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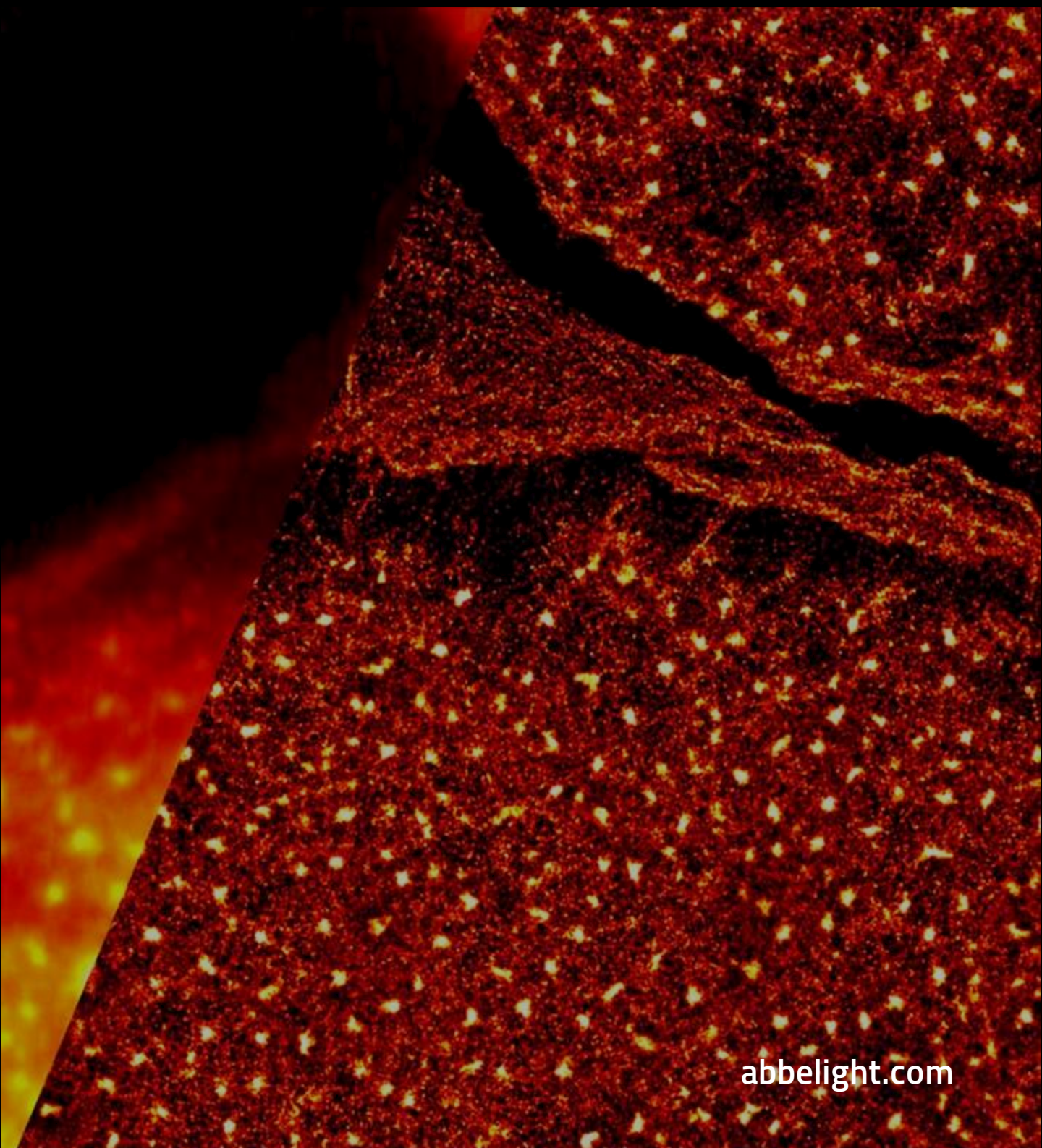
Nanoscale architecture of adhesion structures



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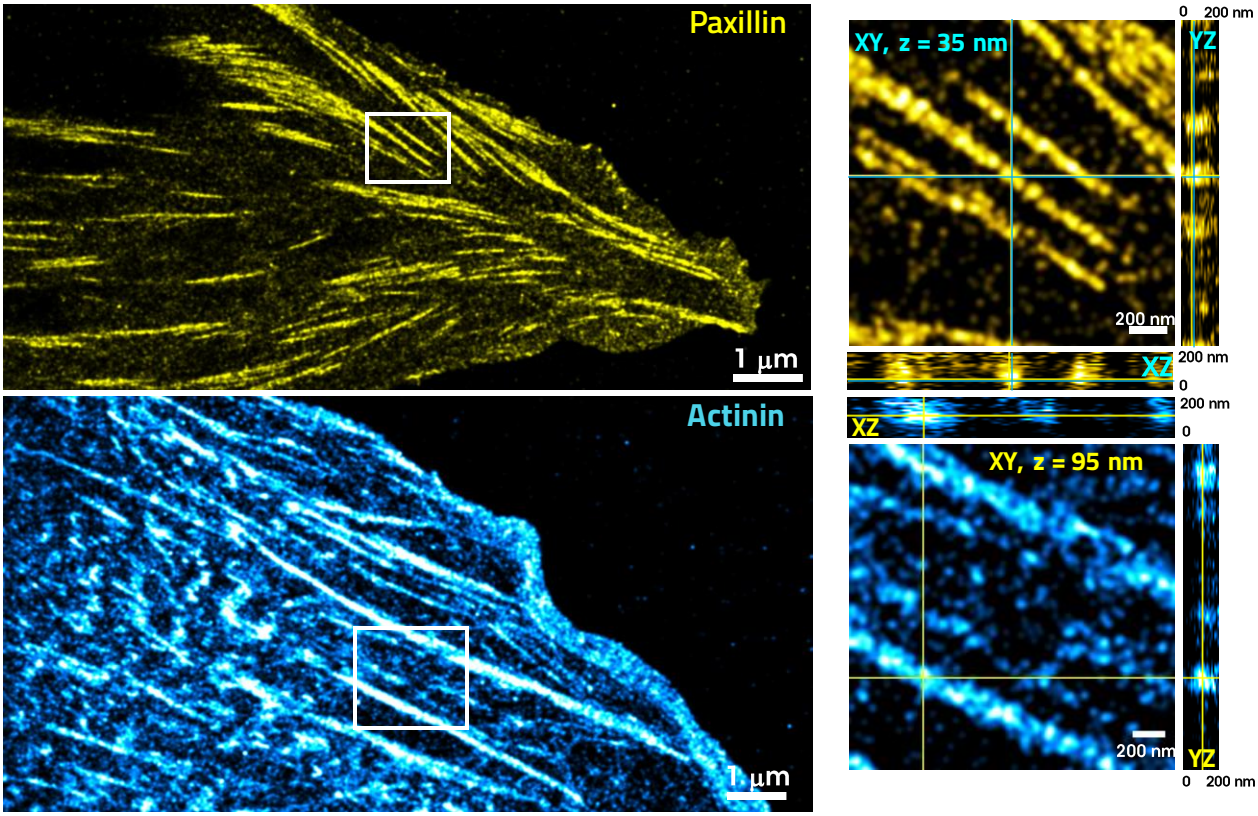
Nanoscale architecture of focal adhesions

Cells maintain contact and dynamically interact with each other and with the surrounding acellular matrix (ECM) via transmembrane adhesion receptors, such as integrins, which provide a physical link between the ECM and the cell cytoskeleton and promote the assembly of large signaling platforms.
The adhesion structures (AS) between cell and ECM take different specialized forms, ranging from classical focal adhesions (FA) to podosomes and invadopodia.
AS have highly complex multiprotein architectures, composed of hundreds of molecules dynamically interacting at scales that are well below the light diffraction limit. Thus, unravelling the nanoscale organization of AS poses a daunting challenge to researchers, which classical light microscopy is unable to overcome.

Super-resolution imaging methods overcome the diffraction limit and allow optical microscopes to achieve unprecedented resolutions. It is now possible to localize single AS proteins in three dimensions, determine their diffusive behaviors, orientations, and how much mechanical force is transmitted across individual components. Among several outstanding achievements, super resolution, and specifically single molecule localization microscopy (SMLM) has been used to dissect distinct functional nanolayers in focal adhesions[1,2]; determine the length of stretched force–sensing proteins in cells[3]; describe the molecular architecture of hemidesmosomes[4] and podosomes[5]; follow protein movements and interactions leading to the formation of AS dynamic architecture[6,7].

Abbelight easy-to-use SMLM imaging platform is ideally suited to study cell adhesion structures, thanks to features such as isotropic 3D super-resolution, variable angle TIRF illumination for homogeneous resolution over large FOVs, and simultaneous multicolor imaging. These features are easily implemented even on the most difficult samples, thanks to a complete and ergonomic software suite designed for super-resolution.



Single Molecule Localization Microscopy reveals the spatial segregation of FA proteins in three dimensions. FAs consist of a complex network of several proteins organized in multilaminar plaques less than <200-nm thick. Abbelight isotropic 3D resolution (15 nm) with absolute axial reference easily enables the localization and tracking of individual FA components at the scale of a few tens of nanometers, showing their axial stratification. Integrin interacting protein Paxillin (upper panel, yellow) is found immediately above the plasma membrane at ~35 nanometers of height, while actin associated alpha-Actinin (lower panel, cyan) localizes around 95 nm above the plasma membrane. In the panels, on the left are shown z-projections of 200 nm thick reconstructions, on the right, 10 nm thin orthoslices of selected ROIs.

Samples and images courtesy of Gregory Giannone and Olivier Rossier, IINS, CNRS, Bordeaux*

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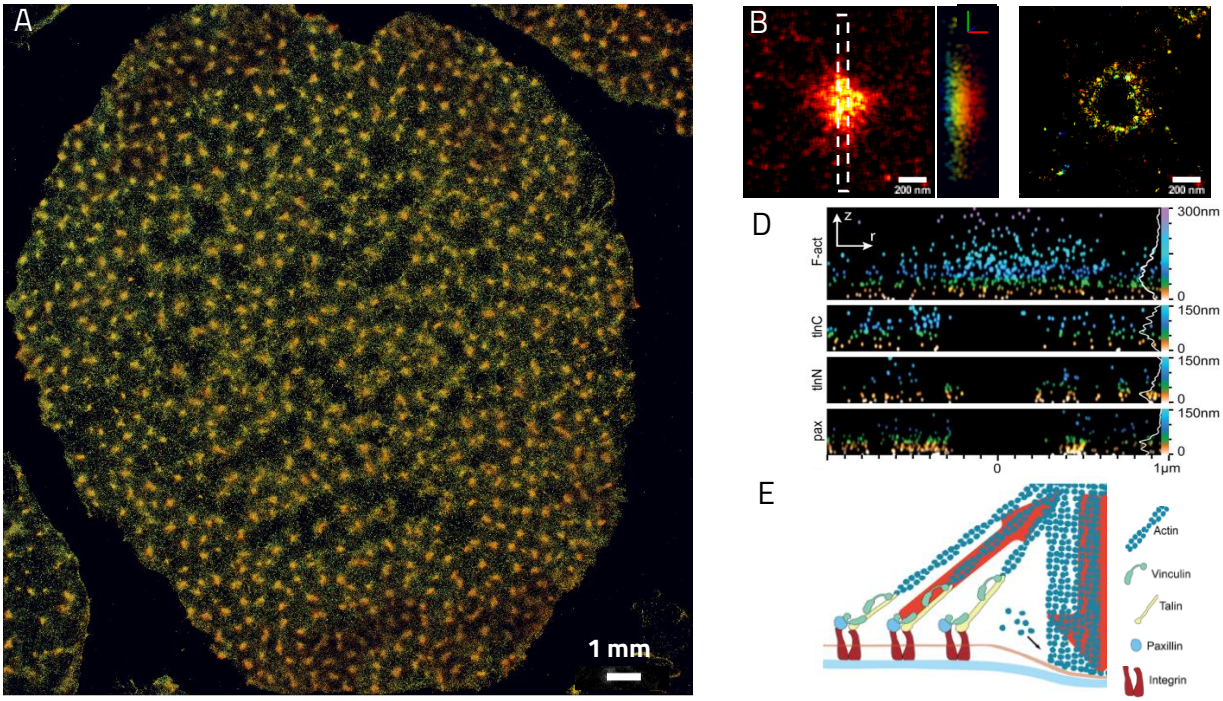
Nanoscale architecture of podosomes



Single Molecule Localization Microscopy reveals ring protein organization and podosome force generation machinery. Podosomes are sub-diffraction structures, with diameter of 0.5-1 µm, depth of 0.2-0.4 µm and a two-part architecture: a core of actin and associated proteins surrounded by a ring of adhesion-related proteins (Talin, Vinculin, Paxillin). Individual podosomes could be dissected thanks to Abbelight isotropic 3D resolution (15x15x15 nm); A and B, F-actin cores; C, Talin ring. In D, nanometric vertical distribution of podosome components, revealing that Talin extension correlates with radial position. In E, a model of podosome force generation machinery obtained with Abbelight super–resolution (Bouissou et al., 2015).

Samples and images courtesy of Renaud Poincloux, IPBS, CNRS, Toulouse

PREPARATION

For FA imaging, mouse embryonic fibroblasts were cultured, fixed and stained with primary antibodies and secondary antibodies conjugated with AF647, as described in[6].

For podosomes, primary human macrophages were prepared as in[5]. Unroofed cells were stained with either phalloidin (F-actin) or primary and secondary antibodies coupled with AF647.

IMAGING INSTRUMENT

For STORM imaging coverslips were incubated in **Abbelight ™ Smart Kit** buffer.

3D imaging was performed on Nikon Ti Eclipse microscopes with a 100X 1.49NA objective, equipped with **Abbelight ™ SAFe360**, implementing DONALD technology for 3D isotropic super-resolution with absolute axial reference,

DATA MANAGEMENT

Single molecule localization and image reconstruction were performed in real time with **Abbelight ™ NEO software**. NEO software was also used for 3D visualization and analysis of single molecule data, that is the spatial coordinates of each detected molecule. Within NEO software, tools such as single particle tracking are especially suited to unravel the detailed interactions occurring within AS.

References

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6. O. Rossier et al., Nat. Cell Biol. 14, 1057–1067 (2012).
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Highlights

How does SMLM contribute to understanding the mechanobiology of adhesion structures?

- **By offering the highest spatial resolution to unravel the molecular organization of AS.**
- **Through quantitative co-clustering of proteins at the nanoscale.**
- **By dynamic tracking of single molecules in crowded and complex structures.**

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