Diamond Quantum Devices

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Diamond Quantum Devices in Biology

Yuzhou Wu, Fedor Jelezko,* Martin B Plenio,* and Tanja Weil**

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The currently available techniques for molecular imaging capable of reaching atomic resolution are limited to low temperatures, vacuum conditions, or large amounts of sample. Quantum sensors based on the spin-dependent photoluminescence of nitrogen-vacancy (NV) centers in diamond offer great potential to achieve single-molecule detection with atomic resolution under ambient conditions. Diamond nanoparticles could also be prepared with implanted NV centers, thereby generating unique nanosensors that are able to traffic into living biological systems. Therefore, this technique might provide unprecedented access and insight into the structure and function of individual biomolecules under physiological conditions as well as observation of biological processes down to the quantum level with atomic resolution. The theory of diamond quantum sensors and the current developments from their preparation to sensing techniques have been critically discussed in this Minireview.

1. Introduction

Molecular-scale imaging and structure-determination methods such as X-ray diffraction, NMR spectroscopy, electron microscopy, and scanning tunneling microscopy play a pivotal role in scientific advancement of various disciplines. Most currently available techniques capable of reaching atomic resolution are limited to low temperatures, vacuum conditions, or the availability of crystals and/or large sample volumes. These requirements limit their potential impact especially in the area of life sciences, where single-molecule optical spectroscopy and atomic force microscopy are the only methods able to gain insight into the molecular structure, although their resolution is not sufficient to detect single atoms.

Quantum sensors based on the spin-dependent photoluminescence of nitrogen-vacancy (NV) centers in diamond offer great potential for imaging with atomic resolution.[1] The advent of diamond quantum sensing promises a solution to the longstanding goal of single-molecule detection with atomic resolution under ambient conditions.[1,2] There is currently no other technique that can offer such features. Therefore, this technique would offer great impact for understanding biomolecules in their native environments as well as fundamental biological phenomena.

This Minireview describes the new and rapidly evolving field of nanoscale quantum sensing based on NV centers in diamond as well as potential applications for super-resolution molecular imaging in living biological systems. The theoretical principles and most advanced techniques of diamond-based quantum sensing are summarized. In particular, the chemical preparation of high-quality diamond quantum devices and the modification of diamonds for sensing in a living biological environment will be introduced. Their great potential and challenges for biosensing under physiological conditions are especially highlighted.

2. The Theory of Diamond Quantum Sensing

A perfect diamond crystal is transparent to visible light because of its large optical band gap of $E = 5.48$ eV (which corresponds to a wavelength of $\lambda \approx 226$ nm). Despite this basic physical property, natural diamond is found on rare occasions to display vivid color. The source of this color can

[*] Dr. Y. Wu, Prof. Dr. T. Weil
Institut für Organische Chemie III, Universität Ulm
Albert-Einstein-Allee 11, 89081 Ulm (Deutschland)
E-mail: Yuzhou.Wu@uni-ulm.de
Tanja.Weil@uni-ulm.de
Prof. Dr. F. Jelezko
Institut für Quantenoptik, Universität Ulm
Albert-Einstein-Allee 11, 89081 Ulm (Deutschland)
E-mail: fedor.jelezko@uni-ulm.de
Prof. Dr. M. B. Plenio
Institut für Theoretische Physik
Albert-Einstein-Allee 11, 89069 Ulm (Deutschland)
E-mail: martin.plenio@uni-ulm.de

be traced back to plastic deformations of the diamond lattice or, more important for our purposes, substitutional and vacancy defects in the lattice structure that are capable of absorbing and emitting visible light. Hundreds of luminescent defects in diamond are currently known[3] and have been analyzed in sufficient detail to reveal their basic optical and electronic spin properties and, in some cases, their chemical composition.[4] Impurity defects may originate from a wide range of elements, including boron, nickel, silicon, and, most commonly in diamond, nitrogen, whose color centers are of particular importance in the context of biological sensing applications.

2.1. Nitrogen Vacancy Centers and Other Color Centers in Diamond.

The NV center[5] is a point defect in the diamond lattice that consists of a substitutional nitrogen atom directly adjacent to a lattice vacancy and which is oriented along the [111] direction in the crystal (Figure 1 a). It is known to exist in several charge states, including a neutral form, NV^0, and the negatively charged NV^-. Although both forms are optically active, the optical and electronic properties of NV^- are far better understood and appear to be more suitable for applications in sensing and quantum technologies.

The NV^- center possesses a sharp optical zero-phonon line at $\lambda = 637$ nm ($E = 1.945$ eV) and broad vibronic side bands. Under illumination, it displays an additional, very weak zero-phonon line at $\lambda = 1042$ nm ($E = 1.190$ eV), thereby supporting the electronic structure shown in Figure 1 b. It is noteworthy that the NV^- and other color centers do not bleach even when hosted in nanometer-sized diamonds and subjected to intensive illumination. Furthermore, their wavelengths are independent of the size of the host diamond down to sizes below 10 nm[6] (even around 2 nm[7] for a silicon vacancy). These properties alone make fluorescent nanodiamonds (NDs) attractive biomarkers.[8] Importantly, both the ground and the excited state of the NV^- center display a zero-field magnetic resonance at 2.88 GHz and 1.42 GHz, respectively, which occurs between the $m_s = 0$ and the $m_s = \pm 1$ magnetic states of an electronic spin triplet. This electron spin exhibits remarkably long coherence (T_2) and relaxation times (T_1), which can reach milliseconds in ultrapure diamond.[9] It is the combined availability of optical and electronic transitions that make the NV^- center particularly suitable for a wide range of sensing applications. Crucially in this respect, even for single NV^- centers, the electronic spin state can be detected by means of electron spin state dependent light scattering, which discriminates between the $m_s = 0$ and the $m_s = \pm 1$ states[10] by making use of the concept of single-molecule optically detected magnetic resonance (ODMR).[11] The underlying principle of diamond quantum sensing and various applications are described in Sections 4.1–4.3. Interestingly, even at room temperature, the same underlying physics allows for the polarization of the electron spin triplet to the $m_s = 0$ state within a few microseconds by means of optical pumping. The resulting hyperpolarization of the electron spin may be transferred to nuclear spins,[12] thereby leading to potential applications in magnetic resonance imaging, which will be described in Section 4.4.

Despite the current emphasis of research on NV^- centers, a multitude of color centers are known to exist, some of which display a similar combination of optical transitions, magnetic resonance from electronic spin states, and spin-dependent fluorescence. Recently, the silicon vacancy (SiV^-) has received growing attention in this respect. It has a strong zero-phonon line at $\lambda = 737$ nm ($E = 1.68$ eV) and an electronic

Yuzhou Wu is group leader at the Institute of Organic Chemistry III at Ulm University. Her research interests focus on bio-nanotechnology, precision nanoarchitectures, and biofunctionalization of nanomaterials for biomedicine and imaging. She has received a number of prizes, including the Forschungsbonus and Promotionspreis from Ulm University.

Martin B. Plenio is Director of the Institute of Theoretical Physics at Ulm University. His work covers a broad range of topics, including quantum information science, quantum effects in biological systems, quantum optics, and quantum technologies. His awards include an Alexander von Humboldt Professorship, the Maxwell Medal and Prize, the Born Medal, and Prize of the Institute of Physics and the German Physical Society, as well as an ERC Synergy grant.

Fedor Jelezko is a director of the Institute of Quantum Optics and fellow of the Center for Integrated Quantum Science and Technology (IQ^{ST}) at Ulm University. His research interests are at the intersection of fundamental quantum physics and application of quantum technologies for information processing, communication, sensing, and imaging. He has received several honors, in particular, the Walter Schottky Prize of the German Physical Society and membership of Heidelberg Academy of sciences.

Tanja Weil is Director of the Institute of Organic Chemistry III at Ulm University. Her work covers precise macromolecular architectures and biohybrid polymers for medicinal applications and bio-imaging. She has been awarded the Otto-Hahn Medal of the Max-Planck Society, the Wissenschaftspreis of the city of Ulm, and an ERC Synergy Grant.

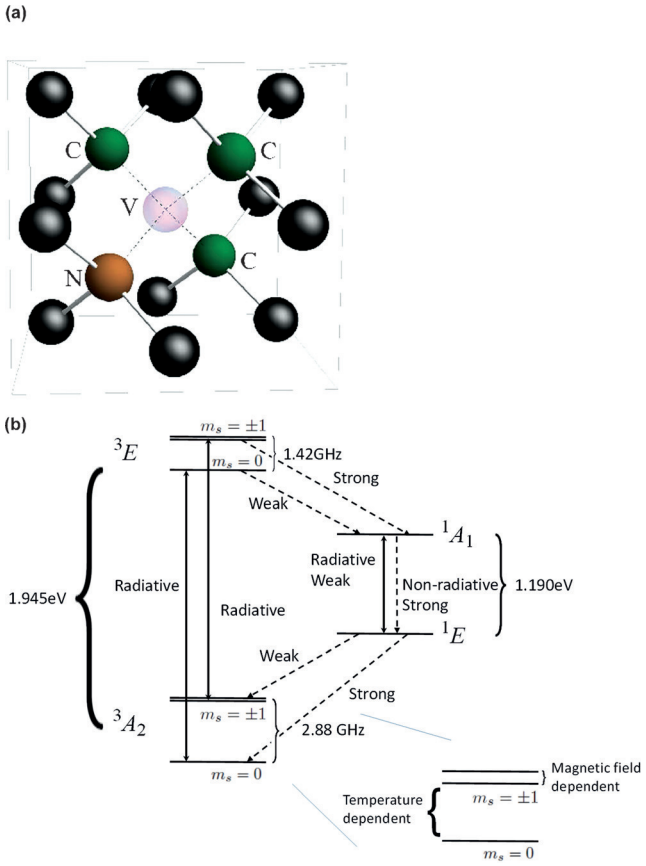

Figure 1. a) The NV center in diamond—a substitutional nitrogen atom adjacent to a vacancy formed by a missing carbon atom. b) The dangling bonds form an electronic spin 1 system with an optical transition at $E = 1.945$ eV. Optical and infrared transitions (solid arrows) and weak nonradiative transitions (dashed lines) allow the observation of spin-dependent fluorescence and optical electron spin polarization.

spin 1/2 system, whose structure was recently elucidated in some detail to demonstrate its application potential.[13]

3. Preparation and Functionalization of Diamond Quantum Sensors

Diamond quantum sensors can be fabricated by implanting color centers in both bulk diamonds and diamond nanoparticles. Bulk diamond refers to diamond crystals with micrometer to millimeter dimensions, which are ideal for developing in vitro biosensing arrays. Their preparation is less challenging since color centers are normally stable if they are located more than 2 nm below the surface. In contrast, nanoscale diamonds (nanodiamonds or NDs) are particularly attractive for biosensing applications, because they could serve as nanoprobes in solution that could be delivered into cells, tissue, and living animals. However, it is much more challenging to prepare high-quality NDs with stable color centers due to their very large surface to volume ratios. The color centers implanted in NDs can be easily perturbed by surface functionalities. These are, however, required to improve the colloidal stability, biocompatibility, as well as biofunctionality of the NDs for biomedical applications. In this section, different methods for the preparation of both bulk diamonds and ND particles with color centers are briefly described and compared. In particular, the challenges for producing high-quality NDs and the functionalization of NDs for in vivo applications will be discussed.

3.1. Preparation of Bulk Diamond for Quantum Sensing.

Bulk diamond crystals can be prepared by several synthetic methods, including high-pressure high-temperature (HPHT) growth and chemical vapor deposition (CVD, Figure 2). HPHT growth is the major manufacturing method for synthetic diamond products and it offers a significant degree of control over the quality and geometry of the diamond obtained. Most diamonds produced by this method consist of small grains of type Ib (the classification of diamonds with isolated nitrogen impurities dispersed in the crystal) with dimensions from a few micrometers up to about 10 mm.[14] These diamonds often already contain substitutional nitrogen atoms (called P1 centers) from the solvent, metal, and carbon source material, as well as from the residual gas left in the HPHT reactor. To produce lattice vacancies, these diamonds can be irradiated by high-energy particles such as electrons, protons, neutrons, ions, and gamma particles. At temperatures of around 800 °C, the substitutional nitrogen atoms produce strain in the diamond lattice and efficiently capture moving vacancies, which then form the NV color center (Figure 2c).[15]

Another popular method of growing synthetic diamond is CVD.[16] At low pressures (below atmospheric pressure),

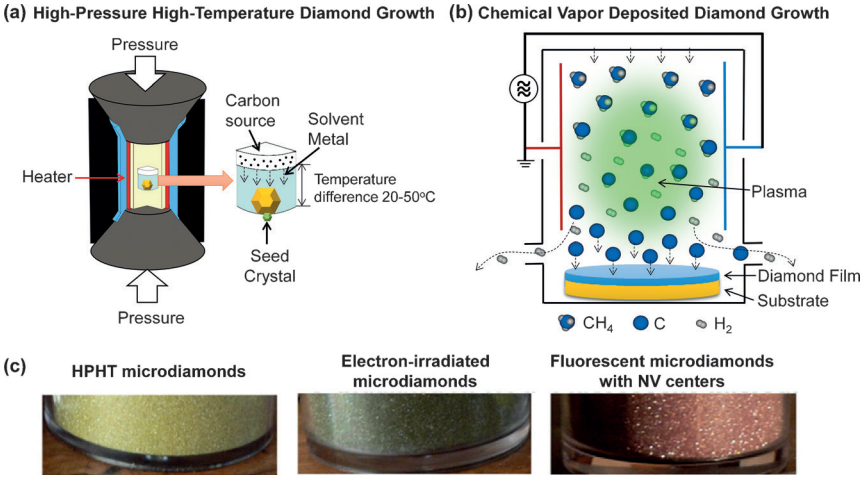

Figure 2. HPHT growth and CVD preparation of diamonds and creation of NV centers in diamonds.

a mixture containing carbon-rich gases (typically, methane/hydrogen 1:99) is broken into fragments between two electrodes by plasma and reassembles into a diamond film on a surface (Figure 2b). Therefore, the CVD diamonds are normally obtained as thin films. NV centers can be created during the CVD process by the growth of diamond film in the presence of a gas mixture with 0–0.1% N_2, 0.7% CH_4, and 99.2% H_2.[17] The concentration of NV centers varies with the nitrogen doping levels. Therefore, even single defects can be created by optimizing the nitrogen ratio.

3.2. Preparation of Nanodiamond Particles for Quantum Sensing

Nanodiamond particles can be produced by several methods, such as detonation,[18] laser ablation,[19] ion irradiation,[20] and chlorination of carbides.[21] However, most of these methods produce polycrystalline NDs and ultra-nanocrystalline NDs (Figure 3) with only a few nanometers of

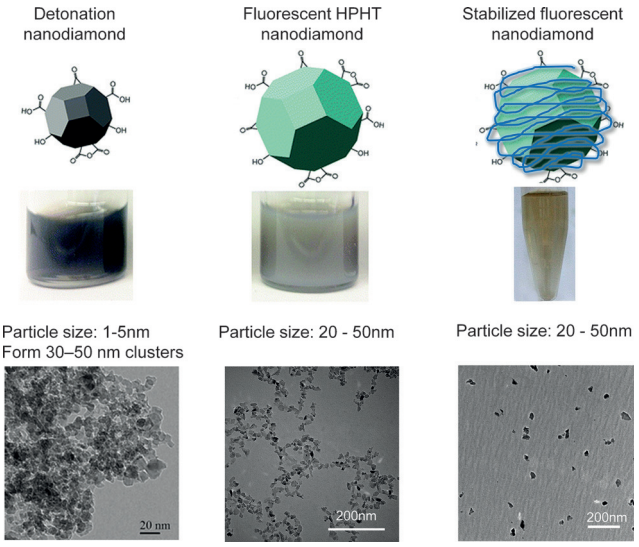

Figure 3. Comparison of detonation ultra-nanocrystalline ND, HPHT fluorescence NDs with NV centers, and fluorescent NDs after stabilization.[26]

crystalline regions, which are not suitable for quantum sensing applications. Ideal NDs for quantum sensing should contain very stable color centers in a highly crystalline diamond lattice, preferably with controllable numbers of color centers at spatially defined positions. Therefore, the NDs used for developing quantum sensors are normally monocrystalline and prepared by high-energy ball milling of HPHT diamond microcrystals[6] and plasma-assisted CVD.[22] As explained in Section 3.1, HPHT growth could produce type Ib diamonds with diameters of a few micrometers (>20–50 μm). The mechanical milling step enables these HPHT microdiamonds to be fabricated into NDs with high purity after acid cleaning (normally with highly oxidative acids such as a 1:1:1 mixture of $HClO_4$, HNO_3, and H_2SO_4) and decontamination.[6b] Purification of these NDs by centrifugation allows them to be separated into different sizes of a few nanometers to tens of nanometers. Since HPHT synthetic diamonds already contain nitrogen defects (P1 centers), they can be used to produce stable and bright fluorescent NV-rich NDs. To create NV centers, the milled NDs can be embedded into graphite tablets to allow convenient irradiation and annealing, similar as for bulk diamonds. Subsequent oxidation procedures, such as acid treatment or thermal oxidation, allow purification of the NDs from the graphite tablets to remove the surface contamination caused by annealing and to further increase the brightness of the NDs. The NV centers obtained by this method are normally stable after implanting; however, it is still currently challenging to render all the NDs fluorescent. The alternative strategy is the irradiation and annealing of HPHT microdiamonds and then milling into NDs,[6] an approach which has been more widely used and which has even been commercialized by several companies.[23] For this method, some NV centers will be destroyed during the milling process and, therefore, an extremely high concentration of NV centers is required in the microdiamond starting material, which is challenging to achieve.

The production of monocrystalline NDs can also be achieved by CVD;[22] however, the incorporation of nitrogen in small nanocrystals by this concept is very inefficient.[24] In comparison, the CVD method is more attractive for preparing SiV-containing NDs, since plasma etching of the silicon substrate on which the CVD diamonds are grown provides the source of the silicon. It is also convenient for introducing other defects by the addition of alternative gases, such as trimethylboron for boron defects.[25]

Although the current methods provide NDs of different sizes and color centers, it is still challenging to fulfil all the requirements for quantum sensing. Ideally, the NDs for quantum devices should have a controlled number of NV centers, and the stable NV centers should be at least 2 nm below the surface. Therefore, NDs should have regular shapes with narrow size distributions. In some cases, when a single NV center is required to act as a spin probe, the diamond nanocrystal needs to have a controlled size and shape, with the NV localized in the center. Therefore, new methods that offer a more controlled synthesis of NDs are still urgently needed. One potential method might be controlling the HPHT growth of diamond crystals to be as small as possible to directly obtain NDs with a regular shape. In principle, if one could control the HPHT growth from a nitrogen-containing molecular seed, it might be possible to produce NDs with only one NV center in the middle, which would be ideal for quantum sensing.

3.3. Functionalization of ND Sensors for Biological Applications

After fabrication of high quality NDs with NV centers, it is crucial for biosensing applications to achieve a surface functionalization that improves the colloidal stability in a biological environment. Biomolecules of interest can simply be absorbed nonspecifically at the surface of the NDs because of their large relative surface area and electrostatic interactions.[27] In first attempts, this approach facilitated the sensing of certain biomolecules. For example, absorbing the

protein ferritin on the ND surface has allowed non-invasive detection of iron levels inside the protein cage.[27b] In addition, different functional groups can also be introduced at the ND surface, thereby facilitating the covalent attachment of the biomolecules (Figure 4), which has been reviewed previously.[8c,28] Such direct surface conjugation is currently the main method for the biofunctionalization of NDs, and it has been applied successfully for conjugating antibodies,[29] DNA,[29] enzymes,[30] and other functional proteins.[31] However, the raw, as-synthesized NDs easily form aggregates, especially in biological media, because of their large surface to volume ratio, which makes it challenging to detect them as individual probes (Figure 3). Surface chemistry is also restricted by uncontrolled aggregation and even precipitation. Moreover, because of the limited number of surface functionalities, only very low densities of biomolecules can be attached to NDs by direct covalent surface modification.

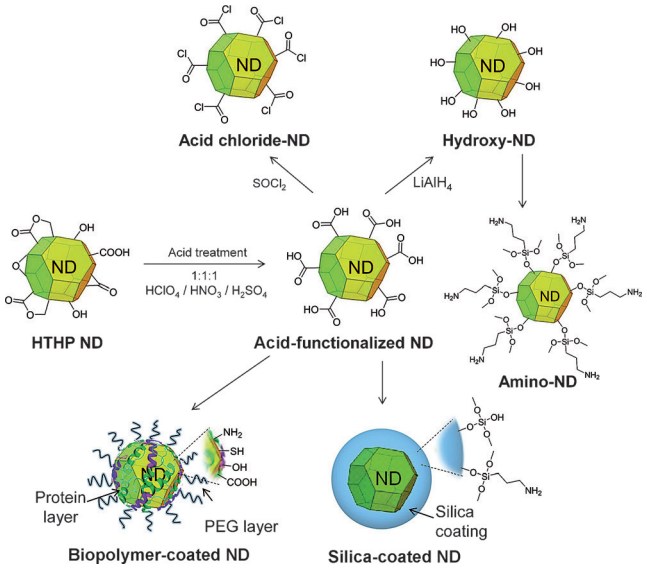

Figure 4. Functionalization of NDs by direct chemical modification and biocompatible coating. The originally prepared HTHP NDs have uncontrolled functional groups on the surface and acid treatment yields NDs with carboxylic acid functionalized groups. Such carboxylic acid groups on NDs could be subsequently derivatized to acid chloride, hydroxy, and amino groups[8c,28] that facilitate further chemical modifications with biomolecules. The acid-treated NDs can also be coated with silica[33] or positively charged biopolymers[35] to alter the surface properties of NDs.

To stabilize NDs in biological media and also to make the functionalization more efficient and reproducible, biocompatible noncovalent coatings have been introduced. Initial studies have demonstrated that NDs with an absorbed layer of the plasma protein serum albumin show an improved stability that facilitates the detection of a single-particle probe inside living cells by stimulated emission depletion (STED) microscopy.[32] Along this line, different coating materials have already been investigated, including inorganic and polymeric materials (Figure 4). For example, a silica shell has been grown on the ND surface to render the particle surface biocompatible and stable, and enable ready functionalization through routine conjugation approaches.[33] The hydrophilic polymer polyethylene glycol (PEG) has been attached to the silica shell to further enhance the colloidal stability over a broad pH range (pH 2–10) and even in 1 M NaCl and cell culture medium.[33a] Alternatively, polymers can also be used as biocompatible ND coatings. For example, PEG-based polymers have also been directly coated onto NDs by covalent conjugation.[34] Since the PEG coating is known to be particularly biocompatible and have low non-specific absorption, such NDs are nontoxic for cells and tissues and are, therefore, promising for in vivo applications.[34b,c] However, PEG polymers do not allow post-functionalization, which is a limitation of this coating method. Recently, a biopolymer-coating strategy has been developed using a PEG hybrid polymer derived from native albumin.[35] NDs coated by these biopolymers are found to be highly stable in all tested biological buffers and also over a broad pH range (pH 2–8) without any aggregation. In addition, this protein-derived biopolymer provides high numbers of orthogonal functional groups from the amino acid residues, which allows easy post-modification of biomolecules (Figure 4).

4. Sensing with Diamonds—Methods and Applications

Unique optical access to a long-living ground state spin is a key element for magnetic sensing at the nanoscale and will be described in this section. Single NV centers are particularly interesting compared to other magnetic field based sensing techniques (such as magnetic resonance force microscopy) because of the extremely weak perturbation, since a single NV center carries only weak magnetic moments (similar to a magnetic moment of a single electron spin). In addition, the absence of fluorescence bleaching allows color centers to be used for sensing applications through fluorescence resonance energy transfer (FRET)

4.1. Optical Sensing with Diamonds

Optical techniques developed during the past two decades nowadays provide the ultimate detection sensitivity of down to single fluorophores.[36] Fluorescence markers even offer ultrasensitive imaging beyond the limits imposed by the diffraction of light.[37] However super-resolution imaging techniques often suffer from a limited photostability of the fluorophores. Stimulated emission depletion (STED) microscopy routinely reaches resolution of 20–50 nm,[38] but higher resolving powers require depletion beams of high intensity to lead to fast photobleaching of the fluorescence markers. Other super-resolution imaging methods are based on the analysis of blinking events of single chromophores, which also requires high photostability (i.e. absence of irreversible bleaching) for achieving high resolution.[39] This is the reason why color centers in diamond with their extreme photostability are very promising probes for super-resolution microscopy when perturbation as a result of the relatively

large size of nanodiamonds is not crucial. Initial experiments performed with NV centers in bulk diamond demonstrated resolution down to 6 nm.[40] More recently, STED microscopy was applied to NV-doped diamond nanocrystals[41] and a resolution of up to 10 nm was demonstrated.[42]

Another very powerful optical technique for sensing at the nanoscale is fluorescence resonance energy transfer (FRET). FRET sensing is based on the sharp distance dependence of energy transfer between individual chromophores. The unique photostability of color centers offers new opportunities for improving FRET-based sensing techniques. It was shown that single NV centers embedded in diamond nanocrystals of 20 nm allow single molecules absorbed at the nanodiamond surface to be detected with high efficiency.[43] Although the dynamic range of FRET microscopy is limited, this drawback can be overcome by scanning-probe FRET techniques where a donor, for example, the NV center in a diamond nanocrystal, is attached to the tip of an atomic force microscope.[44]

4.2. Detection of External Electron Spins by Using NV Centers

Nanometer-sized diamonds containing NV centers are promising nanosensors in biological environments due to their biocompatibility, bright fluorescence, and high magnetic sensitivity under ambient conditions. The triplet ground state of the NV center can be employed as a nanoscale magnetometer.[1,45] The localized nature of the NV defect means that it can be considered as a point magnetic dipole, whose energy is modified by the presence of external spins. Such changes in energy (recorded as a shift of the spectral lines, perturbances of the spin echo, or enhanced relaxation of the NV centers) can be accessed through any optically detected magnetic resonance of the NV center spins. The detection of external electron spins by using such a diamond magnetometer[46] suggested many opportunities for studying biomolecules even in their cellular surroundings. Fast fluctuating external spins have been measured through the enhanced relaxation of the NV centers. In this case, noise from external spins is recorded as a signal (noise or decoherence microscopy).[47]

Typically, the goal in sensing systems is to reduce the noise to gain optimal access to a coherent signal. In decoherence microscopy, noise can also constitute the signal. The sensitivity of this technique can reach the ultimate limit of the detection of a single paramagnetic ion.[48] It can also be extended to the detection of biomolecules, for example, when paramagnetic ions are attached to proteins.[49] The detection of single electron spins can also be applied to proteins, where spins are natively present and carry a particular function, for example, in magnetic proteins such as ferritin.[27b,50]

The detection of ferritin (proteins carrying iron in many organisms) by using magnetic noise induced by the inner paramagnetic iron center as a contrast mechanism has been accomplished with sensitivities reaching the single molecule detection threshold.[27b,50] A significant reduction of both the coherence (T_2) and relaxation time (T_1) as a result of the presence of ferritin on the surface of NDs was obtained (Figure 5). Thus, nanoscopic magnetic field sensors based on

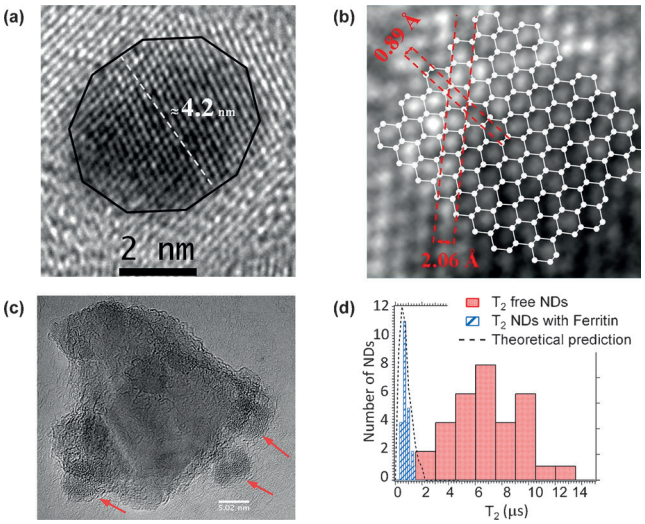

Figure 5. Detection of iron-containing ferritin with single-protein sensitivity by using an NV center in an ND. a) High-resolution transmission electron microscopy (HRTEM) image of an ND. b) The carbon lattice of an ND according to the HRTEM image. c) HRTEM image of ferritin absorbed on an ND (ferritin moieties are indicated by red arrows). d) Detection of ferritin through the significant reduction in the coherence time (T_2).

single defects in diamonds pave the way towards a novel sensing technology that may potentially even resolve important biological processes such as electron transfer in cellular environments. The remarkable sensitivity paired with the non-invasive character of the detection process may also provide access to quantum mechanical properties at the biological level, for example, in electron transfer and radical pair dynamics, which are typically hidden in ensemble and highly invasive measurement schemes.[51]

4.3. Single-Molecule NMR Spectroscopy with Diamond Spins

Nuclear magnetic resonance (NMR) is one of the most powerful imaging techniques in life sciences. Highly informative NMR spectroscopy allows reconstruction of the structure of proteins by unraveling interactions between nuclear spins inside complex molecules.[52] The sensitivity of conventional NMR techniques (based on induction detection) is limited to large spin ensembles. Therefore, there is growing interest in the development of new NMR detection methods. NV-based magnetometry is one of the most promising avenues in this research field.

A single nuclear magnetic moment is weak and is approximately 1000 times weaker than that of an electron. However, when brought into proximity to the NV center (a few nanometers), small spin ensembles can produce fields on the order of microtesla, which allows the detection and localization of such spins.[53] The main challenge of NV-based NMR experiments is placing NV centers close to the molecule of interest and retaining long coherence time. The coherence time of NV defects in the presence of noise originating from parasitic spins located at the diamond interface can be

improved by orders of magnitude by using high-order spin echoes.[54] Careful design of such echo sequences allows the realization of a situation in which a significant part of the noise is cancelled out, but a narrow spectral channel for sensing remains open. Recently, the detection of NMR signals in 100 nm^3 of a liquid sample placed at a diamond surface[55] and in a microfluidic device was achieved with spatial resolution below 500 nm.[56] For solid samples with long coherence times of the nuclear spins (Si^{29} in quartz and nuclear spins associated with hydrogen bonds to the diamond interface), sufficient sensitivity for the detection of a single nuclear spin within a few seconds of measurement time was reached[57] (Figure 6).

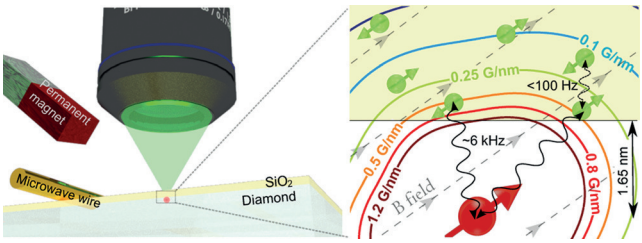

Figure 6. NMR spectroscopy of ^{29}Si nuclei with a strongly coupled sensor. Left: Schematic representation of the experimental setup. Dilute, unpolarized nuclear spins in a silica layer interact with an electronic spin in a (100)-surface diamond, which is readout by optical microscopy. Right: Schematic representation of the strong coupling regime. A shallow NV center in diamond (2 nm from the surface) couples to nearby ^{29}Si nuclei in a silica layer, as a result of a hyperfine interaction. The contour lines show the strength of the effective magnetic gradient experienced by the nuclear spins. Reproduced from Ref. [57] with permission, copyright Nature Publisher Group.

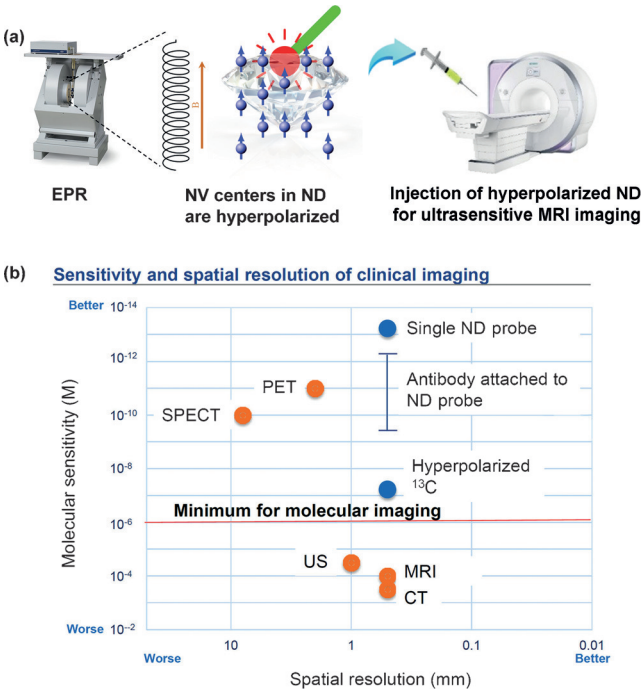

Figure 7. Hyperpolarization of NV centers in an ND for enhanced MRI. a) Illustration of the process of hyperpolarized NV centers for enhanced MRI imaging. b) Comparison of the sensitivity and spatial resolution of clinical imaging techniques and hyperpolarization MRI. PET: positron emission tomography; SPECT: single-photon emission computed tomography; US: ultrasound; CT: computerized tomography. Hyperpolarized ND probes would allow the same spatial resolution as MRI, but with extremely high sensitivity for molecular imaging with existing MRI systems.

To apply the NV-based NMR technique for the structure elucidation of a biomolecule, the spectral resolution of NV magnetometers needs to be improved to detect dipolar couplings and chemical shifts. Such an improvement can be achieved on the one hand by the development of new measurement procedures similar to two-dimensional NMR spectroscopy[58] and on the other hand by improving the coherence time of NV centers close to the interface. New techniques that allow removal of magnetic noise using new procedures developed in the context of quantum computation (such as quantum error correction) are very promising in this respect.[59]

4.4. Hyperpolarization of Diamonds for Molecular Imaging

Magnetic resonance imaging (MRI) is one of the most important imaging techniques in medicine. The long wavelength of radiofrequency photons means that they do not suffer from scattering, thereby allowing non-invasive imaging of tissues. The main drawback of MRI is its low sensitivity, which in part is related to a low polarization of nuclear spins at low temperature. Recently it was demonstrated that the sensitivity of MRI can be improved by using dynamic nuclear spin polarization (DNP) of NDs[60] (Figure 7).

The key element of this technique is optical pumping, which allows polarization of the electron spins of the NV centers on a microsecond timescale (hyperpolarization). Polarization can then be transferred to nuclear spins by using well-established NMR procedures such as the Hartmann–Hahn match,[12] the solid effect,[61] or the energy match between electron and nuclear spins through level anticrossing.[62] The polarization of NDs represents a more challenging task compared to bulk diamond because of the disordered orientation of the NV centers, but advanced DNP approaches addressing this issues were developed recently.[60b]

The main advantage of NV defects for dynamic nuclear spin polarization is related to the optical pumping, which allows the use of the same NV center to polarize multiple nuclear spins on a short timescale.[12] In addition, experiments can be performed in a low field and at room temperature without loss of performance. Hyperpolarized diamond nanoparticles show long (more than a minute) relaxation times[63] and relaxation on the scale of hours was measured in bulk diamond, which is crucial for MRI. Furthermore, NV defects can also be used to polarize external nuclear spins when they are located close to the diamond surface,[64] and may permit the construction of flow channels, in which highly polarized molecules may be obtained at volumes sufficient for NMR detection.[65]

4.5. Self-Assembled ND Structures

The realization of scalable arrangements of NV centers in diamond remains a key challenge on the way towards efficient processing of quantum information, quantum simulation, and quantum sensing applications. Although technologies based on implanting NV centers in bulk diamond crystals or hybrid devices have been developed, they are limited by the achievable spatial resolution and by the intricate technological complexities needed to achieve scalability. A novel approach for creating an arrangement of NV centers is based on the self-assembling capabilities of biological systems, and their beneficial spatial resolution of nanometers has been proposed and demonstrated.[66] The feasibility of interconnecting NDs with biological structures was achieved through the formation of small ND complexes using a SP1 (stable protein 1) variant and the first steps have been made towards the formation of regular arrays of NDs on SP1 arrays[66] (Figure 8). An important result towards the creation of ordered ND structures was the formation of numerous dimers and trimers along with larger ordered structures such as a seven ND hexagon.

Going beyond 1D and 2D periodic patterns, the method of DNA origami, based on the directed folding of a large single-stranded DNA molecule by staple strands, enables the creation of more and complex, highly controllable structures, ranging from aperiodic arrays to real three-dimensional architectures.[67] Precise 2D or 3D DNA nanostructures have designed and manufactured by using selective Watson–Crick base pairings. Composite assemblies based on DNA nanostructures offer an ideal platform to study the distance- and orientation-dependent electronic and optical properties of fluorophores and nanoparticles. The low colloidal stability even of coated NDs at high ionic strengths has so far limited their conjugation to DNA nanostructures. Recently, a biopolymer-based coating was reported that efficiently encapsulates NDs and imparts high colloidal stability even at the high ionic strength required for DNA conjugation (see Section 3.3). Biotin-functionalized and perfectly dispersed nanodiamonds have been realized that were self-assembled in predefined 1D, 2D, and 3D geometries[68] through the tight interaction of biotin and streptavidin (Figure 8). Such assemblies offer unique opportunities for preparing self-assembled spin lattices or plasmon-enhanced spin sensors as well as improved fluorescent labeling for bioimaging.

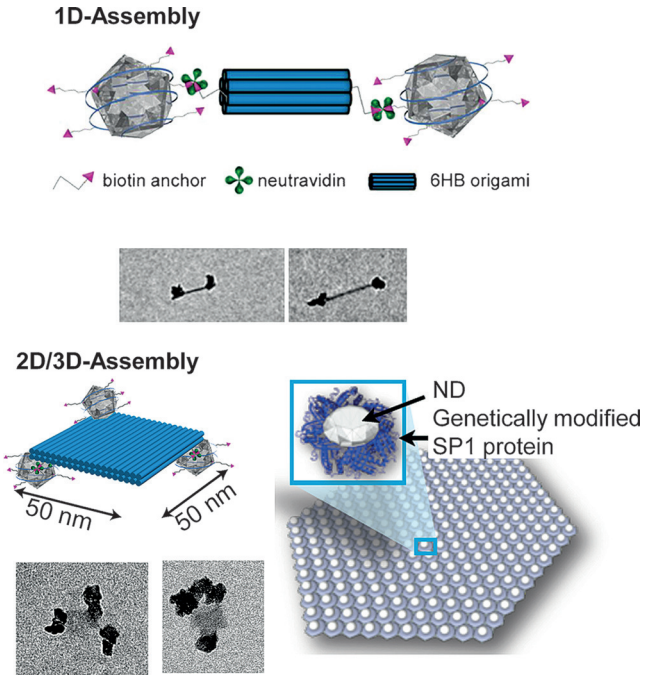

Figure 8. Self-assembly of NDs on a DNA origami template and a protein template (SP1).

5. Diamond Sensing in a Living Biological System—Progress and Challenges

The use of diamond-based sensing to study various biological questions, such as the structure and dynamics of proteins in a cell, represents an emerging area. Beyond the advantages of traditional optical imaging, NDs can be applied, in combination with the techniques discussed in Section 4 in living biological systems as probes to detect the tiniest magnetic fields, electron spins, and temperature with nanoscopic and even atomic precision.[1,69] Applying such diamond magnetic field sensors for biological studies will provide a potentially revolutionary tool to elucidate the structure and dynamics of biomolecules in a biological environment. There are still several major challenges that need to be addressed before these ultimate goals can be achieved. In this section, the current progress of NV-diamond materials for biological applications will be briefly discussed, with the future potential and challenges highlighted.

5.1. Biocompatibility of NDs

Diamonds are generally considered stable, bio-inert, and biocompatible[70] because of their highly stable lattice. However, the biosafety of diamond nanoparticles for in vitro and in vivo applications still needs to be carefully investigated because of their potential "nanotoxicity" (that is, toxicity induced by their nanoscopic size). Until now, many in vitro cytotoxicity studies suggest that, in comparison to other nanomaterials such as carbon nanotubes, semiconductor or metal particles, NDs are the most biocompatible materials with no cytoxicity to different cell lines.[71] Observations of NDs in cell culture over a long period of time also show that NDs do not cause chromosomal or genetic damage and they equally divided into daughter cells without affecting cell division and differentiation.[72] Intensive in vivo studies in mice and *Caenorhabditis elegans* also revealed promising biocompatibility without any liver toxicity, systemic inflammation, or deleterious effects to the offspring.[73]

However, some potential risks of NDs have recently been pointed out, such as the occurrence of increased total reactive oxygen species in human hepatocytes[74] that cause slight DNA damage in embryonic stem cells[75] and long-time accumulation in liver, spleen, and lung with a slow excretion

rate.[76] NDs as any other non-endogenous material might elicit side effects in vitro and in vivo depending on the concentrations applied. However, based on the in vitro and in vivo studies reported to date, NDs are still among the most biocompatible nanoparticles, with a promising biosafety profile. In addition, NDs of different sizes, surface modifications, and administration routes will most likely also have a different biodistribution and potential side effects. Therefore, the biocompatibility of NDs needs to be carefully assessed when new in vivo ND probes are designed. Improving the quality of ND samples by optimizing the diameters, narrowing the size distributions, and enhancing the colloidal stability under in vivo conditions, as well as functionalization of the ND surface will provide NDs that are optimized for in vitro and in vivo applications.

5.2. NDs for Drug Delivery

The current applications of NDs in living biological systems are mainly focused on drug delivery. NDs as carriers for drug delivery offer many advantages, such as their detection by fluorescence imaging as well as surface functionalization to facilitate different drug loading and cell targeting strategies. The first generation of ND–drug delivery systems were mainly prepared by simply absorbing lipophilic drug molecules onto the ND surface.[77] These ND–drug complexes composed of about 4–6 nm primary detonation ND particles cluster in solution into 100–200 nm aggregates, which can substantially absorb drug molecules and significantly enhance their blood circulation half-life and tumor retention.[77,78] Different anticancer drugs have been delivered by this approach,[77,79] and progress has been made to address targeted drug delivery, controlled drug release, intracellular tracking, and evading chemoresistance.[34b,80] However, it is highly challenging to prepare stable and well-defined nanoparticles with a narrow size dispersion by this method, which leads to the risk of nonspecific interactions with plasma proteins and unpredictable side effects.[81] Second-generation ND transporters have been designed by surface modification of single ND particles with stabilizing polymers.[82] Drug molecules[29,83] such as the anticancer drug doxorubicin (DOX)[84] and platinum complexes[85] have been covalently conjugated to these NDs, with some even offering stimulus-responsive drug release.[83b,84,85] Among these covalent ND modification approaches, the introduction of a polyglycerol shell[84,85] significantly increases the stability of NDs and offers great prospects, especially for the delivery of hydrophilic drugs, for example, platinum complexes, since no agglomeration in mammalian cells was observed. However, achieving high colloidal stability after loading hydrophobic drugs still remains a key challenge.[27a] Recently we investigated drug delivery with nanodiamonds coated with albumin biopolymer since they have revealed excellent stability under biological conditions, as introduced in Section 3.3. Drug molecules have been covalently conjugated to the albumin backbone through an acid-labile linker. The resulting ND–drug conjugates show a high solubility in biological fluids, without observable aggregations, even after a high loading of hydrophobic drug molecules. The preliminary in ovo data with breast cancer xenografts on the chick chorioallantoic membrane (CAM) model also indicated the enhanced therapy effect on the tumor.[35] All these attempts show that surface coating and modification of NDs could be an efficient strategy to optimize NDs as efficient drug transporters (Figure 9).

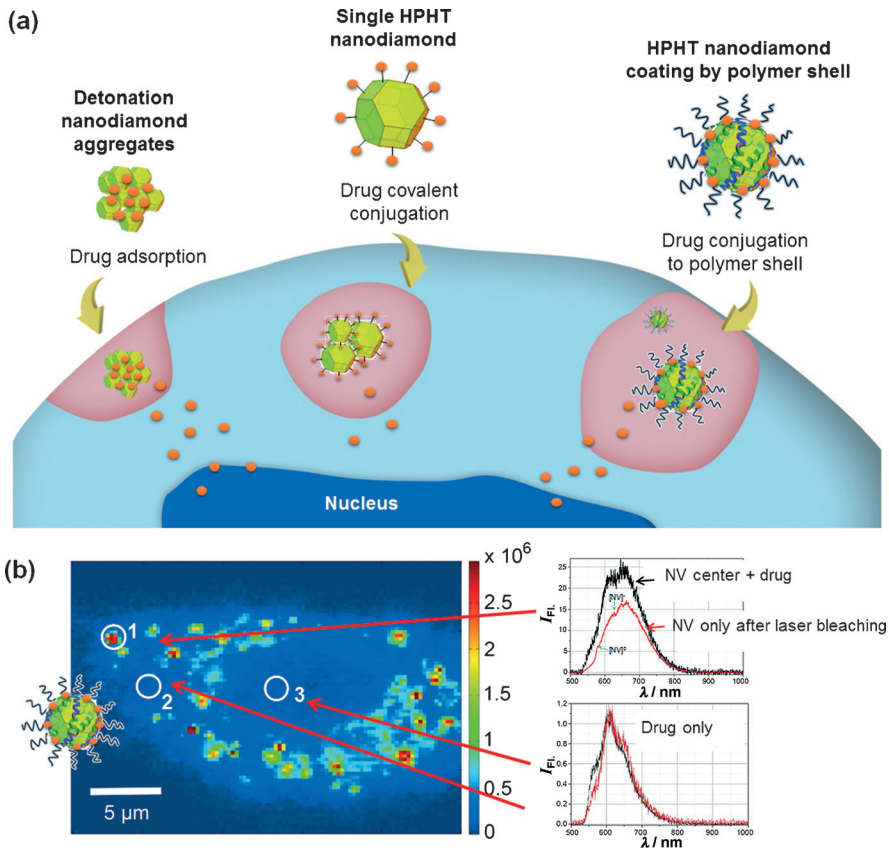

Figure 9. ND-based drug-delivery systems. a) Comparison of different ND-based drug-delivery strategies. Detonation NDs form aggregates that could absorb drug molecules and also allow uncontrolled drug release.[27a,77,78,79b] Uncoated HPHT NDs with covalently linked drugs often reveal aggregation inside biological systems.[29,83a] Polymer-coated HPHT NDs allow loading of drug molecules on a polymer shell and stimuli-responsive drug release.[34c,35] b) Confocal fluorescence microscopy imaging of DOX loaded on biopolymer-coated HPHT NDs inside living cells.[35] Circle 1 highlights the representative emission from cell vehicles, where overlap of the florescence spectra from the NV center and DOX drug can be observed. After laser bleaching, DOX emission can be quenched and nonbleaching fluorescence from the NV center could be clearly recorded. Circles 2 and 3 represent the fluorescence spectra from the cytosol and nucleus, where only DOX emission can be observed, thus indicating drug release from the ND carriers.

5.3. Potential and Challenges of Diamond Sensing in Biological Systems

Evaluation of the biosafety of NDs and drug-delivery studies support the promising potential of using NDs in living biological systems. The unique and powerful technique of quantum sensing based on NV centers has already been discussed above.

The combination of these advantages results in NDs having great potential to be developed into the smallest sensing probes to detect the tiniest magnetic fields and electron spins in vitro and eventually in vivo, and may serve as non-invasive nanosensors to study biological reactions inside living organisms. Although this field is just emerging, several successful examples have already been demonstrated that underline the unique potential of ND quantum sensors. Studies have shown that the magnetic resonance of individual fluorescent NDs can be detected optically inside living human Hela cells, and their location, orientation, spin levels, and spin coherence times can be measured with nanoscale precision.[80d] The energies of the spin levels of individual NV centers served as fingerprints, thus allowing the identification and tracking of ND particles with identical fluorescence. Furthermore, monitoring decoherence rates in response to changes in the local environment may provide unique insights into intracellular processes. One successful example uses ND-based sensitive nanoscale thermometry by measuring changes in the spin coherent time of the NV center in response to a local temperature. In the absence of an external magnetic field, the precise value of the transition frequency has a temperature dependence, which was used to report changes in the local temperature (see Figures 1 and 10). With this technique, it is possible to directly measure the local temperature in living cells on length scales as short as 200 nm.[69] Other examples include the potential for measuring minute forces in cells and proteins by employing analogous principles.[86]

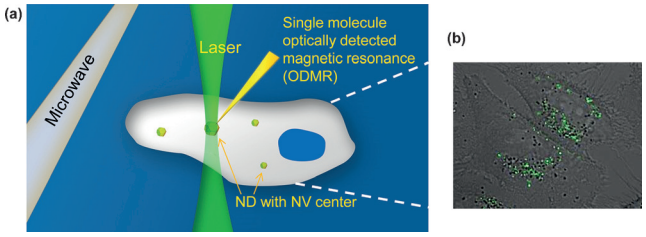

Figure 10. Illustration of ND quantum sensing in living cells. a) The experimental setup for ND quantum sensing inside living cells. b) Confocal fluorescence microscopy image of NDs with NV centers inside living cells (green dots).

If, on the other hand, an external magnetic field is applied in the biological sample, the spin coherence time of the NV center is then dependent on the magnetic environment, which enables magnetic imaging of proteins in vitro[27b] and even inside living cells. As the first example, the magnetosomes produced in living magnetotactic bacteria could be imaged and analyzed using an optically detected magnetic field imaging array consisting of a nanometer-scale layer of NV centers implanted on the surface of a diamond chip.[87] In addition, as discussed in Section 4.2, NV centers can also detect electron spins in a local biological environment. If such an electron spin label was introduced into the biomolecule of interest, this technique would also allow the detection and analysis of this biomolecule through NV centers in a living system with single molecule sensitivity. In addition, the single-molecule NMR technique described in Section 4.3 might eventually also allow the detection of nuclear spins, thus facilitating a deeper understanding of cellular reactions in real time, even in living cells. The recognition of nuclear spins represents a greater challenge than the detection of electron spin because of the higher noise of nuclear spins in a biological system as well as the weak nuclear spin magnetic moment, which must be very close to NV centers. However, promising solutions are already under intensive investigation, as discussed in Section 4.3. Furthermore, the hyperpolarization technique based on NV centers is envisioned to achieve ultrahigh-sensitive MRI, as described in Section 4.5. These studies demonstrated the viability of controlled NV centers as single spin probes for nanoscale magnetometry in biological systems, thus opening up a host of new possibilities for quantum-based imaging in the life sciences.

It should be stressed that a variety of obstacles have to be overcome before these ideas can be brought to full fruition. The sensitivity of the NV sensor to tiny signals also implies that it is easily perturbed by the wide variety of noise sources, such as nuclear and electron spins, charges, and radicals in random motion, which are intrinsically present in biological systems. Furthermore, the ND sensor is not stationary and will move and rotate, thus leading to randomization of the signals. Solutions for these challenges are currently being developed in physics laboratories (see Section 4), but will certainly need to be adapted and transferred to biological environments to allow their successful application initially in vitro and finally in vivo. Although such solutions are certainly challenging, current research has not identified fundamental limitations in this direction. Hence, it can be expected that the application of ND sensing in biology will be realized. Their performance relative to established technologies can then be assessed in detail, and application areas in which they outperform current technologies can be identified to open the door to a new mode for the observation of biological phenomena.

6. Conclusions

The detection and analysis of the structure, dynamics, and interactions of single molecules under native conditions with high sensitivity and spatial resolution on the nanometer scale represents an outstanding challenge with a strong impact on modern science and technology. The NV center in diamond is a unique tool that offers 1) measurement of a nonbleaching fluorescence required for super-resolution fluorescence imaging, 2) detection of electron spin and nuclear spin with a great potential for atomic resolution under ambient conditions, as well as 3) hyperpolarization through simple optical pumping

to enhance the MRI sensitivity. In addition, diamond nanoparticles down to 2 nm could be implanted with stable NV centers, which would allow nanoscale sensing at the single-molecule level. In particular, nanodiamonds could be functionalized with biomolecules and delivered to biological systems with promising biocompatibility. Therefore, the quantum sensing techniques with NV centers could be transferred into cells and would allow nanoscale sensing even inside a living cell environment. Theoretical and quantum optical studies have already demonstrated the power of these quantum sensing techniques in vitro. Some pioneering studies have also shown the great potential for nanoscale sensing inside living cells. We envision that ND-based quantum sensing techniques will become a pioneering imaging method for the broad field of life science research and provide unprecedented access and insight into the structure, dynamics, and function of individual biomolecules under physiological conditions.

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