAm (300 mg), BIS (180 mg), and APMA (300 mg) were dissolved in DI water (2 mL). After filtration through a 0.22 μm filter, the solution was mixed with 250 μL of the APMA-modified TAMRA solution. The mixture was added to a mixture of hexane (43 mL), Brij 30 (3.2 mL) and AOT (1590 mg). After purging with N_2 gas for 20 min, APS (80 μL, 0.1 g/mL) and TEMED (60 μL) were added to initiate the polymerization. The reaction was continued under N_2 gas for 2 h at room temperature. Then, the hexane was removed by vacuum evaporation,

and the residue was washed with ethanol (×5) and lyophilized to obtain PAAT nanogels.

Preparation of PAAT-CCA (PAATC) nanogels:

A mixture of CCA (10 mg), EDC (80 mg) and NHS (120 mg) was first dissolved in MES buffer (20 mL). Then, PAAT nanogels (50 mg, in 20 mL of PBS buffer) were immediately mixed with the activated CCA solution and stirred for 10 h in darkness at room temperature. Subsequently, the solution was filtered through Whatman 1# filter paper and precipitated by adding excessive ethanol (×5). Finally, 10 mg of lyophilized PAATC nanogels were dissolved in 2 mL of PBS buffer to make a stock solution (5 mg/mL) prior to use.

Characterization:

The morphology of the PAATC nanogels was investigated by transmission electron microscopy (TEM, Tecnai G2 spirit BioTwin, FEI, USA). The hydrodynamic diameter and Zeta potential were determined using a Zetasizer Nano ZS90 apparatus (Malvern, UK). Attenuated total internal reflectance Fourier transform infrared spectra were recorded on a Nicolet iS50 ATR FTIR spectrometer (Thermo Fisher Scientific, U.S.). The fluorescence spectra were obtained at room temperature using a Synergy NEO microplate reader (BioTek, USA).

Ionizing radiation from X-rays:

All samples were irradiated using a RS2000 X-ray Biological Irradiator (Rad Source Technologies, FL, USA) at 160 kVp/ 25 mA (dose rate: 1.166 Gy/min).

1. Reproducibility:

The PAATC nanogel sensors (1 mL, 0.2 mg/mL, 1×PBS buffer) were randomly divided into four groups in 2 mL plastic tubes. Then, each group was irradiated by X-rays at a given dose. The fluorescence intensity was measured using a synergy NEO microplate reader at 25 ^{o}C.

2. Temperature stability:

The PAATC nanogel sensors (1 mL, 0.2 mg/mL, 1×PBS buffer) were randomly divided into four groups in 2 mL plastic tubes. Then, each group was irradiated by X-rays at doses of 2, 5, 10, and 20 Gy. Prior to testing the temperature stability, the nanogels were stored at 4 °C. The fluorescence intensity was measured using a synergy NEO microplate reader with a temperature controller.

3. Long-term stability:

The PAATC nanogel sensors (1 mL, 0.2 mg/mL, 1×PBS buffer) were randomly divided into four groups in 2 mL plastic tubes. Then, each group was irradiated by X-rays at doses of 2, 5, 10, and 20 Gy. Prior to testing the long-term stability, the nanogels were stored at 4 °C. The fluorescence intensity was measured using a synergy NEO microplate reader at an interval of 2 days at 25 °C.

Cytotoxicity test:

The cytotoxicity of the PAATC nanogels was evaluated by the CCK8 assay. Briefly, the A549 cells (6000 cells per well) were first cultured in a 96-well culture in an incubator (37 ℃, 5% CO_2). When the cells grew up to 50% of the square of each well, PAATC nanogel solutions at a given concentration were added (100 μL/well) and incubated for another 24 h. Then, a mixture of 10 μL of CCK8 solution and 90 μL of

the medium was added to each well, followed by incubation at 37 °C for another 1 h. The cell viability can be estimated by monitoring the optical density of the mixture at 450 nm:

$$\text{Cell viability (\%)} = (OD_{Treated}/OD_{Control}) \times 100\%$$

where $OD_{Treated}$ and $OD_{Control}$ were obtained in the presence and absence of the PAATC nanogels, respectively.

The morphology of the cells was observed using Leica inverted microscope (DMI1 LED S40).

The measurement of intracellular IR doses:

A549 cells were plated on a 12-well culture with 20 mm glass bottom well in an incubator (37 ℃, 5% CO_2) for 24 h. Then, A549 cells were incubated with PAATC nanogels (0.4 mg/mL) for another 24 h and washed with PBS buffer (pH=7.4) three times to remove the residue nanogels in solution. After that, the cells were irradiated using a RS 2000 irradiator with 2 and 4 Gy and finally imaged using a laser scanning confocal fluorescence microscope (FV 1200, OLYMPUS) with a 20× objective lens.

Results and discussion

Table S1. I_{450}/I_{580} values of PAATC nanogels in a dose range of 0-20 Gy.

Dose (Gy)	I_{450}/I_{580}	Error of the standard deviation (%)
0	0.06156	3.73595
0.1	0.09686	3.92310
0.2	0.12292	1.45212
0.5	0.21361	3.89020
1	0.38846	4.20116
1.5	0.57433	2.50203
2	0.73532	4.09483
5	1.91750	3.68813
10	3.67332	2.12737
15	5.49556	1.62211
20	7.10496	1.46278

Error of the standard deviation= (the standard deviation of $\mathbf{I_{450}/I_{580}}$)/ ($\mathbf{I_{450}/I_{580}}$)

Table S2. The reproducibility of the measurements.

Radiation dose (Gy)	Measured dose (Gy)	Standard deviation (Gy)
0.1	0.09664	0.0055
0.2	0.20568	0.0102
0.5	0.51267	0.0084
1	0.99190	0.0109
1.5	1.51838	0.0178
2	1.92070	0.0203
5	5.21600	0.0237
10	10.23628	0.0263
15	14.93535	0.0247
20	19.96347	0.0197

Error= (Measured dose-Radiation dose)/ Radiation dose

Figure S1

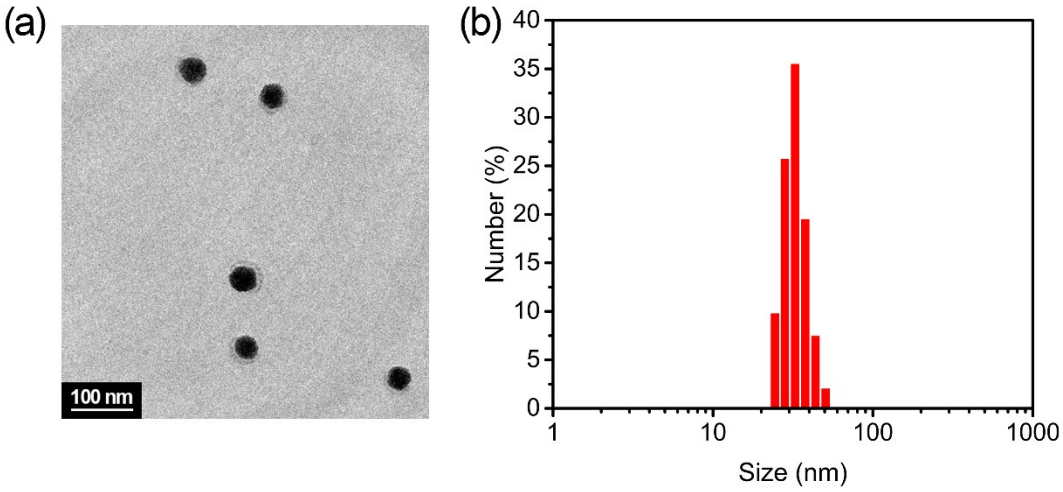

Figure S1. (a) TEM image and (b) DLS of PAATC nanogels.

Figure S2

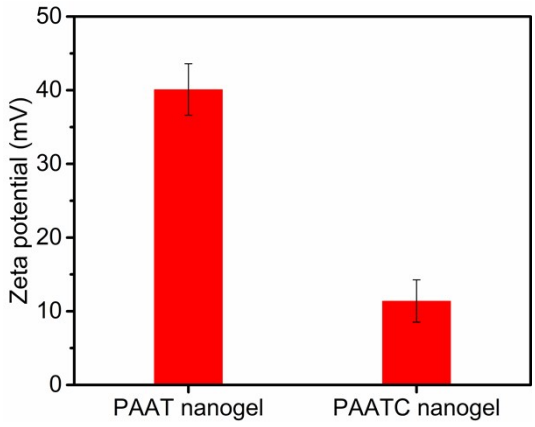

Figure S2. Zeta potential of PAAT and PAATC nanogels. Each data point is the average of 3 measurements with the error bars showing the standard deviation.

Figure S3

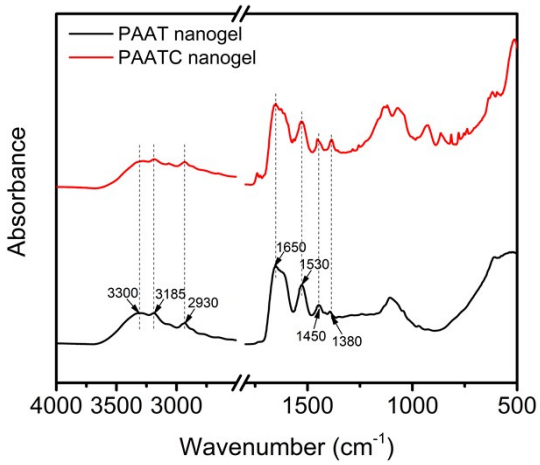

Figure S3. ATR-FTIR spectra of the PAAT and PAATC nanogels.

PAAT nanogels showed peaks centered at 3300 and 3185 $cm^{-1,}$ which can be ascribed to the characteristics peaks of $-NH_2$ and –NH, respectively. The peak at 2930 cm^{-1} can be assigned to the vibration of $–CH_3$. The peaks at 1650 and 1530 cm^{-1} were assigned to C=O (amide I) and C-N, respectively. In addition, the peaks centered at 1450 cm^{-1} and 1380 cm^{-1} were ascribed to the $-CH_3$ bending vibration. These peaks can also be seen in the case of the PAATC nanogels. In addition, the peaks in the 1250-650 cm^{-1} region observed for the PAATC nanogels were assigned to the characteristic peaks of the benzene ring from CCA. Although the TAMRA molecule has a benzene ring structure, the characteristic peak in this region was hardly detected, possibly due to the relatively low concentration.

Figure S4

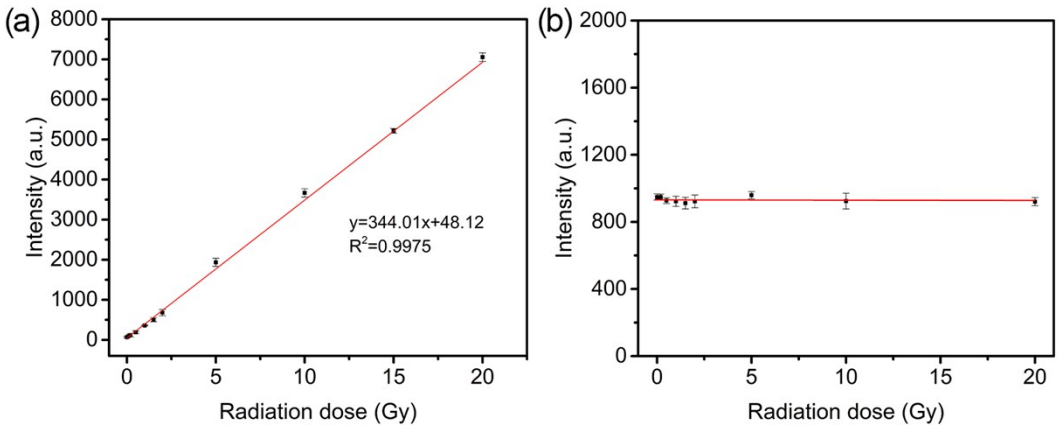

Figure S4. Fluorescence intensity at (a) 450 and (b) 580 nm of the PAATC nanogels after exposure to X-rays. λ_{ex1}=400 nm, λ_{ex2}=530 nm. Each data point is the average of 5 measurements with the error bars showing the standard deviation.

Figure S5

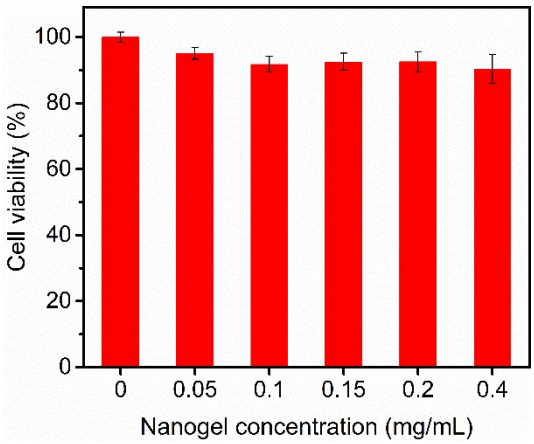

Figure S5. Cytotoxicity of PAATC nanogels. Each data point is the average of 10 (x 3) measurements with the error bars showing the standard deviation.

Figure S6

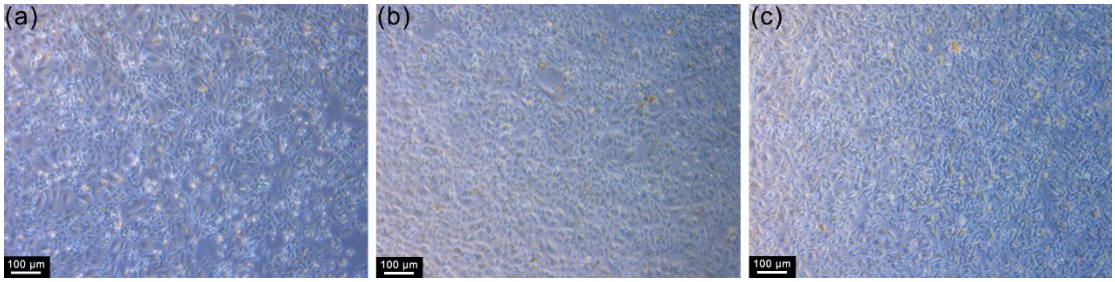

Figure S6. Cell morphology of A549 cells incubated with nanogels for 24 h. Nanogels concentration: (a) 0, (b) 0.2, (c) 0.4 mg/mL.

Figure S7

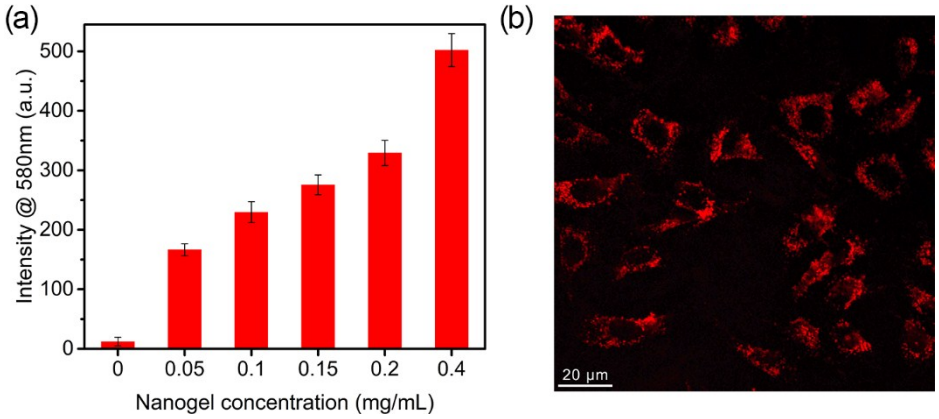

Figure S7. (a) Cell uptake of PAATC nanogels with different concentrations. Each data point is the average of 10 (x 3) measurements with the error bars showing the standard deviation. (b) Fluorescence image of A549 cell incubated with PAATC nanogels (0.4 mg/mL).

References

1. M. King and R. Kopelman, *Sensors and Actuators B: Chemical*, 2003, **90**, 76-81.