O-10.4 Short talk

Single-Molecule and Multiscale Fluorescence Imaging to Investigate PD-L1 Dynamics and its Association with Lipid Rafts in Non-Small Cell Lung Cancer Cells

Simone Civita [1], Martina Ruglioni [1], Matteo Mariangeli [2], Tiziano Salvatori [1], Sofia Cristiani [1], Vittoria Carnicelli [1], Serena Barachini [1], Iacopo Petrini [1], Irene Nepita [2], Marco Castello[2], Stefano Fogli [1], Romano Danesi [3], Alberto Diaspro [2], Paolo Bianchini [2], Barbara Storti [4], Ranieri Bizzarri[1]

[1] Università di Pisa, Pisa, Italy

[2] Istituto Italiano di Tecnologia, Genova, Italy

[3] Università di Milano, Milano, Italy

[4] i NEST, Scuola Normale Superiore and Istituto Nanoscienze-CNR, Pisa, Italy

The membrane protein PD-L1 plays a pivotal role in tumors and is a key target for immunotherapy. While its immunosuppressive function in the PD-1/PD-L1 checkpoint is well characterized, its nanoscale regulation remains unclear and is likely influenced by spatial organization and diffusion dynamics. We employed super-resolution and single-molecule fluorescence techniques to investigate PD-L1 organization and mobility in non-small cell lung cancer (NSCLC) cells. Single-Molecule Localization Microscopy (SMLM) revealed PD-L1 clustering (~30-40 nm) within cholesterol-enriched membrane rafts, confirmed by colocalization with key raft markers. Fluorescence Correlation Spectroscopy (FCS) and spot variation-STED-FCS in live cells uncovered confined diffusion, typical of raft-associated proteins, indicating a tightly regulated mobility. PD-L1 raft association may influence immune checkpoint activity, impacting immune evasion and therapeutic strategies. Additionally, a non-clustered PD-L1 pool may have alternative functions. To validate our findings, we have performing PALM to compare SMLM data from antibody-based and genetically encoded fluorescence labeling. By integrating multiscale fluorescence imaging with single-molecule biophysics, this study provides novel insights into PD-L1 spatial organization and its role in NSCLC pathophysiology and immunotherapy.

O-10.5 Short talk

High-throughput biophysical measurements of cells in health and disease

Cenk Gurdap[1], Dunya Aydos[2], Yagmur Urem[1], Pablo Carravilla[1], Verda Bitirim[2], Luca Andronico[1],Erdinc Sezgin[1]

[1] Karolinska Institute, Stockholm, Sweden

[2] Ankara University, Ankara, Turkey

Changes in the biophysical properties of cells are crucial to various diseases and have considerable potential as a diagnostic or prognostic marker. However, available technologies generally suffer from low throughput. Moreover, low-parametric phenotyping lacks the power of integrating diverse biophysical properties and their underutilized correlations. Therefore, there is still a major gap in our understanding of how collective biophysical properties of the cells alter during both physiological and pathological processes. Here, we describe a high-throughput and multi-parametric platform with single-cell resolution, which combines spectral flow cytometry and environment-sensitive dyes. We establish a unique pipeline to: 1) biophysically phenotype different diseases; 2) integrate biophysical phenotyping with multi-omics technologies 3) predicting heath state of individuals from their immune cells in blood using biophysical remodelling and machine learning 4) explore drug treatments using the biophysical measurements as output. This pipeline may aid the detection of different cellular pathogenic alterations and discovery of their possible drug targets. Thus, our technique has the potential to be widely used in a variety of fields, where biophysical properties can be exploited as complementary to current protein or nucleic acid markers to diagnose and treat diseases.

O-10.6 Short talk

Computational approaches to assess biomolecular interfaces compatibility: exploring its implications in molecular design

Lorenzo Di Rienzo [1], Mattia Miotto [1], Edoardo Milanetti [2], Giancarlo Ruocco [1]

[1] Italian Institute of Technology, Rome, Italy

[2] Department of Physics, Sapienza University, Rome, Italy

Molecular recognition relies on the fine-tuning of physicochemical properties at the interface. Even a single amino acid mutation can disrupt an interaction, often altering a biochemical pathway pathologically. However, a computational rapid and accurate assessment of the impact of mutations on binding remains a major challenge in molecular biophysics. Still, understanding the physical mechanism ruling this phenomenon would enable the design of molecular interfaces for ad-hoc purposes. To this end, we model interaction surface geometry using Zernike polynomials, creating an ordered set of rotationally invariant descriptors and allowing a fast evaluation of compatibility between surfaces. Incorporating electrostatic and chemical complementarity, we developed a novel computational mutagenesis strategy for protein interface optimization. These efficient formalisms allow iterative assessments of residue substitutions in each step of a simulated annealing simulation, generating multi-residue protein mutants with controlled binding compatibility. As an example of this method's results, we modified the native sequence of Ste2, the sole GPCR in haploid Saccharomyces cerevisiae, enabling it to bind adrenaline, as confirmed by independent molecular dynamics simulations. These findings open new avenues for engineering this organism as a biosensor able to react to a user-defined molecule.