

Electronic Supplementary Information

Controlled Loading of Superparamagnetic Nanoparticles in Fluorescent Nanogels as Effective T2-Weighted MRI Contrast Agents

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S1. Chemical Structure Analysis of PBMA-*g*-(C₁₂/Fluorescein)

From the NMR spectrum (**Figure S1a**), the peak at 10.07 ppm was evident for the formation of the amide bond that followed the ring-opening of the anhydride rings after reaction with 1-dodecylamine and fluoresceinamine. The characteristic peaks of fluorescein could be observed from 6.5 ppm to 7 ppm, as well as the resonance due to the alkyl chain (C₁₂) of the hydrophobic side chains at 1.2 ppm and 0.84 ppm, although some of the peaks due to the main chain protons appeared to be obscured by the solvent and water peaks at 2.5 ppm and 3.3 ppm, respectively.

¹H NMR (300 MHz, DMSO-*d*₆, δ): 0.84 ppm (t, -(CH₂)₁₁-CH₃), 0.9 – 1.1 ppm (m, -CH₂-C(CH₃)₂-), 1.23 ppm (s, -CH₂-CH₂-), 2.47 – 2.53 ppm (d, -CH-CH-; t, -NH-CH₂-CH₂-; obscured by solvent peak), 6.5 – 7.0 ppm (m, Ar H), 10.07 ppm (br, -CO-NH-).

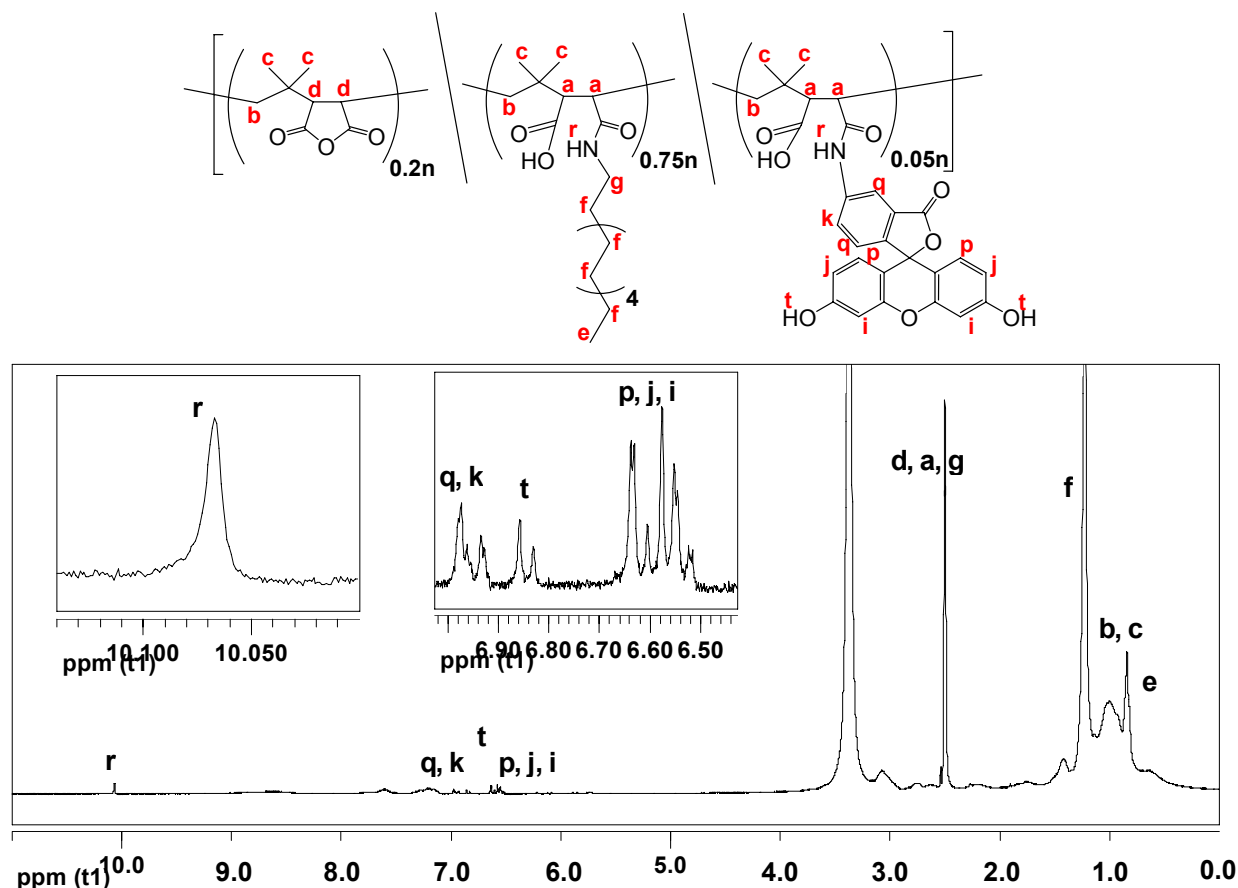


Figure S1a. ^1H NMR spectrum of PBMA-g-(C₁₂/Fluorescein) in DMSO-d₆. (Solvent peaks: DMSO-d₆ at 2.5 ppm, HOD at 3.3 ppm)

Further verification was performed using FT-IR spectral analysis. From the FT-IR spectra (**Figure S1b**), it could be observed the diminishing absorption peaks at 1835 and 1782 cm^{-1} , which were contributions by the stretching of the C=O bonds of the anhydride. The absorbance peaks at 1084 and 924 cm^{-1} due to stretching of the C–O bonds of anhydride were also reduced following reaction. The reduction of these peaks indicated that the maleic anhydrides had reacted. There was also the appearance of broad absorption peak from 3400 – 2400 cm^{-1} which indicated the presence of O–H stretching resonance of the carboxylic acids formed after ring-opening of the anhydrides. These observations were coupled with the appearance of the absorption peaks at 1713, 1605, and 1566 cm^{-1} , attributed to C=O stretching of carboxylic acids, C=O stretching of amides, and N–H bending of the amides, respectively.

This provided evidence for both the ring-opening of the anhydrides rings as well as the formation of the amide bonds between the anhydride and the amines. The double peaks of the amine (N–H stretching) between $3500 - 3200\text{ cm}^{-1}$ became a single diffused peak in the same region verified the reaction of the amine to form the amide bond with the anhydride. Lastly, the stretching of C–H bonds mainly due to the alkyl chain of the grafted side chains were recorded at 2930 and 2855 cm^{-1} . These results provided good evidence for the successful formation of PBMA-g-(C₁₂/Fluorescein) brush copolymer. Finally, the IR spectrum for IONC-6 showed the characteristic peaks of PBMA-g-(C₁₂/Fluorescein) together with the added peak at 609 cm^{-1} , which could be attributed to the Fe–O stretching of crystalline Fe₃O₄. This proved that the spheres were a composite mixture of these two different materials.

FT-IR (KBr): Wavenumber = $3500 - 3200\text{ cm}^{-1}$ (amide N–H stretching), $3400 - 2400\text{ cm}^{-1}$ (carboxylic acid O–H stretching), 2930 and 2855 cm^{-1} (C–H stretching); 1836 and 1782 cm^{-1} (anhydride C=O stretching); 1713 cm^{-1} (carboxylic acid C=O stretching), 1605 cm^{-1} (amide C=O stretching), 1566 cm^{-1} (amide N–H bending), 1084 and 924 cm^{-1} (anhydride C–O stretching), 609 cm^{-1} (Fe–O stretching).

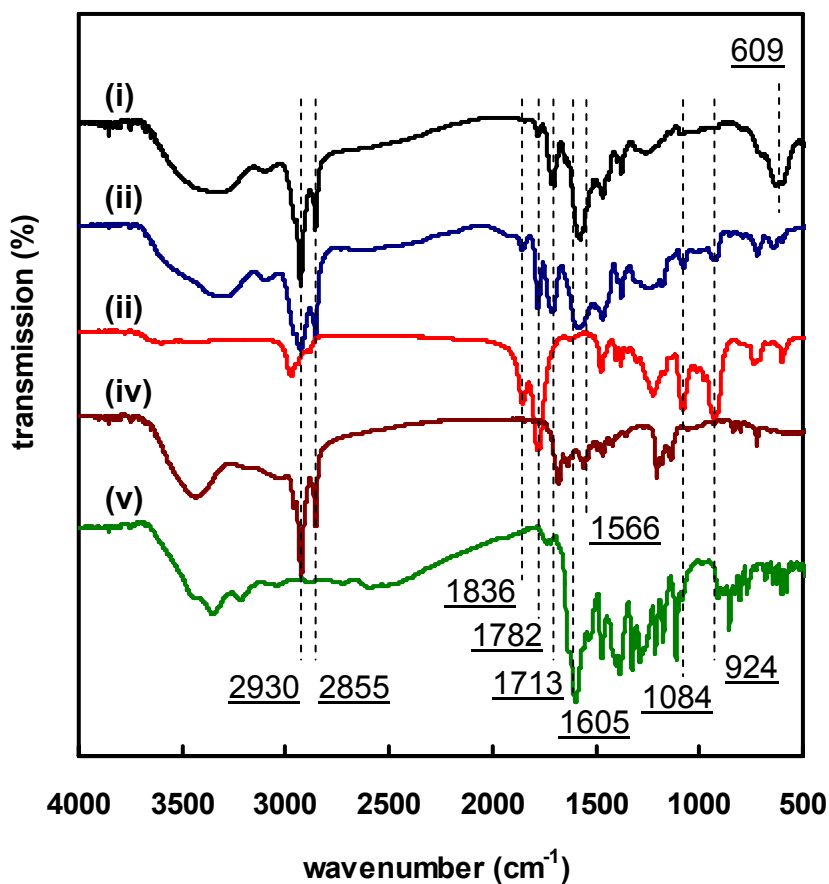


Figure S1b. FT-IR spectra of (i) IONC-6, (ii) PBMA-g-(C₁₂/Fluorescein), (iii) PBMA, (iv) 1-dodecylamine, and (v) fluoresceinamine.

S2. Determination of SPION-loading of IONCs

The iron oxide composition of the IONCs in terms of weight percentage was determined by thermogravimetric analysis (TGA) based on dried mass. The loading percentage increased with increasing amounts of SPIONs injected into the nanoemulsion.

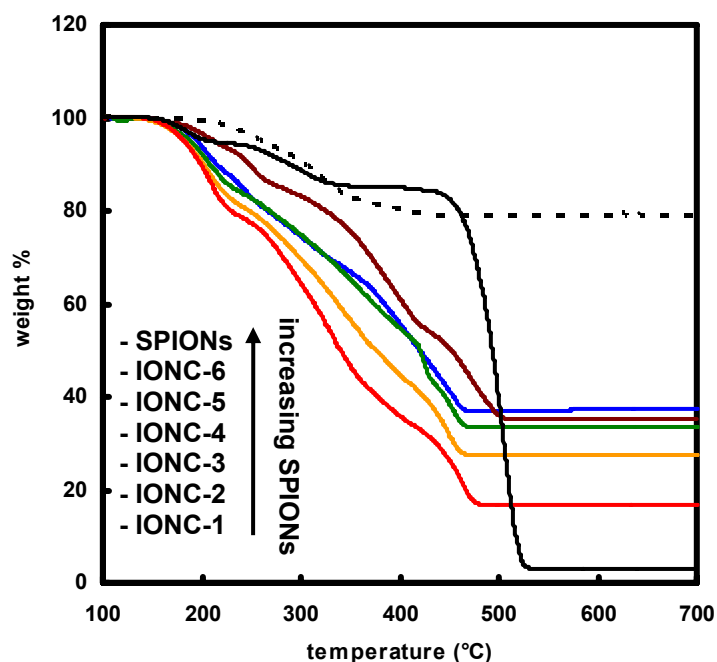


Figure S2. TGA profiles of SPIONs and IONCs in increasing order of iron oxide composition from bottom to top.

S3. Calculation of Intra-Particle Separation between SPIONs

Firstly, the average number of SPIONs in a sphere was estimated on the basis of SPION-loading obtained from TGA measurements. The densities of Fe_3O_4 and the polymer matrix were taken be $5.15 \text{ g}\cdot\text{cm}^{-3}$ and $1.3 \text{ g}\cdot\text{cm}^{-3}$, respectively, as adopted from common online resources. (ρ is density, m is mass, and $\text{wt}\%$ is SPION loading (TGA results))

$$\text{Volume of sphere: } V = \frac{4}{3} \pi r^3 \quad \text{----- (1)}$$

$$\text{Volume: } V = \frac{m}{\rho} \quad \text{----- (2)}$$

$$\frac{V_{\text{SPION}}}{V_{\text{IONC}}} = \frac{m_{\text{SPION}}}{m_{\text{SPION}} + m_{\text{PBMA}}} \cdot \frac{\rho_{\text{Fe}_3\text{O}_4} \text{wt}\% + \rho_{\text{PBMA}} (1 - \text{wt}\%) }{\rho_{\text{Fe}_3\text{O}_4}} = n_{\text{SPION}} \cdot \frac{r_{\text{SPION}}^3}{r_{\text{IONC}}^3} \quad \text{----- (3)}$$

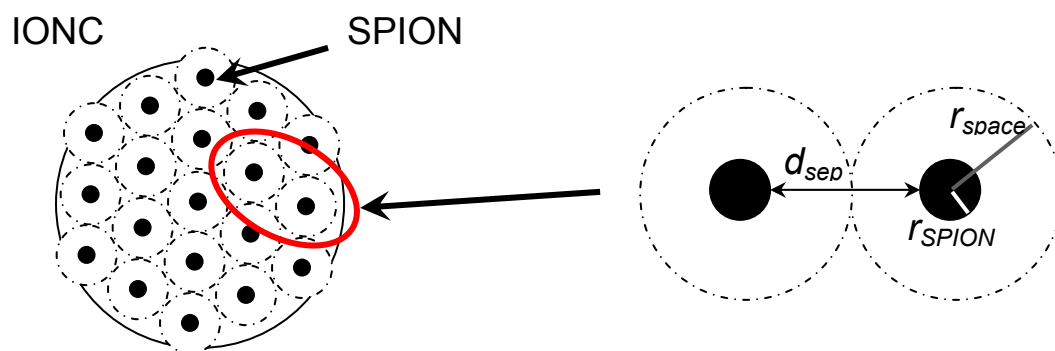
$$\text{wt}\% = \frac{m_{\text{SPION}}}{m_{\text{SPION}} + m_{\text{PBMA}}} \quad \text{----- (4)}$$

$$\frac{V_{SPION}}{V_{IONC}} = n_{SPION} \cdot \frac{r_{SPION}^3}{r_{IONC}^3} \quad \text{-----} \quad (5)$$

No. of SPIONs per sphere:

$$n_{SPION} = wt\% \cdot \frac{r_{IONC}^3}{r_{SPION}^3} \cdot \frac{\rho_{Fe_3O_4} wt\% + \rho_{PBMA} (1 - wt\%)}{\rho_{Fe_3O_4}} \quad \text{-----} \quad (6)$$

The average separation distance (d_{sep}) between the particles was estimated by assuming that the SPIONs were perfectly dispersed within the spherical nanogel matrix. Individual SPIONs occupied a volume of space, V_{space} , within the spherical matrix. The maximum volume fraction occupied by closed-packed spheres in a body was $\frac{\pi}{3\sqrt{2}} = 0.74$.



$$V_{space} = 0.74 \cdot \frac{V_{IONC}}{n_{SPION}} \quad \text{-----} \quad (7)$$

$$r_{space} = \left(0.74 \cdot \frac{V_{IONC}}{n_{SPION}} \cdot \frac{3}{4\pi} \right)^{\frac{1}{3}} \quad \text{-----} \quad (8)$$

$$\boxed{d_{sep} = 2 \times (r_{space} - r_{SPION})} \quad \text{-----} \quad (9)$$

S4. Morphology and Size Distribution Profiles of IONCs

The particles were observed under SEM to be spherical with smooth surfaces, with a uniform size distribution. The size distributions were narrow as confirmed by the DLS intensity-weighted size measurements. The particle sizes observed under the electron microscope were smaller than the DLS measurements. This was evident of the swelling of the nanospheres when dispersed in water during DLS measurements.

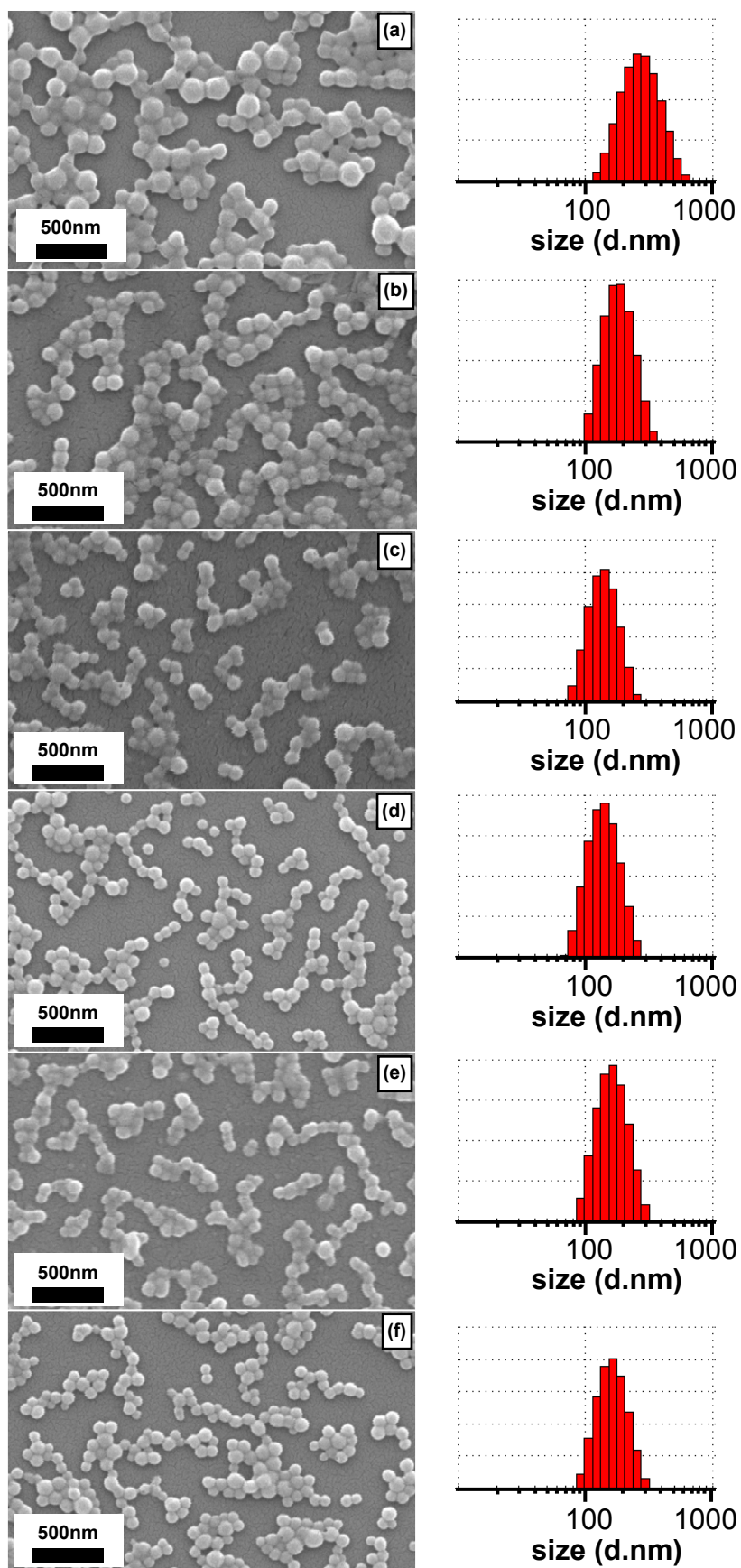


Figure S4. SEM images and the corresponding Z-average size measurements using DLS. (a) IONC-1, (b) IONC-2, (c) IONC-3, (d) IONC-4, (e) IONC-5, and (f) IONC-6.

S5. Morphology of SPIONs Before and After Phase Transfer

The SPIONs remained generally well-dispersed following phase transfer from chloroform to water. DLS size measurements suggested minor clusters that have an average size of 20.2 nm in water.

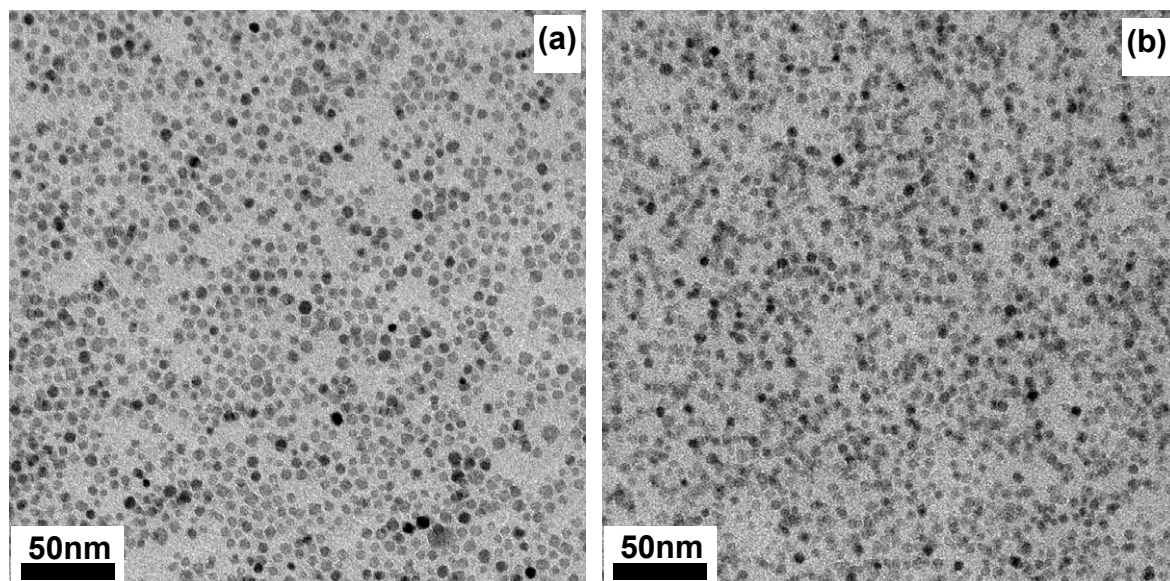


Figure S5. TEM images of (a) the as-synthesized hydrophobic SPIONs dispersed in chloroform, and (b) SPIONs after phase transfer to water using PBMA-*g*-(C₁₂/Fluorescein) as surfactant coating.

S6. Optical Properties of IONCs

The fluorescent properties of the fluorescein-modified IONCs were studied using PL and UV-Vis absorbance spectroscopy. Pure fluorescein-amine had a peak emission wavelength (λ_{em}) centered at 520 nm (green) and there was no observable shift in λ_{em} after conjugation to PBMA or by incorporating SPIONs to the nanospheres. However, there was a marked decrease in the PL intensity after the incorporation of SPIONs as compared to the blank fluorescent nanospheres. This could be attributed to the decrease in absorption intensity as the absorption peaks dropped with respect to increasing SPION-loading. This was likely due to contribution of SPION nanocrystals to the absorption and scattering of light within this range of wavelength, which caused a rise in overall baseline absorption levels and thus a reduction in quantum yield. Nevertheless, the IONCs exhibited substantial photoluminescent qualities and thus the cell labeling tests were performed.

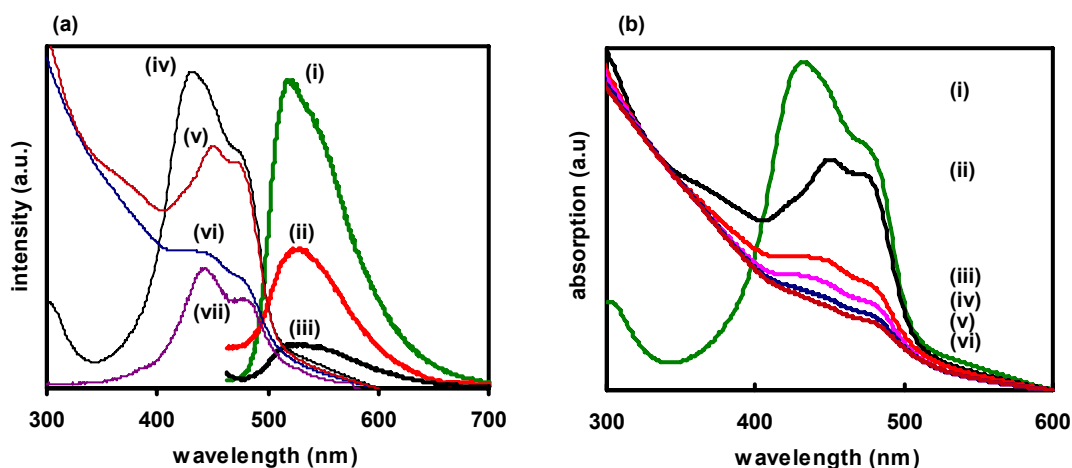


Figure S6. (a) PL spectra of (i) fluoresceinamine, (ii) blank fluorescent nanogels, and (iii) IONC-2 dispersed in water. UV-Vis absorbance spectra of (iv) fluoresceinamine, (v) blank fluorescent nanogels; and (vi) IONC-2 dispersed in water. (vii) The excitation spectrum for IONC-2 with the emission wavelength fixed at 520 nm. (b) UV-Vis absorbance spectra for (i) fluoresceinamine; (ii) blank fluorescent nanogels; (iii) IONC-2; (iv) IONC-3; (v) IONC-4; and (vi) IONC-5 in water.