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Spinning around

Electron spin resonance is emerging as a valuable analytical tool with uses spanning from elucidating structures of protein complexes to characterising materials for hydrogen storage. Michael Gross reports

Most centres carrying out chemistry and life sciences research employ nuclear magnetic resonance (NMR) spectroscopists, who use the spin of certain atomic nuclei as a window into molecular structures ranging from small organic compounds to fairly large proteins. You have to hunt a bit harder, however, to find people who use the analogous study of the spin of the electron, electron spin resonance (ESR), in a comparable way. In the UK, for instance, there are only a dozen laboratories that use ESR beyond the level of routine analysis. However, a few dedicated centres are pushing the boundaries of this technology and making it suitable for an ever widening portfolio of chemical and biological research projects.

The longest-serving practitioner and a pioneer of the field is physical chemist Jack Freed, who started work on ESR at Cornell University, Ithaca, US, in 1963 and is still in full swing. In 2001, he set up the National Biomedical Research Center for Advanced ESR Technology (ACERT) at Cornell, which now features eight ESR spectrometers, all but one self-built. 'This way, we know exactly how we can get the best out of our machines,' Freed says. 'Commercial machines are all black-boxed, so it's hard to improve their performance.'

His research is mostly conducted in collaboration with other chemists, or structural biologists, who come to him when the more widely used structural methods are not giving the complete picture. 'ESR is complementary to NMR,' Freed explains. 'While NMR measures distances between nuclei that are between 1 and 7 Å apart, ESR can measure distances from 10 to 90 Å.' The techniques are similarly complementary in the time regime,

he adds, as they capture dynamics in different ranges of timescales.

Protein architecture

The ability to measure larger distances is a crucial advantage in the study of large functional complexes involving several proteins. These are often membrane-embedded or highly flexible and hard to crystallise. And while there may be NMR or crystal structures of individual subunits, studying the assembly and function of the whole molecular machine is often out of reach for these techniques. Fixing long-range distances by ESR can help researchers to work out how the smaller pieces fit together.

For instance, Freed has – in collaboration with his Cornell colleague Brian Crane – studied the sensory system that allows the heat-loving bacterium *Thermotoga maritima* to swim towards a source of nutrients, a process known as chemotaxis. Like the bacterial motor

In short

- ESR is a complementary technique to the better known NMR
- It can establish long-range distances in protein molecules, aiding structure assignment and understanding of biological processes
- Other uses include quantum information processing, developing hydrogen storage materials and studying the compass of migrating birds

Freed (left), Crane (second from left) and colleagues at Cornell

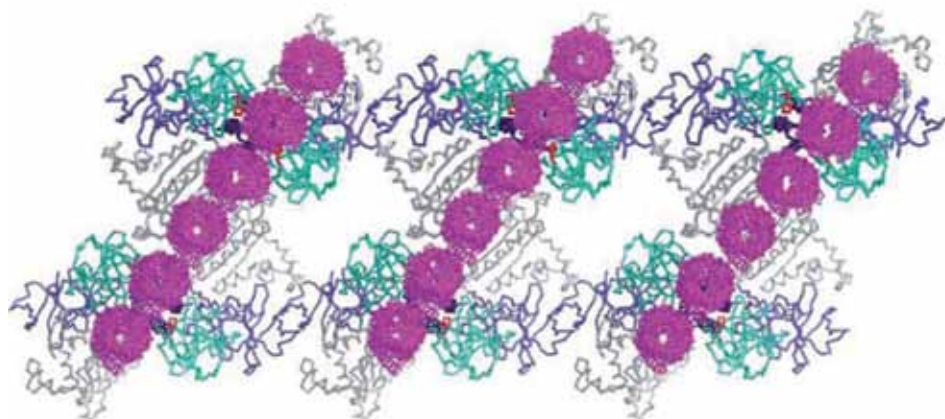
itself, the sensory system that controls it is a complex molecular machine embedded in the bacterial cell membrane. Even though structural biologists using NMR and x-ray crystallography have managed to elucidate the molecular structures of small parts of the machine, the overall assembly and functional architecture has remained unknown.

In order to apply ESR distance measurements to such systems, the researchers have to tag their proteins with molecular groups carrying a stable nitroxide radical, which is a chemical means of introducing an unpaired electron. The ESR spectroscopists call this a spin label, or simply a label. The labels are needed because nearly all electrons in ordinary molecules come in pairs whose spins cancel out and are therefore invisible to the ESR spectrometers. Obviously, they can only attach such spin labels in certain locations, but as it is long-range distances being measured, this isn't too problematic.

Spin labels are what certain atomic nuclei (eg ^1H) are to the NMR spectroscopist. Their spins align with the strong magnetic field and can be flipped by radiation of the right wavelength (in this case the microwave range, NMR uses radiowaves). In both techniques, the interaction of the aligned spins with the radiation is highly sensitive to changes in the atomic environment and to the presence of other spins in the neighbourhood.

In their first analysis of the sensory complex of *T. maritima* chemotaxis, published in 2006, Freed and colleagues measured 12 distances between key locations in a simplified assembly of four protein molecules.¹





This was the first report of ESR measurements of distances between separate protein molecules. They used the resulting distances as constraints to assemble a model of the components with known structures. Using this model they then suggested how the receptor pair should align when added to this four protein complex.

However, further measurements carried out in recent years with the full complex of six molecules showed, as Freed readily admits, that the initial model was wrong. Essentially, two subunits sticking out of the structure like the legs of a table had been attached to the wrong side of the table surface. A full structural analysis based on a large number of constraints between all six proteins has just been accepted for publication.²

Other biological systems that Freed and colleagues have recently studied by ESR include the Snare complexes,³ which facilitate the release of neurotransmitters at the synapse, and the protein α -synuclein, whose change of conformation has been linked to Parkinson's disease.

Shining light on photosynthesis

The laboratory of Gunnar Jeschke at ETH Zurich, Switzerland, is also involved in collaborative projects examining biologically relevant transmembrane complexes. Jeschke has teamed up with Harald Paulsen from the University of Mainz, Germany, to study the folding process of a key light harvesting complex involved in photosynthesis, known as LHCII (one of the most abundant membrane proteins on Earth). This protein starts out unfolded in water, and then simultaneously folds up and inserts itself into a membrane. Recently, the researchers have reported the first

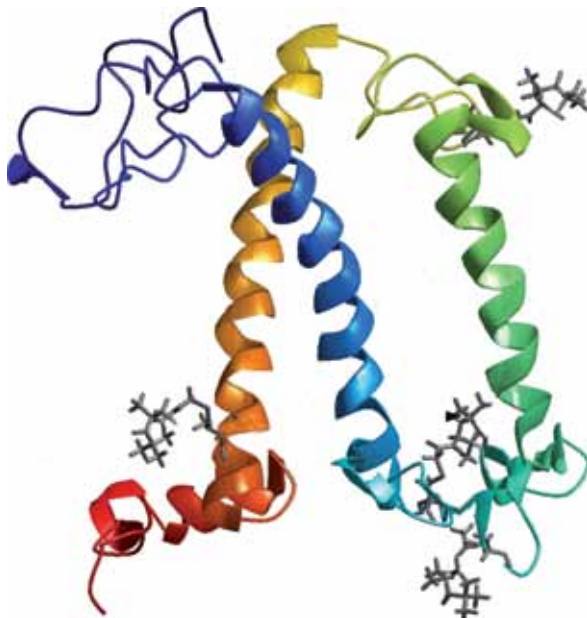
details of both the molecular folding process of this protein complex,⁴ and the insertion step.⁵

'In the first study,' Jeschke explains, 'we measured distances as a function of folding time for two pairs of selected sites in the protein.' In doing so, Jeschke says, the first experimental proof for the hypothesis that transmembrane proteins fold up in two distinct steps was obtained.

In the second study, the researchers exchanged the water the protein is in for heavy water (containing the hydrogen isotope deuterium). 'Deuterium nuclei close to an unpaired electron cause a characteristic modulation of the ESR signal,' Jeschke says. 'The depth of this modulation reveals the local concentration of deuterated water near the spin-labelled site in the protein.' As the membrane is hydrophobic, and so doesn't admit water, by monitoring the modulation the team could observe how fast certain parts of the protein embedded in the membrane.

ESR has aided knowledge of how the bacterium *T. maritima* swims towards nutrient sources

The folding of the LHCII protein is also being explored using ESR



Interdisciplinary design

Freed and Jeschke both acted as advisers when chemists at the University of Oxford, UK, set up a new interdisciplinary research centre dedicated to ESR. The Centre for Advanced ESR (CAESR) started out in 2007, with 11 principal investigators from five departments and with a portfolio of diverse projects. At the biological end of the spectrum, Susan Lea and co-workers at the school of pathology use Deer (a type of ESR called double electron electron resonance) distance measurements to study the folding and assembly of medically relevant proteins such as the von Willebrand factor,⁶ a large multimeric glycoprotein found in the blood plasma and implicated in several diseases.

Over in the physics and materials departments, Arzhang Ardavan and John Morton are using a self-built ESR spectrometer to manipulate the electron spin and use it in quantum information processing. In one project, the researchers are developing methods to use nuclear spins for information storage and electron spins for information input and output.⁷

In between, there are the chemists who are keen to exploit ESR methods for any problem that happens to involve an unpaired electron or two. The group of Peter Edwards, who co-chairs CAESR (with Christiane Timmel), is interested in the mobility of single electrons in solutions. For instance, if metallic lithium is dissolved in liquid, water-free ammonia, the atom splits into a Li^+ ion and a dissolved electron. A deep blue solution forms due to each of the single electrons occupying its own solvation sphere.

At higher concentrations, however, the solution undergoes a transition to a metal-like state with a bronze colour, but its conductivity is proportional to the temperature, which is typical of a semiconductor, not a metal. Postdoc researcher Kiminori Maeda is currently studying the mobility of the single electrons in this fascinating system. 'The electrons hop between solvent shells,' Maeda explains. 'Optical spectroscopies cannot detect this process, as the reactant and the product are the same species.' However, in the ESR observations, the movement leaves a characteristic trace in the so-called hyperfine interactions (a splitting of signals analogous to

Alphabet soup: how to tell X band from W band

ESR spectroscopists are so fond of acronyms that they have two different ones for their own technique. Aside from ESR (electron spin resonance), some researchers in the field prefer the term EPR (electron paramagnetic resonance) – both referring to exactly the same technique.

To specify the field strength and frequency range in which they are operating, NMR spectroscopists tend to cite the frequency of the radiowaves with which their nuclei interact, eg 900MHz would be the



resonance frequency for hydrogen nuclei in a very big and expensive magnet, while 500MHz would describe a more modest one that might serve in routine protein analysis.

In ESR, by contrast, life is more complex with 14 different letter codes given to the different microwave frequencies. For example, the commercial X band instruments (often used for routine analytics) run at 10GHz, while W band means 95GHz and J band 285GHz.

J-coupling in NMR, which reflects cross-talk between spins, as opposed to simple signal shifts caused by the chemical environment). 'The study of these historic metal ammonia systems has a strong link with the spectacular natural phenomenon of superconductivity,' Edwards explains.

Edwards' team hopes that an in-depth knowledge of this elementary process of electron migration might also help to better understand electron movements in more complex systems, for example during redox reactions catalysed by proteins. 'The presence of CAESR has catalysed our own thinking on electron conduction and electron transfer in systems as diverse as doped semiconductors, proteins and metal solutions,' says Edwards. 'I believe there are still many, many surprises hidden in these venerable chemical curiosities,' he adds.

Chemists at CAESR are also using ESR to develop better ways of producing and storing hydrogen, and to develop models for the 'chemical compass' of migrating birds,

which they believe to be rooted in a stable pair of radicals. Last year, Christiane Timmel and Peter Hore demonstrated a model compound that was sensitive enough to detect the inclination of the Earth's magnetic field.⁸ Although it may sound far-fetched, this mechanism is at the moment the only plausible explanation of bird navigation. Exactly how it is implemented in birds, however, remains to be discovered.

Looking for labels

All in all, there is no shortage of ideas regarding what one could do with ESR spectroscopy across a wide range of fields, even though both funders and commercial instrument developers appear to have invested most of their efforts in NMR instead. While Oxford's CAESR has received a substantial grant from the research councils, Freed says Cornell's ACERT is getting the same figure in its ninth year as in its first year, so the financial support in real terms is gradually dwindling.

Cash considerations have also

'We've confined ourselves to 9T magnets, as they get very expensive beyond that'

limited the extent to which Freed's group can explore the use of higher frequencies, which – as in NMR – requires the use of extremely powerful magnets. Standard commercial instruments used in routine analytics have magnets of 0.3 Tesla, corresponding to an excitation frequency of 9GHz. In building more powerful instruments, Freed says, 'we've confined ourselves to 9 Tesla magnets, that's 250GHz, because magnets get very expensive beyond that'.

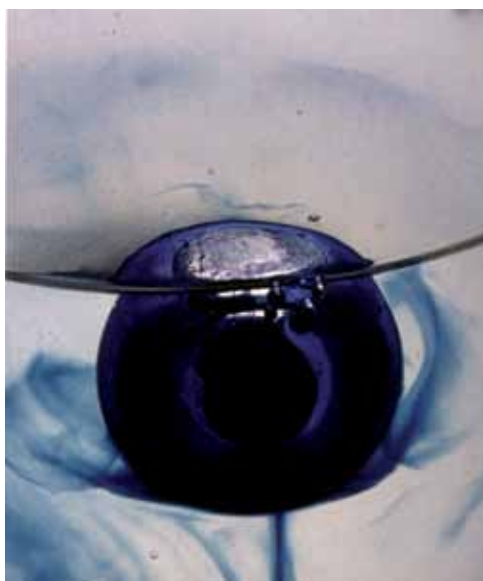
A more significant limitation of ESR research is in the chemistry, however. The widely used nitroxide spin labels, typically stabilised by a few methyl groups surrounding the polar N–O group, are attached to the molecule being studied by a flexible tether.

Therefore, they lead to some uncertainty in translating the distance between the nitroxide labels into the distance between the labelled sites on the molecule, and in studies on molecule dynamics the motions of the tethers complicate the analysis.

'We need organic chemists to design better labels,' Freed declares. That and the funding worries apart, however, he seems to be quite happy with the technique he spent the last 47 years optimising and pushing forward.

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In NH₃, Li dissociates to form a deep blue solution. At higher concentrations it turns bronze



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