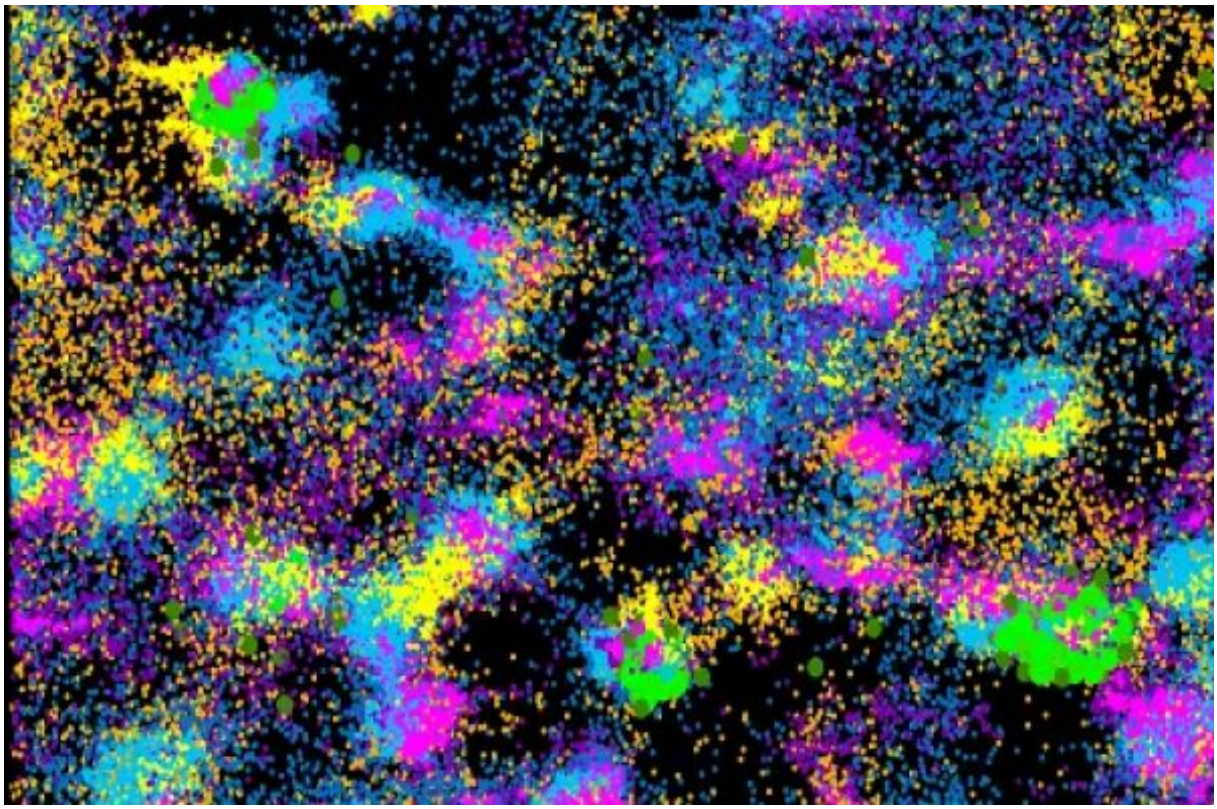


# Mapping individual multi-molecular interactions provides new insights into virus capture

**Researchers have provided a new and powerful single molecule methodology capable of simultaneously monitoring different molecules in living cells, a major technological breakthrough in the single molecule field.**
**The technique successfully uncovered individual interactions between three proteins and two different viruses, HIV-1 and SARS-CoV-2, which resulted crucial to increase their capture.**
**These results suggest there exists a common physical mechanism that enhances viral capture, the first crucial step that ultimately leads to infection.**

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When a virus infects us, dendritic cells detect and capture it. They then present the virus to other immune system cells, who activate the proper immune response to, hopefully, halt the

infection. However, some viruses, like HIV, have learned to take advantage from this situation, and use dendritic cells as "Trojan horses" to spread deeper into the body. Once inside, HIV inhibits the ability of dendritic cells to mature and alert the immune system. This weakens the immune response and can eventually lead to AIDS.

Understanding the early molecular events leading to viral capture and cell entry is critical for designing effective vaccines. To elucidate how a receptor in the cell membrane binds and captures a virus, information at the single molecular level is essential. However, molecules rarely act isolated; their function depends on interactions with other molecules. One can think of them as a society, where individual behavior strongly depends on the interactions with others. i¿½You behave differently when interacting with your parents, siblings, or bos . And most of the time, we need to interact with others to perform a better job, or simp y because we cannot do it alonei¿½, explains ICREA Professor at ICFO, Maria Garcia Par jo. i¿½Exactly the same happens in a cell: interactions between individual molecules lie t the core of their function and can significantly enhance their wo k.i¿½ Therefore, a tool capable of tracking interactions between multiple molecules in real- ime, at the single molecule level and in living cells has long been a priority in biophysics.

ICFO researchers **Dr. Nicolas Mateos**, **Dr. Enric Gutierrez-Martinez**, **Dra. Jessica Angulo-Capel**, **Dr. Juan A. Torreno-Pina**, led by **ICREA Prof. Maria F. Garcia-Parajo**, and in collaboration with the King's College London, have recently presented in ACS Nano a technique that fulfills these conditions. The team has come up with a new and powerful single molecule methodology, which can monitor different labeled molecules simultaneously in living cells, and have used this information to construct spatiotemporal maps. **These maps track the position and the interactions of several bioparticles individually over time,** something that former approaches were not able to resolve.

Using this technique, researchers captured real-time interactions between individual virus-like particles and three different proteins on the membrane of living immature dendritic cells. **This mapping revealed a coordinated action of the three proteins that was crucial to capture two different viruses: HIV-1 and SARS-CoV-2**.

**Multicolor HiDenMaps for visualizing molecular interactions**

To study individual virus-receptor interactions and the role of other individual molecules in real-time, the team developed a multimolecular mapping technique called Multicolor High-Density Maps (HiDenMaps). To showcase it, they studied the HIV-1 virus and its interactions with three proteins (DC-SIGN, CD44 and galectin-9).

HiDenMaps were created by labelling each protein and the virus with fluorescent markers. When illuminated, each marker emitted light of a different color, enabling researchers to track their positions with nanometer precision. Data from each marker was combined into a single image, the HiDenMap. Then, the four HiDenMaps collapsed to a single multicolor map encapsulating the spatial and temporal distribution of all molecules. i¿½The final maps ha

e four colors, one for each molecule. This makes it easier to i¿½seei¿½ where an
when different molecules coincide and, thus, helps us to visualize interactions between th
mi¿½, assures Dr. Nicolas Mateos, first author of the article and main developer of the te

**Revealing how dendritic cells efficiently capture HIV**

HiDenMaps, combined with quantitative tools, allowed researchers to follow the three proteins and the virus in real time, showing how their interactions influence viral binding and capture. For HIV-1, researchers identified four main steps. First, the three proteins explor the environment in a coordinated manner, inspecting the same or adjacent regions, like a cel patrol waiting for enemies. When the virus arrives, it is more likely to bind in areas where th three proteins accumulate in larger amounts and, during viral engagement, the thre proteins cluster closer together, strengthening even more the binding. Then, thes nanoclusters enhance the interaction between the viral receptor (DC-SIGN, one of th proteins being tracked) and the virus. And, finally, the enhanced interaction increases th probability of capturing the virus -the initial step towards dendritic cell infection

The whole process had never been observed at the single molecular level before. i¿?
We found that, while DC-SIGN can capture the virus, it needs two partners -CD44 and galectin 9- to do so efficientlyi¿½, explains Dr. Mateos. i¿½Interestingly, only when DC-SIGN int racted simultaneously with both CD44 and galectin-9, the binding and capture of the virus s were stronger, more stable and more likely to lead to infectioni¿½,

**HiDenMaps: a general tool for tracking multimolecular interactions**

The same process was observed for SARS-CoV-2, the famous responsible for COVID-19. **This suggests a potential generalized mechanism for virus capture in immature dendritic cells, where the three proteins play a central role**. According to Prof. Garcia Parajo: i¿½This h s enormous consequences for vaccine development, since preventing the early stages of vir l capture by disrupting interactions between DC-SIGN and its partners could be a mo e effective strategy than blocking the viral receptor alone.i
?½ Even more generally, multicolor HiDenMaps could be applied to study any multimolec lar interactions in living cells. Moreover, combining HiDenMaps with another existing techn que called frequency multiplexing could increase the number of tracked particles. i¿½Id ally, such a combination would result in a full rainbow-colored HiDenMap! The challenge is th t, at the moment, frequency multiplexing has only been applied to fixed cells. Going to living cells is far from trivial, but certainly not impossiblei¿½, shares Prof. Garcia Parajo. Th s great versatility makes HiDenMaps a major technological breakthrough for the single olecul

**Reference:**

Nicolas Mateos, Enric Gutierrez-Martinez, Jessica Angulo-Capel, Irene Carlon-Andres, Sergi Padilla-Parra, Maria F. Garcia-Parajo, and Juan A. Torreno-Pina. ACS Nano 2024 18 (42), 28881-28893
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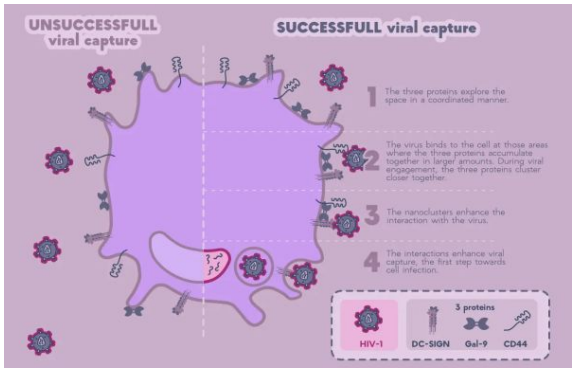


Illustration the HIV capture procedure. Credit: Isabel Santa-Maria.