Supporting information

A Facile Strategy for the Large-Scale Preparation of Starch-Based AIE Luminescent Nanoaggregates via Host-Guest Interactions and Their Versatile Applications

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Materials and Methods

Materials

Corn starch (15-20% amylose content), hydrochloric acid, absolute ethanol, sodium bicarbonate, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, and potassium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Potassium bromide, glucose, dextran, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol))-2000] [DSPE-PEG_{2000}] were obtained from Sigma-Aldrich. 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) and cell counting kit-8 (CCK-8) were purchased from Thermo Fisher Scientific. All the AIEgens were previously synthesized by our lab (CUHK-SZ): B represents TPE-Q2[1]; Y represents IQ-DPA[2]; R represents DCPy[3]. Distilled deionized water was obtained from a Millipore Milli-Q-RO4 water purification system with a resistivity of 18.2 MΩ cm^{-1}. A dialysis membrane with MWCO of 1400 Da was purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China).

Instruments

All fluorescence spectrum measurements were carried out with a Fluoromax-4P spectrometer (Horiba). The absorption spectra were measured with a PerkinElmer Lambda-950 spectrophotometer. Transmission electron microscopy (TEM) studies were performed on an FEI Tecnai G2 Spirit transmission electron microscopy operating at 120 kV. Dynamic light scattering (DLS) was used to determine the hydrodynamic diameter of the nanoparticles in Milli-Q water. Fourier transform infrared spectroscopy (FTIR) characterizations were conducted on a Nicolet Nexus 670 FTIR spectrometer with KBr pellets. Powder X-ray diffraction (XRD) characterizations were performed on a D/max 82400 X-ray powder diffractometer (Rigaku, Japan) by using CuKa (λ = 0.154056 Å) as the incident radiation. Fluorescence photographs were taken under 365-nm excitation by a handheld ultraviolet (UV) lamp. Fluorescence quantum yields were measured by Quantaurus-QY C11347-11. Live-cell fluorescence images were acquired by UltraVIEW VoX (PerkinElmer) confocal laser-scanning microscopy (Nikon C1-Si

TE2000, Japan). Scanning electron microscopy (SEM) was conducted using Hitachi Regulus 8100.

Synthesis of starch nanoparticles (SNPs)

SNPs were prepared by an ultrasonic-assisted acid hydrolysis process. In brief, 30 g corn starch was used to prepare a 30% (*m*/*v*) starch suspension in 100 mL of distilled water followed by sonication at 400 W for 30 min. The supernatant was discarded after centrifugation. Starch in the pellet was mixed with 150 mL of 3 M H_2SO_4 solution. The suspension was incubated in a water bath at 40℃ with mechanical stirring at 200 rpm for 20 h. After hydrolysis, the suspension was centrifuged at 16200 *g* for 30 min. The pellet was washed with deionized water and centrifuged 3 times to ensure the filtrate had a constant pH value of 6.5. The final suspension of SNPs in water was further dialyzed against deionized water for 24 h to remove the residual H_2SO_4. The dialyzed SNP suspension was lyophilized to achieve 4.35 g loose SNP powders.

Fabrication of starch luminescent nanoaggregates (SLNs)

The AIEgen solutions were prepared by dissolving AIEgens (B, Y, or R) in 200 μL dimethyl sulfoxide, which were then added dropwise to a stirring SNP solution and stirred for 10 min to obtain the SLNs. The solution was then centrifuged three times at 10,000 rpm for 10 min, dispersed in a deionized aqueous solution, and stored at 4℃ for further experiments.

Optimal ratio of SNPs and AIEgens

Firstly, a panel of twelve AIEgen solutions at a concentration of 1 μM in 1 mL Eppendorf tubes were prepared. Then, a series of SNPs were added to the solutions, resulting in final SNP concentrations of 0.01, 0.05, 0.1, 0.2, 0.5, 0.8, 1, 2, 3, 4, 5 and 8 $mg \cdot mL^{-1}$, respectively. The solutions were mixed for 5 min by a shaker. The corresponding fluorescence intensity and correlation curve were determined by fluorescence spectrometry.

Photostability of the SLNs

100 μL of the SLN suspension was added to a 96-well enzyme label plate. Each fluorescent dye had six groups in parallel, and the same amount of AIEgen solutions were used as the control group. The enzyme label plate was wrapped in aluminum foil and irradiated with UV light at 365 nm for different periods of time. Subsequently, the fluorescence intensities of the samples were collected by a microplate reader.

CCK-8 and ROS assays

100 μL of HeLa cells were seeded in a 96-well plate at a density of 20 000 cells/well and incubated overnight for adhesion. Cells were then incubated with SNPs for 24 h. For the CCK-8 assay, 20 μL of CCK-8 (5 $mg \cdot mL^{-1}$) was added to each well and the absorbance at 490 nm was measured using a microplate reader. For the reactive oxygen species (ROS) assay, cells were incubated with B/Y/R or B/Y/R SNPs for 4 h. After washing away the unbounded dyes, 150 μL DCFH-DA (10 μM) probe was added to each well and then cells were irradiated with white light for 5 min with a power density of 0.22 $W \cdot cm^{-2}$, followed by culturing, the fluorescence intensity was measured using a microplate reader at designated time points.

Preparation of fluorescence hydrogels

Solution I, containing SNPs (B, Y, or R), 0.4 mg/ml acrylamide (AAM), 25 mM *N*,*N*'-methylene bisacrylamide (MBA), 9.6 mg/ml poly(*N*-isoproplyl acrylamide) (PNIPAM), and 32 mM tetramethylethylenediamine (TEMED), and solution II, containing 50 mM $K_2S_2O_8$, were first prepared. 9 mL of solution I and 1 mL of solution II were mixed and rapidly cast into a Teflon template. Photoluminescent PAAM/PNIPAM-CD hydrogels were obtained after storage at room temperature for 8 h.

Fingerprint development

To extract fingerprints, volunteers first daubed their fingers on greasy areas and pressed on the surfaces of paper, plastic, and glass to obtain latent fingerprints (LFPs). Then, lightly sprinkle fluorescent powder ground with mortar on the LFP. After a few seconds, the excess powder was carefully removed, and LFPs were captured by the camera under natural and UV light at 365 nm.

Cell imaging

HeLa, NIH/3T3, MDA-MB-231, HFF, and 4T1 cells were grown on 18 × 18 mm^2 glass coverslips and cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS), 100 $mg \cdot mL^{-1}$ glutamine, penicillin (100 $units \cdot mL^{-1}$), and streptomycin (100 $U \cdot mL^{-1}$) at 37°C in a humidified atmosphere of 5% CO_2 overnight. The cells were then incubated with 300 $\mu g \cdot mL^{-1}$ SNPs for 24 h. Finally, cell imaging was performed with a Nikon inverted Eclipse Ti microscope equipped with a 20× 0.75 NA objective or confocal laser scanning microscopy (Nikon C1-Si TE2000, Japan).

Lymph imaging

Female BALB/c mice (18-22 g) were provided by the Animal Center of Zhengzhou University (Zhengzhou, China). All animal protocols were evaluated and approved by the Ethics Committee of the Animal Laboratory of Zhengzhou University. SLN-R was injected into the hind footpad of the inoculated side of BALB/c mice with lymph node metastases. An equivalent amount of AIE-R was used as a control. At predetermined times after injection, the mice were anesthetized with tert-amyl alcohol. Mice were shaved, glued to trays, and imaged by a Zoom Stereo Microscope SZX-RFL-2 (Olympus, Tokyo, Japan) equipped with a fluorescence illuminator and a CCD camera (PCO, Kelheim, Germany). The excitation and emission for the red filter set

were 510 to 550 nm and 590 nm, respectively. Images were acquired using the Camware camera-controlling software program (PCO).

Statistical analysis

Each experiment was repeated three times, and the results were expressed as mean ± standard deviation, and statistical analysis was performed using the statistical *t*-test method. Data between different groups were compared via a one-way analysis of variance. The following P-values were considered statistically significant: $P<0.05$: *; $P< 0.01$: **; and $P< 0.001$: ***.

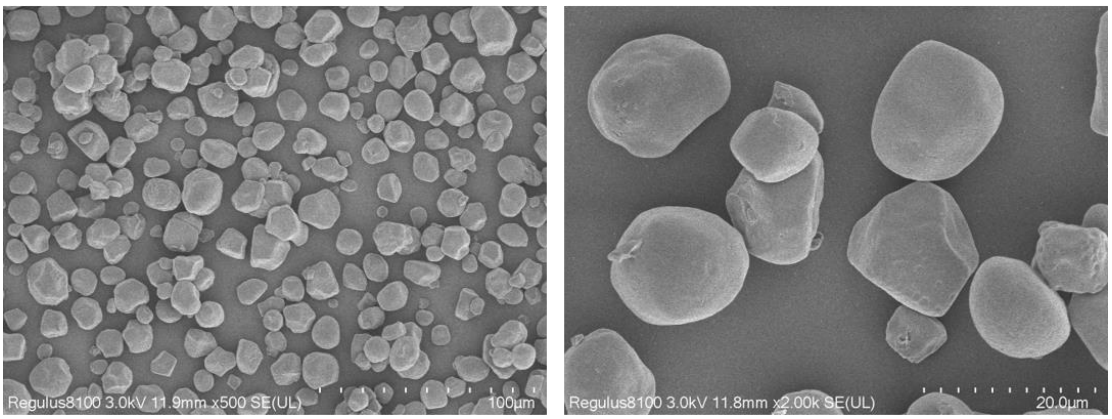

Figure S1 SEM images of starch granules at different magnifications.

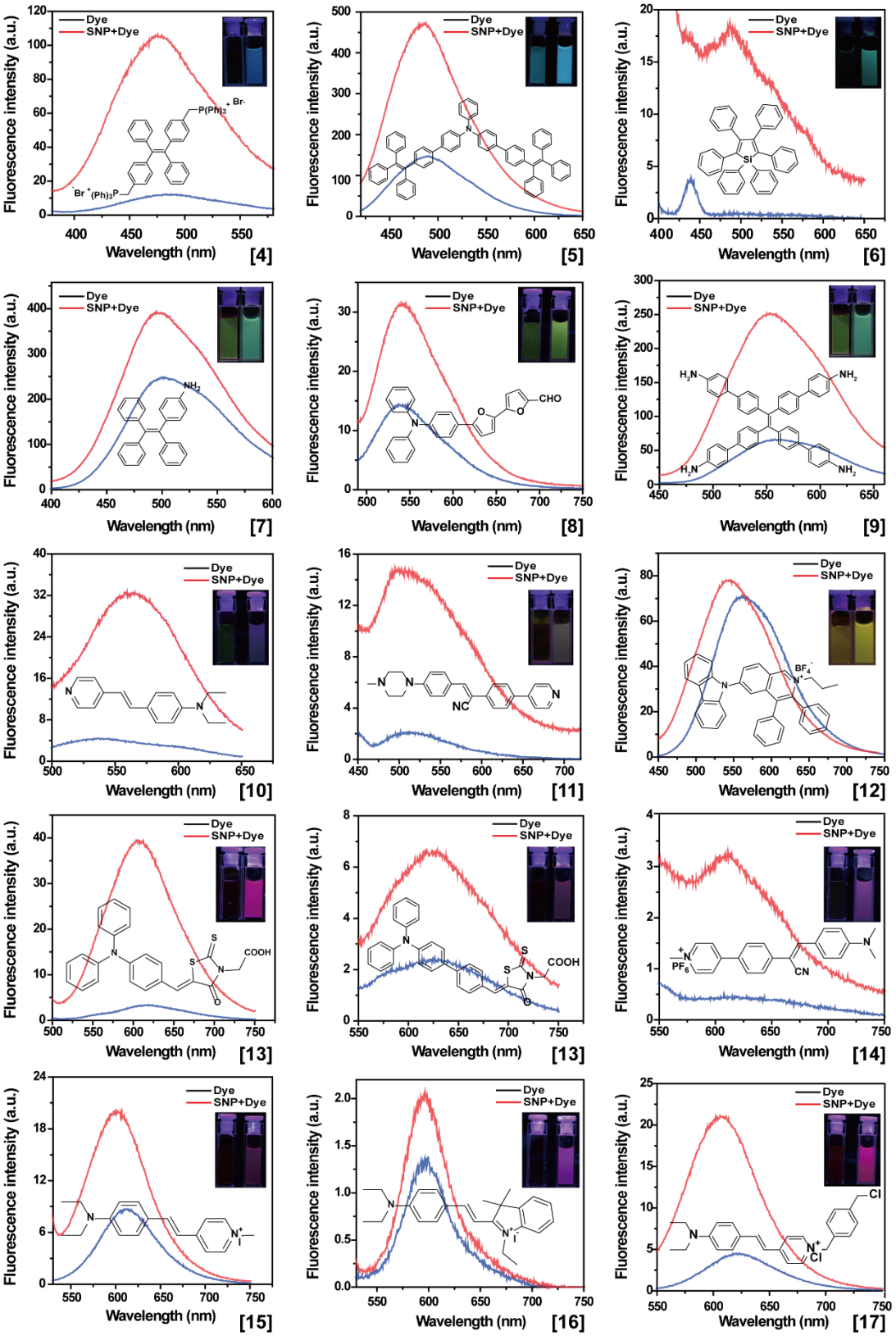

Figure S2 Fluorescence spectra of fluorescence dye solutions before and after addition of SNPs. (Insets are corresponding images captured under a handhold UV lamp). a.u., arbitrary unit. (Tang's Laboratory provides all the AIEgens used above. The synthesis

processes, yields, and purities can be found in previous literatures. [4-17])

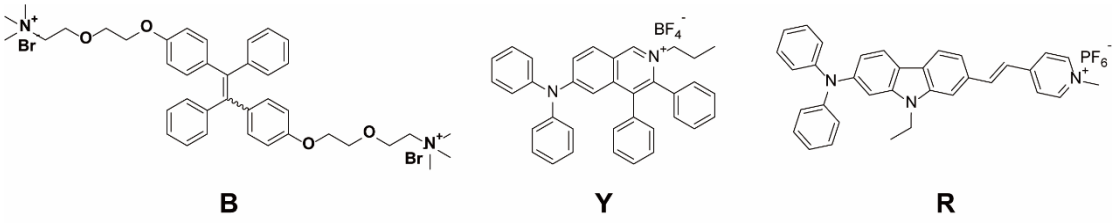

Figure S3 Chemical structures of B, Y, and R.

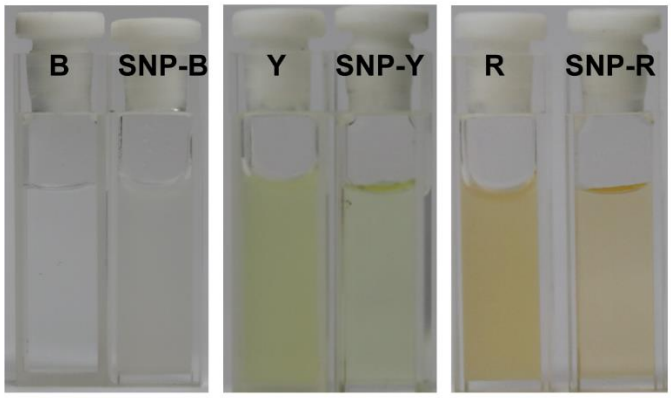

Figure S4 Images of B, SNP-B, Y, SNP-Y, R, and SNP-R solutions.

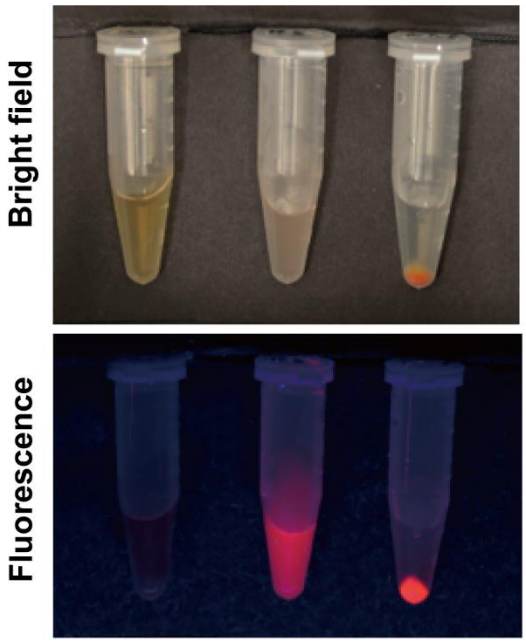

Figure S5 Bright-field and fluorescence images of R, SNP-R (before centrifugation), and SNP-R (after centrifugation).

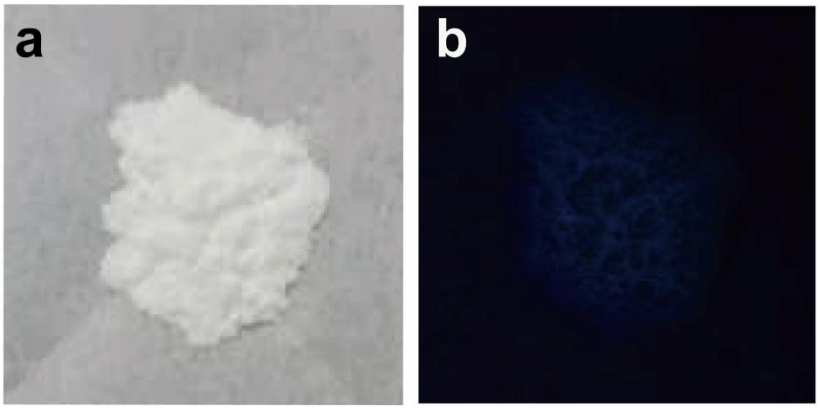

Figure S6 Bright-field (a) and fluorescene images (b) of SNPs.

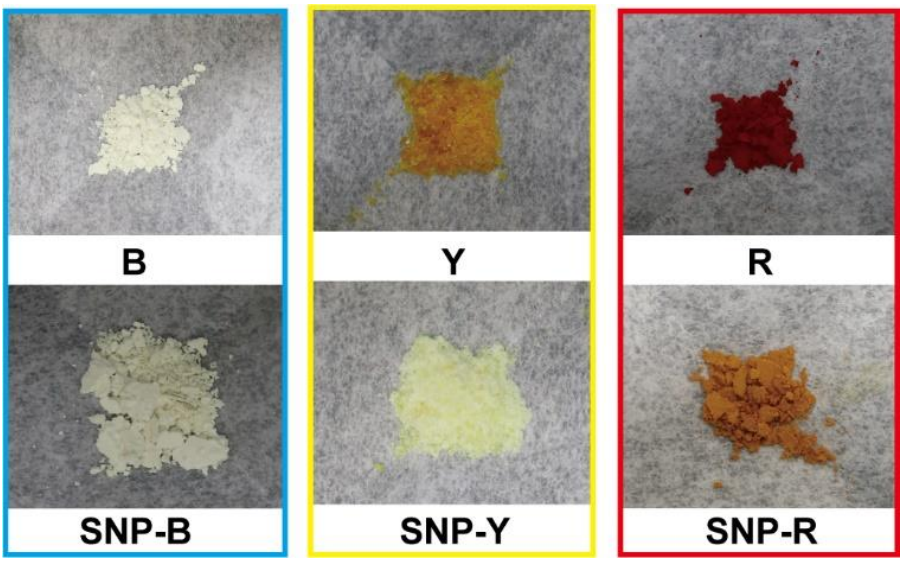

Figure S7 Optical photographs of B, Y, R and SNP-B, SNP-Y, SNP-R powders.

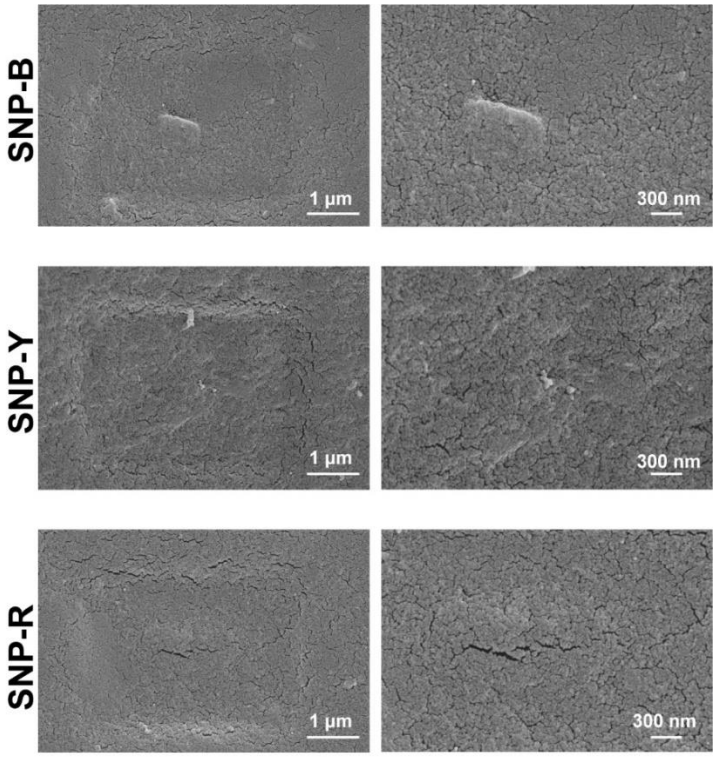

Figure S8 SEM images of SNP-B, SNP-Y, and SNP-R at different magnifications.

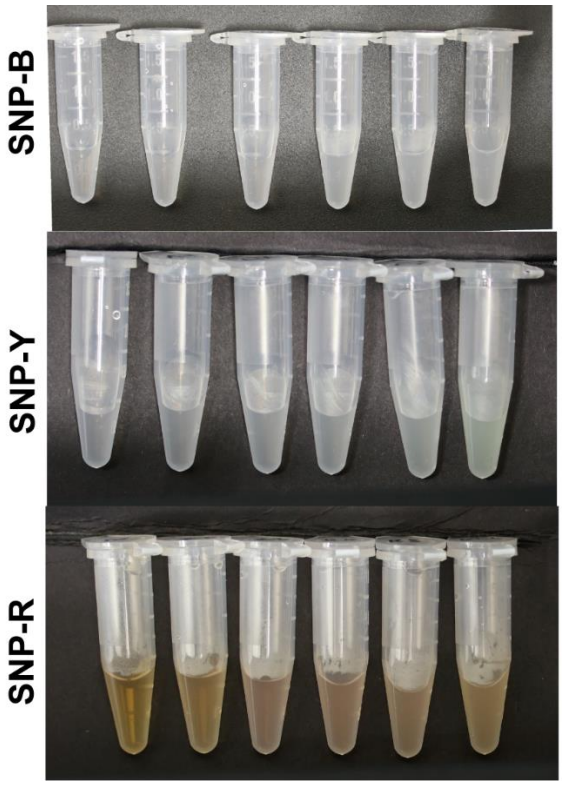

Figure S9 Bright-field images of B, Y, R with a series concentration of starch nanoparticles (0, 0.1, 0.5, 1.5, 3.0, 8.0 mg·mL^{-1}) .

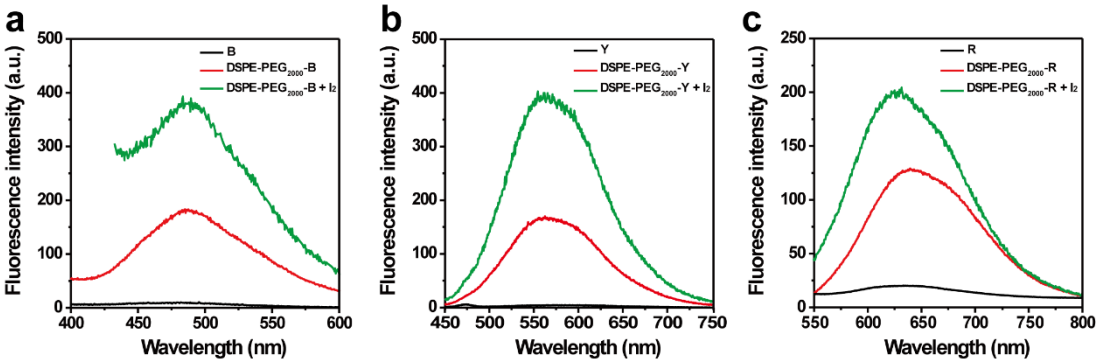

Figure S10 Fluorescence spectra of (a) B, DSPE-PEG$_{2000}$-B, DSPE-PEG$_{2000}$-B + I_2; (b) Y, DSPE-PEG$_{2000}$-Y, DSPE-PEG$_{2000}$-Y + I_2; (C) R, DSPE-PEG$_{2000}$-R, DSPE-PEG$_{2000}$-R + I_2. a.u., arbitrary unit.

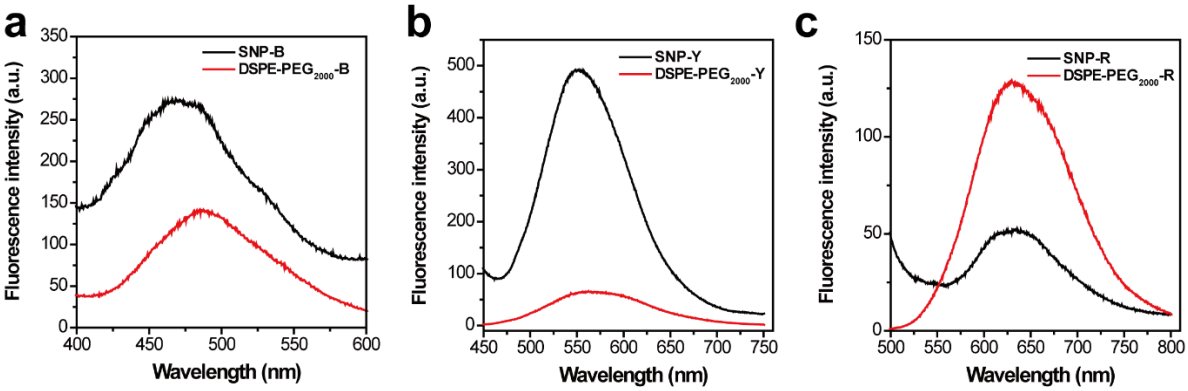

Figure S11 Fluorescence spectra of SNP-B, DSPE-PEG$_{2000}$-B; SNP-Y, DSPE-PEG$_{2000}$-Y; SNP-R, DSPE-PEG$_{2000}$-R. a.u., arbitrary unit.

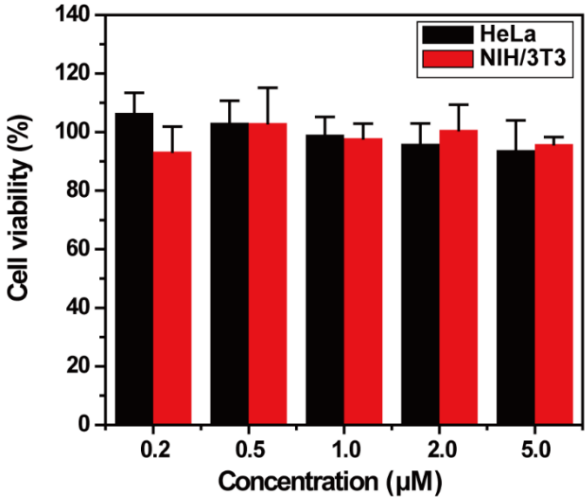

Figure S12 Viabilities of HeLa and NIH/3T3 cells after incubation with SNP for 24h. Data represents mean ± standard deviation. n =6.

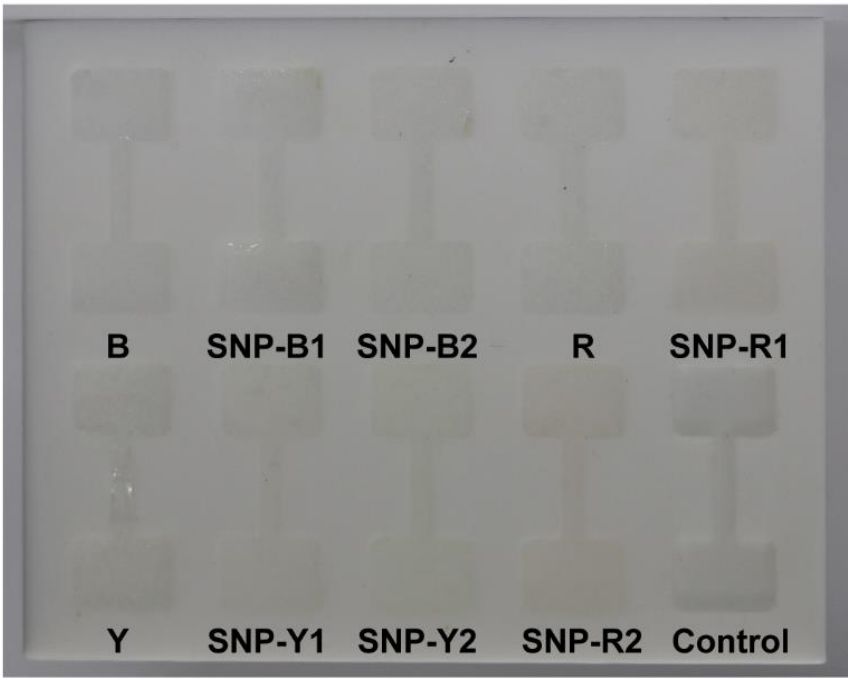

Figure S13 The bright-field image of hydrogel doped with different fluorescent materials.

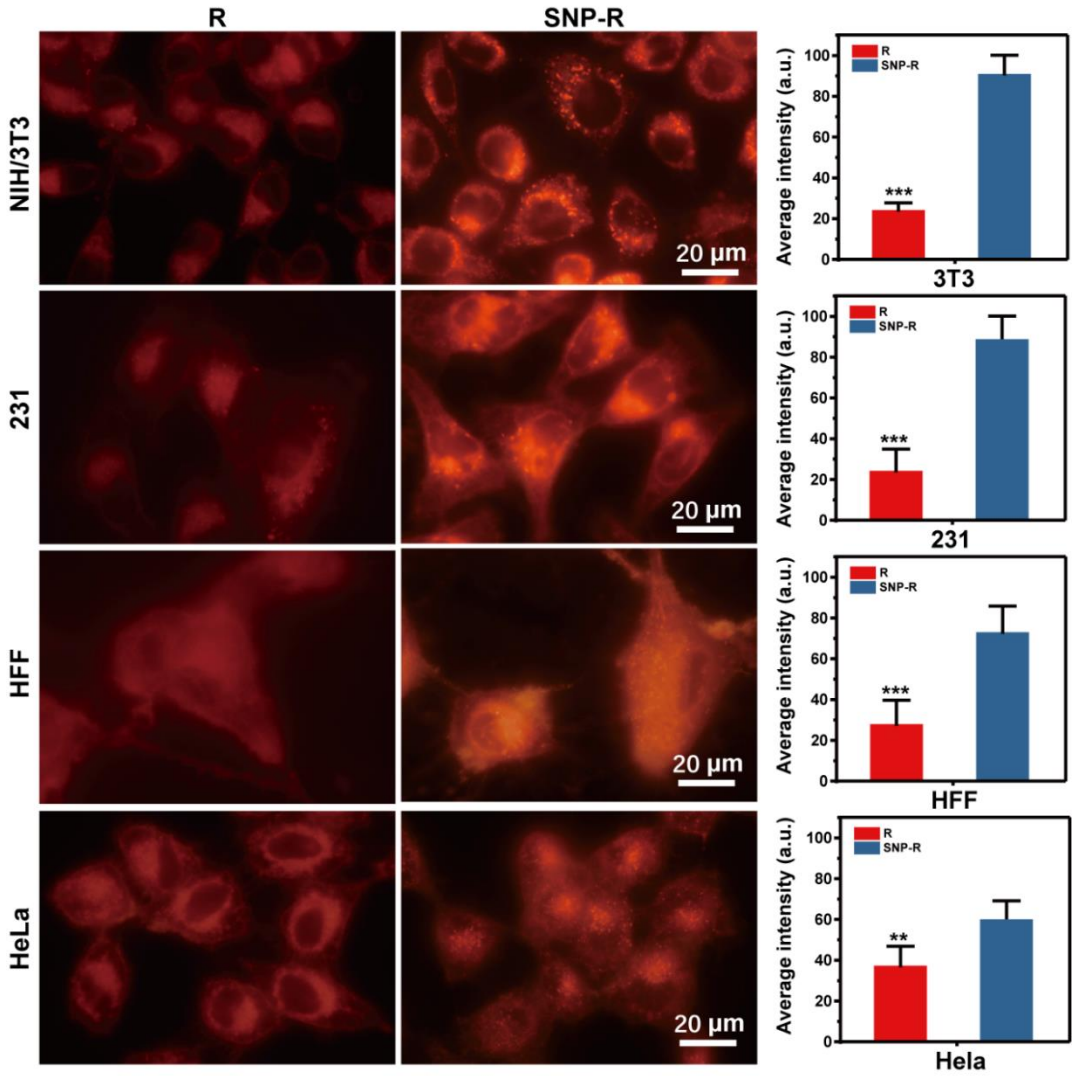

Figure S14 Fluorescence images of R and SNP-R in different cell lines. Data represents mean ± standard deviation. $n = 3$. *t test used,* $^{**}P < 0.01$, $^{***}P < 0.001$.

Table S1 Quantum yields (QY) and fluorescence lifetimes of different materials.

Material	QY solid	QY solution	Lifetime (ns) (solution)
B	0.536	0.015	2.08
SNP-B	0.732	0.030	2.50
Y	0.065	0.005	1.68
SNP-Y	0.373	0.147	2.76
R	0.024	0.017	2.60
SNP-R	0.103	0.218	2.08

Reference:

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