XXV Congresso Nazionale SIBPA 2021

28 June - 1 July 2021

Book of abstracts

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Il Comitato Scientifico e il Comitato Organizzatore, a nome della SIBPA - Società Italiana di Biofisica Pura e Applicata, esprimono un sentito benvenuto agli invitati, ai soci e ai partecipanti del XXV Congresso Nazionale - SIBPA2021, che si svolge in modalità virtuale dal 28 giugno al 1 luglio 2021. Il Congresso SIBPA2021 si articola in sei sessioni tematiche: Molecular biophysics, Cell and tissue biophysics, Systems biophysics /environmental biophysics, Applied biophysics, Biophysics at the nanoscale, Optical and spectroscopic methods applied to biology and medicine.

Numerose sono le relazioni previste, a partire da 5 Keynote lecture da parte di scienziati di fama internazionale. Il programma prevede inoltre 7 comunicazioni su invito, 29 comunicazioni orali, e 35 poster.

Il congresso si aprirà lunedì 28 giugno con la Keynote lecture di Gerhard Thiel, della TU Darmstadt (Germania), a cui farà seguito, nel pomeriggio dello stesso giorno, la Keynote lecture del premio Nobel Martin Chalfie, della Columbia University di New York (USA). La giornata di martedì 29 giugno si aprirà con la Keynote lecture di Gaia Pigino dello Human Technopole e del Max Planck Institut di Dresda (Germania), mentre nel pomeriggio sarà la volta di Catherine Royer del Rensselaer Polytechnic Institute di Troy (USA), Presidente della Biophysical Society americana. Mercoledì 30 giugno l'ultima Keynote lecture del congresso sarà tenuta da Luciano Conti dell'Università di Trento.

Durante il Congresso saranno consegnati i premi "Antonio Borsellino" per la migliore tesi di dottorato di ricerca e il premio "Gianfranco Menestrina" per la migliore tesi di laurea magistrale. Verrà inoltre conferito il premio "Marina Diana Mercurio" ad una personalità scientifica la cui attività nel corso degli anni è stata ispirata da valori di interdisciplinarità e di responsabilità civile e democratica.

Si ringraziano sentitamente tutti gli sponsor il cui contributo ha permesso l'organizzazione del convegno e la partecipazione di studenti e giovani ricercatori, offrendo loro un'importante opportunità per presentare e discutere il loro lavoro.

Il Comitato Scientifico e il Comitato Organizzatore - SIBPA2021

UNIVERSITÀ DI PARMA

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Parma Capitale Italiana della Cultura 2021

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI BIOTECNOLOGIE MEDICHE E MEDICINA TRASLAZIONALE
BIOMETRA

General Program

Monday June 28

10.00-11.00 live session

Welcome Addresses:

Cristiano Viappiani (Università di Parma, Presidente SIBPA)

Chair: Carlo Musio (Istituto di Biofisica, CNR, Trento)

Keynote lecture: **Gerhard Thiel** (TU Darmstadt, Germany); abstract K5.
Ion channel function of E-protein from SARS-CoV2 and its impact on cellular signaling cascades.

11.00-13.00 live session: **Optical and spectroscopic methods applied to biology and medicine**

Chair: Silvia Caponi (Istituto Officina dei Materiali, CNR)

11.00-11.30 Invited lecture: **Dario Polli** (Politecnico di Milano); abstract I6.
Non-linear label-free microscopy of cells and tissues based on broadband coherent raman scattering and artificial intelligence.

11.30-11.45: **Margaux Bouzin** (Università degli Studi di Milano-Bicocca); abstract O24.
Imaging the melanin distribution in murine melanoma biopsies by label-free super-resolution thermography.

11.45-12.00 **Morgana D'Amico** (Università di Catania); abstract O25.
Oncogene-induced alterations of chromatin organization investigated by confocal microscopy.

12.00-12.15 **Martina Alunni Cardinali** (Università di Perugia); abstract O23.
Chemo-mechanical characterization of human bone and cartilage tissues by Brillouin and Raman microspectroscopy in physiological and pathological conditions.

12.15-12.30 **Giulia Pinto** (Università di Genova); abstract O27.
Label-free molecular investigation via spectroscopic ellipsometry: optical detection of a SARS-CoV-2 gene region.

12.30-12.45 **Valeria Libera** (IOM-CNR e Università di Perugia); abstract O26.
A multi-techniques approach to investigate the conformational changes of human telomeric G-quadruplex complexed with ligands.

12.45-13.00. **Stefania Abbruzzetti** (Università di Parma); abstract O22.
A plant gene encoding one- and two-heme hemoglobins with extreme reactivities toward diatomic gaseous ligands.

15.15-16.15 live session: **Systems biophysics/environmental biophysics**

Chair: Vincenzo Martorana (Istituto di Biofisica, CNR, Palermo)

15.15-15.45 Invited lecture: **Chiara Santinelli** (Istituto di Biofisica, CNR, Pisa); abstract I7.
When the biophysics meets the sea: a fascinating trip into marine carbon cycle.

15.45-16.00 **Fabio Lauria** (Istituto di Biofisica, CNR, Trento); abstract O28
Exploring Ribosome heterogeneity at high resolution by next generation sequencing.

16.00-16.15 **Simona Retelletti Brogi** (Istituto di Biofisica, CNR, Pisa); abstract O29
SENSOR project: a fluorescence sensor to detect organic matter contamination in drinking water.

15 minute break

16.30-17:15 live session

Chair: Cristiano Viappiani (Università di Parma)

Keynote lecture: **Martin Chalfie**, 2008 Nobel laureate (Columbia University, New York, USA); abstract K1.
The continuing need for useless information.

Tuesday June 29

9.00-12.10 live session **Molecular Biophysics**

Chairs: Valeria Maria Rondelli (Università di Milano) and Lorenzo Stella (Università di Roma "Tor Vergata")

9.00-10.10 Keynote lecture: **Gaia Pigino** (Human Technopole and MPI Dresden, Germany); abstract K3.
Towards a mechanistic understanding of motile and primary cilia with CLEM and cryo-electron tomography.

10.10-10.25 **Andrea Saponaro** (Università di Milano); abstract O20.
Gating movements and ion permeation in HCN4 pacemaker channel.

10.25-10.40 **Armando Carpaneto** (Università di Genova and Istituto di Biofisica, CNR); abstract O16.
The patch-clamp technique and beyond: characterization of non-electrogenic vacuolar NHX proton/potassium antiporters and inhibition by phosphoinositides.

10.40-10.55 **Rocco Zerlotti** (Università di Parma and Nanion Technologies GmbH); abstract O21.
Oxygen diffusion pathways in mutated forms of a LOV photoreceptor from methylobacterium radiotolerans: a molecular dynamics study.

10.55-11.10 **Matteo de Rosa** (Istituto di Biofisica, CNR, Milano); abstract O17.
The interface between domain G4 and G5 is a novel hotspot of gelsolin instability, toxicity and amyloid propensity.

11.10-11.25 **Maria Grazia Ortore** (Università Politecnica delle Marche); abstract O19.
Dimer-monomer equilibrium of SARS-COV-2 main protease as affected by small molecule inhibitors. A biophysical investigation.

11.25-11.40 **Velia Minicozzi** (Università di Roma "Tor Vergata"); abstract O18.
The odd faces of oligomers: the case of TRAF2.

11.40-12.10 Invited lecture: **Luca Monticelli** (CNRS, University of Lyon, Lyon, FR); abstract I5.
Lipid droplet biogenesis: insight from molecular dynamics simulations.

14.30-16.15 live session: **Applied Biophysics**

Chairs: Antonella Sgarbossa (Istituto Nanoscienze, CNR, Pisa) and Francesco Spinozzi (Università Politecnica delle Marche, Ancona)

14.30-15.00 Invited lecture: **Giovanni Romano** (Università di Firenze); abstract I1.
Innovative light sources in applied biophysics.

15.00-15.15 **Miriam Grava** (Università di Milano); abstract O3.
Internalization of extracellular vesicles in model membranes.

15.15-15.30 **Estella Rao** (Istituto di Biofisica e Cell-Tech HUB, CNR, Palermo); abstract O5.
Extracellular vesicles from microalgae: a renewable and scalable bioprocess.

15.30-15.45 **Samuele Raccosta** (Istituto di Biofisica e Cell-Tech HUB, CNR, Palermo); abstract O4.
Extracellular vesicles from microalgae: biophysical/biochemical characterization.

15.45-16.00 **Ines Delfino** (Università della Tuscia); abstract O2.
Raman micro-spectroscopy and multivariate analysis for monitoring X-rays exposed neuroblastoma and human epithelial cells.

16.00-16.15 **Giulia Borile** (Università di Padova); abstract O1.
DIY platform for optogenetic stimulation.

15 minutes break

16.30-17.15 live session

Chair: Alberto Diaspro (Università di Genova and Istituto Italiano di Tecnologia)

Keynote lecture: **Catherine Royer** (Rensselaer Polytechnic Institute, Troy, USA); abstract K4.
Probing the commitment to division in budding yeast by quantitative imaging of the G1/S transcription factors.

Wednesday June 30

9.00-11:25 live session: **Cell and tissue Biophysics**

Chair: Massimo Vassalli (University of Glasgow, UK)

9.00-9.40 Keynote lecture: **Luciano Conti** (Università di Trento); abstract K2.
In vitro modelling of human neocortex using pluripotent stem cells.

9.40-10.10 Invited lecture: **Sirio Dupont** (Università di Padova); abstract I4.
Unraveling new mechanostransduction pathways linking extracellular matrix mechanics to cell function.

10.10-10.25 **Michael Pusch** (Istituto di Biofisica, CNR, Genova); abstract O14.
Gating defects of endosomal ClC-3 exchangers involved in neurodevelopmental disorders.

10.25-10.40 **Beatrice Senigagliesi** (SISSA e Elettra, Trieste); abstract O15.
Extracellular vesicles as modulators of biomechanical properties in target cells.

10.40-10.55 **Paola Brocca** (Università di Milano); abstract O12.
Mucin based hydrogels as model of mucus tissue covering lungs: structure, dynamics and interaction with nanoparticles.

10.55-11.10 **Antonella Battisti** (Istituto di Nanoscienze, CNR, e Scuola Normale Superiore, Pisa); abstract O11.
Endogenous photosensitizing molecules in antibacterial photodynamic therapy.

11:10-11.25 **Perego Eleonora** (Istituto Italiano di Tecnologia, Genova); abstract O13.
Fluorescence fluctuation spectroscopy with a SPAD array detector to unravel molecular processes in living cells.

11.30-14.30 Poster Session

15:00-16.30 SIBPA Social Assembly

Thursday July 1

9.00-11.15 live session: **Biophysics at the nanoscale**

Chair: Paolo Bianchini (Istituto Italiano di Tecnologia, Genova)

9.00-9.30 Invited lecture: **Aleksandra Radenovic** (Laboratory of Nanoscale Biology - EPFL, Lausanne, CH); abstract I3.
From cells to ions.

9:30-09.45 **Ranieri Bizzarri** (Università di Pisa); abstract O7.
Spatio-temporal image correlation spectroscopy and single molecule localization reveal elusive membrane organization of Ace2, the receptor of SARS-Cov-2.

09.45-10.00 **Pietro Delcanale** (Università di Parma); abstract O9.
Aptamers with tunable affinity for single receptor imaging on living cells.

10.00-10.15 **Patrizia Andreozzi** (Università di Firenze); abstract O6.
Novel core-shell polyamine phosphate nanoparticles self-assembled from pegylated poly(allylamine hydrochloride) with low toxicity and increased in vivo circulation time.

10.15-10.30 **Isotta Cainero** (Istituto Italiano di Tecnologia, Genova); abstract O8.
Novel approaches to reconstruct and analyze structured illumination microscopy.

10.30-10.45 **Chiara Peres** (Institute of Biochemistry and Cell Biology, CNR, Monterotondo, RM); abstract O10.
A commercially-derived multimodal microscope for 2P-STED, 2C-2PE, SHG and CARS imaging.

10.45-11.15 Invited lecture: **Silvia Galiani** (Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK); abstract I2.
Exploring the distribution, the diffusion and interaction dynamics of the cytosolic peroxisomal import receptor PEX5 via advanced microscopy techniques.

15 minutes break

11.30-13.00 live session: **SIBPA awards ceremony**

Chairs: Enrico Di Cera (Saint Louis University), Settimo Termini (Università di Palermo)

11:30-11:45 - Introduction to the “Marina Diana Mercurio - SIBPA award” 2020

11.45-12.15 - **Nicoletta Berardi** (Università d di Firenze); abstract A1.
Environment and brain plasticity.

Chairs: Carlo Musio (Trento), Cristiano Viappiani (Parma)

12.15-12:30 “Antonio Borsellino award” 2020 - **Caterina Ricci** (Università Politecnica delle Marche); abstract A4.
Study of amyloid proteins aggregation process in presence of active biomolecules.

12.30-13.00 “Gianfranco Menestrina award” 2020 -
Mario Marini (Università di Milano Bicocca); abstract A2.
Photo-activated raster scanning thermal imaging at sub-diffraction resolution.

Alessia Pepe (Università Politecnica delle Marche); abstract A3.
G/GMP hydrogels for drug delivery: structure and stability in the presence of intercalating and polymerizing agents.

13:00 - Concluding remarks and farewell

Keynote lectures

The Continuing Need for Useless Information

Martin Chalfie [1]

[1]Columbia University, New York, NY, USA

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.

IN VITRO MODELLING OF HUMAN NEOCORTEX USING PLURIPOTENT STEM CELLS

Luciano Conti[1*]

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The human brain is composed by many distinct neuronal subtypes spatially and functionally organized that originate during development by an elaborate sequence of events that, if altered, can lead to defined pathological conditions. Until recently, efforts to study the development and function of the human cerebral cortex in health and disease have been limited by the availability of appropriate in vitro human model systems. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs), provided opportunities to generate human neural progenitors and neuronal populations cells to the understanding of human brain development and to study the human diseases and regenerative medicine dynamically. Here I will present how by a multistep in vitro process it is possible to generate large numbers of homogeneous defined populations of MGE and cortical progenitors. I will discuss also their further neuronal terminal differentiation to acquire properties of Parvalbumin- and Somatostatin-positive cortical GABAergic interneurons and different cortical glutamatergic neurons. These cultures provide a versatile platform for allowing a detailed molecular and functional interrogation of human cortical development, function and disease.

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TOWARDS A MECHANISTIC UNDERSTANDING OF MOTILE AND PRIMARY CILIA WITH CLEM AND CRYO-ELECTRON TOMOGRAPHY

Gaia Pigino[1,2*]

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[2]Structural Biology Research Centre, Human Technopole, Milano

Research in my lab aims at understanding the molecular principles and processes that drive the self-organization of complex cellular machines, which are relevant for human health and disease. In this talk I will show how we combine EM and molecular cell biology methods to obtain a mechanistic understanding of the molecular machineries required for the assembly and the function of cilia/flagella: conserved organelles that are fundamental for most eukaryotic cells.
Assembly of the cilium requires the rapid bidirectional intraflagellar transport (IFT) of building blocks to and from the site of assembly at its tip. This bidirectional transport of IFT trains is driven by the anterograde motor kinesin-2 and the retrograde motor dynein-1b, which are both bound to a large complex of about 25 IFT adaptor proteins. We developed a millisecond resolution 3D correlative light and electron microscopy (CLEM) approach to show that the spatial segregation of oppositely directed IFT trains on the two microtubules of each axonemal doublet ensures a collision free transport in the cilium. Then, it remained to be explained how competition between kinesin and dynein motors, both found on anterograde IFT trains, is avoided. In other bidirectional transport systems in the cell the presence of opposing motors leads to periodic stalling and slowing of cargos moving along the microtubule. No such effect occurs in IFT. To address this question, we used cryo-electron tomography and sub-tomogram averaging to resolve the 3D structure of IFT train complexes in the cilia/flagella of Chlamydomonas cells. We showed that a tug-of-war between kinesin-2 and dynein-1b is prevented by loading dynein-1b onto anterograde IFT trains in an inhibited conformation and by positioning it away from the microtubule track to prevent binding. We also show that dyneins are released from the train at the ciliary tip, but how this happens and how these motors are activated to power retrograde IFT is not yet understood. Protein complexes that form specialized structures at the ciliary tip are thought to be involved in this process. By mechanically manipulating IFT trains in the cilia/flagella of Chlamydomonas cells, we showed that the structures of the ciliary tip are not necessary for the conversion from anterograde to retrograde IFT. Thus, the conversion process is an intrinsic, calcium-optional ability of IFT machinery.

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PROBING THE COMMITMENT TO DIVISION IN BUDDING YEAST BY QUANTITATIVE IMAGING OF THE G1/S TRANSCRIPTION FACTORS

Catherine Royer[1*]

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The timing of cell division sets the cell size. In budding yeast, deletion of activators of the G1/S transition (START) leads to a large size phenotypes, whereas deletion of START repressors results in a small size phenotype. Likewise when grown on poor nutrients, yeast cells are smaller than when grown in rich medium. The molecular mechanisms that control the commitment to cell division in budding yeast remain ill-defined and controversial. We have investigated these mechanisms by quantifying and localizing with high accuracy and precision the major activating and repressing transcription factors involved in regulating the G1/S transition using scanning number and brightness and super-resolution PALM microscopy. We find that these factors are present in low copy number with respect to their target G1/S promoters in small cells. Moreover they are localized in a small number of clusters. As cells grow, the copy number and the number of clusters increases. We propose that this spatio-temporal titration of the G1/S regulon, in conjunction with a phosphorylation-based positive feedback loop, determines the timing of START.

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Ion channel function of E-protein from SARS-CoV2 and its impact on cellular signaling cascades

Gerhard Thiel[1], Tobias Schulze[1,], Andreas Hartel[2,], Sebastian Höler[1], Clara Hemming[1], Robert Lehn[1], Dominique Tandl[1], Timo Greiner[3], Adam Bertl[1], Kenneth Shepard[2], Anna Moroni[4] and Oliver Rauh[1]

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Many pathogenic viruses employ small ion channel proteins at different stages of their infection/replication cycle. It has been proposed that also the E-protein from SARS-CoV2, the smallest of the four structural proteins, is a cation channel. Its function seems crucial for the replication cycle since its depletion reduces virus release and has an overall positive effects on disease outcome. To mimic the pathophysiological impact of the E-protein from SARS-CoV2 (Ep-CoV2) we overexpressed it in mammalian cells and monitored the effects on key cellular signaling parameters. We found that the protein is mostly present in the endoplasmic reticulum but arrives in small amounts also at the plasma membrane. We observed that the wild type protein and, to a lesser extent its mutants, corrupted some of the most important homeostatic mechanisms in cells: Ep-CoV2 elevates intracellular Ca^{2+} and pH and causes a membrane depolarization. With these scope of effects, Ep-CoV2 has the potential of interfering with the major signal transduction cascades in cells, which may eventually contribute to the pathogenesis of the viral protein. The Ep-CoV2 evoked effects can be at least partially explained by an ion channel activity of the viral protein. Two independent assays, a functional reconstitution of Ep-CoV2 protein in artificial membranes and a rescue of K^+-deficient yeast mutants confirm that Ep-CoV2 generates a cation conducting channel with a low unitary conductance and a complex ion selectivity.

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Applied biophysics

Innovative light sources in applied biophysics

Giovanni Romano[1*], Franco Fusi[1], Giuseppe Tortora[2], Gian Maria Rossolini[3], Lucia Pallecchi[4], Chiara Treghini[1], Alfonso Dell'Accio[1], Elisabetta Dattola[1], Light4Lungs group[5]

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[5]S. Nonell, Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain; G. Romano, University of Florence, Florence, Italy; N. Henry, Sorbonne Université, Paris, France; E. Palomares, Fundacio Privada Institut Catala d'Investigacio Quimica, Tarragona, Spain; E. Reddi, University of Padova, Padova, Italy; G. Taylor, Cardiff Scintigraphics Ltd, Cardiff, UK; A. Kadioglu, The University of Liverpool, Liverpool, UK

Light sources in applied biophysical and biomedical sciences have a myriad of applications and properties, from diagnosis to therapy, from UV to near infrared, from pulsed to continuous emission. If on one side it could be argued that the technical aspects drive and characterize innovation in light sources, a more comprehensive analysis should probably start from the applications themselves and the insurgence of new needs.
Starting from this idea, examples of innovative light sources will be examined and discussed, with a focus on therapeutic applications: a "light pillow" adaptable to the irradiated surface with applications in dermatology, a UVC light barrier for air disinfection, a luminous pill to perform photodynamic inactivation against gastric infections [1], a light-emitting aerosol to control antibiotic-resistant and recalcitrant lung infections [2].
In each case, reasons for innovation will be proposed and discussed, together with the presentation of technical features and application field(s), underlying the importance of a correct light dosimetry and light-biological matter interaction modeling [3,4].

[1] G Tortora, et al., IEEE/ASME TMECH, 21(4) (2016): 1935-1942.
[2] https://light4lungs.eu/
[3] A Gnerucci, et al., Photochem. Photobiol. Sci., 19(1) (2019): 34-39.
[4] F Fusi, G Romano, Physica Medica, 77 (2020): 18-20.

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DIY PLATFORM FOR OPTOGENETIC STIMULATION

Giulia Borile[1,2,3], Andrea Vogliardi[1], Giulia Borella[3,4], Martina Pigazzi[3,4] and Filippo Romanato[1,2]

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Light is an extremely powerful investigation, typically used to reconstruct or characterize materials as living systems. Optogenetics has revolutionized the experimental paradigms, giving the possibility to intervene and interfere with the intrinsic dynamics of these systems. The transition from microscopy-based experiments to multiwell plates photostimulation requires a user-friendly flexible device and do-it-yourself approaches can fulfill these requests. Here, we describe the design and development of an Arduino-controlled LED array, that is mounted on a breadboard to maximize the flexibility of the platform. The use of commercially available components installed on a breadboard allows to avoid the use of a PCB and subsequent soldering, making our device amenable also for installation in biology laboratories. We adopted two wavelengths, 470 nm and 590 nm, that cover most optogenetic actuators and can be combined for double illumination. The system will be characterized and tested on different cells lines, with a particular interest for non-excitable cells, like cancer cells, where optogenetics based applications are increasing.

The project is funded by "Roche per la Ricerca" to Dr. Giulia Borile

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RAMAN MICRO-SPECTROSCOPY AND MULTIVARIATE ANALYSIS FOR MONITORING X-RAYS EXPOSED NEUROBLASTOMA AND HUMAN EPITHELIAL CELLS.

Ines Delfino[1*], Valerio Ricciardi[2,3], Giuseppe Perna[4,5], Maria Lasalvia[4,5], Lorenzo Manti[3,6], Vito Capozzi[4,5], Maria Lepore[2]

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Radiotherapy is one of the most common methods for the treatment of cancer. Despite its popularity, there are still open outstanding radiobiological issues that require further studies. Recently, Raman microscopy and spectroscopy have been increasingly used in this field because of their potentialities, further enhanced thanks to the development of new methods and approaches for data analysis that have considerably increased the relevancy of the information conveyed by the data. Different studies were devoted at investigating radiation-induced effects by means of Raman micro-spectroscopy and different approaches for multivariate analysis were used for an investigation of sub-cellular regions of X-ray exposed single SH-SY5Y human neuroblastoma and human mammary epithelial cells [1-3]. Nucleus and cytoplasm regions of single cells were investigated after the exposure to different doses of X-rays (0, 2, 4, 6 and 8 Gy). Cells were fixed immediately after irradiation and after 24h. In the multivariate analysis Principal component analysis (PCA) and interval-PCA (i-PCA) were used for analyzing the collected spectra to highlight the changes due to the irradiation treatment at different extent. Biochemical changes occurring in the nucleus and cytoplasm regions of single cells upon X-ray irradiation were observed. The analysis of Raman spectra showed changes in the contribute of proteins, nucleic acids, lipids, and carbohydrates of cells. The characteristics of these changes were dependent on the considered cell regions. Biochemical modifications occurring in neuroblastoma cells were also discussed by using an alternative approach, namely the analysis of difference spectra, obtained by subtracting the cytoplasm-related spectrum from the corresponding one detected at the nucleus. The results indicated that the development of accurate data analysis methods enabling to take into account the complexity of the Raman spectra of cells and tissues and the high number of spectra can further enhance the readability of information related to the X-rays effects on the cells embedded in Raman spectra.

[1] I Delfino, et al., J. Biomed. Optics, 20 (2015): 035003.
[2] I Delfino, et al., Sensors, 19 (2019): 3971.
[3] I Delfino, et al., J. Pharm. Biomed. Analysis, 164 (2019), 557-573.

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INTERNALIZATION OF EXTRACELLULAR VESICLES IN MODEL MEMBRANES

Miriam Grava[1*], Sally Abdalla[2], Loredana Casalis[3], Pietro Parisse[4], Paola Brocca[2], Valeria Rondelli[2]

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Extracellular vesicles (EVs) are the main intercellular communication system. They are widely studied as vectors for therapeutic or diagnostic applications, have been proposed as biomarkers for various diseases, and have a crucial role in cancer and neurodegenerative pathologies diffusion.
Previous AFM, neutron reflectometry, SAXS and SANS analysis [1] revealed a strong interaction between Umbilical Cord Mesenchymal Stem Cells EVs and the borders of model membrane protruding liquid ordered phase domains. To analyse deeply EVs to cell internalization mechanisms, we performed differential scanning calorimetry (DSC) measurements on model membrane systems of variable complex composition.
DSC thermograms from unilamellar phospholipid vesicles show two transition peaks, interpreted as separate contributions by the two leaflets of the membrane bilayer [2]. Mixing dimyristoylphosphocholine (DMPC) liposomes with EVs, we measured different effects on the lipids residing in the two target membrane leaflets, being the effect stronger on the lipids residing in the inner one. The corresponding melting temperature and associated enthalpy are increased, showing that EVs components impose to inner target phospholipids a lower mobility. On the contrary, the enthalpy relative to the outer leaflet decreases as we increase the quantity of EVs interacting with model liposomes.
Results suggest that the proteins from EVs inserted in the model membrane give it a more rigid structure and the stronger effect on the inner leaflet lipids is due to a more disordered arrangement, having these lipids a higher packing parameter if compared to that of outer leaflet lipids.
In addition, the melting transition of the inner leaflet becomes less cooperative than the outer one and the specific heat maximum increases as the quantity of EVs in the sample increases. So, adding EVs, the outer leaflet goes to a more disordered state, while the inner leaflet goes to a more ordered one.
In a therapeutic perspective, we studied EVs-loaded vesicles as possible delivery system: drug delivery to several epithelial tissues is challenging, seen the need of carriers able to cross the barrier built by the mucus layer. We studied the interaction with a model bio-hydrogel composed of mucin in semidiluted concentration regime in physiological water solution, by performing X-ray photon correlation spectroscopy (XPCS), in order to measure their dynamics in a viscoelastic medium at ID10 beamline of the EBS synchrotron in Grenoble.

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Extracellular vesicles from microalgae: biophysical/biochemical characterisation

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Extracellular vesicles (EVs) are nanometer-sized particles “made by cells for cells” to shuttle lipids, proteins and nucleic acids. As potent signal transducers and intercellular and inter-organismal communicators, EVs mediate physiological processes and help to spread various diseases, including cancer and infections. We recently identified nanoalgosomes, that are EVs secreted by microalgae, a sustainable natural source [1,2].
Here, we use an integrated biochemical-biophysical approach for an extensive characterisation of their structural properties. Specifically, combined experiments of immunoblotting analysis for commonly used EV-biomarkers (IB), Atomic Force Microscopy (AFM), Small-Angle X-ray Scattering (SAXS) and Small-Angle Neutron Scattering (SANS), Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA), along with a dedicated analysis, are used to cope with the heterogeneity of such complex systems. Our effort is also dedicated to the development of Standard Operating Procedures which may guide the characterisation of size distributions in EV sample and meet the minimal requirements for studies of EVs (MISEV) [3]. In this frame, we have used AFM to assess the biomechanical properties of EVs, along with their size distribution. Our experimental strategy consists in deposit EVs on functionalised substrates with different affinity for specific EVs components (proteins, sugars, lipids), so to perform AFM imaging on selected EVs subpopulations. Our study provides the means for a comprehensive structural characterisation of EVs, which is expected to be crucial in the design of engineered vesicles to be employed in different fields, such as nanomedicine, nutraceutics and cosmetics.

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EXTRACELLULAR VESICLES FROM MICROALGAE: A RENEWABLE AND SCALABLE BIOPROCESS

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Several cell types have the capacity to secrete nanometer-sized extracellular vesicles (EVs) working as potent signal transducers and cell-cell communicators. Nanoalgosomes are a newly characterised subtype of small extracellular vesicles derived from microalgae, which constitute sustainable microbial bioresources [1,2]. We aim to exploit nanoalgosomes as novel and scalable biogenic nanotechnology for application in theranostics, cosmetics, and nutraceutics.
We developed a production platform to manage the harvest, manipulation, storage, characterisation and application of microalgal EVs. First, the best strain/s and optimized culture conditions and EV isolation procedures were selected from different microalgal strains. Small EVs were isolated using Tangential Flow Filtration (TFF) from cultures of microalgal strains [1]. Further separation methods were explored to enhance sample purity and yield, including affinity and size exclusion chromatography. Then, the production and separation processes were optimised to obtain a high yield and good quality of small EVs [2]. Also, we have assessed the feasibility of recycling microalgae cells cultures, suggesting them as a renewable bioresources, suitable for the implementation of a circular bioprocess. We developed Standard Operating Procedures to orientate the physico-chemical and biological characterisation of EVs and meet the minimal requirements for studies of EVs (MISEV2018) [3], which will ensure that the production scalability does not affect the product quality.
Further, the biotechnological potential of nanoalgosomes as carriers of bioactive compounds, such as miRNA, siRNA, mRNA, lncRNA, proteins, peptides, lipids, synthetic drugs or other cargo, is currently under evaluation by optimising different loading strategies, such as electroporation or extrusion.

(This work has been developed under the projects VES4US and BOW, which have received funding from the European Union's Horizon 2020 research and innovation programme, under grant agreements No 801338 and 952183, respectively).

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Biophysics at the nanoscale

Exploring the distribution, the diffusion and interaction dynamics of the cytosolic peroxisomal import receptor PEX5 via advanced microscopy techniques

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Peroxisomes are small organelles that fulfil many anabolic and catabolic functions in mammalian cells by importing all required proteins post-translationally. Dysfunction of the peroxisomal import process leads to severe diseases making the molecules involved in this process a study of utmost importance. Here, we used advanced optical microscopy to characterize the peroxisomal protein import processes. A multicolour super-resolution STED microscope has been implemented to investigate with sub-60-nm resolution the spatial organization of the proteins PEX5 and PEX14 at the peroxisomal membrane, around actively importing peroxisomes. The large degree of heterogeneity common for this complex biological system has been characterized thanks to the robust imaging and complementary analysis, enabling the quantification of an observed compartmentalization of peroxins in a subset of peroxisomes. Furthermore, we combined advanced live-cell microscopy and spectroscopy techniques such as fluorescence correlation spectroscopy (FCS) and super-resolution STED microscopy to present a detailed characterization of the diffusion and thus interaction dynamics of the import receptor PEX5 in the cytosol. In this study we disclose an unexpectedly slow diffusion of PEX5, independent of many factors such as aggregation, target binding or cytoskeleton but associated with larger cytosolic interaction partners via the protein N-terminal half. In addition to these specific insights, our study highlights the potential of using complementary microscopy tools to reveal molecular organization and interactions in the cytosol via studying their nanoscale distribution and diffusion dynamics to a yet unseen level of detail, paving the way for equally complex biological studies in the future.

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From Cells to Ions –

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From the plethora of correlative imaging modalities, SR techniques were most frequently combined with electron microscopy to provide protein-ultrastructure relationships at nanometer-scale resolution[1]. At the other forefront of methods development, scanning probe microscopy techniques aim to capture nanoscale topographical dynamic changes of cells under physiological conditions. To avoid membrane deformation and to provide a method that could unlock long-term monitoring of the biological processes, we recently implemented SICM[2]. The method currently experiences vast leaps in performance due to instrument developments[2] and the ability to fabricate capillaries below tens of nanometers[3] reliably.

In contrast to AFM, SICM is truly non-contact, and represents the soft cell surface much more faithfully[4] In addition to providing accurate topographic imaging with nanometer resolution[5], SICM can be used to measure membrane stiffness[6] surface charges[7] and allows local delivery of material (e.g. fluorescent probes)[8].In parallel, the use of self-blinking dyes in SR microscopy permitted imaging conditions such as low laser excitation intensities and negligible bleaching that are ideal for live-cell imaging. In addition, the high SNR and photophysical properties of self-blinking dyes allow us to extend multiplane cross-correlation analysis to the 4th order using 8-plane volumetric imaging, achieving up to 29 (virtual) planes[9]. Finally, with a combined SICM-SR setup we demonstrate long-term correlated live-cell imaging.

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NOVEL CORE-SHELL POLYAMINE PHOSPHATE NANOPARTICLES SELF-ASSEMBLED FROM PEGYLATED POLY(ALLYLAMINE HYDROCHLORIDE) WITH LOW TOXICITY AND INCREASED *IN VIVO* CIRCULATION TIME

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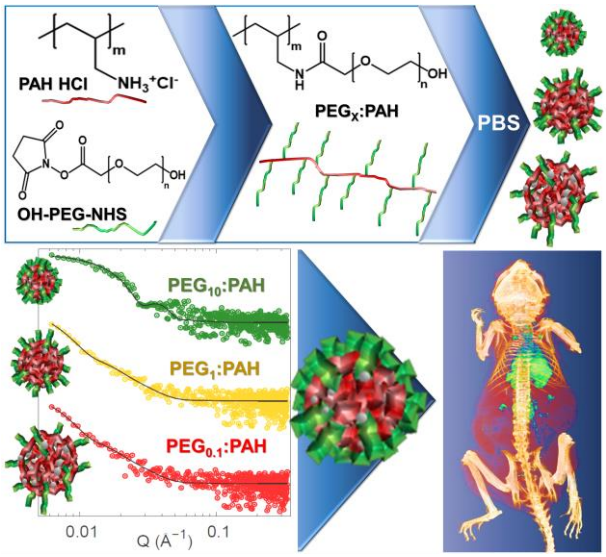
Polyamine phosphate nanoparticles (PANs) are supramolecular assemblies of poly(allylamine hydrochloride) (PAH) and phosphate ions, which display a fascinating response to variations in pH. [1] PANs are stable at neutral and moderately basic pH values, from 7 to 9. Outside of this narrow pH range, PANs disassociate into their molecular components. This pH-responsiveness makes PANs a very appealing vehicle for intracellular drug delivery, as they are stable in physiological media and pH values, but disassemble inside endosomes, i.e. at pH below 6, liberating encapsulated cargo. [2] Besides, the amine groups in the polyamines can protonate inside endosomes, inducing an osmotic swelling that facilitates PANs translocation into cytosol. We have explored the use of PANs prepared with PAH for the delivery of siRNAs and we have shown that PANs are capable of successfully silencing green fluorescent protein (GFP) expression at non-toxic concentrations. An increase in PANs concentration, however, which could be expected to lead to a more effective silencing, resulted in moderate toxicity, thereby limiting therapeutic use.

An approach for reducing toxicity and enhancing therapeutic potential of PANs through PEGylation of polyamines before their assembly into nanoparticles is presented here. [3] We have modified PAH with PEG chains, and unreacted amine groups were used to form nanoparticles through complexation with phosphates. While the association of PAH chains trough phosphate was not prevented by PEGylation, we have observed by Dynamic Light Scattering (DLS), zeta potential measurements and Small Angle X-ray Scattering (SAXS) that the number of PEG molecules per PAH chain play a fundamental role in the organisation of the polyamines into PANs, leading to a core shell structure with PEG forming a shell around a polyamine core, Scheme 1. Interestingly, we observed that PANs retain their ability to disassemble at endosomal pH values, despite changes in their physico-chemical characteristics. PEGylation of PAH affects PANs-protein interactions as observed by Fluorescence Correlation Spectroscopy (FCS). Moreover, PEGylated PANs show a decreased toxicity as evaluated with the MTT assay, and longer circulation times as determined by Positron Emission Tomography (PET), which enhance their potential for drug delivery applications. To resume, we show here novel PEGylated assemblies fabricated through covalently modifying polyamines and induce their association with phosphate ions, and we have been able to correlate their structure with physico-chemical properties and toxicological endpoints.



Scheme 1: PEGylated polyamines are assembled into nanoparticles by complexation with phosphate ions. PEGylated polyamine nanoparticles show a core–shell structure with an external PEG layer as shown by small angle X-ray scattering and display almost no charge. PEGylated polyamine phosphate nanoparticles show lower toxicity and longer circulation time *in vivo* than non-PEGylated ones.

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SPATIO-TEMPORAL IMAGE CORRELATION SPECTROSCOPY AND SINGLE MOLECULE LOCALIZATION REVEAL ELUSIVE MEMBRANE ORGANIZATION OF ACE2, THE RECEPTOR OF SARS-COV-2

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Angiotensin-converting enzyme 2 (ACE2) is a ubiquitous membrane protein that exerts antihypertensive effects by catalyzing the hydrolysis of the vasoconstrictor peptide Angiotensin II into the vasodilator angiotensin (1-7). Actually, ACE2 counterbalances the activity of the related enzyme ACE, which converts the peptide hormone angiotensin I in angiotensin II. Together, ACE2 and ACE are central components of the Renin-Angiotensin system (RAS), which control the blood pressure in the body. ACE2 has several other less-known biochemical functions, and its expression is tightly regulated at transcriptional and post-translational level [1]. Recently, it has been discovered that ACE2 serves as the initial cellular target of at least six coronaviruses (CoVs) [2], including the recent SARS-CoV2, responsible of COVID-19 [3]. SARS-CoV2 engages with ACE2 through its spike (S) protein, which consists of two subunits: S1, which mediates binding to the host receptor through its Receptor Binding Domain (RBD); S2, which induces membrane fusion of the viral envelope delivery of the viral genome. The RBD-ACE2 interaction eventually determines viral host range, and in tandem with the host proteases is responsible for virus tropism in the body.
On account of the remarkable role of ACE2 as multifunctional membrane protein and cross-cutting species “hotspot” harnessed for CoV entry, we set out to investigate its “interactome” on cell membrane by means of single molecule localization methods (SMLM) and spatiotemporal image correlation spectroscopy (STICS). Interestingly, we found out that ACE2 clusterizes predominantly out of lipid raft regions, at odds with previously reported data [4]. Additionally, our data suggest a complex dynamic interplay with the cytoskeleton, that might be mediated by some interactions of ACE2 with integrins [5]. We hypothesized that these features could be relevant also for viral attachment and entry.

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NOVEL APPROACHES TO RECONSTRUCT AND ANALYZE STRUCTURED ILLUMINATION MICROSCOPY

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Structured Illumination Microscopy (SIM) is an important technique among Super-Resolution (SR) Microscopy methods [1]. In comparison to other SR techniques, such as Stimulated Emission Depletion Microscopy (STED), SIM can perform multicolor channel acquisition using conventional antibodies, live-cell imaging due to its low phototoxicity, and optical sectioning. The SIM structured pattern directly encodes sub-diffraction information into the collected data enabling the reconstruction of super-resolved images. In our work, we employ a novel SIM data analysis tool based on the Separation of Photons By Lifetime Tuning (SPLIT) principle [2-3]. We show how the SPLIT phasor approach analyzes information encoded into the raw images acquired at different illumination patterns. As a result, SPLIT-SIM reconstructs the SR image by isolating a fraction of the intensity related to the center of the diffraction-limited point spread function. SIM has become widely used in the investigation of biological samples due to its abovementioned advantages, and here we show that SPLIT-SIM can be used to generate SR images of chromatin organization, with tunable resolution.
This work includes the quantification of nanoscale spatial distributions of target molecules, to introduce a novel analysis approach for multicolor SIM data. To evaluate the spatial distribution of structures we perform colocalization analysis with an object-based technique and using image cross-correlation spectroscopy (ICCS) [4]. We show that by combinging SIM and ICCS a powerful and simple tool to precisely quantify nanoscale distances is obtained. In particular, we validated the method by using a DNA-origami-based model sample using SIM-ICCS to investigate the spatial distribution of nuclear sites in fixed cells.
Using SPLIT-SIM we can generate tunable multicolor SR images of nuclear structures, and by using SIM-ICCS we can get quantitative information about the relative nuclear distribution of the target structures. The final aim of this work is to introduce a solid basis to perform reliable nanoscale distances analysis of chromatin organization with SIM microscopy.

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APTAMERS WITH TUNABLE AFFINITY FOR SINGLE RECEPTOR IMAGING ON LIVING CELLS

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Tumor cell-surface markers are usually overexpressed or mutated membrane protein receptors for which spatiotemporal regulation differs between and within cancers, and are therefore exploited for diagnosis and targeted drug delivery. Single-molecule fluorescence imaging can localize individual markers in different cellular contexts with molecular precision, revealing their distribution and dynamics. However, standard single-molecule imaging methods based on overexpressed genetically-encoded tags or cumbersome probes can significantly alter the native state of receptors [1].
Here we introduce a Points Accumulation for Imaging in Nanoscale Topography (PAINT [2]) method that exploits aptamers as minimally invasive affinity probes, applied to living cells [3]. Localization and tracking of individual receptors are based on stochastic and transient binding between aptamers and their targets exposed the cell surface. We demonstrated single-molecule imaging of a model tumor marker, epidermal growth factor receptor (EGFR), on a panel of living cancer cells. The nucleotide sequence of a well-described high-affinity EGFR aptamer was rationally engineered to generate a variant with faster dissociation rate from EGFR. The use of this reversible probe allowed photobleaching-insensitive detection of both density and motion of receptors, with optimal sampling and minimal perturbation of their endogenous behaviour. These results highlight the possibilities offered by specifically tailored imaging probes with tunable affinity, such as aptamers.

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A COMMERCIALLY-DERIVED MULTIMODAL MICROSCOPE FOR 2P-STED, 2C-2PE, SHG AND CARS IMAGING

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Multimodal microscopy combines multiple nonlinear microscopy techniques to obtain deeper description of the sample, exploiting different optical processes, such as two-photon excitation fluorescence (2PEF), Second Harmonic Generation (SHG) and Coherent Anti-Stokes Raman Spectroscopy (CARS), to generate contrast [1]. Non-linear microscopy is the preferred method for *in vivo* applications because it allows high contrast imaging with sub-cellular resolution even in highly scattering tissues with low phototoxicity, high penetration depth and intrinsic optical sectioning. Since different non-linear optical processes require similar source of excitation [2], it is possible to implement multiple imaging modalities in the same microscopy platform. A powerful tool to further improve imaging resolution in biological samples is two-photon STimulated Emission Depletion (2P-STED), a non-linear optical technique in which resolution enhancement of STED is combined with 2PEF, allowing for high resolution imaging in relatively thick 3D samples [3]. Previous implementations of multimodal or 2P-STED microscopes were based on custom-made architectures, complex and expensive optical systems that require everyday alignment and maintenance [4].

Here, we describe the implementation of a multimodal versatile system to perform stable 2P-STED, 2PE, two-color two-photon excitation (2c-2PE), SHG and CARS imaging in the same optical platform. The 2P-STED multimodal microscope we assembled was based on a commercially available system and our upgraded version was so stable and reliable that guaranteed day-to-day usability even for not-expert users. The components we added included computer-controlled optical and mechanical parts to remotely adjust with high precision the spatiotemporal overlap of two infrared excitation beams. This control was crucially important to generate non-linear processes that required multi-wavelengths interaction, such as 2c-2PE and CARS imaging.

In addition, to perform 2P-STED imaging in thick samples, we carefully aligned spatially the pulsed STED beam on the excitation ones and optimized the STED laser timing relative to excitation pulses. To this end, we used suitable electronics that permitted us to obtain full spatial and temporal control over the STED beam.

Finally, we tested the capabilities of our system by performing 2P-STED microscopy up to 70 µm deep in *ex vivo* and *in vivo* tissue samples stained with Nile Red, a fluorescent dye suitable for STED that labels lipid structures. We proved the suitability of the microscope to perform in-vivo functional calcium (Ca^{2+}) imaging by 2c-2P excitation fluorescence in the earlobe of a murine model expressing the cytosolic Ca^{2+} biosensor GCaMP6 and conducted *in vivo* label-free morphological imaging of mouse skin, exploiting endogenous sources of contrast in SHG and CARS microscopy.

Our work shows that, with little additional equipment, it is possible to dramatically extend the performances of a commercial architecture, turning it into a versatile instrument for multimodal imaging on biological living samples.

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Cell and tissue biophysics

Unraveling new mechanostransduction pathways linking extracellular matrix mechanics to cell function

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Extracellular matrix (ECM) mechanical properties can regulate a wide array of cell phenotypes. While much is known on the mechanosensing mechanisms at the force/cytoskeleton interface, what are the downstream processes and signaling pathways regulated in response to extracellular forces remain less understood. To address this issue in recent years we have been querying the transcriptional and metabolic responses to ECM elasticity [1-3]. We will present new data indicating how ECM stiffness regulates cellular redox metabolism and homeostasis. This response is initiated by the remodeling of peri-mitochondrial F-actin and mitochondrial dynamics. This in turn activates a retrograde transcriptional pathway that empowers antioxidant cell defense systems and makes breast cancer cells resistant to chemotherapy. The same pathway is activated by the ECM of the metastatic niche, so that disabling its key players cooperates with chemotherapy to prevent metastatic relapse in a mouse model of breast cancer.

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ENDOGENOUS PHOTOSENSITIZING MOLECULES IN ANTIBACTERIAL PHOTODYNAMIC THERAPY

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The interaction between light and biological systems is one of the primary processes for life on Earth. It is the basis for phenomena such as photoperception, photosynthesis, photomovement and is usually mediated by highly specialized photoreceptors that undergo a light-induced modification causing the generation of a signal.
In some cases, the interaction with light can be detrimental to biological systems, as in mutagenesis or carcinogenesis: light can either be absorbed by biomolecules such as proteins or DNA, resulting in their direct chemical modification, or by other light-sensitive molecules, namely photosensitizers (PS), which are molecules able to interact with light and to transfer the absorbed energy to other species. When oxygen is present, PS can induce the formation of reactive oxygen species (ROS) that can eventually lead to cell death. The number of known PS is wide [1], and their ability to alter target molecules inside cells has been exploited in medicine to develop novel therapeutic techniques such as photodynamic therapy (PDT), mainly employed in the treatment of certain cancers and skin diseases [2], and antimicrobial photodynamic therapy (aPDT), a version of PDT used to defeat drug-resistant infective microorganisms [3]. Both techniques can require prior administration of PS or exploit the presence of endogenous PS spontaneously produced by the target [4]. Such natural substances include flavins, porphyrins, urocanic acid and some sterols [5].
Helicobacter pylori (Hp) is a pathogen potentially prone to aPDT. It is responsible for severe gastric diseases including cancer, but conventional pharmacological therapy often fails due to increasing antibiotic resistance. Hp naturally accumulates porphyrinic PS such as protoporphyrin IX and coproporphyrin I [6]. The efficacy of photoinactivation obtained by irradiation at different wavelengths is a function of the absorption spectrum of the endogenous porphyrins, which show a strong absorption peak in the 380-420 nm range and some weak bands in the 500-650 nm region. Indeed, irradiation with light at 405 nm produced the best inactivation results among the tested wavelengths. However, fluorescence spectra of bacterial extracts and unexpected inactivation efficiency of blue light (460 nm) revealed the presence of an additional photosensitizer, riboflavin, playing a significant role in Hp photoinactivation [7].

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MUCIN BASED HYDROGELS AS MODEL OF MUCUS TISSUE COVERING LUNGS: STRUCTURE, DYNAMICS AND INTERACTION WITH NANOPARTICLES.

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Mucus is a complex hydrogel that acts as the physiological barrier coating several tissues of our body. The mechanical and rheological properties of mucus mainly depend on its major components, mucins. Mucins show an extended amino acid backbone bearing regularly distributed sugar chain branches, accounting for about the 85% of the total mucin MW. In a complementary X- ray and neutron small angle scattering study, we exploited the contrast matching opportunity offered by neutron spectroscopy to improve the understanding of the nanoscale structure of mucin hydrogel. We distinguished the carbohydrate branches contribution and the contribution of backbone network to the small angle scattering profiles. Studies of the interaction between the main constituent of mucus, the mucins, and molecules involved in topical transmucosal drug or gene delivery, is a prerequisite for nanomedicine design. Nanomedicine formulations for lung delivery purpose diffuse inside an adhesive hydrogel in the presence of interaction forces between particle components and the building blocks of the mesh. We studied [1] a nanocrystal suspension for which, beside a brushed PEG shell that can reduce particle adhesion, shape concerns play an important role in promoting the diffusion in view of the large particle size compared to the typical mucus mesh size. The study was extended to mucus models enriched in DNA, as demanded for mucus/nanoparticles interaction studies, particularly focusing to Cystic Fibrosis disease. On the molecular scale, quartz crystal microbalance with dissipation and neutron reflectometry [2] were applied to thin mucin layer targeted by a polymer intended to contact cell membranes and a new model for mucus-covered epithelial tissues obtained by the deposition of a mucin layer 20 Å thick on top of a glycolipid enriched phospholipid single membrane. Such techniques can unveil the cross structural details of mucus-covered epithelia in interaction with macromolecules with ~~the~~ Å resolution.
Very recently, mucin mesh dynamics have been investigated by X-ray Photon Correlation Spectroscopy (XPCS) at the ID10 beamline of the ESRF-EBS source in Grenoble (France), overcoming the limitations of DLS. The high coherencel of high energy X-ray beams at EBS allowed measuring fast decaying intensity correlation functions on semidiluted concentrations of the bio-gel, ensemble averaging over Q-vector equivalent pixels of a fast 2D pixelated detector. Silica particles of different size and nanoparticles designed for drug and siRNA delivery have been applied as tracer nanoparticles (NPs). NPs have different size tuned to the length scale relevant to the topological entanglements of mucin and mucin/DNA meshes, in order to provide information on the fluctuations of the mesh within their local environment. Different responses were obtained, distinguished in fast and slow decaying components of the correlation function, also showing diverse Q-vector dependences, which are discussed in terms of particle dynamics and mesh stress relaxation, respectively.

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Fluorescence fluctuation spectroscopy with a SPAD array detector to unravel molecular processes in living cells

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Fluorescence Fluctuation Spectroscopy (FFS) is a family of analytical methods that allow quantifying molecular processes by studying the fluorescence intensity fluctuations generated by a population of fluorescent molecules entering and leaving a small microscope focal volume (e.g., the detection volume of a confocal microscope). By employing a novel single-photon-avalanche-diode (SPAD) array detector, we can further enhance the classical confocal FFS methods. In fact, by spatially and temporally tag the single-photons (temporal tag allows also leveraging on fluorescence lifetime spectroscopy), we can quantify not only molecular dynamics but also changes in the cell organization at a single molecule level. In particular, we propose a new class of FFS techniques performed with the SPAD-array detector to better decipher molecular dynamics, interactions and structural changes directly in living cells with microsecond time resolution.

In fact, despite the importance for several cellular processes, molecular dynamics and protein interactions in living cells is yet poorly understood. For example, RNA molecules play a major role in multiple processes, from encoding and regulating gene expression to catalyzing biological reactions. While, alterations of the metabolism of RNAs and RNA-binding proteins are involved in neurodegenerative diseases, such as ALS in neuronal cells.

As test case, we show how combining single-molecule labeling and FFS with SPAD array detector can be employed to investigate different RNA-based molecule processes in living cells.

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Gating defects of endosomal ClC-3 exchangers involved in neurodevelopmental disorders

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The genetic causes of global developmental delay (GDD) and intellectual disability (ID) are diverse and include variants in numerous ion channels and transporters. Loss-of-function variants in all five endosomal/lysosomal members of the CLC family of Cl^- channels and Cl^-/H^+-exchangers lead to pathology in mice, humans, or both [1]. CLC proteins are homodimers with transport occurring independently in each subunit in physically separated transport pathways. We have identified nine variants in *CLCN3*, the gene encoding CIC-3, in eleven individuals with GDD/ID and neurodevelopmental disorders of varying severity. In addition to a homozygous frameshift variant in two siblings, we identified eight different heterozygous *de novo* missense variants. All have GDD/ID, mood or behavioural disorders and dysmorphic features, 9/11 have structural brain abnormalities and 6/11 have seizures. The homozygous variants are predicted to cause loss of ClC-3 function, resulting in severe neurological disease similar to the phenotype observed in $Clcn3^{-/-}$ mice. To characterize the altered function of the exchanger, electrophysiological analyses were performed in Xenopus oocytes and mammalian cells. Two variants, I607T and T570I, had dramatically increased currents at negative cytoplasmic voltages and loss of inhibition by luminal acidic pH. In addition, I607T completely lacked transient currents, that are visible in WT ClC-3 but whose physiological role is unclear. Residue I607 is located at the dimer interface and the mutation likely causes an incomplete closure of a voltage-dependent gating process, that assures almost complete absence of transport at negative voltages in WT ClC-3. This gating effect likely leads to a functionally dominant toxic gain of function in patients. Disease is thus caused by aberrant ionic homeostasis of endosomes leading to neuronal dysfunction and possibly neuronal death. In contrast, two other variants showed no significant difference in the current properties. Overall, our work establishes a role for *CLCN3* in human neurodevelopment and shows that both homozygous loss of ClC-3 and heterozygous gain-of-function variants can lead to GDD/ID and neuroanatomical abnormalities.

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Extracellular vesicles as modulators of biomechanical properties in target cells

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Extracellular vesicles (EVs) mediated communication has been recently receiving a growing attention for its role in the development of cancer metastasis. EVs are small vesicles exchanged among cells and, owing to their biologically active content, EVs promote tumour–induced immune suppression, metastasis and angiogenesis, and constitute a potential target in cancer therapy. Yet, their role in priming the premetastatic niche and helping the spreading of cancer cells is still debated. Here, we focused on the effects of EVs on the biomechanical properties of target cells, since it is recognized that the biomechanical properties play a crucial role during metastatic spreading. To this purpose, we isolated and thoroughly characterized metastatic breast cancer cell derived EVs. We tested their ability to model the pre-metastatic niche by analyzing the biomechanical changes induced by EV addittion on recipient cells through AFM-based nanoindentation and immunofluorescence measurements. Our results indicate that triple negative breast cancer (TNBC)-derived small extracellular vesicles can actively induce cellular stiffness, cytoskeleton, nuclear and Yap activity rearrangements.

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Molecular biophysics

Lipid droplet biogenesis: insight from molecular dynamics simulations

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Lipid droplets (LDs) are key cellular organelles regulating energy metabolism. LD biogenesis occurs in the ER membrane, and starts with the phase separation of synthesized neutral lipids (substantially hydrophobic) from phospholipids (amphipathic, enriched in the ER bilayer), leading to the formation of droplets, which then bud off from the ER towards the cytosol. The fate and biological function of LDs are largely determined during their formation, but the driving forces and mechanism of LD formation remain elusive. Here I will present molecular dynamics simulations at the coarse-grained level exploring two specific questions: the determinants for LD directional budding and for lipid droplet proteome.

THE PATCH-CLAMP TECHNIQUE AND BEYOND: CHARACTERIZATION OF NON-ELECTROGENIC VACUOLAR NHX PROTON/POTASSIUM ANTIPORTERS AND INHIBITION BY PHOSPHOINOSITIDES

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In this work [1], we combined the patch-clamp technique with ratiometric fluorescence imaging using the proton-responsive BCECF dye as a luminal probe. Upon application of a steep cytosol-directed K^+ gradient in Arabidopsis mesophyll vacuoles, a strong and reversible acidification of the vacuolar lumen was detected, while no associated electrical currents were observed, in agreement with electroneutral cation/H^+ exchange. Our data show that this acidification was generated by NHX antiport activity, since i) it did not distinguish between K^+ and Na^+ ions, ii) it was sensitive to the NHX inhibitor benzamil, and iii) it was completely absent in vacuoles from *nhx1 nhx2* double knockout plants. Our data further show that NHX activity could be reversed, was voltage independent and specifically impaired by the low-abundance signaling lipid phosphatidylinositol-3,5-bisphosphate ($PI(3,5)P_2$), which may regulate salt accumulation in plants by acting as a common messenger to coordinately shut down secondary active carriers responsible for ion uptake inside the vacuole. Finally, we developed a theory based on thermodynamics, which supports the data obtained by our novel experimental approach. This work, therefore, represents a proof of principle that can be applied to the study of proton-dependent exchangers from plants and animals, which are barely detectable using conventional techniques.

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The interface between domain G4 and G5 is a novel hotspot of gelsolin instability, toxicity and amyloid propensity

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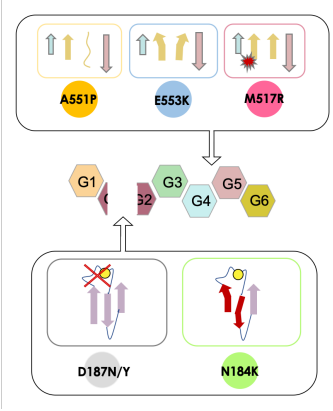

Gelsolin is a protein composed of 6 homologous domains (sequentially named G1-G6). Each module hosts at least one Ca^{2+} binding site and the ion induces subtle local and large global conformational changes that also shift the protein from a compact to an extended and flexible conformation.

Single point mutations in the protein are responsible for a hereditary misfolding disease, named **gelsolin amyloidosis**, characterized by progressive corneal lattice dystrophy, *cutis laxa*, and polyneuropathy [1]. Several different amyloidogenic variants of gelsolin have been identified over the years, but only the most common D187N/Y mutants in G2 have been thoroughly characterized and the underlying functional mechanistic link between mutation, altered protein structure, susceptibility to furin cleavage and aggregative potential resolved.

Little is known about the **recently identified mutations** A551P, E553K and M517R hosted at the interface between G4 and G5 domains, whose aggregation process likely follows an alternative pathway [2-4]. We performed a thorough **structural** and **biophysical** characterization of the three variants, showing that they are not susceptible to furin cleavage, but are characterized by a higher tendency to aggregate in unproteolysed forms. This also results in a higher **proteotoxicity** in *C. elegans*, an assay which we exploited for the first time to quantify toxicity of the full length proteins.

All available evidence points to a **destabilization of the interface between G4 and G5** domains which leads to a relaxation of the structure. We identified three different structural determinants of such destabilization (β-strand breaking, steric hindrance and/or charge repulsion), all implying the impairment of interdomain contacts. The rearrangement of the protein global architecture triggers a furin-independent and likely proteolysis-independent aggregation of the protein, supporting the existence of so-far **undescribed non-canonical pathways** of gelsolin amyloidosis pathogenesis.

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THE ODD FACES OF OLIGOMERS: THE CASE OF TRAF2

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TNF Receptor Associated Factor 2 (TRAF2) is a trimeric protein that belongs to the TNF receptor associated factor family (TRAFs). The TRAF2 oligomeric state is crucial for receptor binding and for its interaction with other proteins involved in the TNFR signaling. The equilibrium between trimeric TRAF2 and its dimeric/monomeric species is crucial for the protein activities. Recently, thanks to the analysis of pressure-induced dissociation measurements and to molecular dynamics (MD) simulation we have found that TRAF2 probably exists in the solution as an “asymmetric” trimer, in which two subunits cluster together while the third becomes more independent from the others [1]. We have argued that this behavior must be strictly dependent on the interactions at the monomer-monomer interfaces, so we have applied dynamic light scattering, circular dichroism, and fluorescence spectroscopy [2] to monitor the structure and conformational dynamics of TRAF2 as a function of temperature. Furthermore, the protein dynamics have been simulated in silico at two different temperatures in the pre-denaturation range and the outputs analyzed by a protein contact network approach [3]. The results demonstrate that in order to reach the physiological temperature (around 35°C) few, critical hotspot areas of the trimers interfaces govern the monomer-dimer-trimer equilibrium and the time-dependent asymmetric approaching and distancing of the subunits. Such dynamics affect the results of molecular docking on the external protein surface using receptor peptides, indicating that the TRAF2-receptor interaction in the solution might not involve three subunits at the same time, as suggested by the static analysis obtainable from the crystal structure. These findings shed new light on the role that the TRAF2 oligomeric state might have in regulating the protein binding activity in vivo.

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DIMER-MONOMER EQUILIBRIUM OF SARS-COV-2 MAIN PROTEASE AS AFFECTED BY SMALL MOLECULE INHIBITORS. A BIOPHYSICAL INVESTIGATION

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The maturation of coronavirus SARS-CoV-2, which is the etiological agent at the origin of the COVID-19 pandemic, requires a main protease M^{pro} to cleave the virus-encoded polyproteins [1]. Despite a wealth of experimental information already available, there is wide disagreement about the M^{pro} monomer-dimer equilibrium dissociation constant [2]. Since the functional unit of M^{pro} is a homodimer, the detailed knowledge of the thermodynamics of this equilibrium is a key piece of information for possible therapeutic intervention, with small molecules interfering with dimerization being potential broad-spectrum antiviral drug leads. Because of this mechanism of action, inhibiting Mpro might lead to an attenuation of the viral infection. Indeed, this enzyme is a very attractive target for anti-CoV drug design: the Mpro sequence is highly conserved among various coronaviruses, as mutations of M^{pro} turn out to be often fatal for the virus. Thus, the risk of mutation mediated drug resistance is very low and inhibitors will display broad-spectrum antiviral activity [3].
We have exploited Small Angle X-ray Scattering (SAXS) to investigate the structural features of SARS-CoV-2 M^{pro} in solution as a function of protein concentration and temperature [4]. A detailed thermodynamic picture of the monomer-dimer equilibrium is derived, together with the temperature-dependent value of the dissociation constant. SAXS studies were performed both in the absence and in the presence of a set of in-silico selected small inhibitors, whose activity was spectroscopically assayed, in order to simultaneously test their therapeutic potential with respect to dimerization inhibition. We find that these inhibitors affect dimerization and enzymatic activity to a different extent and sometimes in an opposite way, likely due to the different molecular mechanisms underlying the two processes. To the best of our knowledge, we show for the first time how structural information about the SARS-CoV-2 M^{pro} in solution in the absence and in the presence of potential inhibitors and as a function of temperature can be obtained from an advanced analysis of SAXS data within an overall thermodynamic picture, complemented by more conventional approaches.
Our results suggest that more experimental evidences about the impairment of monomer and dimer M^{pro} in the presence of inhibitors corroborated by computational information will be necessary for a deeper understanding of the M^{pro} allosteric mechanism.

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GATING MOVEMENTS AND ION PERMEATION IN HCN4 PACEMAKER CHANNELS

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Hyperpolarization-activated cyclic nucleotide-gated (HCN1-4) channels are the molecular correlate of the I_h (or I_f) current, which plays a key role in controlling rhythmic activity in cardiac pacemaker cells and spontaneously firing neurons. HCN channels are activated by voltage and modulated by the direct binding of cAMP to their cytoplasmic C-terminal cyclic nucleotide binding domain (CNBD) [1, 2]. While structural models for the HCN channels with a closed pore conformation have recently become available [3, 4], no information is currently available on the open pore conformation of HCN channels. This is because HCN channels are primarily activated by membrane hyperpolarization, a condition that cannot be reproduce, now days, in structural studies. Additionally, the HCN1 structures provide no insight into the mechanism of cAMP modulation of gating, as negligible conformational changes are seen upon cAMP binding, which may reflect the low cAMP efficacy on HCN1 gating [5].
We present cryo-EM structures of HCN4 in the presence/absence of bound cAMP, displaying the pore domain in closed and open conformations. Analysis of our structures sheds light on how ligand-induced transitions in the channel cytosolic portion mediate the effect of cAMP on channel gating, and highlights the regulatory role of a Mg^{2+} coordination site formed between the C-linker and the S4-S5 linker. Comparison of open/closed pore shows that the cytosolic gate opens through concerted movements of the S5 and S6 transmembrane helices. Furthermore, in combination with molecular dynamics analyses, the open pore provides insights into the mechanisms of K^+/Na^+ permeation, revealing distinctive ion-binding dependent adaptation in the selectivity filter.
Our results contribute new mechanistic understanding on HCN channel gating, cyclic nucleotide-dependent modulation, and ion permeation.

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OXYGEN DIFFUSION PATHWAYS IN MUTATED FORMS OF A LOV PHOTORECEPTOR FROM *METHYLOBACTERIUM RADIOTOLERANS*: A MOLECULAR DYNAMICS STUDY

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*Mr*4511 LOV (Light, Oxygen and Voltage) protein is a blue light sensing photoreceptor from *Methylobacterium radiotolerans*, binding flavin mononucleotide (FMN) as chromophore. Blue light activation of LOV domains triggers the reversible formation of a FMN-cysteine adduct by a photocycle that goes through the FMN excited triplet state. LOV domains can be engineered as fluorescent sensors and actuators for optogenetics and photomedicine [1].
First experimental data on *Mr*4511 LOV protein [2] indicate its high potential as a photosensitizer for singlet oxygen (SO) the cytotoxic reactive excited state of molecular oxygen, produced by diffusion limited energy transfer from the FMN triplet state. This feature is obtained after the single mutation of reactive cysteine C71, a change that prevents formation of the photoproduct. In addition, the lack of a tryptophan, conserved in ca. 75% of LOV domains and shown to strongly quench the FMN triplet lifetime (τ_T) in LOV proteins, allows for *Mr*4511 LOV a longer τ_T than for other LOV domains in C71S and C71G variants [2].
After an homology modeling of *Mr*4511 LOV, that has lead to a dimeric protein stabilized by the presence of a strong leucine zipper in the C-terminal helices, a mutation of the photocycle substrate cysteine into serine (C71S) has been introduced *in silico* to make it a SO photosensitizer, and the mutated form stability was tested by MD simulations.
Afterwards, both transient and persistent oxygen channels were detected and analysed both in the wt and in the mutated protein. Molecular oxygen was then placed both outer and into the chromophore cavity and potential diffusion pathways were explored with MD simulations, showing a high accessibility of the binding cavity and a high persistence of oxygen inside.
Mutations that might favor SO generation were designed based on their position with respect to the FMN and the oxygen channels, taking into account the ability of certain amino acids to quench FMN triplet state and SO. Therefore, C71S/Y61T and C71S/Y61S double mutants were generated *in silico* and their stability was checked. The analysis of their oxygen diffusion pathways showed an increased diffusion and persistence of oxygen molecules inside the binding cavity, indicating a promising model for SO photosensing and its biomedical and biophysical applications.

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Optical and spectroscopic methods applied to biology and medicine

NON-LINEAR LABEL-FREE MICROSCOPY OF CELLS AND TISSUES BASED ON BROADBAND COHERENT RAMAN SCATTERING AND ARTIFICIAL INTELLIGENCE

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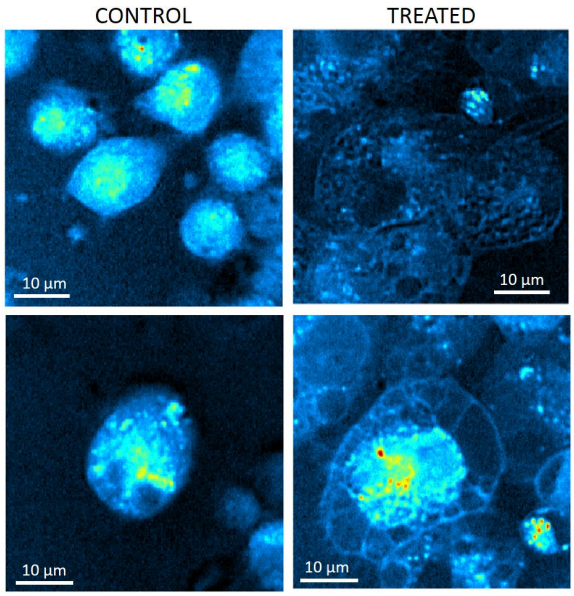

Fluorescence microscopy is a powerful investigation tool for life sciences. It can visualize morphological details in cells and tissues with sub-micrometer resolution and single-molecule sensitivity. However, fluorescent markers can perturb the investigated system and induce phototoxicity. This calls for intrinsic, label-free imaging methods such as Spontaneous Raman (SR) microscopy. SR measures the vibrational spectrum that characterizes every component of a biological specimen, reflecting its molecular structure and providing an endogenous and chemically specific "fingerprint". Its main drawback is the very low scattering cross section, making it difficult to probe diluted species and preventing real-time imaging of dynamical processes in living cells or tissues.
These limitations can be overcome by the use of coherent Raman scattering (CRS) techniques. CRS is a nonlinear optical technique employing a sequence of ultrashort pulses to set up and detect a vibrational coherence within the molecules in the laser focus, which enhances the Raman response, thus allowing high imaging speeds with 3D sectioning capability.
In this talk, I will review the state of the art and recent advances of CRS microscopy, in both coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) modalities [1]. In particular, I will show a few examples of their application in biology, for studying cells and tissues and for tumor identification. Furthermore, I will show how novel artificial-intelligence (deep learning) methods can be applied to enhance image quality and extract the relevant bio-chemical information from congested and noisy hypercubes (three-dimensional datasets of vibrational spectra as a function of x-y position on the sample) [2].

About the speaker: Dario Polli is Associate Professor of Physics at Politecnico di Milano (Italy). His main research focus is on coherent Raman spectroscopy and microscopy, ultrafast and non-linear optics, Fourier-transform spectroscopy and time-resolved pump-probe spectroscopy and microscopy. He is the coordinator of CRIMSON (www.crimson-project.eu), a pan-European H2020 project to develop the next-generation microscopy platform to study the cellular origin of diseases. He published >100 scientific papers on international journals, cited >5500 time. His H-index is 35. He has been awarded an ERC Consolidator grant (www.vibra.polimi.it) in 2015. He is passionate about science divulgation to the public. He is co-founder of NIREOS (www.nireos.com) and Specto Photonics (http://spectophotonics.com/) photonics startups. He is passionate about science divulgation. More info on http://polli.faculty.polimi.it/ and www.linkedin.com/in/dariopolli/

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A PLANT GENE ENCODING ONE- AND TWO-HEME HEMOGLOBINS WITH EXTREME REACTIVITIES TOWARD DIATOMIC GASEOUS LIGANDS

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The recently identified non-symbiotic hemoglobin gene *MtGlb1-2* of the legume *Medicago truncatula* possesses unique properties as it generates four alternative splice forms encoding proteins with one or two heme domains [1]. We investigated the ligand binding kinetics of MtGlb1-2.1 and MtGlb1-2.4, bearing one and two hemes, respectively. Unexpectedly, the overall time-course of ligand rebinding followed by ns laser flash photolysis and a hybrid fs-ps pump–probe setup was unusually fast. Most photodissociated ligands are rebound geminately within a few nanoseconds, but the very peculiar kinetic feature is that the rate constants for ligation and deligation of distal histidine to the heme are the highest reported for any plant or vertebrate globin. The combination of microscopic rates results in unusually high overall ligand binding rate constants, a fact that contributes to explain at the mechanistic level the extremely high reactivity of these proteins toward the physiological ligands oxygen, nitric oxide and nitrite [2].

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CHEMO-MECHANICAL CHARACTERIZATION OF HUMAN BONE AND CARTILAGE TISSUES BY BRILLOUIN AND RAMAN MICROSPECTROSCOPY IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS.

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Bone and cartilage tissue have a complex structure, which is well-designed to ensure their exceptional mechanical performance. Human bone tissue is characterized by a hierarchical architecture, with several levels of organization going from the macroscale down to the microscale [1], while the articular cartilage is molded to form a bearing-like sheet, which prevents the friction between bones involved in joint [2]. Their capability of both resistance and adaptation to the mechanical and chemical stresses acting on them, is strongly dependent on maintaining a correct arrangement of all their constituents already at the microscale. Several orthopaedic pathologies, such as osteoarthritis (OA), arise from the destruction of the tissue structure by inflammatory processes which determine a rearrangement of the overall structure of the whole organ, leading to a complete loss of its functionality. During the OA onset the articular cartilage is progressively degraded, while the structure of the subchondral bone beneath is remodelled to respond to compressive forces acting now directly on the surface. Brillouin and Raman micro-Spectroscopy (BRaMS) is a scattering technique that allows assessing simultaneously the mechanical and the chemical properties of the biological tissues with a micrometric resolution [3]. Moreover, it is not destructive, contact-less and does not require labeling, thus it has the potential for future *in vivo* application. BRaMS has been successfully employed for single-cell study, tissue-phantom characterization, and whole tissue description in both physiological and pathological conditions [4,5]. Here, we report the results obtained by BRaMS in the characterization of the human bone tissue [6] and the first attempt to employ this technique to identify the remodelling processes acting on both the cartilage surface and subchondral bone layers during the OA development in the femoral hip joint.

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IMAGING THE MELANIN DISTRIBUTION IN MURINE MELANOMA BIOPSIES BY LABEL-FREE SUPER-RESOLUTION THERMOGRAPHY

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Screening of melanoma biopsies is typically performed by color- and texture-based histopathological analyses upon tissue staining with specific markers such as hematoxylin and eosin. An additional feature that can be added to this characterization can be retrieved by spatially mapping the distribution and concentration of melanin pigments by active photo-thermal imaging.
In this work, we employ a non-contact photo-thermal super-resolution image acquisition approach we have recently developed[1] to quantify the relative concentration of melanin pigments in murine B16 melanoma biopsies. Sample absorption and heat-release events are sparsely primed by a 514-nm focused Gaussian laser beam, so that the endogenous photo-thermal signal of melanin pigments is exploited to provide contrast in a label-free approach. The coordinates of the absorptive centers get localized by a 2D fit of the temperature peaks imaged by a thermal camera. The procedure of illumination and peak localization is repeated by means of a modulated laser raster scanning, and the final super-resolution image is reconstructed by the localized centers and amplitudes of all the measured temperature peaks. The spatial resolution is pushed to the 10-50 μm range, as compared to the typical ~400 μm diffraction-limited resolution of conventional thermography.
At first, we demonstrate that super-resolved temperature-based maps accurately recover the conformation of melanin-rich regions, and clearly correlate with the morphological information provided by transmitted-light imaging of the same tissue sections. Then, based on the analytical treatment of the 3D heat transfer model, we convert temperature images into quantitative maps of the relative concentration of melanin pigments. Finally, by combining super-resolved thermography with fluorescence Draq5 staining, we highlight a correlation between the detected temperature signal and the size distribution of cell nuclei. Provided melanin concentration is related to the differentiation state of tumor cells, we envision applications of the proposed approach in the assessment of the developmental stage of melanomas imaged at different time points, thereby adding a new feature to the melanoma characterization.

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Oncogene-induced alterations of chromatin organization investigated by confocal microscopy

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Genomic instability is a hallmark of cancer. Oncogenes can induce DNA damage by interfering with fundamental processes such as DNA transcription, replication and DNA damage response [1]. However, the molecular mechanisms associated with oncogene-induced genomic damage are still poorly understood.
Here, we perform confocal microscopy to investigate oncogene-induced alterations of chromatin organization. Specifically, we use the U937-PR9 cell line, an in vitro model of Acute Promyelocytic Leukemia (APL), which allows us to selectively activate the expression of the PML-RARα oncogene and to analyze its effects on the spatiotemporal organization of functional nuclear processes. We perform high-resolution, multi-color, confocal microscopy of DNA replication sites and DNA transcription sites, before and after activation of the PML-RARα oncogene. We take advantage of Image Cross-Correlation Spectroscopy (ICCS)[2] to quantify alterations in the spatial organization of the sites induced by expression of the oncogene.
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A multi-techniques approach to investigate the conformational changes of human telomeric G-quadruplex complexed with ligands

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The human telomeric guanine-rich sequences, known as G-quadruplex (G4), can be considered a new and promising target for anticancer therapeutics. It was increasingly recognized, indeed, that the presence of G4 structures can inhibit the telomerase action. As telomerase is an enzyme able to overcome telomere-dependent cell death, which is present in 85% of the cancer cells making them immortal [1], interfering with its activity is vital in molecular biology and biomedicine. This discovery has lighted up the interest in testing and designing small molecules with high affinity to G4, able to stabilize their structure for therapeutic purposes [2].
The interaction of G4 with binders is really challenging because their folded topology, i.e. the geometry of the loops connecting the guanine segments [2][3], is greatly affected by many environmental factors, such as temperature, ionic strength and DNA/ligand concentration.
To investigate the high degree of polymorphism of G4 complexed with different ligands, a biophysical approach combining small-angle scattering (SAS) and circular dichroism (CD) was used.
We studied the sequence d[AGGG(TTAGGG)$_3$] (Tel22) in K+ solution alone and complexed with several binders suitable for different uses, namely Berberine, Palmatine, Actinomycin D and Porphyrin TMPyP4. CD experiments of the complexes reveal a harvest of changes on the secondary structure. A possible way for interpreting the CD response is to decompose a given signal into the CD curves associated with the three main generic topologies that G4 can adopt, parallel (P), hybrid (H) and anti-parallel (AP). SAS measurements show what happens at a higher structure level. In the condition under study, Tel22 is in a monomeric form, while upon complexation elongated structure are formed, which are compatible with dimeric units.
The four drugs promote both different G4 conformational changes *and structural modifications at the quaternary level:* a resuming view *is provided.*

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LABEL-FREE MOLECULAR INVESTIGATION VIA SPECTROSCOPIC ELLIPSOMETRY: OPTICAL DETECTION OF A SARS-CoV-2 GENE REGION

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The challenge to defeat diseases starting from a rapid diagnosis has been relighted by the last pandemic due to SARS-CoV-2. The development of biosensors able to recognize molecular adsorption on surfaces plays a crucial role in this issue, allowing for example to detect the presence of disease biomarkers, like specific nucleic acid sequences or antibodies, in solution. Currently, viral RNA detection relies almost exclusively on polymerase chain reaction (PCR), requiring an expensive and complex process, or on fluorescent methods using labels that could interfere with the hybridization process. We exploited spectroscopic ellipsometry (SE) label-free experiments to detect UV-Vis molecular absorptions at the monolayer level in order to develop biosensing platforms.
Spectroscopic ellipsometry is a non-perturbative and extremely sensitive method for the analysis of ultrathin molecular films. Changes in polarization of a light beam upon reflection from the sample surface are detected, providing information, both quantitative and molecular-specific, on film thickness and optical properties. In fact, SE difference spectra, obtained as the difference between the spectra acquired after the molecular deposition and the spectra acquired on the bare substrate, clearly show molecular absorption fingerprints in the UV-Vis-NIR spectral range [1].
We investigate in situ and in real time the self-assembly of thiolated single-strand DNA on flat gold, and the following hybridization with specific RNA sequences, like SARS-CoV-2 RdRp-Helicase. DNA-based biosensors are an excellent option to develop micro-nano devices which are reusable thanks to the denaturation property of nucleic acids. By measuring the change in the DNA absorption at 260 nm and the parallel increase of optical thickness, we could monitor the hybridization process with complementary RNA strands. SE results on DNA films before and after hybridization with complementary RNA strands were supported by the detection of variations in film thickness (AFM nanolithography) and coverage (XPS, QCM). Hybridization experiments carried on through optical and mass-sensitive methods as a function of the concentration of RNA solution indicate, at present, a detection limit of 10 nmol/L, which is expected to decrease through an ongoing upgrade of the optical experimental setup.
In conclusion, an accurate optical SE model, supported by AFM thickness data, allowed us to optically characterize ssDNA and dsDNA films in the whole probed wavelength region, providing a new tool for nucleic acid sequence recognition.

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Systems biophysics /environmental biophysics

WHEN THE BIOPHYSICS MEETS THE SEA: A FASCINATING TRIP INTO THE MARINE CARBON CYCLE

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Marine DOM, defined as a complex mixture of organic molecules that passes through a 0.2 µm filter, represents one of the largest (662 Pg C) and the least understood reservoir of organic carbon on Earth and plays a key role in regulating the capability of the oceans to store CO_2 [1] It also represents the main source of energy for the heterotrophic prokaryotes, regulating the marine food web [2]. Most DOM is autochthonous that is it is in-situ produced by photosynthesis and chemosynthesis and is released at all the levels of the food web. External sources (atmosphere, rivers, groundwater, and sediments) may strongly affect DOM concentration and distribution, in particular in marginal semi-enclosed basins like the Mediterranean Sea [3, 4, 5].
Although marine DOM is produced and removed by many different processes, its concentration always falls within a very narrow range (34-80 µM C). The biogeochemical feedbacks that buffer DOM concentrations are unknown, and their understanding is one of the most intriguing and pressing issues in marine science.
One of the most interesting aspects of dissolved organic carbon (DOC, 93% of the DOM pool) dynamics is that it includes molecules with a wide range of biological lability. Different fractions of DOC have therefore been described based on their turn-over time. Labile DOC (LDOC) is defined as the fraction that is immediately used by prokaryotic heterotrophs and does not accumulate [1]. It, therefore, has a very low steady-state concentration, even if its production and removal rates are the highest ones. The fraction of DOC, that escapes rapid mineralization and accumulates, is considered to be recalcitrant [1]. Why some of these molecules persist in the oceans is one of the most pressing questions in marine science, due to its impact on the global carbon cycle.
Our research activities aim at a comprehensive understanding of DOM, we study its dynamics in the open oceans and coastal zone [3, 6] as well as in the rivers [4] and atmospheric depositions [5]. One of the main goals of our research activity is to understand how DOM-microbes interaction regulates the C cycle in the oceans. In order to achieve these goals we measure DOC concentrations and optical properties (absorption and fluorescence) on natural samples, we study their distribution and seasonal variation and we carry out incubation experiments. Radiocarbon analyses allowed to unveils a complex cycle of DOM in the deep oceans changing the idea of a recalcitrant pool dominating the deep oceans [7, 8]. In this talk, I will stress how biophysics can help in understanding the key processes regulating carbon cycle in the ocean.

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EXPLORING RIBOSOME HETEROGENEITY AT HIGH RESOLUTION BY NEXT GENERATION SEQUENCING

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Ribosomes have been classically considered as macromolecular assemblies with fixed composition of four ribosomal RNA (rRNA) and 80 ribosomal proteins (RPs). Recent findings support the intriguing “ribosome heterogeneity hypothesis” based on the existence of distinct populations of ribosomes with diverse composition and called specialized ribosomes. Ribosome heterogeneity acts as a filter of subsets of mRNAs to be translated and derives from a variety of ribosome-associated proteins (RAPs), rRNA variants, post-transcriptional modifications and unique ribosomal proteins composition. Structural information about ribosomes is commonly obtained by Cryo-EM techniques and generation of mean ribosome structures. Despite the advantage of obtaining information at high resolution, this approach cannot distinguish between heterogeneous ribosomes. Hence, complementary approaches are required for the comprehensive investigation and characterization of heterogeneous ribosomes.

Here we exploited RiboSeq and RiboMethSeq, two next-generation sequencing-based techniques, to resolve at single nucleotide resolution the ribosome heterogeneity in terms of RAPs and rRNA post-transcriptional modifications of mouse ribosomes and in the context of the Spinal Muscular Atrophy. SMA is a genetic disease caused by low levels of the Survival Motor Neuron (SMN) protein. SMN is known to be directly implicated in the control of translation. However, the mechanisms linking SMN to regulation of protein synthesis remain unclear.

First, we took advantage of selective ribosome profiling (RiboSeq), and investigated the involvement of the RAP, SMN, in positioning of ribosomes along the transcripts. RiboSeq is based on the identification of short RNA fragments protected by ribosomes (RPFs) coupled with next-generation sequencing. This approach provides exact information on the positioning of ribosomes along single RNAs in control and disease conditions, unravelling the existence of defective ribosome localization or ribosome conformational changes. We observed that SMN-specific ribosomes are located within the first five codons of the coding sequence of a subset of mRNAs. This result suggests a ribosome-based regulation of translation driven by SMN. Importantly, two distinct populations of RPFs lengths were observed, suggesting that SMN may play a role during ribosome translocation. In SMA early-symptomatic mouse brains, we observed that SMN loss causes defective ribosome occupancy on SMN-specific transcripts and ribosome loss at the beginning of the CDS, further reinforcing the role of SMN in controlling translation.

Second, we exploited RiboMethSeq to map rRNA 2’O-methylations. Chemical modifications of rRNA are known to influence ribosome biogenesis, structure and activity by adding a regulatory layer in the modulation of translation. The most widespread modification in rRNA, the methylation of ribose 2-OH moiety, is carried out by fibrillarin methyltransferase, a protein known to directly interact with SMN. This observation prompted us to investigate the potential role of SMN as modulator of rRNA modifications. To this aim, we performed RiboMethSeq in physiological and pathological conditions. First, we obtained the entire 2’O-methyl map of rRNA extracted from mature and purified ribosomes or from total rRNA of wild-type mouse brain. Our analysis confirmed the presence of previously reported methylation sites that match more than 80% of the methylations identified in the total sample. The majority of these sites localize in the internal core of the 3D structure of ribosomes. Remarkably, rRNA from the 80S sample revealed previously unknown methylation sites, resulting in 40% more sites than in the total rRNA. Strikingly, almost all newly-identified methylation sites are located on the surface of the ribosomes, specifically on their expansion segments. These results suggest that additional steps of rRNA methylation may occur directly on mature and already assembled ribosomes. Finally, we investigated the effect of SMN loss on rRNA methylation by RiboMethSeq on SMA early-symptomatic mouse brain. Despite most of the previously reported methylation sites were detected in both samples, we observed a general decrease of methylations in the SMA condition. Remarkably, more than 75% of the methylations on expansion segments are lost in the 80S sample, suggesting that SMN may play an as yet uncharacterized role in regulating translation acting as a modulator of rRNA methylations.

Overall, our results highlight the ability of the high-throughput techniques and dedicated computational pipelines in retrieving meaningful information on ribosome heterogeneity and the regulation of translation at single nucleotide resolution.

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SENSOR project: a fluorescence sensor to detect organic matter contamination in drinking water

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Natural organic matter (NOM) contains all organic contaminants (quinones, tannins, fertilizers, pesticides) present in aquatic environment. In the treatment of drinking water containing NOM, the use of chlorine as a disinfectant leads to the formation of carcinogenic, mutagenic and toxic compounds that represent a potential threat for health. Although the structure and functionality of NOM is not well defined, NOM contains a chromophoric fraction , which is able to absorb light in the visible and UV wavelengths and re-emit a part of it as fluorescence (FDOM). Most of the organic contaminants, due to their chemical structure, fall within the latter fraction, the measurement of fluorescence is therefore able to give information on the type of organic molecules present, representing a sensitive indicator of chemical pollution from organic molecules FDOM measurements are particularly useful in drinking water systems as:

- they can give quick information on the efficiency of NOM removal [1]
- they can improve the predictability of the formation of toxic substances (for example trihalomethanes, bromate, halogenic acids) as a result of chlorination processes [2]
- protein-like fluorescence has been correlated with biological contamination [3]
- the humic-like fluorescence can be related to the Kuber index, a parameter used for determining water potability [4]

In the framework of the SENSOR project (New real time sensors for the determination of chemical and microbiological contamination in environmental and biomedical matrices) co-funded by the POR FESR Tuscany 2014-2020 (FAR-FAS, line 1.1., 2014), we are developing a sensor (currently in the form of a prototype) capable of measuring the fluorescence of NOM in drinking water.

The developed prototype is able to detect the presence, even in low concentrations, of humic and protein-like organic substances. The prototype works with two LED-UV sources having an excitation wavelengths of 285 and 340 nm that send UV radiation towards a quartz cuvette containing the sample. Two optical filters select the ranges of emission wavelengths (λem = 355 ± 15 nm and λem = 445 ± 25 nm), and the fluorescence emitted from the sample is read by two high sensitivity photodetectors. The functioning of the LEDs and the acquisition of the fluorescence signals are controlled by a dedicated hardware and is controlled through a specific software, both developed by the DIELECTRIK S.r.l. company. A prototype testing phase is currently underway at drinking water treatment plants in order to define its functionality and sensitivity. Fluorescence measurements were made on 224 samples of drinking water both with the sensor and with a bench spectrofluorimeter (FLUOROMAX4, Horiba-JobinYvon). These first data showed a very good correlation between the measurements made with the sensor prototype and those made with the spectrofluorimeter (R^2=0.998 for the protein-like signal and R^2=0.999 for the humic-like signal) supporting the good functioning of the prototype.

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Awards

Environment and brain plasticity

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Brain plasticity refers to the property of neuronal circuits to change structure and function in response to experience, a fundamental theoretical theme in the field of basic research and a major focus for neural rehabilitation. Neural plasticity relies on specific molecular and cellular steps, and its outcome can be adaptive or maladaptive, depending on how the process is harnessed. A large body of evidence shows that brain plasticity is strongly affected by the environment: exposure to stimulating environments has beneficial consequences throughout the entire life while exposure to impoverished environments has negative consequences. I shall present past and recent work form our laboratory concerning the influence exerted by Enriched Environment on brain plasticity processes and its ability to enhance plasticity processes and to guide them towards adaptive outcomes, with special emphasis on the underlying cellular and molecular mechanisms. I shall start from environmental effects on brain developmental plasticity, then highlight the striking ability of environmental enrichment to empower adult brain plasticity to conclude with the effects elicited by enriched living conditions on physiological and pathological aging brain processes.

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PHOTO-ACTIVATED RASTER SCANNING THERMAL IMAGING AT SUB-DIFFRACTION RESOLUTION

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Conventional non-contact thermal imaging provides temperature spatial maps based on the intensity of thermal radiation emitted by the sample. Local temperature variations, induced by endogenous or exogenous sources, are monitored by a low-cost microbolometer-based thermal camera, that converts the intensity of the detected far infrared (~7-15 μm) radiation into absolute temperature measurements assuming grey body radiance and an emissivity value. The spatial resolution is theoretically limited at ~0.1-0.5 mm by diffraction at the low (typically ≤0.05) numerical aperture of the collecting Germanium lens, but it is typically lowered to an effective value of ~1 mm by the thermal wave diffusion in the sample. Such a limitation hampers application of thermal imaging in research fields such as biotechnology, nanomedicine, molecular electronics and materials engineering, where fine temperature mapping on extended (mm-/cm-sized) fields of view with tens-of-microns resolution could help characterizing the homogeneity, morphology and functional state of both biological tissues and synthetic materials.

In my master's thesis, I have described and validated a photo-thermal super-resolution image acquisition approach based on a time modulated and spatially sparse laser-light scanning of the sample, and on the automated a posteriori localization of the resulting isolated laser-induced temperature variations. By the surface fit of the temperature peaks in the acquired thermal camera frames, the locations where the employed focused low-power Gaussian laser beam primes a photo-thermal effect get localized and exploited for the reconstruction of the final super-resolution image [1]. Best-fit amplitudes color-code for local temperature values, while peak coordinates provide morphological information on the absorptive centres in the sample. Provided the uncertainty in the peaks localization can be made arbitrarily small by increasing the signal-to-noise ratio of the acquired thermal camera frames, the spatial resolution of the rendered image is ultimately determined by the excitation laser spot size, which can be tuned at will down to the ~μm range by adjustment of the optical path.

Photo-activated super-resolution thermal imaging has been demonstrated at first with proof-of-principle experiments on synthetic ink samples produced by microfiche printing. Comparison of the results with the images obtained by conventional transmitted-light microscopy on the same samples has confirmed accurate imaging capability and quantified the 60-μm attainable resolution on the adopted setup configuration. This proves resolution enhancements of a factor of ~6 and ~20 with respect to the diffraction-limited prediction and the effective (1200±180)-μm resolution of our thermal camera in conventional operation. The applicability of the proposed approach to complex biological samples has been further demonstrated. Explanted murine skin biopsies, treated with Prussian blue 30-nm nanocubes, have been imaged with photo-activated temperature increases as low as 0.1-2 °C, thereby providing temperature-based super-resolution maps of the distribution of the absorbing nanostructures inside the tissue across mm-sized areas. This is a necessary step to develop effective in-vivo imaging tools based on the localization of photo-thermal nanoparticle tracers and for the subsequent nanoparticle-mediated photo-thermal therapy of the tissue in pathological conditions. Potential applications and future impact of photo-activated super-resolved thermal imaging can be envisioned in both the biotechnological and nano-medical fields.

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G/GMP HYDROGELS FOR DRUG DELIVERY: STRUCTURE AND STABILITY IN THE PRESENCE OF INTERCALATING AND POLYMERIZING AGENTS

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It is well known that nucleotides self-assemble into functional nucleic acids and that the structure is organized through specific H-bonds, for example guanine combines with cytosine via Watson-Crick base pairs. However, guanine may also show a different behaviour, because its chemical structure allows to adopt non-canonical Hoogsteen H-bonds with other three guanines, forming planar tetrameric structure called G-quartets. In turn, G-quartets are able to stack one on the top of the other by π-stacking interaction, forming long 4-stranded helices called G-quadruplexes.

In DNA, G-rich sequences promote the formation of such a supramolecular G-quartet organisation. This is the case of arrays of guanine-rich DNA telomeric region, but this fascinating property is observed also in the nucleotide guanosine-monophosphate (GMP). Indeed, GMP self-assembles in water and in the presence of monovalent cations (e.g. K^+) to form G-quadruplex structures. Two points should be underlined: first, the counterion interacts with the inner eight oxygen atoms of two-faced GMP tetramers; second, each G-quartet is rotated of about 30° to the next stacked one, to minimize the hydrophobic repulsion between the lateral phosphate groups. It has been demonstrated that it is possible replace some GMP with guanosine (G), in order to minimize the electrostatic interactions among quadruplexes. The result is the formation of a stable, transparent self-assembled hydrogel, a polymeric material made up by a 3D networks of G/GMP quadruplexes. Several biomedical applications (tissue engineering, drug delivery..) have been imaged for hydrogels, but the G/GMP-hydrogel shows very additional properties: high viscosity, high-stability, biocompatibility, biodegradability, reversibility and adaptability to environmental changes such as pH, temperature, enzymatic activity.

The aim of my thesis work concerned the biophysical and structural characterization of self-assembled guanosine hydrogels, composed by binary mixture of G and GMP at molar ratio 1:2 and 95% v/v of water, in order to verify if it could be used as drug delivery system. This purpose was two-fold: at one side, three fluorescent dyes (ThT, DAPI, EtBr) and three different proteins (BSA, lysozyme and myoglobin) were tested, in order to characterize intercalation within G-quartets and interaction effects on quadruplex stability. On the other side, the study was related to the possibility to increase the gel stability thanks to a non-enzymatic polymerization of GMP by EDC. Several experimental techniques were used, as atomic force microscopy (AFM), fluorescence microscopy, small angle X-ray scattering (SAXS) and UV and FTIR spectroscopy.

Regarding the first thesis purpose, results from UV-vis demonstrated that dyes intercalate among G-quartets and that there is a different fluorescent intensity in the hydrogel compared to that present in water solution. This is an interesting point because indicates that dyes can be used as molecular probe for anticancer drugs. Through SAXS analysis, we demonstrated that intercalated molecules do not alter the ability to form quadruplexes, even if a few changes in G-quadruplex flexibility and thermal stability were detected. Concerning the added proteins, the results of SAXS and AFM depict that the structural effects strongly depend on the type of protein: BSA does not alter the drug delivery system, while lysozyme acts in the opposite manner.

Turning to the gel stability, it was the first time that EDC molecule was demonstrated able to link GMP's phosphates each other and that the gel stability is modified. However, we found that an excessive rigidity of the quadruplexes determines the impossibility to form gel. By using UV-vis it was illustrated the reaction kinetic both in water solution and in the gel system.

In conclusion, it is possible claim that the proposed studies have produced encouraging results. At one side, further researches have been performed on the mode of binding of THT and DAPI to G-quadruplexes, founding out two different behaviour (respectively intercalating and groove binding). On the other side, diffusivity and viscosity experiments are carrying on in order to have a complete biophysical characterization of the guanosine hydrogel as drug delivery system. Finally, a more in-depth analysis based on Atomic Force Microscopy shows that is possible have a strong representation about the helicoidal structure of the G-quadruplexes that enter in the formation of the whole guanosine hydrogel.

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STUDY OF AMYLOID PROTEINS AGGREGATION PROCESS IN PRESENCE OF ACTIVE BIOMOLECULES

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Alzheimer's disease (AD) is, together with Parkinson's disease (PD), the most common form of senile dementia, characterized by progressive impairment in cognitive function and behavior. With the size of the elderly population rising dramatically, the development of new methodologies for diagnosis and treatment of these pathologies represents a fundamental challenge for public health in the 21st century. The onset of the diseases is identified by the incorrect folding and reorganization in beta sheets structures of peptides (Aβ peptide or α-synuclein respectively for AD and PD) that are normally present in cerebral tissues. These misfolded proteins accumulate
and generate insoluble bundle of fibrils and plaques that are considered the hallmark of the pathologies. It is well established, however, that the very onset of the disease is linked to intermediate oligomeric populations, more than mature
fibrils. In this framework, my project concerns the study of the molecular basis of neurodegenerative disorders, to better characterize pattern and interconnections in the amyloid fibrillation process [1]. The intervention in amyloid diseases, towards a therapeutic approach for their treatment, can include different strategies interfering with the route of amyloid formation: from blocking the production of the amyloidogenic proteins, to inhibiting or reverse their misaggregation with exogenous or endogenous compounds, to modulate an auxiliary cellular pathway that affects beneficially one or more of the foregoing approaches. In this thesis, the effect of molecules suspected to interfere with amyloid formation has been investigated, to identify their interrelationship mechanisms with the target proteins. Furthermore, the structures of the selected compounds have been analyzed, to gain a deepen view on their physiological configuration. The chosen molecules belong to different classes: a biomolecular class, the one of molecular chaperones, that are already present in the cellular environments and yet play a role in maintaining cellular homeostasis, and a chemical and exogenous one, the class of curcumin derivatives. After this, the effect of Aβ peptide on model membranes has been analyzed to better understand the mechanisms underlying the toxic action of the peptide.
Molecular chaperones play essential roles in many cellular processes, including protein folding, targeting, transport, degradation and disruption of toxic aggregates by clearance mechanisms. Moreover, they regulate protein functions in order to protect against oxidative stress due to toxicity resulting from amyloid aggregates. Current studies have attributed to the human chaperones Hsp60 and Hsp70 an important role in amyloid neurodegenerative diseases, even if with often controversial mechanisms that need to be clarified. Nevertheless, the oligomeric stability of Hsp70 has been studied only recently while little is known about Hsp60 in the two forms in which it is present in the cellular environment: the naïve and mitochondrial form. Even for the extensively studied GroEL, the bacterial homologue of Hsp60, some concerns remain about its structure and function in solution and, more importantly, under physiological conditions. Since these molecules are readily emerging as therapeutic and diagnostic targets, their comprehensive structural knowledge is an important
field for molecular biology, useful for the development of new drugs and therapies. For this reason, a characterization of these proteins' structures in solution was due and has been firstly obtained [2-4]. Subsequently a study of chaperones-amyloidogenic proteins direct interactions has been carried out for the validation of possible defence pathways based on preferential binding effects [5].
The chemical agents considered to interfere with amyloid peptide are new curcumin derivatives. In fact, in the past years, curcumin seemed to be among the most powerful candidates for the inhibition of the Aβ in vitro aggregation and fibrillization, being a natural compound and being already used as a natural drug for different targets, including primarily as an antioxidant. The idea of exploiting curcumin for therapeutic purposes, however, presents considerable limitations inherent to its poor solubility, stability and ability to permeate the blood-brain barrier. Hence it is necessary to synthesize compounds with the same ability of curcumin to bind Aβ peptide, but that do not present stability and bio-availability problems, allowing a real applicability in the treatment of the pathologies. The leading characters of this part of the study, i.e. curcumin derivatives, have been designed to specifically maintain the merits of curcumin, avoiding its defects, and have been characterized with different techniques until the biological evaluation on a cellular model system [6]. The study of the interactions between amyloidogenic proteins and different chemical and biological agents has been conducted mainly by means of Small Angle X-Ray Scattering (SAXS), but presents a multidisciplinary experimental approach.

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Poster Presentations

Extracellular vesicles from microalgae: *in vitro* and *in vivo* cellular uptake

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Extracellular vesicles (EVs) are lipid membrane nano-sized vesicles secreted by various cell types for intercellular communication. EVs also constitute cross-species communication means and have been found in all kingdoms of life. The exploitation of the biotechnological potential of EVs as nanocarriers of bioactive compounds, such as siRNA, mRNA, lncRNA, proteins, peptides, lipids, synthetic drugs or other cargo, for different theranostic applications is of increasing interest. Here, we describe the newly discovered subtype of EVs derived from microalgae, which we named nanoalgosomes [1,2]. Specifically, we focus on the cellular uptake of the nanoalgosomes, confirming that they bypass mammalian cell membrane and that they are uptaken through an energy dependent mechanism.
Nanoalgosomes were separated from a suspension of *Tetraselmis chuii* cells using differential ultracentrifugation (dUC) or tangential flow filtration (TFF). After the biophysical characterization (according to MISEV-2018 guidelines) [3], they were labelled with membrane-specific fluorescent dyes and validated by F-NTA and infrared-fluorescence assay. The uptake of the nanoalgosomes was monitored by epifluorescence and confocal microscopy in normal and tumoral mammalian cells and *in vivo* using the animal model *Caenorhabditis elegans.*
The studies performed demonstrated that nanoalgosomes can be efficiently taken up by mammalian cells, confirming the cross kingdom communication potential of EVs. These data were also corroborated by *in vivo* studies performed with *C. elegans* in which intestinal cells internalized the labelled EVs. Nanoalgosomes will be further explored as novel and natural delivery systems of high-value microalgal substances (such as antioxidants, pigments, lipids and complex carbohydrates), bioactive biomolecules and/or synthetic drugs.

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Hydrogel blends of k-carrageenan/PVA as 3D printable scaffolds for cartilage reconstruction

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The design of biologically active scaffolds with suitable characteristics is one of the key factors for the success of tissue engineering. Many researches have shown that cells cultured in vitro in 3D resemble the physiology of their counterparts in vivo much better than cells cultured conventionally on flat surfaces of tissue culture plates.
Hydrogels have received a considerable interest as leading candidates for engineered tissue scaffolds due to their unique compositional and similarities to the natural extracellular matrix. In addition to their porous structure allows the incorporation of growth factors, glycans, bioactive peptides and natural proteins necessary for cell proliferation and survival. Most recently, 3D bio-printing, using a mixture of cells, biopolymers and bioactive molecules, has received particular attention due to the possibility of placing the cells in the right positions inside the matrix structure to create complex biohybrid structures.
Human adipose-derived stem cells (hADSCs) spheroids are relatively accessible and attractive as a cell source for cartilage regeneration, due to the simple surgical procedures required to harvest the cells, the repeatable access to the subcutaneous adipose tissue and relatively easy enzyme-based isolation procedures.
In the present work we have prepared and characterized different hydrogel blends composed by k-carrageenan (k-C) and PVA. Their mechanical and morphological properties, as well as their degradation behavior have been characterized, in the prospect of using them as a 3D bio-matrix in which hADSCs can be seeded for cartilage reconstruction.

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Tuning Curvature and Phase Behavior of Monoolein Bilayers by Epigallocatechin-3-gallate: Structural Insight and Cytotoxicity

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The application of Glyceryl Monooleate based NanoParticles (GMO/NPs) containing Epigallocatechin-3-gallate (EGCG) has a great potential as a new anticancer treatment. The EGCG is a green tea polyphenol with antitumoral activity that was reported in different kind of cancer cells, such as breast, lung and liver [1]. [2]. A pronounced chemical and metabolic instability limit the biological activity of EGCG and makes it necessary to protect the molecule by encapsulation into a delivery system. Because the inclusion of different concentrations of a guest molecule can produce supramolecular structures with different curvature and phase behavior, in this work we investigated structural modifications in GMO/NPs stabilized by Poloxamer-407 and loaded with increasing EGCG amount (5, 10, 18 % w/w) by using Small Angle Neutron Scattering, X-Ray Diffraction experiments and Molecular Dynamic Simulations. The size and the morphological characteristics of the different preparations were studied by Dynamic Light Scattering and Atomic Force Microscopy. Measurements on the cytotoxic activity of the GMO/NPs were performed in human lung carcinoma A549 cell lines by means cellular metabolic evaluation (MTT assay). We found that the formulation containing 18% EGCG showed high cytotoxic activity in human lung carcinoma A549 cell lines than other formulations.

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STRUCTURAL INSIGHTS INTO FUSION MECHANISMS OF EXTRACELLULAR VESICLES WITH MODEL MEMBRANES

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Extracellular vesicles (EVs) are a potent intercellular communication system [1]. Such small vesicles transport biomolecules between cells and throughout the body, strongly influencing the fate of recipient cells. Due to their specific biological functions they have been proposed as biomarkers for various diseases and as optimal candidates for therapeutic applications [2]. Despite their extreme biological relevance, their mechanisms of interaction with the membranes of recipient cells are still hotly debated [3]. Here, we propose a multiscale investigation based on atomic force microscopy, small angle X-ray scattering, small angle neutron scattering and neutron reflectometry to reveal structure–function correlations of purified EVs in interaction with model membrane systems of variable complex compositions and to spot the role of different membrane phases on the vesicle internalization routes. Our analysis reveals strong interactions of EVs with the model membranes and preferentially with the borders of protruding phase domains. Moreover, we found that upon vesicle breaking on the model membrane surface, the biomolecules carried by/on EVs diffuse with different kinetics rates, in a process distinct from simple fusion [4]. The biophysical platform proposed here has clear implications on the modulation of EV internalization routes by targeting specific domains at the plasma cell membrane and, as a consequence, on EV-based therapies.

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CHARACTERIZATION OF CASEIN-LOADED PROTEOLIPOSOMES, POTENTIAL INHIBITORS IN AMYLOID FIBRILLOGENESIS

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The Aβ aggregation process follows a typical nucleation pathway, with the exponential growth limited by the initial formation of intermediate oligomeric species presenting the highest cytotoxic activity.These smaller soluble Aβ prefibrillar oligomers are in fact able to interact with membranes by interfering with cellular functioning. Sequestering these oligomers or prefibrillar aggregates, rather than mature fibers, is a therapeutic strategy to recruit dangerous species that can interact with membrane components such as lipids or receptors or onset formation of sticky pathological aggregates.
αs_1-Casein results able to inhibit the amyloid nucleation phase by interacting with the Aβ peptide as well as the insulin peptide with a consequent evident delay of the entire fibrillogenesis process [1,2]. αs_1-Casein due to its amphiphilic nature, being an intrinsically disordered protein, is able to work as a chaperone-like system, by sequestering metastable protein species, having a high tendency to further aggregate. In order to be tested as an inhibitor for the treatment of Alzheimer disease (AD), it is crucial to define a way to efficiently protect αs_1-Casein to be delivered to the brain. On this purpose unilamellar proteo-liposomes, 50nm large, composed of phospholipids, cholesterol and αs_1-Casein (Lipo Cas) were made and purified. Nanosystems were characterized by different biophysical techniques such as multi-angle light scattering, zeta-potential, AFM imaging and small angle x-ray scattering. Lipo Cas have been tested in vivo on a C. elegans AD model, expressing the Aβ peptide in muscle [3]. In these animals, intracellular amyloid deposits form constitutively in muscle cells and the accumulation of aggregates hinders the movement of the animal. Treatment with Lipo Cas, but not empty liposomes, improved this defect in a dose dependent manner and far more efficiently than using free αs_1-Casein. These results suggest that liposomes allow an efficient delivery of αs_1-Casein in a whole animal, without toxic effects.

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Ultrastructural analysis of ribosome organization in large polysomes

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The ribosome is the macromolecular machinery which plays an essential role in protein synthesis, translating an mRNA into a protein. During this process, multiple ribosomes bind to the same mRNA, forming a more complex structure, known as polyribosome, or polysome.
Whilst the structure of ribosomes has been deeply investigated, the overall organization of polysomes and their role in translational control is still poorly understood. Different classes of ribosomal assemblies [1] and recurrent geometrically arranged ribosome-ribosome interactions within small polysomes (less than five ribosomes) have been previously observed. However, the presence of local and non-stochastic assemblies of ribosomes within more complex polysomes is still unexplored.
For these reasons, we propose a pipeline to systematically analyze a large number of polysome images and unravel the presence of recurrent organized ribosome sub-assemblies forming composite polysomes. We visualised and collected images of native mammalian polysomes using Atomic Force Microscopy, and modelled the relative organization of adjacent ribosomes as graph data structures.
First, we searched for previously identified locally-organized small ribosome assemblies, defined “cliques” [1]. This analysis confirmed the presence of repetitively arranged sub-assemblies of ribosomes at the basis of more complex polysomes. Next, we investigated polysome organizations under various physiological and pathological conditions and observed different conformations when translation is inhibited.
Our findings suggest a possible model of the organization of the translation machinery where more complex polysomes might result from ultra-structural arrangements of heterogeneous and defined ribosomal cliques with precise geometric conformations and functionally related features.

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AN AUTOMATED TOOL TO QUANTIFY CHROMATIN COMPACTION IN 3D FLUORESCENCE IMAGES

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Chromatin arrangement undergoes various structural changes during the entire cell life. The spatial structure of the chromatin within the nucleus is not random. Indeed, it is related to the silencing/activation of chromosomal territories, and therefore it is fundamental for proper cellular functioning. However, how the chromatin architecture varies during cellular processes is still partially unknown. The recent development of three-dimensional (3D) fluorescence microscopy facilitates access to the organization of chromatin at the nanoscale by optical means [1]. Here we present an automatic tool to quantify chromatin compaction from 3D image stacks of stained nuclei. The first step in the analysis is nuclei segmentation. Since it is common to have multiple stained nuclei in the same field of view, we can train a neural network to separate the nucleus of interest from the background and the other nuclei. Once the region of interest is determined, we can compute different morphological measures and statistical analysis on fluorescent intensities. Furthermore, the nucleus can be clustered, thus identifying other compactness regions. By providing a parameter-free and automatic numerical quantification of chromatin compaction, this workflow opens the way for biologists to study how chromatin structures form and behave in various cellular processes, from physiological to pathological ones.

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REVERSIBLY SWITCHABLE PROTEINS IN TWO-PHOTON PHOTOACOUSTIC MICROSCOPY

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The photoacoustic (PA) effect is a physical phenomenon that involves the generation of soundwaves following the light absorption allowing deeply penetrating imaging in thick biological samples [1]. Since scattering depends on wavelength the use of near infrared light (NIR) improves the penetration depth. Therefore, two-photon microscopy (TP) represents one of the election techniques to couple with PA. The resulting two-photon photoacoustic imaging (TP-PAI) could be an innovative tool in the study of thick samples. While the spatial resolution decreases at longer wavelengths the two-photon excitation process allows a partial compensation preserving a good compromise in terms of tree-dimensional resolution and signal-to-background contrast [2,3]. The application of reversibly switchable proteins properly designed for PA such as Slr1393 (GAF3) [4] could be use both to overcome resolution limitation in a RESOLFT approach or to increase the contrast.

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INTERACTION OF ALPHA-SYNUCLEIN WITH THE INTERNAL AND EXTERNAL LEAFLET OF THE LIPID MEMBRANES

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ABSTRACT

α-synuclein (α-syn), a peptide involved in Parkinson's disease, is present in both the inner (cytosol) and extracellular space. Both the endogenous and exogenous components seem to have a role in the neurodegenerative process typical of the disorder. The cell membrane has an asymmetric structure, i.e., the lipid compositions of the inner and outer leaflet are different. As a consequence, also the organization of the lipid phase in the two leaflets are different. We prepared supported lipid bilayers (SLBs) with two different compositions that mimick the inner and the outer leaflet of the neuronal membrane, respectively. We studied the interaction between α-syn and these two types of SLBs, to reveal the possible destabilization induced by the pathological peptides. It is known that the diffusion of lipids is affected by the presence of the supporting rigid substrate. In particular, the interaction between the SLB and the subtsrate induces a mechanical stabilization of the membrane, that increases its resistance toward the action of an external agent. We proposed an alternative approach, making the peptides interact with liposomes in solution, forming a planar bilayer after this interaction. In this way, α-syn interacts with free diffusing vescicles, that better resembled the properties of the cell membrane. We characterized the membranes before and after the interaction with α-syn by atomic force microscopy, quartz micor-balance, and fluorescence techniques.

A NOVEL TARGETING APPROACH FOR MELANOMA CANCER TREATMENT BASED ON PHOTODYNAMIC THERAPY

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Photosensitizing molecules have been at the basis of photodynamic therapy (PDT) since its early development in the 1950s. The photosensitizer (PS) is one of the three main PDT components, in addition to visible light and molecular oxygen. The therapy is a selective treatment method based on the administration of the PS to diseased cells: when irradiated with visible light of suitable wavelength, the photo-activated molecule starts a cascade of molecular transitions leading to cytotoxic effects promoted by the formation of singlet oxygen in proximity of the targeted cells [1].
The achievement of effective bio-compounds with photosensitizing capabilities deeply relies not only on carrying capabilities [2] but also on an increased selectivity towards specific types of cancer. Hence, the effective design of targeting properties lies at the heart of this therapy. The aim of the herein research project is to create an all-in-one multi-functional bio-molecule to be used in PDT treatments for melanoma cancer cells, with this compound showing targeting, imaging and photosensitizing features.

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NEAR-INTERFACE BEHAVIOR OF PRIMARY CARDIAC FIBROBLASTS UPON CYTOSKELETAL ACTIN ALTERATION

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Every year highly numerous death due to heart failure in patients with cardiomyopathies are recorded. This is an extremely important sanity problem which requires many efforts for a deeper understanding of the causes. Recently, the alteration of the cell's cytoskeleton has been shown to be at the base of many cardiomyopathies [1]. The cytoskeleton is a vital component of the cells, which imparts them rigidity and elasticity and thus the ability to react under external stimuli. Moreover, it is involved in many cellular processes such as adhesion process through binding proteins employed in the connection with the extracellular matrix [2, 3]. Therefore, in the aim of early diagnosis and successful disease treatment, the study of cell mechanical properties changes occurring as a consequence of this kind of cellular disorder, could lead to the development of future and powerful biomarkers.

For this purpose, we studied the behavior of cardiac fibroblasts at the near-interface with the substrate, upon cytoskeletal alteration induced by Cytochalasin treatment. We have used two complementary techniques: Quartz Crystal Microbalance (QCM) and Digital Holographic Microscopy (DHM).

QCM is a valid solution for monitoring real-time cells variations whereas a frequency resonant quartz sensor allows monitoring the near-interface changes. [4]. Thus, we used the QCM to investigate viscoelastic changes occurring under the alteration of actin filaments by Cytochalasin D (Cyt-D) 5 µM. Primary cardiac fibroblasts from rats (5-6 days) seeded on the QCM AT-cut quartz crystal sensor were monitored for 4 hours after Cyt-D injection. The study of cellular viscoelastic properties variations was based on the interpretation of the QCM frequency (Δf) and dissipation (ΔD) shifts. We observed major variations of Δf and ΔD shifts within the first hour and mostly in the first 10 minutes, in accordance with typical range of Cyt-D action on actin network [5]. The Cyt-D effect on the actin filaments was also checked by fluorescence imaging. From the positive Δf shifts, we determined the cells tendency to lose the adhesion to substrate and to detach from the surface upon treatment. Moreover, we ascertained that cells go towards a more liquid-like state with less energy dissipation and reduced viscosity, confirmed by negative ΔD shifts.

DHM was used to assert the cell morphological changes due to the Cyt-D treatment. Cells showed a progressive decrease in the projected area (about 60% within the first hour of treatment) and an increase in thickness due to the loss of focal adhesion and rounding up. We measured also the cell membrane fluctuation (CMF) but we could not find appreciable differences between healthy and altered cells, because the CMF range was very small, near to that of the instrumental noise.

In order to validate the real-time behavior upon treatment, we performed a series of time lapse at 2 frame per second for 15 minutes on single cells. We observed a decrease of about 20-30% in the projected area within the first 10 minutes of treatment and an overall decrease of about 50% in 15 minutes.

In conclusion, we studied the rheological and morphological changes of fibroblasts treated with a known actin drug (Cyt-D). Even if actin is known as the main cytoskeletal component involved in preserving mechanical and morphological properties in healthy cells [6], to address also the contribution of other cytoskeletal components, such as microtubules and intermediate filaments, we will implement similar experiments with suitable drugs.

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AN ADAPTABLE 3D PRINTED DEVICE FOR CELLULAR MECHANOSENSITIVITY ASSAY

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The ability of cells to sense external mechanical stimuli and exert forces to their surrounding environment is called mechanotransduction and is involved in a variety of physiological processes e.g. growth, motility, stem cells differentiation and tumour progression. Mechanosensitive ion channels (MSCs) are considered having a crucial role in mechanotransduction pathway, in particular Piezo family (Piezo1 and Piezo2), identified by Coste et al. in 2010 [1]. Several technologies have been employed to study cellular response to mechanical stimuli focusing on MSCs role, i.e. exerting mechanical cues on single cells and observing the effects of MSCs opening. We propose an open-science approach for dynamically providing mechanical stimulus to single cells, exploiting 3D printing and open-source computer-aided drafting (CAD) projects adaptability. The device allows the micromanipulation of a glass probe through a monolithic 3D printed flexure translation stage, capable of sub-micron-scale motion [2]. The system's adaptability to different read-out technologies opens up new possibilities to study mechanotransduction process. Integration with fluorescence microscopy has allowed us to test the assay assessing Ca2+ ion flux inward cells membrane, a downstream effect of cellular mechanosensitivity. Mouse embryonic mesencephalic neuron-derived cell line (A1 mes-c-myc) has been transfected with human Piezo1-GFP plasmid (A1 OV) and the correlation between cellular mechanosensitivity and Piezo1 expression has been tested. A wide range of possible applications will be possible by integrating our device with different read-out technologies and will permit to investigate several aspects of mechanotransduction pathway.

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In primary melanoma cells calcium entry through TRPM2 channel activates two distinct potassium currents

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Ion channels play an increasingly recognized role in various aspects of cancer, including migration, proliferation, and apoptosis. In particular, specific alterations of key physiological parameters like a change in the concentration of reactive oxygen species can have direct and indirect effects on the activity of ion channels. Here, we studied the response to an oxidative stimulation in primary human melanoma cells (IGR39) and in metastatic cells from the same patient (IGR37) performing patch clamp recordings, intracellular calcium ($[Ca^{2+}]_i$) imaging and RT-qPCR gene expression analysis. In IGR39 cells, the mild oxidizing agent Chloramine-T (Chl-T, 0.5 mM) activated large K^+ currents (KROS) that were partially sensitive to tetraethylammonium (TEA). A large fraction of KROS was inhibited by Paxilline, a specific inhibitor of large conductance Ca^{2+}-activated BK channels. The TEA-insensitive component was inhibited by Senicapoc, a specific inhibitor of the Ca^{2+}-activated KCa3.1 channel. BK and KCa3.1 activation was mediated by an increase of $[Ca^{2+}]_i$ induced by Chl-T. Both, KROS and $[Ca^{2+}]_i$ increase were inhibited by ACA and Clotrimazole, two different inhibitors of the calcium permeable TRPM2 channel. We performed the same experiments in IGR37; surprisingly, IGR37 cells did not exhibit current increase upon Chl-T application. Expression analysis confirmed that the genes encoding BK, KCa3.1 and TRPM2 are much more expressed in IGR39 compared to IGR37. The potassium currents and increase of $[Ca^{2+}]_i$ observed in response to the oxidizing agent strongly suggest that these three molecular entities play a major role in melanoma progression. Pharmacological targeting of either of these ion channels could be a new strategy to reduce the metastatic potential of melanoma cells, and could complement classical radio- or chemotherapeutic treatments.

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CALCIUM SIGNALING IN PROSTATE CANCER CELLS OF INCREASING MALIGNANCY

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Calcium signaling controls a large variety of cell functions, including proliferation and apoptosis and it is believed to play a role in neoplastic transformation. Perturbed intracellular Ca^{2+} homeostasis is a cause and a consequence of many cell pathological states and a long lasting question in cell signaling is how Ca^{2+} is able to achieve specificity with its abundant and varied intracellular targets. Therefore, it is important to characterize the components that cells express to generate Ca^{2+} signals ("Ca^{2+} signaling toolkit", [1]) and their altered expression or mutation that is observed in several cancer types and in cancer development.

Prostate cancer (PCa) is one of the most common malignancies for men in industrialized countries. Androgen deprivation, the mainstay of treatment for advanced PCa, frequently but not always results in remission of tumor, while many patients progress to castration resistant prostate cancer (CRPC), a lethal form lacking effective cure. The transition to CRPC could be influenced by fine tuning of intracellular Ca homeostasis.

This work investigates internal calcium dynamics in metastatic prostate cancer cell lines that mimic the progression of PCa to androgen resistance: (i) well differentiated LNCaP cells that require dihydrotestosterone (DHT) for survival, and (ii) poorly differentiated, highly aggressive androgen-independent prostate cancer (AIPC) PC3 and DU145 cells that are largely DHT-independent for their growth. $[Ca^{2+}]_i$ was measured in real time and on viable cells by the fluorescent dye Fura-2, which provides a quantitative estimate of $[Ca^{2+}]_i$ variations. Cells are incubated in the esterified form of the dye, Fura-2-acetoxymethyl ester (Fura-2-AM) and subsequently exposed to different stimuli by external solution exchange.

$[Ca^{2+}]_i$ measurements revealed important differences in the behavior of LnCaP cells and that of PC3 and DU145 cells. In AIPC PC3 and DU145 cells, ATP induces a fast rise in intracellular Ca^{2+}, which was entirely released from intracellular stores, was sensitive to phospholipase C (PLC) inhibitors and most probably due to the activation of P2Y receptors. In contrast, androgen-sensitive LnCaP cells do not respond to ATP challenge, suggesting a different functional purinergic receptor profile and sensitivity. This observation is in agreement with the previously reported hypothesis that purinergic receptors may represent a pharmacological target for prostate cancer treatment [2].

A second significant difference it that AIPC cells show a reduced capacity to store Ca^{2+} in thapsigargin-sensitive stores and limited store-operated calcium entry (SOCE), with respect to androgen-dependent LnCaP cells.

The consequences of these differences will be discussed and interpreted with reference to previously proposed models for Ca^{2+} dependence of prostate carcinogenesis.

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NEUROBIOPHYSICS OF VOLTAGE-GATED ION CHANNELS IN SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA)

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Recent scientific literature of neurodegenerative diseases (NDs) reports a particular attention to the role of the alteration of neuronal excitability in the onset of several NDs. Emerging evidence from animal and *in vitro* cellular models of disease indicates that abnormal neuronal function (at single cellular or network level) could contribute to symptoms of the disease. Hence, attenuation and/or recovery of altered neuronal activity could improve the disease phenotype: Therefore, targeting pharmacologically specific ion channels' activity could represent a potential therapeutic strategy.
In polyglutamine (polyQ) NDs the role of ion-channel dysfunction has been ascertained in Huntington's disease and in a variety of spinocerebellar ataxias, while it has been quite neglected in spinal and bulbar muscular atrophy (SBMA). SBMA, also known as Kennedy's disease, is an adult-onset, genetically inherited (X-linked) neuromuscular disorder, characterized by fasciculation, weakness and atrophy of bulbar and limb muscles besides a marked loss of motor neurons in the spinal cord and brainstem which is correlated with the high expression of AR in these neuronal tissues. In SBMA the AR exhibits an abnormal amount of polyQ repeats, which causes its misfolding and cell accumulation leading to motor neurodegeneration. The causative mutation in SBMA is a glutamine-encoding trinucleotide CAG repeat expansion in the first exon of the androgen receptor (AR) gene. In normal conditions, the polyQ-AR tract ranges from 9 to 36 residues. However, a repeat number higher than 38 is considered pathogenic. Under pathological conditions, the stimulation of the AR by the natural agonists testosterone or dihydrotestosterone (DHT) is required for the induction of polyQ-expanded AR-mediated toxicity.
For some years, we have combined patch-clamp electrophysiology, pharmacology and molecular biology to study the key-elements, i.e. ion channels, of the neuronal excitability in a cell model of SBMA in order to demonstrate that defects/alterations of ion currents are a suitable cellular marker of the disease; in particular, we have addressed our attention to the role of voltage-gated channels (VGCs) and their modulation.
i) Recording DHT-stimulated MN-1 cells expressing AR with an expansion of 100 glutamine residues (AR100Q MN-1), which are considered a pathogenic model of SBMA and recognized to recapitulate AR toxicity, our group first has analysed Na^+, K^+, Ca^{2+} and Cl^- voltage-gated currents in SBMA cell model and has showed different alterations of each current. We demonstrated the expression of polyQ expanded AR causes an androgen-dependent reduction of the macroscopic membrane ionic currents in polyQ-AR motor neuron-derived (MN-1) cells [ref. 1]. Furthermore, pharmacological rescuers that ameliorate the SBMA phenotype (the growth factor IGF-1 and the neuroprotector peptide PACAP) in animal models were able to restore VGCs activity. Our results showed that the pathological conductive state predisposes neurons to activity alterations which are reversed by those pharmacological rescuers.
ii) In AR100Q MN-1 cells we have identified anionic currents that resemble those of voltage-gated ClC-2 channels [ref. 2]. We found an alteration of ClC-2 currents in DHT-stimulated AR100Q cells; moreover, the incubation with PACAP produced an attenuation of the chloride current defects in SBMA MN-1 cells, uncovering a functional mechanism by which PACAP might exert its efficacy, indicating that motor neurons may be vulnerable to an excitotoxic damage *in vivo*, and that ClC-2 channels may represent a novel target for therapeutic intervention in SBMA.
iii) In AR100Q MN-1 cells we have identified clenbuterol-sensitive delayed outward potassium currents [ref. 3]. We demonstrated that the incubation with β_2-agonist clenbuterol ameliorates ionic alterations through the modulation of tetraethylammonium (TEA) sensitive delayed rectifier potassium (I_K) currents. These findings indicate TEA-sensitive I_K currents as important determinants in SBMA pathophysiology, and clenbuterol as potential pharmacological option of the disease.
Taken together**,** our results reveal that VGCs are functionally altered in SBMA MN-1 model cells and that the above mentioned pharmacological rescuers ameliorate the SBMA phenotype in animal models restoring the VGCs activity. We propose that specific VGCs currents are involved in the onset of the motor neuron degeneration of SBMA, supported by our findings which have shown that Na^+, K^+, Ca^{2+} and Cl^- currents are indeed affected during the pathophysiology and represent attractive molecular targets for future research on diagnostic and therapeutic approaches. Moreover, the pharmacological rescuing of altered channels' activity holds up that these molecular targets are able to ameliorate or inhibit symptoms of SBMA and, possibly, its outcome.

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expressyouRcell: shooting for time-space changes in gene expression in cells

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expressyouRcell is a unique and easy-to-use R package which provides an intuitive approach for visualizing and presenting time-course variations of gene expression levels in cellular compartments. This tool gives the possibility of generating animations of pictographic representations of cells, or pictograms, providing a convenient method for mapping and visualizing the localization and amount of gene expression levels variations in cellular compartments across time.
Next generation sequencing (NGS) is nowadays widely adopted in several biology applications for investigating RNA changes across different experimental conditions. This led to a massive increase in the amount of NGS data, improving our understanding of complex biological processes. On the other hand, the complexity of NGS data also requires a clear and easy interpretation of biological meaning of data. Biological processes and mechanisms associated with variations in gene or transcript levels are commonly assessed by differential analysis and annotation enrichment analyses. However, effective computational pipelines and dedicated graphical tools to support the communication of time course variations are still lacking and strongly required by the scientific community.
An immediate and intuitive approach for representing variations of variables across different regions of a given area across time is provided by choropleth maps. Likewise, fluctuations of gene and transcripts expression levels can be mapped directly to specific cellular compartments and visualized in cell pictograms. A handful of tools have been proposed to display gene expression data directly on anatomical pictograms, or anatograms, representing several tissues from multiple organisms, or, in one case, on a generic cellular pictogram. However, the available pictograms are not suitable for representing specific cellular types and do not allow the creation of animated pictures for visualizing time-course changes. Filling this gap, expressyouRcell provides a range of customizable options for generating animated cell pictograms based on different gene expression measures, such as the number of reads per gene, CPM or FPKM over time. Alternatively, expressyouRcell can also exploit values obtained from upstream differential analyses, such as fold changes, which is particularly useful to highlight the cellular compartments and structures mostly affected across different experimental conditions. Compared to other tools for data visualization, expressyouRcell can be exploited to generate clearer and more effective representations of time course variations in gene expression levels, leading towards a more comprehensive understanding on several physiological and pathological conditions.

MICROFLUIDIC IMPEDANCE CYTOMETRY: A NEW TOOL TO STUDY ANTIMICROBIALS PEPTIDES AT THE SINGLE-CELL LEVEL

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Antimicrobial peptides (AMPs) represent a promising class of compounds to fight resistant infections. In most cases, they kill bacteria by making their membrane permeable [1, 2]. However, in view of their clinical application, the absence of significant toxicity is almost as important as a good activity. Unfortunately, the interaction of AMPs with bacteria and human cells simultaneously is still not fully understood [3], also due to the need to deal with heterogeneous cell populations in microbiological studies [4]. In addition, standard assays for determining the activity of antimicrobials are based on the analysis of the growth kinetics of a bacterial population and therefore require many hours.
With the aim of understanding the activity and selectivity of AMPs, we are currently developing microfluidic impedance cytometry as a new tool to study the effects of AMPs at the single cell level. This technique involves the measurement of the electric field screening of individual cells flowing over patterned electrodes integrated in a microchannel, as accomplished by electric current variation under an applied AC voltage. The measured frequency-dependent impedance depends on cell features, i.e., volume and dielectric properties [5] and can be analyzed with appropriate signal processing for the characterization of cellular electrophysiology [6].
In our experiments, we analyzed both bacteria and human cells, incubated with different concentrations of the DNS-PMAP23 AMP, using a coplanar-electrode microfluidic impedance chip [6]. For these studies, we selected human erythrocytes (obtained from healthy donors) and *Bacillus megaterium* Bm11 cells, because their relatively large dimensions are compatible with the size of the microfluidic channels. Electrical impedance spectroscopy allows the analysis of thousands of cells per minute and the impedance magnitude and phase data (particularly those collected at 10 MHz) allow the discrimination of healthy cells from those whose membranes were made permeable by the AMP. In addition, our preliminary data indicate that microfluidic impedance cytometry can sense peptide-induced pores that are smaller than those detected by traditional microbiological approaches. Overall, electrical impedance spectroscopy is a very promising approach for the development of next- generation fast and sensitive antimicrobial activity assays.

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The VRAC blocker DCPIB directly activates the Ca^{2+}-activated K^+ channel BK and increases intracellular Ca^{2+} in melanoma and Pancreatic Duct Adenocarcinoma (PDAC) cell lines

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The volume-regulated anion channel (VRAC) is an heteromeric channel which activates in response to cell swelling [1,2]. This channel has been found to play an important role in in different aspects of cancer cells behavior and response to therapies [3,4,5]. For this reason, we decided to study the VRAC current in two types of cancer: pancreatic duct adenocarcinoma (PDAC) and melanoma. PDAC is one of the deadliest types of cancer, as it has a 5-year survival of around 5% [6]. The outcome of melanoma depends on the stage of the disease at the time of diagnosis, with 5-year survival rate dropping from 93% for stage IIIA to 32% for stage IIID [7]. For our study, we employed two PDAC lines (Panc-1 and MiaPaCa-2), as well as a primary (IGR39) and a metastatic (IGR37) melanoma line, obtained from the same patient. To better characterize the VRAC current in those cells, we employed DCPIB, a presumably specific blocker of VRAC [8]. Surprisingly, we found that DCPIB induced a dramatic increase of whole-cell currents in Panc1, MiaPaca2 and IGR39, but not in IGR37 cells. The induced currents were sensitive to tetraethylammonium (TEA) and thus not mediated by K2P channels, known to be activated by DCPIB [9]. Rather, the currents seemed to be mostly mediated by the large conductance Ca^{2+}-dependent BK channel [10], as they were inhibited by paxilline, a known blocker of BK. Gene expression analysis showed that BK mRNA could be detected in Panc-1, MiaPaCa-2 and IGR39 but not in IGR37 that is the only cell line, among those employed, insensitive to DCPIB. We verified DCPIB activation of BK channels also in HEK293 cells transfected with only the α subunit of the channel. Further experiments showed that in IGR39, and to a smaller degree also in Panc-1 cells, DCPIB induces a rapid Ca^{2+} influx. This, in turn, indirectly potentiates not only BK but, in IGR39 cells, additionally activates other Ca^{2+}-dependent channels. However, the Ca^{2+} influx is not required for BK activation by DCPIB. The direct activation of BK by DCPIB involves the extracellular part of the protein, as no effect was detectable when DCPIB was delivered inside the cell via the patch pipette. We conclude that the BK channel is a new target of DCPIB, and that the latter can acutely increase intracellular Ca^{2+}, elongating the list of DCPIB side-effects that need to be taken into consideration for future development of DCPIB-based activators/inhibitors of ion channels and other membrane proteins.

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Pharmacological treatment option for a genetic form of migraine assayed in mouse cortical neuronal cultures

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In vitro cultured neuronal networks are a valuable experimental model to unravel many aspects of brain physiology. However, it is unclear to which extent cultured neurons can reliably model genetic diseases. Indeed the establishment of an *in vitro* model based on cultured neurons presents some limitations needed to be taken into consideration. On the one hand, 2D-cultures cannot represent the full complexity of the brain. Another concern regards the experimental variability among different preparations of neuronal cultures. Taking these limitations into consideration, in this work, we tested the usefulness of cortical neuronal cultures obtained from genetically modified knockin-mice (KI) mimicking a human monogenetic migraine model.

The KI mice recapitulate familial hemiplegic migraine of type 3 (FHM3), a genetic form of migraine with aura caused by gain-of-function mutations in the SCNA1 gene encoding the Nav1.1 channel [1]. In particular, we employed a KI mouse model of FHM3 bearing a mutation in the Scn1a gene encoding the Nav1.1 channel; for this mouse model, neurophysiological phenomena have been recently investigated [2], disclosing key differences between the heterozygous and the wild-type phenotype. In the present work, brain cortices were dissected from WT and FHM3-KI embryos at E17 and dissociated neurons were cultured in defined medium for up to 25 days. Cultures were then studied by whole-cell and outside-out patch clamp recording and population excitability recording using microelectrode arrays (MEAs). By patch-clamp recording, kinetic parameters of Na current activation and inactivation were determined for single neurons, while using MEA devices electrical activity of the whole cortical network was investigated. In particular, the effect of the Na current blocker GS967 on the spontaneous electrical activity of the network was investigated for the different genotypes (WT, heterozygous KI, homozygous KI). In addition, the expression level of brain Nav channel genes, namely Scn1a, 2a, 3a, and 8a, was estimated by RT-qPCR to detect possible dysregulation caused by FHM3 disease. Overall, the results suggest that the *in vitro* model can recapitulate several features observed in the FHM3 mouse model and it is worth emphasizing that, despite its limitation, the use of dissociated culture may be a valid alternative method to reduce the number of animals used in experimental studies.

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LIBRATIONAL MOTION AND DYNAMICAL TRANSITION IN PROTEINS AT DIFFERING HYDRATION LEVEL

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The librational motion is an orientational molecular motion which dominates the dynamics of macromolecules at cryogenic temperatures. It consists of rapid oscillations on the nanosecond timescale of small angular amplitude. This motion is appropriately studied by using spin-label electron paramagnetic resonance (EPR) spectroscopy [1, 2]. The temperature dependence of the mean-square angular amplitude of librations detected from EPR parallels that of the mean-square atomic displacement in diffraction studies and it displays a rapid enhancement at the temperature of the so called dynamical transition [3,4]. Such a dynamical signature of protein dynamics is influenced by hydration water, which in turn is fundamental in the activation of the biological functionality [5].
We investigated the dependence of the librational oscillations and the dynamical transition on the hydration conditions of two globular proteins, namely β-Lactoglobulin (β-LG) and human serum albumin (HSA), by spin-label EPR in the temperature range 120-270 K. The proteins have different shape and size (18 kDa for β-LG vs 66 kDa for HSA) and secondary structure (mainly α-helical for HSA and β-sheet for β-LG). They were spin-labelled with 5-maleimide spin-label on exposed cysteins (Cys121 in β-LG and Cys34 in HSA) to probe the protein surface and prepared at high water content (> 0.55 mg H_2O/mg protein), at low water content (0.10 mg H_2O/mg protein), and in the lyophilized state. The results show that the angular amplitudes of librations are small (ca. 3° in HSA and 6° in β-LG) and almost constant over the entire temperature range for both lyophilized proteins. In these samples, the librational dynamics is therefore inhibited and the dynamical transition is absent. Independently from protein structure, librational oscillations of considerable and similar amplitudes are recorded in the hydrated proteins. They increase first slowly and then more rapidly on increasing the temperature, reaching ca. 10-12° at the highest temperatures. Low and fully hydrated proteins undergo the dynamical transition at about 230 K and cross low energy barriers of about 20 kJ/mol. The overall results indicate that protein dynamics activates even at a such low water content and highlight biophysical properties that are shared by hydrated proteins in the low-temperature phase regardless of their secondary structure.

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THE MOLECULAR-SCALE BIOPHYSICS RESEARCH INFRASTRUCTURE (MOSBRI)

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The MOlecular-Scale Biophysics Research Infrastructure (MOSBRI) enables the cutting-edge integrative investigation of bio-systems at the critical intermediate level between atomic descriptions and cellular-scale observations.
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High“lighting” the effect of solar radiation on collagen fibers

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Collagen is one of the main components of the extracellular matrix and its molecular organization is responsible for mechanical and physical properties of tissues. Due to its ubiquity in almost every biological tissue it is often object of different stresses (mechanical, chemical, photochemical). Understanding the effect of solar radiation on collagen has great implications in skin health, humans’ larger organ. Indeed, UV induces modifications on collagen structure and morphology, which are often linked to early skin aging and to the occurrence of pathological conditions. Here, we present an experimental study on the effects of solar radiation on type I collagen aimed at understanding the solar induced modifications at molecular level and at identifying potential fluorescence markers to characterize them.
The sample is irradiated with a solar simulator, which provides a close spectral match and a comparable intensity to natural sun light but in controlled conditions and analyzed by means of spectroscopy and time resolved non-linear optical imaging. Irradiation is found to induce a clear reduction of the native autofluorescence of collagen which is attributed to aromatic residues and cross-linking stabilizing collagen structure. Following light irradiation, structural modifications are observed by circular dichroism spectroscopy which parallels a reduction of the sample turbidity, attributed to the reduction of supramolecular structure size in solution.
To better understand the nature of solar light-induced damages we use 8-Anilinonaphthalene-1-sulfonic acid (ANS), a well-known fluorescent dye, whose fluorescence depends on the specificity of the molecular environment. ANS is known to bind to collagen fibers, in proximity of the tyrosine residue so that changes in its spectral features (fluorescence lifetime and steady state spectra) provide information on polar domains in collagen triple helix telopeptide. Fluorescence lifetime imaging (FLIM) measurements of ANS-stained samples, analyzed by means of the phasor approach, was used to observe topological modifications and changes at molecular level as the light-exposure increases. FLIM analysis show an overall reduction of ANS fluorescence lifetime and the growth of its lifetime distribution heterogeneity. This reveals that the solar radiation induces a variety of phenomena (from cross-links cleavage to oxidations), that reduce the affinity of collagen ANS binding sites, this effect being the result of the combination of light and thermal effects.
Observed modifications are possibly at the basis of modifications in fibrillar collagen organization involved in skin photo aging.

MAPPING THE FUNCTION OF AROMATIC AMINO ACIDS IN A BLUE-LIGHT PHOTORECEPTOR

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Photoreceptors of the LOV (Light, Oxygen, Voltage) superfamily area ctivated by UVA/Blue (BL) light by forming a covalent adduct between the FMN (flavin mononucleotide) chromophore and a conserved cysteine, via the decay of the FMN triplet excited state. Engineered LOV domains lacking this reactive cysteine are valuable fluorescent tools for microscopy and as genetically encoded photosensitisers for singlet oxygen (SO) [1]. Furthermore, LOV domains naturally void of the reactive cysteine can still be functionally photoactivated via formation of the neutral radical $FMNH^{\bullet}$.Tryptophans and tyrosines are held as the main partners as potential electron donors to the FMN excited states[2]. Here we explore the relevance of aromatic amino acids in determining the photophysical features of the LOV protein *Mr*4511[3] from the plant symbiont *Methylobacterium radiotolerans*, by introducing point mutations into the C71S variant that does not form the covalent adduct. Eleven *Mr*4511 variants were generated for which we measured fluorescence quantum yields and lifetimes, triplet yields and lifetimes, and the efficiency of SO formation. Insertion of Trp residues at edge-to-edge distances between 0.6 and 1.5 nm from FMN, results in efficient quenching of FMN excited triplet state and, at the shorter distances, even of the singlet excited state. Mutation F130W (ca. 0.6 nm) completely quenches the singlet excited state, preventing FMN triplet formation: even when the reactive Cys71 is present, the photo-adduct is not formed. Tyrosines are also quenchers of FMN excited states as demonstrated with their substitution by inert phenylalanines. The variant C71S/Y116F has a prlonged triplet lifetime and the quantum yield of formation for singlet oxygen is 0.44 in aqueous aerobic solution, vs 0.17 for C71S [4]. Based on our results and on literature data for other LOV domains we suggest that Trp and Tyr residues at distances less than 0.9 nm from FMN, would reduce the yield of photoproduct formation, explaining why there are no such amino acids in close proximity to FMN in these photsensing domains. Introduction of inert Phe residues in key positions can also help developing more efficient LOV-based photosensitisers,as demonstrated with the C71S/Y116F variant.

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MONO-RHAMNOLIPIDS AND DI-RHAMNOLIPIDS DIFFERENTLY AFFECT PLASMA MEMBRANES REMODELING

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It has been reported that strains of P. aeruginosa are able to produce biosurfactants called rhamnolipids (RL) that, with significant tensioactive and emulsifying properties, have different applications, which have motivated the growing research activity to characterize their biochemical and structural properties [1]. RLs are formed by a hydrophilic head, composed of one or two molecules of rhamnose, called mono-rhamnolipds (monoRL) and di-rhamnolipids (diRL) respectively, connected to a hydrophobic tail [2]. It is important to evaluate the specific physicochemical and biological features of monoRL and diRL separately, in order to identify which are the components with the most optimal molecular properties that allow them to be used in formulations for tailored technological uses [3]. We have investigated the interactions between mimetic phospholipid plasma membranes in the form of giant unilamellar vesicles (GUV) with monoRL and diRL individually, separated from the commercial mixture. We performed phase contrast and fluorescence mode microscopy experiments on dispersions of GUVs in the presence of monoRL or diRL at different concentrations. GUVs formed by POPC and by ternary mixtures of DOPC, sphingomyelin (SM) and cholesterol (CHOL) in molar ratios 1:1:1, 3:5:2 and 5:3:2 were investigated under the microscope as a function of time after mixing with either monoRL or diRL. Therefore it was possible to evaluate simultaneously changes in lipid rafts organization, membrane remodelling, permeabilization and membrane disruption induced by monoRL and diRL. The images collected for each sample were analyzed using a method implemented through a series of macros under the ImageJ software, then we developed a method for determining the shape of the GUVs. The shape analysis finds the best contour for the GUVs and provides its average radius R, area A and volume V. These parameters were reported as a function of time after for each sample, and then A and V have been analysed with the kinetic model by means of a unique fitting calculation.

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TAURINE: AMINO ACID AGAINST PROTEIN THERMAL UNFOLDING AND AMYLOID AGGREGATION

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The conversion of normally soluble peptides and proteins into intractable amyloid deposits has emerged in the last decades as a subject of fundamental importance in scientific disciplines ranging from physics and chemistry to biology and medicine. The amyloid formation is associated both with the loss of protein functionality and with the generation of toxic intermediates in the process of self-assembly [1]. In this context, the study of osmotically active solutes including sugars [2], amino acids as well as polyols, which have stabilizing effects on the proteins structure [3], could open the door to new therapeutical intervention against neurodegenerative disorders like Alzheimer's Disease or Parkinson's Disease.

We present a study on the effects of the amino acid taurine as stabilizing agent against lysozyme thermal unfolding and amyloid aggregation. Although it has been recently reported that taurine has neuroprotective properties against dementia, including Alzheimer's Disease [4], the molecular mechanisms that determine taurine effectivity are still unknown. The aim of this work is to understand taurine effect in slowing down a model protein thermal unfolding and in altering the protein fibrillogenic process under denaturation conditions. The analysis was performed by a combination of biophysical techniques: Circular Dichroism, UV-Vis absorption spectrophotometry with Congo Red as dye for studying the secondary structures induced together with aggregation, and by Small Angle X-ray Scattering to investigate aggregates overall structure. Furthermore, optical microscopy and Atomic Force Microscopy (AFM) for the study the sample morphology in different solvent conditions had been employed. Results claim that taurine can determine protein stabilization against thermal unfolding and modify amyloidogenic patterns, providing noticeable information on its use in Alzheimer's disease therapy.

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STRUCTURE-CONTROLLED VISCOSITY AND DIFFUSIVITY IN GUANOSINE HYDROGELS FOR DRUG DELIVERY APPLICATION

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Supramolecular hydrogels represent a new class of biomaterials with excellent potential applications in tissue engineering and drug-delivery due to their unique properties such as biocompatibility, biostability, self-assembling, self-healing and external stimuli-responsiveness [1][2].

The case of guanosine is particularly relevant [3]. Guanosine 5'-monophosphate (GMP) in water self-assembles in supramolecular, columnar helicoidal structures (G-quadruplexes), made by stacked GMP planar tetramers (G-quartets). If the hydrophobic guanosine (Gua) is also present in solution, stable and transparent hydrogels, made by a quadruplex 3D network, occur at high hydration (up to 99% w/w of water) and over a large temperature range: in fact, GMP helps to solubilize the insoluble guanosine (that enters in the formation of G-quartets) and the insolubility of Gua promotes the gelation at low concentrations, probably because of the reduction of the number of charges-per-unit-length in the G-quadruplexes. Quadruplex interactions, flexibility and chirality seem to play a unique role in the hydrogel formation and stability.

Here, we present a FRAP analysis of Gua/GMP hydrogels in combination with Small and Wide Angle X-ray Scattering (SAXS/WAXS) and Atomic Force Microscopy (AFM). We analyzed Gua/GMP hydrogels prepared at different molar ratio (1:4, 1:2, 1:1) and at different hydration (90%, 95% and 98 % w/w of water). At one side, SAXS/WAXS results indicate that guanosine hydrogels show strong orientational properties due to their anisotropic nature. Orientational anisotropy strongly depends on hydration level and on Gua/GMP molar ratio, as a result of decreased flexibility due to changes in water composition and in the number on negative charges-per-unit-length in G-quadruplexes. On the other side, AFM results indicate that the 3D nature of Gua/GMP hydrogel is different according to the features discussed before: the hydrogel nano-structure seems to be like a swelling “fishnet” whom is possible regulate mesh size playing with the degree of hydration and with the Gua/GMP molar ratio.

FRAP experiments based on confocal microscopy have been then performed: in particular, we analyzed a series of fluorescent probes presenting different molecular weight and binding affinities (FITC-dextran 4kDa, FITC-dextran 10kDa, FITC-dextran 70kDa, Thioflavin T and DAPI). As a result, the diffusion coefficients of the different fluorescent molecules and the viscosity of the hydrogels were derived. Differences were discussed on the basis of hydrogels structural properties (e.g., hydration level and Gua/GMP molar ratio).

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THE MULTIFUNCTION TRANSLATION FACTOR aIF5A

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The synthesis of proteins is one of the most complex process that all cells undergo during their life, but it is also the most conserved. Even if each realm has developed some variations concerning the translation process, the elongation phase of proteins has been maintained essentially unchanged. Probably for this reason there are several translation factors shared between Archaea and Eukarya. One of these proteins is the translation initiation factor (IF5A), called eIF5A in Eukarya and aIF5A in Archaea. In the past, this factor was considered involved in the initiation phase of protein synthesis, but recently it has been shown that it plays a crucial role during their elongation phase, avoiding ribosomes to stall [1]. Its activity is performed by means of a particular post-translational modification called hypusination, which is catalysed by two enzymes: deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). DHS transfers the 4-aminobutyl moiety of spermidine to the ε-amino group of one specific lysine residue of the protein to form deoxyhypusine residue. The second enzyme, DOHH, hydroxylates the intermediate to form the mature hypusine residue and the mature form of IF5A[2]. Since in humans there is a strong correlation between levels of eIF5A and cancer development, the inhibition of the hypusination pathway is considered as a potential therapeutic target against cancer [3]. To obtain structural information about the interaction between the translation factor and the enzyme, which is the very first step of the hypusination mechanism, we are investigating aIF5A and DHS derived from the model archaea organism *Sulfolobus sofataricus.* We have firstly expressed both proteins by using recombinant techniques in *E. coli* and then we have purified them using affinity chromatography. Afterwards, we have determined the hydrodynamic diameter of solutions of both DHS and aIF5A with dynamic light scattering (DLS) experiments carried out as a function of temperature. Results show that, at increasing temperatures, the size of DHS does not change, whereas a completely different behaviour has been obtained for aIF5A. In fact, its hydrodynamic diameter gradually changes from about 100 nm at 25° C, to 1000 nm at 60° C, with an evident aggregation effect starting at 47° C, which is not reversible by cooling down the sample. Since the aggregation propensity of aIF5A does not allow to extract structural information about its monomeric state, which is retained to be mostly involved in the interaction with DHS, a more extensive structural analysis with Size Exclusion Chromatography (SEC) combined with Small-angle X-rays scattering (SAXS) will be performed in the near future.

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ASSESSING P6 SINGULAR ROLE IN HIV-1 SPREAD

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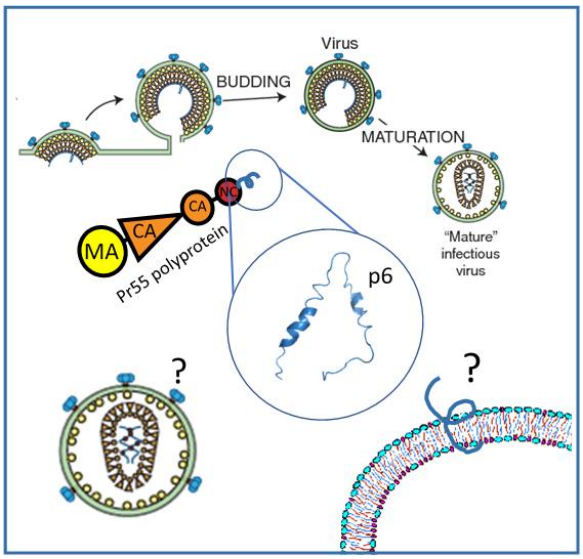
During the HIV-1 replication cycle, the Gag polyprotein mediate the assembly and release of progeny virions from an infected cell membrane. The p6 domain of the Gag C-terminal is composed of 52 amino acids (5.8 kDa) and has different tasks: it facilitates virus release from the plasma membrane and mediates incorporation of accessory proteins like Viral protein R [1, 2]. Up to 10^{10} HIV-1 particles are produced and disintegrated per day in high viraemic HIV-1 infected persons, accounting for 2–5 10^3 p6 molecules per released virion. The fate of each viron is either to disintegrate in the extracellular space or to infect a new host cell [3]. Although free p6 can be found in mature virions, it is hardly detectable in infected cells or in cell-free spaces, raising the question about its outcome outside an HIV-1 particle [4].
The fate of p6 is linked to its ability to interact with membranes, which has not been yet fully undisclosed.
Exploiting Quartz Crystal Microbalance, Small Angle X-ray Scattering and Neutron Reflectometry we investigated p6 structure and its interaction with biomimetic membranes. Our findings reveal that the interplay between protein and lipids is modulated by the lipid composition, giving insight on mature p6 behaviour and shedding light on a possible therapeutic strategy towards the disease.



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Impact of long-wavelength chlorophyll forms in PSII antennae of *Cromera velia* and *Pheodactylum tricornutum* on the photochemical quantum efficiency

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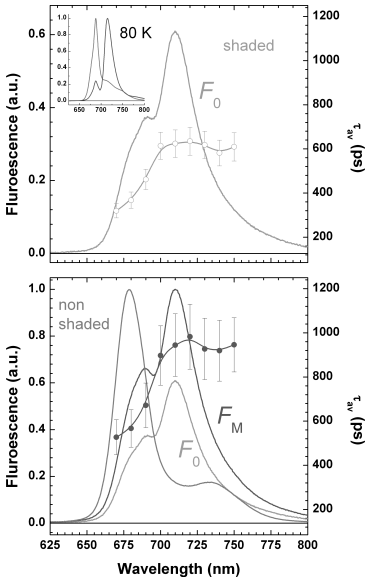
When grown under concomitant limiting light regimes (~20 µE m-2 sec-1) and shading conditions leading to an enrichment in near-infrared radiation in the incident light spectrum, the red algae *Cromera velia* and *Pheodactylum tricornutum* show an intriguing adaptive strategy associated to the synthesis of specific antenna isoforms. These harbour moderately red-shifted Chlorophyll emission forms that have a maximal emission centred at about 710-715 nm at room temperature, which is clearly discernible from the principal emission form of cultures grown under standard (non-shaded) conditions that display a maximal emission at about 684 nm [1,2]. The 684 nm/710 nm emission peaks ratio decreases with increasing culture density and the subsequent near-infrared enrichment in the culture growth light, due to self-shading [3]. In very dense cultures, or in cultures grown under enriched far-red lights, the 715 nm emission might become the dominant emission form [3]. At room temperature, the yield of emission of the 715 nm emission form and the observation that it displays a sizable variable fluorescence yield upon PSII trap closure, indicates that, contrary to the most extensively studied long-wavelength emission forms of green algae, plants and cyanobacteria that are known to be located in PSI, those observed in *C. velia* and *P. tricornutum* grown under shading conditions are, in part or primarily, associated to PSII [3,4].

In order to acquire further insight into the physiological role of PSII-associated red chlorophyll forms in these organisms, comparative studies of the fluorescence emission characteristics in the steady state and dynamics in the picosecond time domain have been undertaken on cultures accumulating different levels of red-form-to-bulk emission. It is shown that, under conditions approaching PSII open centres (F_0'), when red-forms are present in PSII antenna, the average fluorescence lifetime of the cells increases progressively towards the long wavelength emission edge. The extent of this variation (which can range from 100-200 ps in the 660-690 nm window to 300-400 ps in the 700-750 nm window) depends on the extent of red-form accumulation. This process resembles previous findings in the PSI, interpreted in terms of a partial kinetic bottleneck for energy diffusion due to unfavourable energy transfer from these antenna forms to the photochemical trap (i.e. a transfer-to-the-trap limitation). In the case of *P. tricornutum*, however, a similar average lifetime increase is also observed under PSII closed trap conditions (F_M), indicating that some energy diffusion limitation might occur even in the absence of photochemical quenching, and that energy transfer from the 715 nm form to bulk might therefore be relatively slow.



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A MULTIPHOTON INTRAVITAL MICROSCOPY STUDY OF PHOTODAMAGE INDUCED CALCIUM WAVES IN MOUSE SKIN

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BACKGROUND: The mammalian skin, the body's largest single organ, is a highly organized tissue that forms an essential barrier against dehydration, pathogens, light and mechanical injury. Damage triggers perturbations of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$) that spread from cell to cell (known as intercellular Ca^{2+} waves) in different epithelia, including epidermis. Ca^{2+} waves are considered a fundamental mechanism for coordinating multicellular responses, however the mechanisms underlying their propagation in the damaged epidermis are incompletely understood.
AIM OF THE PROJECT: To dissect the molecular components contributing to Ca^{2+} wave propagation in a murine model of epidermal photodamage.
METHODS: To trigger Ca^{2+} waves, we used intense and focused pulsed laser radiation and targeted a single keratinocyte of the epidermal basal layer in the earlobe skin of live anesthetized mice. To track photodamage-evoked Ca^{2+} waves, we performed intravital multiphoton microscopy in transgenic mice with ubiquitous expression of the sensitive and selective Ca^{2+} biosensor GCaMP6s. To dissect the molecular components contributing to Ca^{2+} wave propagation, we performed *in vivo* pharmacological interference experiments by intradermal microinjection of different drugs.
EXPERIMENTAL RESULTS: The major effects of drugs that interfere with degradation of extracellular ATP or P2 purinoceptors suggest that Ca^{2+} waves in the photodamaged epidermis are primarily due to release of ATP from the target cell, whose plasma membrane integrity was compromised by laser irradiation. The limited effect of the Cx43 selective inhibitor TAT-Gap19 suggest ATP-dependent ATP release though connexin hemichannels (HCs) plays a minor role, affecting Ca^{2+} wave propagation only at larger distances, where the concentration of ATP released from the photodamaged cell was reduced by the combined effect of passive diffusion and hydrolysis due to the action of ectonucleotidases. The ineffectiveness of probenecid suggests pannexin channels have no role. As GCaMP6s signals in bystander keratinocytes were augmented by exposure to the Ca^{2+} chelator EGTA in the extracellular medium, the corresponding transient increments of the $[Ca^{2+}]_c$ should be ascribed primarily to Ca^{2+} release from the ER, downstream of ATP binding to P2Y purinoceptors, with Ca^{2+} entry through plasma membrane channels playing a comparatively negligible role. The effect of thapsigargin (a well-known inhibitor of SERCA pumps) and CBX (a recently recognized inhibitor of Ca^{2+} release through IP_3 receptors) support this conclusion.
CONCLUSIONS: The one presented here is an experimental model for accidental skin injury that may also shed light on the widespread medical practice of laser skin resurfacing, used to treat a range of pathologies from photodamage and acne scars to hidradenitis suppurativa and posttraumatic scarring from basal cell carcinoma excision. The results of our experiments support the notion that Ca^{2+} waves reflect chiefly the sequential activation of bystander keratinocytes by the ATP released through the compromised plasma membrane of the cell hit by laser radiation. We attributed the observed increments of the $[Ca^{2+}]_c$ chiefly to signal transduction through purinergic P2Y receptors. Several studies have highlighted fundamental roles of P2Y receptors during inflammatory and infectious diseases, and the initial phase of wound healing involves acute inflammation. In addition, hyaluronan is a major component of the extracellular matrix and its synthesis is rapidly upregulated after tissue wounding via P2Y receptor activation. It is tempting to speculate that response coordination after injury in the epidermis occurs via propagation of the ATP-dependent intercellular Ca^{2+} waves described in this work.
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Binding of hypericin against SARS-CoV-2

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Hypericin is a potent natural photosensitizing agent and, due to its significant antiviral activity against membrane-enveloped viruses [1], is a promising candidate for the treatment of SARS-CoV-2. The fundamental parameters that influence the effectiveness of hypericin against viruses are the binding affinity of the PS to the viral components and the distribution of PS molecules within a population of viral particles [2]. Therefore, in addition to photoinactivation measures to quantify the reduction in infectivity, quantitative measurements of the molecular binding and distribution of hypericin on SARS-CoV-2 virions are crucial to plan better photodynamic therapy treatments.
In this work, we used quantitative approaches to follow the interaction of hypericin with SARS-CoV-2. Hypericin in water is found in aggregates that do not fluoresce. In the presence of viral particles hypericin recovers its fluorescence emission, a fact we exploited to detect binding to SARS-CoV-2. We used fluorescence microscopy to investigate the loading of hypericin onto SARS-CoV-2. Using a combination of spectroscopy techniques, we identified that the main viral component targeted by the PS is the membrane envelope of the virus and we estimated the binding affinity of hypericin for SARS-CoV-2 virions. Furthermore, these results are related to the cytotoxic efficacy of hypericin, both photoinduced and in the dark, observed on Vero E6 cells infected with SARS-CoV-2.
We have provided a quantitative characterization of the interaction between hypericin and SARS-CoV-2, which is the key to understanding the mechanisms of action of the PS and to guide the rational development of antiviral treatments based on photosensitization. Finally, the results obtained demonstrate that hypericin has significant antiviral activity against SARS-CoV-2.

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INTRAVITAL MULTIPHOTON MICROSCOPY OF MELANOMA REVEALS THAT CONNEXIN HEMICHANNELS ARE POTENTIAL TARGETS FOR ENHANCED PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) of cancer involves the photoactivation of a photosensitizing drug (PS) absorbed by tumor cells. The activated PS reacts with molecular oxygen and triggers reactive oxygen species (ROS) formation, followed by a cascade of cellular responses involving calcium (Ca^{2+}) and other second messengers, leading to cell death [1]. Despite its incomparable qualities, the application of PDT to the treatment of pigmented melanomas, the most aggressive skin cancer types [2], is limited by several factors, including melanin antioxidant properties [3] and attenuation of light penetration due to pigment absorbance [4]. To find a strategy to improve therapy efficacy also in deeper layers of tumor mass, we investigated the propagation of calcium (Ca^{2+})-mediated bystander effects induced by PDT with the PS Aluminum Phthalocyanine Chloride (AlClPc) in a syngeneic murine melanoma model [5].

In multiphoton intravital microscopy experiments using genetically encoded fluorescent biosensors expressed by melanoma cells *in vivo*, we showed that following pulsed laser irradiation of a single cell in a photosensitized tumor mass intercellular Ca^{2+} waves propagated from the irradiated to non-irradiated cells, promoting bystander cytosolic Ca^{2+} rise and intracellular Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria. Ca^{2+} waves were paralleled by rapid activation of apoptotic pathways up to 100 µm away from the photostimulated cell. The propagation of bystander processes was mediated by paracrine signaling due to ATP release from connexin (Cx) hemichannels (HCs) and was amplified in the presence of EGTA, that chelates extracellular Ca^{2+}, increasing HC opening probability. Thus, we reasoned that combination treatment with HC openers could potentiate bystander cell killing via enhanced Ca^{2+} signaling. To verify this hypothesis, prior to irradiation we intratumorally administrated S-Nitrosoglutathione (GSNO), an endogenous nitric oxide (NO) donor that biases the HCs towards the open state in our melanoma model. Combination treatment with GSNO greatly improved the efficacy of AlClPc-mediated PDT, leading to 86 % reduction of post-irradiation tumor mass.

These findings strongly indicate that HCs expressed by tumor cells are candidate targets for enhanced PDT and potential application of HC activators as PDT adjuvants for the treatment of melanoma and possibly other cancer types should be further investigated in clinical settings.

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OPTICAL CLEARING IN MULTIPHOTON LABEL-FREE MICROSCOPY

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Label-Free Microscopy is a very powerful technique that can be applied to study samples with no need for exogenous fluorescent probes. It is a method that rely on photophysical processes to generate signals through specific interactions with biological molecules and offer great potential for basic research and clinical applications. Using the same optical path, it can identify three different types of signal: autofluorescence, second harmonic generation and third harmonic generation [1]. Two photon autofluorescence has been widely used to identify and quantify metabolic molecules; second and third harmonic signals (SHG and THG) are non-fluorescent photo-physical conversions dependent on the intrinsic properties of the target biomaterial [2,3]. A variety of molecules have been reported to generate second harmonic signals, such as collagen, myosin, microtubules, silk, starch and cellulose. All these molecules are characterized by non-centrosymmetric architecture or hyperpolarizability, making them SHG active molecules [4].
For some applications, improvement of the 2-photon microscopy penetration depth (250–500 μm) is highly demanded. Optical clearing methods are applied to biological samples to achieve transparent tissue allowing unprecedented three-dimensional views of enormous volumes of specimens. These methods employ dehydrating samples, extracting lipids and refractive index homogenization to a high value (presumably matching the refractive index of remaining proteins) by using, for instance, hydrogel embedding and/or organic solvent. Practically, the optical clearing aims to reduce scatter in thick samples, reduce light absorption and refractive index matching [5,6], however it may come with some alteration of the original tissue. Here, we aim at systematically test different clearing methods, on the same tissue, to identify the protocol ensuring maximal penetration depth when imaging collagen SHG with minimal disruption of signal and fibres distribution.

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Biological lability of dissolved organic matter released by phytoplankton

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[3]Phytoplankton is the primary source of Dissolved Organic Matter (DOM) to the oceans [1]. DOM is mainly released by extracellular exudation and used by heterotrophic prokaryotes to synthesise biomass and recycle inorganic nutrients. DOM released by phytoplankton is mainly composed by carbohydrates, proteins and lipids that are thought to be labile and by humic substances that are thought to be recalcitrant and thus resistant to bacterial degradation [2]. There are a lot of uncertainties regarding the biological lability of exudates and the role of the DOM, released by phytoplankton, in the marine carbon cycle [3]. In this study, cultures of the diatom *P. tricornutum* were produced under axenic conditions and Dissolved Organic Carbon (DOC) concentration, Excitation-Emission matrices (EEMs) and cell density were measured with time to follow the release of DOM during the different growth phases. Exudates from the stationary phase were inoculated with a marine microbial community, DOC removal, FDOM transformation and Heterotrophic prokaryotes abundance were followed for 24 days of incubation. In each subsample, DOM was also separated in Low Molecular Weight (LMW, <3k Da) and High Molecular Weight (HMW, <3k Da) fractions. Reverse-phase HPLC was employed to separate the HMW fraction in compounds with different polarity. Our results show that ~75% of the total DOC pool has LMW. After 24 days, 28% of the initial DOC pool was removed. Fluorescence indicates high lability of protein-like molecules and degradation of bigger proteins into smaller peptides during the first days of incubation and a change in the hydrophobicity of the HMW proteins during the incubation, related to bacterial utilization.

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ATMOSPHERIC DEPOSITION OF ORGANIC MATTER AT THE LAMPEDUSA ISLAND

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Atmospheric deposition of organic matter is an important source of nutrients for marine ecosystems, affecting local, regional and global biogeochemical cycles. However, being a relatively recent field of research, studies focused on dissolved organic matter (DOM) deposition to the Mediterranean Sea (Med Sea) are scarce and fragmented. For this reason, atmospheric fluxes of DOM were studied for the first time on the island of Lampedusa, a remote site in the central Med Sea, between March 2015 and April 2017. Lampedusa was chosen because it is located far from large islands and continental areas and from relevant sources of pollution, allowing the quantification of total DOM atmospheric deposition and the evaluation of the impact of Saharan dust on DOM dynamics in the Med Sea.
We measured dissolved organic carbon (DOC), dissolved organic phosphorus (DOP), dissolved organic nitrogen (DON), ions, metals in both dry and wet depositions as well as PM_{10}. Our data show high variability in DOM deposition rates without a clear seasonality; each deposition event is characterized by a specific elemental ratio, suggesting a high variability in DOM composition and the presence of multiple sources. The data show that Saharan dust can be an important carrier of organic substances, even though the load of DOC associated with dust is highly variable; they also indicate that DOC fluxes from the atmosphere to the Med Sea might be up to 6 times larger than total river input. Longer time series combined with modelling would greatly improve our understanding of the response of DOM dynamics in the Med Sea to changes in aerosol deposition pattern due to human intervention and climate change.

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