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Magnetic amino acids for measuring proteins

Dr. Malte Drescher and Dr. Daniel Summerer, two chemists from Konstanz University, have developed an innovative method for studying protein structures using magnetic labels inside cells. The method is based on non-canonical magnetic amino acids that are directly incorporated into the protein as it is biosynthesised in the cell. A patent has been filed for the method, which could potentially lead to major progress in the field of structural biology.

A protein's three-dimensional structure relies on the interactions between its amino acid building blocks and is crucial for protein function. Despite huge progress in bioinformatics, protein structure is still difficult to predict, which is why the experimental determination of a protein's three-dimensional structure is still of key importance. Several methods are available to determine a protein's structure, including electron spin resonance (ESR; also known as paramagnetic resonance (EPR)) spectroscopy which can be used to study proteins inside cells (see article entitled "Malte Drescher provides insights into cells"). Proteins are labelled with several spin labels (or spin probes) with unpaired electrons, which then interact with each other in relation to the distance of the unpaired electrons from each other. The spin-labelled proteins are subsequently injected into the cells where they take on their natural conformation. The strength of the magnetic labels enables the researchers to deduce information on the distance between the labels and hence on the three-dimensional structure of the protein under investigation.

The cell suspensions used for electron spin resonance spectroscopy are transferred into special sample vials.
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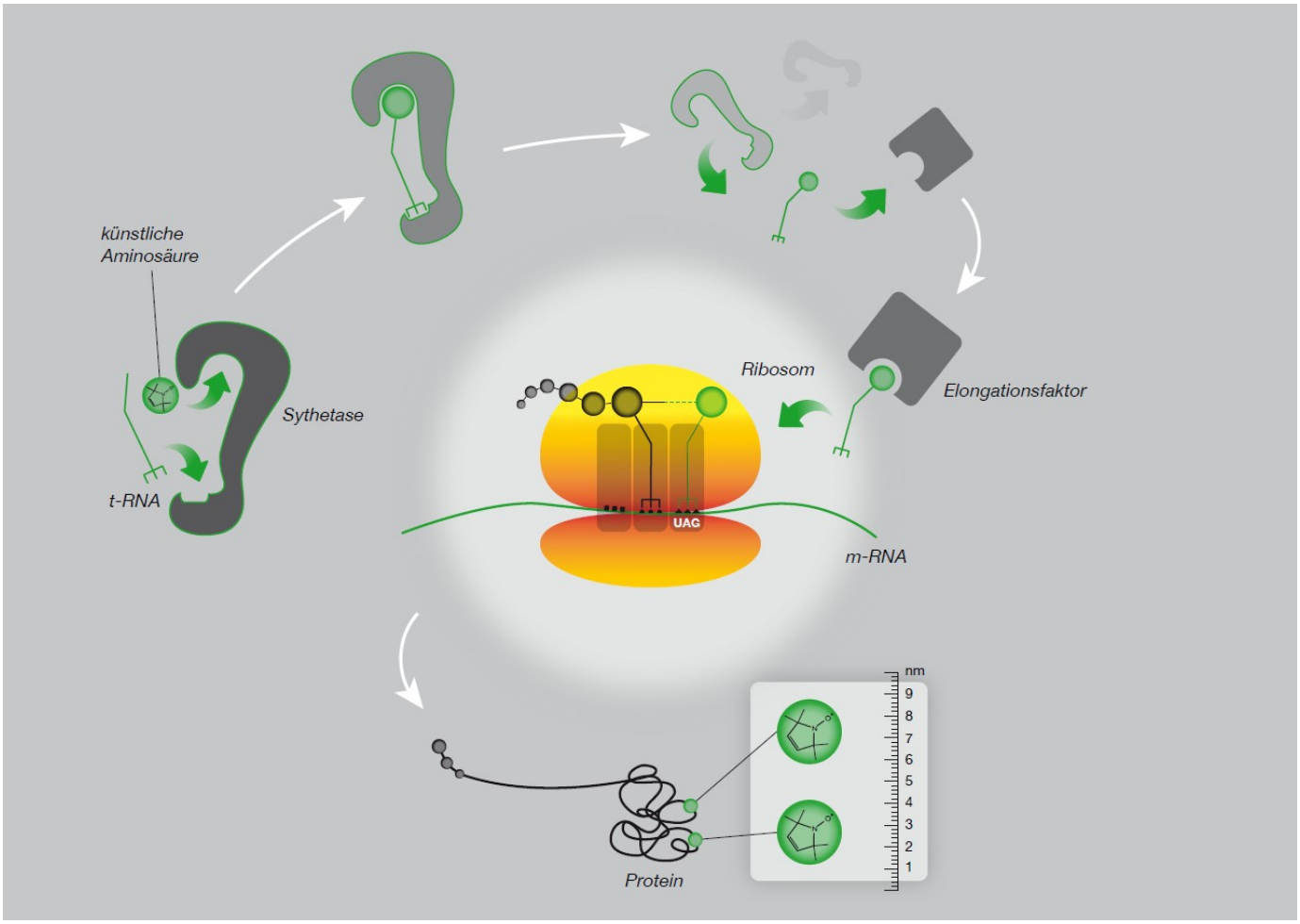
As effective as the method might be, it still has a number of drawbacks. Dr. Malte Drescher from the University of Konstanz explains why: “Conventional spin labelling is a very time-consuming process as we have to specifically prepare the proteins that we want to investigate,” says Drescher. Chemical compounds which can be used as spin labels are usually attached to the amino acid via their cysteine residues. Therefore, the researchers need to remove all cysteine codes in the DNA strand of the protein under investigation and introduce new ones at strategically chosen protein sites. The protein is subsequently expressed and the spin label attached. “However, we have to keep in mind that the native cysteines might play a key role in determining the protein’s three-dimensional structure,” says Drescher, a physical chemist. In order to overcome the method’s limitations, Drescher sought a simpler method which involved the use of genetically encoded non-canonical amino acids, typically employed in the expression of proteins with made-to-measure functions (see articles entitled “Red light to elucidate the regulatory mechanisms of gene expression” and “Daniel Summerer: new ways of biosynthesising proteins with novel functions”).

A clever trick to solve the problem

It was not long before Drescher found cooperation partners who could help make the impossible possible. One of the partners he found was Dr. Summerer, another chemist at the University of Konstanz whose area of expertise is the creation of synthetic amino acids and who is well aware of the potential of magnetic amino acids. “However, we all knew that all of the research groups then working in this area had failed to develop paramagnetic non-canonical amino acids,” says Drescher, referring to the amino acids' reactivity, which is still poorly understood. Although it was known that the lifetime of magnetic compounds is far shorter than is required by protein expression, researchers lacked insights into the concrete chemical reactions associated with the loss of magnetism. The solution to the problem was to reprogramme the protein biosynthesis machinery so that non-canonical amino acids could be incorporated into proteins. This required highly stable amino acids.



Schematic showing how a non-canonical amino acid is incorporated into a protein: (from the left): synthetase enzyme transferring a non-canoncial amino acid to a tRNA, transport of the loaded tRNA to the ribosome where the amino acid binds and is incorporated into the growing polypeptide. Insert: distance between two incorporated amino acids in a native protein.
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In order to improve this situation, Drescher and his group of researchers decided to concentrate on developing magnetic amino acids by specifically modifying protein biosynthesis and establishing new protein labelling processes. "We have now achieved a real breakthrough. We are a lot closer to our goal of being able to precisely measure the structure of proteins directly in the cell," says Daniel Summerer of the research project. Summerer's working group used a special trick to prevent undesired reactions and to stop the magnetic label from losing its reactivity. "Rather than using magnetic amino acids to reprogramme the cellular biosynthesis machinery, we used stable non-canoncial amino acids whose structure is very similar to that of the target amino acids," explains Dr. Summerer. Initial experiments have shown that the modified protein biosynthesis machinery was nevertheless subsequently able to also use magnetic amino acids and reliably incorporate them into different proteins.

Magnetic amino acids were then used to examine protein structures by means of ESR spectroscopy. "We started off by incorporating two amino acids into a protein with a known structure and measuring the magnetic interactions between the two in order to evaluate the efficiency of the method," says Dr. Drescher. The researchers were able to deduce the distance between the two amino acids from the strength of the magnetic interaction. "Our experimental results were identical to the theoretical predictions achieved for the known protein structure. This is a clear indication that the method works," concludes Drescher.

Huge potential for the investigation of biological structures

The non-canonical amino acids possess an unpaired electron, which is predominantly localized to the N-O-bond. This electron makes the amino acid (para)magnetic and enables the amino acid to be detected using ESR spectroscopy. "The use of such amino acids offers completely new options in terms of new protein properties and perspectives that cannot be achieved with other methods; by this I mean the measurement of precise, absolute distance distributions in proteins without the presence of disturbing background noise," says Dr. Summerer with obvious enthusiasm. This is a particularly useful advantage in a complex environment such as the one inside a cell. The researchers were also able to demonstrate that the non-canonical amino acids did not influence the properties of the labelled protein. "Compared to other labels such as fluorescent dyes that are chemically coupled to a protein, our magnetic label is tiny and the potential disturbance is either minor or not present at all," says Drescher. In contrast to conventional spin labelling procedures, the Konstanz researchers' method does not require the removal of the protein's native cysteines, something that may be crucial in terms of protein folding. In addition, the method also enables the researchers to label previously inaccessible sites in the protein.

The researchers will now use their new method for elucidating previously unknown protein structures. They also plan to further optimise the method. "The results we have published so far have all been obtained with E. coli bacteria. However, we have also carried out some experiments with human cells, and the results are quite promising. We therefore believe that we will be able to adapt the method to different mammalian cells," says Dr. Summerer. The chemists have also filed a patent for the method. In addition to using the method for investigating biologically relevant systems, the patent gives them the opportunity to commercially exploit their invention. "We have begun discussions with the industry and we have already initiated a concrete product development project," says Summerer.

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Article

05-May-2014
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BioLAGO
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