Fluorescent Nanodiamonds - Single Particle Optical Characterization & In vivo Imaging in C. elegans

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Introduction: The negatively charged nitrogen-vacancy defect center (N-V), in type Ib diamond exhibits distinct fluorescence features such as extended red emission at ~700 nm, high photostability, diverse surface functionalizability. We extracted smallest possible Fluorescent Nanodiamond (FND) with as many as 3 NV centers within and brighter than single RFP. We explore in this work the possibilities of using FNDs for long-term bio-imaging in vivo. We choose Caenorhabditis-elegans (C. elegans) as our in vivo system because of its size amenable to optical microscopy, short life cycle and well defined behaviors. FNDs are incorporated into wild-type C. elegans by feeding them with FND and could image the whole digestive system of the organism for several days. We investigated the toxicity of FND particles in C. elegans by examining longevity and broad size and Oxidative Stress response and proved FND is nontoxic to the organism.

Single 10-nm FND is 8 times brighter than single RFP

Figure 1. (a) NV center formation in diamond by helium beam irradiation followed by annealing and its characteristic fluorescence spectrum. (b) Differential Centrifuge method followed to extract 10 nm FNDs and characterized by (c) TEM (d) AFM and (e) Confocal microscopy. Figure 2. (a) Size measurement with dynamic light scattering for particles suspended in water. b) Photostability tests of particles spin-coated on glass substrate. c) photon correlation functions of the fluorescence intensities of two single FND particles containing 1 and 3 (N-V) centers respectively. d) Histogram of the number of (N-V) centers in the individual particles and their aggregated. Figure 3. (c) characterization of DsRed-Monomers and FNDs by FCS. b) fluorescence intensity histogram of the particles. single 10 nm FND is ~ 8 times brighter than single 7 nm RFP

Bare-FND remains in the gut but Bio-conjugated FND localizes to intestine tissue

Figure 3. (a) Fluorescence and DIC merge images show bare FND localized inside the Mouth, Pharynx and Lumen of C. elegans. (b) Cartoon depicting development and anatomy of an adult C. elegans. (c) procedure followed to to feed worms with FND. Figure 4. Surface modification of FND with (a) Dextran and (b) BSA protein prevents aggregation of FNDs in the gut lumen of C. elegans and get localized in the intestinal cells by endocytosis. Fluorescence and DIC merge images show surface functionalized FND localized in the potential fat droplets in the intestinal cells of the worms.

Oxidative stress gene response analysis proves FND to be Non-toxic

Figure 5. Comparisons of life span (a), brood size (b), and ROS level (c) of the worms treated with bioconjugated FNDs, and the untreated control show that both the life span and the potential for progeny production are unaffected by the treatments with either dextran- or BSA-coated FNDs. Figure 6. (a) Under Oxidative stress, there is nuclear translocation of DAF-16. Stress by heat shock cause nuclear translocation of gfp::daf-16. (b) gcs1::gfp expresses in the gut under oxidative stress. (Arsenic treatment ). FND treatment does not induce any oxidative stress to the organism.

Conclusion: Single 10-nm FND can have 3 NV centers and is brighter than single Ds-Red RFP. FND could be used for long term bio imaging in vivo. Bare FND remains within the intestinal lumen while surface modified FND get localized within the intestinal cells. Life Span, Brood Size and oxidative stress response assays prove FND is non-toxic to the organism.