DNA-Based Self-Assembly of Fluorescent Nanodiamonds

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ABSTRACT: As a step toward deterministic and scalable assembly of ordered spin arrays we here demonstrate a bottom-up approach to position fluorescent nanodiamonds (NDs) with nanometer precision on DNA origami structures. We have realized a reliable and broadly applicable surface modification strategy that results in DNA-functionalized and perfectly dispersed NDs that were then self-assembled in predefined geometries. With optical studies we show that the fluorescence properties of the nitrogen-vacancy color centers in NDs are preserved during surface modification and DNA assembly. As this method allows the nanoscale arrangement of fluorescent NDs together with other optically active components in complex geometries, applications based on self-assembled spin lattices or plasmon-enhanced spin sensors as well as improved fluorescent labeling for bioimaging could be envisioned.

Fluorescent nanodiamonds (FNDs) constitute a new class of stable fluorescent markers, which show neither photobleaching nor blinking and are at the same time biocompatible due to a chemically robust and inert surface.1 This renders FNDs perfectly suited for a wide range of biocompatible applications including thermometry,3 imaging and tracking,4 or color centers in diamond6 provide a promising platform for long electron spin coherence times of nitrogen-vacancy (NV) nanoscale spin-based quantum technologies such as magneto-

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phobic interactions. After overnight stirring in 0.5 × TBE buffer with NDs and dBSA-PEG and then purification by centrifugation, dBSA-PEG-modified NDs were obtained (SI Methods, Notes S1 and S2, Tables S1 and S2). With our approach we have realized for the first time a biopolymer-based coating that efficiently encapsulates NDs and imparts high colloidal stability even at high ionic strength required for DNA conjugation. Most importantly, multiple functional groups can be introduced to the FNDs’ surface providing now access to site-specific bioconjugation to precisely assemble FNDs with DNA origami.

Due to the steric repulsion effect of the grafted PEG chains, the dBSA-PEG-coated NDs were fully dispersed in TBE buffer even in the presence of 10 mM MgCl2 (Figure 1 and Figure S1). Note that TEM images revealed strong clustering of the untreated NDs after drying on the carbon-coated Formvar grids, and no reliable image analysis was possible. After treatment with dBSA-PEG, however, the NDs were perfectly dispersed, and we measured an average diameter of the NDs of 49.0 ± 14 nm. NDs grafted with PEG750-methoxy. This coating results in perfect dispersion of the NDs and an average surface-to-surface distance of 48 ± 12 nm. TEM images of NDs modified with dBSA-PEG3000-methoxy. The longer PEG-chains increase the observed average surface-to-surface distance to 149 nm (scale bars: 200 nm).

Figure 1. Surface modification of NDs. (a) dBSA-PEG-R (R: methoxy or biotin) is conjugated to the NDs via charge and hydrophobic interactions. (b) TEM images of bare NDs. Note that mainly clusters are observed before coating. (c) TEM images of NDs modified with dBSA-PEG750-methoxy. This coating results in perfect dispersion of the NDs and an average surface-to-surface distance of 48 ± 12 nm. (d) TEM images of NDs modified with dBSA-PEG3000-biotin. The longer PEG-chains increase the observed average surface-to-surface distance to 149 nm (scale bars: 200 nm).

Dynamic light scattering (DLS) measurements were carried out to observe the size changes of the NDs after surface modification in solution (Figure S4). Note that DLS of bare NDs could only be performed after spinning down aggregates. The averaged hydrodynamic diameters of the NDs increased from 97 to 120 nm, while the size distribution decreased from 124 to 107 nm (full width at half maximum, fwhm) before and after coating with dBSA-PEG3000-biotin, respectively. These observations indicate that clusters were still present during the DLS measurement of bare NDs and that the coating procedure homogenized the size distribution. The surface potential increased from −28.2 ± 14.2 mV for untreated NDs to −7.6 ± 7.03 mV after polymer coating, which indicates that the surface functionalization is also based on charge interactions with the positively charged BSA backbone partially neutralizing the negatively charged ND surface. Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) of dBSA-PEG-modified NDs exhibit significant signals of PEG and dBSA molecules and two different types of sulfur atoms, respectively, indicating the successful coating (Figures S5 and S6).

To spatially organize our coated FNDs we chose four different DNA origami structures: a six-helix bundle23 (6HB, 428 nm long), a 14-helix bundle18b (14HB, 210 nm long), a 24-helix bundle27b (24HB, 100 nm long), and a 2-layered sheet (2LS, 50 nm × 50 nm). Biotin-labeled oligonucleotides extending from the DNA structures either at their ends or their sides served as anchor sites for neutravidin molecules, each of which offered four binding sites to biotin (Figure 2a,b and Methods in SI). The remaining unoccupied biotin pockets were then used to conjugate biotin residues of the biotin-functionalized FNDs (dBSA-PEG3000-biotin modified FNDs). Due to the multivalent nature of the biotin-FNDs, varying morphologies were observed dependent on the ratio of the mixed components (Figures S7–S9). By fine-tuning the ratio of biotin-FNDs and DNA origami structures for each batch of FNDs, the assembly yield of FND dimers was optimized. TEM analysis revealed a yield of correctly assembled dimers of up to 27% (Table S3) and confirmed the efficient control over the separation distances of FNDs dimers: 96 ± 24 nm (SD) for 24HBs, 208 ± 28 nm for 14HBs, and 361 ± 40 nm for 6HBs (Figure 2c–e). The 6HBs consist of only six parallel DNA helices and are therefore more flexible than the 14HBs or 24HBs (14 and 24 parallel helices).24 As a result of their flexibility, many of the 6HBs adsorb on the TEM grid in bent configurations that have shorter end-to-end distances than a fully stretched bundle, and thus large deviations from the nominal length are observed. The flexible PEG-linkages that connect the FNDs to the DNA constructs additionally contribute to a random distribution of distances for all bundle types.

As DNA origami allows the formation of virtually any geometry, the spatial organization of FNDs is not limited to dimer assemblies. Figure 2f shows FNDs that were designed to arrange in a triangle and an irregular tetrahedron, respectively. For these geometries, we offered either three or four binding sites at the edges of the two-layered sheet as depicted in the schematic drawings. TEM revealed the successful assembly of clusters of three and four NDs. Note that due to the flattening of the structures during adsorption to the TEM grid, we were not able to prove the three-dimensionality of the tetrahedron design.

Finally, we explored the optical properties of the self-assembled FND dimers containing NV centers in a confocal microfluorescence spectroscopy setup under ambient conditions (SI Methods, Note S3). A representative color-coded fluorescence intensity map of spatially dispersed FND dimer nanostructures (6HBs) on quartz is shown in Figure 3a. The map was recorded with diffraction-limited confocal excitation at 532 nm and collection above 575 nm by raster scanning the sample with respect to the focal spot (fwhm spot size of ~370 nm). Several FND dimer assemblies appear as pairs of fluorescence hotspots in the map. Other assemblies are not clearly resolvable or represent single-labeled 6HBs. Figure 3b,c...
depicts two high-resolution maps of FND dimers exhibiting a separation of 451 and 406 nm, which is well within the limits of an expected maximum center-to-center distance of ∼476 nm (length of 6HB + radius of coated FND: 428 + 48 nm). Figure 3d shows a typical spectrum of an FND assembled on DNA origami (red trace) (see Figure S10 for further spectra). It exhibits spectral characteristics of negatively charged NV center fluorescence, which was unaffected by our procedures. Note that the reduced intensity of the long-wavelength phonon side-bands is a result of the chromatic optical response function of the setup.

Figure 2. Self-assembly of NDs with DNA origami. (a) DNA sequence maps (SI Note S4) exemplarily show design details of binding sites on the six-helix bundle and the two-layered sheet. By mixing the biotin-functionalized NDs with the neutravidin-modified DNA structures, the biotin residues of the modified NDs bind to the remaining free pockets of the neutravidin, which leads to the site-specific assembly of NDs. (b) Schematic conjugation between NDs and DNA origami. (c–g) Top: DNA nanostructure design and histograms (with Gaussian fits) of the NDs surface-to-surface distance. (c) 24-helix bundles yield an average distance of 96 nm (designed distance: 100 nm). (d) 14-helix bundles yield an average distance of 208 nm (designed distance: 210 nm). (e) 6-helix bundle yield an average distance of 361 nm (designed distance: 428 nm). Note that this DNA construct is considerably more flexible compared to the other two. (f, g) NDs trimer and tetramer assembly on a two-layered origami sheet (50 × 50 nm). Bottom: corresponding TEM images of ND assemblies (Scale bars: 100 nm).

Figure 3. Optical analysis of DNA origami-assembled fluorescent NDs. (a) Color-coded confocal fluorescence map of ND dimers (6HB). The dimer assemblies appear as pairs or individual fluorescence hotspots in the confocal map. Scale bar: 2 μm. (b, c) Zoom-in on ND dimers with corresponding line scans fitted with two gaussians. Scale bar: 400 nm. (d) Fluorescence spectrum of a ND assembled on DNA origami (red trace) together with typical spectra of a bare and a dBSA-PEG3000-biotin-modified ND (gray traces). The spectra are offset for clarity to highlight the characteristics of negatively charged NV center fluorescence, which was unaffected by our procedures. Note that the reduced intensity of the long-wavelength phonon side-bands is a result of the chromatic optical response function of the setup. indicating that the fluorescence properties of FNDs are robust against the dBSA-PEG modification and the DNA assembly procedures (see Figure S11 for complementary examples and Figure S12 for a spectral time trace).

In conclusion, we have successfully realized nanoscale positioning of NDs containing fluorescent NV-centers by DNA self-assembly. Bioconjugation between FNDs and DNA could be accomplished by a PEG-labeled biopolymer surface coating derived from the plasma protein BSA that imparts high colloidal stability to the FND. This approach offers the possibility to introduce many different modifications at the same time by attaching the desired end groups to the PEG molecules, as demonstrated herein for biotin. Fluorescence measurements certified correct assembly and that our method does not alter the optical properties of the NV centers. Moreover, using DNA origami, FNDs could be arranged in nanoscale geometries with other optically active nanoparticles. Since NV centers exhibit excellent photostability and remarkably long electron spin coherence times for efficient spin manipulation by optical means, site-specific arrangement of FNDs would ultimately allow investigating the coherent coupling of NV centers assembled in pairs and extended arrays or lattices, which is a step toward the realization of scalable quantum processors and simulators. Before this goal can be approached with the method presented here, however, thinner coating layers for the FNDs have to be developed, and the positioning accuracy as well as efficiency has to be increased further.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b04857.

Methods and materials, additional data including DLS measurements, TEM images, optical analysis, and DNA origami design (PDF).
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