Plasmonic Fluorescent CdSe/Cu₂S Hybrid Nanocrystals for Multichannel Imaging and Cancer Directed Photo-Thermal Therapy

M. Sheikh Mohamed ¹⁺, Aby Cheruvathoor Poulose ¹⁺, Srivani Veeranarayanan ¹, Rebeca Romero Abeurto ², Trevor Mitcham ³, Yuko Suzuki ⁴, Yasushi Sakamoto ⁴, Pulickel M. Ajayan ², Richard R. Bouchard ³ Yasuhiko Yoshida ¹, Toru Maekawa ¹, D. Sakthi Kumar ^{1*}

¹Bio-Nano Electronics Research Center, Toyo University, 2100, Kujirai, Kawagoe, Saitama, Japan- 350-8585

²Department of Material Science and NanoEngineering, Rice University, 6100 Main Street, Houston, TX 77005, USA

³Department of Imaging Physics, University of Texas MD Anderson Cancer Center, Houston, TX 77054, USA

⁴Biomedical Research Centre, Division of Analytical Science, Saitama Medical University, Saitama 350-0495, Japan

SUPPLEMENTARY INFORMATION

Authors Contributed Equally to this Work⁺

Corresponding author* Prof. D. Sakthi Kumar, Ph: 81-492-39-1636 Fax: 81-492-34-2502 E-mail: sakthi@toyo.jp



Fig. S1. ¹H NMR spectra of crude JC oil. Signature peaks corresponding to the mixture of fatty acids and glycerols can be seen. (inset) a tabular representation of the observed peaks and their chemical shifts in correlation to the theoretical values (obtained from Chemdraw ultra spectroscopic prediction).



Fig. S2. The EDS spectra of (a) CdSe (on a Cu microgrid) and (b) Cdse/Cu₂S (on a Ni microgrid) NCs indicating the presence of the respective elements.



Fig. S3. The composition and valence states of the hybrid CdSe/Cu₂S NCs were analyzed by XPS. The spectrum of Cu 2p and S 2p indicated the presence of S and Cu. The doublet features of Cu 2p spectrum arise because of spin orbit splitting, resulting in $2p_{3/2}$ and $2p_{1/2}$ peaks. The Cu 2p spectrum revealed the centering of Cu $2p_{3/2}$ and Cu $2p_{1/2}$ signals at 932.5 eV and 952.5 eV respectively, indicating the Cu (I) state existence of Cu ion. The S 2p level was resolved into single spin orbit splitting at 160.8 eV. The doublet feature of Cd 3D spectrum arising because of spin orbit splitting results into $3d_{5/2}$ and $3d_{3/2}$ peaks. The position of maxima at 403.16 and 409.9 eV for Cd3d_{5/2} and Cd3d_{3/2} respectively was observed. Se 3d peak positions ranged from 52 eV to 55 eV centered at 53.5 eV.



Fig. S4. The XRD pattern matches well with the Zinc Blend (cubic) CdSe (JCPDS 65-2891) and Digenite (Rhombohedral Hexagonal) Cu2S (JCPDS 47-1748) structure further confirmed the purity of the as synthesized core-shell nanostructures.



Fig. S5. To translate the PL emission spectra into visual confirmation of luminescence, we performed an imaging analysis of both the NCs by placing a drop of the respective solutions in PBS on a glass slide and analyzed their luminescence with ClairVivo Opt *In vivo* imaging system. The exposure time was kept to be constant at 15 sec. The images were recorded under Cy 5.5, Cy 7 and ICG filter with excitation of 530, 650 and 810 nm respectively. Both the NCs showed commendable luminescence at Cy 5.5, though the luminescence of core-shell nanostructure was slightly lower. The hybrid CdSe/Cu₂S exhibited similar luminescence potential at Cy 7 filter also. Under ICG filter, the luminescence of core-shell NCs, though noticeable, was nearly halved when compared to that in the other filters. The observations were on par with the PL recordings. It is to be noted that metal sulfide shell quenched the luminescence of core structures only by 14 %, as observed from the comparative intensity values of Cy 5.5 filter images of the respective NCs.



Fig. S6. Control X-Ray micro CT image of the filter paper cutting in the phantom. The orange line marks the boundary of the filter paper and the white circle demarcates the holder phantom.



Fig. S7. The NIR responsiveness of the core/shell materials was tested with the NIR laser irradiation. The temperature of the NCs (1 mg/mL) increased to 60 °C (b) from 29 °C (a) within 2 min of laser exposure.



Fig. S8. The stability of PEGylated NCs in physiological (7.0) and acidic (4.0) pH was investigated by incubating the PEGylated NCs in the respective pH solutions for a period of 7 days. The TEM analysis of NCs in pH 7.0 (a) and pH 4.0 (b) clearly indicate the morphological stability of the NCs which is equally supported by the negative potential values (c) obtained for both the conditions.

Element	рН 4.0	рН 7.0
Cu	8.9	8.7
Cd	7.8	7.21
Se	7.6	7.19

Fig. S9. The release of elemental ions under pH 4.0 and 7.0 was assessed to verify the robustness and stability of the PEGylated core shell structure and whether there is any leaching of Cd or Se ions. Post incubation at the respective pH, the NCs suspension was centrifuged and the supernatant and pellet separated. ICP-MS of the as obtained pellet and supernatant was performed. The results indicated highly insignificant levels of Cu, Cd or Se ions in the supernatant under both the pH conditions, confirming the proper and safe coating of CdSe by the Cu shell.



Fig. S10. To confirm the toxicity as a result of any sulfhydric acid or toxic byproducts due to degradation under continuous incubation of PEGylated NCs in the medium, a simple experiment was devised. The NCs incubated in physiological pH 7.0 and acidic (similar to the acidic cancer environment) pH 4.0 were pelleted and added to glioma and HCN-1A cells at the specified concentrations. Degradation byproducts as sulfhydric acid are critically toxic to cells and biological entities. The cytotoxicity data clearly depicts a considerably higher viability of the cells in exposure to the different pH incubated NCs, thus negating any degradation or toxic byproduct formation.