Waking proteins up from deep sleep to study their motions

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In order to carry out their functions, proteins need to move. Scientists at EPFL have developed a new technique to study motions in proteins with unprecedented accuracy. The method, which is based on NMR, freezes proteins down to immobility, then slowly heats them to 'wake them up' and restart motions individually and in sequence, providing a slow-motion image of real conditions.

Proteins inside a cell are in constant motion, changing shape continuously in order to carry out their functions. In addition, their multiple component atoms each have individual patterns of motion, making the entire protein a system of non-stop highly complex movement. Understanding how a protein moves is the key to developing drugs that can efficiently interact with it. But because of this complexity, protein motion has been notoriously difficult to study. Scientists at EPFL, IBS-Grenoble, and ENS-Lyon, have developed a new method for studying protein motion by first freezing proteins and then slowly "waking them up" with increasing temperature. The breakthrough method, which was developed at EPFL's advanced NMR facility, is published in *Science*.

Protein motion is highly complex

Motion is part of a protein's function, allowing it to adjust its 3D shape and interact with other molecules like biological molecules and synthetic drugs. These "functional" motions however are complex, and can be

thought as the mechanism of a watch, where motions between interlocking cogs and springs, at different timescales, result in the smooth movement of the hands.

In a protein the cogs and springs are the molecules that make it up: amino acids form its backbone each with side-chains of different molecules branching out on all sides in three dimensions. In addition, water molecules on the protein as well as the solution where it exists, e.g. the cell's cytoplasm, add even more layers of motion complexity to the system. But unlike a watch, whose individual movements are all well defined, each of the component motions in a protein are actually random. As a result, protein motion seems almost chaotic, and is practically impossible to study.

Freeze, sleep, wake up, and move

A team of scientists at EPFL, IBS-Grenoble, and ENS-Lyon led by Lyndon Emsley and Martin Blackledge developed an innovative solution to the motion problem: freeze the proteins and then watch them "wake up" from deep sleep. Protein motion depends on energy, and temperature is basically a measurement of the energy of a system. By freezing proteins down to temperatures of -168°C, the researchers were able to completely stop all the motions of interest in the molecules. Then, they slowly raised the temperature to the point where the proteins could regain their natural motions, but at a much slower pace. This way, it was possible to look at each motion a protein makes individually and - more importantly - in sequence.

In order to detect the individual motions of proteins, the scientists used a spectroscopic technique called nuclear magnetic resonance (NMR), which exploits the magnetic properties of certain atoms like hydrogen and carbon. NMR works by placing the sample of the protein to be studied inside a device with a strong magnetic field, and observing how

they respond to different radio frequencies. This response is registered on a computer that produces a diagram of peaks, each representing energy transitions in specific atoms. Depending on the properties of the peaks on the diagram, scientists can determine the degree of motion of each atom in the protein, e.g. its backbone, a side-chain etc.

Because the proteins in this study needed to be frozen down, the team had to adjust their NMR methodology to work with samples at very low temperatures, and keep doing so as the researchers slowly raised the temperature to "wake the proteins up". In addition, samples that are frozen solid are difficult to read in NMR, so the tube containing the proteins had to also be constantly spinning at a specific ("magic") angle to the NMR's magnetic field, to improve resolution. Finally, every NMR experiment took days to perform.

These complications were overcome by using a newly developed device that had been specifically designed to work with NMR at low and changing temperatures. To achieve the necessary high resolution, the scientists combined this device with a precise rotor system that could spin the sample over long periods of time.

A hierarchy of motion

Using their innovative approach, Emsley's team found that the sequence of protein motions follows a specific hierarchy as temperature increases: first the protein's solvent molecules, then the protein's side-chains and water molecules, and finally the protein's backbone. The sequence culminates with a functionally active protein at temperatures even as low as -53°C, well below physiological levels. This means that the "waking up" method is very effective for studying the motions of a protein individually and sequentially.

"Our work shows that we can use this technique, which is called 'variable-

temperature solid-state NMR', to gain unique and novel insights into the role of protein dynamics in biology," says Lyndon Emsley. The team is now interested in using this method to find out just how universal this hierarchy of motions is, and what might cause variations between different molecules.

More information: Lewandowski J.R, Halse ME, Blackledge M, Emsley, L. Direct observation of hierarchical protein dynamics. *Science* May, 1, 2015. www.sciencemag.org/lookup/doi/ … 1126/science.aaa6111

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