INTERNATIONAL SCHOOL OF BIOPHYSICS «ANTONIO BORSELLINO»

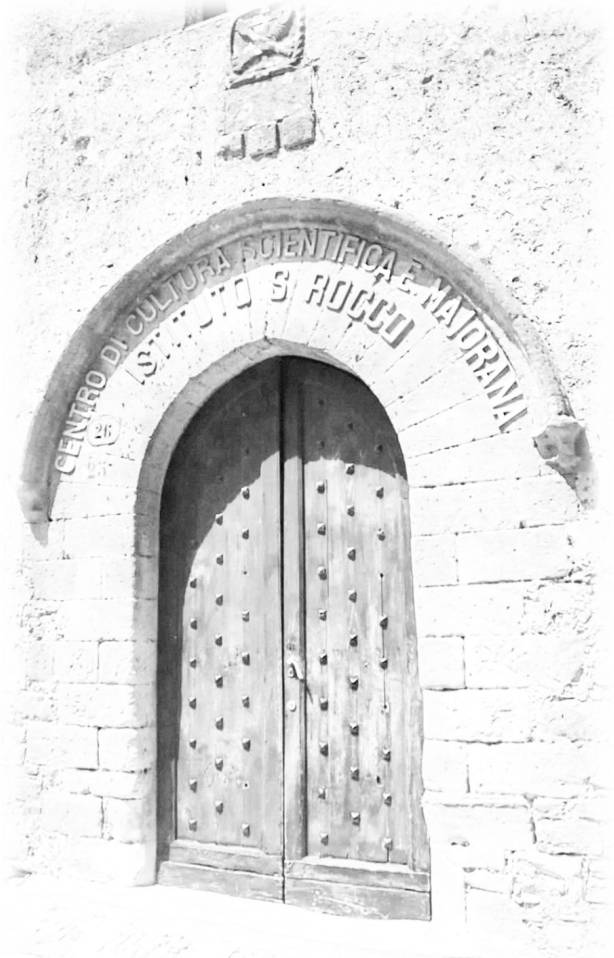
48th Course:

Memos for biophysics into the future: Lightness, quickness, exactitude, visibility, multiplicity, and consistency

Erice - Sicily: 16 – 22 October 2023

DIRECTORS OF THE COURSE:

A. DIASPRO, M. DALLA SERRA, C. VIAPPIANI



50 years SIBPA Società Italiana di Biofisica Pura e Applicata 1973 - 2023

lambs iit NANOSCOPY

IBF Istituto di Biofisica

Consiglio Nazionale delle Ricerche

UNIVERSITÀ DEGLI STUDI DI GENOVA

UNIVERSITÀ DI PARMA

LECTURERS AND ABSTRACTS

Alessio Accardi, Dept. of Anesthesiology, Weill Cornell Medicine, New York, USA

Structural basis of scrambling by TMEM16 proteins

Activation of Ca2+-dependent TMEM16 scramblases induces the externalization of phosphatidylserine, a key molecule in multiple signaling processes. Current models suggest that the TMEM16s scramble lipids by deforming the membrane near a hydrophilic groove, that Ca2+ binding induces opening of a hydrophilic groove that serves as a lipid conduit, thus accounting for the ligand dependence of the activity. However, the molecular rearrangements involved in groove opening are poorly understood. Further, it has been proposed that in some TMEM16 homologues the closed groove is the scrambling competent conformation. However, the arrangement of lipids near the open and closed groove remains unknown, preventing our understanding of how these proteins can catalyze the rapid transbilayer movement of lipids. Using cryogenic electron microscopy, we directly visualize how lipids associate at the open and closed groove of two TMEM16 scramblases reconstituted in lipid nanodiscs. These structures reveal how the lipid membrane becomes severely distorted and thinned near the groove and how lipids rearrange upon groove opening. Functional experiments show that when the groove is open, membrane thinning is critical for scrambling. In contrast, when the groove is closed specific lipid-protein interactions are critical to enable transbilayer lipid movement. Finally, we show that the choice of scaffold protein and lipids can dramatically affect the conformations and their distribution of a TMEM16 scramblase, highlighting a key role of these factors in cryoEM structure determination.

Francisco Balzarotti, Research Institute of Molecular Pathology (IMP), Wien, Austria

Accessing Nanoscale Structure and Dynamics with Light

Super-resolution methods have revolutionized fluorescence microscopy, unlocking access to the sub-diffraction domain by manipulating state transitions of fluorescent emitters. In this lecture, I will introduce MINFLUX, a single-molecule localization strategy that renders each emitted photon more informative by sequentially exciting with tailored light patterns. In its tracking modality, MINFLUX can increase the temporal resolution by 100-fold, while in its imaging modality, it achieves 3D isotropic, single nm precision in biological samples. I will present recent advances in modelling, fluorescent probes, and implementations.

Antonella Battisti, NEST, Istituto Nanoscienze-CNR and SNS, Pisa, Italy
Viscosity exposed: the role of fluorescent molecular rotors

A fluorescent molecular rotor (FMR) is a kind of fluorophore that can undergo twisted intramolecular charge transfer (TICT) upon photoexcitation, therefore exhibiting two competing de-excitation pathways. This peculiar photochemistry allows for the quantification of intracellular parameters on a micrometric scale and for the detection of intracellular phenomena.

Paolo Bianchini, Istituto Italiano di Tecnologia (IIT), Genoa, Italy
Converging multimodal microscopy methods as biophysics tools for nanoscale studies

In recent years, Expansion Microscopy (ExM) has revolutionized the field of cellular imaging by enabling enhanced resolution imaging beyond the diffraction limit without the needs of expensive and complex instrumentation. Expansion microscopy involves physically expanding biological specimens, typically using a swellable polymer, to increase their physical dimensions while preserving the molecular structure. ExM provides a scalable and versatile platform for visualizing subcellular structures. However, accurately quantifying the expansion factor and verifying the correctness of expansion at the nanoscale can be challenging. Here, we discuss about leveraging the well-characterized structure of the nuclear pore complex (NPC), a complex composed of nucleoporins that span the nuclear envelope, to serve as a reference for determining the expansion factor. The regular spacing and known dimensions of the NPC can be used as an internal ruler, providing a reliable measurement for the expansion factor.
To reach this purpose the integration of ExM with advanced and super-resolved imaging modalities such as Image Scanning Microscopy (ISM) and Stimulated Emission Depletion Microscopy (STED) become of fundamental importance and will be furtherly elaborated and discussed.
Additionally, the application of expansion microscopy with ISM and STED microscopy in chromatin studies offers the opportunity to examine the three-dimensional organization of chromatin at an unprecedented level of detail. The expanded chromatin structures reveal the spatial arrangements of DNA, nucleosomes, and higher-order chromatin domains, facilitating the understanding of chromatin architecture and its relationship with gene expression and cellular processes.

Ranieri Bizzarri, Dept. of Surgical, Medical, Molecular Pathology and Critical Care Medicine, University of Pisa, Italy

Is the cell really a machine? (1)

The great Italian biophysicist Mario Ageno once defined life as a coherent chemical system endowed with a program. This paradigm has been largely adopted by modern biology, which often depicts the cell as a (complicated) machine. But is this picture real? Behind the apparently calm and orderly appearance of each cell, on the nanometer scale where most life molecules play their role, chaos lurks and a molecular storm never cease. In this messy and irregular world, billion years' evolution has designed special molecules capable of channeling and directing information and energy in orderly processes, keeping life possible in spite of the universal thermodynamic evolution to a disordered and "dead" state. Among the first to understand this, Jacques Monod identified in chaos and necessity the two roots of what life is. In essence, biophysics witnesses our attempt to model the "effort" of cell existence, because the cell is not really a machine.

"The fair switch project": how single molecules reveal the nanoscale of the cell. (2)

ABSTRACT NOT AVAILABLE

Martino Bolognesi, University of Milan, Italy

The future of structural biology is shaped by electrons and X-ray photons

The Structural Biology domain hosts several biophysical, biochemical and computational approaches whose synergistic action provide us with integrated views of biological matter and answers leading questions in life sciences. X-ray crystallography is one of the main contributors to our understanding of protein structure, protein complexes and their mechanisms of recognition and action. The field has grown to maturity over several decades, but most significantly after the onset of third generation synchrothron sources (ca. 1985-90). The crystallographic method applied to biological macromolecules relies on firm theory and technical developments, which recently extend to free electron laser sources. The development of cryo-conservation techniques and of high efficiency direct electron detectors brough about a revolution in cryo electron microcopy around 2015. Single particle cryoEM relies on careful control of the experimental (sample and instrument) conditions and provides 3D structures of protein assemblies with resolutions comparable to crystallography. A particular filed that saw a booster step through cryoEM concerns the study of amyloid fibrils. In this case, no other experimental technique could provide previously access to the 3D structures of the fibril core region with the near atomic detail provided by cryoEM. The development of Electron Tomography is

proceeding steadily and may provide access to suitable resolution structures within their live environment. Progresses in the field of Electron Diffraction may reserve interesting approaches to the study of crystalline arrays in membranes or within cell organelles.

Carlos Bustamante, University of California, Berkeley, USA

Division of Labor and Mechanism of Translocation in a Ring ATPase

Many transport processes in the cell are performed by a diverse but structurally and functionally related family of proteins. These proteins, which belong to the ASCE (Additional Strand, Conserved E) superfamily of ATPases, often form mutimeric rings. Despite their importance, a number of fundamental questions remain as to the coordination of the various subunits in these rings. Bacteriophage phi29 packages its 6.6 mm long double-stranded DNA using a pentameric ring nano motor Using optical tweezers, we find that this motor can work against loads of up to ~55 picoNewtons on average, making it one of the strongest molecular motors ever reported. Interestingly, the packaging rate decreases as the prohead fills, indicating that an internal pressure builds up due to DNA compression attaining the value of ~3 MegaPascals at the end of packaging, a pressure that is used as part of the mechanism of DNA injection in the next infection cycle. We have used high-resolution optical tweezers to show that the motor packages the DNA in alternating phases of dwells and bursts. During the dwell the motor exchanges nucleotide, whereas during the burst, the motor packages 10 bps of DNA per cycle. We have also characterized the steps and intersubunit coordination of this ATPase. By using non-hydrolyzable ATP analogs and stabilizers of the ADP bound to the motor, we establish where DNA binding, hydrolysis, and phosphate and ADP release occur relative to translocation during the motor's cycle. Surprisingly, a division of labor exists among the subunits: while only 4 of the subunits translocate DNA, all 5 bind and hydrolyze ATP, suggesting that the fifth subunit fulfills a regulatory function. Furthermore, we show that the motor not only can generate force but also torque. We characterize the role played by the special subunit in this process and identify the symmetry-breaking mechanism in the motor. Finally, we use dsRNA, and RNA/DNA hybrids to show that the size of the burst phase adapts to the periodicity of the substrate being translocated. This information, together with recent structural data, allows us to propose a novel mechanism of translocation for this motor.

Josè-Maria Carazo, National Center for Biotechnology CNB-CSIC, Madrid, Spain
Cryo Electron Microscopy informing of the continuous flexibility of biological macromolecules

Part of the success of cryo Electron Microscopy in Structural Biology has been due to the development of new image processing methods that can partly take into account macromolecular flexibility, at least for its most stable states. Still, a decade of practice has indicated that this approach has its limitations. In this talk I will present new methods that can consider the whole data set and produce estimations of conformational macromolecular landscapes (Scheres et al., NatMet 2007; Herreros et al., NatComm, 2023, and unpublished)

Rita Carrotta, CNR - Istituto di Biofisica, Palermo, Italy
Protein assemblies: multiple pathways and structures

Proteins are complex systems whose structure is determined by multiple interactions. Solvent and cosolvent and not only plays a fundamental role in determining processes such as folding and/or protein aggregation. The same protein can organize in very different ways and a multiplicity of structures can be obtained, although the rules of interaction beeing the same.

Loredana Casalis, Elettra – Sincrotrone, Trieste, Italy
Biophysical aspects governing the uptake of extra cellular vesicles by cells.

Small extracellular vesicles (EVs) play a pivotal role in health and disease. Depending on cells of origin and overall physiological conditions, EVs modulate their molecular cargo, size and membrane composition, to promote specific biological effects. Despite the growing scientific interest, the mechanism(s) driving EVs docking on recipient cell membranes and cargo release are still debated. Here, we exploit atomic force microscopy and scattering techniques to highlight the role of biophysical properties of plasma cell membrane in EVs uptake.

Martin Chalfie, Dept. of Biological Sciences, Columbia University, New York, USA
The Continued Usefulness of Useless Knowledge

Over 80 years ago, the educator and first director of the Princeton Institute for Advanced Study Abraham Flexner wrote an article in Harper's Magazine entitled "The Usefulness of Useless Information." In this article he states, "I sometimes wonder. . .whether our conception of what .is useful may not have become too narrow to be adequate to the roaming and capricious possibilities of the human spirit," and he argues that real discoveries are made when scientists are allowed to explore the world without recourse to usefulness. Several Nobel prizes have been given for discoveries tangential to what was initially studied. I will argue that "useless information" is needed as much today as in the past for the advancement of industry and medicine and suggest ways that we can encourage the finding of the unexpected, the discoveries that will enable future revolutions.

Daniel Ciepielewski, Nikon Europe B.V., Amsterdam, NL
Collaboration Academia – Industry & career step

ABSTRACT NOT AVAILABLE

Corrado Dallacosta, Leica Microsystems, Mannheim, Germany
Leica Stellaris and latest portfolio news

ABSTRACT NOT AVAILABLE

Mauro Dalla Serra, CNR - Istituto di Biofisica, Genova, Italy
Drilling holes into cell membranes: the amazing world of pore forming toxins.

Pore-Forming Toxins (PFTs) are normally able to drill poorly selective nanometer-sized holes into cell membranes, causing cell death through osmotic imbalance.
PFTs constitute the largest and best characterized class of bacterial protein toxins, accounting for more than 30% of all known bacterial protein toxins. They are potent virulence factors evolved during ancient competition among organisms for defense and/or attack purposes. Interestingly, similar structures and modes of action are also adopted by components of the immune system, like perforin and complement, and by some antimicrobial peptides. PFP mode of action generally proceeds through a sequential mechanism implicating (i) protein release into the aqueous medium, (ii)

binding to target membrane, (iii) oligomerization, (iv) conformational changes, and (v) pore opening. PFP are good archetype for studying key aspects of protein-protein and protein-lipid interaction, being prone to biotechnological applications. Regarding the pore architecture, two conformations have been described: a purely proteinic channel and a mixed protein-lipid arrangement. In this second case, lipid heads could either intercalate between adjacent protein monomers or constitute the chord of arc shaped pores. In both cases the lipid lamellar structure is destroyed and lipids should bend, assuming a toroidal shape. Here I will discuss how transmembrane pores are formed by gamma-hemolysins from S. aureus. I will then focus on cholesterol-dependent cytolysins (CDCs), a toxin family from pathogenic bacteria, and on actinoporins, cytolysins from sea anemones. I will briefly present experimental evidence supporting the ability of these two unrelated protein families to punch proteolipidic nanopores into lipid membranes.

Alberto Diaspro, University of Genoa, IIT, IBF-CNR, Genoa, Italy
The Makapansgat pebble.

The Makapansgat pebble (ca. > 2.500,000 BP) is a red jasper stone with natural chipping and patterns that make it look like a human face, and it is considered the oldest known manuport. It has been suggested that the Australopithecus realized, by collecting and storing it in a cave, the earliest case of symbolic thinking or aesthetic sense in the human heritage ((A.Tura, 2020, Johan &Levi). In recognition and action of the Australopithecus, there is the naivety that makes a researcher solid and elegant and the ability to try to build a model the "most naive possible" that he considers only those aspects that are essential in determining the trend and the characteristics of the phenomenon that one intends to study (M.Ageno, 1967, Q102, Accademia Nazionale dei Lincei). So far, we can consider biophysics as the «trajectory ideal that connects with a single arch, the Makapansgat pebble to man, " enabling the prediction of both the past and the future.

Jörg Enderlein, Third Institute of Physics – Biophysics, Georg August University, Göttingen, Germany
Lifetime-Multiplexed Image-Scanning Single-Molecule Localization Microscopy

Single Molecule Localization Microscopy (SMLM) (1,2) has become one of the most important methods of super-resolution microscopy, achieving lateral resolutions that can surpass Abbe's classical diffraction limit by more than two orders of magnitude. Usually, SMLM is realized with a wide-field fluorescence microscope equipped with a sufficiently sensitive and fast wide-field camera (e.g. EM-CCD or sCMOS cameras). Here, we

represent an alternative approach to SMLM which is based on a fast laser scanning confocal microscope, equipped with single photon avalanche diode (SPADs) or a SPAD array. By using such a microscope, it is possible to not only image fluorescence intensities, but also fluorescence lifetime. We demonstrate that the latter can be used for multiplexing, i.e. simultaneously imaging several emitters with similar spectral properties but different fluorescence lifetimes, which allows for high-precision co-localization studies without any chromatic aberration artefacts (3,4). The ability to measure not only brightness and position but also the lifetime of single molecule localizations opens also the door to combining SMLM with Metal- and Graphene-Induced Energy Transfer (MIET/GIET) imaging (5,6), which allows for localizing a molecule with nanometer accuracy along the optical axis. This results in a nearly isotropic, nanometric image resolution (7). Finally, when using a SPAD array for detection, we demonstrate doubling of the lateral localization accuracy by combining SMLM with Image Scanning Microscopy (ISM) (8,9).

1. Rust MJ, Bates M, Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nature Methods. 2006 Oct;3(10):793–6.
2. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, et al. Imaging Intracellular Fluorescent Proteins at Nanometer Resolution. Science. 2006 Sep 15;313(5793):1642–5.
3. Oleksiievets N, Sargsyan Y, Thiele JC, Mougios N, Sograte-Idrissi S, Nevskyi O, et al. Fluorescence lifetime DNA-PAINT for multiplexed super-resolution imaging of cells. Commun Biol. 2022 Jan 11;5(1):1–8.
4. Oleksiievets N, Mathew C, Thiele JC, Gallea JI, Nevskyi O, Gregor I, et al. Single-Molecule Fluorescence Lifetime Imaging Using Wide-Field and Confocal-Laser Scanning Microscopy: A Comparative Analysis. Nano Lett [Internet]. 2022 Jul 6 [cited 2022 Jul 6]; Available from: https://doi.org/10.1021/acs.nanolett.2c01586
5. Chizhik AI, Rother J, Gregor I, Janshoff A, Enderlein J. Metal-induced energy transfer for live cell nanoscopy. Nature Photon. 2014 Feb;8(2):124–7.
6. Ghosh A, Sharma A, Chizhik AI, Isbaner S, Ruhlandt D, Tsukanov R, et al. Graphene-based metal-induced energy transfer for sub-nanometre optical localization. Nat Photonics. 2019;13:860–5.
7. Thiele JC, Jungblut M, Helmerich DA, Tsukanov R, Chizhik A, Chizhik AI, et al. Isotropic Three-Dimensional Dual-Color Super-Resolution Microscopy with Metal-Induced Energy Transfer. Science Advances. 2021 Dec 21;8:14190–200.
8. Sheppard CJ. Super-resolution in confocal imaging. Optik. 1988;80(2):53–4.
9. Müller CB, Enderlein J. Image Scanning Microscopy. Phys Rev Lett. 2010 May 10;104(19):198101.

Laura Finzi, Department of Physics, Emory College, Atlanta, GA, USA
DNA torsional state affects transcription and is influenced by macromolecular crowding

Cells are crowded environments with up to 40% of their volume occupied by molecules. Crowding changes inter-and intra-molecular interactions altering entropic forces. I will describe how macromolecular crowding may affect genomic structure and function using in vitro, single-molecule techniques to monitor the structure of DNA and DNA-protein complexes.

Giorgio Giacometti, Dept. of Biology, University of Padova, IVSLA, Venezia, Italy
A touch on biophysical aspects of Photosynthesis

The photosynthetic process, by which photosynthetic organisms use energy from sunlight to convert Carbon Dioxide and Water into Oxygen gas and Glucose is extremely important for virtually all life on earth. The process can be broken into two stages: the light dependent reactions and the dark (light independent) reactions also known as Calvin-Benson Cycle. The first stage, in which light energy is converted to chemical free energy is particularly interesting from a biophysical view point as it includes one of the fastest electron transfer reaction observed in nature and brings about one of the most difficult redox reactions: the splitting of water into its components hydrogen and molecular oxygen. Mimicking this reaction at industrial level would provide virtually unlimited clean energy to power human activities

---

Luca Lanzanò, Dept. of Physics and Astronomy "Ettore Majorana", University of Catania, Catania, Italy, IIT, Genoa, Italy
Lifetime-based super-resolution microscopy and its application to a model of oncogene activation

I will discuss how to take advantage of fluorescence lifetime detection and phasor analysis to make superresolution microscopy more efficient. In particular, I will discuss the separation of photons by lifetime tuning (SPLIT) method and its implementation in different types of imaging modalities. Then, I will discuss application of superresolution microscopy to the investigation of chromatin alterations in a model of PML-RARα oncogene activation.

Mauro Manno, CNR - Istituto di Biofisica, Palermo, Italy
Biophysics consistency in the landscape of biogenic nanoparticles

Extracellular vesicles are considered advanced drug delivery nanocarriers. A complex physicochemical landscape differentiates extracellular vesicles from other nanoparticles, such as protein therapeutics and viral vectors. Several methods are required to determine their structural and functional properties, including optical and scattering techniques. Can biophysical approaches help to cope with the heterogeneity of nanoparticles properties and promote their translation as bioproducts?

Michele Migliore, CNR - Istituto di Biofisica, Palermo, Italy
Exactitude, visibility, and consistency of biophysical models of neurons and brain circuits.

I will discuss several computational models of brain cells and circuits, and how their online open access implementation on the EBRAINS Research Infrastructure enables a unified approach to exactitude, visibility, and consistency, to better understand many fundamental aspects of the biophysical processes underlying cognitive functions and dysfunctions.

Velia Minicozzi, Dept. of Physics and INFN – Univ. of Rome "Tor Vergata", Italy
Simulations meet experiments in Biophysics

In recent years, Molecular Dynamics simulations have reached such a level of development to even become a guide for experiments. These results have been achievable thanks to the development of new algorithms capable of more efficiently sampling the configurations space, to increasingly accurate force fields but also thanks to hardware improvement. The interplay between simulations and experiments assures results consistency.

Valentina Mussi, CNR - Institute of Microelectronics and Microsystems, Rome, Italy
The unexpected diagnostic potential of 3D nano-disorder: epigenetic effects and cancer alterations.

A novel approach will be demonstrated to capture diagnostic information carried by structural conformation and physical characteristics of healthy and malignant DNA molecules, based on Raman mapping of dehydrated aqueous DNA droplets directly deposited onto a disordered nanostructured platform, without any knowledge of the DNA sequence.

Maria Grazia Ortore, Università Politecnica delle Marche, Ancona, Italy
Observing the not-visible biological details: a challenge between quickness, and consistency

The past century has been defined by Richard Panek “the invisible century”, considering that both Einstein and Freud had exploded the myth by leading exploration into the science of the invisible and the unconscious, inspiring a fundamental shift in the history of human thought. On the other side, in this century scientific and technological advancements have led us to new microscopic techniques and resolution unconceivable few decades ago, making visible something unpredictable before. However, not-visible biological details such as protein-protein interaction potential, still need biophysical techniques based on not-visible, assuring the same quickness and consistency of the several microscopies available nowadays. In this lecture I will show the basic of Small Angle X-ray and Neutron Scattering, which provide differential macroscopic cross sections that, after accurate data analysis, can provide a biophysical picture of different biological issues, not substituting microscopy, but being complementary to it. According to a set of examples, I will present how to obtain consistency from this kind of approach.

Giorgio Vallortigara, Centre for Mind/Brain Sciences, University of Trento, Italy
The neurobiology of number cognition

What underlies the ability to deal with numbers and where did it come from? It has been hypothesized that our ability to accurately represent the number of objects in a set (numerosity), and to carry out numerical comparisons and arithmetic, developed from an evolutionarily conserved system for approximating numerical magnitude. Non-symbolic number cognition based on an approximate sense of magnitude has been documented in a variety of species. However, we know little about its origins (i.e., to what extent experience would shape it) and of its neural and molecular bases. To address the first issue we performed single cell recordings in awake young domestic chicks. We found neurons selective to number in the caudal nidopallium (a higher associative area with functional similarities to the mammalian prefrontal cortex), which suggest that an approximate sense of magnitude can be an inborn feature in the avian brain. To address the issue of circuitry and molecular bases of the sense of magnitude we made use of zebrafish, that in recent years became established as ideal developmental and behavioral genetic model system. Using a combination of early gene expression and in-situ hybridization we identified for the first time a small region in the caudal part of the dorso-central division of the zebrafish pallium that shows selective activation upon change in numerosity of visual stimuli. As pallial regions are implicated in number cognition in mammals and birds, these findings support the existence of an evolutionarily conserved system for approximating magnitudes and provide an avenue for exploring its underlying molecular and genetic correlates.

Massimo Vassalli, University of Glasgow, Scotland, UK
Investigating cellular mechanosensing with fluidic force microscopy

Modern medicine has made astonishing progress leaning on genetic and biochemical research. Nevertheless, treatments are still missing for uncountable conditions, indicating that key areas of human biology are still unknown. In addition to chemical signalling, mechanical perturbations are now recognized to influence cell and tissue function. New therapeutic approaches are expected to emerge in the next decades that target the physical basis of many diseases, including atherosclerosis, asthma, osteoporosis, heart failure, or cancer. Moreover, a clear mechanical footprint is recognisable in many age-related disabilities, like lower back pain and irritable bowel syndrome, where irregular responses to mechanical forces leads to over-activation of cells, sustaining long-term inflammation and fibrosis that consolidate pathology. Nevertheless, to properly leverage the fascinating promise of mechanomedicine, we

need to fully understand how single cells respond to mechanical stimuli. In the Cellular Mechanobiology Lab (CML) of the Centre for the Cellular Microenvironment of the University of Glasgow we develop correlative approaches where atomic and fluidic force microscopy are combined with fluorescence confocal microscopy to apply controlled forces on single living cells and observe the response in real time. During the lecture a novel method to study cellular mechanosensing will be presented, based on the use of Fluidic Force Microscopy. This approach is particularly suitable to study the membrane microenvironment, observing how the local tension propagates and impacts on mechanosensitive ion channels.

Alessandro Verri, University of Genoa, Italy
"... and now for something completely different: is AI coming of age?"

ABSTRACT NOT AVAILABLE

Valeria Vetri, Dip. di Fisica e Chimica & ATeN Center, Università di Palermo, Italy
Multiplicity and visibility in the study of amyloid superstructures.

Visual images can be determining factors in understanding natural phenomena which are often spatially heterogeneous. When microscopy and spectroscopy are coupled a high level of exactitude can be reached in deciphering occurring molecular mechanisms. This lecture will focus on the possibility of using Fluorescence Lifetime Imaging Microscopy to non-invasively analyze protein aggregation and amyloid formation phenomena in real time, providing a pixel resolution map of the molecular structure of protein aggregates.

Cristiano Viappiani, University of Parma, Parma Italy
Light-triggers visualize quick biomolecular processes

Several reactions at the basis of fundamental processes in biophysics, occur with rates exceeding the time resolution afforded by conventional methods, and often show extension over several orders of magnitude in time. To overcome this limitation, a few experimental approaches have been proposed, most of which are based on **fast** photochemical triggers. Using **light** pulses from a laser source to introduce the perturbation, it is possible to **watch** relaxation of ground state reactions from nanoseconds (or faster in favorable cases) to seconds, thus achieving unprecedented temporal dynamics. Prototype examples are discussed. The specific needs for these

particular flash-photolysis applications are met by dedicated experimental layouts, affording non-contact perturbations, potentially useful also for cellular applications.

Anthony Watts, Biochemistry Dept., University of Oxford, UK

The importance of water in membrane receptor function – Implications for optogenetics

Water plays a fundamental role in biology. With ultra-high exactitude and resolution, it is now possible to see individual waters at high resolution in the initial (fs) stages of receptor sensitization.

Selected Participants abstracts

NDM29-mediated epigenetic and nuclear architecture remodelling during neuroblastoma retro- transformation

Francesca Baldini[1], Lama Zeaiter[1,2], Lisa Cuneo[1,3], Paolo Bianchini[1], Laura Vergani[2], Aldo Pagano[4,5], Alberto Diaspro[1,3]

1Nanoscopy, Istituto Italiano Tecnologia, Genoa, Italy
2DISTAV, Department for the Earth, Environment and Life Sciences, University of Genoa, Genoa, Italy
3DIFILAB Department of Physics, University of Genoa, Genoa, Italy
4IRCCS Ospedale Policlinico San Martino, Genoa, Italy
5DIMES, Department of Experimental Medicine, University of Genoa, Genoa, Italy

Chromatin organization is a well-orchestrated mechanism regulating cellular physiology and its changes are frequently associated to onset of many diseases, including cancer. Neuroblastoma (NB) is the most common extracranial solid tumor in childhood, and it shows remarkable heterogeneity in the clinical phenotypes and in the evolutionary patterns. Although the molecular mechanisms of this heterogeneity are still unclear, the impairment of the "epigenetic machinery" and the consequent chromatin reorganization might play an important role. We aimed to investigate the alterations in nuclear architecture and epigenetics being associated with neuroblastoma transformation. The cellular model employed in this study is the neuroblastoma cell line SKNBE2 genetically engineered to overexpress the ncRNA NDM29 to differentiate from highly malignant (Mock) into neuron-like (S1.1) cells. By confocal microscopy, we explored the nuclear architecture, evaluating volume, elongation, compactness, and chromatin density. Using super-resolution microscopy (STED) and histone H3 immunolabelling we assessed the epigenetic rearrangement, and by enzyme-linked immunoassay the global DNA methylation. The mRNA expression of the main epigenetic modifying enzymes was assessed by quantitative PCR, and the expression profile of NF- κB-regulated genes was assessed by cDNA microarray. The NDM29 over-expressing cells phenotype exhibited nuclei with reduced volume and more elongated shape compared to malignant cells, together with an altered distribution of H3K9-acetylated and H3K9-methylated domains and DNA hypermethylation. Also, the recovered neuron-like cells exhibit up-regulation of the acetyltransferase KAT2A, down-regulation of the methyltransferase EHMT2, and down-regulation of most NF-κB-regulated genes. Our findings reveal a global remodelling of the nuclear structure and epigenetics during neuroblastoma retro-transformation induced by NDM29, and this structural rearrangement impacts on gene expression. These findings offer potential insights into understanding the mechanism of neuroblastoma malignancy opening exciting prospects for new prognostic and therapeutic approaches.

Laser oscillators for fast label-free polarization-resolved imaging in a laser scanning architecture

F. Callegari1, P. Bianchini1, A. Diaspro1,2

[1] Nanoscopy and NIC @ Italian Institute of Technology (IIT), Genova, Italy;

[2] DIFILAB, Department of Physics, University of Genoa, Genoa, Italy

The development of label-free imaging methods for life-science studies has gained significant attention in recent years due to their capability to provide images of samples in their most native and less altered state. Among these approaches, polarimetry-inspired microscopy is a class of label-free imaging methods which offers images related to sample optical anisotropies, such as intrinsic birefringence or dichroism.
Here, we present the architecture of laser oscillators, enabling the generation of a fast, time- varying polarization state of light. The high polarization generation rate allows to probe the specimen anisotropies on a time scale compatible with the pixel-dwell time of a laser scanning microscope. Moreover, this approach avoids the use of external active devices to control the polarization state, enabling a reduction of the complexity of the optical setup. We discuss the result of our novel approach and how such technological advancement will help the spreading of label-free imaging instruments based on the manipulation of polarized light.

Background subtraction in Single-Molecule Localization Microscopy images by means of Scattering Network as encoder in a Unet architecture

Lisa Cuneo [1,2], Simone Civita[1], Luca Ratti[3], Ivan Trapasso[3], Paolo Bianchini[1,2], and Alberto Diaspro[1,2]

[1] DIFILAB Department of Physics, University of Genoa, Genoa, Italy

[2] Nanoscopy&NIC@IIT, Istituto Italiano di Tecnologia, Genoa, Italy

[3] MALGA, Machine Learning Genoa Center, Genoa, Italy

In Single-Molecule Localization Microscopy (SMLM), tackling background noise is a pivotal challenge with profound implications for image quality and contrast [1]. To confront this issue, we introduce a novel approach based on Scattering Network [2]. Scattering Networks are known for their translation- invariant image representation and stability to deformations, making them suitable for image classification. They require no parameter learning, reducing computational demands, and offer interpretability due to their mathematical foundations. One distinctive aspect of Scattering Networks is their possibility to fix the scale of the filters, a feature that inspired us to incorporate them as a fixed encoder within a UNet framework [3]. This strategic integration harnesses the strengths of Scattering Networks to address the challenge of background removal in SMLM. By combining Scattering Networks with Convolutional Neural Networks (CNNs), our method excels at separating the signal from the background within the scattering representation domain. Additionally, it facilitates the reconstruction of the image, potentially elevating the precision and quality of SMLM image processing, and ultimately, advancing the quality of microscopy outcomes.

[1] H. Deschout, F. Zanacchi, M. Mlodzianoski et al, Precisely and accurately localizing single emitters in fluorescence microscopy, Nat. Methods 11, 253–266 (2014).
[2] J. Bruna and S. Mallat, Invariant Scattering Convolution Networks, IEEE Transactions on Pattern Analysis and Machine Intelligence 35 (8), 2013.
[3] O. Ronneberger, P. Fischer and T. Brox U-Net: Convolutional Networks for Biomedical Image Segmentation , Medical Image Computing and Computer-Assisted Intervention – MICCAI 2015.

Exploiting the detector distance information in image scanning microscopy by phasor SPLIT-ISM

Elisabetta Di Franco[1,5], Paolo Bianchini[2], Giuseppe Vicidomini[3], Alberto Diaspro[2,4], Giulia Tedeschi[5], Lorenzo Scipioni[5], Enrico Gratton[5], Michelle Digman[5] & Luca Lanzanò[1,2]

[1]Department of Physics and Astronomy "Ettore Majorana", University of Catania. [2]Nanoscopy CHT Erzelli, Istituto Italiano di Tecnologia, Genoa, Italy. [3]Molecular Microscopy and Spectroscopy, CHT Erzelli, Istituto Italiano di Tecnologia, Genoa, Italy. [4]DIFILAB, Department of Physics, University of Genoa, Genoa, Italy. [5]Laboratory of Fluorescence Dynamics, University of California Irvine.

Confocal microscopy is an important bio-imaging technique which increases the resolution using a spatial pinhole to block out-of-focus light. In theory, the maximum resolution and optical sectioning is obtained when the detection pinhole is fully closed but this is prevented by the dramatic decrease of the signal reaching the detector. In image Scanning Microscopy (ISM) this limitation is overcome by using an array of point-detectors rather than a single detector. This, combined with pixel reassignment, increases the resolution of $\sqrt{2}$ over widefield imaging, with relatively little modification to the existing hardware of a laser-scanning microscope. Separation of photons by lifetime tuning (SPLIT) is a super-resolution technique, originally introduced in the context of STED microscopy, based on the phasor analysis of the fluorescent signal into an additional channel of the microscope. The phasor is a powerful tool capable of describing the evolution of a signal (as a function of a variable, such as time) as a single point in a plane of coordinates gg (cosine transform) and ss (sine transform), that is the phasor diagram. Our aim is to apply SPLIT technique to the images recorded by an ISM microscope to further improve the resolution, contrast, and SNR (SPLIT-ISM). Application of SPLIT requires an additional channel of the microscope. In SPLIT-ISM, we use the distance between the detectors and the center of the array. We apply the technique on data acquired by two different ISM setups: the SPAD array-ISM and the ZEISS Airyscan. We evaluate the improvement provided by SPLIT-ISM through the QuICS algorithm, a quantitative tool based on image correlation spectroscopy. QuICS allows extracting three parameters related to the resolution, contrast e SNR of the image. We find that the SPLIT method improves the resolution of images acquired with both SPAD array-ISM and the ZEISS Airyscan while retaining the captured image signal. This method can be used for any type of biological application performed with image scanning microscopy by improving image resolution for a better understanding of biological samples.

Raman and SERS combined approach for evaluating HER2 levels in breast cancer cells.

A. Esposito [a], S. Spaziani [c, d], G. Barisciano [b], G. Quero [e], M. Pisco [c, d], V. Colantuoni [b], L. Sabatino [b], A. Cusano*[c, d], A. C. De Luca [a**].

[a] Laboratory of Biophotonics and Advanced Microscopy, Institute of Experimental Endocrinology and Oncology "G. Salvatore", Second Unit, National Research Council, Naples, Italy

[b] Department of Sciences and Technologies, University of Sannio, Benevento, Italy

[c] Department of Engineering, University of Sannio, Benevento, Italy

[d] Centro Regionale Information Communication Technology (CeRICT Scrl), Benevento, Italy

[e] Department of Biosciences and Territory, University of Molise, 86090 Pesche, Italy

The detection and identification of epidermal growth factor receptor 2 (HER2) in breast cancer cells at molecular level is crucial for the clinic therapy of breast cancer [1,2]. This investigation, with common practice requires expensive and metabolomic and genetic profiling analysis, that are far to be applied as routinary diagnostic tool, alimenting the needing for fast cell metabolic evaluation and precise quantitation of HER2 levels. The implementation of Raman and SERS for the detection of disease has increased in recent years, due to the unique features of Raman scattering. Raman spectroscopy is a cutting-edge tool to inspect biological materials. It is an analytical technique, counting on the vibrational fingerprint can easily detects several molecules in a biological sample, in native condition with few or negligible sample preparation. Raman can capture the overall biochemical picture of a cell, distinguishing among the metabolic changes over different conditions, with subcellular precision [3-4]. On the contrary, this multiplexing approach suffers of limited sensitivity and specificity due to the low cross-section of Raman scattering, and the overall contribution of all the Raman active molecules, that may cover and disturbing the signal of the probed analyte, showing a rather difficult applicability in quantitative biology and precision medicine. Finally, SERS labelled approach allows to directly quantify the number of receptors on the cell surface, reaching even single molecule sensitivity [5]. In this study we used a Herceptin-based SERS probe, for fast identification and differentiation of BC cell lines with a differential level of HER2 expression. The same Raman microscope is used for analysing the main biochemical differences between the cell lines and correlating them with cellular HER2 levels. This combined Raman-SERS probe holds a potential for a direct detection of living breast cancer cells with the advantages of easy fabrication, high SERS sensitivity, and biocompatibility.

[1] Arnold M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *The Breast*. 66, 15-23 (2022).

[2] Sajjadi E, et al. *Front Mol Biosci*. 10, 1176309 (2023).

[3] S. Elumalai, S. Managó, De Luca AC. *Sensors.*;20, 5525 (2020).

[4] Mangini M., et al. *Front Bioeng Biotechnol*. 11, 1057216 (2023).

[5] Managò et al. *Sens Actuators B Chem*. 339, 129321 (2021).

Acknowledgement: The support from Italian Association for Cancer Research (AIRC) grant n°21420, and the projects POR CIRO and PON IMPARA are gratefully acknowledged.

Linking molecular-scale to mesoscale dynamics in biomolecular condensates

Nicola Galvanetto[1,2], Miloš T. Ivanović[1], Simone del Grosso[1], Aritra Chowdhury[1], Andrea Sottini[1], Daniel Nettels[1], Robert B. Best[3], and Benjamin Schuler[1,2]

[1]Department of Biochemistry, University of Zurich, Zurich, Switzerland

[2]Department of Physics, University of Zurich, Zurich, Switzerland [3]National Institutes of Health, Bethesda, MD, USA

Biomolecular condensates are physiological structures whose functions span a wide range of length scales. For this reason, they present a new paradigm of subcellular organization. Precise understanding of how the dynamics of molecular constituents contribute to the emergent material properties of the polymer network has been subject of extensive theoretical investigations. However, experimental evidence is largely lacking, primarily owing to limitations in the ability to perform molecular-level measurements in individual phase separated droplets. Here we employ single-molecule techniques to study the conformations and the intramolecular dynamics of intrinsically disordered proteins within single droplets (*1*), combined with microrheology approaches to assess mesoscale properties. By tuning the strength of the interactions among the constituent proteins, we produced condensates spanning almost two orders of magnitude in viscosity, and we observed a concomitant variation of the reconfiguration time of the proteins — ranging from hundreds of nanoseconds to tens of microseconds. We first focused on the microscopic level with all-atom molecular dynamics simulations to pin down how arginines and low salt can slow down residue-residue contact dynamics. We then generalized our experimental findings across length scales providing a quantitative link between local protein reconfiguration, protein spatial diffusion, and macroscopic viscosity of such condensates.

1. N. Galvanetto, M. T. Ivanović, A. Chowdhury, A. Sottini, M. F. Nüesch, D. Nettels, R. B. Best, B. Schuler, Extreme dynamics in a biomolecular condensate. *Nature*. **619**, 876–883 (2023).

A biophysical study of the dynamic properties of glucagon granules in α cells by iMSD and SPT approaches

S. Ghignoli[1], V. De Lorenzi[1], G. Ferri[2], S. Luin[1], F. Cardarelli[1]

[1]NEST Laboratory - Scuola Normale Superiore, Pisa, Italy

[2]G. Ferri is with Fondazione Pisana per la Scienza, Pisa, Italy

Insulin and glucagon are the two essential hormones for maintaining proper blood glucose homeostasis, which is disrupted in Diabetes. A constantly growing research interest has been focused on the study of the subcellular structures involved in hormone secretion, namely insulin- and glucagon-containing granules, and on the mechanisms regulating their behavior. Yet, while several successful attempts were reported describing the dynamic properties of insulin granules, little is known about their counterparts in α cells, the glucagon-containing granules. To fill this gap, we used αTC1 clone 9 cells as a model of α cells, and ZIGIR as a fluorescent Zinc chelator for granule labelling. We started by using spatiotemporal fluorescence correlation spectroscopy in the form of imaging-derived mean square displacement (iMSD) analysis. This afforded quantitative information on the average dynamical and structural properties of glucagon granules having insulin granules as benchmark. Interestingly, the iMSD sensitivity to average granule size allowed us to confirm that glucagon granules are smaller than insulin ones (~1.4 folds, further validated by STORM imaging). To investigate possible heterogeneities in granule dynamic properties, we moved from correlation spectroscopy to single particle tracking (SPT). We developed a MATLAB script to localize and track single granules with high spatial resolution. This enabled us to classify the glucagon granules, based on their dynamic properties, as 'blocked' (i.e., trajectories corresponding to immobile granules), 'confined/diffusive' (i.e., trajectories corresponding to slowly moving granules in a defined region of the cell), or 'drifted' (i.e., trajectories corresponding to fast moving granules). In cell-culturing control conditions, results show this average distribution: 32.9 ± 9.3% blocked, 59.6 ± 9.3% conf/diff, and 7.4 ± 3.2% drifted. This benchmarking provided us with a foundation for investigating selected experimental conditions of interest, such as the glucagon-granule relationship with the cytoskeleton. For instance, if Nocodazole (10 μM) is used for microtubule depolymerization, the percentage of drifted motion collapses to 3.5 ± 1.7% while immobile granules increase to 56.0 ± 10.7% (remaining 40.4 ± 10.2% of conf/diff). This result confirms the clear link between glucagon-granule motion and cytoskeleton structures, a first step towards understanding the intracellular behaviour of this subcellular compartment. The information collected might now serve to support future investigations on glucagon granules in physiology and disease.

[This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 866127, project CAPTUR3D).]

Fluorescence Correlation Spectroscopy (FCS) on a commercial microscope for studying fast dynamics of molecules in solutions and in live cells

Elisa Longo, Luca Lanzanò
Department of Physics, University of Catania, Catania, Italy

A still open question in biophysics is understanding how the complex network of molecular interactions and dynamics inside the cell nucleus is affected by chromatin architecture and its alterations. A powerful technique to study molecular dynamics and interactions in biological systems is Fluorescence Correlation Spectroscopy (FCS). It is commonly employed in fluorescence microscopy to investigate dynamic processes such as diffusion, binding, and reactions of fluorescently labelled molecules within a sample. FCS is typically performed on dedicated confocal microscope setups/modules where the excitation laser is focused on a point of the specimen, the single-point data are acquired and the autocorrelation function is generated by the dedicated software.
Scope of this work is to demonstrate that single-point FCS data can be extracted in any confocal laser scanning microscope, even in the absence of the dedicated FCS module. We show that data can be acquired in line or raster scan mode and then exported and processed with a custom script in Matlab to generate the corresponding autocorrelation function. We test the method on the Leica SP8 confocal microscope available at the Bio and Nano-tech Research and Innovation Tower (B.R.I.T.) facility of our institution. We first validate the method measuring the diffusion constant of probes of different size in solution. Then, we apply the method to measure the diffusion of fluorescent proteins inside the nucleus of live cells. As expected, we find that the diffusion coefficient of GFP was slower in the nucleolus (D = 10 ± 2 $\mu m^2/s$) relative to the nucleoplasm (D = 29 ± 5 $\mu m^2/s$), presumably because of higher crowding.

A CORRELATIVE STUDY OF THE PHOTOSENSITIZER HYPERICIN IN LIPID BILAYERS MIMICKING VIRAL ENVELOPES

Matteo Mariangeli[1,2], Simone Civita[1,2], Silvia Dante[4], Pietro Delcanale[2], Stefania Abbruzzetti[2], Alberto Diaspro[1,3], Cristiano Viappiani[2], Paolo Bianchini[1, 2, 3]

[1]Nanoscopy and NIC @ Istituto Italiano di Tecnologia - Genoa, Italy
[2]Department of Mathematical, Physical and Computer Sciences, University of Parma - Parma, Italy
[3]DIFILAB, Department of Physics, University of Genoa - Genoa, Italy
[4]Materials Characterization Facility, Istituto Italiano di Tecnologia - Genoa, Italy

Supported lipid bilayers (SLBs) are an excellent model system to simulate the behavior of biological membranes1. Atomic Force Microscopy (AFM) is one of the best and most used techniques to study such systems since it can access morphological and structural information with high spatial resolution while working at physiological conditions and maintaining the sample intact.

Fluorescence microscopy can be implemented in correlation with AFM to extract complementary information2 regarding chemical interactions between lipids and specific molecules.

We focus our attention on SLBs composed of a lipid mixture similar to the one constituting enveloped viruses (e.g. coronaviruses, HIV), and we are interested in studying the interaction between SLBs and hypericin (Hyp). Hyp is a small lipophilic molecule, a fluorophore with photosensitizing properties, that was discovered to be a potent antiviral either upon illumination or in the dark3.

Our study aims to highlight the mechanism involved in the Hyp virucidal activity by using a correlative AFM- fluorescence approach. So far, with AFM, we highlighted a morphologic change of SLBs when Hyp is added in the presence of absorbable light. This is most likely due to Hyp's photodynamic action, which indirectly damages double bonds in lipids resulting in a structural modification of the whole SLB. Confocal microscopy will help us to check if Hyp has a preferential interaction with specific lipids.

On the other hand, when the SLB with Hyp is not exposed to light, we do not see any evident change with AFM, despite the known Hyp antiviral effect in viruses in this condition. Under the assumption that Hyp induces a fluidity change4, we are applying Atomic Force Spectroscopy to extract nanomechanical properties of the SLB. We aim to correlate such results with fluorescence methods, i.e., fluorescence correlation spectroscopy (FCS) and fluorescence lifetime imaging microscopy (FLIM), to decipher the influences of Hyp on the molecular dynamics of the system.

1) E.T. Castellana, P. S. Cremer, Surface Science Reports, 61 (10), 429-444, (2006).
2) B. Harke, et al. Optical Nanoscopy, 1(1), 3, (2012).
3) A. Kubin Curr. Pharm. Des., 11, 233–253 (2005).
4) P. Delcanale et al. ACS Applied Materials & Interfaces 14 (12), 14025-14032, (2022).

Visible light-mediated photocatalysis for antibacterial photodynamic therapy: photodegradation of calf thymus DNA

Giorgia Puleo,[1,3] Vittorio Ferrara,[2] Giuseppe Sancataldo,[2] Mariano Licciardi,[1] Vito Foderà,[3] Valeria Vetri[2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze Bld.18, Palermo (Italy)
[2] Department of Physics and Chemistry, University of Palermo, Viale delle Scienze Bld. 18, Palermo (Italy)
[3] Department of Pharmacy, University of Copenhagen, Universitetsparken 2, Copenhagen, 2100, Denmark

Antibacterial photodynamic therapy (aPDT) is an innovative approach to treating increasingly widespread multi-resistant bacterial infections. aPDT exploits radical oxygen species (ROS), which are formed when photosensitizers are exposed to light. In this study, the photocatalytic properties of nitrogen-doped titanium dioxide (N-TiO_2), a photodynamic agent, were combined with gold nanoparticles (AuNPs) as suitable enhancers. Indeed, noble metals such as gold and platinum are known to stabilize charge carriers on their surface, preventing electron-hole pairs recombination. To make the treatment activable by visible light and thus modifying the TiO_2 bandgap, the synthesis was appropriately modified with nitrogen doping, making this material activable by blue light to trigger ROS production. The photocatalytic activity of N-TiO_2 alone and with AuNPs was evaluated by spectroscopic analysis on Methylorange as a standard dye, showing excellent photodegradation yields under blue light. Then, to evaluate the photodegradation properties of biomolecules, we focused on the damage induced on the DNA structure as a major target, since it is essential for bacteria survival and replication. A deep spectroscopic analysis shows critical modifications in DNA structures upon treatment, which is enhanced in the presence of AuNPs as a consequence of the synergetic effect of the N-TiO_2 photocatalyst with AuNPs. These results were confirmed when the materials were embedded into a chitosan hydrogel matrix, which allowed a more in-depth study of the phenomena.

Multimodal Nanoscopy Imaging Determines the Genomics and Epigenomics pattern involved in Adipocyte Differentiation and Hypertrophy

Lama Zeaiter[1,2], Francesca Baldini[2], Lisa Cuneo[2,4], Farah Diab[1], Paolo Bianchini[2], Piero Portincasa[3], Laura Vergani[1] and Alberto Diaspro[2,4]

[1]DISTAV, Department for the Earth, Environment and Life Sciences, University of Genoa, Genoa, Italy

[2]Nanoscopy, Istituto Italiano Tecnologia, Genoa, Italy

[3]Clinica Medica "A. Murri", Department of Biomedical Sciences & Human Oncology, University of Bari Medical School, Bari, Italy

[4]DIFILAB Department of Physics, University of Genoa, Genoa, Italy

White adipose tissue plays a central role in regulating energy homeostasis by both storing fat and secreting adipokines which mediate endocrine functions. Obesity is mainly due to adipocyte hypertrophy which is defined as an increase in cell size resulting from the enlargement of cytosolic lipid droplets and which results in more or less severe metabolic dysfunction. Our aim is to clarify the possible changes in nuclear morphology and epigenome (histone acetylation and DNA methylation) occurring during adipocyte maturation and hypertrophy. Moreover, the interplay between chromatin domains and nuclear lamina is investigated. To this aim we employed a cellular model of pre-adipocytes 3T3-L1 that were firstly differentiated into mature adipocytes, then cultured with fatty acids for hypertrophy induction. We employed advanced optical nanoscale microscopy such as confocal and super-resolution stimulation emission depletion (STED), combined to molecular biology immunoassay (ELISA) and immunofluorescence techniques, to investigate the nuclear and chromatin remodeling at nanoscale level focusing on nuclear morphometry, chromatin epigenome being associated to heterochromatin/euchromatin rearrangement and chromatin/nuclear lamina interaction. The preliminary results of this multidisciplinary approach seem to indicate a significant rearrangement of nuclear architecture, chromatin condensation and distribution, chromatin methylation/acetylation, and nuclear lamina landscape during adipocyte differentiation and hypertrophy.

Selected Participants "late" abstracts

Image Correlation Spectroscopy Algorithms To Map Diffusion In Biological Samples

Simone Civita, [1,2] Ranieri Bizzarri[3,4], Barbara Storti[3], Alberto Diaspro[1,5], Paolo Bianchini[1,2,5]

[1]Nanoscopy & NIC@IIT, CHT Erzelli, Istituto Italiano Tecnologia, Genoa, Italy

[2]Dept. of Mathematical, Physical and Computer Sciences, University of Parma, Parma, Italy

[3]NEST Scuola Normale Superiore & Istituto Nanoscienze-CNR, Pisa, Italy

[4]Dept. of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Italy

[5]DIFILAB Department of Physics, University of Genoa, Genoa, Italy

Fluorescence correlation spectroscopy (FCS) is one of the effective techniques used to study molecular dynamics in cellular experiments[1]. It provides valuable insights into the diffusion of fluorescent molecules within a well-defined volume by analyzing fluctuations in fluorescence intensity through auto/cross-correlation. However, when dealing with complex landscapes, a single observation point may not be sufficient[2]. In such cases, the spatio-temporal image correlation spectroscopy (stICS) method[3] can be employed. This approach involves capturing a sequence of images in the same functional biological area and computing auto and cross-correlation over the entire region. Since the diffusive parameters are extracted from the autocorrelation map as an average over a region of interest, stICS can be susceptible to spatial inhomogeneities of the fluorescent moiety in the same area. However, vesicles, membranes, and confinements are sometimes poorly detectable from the sole fluorescence intensity. If different diffusion pools are present, some of them may be missed from the analysis of the global autocorrelation curve on account of the signal-to-noise ratio and/or their dynamic characteristics. This work explores different independent approaches to reveal the spatial features of the molecular dynamics inside the cell and strategies to uncouple different diffusive pools in a region. These approaches required a sequence of images to perform FCS analysis pixel by pixel. The evaluation of the autocorrelation in each pixel avoids mixing contributions reulting from averaging dynamics pools over a large field of view. To this aim, one of the most promising approaches is based on the maximum entropy method (MEM), which, for each point, gives a spectrum of Brownian diffusion coefficients as the description of the autocorrelation curve. As an advantage, such an approach avoids the use of a priori hypothesis about the amount of dynamics pools in a single pixel. Therefore, the fitting is not anymore based on the fitting of few potential components and their weight, but it considers a fixed quasi-continuum number of components and fits their weigths maximasing the entropy. This work first presents a comparison among MEM, classical fitting methods, and phasor transformation (PLICS[4]). The spatial information about the local dynamics allows a precise quantification, improves the signal-to-noise ratio by merging all the autocorrelation curves with the same defined proprieties, and outlines the dynamics feature of a region without prior knowledge. We used this procedure to identify diffusive coefficients of the membrane protein ACE2, a key host by which SARS-CoV-2 enters within cells, in physiological conditions, and after different cell treatments. This new local dynamics information can help link morphological, and dynamics feature, also through correlative analysis with other spatial techniques, enabling new imaging schemes.

Unveiling surface-triggered LLPS phenomena during amyloid self-assembly in microscale compartments

G. De Luca[1], G. Sancataldo[2], V. Vetri[2]

[1]Dipartimento di Scienze e Tecnologie Biologiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Viale delle Scienze Ed. 16, 90128, Palermo, Italy

[2]Dipartimento di Fisica e Chimica – Emilio Segrè Scienze e Tecnologie Biologiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Viale delle Scienze Ed. 16, 90128, Palermo, Italy

Liquid-liquid phase separation (LLPS) has emerged as a key phenomenon in the formation of membraneless structures within the cell with different roles. These structures appear as liquid biomolecular condensates in dynamic equilibrium with the cellular environment. Protein condensates are the most studied and have the tendency to evolve, following a phase transition. This aspect makes them very interesting systems to investigate in the framework of protein aggregation processes and amyloid formation, a peculiar class of protein supramolecular structure. We present an experimental study on the supramolecular association of Human Insulin (HI) in microscale aqueous compartments at room temperature by means of quantitative microscopy techniques. In the presented conditions it is possible to image in real-time the formation of spherical amyloid-like superstructure. The formation and development of these superstructures is a combination of a LLPS-driven process and a liquid-to-solid transition. The process is triggered at the interface between the aqueous compartment and the glass coverslip onto which it is cast. The effects of surface are explored by increasing the surface-volume ratio of the aqueous compartments, showing an increase in the aggregation rate and a reduction of the particles' size. Data on the molecular organization was provided by FLIM and FRAP showing that the particles present a more amorphous solid-like core, and fluid-like edges, characterized by a higher affinity towards Thioflavin T. Our experimental study sheds light on the intricate interplay between liquid LLPS, surface effects, and the formation of amyloid-like superstructures in the context of protein aggregation.

Natural compounds can influence amyloid aggregation: focus on the hop extract from waste and its bioactive compounds

Eleonora Mari[1], Maria Rosalia Mangione[2], Silvia Vilasi[2] and Maria Grazia Ortore[1]
[1]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona, Italy
[2]Istituto di Biofisica, CNR, Via Ugo La Malfa, Palermo

In the last decades, the scientific world has turned its focus on exploring the biological effects of plants used in traditional medicine and hop is one of them[1]. In fact, the biomass of hops can be a precious resource, having several application potentialities, for the pharmaceutical and nutraceutical industries.
In this framework, the search for natural compounds, whose intake, even though diet, can be useful to prevent the main biochemical mechanisms responsible for the amyloid aggregation, correlated with several neurodegenerative diseases, together with the aim to pursue new circular economy patterns, led us to investigate extracts from leaves from hops, one of the main ingredients of beer. It is known that proteins can denature and progressively result in insoluble aggregates that, together with intermediate oligomeric species, modify extracellular environment, and that some natural compounds can maintain protein-functionality in denaturing conditions[2]. In this work we investigate the influence of the bioactive compounds present in hop and of the whole hop extract (produced from plant waste) on fibrillation of model proteins.To monitor the kinetic of amyloid aggregation we performed UV-Visible absorption and fluorescence spectroscopy, using Thioflavin T and Congo Red as amyloid specific probes[3]. To study the overall structural features and size of aggregates we performed Synchrotron Small Angle X-ray Scattering and Dynamic Light Scattering experiments, while to monitor secondary structure changes (from α-helix to β-sheets) we performed Circular Dichroism experiments.Our experimental results prove that the whole hop extract and saponins (a bioactive compound contained on the hop plant) interfere with the amyloid aggregation by inhibiting or reducing the final number of fibrils. We are going to perform further aggregation experiments with other bioactive compounds from hop plant.

[1] Judy L. Bolton, Tareisha L. Dunlap, Atieh Hajirahimkhan, Obinna Mbachu, Shao-Nong Chen, Luke Chadwick, Dejan Nikolic, Richard B. van Breemen, Guido F. Pauli, and Birgit M. Dietz. The Multiple Biological Targets of Hops and Bioactive Compounds. Chem. Res. Toxicol. Vol. 32, pages 222−233. 2019.
[2] Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. 2016;8(6):595-608. Published 2016 Jun 1. doi:10.15252/emmm.201606210
[3] Mari E., Ricci C., Pieraccini S., Spinozzi F., Mariani P. and Ortore M.G. Trehalose Effect on The Aggregation of Model Proteins into Amyloid Fibrils. Life 2020**,** 10,60.

Towards understanding the interaction between the SARS-CoV-2 nucleocapsid protein and viral RNA

Yessica Roque-Diaz [1], Paolo Moretti [1], Francesco Spinozzi [1], Paolo Mariani [1]
[1] Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy

With the Covid-19 outbreak, great attention has been paid to the mechanism of the SARS-CoV 2 virus infection to develop new therapies. In particular, the nucleocapsid (N) protein has gained immense interest as it is structurally associated with the RNA of the coronavirus and therefore represents a potential molecular target [1]. In recent studies, the ability of the N protein to phase- separate in the presence of viral RNA has been reported to play a key role in the viral replication process [2]. However, the mechanism through which this occurs is not yet understood. Our purpose is to study the structural features of the nucleocapsid protein and to understand the mechanistic basis for the nucleocapsid-mediated RNA packaging. In this work, we focused on the study of the structural features of the N protein using Small-Angle X-ray Scattering (SAXS). For this purpose, we first purified the recombinant full-length N protein using the expression vector R619-X67-527 (Addgene plasmid # 170204), transformed into BL21DE3pLys *Escherichia coli* cells. SAXS experiment was carried out at ID02 beamline, at the European Synchrotron, Grenoble, France. Our preliminary results show that the protein appears to have an elongated conformation and complex supramolecular structures, probably in the form of fibril-like structures.

1. Wu, W., et al., *The SARS-CoV-2 nucleocapsid protein: its role in the viral life cycle, structure and functions, and use as a potential target in the development of vaccines and diagnostics.* Virol J, 2023. **20**(1): p. 6.
2. Chen, H., et al., *Liquid–liquid phase separation by SARS-CoV-2 nucleocapsid protein and RNA.* Cell Research, 2020. **30**(12): p. 1143-1145.


Nikon


NIKON
IMAGING
CENTER
iit
ISTITUTO
ITALIANO DI
TECNOLOGIA


EURO
BIOIMAGING
Image life, discover the future


SeeLife


SIBPA
Società Italiana di Biofisica Pura e Applicata
fondata nel 1973


SIOF
Società Italiana
di Ottica e Fotonica


Università
degli Studi
di Palermo
DiFC


Genoa
Instruments


Leica
MICROSYSTEMS


schaefer


ZEISS


EVIDENT


CHROMA®


PicoQuant


abberior

INTERNATIONAL SCHOOL OF BIOPHYSICS «ANTONIO BORSELLINO»

48th Course: MEMOS FOR BIOPHYSICS INTO THE FUTURE: LIGHTNESS, QUICKNESS, EXACTITUDE, VISIBILITY, MULTIPLICITY, AND CONSISTENCY.

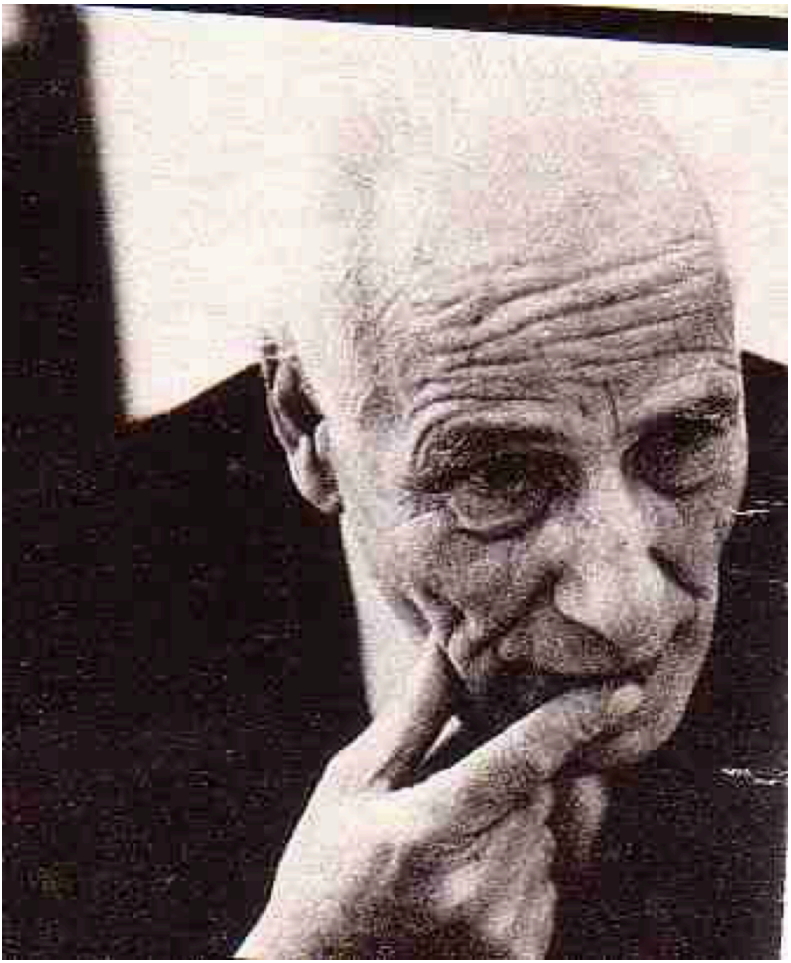
Erice - Sicily: 16 – 22 October 2023

DIRECTORS OF THE COURSE: A. DIASPRO, M. DALLA SERRA, C. VIAPPIANI

DIRECTOR OF THE SCHOOL: A. ZICHICHI - EMFCSC PRESIDENT A. ZICHICHI

ETTORE MAJORANA FOUNDATION AND CENTRE FOR SCIENTIFIC CULTURE

Via Guarnotta, 26 - 91016 ERICE (Sicily) – Italy Tel: +39-0923-869133 Fax: +39-0923-869226



Antonio Borsellino (Reggio Calabria, 11/6/1915 – Trieste, 23/11/1992)