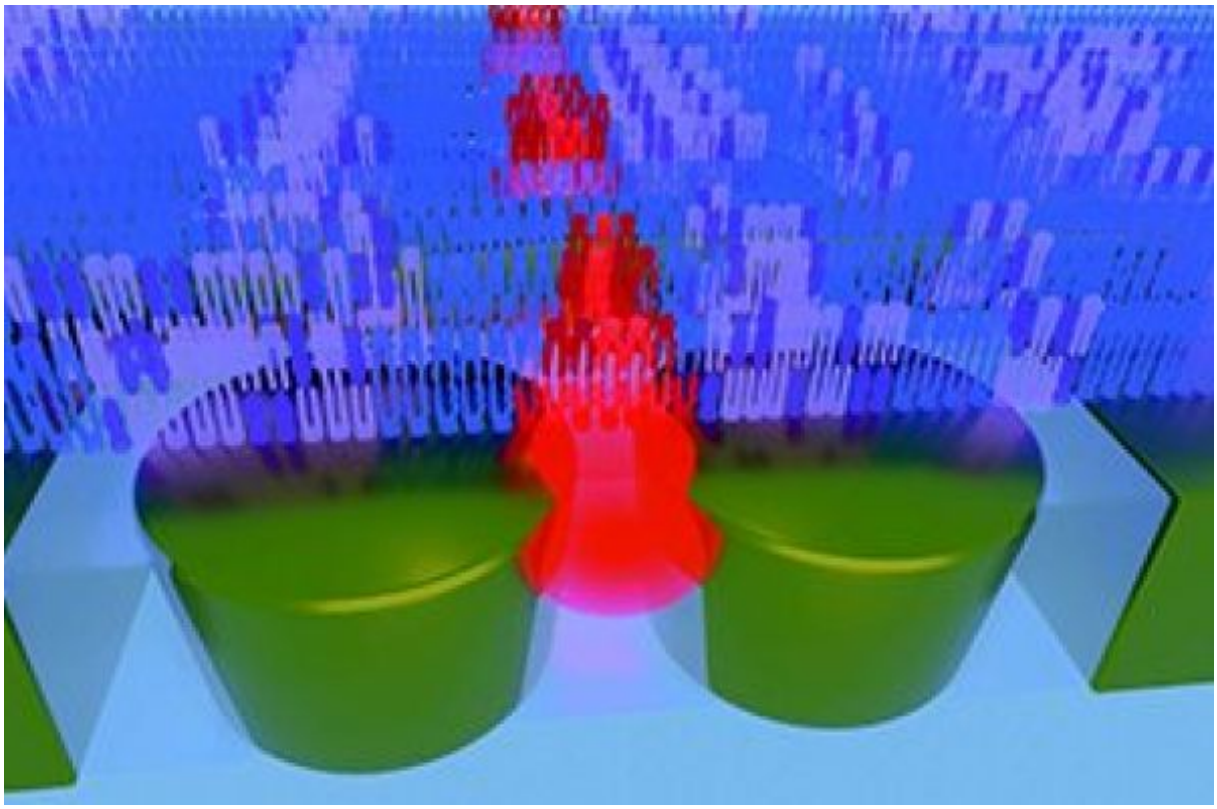

PhD THESIS DEFENSE: Novel planar photonic antennas to address the dynamic nanoarchitecture of biological membranes

PAMINA WINKLER

November 27, 2020

11:00

ICFO Auditorium and Online (Teams)

The cell membrane is the encompassing protective shield of every cell and it is composed of a multitude of proteins, lipids and other molecules. The organization of the cell membrane is inextricably intertwined with its function, and sensitive to perturbations from the underlying actin cytoskeleton and the extracellular environment at the nano- and the mesoscale. Elucidating the dynamic interplay between lipids and proteins diffusing on the cell membrane, forming transient domains and (re)organizing them according to signals from the juxtaposed inner and outer meshwork, is of paramount interest in fundamental cell biology. The overarching goal of this thesis is to gain deeper insight into how lipids and proteins

dynamically organize in biological membranes at the nanoscale.
Photonic nano-antennas are metallic nanostructures that localize and enhance the incident optical radiation into highly confined nanometric regions (< 20 nm), leading to greatly enhanced light-matter interactions. In this thesis, we exploit an innovative design of planar gold nano-antenna arrays of different gap sizes (10-45 nm) and embedded in nanometric-size boxes. To elucidate nanoscale diffusion dynamics in biological membranes with high spatiotemporal resolution and single-molecule detection sensitivity, we further combine our nanogap antenna arrays with fluorescence correlation spectroscopy (FCS) in a serial and multiplexed manner.
In this dissertation, we first describe the fabrication process of these planar gold nanogap antennas and characterize their performance by means of electron microscopy and FCS of individual molecules in solution. We demonstrate giant fluorescence enhancement factors of up to 104-105 times provided by our planar nanogap antennas in ultra-confined detection volumes and with single molecule detection sensitivity in the micromolar range.
Second, we apply these planar plasmonic nano-antennas in combination with FCS for assessing the dynamic organization of mimetic lipid membranes at the nanoscale. For a ternary composition of the model membranes that include unsaturated and saturated lipids together with cholesterol, we resolve transient nanoscopic heterogeneities as small as 10 nm in size, coexisting in both macroscopically phase-separated lipid phases.
Third, we add a Hyaluronic Acid (HA) layer on top of the model lipid membranes to emulate the effect of the extracellular environment surrounding native biological membranes. We extend our nano-antenna-FCS approach with atomic force microscopy and spectroscopy. We reveal a distinct influence of HA on the nanoscale lipid organization of mimetic membranes composed of lipids constituting the more ordered lipid phase. Our results indicate a synergistic effect of cholesterol and HA re-organizing biological membranes at the nanoscale.
Fourth, we apply our planar nano-antenna platform combined with FCS to elucidate the nanoscale dynamics of different lipids in living cells. With our nanogap antennas we were able to breach into the sub-30 nm spatial scale on living cell membranes for the first time. We provide compelling evidence of short-lived cholesterol-induced ~10 nm nanodomain partitioning in living plasma membranes.
Fifth, we demonstrate the multiplexing capabilities of our planar gold nanogap antenna platform combined with FCS in a widefield illumination scheme combined with sCMOS camera detection. Our approach allows recording of fluorescence signal from more than 200 antennas simultaneously. Moreover, we demonstrate multiplexed FCS recording on 50 nano-antennas simultaneously, both in solution as well as in living cells, with a temporal resolution in the millisecond range. The dissertation finishes with a brief discussion of the main results achieved in this research and proposes new avenues for future research in the field.

Friday November 27, 11:00 - ICFO Auditorium / Teams

Thesis Advisor: Prof Dr Maria Garcia - Parajo

Due to recommendations in place to contribute containing the spreading of COVID-19, th defence will be carried out semi presencial with a maximum of 66 Icfonians in t e Auditorium, and partly remotely via MS Team

This is the link to follow the Thesis Defense online Click here to join the meeting

If you are interested in attending in person, please address your request to mery.gil@icfo.eu by Wednesday November 23.

Hosted by: Maria Garcia - Parajo

Pamina Winkler's Thesi Cover