SUPPORTING INFORMATION

Photoswitching Near-Infrared Fluorescence from Polymer Nanoparticles Catapults Signals over the Region of Noises and Interferences for Enhanced Sensitivity

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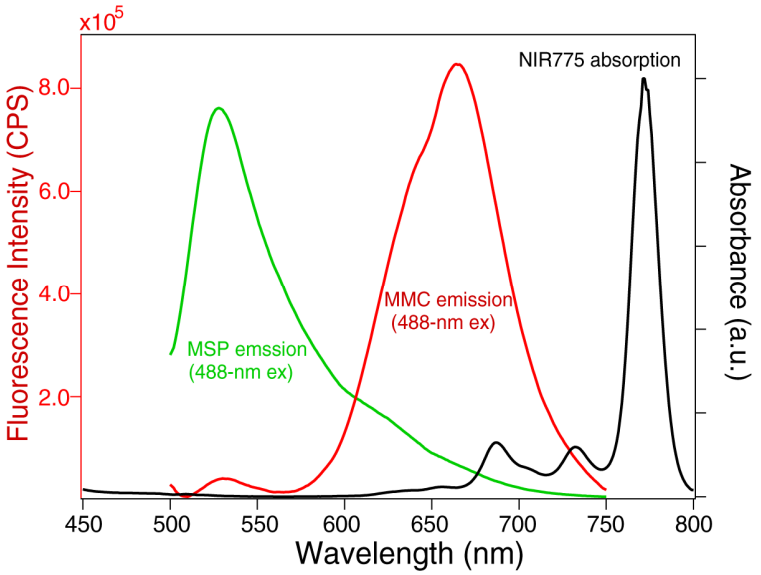

Figure S-1. Fluorescence emission spectra of MSP-containing polymer nanoparticles (NPs) aqueous sample before (green solid curve) and after (red solid curve) illumination of 365-nm UV light and the absorption spectrum of the NIR775 fluorescent dye in THF solution (black dot curve).

MSP-containing polymer nanoparticles (NPs) before UV irradiation emit vivid green fluorescence with maximum at 530 nm. Upon illumination of 365-nm light, MSP moiety residing in the hydrophobic core of polymer NPs is converted to its counterpart isomer MMC, which typically emits intense red fluorescence in the region of 560-750 nm with the maximum at 665 nm. Owing to such photochemical reaction based one-to-one MSP-to-MMC conversion, the strong red fluorescence emerges at the expense of the green emission originating from MSP moiety, giving rise to ~95% of the original intensity (at 530 nm) attenuated. The absorption spectrum of NIR775 dye in THF solution displays an intense peak at 771 nm and noticeable broad absorption band in the region of 600-750. Specifically, such broad absorption band appreciably overlaps with the emission band of the MMC-containing polymer NPs and therefore potentially enables Förster resonance energy transfer (FRET) from the MMC moiety to NIR775 molecule. In contrast, the absorption band of NIR775 dye does not display noticeable overlap with the emission band of the MSP-containing polymer NPs, thus inhibiting the FRET process from MSP moiety to NIR775 molecule.

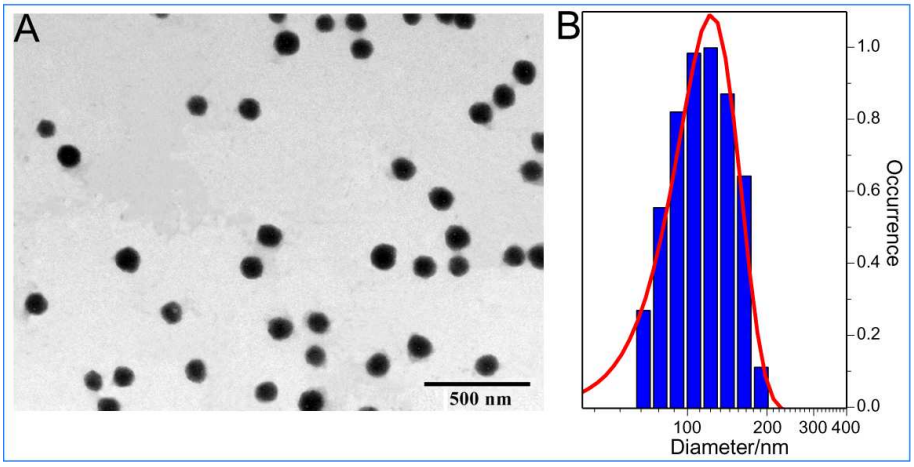

Figure S-2. TEM (A) and DLS (B) characterization results of a representative version of polymer NPs with relatively large size.

TEM (JEM 1200EX, JEOL) and DLS (Nicomp 380 ZLS Particle Size Analyzer) techniques were applied to characterize the as-prepared polymer NPs. Two representative versions of NPs were measured with the characterization results shown in Figure 1 in the main text and the Figure S-2. Note that for the DLS results, the counterpart TEM characterization report average diameter results of ~50 nm and ~100 nm, respectively. Such discrepancy is probably attributable to the hydrodynamic swelling effect as the DLS measurement was carried out with the core-shell NPs carrying hydrophilic shell suspended in an aqueous environment while the TEM measurement was made with dry NPs under high vacuum conditions.[1] In the radical-initiated microemulsion polymerization, the water soluble ABVA is used to firstly initiate polymerization of water-soluble NIPAM monomeric units. Owing to its thermosensitive nature, poly(2,2'-*N*-isopropylacrylamide) (PNIPAM) self-organizes into micelles with the assistance of Tween 20 at temperatures above its low critical transition temperature. Following the formation of micelle structures, hydrophobic monomers including ST, BA, MSP, and DVB begin to participate in the polymerization and are incorporated into the growing polymer chains. Thus, core-shell type polymer NPs with hydrophilic PNIPAM shells and hydrophobic cores with MSP moiety covalently incorporated in the polystyrene (PST) skeletons that are cross-linked with DVB are eventually obtained. Specifically, hydrophobic fluorescent NIR775 dyes are also incorporated into the cross-linked PST matrix and therefore reside in the hydrophobic cavities of the particles. Such core-shell polymer nanostructures have been experimentally validated, confirming that the hydrophobic dyes reside in the hydrophobic cores of the NPs.[2] Owing to the core-shell structure,

in aqueous solutions the photoactive component MSP and NIR775 are protected in the hydrophobic core of the NPs and their fluorescence is unlikely to be quenched by components of biological milieu that cannot permeate through the hydrophilic shell. Additionally, the hydrophobic PST matrix in the core part of the NPs surrounds the photoactive MSP and NIR775 components, providing another protective layer for them.

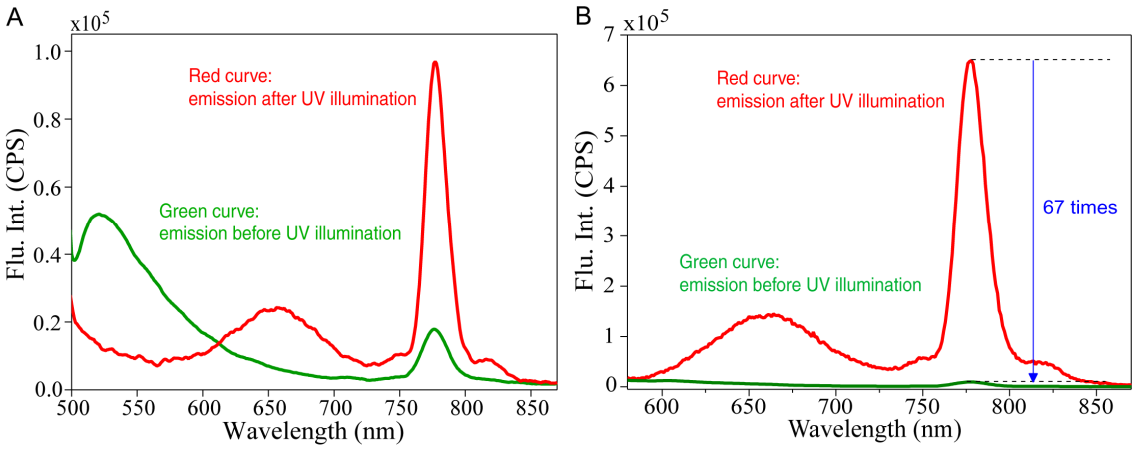

Figure S-3. NIR fluorescence photoswitching features of the aqueous dispersion sample containing suspended photoswitchable polymer NPs. (A) Fluorescence emission spectra of the sample before (green curve) and after (red curve) UV illumination upon excitation of 473-nm light. (B) Fluorescence emission spectra of the sample before (green curve) and after (red curve) UV illumination upon excitation of 561-nm light.

Upon 473-nm excitation, the aqueous NPs sample before UV illumination emits green fluorescence with maximum around ~530 nm and a relative weak peak at 776 nm. Taking the negligible overlap between the absorption band of NIR775 dye with the green fluorescence band of MSP-containing NPs, as shown in Figure S-1, such NIR emission feature may not originate from the FRET from MSP to NIR775. It is noted that NIR775 dye exhibits relatively weak but noticeable absorption at 473 nm, which is therefore responsible for the observed weak NIR emission feature of the sample upon 473-nm excitation. In contrast to the weak emission feature of the sample before UV illumination, the NPs sample after pre-illumination of 365-nm light displays intense fluorescence emission feature with maximum around ~776 nm and a moderate peak around ~660 nm upon 473-nm excitation. Upon UV-illumination, MSP moiety is converted to its counterpart isomer MMC and the latter emits vivid red fluorescence upon excitation. For polymer NPs with embedded MSP moiety and NIR775 dye, the overlap between the absorption band of the NIR dye and the emission band of the MMC moiety activates FRET process from the

MMC to NIR775 molecule within the close proximity. As a result, the NIR fluorescence (at 776 nm) increases at the expense of the green fluorescence (at 530 nm). Alternating UV and visible illumination induces the reversible interconversion between MSP and MMC, thus enables the reversible NIR fluorescence photoswitching on and off.

It is noted that for abovementioned NIR fluorescence photoswitching, the "off" state, namely the sample before UV pre-illumination, exhibits unnegligible "background" noise owing to the weak but noticeable absorption feature of NIR775 dye at the excitation wavelength. Actually, upon excitation with light in the region of 470-500 nm, the polymer NPs sample display NIR fluorescence photoswitching with on/off ratio varying roughly from 5 to 8 depending on the excitation wavelength. For photoswitching-based biological fluorescence imaging, fluorescence probes with high on/off ratio are undoubtedly desired for improved imaging resolution. For the photoswitchable polymer NPs with optimized format, NIR fluorescence photoswitching with significantly improved on/off ratio was obtained by shifting the excitation wavelength to the region that MMC has appreciable absorption while NIR775 dye has lowest absorbance, around 563 nm. Taking the availability of the laser source in biological fluorescence imaging, we evaluated photoswitching performance of the polymer NPs using 561-nm light as the excitation source, which yielded photoswitching feature with on/off ratio up to 67, as shown in Figure S-3B.

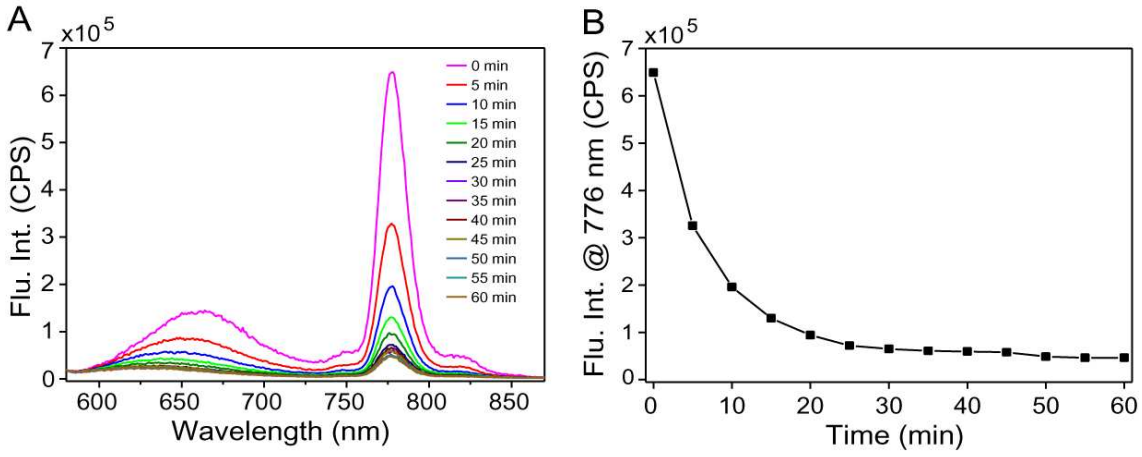

Figure S-4. (A) Thermally driven fluorescence emission spectrum evolution of the polymer NPs after UV preillumination at room temperature over time. (B) Evolution of the NIR fluorescence intensity (at 776 nm) of the polymer nanoparticle aqueous sample after pre-illumination of 365- nm light.

MSP-containing NPs after UV preillumination display strong NIR emission centered at 776 nm upon excitation of 561-nm light, indicating that most MSP molecules within the NPs were converted into the MMC and efficient FRET from MMC to NIR775 dyes. As time elapses after remove of the UV-illumination, the ring-opened MMC molecules within the NPs at room temperature undergo thermally driven (spontaneous) reversion back to the ring-closed MSP. As a result, NIR fluorescence together with the red emission features of MMC (centered at 665 nm) gradually decreases with time. Such NIR fluorescence On-to-Off conversion occurs thermally, but illumination of visible light may greatly accelerate the kinetics. Additionally, such observed thermally driven NIR fluorescence On-to-Off evolution is similar to that of thermally driven MMC-to-MSP conversion. [3]

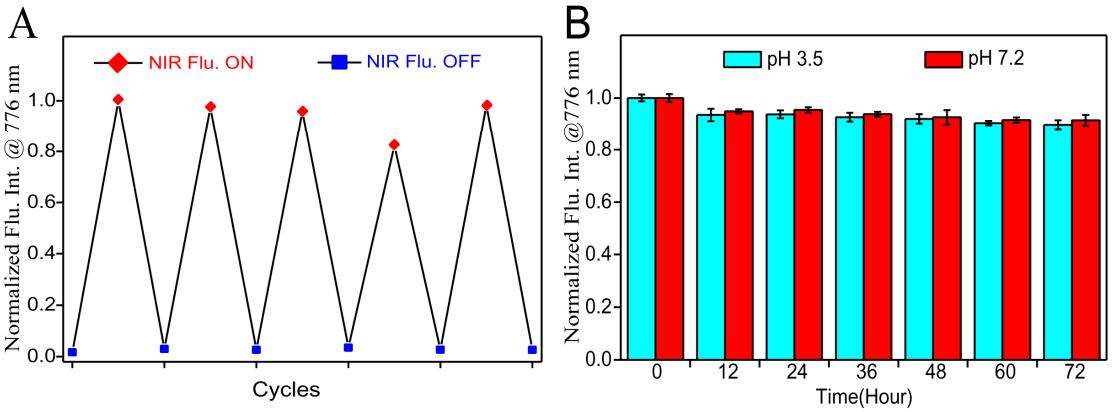

Figure S-5. (A) Modulation of the intensity of fluorescence at 776 nm with alternating UV and visible light; the concentration of nanoparticles dispersed in water is 1.0×10^{-8}M. (B) Investigation on potential NIR775 dye leaching from the hybrid polymer nanoparticles with averaged diameter of ~50 nm. The dye leaching problem of hybrid nanoparticle sample was gauged both in 10 mM PBS buffer (pH 7.2, red columns) and in acidic aqueous milieu (pH 3.5, cyan columns) at room temperature.

The repeatability of NIR fluorescence photoswitching of the polymer nanoparticle ensemble sample was evaluated by monitoring the NIR fluorescence intensity (at 776 nm) of the nanoparticle aqueous dispersion sample subsequent to illumination of alternating UV and visible light. Specifically, the intensities of the sample in "On" state were acquired following 2 minutes illumination of UV (365 nm) light from a handheld lamp; and those in "Off" state was acquired 10 minutes illumination of LED light using a filter for blocking the light with wavelength less than 525 nm. It can be seen from Figure 5-SA that for several cycles, at least, the modulation of NIR fluorescence was nearly fully reversible, demonstrating the well-behaved on-and-off

fluorescence switching properties and photostability of such hybrid nanoparticles.

Owing to their high hydrophobicity, NIR775 molecules are expected to self-aggregate in the aqueous milieu following their leaching from the hydrophobic core of the polymer nanoparticles, which in generally resulting in great decrease in the intensity of NIR fluorescence. To gauge the potential dye leaching, NIR fluorescence of the hybrid polymer nanoparticles in 10 mM of PBS buffer and in acidic aqueous milieu, respectively, were monitored with time up to 72 h. As shown in Figure S-5B, no appreciable leakage of NIR775 dye into the aqueous milieu was observed in both cases, suggesting the NIR775 dyes were tightly trapped within the hydrophobic core and did not suffer from dye leaching problem under our experimental conditions.

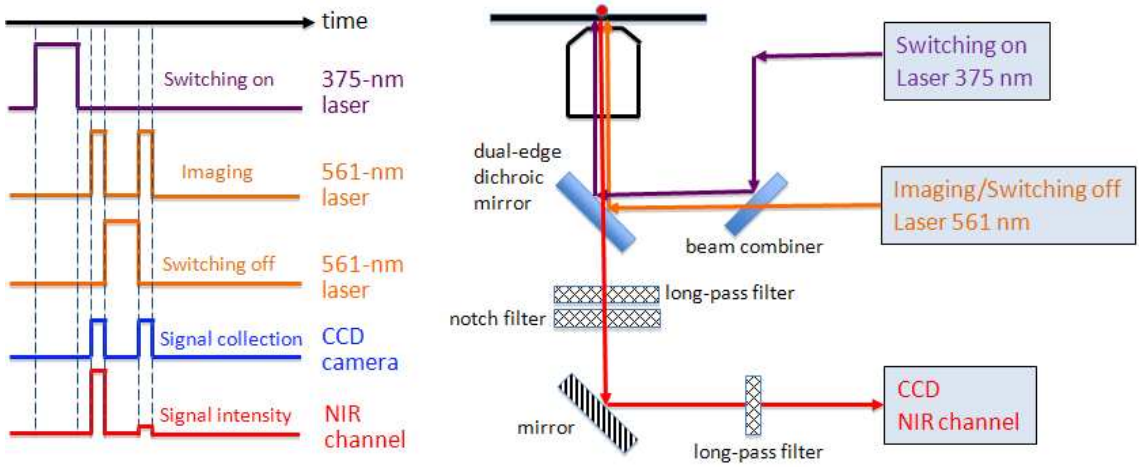

Figure S-6. Optical setup (right) for NIR-photoswitching fluorescence imaging: two lasers are sequentially brought to the microscope objective following the pattern shown on the left. The UV laser (375- nm) optically converts the MSP into MMC, thus turning on NIR fluorescence. Immediately, the 561-nm imaging laser will record the NIR fluorescence. The imaging excitation plus an additional 561-nm illumination convert the MMC into the MSP and therefore photoswitched the NIR NPs to the dark state, thus completing one switching cycle.

The 375-nm laser was switched on for 300 ms to photochemically convert MSP to MMC, thus turning on NIR fluorescence. Then the 561-nm imaging laser and CCD camera were turned on simultaneously for 100 ms to capture the bright NIR fluorescence image. To switch the MMC to MSP, the 561-nm yellow laser was turned on for additional 220 ms. Then again, the 561-nm imaging laser and CCD camera were turned on simultaneously for 100 ms to capture the dark NIR fluorescence image. The above laser and camera pulse sequences complete a periodic cycle that comprises of both the bright and dark states of the NIR fluorescence. This cycle was typically repeated 25 times to enhance the signal to noise ratio and image quality.

Reference

1. Zhu, M. Q.; Zhu, L. Y.; Han, J. J.; Wu, W. W.; Hurst, J. K.; Li, A. D. Q., Spiropyran-Based Photochromic Polymer Nanoparticles with Optically Switchable Luminescence. *J. Am. Chem. Soc.* **2006,** 128, 4303-4309.

2. Tian, Z. Y.; Wu, W. W.; Wan, W.; Li, A. D. Q., Single-Chromophore-Based Photoswitchable Nanoparticles Enable Dual-Alternating-Color Fluorescence for Unambiguous Live Cell Imaging. *J. Am. Chem. Soc.* **2009,** 131, 4245-4252.

3. Tian, Z. Y.; Li, A. D. Q.; Hu, D. H., Super-Resolution Fluorescence Nanoscopy Applied to Imaging Core-Shell Photoswitching Nanoparticles and Their Self-Assemblies. *Chem. Commun.* **2011,** 47, 1258-1260.