## ASSEMBLY, MODIFICATION AND CHARACTERIZATION OF NANODIAMOND QUANTUM EMITTERS

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Henry J. Shulevitz

To Noalle

#### ACKNOWLEDGEMENT

If this thesis were a diamond it would be a chemical vapor deposition diamond: slowly built up piece by piece under the proper conditions. Thankfully, there were a few moments of the explosive potential of the denotated nanodiamonds and fewer moments high pressure high temperature growth. Instead I have mostly been allowed to grow layer by layer little by little. There are many people I have to thank for maintaining and allowing the conditions of that growth. (That was actually a very good analogy, but it might not make sense if you have not read the rest of the thesis.)

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#### ABSTRACT

# ASSEMBLY, MODIFICATION AND CHARACTERIZATION OF NANODIAMOND QUANTUM EMITTERS

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Fluorescent milled nanodiamonds are one of the most promising physical bases for quantum devices. The nitrogen vacancy (NV) center in diamond acts a room temperature, optically addressable quantum system which significantly decreases the infrastructure costs of developing quantum devices. Since nanodiamonds are nontoxic and colloidally stable, the nanoparticles are particularly attractive to the fields of sensing and nanomedicine where environmental changes must be detected with nanoscale resolution. Particle to particle irregularity, however, alters the spin and optical properties of each nanodiamond and complicates their integration into more complex systems. In this dissertation, we address the inhomogeneity of nanodiamonds and provide multiple paths towards fully utilizing these quantum emitters. First, we develop a technique for the self-assembly of nanodiamonds into arrays. We then utilize these arrays to perform a statistical study of the nanodiamonds and characterize the impact of the material's surface and crystalline structure on the NV center's optical and spin properties. Next, we establish a surface modification method that both improves the general properties of the nanodiamonds and enables chemical conjugation reactions. Together, these results deliver a newfound level of control and uniformity over an otherwise uncontrollable and polydisperse material.

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#### CHAPTER 1

#### Introduction

In the late 19th century, James Clark Maxwell demonstrated that light could be modelled as an electromagnetic wave. In 1926, Erwin Schrodinger arrived at an equation that described quantum mechanics as a the wave function. Today, nearly a century later, we are just realizing the revolutionary potential of quantum physics. Quantum computers are theorized to solve problems currently impossible through classical means [45, 126, 79, 26], while quantum communication could provide unparalleled levels security and encryption [43, 51, 149], and quantum sensors could reveal presently undetectable environmental minutiae [120, 38]. Governments and industries have committed billions of dollars to construct these devices. Yet, to fully realize the big future of quantum technology, we must approach its potential on the small scale. This thesis focuses on nanodiamonds containing nitrogen-vacancy (NV) centers as the basis for quantum devices and addresses engineering challenges associated with the material.

Quantum mechanics emerged to describe certain phenomena that could not be explained by classical physics, particularly related to light and nanoparticles. Famous experiments include the Double-slit and Stern–Gerlach experiments. What binds these results together can be described as probabilistic or a wave nature of matter. Unlike in classical physics, where a particular variable, like momentum or position, takes an a single value at a given time, a quantum variable is described by a complex probability amplitude. As quantum features are measured or interact with the larger world, the coherent state collapses into a classical singular value that we can observe. The likelihood of observing a post-collapse state is described by the wave function of the quantum variable and the Schrodinger Equation. In every day life coherent states are constantly collapsing so we experience the world classically. But by basing new technologies around maintaining and manipulating quantum features, we hope to provide not only new functionality but a deeper understanding of the physical world.

Modern nanotechnology already blurs the distinction between classical and quantum devices. While quantum mechanics explains the movement of an electron in tunnel field effect transistor or the emission from a quantum dot, these devices do not need to maintain a specific quantum state. Conversely, in a quantum device, coherent states must be maintained and manipulated [79, 40].

Several competing material bases for quantum devices exist, including trapped ions [21], superconducting circuits[49, 144, 48], photonics [128, 134], and semiconductors [9]. To maintain quantum coherence, each of these platforms depends on a different quantum property and requires different types of environmental isolation [79]. For example, trapped ions and superconducting circuits require cryogenic temperatures. Photon-based quantum systems commonly rely on either the polarization of the photons or the very presence of a photon and require shielded optical components to avoid decoherence. These additional materials complicate the fabrication and design of quantum systems. Alternatively, by resting within the bandgap of a semiconducting material, point defects are naturally shielded from decoherence without the need for additional infrastructure. These semiconductor systems often rely on electron or nuclear spin state.

Diamond is one of the most promising hosts for point defect quantum systems. Historically, natural diamonds have been coveted as prized gemstones but with the advent of synthetic methods, laboratory grown diamonds are now commercially accessible and can exhibit enhanced mechanical and electronic properties. There are two main methods for synthesizing diamond: high pressure high temperature (HPHT) and chemical vapor deposition (CVD). Bundy *et al.*, first reported the HPHT process in 1955 by replicating the conditions for natural diamond formation in a crucible [22]. Alternatively, chemical vapor deposition allows for the layer-by-layer growth of an extremely chemically pure diamond crystal; Kamo *et al.*, pioneered this technique in the 1980s [73]. In both methods, the introduction of dopants into the synthesis chamber can create defect centers, thereby modifying the physical appearance of the diamond along with their optical, mechanical, and electrical properties. Hundreds of defect centers have been discovered, documented and fabricated to date, with many more likely to come [155]. This thesis will focus on the nitrogen vacancy (NV) center in diamond, a combination of a substitutional nitrogen dopant coupled with a vacancy in the carbon lattice.

Critically for this work, the NV center can act as a room temperature, optically addressable quantum system [55, 68]. Researchers have utilized NV centers in diamond to test fundamental quantum mechanics theories [59], to build quantum memories [42] and as the basis for quantum communication networks [72]. Perhaps the most promising application of the NV center is as a quantum sensor [120]. Without the need for cryogenic temperatures or additional integrated infrastructure, the NV center functions in a variety of sensing environments. Furthermore, diamond is biocompatible and can be utilized for biological sensing in living organisms [94, 78, 153]. Quantum sensing relies on mapping the decoherence of the sensor to environmental perturbations. For example, an NV center would transition from an initialized coherent state to a mixed decoherent state more rapidly in the presence of a strong magnetic field. Surprisingly, in many instances, the distance between the NV center and the surface environment in bulk diamonds can provide too much shielding and therefore hinder sensitivity [82]. Nanodiamonds instead allow for nanoscale spacial resolutions by placing the particle directly in the sensing environment while still maintaining adequate shielding of the NV center for spin measurements. Researchers have demonstrated many examples of nanodiamond based quantum sensing in both inorganic and living environments [120, 13, 100, 75, 88, 11, 86]. Many diamond quantum sensing applications, along with the majority of work in this thesis, utilize particles with sizes ranging from 10-100 nm in diameter with approximately 1-20 NV centers per particle. Contemporary synthesis methods for these particles are described in Chapter 2 section 2.2

The major drawback of nanodiamonds is the inconsistency of the material. For example, chemically identical particles can exhibit significant optical and spin inhomogeneity due to morphological irregularity [127, 112, 137, 104]. Furthermore, surface termination irregularity impedes efforts to functionalize the nanodiamond and integrate the particles into more complex systems [101, 123, 119, 5, 150]. This inherent inhomogeneity significantly limits the potential of nanodiamonds as quantum sensors.

In this thesis, I develop methods for assembling, controlling and modifying nanodiamonds to ultimately produce improved NV center-based sensing platforms. Chapter 2 expands on the physics of the NV center and the synthesis of nanodiamonds. Chapter 2 also details additional experimental laboratory methods and techniques with an emphasis on confocal microscopy and templated self-assembly techniques. Chapter 3 discusses the self- assembly of nanodiamond arrays. These arrays enable us to study over 200 nanodiamonds and identify relationships between the optical and morphological properties of the particles. Chapter 4 introduces a novel method for modifying the surface termination of nanodiamonds by the formation of emulsions. We then establish chemical conjugation procedures for these emulsions. Chapter 5 describes in-progress and future projects for understanding the unique properties of quantum emitters and capitalizing on the techniques outlined in the previous chapters. Specifically, Chapter 5 proposes the expansion of the templated self-assembly technique to multiparticle heterogeneous assemblies, a longitudinal study of the optical properties of nanodiamonds ranging from 10-100nm and further methods and challenges in transforming nanodiamonds into nanomedical devices. Chapter 6 concludes this thesis.

#### CHAPTER 2

#### MATERIALS AND METHODS

Most materials and methods utilized throughout this thesis will be familiar to those working within the field of nanotechnology. In principle, anyone with access to nanofabrication facilities, a confocal microscope, and commercially available nanodiamonds can replicate the results reported here. In practice, though, even minor tool to tool variations will require slight modifications to the methods and procedures described below. Specific methodological details, such as commercial sources of materials, equipment names, device settings and tool recipes, are described in Chapters 3 and 4 as needed. This chapter instead provides generalized knowledge and techniques that address laboratory specific roadblocks related to studying irregular, nanoscale quantum emitters.

The chapter is organized as follows: Section 2.1 presents the electronic structure and charge properties of the NV center; Section 2.2 discusses the differences between milled and detonated nanodiamonds; Section 2.3 details the implementation of a spin lifetime measurement on a confocal microscope; Section 2.4 describes performing measurements on solutions in a confocal microscope; Section 2.5 outlines the general techniques for templated self assembly of a novel material.

### 2.1. The Nitrogen Vacancy Center



Figure 2.1: Solid lines indicate radiative transitions (with corresponding rate  $\gamma^r$ ), and dashed lines represent nonradiative intersystem crossing (ISC) transitions (with rates  $\gamma_i^{nr}$  and  $\kappa_i$  for the excited and ground-state spin projections respectively). Solid black arrows represent the zero phonon lines of the triplet and singlet manifolds. Modified with permission from Hooper *et al.*, [61].

As stated in Chapter 1, this thesis is primarily focused on the applications of NV center nanodiamonds. To put later quantum and sensing experiments into their proper context, two key features of the NV center – electronic diagram and charge properties of the NV center – are discussed in the following sections. A deeper look into the physics of the NV center can be found in Doherty*et al.*[41], and Maze *et al.* [87].

#### 2.1.1. Electron state diagram

Figure 2.1 depicts a simplified electronic diagram of the NV center. A more detailed model can be found in Hopper *et al.* [62]. Under green excitation at room temperature ( $\lambda = 532 \text{ nm}$ ), the NV center emits photons in the 550-800nm range with a peak at 700nm. What separates the NV center from most emitters is the presence of a spin dependent photoluminescence (PL). Once excited, NV center electrons have the potential to go through an intersystems crossing (ISC) to a nonradiative relaxation path. The ICS rate ( $\gamma_i^{nr}$ ) is spin dependent and occurs significantly more in the spin 1 state (10  $\gamma_0^{nr} \approx \gamma_{\pm 1}^{nr}$ ) [52]. Therefore, the  $m_s = 0$ state is significantly brighter than the  $m_s = 1$  state. This allows us to detect the spin of the NV center optically through simple photon counting. Additionally, after going through the nonradiative path, the spin state is shelved preferentially into the  $m_s = 0$  state through an initial optical pumping step. Accordingly, we have a method for optically detecting spin and a method for optically initialize spin. These two features are key to NV center quantum engineering and will be utilized both directly and indirectly throughout this thesis.

#### 2.1.2. Charge state

The NV center is a five electron system: three from the dangling bonds surrounding the vacancy and two donated from the nitrogen atom. Technically, the electronic structure described in the previous section refers to the negative  $(NV^-)$  charge state, where an additional electron is captured by the defect. While other charge states exist, the unique quantum properties described in the previous section only occur in the negative charge state. Therefore, reference to the NV center are almost always referring to the NV<sup>-</sup> state. Recent work has

highlighted the benefits of controlling the neutral [63] and positive [108] charge state, but here we will mostly be focused on the  $NV^-$  state.

#### 2.2. Milled Vs. Detonated Nanodiamonds

There are two major commercially available methods for the synthesis of nanodiamonds: detonation and milling.

Detonation nanodiamonds (DNDs) were first discovered in 1963 but forgotten until their rediscovery in 1982 [34]. The synthesis of DNDs involves controlled explosions in which rapid increases in pressure and temperature create a supersaturated carbon vapor that then condenses into nanodiamond form [94, 56]. DNDs result in uniform spherical particles with sub 10nm diameters. However, DNDs consist mainly of  $sp^2$  hybridized carbon which is highly reactive and can hinder quantum measurements[120, 94, 14]. The explosive method of synthesis also results in uncontrollable adoption of defects within the crystal lattice. These defects hinder the formation and measurement of isolated colors centers that are necessary for quantum applications [94, 120, 129, 25]. Accordingly, DNDs are currently utilized for their mechanical [67, 30, 81], and electrical [130, 157, 105] properties, and as drug delivery platforms[31, 91] but rarely for quantum sensing applications.

Alternatively, milling - the act grinding bulk diamond into nanoparticles - results in high levels of chemical purity at the cost of morphological inhomogeneity [98, 123]. After milling, centrifugation is necessary to sort the milled particles by size. Through proper centrifugation, most size distributions can be achieved, although selectivity comes at the cost of excluding material and does not account for the distribution in particle shape [97]. For these nanodiamonds, the presence of dopants or defects is determined by the purity of the bulk diamond crystal. NV centers are often generated by introducing nitrogen dopants into the growth chamber of either CVD or HPHT bulk diamond and then irradiating the diamond to generate vacancies [124, 10, 74]. The extent of irradiation determines the density of NV centers, while the extent of the milling determines the ultimate size of the particles. Eventually, new and improved methods for nanodiamond synthesis may eliminate the need to choose either size selectivity and regularity or chemical purity. For now, since we are focused on quantum applications, we exclusively utilize milled nanodiamonds.





Figure 2.2: Idealized  $T_1$  Measurement Procedure

A spin lifetime measurement tracks the transition from a pure spin state into a mixed spin state. Since the spin state of the NV center can be optically polarized and detected, this measurement is easily performed by following the procedure in Figure 2.2. An initializing laser pulse pumps the NV center into a pure ms = 0 state. Then, after a certain delay time ( $\tau$ ), a shorter laser pulse probes the NV and a detector counts the number of emitted photons. This procedure is repeated to achieve the desired resolution and is applied for each  $\tau$  that is measured. The procedure for each  $\tau$  is referred to as a line and the summation of all the lines can be referred to as a sequence and fed into an arbitrary-waveform generator (AWG) to control the laser and detectors. The total time for a measurement is equal to the length of each line multiplied by the number of repetitions and is related to the laser pulse widths and the  $\tau$  times examined.



Figure 2.3: Practical  $T_1$  Measurement Procedure

To apply this sequence to an actual confocal microscope, we must make several modifications to account for experimental limitations. We will now discuss the sequence utilized for  $T_1$ measurements in this thesis (Figure 2.3). Some of these modifications may be irrelevant to the reader's experimental setup but should provide a guide for the considerations necessary when adding this functionality to any microscope. Our first modification is the addition of an electronics delay time (ED). The ED accounts for the time the laser takes to actually turn on and off after a switch signal is sent. In our setup, a typical ED time is about 800ns which could significantly skew the measurement for nanodiamonds with a typical  $T_1$  time in the 10-100 $\mu$ s range. Next, we can account for long measurement sequences by adding two additional detector measurements per line. Detector 1 remains unchanged and measures the system after  $\tau_1$ . Detector 2 and  $\tau_2$  account for the delay between the end of one line and the start of another. For some setups, this delay may be negligible. Here, we utilized a Tetronix AWG 520 that requires each line of a sequence to have the same length. This requirement can significantly increase the total measurement time but increases the utility of  $\tau_2$  which measures a delay time of:

$$\tau_2 = \tau_{Max} - \tau_1 + linedelay. \tag{2.1}$$

By properly choosing  $\tau_{Max}$  and  $\tau_1$ , we can cut our required number of sequences in half. Detector 3 measures the brightness of the NV center in the pure ms = 0 state as a reference immediately after initialization pump step. By recording the photon count rate for ms = 0 for each line, we can normalize the measurement and account for any microscope drift or sample blinking during a measurement. We ensure that each additional detector counts photons at the same rate. Similar to the ED time, there may be some delay in the rise and fall times for each detector pulse. A discrepancy can be detected by sending a function generated pulse train with a fixed frequency in place of the PL signal from an NV. Any difference in the count rate detected by the three detectors can be accounted for by modifying the integration time of an individual detector.

Finally, we must consider the non-idealities of the laser. A non-ideal extinction ratio  $\left(\frac{Power_{Off}}{Power_{On}}\right)$  means that the NV centers are actually polarizing, albeit very slowly during each  $\tau$ . Accordingly, the extinction ratio roughly determines the maximum achievable  $\tau$ :

$$\tau_{Max} = PumpTime * \frac{Power_{On}}{Power_{Off}}$$
(2.2)

With a reasonable extinction ratio,  $\tau_{Max}$  time will likely be orders of magnitude longer than necessary.

#### 2.4. Solution Measurements in a Confocal Microscope

This section reviews the general considerations for measuring a colloidal solution in our confocal microscope. Typically, individual nanoparticles are dispersed on substrates and measured in a confocal microscope whereas colloidal solutions are transferred to a cuvette and measured in specialized tools such as a fluorometer. In some cases, however, an alternative solution measurement tool does not exist, most noticeably for the  $T_1$  measurement. Instead of designing new microscope, which would be a significant endeavor, we can measure a colloidal solution in our current confocal microscope by introducing slight modifications. For these colloidal solutions, we must consider the type of sample holder, the average number of nanoparticles being probed, the excitation efficiency and the collection efficiency.

Due to the orientation of our confocal microscope the chosen cuvette must be able to hang horizontally without spilling any of the solution. We note that when working with an aqueous solution and a suitably small cuvette, a capillary force will prevent the solution from spilling, eliminating the need for a cap. An unstable solution complicates the measurement as liquid can flow in and out of the collection volume. Unfortunately, the same capillary force makes it difficult to empty and clean the cuvettes. For these smaller volume containers, we utilized overnight soaks in 5% Hellmex solutions. With a glass cuvette, proper focus levels can then be attained by sweeping the laser focus through the first two focus points of the lower wall of the cuvette.

Next, we must consider the average number of nanoparticles within the excitation/collection path during a measurement. By first considering a Gaussian beam, we can define our excitation width (wo) using the  $\frac{1}{e^2}$  criteria as:

$$wo = \frac{.41 * \lambda}{n * NA} \tag{2.3}$$

where NA is the numerical aperture of the objective, n in the index of refraction of the solution and  $\lambda$  is the wavelength of our laser. Next, we can calculate the Rayleigh length

 $(\mathbf{z}_r)$  as:

$$z_r = \frac{2 * \pi * wo^2 * n}{\lambda} \tag{2.4}$$

Equations 2.3 and 2.4 describe a cylindrical beam with radius: wo and height:  $z_r$ . Note that we could have utilized the airy disc diameter or the full width half maximum spot size to approximate wo or directly calculate the entire volume of the Gaussian beam as it passes through the cuvette but equation 2.3 and 2.4 are sufficiently accurate for our purposes. Now we can estimate the number of nanodiamonds per millimeter (ND/mL) by assuming perfectly spherical nanoparticles with a fixed radius ( $R_ND$ ):

$$ND/mL = \frac{4}{3}\pi R_{ND}^3 * \rho_{Diamond} * \rho_{ND}$$
(2.5)

where  $\rho_{Diamond}$  is the density of diamond (3.5  $g/cm^3$ ) and  $\rho_{ND}$  is the mass concentration of the nanodiamond solution. The total number of excited nanodiamonds (NDs(#)) is given by multiplying the excitation volume and ND/mL:

$$NDs(\#) = ND/mL * \pi * wo^2 * z_r$$
 (2.6)

Table 2.1 provides an example of these calculations for various objectives assuming a water n of 1.33.

Objective	NA	wo	$\mathbf{Z}_r$	$R_{ND}$	$ ho_{ND}$	NDs(#)	MP
		nm	nm	nm	mg/ml	#	mW
5X	.15	1093	18777	30	1	178	10.4
10X	.3	547	4694	30	1	11	2.6
60X	.7	234	862	30	1	0.4	.5
100X	.9	182	522	30	1	0.1	.3

 Table 2.1: Solution Measurement Estimates

NDs(#) is highly dependent on NA of the objective  $R_{ND}$  and  $\sigma_{ND}$ . With the 5X objective

and a little control over  $\rho_{ND}$ , our setup can measure most nanodiamond sizes at NDs(#)  $\geq 100$ . We must also consider the energy that is delivered to these excited nanodiamonds. In our setup, the laser power can range from  $6\mu$ W to  $\approx 45$ mW. In Chapter 3, we will see that, on average, optical saturation requires power densities ( $OP_{Sat}$ ) of  $\approx 4 * 10^8 \frac{mW}{cm^2}$ . With this relationship in mind, we can calculate the minimum power (MP) necessary to optimally excite the nanodiamonds:

$$MP = OP_{Sat} * \pi wo^2 \tag{2.7}$$

We should be careful to remember that excitation power will not be uniform throughout the laser path. we represents the distance at which laser intensity falls to  $\frac{1}{e^2}$  of the maximum value. Therefore, exceeding the MP is recommended to maximize the number of optically saturated nanodiamonds. Emission from unsaturated NV centers will contribute to lower optical contrasts for the  $T_1$  measurement.

Improper configurations may result in insufficient power reaching the solution. With nanodiamonds, however, we observe the opposite problem whereby an excess amount of emission counts saturates our microscope's detectors. Accordingly, we add a neutral density filter (Newport) before our photon detectors.

By incorporating the aforementioned adjustments, we can utilize our confocal microscope for solution measurements and probe hundreds of colloidal particles simultaneously.

#### 2.4.1. Electro-Chemical Cell

The solution measurement also enables the use of an electrochemical cell. The specific physics of the electrochemical cell can be found in the following sources: [75, 16, 80, 159]. Here, it is enough to know that by utilizing a specialized cuvette (Pine Research Quartz), and electrochemical cell (Pine Research Honeycomb Spectroelectrochemical Cell) we can apply electric fields to nanodiamond solutions and control the NV center's charge state during a measurement. The cell itself consists of a metal honeycomb lattice that the solution fills. Unlike with the traditional cuvette, we must position the laser to have the focal point be within the honeycomb (Figure 2.4). Otherwise, background emission from the metal electrodes can overwhelm any emission from the particles. Due to this background PL the cell cannot be used to measure NV centers in the fluorimeter or UV-VIS measurement systems.



Figure 2.4: Proper Electrochemical Cell Laser Alignment

We must also add an electrolyte to the colloid solution to enable the electrochemical cell to function. At high concentrations, this electrolyte may lead to nanodiamond agglomeration. Good results were obtained in the past with a 0.1mM solutions of potassium chloride.



Figure 2.5: Difference between emission spectrum of 1 mg/ml 60nm fluorescent nanodiamond in the electrochemical cell at a -1V applied bias and a +1V applied bias.

When using the electrochemical cell, we can detect the change in the NV centers' charge state by directly measuring the emission spectra of the solution at a positive and negative applied bias. The differences between these two spectra (Figure 2.5) clearly displays the shift between NV<sup>-</sup> ( $\geq 650$ nm) to NV<sup>0</sup> ( $\leq 650$ nm) as the voltage increases. In the future, we could apply a filter at the emission wavelength from a certain charge state and track the shift in charge exclusively through a change in PL rates.

#### 2.5. Template Self-Assembly of Novel Particles

Chapter 3 describes in greater detail the template assisted self-assembly (TASA) of milled nanodiamonds. However, like many papers on the TASA method, Chapter 3 only describes the optimal assembly conditions for these nanodiamonds. Since optimal assembly conditions will vary between material, researchers can be at a loss when trying to assemble a new material. In this section, we describe essential strategies and procedures for developing assembly conditions for any material.

For a given colloidal particle, the assembly variables include hole shape, hole size, hole depth, template surface, template temperature, chamber temperature, chamber humidity and assembly speed. Some of these variables are determined by particle geometry. The holes must be large enough to fit the particles but not too large to lead to uncontrolled deposition. Typically, 1-1.5X particle diameter is optimal for hole diameter and depth. Although modifying the hole shape can alter the assembly process [54], hole design is often limited by lithography considerations. The template surface can significantly impact the assembly depending on how the colloidal solution wets the material. For polar solvents, such as aqueous colloidal solutions, light O<sub>2</sub> plasma treatments can usually increase the wetting on a PMMA template. Plasma treatments also have the added benefit of removing any remaining polymer in the template holes. However, surface interaction can usually be accounted for by varying the conditions within the assembly chamber. Temperature, humidity and assembly speed combine to govern the evaporation rate which will ultimately determine the success of the assembly. Too high of an evaporation rate will lead to uncontrolled deposition on the substrate. Too low of an evaporation rate will inhibit the capillary force and keep the particles in solution and out of the templates. Many combinations of the assembly conditions can lead to the successful assembly. High humidity, which inhibits evaporation, for example, can be counteracted by increasing chamber and template temperature or altering the assembly speed.



Figure 2.6: Templated Self Assembly Chamber. (A) Sample stage. (B) Glass slide holder.(C) Birds eye view of assembly process. (D) Vertical microscope for monitoring assembly.(E) Side view camera for monitoring assembly.

In our assembly apparatus (Figure 2.6), humidity is the easiest condition to control. Within the sealed chamber, humidity can be increased by spritzing a small atomizer or adding damp paper towels to the chamber. Alternatively, an air gas valve can be utilized to decrease humidity. Controlling humidity during an assembly can not only ensure a successful assembly but also aid in the identification of the optimal assembly conditions. If the chosen nanoparticles have a large enough scatter coefficient, a vertically oriented microscope (Figure 2.6D) can be utilized to monitor the assembly. This microscope is aligned to the back edge of the assembly, which is known as the accumulation zone. The chamber conditions can then be altered in real time to encourage proper assembly. However, for particles with lower scattering coefficients, such as nanodiamonds, the vertical microscope does not detect differences during a successful or unsuccessful assembly. In such cases, a horizontally oriented camera (Figure 2.6E) can instead observe the angle between the accumulation zone makes and the substrate. A narrow angle (Figure 2.7 A) often results in nonspecific deposition, whereas a sharper angle (Figure 2.7 B) is indicative of a successful assembly. By varying the humidity and assembly speed, a nearly vertical accumulation angle can be achieved, and a new material successfully assembled.



Figure 2.7: (A) Improper, assembly angle between the solution and the substrate (dotted line) which will likely result in nonspecific deposition of particles. (B) Proper, sharp assembly angle between the solution and the substrate (dotted line) which will likely result in a successful assembly.

#### CHAPTER 3

#### SELF ASSEMBLY AND CHARACTERIZATION OF NANODIAMONDS

With the general properties and theoretical potential of the NV center established, we now turn our attention to working with actual nanodiamonds. In this chapter, we address the core issue of nanodiamond irregularity by developing a large scale self-assembly technique. Then, we establish a statistical understanding of how crystalline inhomogeneity impacts the optical and spin properties of individual milled nanodiamonds.

This chapter, along with supplemental material, was published previously in ACS Nano[127]. Reproduced with permission from HJ Shulevitz *et al.*, "Template-Assisted Self-Assembly of Fluorescent Nanodiamonds for Scalable Quantum Technologies" ACS Nano 16, 2, 1847–1856 Copyright 2022 American Chemical Society.

#### 3.1. Introduction

The nitrogen-vacancy (NV) center in diamond functions as an optically-addressable spin qubit with room-temperature spin coherence and sensitivity to environmental perturbations such as magnetic and electric fields, strain, temperature, and pressure [41, 116, 120, 62]. Although the best spin coherence properties are achieved in bulk diamond crystals [12, 118], nanodiamond particles with diameters < 100 nm still shield the spin qubit from decoherence [137], and unlike bulk diamond, they can be dispersed in solvents to form colloids and placed within nanoscale distances of biological or inorganic structures. As an optically robust, biocompatible, quantum sensitive colloid, nanodiamonds provide a platform for sub-diffraction limited imaging, *in vivo* and *in vitro* sensing, and integration with complex heterojunction devices [136, 120, 78].

Two methods are typically used to prepare nanodiamonds: detonation synthesis and milling bulk crystals. Detonation nanodiamonds are useful as dyes and biomarkers; they offer good size uniformity, but the outer layer of sp<sup>2</sup> carbon and high background impurity levels associated with their synthesis inhibit the formation and stability of NV centers [120, 94]. On the other hand, milled nanodiamonds inherit the chemical purity of their source crystal, at the cost of uniformity [98]. Milled nanodiamonds vary widely in size, shape, surface chemistry, and number of NV centers [120]. These chemical and structural variations are reported to give rise to inhomogeneity in the nanodiamonds' optical and quantum properties [137, 104, 112]. Statistical studies of individual, isolated nanodiamonds are therefore necessary to correlate their structure and properties and ultimately to optimize nanodiamond quantum devices.

The variation in nanodiamond size typically exceeds 10-50% depending on the milling parameters [104, 123, 112]. This variation, in addition to heterogeneity in shape and surface chemistry hinders the placement or assembly of individual particles and their incorporation into devices. In past studies, researchers have used atomic force microscopes (AFMs) to serially position individual nanodiamonds [121, 125, 132, 19], or chosen to forgo ordered assemblies and search for randomly dispersed particles [20, 104, 61, 112]. Self-assembly techniques have been employed to assemble clusters of several nanodiamonds [58]. Nanodiamond placement has been achieved through electrostatic interactions between the particles and specific substrates [70]. These approaches however place limits on the type of templates that can be used and hinder large statistical studies of individual nanodiamonds.

Here, we employ capillary-driven, template-assisted, self assembly (TASA) to form millimeterscale area arrays of individual, milled nanodiamonds. TASA combines the flexibility and precision of nanolithography with the scalability of colloidal assembly. It has been used to position individual nanoparticles, as well as to direct the orientation of anisotropic particles, of various materials [53, 54, 83, 90, 47, 6, 102, 110]. By using these arrays and an automated optical measurement system, we study the statistical heterogeneity of the nanodiamonds' structural, optical, and spin properties. Our studies uncover statistical variations in nanodiamond properties correlated with characteristics of the underlying diamond material, the nanodiamond creation process, and the NV centers' local environment. This understanding will inform the realization of improved nanodiamond materials, and the versatile TASA method can be utilized in fabricating devices for quantum sensing and quantum photonics.

#### 3.2. Results and Discussion

#### 3.2.1. Assembly Process

We utilize commercially-available, low-fluorescence, milled nanodiamonds from Adámas Nanotechnologies. The nanodiamonds are specified to contain an average of 1-4 NV centers (with approximately 13% containing a single NV center), have an average size of  $\sim 40$  nm, and have surface carboxylate anions that enable their stable colloidal dispersion [123, 1].

Figure 3.1 depicts the TASA process. Electron-beam lithography is used to fabricate templates with cylindrical traps, ranging in design diameter from 32-200 nm and having heights of 62 nm, in poly-(methyl methacrylate) (PMMA) thin films deposited on 250 nm SiO<sub>2</sub> on Si substrates. Larger trap diameters (> 50 nm) result in multiple particle assembly (Figure fig: Large Template) and smaller templates (< 35 nm) show limited particle assembly. Here, we focus on 35-48 nm trap diameters with a 2.6 µm pitch to simplify single-nanodiamond spectroscopic and morphological characterization, and we fabricate  $26 \times 26$  nanodiamond arrays.



Figure 3.1: Schematic of the capillary-driven, template-assisted self assembly (TASA) of milled, fluorescent nanodiamonds into lithographically defined, PMMA templates(purple) on a Si/SiO<sub>2</sub> substrate (grey).

To assemble the nanodiamonds, we use a home-built apparatus in which an aqueous nanodiamond dispersion is deposited between a glass slide and the template surface (Figure 3.1). A motororized stage translates the nanodiamond dispersion at a velocity of 3.5 µm/s across the template surface. An accumulation of particles forms at the meniscus and capillary forces drive the nanodiamonds into the trap sites [53, 152, 90, 47, 6]. The assembly apparatus is enclosed in an environmental chamber to maintain a dew point of 8.6-9.5 °C. A recirculating chiller and heater is used to pump water through a copper block that serves as a sample holder and maintains a substrate temperature of 24-25 °C. Drier chamber conditions yield assemblies with excess nanodiamond deposition, overfilling the trap sites. A wetter chamber results in limited assembly. After assembly, the PMMA template is removed by sequential immersion of the samples in N-Methyl-2-pyrrolidone (NMP) and acetone baths followed by
an isopropanol wash.



Figure 3.2: (A) Stitched AFM images of an assembly based on a  $26 \times 26$  array of 42-nmdiameter trap sites. (B) Higher resolution AFM image of the  $5 \times 5$  subarray indicated by a white box in (A). The lateral dimensions of the individual nanodiamonds are enlarged due to convolution with the AFM probe tip. (C) Assembly yield vs. trap diameter. The box plot statistics are calculated by dividing the array into  $5 \times 5$  nanodiamond subarrays to account for regional variations. (D) Histogram of AFM-measured, assembled nanodiamond heights over all templates (258 individual nanodiamonds) and a DLS measurement (red line) of the parent nanodiamond dispersion. (E) Nanodiamond size distribution as a function of template trap diameter. Outliers (red crosses) in (C) and (E) indicate measurements whose values are >1.5 times the interquartile range from the bottom or top of the box.

We employ AFM measurements to characterize the yield of our large-area nanodiamond assemblies. The piezo-driven stage on the AFM has a maximum 40  $\mu$ m × 40  $\mu$ m scan area. We stitch together sequential scans to view the full arrays [109]. Figure 3.2A shows a representative AFM image of a TASA assembly of nanodiamonds in a  $26 \times 26$  array. Although assembly yield is high (see below), we still observe instances of multi-particle and no-particle assembly. These imperfections are expected in part due to the polydispersity in size, shape, and surface chemistry of the nanodiamonds which can both impede assembly and lead to particle agglomeration. We also hypothesize that regional variations in assembly arise from fluctuations in chamber conditions, which alter the capillary forces, and incomplete liftoff, which may fail to remove larger nanodiamond deposits. Dividing the larger array (Figure 3.2A) into subarrays, as shown in Figure 3.2B, accounts for these regional variations and allows for statistical characterization of the assembly yield.

We repeat the assembly and AFM characterization for five different template trap diameters (Figure 3.6). Collectively, we observe a median yield of individually occupied trap sites of 76%, with individual regions in most arrays reaching 100% yield (Figure 3.2B). Figure 3.2B represents an approximate bound on assembly yield, since higher resolution AFM scans occasionally reveal previously unseen nanodiamonds or the presence of multiple co-assembled particles. No significant statistical variation in yield is observed for trap diameters ranging from 38–48 nm, however we see a ~15% drop in yield for the 35 nm trap diameter template compared to those for the larger traps.

### 3.2.2. Nanodiamond Size Distribution

In addition to detecting the presence of assembled nanodiamonds for yield estimates, we utilize higher resolution AFM measurements to identify individual nanodiamonds and quantify their heights. Tip convolution prevents reliable measurement of the lateral dimensions of individual nanodiamonds and could obscure the presence of multiple nanodiamonds within a single trap site. The nanodiamonds are also irregularly shaped, which complicates the relationship between height and particle size. However, because of the fidelity between the AFM and DLS measurements, we posit that most trap sites contain only a single nanodiamond, and that high-resolution AFM scans serve as a suitable method for identifying individual nanodiamonds and measuring their size. The distribution of particle heights (histogram, Figure 3.2D), with average size and standard deviation of  $39 \pm 12 \text{ nm}$ , is consistent with dynamic light scattering (DLS) measurements (red curve, Figure 3.2D;  $36 \pm 12 \text{ nm}$ ) and with the manufacturer's specifications [123, 1]. Although the nanodiamonds are irregular in shape, we posit that the AFM height serves as a suitable measure for individual nanodiamond size.

Each template design yields arrays of nanodiamonds with a similar median size (Figure 3.2E). The smallest 35 nm template displays a tighter particle size range, while the larger templates have extended tails, especially towards larger particle sizes. The observation of particles larger in height than the trap diameter is consistent with the likelihood of irregularly-shaped nanodiamonds assembling with their long axis perpendicular to the substrate surface.

## 3.2.3. Optical Characterization

We use a custom-built, automated, confocal microscope (see Methods) to probe the optical and quantum properties of the nanodiamond arrays. We stitch together multiple photoluminescence (PL) images to map the fluorescence of nanodiamonds in large arrays (Figure 3.3A). Using an automated procedure, we characterize each fluorescent nanodiamond within a subarray (Figure 3.3B, Figure 3.7, and Figure 3.8), recording measurements of the particles' PL spectrum, photon autocorrelation function, PL saturation as a function of excitation power, and spin lifetime. Figures 3.3C-F depict the results of these measurements for four sites (I-IV) that exemplify the variation of observed optical properties. All optical measurements are performed using 532 nm excitation. The excitation power is 0.425 mW, measured before the objective lens for all measurements except for saturation where the power varies. AFM scans confirm that each of the four sites contains a single fluorescent nanodiamond (see Figure 3.2C Figure 3.7).



Figure 3.3: (A) PL scan of the nanodiamond array shown in Figure 3.2A. (B) Highermagnification PL scan of the 5×5 subarray indicated by a black box in (A). Labels I-IV indicate individual nanodiamonds featured in panels C-F. (C) PL spectra of individual nanodiamonds I-IV and their decomposition into NV<sup>-</sup>emission (red) and NV<sup>0</sup> emission (blue). The overall spectra are color coded to represent the charge ratio,  $(F_{NV^-})$ . This color code is maintained for fits in panels D-F. (D) Autocorrelation, (E) PL saturation, and (F) spin relaxation time ( $T_1$ ) measurements of nanodiamonds I-IV (black points), with corresponding fits to the models described in the text (colored curves). Error bars represent experimental uncertainties due to shot noise and slow signal variations.

	Individual Nanodiamonds				Ensemble Results	
	Ι	II	III	IV	Mean	Standard
						Deviation
Height (nm)	21.4	32.9	41.5	41.7	$38.7 \pm 0.1^{a}$	$12.4^{a}$
$N_{\rm Eff}$	$0.8 {\pm} 0.2$	$2.2{\pm}0.2$	$4.1 \pm 0.4$	$9.3{\pm}2.6$	$0.5 {\pm} 0.1^{a}$	$1.2^{a}$
					$2.1\pm 0.1$	1.4
$F_{\rm NV^-}$	0.58	0.25	0.48	0.63	$0.61\pm0.1$	0.16
$C_{\rm Sat}~({\rm kCts/s})$	$14.0 \pm 0.5$	$77.0 \pm 3.1$	$111.0 \pm 2.8$	$106.0 \pm 2.0$	$28.6 \pm 0.1$	31.0
$P_{\rm Sat} ({\rm mW})$	$0.12 \pm 0.02$	$0.25\pm0.06$	$0.26 \pm 0.04$	$0.13{\pm}~0.01$	$0.29 \pm 0.01$	0.42
$T_1$ (µs)	$1.7{\pm}1.0$	NA	$125.4 \pm 55.6$	$65.0{\pm}14.7$	$11.58 \pm 0.35$	24.5

Table 3.1: Nanodiamond Characteristics

#### <sup>a</sup>Including non-fluorescent nanodiamonds.

Figure 3.3C shows single nanodiamond PL spectra. Wavelengths below 550 nm are cut off by the long pass filter used to exclude the 532 nm excitation. The NV center's PL spectrum consists of a linear combination of spectra associated with the NV<sup>0</sup> and NV<sup>-</sup> charge states. Under optical excitation, the NV center cycles between these two charge states through processes of ionization and recombination [7], resulting in different spectral weightings as shown by the examples of nanodiamonds I-IV. We use a nonnegative matrix factorization method to decompose the spectra of individual nanodiamonds into their corresponding  $NV^0$  (blue) and  $NV^-$  (red) charge components [112]. From these decomposed spectra we calculate the charge ratio,  $F_{\rm NV^-} = S^-/(S^- + S^0)$ , where  $S^-$  ( $S^0$ ) is the integrated spectral intensity associated with the NV<sup>-</sup> (NV<sup>0</sup>) charge state. We graphically represent  $F_{\rm NV^-}$  using a blue (predominantly NV<sup>0</sup>) to red (predominantly NV<sup>-</sup>) color scale throughout Figure 3.3. Numeric results for nanodiamonds I-IV can be found in Table I. Observations of mixed charge states,  $0 < F_{\rm NV^-} < 1$ , can result from dynamic ionization and recombination of individual NV centers or from ensemble averaging over multiple NV centers within a nanodiamond.

We measure the photon autocorrelation function,  $g^{(2)}(\tau)$ , which probes the likelihood of two fluorescence photons being detected at varying temporal delays,  $\tau$  [141, 46]. Figure 3.3D shows examples of  $g^{(2)}(\tau)$  for individual nanodiamonds.  $g^{(2)}(\tau)$  is corrected for background fluorescence, detector dark counts, and detector timing jitter. The zero-delay value,  $g^{(2)}(0)$ , is used to quantify the number of NV centers in each nanodiamond. Specifically, we calculate the effective number  $(N_{\text{Eff}})$  of NV centers using the equation:

$$N_{\rm Eff} = \frac{1}{1 - g^{(2)}(0)} \tag{3.1}$$

The progressively shallower dips seen in nanodiamonds I-IV for small delays, where  $g^{(2)}(\tau) < 1$  as  $\tau \to 0$ , indicate quantum fluorescence from a progressively larger number of discrete emitters. Nanodiamond I exhibits  $g^{(2)}(0) < 0.5$  which indicates that the emission is dominated by a single NV center.

This relationship assumes identical PL brightness from each emitter, which is not strictly accurate for nanodiamonds given their morphological inhomogeneities, varying crystal orientations, and different charge state populations. Furthermore, measurements of  $g^{(2)}(0)$  can be biased by systematic errors such as incomplete background correction, particularly for dim emitters (typical of small  $N_{\text{Eff}}$ , e.g., for nanodiamond I) or when  $g^{(2)}(0) \rightarrow 1$  (as  $N_{\text{Eff}} \rightarrow \infty$ , e.g., for nanodiamond IV). As described further in the Statistical Characterization Section, we find that  $N_{\text{Eff}}$  is linearly related to the saturation PL brightness of the nanodiamonds (Figure 3.3E), especially for the range  $1 < N_{\text{Eff}} < 10$  and we therefore interpret  $N_{\text{Eff}}$  as a reasonable quantitative estimate for the number of NV centers at each location.

While each nanodiamond varies in brightness (Figure 3.3B), by varying the illumination power, P, and recording the PL intensity,  $I_{\rm PL}$ , we find that the nanodiamond  $I_{\rm PL}$  displays a similar characteristic power dependence (Figure 3.3E). As expected, the observed brightness increases with  $g^{(2)}(0)$  and the number of emitters,  $N_{\rm Eff}$ . We perform this PL saturation measurement multiple times to check for stability, subtract a background measurement from a location on the sample nearby each nanodiamond, and fit the resulting data to the empirical model,

$$I_{\rm PL}(P) = \frac{C_{\rm Sat}}{1 + \frac{P_{\rm Sat}}{P}},\tag{3.2}$$

from which we quantify the nanodiamond's saturation PL brightness  $(C_{\text{Sat}})$  and saturation power  $(P_{\text{Sat}})$ .

Finally, to probe the quantum features of the nanodiamonds, we measure the electron spin lifetime,  $T_1$  (Figure 3.3F), using a time-resolved fluorescence technique. We initialize the NV centers into their  $m_s = 0$  spin-triplet sublevel using a 1.6 µs, 532 nm laser excitation pulse and then count PL photons during a 300 ns readout laser pulse at delay times ranging from 50 ns to 900 µs. Due to the NV center's spin-dependent optical dynamics, the PL amplitude represents the probability that the NV centers remain in the  $m_s = 0$  spin sublevel of the  $NV^-$  ground state at the time of the readout pulse. Longer  $T_1$  lifetimes indicate higher levels of quantum isolation and enable improved quantum sensitivity [137, 120]. Variations at short ( $\leq 200$  ns) times can result from relaxation of the metastable spin singlet, whereas longer-time variations can reflect charge or spin relaxation [137, 120, 61]. We fit the data using a set of mathematical functions that account empirically for the rates of these disparate processes as well as for the potential presence of multiple NV centers within a nanodiamond (see Methods). In some cases, such as nanodiamond II, the analysis is inconclusive; in these cases we do not report a  $T_1$  lifetime. Nanodiamond II's small PL contrast is consistent with its low  $F_{\rm NV^-}$ , since spin relaxation is only observed for NV centers in the negative charge state.

## 3.2.4. Statistical Characterization

Ordered arrays of nanodiamonds significantly simplify experiments, allowing us to study the statistical properties of large numbers of nanodiamonds, correlating optical and structural measurement modalities. We collect AFM scans and the suite of optical measurements, as described for the example nanodiamonds in Figure 3.4, but now for 219 nanodiamonds assembled in multiple arrays, to build statistical data sets of nanodiamond size and emitter number, brightness, charge state, and spin lifetime (Figure 3.4). Table 1 summarizes the ensemble properties of the nanodiamonds. In this study, we report the optical properties of individual nanodiamonds and exclude array spots that contain multiple nanodiamonds based on high-resolution AFM scans. Section 3.5 also includes an analysis of correlations between these variables (Figures 3.9–3.13).



Figure 3.4: Throughout the figure, color-coded histograms indicate nanodiamonds with  $N_{\rm Eff} = 0$  (purple),  $0 < N_{\rm Eff} < 1.5$  (yellow-green),  $1.5 < N_{\rm Eff} < 2.5$  (green), and  $N_{\rm Eff} > 2.5$  (blue). (A) Distribution of  $N_{\rm Eff}$ , calculated from autocorrelation measurements (colored bars) or estimated from  $C_{\rm Sat}$  (grey bars). The red curve is the result of a fit to the model described in the text. (Inset) AFM height distribution for the non-fluorescent nanodiamonds only. Panels (B), (C), and (D) respectively show the measured distributions of  $C_{\rm Sat}$ ,  $F_{\rm NV^-}$ , and  $T_1$ , respectively, for the ensemble of fluorescent nanodiamonds. The inset to panel (B) shows  $C_{\rm Sat}$  as a function of  $N_{\rm Eff}$  (data points), along with a linear fit (red line).

### Emitter Number Distribution

We study the number of NV centers hosted within each nanodiamod (Figure 3.4A). A significant fraction (68 out of 219, or 31%) of nanodiamonds are observed in AFM measurements but invisible in optical measurements. We set  $N_{\rm Eff} = 0$  for these non-fluorescent nanodiamonds, as indicated by the purple bar in Figure 3.4A. The non-fluorescent nanodiamonds are excluded from the other datasets in Figure 3.4A. For the fluorescent nanodiamonds, we calculate  $N_{\rm Eff}$  from autocorrelation measurements of  $g^{(2)}(0)$ , as in Figure 3.4D, using eq. (3.1). Throughout Figure 3.4, we indicate the nanodiamonds likely to contain one  $(0 < N_{\rm Eff} < 1.5)$  or two  $(1.5 < N_{\rm Eff} < 2.5)$  NV centers using separately colored bars. Of the 151 fluorescent nanodiamonds, four are too dim and sixteen are too bright to reliably determine  $N_{\rm Eff}$  from  $g^{(2)}(0)$ ; we estimate  $N_{\rm Eff}$  for these nanodiamonds using the observed linear relationship between  $N_{\rm Eff}$  and  $C_{\rm Sat}$ , as described below (Figure 3.4B, inset) and add the results to Figure 3.4A as grey bars.

The observed fractions of non-fluorescent nanodiamonds (31%) and single emitters (12%) in this sample are in close agreement with the manufacturer's product specifications (25% and 13%, respectively)[1]. This agreement provides further evidence that we are successfully identifying individual nanodiamonds. However, we show below that the observed  $N_{\text{Eff}}$  distribution in Figure 3.4A is not consistent with a simple stochastic model assuming an isotropic distribution of NV centers within the nanodiamonds. We develop a quantitative model for the observed distribution, motivated by the mechanisms of diamond growth, irradiation, and milling.

A model for the  $N_{\text{Eff}}$  distribution must account for the variance in particle size and the stochastic incorporation of NV centers within each particle. First, we consider a model in which the probability of observing N emitters in a spherical nanodiamond of diameter d obeys a Poissonian distribution,  $P(N|d) = \text{Poiss}(N; \langle N \rangle)$ , where the mean,  $\langle N \rangle = \rho V$ , is determined by the NV density,  $\rho$ , and the particle volume,  $V = \pi d^3/6$ . The probability distribution for N is subsequently given by  $\sum_i P(N|d_i)\phi(d_i)$ , where  $\phi(d)$  is the probability of finding a particle with diameter d, as characterized by AFM measurements (Figure 3.2D). This model fails to match the observed distribution, with a marked discrepancy especially at low  $N_{\text{Eff.}}$  (The best fit is rejected on statistical grounds with reduced chi-squared  $(\chi_r^2) =$  $3.05, p < 10^{-10}$ , as shown in Figure 3.14.) However, when we exclude the non-fluorescent nanodiamonds, this model can explain the distribution of remaining fluorescent particles  $(\chi_r^2 = 1.2; p = 0.29)$ . We conclude that the relatively large fraction of non-fluorescent particles in our data cannot be explained using this simple model.

To reconcile this discrepancy, we hypothesize that the nanodiamonds can be divided into two groups: one where the NV centers are distributed isotropically with non-negligible density, resulting in the observed  $N_{\rm Eff}$  distribution of fluorescent nanodiamonds, and another group where the density is so low that there is a negligible probability of finding an NV center. Nitrogen is incorporated inhomogeneously during high-pressure, high-temperature growth of bulk diamond crystals [124, 10, 74]. When the crystals are milled, the resulting nanodiamonds have an inhomogeneous nitrogen concentration. Subsequent electron irradiation creates a homogeneous distribution of carbon vacancies, which become mobile upon annealing to form NV centers. In regions with high nitrogen content, the NV-center formation will be vacancy limited and hence the NV-center density is proportional to the radiation dose. In nitrogen-depleted regions, the NV-center formation will be nitrogen limited, and the density will be lower. This hypothesis is supported by the observation that Adámas Nanotechnology produces "highly-fluorescent" nanodiamonds with the same size distribution, using an identical synthesis and milling procedure but significantly higher irradiation levels. These samples also contain a 30% fraction of non-fluorescent particles. Furthermore, the height distribution of non-fluorescent particles (Figure 3.4A, inset) is similar to the full distribution (Figure 3.2D), whereas an exclusively isotropic NV-center creation model would predict a narrowed distribution of primarily non-fluorescent smaller particles. The invariance of the non-fluorescent fraction with respect to milling and irradiation conditions supports the conclusion that the heterogeneity in NV-center creation derives from a nonuniform nitrogen distribution.

To account for this effect in our model, we adopt a bimodal distribution of NV-center density, where  $\rho = \rho_1$  with probability  $\alpha$ , and  $\rho = \rho_2$  with probability  $1 - \alpha$ ; see Methods, Equation (3.11). A fit to the data using this model yields an acceptable result as shown by the curve in Figure 3.4A ( $\chi_r^2 = 1.3$ , p = 0.17), where  $\rho_1 = 14 \pm 1.4 \times 10^{16}$  cm<sup>-3</sup>,  $\rho_2 = 0.001 \pm 0.45 \times 10^{16}$ cm<sup>-3</sup>, and  $\alpha = 0.77 \pm 0.05$ . The implication of this analysis is that the observed 31% of dark nanodiamonds arise from a combination of the underlying material inhomogeneity (accounting for 23% of dark nanodiamonds in the sample) and the stochastic creation of NV centers (accounting for the remaining 8% of dark nanodiamonds). Accounting for the expected ranges of NV-center creation efficiency, these fits place lower (upper) limits on the nitrogen content in the nitrogen-rich (nitrogen-poor) particles of 7.1 ppm (2.5 ppm). See section 3.5.3 for more details on the nitrogen content and NV-center creation estimates.

### Nanodiamond Brightness

The empirical saturation brightness distribution is shown in Figure 3.4B. We observe a clear linear relationship between  $C_{\text{Sat}}$  and  $N_{\text{Eff}}$  (Figure 3.4B inset), with a slope of 22.8  $\pm$  2.2 kCts/s per NV center. The linear relationship implies that NV centers in these nanodiamonds have nearly identical apparent brightness, despite wide variations in charge stability and spin stability, as discussed in the following sections. The strong linear relationship between  $C_{\text{Sat}}$ and  $N_{\text{Eff}}$  implies that more time-efficient measurements of  $C_{\text{Sat}}$  in comparison to  $g^{(2)}(0)$  can serve as a robust indicator for  $N_{\text{Eff}}$  without the need for a time-correlated single-photon counting module. We use the relationship to estimate  $N_{\text{Eff}}$  values for the 20 nanodiamonds with an ambiguous  $g^{(2)}(0)$  in our dataset (Figure 3.4, grey bars and Figures 3.15–3.16). The distribution for  $P_{\text{Sat}}$  can be found in Figure 3.17.

## **NV-Center Charge-State Distribution**

Figure 3.4C shows the  $F_{\rm NV^-}$  distribution for fluorescent nanodiamonds in our assemblies. The mean value ( $\langle F_{\rm NV^-} \rangle = 0.6 \pm 0.1$ ; see Table 3.1) agrees with previous measurements of randomly dispersed, ~150-nm-diameter, milled nanodiamonds [112]. However, it is significantly lower than the measurement of  $F_{\rm NV^-} = 0.75$  for the parent nanodiamond dispersion used in assembly (Figure 3.18). The dispersion and individual nanoparticle measurements are performed at similar optical excitation power densities, however we note that dispersed particles can exchange freely in and out of the excitation path during the measurement, whereas assembled, individual particles are continuously probed. Although ionization and recombination rates depend on excitation power density, the ratio  $F_{\rm NV^-}$  generally does not vary strongly with power under 532 nm excitation [7, 57, 146]. We did not observe a power dependence in the qualitative shape of the PL spectra of individual nanodiamonds for excitation powers ranging from 0.1 to 1.6 mW before the objective.

Accordingly, the nanoparticles' environment may play the dominant role in determining  $F_{\rm NV^-}$ . A bias towards the negative charge state is often explained by a combination of core nitrogen impurities, which are reported to donate electrons to NV centers [32, 146], together with the presence of carboxylate anions on the nanodiamond surface [115, 112, 107]. Once assembled, we hypothesize that silanol groups or water of hydration on the SiO<sub>2</sub> surface, known to act as electron traps [3, 114, 122], can neutralize the negative surface charge of the nanodiamonds and thereby decrease  $F_{\rm NV^-}$ .

A closer examination of the  $F_{\rm NV^-}$  distribution of individual nanodiamonds containing small

numbers of NV centers reveals a bimodal shape (Figure 3.4C). Nanodiamonds with one or two NV centers exhibit  $F_{\rm NV^-}$  values clustered either around 0.4 or around 0.9. A few particles exhibit  $F_{\rm NV^-} > 0.95$ , essentially a pure negative charge-state configuration. The bimodal distribution for nanodiamonds with  $N_{\rm Eff} \sim 1-2$  suggests the NV centers fall into two distinct categories based on  $F_{\rm NV^-}$ . The more mixed charge states ( $F_{\rm NV^-} \sim 0.6$ ) seen in particles with larger  $N_{\rm Eff}$  therefore arise from averaging the signals from multiple NV centers. The bimodal distribution could reflect distinct local environments, for example, distinguishing NV centers near the surface from those within the nanodiamond core. We checked for a correlation between  $F_{\rm NV^-}$  and particle size, which would support this interpretation, but any such correlation is not significant in our data (see Figures 3.9–3.11. However, if environmental effects are limited to NV centers within a few nanometers from a particle's surface, we would not expect to observe a strong correlation for this range of particles sizes. To quantify this relationship, we compared the fraction of nanodiamonds with  $F_{\rm NV^-} < 0.5$  with the predicted shell-volume fraction as a function of particle size (see Figure 3.19). We find that our data are consistent with this interpretation, where NV centers located within 2-3 nm of the surface have reduced  $F_{\rm NV^-}$ , however even larger sample sizes would be required to establish a direct correlation between  $F_{\rm NV^-}$  and particle size.

The observation of a bimodal charge-state distribution has important practical implications. The pure negative charge state is typically desired for quantum spin sensing applications, whereas mixed charge states may be more sensitive to variations in electrochemical potential [75]. Hence, deeper understanding of this phenomena that leads to manufacturers' ability to promote one category over the other, or to efficiently sort the particles based on  $F_{\rm NV^-}$ , would improve the material's performance in these key applications.

## Spin Lifetimes

Spin relaxation measurements are performed as described in the Optical Characterization section, and those nanodiamonds whose measurements are adequately fit by our models are included in Figure 3.4D; we are unable to resolve  $T_1$  values for 48 nanodiamonds. For the remaining nanodiamonds, we observe a wide distribution of  $T_1$  values, ranging from  $1.0 \pm 0.5 \ \mu s$  to  $608.0 \pm 211.5 \ \mu s$ ; see Figure 3.4D. We also observe a positive correlation between  $T_1$  and  $F_{NV^-}$  but no correlation between  $T_1$  and height (see Figures 3.9, 3.13 and 3.12). These correlations are consistent with the interpretation proposed in the previous section that NV centers close to the nanodiamond's surface display higher environmental sensitivity, as seen by lower  $F_{\rm NV^-}$  and  $T_1$ , according to a core-shell relationship. Other studies have observed that a correlation between  $T_1$  and particle size becomes apparent for nanodiamonds with diameters less than 20 nm [137, 104]. These works stress the importance of NV center placement rather than particle size for these nanodiamonds. However, other interpretations are possible. For example, proximal fluctuating charges that lower  $F_{\rm NV^-}$ for an NV center in the nanodiamond core could also limit  $T_1$ . Charge fluctuations have been observed for NV centers in nanodiamonds in the dark following a laser pulse [61]; such fluctuations could systematically bias the  $T_1$  measurements.

As for the case of charge-state stability, understanding and controlling the spin lifetime has important implications for applications. Particularly for spin relaxometry sensing and imaging, an increased  $T_1$  translates directly to improved sensitivity [116, 120, 137, 61]. We note that several of the longest  $T_1$  values observed in our sample occurred in nanodimaonds with only one or two NV centers, boding well for the prospects of single-spin applications.

## 3.3. Conclusion

We describe the use of the TASA method to assemble large arrays of single nanodiamonds with high yield. We exploit these arrays to perform automated measurements of the morphological, optical, and quantum properties of nanodiamonds from 219 isolated particles. We observe a wide distribution of emitters in the particles, ranging from zero to >20 NV centers per particle, with 31% dark nanodiamonds and 12% single NV centers. We attribute the distribution to spatial heterogeneity in the nitrogen incorporation during growth of the parent, bulk crystal as well as stochastic creation of NV centers within nanodiamonds. Greater control over the nitrogen incorporation rates during crystal growth, vacancy generation during electron irradiation, and the particle size uniformity could provide tighter control over the NV center distribution. The fluorescent nanodiamonds show a wide distribution in their charge states and spin lifetimes. We find that  $F_{\rm NV^-}$  and  $T_1$  are not correlated with nanodiamond size in this sample, but instead indicate a heterogeneity in the NV-center environment, which could arise from variations in the internal crystal structure and composition or the NV center distance from the nanodiamond surface. Controlling the surface conditions should therefore enable more uniform charge states and longer spin lifetimes. Here we have only considered one type of nanodiamond. Replicating this study with a nanodiamonds of different sizes, fabricated under different conditions, and with a variety of surface terminations could expand our understanding of the assembly process and the sources of inhomogeneity amongst these quantum emitters.

Building on the TASA method and leveraging our understating of the NV center's properties, we see a path for integrating nanodiamonds and other nanoscale quantum materials into photonic and electronic quantum devices. We envision extending the TASA method for the placement of individual nanodiamonds to that of other irregularly shaped nanoparticles. Templates can also be fabricated on functional substrates to better optimize the nanodiamonds' surface environment. We can adapt the TASA method to create multiparticle heterostructure assemblies [53, 20, 19], where plasmonic, magnetic, or dielectric nanoparticles could act as optical or magnetic antennas and enhance the NV-center's optical signal and quantum sensitivity.

## 3.4. Methods

## Template Fabrication and Nanodiamond Assembly

The starting material for these studies is an aqueous suspension of 40 nm, milled, fluorescent nanodiamonds (Adámas Nanotechnology, NDNV40nmLw10ml).

We fabricate templates on single-side-polished Si wafers with a 250 nm SiO<sub>2</sub> thermal oxide layer (Virginia Semiconductor). Poly(methyl methacrylate) (PMMA) positive resist, 950 K molecular weight PMMA in anisole (MicroChem), is spun onto the substrates at 3000 rpm for 60 s followed by a soft bake at 180 °C for 90 s. Electron-beam lithography (Elionix ELS-7500EX) is used to pattern the template at a 50 kV accelerating voltage and a 50 pA beam current. Circular traps are designed with diameters ranging from 35–200 nm. The PMMA is developed in a 1:3 water:isopropanol solution for 90 s. Prior to assembly we perform a 6 s, 75 W, 20 sccm, O<sub>2</sub> plasma (Gatan Solarus) descum to remove any remaining polymer within the trap sites. This treatment results in an approximately 62-nm-thick PMMA assembly template.

The assembly is performed using a custom-built capillary assembly apparatus [53]. A 35  $\mu$ L volume of a 0.001 mg/mL nanodiamond dispersion in a 1% Sodium dodecyl sulfate (Sigma-Aldrich) aqueous dispersion is dropped between the template and a glass slide. The substrate is translated by a motorized linear stage (New Focus Picomotor) at a rate of 3.5  $\mu$ m/s. The assembly apparatus is enclosed in a home-built, humidity-controlled environmental chamber, and the substrate temperature is regulated by a water-cooled stage and monitored by a

thermocouple. During assembly, the substrate temperature is 24 °C, and the ambient dew point is maintained between 8.5 and 9.5 °C. These temperature and humidity conditions maintain a contact angle >24° throughout the assembly. The PMMA template is then lifted off by soaking the assembled samples in N-Methyl-2-pyrrolidone (NMP) for 1 min, followed by 5 min acetone and IPA washes.

## **AFM** Measurements

Atomic force microscopy (AFM) measurements are taken using a MFP-3D-BIO AFM from Asylum Research. All measurements are collected using a standard Si probe (AC240TS-R3, Olympus). For quantifying yields, large-area scans ( $40 \mu m \times 40 \mu m$ ) are taken at a scan rate of 0.3 Hz. Mid-area scans ( $20 \mu m \times 20 \mu m$ ) for mapping subarrays are taken at a scan rate of 0.27 Hz and individual nanodiamond measurements are collected using a 1.5  $\mu m \times 1.5 \mu m$ scan area at a scan rate of 0.3 Hz. Igor Pro version 6.38B01 is used to analyze AFM data. Composite images are formed from the AFM scans using ImageJ and the program described by Preibisch et al. [109] Total yield counts and are analyzed by hand from the resulting images.

## **Optical Measurements**

Optical measurements are taken using two custom-built confocal microscopes. In both microscopes a fast steering mirror (FSM, Optics in motion, OIM101) projects a beam onto to the back of a 100x objective (0.9 NA 100x objective Olympus, MPI Plan Fluor/OL, or Nikon, Plan Flour x100/0.5-1.3), allowing us to raster a 532 nm (green) continuous-wave laser (Coherent, Compass 315M-150) over the sample and collect PL emission on single-photon avalanche diode detectors. We employ two microscopes to allow multiple measurements to run in parallel and to extend the spectral bandwidth. The first microscope, described by Exarhos *et al.*,[44] is used to collect saturation and autocorrelation measurements. The second microscope, described by Huang *et al.*,[64] is modified with a 532 nm low pass dichroic

mirror (Semrock), to extend the spectral bandwidth and collect PL maps, spectra, and  $T_1$  measurements. Throughout each measurement, a periodic tracking PL scan is used to maintain alignment for optimal collection from individual nanodiamonds. During tracking, PL line scans in the x and y directions (controlled by the FSM) are fit using a Gaussian peak plus a constant background. The FSM is then adjusted to align the microscope to the Gaussian's mean position. A similar tracking scan and Gaussian fit in the z (focus) direction is also performed utilizing a piezoelectric stage (Thorlabs MZS500-E) connected to either the sample mount or the objective, depending on the microscope, to optimize focus.

Spectral measurements are taken on a Princeton Instruments IsoPlane-160 spectrometer with a 700-nm blaze, 300 G/mm grating and a thermoelectrically cooled charge-coupled device (CCD) camera (Princeton Instruments PIXIS 100BX) yielding a spectral resolution of 0.7 nm. Measurements consist of one 90 s background measurement combined with multiple 90 s signal measurements. We then perform cosmic ray rejection and background subtraction on each scan before combining the signals and correcting for wavelength-dependent photon detection efficiencies.

The power-dependent PL measurements are achieved by placing a variable neutral density filter (Thorlabs NDC-50C-2-A) in the excitation path, with pre-objective powers calibrated with a Thorlabs PM100D power meter.

 $T_1$  lifetime measurements are implemented by programming the required pulse sequences (described in the Optical Characterization subsection) onto an arbitrary-waveform generator (AWG; AWG520 Tektronix). The AWG control signals are passed to the microscope's acousto-optic modulator (AOM) for generating optical pulses, and to three high isolation switches (ZASWA-250DR Mini-Circuits) for time-gating photon detection events recorded by counters in a data acquisition card (National Instruments PCIe-6323). DLS measurements are conducted on a Malvern Instruments Zetasizer Nano-s and analyzed using Malvern's software.

## Autocorrelation analysis

Autocorrelation data is collected using a Hanbury Brown-Twiss setup with a time correlated single-photon counting module (PicoQuant PicoHarp 300) in time-tagged, time-resolved collection mode. Background correction is performed by determining  $\rho = S/(S+B)$  from the signal (S) and background (B) levels for each spot (both determined from Gaussian fits during tracking scans) and then calculating the corrected autocorrelation function:

$$g_{\rm corr}^{(2)}(\tau) = \frac{g_{\rm meas}^{(2)}(\tau) - (1 - \rho^2)}{\rho^2}$$
(3.3)

The background corrected autocorrelation data are then fit using the empirical model:

$$g_{\rm corr}^{(2)}(\tau) = 1 + A - De^{-|\tau - t_0|/\tau_1}$$
(3.4)

Here, A and D represent the bunching and antibunching amplitudes, respectively,  $t_0$  accounts for signal delays, and  $\tau_1$  represents the antibunching timescale [46].

The signal-to-noise ratio of autocorrelation measurements depends on S,  $\rho$ , and on the acquisition time. Therefore, we dynamically adjust the acquisition time for each nanodiamond in order to achieve a desired uncertainty in  $g^{(2)}(\tau)$  ( $\Delta g$ ), given a particular time resolution ( $\Delta \tau$ ):

Measurement Time = 
$$4 \frac{(S+B)^2}{S^4 (\Delta g)^2 \Delta \tau}$$
 (3.5)

For this study, we use  $\Delta \tau = 2$  ns, and we set  $\Delta g = 0.025$  in order to achieve sufficiently high resolution, however we limit the total measurement time for individual spots to a maximum of 1 h. For most instances, this results in a resolvable  $g^{(2)}(0)$  value. For some exceptionally bright or dim nanodiamonds, the antibunching dip is smaller than  $\Delta g$ , and we cannot reliably determine  $N_{\text{Eff}}$  values from  $g^{(2)}(\tau)$ .

## Fitting Spin Relaxation Data

Recorded spin lifetime measurements for single NV centers are typically fit to a four-level rate model [137]:

$$I(\tau) = I(\infty)[1 - C_m e^{\frac{-\tau}{T_m}} + C_1 e^{\frac{-\tau}{T_1}}]$$
(3.6)

where  $T_m$  accounts for the relaxation of the metastable singlet spin state. To account for the presence of multiple NV centers, we expand our empirical fitting model to account for a second spin relaxation rate:

$$I(\tau) = I(\infty) \left[1 - C_m e^{\frac{-\tau}{T_m}} + C_1 e^{\frac{-\tau}{T_1}} + C_2 e^{\frac{-\tau}{T_2}}\right]$$
(3.7)

Due to signal-to-noise constraints and the potential overlap with  $T_1$ , we may not always observe the  $T_m$  term. Accordingly, we add two more potential models to describe the spin relaxation dynamics of the nanodiamonds.

$$I(\tau) = I(\infty)[1 + C_1 e^{\frac{-\tau}{T_1}}]$$
(3.8)

$$I(\tau) = I(\infty) \left[ 1 + C_1 e^{\frac{-\tau}{T_1}} + C_2 e^{\frac{-\tau}{T_2}} \right]$$
(3.9)

We fit the data for each nanodiamond using all four models. We exclude fits where uncertainties for the  $T_1$  parameter exceed the  $T_1$  result, where parameters are inconsistent with known physical properties ( $T_m > 10 \ \mu s$  or  $C_1 < 0$ ), or where  $\chi_r^2$  is greater than  $1 + 2\sqrt{\frac{2}{DoF}}$ . Here DoF is the number of degrees of freedom in the model. If no valid fits remain, we exclude that data point from later statistical analysis. In the event of multiple acceptable fits,  $\chi_r^2$  is within  $1 \pm \sqrt{\frac{2}{\text{DoF}}}$ , we choose the model with the smallest number of parameters.

#### Modeling the Emitter Number Distribution

We fit the  $N_{\text{Eff}}$  data by modeling the total probability of observing N emitters given a spherical nanodiamond of diameter d weighted by the likelihood of observing each d value:

$$P(N) = \sum_{i} P(N|d_i)\phi(d_i)$$
(3.10)

Here, the conditional probability of N given d is modeled as a Poissonian distribution:  $P(N|d) = \text{Poiss}(N; \langle N \rangle)$ , where the mean,  $\langle N \rangle = \rho V$ , is determined by the NV density,  $\rho$ , and the particle volume,  $V = \pi d^3/6$ . The unconditional probability of finding a particle with diameter d,  $\phi(d)$ , is determined using AFM measurements as shown in Figure 3.2D. To account for a variation in nitrogen content, we assume two different  $\rho$  values with their own probabilities  $\alpha$  and  $1 - \alpha$ . The model of P(N) then becomes:

$$P(N) = \sum_{i} \left[ P(N|d_i, \rho_1)\phi(d_i)\alpha + P(N|d_i, \rho_2)\phi(d_i)(1-\alpha) \right]$$
(3.11)

The results of these models can be found in Figure 3.14.

# 3.5. Supplementary Information



3.5.1. Additional SEM, AFM, and PL Images

Figure 3.5: Scanning electron microscope images of a 250 nm diameter template with assembles of nanodiamond clusters.



Figure 3.6: AFM images mapping  $26 \times 26$  nanodiamond arrays. (A) 48 nm diameter template, (B) 45 nm diameter template, (C) 42 nm diameter template, (D) 38 nm diameter template, (E) 35 nm diameter template.



Figure 3.7: AFM images of the subarrays probed in automated measurements.



Figure 3.8: PL images of the subarrays probed in automated measurements.

	Height	$N_{\rm Eff}$	C <sub>sat</sub>	F <sub>NV-</sub>	P <sub>sat</sub>	$T_1$
Height	1.00	0.25	0.25	0.07	0.08	-0.08
	0.25	1.00	0.99	0.08	-0.05	-0.07
C <sub>sat</sub>	0.25	0.99	1.00	0.05	-0.02	-0.07
FNV	0.07	0.08	0.05	1.00	-0.02	0.25
P <sub>sat</sub>	0.08	-0.05	-0.02	-0.02	1.00	-0.19
	-0.08	-0.07	-0.07	0.25	-0.19	1.00

3.5.2. Results of Statistical Analysis

Figure 3.9: Pearson correlation coefficients for different optical and morphological properties of the nanodiamonds. A magnitude  $\geq 0.2$  indicates a potentially significant correlation.

	Height	N <sub>Fff</sub>	C <sub>sat</sub>	F <sub>NV-</sub>	P <sub>sat</sub>	<i>T</i> <sub>1</sub>
Height	1.00	0.00	0.00	0.38	0.34	0.44
N <sub>Eff</sub>	0.00	1.00	0.00	0.32	0.57	0.52
C <sub>sat</sub>	0.00	0.00	1.00	0.55	0.78	0.49
F <sub>NV</sub>	0.38	0.32	0.55	1.00	0.80	0.02
P <sub>sat</sub>	0.34	0.57	0.78	0.80	1.00	0.08
$T_1$	0.44	0.52	0.49	0.02	0.08	1.00

Figure 3.10: Pearson correlation coefficients p-Values for different optical and morphological properties of the nanodiamonds. A p-Value  $\leq 0.05$  indicates a potentially significant correlation.



Figure 3.11: Height vs  $F_{\rm NV^-}$ .



Figure 3.12: Height  $vs T_1$ 



Figure 3.13:  $T_1 vs F_{NV^-}$ 



Figure 3.14:  $N_{\rm Eff}$  histogram with three fits based on different models. The red curve represents a model with a constant NV density,  $\rho$ , and fits  $N_{\rm Eff}$  data for all nanodiamonds. The magenta curve is a fit using the same model but only fitting data for fluorescent nanodiamonds. The green curve assumes a bimodal distribution of  $\rho$  and utilizes all  $N_{\rm Eff}$  data.

We estimate a rough NV center density in the final nanodiamonds using the specifications provided by Adamas Nanotechnology. The nanodiamonds were irradiated with 2-3 MeV electrons to a dose of  $10^{18}$  cm<sup>-2</sup> [123]. For this energy and dose, we assume a vacancy creation efficiency of order 1 cm<sup>-1</sup>[23, 65]. While NV center creation efficiency depends on annealing temperature, nitrogen content, and diamond size, recent studies employing similar synthesis conditions demonstrate conversions efficiencies on the order of 1-10%[93, 27]. Based on these assumptions, we estimate a final NV-center density of  $\sim 10^{17}$  cm<sup>-3</sup>, which is consistent with the value found in the vacancy limited region (Figure 3.14).

From the best fit values of  $\rho_1$  and  $\rho_2$  we can estimate the nitrogen distribution within the premilled diamond. In the vacancy limited region, the lower bound of  $\rho_1$  divided by the NV-center creation efficiency of 10% (1%) provides a lower limit on nitrogen density of 7.1 (71) ppm. In the nitrogen limited region, the upper bound of  $\rho_1$  multiplied by the NV-center creation efficiency provides an upper bound for nitrogen density of 0.25 (2.5) ppm. The bounds for both regions are consistent with the reported distributions of accessible nitrogen within bulk high temperature high pressure synthetic diamonds[124, 10, 74].



3.5.4. Relationship between Brightness and Emitter Number

Figure 3.15: Plot of  $C_{\text{Sat}}$  vs  $N_{\text{Eff}}$  for 131 nanodiamonds with reliable  $N_{\text{Eff}}$  values, with linear fit (red).



Figure 3.16: Plot of  $C_{\text{Sat}}$  vs  $N_{\text{Eff}}$  for the 20 nanodiamonds with unreliable  $N_{Eff}$  values. Uncorrected data (blue), corrected data (red),  $C_{\text{Sat}}$  vs  $N_{\text{Eff}}$  curve (magenta).



Figure 3.17: Distributions of  $P_{\text{Sat}}$ 

By utilizing exclusively the 131 nanodiamonds where the  $N_{\text{Eff}}$  was deemed reliable, we fit our data using the linear function:  $C_{\text{Sat}} = AN_{\text{Eff}} + B$  (Figure 3.15). From this fit we find that individual NV centers ( $N_{\text{Eff}} = 1$ ) have  $C_{\text{Sat}} = A + B = 22.3 \pm 8.35$  kCts/s. Each additional emitter adds  $A = 20.72 \pm 1.13$  kCts/s.



Figure 3.18: Spectral measurement (black) and nonnegative matrix factorization (red  $NV^-$ , blue  $NV^0$ ) decomposition for a 1 mg/mL dispersion of nanodiamonds.  $F_{NV^-} = .75$ .

The dispersion spectra are acquired by placing 300 µL of 1 mg/ml nanodiamond disperion loaded in a glass cuvette (SpectroCell R-403) in the confocal excitation path and illuminating with a 10x objective (0.3 NA 10X objective Nikon) at power of 4.7 mW before the objective. We perform a nonnegative matrix factorization analysis to compare this data with the spectral data of the assembled nanodiamonds.

#### 3.5.6. Core-Shell Model

To better understand the correlations between  $F_{NV^-}$ ,  $T_1$  and, size, we model the nanodiamonds as spherical particles with a core radius  $(R_C)$  and a shell thickness (S) such that  $R_C + S$  is equal to the total radius of the sphere (R). The shell volume ratio  $(V_{S/R})$  is then given by:

$$V_{S/R}(R,S) = 1 - \frac{(R-S)^3}{(R)^3}$$
(3.12)

This relationship is plotted in Figure 3.19 for a series of S and R values. We can compare these volume ratios to the distribution of observed  $F_{\rm NV^-}$  and  $T_1$  values, assuming that NV centers residing in the shell region are more affected by the environment then defects in the core region, and hence have a lower  $F_{\rm NV^-}$  and smaller  $T_1$ . In this model, the likelihood of observing an NV center in the shell takes the form of a binomial distribution with a success probability  $V_{S/R}$ . To perform this analysis, we sort the nanodiamonds by height and calculate the observed fraction of measurements that fall below a given threshold value for  $F_{\rm NV^-}$  or  $T_1$ . From this observed fraction and the number of measurements, we calculate the Wilson score interval as an estimate for the underlying binomial probability and corresponding uncertainty. In Figure 3.19, we plot the Wilson score intervals alongside the shell volume fraction for particular threshold values. Although the uncertainties are large, the results are consistent with the interpretation that both  $F_{\rm NV^-}$  and  $T_1$  are reduced for NV centers located within a few nanometers of the nanodiamond surface.


Figure 3.19: Throughout the figure, colored lines indicate the shell volume ratio  $(V_{S/R})$  for shell sizes ranging from 1-10 nm. In each panel, circles and error bars represent the Wilson score interval estimate for  $V_{S/R}$  based on the number of  $F_{NV^-}$  (A,B) or  $T_1$  measurements (C,D) that fall below the threshold listed in each plot label, for nanodiamonds binned according to their height. The inset of (A) shows a core-shell structure with total radius R, core radius  $R_C$  and shell thickness S.

# CHAPTER 4

# NANODIAMOND SURFACE MODIFICATION THROUGH EMULSIONS

In the previous chapter, we established that an NV center's spin and optical characteristics are more dependent on nanodiamond's surface termination and nanoscale environment than the particle size and shape. Accordingly, Chapter 4 details a method for modifying a nanodiamond's surface termination and describes how further functionalization could transform nanodiamonds into quantum nanomedical platforms.

# 4.1. Introduction

Nanomedicine has the potential to dramatically improve the way we detect, treat, and prevent disease. An ideal nanomedicine platform should include three key functionalities: targeting, the ability to address and co-assemble with specific biological components such as proteins, antibodies, or cancerous cells; drug delivery, the ability to distribute treatment compounds; and diagnostics, the ability to detect nanoscale changes in biological environments. For example, future nanomedicine-based chemotherapy could involve injecting a patient with fluorescent nanoparticles attached to cancer antibodies, to exclusively target tumorous cells; delivering nano-molar doses of chemotherapy drugs; and then optically imaging the tumor to determine the efficacy of the treatment [31, 119]. Despite substantial progress on each of these goals, existing nanomedicine platforms suffer from drawbacks including lack of specificity, lack of sensitivity, and side effects from nanoparticle toxicity.

Nanodiamonds containing nitrogen-vacancy (NV) centers represent a promising solution. The carbon nanoparticles are nontoxic while being robust and unlikely to degrade within the body[153]. The NV center is a nonbleaching emitter in the near-infrared biological window, allowing for fluorescence imaging within living biological systems [148, 66, 95]. And most importantly, the NV center hosts an optically addressable spin state, allowing for nanoscale quantum measurement of a variety of external fields and perturbations *in vitro* or *in vivo*[147, 120, 116, 107].

Unfortunately nanodiamonds, and in particular the milled nanodiamonds that are of interest to quantum sensing, are not easily functionalized[115, 76, 119]. Although several surface terminations are possible [112, 107, 76, 123], most work has focused on carboxylated nanodiamonds that are stable in aqueous environments[119, 5, 150, 120, 147]. However, the coverage of carboxyl groups on the surface of milled nanodiamonds is patchy as they are reported to occur predominantly at undercoordinated carbon sites at the edges between (111) and (100) crystalline facets [101, 36]. With a limited number of surface carboxyl groups to stabilize the particles, the nanodiamonds tend to agglomerate when added to non-neutral solutions or biological fluids [142, 154, 119]. While techniques exist for coating nanodiamonds with materials such as silica, polyethylene glycol, polymers, or lipids [99, 135, 145, 131, 113, 143, 158], these methods are specific to the type of coating and typically require complex synthetic processes that can degrade the quantum and optical properties of the NV center.

Here we present a one-pot method for functionalizing milled nanodiamonds through the formation of emulsions. By sonicating hydrophobic nanodiamonds with amphiphilic small molecules, we form stable aqueous nanoparticle dispersions [4]. By selecting the composition and tailoring the concentration of the amphiphile, we control the ligand density and chemistry. This method maintains nanodiamonds' unique material, optical, and spin properties while enhancing colloidal stability and enabling carbodiimide crosslinker and click-chemistry conjugation reactions.



Figure 4.1: Schematics of (A) a carboxyl-terminated, hydrophilic nanodiamond containing NV centers (ND-COOH); (B) an octadecane-terminated, hydrophobic nanodiamond containing NV centers (ND-C18); (C) an ND-C18 nanodiamond coated with amphiphilic hemin, formed as an emulsion (EM-Hem); and (D) an ND-C18 nanodiamond coated with hemin and cholesteryl-TEG azide, formed as an emulsion (EM-Hem/Chol).

# 4.2. Results and Discussion

## 4.2.1. Emulsion Synthesis and Characterization

In this study, we examine four types of colloidal nanodiamond samples. These include commercial, aqueous dispersions of carboxyl-terminated, milled fluorescent nanodiamonds (ND-COOH, shown in Figure 4.1A) from Adámas Nanotechnologies, which have an average diameter of 53 nm (Figure 4.7) and contain less than 1 ppm of NV centers. Based on previous statistical studies of the heterogeneous distribution of NV centers in this material [127], we estimate that each nanodiamond contains between 0-20 NV centers. We also study commercial hydrophobic, octadecane-terminated nanodiamonds (ND-C18, shown in Figure 4.1B), fabricated by Adámas Nanotechnologies using the same conditions as those for the ND-COOH samples. The ND-C18 have the same NV center density, but a larger average diameter of 68 nm, due to variability in the milling and size-purification process. We create two emulsions from commercial ND-C18 material, one with hemin (EM-Hem, shown in Figure 4.1C), and another with equal weight of hemin and cholesteryl-TEG azide (EM-Hem/Chol, shown in Figure 4.1D).

To form emulsions, we adapt a previously reported procedure to coat superparamangetic iron oxide nanoparticles with protoporphyrin IX [151]. We first mix 1 mg of dry ND-C18 nanodiamonds with the amphiphilic compound(s) in 100–200 µL of toluene. We then add this mixture to 4 mL of water by pipette, while sonicating and using the pipette tip to vigorously stir until a homogeneous mixture is observed (see Methods). The mixture is left overnight, uncovered, to allow the toluene to evaporate, and finally, the samples are dialyzed to remove any remaining organic solvent or free amphiphile. For EM-Hem, we utilize hemin as the amphiphilic material at a weight ratio of 10:1 nanodiamond to hemin. We used nanodiamond to hemin ratios ranging from 20:1 to 5:1 (Figures 4.8-4.14), and we found that 10:1 results in the most stable and consistent single-particle emulsions. For EM-Hem/Chol, we modify the EM-Hem synthesis procedure by replacing half of the weight of hemin (MW = 651.94 g/mol) with cholesteryl-TEG azide (MW = 630.90 g/mol) to provide an azide termination for subsequent click-chemistry conjugation. We are unable to form stable emulsions using cholesteryl-TEG azide as the sole amphiphilic material, likely due to the lower solubility of cholesteryl-TEG azide, compared to hemin, in water.



Figure 4.2: (A) Box plots of the particle size distribution measured by dynamic light scattering (DLS) for the four nanodiamond samples in Figure 4.1. (B) Box plots of the particle size distribution measured by DLS for EM-Hem (brown) and EM-Hem/Chol (yellow) monitored over 10 months. (C) UV-Vis extinction (left axis; dashed curves) and photoluminescence (right axis; solid curves) spectra under 532 nm excitation for ND-COOH, ND-C18, EM-Hem, EM-HChol and hemin micelle aqueous dispersions.

Dynamic light scattering (DLS) is used to measure the particle size distribution for the four types of samples (Figure 4.2A and Figure 4.7). The uncoated nanodiamond samples, ND-COOH ( $53 \pm 15$  nm) and ND-C18 ( $68 \pm 23$  nm), exhibit average diameters and standard deviations consistent with the vendors specifications. For EM-Hem ( $75 \pm 23$  nm) and EM-Hem/Chol ( $87 \pm 26$  nm), we observe an increased average diameter of 7 nm and 19 nm, respectively, compared to the ND-C18 sample. Since the molecular lengths of hemin and cholesteryl-TEG azide are 1.5 nm and 3 nm, we expect a monolayer coating to increase the particle diameter by only 3 nm and 6 nm, respectively. The larger increase we observe is consistent with a coating comprised of multiple amphiphilic layers. While we consider the possibility that multiple nanodiamonds might also cluster within a single coating layer, the marginal increase in average particle size, with no significant change in standard deviation, suggests that clustering does not play a significant role. Moreover, van der Waals interactions would cause larger particles to preferentially agglomerate, which is not consistent with our observations. Transmission electron microscopy (TEM) images also show the particles remain separated (Figures 4.15-4.17). Figure 4.2B shows the size distributions for EM-Hem and EM-Hem/Chol dispersions monitored over 9 months, demonstrating their long-term stability.

We measure the optical extinction and photoluminescence (PL) spectra of the emulsions to confirm that we can still optically address and measure NV centers in EM-Hem and EM-Hem/Chol dispersions (Figure 4.2C). Figure 4.2C also displays, for comparison, the spectra of ND-COOH and ND-C18 dispersions and the spectra of free hemin micelles (see Methods). ND-COOH and ND-C18 exhibit monotonically decreasing extinction, arising from a combination of absorption and scattering at shorter wavelengths, while EM-Hem and EM-Hem/Chol feature an additional absorption peak around 325 nm associated with hemin. As expected, EM-Hem shows a more pronounced absorption peak than EM-Hem/Chol arising from the larger amount of hemin present. In the red and near-infrared regions under 532 nm excitation, the hemin control sample exhibits almost no emission, while all nanodiamond samples feature the characteristic NV-center PL emission spectrum.

#### 4.2.2. Effects on the NV-Center Charge State

Under optical excitation at 532 nm, the NV center fluctuates between the neutral (NV<sup>0</sup>) and negative (NV<sup>-</sup>) charge-state configurations through a process of ionization and recombination [7]. Moreover, the time-averaged charge configuration of individual NV centers depends on the local chemical potential, which varies spatially due to inhomogeneities in the nanodiamonds' impurity concentrations and their surface chemistry [127, 104, 112, 75]. The nanodiamond ensemble emission spectra are therefore a linear combination of the characteristic spectra for NV<sup>0</sup> and NV<sup>-</sup>, due to both temporal and spatial averaging. Since



Figure 4.3: (A) PL spectrum of a representative, EM-Hem/Chol emulsion (black) and its decomposition into NV<sup>-</sup> emission (red) and NV<sup>0</sup> emission (blue) spectra *via* nonnegative matrix factorization. (B)  $F_{\rm NV^-}$  for the nanodiamond samples.

the spin properties required for quantum sensing are only accessible in the NV<sup>-</sup> state, it is important to maximize the charge ratio,  $F_{\rm NV^-} = S_{\rm NV^-}/(S_{\rm NV^0} + S_{\rm NV^-})$ , where  $S_{\rm NV^0}$  and  $S_{\rm NV^-}$  are the integrated PL intensities of the neutral and negative charge states. The quantity  $F_{\rm NV^-}$  is extracted directly from measured ensemble emission spectra using nonnegative matrix factorization[17] (see 4.5.1), which decomposes each spectrum into its NV<sup>0</sup> and NV<sup>-</sup> components, as shown in Figure. 4.3A. Figure 4.3B shows  $F_{\rm NV^-}$  for the four nanodiamond samples. ND-C18 exhibits a significantly lower  $F_{\rm NV^-}$  than ND-COOH, which is consistent with prior works showing that surface carboxyl groups donate electrons and raise the chemical potential [89, 115, 112, 107, 75]. Interestingly, addition of the organic coatings in the emulsion samples acts to increase  $F_{\rm NV^-}$ , compared to the ND-C18 source material. This observation suggests that the coating of hemin molecules, which also feature carboxyl groups, could similarly increase the chemical potential. It is also possible that the hemin coating modifies the nanodiamond emission spectrum through preferential absorption or energy transfer of the shorter wavelengths associated with NV<sup>0</sup>. As shown in Fig 4.2C, hemin features a broad absorption peak between 300-450 nm, with a tail extending to  $\approx 600$  nm. In either case, the slightly higher value of  $F_{\rm NV^-}$  for EM-Hem compared to EM-Hem/Chol is consistent with the larger hemin concentration. Regardless of the mechanism, the increased  $F_{\rm NV^-}$  is desirable, since it improves the signal-to-noise ratio and sensitivity for quantum sensing experiments.

#### 4.2.3. Effects on the NV-Center Spin Lifetime

The NV center's spin lifetime  $(T_1)$  is an important figure of merit for nanodiamond quantum sensors. Variations in  $T_1$  are used to detect broadband magnetic noise associated with free radicals, and  $T_1$  represents an upper limit on the spin coherence time,  $T_2$ , which is relevant for detecting ac fields. For NV centers in nanodiamonds,  $T_1$  is typically limited by fluctuating spins on the nanodiamond surface; the strong  $\approx 1/r^6$  scaling of this coupling, where r is the distance between the fluctuating spin and an NV center, means that  $T_1$  can vary over orders of magnitude for NV centers in nanodiamonds depending on their placement relative to the nanodiamond surface. As a consequence, the ensemble average spin lifetime,  $\langle T_1 \rangle$ , depends strongly on nanodiamond size as well as on surface termination [127, 137, 106, 104, 118, 139]. Because the measurement can be performed optically, without the need for microwave infrastructure,  $T_1$  is the preferred spin measurement for *in vivo* sensing. Larger  $T_1$  typically



Figure 4.4: (A) PL as a function of delay time,  $\tau$ , between an optical initialization pulse and subsequent readout pulse, for ND-COOH (dark green stars) and EM-Hem (brown circles). Solid curves are fits using Equation (4.2) of the main text. The PL signal is normalized to the intensity at  $\tau = 9$  ms, and error bars represent the uncertainty from photon shot noise. (B) Best-fit  $T_1^{\rm S}$  for the four nanodiamond samples. Error bars represent the standard deviation of  $T_1^{\rm S}$  from measurements of multiple samples (for ND-COOH, ND-C18, and EM-Hem) or the fit confidence interval from a single measurement for EM-Hem/Chol (Figure 4.18). (C)  $\langle T_1 \rangle$  as a function of [Gd<sup>3+</sup>] added to EM-Hem (brown circles). Error bars represent the uncertainty in  $\langle T_1 \rangle$  through propagation of the best-fit confidence intervals for  $T_1^{\rm S}$  and  $\beta$ through Equation (4.3). The curves represent models for the effect of  $[\mathrm{Gd}^{3+}]$  on  $\langle T_1 \rangle$  that respectively assume no surface adsorption (black dashed curve) or adsorption according to a Langmuir model (solid curve for best fit and shaded region for 68 % confidence intervals). (D) Best-fit  $T_1^{\rm S}$  vs. [Gd<sup>3+</sup>] for ND-COOH (green stars) and EM-Hem (brown circles) (left axis). Error bars represent 68% confidence intervals. Dotted curves are fits using Equation (4.5). The right axis shows  $\eta_{[Gd^{3+}]}$  for ND-COOH (green solid curve) and EM-Hem (brown solid curve) as a function of  $[Gd^{3+}]$  calculated according to Equation (4.6).

correlates with increased spin coherence times. Moreover, increased  $T_1$  lowers the threshold for detecting magnetic field noise, potentially improving the sensitivity to free radicals.

We measure  $\langle T_1 \rangle$  for nanodiamond dipsersions using an all-optical protocol. In each measurement sequence, we initialize the NV centers into their  $m_s = 0$  spin-triplet sublevel using

a 3 µs, 532 nm laser excitation pulse and then count PL photons during a 350 ns readout laser pulse at delay times ( $\tau$ ) ranging from 50 ns to 9 ms. Based on the optical configuration and nanodiamond concentration, we estimate that each measurement probes approximately 200 nanodiamonds, each containing 0-20 NV centers. Figure 4.4A shows the results of this measurement for ND-COOH and EM-Hem. The PL signal is initially bright due to polarization in the  $m_s = 0$  state, and it decays as a function of  $\tau$  due to relaxation into the  $m_s = \pm 1$ dark states. For an ensemble measurement, the shape of the observed PL signal,  $I(\tau)$ , results from the summed contribution of several thousand NV centers,

$$I(\tau) = \sum_{i} I(\infty) \left[ 1 + C \exp\left(-\frac{\tau}{T_{1,i}}\right) \right], \qquad (4.1)$$

where  $T_{1,i}$  is the spin lifetime of the  $i^{\text{th}}$  NV center, C is the spin-dependent PL contrast, and  $I(\infty)$  is the brightness associated with a spin-unpolarized state. To account for the ensemble average over many spin lifetimes, we model the data using a stretched exponential function:

$$S(\tau) = S(\infty) \left[ 1 + C_{\rm S} \exp\left(-\frac{\tau}{T_1^{\rm S}}\right)^{\beta} \right].$$
(4.2)

Here,  $S(\infty)$  and  $C_{\rm S}$  represent the asymptotic long-delay signal and PL contrast, respectively. The stretched-exponential spin lifetime,  $T_1^{\rm S}$ , describes the effective ensemble decay rate, whereas the stretching exponent,  $\beta$ , accounts for averaging over the distribution of lifetimes. The variables  $T_1^{\rm S}$  and  $\langle T_1 \rangle$  are related by the formula[71]:

$$\langle T_1 \rangle = T_1^{\rm S} \frac{\Gamma(\frac{1}{\beta})}{\beta},$$
(4.3)

where  $\Gamma$  represents the gamma function. For  $\beta$  in the range 0.5–1, as we typically observe,  $\langle T_1 \rangle$  is larger than  $T_1^{\rm S}$  by a factor between one and two. Figure 4.4B shows  $T_1^{\rm S}$  for the four samples. The 87% increase in  $T_1^{\rm S}$  of ND-C18 compared to ND-COOH may reflect the larger average size of ND-C18 (see Fig. 4.2A), since there will be a greater average distance between NV centers and the surface of a larger particle, or the difference in surface chemistry, which may result in a lower surface noise density [137]. The difference in  $T_1^{\rm S}$  for EM-Hem and EM-Hem/Chol, in comparison to ND-C18, are within the measurement uncertainty. This observation is surprising, since hemin contains paramagnetic Fe<sup>3+</sup> ions that would be expected to reduce  $T_1$ ; however, this effect is small given Fe<sup>3+</sup>'s low intrinsic spin relaxation rate and spin number. In the Section 4.5, we extract the hemin concentrations from the extinction measurements (Figure 4.2C) and utilize a model for  $T_1$ , described below, to quantify the impact of the hemin coating on  $T_1$  (Figures 4.19 and 4.20).

In order to examine the emulsions' sensitivity to free radicals, we prepare  $\approx 5$  nM Em-Hem dispersions and track  $T_1$  as a function of increasing concentration of a gadolinium (Gd<sup>3+</sup>) chelate with butylamine termination, with [Gd<sup>3+</sup>] varying from 0.05 µM to 2000 µM (see Figure 4.4C). We observe that  $\langle T_1 \rangle$  begins to decrease for [Gd<sup>3+</sup>]  $\gtrsim 10$  µM before saturating when [Gd<sup>3+</sup>]  $\gtrsim 1000$  µM. To interpret these observations, we adapt a model by Tetienne *et al.*,[137] to calculate the effect of fluctuating spins on  $T_1$  for NV centers inside nanodiamonds. The drop in  $T_1$  will depend on both [Gd<sup>3+</sup>] as well as the distance of the NV centers from the outside spin bath. We model the nanodiamonds as 68 nm diameter spheres, each containing a random spatial distribution of NV centers. We initially assume that no NV centers exist within 3 nm of the nanodiamond surface [18]. By integrating over possible locations for the NV centers and assuming a uniform [Gd<sup>3+</sup>] in the surrounding medium, we calculate  $\langle T_1 \rangle$  as a function of [Gd<sup>3+</sup>]. The result of this calculation is shown as a dotted line in Fig. 4.4C. This model significantly underestimates the impact of [Gd<sup>3+</sup>] on  $\langle T_1 \rangle$ . As shown in Figure 4.21, we can improve the model's agreement with the data in the range of [Gd<sup>3+</sup>]  $\approx 10$ -100 µM by allowing for NV centers to reside within 0.1 nm of the nanodiamond surface, however, this is inconsistent with the proposed instability of near-surface NV centers and, moreover, this explanation does not reproduce the saturation of  $\langle T_1 \rangle$  for larger [Gd<sup>3+</sup>]. To resolve this discrepancy, we hypothesize that some of the Gd<sup>3+</sup> chelates adsorb to the particle surface, resulting in a Gd<sup>3+</sup> surface density  $\sigma([Gd^{3+}])$  that follows the Langmuir model:

$$\sigma([\mathrm{Gd}^{3+}]) = \sigma_{\infty} \frac{K[\mathrm{Gd}^{3+}]}{1 + K[\mathrm{Gd}^{3+}]},$$
(4.4)

where  $\sigma_{\infty}$  and K are empirical constants. The solid curve and shaded region in Figure 4.4C is the result of a fit using this model, with  $\sigma_{\infty} = 0.10 \pm 0.04 \text{ nm}^{-2}$  and  $K = 6000 \pm 4000 \text{ M}^{-1}$ . These values correspond to a maximum of  $1500 \pm 600 \text{ Gd}^{3+}$  adsorbed to a 68 nm diameter nanodiamond. This maximum is < 10% of the  $\approx 20000$  surface sites for a perfectly packed Gd<sup>3+</sup>-chelate monolayer on a 68 nm diameter sphere. Although this model makes many simplifying assumptions, the results distinctly show how NV centers can serve as sensitive reporters of variations in their nanoscale local environment, in this case distinguishing the effects of adsorbed free radicals from those in solution.

### 4.2.4. Chemical sensitivity

A typical sensing experiment with NV centers involves tracking the change in PL as a function of variations in the environment. The chemical sensitivity,  $\eta_{[Gd^{3+}]}$ , quantifies the minimum detectable change in  $[Gd^{3+}]$  that can be identified in a given measurement bandwidth. Here, we consider the case of  $T_1$  relaxometry, where the PL signal is given by Equation (4.2) for a fixed delay time,  $\tau_0$ . The variables  $T_1^S$ ,  $\beta$ , and  $C_S$  are, in principle, all functions of  $[Gd^{3+}]$ . In practice, however, we empirically find that the predominant effect is on  $T_1^S$  (Figures 4.22-4.26). Figure 4.4D shows the variation in  $T_1^S$  as a function of  $[Gd^{3+}]$  for ND-COOH and EM-Hem. Based on the discussion in the previous section, and similar to previous works [143], we utilize an empirical model:

$$T_1^{\rm S}([{\rm Gd}^{3+}]) = T_{1,{\rm Sat}} + \Delta T_1 \exp\left(\frac{-[{\rm Gd}^{3+}]}{[{\rm Gd}^{3+}]_0}\right)$$
(4.5)

Assuming the measurement uncertainty is dominated by photon shot noise and that the duration of each measurement cycle is dominated by  $\tau_0$ , the chemical sensitivity is given by:

$$\eta_{[\mathrm{Gd}^{3+}]} = \sqrt{\frac{\tau_0 S(\tau_0)}{Rt_{\mathrm{m}}}} \left(\frac{\partial S}{\partial T_1^{\mathrm{S}}} \cdot \frac{dT_1^{\mathrm{S}}}{d[\mathrm{Gd}^{3+}]}\right)^{-1},\tag{4.6}$$

where R is the steady-state photon count rate, and  $t_{\rm m}$  is the photon integration time per cycle.

Figure 4.4D shows  $\eta_{[Gd^{3+}]}$  as a function of  $[Gd^{3+}]$ , for the ND-COOH and EM-Hem samples. In evaluating Equation (4.6), we assumed a fixed  $\tau_0 = T_1^S/2$ , and R = 500 kCts/s. This count rate represents the typical brightness of a single nanodiamond containing  $\approx 20$  NV centers measured using a high-NA optical collection system. It is also similar to the signal levels in our ensemble measurements, where we probed  $\approx 200$  nanodiamonds and attenuated the signal in order to remain within the dynamic range of our single-photon detector. In an optimized sensing experiment, orders-of-magnitude improvements in sensitivity can be achieved by collecting the fluorescence of N nanodiamonds, with a scaling according to  $\eta_{[Gd^{3+}]} \propto 1/\sqrt{N}$ .

For small [Gd<sup>3+</sup>], we observe that  $\eta_{Gd^{3+}}$  for ND-COOH is approximately three times smaller than  $\eta_{Gd^{3+}}$  for EM-Hem, however this advantage rapidly declines for [Gd<sup>3+</sup>] > 10 µM and reaches parity at [Gd<sup>3+</sup>]  $\approx$  500 µM. The initially smaller  $\eta_{[Gd^{3+}]}$  results from ND-COOH's shorter  $T_1^S$ , which improves sensitivity by reducing the required delay time in each measurement. The spin lifetime can be tuned for the needs of particular applications. Since past work [137, 104, 127] and the previous section shows that  $T_1^{\rm S}$  increases with particle size, the emlusions'  $\eta_{[{\rm Gd}^{3+}]}$  could be improved by employing smaller initial ND-C18 nanodiamonds. On the other hand, ac-field sensing experiments benefit from longer  $T_1^{\rm S}$ . The interaction of nanodiamonds to the Gd<sup>3+</sup> spins could also be improved by employing conjugation techniques instead of relying indirectly on adsorption, as we explore in the next section.

### 4.2.5. Conjugation

The application of nanodiamonds as sensor platforms for nanomedicine requires chemical conjugation of various biomolecules to the nanodiamonds. Examples include DNA oligos or antibodies for selective binding and targeting, or labels and transducers such as dyes or paramagnetic species. Carboxyl-terminated nanoparticles (ND-COOH, EM-Hem, and EM-Hem/Chol) can be conjugated to amine-terminated compounds through carbodiimide crosslinking chemistry [60]. In this method, a combination of N-(3-dimethylaminopropyl)-N'ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (Sulfo-NHS) is utilized to mediate the conjugation reaction. To crosslink the carboxyl-terminated particles to amineterminated compounds, we first add equal amounts of EDC and Sulfo-NHS to the carboxyterminated nanoparticles in a 5% N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) buffer, used to maintain a neutral pH. We add excess crosslinker (EDC and Sulfo-NHS) and target conjugates, utilizing concentrations  $\approx 100$  M higher than the number of surface -COOH groups, in order to compensate for EDC hydrolysis and increase the likelihood conjugation. We shake the mixture for 25 min before adding a chosen conjugate and then continue to shake the mixture overnight. Although previous studies have demonstrated EDC-based crosslinking to nanodiamonds [84, 33, 29, 50], we find it challenging to reproduce these results for ND-COOH without observing significant agglomeration (Figure 4.5B, Figure 4.27). In contrast, EM-Hem exhibits greater stability toward the crosslinking reaction. The samples are cleaned using centrifugation or dialysis to remove any unconjugated target

particles and crosslinker by-products.



Figure 4.5: (A) Model of EDC-enabled crosslinking of amine-terminated  $\mathrm{Gd}^{3+}$  chelate to EM-Hem. (B) DLS size distribution for ND-COOH (dark green) and EM-Hem (brown) before (upper) and after (lower) addition of crosslinkers. (C)  $T_1^{\mathrm{S}}$  values as a function of [EDC]:[Gd<sup>3+</sup>] ratio following the EDC-Gd<sup>3+</sup> conjugation experiment; after conjugation (dotted bars), initial after centrifugation washing (striped bars), and after a two-day dialysis step (solid bars). Error bars represent the best-fit 68 % confidence intervals for  $T_1^{\mathrm{S}}$  through Equation (4.2). (D) ICP-OES measurements of remaining [Gd<sup>3+</sup>] after dialysis. Error bars represent propagated uncertainty from the best-fit confidence intervals for ICP-OES Gd<sup>3+</sup> calibration curve.

The crosslinking reaction is general, allowing the addition of amine-terminated molecules of interest. We considered amine-terminated dyes (Figures 4.28), and as we focus here on spin-based sensing, conjugation of the butylamine-terminated,  $\mathrm{Gd}^{3+}$ -chelate spin label (Figure 4.5A). The  $\mathrm{Gd}^{3+}$  conjugation is especially useful since the spin label is expected to decrease  $T_1$  without altering other nanodiamond optical properties. We performed two conjugations using 1.5 mL samples of 0.25 mg/mL EM-Hem ( $\approx 1$  nM of 68 nm spherical nanodiamonds) and 3 mg of  $\mathrm{Gd}^{3+}$  (3 mM), with different molar ratios of EDC to  $\mathrm{Gd}^{3+}$ , i.e., [EDC]:[ $\mathrm{Gd}^{3+}$ ] of 2:1 and 3:1. A control sample (0:1) is also made in parallel to probe the extent of  $\mathrm{Gd}^{3+}$  chelate adsorption in the absence of the EDC crosslinker. Figure 4.5C shows  $T_1^{\mathrm{S}}$  of each sample immediately after conjugation (dotted) and after washing *via* either centrifugation (striped) or dialysis (solid). As expected, after the initial mixing with  $\mathrm{Gd}^{3+}$ , all samples exhibit a significant drop in  $T_1^{\mathrm{S}}$ . The washing results in an increase in  $T_1^{\mathrm{S}}$  for the control sample as the free  $\mathrm{Gd}^{3+}$  chelate is removed from the solution. Conversely, regardless of the cleaning method, the two conjugated samples retain a shortened  $T_1^{\mathrm{S}}$ , indicating successful chemical bonding.

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) measurements are used to quantify the amount of  $Gd^{3+}$  chelate in each sample after dialysis (Figure 4.5D). We observe significantly more  $\mathrm{Gd}^{3+}$  in the two conjugated samples than in the control, and the amount of retained  $Gd^{3+}$  is proportional to the amount of EDC used. Since the amount of EDC and  $Gd^{3+}$  spin label are added in significant excess of the number of hemin -COOH groups, this trend implies that the conjugation is limited by the efficiency of the EDC reaction. By once again assuming spherical 68 nm diameter nanodiamonds, the ICP-OES measurements imply 1600  $\pm$  1000, 12000  $\pm$  2000 and 27000  $\pm$  5000  $\rm Gd^{+3}$  chelates per nanodiamond for the control and two conjugated samples, respectively. The residual Gd<sup>3+</sup> in the control sample likely reflects an incomplete dialysis of the samples. Some of the remaining  $\mathrm{Gd}^{3+}$  may be adsorbed to the nanodiamonds' surfaces, but the long  $T_1^{\mathrm{S}}$  implies that the majority of Gd<sup>3+</sup> remains unbound. The approximately 10-fold to 20-fold increase in retained  $\mathrm{Gd}^{3+}$  for the two conjugated samples over the control highlights the success of crosslinking conjugation. In fact, here we observe higher retained  $[\mathrm{Gd}^{3+}]$  than the observed decrease in  $T_1^{\mathrm{S}}$ would imply. From the relatively small decrease in  $T_1^{\rm S}$ , we infer that the conjugated  ${\rm Gd}^{3+}$ chelates are positioned farther from the nanodiamond surface than the adsorbed chelates responsible for the reduced  $T_1$  in Figure 4.4D. We did not notice agglomeration in DLS measurements from any of the samples immediately after the conjugation process. After dialysis, however, the conjugated samples showed signs of agglomeration (Figure 4.29), which suggests that the conjugation process destabilizes the emulsions.



Figure 4.6: (A) Model of click conjugation of a DBCO-terminated  $\mathrm{Gd}^{+3}$  spin label to EM-Hem/Chol. Data shown for conjugations at 0:1 (yellow) 1:4 (orange), 2:1 (lavender) and 10:1 (purple) molar ratios of dibenzocyclooctyne (DBCO) to azide (N<sub>3</sub>). FTIR transmittance curves of (B) the CH and (C) the N<sub>3</sub> stretching regions of the four samples. (D) Ratio of the integrated areas of N<sub>3</sub> to CH stretches for each sample. (E)  $T_1^{\mathrm{S}}$  for the samples. Striped bars refer to measurements taken before dialysis. Error bars represent the best-fit 68 % confidence intervals for  $T_1^{\mathrm{S}}$  through Equation (4.2). (F) ICP-OES measurements of remaining [Gd<sup>3+</sup>] in the click-conjugated samples after dialysis. Error bars represent propagated uncertainty from the best-fit confidence intervals for ICP-OES gadolinium calibration curve.

To overcome the limited stability of EDC and the complexity of adding buffer and Sulfo-NHS in the crosslinking reaction, we instead exploit copper-free click chemistry reactions, which are becoming a widely accepted standard for biological conjugation [138, 92, 15, 39, 69]. Here,

we focus on the reaction of dibenzocyclooctyne (DBCO) compounds with terminal-azide ( $N_3$ ) groups [37, 2, 24]. The emulsion technique provides a simple route to introducing  $N_3$  groups around the nanodiamond surface, as seen in Figure 4.1C for the example of EM-Hem/Chol. Again, this conjugation reaction is general, and allows EM-Hem/Chol to be conjugated with any DBCO-terminated particle including: dyes (Figure 4.30) and DBCO-terminated Gd<sup>+3</sup> spin labels (Figure 4.6A). In contrast to the crosslinking reaction, which involves multiple steps where pH must be carefully controlled, the click-chemistry conjugation of Gd<sup>3+</sup>-DBCO with EM-Hem/Chol simply consists of mixing the two materials together overnight.

To test the reaction efficiency, we carry out the conjugation reaction with increasing  $\mathrm{Gd}^{3+}$ -DBCO to  $N_3$  molar ratios (1:4, 2:1, 10:1), and compare it with a nonconjugated control sample (0:1). Figure 4.6B,C shows Fourier transform infrared transmission (FTIR) measurements used to monitor the CH and  $N_3$  stretching regions for the four samples. Successful conjugation is evidenced by the increase in the CH stretching modes and a corresponding reduction in the  $N_3$  modes, as quantified by the ratio of their integrated areas in Figure 4.6D. Similar to the crosslinknig conjugation, we require an excess of DBCO, here only 10X, to completely quench the  $N_3$  signal and ensure complete conjugation. The greater conjugation as DBCO:N<sub>3</sub> increases is also reflected by a decrease in  $T_1^S$  (Figure 4.6E), which remains low before (striped) and after dialysis (solid), and by an increase in  $[Gd^{3+}]$  measured by ICP-OES after dialysis (Figure 4.6F). The ICP-OES measurements imply concentrations of 4000  $\pm$  2000, 5000  $\pm$  3000 and 11000  $\pm$  4000  $\rm Gd^{3+}$  per spherical 68 nm nanodiamond for the conjugated samples with increasing  $DBCO:N_3$ . The maximum coating is less than we observed for crosslinking, which we expected due to the smaller number of potential conjugations sites. Notably,  $T_1^S$  for the click-conjugated reactions are lower than those of the crosslinked samples in Figure 4.5 and the unconjugated samples in Figure 4.4, even though the distance between the conjugated chelate and the nanodiamond surface is larger and the amount of initially added conjugation material is smaller. This result demonstrates the efficiency of the click conjugation reaction. To control for possible adsorption of  $\mathrm{Gd}^{3+}$ -DBCO to the nanodiamonds, we repeated the experiments for ND-COOH and EM-Hem samples (Figures 4.32-4.40), where we do not expect any click reactions to occur. We observe longer  $T_1^{\mathrm{S}}$ times for these samples after dialysis and significantly lower concentrations of  $\mathrm{Gd}^{3+}$  detected by ICP-OES, consistent with the 0:1 control sample of Figure 4.5.

We do not observe agglomeration of the samples after click-chemistry conjugation (Figure 4.41). We hypothesize that the hybrid EM-Hem/Chol surface coating allows the functional separation of stabilization, imparted by the COOH groups of hemin, with conjugation, achieved using the azide-terminated cholesteryl-TEG. Our results illustrate a simple and efficient nanodiamond emulsion click-chemistry procedure that could not be achieved with commercially available carboxyl-terminated nanodiamonds.

### 4.3. Conclusion

We report nanodiamond emulsions as versatile building blocks for quantum sensing and nanomedicine platforms that maintain the beneficial optical and quantum properties of the NV center, while allowing for generalized chemical conjugation. The hybrid surface termination enables multiple simultaneous yet distinct conjugation reactions without jeopardizing the colloidal stability of the nanoparticles. By separating the tasks of stabilizing and conjugating across two distinct surface groups, the EM-hemin/chol nanoparticles outperformed the singularly terminated alternatives. We also observed that the charge and spin properties of the NV center can be modified by altering the composition of the coating materials. These controls will enable higher signal-to-noise ratios and tunable sensitivity to external perturbations.

The polydispersity of milled nanodiamonds currently hinders biological sensing applications.

In our measurements and modeling, we averaged over a large number of nanodiamonds to account for the variations in size, NV-center density, and surface termination. A more precise quantification of these parameters, and their variations across individual nanodiamonds, will likely be necessary before performing *in vivo* experiments. Nevertheless, in their current form, EM-Hem and EM-Hem/Chol represent a major improvement over commercially available milled nanodiamonds (ND-COOH and ND-C18) and demonstrate the utility of forming emulsions to develop chemical quantum sensors.

While we have focused here on the imaging and sensing aspects of nanomedicine, our work lays the foundation for advances in targeting and drug delivery. These functionalities could be achieved through the addition of amphiphiles with specifically chosen functional groups, such as folic-acid-terminated lipids for targeting, or the simultaneous conjugation to biomolecules, such as proteins, antibodies, or DNA oligos. In this way, a complete nanomedicine platform can be designed, combining targeting and drug delivery functionality with the quantum sensitivity of biocompatible nanodiamonds. Although we have only provided an initial step, these quantum nanomedical devices could drastically change the way we detect and treat disease.

### 4.4. Methods

#### **Optical Measurements**

Spin relaxation measurements are taken using a custom-built confocal microscope described by Huang *et al.*,[64] and Shulevitz *et al.*,[127] configured to preform nanodiamond solutions. A 532 nm (green) continuous-wave laser (Coherent, Compass 315M-150) is sent through a 5X objective (Olympus UMPlanFL) focused into the approximate center of a 300 µL cuvette (Spectrocell). To average over a sufficient number of nanodiamonds, we calculate the beam width of this laser and modify the concentration of nanodiamond material by a factor of 5X, via vacuum evaporation or centrifugation, to ensure at least 150 nanodiamonds are within the excitation volume, assuming uniform spherical nanodiamonds.

 $T_1$  lifetime measurements are implemented by programming the required pulse sequences onto an arbitrary-waveform generator (AWG; AWG520 Tektronix). The AWG control signals are passed to the microscope's acousto-optic modulator (AOM) for generating optical pulses and to three high isolation switches (ZASWA-250DR Mini-Circuits) for time-gating photon detection events recorded by counters in a data acquisition card (National Instruments PCIe-6323).

Spectral emission measurements are taken on an Edinburgh Instruments FLS1000 spectrometer with a PMT-980 photodetector. We utilized a 450 W Xe lamp for the 532 nm excitation source.

Absorption spectra were measured with an Agilent Cary 5000 spectrometer.

ICP-OES measurements were performed on a SPECTRO GENESIS ICP-OES spectrometer.

DLS measurements are conducted on a Malvern Instruments Zetasizer Nano-s and analyzed using Malvern's software.

FTIR transmittance measurements are performed on a Nicolet 6700 (Thermo Scientific) spectrometer with a mercury cadmium telluride detector. To perform the measurements, samples were drop cast on double-side polished Si chips and vacuum dried. Measurements were then taken at multiple spots on the film to account for potentially uneven distributions.

4.4.1. Synthesis Methods

To form the emulsions, 1 mg of ND-C18 is dispersed in 60  $\mu$ L toluene and mixed with 0.05 mg-0.1 mg, depending on the emulsion type, of hemin (Sigma Aldrich) and Cholesteryl-

TEG azide (Sigma Aldrich) dissolved in 10:1 toluene to dimethyl sulfoxide (DMSO). The DMSO ensures the hemin fully dissolves. The final volume of ND-C18/amphiphilic mixture should be between  $100\mu$ L-  $200\mu$ L.

The mixture is then pipetted into a glass vial containing 4 mL of Milli-Q (18.2 M $\Omega$ ·cm) water, during sonication and stirred vigorously for at least 5 minutes until a homogeneous mixture is observed. The toluene is then allowed to evaporate overnight. Dialysis is then performed with 4 L of Milli-Q (18.2 M $\Omega$ ·cm) water to remove DMSO and free amphiphilic compounds. Hemin micelle can be formed in the same manner but without the addition of nanodiamonds.

To synthesise the GD-DBCO chelates we first dissolved 15 mg of DO3A-DBCO (Macrocyclics B-283) in a 0.5 mL H2O/MeOH= 1/1 solution. We then added 2Eq of GdCl3 (Sigma) and 10Eq of N,N-Diisopropylethylamine (DIPEA) and stirred at room temperature for 3 hours to initially mix and then overnight at 40C. We then evaporated to dryness under vacuum and purified by High-performance liquid chromatography (HPLC).

#### Conjugations

For carbodiimide crosslinking conjugation we add equal amounts of EDC (Thermo Fisher) and Sulfo-NHS (Thermo Fisher) to 1.5 mL of EM-Hem at a 0.25 mg/mL concentration (nanodiamond mass) in a 5% HEPES solution. The crosslinkers are measured out in powder form and added directly to the EM-Hem solutions to minimize the impact of hydrolysis. The solution is then shook for 25 minutes before adding the  $Gd^{3+}$  chelate (Macrocyclics X-287) and then is left to shake overnight to complete the conjugation.

For click chemistry conjugation, GD-DBCO chelates are dissolved in Milli-Q water and then mixed with 0.6 mL samples of EM-Hem/Chol at a 0.25 mg/mL concentration, (nanodiamond mass) with the proper molar ratio and shaken overnight.

# 4.5. Supporting Information



Figure 4.7: Dynamic light scattering (DLS) data for aqueous, carboxyl-terminated, hydrophilic nanodiamonds containing NV centers (ND-COOH, dark green), octadecaneterminated, hydrophobic nanodiamonds containing NV centers (ND-C18, light green), ND-C18 nanodiamonds coated with amphiphilic hemin, formed as an emulsion (EM-Hem, brown), and ND-C18 nanodiamonds coated with hemin and cholesteryl-TEG azide, formed as an emulsion (EM-Hem/Chol, yellow).

### 4.5.1. Varying Nanodimaond Size and Surface Coating

In this subsection, in addition to the materials described in the previouse sections, we also consider smaller milled nanodiamonds (Adámas Nanotechnologies), emulsions (EM-OA) formed with oleic acid (Thermo Fisher), and emulsions formed with varying amounts of hemin and hemin-cholesteryl-TEG azide. Accordingly, the samples are labeled by the material type and either the advertised size for ND-COOH and ND-C18 or the weight ratio of amphiphilic molecules to nanodiamonds utilized in preparing the emulsions. Due to the variability of the milling process the advertised size of the nanodiamonds is often different than the measured size as seen in Figures 4.7 and 4.8 and Main Text Figure 4.1.



Figure 4.8: Dynamic light scattering measurements (DLS) of the mean diameter for nanodiamond and emulsion samples. The top row of the X axis label indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, or hemin-cholesteryl-TEG azide to the ND-C18 nanodiamonds used in synthesizing the emulsions. Error bars represent the standard deviation of the DLS size distribution.

## Non-negative Matrix Factorization

We employ the MATLAB function *nnmf* to perform a rank two non-negative matrix factorization (NNMF) of the measured nanodiamond and emulsion spectra.[17]NNMF allows us to extract the NV<sup>0</sup> and NV<sup>-</sup> emission spectra and the PL intensities of those states ( $S_{\rm NV^-}$ and  $S_{\rm NV^0}$ ) relative to the total emission spectra such that:

$$MeasuredSpectrum \approx S_{\rm NV^0} * NV^0 + S_{\rm NV^-} * NV^-$$
(4.7)

The results of this factorization can be seen in Figure 4.3 and Figure 4.9.



Figure 4.9: Photoluminescence spectra of nanodiamond samples and the nonnegative matrix factorization into NV<sup>-</sup> (red) and NV<sup>0</sup> (blue) charge state spectra, with the color of the measured spectra indicating the charge ratio ( $F_{\rm NV^-}$ ) for commercial and emulsion nanodiamond samples. The top row of the labels indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, and hemin-cholesteryl-TEG azide to the nanodiamonds used in synthesizing the emulsions.



Figure 4.10: Charge ratio  $(F_{\rm NV^-})$  for commercial and emulsion nanodiamond samples. The top row of the X axis label indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, or hemin-cholesteryl-TEG azide to the ND-C18 nanodiamonds used in synthesizing the emulsions.



Figure 4.11: Stretched-exponential spin lifetime  $(T_1^S)$  for commercial and emulsion nanodiamond samples. The top row of the X axis label indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, or hemin-cholesteryl-TEG azide to the nanodiamonds used in synthesizing the emulsions. Error bars represent the standard deviation of  $T_1^S$  from measurements of multiple samples (for ND-COOG, ND-C18, EM-Hem 15:1, and EM-Hem 10:1) or the fit uncertainty from a single measurement (for EM-OA and EM-Hem/Chol).



Figure 4.12: Photoluminescence contrast  $(C_S)$  from stretched-exponential spin lifetime fits for commercial and emulsion nanodiamond samples. The top row of the X axis label indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, or hemin-cholesteryl-TEG azide to the nanodiamonds used in synthesizing the emulsions. Error bars represent the standard deviation of  $C_S$  from measurements of multiple samples (for ND-COOH, ND-C18, EM-Hem 15:1, and EM-Hem 10:1) or the fit uncertainty from a single measurement (for EM-OA and EM-Hem/Chol).



Figure 4.13:  $\beta$  factor from stretched-exponential spin lifetime fits for commercial and emulsion nanodiamond samples. The top row of the X axis label indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, or hemin-cholesteryl-TEG azide to the nanodiamonds used in synthesizing the emulsions. Error bars represent the standard deviation of  $\beta$  from measurements of multiple samples (for ND-COOH, ND-C18, EM-Hem 15:1, and EM-Hem 10:1) or the fit uncertainty from a single measurement (for EM-OA and EM-Hem/Chol).



Figure 4.14: Average spin lifetime  $(\langle T_1 \rangle)$  for commercial and emulsion nanodiamond samples. The top row of the X axis label indicates the material type and the bottom row details the advertised milled nanodiamond diameter, for ND-COOH and ND-C18 commercial samples, or the weight ratio of the amphiphilic molecules hemin, oleic acid, or hemin-cholesteryl-TEG azide to the nanodiamonds used in synthesizing the emulsions. Error bars represent the standard deviation of  $\langle T_1 \rangle$  from measurements of multiple samples (for ND-COOH, ND-C18, EM-Hem 15:1, and EM-Hem 10:1) or the fit uncertainty from a single measurement (for EM-OA and EM-Hem/Chol).

### 4.5.2. Transmission Electron Microscopy

Transmission electron microscopy (TEM) images are taken on a JEOL F200 scanning /transmission electron microscope operating at 200 kV. The nanodiamond samples are drop-cast onto 300 mesh copper TEM grids and dried overnight in a vacuum chamber. As expected, the milled nanodiamonds have an irregular shape and size. The milled nanodiamonds exhibit varying orientations and thicknesses, which are evident from the varying contrast and focus observed in different areas of the TEM images. We did not observe any significant difference between the images for the four nanodiamond samples.



Figure 4.15: Low-resolution transmission electron microscopy (TEM) images of ND-COOH, ND-C18, EM-Hem, and EM-Hem/Chol.



Figure 4.16: Intermediate-resolution TEM images of ND-COOH, ND-C18, EM-Hem, and EM-Hem/Chol.



Figure 4.17: High-resolution TEM images of ND-COOH, ND-C18, EM-Hem, and EM-Hem/Chol.



Figure 4.18: Photoluminescence (PL) intensity as a function of delay time,  $\tau$ , between an optical initialization pulse and subsequent readout pulse, for (A) ND-C18, (B) ND-COOH (C) EM-Hem and (D) EM-Hem/Chol dispersions. Solid curves are fits using Equation 2 of the Main Text. The PL signal is normalized to the intensity at  $\tau = 9$  ms, and error bars represent the uncertainty from photon shot noise. The curves within panel A-C represent multiple measurements of the same sample.
#### 4.5.3. Estimating Hemin Concentration

The hemin concentrations for the emulsions are calculated from the extinction curves of the samples (Main Text Figure 2) and a molar extinction coefficient of  $16 \pm 3 * 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ , which is generated by measuring the concentration dependence of the hemin absorbance. Here we fit the extinction curves for EM-Hem and EM-Hem/Chol to a linear combination of ND-C18 and hemin extinction curves to isolate the hemin contribution for each emulsion. If we assume spherical 68 nm diameter nanodiamonds and propagate the uncertainty from the hemin concentration analysis, for EM-Hem we estimate 9300  $\pm$  3600 hemin molecules per nanodiamond and for EM-Hem/Chol we estimate 6800  $\pm$  3300 hemin molecules per nanodiamond.

#### 4.5.4. Additional Information for NV-Center Spin Lifetime

Our modeling of  $T_1$  for a NV center in a nanodiamond builds on the analysis reported by Tetienne *et al.*[137]. The relaxation rate  $1/T_1$  for an NV center placed in a zero mean magnetic field B(t) with a variance of  $B_{\perp}^2$  can be expressed as follows:

$$\frac{1}{T_1} = \frac{1}{T_1^0} + 3\gamma_e^3 B_\perp^2 \frac{\tau_c}{1 + \omega_0^2 \tau_c^2}$$
(4.8)

where  $T_1^0$  is the spin lifetime in the absence of a magnetic field,  $\tau_c$  is the correlation time of the bath,  $\gamma_e$  is the electron gyromagnetic ratio, and  $\omega_0$  is the electron spin resonance frequency ( $\omega_0 = 2 * \pi * 2.87 GHz$ ). The bath's correlation time is given by:

$$\tau_c = \frac{1}{R_{\rm dip} + R_{\rm vib}} \tag{4.9}$$



Figure 4.19: Schematic representation of a nanodiamond (grey-filled circle) with radius  $R_{ND}$ and a surface coating of thickness  $r_{sl}$ , denoted by the blue circle. NV centers (blue-filled circles) are randomly distributed in the nanodiamond, but consistent with literature, do not form within an exclusion zone  $r_{excl}$  of the nanodiamond surface, denoted by the red circle. The exterior spin bath (gold-filled circles) can exist either as a surface distribution along the blue circle (denoted as  $\sigma$ ) or as a volumetric distribution (denoted as  $\rho$ ). The minimum distance between exterior spins is given by  $D_{min}$ .

where the rate  $R_{\rm vib}$  arises from the intrinsic vibrational spin relaxation of the bath and the rate  $R_{\rm dip}$  originates from inter-spin dipolar interactions, which can be approximated by  $\hbar R_{\rm dip} = \sqrt{\sum_{j \neq i} \langle H_{ij}^2 \rangle}$  where  $H_{ij}$  is the magnetic dipolar interaction between spin *i* and *j* of the bath.

In our analysis, we treat the nanodiamond as a sphere with a radius  $R_{\rm ND}$ . Inside this sphere, the NV centers are distributed randomly, but they are restricted to a maximum radius that excludes a shell of thickness  $r_{\rm excl}$ . The exclusion thickness  $r_{\rm excl}$  for NV centers in nanodiamonds is estimated to range from 2 nm to 3 nm [18]. Exterior spins can be excluded from a shell of thickness  $r_{\rm sl}$  defined by the thickness of the coating layer. Each of these exterior spins cannot be positioned closer to each other than a threshold  $(D_{\rm min})$  which is defined by the spin molecule's size and packing density. For a surface coating of spins, we can model the average variance  $B_{\perp}^2$ 

$$B_{\perp}^{2} = \left(\frac{\mu_{0}\gamma_{e}\hbar}{4\pi}\right)^{2} \frac{S(S+1)}{3}$$
$$\sigma \int_{0}^{2\pi} d\phi \int_{0}^{\pi} d\theta \sin \theta \frac{2+3\sin(\alpha(\phi,\theta)^{2})}{r(\phi,\theta)^{4}}$$
(4.10)

where  $\sigma$  is the density of surface spins with spin S. For an NV center at the center of a nanodiamond we set  $\alpha(\phi, \theta) = \theta$  and  $r(\phi, \theta) = \frac{D_0}{2}$ . To simplify the calculations, we consider only NV centers moving along the line parallel (P<sub>Parr</sub>) or perpendicular (P<sub>Perp</sub>) to the NV center's spin axis. These two paths provide the highest and lowest sensitivity to the external fields respectively.

For NV centers along  $P_{Parr}$  we set  $r(\phi, \theta) = \sqrt{\frac{D_0^2}{2} - \delta r^2 \sin(\theta)^2} + \delta r \cos(\theta)$  and  $\alpha(\phi, \theta) = \theta$ . For NV centers along  $P_{Perp}$  we set  $r(\phi, \theta) = \sqrt{\frac{D_0^2}{2} - \delta r^2 \sin(\theta)^2} + \delta r \cos(\theta)$  and  $\alpha(\phi, \theta) = \cos(\theta)^2 + \sin(\theta)^2 \sin(\phi)^2$ , where  $D_0$  is the diameter give by  $2(R_{ND} + r_{sl})$ .

We can model the  $B_{\perp}^2$  experienced by an average NV center by integrating along both paths from  $\delta r = 0$  to  $\delta r = R_{ND} - r_{\text{excl}}$ .

The results are weighted based on the differential volume at each  $\delta r$ , taking into account the random spatial distribution of NV centers in a milled nanodiamond.

The average  $B_{\perp}^2$  seen by an average NV center is then given by:

$$\left\langle B_{\perp}^{2} \right\rangle = \frac{\int_{0}^{R_{ND} - r_{\text{excl}}} B_{\perp}^{2}(D_{0}, \delta r) \delta r^{2} d\delta r}{\int_{0}^{R_{ND} - r_{\text{excl}}} \delta r^{2} d\delta r}$$
(4.11)

R<sub>dip</sub> can be calculated according to:

$$R_{\rm dip} = \frac{1}{\hbar} \sqrt{\sum_{j \neq i} \langle H_{ij}^2 \rangle}$$
$$= \frac{\mu_0 \gamma_e^2 \hbar S(S+1)}{2\sqrt{6\pi}} \sqrt{\sigma \int_{D_{\rm min}}^\infty \frac{2\pi dr}{r^5}}$$
(4.12)

To model the impact of the  $\mathrm{Fe}^{3+}$  present in the hemin molecules on the  $T_1$  of the emulsions, we set  $R_{Vib} = 0.1$  GHz [133], S = 5/2 [8],  $r_{excl} = 3.0$  nm, and  $T_1^0$  as 505  $\pm$  113  $\mu s$ , the  $\langle T_1 \rangle$  of ND-C18. The results of this model for 68 nm diameter nanodiamonds,  $r_{sl}$  ranging from 0.5 nm to 4 nm, and  $D_{min}$  ranging from 0.34 nm (the intermolecular distance in hemin assemblies [85]) to 1.57 nm (the length of the hemin molecule), studied to account for the potential orientations of the amphiphiles on the nanodiamond surfaces, are shown in Figure 4.20. Here we see that the upper bound of our model, representing lower sensitivity of the NDs to the ions, (Figure 4.20) offers a more accurate description of the hemins' impact on  $\langle T_1 \rangle$ . This result implies that the hemin molecules are held slightly farther from the diamond surface than we initially expected. Alternatively, we could be observing a competing force where the emulsion process quenches some surface spins on the nanodiamonds thereby counteracting the reduction from the addition of  $Fe^{3+}$  spins. We note an alternative amphiphilic molecule could reduce or eliminate this observed reduction in  $T_1$  (Figure 4.14). Simply changing hemin-chloride to hemin-bromide, which is structurally similar and thus should not impact the stability of the emulsions, is reported to have a lower  $Fe^{3+}$  spin[111], and thus is expected to decrease the hemin's effect on  $\langle T_1 \rangle$ .



Figure 4.20: Modeled impact of Fe<sup>3+</sup> on  $\langle T_1 \rangle$  for a 68 nm diameter nanodiamond. The upper bound of the shaded region describes a model where  $r_{sl} = 4.0$  nm,  $D_{min} = 1.57$  nm, and  $T_1^0 = 618 \ \mu$ s and the lower bound is a model for  $r_{sl} = 0.5$  nm,  $D_{min} = 0.34$  nm, and  $T_1^0 = 392 \ \mu$ s.  $\langle T_1 \rangle$  are shown for ND-C18 (green star), EM-Hem (brown dot), and EM-Hem/Chol (yellow triangle) dispersions. Vertical error bars represent the standard deviation of  $\langle T_1 \rangle$  from measurements of multiple samples for ND-C18 and EM-Hem or the fit uncertainty from a single measurement of EM-Hem/Chol. Horizontal error bars represent the propagated uncertainty of the hemin concentration estimate.

To model the impact of the  $\mathrm{Gd}^{3+}$  chelates, we set  $R_{Vib} = 1$  GHz [28], S = 7/2, and  $D_{min} = 0.5$  nm. According to Equation 4.8, the higher S and  $R_{Vib}$  values of the  $\mathrm{Gd}^{3+}$  chelates is expected to reduce  $T_1$  twice as much as the Fe<sup>3+</sup> in the hemin for the same ion to nanodiamond ratio. For chemical sensitivity experiments, where  $\mathrm{Gd}^{3+}$  chelates are in solution, and thus present beyond the surface of the nanodiamond, we must modify  $B_{\perp}^2$  and  $R_{dip}$  to account for these additional spins. When the  $Gd^{3+}$  are not only bound to the nanodiamond surface,  $B^2_{\perp_{vol}}$  takes the form:

$$B_{\perp,\text{vol}}^{2}(\xi,\theta) = \left(\frac{\mu_{0}\gamma_{e}\hbar}{4\pi}\right)^{2} \frac{S(S+1)}{3} \rho \int_{\frac{D_{0}}{2}}^{\infty} dr \int_{0}^{2\pi} d\phi \int_{0}^{\pi} d\theta \sin\theta \frac{2+3\sin(\alpha(\phi,\theta)^{2})}{r(\phi,\theta)^{4}}$$
(4.13)

Once again, we consider NV centers along  $P_{Parr}$  and  $P_{Perp}$ , as described above, and take a weighted average of the components to derive  $\langle B_{\perp}^2 \rangle$ . Similarly, for unbounded spins,  $R_{dip}$  takes the form:

$$R_{\rm dip} = \frac{1}{\hbar} \sqrt{\sum_{j \neq i} \left\langle H_{ij}^2 \right\rangle}$$
$$= \frac{\mu_0 \gamma_e^2 \hbar S(S+1)}{2\sqrt{6\pi}} \sqrt{\rho} \int_{D_{\rm min}}^{\infty} \frac{4\pi dr}{r^4}$$
(4.14)

In Figure 4.21, the results of this model are presented for a  $T_1^0$  of 375  $\mu$ s, taken as the  $\langle T_1 \rangle$  measured for EM-Hemin, and a range of  $r_{\text{excl}}$  from 0.1 nm to 5 nm. However, since a  $r_{\text{excl}}$  value of 0.1 nm is not physical, we conclude and describe in the Main Text that the Gd<sup>3+</sup> chelates must adsorb to the nanodiamond surface, and the dependence of  $T_1$  on the concentration of Gd<sup>3+</sup> chelates is well described by a simple Langmuir model.



Figure 4.21:  $\langle T_1 \rangle$  vs  $[Gd^{+3}]$  for EM-Hem (brown), along with  $T_1$  curves calculated using Equations 4.8, 4.13, and 4.14, with values of  $r_{\text{excl}}$  ranging from 0.1 nm to 5.0 nm.



Figure 4.22: Photoluminescence contrast  $(C_S)$  vs  $[Gd^{3+}]$  for different concentrations of the  $Gd^{3+}$  chelate added to aqueous dispersions of ND-COOH (green stars) and EM-Hem (brown circles) nanodiamonds. Error bars represent 68% confidence intervals from fit.



Figure 4.23:  $\beta vs$  [Gd<sup>+3</sup>] for different concentrations of the Gd<sup>3+</sup> chelate added to aqueous dispersions of ND-COOH (green stars) and EM-Hem (brown circles) nanodiamonds. Error bars represent 68% confidence intervals from fit.



Figure 4.24:  $\langle T_1 \rangle$  vs [Gd<sup>+3</sup>] for different concentrations of the Gd<sup>3+</sup> chelate added to aqueous dispersions of ND-COOH (green stars) and EM-Hem (brown circles) nanodiamonds. Error bars represent 68% confidence intervals from fit.



Figure 4.25: Photoluminescence (PL) intensity as a function of delay time,  $\tau$ , between an optical initialization pulse and subsequent readout pulse, for a ND-C18 dispersion before (green, stars) and after (purple boxes) the addition of 2 mM of Gd<sup>3+</sup> chelate. Solid curves are fits using Equation 2 of the Main Text. The PL signal is normalized to the intensity at  $\tau = 9$  ms, and error bars represent the uncertainty from photon shot noise.



Figure 4.26: Photoluminescence (PL) intensity as a function of delay time,  $\tau$ , between an optical initialization pulse and subsequent readout pulse, for an EM-Hem dispersion before (brown, dots) and after (purple boxes) the addition of 2 mM of Gd<sup>3+</sup> chelate. Solid curves are fits using Equation 2 of the Main Text. The PL signal is normalized to the intensity at  $\tau = 9$  ms, and error bars represent the uncertainty from photon shot noise.



4.5.5. Additional Information for Carbodiimide Crosslinker Conjugation

Figure 4.27: Average particle diameters from DLS measurements of samples of ND-COOH and EM-Hem unmodified (red), and upon addition of the various carbodiimide crosslinking chemicals: with EDC (blue), with HEPES buffer (orange), and with EDC and HEPES buffer (purple). Error bars represent one standard deviation of the size distribution.

We perform carbodiimide crosslinker conjugation between EM-Hem (ND-Hemin) emulsions and the dye molecule n-succinimidlyl ester (TAMRA amine, 5-isomer), purchased from Lumiprobe, under similar conditions to those found in the Main Text.



Figure 4.28: UV-Vis extinction spectra of ND-Hemin emulsions conjugated to the TAMRA dye (cyan, TAMRA dye), with EDC (blue, ND Hemin Conjugate) without EDC (magenta, ND Hemin Control) after dialysis.



Figure 4.29: DLS measurements of EM-Hem conjugation to the  $Gd^{3+}$  chelate using EDC, pre- (dotted) and post- (solid) dialysis for the [EDC]:[Gd<sup>3+</sup>] molar ratios discussed in the main text.

4.5.6. Additional Information for Click Chemistry Conjugation

We perform click chemistry conjugation with EM-Hem/Chol emulsions and fluorescein dibenzocyclooctyne (FAM-DBCO), purchased from Lumiprobe. Control measurements are taken with EM-Hem emulsions which do not have an choesteryl-TEG azide, and thus do not have the requisite azide termination for conjugation.



Figure 4.30: Photoluminescence (PL) spectra for FAM-DBCO click conjugation: EM-Hem/Chol conjugated to FAM-DBCO after washing *via* centrifugation (blue), EM-Hem mixed with FAM-DBCO after washing carried out as a control (red), and FAM-DBCO at a concentration 1x EM-Hem/Chol molarity (yellow).



Figure 4.31: Full FTIR spectra for EM-Hem/Chol dispersions conjuagted to  $\mathrm{Gd}^{3+}$  dibenzocyclooctyne (DBCO) at 0:1 (yellow), 1:4 (orange), 2:1 (lavender), and 10:1 (purple)  $\mathrm{Gd}^{3+}$ dibenzocyclooctyne (DBCO) to cholesteryl-TEG azide  $(-N_3)$  ratios after dialysis.

As a control, here we compare the click chemistry conjugation of EM-Hem/Chol emulsions with DBCO-terminated  $Gd^{+3}$  chelate spin labels to samples of ND-COOH and EM-Hem, which do not have surface species with azide termination, that are requisite for click chemistry conjugation.



Figure 4.32:  $T_1^S$  for click chemistry conjugation experiments with NC-COOH, EM-Hem, and EM-Hem/Chol dispersions and the Gd<sup>3+</sup> dibenzocyclooctyne chelate. For EM-Hem/Chol dispersions, experiments are carried out for dibenzocyclooctyne (DBCO) to azide  $(-N_3)$  molar ratios of 0:1 (blue), 2:1 (before dialysis: orange; after dialysis: yellow), and 10:1 (before dialysis: purple; after dialysis: green). For ND-COOH and EM-Hem, which do not have surface functional groups with azide termination, the ratios refer to the addition of identical Gd<sup>3+</sup> concentrations as used for EM-Hem/Chol conjugation reactions. Error bars represent the best-fit confidence intervals.



Figure 4.33:  $\beta$  for click chemistry conjugation experiments with NC-COOH, EM-Hem, and EM-Hem/Chol dispersions and the Gd<sup>3+</sup> dibenzocyclooctyne chelate. For EM-Hem/Chol dispersions, experiments are carried out for dibenzocyclooctyne (DBCO) to azide ( $-N_3$ ) molar ratios of 0:1 (blue), 2:1 (before dialysis: orange; after dialysis: yellow) and 10:1 (before dialysis: purple; after dialysis: green) Dibenzocyclooctyne (DBCO) to azide ( $-N_3$ ) molar ratios samples, for ND-COOH, EM-Hem, and EM-Hem/Chol. ND-COOH and EM-Hem do not have azide termination. Therefore ratios refer to the addition of identical Gd<sup>3+</sup> concentrations as EM-Hem/Chol. Error bars represent the best-fit confidence intervals.



Figure 4.34: Photoluminescence contrast  $(C_S)$  for click chemistry conjugation experiments with NC-COOH, EM-Hem, and EM-Hem/Chol dispersions and the Gd<sup>3+</sup> dibenzocyclooctyne chelate. For EM-Hem/Chol dispersions, experiments are carried out for dibenzocyclooctyne (DBCO) to azide  $(-N_3)$  molar ratios of 0:1 (blue), 2:1 (before dialysis: orange; after dialysis: yellow), and 10:1 (before dialysis: purple; after dialysis: green). For ND-COOH and EM-Hem, which do not have surface functional groups with azide termination, the ratios refer to the addition of identical Gd<sup>3+</sup> concentrations as used for EM-Hem/Chol conjugation reactions. Error bars represent the best-fit confidence intervals.



Figure 4.35:  $\langle T_1 \rangle$  for click chemistry conjugation experiments with NC-COOH, EM-Hem, and EM-Hem/Chol dispersions and the Gd<sup>3+</sup> dibenzocyclooctyne chelate. For EM-Hem/Chol dispersions, experiments are carried out for dibenzocyclooctyne (DBCO) to azide ( $-N_3$ ) molar ratios of 0:1 (blue), 2:1 (before dialysis: orange; after dialysis: yellow), and 10:1 (before dialysis: purple; after dialysis: green). For ND-COOH and EM-Hem, which do not have surface functional groups with azide termination, the ratios refer to the addition of identical Gd<sup>3+</sup> concentrations as used for EM-Hem/Chol conjugation reactions. Error bars represent the best-fit confidence intervals.



Figure 4.36: ICP-OES measurements of remaining  $[Gd^{3+}]$  for click chemistry conjugation experiments with NC-COOH, EM-Hem, and EM-Hem/Chol dispersions and the  $Gd^{3+}$  dibenzocyclooctyne (DBCO) chelate. For EM-Hem/Chol dispersions, experiments are carried out for DBCO to azide  $(-N_3)$  molar ratios of 0:1 (blue), 2:1 (orange), and 10:1 (yellow). For ND-COOH and EM-Hem, which do not have surface functional groups with azide termination, the ratios refer to the addition of identical  $Gd^{3+}$  concentrations as used for EM-Hem/Chol conjugation reactions. Error bars represent propagated uncertainty from the best-fit confidence intervals for the OES-ICP gadolinium calibration curve.

Here we compare the sensitivity of EM-Hem/Chol emulsions to DBCO-terminated and a mine-terminated  $Gd^{+3}$  chelate spin labels.



Figure 4.37: Comparison of  $T_1^S$  for samples of EM-Hem/Chol without any Gd<sup>3+</sup> (0:1), with 10  $\mu$ M and 200  $\mu$ M concentrations of amine-terminated Gd<sup>3+</sup>, and post dialysis of EM-Hem/Chol conjugated to DBCO-terminated Gd<sup>3+</sup> at initial DBCO to azide ( $-N_3$ ) molar ratios of 1:4, 2:1 and 10:1 respectively. These ratios translate to initially added [Gd<sup>3+</sup>] of approximately 25  $\mu$ M, 200  $\mu$ M, and 1000  $\mu$ M, respectively. See Figure 6 in the Main Text for ICP-OES measurements of remaining Gd<sup>3+</sup> in the click conjugated samples. Error bars represent the best-fit confidence intervals.



Figure 4.38: Comparison of  $\beta$  for samples of EM-Hem/Chol without any Gd<sup>3+</sup> (0:1), with 10  $\mu$ M and 200  $\mu$ M concentrations of amine-terminated Gd<sup>3+</sup>, and post dialysis of EM-Hem/Chol conjugated to DBCO-terminated Gd<sup>3+</sup> at initial DBCO to azide ( $-N_3$ ) molar ratios of 1:4, 2:1 and 10:1 respectively. These ratios translate to initially added [Gd<sup>3+</sup>] of approximately 25  $\mu$ M, 200  $\mu$ M, and 1000  $\mu$ M, respectively. See Figure 6 in the Main Text for ICP-OES measurements of remaining Gd<sup>3+</sup> in the click conjugated samples. Error bars represent the best-fit confidence intervals.



Figure 4.39: Comparison of photoluminescence contrast  $(C_S)$  for samples of EM-Hem/Chol without any Gd<sup>3+</sup> (0:1), with 10  $\mu$ M and 200  $\mu$ M concentrations of amine-terminated Gd<sup>3+</sup>, and post dialysis of EM-Hem/Chol conjugated to DBCO-terminated Gd<sup>3+</sup> at initial DBCO to azide  $(-N_3)$  molar ratios of 1:4, 2:1 and 10:1 respectively. These ratios translate to initially added [Gd<sup>3+</sup>] of approximately 25  $\mu$ M, 200  $\mu$ M, and 1000  $\mu$ M, respectively. See Figure 6 in the Main Text for ICP-OES measurements of remaining Gd<sup>3+</sup> in the click conjugated samples. Error bars represent the best-fit confidence intervals.



Figure 4.40: Comparison of  $\langle T_1 \rangle$  for samples of EM-Hem/Chol without any Gd<sup>3+</sup> (0:1), with 10  $\mu$ M and 200  $\mu$ M concentrations of amine-terminated Gd<sup>3+</sup>, and post dialysis of EM-Hem/Chol conjugated to DBCO-terminated Gd<sup>3+</sup> at initial DBCO to azide ( $-N_3$ ) molar ratios of 1:4, 2:1 and 10:1 respectively. These ratios translate to initially added [Gd<sup>3+</sup>] of approximately 25  $\mu$ M, 200  $\mu$ M, and 1000  $\mu$ M, respectively. See Figure 6 in the Main Text for ICP-OES measurements of remaining Gd<sup>3+</sup> in the click conjugated samples. Error bars represent the best-fit confidence intervals.



Figure 4.41: DLS measurements of EM-Hem/Chol dispersions before conjugation (0:1), after click conjugation (10:1), and post dialysis for the 10:1 conjugated sample (10:1 post dialysis), where 10:1 is the DBCO to azide  $(-N_3)$  molar ratio sample.



Figure 4.42:  $T_1^S$  vs average number of Gd<sup>+3</sup> per nanodiamond for adsorbed chelates from solution and for both carbodiimide and click chemistry conjugation. A model of  $T_1^S$  with  $T_1^0$ = 269 ± 36  $\mu$ s and  $R_{ND}$  =34 nm and with  $r_{sl} < 4$  nm (light green region) and with  $r_{sl}$  is between 4 nm and 8 nm (dark green region). Brown triangles display values of  $T_1^S$  for the adsorbed Gd<sup>3+</sup> chelates on EM-Hem samples, as seen in Figure 4 of the Main Text. Error bars represent represent 68% confidence intervals for  $T_1^S$  and the 68% confidence intervals for Langmuir model fit described in the Main Text. Brown stars depict values of  $T_1^S$  for the EDC-mediated conjugation at 0:1, 2:1 and 3:1 [EDC]:[Gd<sup>3+</sup>] ratios respectively. Error bars represent represent 68% confidence intervals for  $T_1^S$  and uncertainty from ICP-OES measurements, assuming a 68 nm diameter spherical nanodiamond. Yellow circles depict values of  $T_1^S$  for the click chemistry conjugation at 1:4, 2:1 and 10:1 DBCO to N<sub>3</sub> ratios. Error bars represent represent 68% confidence intervals for  $T_1^S$  and uncertainty from ICP-OES measurements, assuming a 68 nm diameter spherical nanodiamond. Yellow circles depict values of  $T_1^S$  for the click chemistry conjugation at 1:4, 2:1 and 10:1 DBCO to N<sub>3</sub> ratios.

The results of the ND-Gd<sup>+3</sup> chelate experiments and the previously described  $T_1$  model are

in close agreement (Figure 4.42). The values of  $T_1^{\rm S}$  for nanodiamonds with unconjugated Gd<sup>+3</sup> chelates are found in the region where  $r_{sl} < 1$  nm, consistent with surface adsorption. In contrast, the values of  $T_1^{\rm S}$  for the conjugated nanodiamond emulsions are consistent with the Gd<sup>+3</sup> spin labels spaced from the nanodiamond surface by the nanometer-scale thickness of the the emulsion coatings.

## CHAPTER 5

## FUTURE WORKS

Chapter 5 describes ongoing and future work. Section 5.1 expands the templated self assembly method to multiparticle assemblies. Section 5.2 describes the plans to study an even larger distribution of nanodiamonds ranging from 10nm to 100nm to better understand the properties of the quantum emitters. Section 5.3 suggests new methods for modifying the NV center through emulsions. Finally, Section 5.4 presents challenges in deploying nanodiamond emulsions in biological systems to act as quantum sensors and nanomedical devices.

## 5.1. Expanding Template Self Assembly

Chapter 3 discussed the self-assembly of individual nanodiamonds into arrays, but this same procedure could easily be extended to multiparticle assembles. The general idea would be to add additional nanoparticles that would interact in a positive way with the nanodiamonds. For example, 50nm gold nanoparticles have a plasmonic resonance that overlaps with the absorption spectra of the NV center. Therefore, the co-assembled gold could act as a plasmonic antenna and increase the absorption cross section of the diamond. Beside nanolithography, the major limiting factor for these assemblies is the size of the particles. Sequential templated self-assemblies must occur from largest to smallest nanoparticle to ensure initial particles do not assemble into spots designed for larger particles. This requirement will make working with the polydisperse milled nanodiamonds somewhat difficult. One solution would be to always assembly the nanodiamonds last. However, assembling the nanodiamonds last makes it difficult to determine a direct impact of previously assembled materials.



Figure 5.1: SEM images of 50nm gold nanoparticle and nanoidamond assembly. (A) SEM wide area image of template. (B) SEM image of assembly of two nanodiamonds (ND) with gold nanoparticles (Au)

Figure 5.1 shows an early effort to co-assembly multiple gold particles to a nanodiamond. Here, multiple gold particles and nanodiamonds assemble in an uncontrolled manner in the template holes. More work is required to standardize this process and characterize the impact on the NV center's optical and spin properties.

The templates themselves could also be modified to change the optical features of the NV center. Templates could either be made out metal with plasmonic nanoholes [117] or templates could be utilized to first construct nanopillars which particles would then be assembled on. These proposals will all require adjustments to the assembly procedure but will offer additional control over the optical and quantum properties of the NV center.

#### 5.2. Longitudinal Nanodiamond Solution Study

All of the studies describe in the previous chapters could be replicated and expanded to cover the full range of commercially available milled nanodiamonds. To start, I would recommend combining the statistical goals of Chapter 3 with the solution averaging methods of Chapter 4. In such a study, nanodiamonds ranging from 10nm-100nm in diameter, with various surface terminations, and NV center densities would be purchased and subjected to the following measurements: DLS (size), fluorimeter (emission), UV-Vis (absorption),  $T_1$ (quantum isolation) and  $T_1$  vs. Gd<sup>+3</sup> (sensitivity). All of these measurements can be easily done in solution with the methods and procedures found in this thesis. Once these initial experiments are performed, more time-consuming measurements such as electrochemical measurements and AFM and SEM measurements, to better characterize the morphology of the nanoparticles, can be performed. This study would allow us to expand on the relationships observed in Chapter 3 and better understand the impact of surface conditions and morphology on the optical and spin properties of the NV center. Similar relationships could be found between size  $T_1$  and  $F_{\rm NV^-}$  as were described in Chapters 3 and 4; though, it will be interesting to see how these dependencies change with the surface to volume ratio of the nanodiamonds and the NV center density.

## 5.3. NV Center Emulsions Enhancement

Here, we have studied three amphiphilic materials for emulsions. PPIX was discarded due to its florescent overlap with the NV center and oleic acid was only studied briefly in comparison to hemin. There is, however, a massive library of amphiphilic materials that could be used to form nanodiamond emulsions. Each of these materials will require different ratios of amphiphilic to nanodiamond. Initially, we could examine chlorophyll A to isolate the role that iron plays in the hemin emulsions. By expanding the library of amphiphilics, we can not only increase the functionality of the emulsions but also better understand the properties of emulsions as a platform.

Chapter 4 touched on the impact of the emulsion process on NV center charge state. Emulsions made with hemin amphiphilics demonstrated larger increases in  $F_{\rm NV^-}$  then oleic acidbased emulsions. Expanding on this observation, it should be possible to tune the charge state of the NV centers with the emulsion process. But first we must determine the exact cause of this increase in charge state. The additional carboxyl groups on the hemin may be lowering the fermi level on the diamond surface and thereby decreasing the ionization

rate of the NV center. The hemin carboxyl groups could also be donating electrons to the nanodiamond surface. Both of these actions would explain why we observe a relationship between number of carboxyl groups in an emulsion and  $F_{\rm NV^-}$ . Alternatively, the hemin molecules could be quenching the NV<sup>0</sup> emission through either an absorption or nonradiative process; although, the low levels of absorption observed from free hemin molecules does not support this explanation. To test these hypotheses, we can study the hemin emulsions in an electrochemical cell. The cell will allow us to externally control the charge state of the emulsions. If we are merely observing a quenching of the  $NV^0$  emission, forcing the NV centers into the positive charge state should lead to a significant drop in PL intensity. Conversely, if we are observing an emulsion-based increase in the  $F_{\rm NV^-}$ , the application of a negative voltage should not impact the emission spectra or intensity. Unfortunately, hemin suffers from a narrow electrochemical potential window compared to the NV centers and may break down during these experiments. [35] Instead, we could fabricate emulsions with different amphiphilic materials that can better handle an oxidation and reduction cycle. We could also focus on an amphiphilies that do not contain carboxyl surface groups. Ammonium type amphiphilic molecules, for example, could provide a positive surface charge to nanodiamonds assuming they could form stable emulsions [77]. Alternative amphiphilic materials may be less conducive to crosslinking conjugation but could help shed light on the impact of surface termination on NV properties.

## 5.4. Biological Studies with Emulsion Nanodiamonds

Finally, we should work towards deploying the nanodiamond emulsion into biological samples. Establishing targeting techniques and developing the nanodiamond emulsions into living cells is a major priority. Our first attempt has been the creation of folic acid capped emulsions. Folic acid is readily absorbed by KB cells [140, 96] and folic acid terminated lipids (DSPE-PEG(5000) Folate, Avanti) can be utilized to form stable emulsions in combination with hemin. A major struggle here has been the small concentrations of nanodiamonds and low emission rates of the NV center, which makes it difficult to detect if the nanodiamonds are being absorbed by the cells. Working at higher concentrations and with brighter, more NV center rich, nanodiamonds could solve this problem.

An alternative approach would be directly conjugating biological molecules to the emulsions. Proteins and antibodies with amine or azide terminations exist and could be conjugated to nanodiamond emulsions. While the conjugation should be simple, the growing of the biological material is itself a timely process.

# CHAPTER 6

# CONCLUSION

While the potential for quantum engineering to transform the fields of computation, communication and sensing is clear, engineers must first identify and develop the physical basis for these rapidly developing technologies. Here, we have focused on NV centers in nanodiamonds as a particularly compelling basis for these technologies due to the nanoparticles' room temperature, optically-addressable quantum states, nontoxic nature, and ability to form stable colloidal dispersions. These properties make nanodiamonds valuable for quantum sensing applications wherein the NV center's quantum sensitivity to magnetic, electrochemical and thermodynamic fields can be measured with nanoscale resolution in *in vivo* and *in vitro* environments with little measurement overhead. However, as described in Chapter 2, the benefits of NV center rich nanodiamonds comes at the cost of significant irregularity. The milling process inherently results in a jagged polydisperse nanoparticle, while electron irradiation leads to uncontrolled placement of NV centers within the diamond lattice. Additionally, techniques for functionalizing nanodiamonds often depend on morphology of therefore vary between particles. Together, these features complicate any effort to capitalize on the beneficial properties of nanodiamonds and their integration into more complex quantum systems. Improvements to the milling and irradiation process could mitigate some of these issues. More regular nanodiamonds could be fabricated by applying diamond CVD growth techniques to the nanoscale. [156, 103] Proper templates could act both to assemble seed diamond crystals and to limit CVD growth to specific sizes and shapes. Despite these potential future improvements, milled nanodiamonds are currently the best commercially viable method for synthesizing materially pure nanodiamonds. With particle irregularity identified as a major obstacle, the bulk of this thesis focused on new methods for controlling, modifying and characterizing nanodiamonds.

Chapter 3 established methods for self-assembly of nanodiamonds over large areas. We found that the irregularity of the nanodiamonds and tendency to agglomerate proved a major, but solvable, problem for the capillary assembly-based methods. The arrays were utilized to perform a large scale study of the optical and spin properties of 40nm nanodiamonds. From this study we identified surface and environmental conditions as the major driver of the nanodiamonds optically and spin inhomogeneity. Our method will allow researchers to place nanodiamonds with nanometer precision over millimeter scale areas. This work has applications in the field of sensing and overall nanodiamond engineering.

With these results in mind, Chapter 4 focused on the surface modification of colloidal nanodiamonds through the formation of emulsions. The emulsion method, allowed us to tailor the nanodiamond surface termination without the need for long or complex treatments that can detrimentally impact the spin and optical characteristics of the NV center. The fabricated emulsions exhibited decreased levels of agglomeration in non-ideal and acidic solutions. The emulsions also enabled crosslinking and click chemistry conjugation reactions that are currently unachievable for commercially available nanodiamonds.

Chapter 5proposed immediate research projects to capitalize on the previously mentioned results.

Collectively, this dissertation lays the groundwork for the construction of tailor-made quantum platforms. Through sequential multi-particle self-assembly, quantum heterostructures consisting of nanodiamonds and additional enhancement particles, such as plasmonic metal nanoparticles or upconverting nanophosphors, will allow researchers to engineer the absorption and emission characteristics of the NV center. The heterostructures could then be lifted off the templates, redispersed and encapsulated in emulsions to define heterostructures'
surface termination, avoid agglomeration, and enable additional conjugation. Alternatively, nanodiamond emulsions could be conjugated to particles too large or too small for templated assembly such as magnetic ions, DNA oligos or proteins and subsequently self-assembled into template arrays to form large area devices. In either direction, with these two methods, we can precisely design characteristics of NV center nanoparticles and deploy these quantum systems into any environment. While we cannot anticipate every future application, we have developed the tools necessary for realizing the potential of irregular nanodiamonds.

With these tools and this dissertation, I hope to have contributed to the future development of quantum mechanical devices and technology.

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