Optical Detection of Charged Biomolecules: Towards Novel Drug Delivery Systems

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Abstract

This paper presents work done on developing optically-traceable intracellular nanodiamond sensors, where the photoluminescence can be changed by a biomolecular attachment/delivery event. Their high biocompatibility, small size and stable luminescence from their color centers make nanodiamond (ND) particles an attractive alternative to molecular dyes for drug-delivery and cell-imaging applications. In our work, we study how surface modification of ND can change the color of ND luminescence (PL). This method can be used as a novel detection tool for remote monitoring of chemical processes in biological systems. Recently, we showed that PL can be driven by atomic functionalization, leading to a change in the color of ND luminescence from red (oxidized ND) to orange (hydrogenated ND). In this work, we show how PL of ND changes similarly when interacting with positively and negatively charged molecules. The effect is demonstrated on fluorinated ND, where the high dipole moment of the C-F bond is favorable for the formation of non-covalent bonds with charged molecules. We model this effect using electrical potential changes at the diamond surface. The final aim of the work is to develop a "smart" optically traceable drug carrier, where the delivery event is optically detectable.

Keywords: nanodiamond, drug delivery, nitrogen-vacancy center, luminescence properties, charged molecules.

1 Introduction

Fluorescent cellular biomarkers play an essential role in biology and medicine for in-vitro and in-vivo imaging in living cells. Luminescent nanodiamond (ND) has recently been suggested as a novel optical marker for cellular imaging [1, 2]. ND offers advantages over classical fluorescent markers used for in vivo and in vitro imaging in living cells. It offers a cellular delivery combined with strong and stable photoluminescence (PL) originating from the nitrogen-vacancy (NV) or other lattice point defects. ND is biocompatible, and its surface can easily be terminated with various groups and additionally functionalized with biomolecules [3, 4], making ND a suitable carrier for cellular targeting or drug delivery. In this work we show a method for changing the PL properties of ND in a biological environment by surface termination-induced changes leading to electric field development close to the ND surface.

The NV center is observed in two charge states, negative and neutral, each with different PL properties [5]. A negatively charged NV center (NV^-) emits around 637 nm and a neutral center (NV^0) emits around 575 nm. Under standard conditions, both states are observed, but NV^- related luminescence is more intense. A single NV center can exist in both states [6], depending on its surroundings. In this work we demonstrate the influence of the presence of charged molecules (biopolymers) on changes in the occupation of NV^- and NV^0 states in fluorinated ND, with NV^- quenching upon interaction with positively charged polymers. The high dipole moment of F-terminated diamond attracts positively charged molecules, leading to the creation of a hole accumulation layer and therefore a charge transfer from the diamond to the surrounding polymer, i.e. an upward surface band bending. We use these effects to induce changes in the occupation of $NV^{0/-}$ centers lying in the band-bending zone.

We demonstrate these effects on NV centers in high-pressure high-temperature ND of 20–50 nm in size produced by irradiation and annealing. The results are supported by theoretical modeling of the density of the state distribution for various surface interactions. The effect is compared with oxidized ND.

2 Experimental

The effects presented in this work were studied on HPHT type Ib ND 40–50 nm in size, sourced from Microdiamant AG, Switzerland (size selected from commercial product MSY 0–0.05 GAF) containing about 100–120 ppm nitrogen (Figure 1).

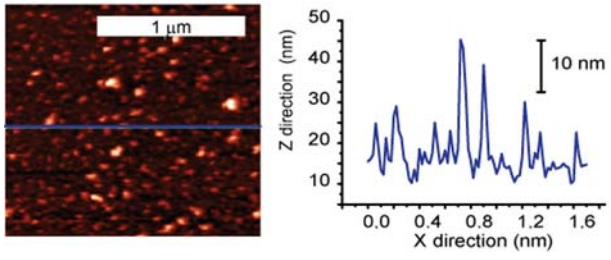

Fig. 1: AFM picture of the ND particles

A commercial solution of ND was lyophilized and heated in air at 425 °C for 5 h to remove any sp^2 carbon-containing layer. The resulting pale grey powder was dispersed in water and deposited in the form of a thin film on a target backing (10 mg·cm^{-2}) for ion implantation. The ND was then irradiated using an external proton beam produced by a U-120M isochronous cyclotron. The angle of the target backing with respect to the beam direction was 10 °. The fluency of the delivered beam was $9.2 \cdot 10^{15}$ cm^{-2}, beam energy 5.4 MeV and beam current 0.6 μA. The irradiated ND was thermally annealed *in a vacuum* at 710 ° C for 2 h to create NV centers by trapping the vacancies next to the nitrogen atom. NDs were then oxidized in a mixture of concentrated H_2SO_4-HNO_3 (9 : 1, vol/vol) at 80 °C for 7 days. The reaction mixture was diluted with deionized water, the NDs were separated by centrifugation and was subsequently washed with 0.1 M NaOH, 0.1 M HCl, and finally washed three times with water. The solution was lyophilized, providing highly fluorescent NDs in the form of a stable colloidal monodispersion in water, as confirmed by AFM and DLS (final size 20–25 nm). The colloidal dispersion was stable after 2 months, with no sedimentation. After the measurement, the quartz plate with ND grains was exposed to microwave-excited hydrogen plasma for 30 minutes at a temperature of 500 °C and at pressure of 1 mbar to produce an H-terminated surface. Finally, the H terminated samples were fluorinated by ionic fluorination in a mixture with an aqueous solution of hydrogen fluoride and fluorine gas. After saturation of the solution, the suspension reacts under pressure for 2 days.

Raman and PL (514 nm) spectra were recorded using a Renishaw InVia Raman Microscope. Spectra were taken at room temperature. All spectra were normalized to the diamond Raman peak. The AFM measurements were performed in tapping mode (111 kHz) with an NTEGRA Prima NT MDT system equipped with a soft HA_NC etalon tip.

Polymers were chosen as positively charged molecules: poly diallyldimethyl ammonium chloride (PDADMAC) and polyallylamine (PAA.HCl). Polyacrylic acid sodium salt (PANa) and polystyrene sulfonic acid sodium salt (PSSNa) were used as negatively-charged molecules.

3 Results

3.1 Comparison of PL on variously terminated ND

The PL of NV centers is sensitive to the surface termination, as shown in our previous work [7]. However, the effect of surface fluorination has not been described yet. Figure 2a shows the PL spectra of fluorinated, oxidized and hydrogenated ND, where we clearly see the difference in the NV^-/NV^0 ratio, where the NV^- luminescence is most pronounced in fluorinated ND.

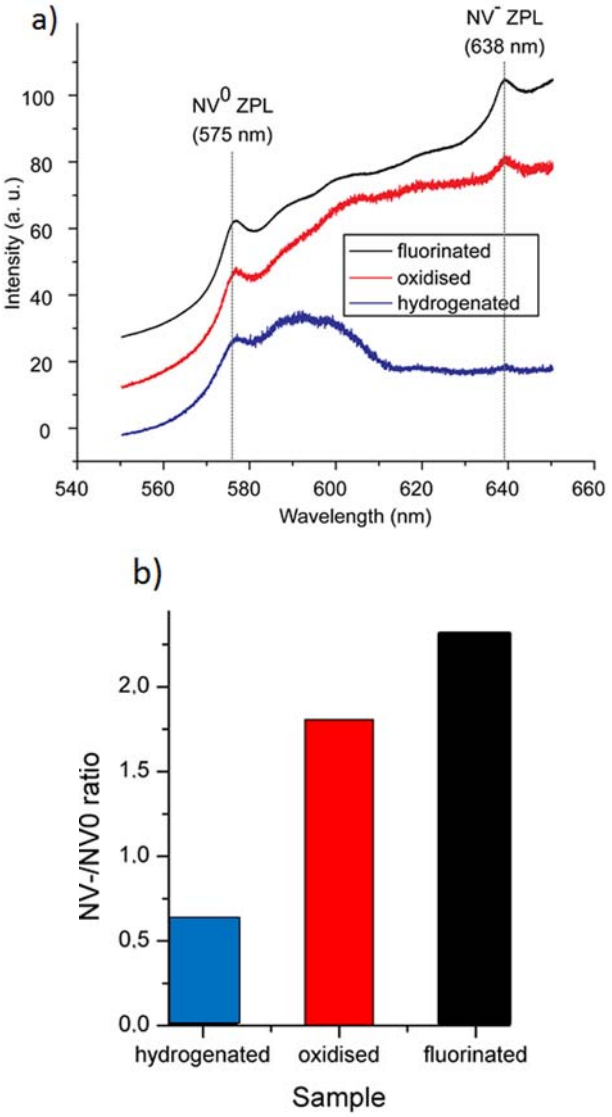

Fig. 2: Comparison of PL of variously treated ND. a) PL spectra taken at 514 nm excitation in a liquid colloid solution showing increased NV^- related luminescence for fluorinated ND in comparison to oxidized and hydrogenated ND. b) Counted NV^-/NV^0 ratio of treated ND. The ratio was counted from the NV^- and NV^0 zero phonon line (ZPL) — gray line in Figure 2a

3.2 Interaction with charged polymers

In a mixture of nanoparticles with charged molecules, non-covalent bonds are always formed between the charged molecule and the nanoparticle. The strength of the bond differs, and depends on many factors, e. g.

the ability to form hydrogen bonds or the size and polarity of the surface dipole moment of the nanoparticle. If the charged molecule is strongly attracted, charge transfer can occur, depending on the HOMO and LUMO energetic levels of the nanoparticle and the chemical potential of the molecule.

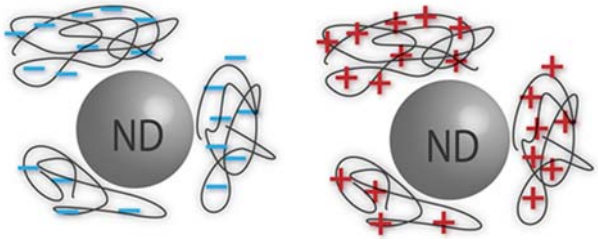

Fig. 3: The negative and positive electric field formed in the close surface proximity of ND after the interaction with charged polymers

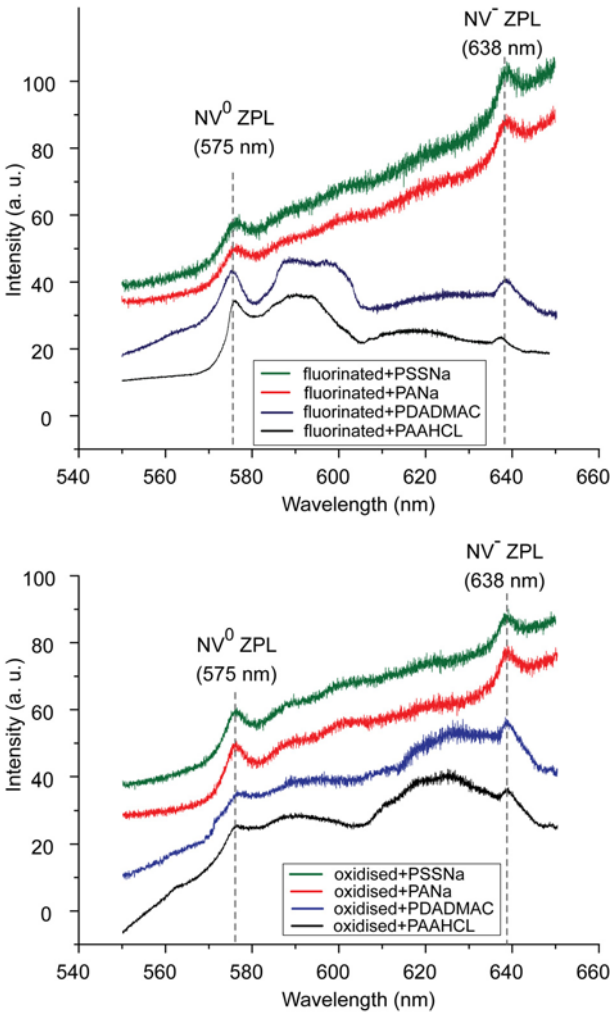

Fig. 4: The PL spectra of fluorinated ND on interacting with the negative and positive electric field formed in the close surface proximity of ND after the interaction with charged polymers

Four different types of polymers were chosen for this experiment (see Experimental for details). The polymer size did not exceed the size of ND, favoring the creation of stronger bonds. The colloidal solution of ND was mixed with polymers in 10× higher concentration, enabling saturation of the surface with polymers. The interaction between polymers and ND is schematically shown in Figure 3.

Figure 4a shows the changes in the PL spectra of fluorinated ND when interacting with charged polymers. Figure 4b shows the PL of the interaction with the same charged polymers, but using oxidized ND. The luminescence of the NV-centers clearly decreased on interacting with positively charged molecules, while after adding negatively charged polymers the luminescence was restored to the original level.

When comparing the effects observed on fluorinated ND with the effects on oxidized ND, we find that the effect is much stronger for fluorinated ND. This difference could be due to the different properties of the carbon-fluor (C-F) and carbon-oxygen (C-O) bond. The electron affinity of the C-F bond is much higher (1.45) than the affinity of the C-O bond (0.9). This leads to stronger attraction of positively charged polymers to the fluorinated surface. However, concerning the hydrogen bond, a fluorinated surface can be only an acceptor of a hydrogen bond, oppose to the oxidized surface (containing carboxyl, carbonyl, hydroxyl or lactone groups) can be both donor and acceptor of the hydrogen bond.

3.3 In-vivo imaging in chicken embryos

Fluorinated NDs were used for in-vivo luminescence imaging in chicken embryos. The results (Figure 5) show an important fact that the intensity of the luminescence from fluorinated ND is strong enough to be detected in a commercially available confocal microscope used for standard luminescence imaging. The method is therefore suitable for optically traceable drug delivery systems.

4 Modeling the effect

To explain the observed effect, we modeled the energetic balance near the surface by numerical solution of the Poisson equation using the Boltzmann distribution. We can write for the depth (x) dependent total space charge density $\rho(x)$:

$$\rho(x) = eN_V \exp\left(-\frac{(E_F - E_V)_x}{kT}\right), \qquad (1)$$

where N_V is the temperature dependent effective density of states at E_{VBM}, $N_V = 2.7 \cdot 10^{19}$ cm^{-3} at room temperature, e is elementary charge, k is Boltzmann constant and T is thermodynamic tem-

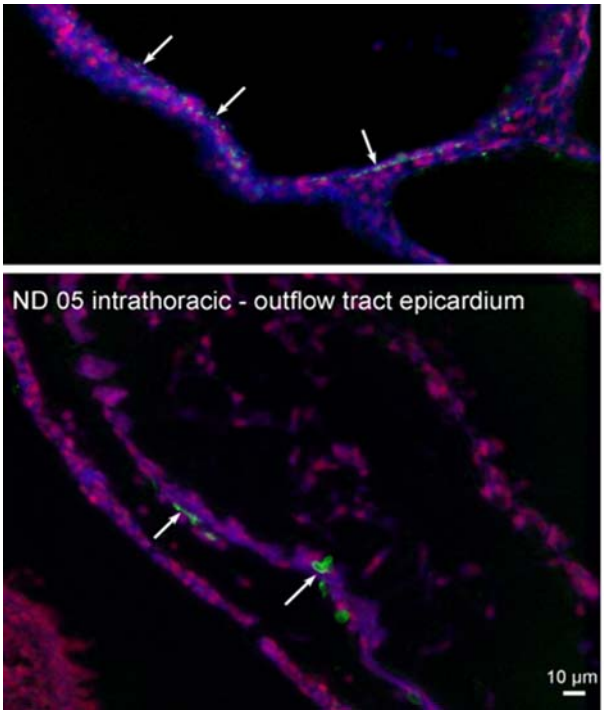

Fig. 5: Luminescence confocal image of fluorinated ND in a chicken embryo. ND visible as green dots in the picture

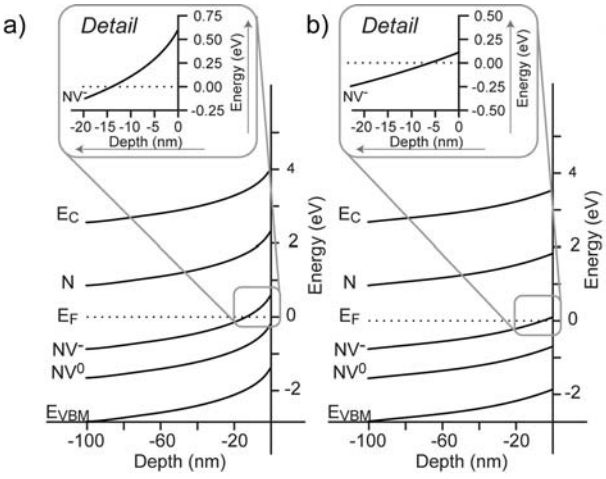

Fig. 6: DOS calculations of surface band bending for two different situations: a) fluorinated ND, b) oxidized ND. The unoccupied (dark) state where luminescence of the NV- centers cannot occur is larger for the fluorinated surface than for the oxidized surface

perature. E_F and E_V are the energetic levels of the Fermi and valence band.

$$\frac{\mathrm{d}^2(E_F - E_V)_x}{\mathrm{d}x^2} = \frac{e}{\varepsilon\varepsilon_0}\rho(x) \qquad (2)$$

where $\varepsilon\varepsilon_0$ is the permittivity of the material.

The Poisson equation was solved numerically with boundary conditions

$$(E_F - E_V)_0 \;=\; kT \ln\left(\frac{N_V}{p_0}\right) \qquad (3)$$

$$p_0 \;=\; e\,(N_A \cdot \zeta) \qquad (4)$$

p_0 is the total unscreened positive charge at $x = 0$ from (1), and N_A is the density of the surface acceptors. The density of the surface acceptors was calculated by solving the Boltzmann-Poisson equation, taking into account the different electron affinity values described above. The electron affinity was introduced into the model as parameter ζ. In our calculations, ζ was set to 1.4 and 0.9.

The results of mathematical modeling are shown in Figure 6. The modeling clearly explains the reduced effect.

5 Conclusions

The luminescent properties of NV defects engineered in HPHT ND when interacting with charged molecules have been studied. It was found that the luminescence of NV centers is sensitive to surface treatment. The NV- luminescence fell significantly after hydrogenation of the surface and increased after fluorination, in comparison with the standardly used oxidized surface. Additionally, it was found that the PL of fluorinated ND can be strongly influenced by the presence of charged molecules. This can be further used for in-vivo optical detection of charged molecules in cells/smart drug delivery systems. The observed effects have been explained by numerical modeling. The final result of this study was an in-vivo application of luminescence ND in a chicken embryo, showing the detectability of luminescence ND in a standard confocal microscope.

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About the author

Vladimíra Petráková was born in Prague, Czech Republic. She graduated with a master degree from the Faculty of Biomedical Engineering, Czech Technical University (FBE, CTU) in June 2009. In October 2009 she joined the Laboratory of Materials for Nanosystems and Biointerfaces at the Institute of Physics, Academy of Sciences. Currently she is in the second year of her doctoral studies at FBE, CTU. She received twice Josef Hlávka prize for the Best Students and Graduates (2007, 2009), and received the Dean's prize for an excellent bachelor thesis in 2007. Her professional interests are luminescence centers in nanodiamond particles, high-resolution optical systems, surface chemistry, biosensors, and also neural circuits and data analysis. In 2010 she received a Young Investigator Award for the best oral presentation in the European Diamond Conference in Budapest, Hungary and also at the MRS Fall Meeting at the Boston, USA for her work describing the mechanism and control of switching between the neutral and negative charge state of the NV center in diamond. Other interests are family, backpacking and music.

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