Supporting Information

Novel Green and Red Autofluorescent Protein Nanoparticles for Cell Imaging and in vivo Biodegradation Imaging and Modeling

Xiaoyu Ma\textsuperscript{a}, Derek Hargrove\textsuperscript{b}, Qiuchen Dong\textsuperscript{a}, Donghui Song\textsuperscript{b}, Jun Chen\textsuperscript{a}, Shiyao Wang\textsuperscript{c}, Xiuling Lu\textsuperscript{b}, Yong Ku Cho\textsuperscript{c}, Tai-Hsi Fan\textsuperscript{d}, Yu Lei\textsuperscript{ac}\textsuperscript{*}

\textsuperscript{a} Department of Biomedical Engineering, University of Connecticut
\textsuperscript{b} Department of Pharmaceutical Sciences, University of Connecticut
\textsuperscript{c} Department of Chemical and Biomolecular Engineering, University of Connecticut
\textsuperscript{d} Department of Mechanical Engineering, University of Connecticut

*Corresponding authors
Figure S1. Fluorescence emission scan of the glutaraldehyde cross-linked BSA nanoparticles without autoclave treatment.

Figure S2. Fluorescence emission spectra of the BSA aggregation dispersion.
Figure S3. Control cell images (293FT cells without exposure to autofluorescent BSA nanoparticles). Scale bar = 10 μm. a) The merged image by overlaying transmission light cell image with green and red fluorescence confocal images; b) The green fluorescence image acquiring at the GFP wavelength range (~510 nm) with a 488 nm excitation laser; c) The red fluorescence image acquiring at the Texas red wavelength range (~615 nm) with a 560 nm excitation laser; and d) The cell image under a transmission light.

Figure S4. Z-stacking of the confocal images focusing on one single cell at different focal planes. Scale bar = 20 μm. a) The merged image by overlaying the transmission light, green, and red fluorescence images at a height of 3 μm from the bottom; b and c) The green and red fluorescent image at a height of 3 μm from the bottom, respectively; d) the merged image by overlaying the transmission light, green, and red fluorescence images at a height of 7 μm from the bottom; e and f) The green and red fluorescent image at a height of 7 μm from the bottom, respectively; g) the merged image by overlaying the transmission light, green, and
red fluorescence images at a height of 15 μm from the bottom; h and i) The green and red fluorescent image at a height of 15 μm from the bottom, respectively.

Cross-sectional confocal image of the cell was obtained to observe the inner cytoplasm fluorescence. As the corresponding result presented in Figure S5, the obvious green and red fluorescence signal comparing with the background signal also indicates the uptake of autofluorescent nanoparticles by the cell.

Figure S5. Confocal slice images indicate the intersection fluorescence. a is the cell image under transmission light, and the intersection of the yellow line was used for confocal slice images to observe the inner cytoplasm fluorescence, while in b) and c), the bottom intersection of the yellow line presents the green and red fluorescence of the cutting section of the intersection shown in a.

Figure S6. a) Temporal effective diffusivity represented by the correction factor: \( g(t) = \frac{D_{\text{eff}}(t)}{D_0} = 0.1 + 0.9 \exp(-10^{-21} t^9) \); and b) initial condition fitted by an exponential function: \( c(r, 0) = 0.15 + 0.85 \exp(-10^{6.4} r^7) \).
Figure S7. The results of the histology study. a) A skin tissue image (40×); b) A pancreas tissue image (40×); c) A liver tissue image (40×); and d) A kidney tissue image (40×).