Tracking Single Molecules

French biologists and physicists at the University of Bordeaux have teamed up to develop a super resolution imaging technique called uPAINT - and revolutionised single molecule imaging in the process.

It all started one and a half years ago when biologists Gregory Giannone and Eric Hosy from Daniel Choquet’s neurobiology lab joined physicist Laurent Cognet at the University of Bordeaux. The researchers wanted clearly to visualise a single molecule moving in the cell membrane. The collaborative study that followed involved input from Germany and America and culminated in the development of a super-resolution technique called uPAINT (Biophys J. 99(4):1301-10).

With the aid of this technique, researchers are, for the very first time, able to monitor the traffic of endogenous protein molecules at ultra-high densities in the living cell membrane.

So what is uPAINT all about? The idea is to observe live cells placed in a solution of fluorescent probes (ligands or antibodies that specifically bind to the molecule of interest) under the microscope and to visualise these molecules by constantly labelling them. By adding a dilute solution of probes, the researchers ensure that not all the molecules to be observed are labelled at once. Instead, only a fraction of the moving molecules get labelled at a time and, rendered fluorescent, can be tracked in real time. As these labels fade, other molecules become freshly labelled in a similar manner, and their movement can then also be followed.

The birth of uPAINT

Giannone explains the history of the technique, “To follow the dynamics of proteins in the confined cellular environment, especially at structures like neuronal synapses or adhesion sites, one needs to use a very small probe and a small linker to join the probe to the protein. The technique that we usually use is based on quantum dots that are almost 20 nm in diameter, making it difficult for them to enter intricate structures like the synapses. Moreover, they may also influence the protein dynamics.” The scientists therefore concentrated on reducing the overall size of the probes by using smaller kinds, such as gold nanoparticles and fluorophores like the Atto dye.

Hosy, who worked on characterising the ligands, their binding and photo-stability, explains the experiment. “During a single molecule tracking experiment, one first labels the cell and then rinses everything out. During our experiments, we incubated the cell with the dye label and then photo-bleached an area. At this point, we saw single protein molecules arriving as a function of time and also observed that some of the dye bound to the protein molecules was coming from the medium or the neighborhood of the cell.” Due to this residual population in the solution, the researchers were able to repeatedly label the protein molecule and, rendering the overall size of the probe, the possibility to label each membrane protein or receptor if we have a good antibody against it.

Research at the biophysical interface

A cell biologist by training, Giannone studied cell motility during his PhD in Strasbourg and the influence of calcium on the stability of integrin-dependent adhesion sites. He followed this up with a postdoctoral stint at Michael Scheetz’s lab in New York. His interest in cellular dynamics of micron scale structures prompted his move to the laboratory of Daniel Choquet at Bordeaux, where Giannone now holds a tenured position as an associate researcher.

Giannone’s research addresses the study of cell motility, cell adhesion and the actin cytoskeleton using single molecule techniques, many of which like uPAINT have been developed in Bordeaux.

Eric Hosy began his scientific career with a PhD in plant science in Montpellier, France, where he studied the electrophysiology of the plant stomata and later the KATP channel, a complex structure that is involved in diabetes regulation. His post doctoral study in Rotterdam helped him to discover his interest in neuroscience and this brought him to the Choquet lab. “My job was to establish electrophysiology-based techniques in Daniel’s lab. In turn, I was introduced to various optical techniques and this ultimately led to the discussion with Greg and Laurent that initiated work on the uPAINT project,” recalls Hosy.

His research interests involve the study of neuronal synapses and receptor distribution using two approaches: optical techniques like uPAINT as well as electrophysiology.

As a physicist, Laurent Cognet’s research interests deal with the ultrasensitive optical detection and spectroscopy of individual nano-objects (“Nanophotonics”) and their applications to the biological sciences. “This includes the optical study of..."
molecules, quantum dots, gold nanoparticles or carbon nanotubes at the single nano-object level, as well as the development of novel single molecule super-resolution microscopy methods for biology as exemplified by this work,” he adds. Applications in fundamental biology (mainly neurobiology) are then performed with biologists on a collaborative basis. Says Cognet, “In particular, this highly interdisciplinary work has been performed for the last ten years in very close collaboration between our group (the ‘Nanophotonics group’ headed by Brahim Lounis in the physics department of University of Bordeaux) and the group of Daniel Choquet, with whom we have pioneered the application of single molecule microscopy in neurosciences.”

Good wine and great minds

Bordeaux is world renowned for its wine. And now its university provides a flourishing academic landscape that is equally inviting. “The Bordeaux research environment is very rich in several fields. Focussing on these two fields, there are many possibilities for talented PhD students seeking research subjects at the best international level. Possibilities range from fundamental physics such as nano-optics, quantum optics and applied optics, biophysics, advanced microscopy as well as neurosciences”, shares Cognet. Hosy and Giannone second his opinion. “Due to our strength in optics and neurosciences, many new techniques are available here in Bordeaux. This in turn creates interesting projects that attract international graduate students and post docs,” they say.

Giannone, who has studied in America, adds a fresh perspective to the research opportunities available in Europe. In his opinion, research on both sides of the Atlantic offers a stimulating academic atmosphere, good support and funding. “I see no major difference between the way I work here and the way I worked in the US. The situation for me in France is the same because Daniel Choquet manages the group really well and this makes a big difference. We have good funding and develop very interesting projects here,” he concludes.

Science as a way of life

“Intellectual freedom, innovation, pushing the limits of knowledge, excellence and the international community are the various facets of research that keep Laurent Cognet in science. “What I like about science is being able to dissect the mechanism behind uncommon observations that provide an insight into the phenomena itself. One can then start to change the parameters in the experiment and really understand what is happening,” says Giannone.

Similarly, Hosy enjoys being able to satiate his curiosity in the lab. “When I wake up in the morning and have a question that I want to answer, this process is pretty great for me.” He also feels that besides hard work, chance has some role to play in scientific success. “Often, scientists looking for a solution never find it or work on the wrong hypothesis and don’t find their way out. In case of the uPAINT technique, all of us worked together but without even one person it may not have worked,” he adds, modestly.

uPAINT in the future

What is the future for uPAINT? Cognet answers, “We plan to apply this method to answering fundamental biological questions concerning neurobiology and other fields. Further developments of the technique will concern extending the method to 3D and multicolour super-resolution imaging.”

Giannone and Hosy elaborate, “This paper is a technical paper and the very first step in this direction. We are now working on applying uPAINT to the labelling of various synapse proteins and already have three to four people working in this direction who would combine the observations from different optical techniques to understand the ‘bigger picture’. Having the possibility of multicolour imaging, would allow us to label different proteins and perhaps observe the interactions between them in a living cell membrane. As we said, this is just the beginning.”

Latika Bhonsle