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Carbon Dots for Optical Imaging *in vivo*

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There has been significant recent interest in the development of highly fluorescent nanomaterials as contrast agents for optical imaging *in vivo*.[1] The imaging agents should ideally be bright, nontoxic, biocompatible, and stable against photobleaching. Among the extensively studied are those based on semiconductor quantum dots (QDs) such as CdSe/ZnS.[2] The rationale for the use of QDs over conventional organic dyes is now generally accepted in the literature.[3] There are already successful *in vivo* imaging demonstrations of QDs on tumor vasculature, tumor-specific membrane antigens, sentinel lymph nodes, etc.[2,4]

The semiconductor QDs containing cadmium or other heavy metals are unfortunately known for their significant toxicity even at relatively low concentrations,[5,6] which may prove prohibitive to any patient studies. Therefore, the search for benign alternatives has continued. Of particular interest and significance was the recent finding that small carbon nanoparticles could be surface-passivated by organic or bio-molecules to become strongly fluorescent.[7] These fluorescent carbon nanoparticles,[7,8] dubbed "carbon dots" (C-Dots, Scheme 1), were found to be physicochemically and photochemically stable and non-blinking. The carbon particle core could also be doped with an inorganic salt such as ZnS before the surface functionalization to significantly enhance the fluorescence brightness (C_{ZnS}-Dots, Scheme 1).[9] These carbon dots have been successfully used for *in vitro* cell imaging with both one- and two-photon excitations.[7,9,10]

Carbon is hardly considered as an intrinsically toxic element. Available results from the ongoing toxicity evaluation of the oligomeric PEG-functionalized C-Dots[7] in mice have suggested no meaningful toxic effects,[11] raising the prospect for *in vivo* biocompatibility and uses of carbon dots. Here we report the first study of carbon dots for optical imaging *in vivo*. The results suggest that the carbon dots are not only brightly fluorescent in solution, as reported previously,[7,9] but also well-behaved as contrast agents in live mice.

The C-Dots and C_{ZnS}-Dots with the PEG diamine, $H_2NCH_2(CH_2CH_2O)_nCH_2CH_2CH_2NH_2$ ($n \sim 35$, PEG_{1500N}), as the surface passivation agent were prepared and characterized as previously reported.[7,9,10] Shown in Figure 1 are representative AFM and HR-TEM imaging results of the carbon dots. Both samples were readily soluble in water to form stable aqueous solutions, suitable for the various injections as follows.

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Supporting Information Available: Complete ref [7] and more experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

For subcutaneous injection, female DBA/1 mice (~25 g) were shaved in the back area surrounding the injection point. Upon the injection of a C-Dots solution (30 μg carbon core-equivalent in 30 μL) or a C_{ZnS}-Dots solution (65 μg in 30 μL), the mice were imagined in a Lumazone FA in vivo Imaging System (MAG Biosystems) with 470 nm (FWHM ~ 40 nm) excitation and 525 nm (FWHM ~ 47 nm) emission filters. As shown in Figure 2, the fluorescence images of the subcutaneously injected mice exhibited bright emissions from C-Dots and C_{ZnS}-Dots. The relatively stronger fluorescence from the latter is consistent with the previously reported solution-phase results.[9] The injected carbon dots in mice diffused relatively slowly, with the fluorescence faded about 24 h post-injection.

The carbon dots could be excited at longer wavelengths for red fluorescence emissions. For the same subcutaneous injection into mice, the imaging results with 545 nm (FWHM ~ 29 nm) excitation and 620 nm (FWHM ~ 59 nm) emission filters also exhibited significant fluorescence from both C-Dots and C_{ZnS}-Dots (Figure 2).

The brighter green fluorescence of C_{ZnS}-Dots was used in the imaging to track the migration through lymph vessels. Upon interdermal injection into the front extremity (10 μg in 10 μL), the carbon dots could migrate along the arm (Figure 3). Unlike in semiconductor quantum dots such as CdSe/ZnS, which could migrate to axillary lymph nodes in minutes,[4c] the observed migration of the carbon dots was slower. This could be due to the small sizes of carbon dots (on the order of 4–5 nm) and/or the surface functionalization by the PEGs, whose protein resistance characteristics might reduce interactions of the carbon dots with lymph cells. The axillary lymph nodes were harvested and dissected at 24 h post-injection, in which fluorescence from the carbon dots could readily be detected (Figure 3).

A C-Dots solution (440 μg in 200 μL) was intravenously injected into mice for whole body circulation. The abdomen was shaved for fluorescence detection of the dots trapped in organs during the circulation, but only emissions from the bladder area were observed (Figure 4). About 3 h post-injection, bright fluorescence in the urine became visible in the imaging facility (Figure 4). The results suggest primarily urine excretion for the intravenously injected carbon dots, which has been a widely observed excretion pathway in the literature for PEGylated nanoparticles, especially for very small particles like the ones used here.[12]

The organs were harvested 4 h post-intravenous injection for imaging analyses *ex vivo*. Only the dissected kidneys and liver exhibited meaningful fluorescence from the carbon dots, brighter in the former (Figure 4), consistent with the urine excretion pathway. The relatively weak fluorescence in the dissected liver was an indication for low accumulation level of the carbon dots. While generally significant hepatic uptake of nanoparticles and nanorubes was widely observed and discussed in many studies,[13] the low accumulation here might again be attributed to the effective surface PEGylation that probably reduced the protein affinity and made the carbon dots stealth with respect to the hepatic uptake.

All of the reported animal experiments were performed at Clemson University by strictly following the IACUC approved protocols. During the experiments, no animal exhibited any sign of acute toxicological responses.

In summary, the results reported here demonstrate that carbon dots, injected into mice via various ways, remain strongly fluorescent *in vivo*, which coupled with their biocompatibility and nontoxic characteristics might offer great potentials in optical imaging and related biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. De M, Ghosh PS, Rotello VM. Adv. Mater 2008;20:4225–4241.
2. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S. Science 2005;307:538–544. [PubMed: 15681376]
3. (a) Alivisatos AP. Science 1996;271:933–937. (b) Chan WCW, Nie S. Science 1998;281:2016–2018. [PubMed: 9748158]
4. (a) Smith BR, Cheng Z, De A, Koh AL, Sinclair R, Gambhir SS. Nano Lett 2008;8:2599–2606. [PubMed: 18386933] (b) Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. Nat. Biotechnol 2004;22:969–976. [PubMed: 15258594] (c) Kim S, Lim YT, Soltesz EG, Grand AMD, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV. Nat. Biotechnol 2004;22:93–97. [PubMed: 14661026]
5. Hardman R. Environ. Health Perspect 2006;114:165–172. [PubMed: 16451849]
6. Lin P, Chen J-W, Chang LW, Wu J-P, Redding L, Chang H, Yeh T-K, Yang CS, Tsai M-H, Wang H-J, Kuo Y-C, Yang RSH. Environ. Sci. Technol 2008;42:6264–6270. [PubMed: 18767697] (b) Geys J, Nemmar A, Verbeken E, Smolders E, Ratoi M, Hoylaerts MF, Nemery B, Hoet PHM. Environ. Health Perspect 2008;116:1607–1613. [PubMed: 19079709]
7. Sun Y-P, et al. J. Am. Chem. Soc 2006;128:7756–7757. [PubMed: 16771487]
8. (a) Liu H, Ye T, Mao C. Angew. Chem. Int. Ed 2007;46:6473–6475. (b) Zhou J, Booker C, Li R, Zhou X, Sham T-K, Sun X, Ding Z. J. Am. Chem. Soc 2007;129:744–745. [PubMed: 17243794] (c) Zhao QL, Zhang ZL, Huang BH, Peng J, Zhang M, Pang DW. Chem. Comm 2008:5116–5118. [PubMed: 18956040] (d) Bourlinos AB, Stassinopoulos A, Anglos D, Zboril R, Karakassides M, Giannelis EP. Small 2008;4:455–458. [PubMed: 18350555] (e) Hu S-L, Niu K-Y, Sun J, Yang J, Zhao N-Q, Du X-W. J. Mater. Chem 2009;19:484–488.
9. Sun Y-P, Wang X, Lu F, Cao L, Meziani MJ, Luo PG, Gu L, Veca LM. J. Phys. Chem. C 2008;112:18295–18298.
10. Cao L, Wang X, Meziani MJ, Lu F, Wang H, Luo PG, Lin Y, Harruff BA, Veca LM, Murray D, Xie S-Y, Sun Y-P. J. Am. Chem. Soc 2007;129:11318–11319. [PubMed: 17722926]
11. Yang S-T, Wang X, Wang H, Lu F, Luo PG, Cao L, Liu J-H, Liu Y, Chen M, Huang Y, Sun Y-P. unpublished results
12. Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Ipe BI, Bawendi MG, Frangioni JV. Nat. Biotechnol 2007;25:1165–1170. [PubMed: 17891134]
13. Li S-D, Huang L. Mol. Pharmaceutics 2008;5:496–504.

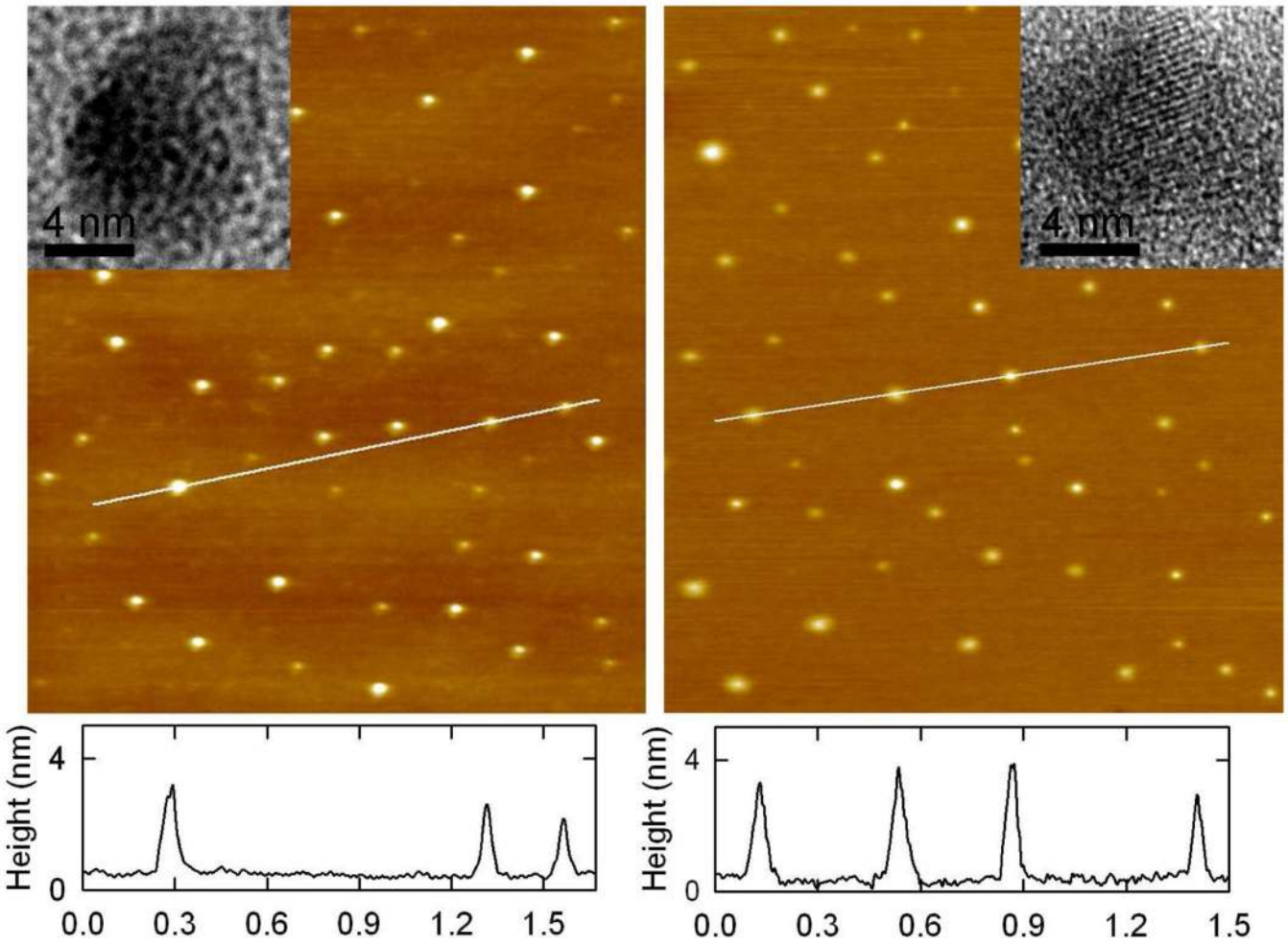


Figure 1.
AFM topography images of C-Dots (left) and C_{Zns}-Dots (right) on mica, with the insets showing the corresponding HR-TEM images of individual dots.

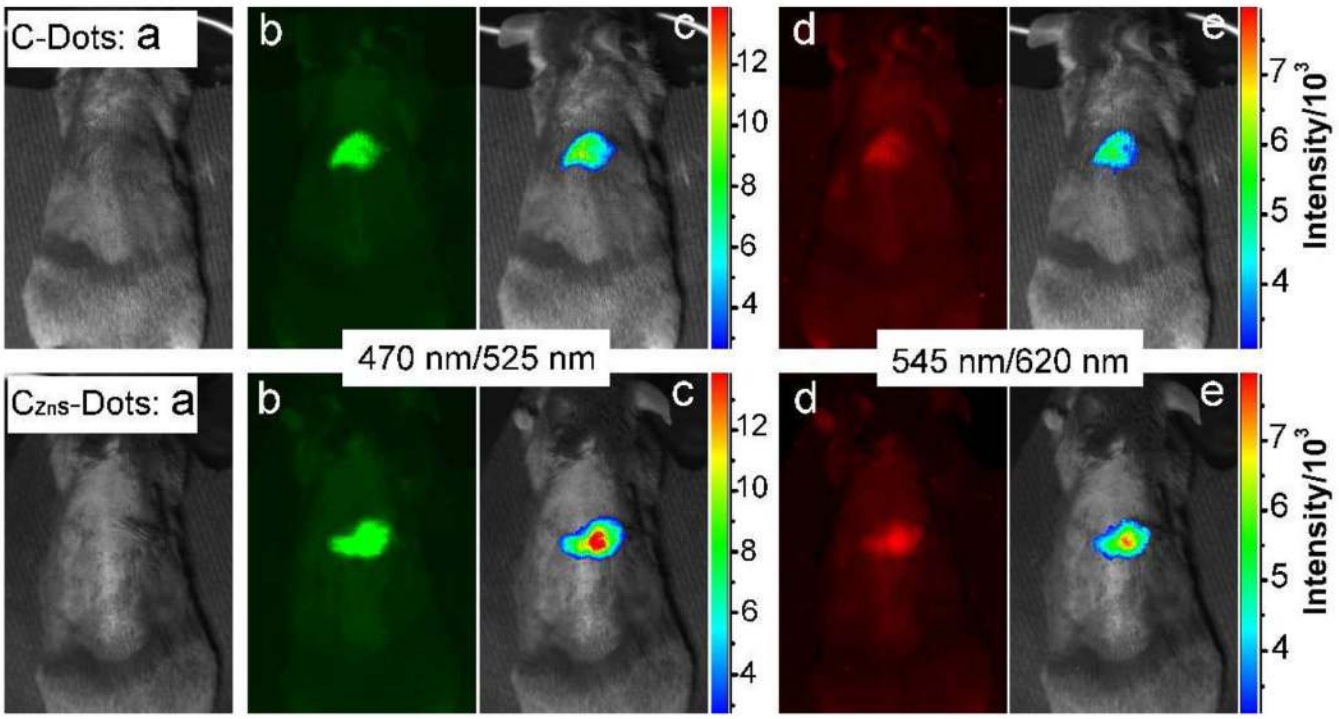


Figure 2.
Subcutaneous injection: (a) bright field, (b,d) as-detected fluorescence (excitation/emission wavelengths indicated), and (c,e) color-coded images (ImageJ from NIH).

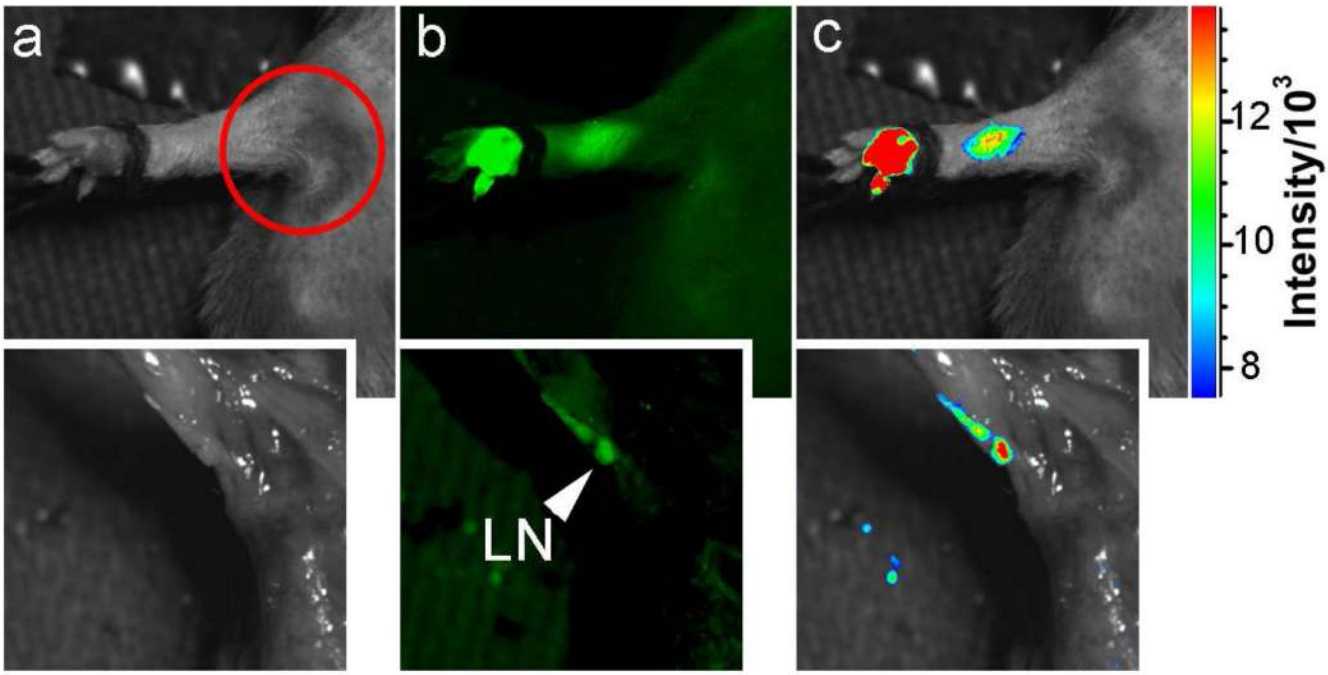


Figure 3.
Interdermal injection: (**a**) bright field, (**b**) as-detected fluorescence, and (**c**) color-coded images. Insets: the dissected (in the circled area) axillary lymph node (**LN**).

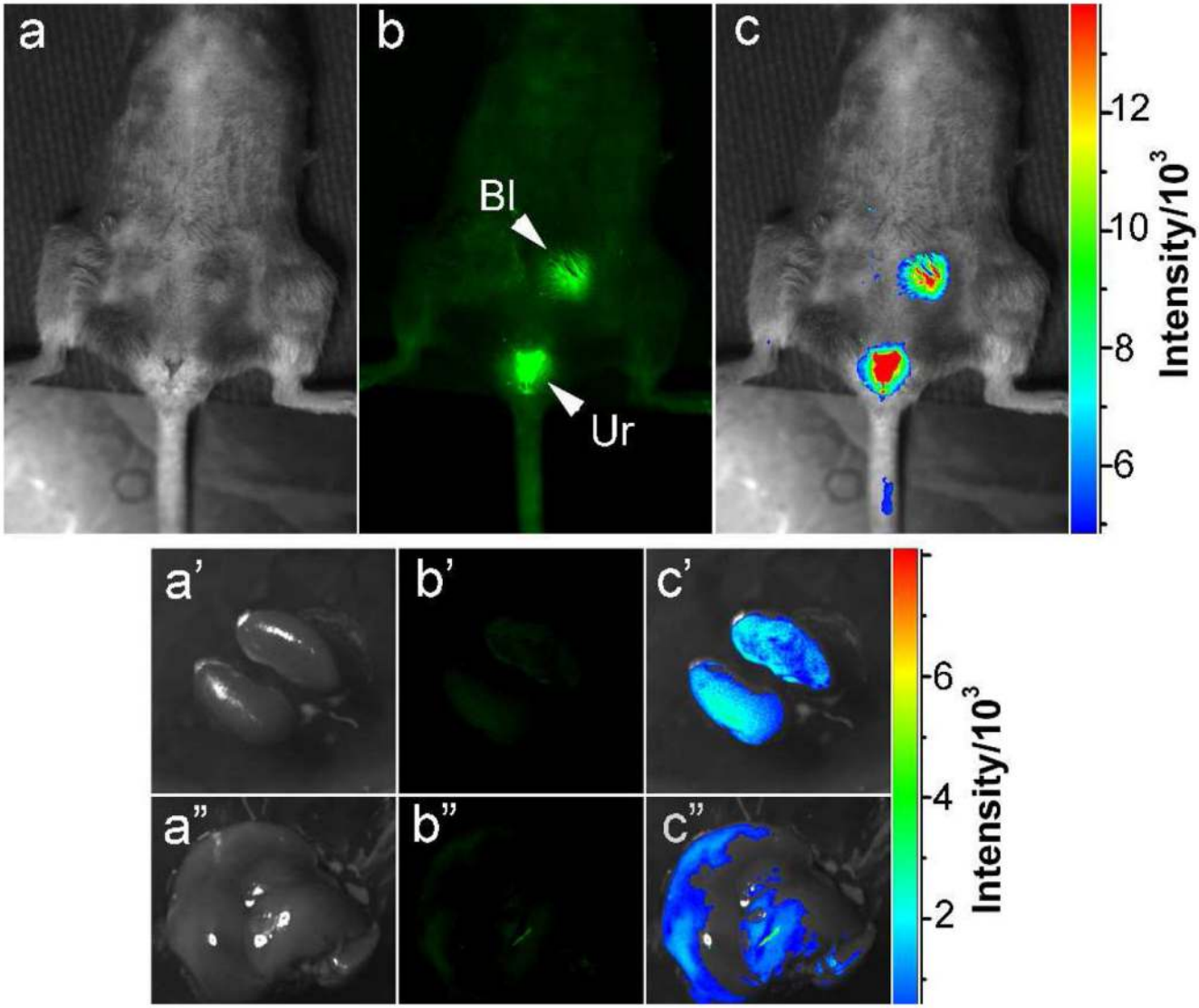
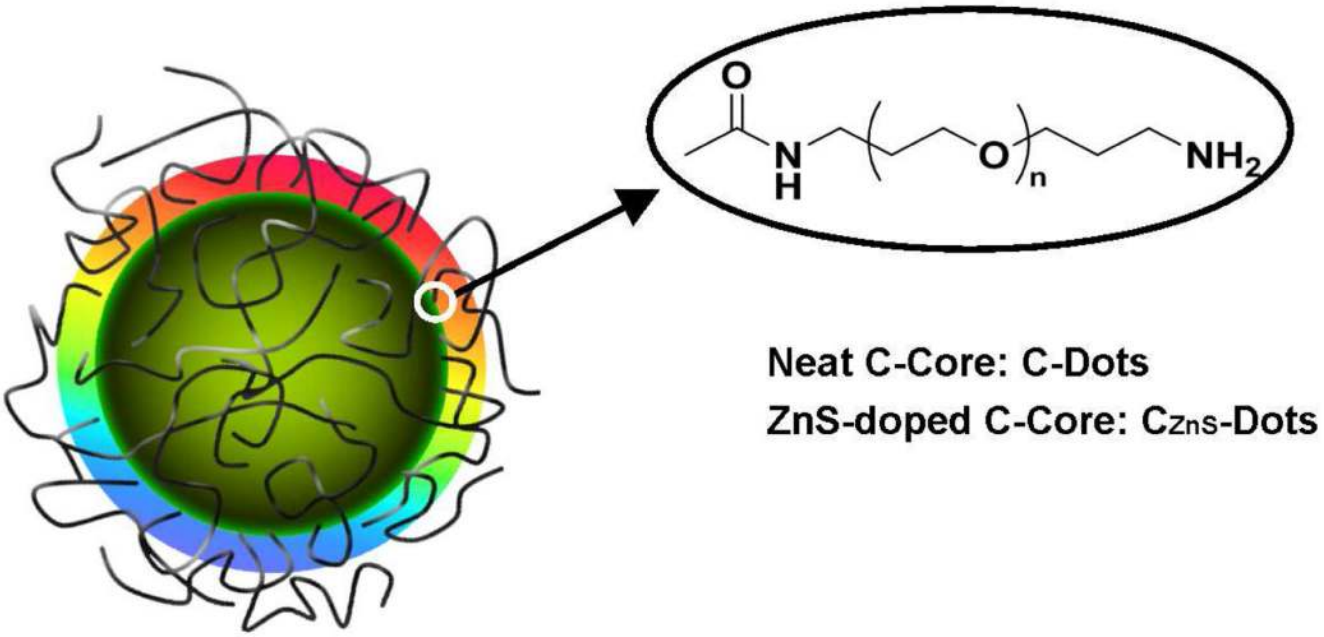


Figure 4.

Intravenous injection: (**a**) bright field, (**b**) as-detected fluorescence (**Bl**: bladder and **Ur**: urine), and (**c**) color-coded images. The same order for the images of the dissected kidneys (lower left) and liver (lower right).



Scheme 1.