Supporting Information

The effect of nanoencapsulation of ICG on two-photon bioimaging

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Self-assembly process was used for the fabrication of the ICG-PLA NPs. As shown in Figure S1 the PLA is a homopeptide with a positively charged side chain. Moreover, EDTA is a multivalent anionic salt; when PLA and salt were mixed systematically, they result in the formation of the PLA NPs. Further, the addition of the ICG in this process resulting in the encapsulation of the ICG in these NPs. The pellet of bare PLA NPs and ICG-PLA NPs were shown in Figure S1.



Figure S1: The schematic representation of the fabrication process for ICG-PLA NPs. The self-assembly process was prominently depending on the molar charge ratio (MCR), incubation time, and aging temperature of the solution. The MCR of this synthesis was optimized for the fabrication of the PLA NPs and was calculated by Equation S1.

For the fabrication of the ICG-PLA NPs, the MCR is set to be 9.

Further, the absorption spectrum of free ICG was recorded by UV-Vis-NIR spectrophotometer. Utilizing the absorption spectrum at different concentration of the ICG the calibration curve was plotted between absorbance and concentration as shown in Figure S2.



Figure S2. Calibration curve of indocyanine green (ICG) in dimethyl sulfoxide (DMSO).

This calibration curve was used to measure the encapsulation efficiency of the ICG-PLA NPs. For concentration measurement, ICG-PLA NPs were dissolved in DMSO which disassembles the self-assembled NPs into amino acids, salts, and free ICG, the absorption after the release was measured to find out the concentration of the ICG encapsulated in PLA NPs.



Figure S3. Fluorescence emission of the free and nanoencapsulated ICG upon 420 nm excitation (a) Original emission (b) After deconvolution of water Raman scattering peak from emission spectra and smoothening of the data.

The fluorescence emission was collected after the excitation of 420 nm. The spectra of free ICG, nanoencapsulated ICG, and water are shown in **Figure S3**. The emission spectra have the water Raman peak at 484 nm as shown in **Figure S3(a)**, which was removed after post-processing using Origin 8.0 for better understanding of emission as shown in **Figure S3(b)**.