Supporting Information

Electrochemically Generated Fluorescent Fullerene[60] Nanoparticles as a New and Viable Bioimaging Platform

Yifeng E, Linling Bai, Louzhen Fan*, Mei Han*, Xiaoyan Zhang, Shihe Yang*

Department of Chemistry, Beijing Normal University, Beijing, 100875, China; Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

Quantum yield calculations

The quantum yield (Φ) of the fullerene (C₆₀) nanoparticles was obtained by comparing the integrated photoluminescence intensities (excited at 350 nm) and the absorbency values (at 350 nm) of the C₆₀ nanoparticle sample with the reference Rhodamine B. Five concentrations of Rhodamine B and C₆₀ nanoparticles solutions were made, all of which had absorbance less than 0.1 at 350 nm. Rhodamine B (literature $\Phi = 0.90$) was dissolved in ethanol (refractive index (η) of 1.36). The C₆₀ nanoparticles were dissolved in water ($\eta = 1.33$). An UV-Vis absorption spectrometer was used to determine the absorbance of the samples at 350 nm. The respective solvents were used as the references. A quartz cuvette with a path length of 1.00 cm was used to contain the samples during the UV-Vis and fluorescent experiments. The data was plotted and the slopes of the sample and the standards were determined. The quantum yield was calculated using the below equation:

$$\Phi_{\rm X} = \Phi_{\rm ST} \left(\frac{m_{\rm X}}{m_{\rm ST}}\right) \left(\frac{\eta_{\rm X}^2}{\eta_{\rm ST}^2}\right)$$

where Φ is the quantum yield, m is slope, η is the refractive index of the solvent, ST is the standard and X is the sample.

Cytotoxicity Measurements

Cytotoxicity of the fluorescent C_{60} nanoparticles was tested by a colorimetric cell viability assay (MTT assay). Cells were seeded into 96-well microculture plates in densities of 4000 cells/well. The cells were incubated with the C_{60} nanoparticles of different concentrations. After 72 hrs of incubation, the cells were treated with MTT according to the manufacturer's protocol. The absorbance values were read out on a VICTOR3TM microplate reader (Perkin– Elmer, Waltham, MA, USA) at 570 nm. The signals were averaged over readings from five independent wells.



Figure S1. Cyclic voltammograms (CVs) of C_{60} film on Pt electrode in an acetonitrile solution containing 0.1 M tetrabutylammonium perchlorate (TBA)ClO₄. Continuous scanning over the third reduction wave causes the continuous decrease in peak currents, indicating the slow dissolution of the film. The inset is the first CV, clearly showing that the first two reductions are irreversible and the third reduction is reversible corresponding to the dissolution of $C_{60}^{3^2}$.



Figure S2. Time- resolved fluorescence spectrum of C_{60} nanoparticle aqueous solution. The time- resolved fluorescence of C_{60} nanoparticles showed multi-exponential decay with the time constants of 0.72 ns (13.99%), 2.94 ns (42.91%), and 8.03 ns (43.10%).



Figure S3. XRD pattern of the fluorescent C_{60} nanoparticles.



Figure S4. FT-IR spectra of the fluorescent C_{60} nanoparticles (a) and pristine C_{60} .



Figure S5. SEM image of C_{60} nanoparticles with large sizes obtained also by constant potential electrolysis of a C_{60} covered Pt foil in acetonitrile solution with 0.01 M (TBA)ClO₄ but without introducing the series resistor into the working electrode

A549



Figure S6. Cross-sectional confocal fluorescence images of A549 cell and MFC-7 cells with the fluorescent C_{60} nanoparticles excited at 405, 488, and 543 nm. The cross-sectional images (from left to right) were captured at vertical thickness from top of the cell (upper membrane) toward the bottom.



Figure S7 The photostabilities of C_{60} nanoparticle aqueous solution. It can be seen that the fluorescent intensity keeps almost unchanged for about 20 days.