Malte Drescher provides insights into cells

Electron spin resonance (ESR) spectroscopy can deliver information about the structure and dynamics of large molecules and was used by Dr. Malte Drescher from the University of Konstanz to develop a method for the structural analysis of biological macromolecules inside cells. It provides insights into the complex structure of molecules under physiological conditions and is a promising approach for the development of cancer drugs.

Biological macromolecules often form complex three-dimensional structures, which cannot be predicted from their constituents alone. Techniques such as X-ray diffraction analysis and magnetic resonance spectroscopy are used to analyse the structures, but require the macromolecules to be present in crystalline or highly concentrated form. However, the conformation of macromolecules can vary depending on the environment. It is therefore hugely important that macromolecules are not only analysed in isolated form, but also in the physiological environment of cells. However, the overwhelming number of molecules in cells makes it rather difficult to specifically analyse a single macromolecule without interference from other signals. Physicochemist Dr. Malte Drescher of Konstanz University has now made this possible. Working with chemist Prof. Dr. Jörg Hartig and biologist Prof. Dr. Daniel Dietrich, Drescher has developed a new technique based on electron spin resonance (ESR) spectroscopy that enables its interference-free application in complex environments. ESR spectroscopy provides distance information on the nanometre scale, which enables conclusions to be drawn on the three-dimensional structure of the macromolecule under investigation. “As we deal specifically with biological macromolecules, we wanted to find a way that enables intracellular measurements free of background noise,” said Drescher explaining the origin of his idea.

Drescher was dependent on the cooperation of colleagues from other scientific disciplines in order to turn his idea into reality and quickly found the necessary support from colleagues in the faculties of chemistry and biology at the University of Konstanz. He already knew Jörg Hartig, an expert in chemical synthesis, from previous successful collaborations and from the Research School Chemical Biology at the University of Konstanz. The two scientists quickly agreed that the project was of high scientific relevance and they managed to bring cell biology expert Daniel Dietrich on board. “Konstanz is ideal for projects on chemical biology issues,” said Drescher whose decision to focus on scientific research was made easy when the German Research Foundation decided to fund his research with an Emmy Noether grant in 2008. “The conditions for continuing my research projects were so good that it was an opportunity not to be missed,” says Drescher. The grant was not the only reason why he decided against a career in industry. “A career in industry would not have given me the student contact that I really enjoy,” says the physicochemist.

Spin labels for measuring distances

Dr. Malte Drescher of the University of Konstanz deciphers the structure of macromolecules inside living cells.
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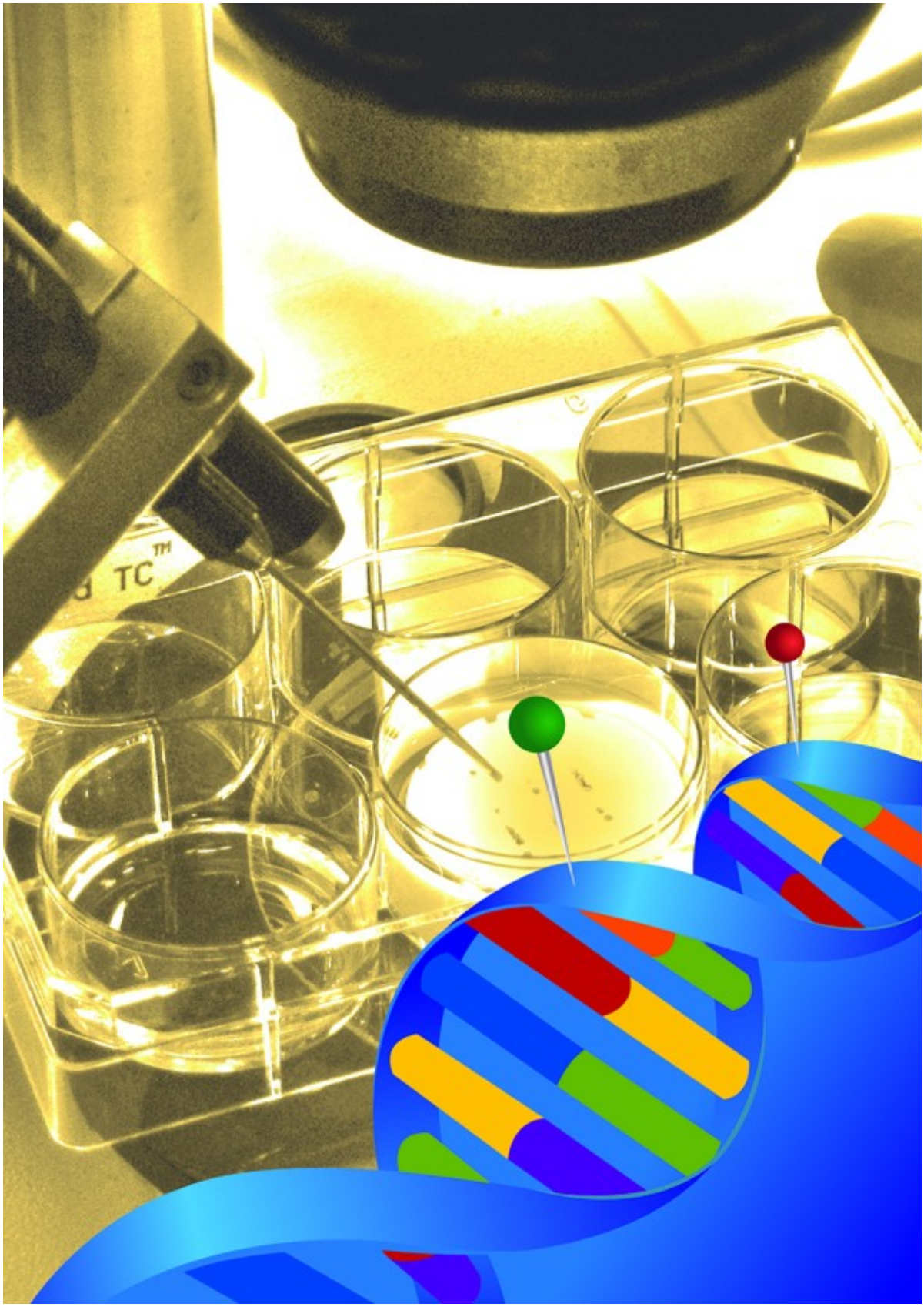
ESR spectroscopy involves the use of spin labels. “Spin labels are small marker molecules that possess an unpaired electron and the ability to bind to another molecule,” says Drescher, going on to explain that DNA investigation involves the use of synthetic, spin-labelled building blocks to build DNA nucleotide by nucleotide using solid-phase synthesis. In order to study the conformation of proteins, cysteine mutants to which spin labels can be attached in a specific reaction are introduced into the amino acid chain.

Unfolded, spin-labelled macromolecules are injected into the cell where they adopt a conformation that corresponds to prevailing environmental conditions. The distance between the spin labels can



Spin-labelling enables scientists to investigate the structure of macromolecules such as DNA and proteins in cells.
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subsequently be measured using ESR. The spin labels are detected by way of their magnetic momentum that is linked to their spin. This is similar to NMR spectroscopy where a particle is placed into the outer magnetic field. However, the magnetic momentum is considerably stronger, making the processes much quicker. A dipole-dipole interaction occurs between two spin labels whose strength depends on the distance between the two labels. “Our method is not targeted at highly

resolved three-dimensional structures; the approach is in fact more similar to the FRET method," says Drescher (ed. note: FRET: fluorescence resonance energy transfer). Selected characteristic sites of the molecule are spin-labelled and measured with the aim of drawing conclusions on the molecule's conformation by comparing these data with crystal structure data. With regard to intracellular measurements, the technique has the advantage that it only detects unpaired electrons, which are quite rare in cells.

Following the injection of a spin-labelled macromolecule, measurements can be carried out free of background noise. Moreover, the technique's high sensitivity enables researchers to work with low macromolecule concentrations. "This helps us to study the protein alpha synuclein, which is associated with Parkinson's disease, under physiological concentrations in the cells," Drescher pointed out.

From molecule structure to new therapeutic approaches

Intracellular ESR is particularly suitable for studying polymorphic molecules whose conformation can differ considerably depending on the physiological situation. The molecules can be present as DNA quadruplexes, i.e. nucleic acid sequences that are rich in guanine and are capable of forming a four-stranded structure by way of hydrogen bridges between the four strands. These quadruplexes can be present in numerous different conformations and can also be found in the telomere ends of chromosomes. The formation of quadruplexes in telomeres has been shown to affect the activity of the enzyme telomerase. This enzyme is responsible for maintaining telomere length by restoring chromosome end and maintaining the DNA's ability to replicate correctly. Telomerase is involved in around 85 percent of all cancers. Knowledge of the physiological conformation of the DNA quadruplexes might therefore lead to the identification of cancer therapy targets. "Intrinsically unordered proteins are even more polymorphic as they can adopt different conformations under various environmental conditions. Alpha synuclein, which plays a role in the pathogenesis of Parkinson's disease, is one such unordered protein," says Drescher.

Up until now, Drescher and his team have mainly worked with oocytes of the African clawed frog (Xenopus) as they are cells that are relatively large and easy to manipulate. In addition, customised transfection methods also enable the researchers to study mammalian cells. Drescher believes that the expression of spin-labelled proteins using genetically encoded, spin-labelled synthetic amino acids remains a great challenge in the future. The researchers will now gradually optimise the technique in order to make it suitable for investigating the structure of proteins.

About

Malte Drescher did his doctoral thesis at the Institute of Physics in Karlsruhe on "Site-resolved electron spin resonance". Supported by a DFG research fellowship, he went on to work at the Institute of Molecular Physics at the University of Leiden (NL) from 2006 – 2007. In 2008, Drescher was awarded an Emmy Noether scholarship by the German Research Foundation (DFG) and has since been the head of the Emmy Noether research group Physical and Biophysical Chemistry in the Department of Chemistry at the University of Konstanz. His research group is mainly focused on the development of methods and applications based on electron spin resonance (ESR; also known as electron paramagnetic resonance (EPR)) spectroscopy. Drescher is a member of the DFG-funded collaborative research centre 969 and of the executive committee of the Research School Chemical Biology at the University of Konstanz.

Original publication:

Site-directed spin-labeling of nucleotides and the use of in-cell EPR to determine long-range distances in a biologically relevant environment", Mykhailo Azarkh, Vijay Singh, Oliver Okle, Isabelle T Seemann, Daniel R Dietrich, Jörg S Hartig, Malte Drescher, Nature Protocols, *8*, 131–147 (2013)

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