Thousands on one chip: New method to study proteins

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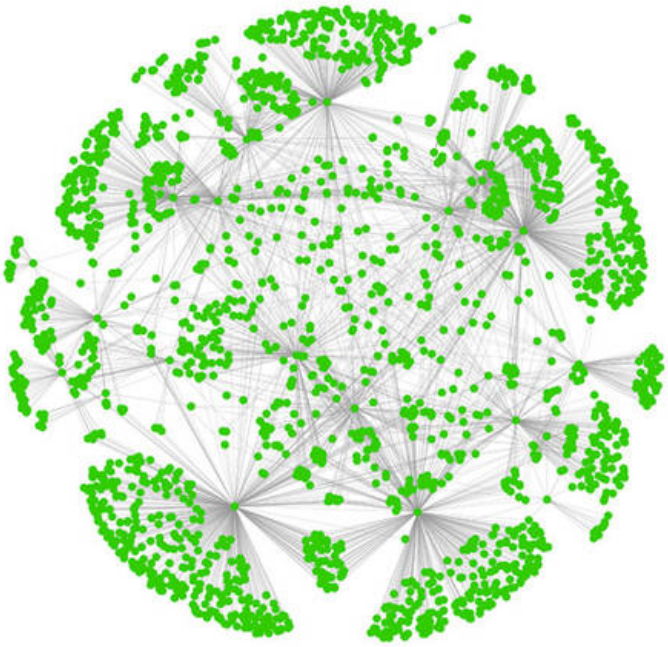

Proteins in plants and in man do not act in isolation but have mutual regulatory relationships and act together in complex networks – to see in this picture. Credit: TUM/Falter-Braun

Since the completion of the human genome an important goal has been to elucidate the function of the now known proteins: a new molecular method enables the investigation of the function for thousands of proteins in parallel. Applying this new method, an international team of

researchers with leading participation of the Technical University of Munich (TUM) was able to identify hundreds of previously unknown interactions among proteins.

The human genome and those of most common crops have been decoded for many years. Soon it will be possible to sequence your personal genome for less than 1000 Euros. At yet, there is a well-kept secret: for thousands of the roughly 20,000 - 30,000 proteins encoded in the genome it is not clear what they do in the body, which function they have. This makes it difficult to interpret many upcoming data and understand the underlying molecular processes - and this is the case in diverse fields such as medical research, plant research or the development of alternative energy sources.

The function of a protein is a composite of many different aspects: with which proteins does it work together? How are its functions regulated and which processes are affected by it? Even for the reference plant thale cress (*Arabidopsis thaliana*) the function for about 10,000 proteins remains enigmatic. Filling this knowledge gap will take a long time using current methodologies. Elucidating these molecular functions is therefore of preeminent importance.

Microarrays enable the Investigations of Thousands of Proteins

Protein microarrays allow the investigation of thousands of proteins in a single experiment. Microarrays are only a few centimeters in size and host thousands of individual test spots on very small space. To produce standard protein microarrays small amounts of proteins are printed to a glass slide and chemically fixed in each spot where they are then available for experiments. However, this approach requires the prior production and purification of thousands of proteins, which is time

consuming and expensive. Together these costs have prevented the widespread use of protein microarrays despite their enormous potential.

The research group of Pascal Falter-Braun of the Chair of Plant Systems Biology at TUM together with colleagues from the USA and Japan now achieved a possibly decisive breakthrough: DNA, which is much easier and cheaper to produce, is printed instead of proteins and the protein arrays are subsequently 'developed'. DNA contains the information that specifies the shape of proteins. After printing the DNA on the array the latter is submerged in a reaction mixture that synthesizes the proteins specified by the printed DNA. A chemical anchor that is attached to the glass surface rapidly and tightly captures the so developed proteins, which are then available for functional studies.

The method is called 'nucleic acid programmable protein array' which, in conjunction with the employed capture agent, is abbreviated Halo-NAPPA. By using the new capture chemistry the researchers were able to increase the density of the arrays such that it is now possible to accommodate all proteins encoded in a genome on just a few arrays. The scientists could demonstrate the potential of the protein arrays in the context of plant hormone signaling pathways, which, for example, mediate responses to drought stress or against pathogens.

1000 novel Protein-Protein Interactions discovered

For the study now published in *PNAS* interactions of 38 of some of the most important transcription factor proteins of thale cress were investigated. Transcription factors determine which genes are active at what time and in which conditions and consequently have a critical role in organisms. The transcription factors themselves can be activated or inactivated by interacting with other proteins - in the present study nearly 1000 new interactions for the investigated transcription factors were detected using the protein microarrays. "Many of the now observed

interactions have never been documented. They will help us to understand how biological systems and the underlying molecular networks function", says Falter-Braun.

Proteins in plants and in man do not act in isolation but have mutual regulatory relationships and act together in complex networks - the research focus of the TUM team around Falter-Braun. In all organisms proteins have key roles and execute nearly all biological processes. "Possibly, the new method is a milestone towards understanding which proteins interact with which other proteins or other molecules in cells. Because it is cheaper and simpler a wider range of researchers can now work with these protein arrays to investigate protein functions" says Falter-Braun.

The scientist is convinced that the new method will also help to accelerate research in the research on renewable energies and the understanding of diseases.

More information: Junshi Yazaki et al, Mapping transcription factor interactome networks using HaloTag protein arrays, *Proceedings of the National Academy of Sciences* (2016). DOI: 10.1073/pnas.1603229113

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