In order to carry out their functions, proteins need to move. Scientists at the IBS Grenoble and EPFL, in collaboration with ENS Lyon, have developed a new approach to the study protein motions with unprecedented accuracy. By freezing proteins down to very low temperatures, researchers slowly heat up the protein and watch the different components of the protein as they combine to produce the dynamics that are essential for function.

Proteins are complex machines that are in constant motion, moving continuously in order to carry out their functions. Their multiple component atoms also have their individual motion patterns, 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 its complexity, protein motion has been notoriously difficult to study. Scientists at IBS-Grenoble, EPFL 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 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 bound to the protein as well as in the solution where it exists, e.g. the cell's cytoplasm, add even more layers of motional detail to the system. Protein motion seems almost chaotic in its complexity, but somehow combines to provide the biological function essential for life.

Freeze, sleep, wake up, and move

The team of scientists led by Martin Blackledge and Lyndon Emsley have 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 allows the researchers to dial more energy into the system in a controlled way. By freezing proteins down to temperatures between -168°C to 7°C, they were able to almost completely stop all motion, then slowly raise the temperature to the point where the protein could regain their natural motions, measuring the dynamics of the protein along the way. This way, it was possible to look at each motion a protein experiences individually and – more importantly – in the right order.

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, nitrogen and

carbon. Because the proteins in this study needed to be frozen down, the research team had to adjust their NMR methodology to work with samples at very low temperatures, allowing consistent readings, and keep doing so as the temperature increased to "wake the proteins up". To make life more difficult, samples that are frozen solid are difficult to read in NMR, so in this study the tube containing the proteins had to also be constantly spinning at a specific ("magic") angle to the NMR's magnetic field, in order to improve resolution. Finally, every NMR experiment took days to perform. These complications were overcome by using a newly developed device that has been specifically designed to work with NMR at low and changing temperatures, combined with a precise rotor system that could spin the sample over long periods of time.

A hierarchy of motion

Using their innovative approach, the Blackledge and Emsley teams found that the sequence of protein motions follows a specific hierarchy as temperature increases: first the protein's solution molecules, then the protein's side-chains and water molecules, and finally the protein's backbone. The sequence culminates at 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, without deviating from realistic conditions in a cell.

This work represents a collaboration between IBS Grenoble CEA/CNRS/UJF, EPFL's Laboratory of Magnetic Resonance and CNRS/ENS-Lyon.

Reference

Lewandowski J.R, Halse ME, Blackledge M, Emsley, L. **Direct observation of hierarchical protein dynamics.** *Science* XX XXXX. DOI: XXXXXXXXX