

Crowded cells: how the cellular environment affects the function of proteins

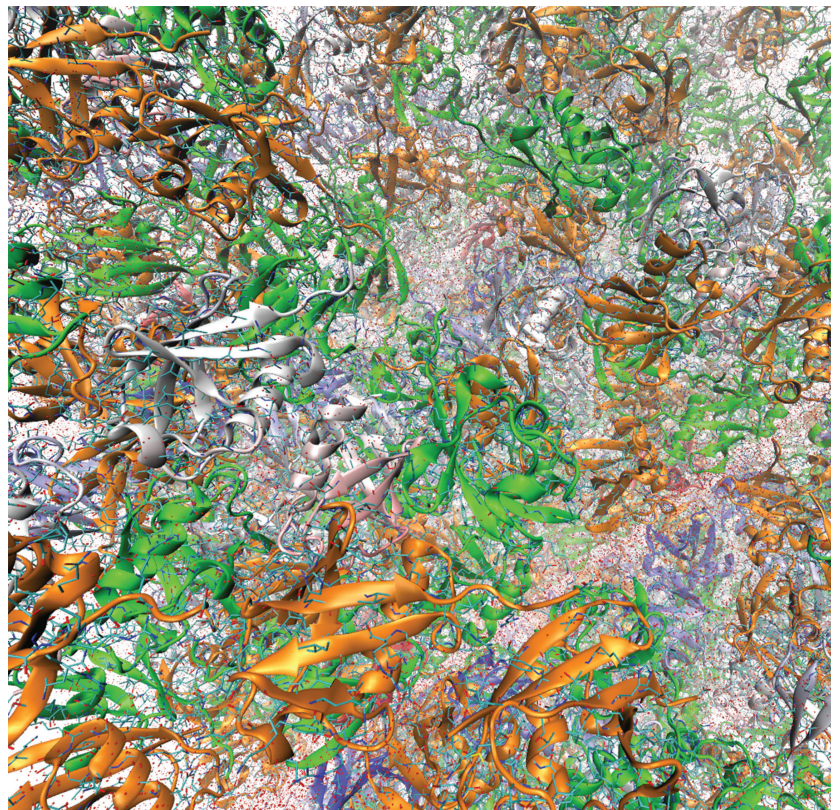
Studies of proteins are generally carried out in dilute conditions, but in reality they exist in the extremely crowded environment found inside cells. **Professor Matteo Dal Peraro** of EPFL in Switzerland has been investigating how this may affect experimental results by carrying out simulations of proteins in conditions more similar to those in the cell.

Proteins are essential to the function of living organisms, but the way we study them in vitro might be ignoring an important fact about how they exist in the real world.

Biochemical studies of proteins are performed in simplified in vitro systems, in which proteins are placed in diluted conditions so that they do not interact with other molecular entities. Although these conditions make it easier to understand properties of the protein in question, they do not accurately portray the dense and crowded mixture of molecules found in cells. This raises the question of how much these studies are actually able to tell us about the physiological function of proteins in living systems.

The notion is not a new one. Scientists have long known that conditions inside cells are more cramped than standard experimental conditions. In the past, the function of proteins has been studied through simulations that use extremely simplified versions of the other molecules present in the cell. Now, a group from EPFL (École Polytechnique Fédérale de Lausanne) in Switzerland has taken things one step further by painstakingly simulating the atomic structure and properties of each molecule, and have garnered some interesting results regarding the dynamics and possible interactions of proteins in the cellular environment.

“When a protein is moving through a cell, it is inevitably going to interact with a huge plethora of other molecules with various properties that will affect it differently,” explains Matteo Dal Peraro. “This is likely to have an effect on the ultimate function of the protein. To give just one example, experiments done in vitro do not contain membranes, which all molecules in the cell must interact with eventually. So there are obviously a myriad of different aspecific interactions going



Model of a solution containing protein (ribbon-like structures) and small solutes (stick-like structures) in water (red spheres)

on in the cell, each one unique due to each molecule’s specific physicochemical properties, which are being overlooked in in vitro studies.”

Dal Peraro and his colleague Luciano Abriata have been investigating this issue through simulations of ubiquitously in the membrane-bound cells of eukaryotic organisms, a small protein that, as its name suggests, occurs ubiquitously in the cellular tissues of eukaryotic organisms. “Ubiquitin has been extensively studied since its discovery in 1975, so there is a lot of literature related to it,” says Dal Peraro. “It is also an ideal molecule to use in simulations, as its small size means we can work with multiple copies of it and longer time scales.” Along with ubiquitin, the simulations involved smaller molecules such as amino acids with various chemical properties such as positive or negative charge, as well as small carbohydrates and large macromolecular crowders. Using NAMD, a parallel molecular dynamics code, each simulation used between 256 and 1024 cores for periods that extended over approximately 2-3 months of continuous running.

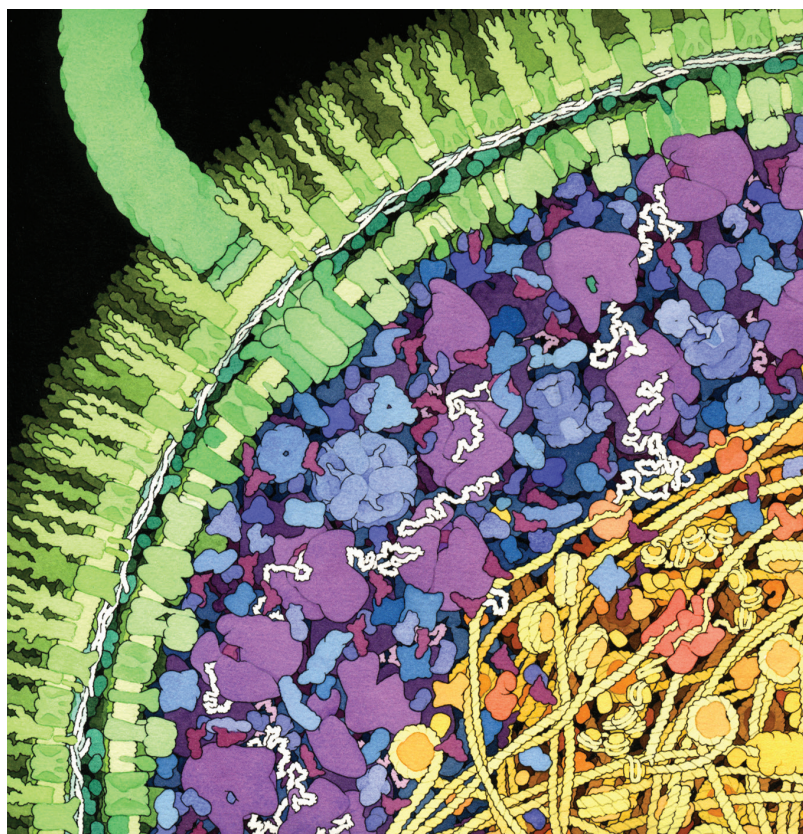
Studies about the effects of crowding in cells on proteins have historically focused on “coarse” protein traits. Excluded volume — the space taken up by other molecules that a protein therefore cannot move into — is one obvious factor taken into consideration. Viscosity — the resistance to flow and movement — of cellular contents is also a key parameter when considering the movement and function of a protein. However, recent evidence has suggested that protein traits seen at the atomic level are also affected by crowding, and this is what the EPFL researchers focused on in their project.

The simulations showed that the addition of small charged molecules altered the internal electrostatic properties of ubiquitin. “We are still unclear as to whether altering this property has any effect on the function of ubiquitin, but it is interesting to note that it is taking place. Further studies will be able to elucidate whether this is meaningful in terms of the protein’s function.”

Some molecules such as sugars were shown to stick to the protein, forming a cage around and effectively slowing down its internal dynamics. “This means that any function that ubiquitin has will happen a lot slower in the cell than when we observe it in dilute conditions in test tubes,” says Dal Peraro. “Again to really see how function is affected, we will have to carry out further investigations, but we can already see a number of new physicochemical effects of proteins in the cell that we have not seen before.”

Interactions with macromolecular crowders were shown to be favoured mainly through hydrophobic, but not through polar, surface patches. All the tested small solutes strongly slowed down water exchange at the protein surface, whereas macromolecular crowders did not exert such strong perturbation.

An illustration depicting a cross-section of a small portion of an E. coli cell.
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Following the work on the supercomputer, Abriata then called upon his experience in NMR spectroscopy to reproduce the same crowded environment that the ubiquitin experienced in the simulations. “We used the same concentrations of small and large crowder molecules and the same concentration of ubiquitin,” says Abriata. “This gave us parallel datasets of simulated and experimental data, allowing us to explore the physicochemical properties of the whole system more extensively. The molecular dynamics simulations have in turn allowed us to explain some of our NMR observations and propose other atomic-level effects that cannot be probed by NMR.”

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Although the researchers intentionally used a simplified system in which to study these molecules, the nature of atomistic molecular dynamics simulations meant that they were still hugely computationally expensive. “We were painstakingly observing the effects of every atom in the system over a fairly long time period and with multiple replications,” says Dal Peraro. “Needless to say, without support from PRACE and the computers we used we would never be able to carry out this work.”

The two researchers believe that their research carries an important message. “What we have shown is that whenever you are doing simulations or experiments about protein function, you must take the physiological conditions in which they exist into account,” says Abriata. “Maybe in the future, people can use work like ours to implicitly model the effects of crowding when studying proteins. We believe this needs to become the status quo.”

For more information

<http://lbm.epfl.ch/>

Resources awarded by PRACE

Matteo Dal Peraro was awarded 45 000 000 core hours on JUGENE, and 7 300 000 core hours on MareNostrum