

# 10-11 JUNE 2019 HIGHLIGHTS IN NANOSCIENCE

### **BOOK OF ABSTRACTS**



Elaborazione a cura del Servizio

# **HIGHLIGHTS IN NANOSCIENCE**

## **BOOK OF ABSTRACTS**

### JUNE, MONDAY 10<sup>th</sup>

### 9.15 Introduction

LUIGI ROLANDI (Direttore NEST) also on behalf of: LUCIA SORBA (Istituto NANOSCIENZE-CNR); MAURO GEMMI (CNI-IIT); PIERDOMENICO PERATA (NanoPlant-SSSA);

FABIO BELTRAM (NEST-SNS)

- 9.45 CAMILLA COLETTI Synthesizing 2D materials for optoelectronics: approaches and prospects
- 10.15 FRANCESCO ROSSELLA Growth of III-V Semiconductor Nanowires and Transport Experiments in Nanowire-based Devices

Coffee break

- 11.15 STEFAN HEUN The importance of surfaces for the properties of 2D materials: From graphene to phosphorene and back to III-Vs
- 11.45 FRANCESCO GIAZOTTO Superconducting quantum electronics lab @NEST
- 12.15 Lunch break
- 14.00 MAURO GEMMI Electron Crystallography
- 14.30 ANDREA CAMPOSEO Additive technologies for hybrid light-emitting and photo-responsive nanowires and networks
- 15.00 MIRIAM VITIELLO Terahertz photonics and nanoelectronic

### Coffe break

### Poster session I

- 15.30 ALESSANDRO PITANTI Nano-Mechanics at NEST: NIR and THz light control and strain engineering of 2D Materials
- 16.00 VALENTINA TOZZINI Theory & Computer Modeling @NEST

### JUNE, TUESDAY 11<sup>th</sup>

- 9.15 FRANCESCO CARDARELLI New paradigms in nanoscale biophysics: looking at life inside cells
- 9.45 VALERIO VOLIANI The ultrasmall-in-nano design
- 10.15 GIANPIERO GARAU Nano-BioStructures: Complex Design & Applications

### Coffee break

### Poster session II

- 11.15 GIANMICHELE RATTO Exploring the brain in space and time
- 11.45 RANIERI BIZZARRI Intracellular nanoscale biophysics by design
- 12.15 Lunch break
- 14.00 MARCO CECCHINI Microfluidics and nanomaterials for biosensing and the nervous system
- 14.30 CHIARA PUCCIARIELLO PlantLab: physiological and molecular basis of plants' adaptation to dynamic environments
- 15.00 ANTONINO CATTANEO Synthetic Biology approaches to Neurosciences: an opportunity for Bio@SNS and Nest?
- 15.30 17.30 Closing discussion

#### Synthesizing 2D materials for optoelectronics: approaches and prospects

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One of the major issues in graphene-based optoelectronics is to scale-up high-quality 2D materials and to demonstrate high-performing devices over large areas. In this talk different synthetic approaches for obtaining graphene (and other 2D materials) over large areas will be discussed and the scalable fabrication of optoelectronic devices presented.

Single-crystal graphene arrays obtained via deterministic seeding on Cu foil and chemical vapor deposition (CVD) growth [1] will be discussed as an appealing approach for wafer scale integration of graphene with high mobilities. Optoelectronic devices fabricated on arrays will be introduced.

Also, the electronic and tribological properties of scalable tungsten disulfide/graphene heterostructures synthesized via CVD [2,3] and the fabrication of photodetectors [4] will be presented. The photodetectors present, when illuminated with red light, a maximum responsivity R  $\sim 220 \text{ A} \cdot \text{W}^{-1}$ , a detectivity D\*  $\sim 2 \times 10^9$  Jones and a -3 dB bandwidth of 250 Hz [4]. Also, they display wavelength-selective memory which makes them of interest for the implementation of 2D-based data storage devices.

Finally, it will be presented an approach to obtain high-quality graphene on the c-plane of  $Al_2O_3(0001)$  substrates with a metal-free and single-step approach in a commercially available CVD reactor. The graphene grown displays a preferential orientation which is 30° rotated with respect to the sapphire substrate. The carrier mobility is above 2000 cm<sup>2</sup>/V·s at room temperature. The presented CVD approach is of appeal in virtue of its implementation in a commercial system, ease of scalability, and as it yields metal-free graphene.

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Figure 1: scalable graphene arrays with homogeneous electrical performance.



Figure 2: scalable WS2/graphene photodetectors with light-dependent behaviour.

## Growth of III-V Semiconductor Nanowires and Transport Experiments in Nanowire-based Devices

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**Nanowire Growth** (<u>lucia.sorba@nano.cnr.it</u>) The main activity of the group is the growth of III-V semiconductor nanowires (NWs) with the chemical beam epitaxy (CBE) technique.

InAs NWs are the focus of the activity (Figure 1(a)): the small band gap, small electron effective mass, large Landè g-factor, and the ease to form good Ohmic contacts make them ideal to study low temperature transport phenomena and for the fabrication of quantum electronic and photonic devices. The NW geometry gives the intriguing possibility to combine dissimilar materials in defect-free axial heterostructures like InAs/InSb, InAs/GaAs and InAs/inP axial NWs [1]. Moreover, engineering the material band gap and the segment thickness [2], quantum dot systems can be realized and manipulated (Fig. 1 (b)). Radial heterostructures (core-shell NWs) can also be grown. In this case, the controlled growth of one or more shells can introduce new electronic functions [3]. As an example, InAs/InP/GaSb core/barrier/shell NWs obtained by catalyst-free growth are shown in Fig. 1 (c).

Another activity of the CBE group is the selective area growth (SAG). SAG proceeds similarly to planar epitaxy, but only inside lithographically defined openings in an amorphous mask on a crystalline substrate. This approach allows the formation of scalable in-plane NW networks (Fig. 1 (d)) as a platform to realize topological qubits, when proximized by superconductors. This activity is performed in collaboration with the Microsoft team of Copenhagen.

Nanowire-based System and Device Applications (francesco.rossella@sns.it) We realize electronic devices for the study and exploitation of the electrical and thermal transport properties of individual nanowires and nanowire heterostructures. Our prototypical devices fully exploit the III-V semiconductor nanowire technology and implement unique functionalities that make them extremely promising for applications across physical sciences and engineering at the nanoscale, in particular: <u>nanowire-based iontronics</u> [4], <u>classical- and quantum-field effect transistors</u> [3-5], <u>thermoelectric conversion</u> [5,6].

Figure 2a reports the front-inner cover of a materials science top-journal showing a pictorial view of one of our InAs-nanowire based field effect transistors gated by a ionic liquid. Figure 2b shows a prototypical device based on a single core-shell InAs-GaSb nanowire where the inner InAs core and the outer GaSb shell have been contacted independently. In Figure 2c, we depict a single InAs-InP nanowire quantum dot in presence of a thermal bias: the occurrence of Coulomb blockade in our single electron transistor (inset, top-panel) induces sign reversal of the Seebeck coefficient (inset,

bottom-panel). Using nanowire quantum dots we are also investigating studying microwave assisted tunneling in YBCO/sapphire coplanar cavity resonator [7].

Besides, we engineer individual semiconductor nanostructures and nanowire arrays to study lightmatter interaction and the optical response in view of their use as building blocks for <u>quantum light</u> <u>emitters</u> [8], as well as <u>all-optical sensors</u> and <u>photonic machine learning processors</u> [9]. Figure 2e reports the polychromatic emission in a wide energy range from individual InP-InAs-InP multi-shell nanowires (top-panel), and magneto-photoluminescence spectra of a crystal-phase InP quantum dot (bottom panel). Optical reflectance measurements are instead carried out in arrays of nanowires, using both continuous wave and time-resolved spectroscopies.



**Figure 1**: (a) 45°-tilted SEM image of InAs NWs. (b) InAs NW with built-in InP barriers defining a double QD system. (c) InAs/InP/GaSb core/barrier/shell NWs (STEM and EDX compositional map (top), HRTEM image of a cross section (bottom)). (d) In-plane NW networks obtained by SAG (EBL-defined patterns on InP substrate with SiO<sub>2</sub> mask (left) and InP/InAs grown structure (right)).



Figure 2: (a) field effect transistors based on InAs nanowires gated by ionic liquids. (b) Core-shell InAs-GaSb nanowire-based device displaying negative differential resistance. (c) Thermally biased InAs-InP nanowire quantum dots displaying sign reversal of the Seebeck coefficient. (d) InP-InAs-InP multishell nanowires as quantum light emitters.

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## The importance of surfaces for the properties of 2D materials: From graphene to phosphorene and back to III-Vs

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Since the isolation of graphene in 2004, 2D materials have attracted the attention of the research community. Initially the focus was of course on graphene, a one atom-thick carbon sheet arranged in a honeycomb structure. More recently, research has widened to other 2D materials. Among these, phosphorene, the monolayer of black phosphorus, has attracted attention due to its tunable band gap and the intrinsic crystalline anisotropy.

Evidently, in these one atom-thick materials, all atoms belong to the surface of the crystal, and therefore its influence on the properties of the material is crucial. In fact, surface functionalization is a common way to modify and to control their performance.

I will review our recent research activities at the interface between quantum transport and surface science. I will present recent results obtained on the properties of black phosphorus (bP) [1-3], on the interactions between hydrogen and graphene [4-7], and on our attempts towards observation of the coexistence of quantum Hall effect and superconductivity [8,9].

**Black Phosphorus.** Our activities on black phosphorus cover a wide range, from STM studies of the surface of bP flakes as shown in Fig. 1 [2] and their functionalization with Cu for a possible n-type doping of bP towards exploitation of these results in device applications [3]. Besides, we work on chemical exfoliation and functionalization of bP (in collaboration with CNR-ICCOM) [1] and on low-temperature magneto-transport characterization of bP-based devices (in collaboration with McGill University, Montreal, Canada). The activities on bP are funded by an ERC grant.

**Hydrogen and Graphene.** We explore the interactions between hydrogen and graphene for possible applications in hydrogen storage. Being lightweight and mechanically as well as chemically stable, graphene could be a formidable storage medium for hydrogen, towards mobile fuel cell applications. Using a combination of surface science techniques and transport studies, both in ultra-high vacuum, we explore both chemisorption [7] and physisorption [6] of hydrogen on graphene. Recently we have demonstrated control of hydrogen adsorption via gate voltage [7]. Furthermore, we have developed a sensitive calorimetric technique, which allows measuring directly the heat release related to hydrogen adsorption on functionalized graphene [4]. We also study the covalent functionalization of graphene, as a precursor for building 3D graphene structures

for gas storage. Besides, we study the details of hydrogen intercalation in epitaxial monolayer graphene [5].

**Quantum Hall Effect and Superconductivity.** After 10 years of research on quantum Hall systems with scanning gate microscopy and low-temperature magneto-transport, we have recently started to investigate superconductivity and Josephson junctions in the presence of a magnetic field [9]. The goal of these studies is to explore the coexistence of both quantum effects in one sample (see Fig. 2) [8], which might eventually lead to the observation of Majorana fermions or their fractional generalization, the parafermions.



**Fig. 1:** Layer-by-layer sublimation of bP after annealing at 400 °C for 2 h. (a) STM image, showing aligned craters on bP. (b) Atomic resolution image obtained after zooming into the region marked in (a), identifying the zigzag-direction and therefore providing information of the crystallographic directions of the bP flake. (c) Height profile across the crater along the dashed line in (a), showing a 0.5 nm step height, compatible with monolayer desorption. (d) Schematics showing crater formation due to bP desorption—bottom layer (green) visible under desorbing top layer (red). From Ref. [2].



**Fig. 2:** Toward quantum Hall effect in a Josephson junction. (a) False color SEM image of a device. The mesa is yellow, side gates are green, and niobium is blue. (b) The critical field of Nb is about 2.8 T. (c) I-V curve showing well-developed supercurrent (the flat central region with zero  $V_{SD}$ ) and hysteretic behavior typical for JJs. (d) Conductance (in units of  $e^2/h$ ) as a function of magnetic field, clearly showing four quantum Hall plateaus. The quantum Hall regime is reached already at B = 1.5 T. From Ref. [8].

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#### Superconducting quantum electronics lab @NEST

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The activity of superconducting quantum electronics lab (SQEL) @ NEST is focused on the investigation of the properties of mesoscopic superconducting devices, which range from hybrid semiconductor-superconductor to fully metallic systems. Using state-of-the-art cryogenic techniques we explore condensed matter physics at low temperatures, typically down to 10 mK. Our activity is mainly experimental but strong effort if given to theoretical aspects as well. Being located within NEST laboratory, most of our research is carried out on in-house fabricated devices. NEST facilities include an ISO 6 rated clean room featuring modern electron-beam lithography systems. Our electron-beam shadow-mask deposition system allows the fabrication of high-quality nanoscale devices consisting of several different materials under ultra-high vacuum conditions with a high degree of control over optional insulating oxide barriers. These nanodevices are then loaded for full investigation in one of our high-performance dilution refrigerators that allow reaching low temperatures.

From the *experimental* side, our interests range from coherent caloritronics [1,2], where coherent thermal effect existing in hybrid systems are exploited to have a precise and fast control of heat currents at the nanoscale, to hybrid and topologically-protected systems for solid-state quantum technology [3,4], where the emergence of Majorana bound states in semiconducting nanowires with strong spin-orbit coupling [5] placed in closed proximity to superconductors is studied via charge and heat transport measurements through normal superconductor interfaces. Moreover, field effect in superconductors is explored [6,7] showing that intense electrostatic fields can be used to manipulate and dramatically affect the superconducting state of metallic Bardeen-Cooper-Schrieffer nanostructures, while ferromagnetic insulator-superconductor devices are researched for the implementation of novel-concept spin valves [8] and radiation sensors in the context of superconducting spintronics.

From the *theoretical* side, our interests span from the study of solid-state Majorana fermions [9] and mesoscopic transport in hybrid superconducting quantum structures, by developing novel designs and concepts and using a complete set of complementary techniques both at the analytical and numerical level, to coherent Josephson thermal transport [10], by conceiving new hybrid device designs such as thermal transistors, thermal logic elements and heat interferometers. In addition, heat and charge transport in topological nanosystems, where the presence of topological insulators is exploited to enhance the capabilities of Josephson-based devices, is investigated [11]. Finally, novel-concept superconducting detectors for radiation sensing are developed [12] at the theoretical level towards the improvement and the optimization of the existing figures of merit of actual ultrasensitive photon sensors.



Left: Pseudo-color scanning electron micrograph of a prototype Ti-based all-metallic superconducting field effect transistor. Right: Pseudo-color scanning electron micrograph of a Josephson thermal router.

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#### **Electron Crystallography**

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At CNI@NEST we developed a state of art electron diffraction station which allows 3D electron diffraction (3D ED) data collections at fast speed and in low dose conditions on nanocrystals. This will change our view of crystalline materials because for the first time we obtain 3D diffraction signals on a scale which was previously unexplored. We can shed a very weak electron beam on a beam sensitive nanocrystal and in few seconds we obtain a 3D reconstruction of its reciprocal space by recording diffraction patterns during the crystal rotation. From the integrated diffracted intensities we have access to its crystal structure, on a crystal three order of magnitude smaller that those studied with single crystal x-ray diffraction.

The dose reduction due to the new collection method, and to the development of new detectors which are sensitive to the single electron, has widened the class of materials that can be studied. At the beginning only inorganic hard materials like oxides and ceramics could stand the electron dose necessary for one entire experiment. Our developments show that it is possible to obtain 3D electron diffraction data on metal-organics and organics at room temperature and on macromolecular crystals under cryo condition.

We expect a great impact on all the fields where the crystallization is an issue. If with 3D ED we can analyze as single crystals, grains that cannot be seen at the optical microscope, maybe our crystallization effort will become less demanding. Pharmaceutical chemistry, macromolecular chemistry but also materials chemistry will all greatly benefit from the option of such a nanocrystallography technique.

Our biggest successes are: the identification of new intermetallic phases in contacted GaAs nanowires; the first new unknown polytype of a protein discovered with 3D ED (monoclinic

lysozime); the crystal structure of complex inorganic structures that crystallize only in nanocrystalline form (Cu<sub>2-x</sub>Te); the crystal structure of metal-organic framework with biological linkers obtained from nanocrystals at room temperature; the first crystal structure of a unknown pharmaceutical solved with 3D ED (orthocetamol); an entire 3D ED data collection obtained in less than 30s with a total dose of less than 1el Å<sup>-2</sup>, two orders of magnitude less than the dose for cryo-EM microscopy; the crystal structure of a new polymorph of Ca carbonate from water drops collected on an alpine cave.

A second activity of our group concerns the development of new staining agents for TEM sample preparation of biological specimens. We have developed and patented, in collaboration with SNS, a new compound that is more efficient in staining biological samples than any available commercial product and that does not have the chemical and safety hazard of the widely used uranyl acetate. We expect our reagent to become the leader on the market.

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## Additive technologies for hybrid light-emitting and photo-responsive nanowires and networks.

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Additive technologies have emerged as innovative methods for designing and manufacturing functional objects, devices and actuators with complex architectures [1]. Additional opportunities are opened by the possibility to add time-changing shape and properties to the manufactured components. To this aim, the manufactured systems have to be composed by multi-materials, have to feature specific properties responsive to external stimuli, and have to display anisotropic physical properties. Such requirements demand for specific manufacturing approaches capable of processing heterogeneous materials simultaneously, of preserving the optical properties of the incorporated active molecules and nanostructures, and of conferring anisotropic properties to the processed hybrid materials.

In our group we developed a platform of additive technologies for light-emitting and photoresponsive materials, which includes 3D printing technologies based on UV photo-polymerization and on the extrusion of melted polymers and viscous inks, and electrospinning. Examples of nanostructured materials, which can be manufactured by those technologies include polymer nanofibers and 3D layered structures, which can be fabricated by using various synthetic polymers and biopolymers. Nanofibers and 3D printed layers can feature various advantages compared to bulk systems, as the possibility to achieve anisotropic optoelectronic properties such as polarized emission and optical birefringence. In addition, nanoscale dielectric filaments provide also enhancement of local electromagnetic fields that can intensify the optical and emission properties of nanostructures and molecular systems incorporated in the fibers [2]. The optical properties of fibers arrays and 3D printed structures can be also designed to achieve transport of light over macroscopic distances and tailored scattering of light [3,4], which allows various regime of random lasing to be observed [4,5]. Moreover, optical properties can be controlled also by external light signals. We demonstrated photo-induced birefringence and all-optical switching of light on timescales of ms, corresponding to switching rates of the order of kHz [6] in nanofibers of DNA and 3D printed structures doped with photo-responsive molecules. Interestingly, the manufactured systems can be assembled in photonic networks [7-8], where the scattering, waveguiding and amplification of light, and the resulting optical modes are determined by the network topology.

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**Figure**. (a) Scanning transmission electron microscopy micrograph of hybrid nanofibers incorporating  $MoS_2$  nanoparticles. The particles are highlighted by yellow arrows. Scale  $bar = 10 \ \mu m$ . The corresponding energy-dispersive X-ray spectrometry profile is displayed in (b). Reproduced with permission from [3]. Copyright 2018, Royal Society of Chemistry. (c)-(d) Scanning transmission electron micrographs of dye-doped polystyrene fibers without (c) or with (d) TiO<sub>2</sub> nanoparticles. Scale bar: 5  $\mu m$ . Inset: schemes of the obtained structures. (e)-(f) Pictures of luminescent nanofiber mats of fibers under white (e) and UV illumination (f). Scale bar: 1 cm. Reproduced with permission from [5]. Copyright 2018, American Chemical Society. (g) Examples of single-shot random lasing spectra from a biosilica templated nanofibers. Reproduced with permission from [4]. Copyright 2018, The Authors. Published by WILEY-VCH Verlag GmbH & Co. KGaA. (h) Optical coupling between two wires made of silylethyne-substituted anthracene single crystals. The inset shows the arrangement of the molecules in the crystalline wires. Reproduced with permission from [8]. Copyright 2019, American Chemical Society.

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#### Terahertz photonics and nanoelectronics

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Terahertz (THz) technology has prompted in the last decade a major surge of interdisciplinary researches, inspiring fundamental insights and amazing applications in microscopic and macroscopic systems. Being a transition region between electronics and photonics, between component sizes that are smaller and larger than the radiation wavelength, the THz frequency "gap" offers unusual possibilities in borrowing concepts and technologies from fundamentally different worlds.

Recent technological innovation in photonics and nanotechnology is now enabling Terahertz frequency research to be applied in an increasingly widespread range of applications requiring systems with targeted sensitivity and resolution and exploiting advanced devices and optical components making use of novel materials and architectures.

In this perspective, the availability of compact THz devices that are conveniently single frequency, high-power, low divergent and narrow linewidth laser sources, as well as high-speed and ultrasensitive nanodetectors, is matching increasing demand for spectroscopic applications encompassing environmental monitoring, security and biomedical sensing, frequency metrology. This talk will provide an overview on our recent developments in the field of broadband high-power, low divergent, 1D and 2D continuous wave THz lasers (Fig. 1a-d), miniaturized frequency combs (Fig. 1e-f), THz ultrafast nano-detectors (Fig.2), and near-field optical system and components.



*Figure 1*: (a) Scanning electron microscope (SEM) image of a corrugated in plane emitting THz wire laser; Inset: Far-field intensity pattern. (b) SEM image of a random laser having area 0.06mm<sup>2</sup>, and filling fraction 25%; (c) Current density – voltage (J-V) and light-current density (L-J) characteristics measured in continuous wave on a random THz laser; (d)

corresponding far field intensity pattern. (e) Intermode beatnote map as a function of driving current of a THz quantum cascade laser frequency comb; FTIR spectra collected in rapid scan mode, under vacuum with a 0.075 cm<sup>-1</sup> resolution at 15 K, while driving the QCL in continuous wave.



**Figure 2**: (a) Schematic representation (right; not to scale) of the antenna-integrated pn-junction device and a zoom of the central part of the THz phototermoelectric detector (left; to scale), consisting of an "H-shaped" graphene channel, contacted by source and drain electrodes; (b) Side view of the device design, with the superimposed color map again indicating the normalized power profile. (c) Same as panel a, now indicating how the antenna branches serve as local gates by applying voltages  $V_L$  and  $V_R$ . Appropriate voltages will create a pn-junction in the central part of the graphene channel, directly above the antenna gap (which is where incident THz light is concentrated by the antenna). The color map superimposed on the device is a simulation that shows the photoresponse created by local photoexcitation, varying the position of photoexcitation. (a) Photovoltage signal measured as a function of the time. The inset shows how the photoroltage VPTE follows the switching of the pulsed laser. The red and blue open dots show the obtained photovoltage in a small time window marked in the inset, with the black line giving the result of exponential fits with timescales of 40 (24) ns for the red (blue) curve

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#### Nano-Mechanics at NEST: NIR and THz light control and strain engineering of 2D Materials

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In this talk I will briefly present the main activities in nanomechanics that are currently being pursued at NEST Lab. The two main research themes consider either static or dynamical control of mechanical objects interacting with electrons or photons.

Polarization control and polarimetry has been recently demonstrated by us in GaAs artificial photonic materials (metasurfaces) defined on a square nanomembrane (see Fig. 1). By activating the 350 kHz fundamental "drum mode" and exploiting the chirality of the metasurface, pure polarization modulation has been demonstrated [1]; conversely, by using the back-action of light on the mechanical resonance, we have showed how the impinging light polarization state can be readily extracted, enabling a novel technique for fast and precise polarimetry. On a different spectral range, mechanical elements are integrated in external or internal THz cavities to ultimately control and modulate the emission of THz lasers. Demonstrating, as a preliminary result, high resolution detection of the motion of a nanomembrane through THz self-mixing interferometry [2], we are currently investigating LC-like resonators [3] where the inductive element could be mechanically actuable, possibly enabling radiation pressure based self-modification of the laser state itself.



Fig.2: (a) SEM micrographs and simulation of the mechanical (top) and electromagnetic (bottom) modes investigated. (b): 350 kHz polarization modulation as a function of laser wavelength. (c): Mechanical resonance characteristics (intensity, peak frequency) as a function of polarization state of impinging light beam. A good agreement between experiment (top) and simulations (bottom) is found.

Static architectures for strain engineering have proven to be exceptional tools for manipulating the electronic and opto-electronic properties of 2D materials. The planar nature of these objects joined to their high cracking resistance makes them perfectly suitable for mechanical deformations, which are routinely applied through the use of external components, resulting in reduced strain gradients and a challenging local control of the strain profile. As a different approach, we developed the use of polymeric actuators made of cross-linked PMMA to locally create strain profiles at the nanoscale. By exposing a pattern using a high-dose electron beam, PMMA undergoes shrinkage, pulling the 2D material underneath it, which is strongly bonded to the polymer by the cross-linking

process itself. By relaxing the actuators with thermal treatments, several, custom profiles can be applied on the same material. As a first result, we employed this technique for applying a pure uniaxial strain on a graphene membrane [1]. The versatility of the technique is highlighted by a second experiment where we apply tensile strain on a van der Walls heterostructure with WS<sub>2</sub> as the topmost layer. The applied stress of a few percent translates in a strong red shift of the excitonic photoluminescence (PL) peak [4]. Furthermore, the superlubric sliding between WS<sub>2</sub> and the graphene layer underneath makes it possible to realize strain profiles without suspending the material, with added easiness in fabrication and robustness of the device.



Fig.2: (a)-(b) SEM micrographs with a superimposed spatial map of PL peak position, pre and post actuation. (c): Full PL spectra pre and post actuation extracted from the central region of the device

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#### Theory & Computer Modeling @NEST

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The activity of the theoretical and computer modeling @NEST is transversal to all research lines of the Lab. Starting from the most basic activities, the quantum information research lines focuses on the problem of optimizing the encoding/decoding-information transferring processes, and related problems in quantum metrology (1). This research lines involves also studies of fundamental aspects of quantum thermodynamics aimed at enhancing the performances of quantum devices for efficient energy storage (2). A general approach, involving super-operator treatment of open quantum system dynamics was developed to deal with energy/heat flows and entropy current, and quantum violation of thermodynamic bounds.

Within the field of quantum transport and non-equilibrium dynamics our activity (often related to experimental projects) is focused on the following subjects: fractional and topological edge states even in the presence of external time-dependent sources; investigation of the interplay between superconductors and semiconductor heterostructures aimed at finding new topological states of matter; topological properties of nanostructures (topological insulator and topological superconductors); modeling intrinsic properties of non-trivial topological states; thermoelectricity and thermodynamics in nanostructures (3-4); Transport properties in ultra-clean graphene systems in the phase-coherent regime (5); entanglement manipulation is solid state devices (6).

An intense computer modelling activity is also present. A multi-scale approach encompassing coarse-grained and atomistic molecular dynamics and *ab initio* techniques is used to study the molecular and electronic structure of fluorescent dies and proteins for bio-imaging, to clarify the interaction of these molecules with the cytoplasm components and how these affect the fluorescence (7). Combinations of atomistic, coarse grained and meso-scale models are used to investigate the interactions of functionalised metal nano-particles with amyloid proteins for therapeutic applications (8). On the more fundamental level, low-resolution models are optimized to simulate large size and time scale transitions of switching proteins. On the side of materials, computer modelling combining Density Functional Theory and classical molecular dynamics is used to investigate several properties of the graphene-based systems, in synergy with the experimental groups of the lab: the capability of the substrate of modulating the electronic and chemical properties of SiC supported graphene at the nano-scale (9); the adsorption and diffusion of

gases and electrolytes within the graphene nanoporous scaffolds for energy storage applications; the electronic properties and superlubricity of composite systems such as graphene/metal dichalcogenide stacks (10).



**Fig 1**: A sample if applications (a) hybrid quantum/classical representation of the Green Fluorescent Protein chromophore (green charge isosurface) embedded within the protein environment represented with a polarizable force field; (b) the  $A\beta$ I-42 amyloid protein organizing its structure upon interaction with a gold surface represented with an polarizable force field; (c) suspension of bio-functionalized gold nanoparticles in atomistic (center) and coarse grained (yellow/green) representations; (d) electrolyte (green and dark red iso-density surfaces representations)diffusing within a nanoporous graphene scaffold from left to right; (e) Schematic of the double TI Josephson junction: by applying the gate voltage is possible to manipulate the non local entanglement of Cooper pairs.

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#### New paradigms in nanoscale biophysics: looking at life inside cells

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Capturing life (mis)regulation at the nanoscale is a crucial challenge for present and future biophysics. At this scale, the main actors are the molecules. To successfully tackle molecular behavior within living matter, two crucial requisites are typically needed: (1) nanometer spatial resolution, (2) micro-to-millisecond temporal resolution. In this context, optical microscopy is a valuable methodological platform: by using fluorescence as readout, spatial and temporal details of molecular processes can be investigated directly within living matter. In this regard, in the last few years, our Group introduced/developed a number of methods, based on 'fluctuation' (Fig. 1) or 'localization' (Fig. 2) analysis, in order to increase the amount of quantitative information that can be extracted from optical microscopy movies. After the development/engineering, if needed, of suitably labelled biomolecular toolboxes<sup>1</sup>, methodological demonstrations were provided in a series of applications of relevant biological or biomedical interest, including the spatiotemporal organization of biological membranes, the regulation of intracellular transport, dynamics and oligomerization of single membrane receptors and their ligands, the intracellular trafficking and fate of drug-delivery vectors (see, for instance, Fig. 2 and Refs. 2-7). In spite of these technological achievements, molecular details of life regulation are still somehow elusive within subcellular membrane-enclosed nanosystems, such as vesicles, organelles, or even entire subcellular protrusions. At this scale, the molecular actors are part of a reference nanosystem that is endlessly changing position in space and time in the complex 3D cellular environment. This condition imposes a third requisite to be concomitantly met in the same experiment, which is: (3) large volume sampling to localize the target nanosystem. Unfortunately, no method has the capability to subtract the 3D evolution of the entire nanosystem while preserving the temporal resolution needed to probe molecular details on it. Our Group tackled this bottleneck by sending the excitation beam in a periodic orbit around the nanostructure of interest, with the recorded signal (e.g. fluorescence or scattered light) used as feedback to localize the nanostructure position with unprecedented spatial and temporal resolution. At this point, state-of-the-art imaging/analytical approaches (e.g. fast spatiotemporal correlation spectroscopy) can be used along the orbit to push biophysics to an entirely new level: molecular analysis on a moving, nanoscopic, reference system. The potential of this strategy have been demonstrated in a number of recent applications to paramount biological processes, including: the vesicle-mediated intracellular transport of nanocarriers (e.g. for gene delivery)<sup>8</sup>, the mechanism by which insulin is packed into nanoscopic granules and secreted by pancreatic β-cells<sup>9</sup>, the metabolism-dependent solvent polarity fluctuations in the lumen of a trafficking lysosome<sup>10</sup>. Overall, by such a toolbox of techniques we propose a paradigm shift in the way we address the natural physiopathology of living matter at the sub-cellular scale, where

molecular information is still hidden behind a plethora of dynamic intracellular nanostructures. The direct involvement of these latter in the processing of drug-delivery vectors suggests them as a due target for a new era of *theranostic* strategies using engineered nanoarchitectures. Preliminary studies are actively carried out in our Group, also in collaboration with external biomedical research units and medical clinics<sup>8,11-13</sup>. If successful, this research activity is expected to open new perspectives in biophysics and related fields, nanomedicine above all.



From molecules to organelles...

**Figure 1. From molecules to organelles:** Schematic representation of the iMSD-based dynamic fingerprint analysis. **a**) A stack of images of fluorescently-labelled intracellular structures is acquired by time-lapse confocal microscopy. **b**) Spatiotemporal correlation function is derived from image analysis by the iMSD algorithm (see Materials and Methods for equations). **c**) Gaussian fitting of correlation functions allows to extract the iMSD plot, which in turn depicts the average diffusion law of the structure of interest (exemplary cases are reported: super-diffusion, dotted red line; isotropic diffusion, dashed red line; sub-diffusion, solid red line). **d**) The short-range diffusion coefficient ( $D_m$ ), the anomalous diffusion coefficient (a), and the y-axis intercept of the iMSD plot, indicating the average size of the diffusing structures. These three parameters are organized in a 3D plot, used to identify the 'dynamic fingerprint' of the diffusing structure. **From organelles to molecules:** Here, lysosome lumen is labelled by the polarity-sensitive 6-acetyl-2-dimethylaminonaphthalene (ACDAN) probe (e). 3D orbital tracking affords the trajectory of single organelles (**f**) while detecting luminal polarity (**g**). Fluctuation analysis finally yields the amplitude and timing of polarity fluctuations (**h**), which in turn are informative of lysosome metabolic status (**i**). Adapted from Begarani et al. ACSNano 2019 (Ref. 10).



Figure 2. Examples of possible results from single particle tracking (SPT): application to neurotrophin receptors and their ligands. a-f) membrane p75<sup>NTR</sup> moves as a monomer with at most transient interactions: membrane located wild type (wt) p75<sup>NTR</sup>, a monomeric (mut p75NTR) and a dimeric (dim p75NTR) controls where labeled with Abberior635P exploiting a peptidic tag and imaged with TIRF microscopy. a) Reconstructed single receptor trajectories (blue) superimposed on a TIRF movie frame. Scale bar: 5 µm. b) Distribution of diffusion coefficient D for the constructs. c-d) Analysis of transient dimerization: c) transient dimer (Td) trajectory superimposed on a region of a TIRF movie frame and enlarged on bottom (M: merge, S: split events); d) distribution of the cell-average duration of Td trajectories for wt (grav), mut (black) and dim (blue) p75<sup>NTR</sup> constructs. All data are from cells with [0.18-0.36] spots/ $\mu m^2$ . f) Examples of intensity profile traces of monomers and dimers. IPRE: particle average intensity before the first bleaching step; red arrows: counted single photobleaching steps. g) Histograms of the number of photobleaching steps per trace for wt, mut and dim p75<sup>NTR</sup>. g-h) Example of single-vesicle tracking for the transport of fluorescent NGF and proNGF in neuronal axons (aligned using a microfluidic chamber): g) examples of reconstructed trajectories; their motion and number of contained fluo-proNGF and fluo-NGF can be analysed; h) example of histogram of average velocities of moving vesicles.

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#### The ultrasmall-in-nano design

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The intriguing features of plasmonic noble metal nanoparticles (NPs) for healthcare and catalysis applications are mostly relegated to laboratory.<sup>[1,2]</sup> Indeed, accumulation issues and stability hurdles have prevented, respectively, their clinical translation and efficient use in catalysis.<sup>[3,4]</sup>

A groundbreaking advance to bring again NPs to the forefront of cancer theranostics relies on the ultrasmall-in-nano approach.<sup>[1]</sup> Within this approach, we have designed a family of all-in-one biodegradable nano-platforms that jointly combine the appealing features of NPs with metal excretion: the nature-inspired passion fruit-like nano-architectures (NAs).<sup>[2,5]</sup> The versatility of NAs production will be presented together with the biokinetics and whole-body biosafety assessments.<sup>[6,7]</sup> Some of the most promising applications of NAs for healthcare applications will be also discussed.<sup>[8–11]</sup>

Last but not least, the potential employment of calcined NAs – nanoplatforms that offer a high catalytic efficiency together with a good stability – will be introduced.



Scheme for the formation of the biodegradable ultrasmall-in-nano architectures (NAs, upper panel). Ultrasmall noble metal nanoparticles consist of silver or platinum or gold. Bottom panel: typical TEM image of NAs before (left) and after (right) calcination.

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#### Nano-BioStructures: Complex Design & Applications

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#### **Biostructures Design**

A major goal of biotechnology is the development of novel nano-biomaterials. The last frontier on the road is designing and creating novel protein-based bioarchitectures, with desired geometries and specific chemical-physical properties, starting from protein scaffolds that can selfassemble into stable/dynamics biostructures. The BIOSTRUCTUREs Lab at IIT@NEST generates engineered scaffolds of (metallo-)proteins that offer a simple and flexible strategy to regulating selectivity and dynamics in assembly-based processes, and getting (ii) specific biotechnological needs (Figure 1). Resulting biostructures achieve highly specific properties from pre-existing modular components. The potential applications in material sciences are huge, spanning from crystalline-like biomaterials and controllable symmetrically bonded oligomers, to biocages for nanomedicine or biotransformation. Recently, our research has been mainly directed to the confinement of biostructures into designed 3D nano-bioarchitectures. This confinement represents a powerful and innovative strategy for: (1) Structure determination of macromolecules (by crystallography and single particle cryo-electron microscopy), and (2) Enzymatic cascades, which constitute an efficient & eco-friendly processes of biotransformation, a hot-topic in green chemistry and biopharma.

#### **Biostructures Targeting**

We are drawn also to research problems in which we can solve fundamental problems in biology and human disease using



Figure 1. Design of novel protein-based pyramidal bioarchitectures.



**Figure 2.** Journal Cover of ACS Chem Biol and discovery of a novel lipid signaling pathway in human.

different approaches of structural biophysics, cell biology and chemistry. We have concentrated our efforts mainly on membrane proteins that modulate lipid signaling in metabolic and neurological disorders. Our studies have allowed us to unveil a novel lipid signaling pathway (Figure 2), inferred from the crystal structure of the membrane enzyme human NAPE-PLD, and discover specific target ligands to define its role in disorders and to validate its therapeutic potential. Finally, I will present briefly other projects and future perspectives in collaboration with different groups.

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#### Exploring the brain in space and time.

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Excitation and inhibition play a never ending equilibrium game throughout all brain states: neuronal activity is finely tuned during sleep, wakefulness and cognitive and sensory tasks by the balancing act of pyramidal cells and interneurons. This theme is particularly relevant in pathological conditions: epilepsy has been long associated with impaired inhibitory feedback but it is gradually emerging that a distortion of the dialogue between excitatory and inhibitory neurons is likely to be at the basis of most, if not all, cognitive deficits. Indeed, a telltale sign of this fact is the high co-morbidity between epilepsy and diseases of the autistic spectra (ASD). We explore the relationship between excitation/inhibition and cognitive impairment in a variety of genetic and pharmacological models of brain pathology by means of in vivo electrophysiology and imaging. 2 photon imaging allows to peer into neuronal function in the intact brain at the level of cell population, individual cells and at the subcellular level: indeed, in vivo imaging is providing novel insights on brain structure and function with unprecedented temporal and spatial resolution. Functional 2-photon imaging relies on genetically encoded proteins that transduces the intracellular environment in an optical signal. In the lab we employ three different classes of opto-genetic sensors. Beatrix: is a novel sensor for genetic expression that allows to identify the presence of a specific genotype in vivo; Ca-sensors transduce changes of the intracellular concentration of Ca<sup>2+</sup> in fluctuations of fluorescence: this signal is meaningful because, each time a neuron becomes active, intracellular Ca2+ increases. Cl-sensors transduce changes in intracellular Cl<sup>-</sup> in changes of the emission spectra: this signal is essential to understand the effects of inhibitory inputs on neurons. These genetic tools, together with the availability of genetic and pharmacological models of brain pathologies, allows to investigate how a brain disease affects computation at the neuronal level, and hopefully this will contribute to enlighten the relationship between the biophysics of neuronal computation and cognitive deficits.

Imaging of the intracellular  $Ca^{2+}$  concentration and the measurement of the local field potential (LFP) report two complementary aspects of neuronal computation:  $Ca^{2+}$  increments are a proxy for neuronal depolarization and firing and report neuronal activity at a high spatial resolution but at a low temporal resolution. The LFP provides an integrated report of brain activity with very high temporal resolution but it does not contain any information regarding the localization of the sources of electrical activity. We are developing a new approach to the quantitative analysis and interpretation of simultaneous recording of LFP and 2-photon imaging. In this study, we employ zebrafish larvae expressing the  $Ca^{2+}$  sensor GCaAMP6f to identify the sources of the LFP and

the temporal evolution of the neuronal territories recruited during physiological and pathological activity. These experiments produces very large data sets and their processing and quantification is not a trivial task but we can exploit the statistical properties of the LFP and of the temporal sequences of images for the extraction of the features of interest in both space and time domain. In this task, we have exploited the convergence of widely different notions originating from the field of linguistic, image analysis, thermodynamics and information theory. We will address three questions that are at the core of the problem of linking optical imaging to brain function: 1) What can we learn from the statistics of the fluorescence fluctuations? 2) What are the 'interesting' events in the fluorescence and LFP data and how can we extract them when hidden in a noisy environment? 3) How can we identify the cortical territories that are recruited during transient burst of activity identified by the LFP? We will provide an overview of the methodology that we are developing for the interpretation of Ca<sup>2+</sup> imaging data and LFP in zebrafish models of epilepsy.



**Figure 1.** In vivo 2-photon imaging of the mouse visual cortex after transfection with the sensor of gene expression Beatrix. The neuronal staining with the red and green fluorescent proteins labels normal neurons and neurons expressing the recombinase CRE. When this tool is used in a transgenic conditional mouse line, the floxed gene is edited away and that event is signaled by the green fluorescence. We are employing this tool for the production of mosaic models of brain disorders and cancer. The left panel shows the full width of the cranial window and it is about 2mm wide. The other panels shows details of the tagged neurons and dendrities.



**Figure 2.** Calcium dynamics in a murine model of Glioblastoma. The model is obtained by transplantation of glioma cell lines in vivo. The cell line has been engineered at NEST in order to express the  $Ca^{2+}$ -sensor GCaMP6 and have been imaged after 2 weeks of proliferation. Glioma cells placed in areas of tumorur infiltration shows a strong spontaneous  $Ca^{2+}$  activity.

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#### Intracellular nanoscale biophysics by design

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Former Enrico Fermi's student and biophysicist Mario Ageno once stated that "*Life is a coherent molecular system ruled by a program*". From this perspective, biological concepts such as genotype, phenotype, and metabolism reconcile with physics and set the basis for what is called *biophysics*. Remarkably, at nanoscale the energy of many biophysical processes (e.g. protein folding, molecular interactions) is near KT, and Nature ably *rectifies* thermal energy to help the activities of the biological organism. Accordingly, our group is committed to *nanoscale biophysics*, i.e. we aim at the spatiotemporal description of biomolecular processes such as transport-coupled protein-protein or protein-DNA interactions. Our main biological targets are biomolecules that coherently orchestrate several biological activities, such as membrane signaling (A1) or gene transcription (A2). Notably, our approach relies upon the exquisite sensitivity of fluorescence microscopy, which nowadays enables the real-time monitoring of biological specimens down to a few nanometers. For this reason, part of our activity is devoted to the development of novel fluorescent reporters and imaging schemes (A3, A4) that can help us addressing our main biological issues.

#### A1. Membrane organization of Transient Receptor Potential Vanilloid 1 (TRPV1)

TRPV1 is a nonselective membrane cation channel involved in the transmission/modulation of nociception. By means of a high-sensitivity multiplex imaging approach, combining FRET and *i*MSD analysis (Fig.1), we demonstrated the directional diffusion of TRPV1 along with microtubules structures as well as TRPV1 desensitization by caveolar internalization [1, 2]. These findings were complemented by nanoscopy imaging to afford a comprehensive picture of TRPV1 nanoscale organization in living cells.

#### A2. Epigenetic control by nanoscale organization of polycomb body proteins (PcG)

PcGs oversee the dynamic organization of the nuclear architecture, thereby modulating the transcriptional (epigenetic) program that commits cells to their state. PcG protein BMI1 has attracted strong interest as main component of polycomb regulatory complex 1 (PRC1) and crossroad in at least 16 different types of cancer, whose could represent a promising drug target. We studied the nanoscale nuclear organization of BMI1 by PALM/STORM nanoscopy (Fig.2) and FRET/iMSD, showing that transcription or post-translational modifications of BMI1 have different outcomes as polycomb body size and stoichiometry. Our study is now extending to other PcG proteins.

#### A3. Development of reversibly-switchable fluorescent proteins (Q-RSFPs)

RSFPs admirably combine the genetic encoding of fluorescence with the ability to repeatedly toggle between a bright and dark state, adding a new temporal dimension to the fluorescence signal. Accordingly, RSFPs enable many techniques such as F-PALM, RESOLFT, and SOFI that provide nanoscale pictures of the living matter. We are committed to the rational design of RSFPs, and we recently reported how the E222Q replacement is a *single photoswitching* mutation that restores the intrinsic chromophore *cis-trans* photoisomerization in otherwise non-switchable proteins (Q-RSFPs) [3-5]. Q-RSFPs are now in use for A1-A2.

#### A4. Development of local polarity/viscosity sensors

The rational tuning of the excited-state physicochemical properties allowed us to transform fluorescent compounds into efficient biosensors of nanoscale dielectric and viscosity properties [6, 7]. We shed light on cell drug delivery mechanisms and chromatin compaction upon nuclear lamina misassembly in the Hutchinson-Guilford progeria syndrome [8], as well as modification of membrane raft composition in Krabbe leukodystrophy [9].

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Figure 1. Steady-state and dynamic FRET analysis of TRPV1-microtubules interaction. Panels a-e refer to sensitized emission (SE) FRET. (a) Donor (TRPV1-EGFP) emission image; (b) acceptor (tubulin-RFP) emission image; (c) SE-FRET intensity image (scale bar:  $5 \mu m$ ); (d) zoom of a cellular region where FRET-iMSD analysis is later performed (scale bar:  $1 \mu m$ ); (e) donor-normalized apparent FRET efficiency  $E_D$  Panel f refers to FRET-iMSD analysis. (f) iMSD vs. time plot for FRET signal between TRPV1-EGFP and RFP-tubulin in physiological condition. From ref. [2].



Figure 2. Super-resolution map of BMI1 cluster in cell nucleus by PALM/STORM analysis.

#### Microfluidics and nanomaterials for biosensing and the nervous system

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Surface acoustic waves (SAWs) are acoustic waves that travel along the surface of an elastic material, with an amplitude that typically decays exponentially with depth into the substrate. Given their very superficial nature, SAWs are highly sensitive to surface perturbations of the substrate along which they propagate. For example, they can interact with liquid droplets or streams inducing macroscopic fluid manipulations or, in a different configuration, be exploited for sensing applications. The interest of my research group in this field is to explore and study novel SAW-driven microfluidic phenomena, and apply this new knowledge to the fields of biosensing and cell biology.

The nervous system (NS) is in some ways the most complex and fascinating organ of the human body. Unfortunately, NS pathologies that lead to tissue loss are dramatically difficult to treat because of the negligible regenerative potential of the central nervous system (CNS) from one side, and of the very slow and ineffective repair mechanisms of peripheral nervous system (PNS) from the other side. The interest of my research group in this field is to develop biocompatible nanostructured materials to help the heal of the PNS and cure and study CNS diseases. In particular, at the moment our attention is focused on textured surfaces for helping nerve regeneration and on nanotechnological methods for treating Globoid Cell Leukodystrophy (or Krabbe disease; OMIM #245200).



Example of a Full-Surface Acoustic Wave (SAW) Lab-on-Chip (LOC). A) Exploded illustration of the biochip. From the bottom to the top layer: Lithium Niobate (LN) substrate, 500hm matched waveguides, resonator-sensors and mixing interdigital transducers (IDTs), PDMS microfluidic channel. The PDMS microchannel is sealed at the top side; the sealing is here removed for clarity of visualization. B) Four resonators (green rectangles) are identified by NW-NE-SE-SW labels, and connected to the electric signal (red) and ground (white). The two IDTs (W and E) send a 50 MHz SAW towards two microchambers respectively for mixing (black arrows). The liquid-filled and air-filled microchambers contours are represented by the blue dashed lines. Scale bar is 2.5 mm. C) Photo of a mounted LoC.

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# PlantLab: physiological and molecular basis of plants' adaptation to dynamic environments.

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The PlantLab activities focus on the physiology of plants, from the responses to endogenous stimuli to adaptation to stress. A molecular and post-genomic approach characterizes all the research activities. These include several topics:

• *Plant hypoxia responses*: we study physiological and molecular mechanisms that enable plants to respond to developmental and environmental hypoxia. Recent findings identified oxygen as a diffusible molecule that has signaling functions connected to developmental processes (**Figure 1a**, Weits *et al.*, 2019, Shukla *et al.* 2019). In parallel, the survival of crops to environmental low oxygen occurring under flooding events, which are predicted to dramatically increase with global climate change, is crucial. Our activities focus on the characterization of molecular traits that promote tolerance and therefore may represent a major target for breeding purposes (**Figure 1b**, Ho *et al.*, 2017, Nghi *et al.*, 2019). In this context, we are interested in the cross-talk between the sugar-sensing pathways and the low oxygen one, which contributes to plants acclimation (Loreti *et al.*, 2018). Our activities in this area of research are also dedicated to the engineering of signaling module derived from different kingdoms of life to produce orthogonal reporters for oxygen (Iacopino *et al.*, 2019). These newly derived signaling components can be exploited to improve plant performances and resilience to the stress.

• *Quality and nutraceutical properties of crops:* our activities in this area of research are dedicated to study the nutraceutical properties of tomato, which are mainly related to the antioxidant potential of the fruit. The aim of our research is the production of high-anthocyanin containing tomatoes through joint efforts in horticulture, crop physiology, genetics and breeding (Colanero et al., 2018). We also provide new technologies to increase the iodine content in

plants. Iodine deficiency is common throughout the less developed world, resulting in illness and disabilities. To reach this goal we use an integrated approach that includes the study of iodine uptake in plants, the development of a protocol for plant iodine applications, and the production of transgenic plants (Gonzali *et al.*, 2017).

• *Biostimulants impact on plant physiology*: in collaboration with Valagro, we focus on the study of physiological responses induced by biostimulants. These are cocktails of molecules of biological origin that can be used in agriculture to increase abiotic stress tolerance in plants (Santaniello *et al.*, 2017).



**Figure 1** – (a) GFP expression driven by the hypoxia-responsive promoter (pHRPE) in the Arabidopsis inflorescence meristem; scale bars, 20  $\mu$ m (bottom), 25  $\mu$ m (top) (Weits et al., 2019). (b) Manhattan plots of genome wide association study (GWAS); the grey straight line shows the threshold for  $p < 10^4$ , the red straight line shows the threshold for FDR<0.05 (Nghi et al., 209).

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