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Supporting Information

Versatile Platform for Bioimaging Based on Colominic Acid-Decorated Upconversion Nanoparticles

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SI-1. X-ray diffraction (XRD) analysis of $NaYF_4:Yb^{3+}/Tm^{3+}$ UCNPs

The phase composition of crystal UCNP was studied by powder XRD analysis in the range of angles 10 ° ≤ 2θ ≤ 100 ° (Rigaku Miniflex 600). XRD analysis showed that a hexagonal crystal phase of $NaYF_4:Yb^{3+}/Tm^{3+}$ was in an agreement with literature data (ICDD PDF card # 01-072-4800).

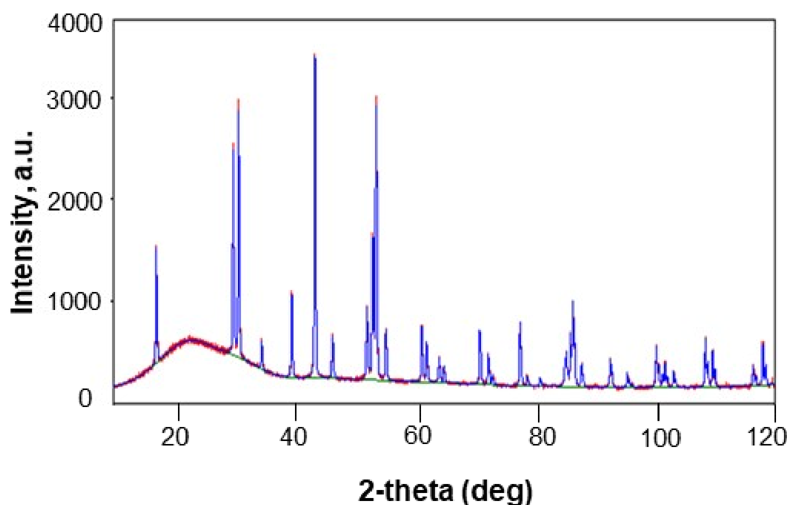

Fig. SI-1. XRD pattern of $NaYF4:Yb^{3+}/Tm^{3+}$ UCNPs.

SI-2. UCNP hydrophilization with poly(ethyleneimine)

Partial substitution of oleic acid on the UCNP surface was confirmed by FTIR-spectroscopy in samples which were mixed with KBr, grinded and pressed (see Experimental section). After hydrophilization with PEI, the bands, corresponded to the C-C bond of the OA, at 1496 and 1459 cm^{-1} are amplified owing to the appearance of the -C-C- and -C-N- bonds of PEI chains. The presence of the -C-N- bond is confirmed by a peak at a wavelength of 1181 cm^{-1}. The band at the wavelength of 1562 cm^{-1}, associated with COOH coordinated to rare-earth metals on UCNP surface, is significantly decreased. In UCNPs-PEI the bands appear at 1737 and 1269 cm^{-1}, attributed to -C = O and OH-, respectively, indicating the presence of protonated COOH, which confirms the destruction of coordination bonds and the partial removal of OA from the surface.[S1] The bands at 1657 and 989 cm^{-1} point out the presence of free amine groups, and band set in vicinity of 1800 cm^{-1} is corresponded to imine-containing compound in UCNPs-PEI. The band at 1544 cm^{-1} can be referred to the protonated form of the amine group, which indicates the formation of a coordination bond with the metal ions on the UCNP surface.

Thus, it can be assumed that the UCNP modification with PEI takes place both due to hydrophobic interactions and through the partial removal and replacement of OA molecules by PEI chains, thus

forming a coordination bond between amine groups of PEI and metal ions on the UCNP surface (Fig. SI-2a).

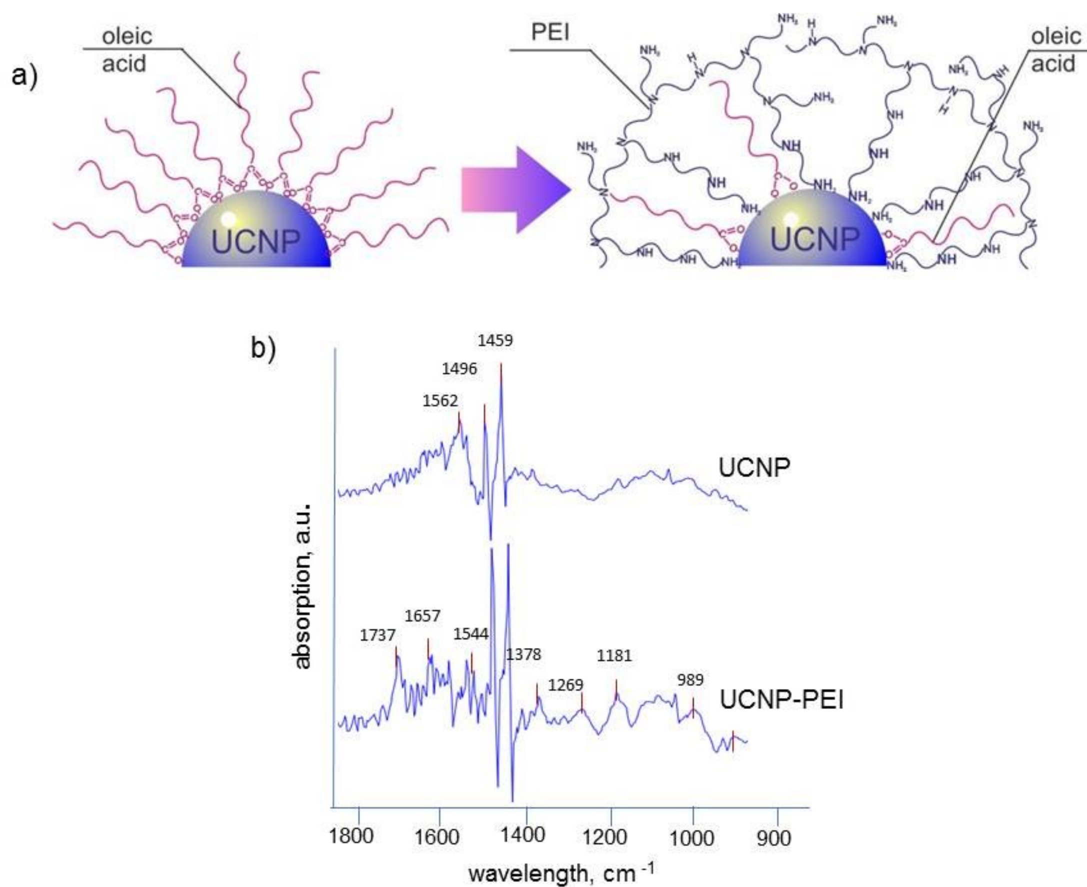

Fig. SI-2. Schematic representation of UCNP modification with poly(ethyleneimine) (a); FTIR spectra of UCNPs and UCNPs modified with poly(ethyleneimine).

SI-3. Photoluminescent spectra of UCNPs-CA

The fluorescence spectra of UCNPs, acquired before and after the modification with CA by using the carbodiimide activation and electrostatic interaction (Fig. SI-3), showed no significant shift of peaks and changes in their shape. This indicates that modification based on both approaches does not affect UCNP photoluminescent properties.

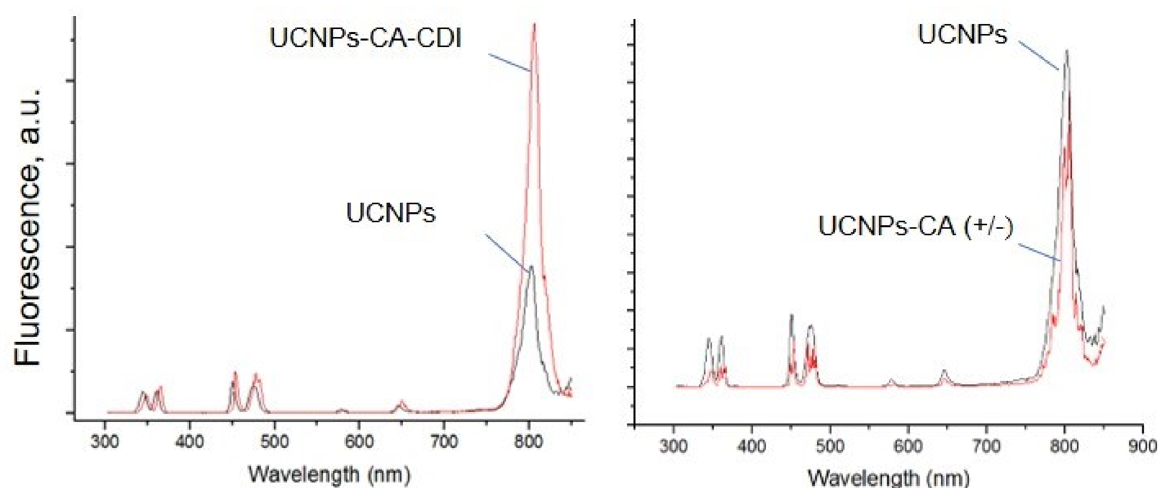

Fig. SI-3. The photoluminescence spectra of UCNPs and UCNPs, modified with colominic acid using carbodiimide activation (UCNPs-CA-CDI) (a) and electrostatic interactions (UCNPs-CA(+/-)) (b) at an excitation radiation power 3 W cm^{-2}.

SI-4. The detection of colominic acid concentration on the UCNPs-CA-CDI surface

CA concentration on the UCNPs-CA-CDI surface was evaluated spectrophotometrically by measuring optical absorption of activated CA in the supernatant (Fig. SI-4). The samples were centrifuged at 20.8 kg for 10 min, the supernatants were collected and measured at the wavelength 270 nm. Using the calibration graph representing the dependence of optical absorption via CA concentration activated with CDI, the CA amount in the supernatant after centrifugation using Amicon® Ultra-0.5 Centrifugal Filters (cut off 3000) was evaluated. The CA amount on the surface of nanocomplexes was calculated as the difference between the amount of added CA and free CA in the supernatant. It was found that nanocomplexes (0.8 mg mL^{-1}) contain 5.47 mg mL^{-1} of CA in UCNPs-CA-CDI, which corresponds to approximately 350 CA molecules per nanoparticle.

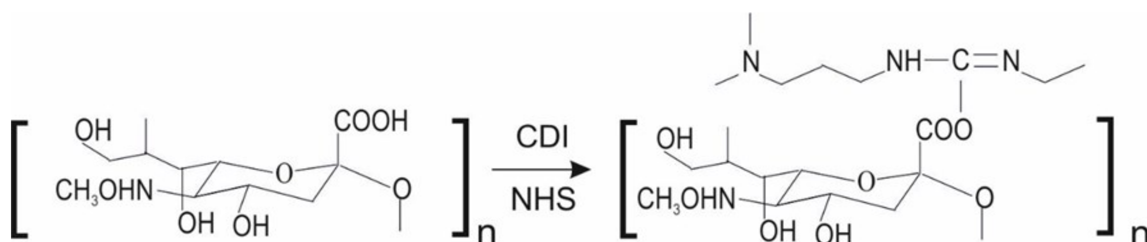

Fig. SI-4. Scheme of colominic acid activation using 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (CDI) in the presence of N-hydroxysulfosuccinimide (NHS).

SI-5. Blood protein adsorption on UCNP samples

We studied the protein adsorption on the CA shell of UCNP samples UCNPs-CA(+/-) and UCNPs-CA-CDI after incubation with mouse serum (see Experimental Section). Both UCNP samples appeared to adsorb insignificant amount of serum proteins that is lower than in the case of UCNPs modified with PEG, which is leader for NP surface modification for *in vivo* application.[S2] Cryo-TEM images of UCNPs-PMAO-PEG and UCNPs-CA (+/-) after incubation with mouse serum confirm the results obtained using Bradford's method (Fig. SI-5).

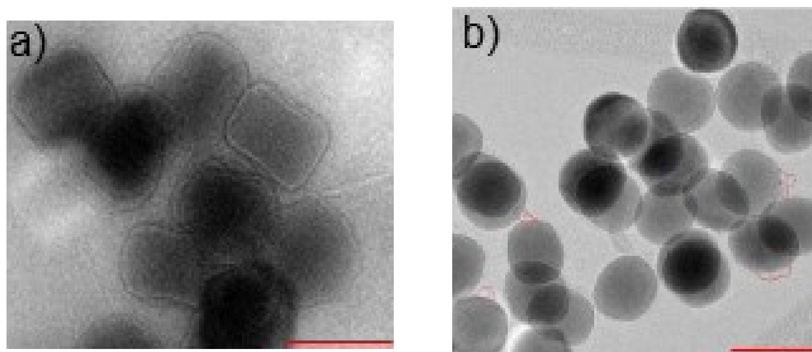

Fig. SI-5. Cryo-TEM images of UCNPs-CA(+/-) (a) and UCNPs-PMAO-PEG (b) after incubation with mouse serum (the protein adsorbed on the surface is highlighted with red line). Scale bar, 100 nm.

SI-6. Cellular uptake of UCNPs-CA

Nonspecific cellular uptake of UCNPs-CA was assessed by the level of phagocytosis of mouse macrophages RAW264 using confocal microscopy (see Experimental Section). Rhodamine-loaded samples UCNPs-CA were resuspended (1:20) in full RPMI medium and added to RAW 264.7 cells. The cells were stained with Hoechst and Calcein AM dyes for 10 min to visualize cell nucleuses and cytoplasm, respectively. The cells were incubated with UCNP probes for 5 min, 30 min and 1 h.

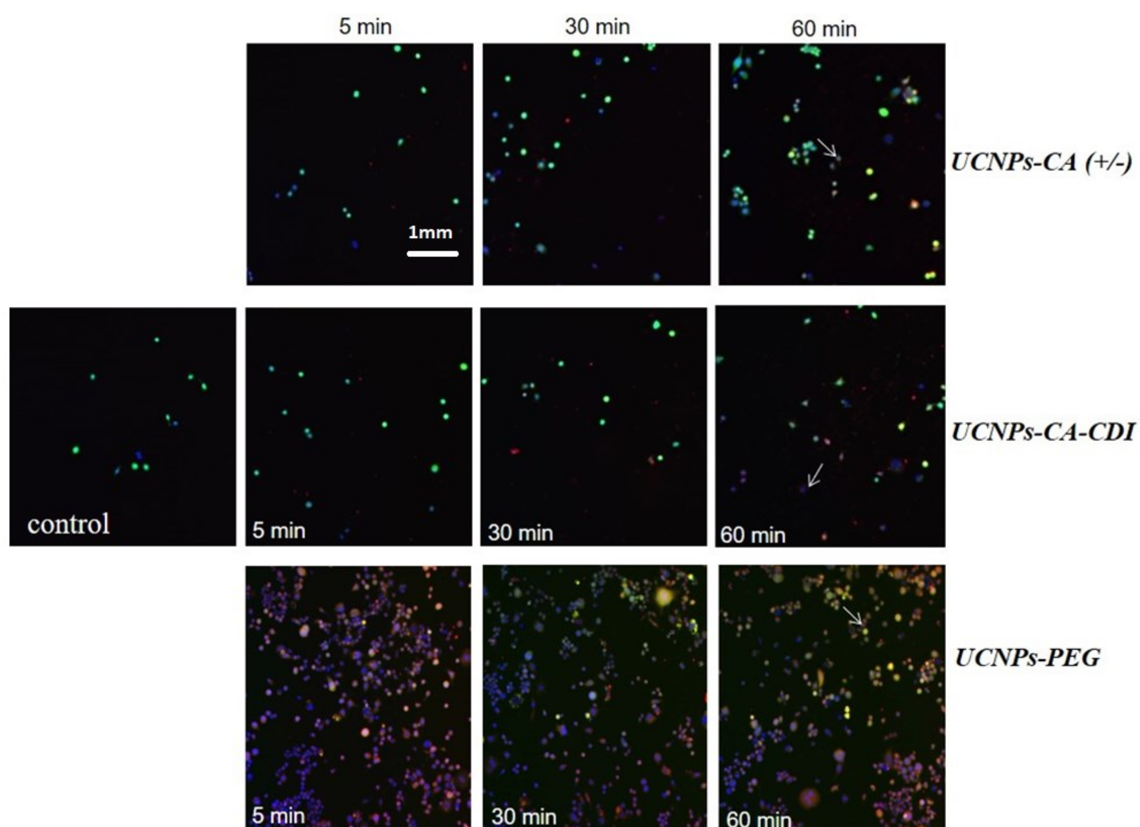

Fig. SI-6. Confocal microscope images of RAW 264.7 cell line after their incubation with UCNPs, modified with PMAO-PEG and UCNPs, modified with colominic acid using carbodiimide activation (UCNPs-CA-CDI) and electrostatic interactions (UCNPs-CA(+/-)) for 5, 30 and 60 min. Cells without incubation with UCNP probes were used as a control.

SI-7. *In vitro* UCNPs imaging

To demonstrate the *in vitro* imaging by using UCNPs-CA nanocomplexes, we incubated human adenocarcinoma MCF-7 cells with UCNPs-CA suspensions Human adenocarcinoma MCF-7 cells were seeded in a 96-well plates (5 × 103 cells per well) followed by overnight incubation. Then UCNPs-CA samples were resuspended (1:20) in full RPMI medium and added to MCF-7 cells for 1 h. After the incubation, the cells were washed three times with PBS pH 7.2 to remove unbounded particles. The images were taken using the anti-Stokes fluorescence microscope equipped with 975 nm-cw diode laser (ATC-10H-975, ATCSD, Russia). Objective (LWD 40X/0.5 NA, Motic, China) was used for bright-field and UCNP-luminescent imaging. It was found that UCNPs-CA accumulated in MCF-7 cells and demonstrated excellent bioimaging properties.

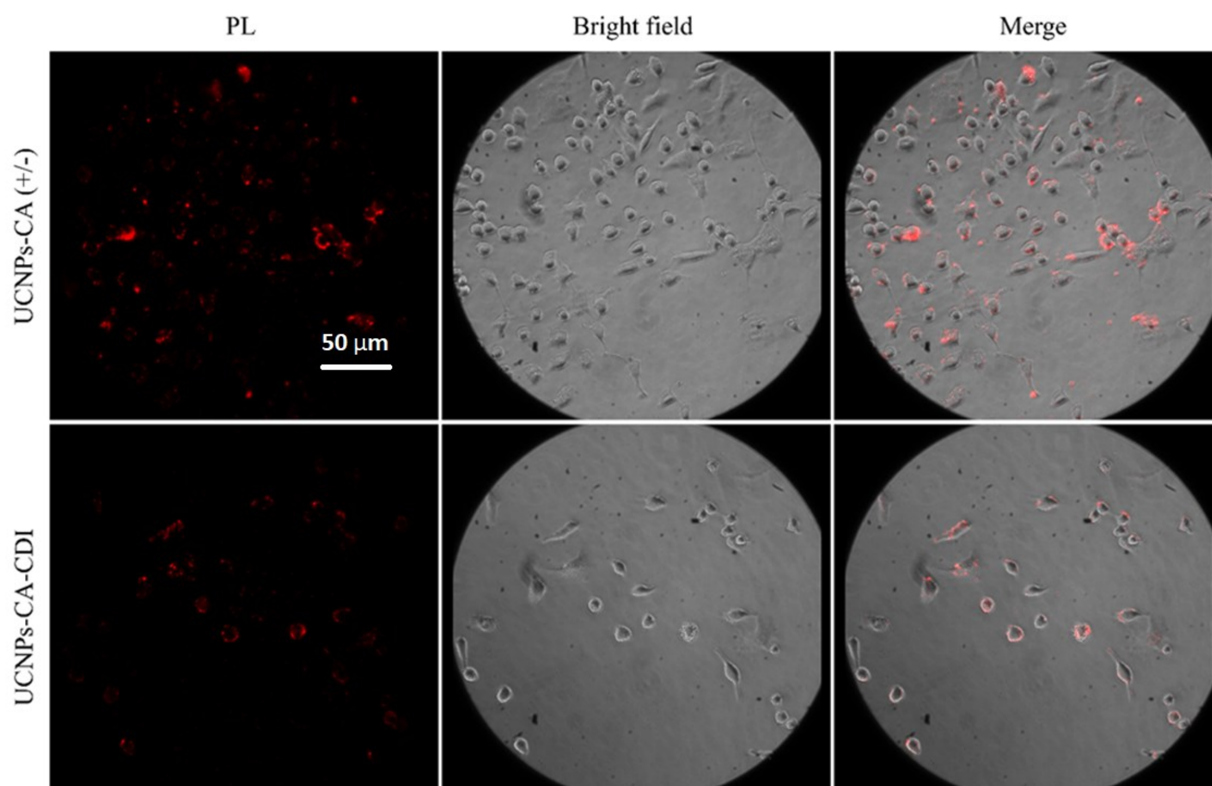

Fig. SI-7. Photoluminescent and phase contrast images of the human adenocarcinoma MCF-7 cells incubated with UCNPs-CA(+/-) (upper row) or UCNPs-CA-CDI (lower row) nanocomplexes (1 h, 4 ºC). The images were taken using the anti-Stokes fluorescence microscope equipped with 975 nm-cw diode laser.

SI-8. UCNP distributions in collected blood samples performed by a home-built anti-Stokes fluorescent microscope

We studied *in vivo* circulation time in blood of UCNPs-CA (+/-) and UCNPs-CA-CDI probes in comparison with UCNPs, modified with PEG. Fig. SI-8 shows the UCNP distributions in blood samples performed by a home-built anti-Stokes fluorescent microscope. Blood samples were collected from tail vein at time intervals from 1 to 60 min after intravenously injection in Balb/c mice as we previously described.[S3] Images were taken from four random areas, and number of UCNPs in all areas was calculated. Excellent UCNP photoluminescent properties made it possible to detect intense PL signal of UCNPs-CA nanocomplexes in whole blood. Note, the PL signal of UCNPs-PMAO-PEG in blood samples significantly decreases after 1-h circulation in comparison with signal of both UCNPs-CA. Besides, UCNPs-CA were detected in blood samples after 3 h, and separate nanocomplexes were found even after 6 h of circulation.

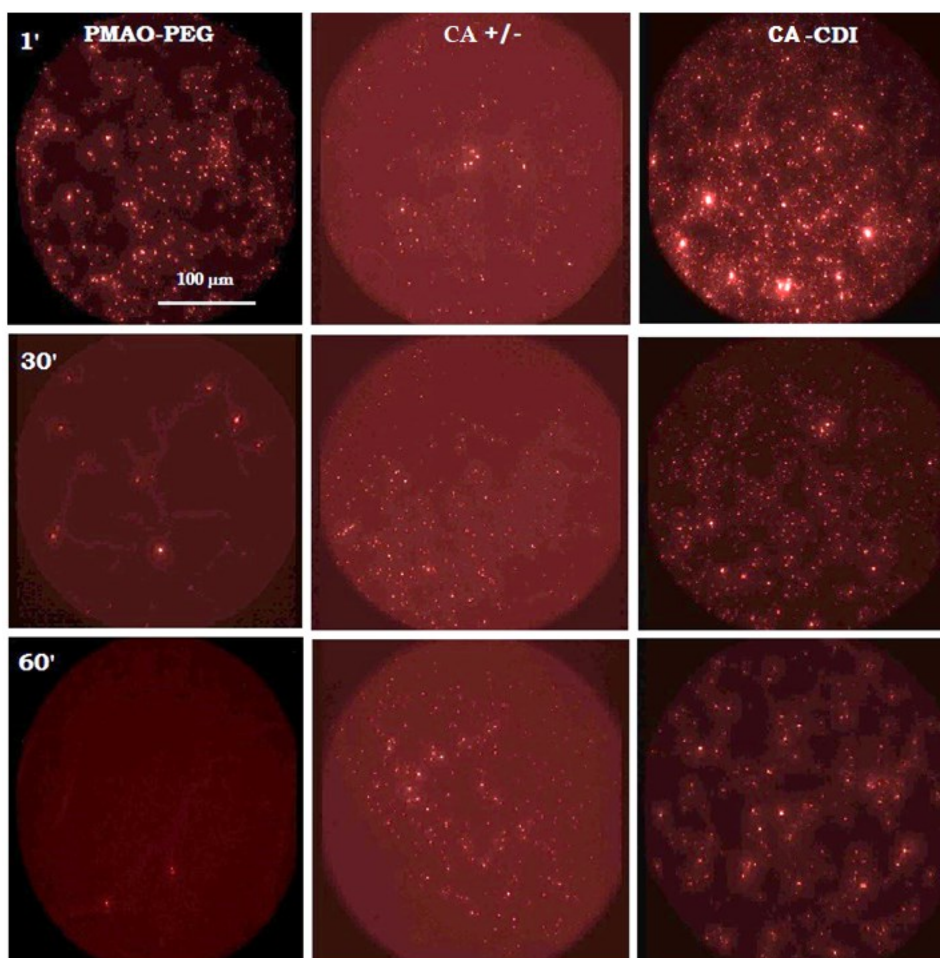

Fig. SI-8. Photoluminescent images of mouse blood samples taken at 1, 30 and 60 min-time intervals after intravenous injection of UCNPs, modified with PMAO-PEG, CA (+/-) and CA-CDI.

SI-9. Chronical cytotoxicity of UCNPs modified with CA using carbodiimide method

In vitro experiment on chronical cytotoxicity (see Experimental Section) showed almost 100%. BJ-5ta fibroblast viability after 72-h incubation with UCNPs-CA in concentrations below 100 μg mL^{-1}, which is comparable with the cell viability after incubation with UCNPs-PMAO-PEG. It is worthy to note that more than 80% of cell viability was observed after 24-h incubation at UCNPs-CA concentration 200 μg mL^{-1}, while similar viability after 72-h incubation was demonstrated for twice lower concentration of UCNPs-CA (100 μg mL^{-1}). These results indicate low level of chronical cytotoxicity of UCNPs-CA prepared using carbodiimide activation.

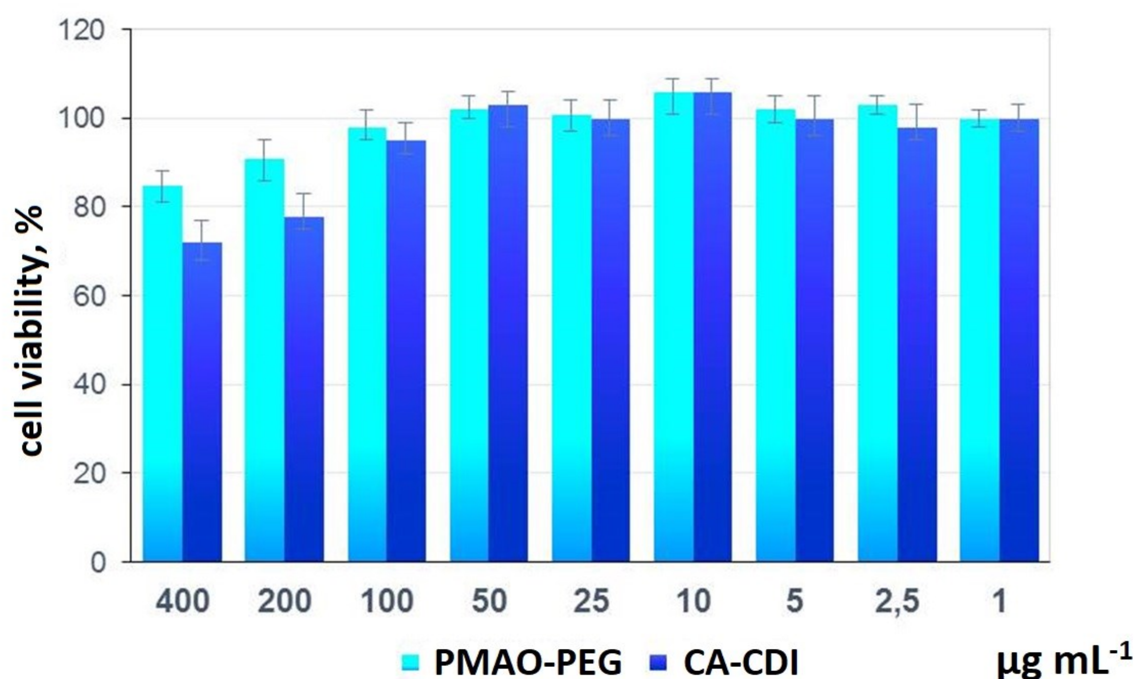

Fig. SI-9A. The viability of BJ-5ta fibroblasts (hTERT) after 72 h - incubation with nanocomplexes of UCNPs with PMAO-PEG and with CA based on carbodiimide activation (CA-CDI).

Images of BJ-5ta fibroblasts (hTERT) after 72 h - incubation with nanocomplexes show viable cells with aggregates of UCNPs-PMAO-PEG on their surface (Fig. SI-9B,b) instead of separate nanocomplexes of UCNPs-CA (Fig. SI-9B,a). The nanocomplex circulation time in blood system can be associated with their sedimentation on cellular surface. The more nanocomplexes are retained on cellular surface, the less amount remains in circulatory system, resulting in the accumulation decrease in the site of interest. This behavior is likely lead to the decrease of UCNPs-PMAO-PEG circulation time, which we observed in experiments.

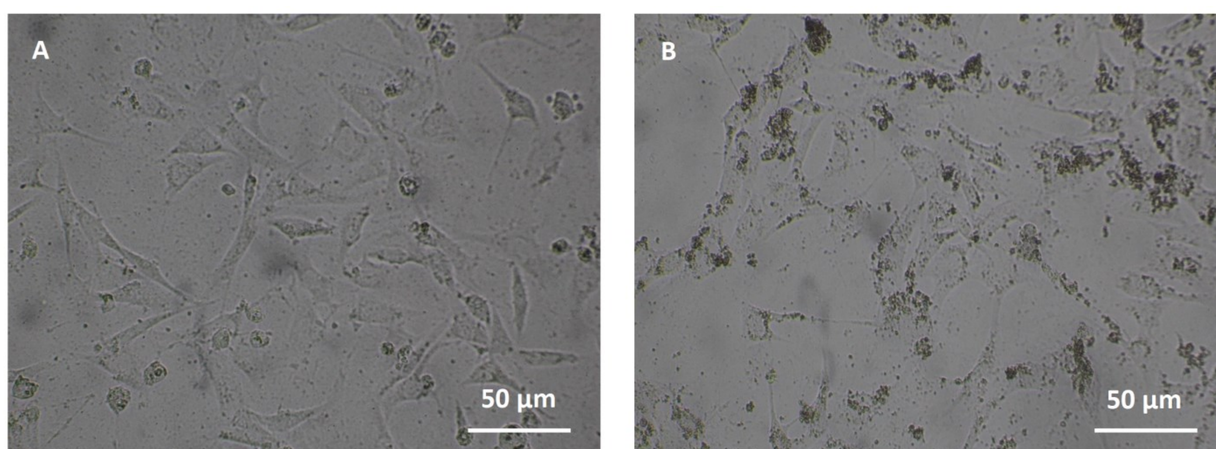

Fig. SI-9B. Images of BJ-5ta fibroblasts (hTERT) after 72 h - incubation with nanocomplexes of UCNPs with CA based on carbodiimide activation (CA-CDI) (a) and with PMAO-PEG (b).

SI-10. Blood vessel visualization

We studied *in vivo* UCNP-CA-CDI circulation after intravenous injection and have shown the possibility of UCNP assisted visualization of macro and micro blood vessels (Fig. SI-10). Apparently, injected UCNP nanocomplexes prevailed in the place near the vessel walls, probably in the cell-free layer in the inflammation site.

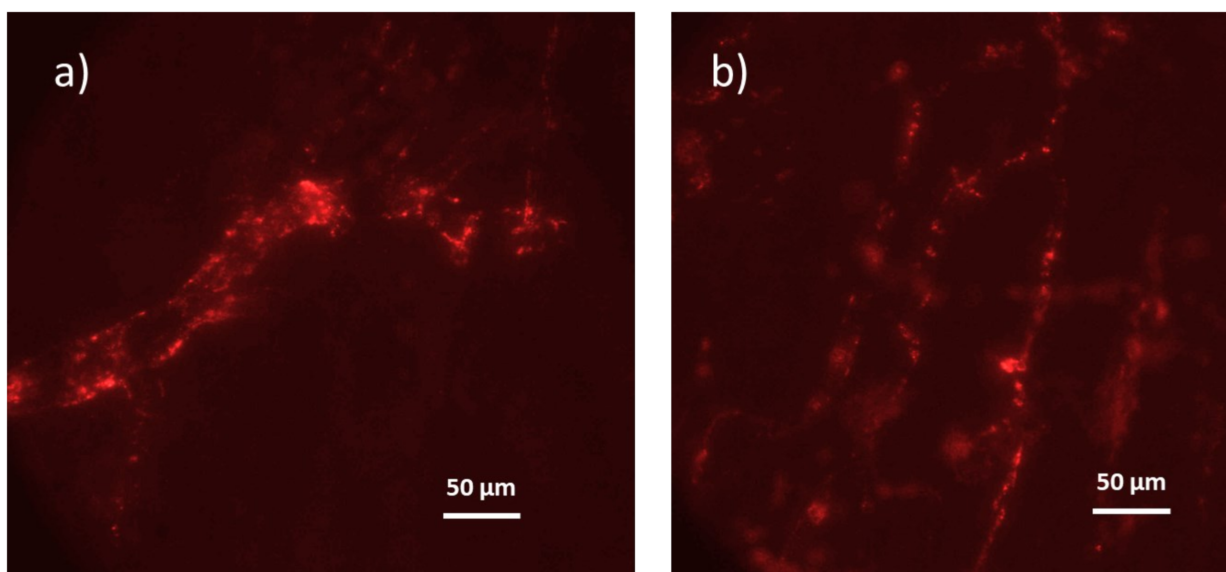

Fig. SI-10. UCNP assisted PL images of macro (a) and micro (b) blood vessels in the inflammatory tissue.

References

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