Supporting Information

Red Fluorescent Carbon Nanoparticle-Based Cell Imaging Probe

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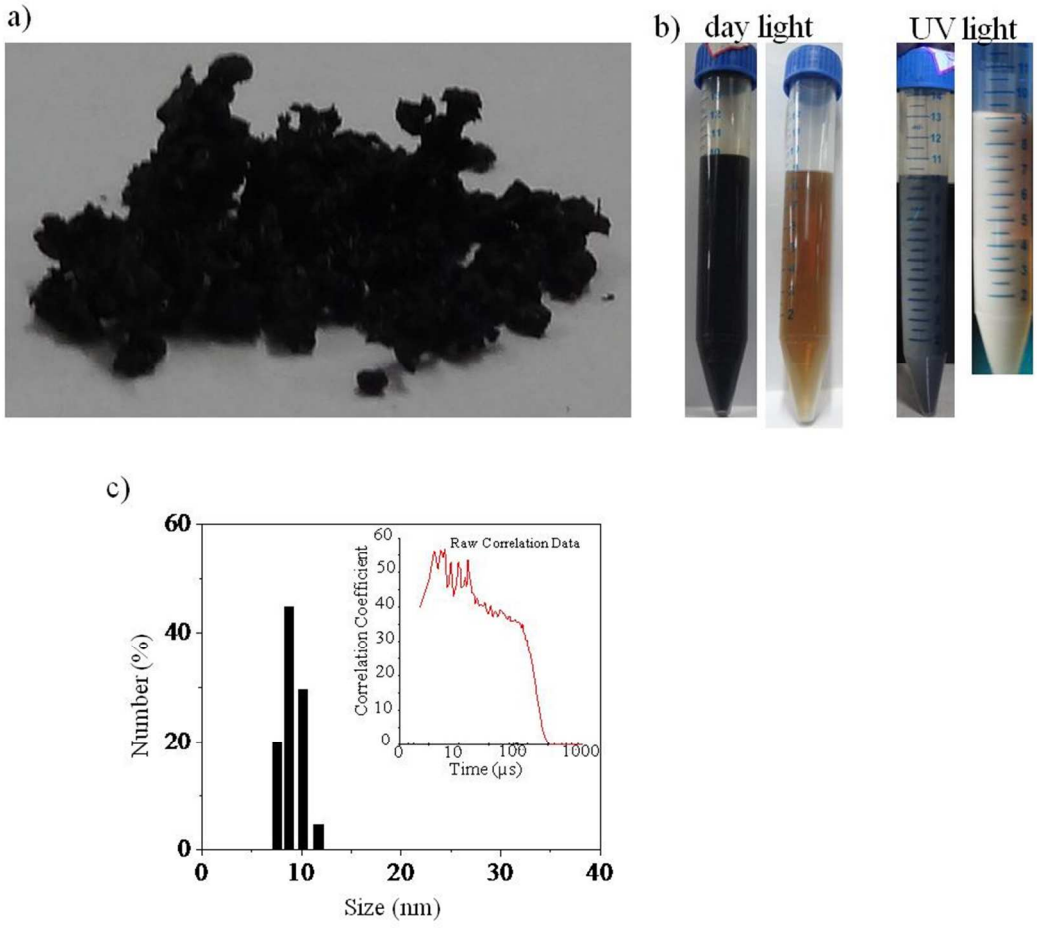

Figure S1. a) As synthesized hydrophobic FCN in solid form after purification, b) Concentrated and dilute chloroform solution of hydrophobic FCN under day light and UV light and c) Hydrodynamic size of hydrophobic FCN as observed from dynamic light scattering (DLS) study.

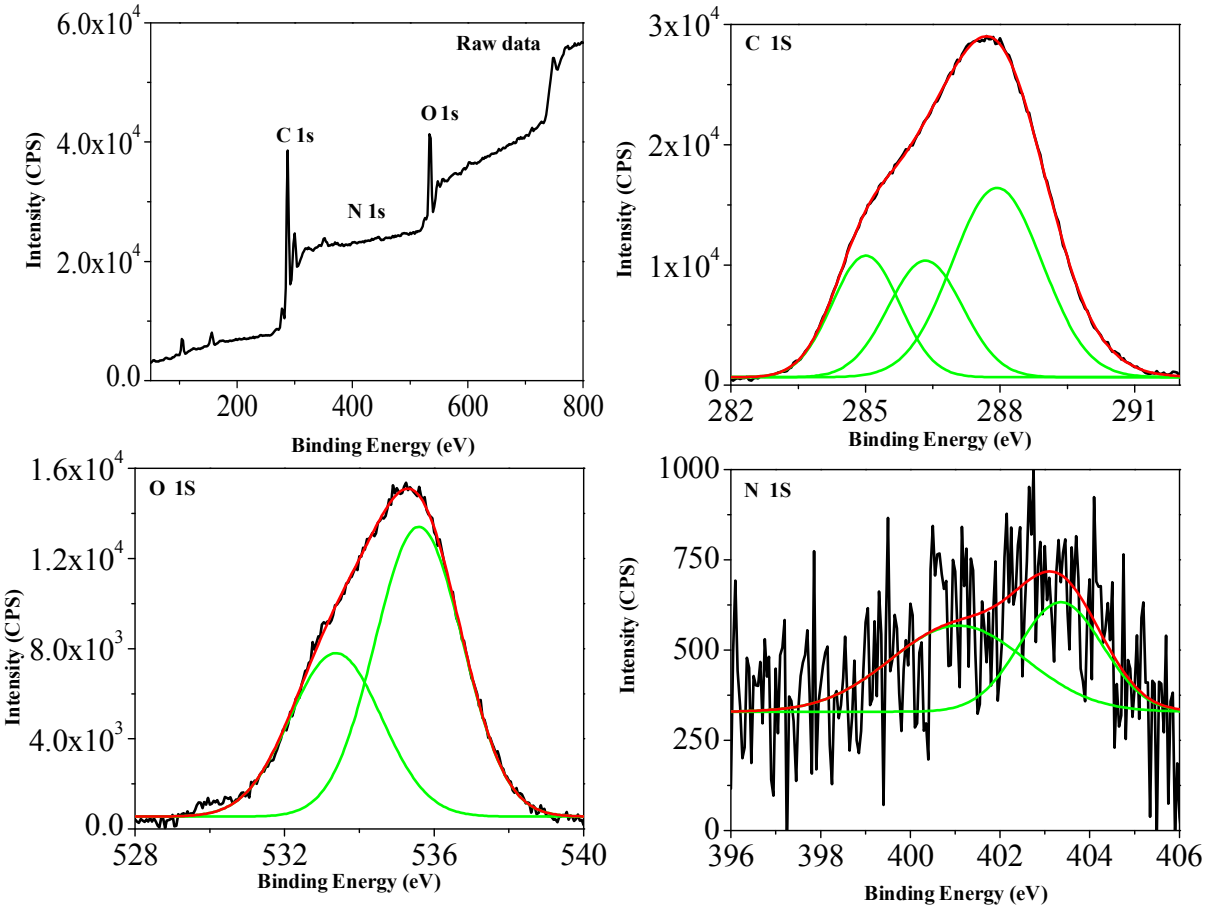

Figure S2. XPS characterization of hydrophobic FCN. The deconvoluted C 1s XPS spectrum shows C=C at ~ 285.0 eV, C–OH at ~286.3 eV and C=O (carbonyl groups) and/or -COO- (carboxylic acid groups, carboxylates, esters) and/or -CONH- (amides) at ~288.1 eV. The O 1s XPS spectrum shows two deconvoluted peaks at 533.5 eV and 535.8 eV. They are assigned as the chemically converted species of C=O and C–OH groups, respectively. The N 1s is fitted with two Gaussian peaks at 401 eV and 403.1 eV and are assigned as the N–C (sp2 bonding) and N–O, respectively.

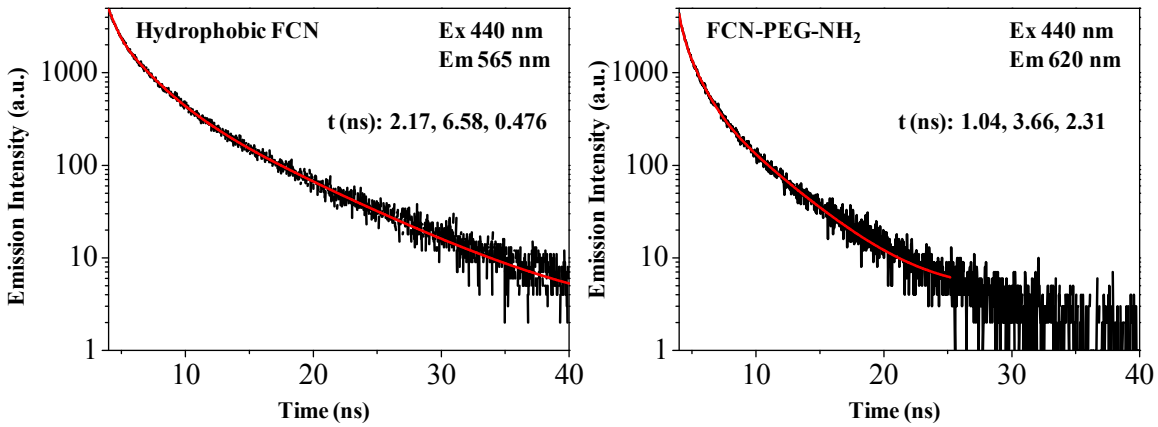

Figure S3. Fluorescence lifetime decay spectra of solutions of hydrophobic FCN and FCN-PEG-NH_2. Black line represents experimental data and red line represents fitted data. Experimental excitation/emission wavelength and resultant lifetime values are displayed inside each graph. The lifetime decay curve represents three lifetime components, pointing out the presence of multiple radiative species.

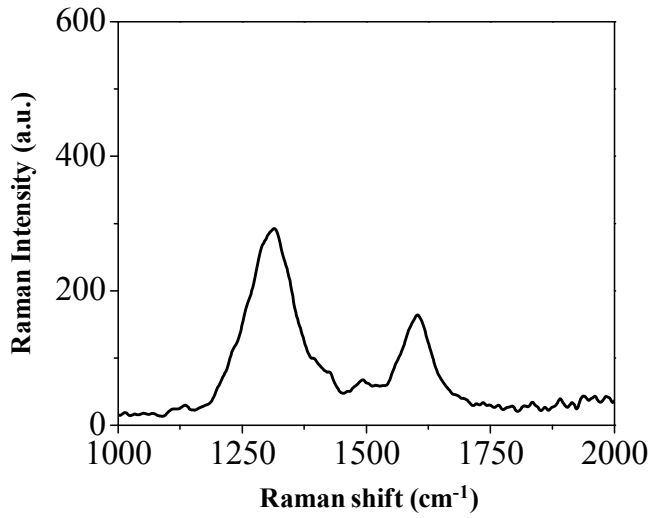

Figure S4. Raman spectra of hydrophobic FCN measured at 785 nm laser excitation showing prominent D band at 1305 cm^{-1} and G band at 1605 cm^{-1} with their intensity ratio of 1.8.

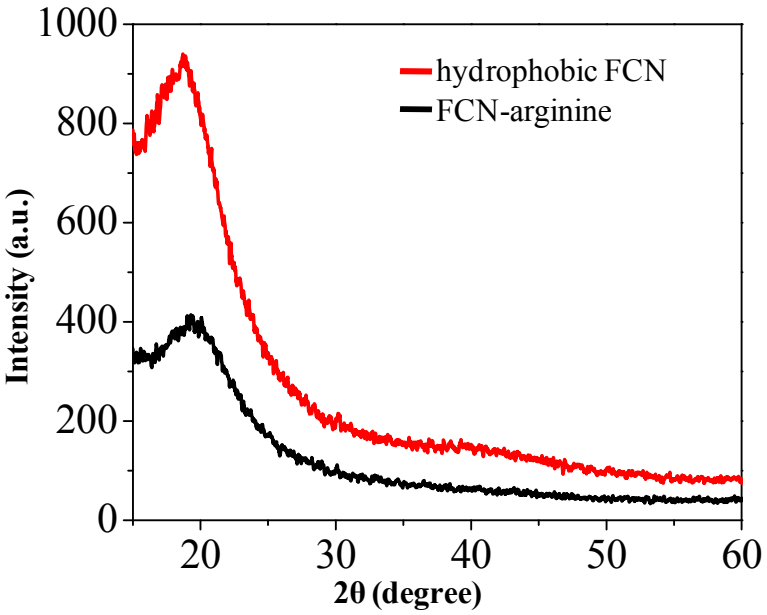

Figure S5. XRD of hydrophobic FCN and FCN-arginine showing reflections at 2θ~ 19° corresponding to amorphous carbon particles and the hump at 2θ~ 40° corresponding to (100) plane of particles.

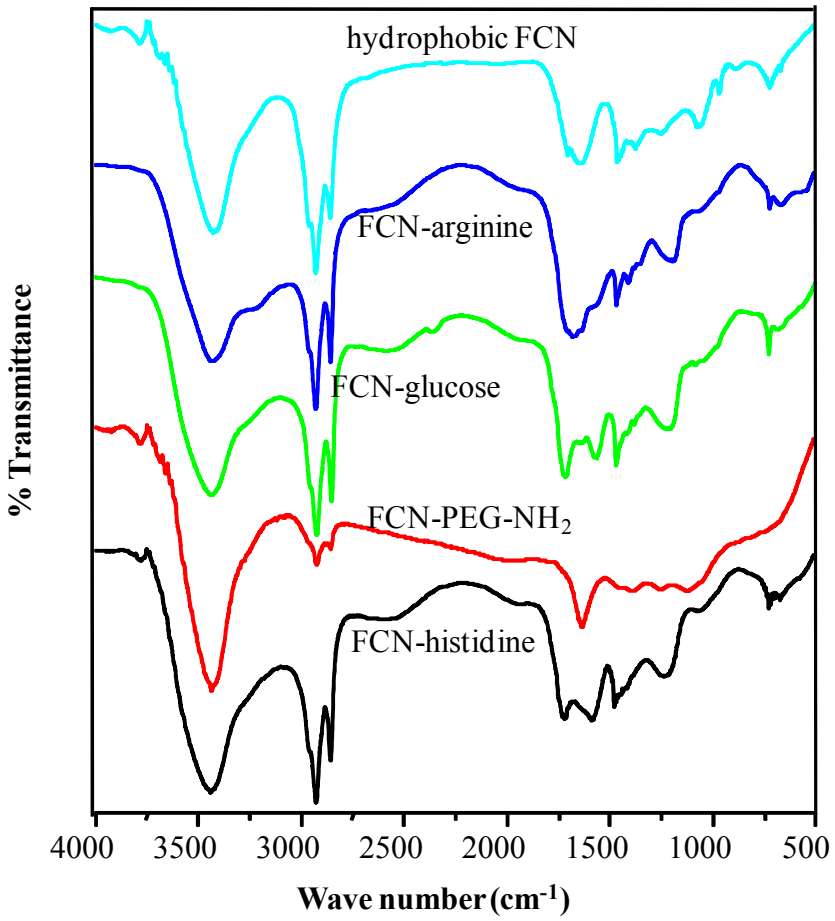

Figure S6. FTIR spectra of red fluorescent FCN before and after functionalization with PEG, histidine, glucose and arginine. Hydrophobic FCN shows intense O-H/N-H stretching band at 3400 cm^{-1} and C-H stretching band at 2900 cm^{-1}, suggesting the presence of hydrophobic oleylamine capping. FCN-PEG-NH_2 shows weaker C-H stretching band at 2900 cm^{-1}. Other functional FCN show N-H stretching band and C-H stretching band in addition to some changes in the fingerprint region.

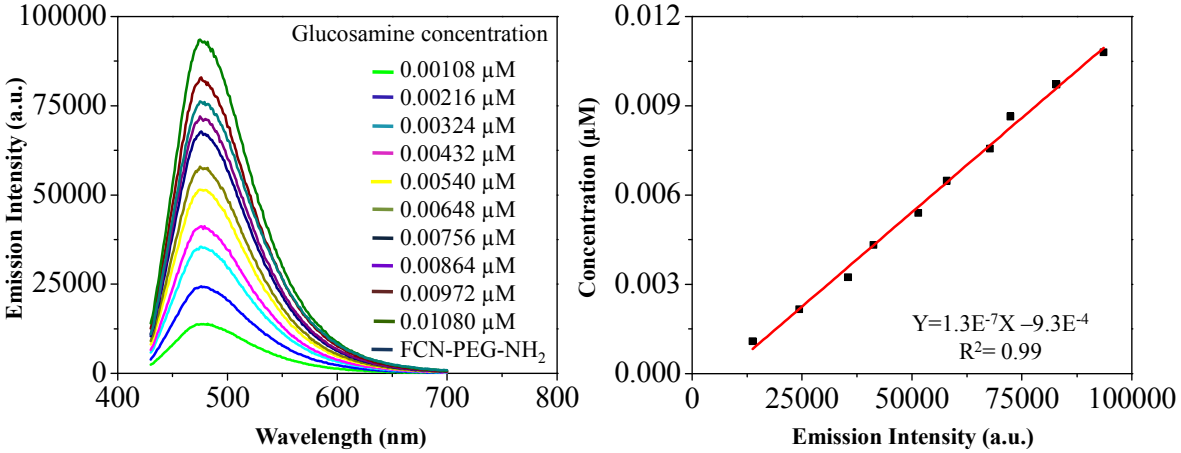

Figure S7. Determination of the concentration of primary amine in FCN-PEG-NH_2. Fluorescence spectra were obtained after reaction of glucosamine of varied concentration with the excess of fluorescamine. Next, linear fitting curve was obtained by plotting fluorescence intensity at 485 nm against glucosamine concentrations and used for measuring concentration of primary amine in FCN-PEG-NH_2.

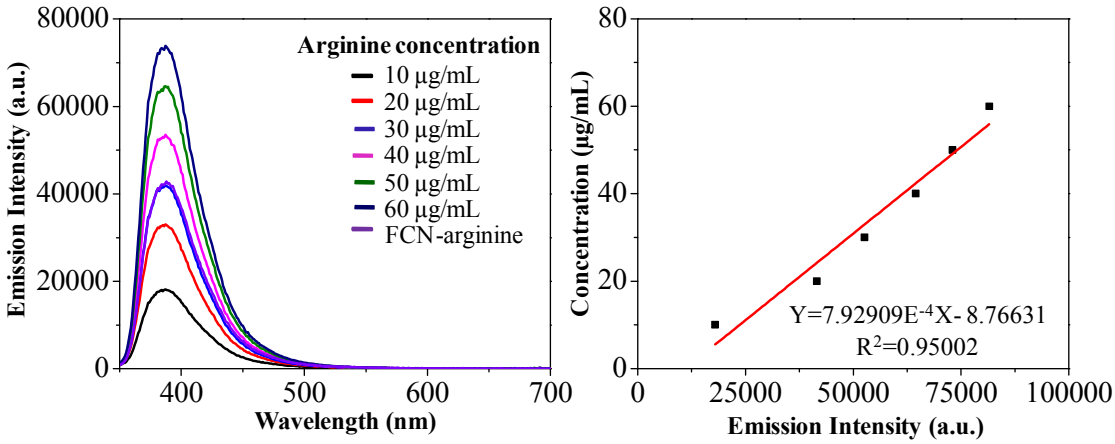

Figure S8. Determination of the concentration of arginine in FCN-arginine. Fluorescence spectra were obtained after reaction of arginine of varied concentration with the excess 9,10-phenanthrenequinone. Next, linear fitting curve is obtained by plotting fluorescence intensity at 387 nm against arginine concentrations and used for measuring concentration of arginine in FCN-arginine.

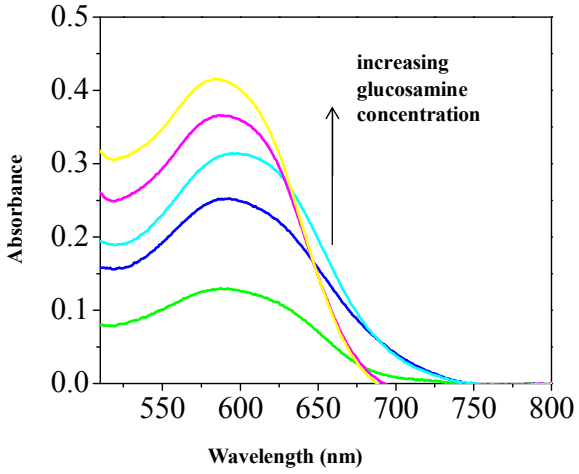

Figure S9. Method for confirmation of glucosamine conjugation with FCN. Absorbance spectra are obtained after adding excess anthrone to the glucosamine solution of varying concentration.

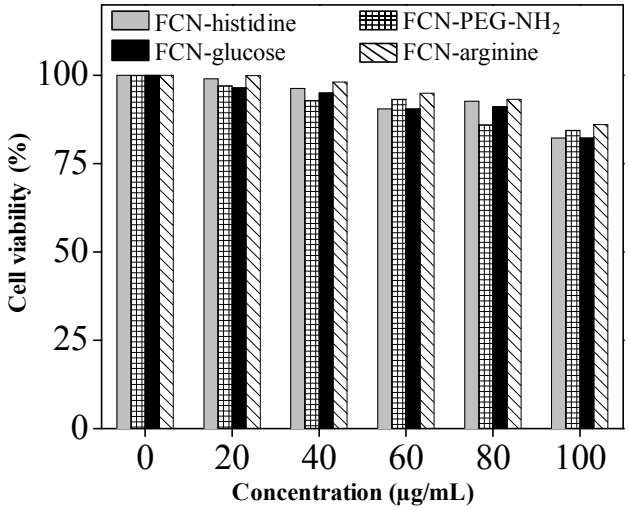

Figure S10. MTT based cytotoxicity assay of functionalized FCN, showing that they are non-toxic at dose comparable to the concentration used for labeling applications.

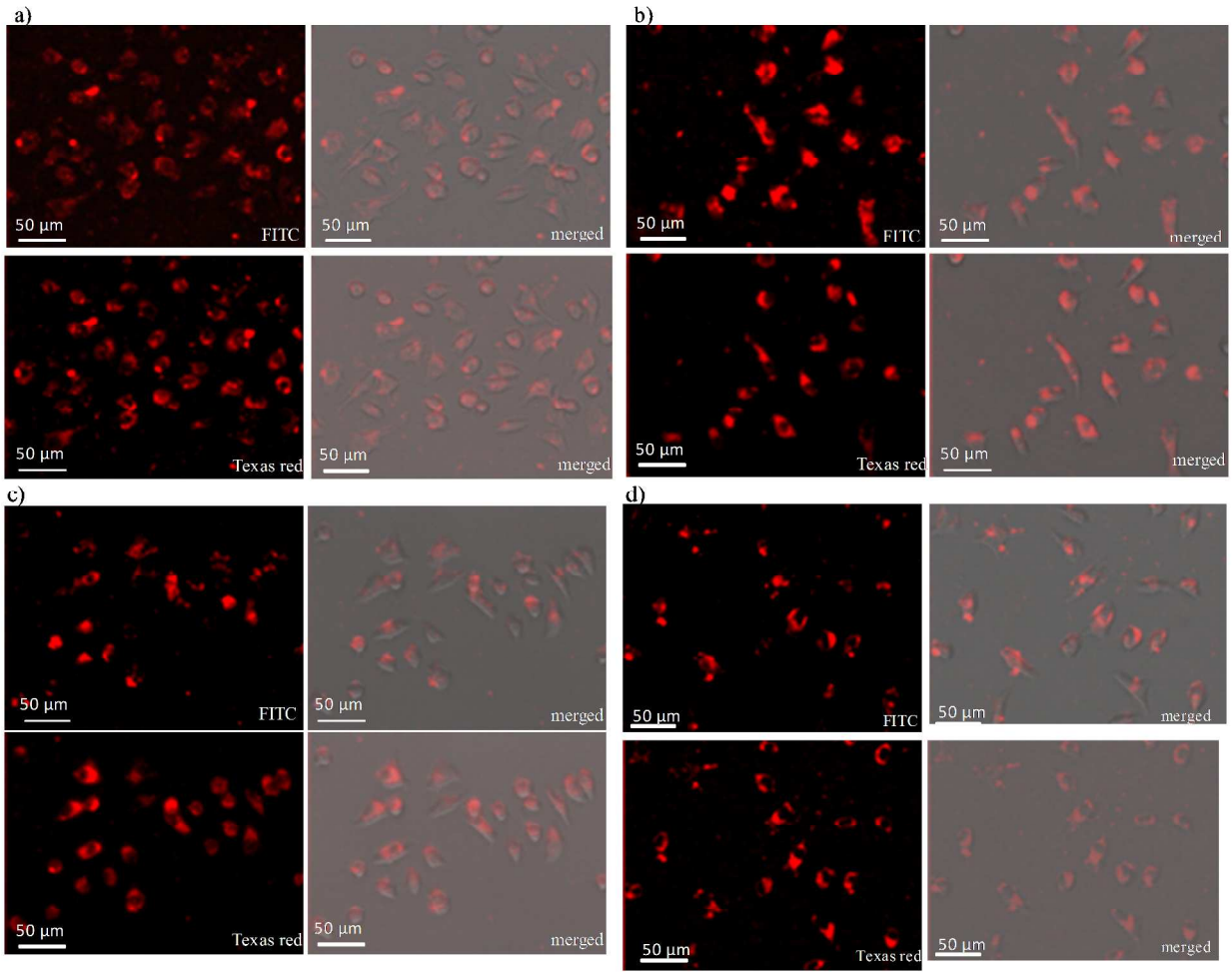

Figure S11. a-d) Additional fluorescence microscopic image of FCN-folate labeled KB cells under blue (FITC) and green (Texas red) excitations, showing that both conditions generate similar quality images. Typically nanoparticles are incubated with cells for one hour and then labeled cells are washed and imaged at different areas (a-d) and each area is imaged under blue (FITC) and green (Texss red) excitations.

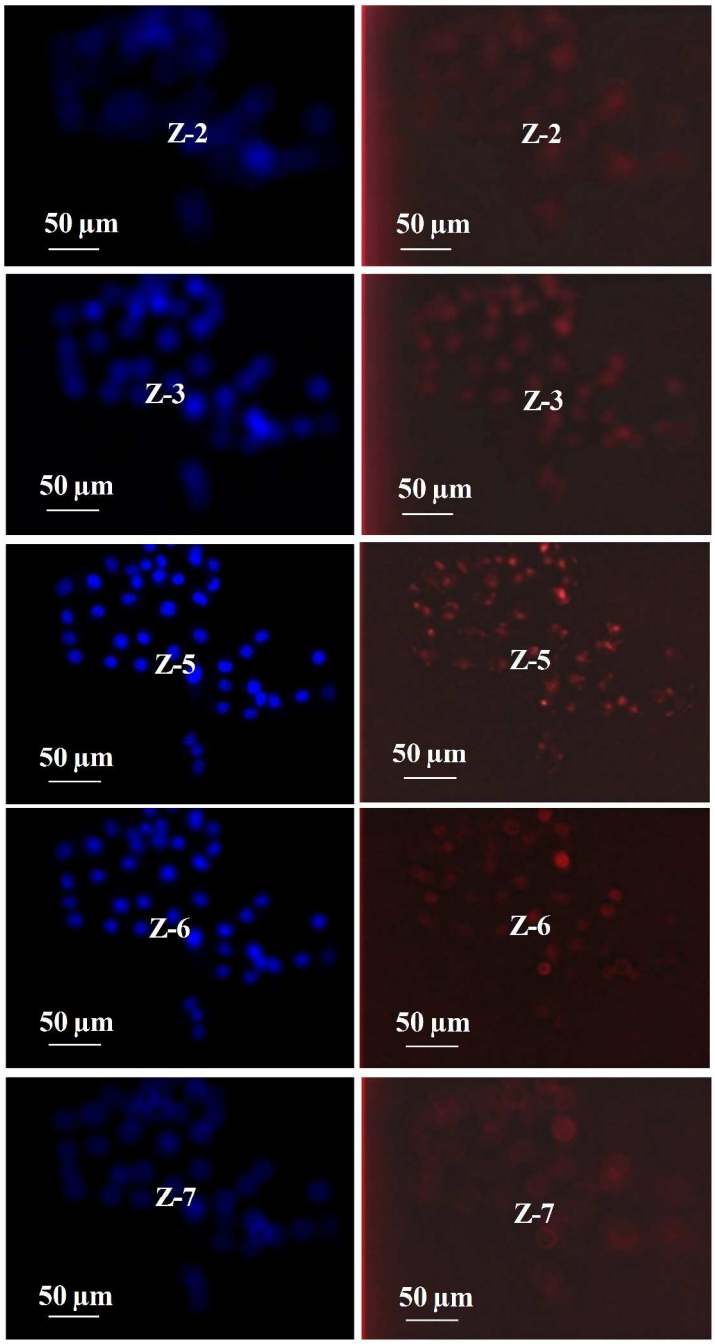

Figure S12. Fluorescence images of FCN-folate (red color) and hoechst (nuclear probe, blue) labeled KB cells taken at different z planes (top to bottom with consecutive z axis slices of 0.75 µm starting from Z-1 to Z-10). Here FCN-folate is incubated with cells for 3 hrs and washed cells are further treated with media for 6-8 hrs prior to imaging. From the images it is found that FCN-folate mostly localize inside cells and in the nuclear plane.

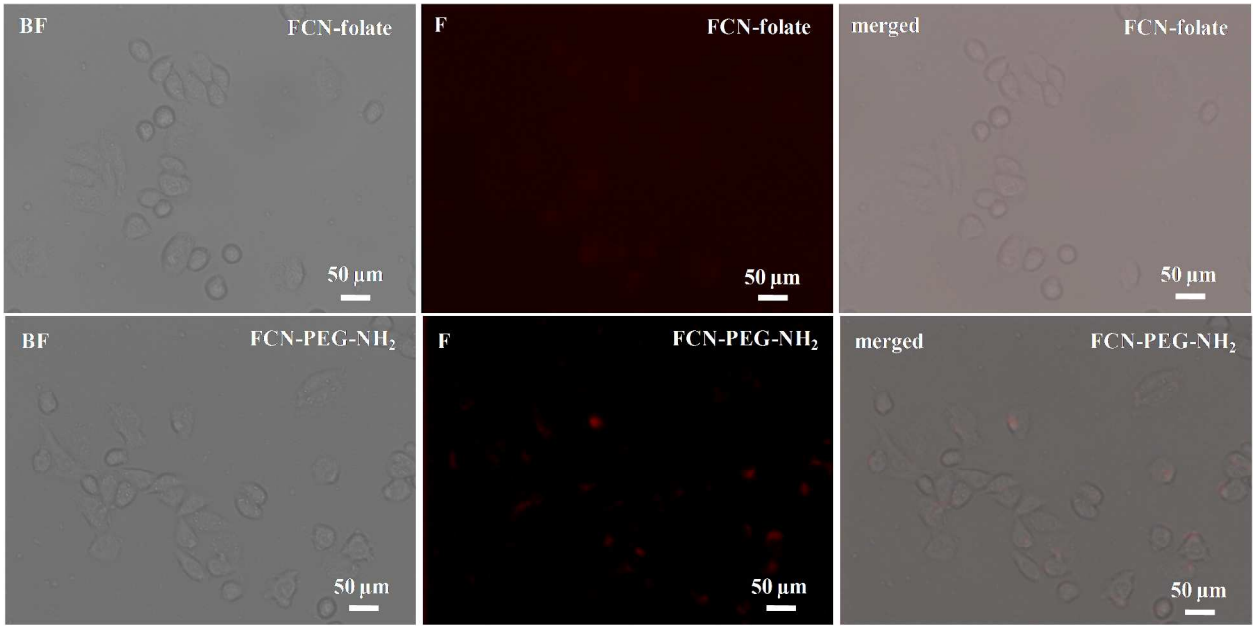

Figure S13. Fluorescence images of CHO cells labeled with FCN-folate and FCN-PEG-NH_2, showing that they cannot label cells having low over expressed folate receptors.

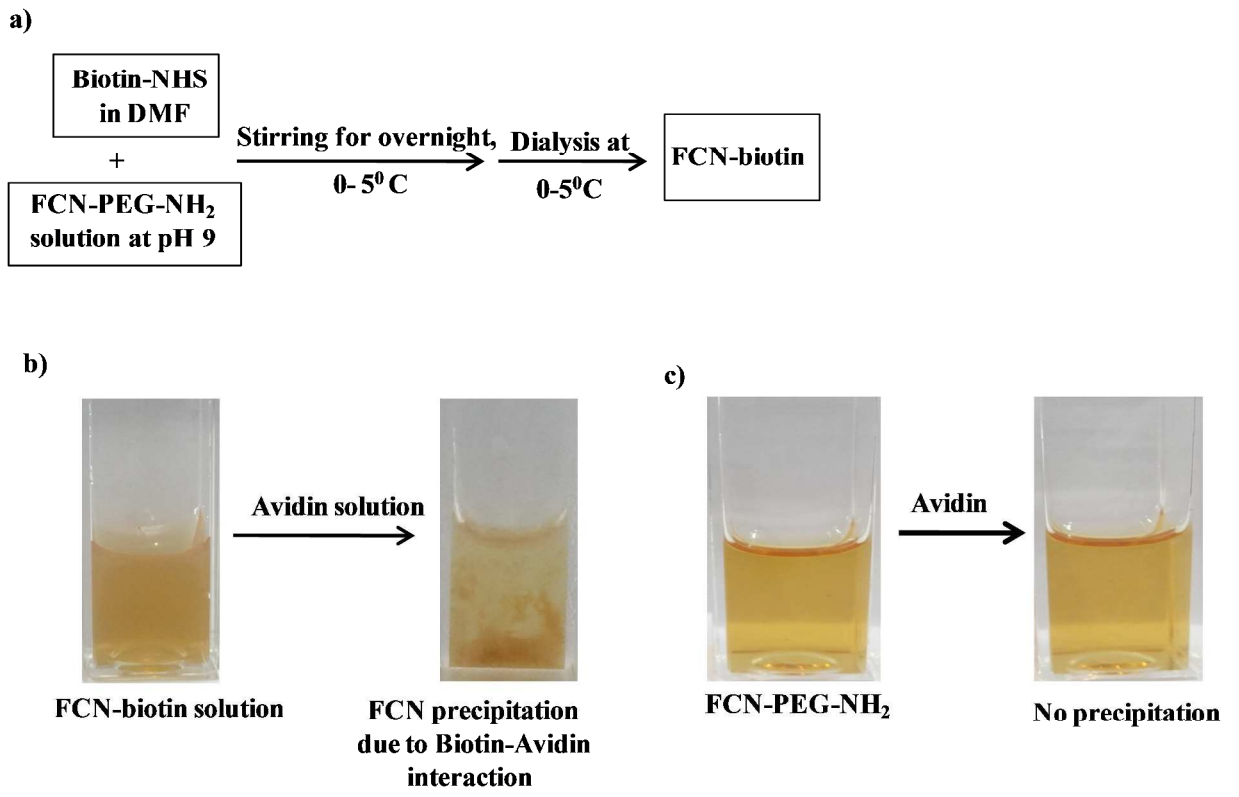

Figure S14. a) Schematic representation of synthesis of biotinylated FCN (FCN-biotin) from FCN-PEG-NH_2. b) Precipitation of colloidal FCN-biotin after reaction with Avidin. Avidin induces cross linking between FCN and as Avidin has four biotin binding sites and each FCN has multiple number of biotin. c) No precipitation occurs after adding avidin solution into the FCN-PEG-NH_2 solution.