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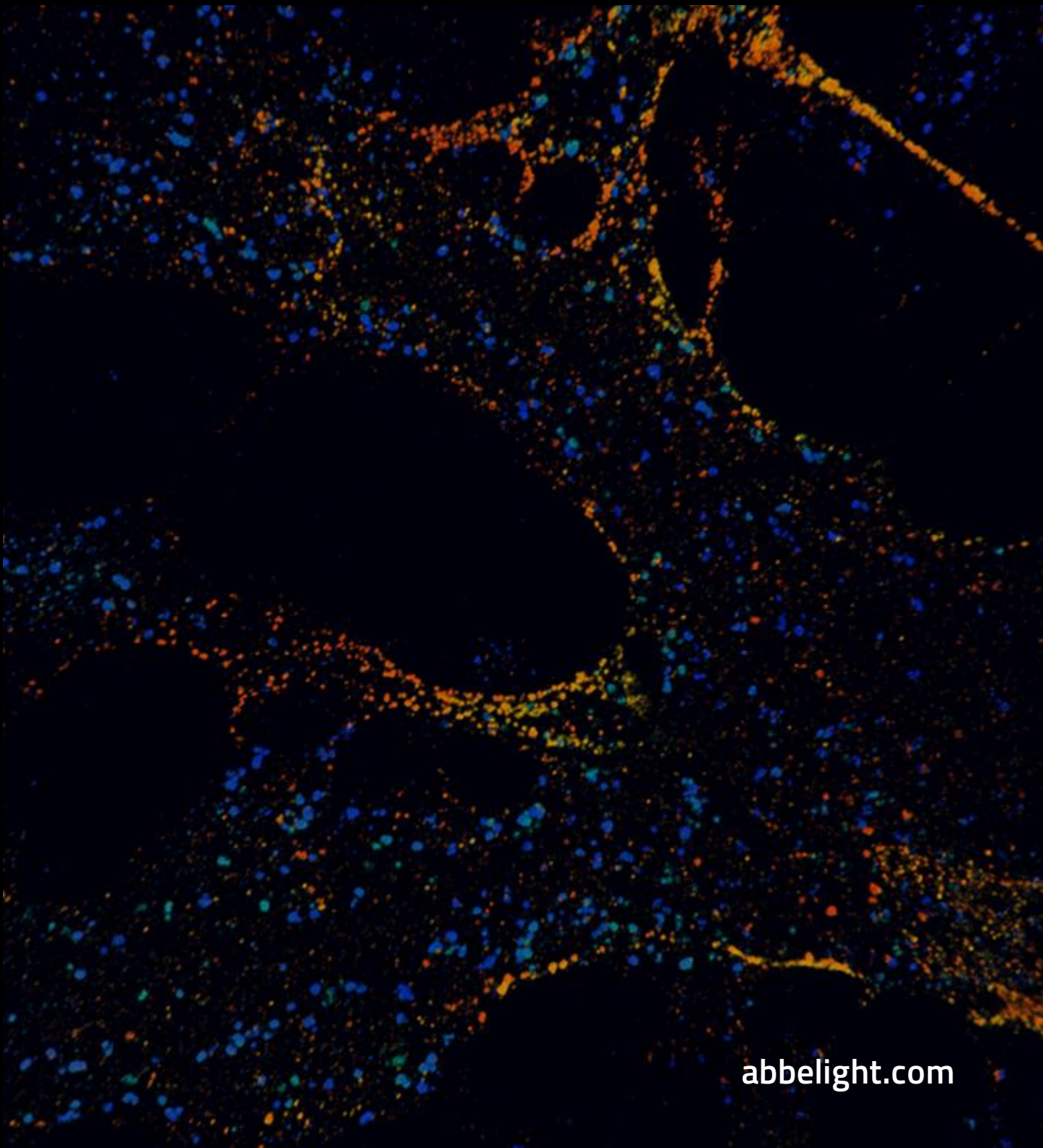
The plasma membrane at the nanoscale



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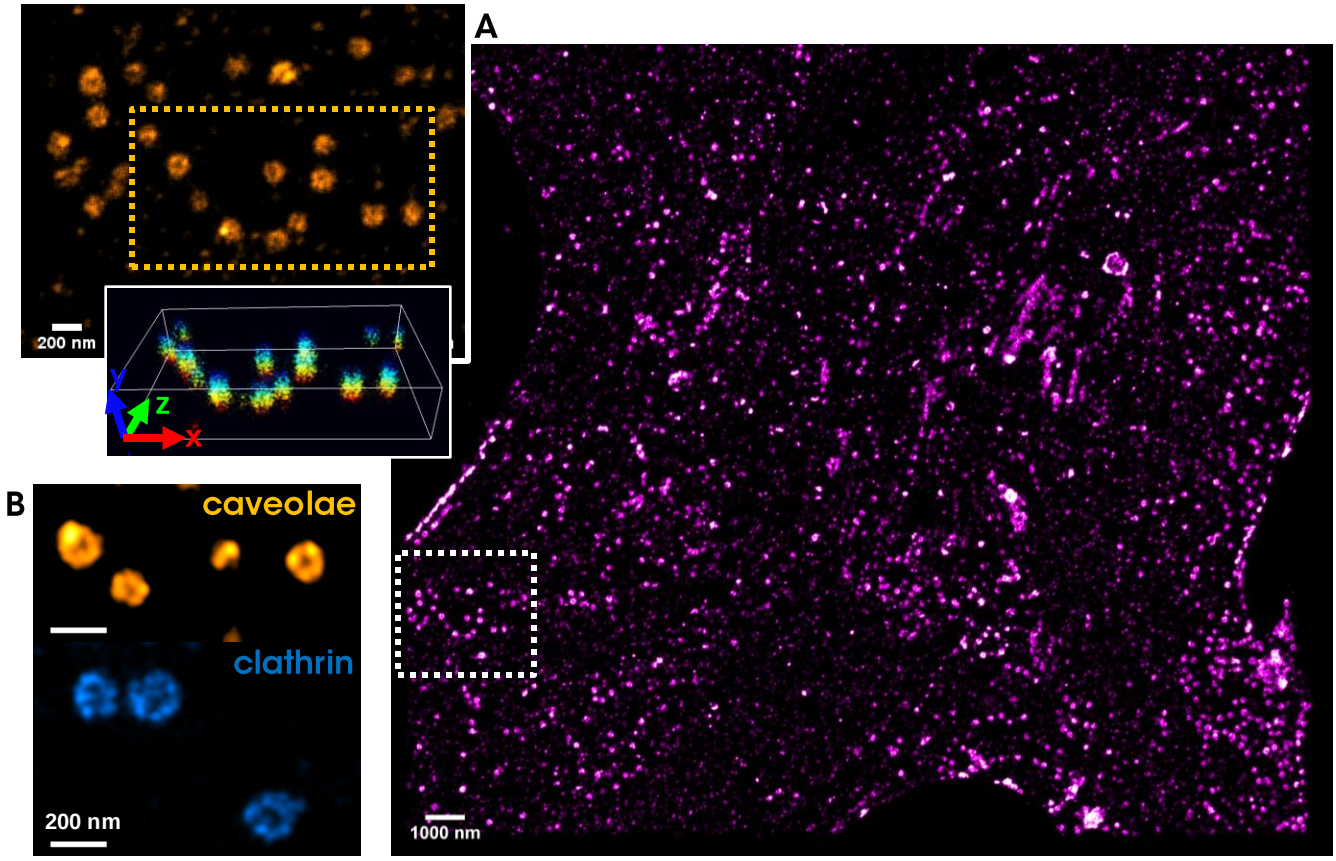
The plasma membrane of eukaryotic cells is primarily heterogeneous, featuring a wide variety of distinct subdomains that differ in lipid composition and biophysical properties [1-6]. Some of these proteo-lipidic subdomains organize plasma membrane invaginations that have long been known to play a major role in the cellular uptake of cargo through a process known as endocytosis. Over the years, there has been an increased focus on understanding the basis for this heterogeneity and the physiological relevance of these different endocytic pathways. Among these different membrane domains, clathrin-coated pits (CCP) and caveolae are the most readily recognizable features when visualized through electron microscopy due to the presence of a distinct coat.

Clathrin coated pits are 80-120 nm farly spherical structures featuring a characteristic coat composed of the heavy and light chains of clathrin along with a host of adaptor proteins [7-10]. The clathrin-mediated endocytic pathway (CME) is one of the most extensively studied and well-understood pathway that utilizes clathrin-coated pits for cargo uptake. On the other hand, caveolae are 50-80 nm invaginations in the plasma membrane that are implicated in various biological functions including signal transduction, membrane trafficking, cholesterol transport, mechanosensing, and are particularly abundant in cells experiencing mechanical stress such as adipocytes, endothelial and muscle cells [11-13]. Although the budding of caveolae from the plasma membrane and fusion with early endosomes has been observed, a role for caveolae in endocytosis has long been debated [14].

In most cells, caveolae and clathrin coated pits densely decorate the plasma membrane and due to their sub-diffraction limit size (<200 nm), conventional light microscopy techniques lack the resolution to facilitate investigations aimed at elucidating the various functions of these nanodomains. In this preliminary study, we used Abbelight's dSTORM super-resolution and spectral demixing techniques [15] to reconstruct and differentiate between caveolae and CCPs in endothelial cells. The results reveal a non-overlapping and distinct distribution as observed through conventional EM thereby enabling and opening new investigative avenues to dissect the specific roles played by caveolae and other proteo-lipidic membrane nanodomains in cells such as the clathrin-independent carriers (CLIC) [16].



Single Molecule Localization Microscopy reveals the fine details of proteolipidic nanodomaines at the plasma membrane. Abbelight features the highest 3D resolution (15 nm) over the largest field of view (up to 200x200 mm) in single molecule imaging, allowing the dissection of sub-diffraction caveolae invaginations at the plasma membrane of endothelial cells. **A**: Caveolae (magenta) are abundantly distributed throughout the membrane; the detailed region (yellow) reveals finer details of the caveolar scaffold, in 2D and 3D view. **B** illustrates the structural similarities and differences of Caveolae and Clathrin Coated Pits.

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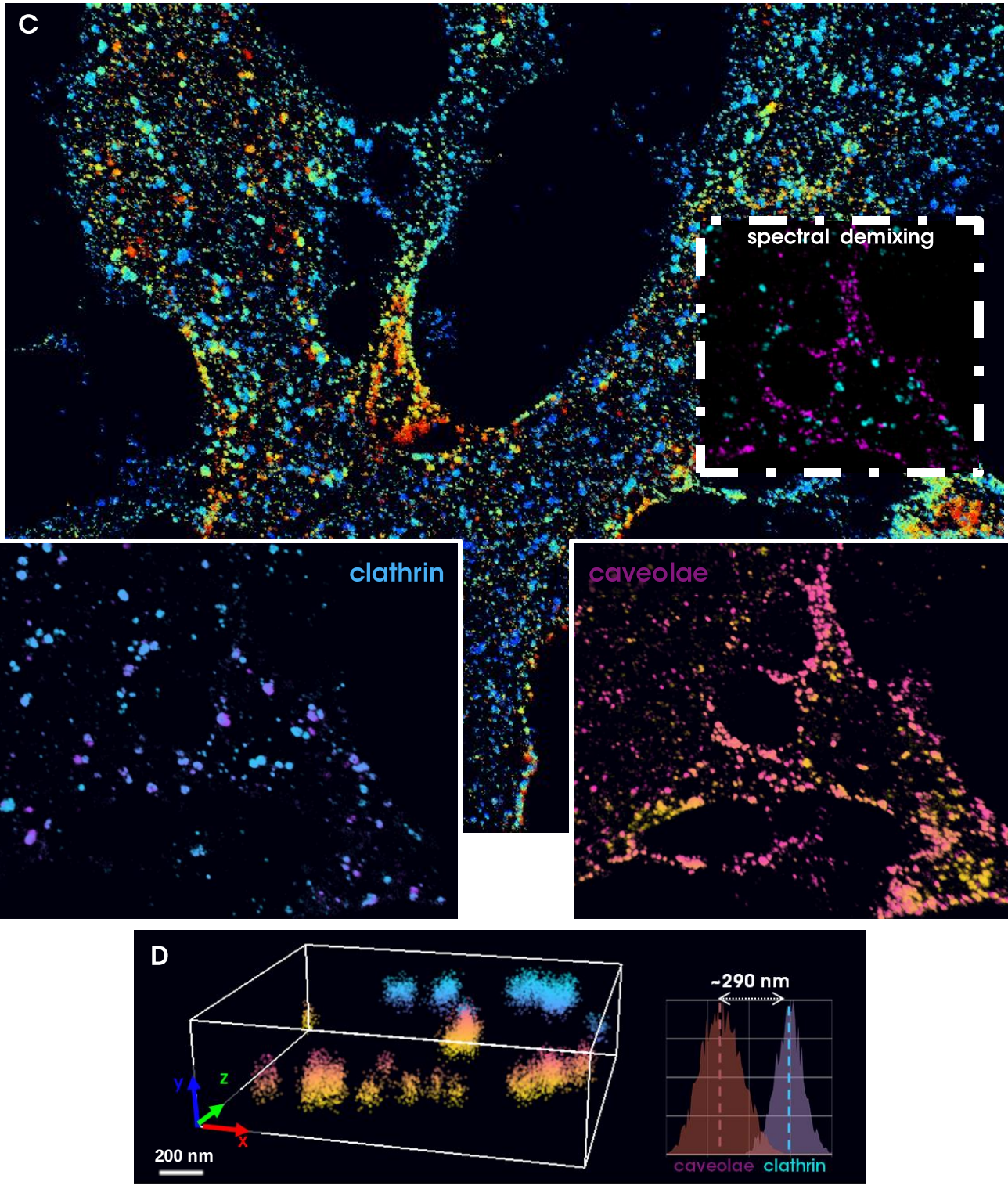

Simultaneous multicolor imaging via spectral demixing of Caveolae and Clathrin nanodomains.
Abbelight spectral demixing strategy reveals non-overlapping and distinct distributions of Caveolae and Clathrin Coated pits and vesicles (C) and allows to investigate the crosstalk between clathrin- and caveolin- endocytic pathways. The 3D detailed view in D shows that seemingly close Caveolae and Clathrin scaffolds are in fact localized in completely distinct submembrane domains.

Samples courtesy of Christophe Lamaze and Satish Kailasam Mani,
Membrane mechanics and dynamics of intracellular signaling, UMR3666/U1143 Institut Curie, Paris

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PREPARATION

Murine Lung Endothelial cells (MLECs) were cultured on precision coverslips and fixed with freshly prepared PFA (4%). The fixed cells were then stained for either Caveolin-1 (Rabbit anti-Cav1; CST 3328S) or Cavin-1 (Rabbit anti-PTRF; abcam 48824) to visualize Caveolae and Clathrin heavy chain (Mouse anti-CHC [X22]; abcam 2731) to visualize Clathrin coated pits/vesicles. Secondary antibodies conjugated with **AF647** and **CF680** fluorophores and compatible with simultaneous multicolor STORM via spectral demixing were used.

IMAGING INSTRUMENT

3D imaging was performed on an Olympus Ix83 microscope with a 100x/1.5NA objective and equipped with the **Abbelight ™ SAFe360** module that implements enhanced astigmatism and **DAISY** technology for 3D isotropic super-resolution and ASTER technology for homogenous TIRF/HILO illumination. The coverslips were incubated in **Abbelight ™ Smart Kit** buffer during STORM acquisitions.

Simultaneous 2-color STORM imaging was performed with a 640 nm laser (for AF647 and CF680) in HILO and a total of 60000 frames were collected at 100FPS (50x50 µm datasets).

DATA MANAGEMENT

Single molecule localization, image reconstruction and spectral demixing of simultaneous multicolor datasets were performed in real time with **Abbelight ™ NEO software**.

NEO software was also used for 3D visualization and analysis of single molecule data i.e. the spatial co-ordinates of each detected molecule.

Tools such as cluster analysis and single-particle tracking are well suited for nanoscale co-localization studies in dense cellular compartments as in the plasma membrane.

Highlights

What can single molecule localization microscopy bring to the study of plasma membrane subdomains?

- *The highest spatial resolution to investigate the molecular organization of membrane associated structures (cytoskeleton; endo/exocytotic scaffolds; organelles), in cell cultures and tissue slices.*
- *Quantitative co-clustering of proteins at the nanoscale.*
- *Dynamic tracking of single molecules and organelles.*

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