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Quantum probes for biology: Unlocking single molecule dynamics

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ABSTRACT

The dynamics of single molecules are critical in determining how biological systems function. The number of measurement techniques to image and characterise single molecules is growing rapidly, however very few techniques are applicable in biologically relevant settings. Here, I provide a perspective on a relatively new field of research focused on the development of diamond-based quantum probes to measure the dynamics of single paramagnetic molecules in ambient aqueous solutions. The magnetic sensitivity of these quantum probes coupled with the biocompatible sensing platform offers a potential pathway towards 3D single molecule microscopy.

Quantum technology is expected to change the way we communicate, compute and sense the world around us. This quantum technology revolution will be enabled by quantum systems and probes which can be coherently controlled and manipulated with extreme precision. The emergence of solid state quantum systems based on defect centres in semiconductor materials has provided a robust platform to build and develop novel quantum sensors. The operating principles of quantum sensors are governed by the laws of quantum mechanics and bring about several advantages when compared to their classical counterparts, for example in the size and sensitivity of the sensor itself. One interesting application area in which quantum sensors are being actively pursued is nanoscale magnetic resonance spectroscopy and the characterisation of individual molecules and nuclei. The dynamics and reaction kinetics of single molecules are of enormous interest in biology: from DNA coding and transcription through to energy production in our cells. Over the past two decades, tremendous advances in imaging techniques have enabled the study and characterisation of individual proteins and biomolecules. Such techniques include: X-ray crystallography [1], Cryo-EM [2], and Optical Tweezers [3], to name a few. Whilst these techniques provide unprecedented access to complex molecular structure and behaviour, they are limited in their ability to operate under physiological conditions. X-ray crystallography, for example, often requires both crystallisation of the target protein and multiple recordings from replicate biomolecules to capture an overall molecular fingerprint. Cryo-EM, on the other hand, can image fixed and immobilised proteins in highly controlled environments (i.e. under vacuum); and optical tweezers rely on functionalising and trapping large spherical particles to probe molecules of interest. Therefore, one of the grand challenges for single molecule imaging is to capture dynamic information from single molecules in their natural state. In this endeavour, magnetic resonance spectroscopy techniques are leading the way. Magnetic resonance techniques such as nuclear magnetic resonance (NMR) and electron spin resonance (ESR) interrogate the magnetic properties of molecules as determined by their composition and coordination environment. Structural NMR allows the chemical composition and structure of ensembles of molecules to be determined based on the chemical shift experienced by nuclei. At present, however, state-of-the-art NMR spectrometers require 10^{11} or 10^{12} nuclear spins to generate sufficient signal to noise ratios for typical acquistion times < hours. A similar story exists for ESR spectrometers, in which molecular dynamics are revealed by attaching electron spin labels, such as nitroxides, to particular components of a molecule, termed site directed electron spin labelling (SDSL). Given the magnetic moment of an electron spin is a factor of $\sim$ 1000 times larger than nuclear spins, the detection limit of traditional ESR spectrometers remains constrained at $\sim 10^8$-10^9 electron spins under ambient conditions in which molecular dynamics can be observed. Therefore, a transformative shift in technology is needed in terms of magnetic sensitivity to realise magnetic resonance studies of individual molecules. Quantum technology, and quantum sensors in particular, offer a promising approach to this challenging problem and have made staggering progress over the past 5–10 years [4–8].

One promising solid state quantum sensing platform in this regard is based on atomic defects in diamond known as nega-

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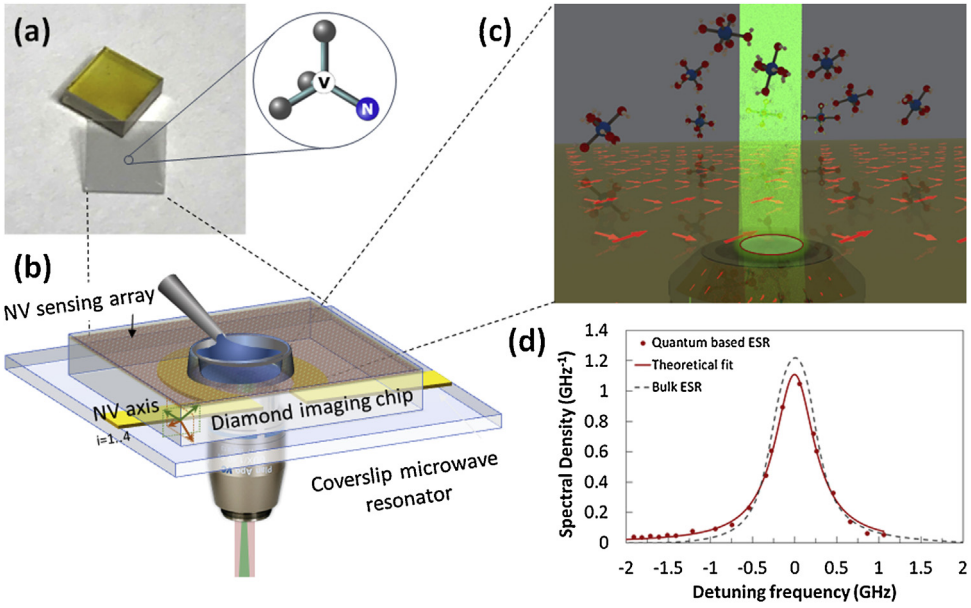

Fig. 1. Quantum based ESR microscopy. (a) Single crystal diamonds hosting NV centres. The yellow diamond has a high nitrogen concentration [N] > 100 ppm while the clear diamond contains [N] < ppb. The expanded image shows the crystal structure of the NV centre in diamond. (b) Diamond-based quantum ESR microscope. The 2D NV sensing array is engineered via nitrogen ion implantation across the entire surface of the diamond. The 2D sensing array is optically excited and the NV fluorescence imaged via an inverted widefield optical microscope. The diamond is adhered to a microwave resonator fabricated on top of a glass coverslip to drive microwave transitions between the NV $m_s = 0$ and $m_s = -1$ states. Paramagnetic target molecules are introduced on the top surface of the diamond ~10 nm from the NV sensing layer. (c) Schematic of the sample interface showing the green excitation used to excite the NV centres and paramagnetic Cu^{2+} molecules diffusing through the sensing volume. Red arrows represent the NV spins below the diamond surface. (d) Ambient hexaaqua copper (Cu^{2+}) ESR spectrum from quantum-based ESR of ~1000 Cu^{2+} molecules compared to bulk ESR ensemble measurements.

tively charged nitrogen vacancy (NV) centres [9]. The NV centre in diamond comprises a substitutional nitrogen atom with an adjacent vacancy aligned along the <111> crystallographic axes. This particular defect can be engineered into the diamond lattice by accelerating nitrogen ions into the diamond or via chemical vapour deposition techniques. The ground state of the NV centre is a spin 1 system whose spin levels are optically addressable. Magnetic detection in these systems is thus achieved by optically monitoring the populations and quantum phases of these spin states under magnetic dipole-dipole coupling to target molecules. One of the most attractive features of these quantum probes is that they operate at room temperature and can be engineered in close proximity to the diamond surface (less than 10 nm) leading to nanoscale spatial resolution [10]. The intimate contact between the NV probes and target molecules results in single electron spin sensitivity at distances of order ~10 nm [8]. This sensitivity, spatial resolution and ambient operation is ideal for SDSL studies of single proteins and molecules.

The first demonstration of single site directed electron spin resonance (ESR) using an NV quantum probe came in 2015 when Shi et al. used techniques adapted from magnetic resonance spectroscopy (double electron-electron resonance or DEER) to demonstrate the direct detection and spectroscopy of a single nitroxide spin label attached to a single spindle checkpoint protein (MAD2) [11]. Spectroscopy of this individual protein allowed the orientation of a single spin label to be determined, marking an important first step for future functional studies targeting confirmation changes of individual proteins. This work was followed up recently with a study on single DNA hybridisation [12]. In this work the Du group utilised nanostructured diamond pillars, which act to enhance the NV fluorescence collection efficiency, in order to improve the acquistion time for a single spin label ESR spectrum. This speed-up is critical for room temperature applications where the spin labels are prone to ionisation from the aqueous environment, as well as photo-ionisation from the optical pumping of the NV centres. Additional challenges faced by this form of nanoscale ESR include the co-localisation of the single molecules with respect to the NV atomic sensors. Current studies are performed on a statistical basis and rely on the NV centres being sufficiently close to the proteins or molecules of interest.

A possible solution to the localisation of molecular targets may lie in the development of quantum-based ESR microscopes. Instead of interrogating a single NV centre in diamond, an alternate approach is to engineer NV centres uniformly across the diamond surface to create a 2D near surface sensing layer which can be addressed and readout in parallel using conventional CCD cameras. These 2D NV sensing arrays have been used for electron/nuclear spin imaging applications [13–15] and provide a possible solution to the localisation of the target molecules with respect to the NV probe, since the average NV spacing in these layers can be of order 20 nm. At present, the imaging resolution in these quantum-based ESR microscopes is limited by optical diffraction to around ~ 300 nm, however super resolution imaging techniques [16] have demonstrated possible pathways toward ESR nanoscopy which would enable single molecule reconstruction of individual spin labels. Another important consideration in the development of ESR microscopy is an appropriate sensing protocol which can operate over large fields of view to provide high-resolution ESR spectra over MHz-GHz spectral range. Hall et al. identified a microwave-free, ESR measurement protocol using NV centres in diamond which uses an applied magnetic field to Zeeman split the energy states of the NV to cover this measurement bandwidth [17]. The applied magnetic field modifies the paramagnetic ground and excited states of the NV centres to tune them into resonance with target paramagnetic molecules. When the resonant condition is reached the target molecules and NV centre exchange magnetisation efficiently, resulting in a strong reduction in the spin lattice relaxation time (T_1) of the NV centres. By implementing this approach using the 2D NV sensing arrays, the ambient ESR spectrum of hexaaqua copper (Cu^{2+}) molecules in aqueous solution was obtained with

diffraction limited spatial resolution (Fig. 1) and spin sensitivity in the zeptomol ~1000 spins/pixel regime [15].

One of the current hurdles facing quantum-based ESR microscopy is the introduction of paramagnetic defects, both at the surface and within the NV sensing layer, resulting from the nitrogen implantation used to engineer the sensing array. These paramagnetic defects have g-factors of ~2 and, due to their close proximity, can quench the NV relaxation time which reduces the electron spin sensitivity to targets with g-factors of 2, most notably free radicals. It is anticipated that alternate fabrication strategies such as chemical vapour deposition, together with specific annealing strategies to remove these parasitic spins [18], will overcome this issue. Finally, for near surface NV centres the sensing volume is limited ($\sim 10 \times 10 \times 100\,nm^3$) by the T_1 and T_2 relaxation and coherence times, respectively. Therefore, the NV centre is subject to statistical variations in the magnetic signal from individual molecules in aqueous solution, this is particularly problematic for DEER protocols that are susceptible to orientation changes within the sensing volume. A promising zero magnetic field approach has been proposed to eliminate this orientation dependence, by matching the NV driving frequency to the target molecules hyperfine transition frequency [19]. Such protocols will be critical to the development and future application of ESR microscopy in studying processes such as cell metabolism, and the production and reactivity of organic free radicals. The maturation of quantum sensing techniques into a robust platform for nanoscale magnetic resonance spectroscopy represents an exciting time for both quantum and biomolecular scientists. In the coming years, we hope to see single molecule dynamics unravelled by these sensitive quantum-based ESR and NMR microscopes.

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