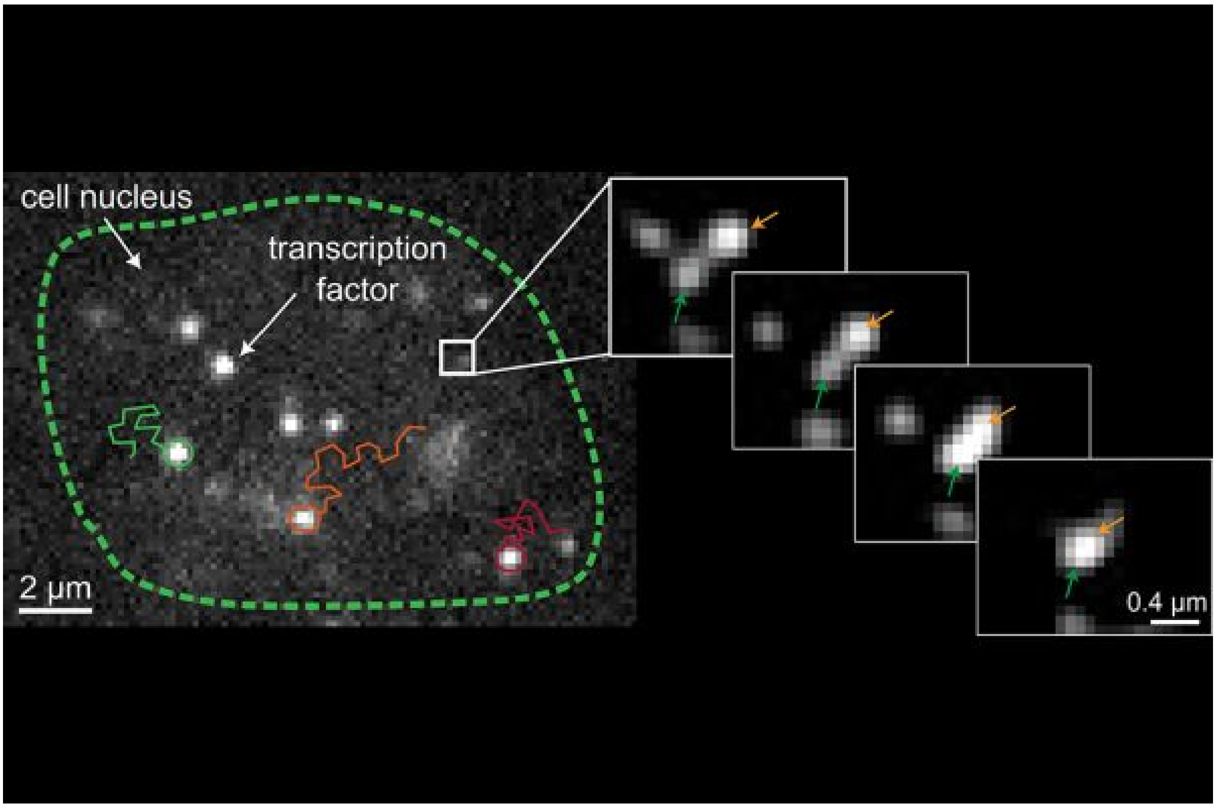

Understanding the dynamics of molecular condensates in living cells

Researchers from ICFO, the Centre for Genomic Regulation
nd the Pompeu Fabra University, report in PNAS about the phys
cs of molecular condensates by combining single-mo
ecule experiments with theory and simulations.

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Phase separation is a physical mechanism by which two mixed liquids form distinct phases, just as the oil separates from water to form droplets. This phenomenon occurs in many different scenarios, ranging from biological systems to quantum matter. Recently, scientists have discovered that liquid-liquid phase separation regulates multiple cells' biochemical processes by creating membrane-less compartments. For instance, biomolecules aggregate together forming phase-separated molecular condensates in the nucleus, cytoplasm and membranes of cells. These operate as versatile biomechanical hubs intervening in several aspects of the cellular processes, promoting or damping some biological reactions.?

An important example of such aggregated molecules is the transcription factors, protein that bind to specific DNA sequences to control the turn on and off of genes to make sure tha they are expressed in the cells at the right time and in the right amount. It has been propose that by forming phase-separated condensates of these molecules, the cell can promote gen expression at will. Although there has been some progress in understanding thi phenomenon, studying phase separation in living cells at the required spatial and tempora resolution remains highly challenging.

A new experimental and analytical approach

So far, the dynamics of transcription factors condensates have been studied either in fixed cells or in in-vitro settings. Now, in a study published at PNAS, ICFO researchers at the Single Molecule Biophotonics group **Juan Torreno**, **Nicolas Mateos** and **Felix Campelo,** led by ICREA Prof. at ICFO **Maria Garcia-Parajo** together with **Gorka Munoz-Gil** and ICREA Prof. at ICFO **Maciej Lewenstein** from the Quantum Optics Theory group, in collaboration with researchers from the Centre for Genomic Regulation and the Pompeu Fabra University, have investigated the physics of those condensates by combining single-molecule experiments with theory and simulations.

In their experiments at the single molecule level, the team studied the growth and diffusion dynamics of a particular transcription factor in the presence of a hormone that promotes its binding to DNA. This allowed them to have a controlled scenario where they could tune the occurrence of phase separation at their will. Unfortunately, a common difficulty when dealing with trajectories arising from single molecules is their very short length and the presence of noise. To overcome this, researchers developed a battery of machine learning techniques, which allowed them to study, with unprecedented accuracy, some of the properties of these individual transcription factors, inside and outside condensates.

Describing the molecular dynamics

Researchers found that, although the initial growth of the phase-separated condensates followed the expected theories, at longer times the growth stopped, limiting the sizes of the condensates at the nanoscale. Previously, such deviations were associated with a complex mechanism occurring in the cells.

By performing simple tweaks to the existing theories, the team showed that such strange behavior could indeed be related to the properties of the transcription factor molecules, pointing out how the constant size of the condensate is maintained thanks to the interaction between molecular escaping and condensation assembly. Then, they showed how their newly developed model could reproduce the experimental observations.

Potential applications in cancer drug development

These findings have a potential impact on drug development and treatment assessment. A clear understanding of the molecular events leading to phase separation of those biological components could guide, for example, an improvement in the design of anti-breast cancer cell therapies. In a similar scenario as the one presented in this work, one could test different treatments and see their effect on, for example, the lateral behavior of different transcription factors in breast cancer cell models. Relating these findings to the turning on or off of specific genes can provide avenues to treat cancer malignancies associated with the deregulation of transcription factors.

The study also sheds some light on the single molecule dynamics of the condensates in living cells, providing a general framework for such studies. Nevertheless, it highlights the importance of developing new analysis tools, which are vital to characterize the behavior of transcription factors or other DNA-binding proteins. To accomplish this task researchers need new machine learning-based approaches, due to the challenging experimental data they often deal with. In the context of diffusion experiments, combining these powerful tools with theoretical models can lead to unprecedented discoveries in the field.