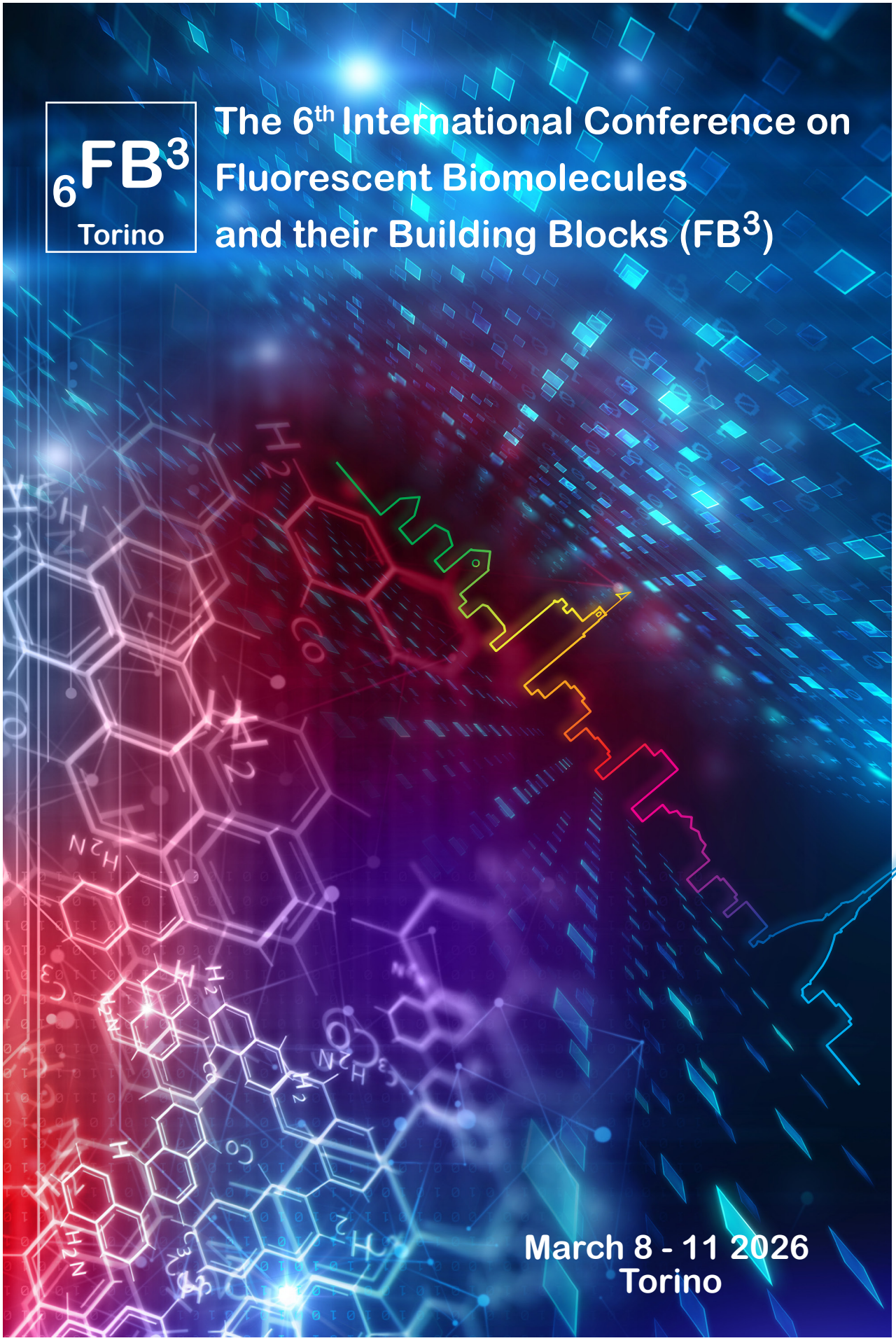
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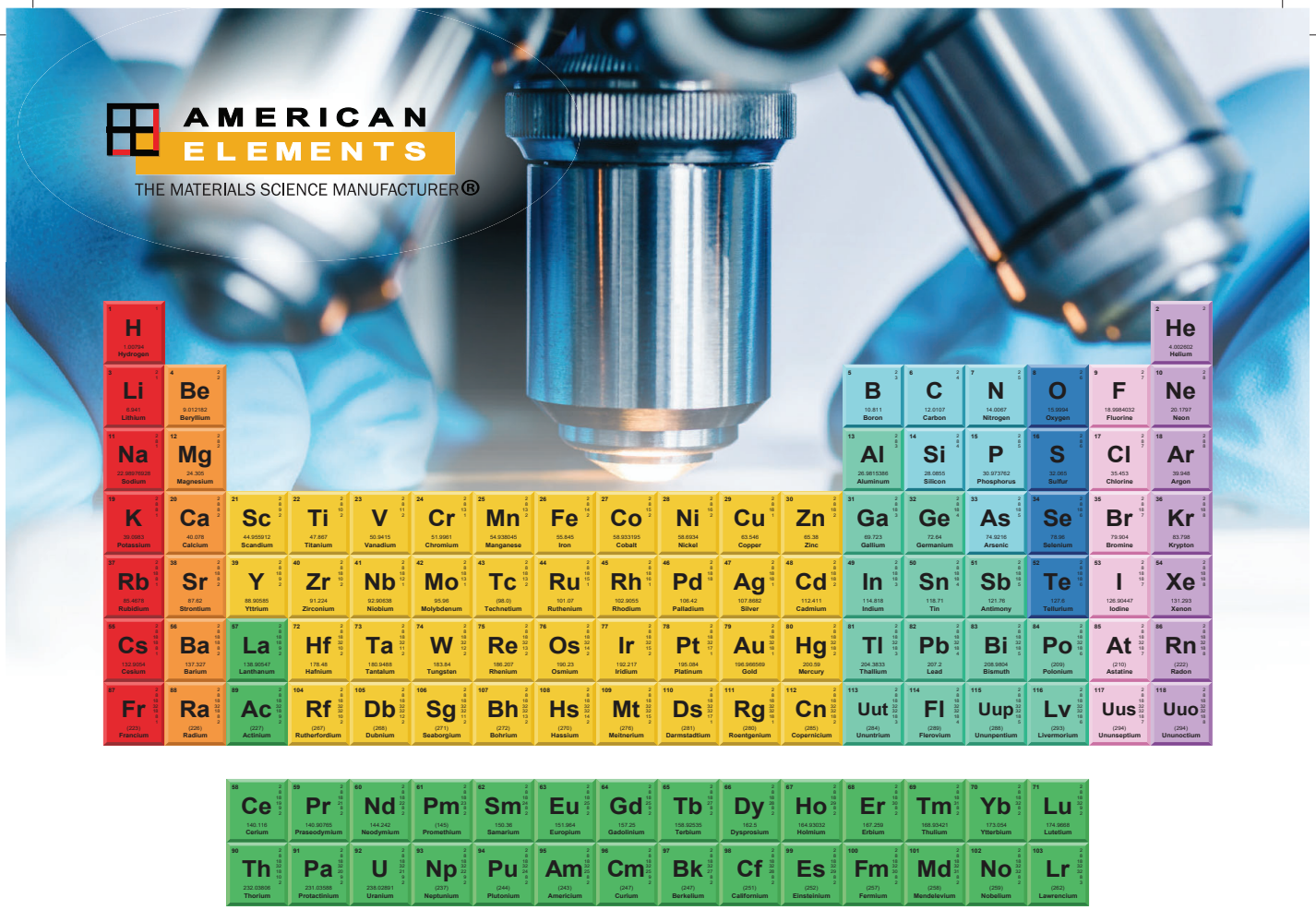

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ABOUT FB3

The 6th International Conference on Fluorescent Biomolecules and their Building Blocks (FB3) will be hosted in Torino from 8-11 March 2026.

FB3 is a biannual international conference series since 2012 (2012 in Gothenburg, Sweden; Keynote speaker: Prof Roger Tsien; 2014 in San Diego, USA; Keynote speaker: Prof William E. Moerner; 2016 in Tianjin, China; Keynote speaker: Prof Eric Kool; 2018 in Glasgow, UK; Keynote speaker: Prof Taekjip Ha; 2024 in Hong Kong, China; Keynote speakers: Prof. Christopher J. Chang, Prof. Kai Johnsson and Prof.Markus Sauer. The 2026 round will be hosted by the University of Torino in synergic collaboration with the University of Napoli Federico II, the University of Catania and the University of Piemonte Orientale.

The FB3 conference brings together established scientists, younger investigators, students and postdocs from around the world to discuss the state-of-the-art developments and applications of fluorescent molecules in chemical biology, bioimaging and biomedical applications. This rapidly evolving field deserves a dedicated venue, and our community would benefit from a vigorous discussion and exchange of ideas related to its progress and future directions.

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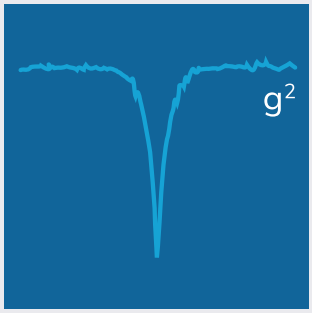

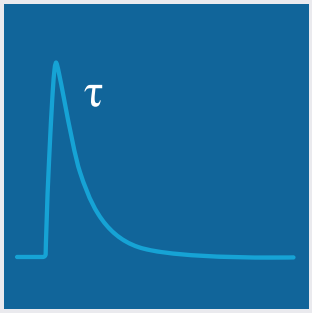

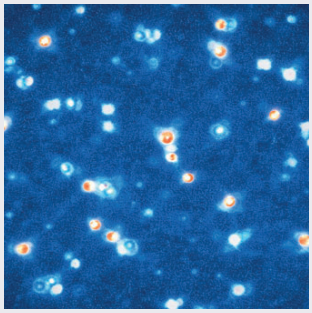

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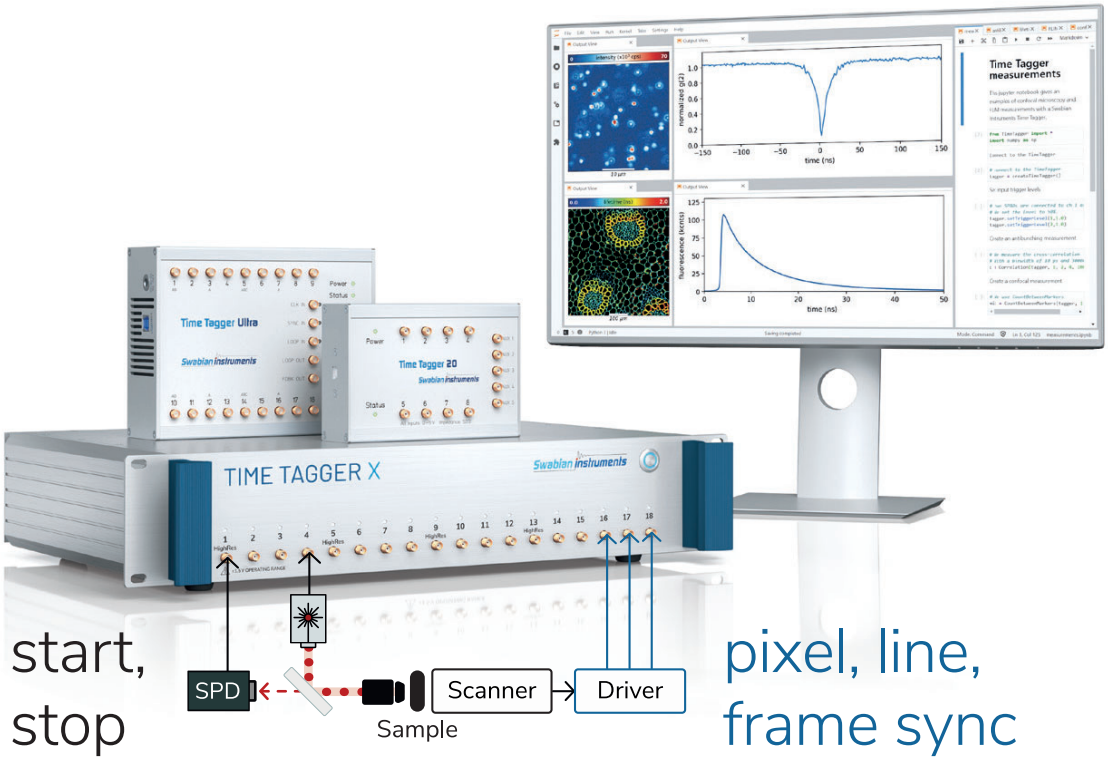

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PROGRAMME

8 March 2026 (Sunday)		
12:30-14:00	**Registration**	
14:00-14:30	**Opening** *Andrea Fin (University of Torino)* **Opening Remarks** *Claudia Barolo (Director of the Department of Chemistry, University of Torino)*	
Session A1 *Chairperson: Mattia Bartoli (Italian Institute of Technology)*		
14:30-15:00	IL-1	**NIR polymethine dyes for photodynamic therapy** *Nadia Barbero (University of Torino)*
15:00-15:15	OC-1	**A computational protocol for uncovering photoinduced electron transfer mechanisms in fluorescent molecules** *Syed Ali Abbas Abedi (Singapore University of Technology and Design - Nanyang Technological University.)*
15:15-15:30	OC-2	**Optical spectroscopy and multiphoton microscopy as tools for drug delivery** *Cristina Sissa (University of Parma)*
15:30-15:45	OC-3	**A fluorescent probe with an ultra-rapid response to nitric oxide in living cells** *Cristina Parisi (University of Catania)*
15:45-16:00	OC-4	**The photophysics of isothiazologuanosine, an isofunctional fluorescent analogue of guanosine: insights from Quantum Mechanical calculations** *Roberto Improta (Consiglio Nazionale delle Ricerche (CNR) Istituto di Biostrutture e Bioimmagini, Napoli)*
16:00-16:30	**Coffee Break**	
Session A2 *Chairperson: Ivana Miletto (University of Piemonte Orientale)*		
16:30-17:00	IL-2	**Design of fluorescent halloysite nanotube based nanomaterials: supramolecular and covalent strategies for biomedical applications** *Marina Massaro (University of Palermo)*
17:00-17:15	OC-5	**Synthesis and characterization of a lanthanide probe for Time-Resolved Forster Resonance Energy Transfer** *Filippo Valloni (University of Piemonte Orientale)*
17:15-17:30	OC-6	**Supporting science across the globe: EURO-BIOIMAGING Research Infrastructure** *Erika Cerutti (Euro-BioImaging ERIC, IBB-CNR)*
17:30-18:30	**Welcome Cocktail**	

9 March 2026 (Monday)		
8:30-9:00	**Reception**	
Session B1 *Chairperson: Yitzhak Tor (University of California San Diego)*		
9:00-9:45	KN-1	**Computation aided fluorophore development** *Xiaogang Liu (Nanyang Technological University)*
9:45-10:15	IL-3	**Fluorescent probes for translational bioimaging** *Marc Vendrell (University of Edinburgh)*
10:15-10:30	OC-7	**Biomimetic and responsive fluorescent lipid microparticles to study phagocytosis** *Blaise Dumat (Ecole normale supérieure, PSL University, Sorbonne Université, CNRS)*
10:30-10:45	SP-1	**Beyond histograms: advanced FLIM and FCS enabled by time taggers** *Eric Schmidt (SWABIAN INSTRUMENTS)*
10:45-11:15	**Coffee Break**	
Session B2 *Chairperson: Salvatore Sortino (University of Catania)*		
11:15-11:45	IL-4	**Fluorescent probes for biomembranes and organelles: from lipids to proteins** *Andrey S. Klymchenko (Université de Strasbourg - CNRS)*
11:45-12:15	IL-5	**Studying RNA biology and therapeutics using fluorescent base analogues** *Marcus Wilhelmsson (Chalmers University of Technology)*
12:15-12:30	OC-8	**Precise sequence-specific fluorescent labeling of DNA: illuminating genomic loci for optical mapping applications** *Xiong Chen (KU Leuven)*
12:30-12:45	OC-9	**Molecular rotors detect the formation and conversion of WT α-synuclein oligomers and its' pathological mutant variants** *Siân Allerton (Imperial College London)*
12:45-13:00	SP-2	**Fast and easy ultrafast spectroscopy: enabling more time for research** *Robertas Grigutis (Light Conversion)*
13:00-14:15	**Lunch**	
Session B3 *Chairperson: Serena Riela (University of Catania)*		
14:15-14:45	IL-6	**Biocompatible lanthanide complexes for responsive molecular imaging** *Ka-Leung Wong (Gary) (City Universiy Hong Kong)*
14:45-15:15	IL-7	**New insights in the photophysics and applications of thienoguanosine** *Yves Mély (Université de Strasbourg)*
15:15-15:30	OC-10	**Chemigenetic fluorescent sensors to visualize Na^+ and K^+ in cells** *Takuya Terai (The University of Tokyo)*
15:30-15:45	OC-11	**Novel quantitative methodology for studying inhibition of protein-protein interactions** *Thomas Mills (Imperial College London)*
15:45-16:00	OC-12	**Observing bioorthogonal macrocyclization in live cell nuclear envelope using on/on fluorescence lifetime microscopy** *Sebastian Pim (Royal College of Surgeons in Ireland)*
16:00-16:30	**Coffee Break**	
16:30-18:30	**Poster Session**	

10 March 2026 (Tuesday)		
8:30-9:00	**Reception**	
Session C1 *Chairperson: Yves Mély (University of Strasbourg)*		
9:00-9:45	KN-2	**Isomorphic fluorescent nucleosides, nucleotides and oligonucleotides** *Yitzhak Tor (University of California San Diego)*
9:45-10:15	IL-8	**Cellular labeling of DNA and RNA with fluorogenic dyes** *Hans-Achim Wagenknecht (Karlsruhe Institute of Technology)*
10:15-10:30	OC-13	**Synthesis and characterization of RNA containing emissive isomorphic nucleotides** *Julia Dietzsch (University of Würzburg)*
10:30-10:45	SP-3	**Techniques to characterise biomolecules using fluorescence spectroscopy** *Grant Cumming (HAMAMATSU-EDINBURGH INSTRUMENTS-NKT PHOTONICS)*
10:45-11:15	**Coffee Break**	
Session C2 *Chairperson: Giorgia Oliviero (University of Napoli Federico II)*		
11:15-11:45	IL-9	**Supramolecular dynamic chemistry for membrane transport and biomimetic systems** *Javier Montenegro (Universidad de Santiago de Compostela, CIQUS)*
11:45-12:15	IL-10	**4D single particle microscopy and AI synergy as a new era in studying nanoparticles cell entry mechanisms***Nikos Hatzakis (University of Copenhagen)*
12:15-12:30	OC-14	**Exchangable fluorescent probes for long-term imaging of plasmic membrane** *Rémi Pelletier (CNRS)*
12:30-12:45	OC-15	**Time-resolved FRET for multiplexed detection of antibodies** *Arthur Charasson (McMaster University)*
12:45-13:00	OC-16	**FLIMBDs – benzodiazoles for fluorescence lifetime imaging** *Deborah Seah (University of Edinburgh)*
13:00-14:15	**Lunch**	
Session C3 *Chairperson: Nadia Barbero (University of Torino)*		
14:15-14:45	IL-11	**Nitric oxide photoreleasing nanoconstructs with fluorescent reporting** *Salvatore Sortino (University of Catania)*
14:45-15:00	JIL	**Benzoxanthene lignans: from discovery to novel bioactive derivatives** *Claudia Sciacca (University of Catania)*
15:00-15:15	OC-17	**ZnO nanotetrapod synthesis and application in optoelectronics** *Simas Rackauskas (Kaunas University of Technology)*
15:15-18:30	**Social Activity** *City Tour and Royal Palace*	
20:00-23:00	**Conference Dinner** *Arcadia*	

11 March 2026 (Wednesday)		
8:30-9:00	**Reception**	
Session D1 *Chairperson: Marcus Wilhelmsson (Chalmers University of Technology)*		
9:00-9:45	KN-3	**Illuminating biological systems using chemistry** *Luke D. Lavis (Janelia Research Campus, Howard Hughes Medical Institute)*
9:45-10:15	IL-12	**BF_2-Aazdipyrromethene fluorophores research tools with the potential for clinical use** *Donal F. O'Shea (Royal College of Surgeons in Ireland)*
10:15-10:30	OC-18	**Teaching new tricks to old dyes: engineering and imaging strategies for high-resolution biology** *Saumya Saurabh (New York University)*
10:30-10:45	SP-5	**TBD** *Erika Tarro Genta (BUCHI)*
10:45-11:15	**Coffee Break**	
Session D2 *Chairperson: Francesca Cardano (University of Torino)*		
11:15-11:45	IL-13	**Photopharmacology: towards image-guided pharmacotherapy** *Wiktor Szymanski (University of Groningen)*
11:45-12:00	OC-19	**A red emitting carbon dot and aptamer- functionalized alginate system for targeted triple-negative breast cancer imaging** *Letizia Sambri (University of Bologna)*
12:00-12:15	OC-20	**Deep eutectic solvent-derived thermoresistant fluorescent material: development and characterization** *Giulia Adriana Bracchini (University of Rome Tor Vergata)*
12:15-12:30	OC-21	**Modeling light-controlled aspartate transport** *Gülbahar Bozan (University of Groningen)*
12:30-12:45	OC-22	**Development of tunable and STED-compatible NIR fluorophores for super-resolution imaging in live cells** *Celia Paramio (Royal College of Surgeons in Ireland)*
12:45-13:00	OC-23	**Measuring photoluminescence quantum yield of difficult samples** *Grant Cumming (HAMAMATSU-EDINBURGH INSTRUMENTS-NKT PHOTONICS)*
13:00-14:15	**Lunch**	
Session D3 *Chairperson: Andrea Fin (University of Torino)*		
14:15-14:45	IL-14	**Bright tools in chemical biology** *Francesca Cardano (University of Torino)*
14:45-15:00	OC-24	**Exploring Si-phthalocyanines with different valency for PSMA-targeted photodynamic therapy: synthesis and preclinical validation** *Rachele Stefania (University of Piemonte Orientale)*
15:00-15:15	OC-25	**Sustainable synthesis of fluorescent furano-chalcones** *Laura Tedesco (University of Torino)*
15:15-15:30	OC-26	**Naphthalene-diimide-based down-converters and other approaches towards bio-based lighting** *Sara Ferrara (University of Torino)*
15:30-15:45	**Prizes and Closing Remarks** *Francesca Cardano (University of Torino)*	

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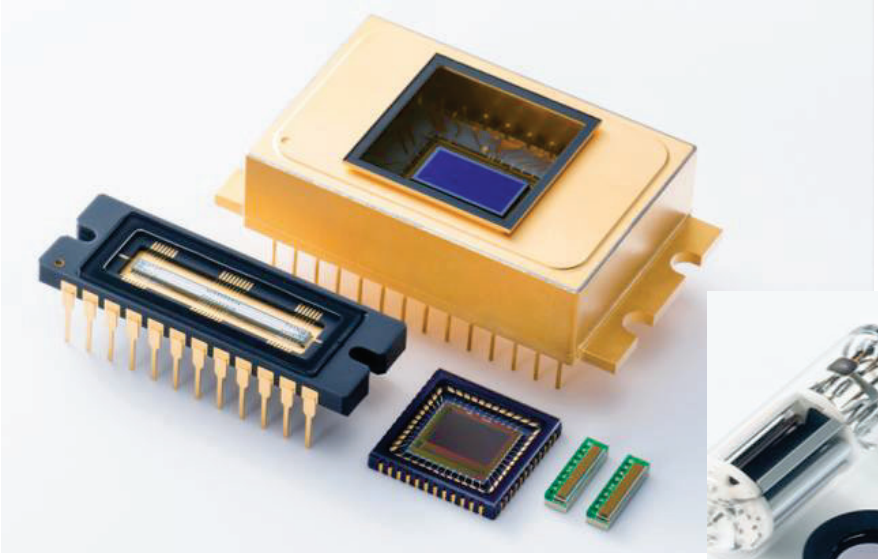

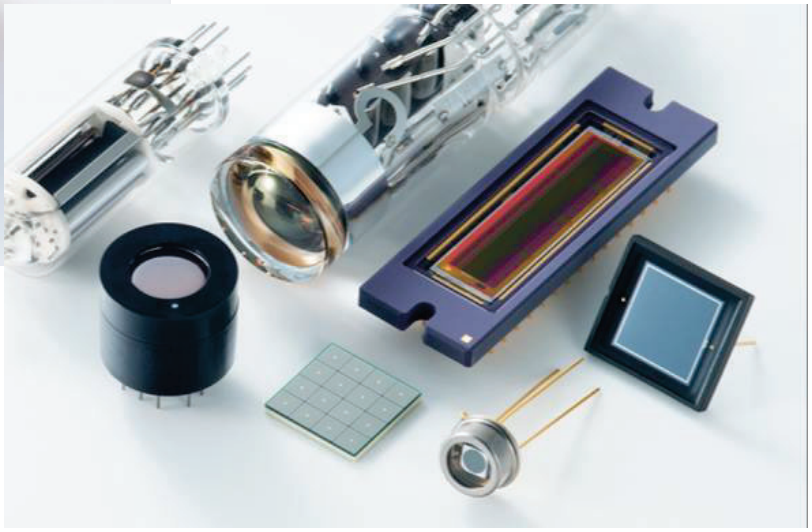

KN-1

Computation aided fluorophore development

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Fluorescent dyes are indispensable tools in bioimaging and clinical diagnostics, yet their development remains largely dependent on empirical, trial-and-error approaches. This lack of molecular-level design principles hampers both the efficiency and precision of dye discovery. To overcome these limitations, we establish a computational-experimental workflow that leverages quantum chemical calculations to unravel structure–property relationships in fluorophores. By correlating electronic structure parameters with key photophysical properties, we develop predictive molecular descriptors that guide the design of high-performance dyes. Moreover, through iterative computation-driven refinement and synthetic validation, we successfully engineered a new class of compact near-infrared dyes with tunable optical properties and good biological compatibility. These "miniature" NIR dyes exemplify how rational design can yield molecules with reduced size, and targeted performance for in vivo imaging.

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KN-2

Isomorphic fluorescent nucleosides, nucleotides and oligonucleotides

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Email: ytor@ucsd.edu

The photophysics of RNA and DNA is unique among biomolecules that possess aromatic building blocks. Unlike proteins, which contain intrinsically fluorescent amino acids, the canonical nucleosides, and hence nucleotides and oligonucleotides, are practically non-emissive. We develop emissive nucleoside surrogates that facilitate the monitoring of nucleoside-, nucleotide- and nucleic acid-based transformations at a "nucleobase-resolution" in real time. The lecture will articulate the fundamental challenges and will present the design, synthesis and photophysical features of emissive nucleosides as well as selected examples for their utilization in fluorescence-based biophysical and discovery assays.[1]

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KN-3

Illuminating biological systems using chemistry

Luke D. Lavis
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Fluorescence microscopy has transformed our understanding of cellular processes, but progress is limited by the brightness and photostability of available fluorescent labels. Over the past 15+ years, our laboratory has developed a comprehensive synthetic toolkit that fundamentally changed how small-molecule fluorophores are designed and accessed by the scientific community. Our key breakthrough was the discovery that incorporation of four-membered azetidine rings into fluorophores dramatically enhances both quantum efficiency and photostability.[1] Building on this foundation, we developed the Janelia Fluor® (JF) platform, creating an optimized palette of dyes that spans the visible spectrum with exceptional brightness and photostability.[2] We also elucidated key structure–activity relationships that enable rational design of fluorogenic and bioavailable dyes. These advances enabled us to pioneer chemigenetic indicators—hybrid small-molecule:protein sensors that combine the brightness of chemical dyes with the genetic specificity of fluorescent proteins—as well as develop deuterated rhodamines for enhanced photostability, spontaneously blinking fluorophores for super-resolution microscopy, and bespoke dyes tailored for specific biological applications. Recognizing that scientific tools only matter if scientists can access them, we established Open Chemistry—a unique initiative that has distributed aliquots of fluorescent dyes to thousands of scientists in 40 countries, representing over $250,000,000 in value to the community. This open approach has democratized access to cutting-edge fluorophores and accelerated discoveries across biology and medicine. Our portfolio of enhanced fluorophores now enables advanced microscopy experiments from single-molecule tracking in individual cells to population-scale voltage imaging *in vivo*, allowing scientists to fully harness the power of imaging in biological research.

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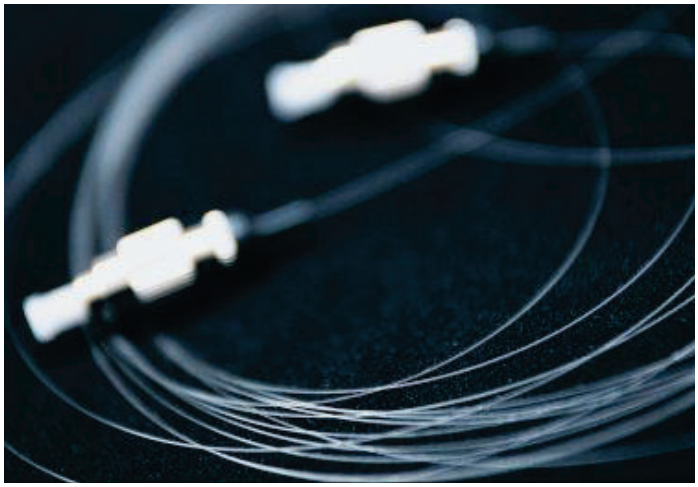

Optical fibers and modules

Photonic crystal fibers, cords, and gain modules

IL-1

NIR polymethine dyes for photodynamic therapy

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Significant efforts have been dedicated to develop near-infrared (NIR) dyes for both biological and optoelectronic applications, such as imaging, photodynamic therapy (PDT), solar cells and biological sensors. Among many, polymethine dyes can be considered as interesting photosensitizers due to the easy and low-cost synthesis along with remarkable absorption property in the far-red NIR region.[1] Squaraines and cyanines are notable for their high molar absorption coefficients, remarkable brightness, fluorescence and photostability, especially in organic media.

This contribution focuses on the design and synthesis of various series of NIR absorbing polymethine dyes. A preliminary structure-properties relationship is presented to highlight the most relevant molecular features for the interactions with light and biomolecules. Among the different applications, results on their use as photosensitizers in PDT[2] and antimicrobial[3] will be discussed. Additionally, the encapsulation of these dyes in organic and inorganic nanomaterials to enhance their optical properties, photostability, biocompatibility, and efficient cellular internalization will be examined.[4]

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IL-2

Design of fluorescent halloysite nanotube based nanomaterials: supramolecular and covalent strategies for biomedical applications

Marina Massaro,[1] F. Leone,[2] N. Borbone,[3] G. Cinà,[2] A.P. Falanga,[3] M. Notarbartolo,[1] G. Oliviero,[4] F.M. Raymo,[5] R. Sanchéz-Espejo,[6] C. Viseras,[6,7] S. Riela[2]

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Fluorescence imaging has become an indispensable tool in biomedical laboratories for elucidating the fundamental dynamic and structural factors that regulate cellular processes. The development of fluorescent nanoprobes represents a major challenge for the detection of cellular processes under a microscope. In addition, the combination of these fluorescent nanoprobes with species possessing therapeutic potential enables the design and development of theranostic nanomaterials that can be efficiently used for the treatment of several pathologies.

Herein, we report the development of fluorescent materials based on the combination of suitable organic molecules with appealing photophysical properties and clay minerals, such as halloysite nanotubes (HNTs), as biomedical tools.[1-3] HNTs are phyllosilicate clay minerals with interesting biological properties, including high biocompatibility and the ability to cross cellular membranes and localize in perinuclear regions, and have been widely used in biological applications.[4]

Their modification, both supramolecular and covalent, with fluorescent probes represents an attractive strategy for the development of multifunctional nanomaterials with potential therapeutic applications. In the present communication, the two different synthetic approaches are discussed and compared in terms of species loading and photophysical properties. Furthermore, a possible application is proposed, highlighting the advantages of using clay minerals for the delivery of this type of species.

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IL-3

Fluorescent probes for translational bioimaging

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Fluorescent activatable probes are valuable tools for live-cell imaging because of their tunability and target specificity.[1] Over the last few years, our group has designed a collection of fluorogenic amino acids and peptides for high-resolution biological imaging and translational medicine, which was recognised with the RSC Bader Prize 2023. Our team have demonstrated that this approach can be used to generate probes to visualize infection and immune cells in human biosamples,[2] *in vivo*[3] and in *ex vivo* human biopsies.[4] We have designed fluorescent amino acids to: 1) be compatible with conventional solid-phase peptide synthesis, 2) maintain the biomolecular recognition features of the native peptides and 3) emit fluorescence preferentially after target binding, improving signal-to-noise ratios for imaging. Recently, we have included the smallest turn-on fluorescent amino acids for peptide-PAINT imaging and super-resolution microscopy,[5] and fluorogenic tags for proteins associated with immune cell function like interleukins,[6] immunophilins and chemokines[7] as well as nanobodies and antibodies.[8] Finally, the talk will also briefly discuss our efforts to establish these fluorescent probes for clinical applications.

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IL-4

Fluorescent probes for biomembranes and organelles: from lipids to proteins

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Biomembranes are important targets for fluorescent probes because they constitute a multifunctional frontier of the cells in form of plasma membranes and they delimit major intracellular organelles.[1] To address limitations of existing plasma membrane probes,[1] we developed lipid-driven covalent labelling of membrane proteins, which enabled permanent labelling of cell surface.[2] Solvatochromic dyes that change their emission color in response to their local environment[3] are key building blocks for development of smart probes for lipids and proteins. To study lipid organization in membranes of organelles under oxidative and mechanical stress, we developed a series of solvatochromic probes with organelle-targeting ligands.[4] Genetic targeting of a solvatochromic dye to proteins enabled sensing their nanoscale environment,[5] as well as monitoring cell-cell contacts and the packing state of chromatin. Lipid droplets are particularly interesting targets for solvatochromic dyes, which allow tracking their core composition and heterogeneity.[6] Finally, artificial lipid droplets are nanoscale reactors that can be used to construct supramolecular sensors (artificial receptors) for small molecules, such as neurotransmitters.[7]

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IL-5

Studying RNA biology and therapeutics using fluorescent base analogues

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We develop and apply a class of minimally perturbing labels for nucleic acids known as *f*luorescent *b*ase *a*nalogues (FBAs), which are gaining increasing significance in molecular biology. Recently, we have employed these FBAs to track the cellular uptake of mRNA-based therapeutics.[1] A major challenge for the broader application of such therapeutics is their inefficient productive cellular delivery. The underlying causes of this low delivery efficiency remain unclear, highlighting the need for novel, non-invasive analytical tools. To address this, we have established FBA-based methods that enable live-cell spatiotemporal tracking of mRNA labeled with our FBAs, tC^O and 2CNqA, using *in vitro* transcription.[1,2] We have also developed similar methods to allow for live-cell imaging of short RNAs and antisense oligonucleotides (ASO gapmers).[3] Comparative analyses of various FBA labels alongside conventional external fluorescent tags in these applications will be presented. Additionally, recent advances from our lab will be discussed, including the spontaneous uptake of fluorescent ATP analogues.[4] One of these ATP analogues facilitates endogenous metabolic labeling of cellular RNAs in non-engineered cell,[4] and we are able to monitor this cellular RNA using live-cell confocal microscopy and fluorescence lifetime imaging (FLIM)[5].

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IL-6

Biocompatible lanthanide complexes for responsive molecular imaging

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Lanthanide ions possess exceptional photophysical characteristics, most notably their sharp near-infrared emission fingerprints and long luminescence lifetimes, which have made them highly attractive for biosensor development. In this lecture, I will present a decade of research dedicated to engineering molecular lanthanide complexes tailored for detecting cancer-specific biomarkers such as hydrogen sulfide (H_2S) and carbonic anhydrase, as well as oncoproteins including LMP1 and EBNA1. Our work not only demonstrates the diagnostic potential of these complexes but also addresses a longstanding challenge in organic lanthanide chemistry: the synthesis of macrocyclic ligands equipped with efficient antenna systems. We introduce an optimized methodology that enables the simple construction of such ligands, paving the way for their integration into biomedical applications.

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IL-7

New insights in the photophysics and applications of thienoguanosine

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To investigate the dynamics of oligonucleotides in their free form or on interaction with proteins, fluorescence techniques are the tool of choice because of their high sensitivity and spatiotemporal resolution. Unfortunately, the very low fluorescence quantum yield of natural nucleobases does not facilitate their use for this purpose, requesting the need of external labelling. Of special interest are emissive nucleobase analogues capable of perfectly replacing natural nucleobases while retaining bright and environmentally-sensitive emission. Thienoguanosine (thG) represents a breakthrough in this direction, since it has been shown to replace natural G almost perfectly in RNA and DNA sequences, while retaining optimal brightness and environmental sensitivity when incorporated into oligonucleotides. Therefore, thG offers unique potential for the selective and reliable monitoring of the conformation and dynamics of a specific G residue within a nucleic acid sequence. In this context, we recently evidenced that the fluorescence of thG and its environment-driven changes in DNA duplexes largely rely on changes in its absorption properties, rather than on changes of its fluorescence quantum yield. Taking benefit of these properties, we have been able by using a combination of fluorescence stopped-flow techniques together with molecular dynamics simulations and quantum mechanical calculations to decode the base flipping mechanism of the epigenetic UHRF1 protein. Moreover, we have also investigated the potential of thG to replace G residues in G-quadruplexes in order to monitor their structure and dynamics. Using telomeric sequences, we evidenced that thG selectively incorporated at several positions appears as a highly promising and sensitive reporter for studying the folding/unfolding dynamics of G4s.

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IL-8

Cellular labeling of DNA and RNA with fluorogenic dyes

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Cellular or metabolic labelling is an important method to attach fluorophores to DNA and RNA in their native environment in cells.[1] The Diels-Alder reaction with inverse electron demand of tetrazines and the photoclick reaction of tetrazoles, both with alkenes or alkynes are applied as bioorthogonal reactions for cellular labelling. One of the critical points is the size of the bioorthogonally reactive group, especially for the cellular kinases. We systematically investigated the efficiency of cellular DNA labelling using synthetic 2-deoxyuridines with dienophiles of different size.[2] Fluorogenic cyanine-styryl dyes are developed for the Diels-Alder reaction[3,4] and pyrene-tetrazoles for the photoclick reactions.[5] Using both, dual and orthogonal labeling of DNA was demonstrated in HeLa cells.[6] RNA labelling in Vero cells was achieved with the TriPPPro approach.[7]

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IL-9

Supramolecular dynamic chemistry for membrane transport and biomimetic systems

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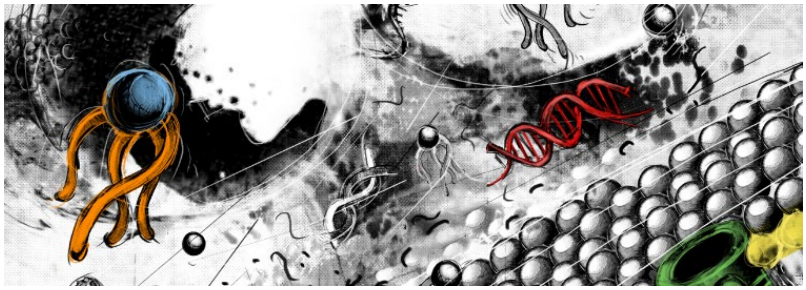
Our research group is interested in the application of supramolecular chemistry to understand and manipulate biology.[1,2] Our work philosophy is based in the importance of weak and non-covalent forces to control the shape and the topology of biomolecules, which are governed by the principles described by supramolecular chemistry. These supramolecular lessons can then be applied to control the properties and function of biomolecules. We believe that by modulating the shape we can mimic, control and improve functional behaviour. With focus in supramolecular interactions for artificial membranes and tubular composites, we investigate the construction of synthetic systems for controlling and emulating biology and life-like soft systems.[3-8]



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IL-10

4D single particle microscopy and AI synergy as a new era in studying nanoparticles cell entry mechanisms

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Current advances in imaging technologies compounded with, machine learning drivel analysis, have allowed the direct observation and interpretation of biological phenomena in real time.

I will discuss some of my labs recent breakthroughs pushing the boundaries of biological imaging and analysis to observe directly biological process as they happen, and to rapidly and precisely extract quantitative mechanistic information. I will focus on a) the advanced the imaging toolboxes offering parallelized tracking of hundreds of individual biological particles (viruses, nanocarriers or Lipid nanoparticles) in live cell in 3D, b) our deep learning approaches that map with up to 95% accuracy -in milliseconds instead of days- the key timepoints of virus or nanoparticle internalization and genetic material releases. using exclusively diffusional behavior, as well as c) how the parallelized readouts of nanoparticles properties, such as loading efficiency, dimensions, lipid composition, enable quantitative understanding and improving of cellular entry and genetic material release.

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IL-11

Nitric oxide photoreleasing nanoconstructs with fluorescent reporting

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Nitric oxide (NO) has great potential as an unconventional therapeutic for a variety of diseases.[1] However, the NO's biological effects are strictly dependent on its concentration and generation site. Light-activatable NO precursors, namely NO photodonors (NOPs), are stable in the dark and permit NO to be delivered exclusively under light input with a very high spatiotemporal control.[2] Nevertheless, monitoring the NO released in the cell environment with the aid of non-invasive fluorescence techniques, without the use of additional external NO probes, is highly demanding to avoid either false positives or negatives. A suitable strategy to overcome this drawback is built on the concept of "*photorelease with fluorescent reporting*".[3] This elegant approach exploits fluorogenic units as a part of the covalent skeleton of the NOPs, whose emission switches "on" after the NO release. In this way, the release process can be easily monitored in real time by following changes in the emission of the stable photoproduct formed, "*the reporter*", which serves as an optical counter of the NO liberated.
In this contribution, we shall illustrate some of the most recent molecular and supramolecular constructs and nanomaterials developed in our laboratory that can photogenerate NO with fluorescent reporting functionalities, highlighting their rationale design and potential therapeutic applications in cancer and bacterial infections.[4-8]

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IL-12

BF_2-Aazdipyrromethene fluorophores research tools with the potential for clinical use

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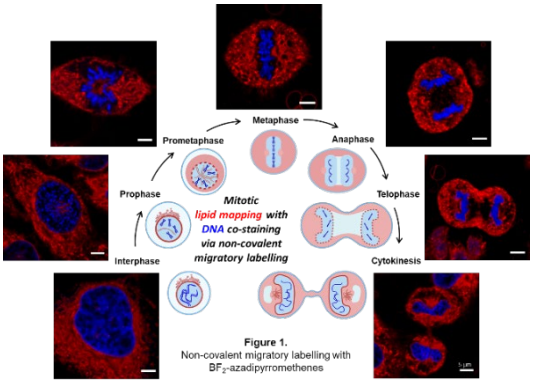

Figure 1.
Non-covalent migratory labelling with BF_2-azadipyrromethenes

Fluorescence imaging, utilizing molecular fluorophores, often acts as a central tool for the investigation of fundamental biological processes. It also offers huge future potential for human imaging coupled to therapeutic procedures such as fluorescence guided surgery.[1] Our research has pioneered the BF_2-azadipyrromethene class of near infrared fluorophores from which *in vitro* and *in vivo* imaging probes have been developed.[2] This class has excellent photophysical characteristics such as tuneable emission maxima between 650 and 820 nm, exceptional photostability and high quantum yields. Their suitability for time-lapse live or fixed cell microscopy employing widefield, confocal, STED super-resolution or fluorescence lifetime (FLIM) techniques allows their application to complex chemical-biology investigations. This presentation is concerned with our ongoing development of this emitter class for investigating the chemical-biology of intracellular lipid structures. Non-covalent migratory fluorescence labelling has been introduced to spatially and temporally map intracellular lipid structures throughout a cell division cycle (mitosis). This less-invasive approach utilizes BF_2-azadipyrromethene fluorophores to first non-covalently label known intracellular lipid compartments at cell interphase which then migrate with the lipid components of these structures as they disassemble, redistribute and reassemble prior to daughter cell separation. Through this unique approach to image capture, key prometaphase events such as lipid intrusion into the nucleus and nuclear membrane disassembly are observable, as are the stages of nuclear membrane reassembly in telophase and lipid distribution during cytokinesis (Figure 1).[3] Remarkably, the non-covalent BF_2-azadipyrromethene label remains associated with the originating lipid components as they undergo these architectural reorganizations and changes in subcellular localization associated with mitosis. To further address the challenges of lipid structure differentiation during mitosis a novel fluorescence lifetime encoded STED-FLIM approach has been developed. This utilises two spectrally overlapping STED compatible fluorophores, with differing lifetimes, to non-covalently label lipid structures which can be image separated from each other with phasor plot analysis, with corresponding STED images confirming their structural identities. As lipid-based cell structures are influenced by numerous biological processes, our approaches to their fluorescence imaging could offer novel perspectives into their multifaceted roles.

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IL-13

Photopharmacology: towards image-guided pharmacotherapy

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Molecular photomedicine holds the promise for precise treatments, which avoid systemic adverse effects and development of drug resistance. This promise is supported by current medical imaging modalities that reveal the nature and location of malignancies. At the same time, biomedical engineering has recently created methods to deliver light deep into human body. The photomedicine puzzle is currently missing its final piece – the way of translating light into a therapy. To address this challenge, drugs are introduced whose activity could be reversibly or irreversibly turned on with light.

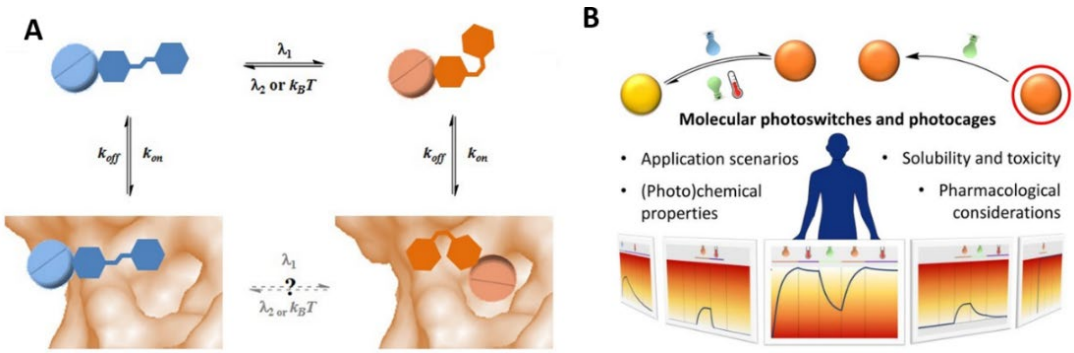

Fig. The principle of photopharmacology (**A**) and its key molecular tools (B).

The aim of this presentation is to describe the emerging concept of photopharmacology (Figure A),[1] which is currently being developed and applied to precisely control the activity of drugs using light. The presentation will focus on our efforts towards bridging light and medicine, focusing first on new light-operated tools[2] (molecular photoswitches[3] and photocages[4], Figure B). Next, I will highlight the synergies between medical imaging and therapy, offered by light, through photo-responsive optical[5] imaging agents. Finally, using those examples, I will highlight the applications[6] and structural aspects[7] of photopharmacology.

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IL-14

Bright tools in chemical biology

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Understanding biochemical structures and their functionalities is closely linked to their accurate visualization. The development of fluorophores featuring straightforward synthetic routes, specific optical read-outs and efficient biological targeting is still highly desired.[1] Likewise, the development of novel emissive chemical biology tools as synthetic emissive analogs of nucleic acids remain a research area deserving further exploration.[2] In our group we focus on the synthesis and systematic investigation of structure-related-properties of different fluorophores. These include symmetrical and unsymmetrical NIR Squaraine dyes,[3] core-substituted 1,4,5,8-naphthalenediimides (c-NDI)[4] spanning the whole visible spectrum and differently substituted PEGylated-BODIPY with the aim of optimizing the design of bioimaging probes targeted to biological membranes. In parallel, we are exploring the implementation of peptide nucleic acids (PNA), well-established and promising platforms for antisense/antigene strategies, gene editing, and nucleic acid sensing, making them easy to visualize. In this context, we are studying either isomorphic fluorescent thieno[3,4-*d*]pyrimidine nucleobases known for their intrinsic optical properties[5] as well as specific designed PNA-cNDI conjugates to evaluate novel PNA's functional properties and related applications.

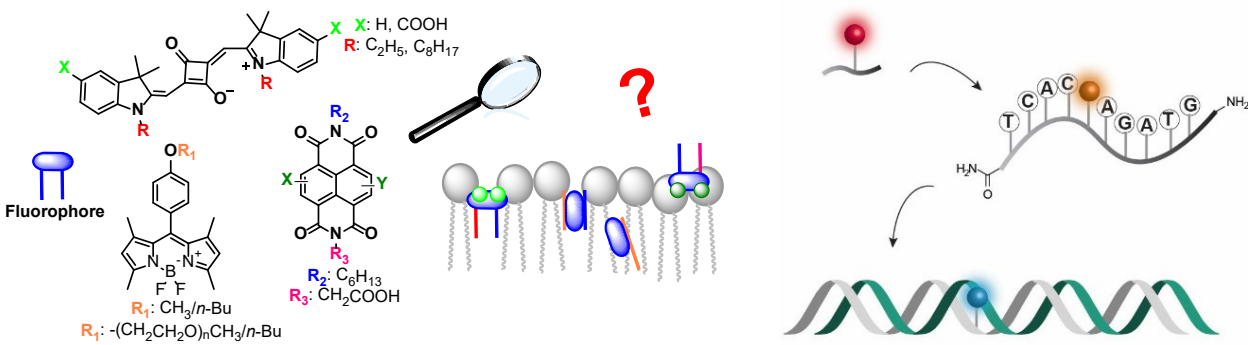

Fig. Fluorophores investigated for bioimaging and novel emissive chemical biology tools.

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Benzoxanthene lignans: from discovery to novel bioactive derivatives

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Lignans are plant secondary metabolites normally synthesized from two phenylpropanoid units (C_6C_3) by oxidative coupling.[1] Benzoxanthenes are fluorescent phenolic compounds belonging to the class of lignans. Less than a dozen natural molecules with this structure have been discovered nowadays. Biomimetic coupling reactions carried out on natural or natural derived phenylpropanoids can afford "unnatural" products through mechanisms that mimic the biosynthetic processes.[2] A library of fluorescent benzoxanthene lignans was obtained through a Mn^{3+} ion-mediated radical coupling reaction according to the mechanism illustrated in **Figure 1**. The process proved to be regioselective, leading to the formation of an 8–8′ dimeric structure that evolves *in situ* into a xanthene core. Several efforts have been employed to deepen the biological profile of this natural-based scaffold, which was evaluated for biological activities and various applications in delivery systems.[3]

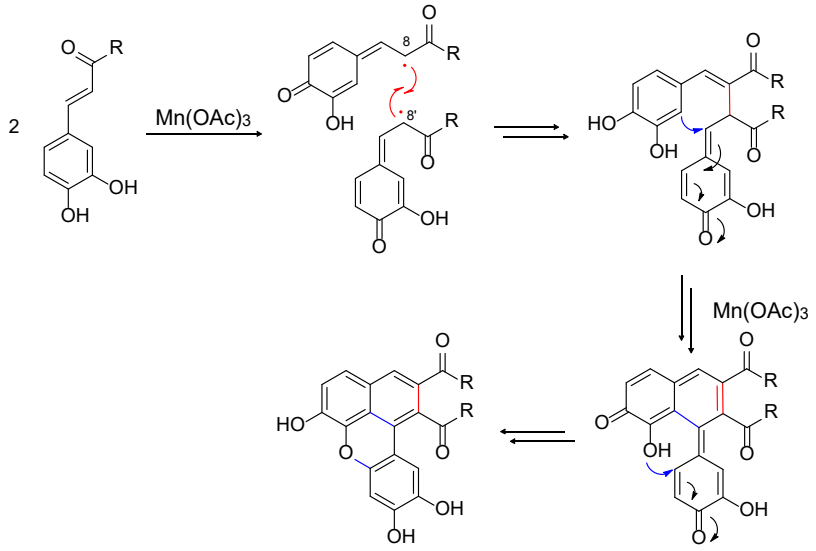

Fig. 1: Synthetic routes to achieve fluorescent benzoxanthene.

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OC-1

A computational protocol for uncovering photoinduced electron transfer mechanisms in fluorescent molecules

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Photoinduced electron transfer (PET) is a cornerstone mechanism in the design of fluorescent probes for bioimaging and sensing.[1] However, the lack of standardized computational protocols for modelling excited-state PET has hindered rational molecular design. Traditional frontier molecular orbital models often fail to predict quenching efficiency as they neglect conformational dynamics and state-specific solvent effects. Here, we present a reproducible, six-stage computational protocol that integrates density functional theory (DFT) and time-dependent DFT (TD-DFT) to accurately characterize PET mechanisms. The workflow features a dual-functional strategy, utilizing B3LYP for robust charge-transfer (CT) state geometry tracking, followed by energy refinement using range-separated functionals (e.g., ωB97X-D or M06-2X). Crucially, the protocol incorporates exhaustive conformational sampling and the corrected linear-response (cLR) formalism within the SMD solvation model to account for the significant polarization of CT states.[2] We demonstrate the protocol's efficacy through case studies of rigid (BODIPY, Rhodamine) and flexible (BN-1) scaffolds, successfully reproducing experimental fluorescence quenching trends where static models fail. This accessible workflow empowers researchers to quantify the thermodynamic driving force of PET, facilitating the development of high-performance optoelectronic materials and sensors.[3,4]

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OC-2

Optical spectroscopy and multiphoton microscopy as tools for drug delivery

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The delivery of poorly water-soluble drugs often relies on (nano)carriers capable of transporting and releasing the active compound at the target site. When a carrier is used, a detailed understanding of its interaction with biological tissues is essential for formulation optimization. In the case of topical administration, such as skin an ocular delivery, permeation and retention studies carried out in validated *ex vivo* animal models, *i.e.* in biorelevant conditions, can reveal carrier behaviors that are not accessible through *in vitro* experiments. These studies can also take advantage of imaging techniques, but unfortunately optical imaging of thick biological tissues remains challenging due to limited transparency and strong light scattering. In this context, multiphoton microscopy (MPM) represents a powerful approach to investigate the distribution and behavior of fluorescently labelled nanocarriers deep inside biological samples, benefiting from intrinsic three-dimensional resolution and enhanced penetration enabled by near-infrared excitation.[1,2]The characterization of nanocarriers such as micelles and nanovesicles is inherently complex, as their formation and stability strongly depend on the surrounding environment. Particularly, solvent removal - sometimes required for analytical purposes - can disrupt the weak intermolecular interactions responsible for nanocarrier stability. Advanced optical spectroscopic techniques allow for the investigation of supramolecular assemblies under native conditions, providing information on the embedded small molecules, carrier integrity, and nanocarrier diffusion without perturbing the system. Absorption and emission spectroscopy are applied to study nanocarriers loaded with small organic probes; special care must be taken to account for scattering effects, which can significantly influence the spectra, especially for larger particles. Fluorescence anisotropy and time-resolved fluorescence anisotropy offer further insight into the mobility of fluorescent probes interacting with the carrier,[3] while solvatochromic dyes (*e.g.* Nile red) enable the assessment of local polarity both in suspension and within biological environments, including *ex vivo* tissues. In this contribution, different case studies are presented in which optical spectroscopy in combination with MPM are applied to investigate nanocarrier permeation in thick biological tissues. Despite the intrinsic complexity of *ex vivo* models, MPM proves to be a robust and versatile technique for deep-tissue imaging, with the additional capability of acquiring hyperspectral data that provide detailed information on nanocarrier fate. Overall, the combined use of optical spectroscopy and MPM allows a comprehensive evaluation of carrier–tissue interactions, offering insight into carrier integrity and probe release mechanisms relevant to drug delivery applications.

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OC-3

A fluorescent probe with an ultra-rapid response to nitric oxide in living cells

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Nitric oxide (NO) is a vital signaling molecule involved in the regulation of many physiological and pathological processes.[1,2] The strict dependence of the biological effects of NO on its concentration makes its real-time monitoring crucial.[3] In view of the reactivity of NO with multiple bio-targets, the development of NO sensors that associate a fast response rate with selectivity and sensitivity is very challenging. In this work, we present an innovative fluorescent probe, featuring a BODIPY fluorophore covalently linked to a trimethoxy aniline derivative, designed for rapid and selective NO detection.[4] Nitrosation of the probe's electron-rich amino site through the secondary oxide N_2O_3 results in a fluorescence enhancement of the BODIPY unit and in a significant change in the relative amplitude of the fluorescence lifetimes. This probe demonstrates rapid response time ($\leq$ 0.1 s), high sensitivity (LOD = 35 nM), responsiveness also to $ONOO^-$, excellent selectivity against many analytes and small interference by physiological concentrations of glutathione. Validation in melanoma cell lines confirms its potential for real-time NO detection in complex biological systems, making it a valuable tool for biomedical research.

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OC-4

The photophysics of isothiazologuanosine, an isofunctional fluorescent analogue of guanosine: insights from Quantum Mechanical calculations

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An important advance in the development of Fluorescent Nucleoside Analogues has been recently achieved with the thieno-guanosine analogues thG and ^{tz}G (Fig. 1), which perfectly replace G in DNA duplexes, while keeping high quantum yields environmental sensitivity. thG, which has been already profitably used to monitor the dynamical behaviour of NA,[1,2] is, however, not perfectly isofunctional to G, lacking of the N7 atom (see Fig. 1). As a consequence, we have recently focussed on the photophysics of ^{tz}G, which is instead both isomorphic and isofunctional to G,[3,4] studying it in different solvents and at different pH. ^{tz}G, though fluorescent, is less bright than thG, but its properties are more sensitivite to the polarity and H-bonding properties of its surroundings.[3] Moreover, depending on the pH, different excited state reactions can occur, affecting the tautomerization equilibria observed in the ground state.[4] In this contribution we shall discuss the role of Quantum Mechanical calculations to rationalize these experimental results, associating any change in the photophysical properties of ^{tz}G with a well-defined electronic process, thus making important steps for its use as fluorescent label in Nucleic Acids.

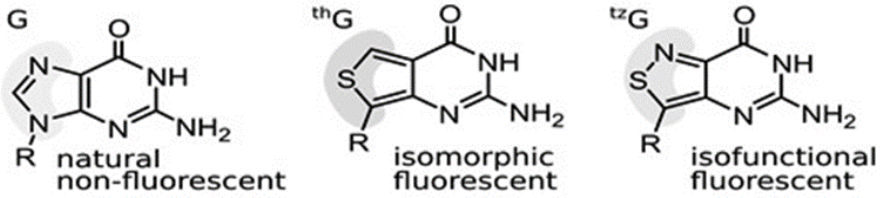

Fig. 1: Structures of deoxyguanosine (G), deoxythienoguanosine (thG) and deoxyisothiazologuanosine (^{tz}G). R = 2'-deoxyribose.

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OC-5

Synthesis and characterization of a lanthanide probe for Time-Resolved Forster Resonance Energy Transfer

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Time Resolved-Foster Resonance Energy Transfer (TR-FRET) is an analytical technique that combines Time-Resolved Fluorescence (TRF) and Foster Resonance Energy Transfer (FRET). These two spectrofluorimetry use fluorophores that, when exposed to a light source set at a specific wavelength, emit fluorescence at a higher wavelength with less energy, obtaining a fluorescence spectrum. Classical fluorophores (like fluorescein) have a very short emission time (nanosecond scale)[1]. Since proteins also exhibit fluorescence, their emission produces a high background that interferes with the emission of the fluorophore. Unlike classical fluorophores, stable lanthanide chelates (typically europium and terbium) are used, which have large stokeshifts and longer emission times (microseconds to milliseconds). All this makes it possible, after excitation of the fluorophore, to wait for the background fluorescence switched off before making the measurement, thus improving the signal-to-noise ratio. The lanthanide probes, in addition to the chelanting site, has two important sites: the “antennae” site and the bioconjugation site. The antenna is characterised by a system conjugated to the chelating portion of the compound which, depending on its structure, allows light to be emitted at specific wavelength[2]. For the bioconjugation component, there are several approaches depending on the type of macromolecule (usually protein) to be bound[3]. In TR-FRET there are the fluorophore donor and the fluorophore acceptor [4]. The aim of the work is to synthesise lanthanide chelates by modifying the antenna and the bioconjugation part, while keeping the size of the chelating site unchanged.

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OC-6

Supporting science across the globe: EURO-BIOIMAGING Research Infrastructure

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Euro-BioImaging ERIC is the European research infrastructure dedicated to providing open access to state-of-the-art biological and biomedical imaging technologies across all scales, from the nano-to the tissue-and organism scale to human imaging. Euro-BioImaging mission is to support and empower researchers with advanced and innovative imaging technologies, expertise, data services and training enabling cutting-edge research and contributing to addressing pressing societal challenges. The infrastructure is organised as a distributed network of 295 imaging facilities across Europe (Euro-BioImaging Nodes) offering access to scientists to the full range of imaging technologies. All imaging technologies are available, from light and electron microscopy to the various biomedical techniques for ex vivo or in vivo imaging in animals or humans or even biological systems like plants. Image data services are also offered supporting the user in adopting FAIR (Findable, Accessible, Interoperable, Reusable) image data practices, image analysis services and tools developed to manage and process image datasets. In addition to access provision, Euro-BioImaging offers funding opportunities for access and also high quality training courses delivered by imaging experts at the numerous imaging facilities (see www.eurobioimaging.eu). By enabling open access to cutting-edge imaging technologies, Euro-BioImaging fosters interdisciplinary collaborations promoting excellence in science and scientific innovation.

OC-7

Biomimetic and responsive fluorescent lipid microparticles to study phagocytosis

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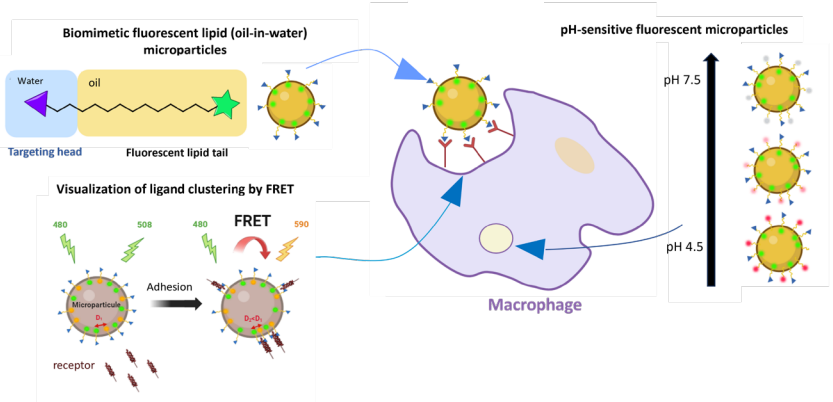
Phagocytosis is a receptor-mediated endocytic processes by which cells internalize objects larger than 0.5 µm (bacteria, cell debris,…).[1] To visualize phagocytosis and the subsequent phagosome maturation, we have developed fluorescent responsive microparticles based on oil-in-water emulsion droplets.[2] Their surface can be easily functionalized with amphiphilic lipids to enable selective membrane receptor targeting and/or environmental sensing and they constitute a modular platform to build up multifunctional biosensors.[3] We have designed a series of fluorescent lipids including a FRET pair of fluorescent glycolipids to visualize cellular adhesion and a pH responsive lipid to monitor phagosomal acidification.[4]



We anticipate that these simple cellular mimics incorporating various fluorescence sensing mechanisms can become versatile tools to study cellular adhesion and internalization processes such as phagocytosis.

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SP-1

Beyond histograms: advanced FLIM and FCS enabled by time taggers

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Time-correlated single-photon counting provides a unified data representation that enables advanced analysis methods across fluorescence microscopy and spectroscopy. This presentation introduces the concept of time tagging and demonstrates how Time Taggers can be used to perform fluorescence lifetime imaging (FLIM) and fluorescence correlation spectroscopy (FCS) within the flexible framework provided by Swabian Instruments. A central focus will be on real-time ("on-the-fly") data processing, which allows lifetime histograms, correlation functions, and photon statistics to be evaluated during acquisition. This enables rapid experimental feedback and advanced measurement schemes such as lifetime-based photon gating for gated FCS, where fluorescence dynamics can be selectively analyzed based on fluorescence lifetimes. Beyond real-time analysis, the full time-tagged data stream can be recorded and replayed, allowing measurements to be reprocessed with different gates, models, or analysis parameters after the experiment. This capability opens new possibilities for exploratory data analysis and method development. Through examples from FLIM and FCS, this talk will highlight how time tagging, real-time processing, and data replay together enable more flexible, information-rich, and fast workflows in life science research.

References

Swabian Instruments: https://www.swabianinstruments.com/

OC-8

Precise sequence-specific fluorescent labeling of DNA: illuminating genomic loci for optical mapping applications

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Precise sequence-specific DNA labeling is central to optical DNA mapping (ODM), a long-read technique for genomic analysis. One prominent example is the DNA methyltransferase (MTase) that recognizes a specific DNA motif (4-6 base pairs) and transfers functional tags of interest to the DNA through the use of cofactor (*S*-adenosylmethionine, SAM) analogues.[1] However, existing MTase-guided systems are constrained by limited enzyme-ligand compatibility, cofactor instability and methylation sensitivity. To address these challenges, we developed a versatile labeling toolbox that expands current biochemical and synthetic strategies for targeted DNA recognition and modification. First, we systematically evaluated SAM analogues across various MTases and identified robust enzyme-cofactor pairs (**Fig. 1a**, left). We further introduced GLOW, a proximity-labeling approach that leverages MTase guiding for methylation-independent labeling adjacent to canonical recognition sites using chemically stable ligands (**Fig. 1a**, right).[2] Complementing the enzymatic platforms, we established an enzyme-free approach using sequence-programmable pyrrole–imidazole hairpin polyamides for sequence-specific labeling and single-molecule analysis of DNA (**Fig. 1b**).[3] Together, these advances delivered promising multicolor labeling strategies for high-resolution and high-accuracy ODM in the near future.

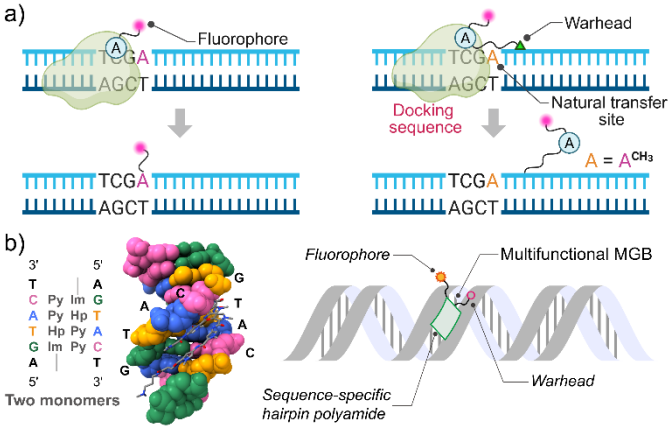

Fig. 1: Various sequence-specific DNA labeling approaches investigated and developed in this work (MGB: minor groove binder).

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OC-9

Molecular rotors detect the formation and conversion of WT α-synuclein oligomers and its' pathological mutant variants

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α-Synuclein is an intrinsically disordered protein that forms amyloid fibrils in Parkinson's disease. Currently, detection methods predominantly report on the formation of mature amyloid fibrils but have poor sensitivity to the early-stage, toxic oligomers.[1] Molecular rotors are fluorophores that sense changes in the viscosity of their local environment.[1,2] We present a novel method to detect the *real-time* formation and conversion of oligomers during α-synuclein aggregation. Our method provides a quantitative approach to unveiling the complex mechanism of α-synuclein aggregation which is key to understanding the pathology of Parkinson's disease.

We monitored changes in the fluorescence lifetime and intensity of two well-known molecular rotors, Thioflavin-T (ThT) and 3,3'- diethylthiacarbocyanine iodide ($DiSC_2$), in the presence of aggregating *wild-type* α-synuclein.[1] Additionally, we compared the fluorescence lifetime of these rotors in the presence of specific α-synuclein aggregates (Type-B oligomers[3] and mature fibrils). This enabled us to attribute lifetime changes observed in the aggregation pathway to distinct aggregation species. Once well established, this method was applied to different pathological mutant variants of α-synuclein. This included investigating A30P α-synuclein which has been reported to aggregate slower than *wild-type* α-synuclein and enable a build-up of oligomers.[4]

We showed that ThT is sensitive to Type-A and Type-B oligomers, whereas $DiSC_2$ is specific to only Type-B oligomers. These differences enabled us to understand the variations in aggregation mechanisms of the α-synuclein variants. For example, A30P α-synuclein shows a similar rate of oligomer formation compared to *wild-type* α-synuclein, however, A30P α-synuclein demonstrates a slower conversion of oligomers to amyloids.

These molecular rotors can act as a powerful tool to reveal mechanistic differences in the aggregation of *wild-type* α-synuclein and its' variants. These differences are key to furthering our understanding of the key mechanisms underlying Parkinson's disease.

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SP-2

Fast and easy ultrafast spectroscopy: enabling more time for research

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Ultrafast spectroscopy has become an essential tool in materials science and photophysics. However, traditional home-built systems often require extensive setup time, expert alignment, and constant maintenance - taking valuable hours away from actual research. In this talk, I will present how we have addressed these challenges by developing transient absorption systems that prioritize speed, reliability, and ease of use.

I will introduce the HARPIA ultrafast spectroscopy system, built around a transient absorption spectrometer and expandable with dedicated modules for time-resolved fluorescence measurements. In particular, the HARPIA-TF module integrates Kerr gating, fluorescence upconversion (FU), and time-correlated single-photon counting (TCSPC), enabling the investigation of fluorescence dynamics across different time scales within a single experimental platform. High-repetition-rate excitation with low pulse energies further supports stable and reproducible measurements while minimizing experimental complexity.

By minimizing technical overhead, we aim to help researchers focus on what really matters: the science.

OC-10

Chemigenetic fluorescent sensors to visualize Na^+ and K^+ in cells

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Metal ions such as Na^+, K^+, and Ca^{2+} play pivotal roles in many biological processes including neuronal activation, muscle movement, and metabolite transport. To spatiotemporally visualize these ions in living cells or tissues, a number of fluorescent sensors that bind to the target metal ions and then change their fluorescence properties have been developed. So far, they are mainly classified into two categories: synthetic small molecule-based sensors and genetically-encoded protein-based sensors. Although the former has advantages in response kinetics, brightness, and photostability, the latter has advantages in organelle localizability, cell specificity, and compatibility to directed evolution, which is a versatile and powerful technology to optimize the proteins for a given purpose.[1] To combine the advantages of both types of sensors, a chemigenetic approach, which utilizes the chimera of synthetic molecules and genetically engineered proteins, is lately attracting attention of sensor developers.[1] For example, the Schreiter lab have reported pioneering sensors for voltage[2] and Ca^{2+}.[3] Those works prompted us to develop a high-performance K^+ sensor called HaloKbp-1, using HaloTag, a K^+-binding protein, and a far-red emitting synthetic dye.[4] We also developed a different kind of chemigenetic Na^+ sensor called HaloGFP-Na, using a green fluorescent protein (GFP) as a fluorophore and a crown-ether ligand as a sensing domain.[5] This is a unique design based on our pioneering example targeting Ca^{2+}.[6]
Importantly, the performances (i.e., brightness and fluorescence response) of these sensors have been substantially improved using directed evolution of the protein. Also, we successfully imaged the dynamics of intracellular Na^+ and K^+ under microscope. We believe that the developed sensors can be good tools to answer the biological questions related to the target ions.

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OC-11

Novel quantitative methodology for studying inhibition of protein-protein interactions

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Protein-protein interactions (PPIs) are central to biological processes, but their deregulation can result in disease.[1]

A significant challenge faced is the ability to monitor PPI binding and inhibition in real-time in complex cellular environments.[2] Herein, we report the conjugation of a small environmentally sensitive fluorophore, known as a molecular rotor (MR), to a permeability-enhanced Bcl-x_L binding peptide. Upon binding to Bcl-x_L, we note an increase in fluorescence intensity and lifetime, with the latter providing a unique, concentration-independent method for intracellular quantitative measurements of protein inhibition. We investigate a small library of peptide-MR conjugates: varying the MR, coupling chemistry, attachment position, and the peptide sequence, to optimise and enhance the sensitivity of this novel phenomenon. Cells were treated with leading peptide-MR constructs and imaged with Fluorescence Lifetime Imaging Microscopy (FLIM) to monitor PPI inhibition, in real-time, in a cellular environment. Initial results suggest that combining the use of peptide-MR conjugate with FLIM provides novel insight into PPI inhibition. Further, we show that this methodology can be easily applied to alternative PPIs, demonstrating the potential value of this technique to be more widely applied.

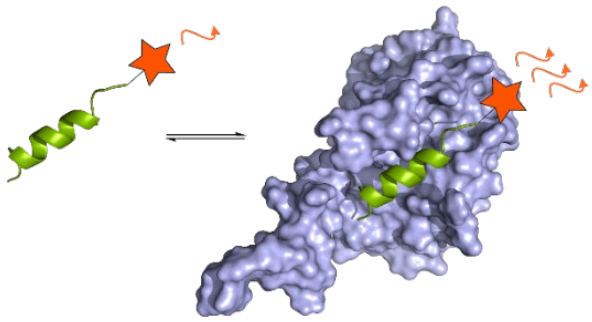

Fig. 1: Cartoon representation of the change in fluorescence intensity and lifetime of an environmentally sensitive peptide-fluorophore conjugate upon binding to a target protein.

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OC-12

Observing bioorthogonal macrocyclization in live cell nuclear envelope using on/on fluorescence lifetime microscopy

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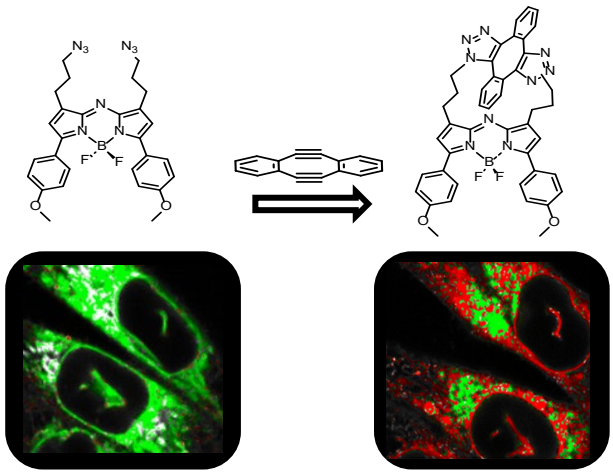
Bioorthogonal fluorescence imaging is an effective way of monitoring dynamic events in sub-cellular compartments in a non-destructive manner. The Sondheimer diyne allows for two sequential 1,3-dipolar cycloadditions under mild conditions without the need for a catalyst.[1] Previous work from the O'Shea research group has shown that this diyne can be used for bioorthogonal imaging in live cells.[2] To expand on these initial findings, the bis-azide substituted BF_2-azadipyrromethene **1** was selected as an attractive candidate for bioorthogonal fluorescence imaging as it would emit in the advantageous near infrared spectral region and has bis-azide functionality allowing for two cycloaddition reactions (Figure). The synthesis of 1 was achieved in 10 steps starting from butan-1,4-diol and 2-bromo-4'-methoxyacetophenone. Photophysical characterization of 1 showed an emission λ_{max} at 677 nm with quantum yield of 0.49 in methanol. The reaction of **1** with Sondheimer diyne gave a mixture of cis and trans macrocyclization products **2** in excellent yield under mild room temperature conditions (Figure). The bioorthogonal reaction between **1** and the Sondheimer diyne has been observed in the nuclear envelope and nuclear invaginations of live cells, with the progress of these reactions being tracked by Fluorescent Lifetime Imaging Microscopy (FLIM).[3]



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OC-13

Synthesis and characterization of RNA containing emissive isomorphic nucleotides

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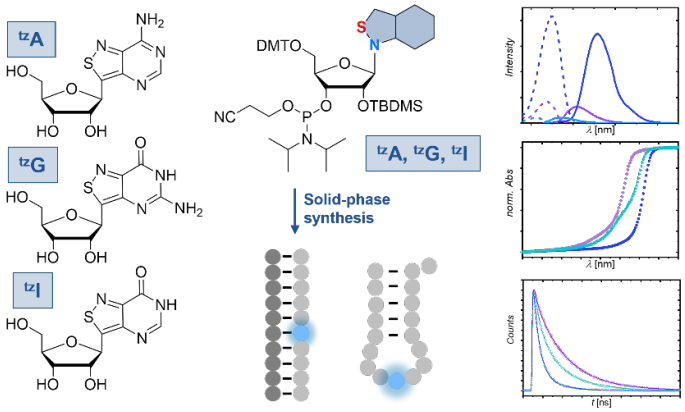
Fluorescence-based tools are crucial for modern biochemistry to study structure, functionality and interplay of biopolymers, small molecule cofactors and messengers. Since canonical nucleobases are intrinsically non-emissive, incorporation of artificial fluorescent nucleosides is a valuable method to explore fundamental biochemical transformations and facilitate the development of biophysical assays. Ideally, such fluorescent nucleobase surrogates are environment-responsive and mimic the size, structure and function of their native counterparts to minimize structural perturbations. The members of the emissive RNA alphabet published by the Tor group perfectly fulfil these criteria as they are based on a purine-like isothiazolo[4,3-d]pyrimidine skeleton, are highly responsive to changes in their microenvironment and therefore excellent substrates for metabolic and catabolic enzymes.[1,2]

Here, we report the first synthetic route towards the phosphoramidite building blocks of ^{tz}A, ^{tz}G and ^{tz}I and their incorporation into RNA by solid-phase synthesis, providing access to site-specifically modified oligonucleotides. We prepared different hairpin and duplex structures to explore the response of ^{tz}N to different base-pairing partners. By detailed thermal denaturation analysis, steady-state fluorescence spectroscopy and fluorescence lifetime analyses we demonstrate the profound sensitivity of these emissive nucleotides towards environmental changes. We were furthermore able to show by gel- and cuvette-based readout assays that the RNA-cleaving deoxyribozyme 8-17 readily accepts ^{tz}G-modified substrates, further proving the high value of the ^{tz}N building blocks as fluorescent nucleoside mimics.



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SP-3

Techniques to characterise biomolecules using fluorescence spectroscopy

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This talk will present fluorescence techniques for the characterisation of biomolecules, with emphasis on how instrument configuration and measurement strategy can be adapted to different systems. Examples will include spectral, time-resolved, and quantum yield characterisation of novel fluorescent probes; high-throughput screening of sensing probes using combined spectral and time-resolved plate-reader measurements; and the application of fluorescence anisotropy and time-resolved emission spectra (TRES).

The presentation will illustrate how flexible fluorescence instrumentation supports the development, evaluation, and application of fluorescent building blocks in biomolecular research.

OC-14

Exchangable fluorescent probes for long-term imaging of plasmic membrane

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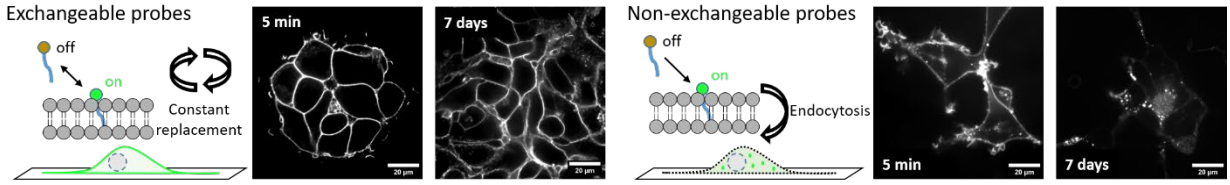
The plasma membrane (PM) is one of the main organelles of the cells that play key roles in many cellular mechanisms such as cell adhesion, cell-cell interaction, apoptosis or cell migration. Its fluorescent labelling is often use to observe changes of cellular morphology or visualize cells limits combined with another probe. Many examples of such fluorescent PM probes have been reported in literature[1] and some of them are now commercially available. However, most of them do not allow long-term imaging for more than several hours due to some limitations such as a toxicity, a loss of signal with cell proliferation (dye dilution) or a rapid probe internalization (strong intracellular signal). Several strategies have been reported to stain PM for a prolonged time including conjugated polymers, zwitterionic anchors and protein covalent binding but the longest are reported for up to 29 h.[2–4] To overcome these issues, we propose an alternative strategy based on fluorogenic exchangeable probes limiting strong accumulation on PM thus preventing cellular internalization and forming a large pool of dye maintaining staining after cell division. Such probes can be simply supplemented in complete growth medium allowing PM imaging for more than a week with limited background and in cells signal (cf. figure).



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OC-15

Time-resolved FRET for multiplexed detection of antibodies

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IgE antibodies drive allergic responses, including anaphylaxis. However, it remains unclear whether IgEs are generated by long-lived memory B cells (MBCs), newly activated naïve B cells (NBCs), or a combination of both.[1] To address this question, we use a mouse model in which antibodies carry a flag-tag when produced by NBCs and a strep-tag when produced by MBCs.[2] This system enables fate-mapping of antibody origins during the allergic response. The currently used ELISA to detect serum antibodies based on allergen specificity, isotype, and tag expression is time-consuming and requires multiple separation steps, often leading to significant sample loss. Here, we propose a multiplexed time-resolved Förster Resonance Energy Transfer (TR-FRET) assay for the highly specific and sensitive quantifications of precise IgEs from murine serum samples based on their specificity, isotype, and tag (Figure 1). The assay provides a straightforward mix-and-read approach that minimizes background noise in serum while limiting the need for separation steps. Multiplexing and optimization strategies are further discussed.

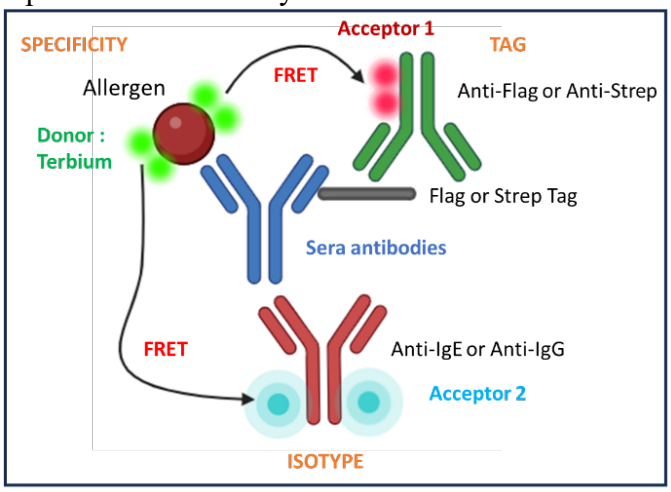

Figure 1 : Principle of sera antibodies detection by multiplexed TR-FRET

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OC-16

FLIMBDs – benzodiazoles for fluorescence lifetime imaging

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The development of organic fluorophores for bioimaging is a growing field due to the widespread application of optical imaging, from interrogating molecular events in cells to clinical diagnosis of disease states.[1] Organic fluorophores offer modular scaffolds for chemical modification, allowing them to become responsive to biostimuli.[2] In particular, small scaffolds are of interest due to their tight binding to target molecules and minimal perturbation to biological systems.[3] Benzodiazoles are compact push-pull fluorophores that are widely used for bioimaging due to their biocompatibility and photostability. Some relationships between their chemical structures and spectral properties has been well documented,[4-6] including the generation of selenium-bridged photosensitisers.[7] However, the impact of structural modifications on fluorescence lifetime has not been yet explored. Fluorescence lifetime is a powerful parameter for multiplexed imaging as lifetime readouts are independent of fluorophore concentration and intensity. Fluorescence lifetime is also sensitive to changes in the microenvironment and the proximity of FRET quenchers, making them invaluable tools for the monitoring of cellular events. To understand the molecular determinants of fluorescence lifetime for the benzodiazole core, we systematically modified the benzodiazole core to understand how defined substitutions modulate photophysical behaviour. This structure–lifetime study reveals principles that decouple lifetime from spectral properties, resulting in the creation of FLIMBDs, a toolbox of benzodiazole fluorophores with a broad dynamic range of fluorescence lifetimes. The FLIMBDs enable multiplexed fluorescence lifetime imaging within a single spectral channel, overcoming a key limitation of traditional spectral multiplexing. We further demonstrate their utility in live-cell microscopy by tracking neutrophil phagocytosis of bacteria. We exploit the fluorogenic nature of the FLIMBD toolbox to monitor bacterial phagocytosis. Taken together, this work provides a framework for lifetime-engineered benzodiazoles optimised for FLIM imaging and demonstrates their application for bioimaging.

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OC-17

ZnO nanotetrapod synthesis and application in optoelectronics

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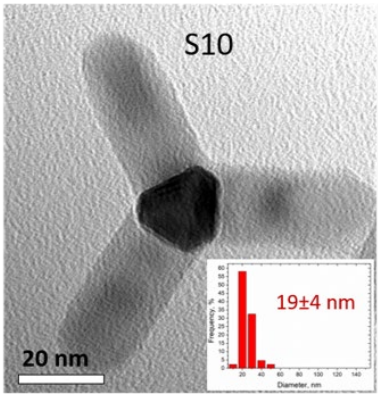
ZnO nanotetrapods are low-cost and low-toxicity semiconductor nanostructures with strong potential for biointerfaces and optoelectronic applications. Unlike simple nanoparticles, ZnO nanotetrapods are three-dimensional, four-legged architectures composed of interconnected nanowire arms, providing large accessible surface area and efficient network formation.[1] In this work, we focus on scalable, high-yield synthesis of ZnO nanotetrapods and their integration into sensing platforms based on a chemoresistive mechanism. Using low-temperature processing, ZnO nanotetrapod networks can be deposited on a wide variety of substrates with controllable thickness and morphology, enabling flexible device design and broad application opportunities.[2,3] We also investigate how packing of tetrapod networks governs pore size and correlates with electrochemical and sensing performance. These results support ZnO nanotetrapods as a versatile platform for optoelectronic sensing, and they motivate future studies where surface functionalization could enable selective interactions with biomolecules and polymers, including the possibility to induce optical responses in ZnO-based hybrid materials.



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OC-18

Teaching new tricks to old dyes: engineering and imaging strategies for high-resolution biology

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Small-molecule fluorescent dyes are essential tools for biological microscopy, but their utility is often limited by their intrinsic properties, such as poor photostability or providing only intensity-based information. Here, I will present two complementary strategies from our lab that unlock new capabilities from well-established dyes, one based on biomolecular engineering and the other on advanced photophysical imaging.

First, we address the challenge of photostability for long-term, live-cell imaging. We developed PEPCy (Photostability Enhancing Proteins against Cyanine dyes), a set of genetically encoded tags evolved from single-chain variable fragments (scFv) that non-covalently bind off-the-shelf sulfonated cyanine dyes like Cy3 and Cy5 with high affinity and specificity.[1] This interaction dramatically enhances the dyes' photostability and molecular brightness, enabling wash-free, multi-color, single-molecule localization microscopy of surface proteins in live cells.

Second, we tackle the challenge of characterizing complex, heterogeneous intracellular environments, such as biomolecular condensates. We introduce ConSTEL (Condensate Spatial Topography via Emission Lifetimes), a fluorescence lifetime imaging (FLIM) approach that repurposes common solvatochromic dyes[2] like Nile Red. ConSTEL uses the dye's environment-sensitive lifetime to quantitatively map the solvent architecture inside condensates with nanoscale resolution.[3,4] This method reveals that condensates are not uniform but are mosaics of discrete aqueous niches with distinct polarities, which in turn dictate local material properties, molecular diffusion, and the selective partitioning of small molecules. These approaches demonstrate how combining existing fluorophores with protein engineering and advanced imaging provides powerful tools to probe biological systems with high spatiotemporal and chemical resolution.

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OC-19

A red emitting carbon dot and aptamer- functionalized alginate system for targeted triple-negative breast cancer imaging

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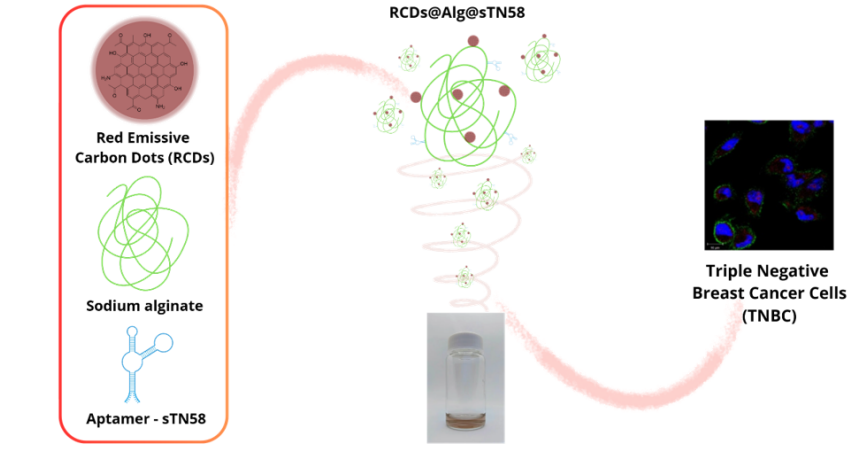
The targeted detection and imaging of cancer cells is crucial for tumour diagnosis and therapeutic treatment. Common imaging agents suffer from various limitations, such as poor biocompatibility, inadequate optical properties and lack of stability. Recently, luminescent carbon dots have generated wide interest in biomedical applications, thanks to their unique properties such as biocompatibility, tuneable emission, water solubility and the possibility of surface functionalization.[1,2] Herein, we report the conjugation of our recently obtained red emitting carbon dots (**RCDs**)[3] to alginate and the sTN58 aptamer to obtain systems able to selectively recognize cancer cells that can be exploited in bioimaging and potentially as photothermal agents.[4]



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OC-20

Deep eutectic solvent-derived thermoresistant fluorescent material: development and characterization

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Photoluminescent materials, capable of maintaining stable fluorescent properties even at elevated temperatures, are of significant interest for their potential application in a variety of advanced technological fields[1] and biological systems. The development of such materials is particularly challenging due to the phenomenon known as *thermal quenching*, which often results in a decrease in fluorescence intensity or even its complete disappearance at high temperatures. In this context, the present work focuses on melamine cyanurate (MCA), a crystalline supramolecular structure formed through hydrogen-bonded interconnections between triazine units[2], which exhibits blue light emission from room temperature up to 200 °C[3]. Compared with conventional methods reported in the literature, where urea is almost exclusively employed as the starting material for the preparation of luminescent MCA systems[4], this study proposes an alternative synthetic approach based on the use of a Deep Eutectic Solvent (DES), composed of urea and zinc chloride ($ZnCl_2$) as the precursor[5]. Within this eutectic medium, the supramolecular self-assembly process can occur in a more controlled manner, promoting the formation of a material with enhanced luminescent properties and improved resistance to thermal quenching. Following the synthesis of the DES-derived MCA, its fluorescence properties were first optically characterized by analyzing the main emission bands and estimating the corresponding quantum efficiencies and fluorescence lifetimes, consequently these results were compared with those obtained for the same structure but synthesized solely from urea. A temperature-dependent structural characterization of the MCA was then performed in the 30–70 °C range, using Raman spectroscopy and X-ray diffraction (XRD). Finally, within the same temperature interval, the temperature-induced variation of the fluorescence emission intensity of the system, was also investigated. A comprehensive set of spectroscopic and structural data, that elucidate the thermal behavior of DES-derived MCA, is essential in view of its potential application in technological fields, requiring materials that ensure stability and efficiency under different operating conditions, while exploiting photoluminescence phenomena.

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OC-21

Modeling light-controlled aspartate transport

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GltTk is a sodium-dependent neurotransmitter transporter essential for aspartate uptake and implicated in neurodegenerative diseases such as Parkinson's. Its alternating access mechanism involves large-scale conformational changes that expose the substrate-binding site to either side of the membrane. Studying these fast transitions requires precise control of substrate availability. We developed a novel caged aspartate analog utilizing a coumarin photoremovable protecting group, which enables the controlled release of aspartate upon light activation.

To understand the molecular basis of this process, we designed a light-activated aspartate analog employing a coumarin photoremovable protecting group. Synthesis and computational analysis were carried out to confirm its suitability for probing GltTk kinetics and dynamics. Using DFT/TD-DFT quantum calculations, we evaluated the electronic properties and photoreactivity of the caged compound. Molecular docking and molecular dynamics simulations were then applied to characterize the binding interactions and conformational effects within the coumarin-Asp and GltTk. The fast uncaging of the coumarin photocage will allow precise stimulation of GltTk and enable tracking of aspartate release and conformational changes on short timescales. Together, these computational approaches provide mechanistic insight into substrate release and transporter dynamics, laying the groundwork for future experimental validation.

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OC-22

Development of tunable and STED-compatible NIR fluorophores for super-resolution imaging in live cells

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Live-cell STED nanoscopy requires fluorophores that combine red-shifted emission, high photostability, and efficient depletion at 775 nm, yet the number of available probes fulfilling these requirements remains limited.[1,2]

Here, we introduce a family of tert-butyl-substituted aza-BODIPYs, comprising both symmetric and asymmetric structures, and featuring a scaffold that enables straightforward post-synthetic functionalization. These non-functionalized dyes display balanced lipophilicity and preferentially localize to intracellular membranes, including mitochondria, the endoplasmic reticulum, the nuclear membrane, and/or lipid droplets, depending on the derivative. Importantly, all members of this dye family proved fully compatible with 775 nm STED depletion, enabling reliable sub-diffraction imaging in live cells across the entire series. The versatility of this scaffold was further demonstrated through selective post-synthetic modification, which afforded a bis-sulfonic acid derivative capable of selectively staining the plasma membrane while fully retaining STED performance.

Altogether, these results establish tert-butyl aza-BODIPYs as a synthetically accessible and adaptable family of red-emissive fluorophores for live-cell STED microscopy. The combination of compact molecular design, photostability, STED compatibility, and facile functionalization highlights this platform as a practical starting point for the development of organelle-targeted probes for super-resolution imaging.

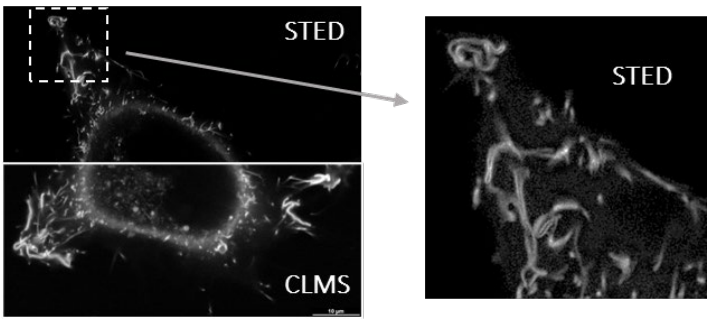

Fig. 2: Left: CLMS vs STED image. Right: STED image showing filopodia structures in HeLa cells.

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OC-23

Measuring photoluminescence quantum yield of difficult samples

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Photoluminescence quantum yield (PLQY) is a critical parameter in the complete characterisation of luminescent materials. Accurate PLQY determination regularly presents significant challenges for some samples such as those with low PLQY; small Stokes shift; or high absorbance.

Small Stokes shift samples: Materials which have substantial overlap between absorption and emission spectra show increased secondary inner-filter effects when measured in an integrating sphere. This leads to the observed PLQY being an underestimation of the true PLQY. This talk discusses how to identify this effect and perform reabsorption correction.

Low PLQY materials: For weakly emissive samples, achieving an adequate signal-to-noise ratio (SNR) for the sample emission requires the use of high-intensity excitation. If the necessary power exceeds the dynamic range of the detection system, it causes signal saturation. This leads to inaccurate determination of the number of photons absorbed resulting in the calculated PLQY being incorrect. This submission discusses a filter-based experimental method to mitigate saturation while maintaining sufficient SNR to determine PLQY.

Highly absorbing samples: Samples with high absorbance can suffer from increased background signal in the blank measurement of a PLQY experiment. This arises because the sample absorbs a significant portion of the excitation flux, while the blank absorbs none, leading to increased stray light. If not accounted for, the observed PLQY will be an underestimation of the true PLQY. Identifying the need for sphere background correction and how to mathematically perform it are discussed in this talk.

This submission outlines some common challenges faced when characterising the PLQY of demanding samples. It discusses how to identify these phenomena and demonstrates some strategies to overcome them.

OC-24

Exploring Si-phthalocyanines with different valency for PSMA-targeted photodynamic therapy: synthesis and preclinical validation

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Prostate cancer remains a significant health concern, with existing treatments often proving invasive or inadequate in preventing recurrence. This study explores the development and preclinical validation of siliconphthalocyanine (SiPc)-based photosensitizers (PSs) targeted at prostate-specific membrane antigen (PSMA) for photodynamic therapy (PDT). Two PSMA-targeted SiPcs, monovalent and bivalent, were synthesized with axial conjugation through Si–O–C linkages to evaluate their efficacy and specificity.[1] The bivalent SiPc-$(PSMAi)_2$ (Fig.1) demonstrated superior optical properties, reduced aggregation, and enhanced target specificity compared to the monovalent SiPc-PSMAi. Cellular and *in vivo* assays confirmed its high PSMA-specific uptake, potent photoinduced cytotoxicity mediated by reactive oxygen species, and significant tumor growth inhibition post-PDT. These findings underscore the potential of bivalent SiPc-$(PSMAi)_2$ as an effective agent for targeted PDT, combining imaging and therapeutic capabilities for improved prostate cancer management. Further optimization and clinical evaluation could establish its role in theranostic strategies to enhance surgical outcomes and reduce recurrence.

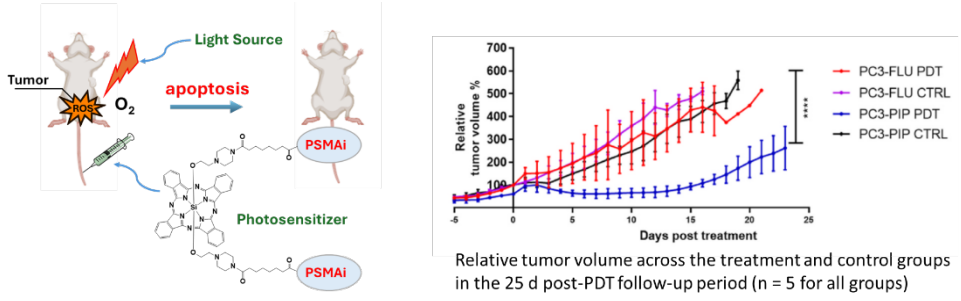

Fig. 1

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OC-25

Sustainable synthesis of fluorescent furano-chalcones

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Developing sustainable synthetic methodologies is crucial to reduce the environmental impact of fine chemicals while enabling access to functional molecules with advanced optical properties.[1] In this work, we report a green approach for the synthesis of fluorescent furano-chalcones from renewable resources. Bio-derived furanic aldehydes, namely furfural, 5-methylfurfural, and 5-hydroxymethylfurfural, derivable from the dehydration of polysaccharidic fractions of lignocellulosic biomass, were used as key building blocks.[2] Furano-chalcones were prepared via Claisen-Schmidt condensation using **anion-exchange resin** Amberlyst™ A-26(OH) as readily recoverable heterogeneous catalyst (**Fig. 1a**).[3] Reactions were carried out at **room temperature** under **solvent-free** conditions and activated by **ultrasound irradiation**, which improved catalyst-substrate contact, enabled mild conditions with reduced energy input and afforded good yields (up to 95%).[4]

Extended conjugation between the aromatic rings and the α,β-unsaturated carbonyl system resulted in intense and tunable fluorescence (**Fig. 1b**).[5] UV-Vis absorption maxima were dependent on both the substituents and the solvent, with methyl groups inducing the largest bathochromic shifts (20 nm). Emission spectra recorded at the absorption maxima revealed significant Stokes shifts (up to 100 nm), highlighting the potential of these molecules for optical and sensing applications.

The proposed methodology aligns with green chemistry principles and offers a sustainable strategy for the development of eco-friendly fluorescent materials.

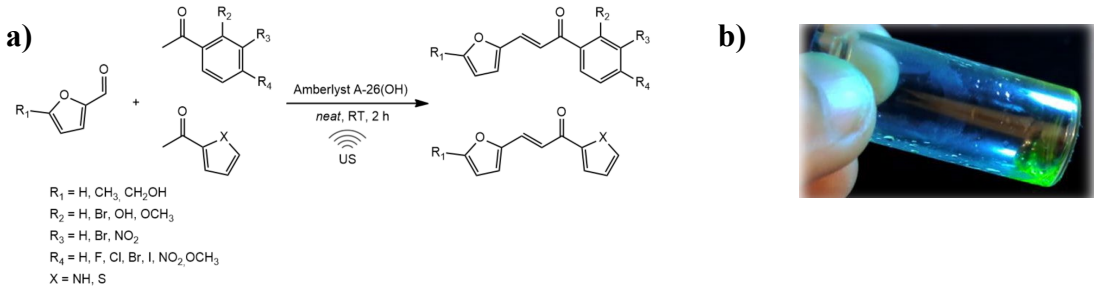

Fig. 1: a) Sustainable ultrasound-assisted synthesis of furano-chalcones via Claisen-Schmidt condensation. b) Fluorescence of furfural-acetophenone furano-chalcone.

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OC-26

Naphthalene-diimide-based down-converters and other approaches towards bio-based lighting

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Low-energy emitting organic phosphor-converted hybrid light-emitting diodes (pcHLEDs) have not met stability, external quantum efficiencies (EQE), and color requirements for phototherapy and indoor farming yet. To date, they are restricted to perylene diimides, while other related families like naphthalenediimide dyes (NDIs) are prone to strong aggregation induced quenching and photon-induced radical degradation. Thus, their use in lighting has always been discouraged. Herein, we explored novel green-, yellow- and red-emitting core-substituted NDIs to fabricate rainbow pcHLEDs. Sadly, the emission of the NDI-polymer coatings shows a concentration-dependent PLQY reduction and a low photostability regardless of i) device operation conditions and ii) chemical cross-linking to polymer matrix. However, a molecular redesign of the NDI combining branched aliphatic and hydrophilic groups at the core nitrogen atoms led to pcHLEDs with EQE ~2 % and stabilities of 1,800 h and 900 h under continuous 55 mW/cm^2 and 130 mW/cm^2 irradiation. Finally, we report the design and characterization of an innovative bio-bases down-converter, reaching PLQYs ~30% and device stabilities >1500 h under high power (130 mW cm^{-2}). This nicely outperforms devices with both organic dyes (<1,000 h)[1] and artificial/natural biogenic phosphors (25/2600 h at 55 mW cm^{-2}).[2,3]

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PP-1

Rational design of bright fluorophores for membrane bilayer imaging

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Bright small molecules for bioimaging read-outs are constantly required to visualize and investigate biochemical structures and functionalities.[1] In particular, new fluorophores that combine straightforward synthesis with efficient biological membrane visualization are highly sought after, given the membranes' functions in chemical biology. The precise design of well-known membrane-targeted fluorophores belonging to structural families such as Squaraine, BODIPY and core-substituted 1,4,5,8-naphthalenediimides (c-NDI) ones is of high interest due to the intrinsic optical properties and synthetic accessibility of those compounds.[2,3] Herein we present the synthesis and investigation of two small dyes' libraries: *a)* symmetrical and unsymmetrical NIR Squaraine dyes[2] and *b)* differently substituted and PEGylated-BODIPY where the hydrophobic-hydrophilic character has been synthetically rationally tuned by the insertion of specific functional groups.

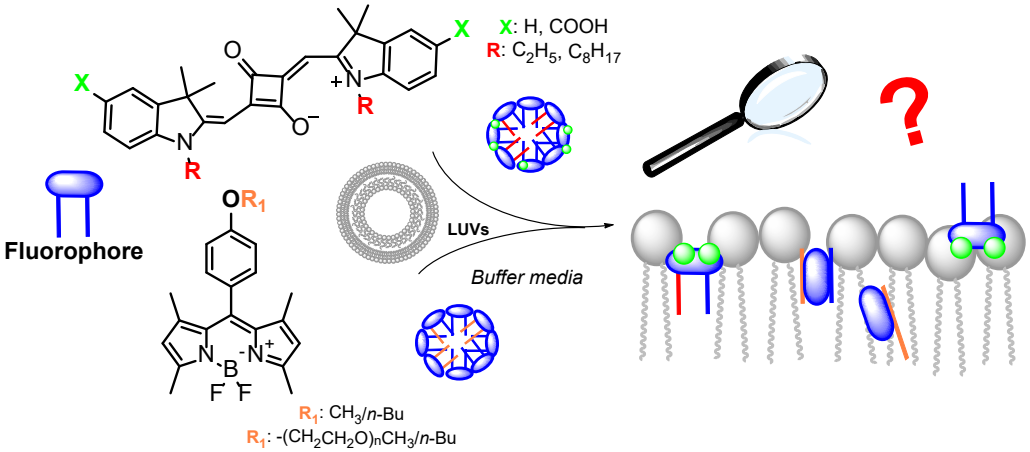

Fig. Squaraine and BODIPY investigated for bioimaging outlooks.

The complete photophysical characterization together with the photophysical properties response and the kinetic of the insertion into different large unilamellar vesicles (LUVs) models have been analysed with respect to the probes' structures. The discussed results represent versatile key data to support the design of new bright sensors for bioimaging.

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PP-2

Enhancing cellular uptake of fluorescent nucleoside analogues for labeling of cellular RNA

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Improved understanding of RNA biology, such as RNA synthesis, intracellular dynamics, and the cellular uptake and distribution of RNA therapeutics is vital for the development of drugs in this class. Such insight can be achieved using fluorescent base analogue (FBA) labelling strategies compatible with the cellular environment and allowing visualization in living cells without perturbing native RNA properties. Previous studies showed that the fluorescent tricyclic cytosine analogue tCO can be enzymatically incorporated into RNA;[1] however, its triphosphate form (tCOTP) unlike a fluorescent ATP analogue of ours, 2CNqATP, was unable to traverse cellular membranes.[2] Here, we evaluate derivatization of tCO into 5′-monophosphate prodrugs to enable membrane permeation and to potentially improve labelling efficiency without using complex genetic engineering or post-labeling bio-orthogonal chemistry. Among these, we found that ProTide-tCO demonstrated significantly enhanced cellular uptake compared to tCOTP. Our preliminary data suggest that ProTide-tCO is even more efficiently taken up into cells than our previously developed metabolic label for cellular RNA 2CNqATP.[2]

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PP-3

Fluorescence-based cellular insights into the mitochondrial impact of Iron(III)–NHC complexes

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Metal-based systems with intrinsic fluorescence can provide valuable insights at the interface between photophysics and chemical biology. In this work, we report a series of iron(III) N-heterocyclic carbene (Fe(III)–NHC) complexes originally investigated as potential photosensitizers,[1,2] which instead revealed a pronounced light-independent cytotoxic activity accompanied by exploitable photophysical properties. All complexes display broad visible absorption centred around 500 nm, assigned to ligand-to-metal charge transfer transitions, and weak emission in the red–near-infrared region ($\lambda_{em} \approx 645$ nm) with nanosecond excited-state lifetimes under aerobic conditions. Although their fluorescence quantum yields are modest, the intrinsic emission is sufficient to allow detection by confocal fluorescence microscopy, enabling label-free tracking of selected compounds inside living cells.

Biological studies showed that the most active Fe(III)–NHC complexes accumulate efficiently in glioblastoma cells and induce strong cytotoxic effects in the low micromolar range, even in the absence of light activation. Fluorescence imaging revealed a heterogeneous cytoplasmic distribution without clear colocalization with major organelles. Notably, co-staining experiments with the mitochondrial probe MitoTracker unveiled a striking alteration of the mitochondrial staining pattern, characterised by a loss of the typical filamentous morphology.

This unexpected imaging signature provided the first indication of mitochondrial dysfunction induced by the Fe(III)–NHC complexes. Subsequent bioenergetic analyses confirmed a severe impairment of mitochondrial respiration, with compromised oxidative phosphorylation and a metabolic shift towards glycolysis. These results suggest that mitochondrial damage, potentially associated with iron-mediated redox processes, plays a central role in the cytotoxic mechanism of action. Overall, this study illustrates how weakly fluorescent metal complexes can act as informative biomolecular probes, where photophysical properties and fluorescence-based imaging contribute to the identification of subcellular targets and to the elucidation of anticancer mechanisms.[3]

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PP-4

NanoBRET reporter RNA delivery quantification

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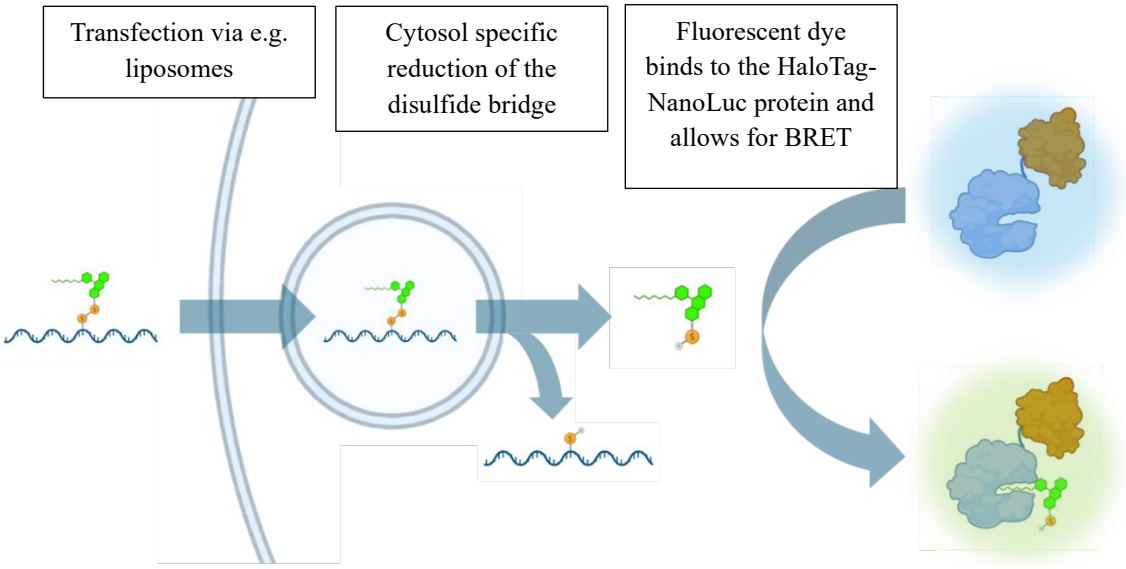
RNAs offer huge potential for therapeutic applications[1]. Despite promising characteristics, few RNA-based pharmaceutical products are available. Due to unreliable and/or labor-intensive methods for cytosolic RNA delivery quantification[2], accurate results for RNA delivery are scarce.
The NanoBRET reporter RNA Delivery Quantification project focusses on establishing a method to quantify cytosolic RNA delivery in real time. RNA is synthesized via in-vitro transcription with co-transcriptional phosphorothioate modified nucleotide incorporation. The modified nucleotide is conjugated with a compound containing a HaloTag ligand and a fluorescent dye, via a disulfide linkage. The RNA can be delivered into cells via a nanoparticle-based vector of interest. Only when the RNA reaches the reducing environment of the cytosol, the disulfide bond is cleaved, and the tag is set free. The oxidizing nature of endosomal compartments and thus the specificity of this assay for cytosolic delivery is confirmed via a RoGFP based assay. In cells expressing a NanoLuc-HaloTag fusion protein, the tag binds to the NanoLuc-HaloTag protein and causes a red-shift in the emission wavelength via BRET. Repeated BRET measurements are taken to gather real time data on RNA delivery for the selected delivery system. In the future, the potential of multicolor BRET may be explored to be able to measure delivery of multiple RNAs in co-delivery drug formulations.



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PP-5

Efficient CNOs-triggered diiodo-BODIPY phototoxicity modulation for cancer therapy and theranostics

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Photodynamic therapy is currently one of the most promising key approaches for targeted cancer treatment. It is based on responses of vital physiological signals such as reactive oxygen species (ROS), which are associated with high-impact diseased condition, e.g. cancers.[1]

Herein, we focus on synthesis, incorporation and application of a diiodo-BODIPY–based photosensitizer,[2] used for a non-covalent functionalization of carbon nano-onions (CNOs). *In vitro* studies evidenced that both the diiodo-BODIPY molecules and their CNO nanohybrids can be successfully delivered to HeLa cells.

Following cell internalization and light exposure, the pyrene–diiodo-BODIPY molecules induce an increase of the ROS level of HeLa cells, resulting in remarkable photomediated cytotoxicity and severe apoptosis.

Viceversa, when the diiodo-BODIPY/CNO nanohybrids are delivered to HeLa cells, no significant cytotoxicity or ROS basal level increase can be detected.

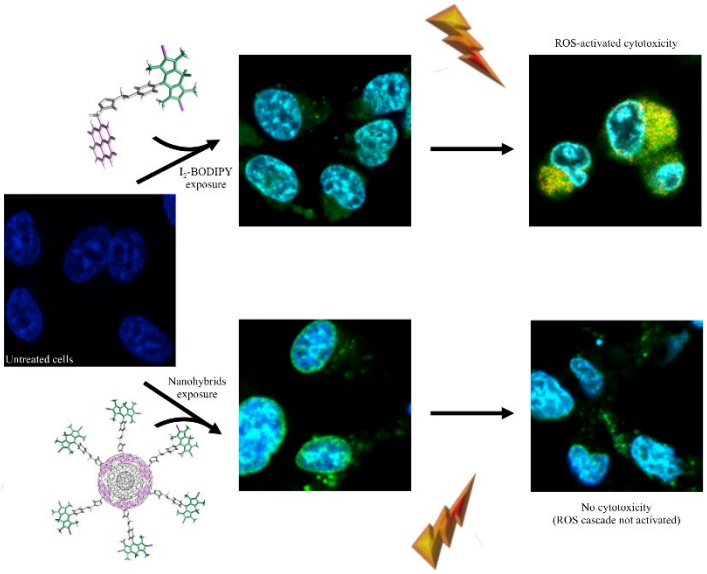

Fig. 1: Schematic including confocal live images of HeLa incubated for 24 h, after 15-min illumination.

These results define a first step toward the understanding of carbon nanomaterials that function as molecular shuttles for photodynamic therapeutics, still tracking the therapeutic agent, hence proposing as innovative approach in theranostics.[3]

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PP-6

Light-induced charge conversion in fluorescent cyclodextrin nanoparticles for deep tumor theranostics

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Fluorescent biomolecules provide a powerful means to couple imaging and therapy within nanomedicine.[1] In this study, we report the design of novel photobleaching-mediated charge-convertible cyclodextrin nanoparticles that exploit fluorescence chemistry to achieve precise tumor targeting and deep penetration for rectal cancer theranostics. The nanoparticles were constructed by complexing a zwitterionic near-infrared cyclodextrin derivative, containing a heptamethine cyanine fluorophore and phenylboronic acid ligand, with a pheophorbide–ferrocene conjugate. Under 808 nm near-infrared laser irradiation, photobleaching of the heptamethine cyanine moiety induced oxidative cleavage, converting the nanoparticle surface charge from zwitterionic to cationic. Fluorescence imaging further validated this conversion in tumor tissue, as evidenced by a rapid decrease in NIR fluorescence intensity toward the autofluorescence level. This rapid and localized charge conversion promoted transcytosis-mediated tumor penetration, a major challenge in solid tumor therapy.[2,3] Subsequent 660 nm laser exposure activated the pheophorbide–ferrocene complex to trigger dual photodynamic and chemodynamic therapy, producing singlet oxygen and hydroxyl radicals for potent cytotoxicity. The nanoparticles achieved selective imaging of rectal tumors with tumor-to-rectum signal ratios up to 7.8, and completely eradicated both heterotopic and orthotopic rectal tumors *in vivo*. These findings demonstrate how fluorescent biomolecules can act as both optical reporters and functional triggers in nanotherapeutics. By integrating photochemical charge modulation with fluorescence-guided imaging, this platform exemplifies a see-and-treat strategy for precision cancer nanomedicine, offering spatiotemporal control over diagnosis and therapy within the tumor microenvironment.

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PP-7

Functional nucleobases and monomers in PNA chemistry

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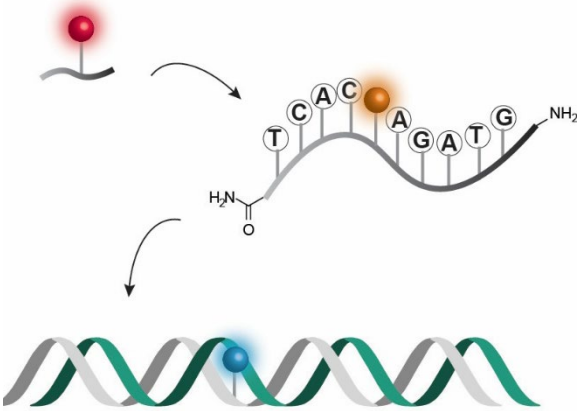
Peptide nucleic acids (PNA) are key tools in chemical biology.[1,2] As artificial oligonucleotide mimetics, they efficiently interact with DNA and RNA through the use of the natural nucleobase alphabet, while replacing the traditional phosphoribosyl backbone with a pseudopeptide framework.[1] This unique combination makes PNA promising platforms for antisense and antigene strategies, gene editing, and nucleic acid sensing and imaging.[2] The replacement of natural PNA nucleobases with synthetic and rationally designed ones is a unique and successful strategies to impart different chemical and physical properties aiming at enhancing PNA's chemical biology applications including the interactions with targeted pathological relevant nucleotide sequences or to confer intrinsic luminescence. In this context, isomorphic fluorescent bases have shown significant emissive read-outs upon variation of pH and polarity and in the imaging of biological relevant events such as duplex formation.[3,4]



For this reason, we have here studied modified thieno[3,4-*d*]pyrimidine nucleobases and their applications in PNA chemistry to evaluate overall novel PNA's functional properties. In parallel we are currently developing the synthesis of lipophilic monomers for tuning overall PNA stability.

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PP-8

Lifetime-encoded STED–FLIM resolves nanoscale lipid organization in cells

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Resolving the nanoscale organization of lipid-rich cellular compartments remains challenging when fluorophores exhibit strong spectral overlap and conventional orthogonal contrast mechanisms are limited. Multiplexed super-resolution imaging within a single spectral window, such as STED, is particularly constrained, preventing clear discrimination of closely associated lipidic regions. Here, we introduce a fluorescence lifetime–encoded STED–FLIM approach that leverages differences in fluorescence lifetime, rather than spectral separation, to resolve lipid-rich structures.

We employ two spectrally overlapping probes—a BF_2-azadipyrromethene dye[1] and a bisthiophene BODIPY—whose emission maxima differ by only ~10 nm but both exhibit high STED compatibility. This combination achieves lateral resolutions sufficient to resolve mitochondrial cristae separated by ~83 nm, surpassing the capabilities of conventional fluorescence imaging. Individually, the BF_2-azadipyrromethene (lifetime ~3 ns) preferentially labels the endoplasmic reticulum, nuclear membrane, and mitochondria, whereas the bisthiophene BODIPY (lifetime ~6 ns) additionally stains lipid droplets. When applied together, the two fluorophores generate a continuous lifetime distribution (3–6 ns) reflecting probe-specific affinities and variations in local lipid environments. By selecting defined regions in the phasor plot, photons corresponding to distinct lifetime populations can be isolated and reassigned, reconstructing high-resolution images of individual lipid-rich compartments. Importantly, this photon reassignment is fully compatible with STED conditions.

Focusing on mitochondria as a representative lipidic region, we demonstrate that lifetime-encoded STED–FLIM can resolve internal membrane architecture while distinguishing overlapping lipid compartments. These results establish fluorescence lifetime as a powerful contrast mechanism for multiplexed, nanoscale imaging of lipid organization in living cells, expanding the capacity of super-resolution microscopy beyond traditional spectral constraints.

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PP-9

Alternative labelling moiety for Peptide Nucleic Acids

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Peptide Nucleic Acids (PNAs) are powerful nucleic-acid recognition tools, and their use in diagnostics as well as imaging and analytical applications strongly depends on labelling strategies that are efficient and minimally perturbing.[1,2] Fluorescent nucleoside analogues (FNAs), structurally diverse mimics of the naturally non-fluorescent nucleosides, provide an important framework for understanding how compact chromophores can be incorporated into nucleic-acid–based probes. To limit disturbance within labelled sequences and improve the stability of the complexes formed with complementary natural counterparts, FNA chromophores must resemble natural bases in size and hydrogen-bonding patterns; in this regard, isomorphic and expanded FNAs represent the most suitable minimally perturbing fluorescent labels. Significant advances in their photophysics have clarified how structural and environmental factors shape fluorescence behaviour, offering principles for rational chromophore design.[3]

Inspired by this paradigm, compact fluorescent moieties, such as the thiophene derivative of the uracil nucleobase (thU), are emerging as promising alternatives for PNA labelling. In this work, we investigate how the sequence context and PNA length influence the fluorescence behaviour of this emerging fluorophore and its compatibility with PNA hybridization. This study is expected to provide useful insights for the future design of thU-tagged PNAs for applications in biosensing, imaging, and nucleic acid-based analysis.

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PP-10

Development of an emission ratiometric chemigenetic Ca^{2+} indicator based on solvatochromism

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Calcium ion (Ca^{2+}) is a second messenger regulating crucial bioactivities in animals and plants, and quantitative measurement of Ca^{2+} can reveal detailed dynamics of Ca^{2+} signals. Ratiometric indicators are commonly used to observe the Ca^{2+} dynamics in living organisms by calculating the intensity ratio of two fluorescence channels, which minimizes the artifacts caused by indicator concentration, photobleaching, or sample thickness. In particular, the emission ratiometric indicators, which use a single excitation and two emission channels, allow continuous imaging for fast Ca^{2+} signaling by detecting two emission channels simultaneously. However, existing emission ratiometric indicators have drawbacks, such as limited control over localization to specific subcellular compartments (for synthetic indicators) and limited signal change due to low FRET efficiencies (for genetically encoded indicators). Recently, a new design of Ca^{2+} indicators based on a chemigenetic approach has emerged by combining a fluorophore that undergoes intramolecular spirocyclization, Ca^{2+}-binding protein calmodulin (CaM), and a self-labeling tag protein as HaloTag.[1,2] This approach can overcome the drawbacks of current indicators by integrating the advantages of both synthetic fluorophores and protein-based domains. Inspired by these works, we hypothesized that introducing a solvatochromic fluorophore with a large emission wavelength shift in response to a protein structural change, could yield ratiometric indicators with controllable subcellular localization and higher ratiometric changes.

To test the hypothesis, we designed a new emission ratiometric Ca^{2+} indicator by combining a HaloTag-CaM fusion protein and the solvatochromic fluorophore AggRetina (**Fig. 1a**).[3] AggRetina-based HaloTag ligands (AggRetina-HTL) with four different polyethene glycol (PEG) linker lengths were synthesized (**Fig. 1b**). Then, we optimized the sequence of HaloTag-CaM protein by directed evolution to achieve $\Delta R/R_0 = 7.9$ with a substantial Ca^{2+}-dependent peak shift ($\Delta\lambda_{em} = 48$ nm) and an appropriate affinity for bioimaging ($EC_{50} = 188$ nM). Further research is ongoing to establish robust chemigenetic bioimaging tools for emission ratiometry.

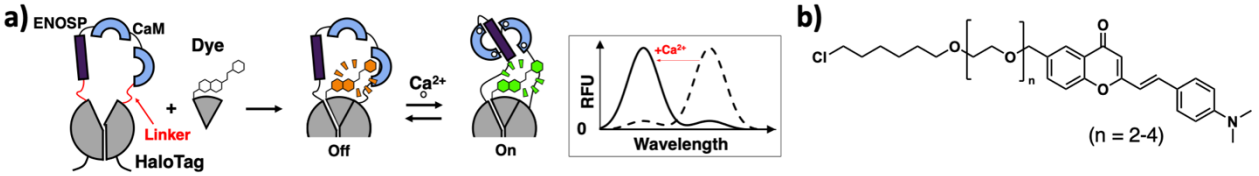

Fig. 1: a) Schematics of chemigenetic emission ratiometric indicator. b) Structure of AggRetina-HTL.

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PP-11

Fluorescent probes targeting the tumor microenvironment for fluorescence imaging application

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Tumor microenvironment is characterized by hypoxia and upregulation of Carbonic Anhydrase IX (CAIX)[1], a transmembrane validated biomarker for imaging and therapeutic purposes. Several heptamethine cyanines (Dye1-5) combined with different CAIX-targeting moieties were synthesized for near infrared fluorescence tumor imaging[2]. The detailed biological characterization of these probes, all containing a modified acetazolamide targeting moiety with an aminoctanoic linker (C8-AZA) but different dyes, is reported in this study. The structural features of heptamethine cyanines modulated the optical properties of resulting probes, their affinity to CA enzymes (measured through a fluorescence-based assay), binding and uptake to CAIX-expressing cells (estimated by flow cytometry), and affinity to human albumin. All C8-AZA-based probes exhibited high affinity to the target: Dye1-C8-AZA, for example, displayed low K_D values of 6.1±1.6 nM and 58±9 nM toward a recombinant CA enzyme and CAIX-expressing HT-29 cells, respectively. Flow cytometry evaluation of HT-29 cells treated with C8-AZA-based probes revealed that cell-associated fluorescence decreased in presence of an excess of unlabeled competitor for CAIX, indicating specificity for the target. Tumor spheroids were then adopted to mimic the hypoxic tumor microenvironment. Indeed, all C8-AZA based probes displayed selective accumulation in CAIX-rich hypoxic cores, identified by pimonidazole staining and fluorescence microscopy analysis, indicating that they were able to reach and bind the target in a 3D structure. Finally, *in vivo* optical imaging efficacy assessment was conducted in mice bearing HT-29 tumors, where C8-AZA-based probes demonstrated strong tumor localisation and clearance from non-target tissues. The *ex vivo* tumour-to-background ratio (TBR) value, 24 h post-injection, reached by Dye1-C8-AZA was 23.1±6.7, indicating excellent contrast and specificity, confirmed by detection of fluorescent signal in tumor sections, in CAIX-positive areas of tumor parenchyma. Overall, the development of fluorescent probes targeting acknowledged biomarkers of the tumor microenvironment might provide powerful tools for tumor imaging, applicable to a wide variety of cancers.

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Conflict of interest: all authors are employees of Bracco Imaging S.p.A., which has filed patent applications on CAIX targeted NIR fluorescent dyes for optical imaging.

PP-12

Luminescent uridine nucleoside analogues (LUNA) for enzymatic DNA synthesis

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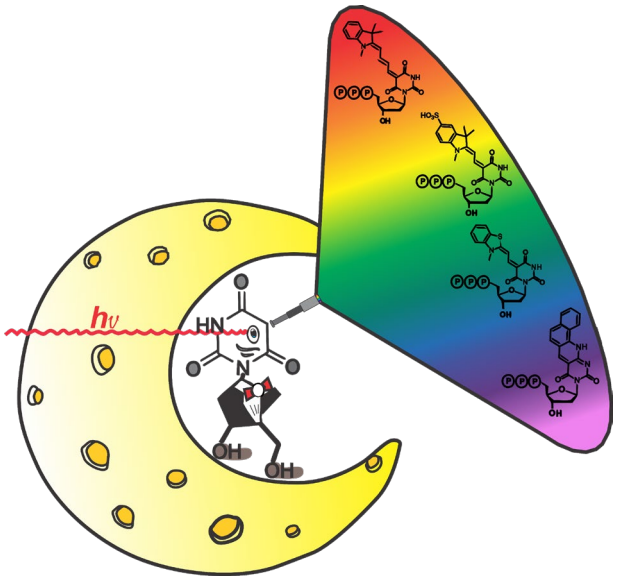
Fluorescent nucleosides are a valuable tool for investigating nucleic acid structures and interactions through spectroscopic analysis. Since naturally occurring nucleosides are essentially non-fluorescent, numerous structural modifications have been developed, leading to a wide array of fluorescent DNA and RNA analogues.[1] Additionally, fluorescent labelling techniques have been established not only for the four canonical nucleosides but also for epigenetic and artificial analogues.[2]



This work builds upon our previous report of barbituric acid-derived merocyanine nucleobase mimics, which were embedded into DNA via solid-phase synthesis.[3] Here, we present the enzymatic incorporation of various Luminescent Uridine Nucleoside Analogues (LUNA) using a convenient triphosphate synthesis and modification method. The resulting LUNA triphosphates exhibit broad spectral coverage within the visible spectrum and possess unique structural properties that support both template-independent and template-dependent DNA synthesis. We demonstrate rapid single-nucleotide 3'-labeling by terminal deoxynucleo-tidyl transferase (TdT), while template-dependent polymerisation allows for the generation of fluorescently labelled longer DNA strands that are challenging to achieve through solid-phase synthesis. Barbituric acid-based dye nucleobases exhibit strong environmental sensitivity when incorporated into oligonucleotides,[4] making them excellent probes for the investigation of DNA-enzyme interactions. The presented results form the basis for establishing LUNA as a key precursor for studying DNA dynamics and dye-dye interactions within DNA.

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PP-13

3D fluorescent imaging of anticancer molecule in the transparent tumor as a function of intratumoral vascular distance

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The aim of this study was to investigate the spatial distribution of anticancer fluorescent molecule in intact transparent breast tumor tissues as a function of the intratumoral vascular distance. The spatial distribution of anticancer fluorescent molecules showed an apparent lack of penetration into the deep tumor site, hypoxic region where drug resistant cancer cells are located.[1] The anticancer fluorescent molecule was mostly accumulated inside the tumor blood vessel and some were extravasated into tumor tissue. Quantitative verification indicated that the penetration depth of anticancer fluorescent molecule was found to be up to ~65 μm. However, the maximum percentage of distribution was observed only within 30 to 40 μm distance from the nearest vessels. To validate the existence of viable cells deep inside the tumor, we attempted to measure the spatial distribution of hypoxic and clonogenic cancer cells in intact transparent breast tumor tissues. This study presents a direct demonstration for the spatial distribution of hypoxic and clonogenic cells as well as anticancer fluorescent molecules with respect to the intratumoral blood vessels. HIF1α was selected as a marker to observe hypoxia region, and CD44 was selected as a marker to identify clonogenic cells in the tumor microenvironment. Characteristic distance mapping illustrated that HIF1α expression in the tumor ranged from 72 to several hundred μm (~450 μm), which verified that HIF1α overexpressed cancer cell was expressed far away distance from the intratumoral blood vessel. Whereas, the distribution of CD44 overexpressed cancer cell was decreased with increasing distance from blood vessels. However, CD44 was still expressed in a significant amount ranging from around 70 μm to 200 μm, and the maximum distribution of CD44 expressed cancer cell was observed to be around 300 μm from the nearest blood vessels. These findings apparently revealed that hypoxic and clonogenic cells did not uptake enough anticancer fluorescent molecules. This study demonstrates that the penetration ability of the anticancer fluorescent molecule into the tumor deeply depends on particular features such as interstitial fluid pressure and extracellular matrix in the tumor microenvironment, and is not sufficient to diffuse deep inside the tumors and target viable cancer cells.

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PP-14

Characterization of endosomal damage and pDNA delivery using fluorescence imaging methods

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Currently, less than 2% of administered oligonucleotide-based drugs encapsulated in lipid nanoparticles (LNPs) reach the cytoplasm.[1] This makes the delivery of the oligonucleotides for translation and transcription highly inefficient. One of the main bottlenecks contributing to the inefficient delivery is endosomal escape. Here, we propose a new method to detect endosomal escape, by utilizing fluorescent lifetime imaging together with traditional endosomal rupture markers (Galectin-9). By labeling the payload, we can combine the methods of lifetime imaging and protein markers to obtain a better understanding of how and when the payload escapes the endosome. Plasmid DNA (pDNA) was used as the model payload and labeled with MFP488 (similar to AF488) via covalent binding to the oligonucleotides. pDNA–MFP488 was then encapsulated in lipid particles (LPs) and transfected into the cells. HEK293 cell line was used, which had been modified to continuously express mCherry–Galectin9 fusion protein (mCherryGAL9) for endosomal rupture detection.[2]

Imaging of Galectin9 expressing HEK293 cells after pDNA-MFP488:LP transfection, shows an increase of high intensity spots of mCherry-GAL9, indicating endosomal rupture. From FLIM data of labelled particles delivered to cells, we can see a clear shift in lifetime over two hours, indicating a significant change in the particle microenvironment. Particles co-localized with GAL9 have consistently a longer lifetime (2.63 ns for DNA-MFP488-LP) than particles non-colocalized (Fig 1).

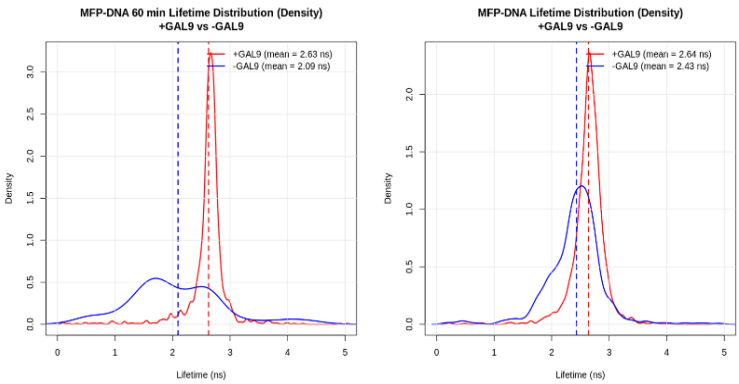

Fig 1. Fast FLIM lifetime distribution of particles colocalized and non-colocalized with Galectin9 endosomal rupture marker at 60min and 120min after transfection.

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PP-15

Targeted NIR Probes for tumor imaging

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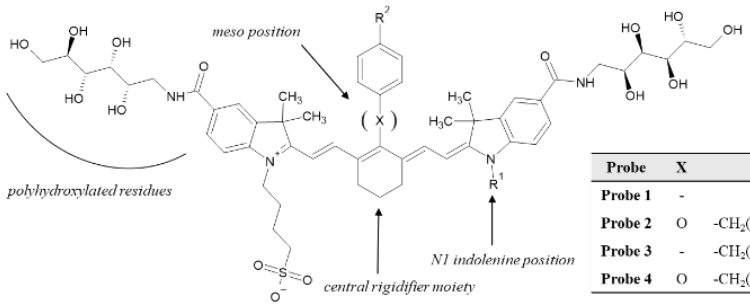
Near-infrared (NIR) fluorescence-guided surgery is becoming increasingly important in surgical oncology, offering enhanced precision in tumor resection and helping to reduce recurrence rates.[1] This technique relies on fluorescent tracers that selectively accumulate in tumor tissues. Among these tracers, a central role is played by cyanine dyes (Cy) in which a polyene chain connects two nitrogen-containing heterocycles, typically indolenines or benzoindolenines.[2] Over the past decade, Bracco Imaging discovered that introducing polyhydroxylated groups, such as D-glucamine, onto the cyanine backbone significantly affects pivotal features of the molecular probe, such as tissue distribution and target engagement.[3] Building on this insight, we investigated the impact of additional chemical modifications on the optical and biological properties of cyanine dyes. Specifically, a heptamethine cyanine bearing a central cyclic hexenyl group, known to red-shift absorption and emission into the NIR region, was differently decorated (Figure 1) by introducing: *i)* a phenyl or phenol moiety at the *meso* position, *ii)* sulfonate groups to improve water solubility, and *iii)* a carboxylic acid, used for conjugation with c(RGDfK), a synthetic cyclic peptide that efficiently targets $\alpha_V\beta_3$ integrin receptors which are overexpressed in many cancer cells.[4] From these combinations, four new targeting probes were synthesized and fully characterized in terms of optical properties, HSA affinity, binding to $\alpha_V\beta_3$ receptors, and cellular uptake on $\alpha_V\beta_3$-expressing melanoma cells. The tumor imaging performance of selected probes was then assessed *in vivo* using a subcutaneous glioblastoma mouse model.



Probe	X	R^1	R^2
Probe 1	-	$-(CH_2)_4-SO_3H$	$-CH_2CH_2CONH$-c(RGDfK)
Probe 2	O	$-CH_2(CH_2)_4CONH$-c(RGDfK)	$-SO_3H$
Probe 3	-	$-CH_2(CH_2)_4CONH$-c(RGDfK)	-
Probe 4	O	$-CH_2(CH_2)_4CONH$-c(RGDfK)	-

Fig. 1: Structures of all synthesized $\alpha_V\beta_3$-targeting probes functionalized with c(RGDfK).

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Conflict of Interest: all authors are employees of Bracco Imaging S.p.A., which has filed patent applications on this class of dyes for optical imaging.

PP-16

ZnO nanotetrapods as functional platforms for optical biosensing

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ZnO nanotetrapods are increasingly attracting attention as functional platforms for label-free optical biosensing due to their three-dimensional morphology, high surface-to-volume ratio, and intrinsic optical properties. In this work, we present recent progress in the synthesis, physicochemical characterization, and biosensing-oriented functionalization of ZnO tetrapods, emphasizing their role as optical transducers for bioanalytical applications.

ZnO tetrapods were synthesized using scalable combustion-based approaches, resulting in highly crystalline nanostructures with reproducible morphology and optical response. Detailed physicochemical characterization revealed a high density of surface hydroxyl groups, enabling efficient chemical functionalization and stable immobilization of biomolecular recognition elements. These features make ZnO tetrapods particularly suitable for label-free biosensing schemes based on photoluminescence signal modulation induced by biomolecular interactions.

The functionalized ZnO tetrapods demonstrate sensitive optical response to biological binding events without the need for external labels, allowing rapid and low-complexity detection. Recent applications include photoluminescence-based biosensors for virus-related biomarkers, where ZnO tetrapods were integrated into hybrid and microfluidic sensing platforms. Beyond biosensing, the same material system shows strong potential for broader biotechnological and optical applications, including surface-engineered coatings and multifunctional nanostructured interfaces.

Overall, ZnO tetrapods emerge as a versatile nanomaterial platform that bridges scalable synthesis, surface chemistry, and functional optical biosensing, with promising prospects for future bioanalytical and biotechnological systems.

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PP-17

Development of a novel near-infrared sensor for monitoring lipid peroxidation-mediated viscosity changes in ferroptosis

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Ferroptosis, a distinct form of regulated cell death characterized by the lethal accumulation of lipid peroxides, provides a potential therapeutic strategy to combat drug-resistant cancers. Two major antioxidant systems, involving glutathione peroxidase 4 (GPX4), which reduces toxic lipid peroxides to their corresponding alcohols in a glutathione-dependent reaction, and ferroptosis suppressor protein-1 (FSP1), which catalyses the regeneration of non-mitochondrial Coenzyme Q10 using NAD(P)H,[1] have been demonstrated to suppress ferroptosis. Understanding where lipid peroxidation occurs and how lipid peroxides are trafficked between organelles during ferroptosis is critical for elucidating its underlying mechanisms.

Here, we developed TM as a highly sensitive near-infrared (NIR) sensor which exhibited turn-on fluorescence upon viscosity change, enabling visualization of lipid peroxidation in ferroptotic hepatocytes with greater sensitivity than BODIPY 581/591 C11. Time-lapse live-cell imaging of erastin-treated cells revealed real-time lipid peroxidation dynamics involving cytosolic lipid droplets (cLDs), endoplasmic reticulum, and nuclear LDs (nLDs) in erastin-treated ferroptotic HepG2 cells. In addition, a strong fluorescence was detected in the PA-induced LD accumulation model, indicating the accumulation of lipid peroxides. Our findings suggest that TM can be a valuable tool for tracking the progression of chronic liver diseases associated with ferroptosis.

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PP-18

Amphiphilic cNDIs: versatile probes in chemical biology

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Core-substituted 1,4,5,8-naphthalenediimides (cNDI) are highly versatile fluorophores that have been extensively explored over the past century.[1,2] Their applications span a broad range of fields, from supramolecular chemistry and anion–π catalysis to organic electronics and chemical biology, including membrane sensing.[1,3]

In this work, we investigate a small library of non-symmetric amphiphilic cNDIs with the aim of developing new bioimaging probes.[4]

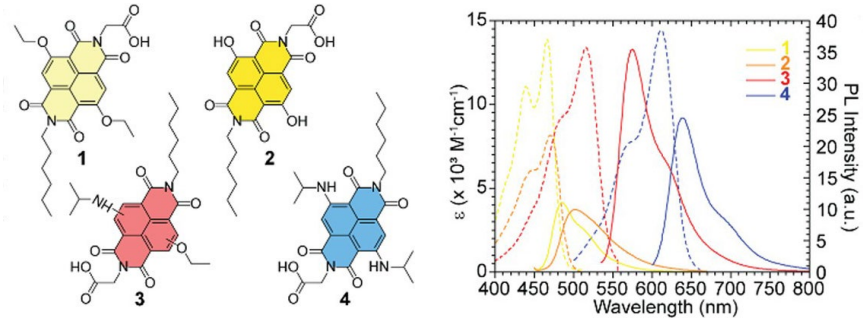

Fig. The library of cNDIs and their optical properties.

Four novel derivatives were synthesized and fully characterized. The influence of their molecular architecture, hydrophilic/hydrophobic balance, and electron-donating substituents, tuned to cover the entire visible spectrum, was evaluated in large unilamellar vesicles (LUVs). Finally, immunofluorescence imaging experiments revealed their selective staining of intracellular membranes. Overall, these findings provide valuable insights into the design of next-generation amphiphilic fluorescent sensors for bioimaging applications.

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ABOUT THE SPEAKERS

KN-1 Xiaogang Liu

Dr. Liu Xiaogang is an Associate Professor (tenured) in the School of Chemistry, Chemical Engineering and Biotechnology (CCEB) at Nanyang Technological University (NTU). He earned his B.Eng. in Electrical and Electronic Engineering from Nanyang Technological University in 2006. He then completed his PhD in Physics at the Cavendish Laboratory, University of Cambridge, in 2014. Following his doctoral studies, Dr. Liu was a SMART Scholar Fellow at the Singapore-MIT Alliance for Research and Technology (SMART) from 2014 to 2017. He began his assistant professorship at the Singapore University of Technology and Design (SUTD) in April 2017, before moving to NTU in August 2025. Dr. Liu's research focuses on understanding the fundamental structure–property relationships of organic fluorophores and fluorescent probes. His group designs novel molecular systems for diverse applications in bioimaging, diagnostics, and optoelectronics. His overarching vision is to transform fluorophore development from an empirical approach to a predictive, design-driven method, enabling the precise tailoring of photophysical properties for specific uses. In addition to his research, Dr. Liu is dedicated to academic service and science education. He has served as a Council Member of the Singapore National Institute of Chemistry and, since 2019, has been the Vice-Chair of the Singapore Junior Chemistry Olympiad.

KN-2 Yitzhak Tor

Yitzhak Tor carried out his doctorate work at the Weizmann Institute of Science earning his PhD in 1990. After a postdoctoral stay at the California Institute of Technology (1990–1993), he took his first faculty position at the University of Chicago. In 1994, he moved to the University of California, San Diego, where he is currently a Distinguished Professor of Chemistry and Biochemistry. He was the Teddy Traylor Scholar in Organic Chemistry (2006–2011) and the George W. and Carol A. Lattimer Professor (2013–2017). His research interests are diverse and include chemistry and biology of nucleosides, nucleotides and nucleic acids, the discovery of novel RNA-targeting antiviral and antibacterial agents, as well as the development of cellular delivery agents and biomolecular fluorescent probes. He was the founding Editor-in-Chief of Perspectives in Medicinal Chemistry and is currently serving as a Section Editor of the Journal of Antibiotics and an Associated Editor of the Journal of Molecular Evolution.

KN-3 Luke D. Lavis

Luke D. Lavis is a Senior Group Leader and Head of Molecular Tools & Imaging at HHMI's Janelia Research Campus. He developed the widely used Janelia Fluor (JF) dyes platform and cofounded Eikon Therapeutics in 2019. Dr. Lavis was named one of C&E's "Talented 12" in 2015 and received the 2025 Gregorio Weber Award for Excellence in Fluorescence Theory and Applications.

IL-1 Nadia Barbero

Nadia Barbero is Associate Professor of Organic Chemistry at the University of Turin, Italy. She has recently been appointed Full Professor starting in 2026 at the same university. She leads the organic synthesis division within the MOF Lab (Functional Organic Materials). Her research focuses on the design, synthesis, and characterization of functional organic compounds capable of interacting with light. Her current interests include the development of organic small molecules for bioLEDs, photovoltaics, photodynamic therapy, and protein detection.

IL-2 Marina Massaro

Dr. Marina Massaro is an Assistant Professor at the Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) of University of Palermo. Her principal research interests include the modification of clay minerals for application in several fields. In 2019 she received the “Vincenzo Caglioti International Award for Chemistry” from Accademia Nazionale dei Lincei at the presence of the Italian President of the Republic, for the excellent results obtained in the study of clay minerals for application in catalytic, biological and medicinal field. Later she was awarded by the "Gian Giacomo Drago e Fausta Rivera Drago Award" from Istituto Lombardo di Scienze e Lettere for relevant researches in the field of natural materials for innovative application in biomedicine and physic. She is author of ca. 79 international publications (h-index 35) on peer review journals.

IL-3 Marc Vendrell

Marc Vendrell is Professor of Translational Chemistry and Biomedical Imaging at the College of Medicine and Veterinary Medicine in Edinburgh. His team has pioneered the design of smart chemical fluorophores for high-resolution optical imaging (>150 papers) and built an ambitious research programme linking physical and biomedical sciences through competitive funding -over £10M as PI (>£40M as co-I), including prestigious European ERC Consolidator and EIC Transition grants-. Vendrell is co-inventor of 12 patents, PI for 9 licensed technologies and collaborative projects with industry and Pharma, and he has contributed to founding two spin-out companies on therapeutics (InPepcide) and diagnostics (IDxSense). Vendrell has a strong record of mentoring and training the next generation of translational scientists, with several alumni holding independent positions in academia and industry. He has won some awards and distinctions, the latest being the Bader Prize for eminence in Organic Chemistry by The Royal Society of Chemistry in 2023 and elected Fellow of the Royal Society of Edinburgh, the Scottish national academy, in 2024. Vendrell currently heads the IRR Chemistry Hub at the College of Medicine in Edinburgh as one of the first global hubs for collaborative non-siloed chemical research to catalyse innovation in biomedical sciences and accelerate translational outputs.

IL-4 Andrey S. Klymchenko

Andrey Klymchenko obtained his PhD degree in 2003 from Kyiv National University. He worked as post-doctoral fellow in the University of Strasbourg and Catholic University of Leuven. Then, he joined CNRS in 2006, received CNRS Bronze Medal in 2010 and was promoted to Director of Research in 2014. In 2015, he obtained ERC consolidator grant BrightSens to work on fluorescent nanoparticles for biosensing. In 2021, he received Prix du Dr et de Mme Henri LABBE from French Academy of Sciences and he was elected a member of Academia Europaea. In 2025, he obtained ERC consolidator grant CaptuRel on capture/sensing & photorelease materials. He is a leader of "Photoactive Materials and Bioimaging" group. His research interests include functional fluorescent molecules and nanomaterials for biosensing, imaging and in vitro diagnostics. He is a co-founder of a start-up BrightSens Diagnostics focused on molecular in vitro diagnostics based on fluorescent nanoparticles and AstraNICE dedicated to fluorescent biomaterials for image-guided surgery. He is a co-author of over 285 peer-reviewed articles and 13 patents.

IL-5 Marcus Wilhelmsson

Marcus Wilhelmsson (1974) was awarded his Ph.D. in 2003 and is now a Full Professor of Physical Chemistry and head of the division of Chemistry and Biochemistry at Chalmers University of Technology. Wilhelmsson's research concerns development and characterization of bio-mimicking fluorophores for nucleic acids and methodologies for their utilization. He is a key player in the field of fluorescent nucleobase analogues and has pioneered their use in FRET applications. Recently, he expanded the utilization of fluorescent nucleobase analogues as nature-mimicking labels by using them in in-vitro transcription into mRNA, subsequent in-cell translation to the corresponding protein, and use in live-cell imaging. Additionally, he has demonstrated that they can be used in metabolic labelling of RNA in non-engineered cells. This has important applications in studies of mRNA-based therapeutics and cellular RNAs.

IL-6 Ka-Leung Wong (Gary)

Professor Ka-Leung Wong, Chair Professor at CityUHK, is an expert in lanthanide chemistry, spectroscopy, and cancer theranostics. He earned his Ph.D. from the HKU and held a Royal Society Fellowship at Durham. He joined CityUHK from HKPolyU in 2026. With over 200 publications, an h-index of 65, and 11,000+ citations (Google Scholar), his work has led to multiple U.S. patents. He's received prestigious awards, including the Royal Society Incoming Fellowship and the European Rare Earth and Actinide Society Junior Award, and serves as Main Editor of Journal of Luminescence.

IL-7 Yves Mély

Yves Mély carried out his doctorate work at the University Louis Pasteur in Strasbourg, earning his PhD in 1988. He took his first faculty position at the same University in 1989 and a sabbatical at the Max Planck Institute for Biophysics in Frankfurt, Germany, in 1997, as a von Humboldt Fellow. He got a full professor position at the University of Strasbourg in 1998. He is team leader since 1999 and was the head of the laboratory from 2009 to 2023. He is deputy director of the national Infrastructure France BioImaging since 2024 and the chair of the international conference series "Methods and Applications in Fluorescence" since 2011. His research activity can be divided into two axes. The first relates to the development of fluorescence microscopy techniques and innovative fluorescence probes for membranes, nucleic acids and proteins. The second axis deals with the application of these techniques and probes to characterize viral and epigenetic proteins, in order to discover new therapeutic avenues. He was senior member of the Institut Universitaire de France (2018-2023) and received several awards such as the “Gregorio Weber Award” in 2017.

IL-8 Hans-Achim Wagenknecht

HansAchim Wagenknecht studied chemistry in Freiburg (Germany), obtained his diploma in 1995 on glycoside inhibitors, received his doctoral degree in 1998 in Basel (Switzerland) on porphyrine enzyme models), and worked until 2000 as postdoc at Caltech (USA) on DNA charge transfer. In 2003, he obtained the habilitation at the Technical University Munich (Germany), and in 2005 he received a professorship at Regensburg (Germany). He has held the chair for organic chemistr yat the Karlsruhe Institute of Technology (KIT) since 2010. His research focuses on nucleic acid and peptide chemistry, fluorescent DNA architectures, photochemistry and photocatalysis.

IL-9 Javier Montenegro

Javier Montenegro received his master degree (2003) and PhD (2009) from the University of Santiago de Compostela, under the direction of Prof. Susana López, working in the field of synthetic retinoids. In 2005 he was a visiting scientist in the group of Prof. Steven Ley at Cambridge University (Total Synthesis). In 2007 he was a visiting scientist at the Scripps Research Institute (La Jolla) working with Prof. Reza M. Ghadiri in prebiotic chemistry and supramolecular dynamic polymers. In 2009 he moved to the University of Geneva for postdoctoral studies with Prof. Stefan Matile in biosensing, synthetic transport systems and controlled delivery. In 2012 he returned to Spain with Juan de la Cierva and a Ramon y Cajal at Organic Chemistry Department and then promoted to Profesor Titular and Oportunius Researcher (2020) as PI in the CIQUS at the University of Santiago de Compostela. He has received different prestigious awards including: a Starting Grant from the ERC (2015), a Young Investigator Grant of the Human Frontier Science Program (2017), JSP Fellow 52th Bürgenstock Conference (2017), the Prize of Young Investigators of the Spanish Royal Society of Chemistry (2018) and the Prize of Young Research Group Leaders in Chemical Biology of the RSEQ (2019).

IL-10 Nikos Hatzakis

Nikos Hatzakis is Professor at the University of Copenhagen, Department of Chemistry and NovoNordisk Center for Optimised Oligo escape Leader.

IL-11 Salvatore Sortino

Full professor of chemistry at the University of Catania. His research activity mainly focuses on the design and fabrication of light-responsive molecular and supramolecular systems and nanomaterials for therapeutic and imaging applications, with particular emphasis on NO photodelivery, photodynamic and photothermal therapy and their combinations. He is the author of 240 publications in peer-reviewed journals and 3 patents.

IL-12 Donal F. O'Shea

Donal O'Shea received his PhD degree in Chemistry from University College Galway, Ireland. He held post-doctoral positions in the University of Edinburgh and Carnegie Mellon University, Pittsburgh following which he was a research scientist at Eastman Kodak Company in Rochester, New York. In 1999, he returned to academia to a position in University College Dublin and was promoted to Professor of Chemistry in 2007. In 2013, he moved to the RCSI where he is the current Professor and Head of the Department of Chemistry. He has received the Institute of Chemistry of Ireland Annual Award for Chemistry, the RSC Inaugural North/South of Ireland lectureship award. He has held visiting professorship positions at Donghua University Shanghai, University of Rennes, École Nationale Supérieure de Cachan Paris, Université Bordeaux and Université Paris-Saclay.
His research interests include synthetic organometallic chemistry, chemical biology, fluorescence microscopy, fluorescence guided surgery and the chemistry of vaping.

IL-13 Wiktor Szymanski

Wiktor Szymanski received his PhD degree from The Warsaw University of Technology, Poland, in 2008. He spent two years working on the use of biotransformations in organic chemistry with Prof. Dick B. Janssen at the University of Groningen. Since 2010 he has been working on the construction of photoactive protein- peptide- and DNA-bioconjugates and photopharmacology in the Feringa Labs. In 2014, he joined the University Medical Center Groningen, where he was appointed in 2015 as tenure track assistant professor and in 2019 as associate professor. In 2023, he became a full professor at the Groningen Research Institute of Pharmacy, where he holds the chair of Medicinal Chemistry, Photopharmacology and Imaging.

IL-14 Francesca Cardano

Francesca Cardano received her PhD in 2020 from the University of Genova in affiliation with the Italian Institute of Technology and the University of Miami where she spent one year working under the supervision of Prof. F.M. Raymo. Afterwards, she moved to the Universities of Torino and Milano as a Postdoc. Since December 2022, she is a Research Fellow at the University of Torino working in Prof. A. Fin research group and collaborating with Prof. W. Szymanski at the University of Groningen. Her research interest focuses on the synthesis and study of molecular photoswitches, functional dyes and fluorophores for application in chemical biology and photopharmacology.

J INV Claudia Sciacca

Claudia Sciacca is a Postdoctoral researcher at the Department of Chemical Sciences of the University of Catania under the supervision of Prof. Vera Muccilli. She gained her Ph.D. in Chemical Sciences in 2023 with a thesis focused on the synthesis of polyphenols as bioactive compounds. She spent six months as a visiting researcher in the laboratory of Prof. Stephane Quideau at the University of Bordeaux, working on the synthesis of neolignans and their derivatives. Her main research interest includes the chemoenzymatic synthesis of polyphenols and the development of new platform for the enzyme immobilization.

Eric Schmidt-SWABIAN INSTRUMENTS (SP-1)

Eric Schmidt is an Application Scientist at Swabian Instruments, specializing in life-science applications of TCSPC methods, particularly fluorescence lifetime and fluorescence correlation spectroscopy.

Robertas Grigutis-LIGHT CONVERSION (SP-2)

Dr. Robertas Grigutis is a Sales Engineer at Light Conversion. He received his Ph.D. in Physics from Vilnius University in 2024. His academic work involved nonlinear light propagation, supercontinuum generation in bulk media, light-induced structural modifications, and laser-induced damage processes. At Light Conversion, he collaborates with regional partners and researchers to support the implementation of femtosecond laser and ultrafast spectroscopy systems across a wide range of scientific applications.

Grant Cumming HAMAMATSU-EI-NKT Photonics (SP-3)

Grant Cumming joined Edinburgh Instruments as a Project Scientist in 2023, with a primary focus on the radiometric calibration of photoluminescence spectrometers. His work centres on the calibration of integrating spheres and enhancing the robustness of quantum efficiency measurements.

Syed Ali Abbas Abedi (OC-1)

Dr. Syed Ali Abbas Abedi is currently a Postdoctoral Research Fellow at the Singapore University of Technology and Design (SUTD) and a Visiting Researcher at Nanyang Technological University (NTU), Singapore. He received his Ph.D. in Science, Mathematics and Technology from SUTD in 2024, where his research focused on the computational design and mechanistic investigation of fluorophores based on excited-state conformational dynamics. His expertise integrates advanced quantum chemistry methods, such as DFT and TD-DFT, with machine learning to elucidate photophysical properties and accelerate the design of next-generation optical materials.

Siân C. Allerton (OC-9)

Siân C. Allerton completed her PhD at Imperial College London under the supervision of Dr Francesco A. Aprile and Professor Marina K. Kuimova. Her doctoral research focused on utilising molecular rotors to monitor α-synuclein aggregation and detect the formation of toxic α-synuclein oligomers. She is currently an EPSRC Doctoral Prize Fellow working within the Aprile and Kuimova groups, where her research now focuses on understanding amyloid-β aggregation using molecular rotors.

Gülbahar Bozan (OC-21)
Gülbahar earned both BSc and MSc degrees in Chemistry from Istanbul Technical University, with stays at the Wroclaw University of Science and Technology and the University of Tartu, where she was involved in several projects regarding the molecular design of potential drug candidates for different biological targets. In 2021, she started her doctoral studies at the University of Regensburg, focusing on pericyclic reactions in open-shell systems within the field of physical organic chemistry. Since 2024, she has been working on the optimization of light-responsive molecules at the University of Groningen.

Erika Cerutti (OC-6)
Dr. Erika Cerutti is a Scientific Project Manager at Euro-BioImaging ERIC, where she is responsible for managing the user access to Euro-BioImaging services. Before joining Euro-BioImaging, Erika graduated in Chemistry at the University of Turin and completed her PhD in biochemical science working on the synthesis of bioconjugates for diagnostic applications. She subsequently held a postdoctoral position at the University of Turin and later in the private sector.

Xiong Chen (OC-8)
Xiong Chen completed his PhD at KU Leuven in the laboratory of Prof. Johan Hofkens, focusing on DNA labeling chemistry and biotechnology, and is currently a postdoc in Prof. Hofkens' lab.

Sara Ferrara (OC-26)
Dr. Ferrara got her B. Sc. in Chemistry and Chemical Technologies in 2017 at the University of Turin, Italy, where she also got her M. Sc. in Industrial Chemistry in 2019 with a thesis about the use of novel squaraine dyes for Dye Sensitized Solar Cells. After a period as research assistant at IMDEA Materials in Getafe, Madrid, Spain, she moved to Germany in 2020, where she carried out her PhD pathway at the Technical University of Munich. Her research focused on the application of natural and artificial fluorescent proteins in Bio-Hybrid Light-emitting Diodes, at the interface between material science, polymer chemistry, biology and physical chemistry. Her main expertise falls in the stabilization of organic and biological species in polymer materials for optoelectronic and photovoltaic applications, with associated molecular, material and device characterization. She is currently covering a Post Doc position at the University of Turin, with a focus on the synthesis and characterization of organic UV-VIS and NIR-IR organic emitters and relative polymeric films for Luminescent Solar Concentrators.

Thomas Mills (OC-11)
About the Speaker: Tom Mills is a final-year PhD student working within the Barnard and Kuimova research groups at Imperial College London, developing a novel methodology to study the inhibition of protein-protein interactions. Tom studied Chemistry at the University of Oxford before completing an MRes at Imperial College London, earning cohort prizes on both occasions.

Letiza Sambri (OC-19)
Letizia Sambri is Associate Professor of Organic Chemistry at University of Bologna with an established experience in luminescent and organic materials.

Saumya Saurabh (OC-18)

Dr. Saumya Saurabh is an Assistant Professor in the Department of Chemistry at New York University, with courtesy appointments in the Department of Physics and NYU's Pain Research Center. His lab seeks to reveal the complex physical and chemical principles that organize cellular processes, from single-molecule signalling to the formation of biomolecular condensates. He conducted his postdoctoral research in the laboratory of W.E. Moerner at Stanford University, where he pioneered super-resolution imaging methods for live-cell studies in bacteria. Building up on this work, his lab develops and applies next-generation optical tools, as highlighted in this talk. When he can, Saurabh enjoys making DIY circuits, hiking, and cycling.

Takuya Terai (OC-10)

Takuya Terai received his PhD from The University of Tokyo in 2012, under supervision from Prof. Tetsuo Nagano and Prof. Yasuteru Urano. After working as an Assistant Professor in the Department of Pharmaceutical Sciences, The University of Tokyo, a JSPS special postdoctoral fellow, and a Project Associate Professor in Saitama University, he became an Associate Professor in the Department of Chemistry, The University of Tokyo in 2020.

Eliza Baranska (PP-2)

Eliza Baranska is a PhD student in the Division of Chemistry and Biochemistry at Chalmers University of Technology, working under the supervision of Prof. Marcus Wilhelmsson and Prof. Morten Grøtli at Gothenburg University. Her research focuses on the design and development of novel fluorescent base analogues for DNA and RNA labeling, enabling studies down to the single-molecule level within the MSCA-funded MeChaNiSM project, as well as on the functionalization of FBAs using prodrug strategies for RNA labeling in living cells.

Maria Grazia Nolli (PP-9)

Maria Grazia Nolli is a skilled researcher with a strong background in pharmaceutical biotechnology. She has developed expertise in the synthesis of oligonucleotides, including DNA and PNA, as well as in the functionalization of biomaterials for biomedical applications. Her research focuses on designing and optimizing advanced materials for drug delivery and diagnostic applications, bridging the gap between molecular design and practical therapeutic or imaging tools. With extensive laboratory experience and a multidisciplinary approach, Maria Grazia combines chemical, biological, and biotechnological knowledge to develop innovative healthcare solutions. She is dedicated to advancing translational research and applying cutting-edge methodologies to real-world biomedical challenges.

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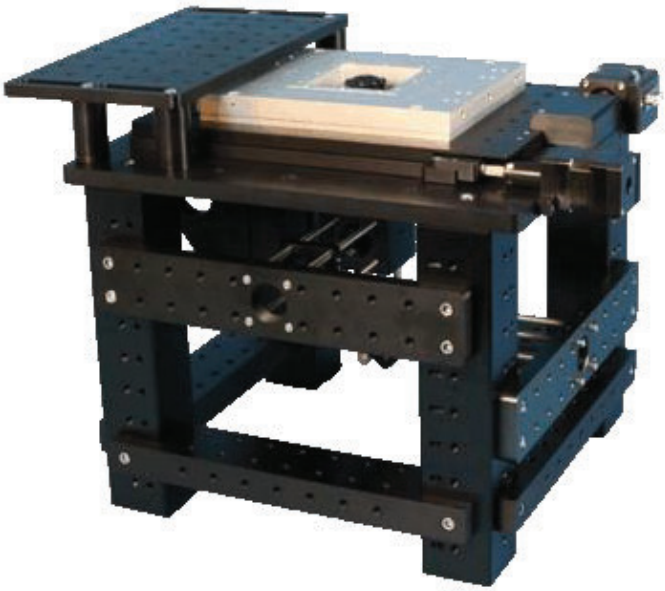

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ENRICO BRUNO s.r.l.
APPARECCHI SCIENTIFICI
SANITARI – INDUSTRIALI

1960 50° 2010

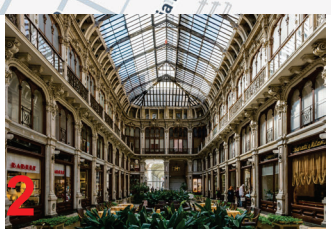
Oltre 50 anni in collaborazione con il mondo della sanità, della ricerca scientifica, del controllo ambientale e di qualità

CAGE Chemicals

Chemistry Europe


Palazzo Reale di Torino
Piazzetta Reale
Giardini Reali di Torino
Via Santa Giulia
Corso Regina Margherita
LAO 老
Via Santa Maria
Via Cernaia
Corso Vinzaglio
Corso Giuseppe Siccardi
Antonio Bertola
Ziccat - L'arte del cioccolato dal 1958
Ziccat: Chocolat depuis 1958
Palazzo Madama - Museo civico d'arte antica
3
Mastio della Cittadella
Via Stampatori
Via Luigi Tarino
Via Sant'Ottavio
Via Guastalla
Santa Giulia
Corso S. Maurizio
Mik Sushi
Via Santa Teresa
Via Raimondo Montecuccoli
Via Ettore De Sonnaz
Via Vittorio Amedeo II
Galleria Subalpina
2
Università degli Studi di Torino
Mole Antonelliana
4
Piazza Solferino
Palazzo Carignano
7
Museo della Radio e della Televisione Rai
Museo Egizio
8
Via Principe Amedeo
Palazzo Nuovo
Via Giulia di Barolo
Via Eusebio Bava
C.so Galileo Ferraris
Corso Giacomo Matteotti
Virgin Active Torino Collection
Fitness e Equilibrio - Cogli l'offerta
9
Via Maria Vittoria
Via Po
Via S. Massimo
Via Confienza
Piazza CLN
Rocca 1794 - Rivenditore autorizzato Rolex
Rocca 1794
Dior Beauty Counter - La Rinascente Torino
Makeup di Lusso Dior
6
Pizza Speedy Rosine Consegne A Domicilio
Pizza da asporto - Pizza Speedy Rosine
Via S. Quintino
Via Santa Croce
GAM - Galleria Civica d'Arte Moderna e...
Piazzetta Madonna degli Angeli
Sushisound Restaurant
Siamo un ottimo ristorante
V. Cavour
Via Giovanni Plana
5
Re Umberto
Jumping Jester Pub
Via Andrea Doria
San Giovanni Antica Sede
Ponte Vittorio Emanuele I
Corso Stati Uniti
Via Massena
Via Vincenzo Gioberti
Porta Nuova
Chiesa Della Gran Madre di Dio
C.so Vittorio Emanuele II
1
Via Carlo Alberto
Via S. Francesco da Paola
P.za Cavour
Via della Rocca
Via dei Mille
Tarì - cake lab viennoiserie
Via Nizza
Via Paolo Sacchi
Via Legnano
Corso Moncalieri


1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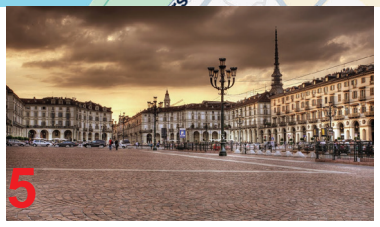
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