University of Trieste
Department of Chemical and Pharmaceutical Sciences

Doctorate in Chemistry

2021

Cycle 37

Research Projects

Positions MD/1 – MD/4: co-funded projects of predetermined topics

Position M/5: project on a topic of Pharmaceutical Technology (SSD CHIM/09)

Position M/6: "free project", to be chosen among those listed there

Project for position MD/1 CHIM/06

Supramolecular short-peptide systems based on dynamic combinatorial libraries

Supervisor: Prof. Silvia Marchesan, Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: smarchesan@units.it

Dynamic cominatorial chemistry (DCC) is an interesting approach for the investigation of multi-component systems towards the emergence of complexity in the form of defined compounds, out a library originated from simple reagents.[1] Ultimately, this approach could be envisaged to provide smart materials that are dynamic and respond to external stimuli with a change in their properties. Amongst the various building blocks used to develop such systems, self-assembling short peptides are very attractive to achieve biodegradable supramolecular materias based in water for an overall low-impact on the environment.

Nature has chosen homochirality to build biomolecules, meaning that, for example, the vast majority of proteins are composed of L-amino acids. Our research group developed interest towards the effects of the substitution of L-amino acids with their rarer D-mirror images at specific positions in very simple, short peptide sequences.[2] Such heterochiral peptides, composed of both D- and L-amino acids, can display peculiar properties, including the adoption of turn-like conformations, and the ability to self-organize into amphipathic superstructures that give rise to nanostructured hydrogels.[3] Applications vary, and those studied within the group span from innovative therapy (e.g., inhibition of amyloid pathological fibrillization as it occurs in neurodegenerative diseases),[4] to drug delivery,[5] tissue regeneration,[6] enzyme mimicry[7] and even small-molecule separation.[8]

One of the features that renders these systmes very interesting is their ambivalent character, for they have a high-degree of similarity with natural biomolecules that they can mimic,[6-7] yet their heterochirality often leads them to diverge from them in their supramolecular behaviour.[3,4] In order to open new horizons in this field, this project aims at the detailed study of dynamic combinatorial libraries that evolve from simple peptides as starting materials, to oligomers based on intermolecular disulfide bridges (Figure 1). In particular, the study will compare the evolution of homochiral peptides (as those found in nature), with that of heterochiral systems. Preliminary data has shown that it is possible to drive the library evolution towards different outcomes, meaning not only different ratios of the same products, but also different products, depending on the experimental conditions. Further, new methods will be developed to provide "stop" and "go" signals to achieve full control over the evolution of these dynamic systems.

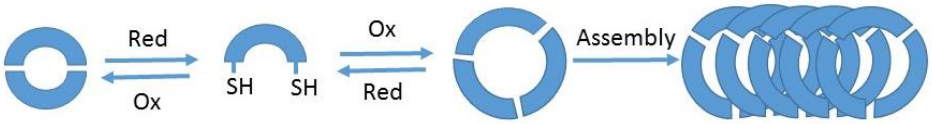

Figure 1. Thiol redox chemistry can be used to control the fate of a short peptide (middle) towards different products originated from a library of oligomers, such as dimers (left), or self-assembling trimers (right).

References:

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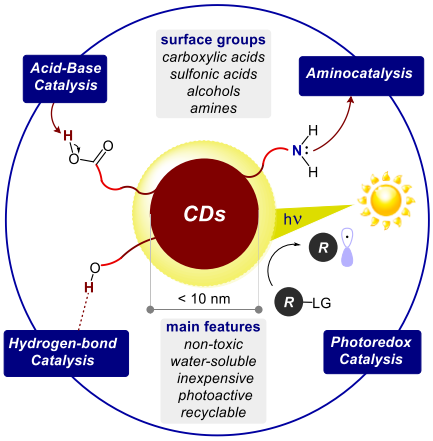
Project for position MD/2 CHIM/06

Carbon Dots as Nanocatalysts for Synthetic Applications

Supervisor: Prof. Maurizio Prato, Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: prato@units.it
Website: https://pratomaurizio.wixsite.com/labcarbon

This project aims at designing and producing novel Carbon Dots to use them as nano-organocatalysts for the development of relevant organic transformations.

Carbon dots (CDs) are a relatively new class of carbon-based materials that consist of quasi-spherical nanoparticles with dimensions smaller than 10 nm.[1] These nanomaterials typically exhibit fluorescence properties, low toxicity along with high solubility in polar solvents including water. These features have generated a broad research interest that spans over various fields such as chemistry, materials science, biology and medicine. Among these areas of application of CDs, an emerging development is their use as green organocatalysts.[2] With this in mind, our research group has recently demonstrated that amine-rich CDs, prepared from arginine and ethylenediamine, are capable of acting as (photo)catalysts to carry out organic transformations under mild operative conditions.[3]



Carbon dots as organocatalyst: CDs application in nano-organocatalysis and photocatalysis.

Our objective is to develop novel CDs-based catalysts for driving valuable synthetic reactions. This project involves the design and synthesis of new photoactive CDs bearing reactive surface groups such as carboxylic acids, sulfonic acids, alcohols, and amines. These groups will be used to catalyse the derivatisation of numerous organic compounds. Moreover, enantioselective catalysis may be achieved by using chiral CDs. In this way, the PhD student will become fluent in organic synthesis, catalysis and materials science. The produced nanomaterials will be diligently and comprehensively characterised using state-of-the-art spectroscopic and microscopic techniques. The PhD work will start from an ongoing project. Then, the individual interests and attitudes of the student will come into play and shape the development of her/his path into research.

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[**2**] "Carbon Dots as Nano-Organocatalysts for Synthetic Applications", C. Rosso, G. Filippini, M. Prato, *ACS Catal.* **2020**, *10*, 8090-8105.
[**3**] (a) "Mapping the Surface Groups of Amine-Rich Carbon Dots Enables Covalent Catalysis in Aqueous Media", G. Filippini, F. Amato, C. Rosso, G. Ragazzon, A. Vega-Peñaloza, X. Companyó, M. Bonchio, M. Prato, *Chem*, **2020**, *6*, 3022-3037. (b) "Use of Nitrogen-Doped Carbon Nanodots for the Photocatalytic Fluoroalkylation of Organic Compounds", C. Rosso, G. Filippini, M. Prato, *Chem.-Eur. J.*, **2019**, *25*, 16032-16036.

Project for position MD/3 CHIM/08

Characterization of antibody fragments

Supervisor: Prof. Sara Fortuna, Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: s.fortuna@units.it
Co-supervisor: Prof. Rita De Zorzi, Department of Chemical and Pharmaceutical Sciences, University of Trieste, rdezorzi@units.it

Goal of this project is to push forward the boundaries of targeted medicine by providing an alternative method for selecting binders with features difficult to obtain by conventional approaches based on immunization and antibody *in vivo* maturation.

The development of specific antibodies with the required features has been recently sped up by our team. We have replaced the process of monoclonal antibody fragment isolation with a computational based protocol[1]: we can now design antibody fragments (or VHH, Fig. 1) completely *in silico*. VHHs are the smallest antibody fragments which still preserve the binding capacity of whole antibodies[2]. VHHs are easy to engineer, and already widely used in the development of diagnostic and therapy reagents (in 2019 the first therapeutic VHH, blocking platelet aggregation, was approved by FDA[3]). They can be unctionalized for effective cell unctionalized [4].

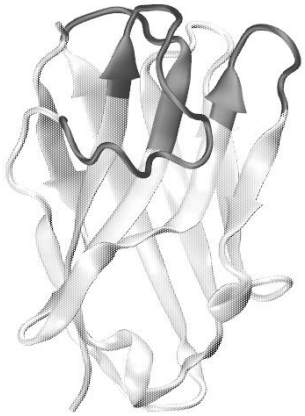

Fig. 1: VHH structure. In silico optimised antigen-binding complementarity determining regions (CDRs) are highlighted.

Specifically, this PhD project will require the expression and thorough unctionalized n a set of ex-novo designed VHHs specifically engineered to be selective towards relevant protein biomarkers and pharmaceutical targets. The activity will be carried out in De Zorzi lab (DSV) in collaboration with Ario De Marco (University of Nova Gorica, Slovenia) and Paola Storici (Elettra, Trieste). *In vitro* expression of *in silico* unctiona VHHs will be carried out in bacterial hosts. VHHs will be isolated, and purified. They will be characterized by standard biophysical techniques (e.g. size exclusion chromatography SEC, dynamic light scattering DLS). VHH/target interaction will be unctionalize by surface plasmon resonance (SPR), enzyme-linked immunosorbent assay (ELISA), and pull-down experiments. Crystallization experiments will be performed, X-ray diffraction data will be collected and the corresponding 3D structural models will be refined and analysed. Synthesis and characterization of GFP-tagged antigen-binding VHH will be performed [4]. Constructs will be unctionalized for cell internalization. In collaboration with Prof. Maurizio Romano (DSV) imaging in living cells will be carried out by confocal microscopy at the Fluorescence Microscopy Core Facility (ICGEB).

References:

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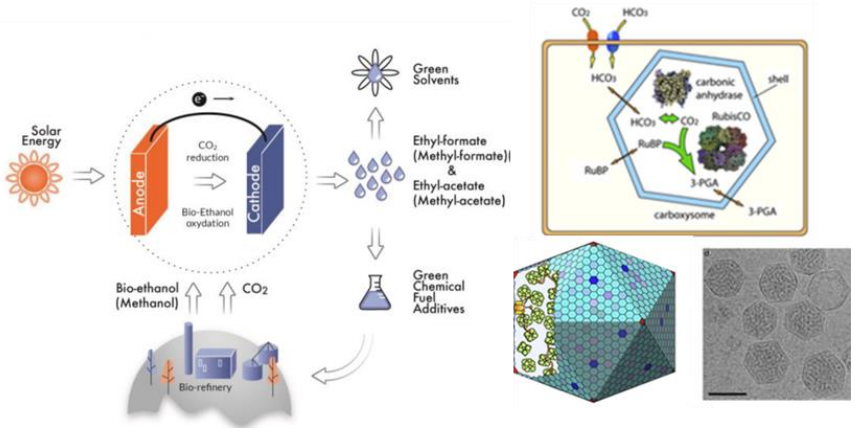
Project for position MD/4 CHIM/03

Paired Electrocatalysis for Efficient CO_2 Valorization

Supervisor: Prof Paolo Fornasiero, Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: pfornasiero@units.it
Co-supervisor: Prof. Marcella Bonchio (University of Padova)

CO_2 fixation into valuable chemicals and fuels is a winning strategy to neutralize the anthropogenic carbon emissions, however even the key player evolved by Nature for carbon-fixation, i.e. the RuBisCO enzyme, is highly inefficient in terms of reaction kinetics (few turnovers per hour) and selectivity (O_2 vs CO_2 processing). To overcome these limitations, the biological machinery adopts a combined strategy of spatial organization and separation of functions with cascade catalysis principles. This is the case of natural carboxysomes, i.e. microcompartments made by a self-assembled porous shell that allows the co-localization of RuBisCO and other catalysts while providing an an efficient capture and activation of CO_2 within a confined space, to boost rates and selectivity (Figure 1). Following a bio-inspired approach, CO_2 fixation by (photo)-electrocatalysis (PEC) in water has been the focus of an intensive research effort. However, the efficiency of CO_2 electrolysis is hampered by: (i) the competitive reduction of water at the cathodic side, (ii) the high energy cost of water oxidation at the anodic side; (iii) the limited commercial value of the O_2 by-product formed at the anodic compartment.



A promising alternative is to combine CO_2 reduction with a paired oxidative reaction that contribute to the production of value-added chemicals at both sides of the PEC device. The project will thus explore the electrocatalytic processing of CO_2 in ethanol solutions, with the aim of pairing ethanol oxidation and CO_2 reduction for the simultaneous production of the value-added chemicals, including ethylacetate and ethylformate. The research activity will focus on the design of

1) selective electrocatalysts for the partial oxidation of alcohols to aldehydes and carboxylic acids, while avoiding the total combustion to CO_2. In particular, multi-redox molecular catalysts will be considered in combination with functionalized carbon nanostructures (CNS)-scaffolds to tune the electrocatalytic mechanism and the anodic process (*Nat. Chem* 2010, 2, 826; *Nat. Chem* 2018, 10, 24; *Nat. Chem* 2019, 11, 146, *JACS* 2016, 138, 2617)

2) multi-functional reticular systems for CO_2 fixation, based on bio-inspired guidelines including: (i) porous covalent organic frameworks; (ii) molecular catalysis for CO_2 activation; (iii) photo-redox cascade mechanisms.

The PhD student will receive a thorough training on state-of-the art synthetic methods for organic and inorganic building blocks, supramolecular catalysis, solution and solid state characterization analysis (FT-IR, X-Ray techniques, NMR spectroscopy, mass spectrometry, chromatography and HPLC, circular dichroism) electrochemistry and advanced technology for light management and non conventional microwave assisted protocols for materials synthesis. The research activity will be performed in the frame of a European Project H2020-NMBP-ST-IND-2019 and in collaboration with academic and industrial experts for advanced photo-electrocatalysis and functional nanomaterials.

References: C. P. Kubiak, K. D. Moeller et al. "Paired Electrolysis in the Simultaneous Production of Synthetic Intermediates and Substrates" *J. Am. Chem. Soc*. **2016**, *138*, 46, 15110–15113**.**

Project for position M/5 **CHIM/09**

Study of mechanism of formation of crystalline polymorphs, solvates and hydrates of active pharmaceutical ingredients by mechanochemistry

Supervisor: Prof.ssa Beatrice Perissutti, Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: bperissutti@units.it

Physical property improvement is of particular interest to pharmaceuticals as the vast majority of active pharmaceutical ingredients (API) are delivered as solid forms. The physical properties of the solids contained within a pharmaceutical drug product will have a direct impact on the processing, delivery, and performance of the medicine. To provide a classical example, it is estimated that 40% of existing drug products and up to 90% of new chemical entities have limited aqueous solubility, and it is well known that crystal structure has a direct influence on the solubility of a given solid in solution. Research in mechanochemical techniques and applications in the preparation of new crystalline forms for APIs, or in alternative routes to obtain functional solid materials has shown an exponential increase in the past decade. Solvent-free techniques such as neat grinding and liquid-assisted grinding have demonstrated their tremendous potential for inducing selective polymorph transformations. At the same time, it is evident from literature that further work is still required to establish the precise mechanism by which these selective transformations occur. In addition, mechanochemical techniques, cheap, rapid and easy to be performed, are paradoxically influenced by a plethora of process variables, dramatically affecting the outcome of the mechanochemical reaction itself. Therefore, a combined mechanochemical and theoretical (mechanistic) approach is needed to design efficient and selective preparation routes for the desired crystal structure.

The aim of this Ph.D. project is to give a contribution in this context, with the aim to study the mechanisms and process conditions leading to different crystal forms of an API. The mechanochemical approach conducted on the essential drug praziquantel has permitted to discover 5 new solid forms of the API, not previously indexed in CSD (see aforementioned bibliography), even though praziquantel was far from a new chemical entity. The design of experiments (DOE) has been frequently applied to drive the mechanochemical reaction towards the desired polymorph or to understand the outcome of the grinding procedure in peculiar process conditions. To fully comprehend the often complex behavior of a mechanochemical-prepared polymorphic, hydrated or solvated form, a multidisciplinary investigation is paramount. Therefore, besides the suitable DOE, the research will also include chemical, thermal, structural, spectroscopic, morphological, biopharmaceutical and stability evaluations of the obtained crystal structures, also comprehending the study of phase interrelations amongst different solid forms. This will let the student to interact with experienced researchers belonging to different research fields gaining a very broad knowledge over the three years' period. The collaboration network across Europe involved in this project is wide and in continuous growth, enclosing researchers from Elettra-Sincrotrone, University of Bologna, Turin, Padua, Cambridge, Basel, Oslo, Zagreb, Ljubliana, Warsaw, Limerick and Granada. This Ph.D. project will combine several hot topics in the current chemical and pharmaceutical research, namely, crystal engineering, mechanochemistry, poor bioavailability and physical stability issues while presenting a new approach to the development of active pharmaceutical materials and suitable pharmaceutical drug products.

References:

Zanolla, D,..,Perissutti, B, Mechanochemical Formation of Racemic Praziquantel Hemihydrate with Improved Biopharmaceutical Properties Pharmaceutics 2020, 12, 289; doi:10.3390/pharmaceutics12030289;

Zanolla, D., Perissutti, B. et al. Exploring mechanochemical parameters using a DoE approach: Crystal structure solution from synchrotron XRPD and characterization of a new praziquantel polymorph. Eur. J. Pharm. Sci. 2019, 140, 105084; Zanolla, D. Perissutti, B., A new soluble and bioactive polymorph of Praziquantel. Eur. J. Pharm. Biopharm. 2018, 127, 19–28.

University of Trieste
Department of Chemical and Pharmaceutical Sciences

Doctorate in Chemistry

2021

Cycle 37

Research projects

(in alphabetical order of the proposer)

for the “free fellowship”

Position M/6

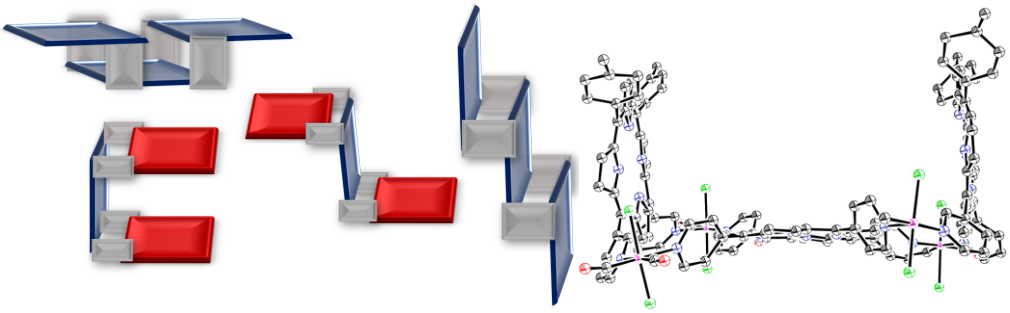
Project 1 for position M/6 CHIM/03

Novel heteroleptic metallacycles of porphyrins for supramolecular architectures

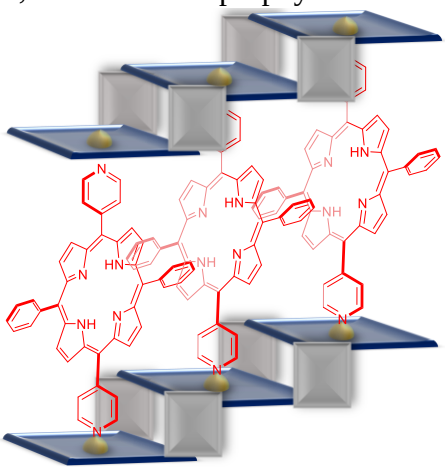
Supervisor: Prof. Enzo Alessio, Department of Chemical and Pharmaceutical Sciences, UniTS, email: alessi@units.it

Nature uses sophisticated arrays of tetrapyrrolic rings (e.g. chlorophyll, cytochromes,…) for performing precise energy and electron transfer processes. Beside the specific nature of the macrocycles, also their number and relative orientations are of paramount importance for determining the properties of such assemblies. The development of simple procedures for preparing synthetic arrays of tetrapyrrole macrocycles with full stereo-control is one of the challenges of supramolecular chemistry.

Recently, we developed a new flexible stepwise synthetic strategy that can be used for the construction of ruthenium-mediated heteroleptic arrays of *meso*-pyridylporphyrins (PyPs) that differ in the number of peripheral pyridyl rings (from 2 to 4) and/or in the position of the pyridyl N atom (3′ or 4′). The strategy requires the initial preparation of a reactive polytopic "acceptor" intermediate, i.e. a pyridylporphyrin bound to at least two ruthenium fragments, each having one residual readily-available coordination site, that is then reacted with the second porphyrin to yield the final heteroleptic assembly.[1,2] This route opens the way to new extended arrays as well as to **unprecedented geometries**, such as the **3+4 metallacycles** schematically shown in the Figure. They are made of 3 porphyrins (either 4′PyPs, red, or 3′PyPs, blue) and 4 Ru connectors (grey). The X-ray structure of one such heteroleptic metallacycles is also shown.



This project aims to consolidate the recent achievements, for example by obtaining additional crystals suitable for X-ray analysis. The zincated derivatives (panels) will be treated with appropriate linkers for the construction of higher-order assemblies, such as the 9-porphyrin adduct shown in the Figure.



The successful candidate will perform the design, synthesis (including microwave-assisted procedures) and characterization of organometallic and coordination compounds, porphyrins and metallo-porphyrins, and the stepwise preparation and purification of the novel metallacycles. The characterization will involve extensive use of 1D and 2D NMR, UV-vis and fluorescence spectroscopy, and the preparation of X-ray quality crystals. Collaborations with other groups for photophysical and X-ray structural characterization of the assemblies are to be expected.

As a final piece of information: my last two PhD students (one finished in March 2021) have already published with me 11 and 7 papers, respectively (+ one under submission).

References

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2. A. Vidal PhD Thesis, University of Trieste, **2021**.

Project 2 for position M/6 CHIM/12

Field and aerosol chamber studies for an extended physical-chemical and genetic characterization of bio-aerosol

Supervisor: Prof. Pierluigi Barbieri, Department of Chemical and Pharmaceutical Sciences, UniTS, email: barbierp@units.it
co-Supervisor: Alberto Pallavicini, email: pallavic@units.it

Atmospheric particulate matter (PM) derives from natural and anthropogenic processes, has composition and size distribution highly variable in time and space, and effects relevant to both climate changes and health. Recently ambient aerosol has received special attention as a potential carrier for SARS-CoV-2 [1]. An interdisciplinary group at the University of Trieste has succesfully searched and identified viral RNA on PM collected in the Po Valley during the initial COVID-19 outbreack by RT PCR [2]. Standardized methods for bioaerosol sampling and analyses are not available yet, even if literature is growing for both outdoor and indoor air particulate matter [3], also in relation to residual infectivity of environmental samples [4].
Bioaerosol components (bacteria, viruses) and variability are still largely unknown, and their viability is related to chemical composition of PM and environmental physical conditions [5]; extended microbiome characterization by shotgun sequencing analysis on particulate matter extracts [6] can provide insight on environmental factors governing the spread of airborne diseases [7] (as common influenza). Relevant aspect to be accounted for are also the molecular evidences of antibiotic resistance in bioaerosol [8]. During the research project, the student will operate in a multidisciplinary team of environmental chemists, genetists, microbiologists and biostatisticians, setting bioaerosol sampling and analysis protocols, performing experiments in aerosol chamber for the study of saline and organic PM with airborne microorganism to study coalescence and possible biological viability stabilization of aggregates, designing and implementing nodes of a monitoring network, and mining data derived from both chemical composition and DNA and RNA analyses, also in relation to seasonal disease dynamics.

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Project 3 for position M/6 CHIM/02

Time-resolved quantum approach to ultrafast spectroscopies: role of correlation and circular dichroism in molecular electron dynamics

Supervisor: Prof. Emanuele Coccia, Department of Chemical and Pharmaceutical Sciences, UniTS
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Recent impressive advances in laser technology are continuously triggering the introduction of new time-resolved spectroscopies which offer the opportunity to investigate electron dynamics with unprecedented time resolution. The optical response of a molecular system in intense ultrashort laser fields is a subject of increasing interest since the advent of attosecond (10^{-18} s) laser pulse generation, characterisation and application [1].

Attosecond pulses may be obtained via the nonlinear optical process high-harmonic generation (HHG) [2]. The many-electron dynamics implicated in the HHG process encodes structural and dynamical information of the spectroscopic target. Indeed, when the laser interacts with the system a non-stationary electronic wavepacket is generated. The wavepacket dynamics is strongly determined by parameters of the laser such as intensity, duration, polarization and phase of pulse frequency.

The proper treatment of the time-dependent electronic wavepacket, under the influence of the laser field, is obtained by solving the time-dependent Schrödinger equation (TDSE) [3].

The aim of the present Project is to develop and use TDSE in laser fields to study several effects encoded in the HHG spectrum and in the electron dynamics of molecules. In particular, we shall investigate:

i) the role of electron correlation among different ionisation channels during a strong-field dynamics generating HHG. This can be accomplished by an accurate analysis of the various components of the wavepacket;

ii) time-resolved circular dichroism when a chiral molecule is irradiated by a circularly polarized laser field, as experimentally shown for limonene [4];

iii) circular dichroism in chiral metallic structures, as coiled nanowires, in both linear and nonlinear optical response.

The computational approach will be based on a combination of electronic-structure simulations, at time-dependent density functional theory (taking advantage from the expertise of the group of Theoretical Chemistry [5]) and quantum-chemistry level, and a real-time propagation of TDSE [6]. A full quantum approach will be therefore employed. Points i) and ii) will require theory and code (Fortran90) development. A direct comparison with experiments will drive the proposed work.

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Project 4 for position M/6 CHIM/08

Graphene Quantum Dots: bio- and electrochemical applications

Supervisor: Prof. Tatiana Da Ros, Department of Chemical and Pharmaceutical Sciences, UniTS
email: daros@units.it

Graphene is a one-atom thick two-dimensional material and the studies on its excellent conductivity and optical properties boosted the research activities in this field and brought forward novel applications for graphene and its derivatives.[1]
In recent years, very promising derivatives of graphene, *i.e.* Graphene Quantum Dots (GQDs), have gained significant interest due to the potential for biomedical and electrochemical applications. GQDs are zero-dimensional small fragments of graphene in the size range.[2] Intrinsically, graphene is a zero-energy band-gap material but band-gap can be opened by size reduction and introduction of defects into the graphene moiety, and this leads strong photoluminescence that normally is not present in semi-conducting graphene.[3] So GQDs basically combine the structure of graphene with the quantum confinement and edge effects of QDs and possess unique properties, which are important for the applications in medicine, electronic, photoluminescence, electrochemical and electrochemiluminescence.[4] GQDs generally consist of 1 - 3 layers of graphene flakes with the diameter of less than 20 nm. Their surface groups of GQDs may vary due to the synthetic methodology, and photoluminescence may change depending on the surface functionalization.
The different processes to obtain GQDs may confer different properties to the materials.
GQDs have potential applications in biomedical,[5] optoelectronic, and energy-related fields especially thanks to their properties such as water solubility and luminescence, which make them excellent candidates for bioimaging and drug delivery.
However, there is the necessity to find appropriate methodology to tailor the size and the properties of the materials, both by optimizing their preparation and/or their functionalization. In this respect new approaches will be explored and will be devoted to ameliorate the preparation of new GQDs derivatives, especially in combination with organic dyes to be used for biological purposes and in electrochromic devices.

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Project 5 for position M/6 CHIM/03

Crossing the membrane: a structural and functional study of a protein involved in lipid translocation

Supervisor: Prof. Rita De Zorzi, Department of Chemical and Pharmaceutical Sciences, UniTS
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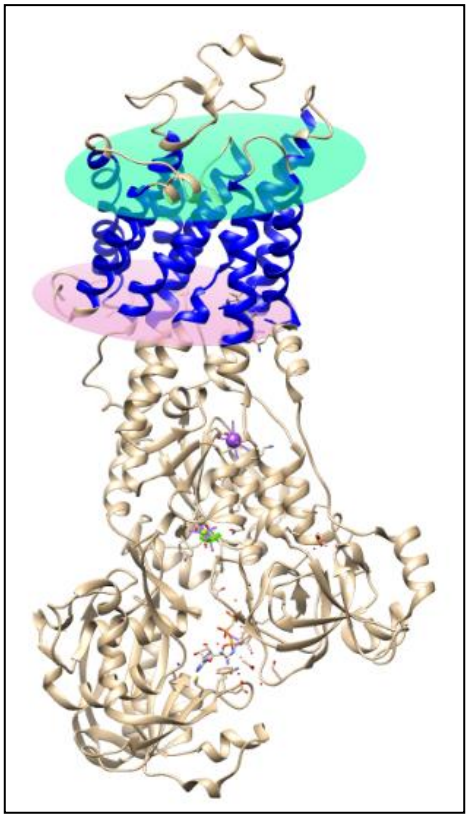

In biological systems, vesicle trafficking is crucial for material exchange between the cell and the external environment and between different cell compartments. Vesicle formation relies on a change in membrane curvature that contributes to recruiting coating proteins on the membrane surface. The asymmetry of lipid and protein composition across the membrane leaflets is a fundamental aspect of all biological membranes and is involved in the change of membrane curvature. While the spontaneous diffusion of lipids between the leaflets would lead to a symmetrization of the membrane, cells employ specific proteins to actively transport lipids from one side of biological membranes to the other. The role of these proteins in determining membrane curvature and, ultimately, in controlling vesicle formation makes them a crucial component whose mutations induce a wide range of serious diseases, from hepatic cholestasis to diabetes, cancer, and Alzheimer's disease.

P4-ATPases are integral membrane proteins responsible for the translocation of phospholipids from the external to the internal leaflet of cell membranes, thus maintaining lipid asymmetry. The overall structure of P4-ATPases is conserved among P-ATPases, a large class of proteins involved in active transport. However, specific features of the P4-ATPases determine the recognition and translocation of their large, amphipathic substrates. Recently, two pivotal studies characterized a human and a yeast P-IV ATPases using cryo-Electron Microscopy (cryo-EM) on single particles [1-2], contributing to our understanding of mechanisms involved in lipid translocation.

Our laboratory has successfully expressed and purified a yeast P-IV ATPase that shows unique features compared to its cognates. Preliminary results show that the protein is stable in the conditions used for purification and negative staining Electron Microscopy images of this sample open the way to a thorough structural study by cryo-EM.

The Ph.D. student involved in this project will collect Electron Microscopy data in collaboration with Prof. Venien-Bryan of the University Sorbonne (Paris, France) and analyze the data to elucidate the structural details of Neo1, an atypical P4-ATPase. Also, mutagenesis will be used to identify residues involved in substrate specificity and lipid transport [3].

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Project 6 for position M/6 CHIM/08

Computational design of theragnostic nanobodies

Supervisor: Prof. Sara Fortuna, Department of Chemical and Pharmaceutical Sciences, UniTS
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Goal of this project is to push forward the boundaries of targeted medicine by providing an alternative method for selecting binders with features difficult to obtain by conventional approaches based on immunization and antibody *in vivo* maturation.

Specifically, this PhD project will require the computational design of a set of ex-novo designed VHHs specifically engineered to be selective towards relevant protein biomarkers and pharmaceutical targets. VHHs are the smallest antibody fragments which still preserve the binding capacity of whole antibodies[1]. VHHs are easy to engineer, and already widely used in the development of diagnostic and therapy reagents (in 2019 the first therapeutic VHH, blocking platelet aggregation, was approved by FDA[2]).

The activity will be carried out in collaboration with Nikola Minovski (National Institute of Chemistry, Slovenia) and Miguel Soler (Italian Institute of Technology, Genova). The project will involve the use of homology modelling, docking, molecular dynamics (MD) and Monte Carlo based methods to introduce and develop novel strategies for the design and optimisation of VHHs (Fig. 1). The ***in silico* optimization** will be carried out with an artificial evolutionary algorithm [3] [4]. The **computational screening** for the identification of stable VHH candidates will take advantage of novel computational protocols developed by our team [5]. Both protocols are based on state-of-the art computational techniques to predict protein-protein interactions, and full-solvent MD simulations coupled with atomistic force fields for an accurate sampling of their conformations. The VHHs will be **characterized** with the same methods to ease the interpretation of the experimental results. Computational predictions will be validated by the collaborators of our team.

Fig. 1 Our pipeline for the in silico design of specific VHHs allows to design and screen, completely in silico, candidate binders then expressed in bacterial cell cultures.

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Project 7 for position M/6 CHIM/06

Natural products and their Semisynthetic derivatives for Animal Health

Supervisor: Prof. Cristina Forzato, Department of Chemical and Pharmaceutical Sciences, UniTS
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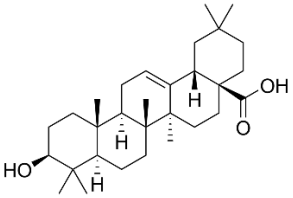
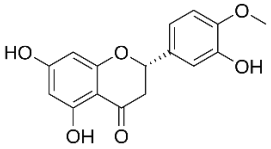
The project will focus on **helminthiases**,[1] a condition regarding animal health, since it is spreading among the EU territory and it is connected with the climate change and the diffusion of vectors.

Helminthiases are treated with three broad-spectrum drugs, the only reliable way to control them in livestock, but resistance is spreading due to erroneous use and/or abuse, greatly impairing animal health and welfare.[2] This is the case of Gastrointestinal nematodes (GINs) affecting sheep and horses. Small strongyles (cyathostomes) are the most important helminth parasites of horses, causing often life-threatening intestinal syndromes. Several anthelmintic have a negative impact on the environment and/or high toxicity, and their use may cause relevant loss. Few anthelmintic are fully metabolized in inactive molecules, and the suspension times, even when respected, may be insufficient as they would need more time to be eliminated. Their broad spectrum has enhanced also the risk to damage the ecosystem. Hence, the control of sheep and equine GINs has become problematical, and climate change could represent a driving force for soil-transmitted helminths. Strong efforts are essential to discover new compounds for preserving animal welfare and reducing pollution.

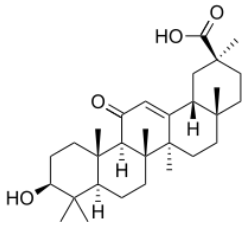
To overcome these problems, in the present project new anthelmintic products will be developed starting from natural sources such as *Cornus mas* (cornelian cherry) and *Hippophae rhamnoides* (sea buckthorn) which are plants commonly present in Europe. Semi-synthetic derivatives of commercially available natural products will be also synthetized to obtain new compounds with higher anthelmintic activity. Triterpenes such as oleanolic acid, ursolic acid and glycyrrethinic acid, which are present in agricultural waste products, will be evaluated while among phenolic compounds hesperetin and cinnamic acids will be considered. Natural products as well as semisynthetic derivatives will be tested at the University of Pisa and the University of Padova against the main helminths affecting horses and sheep, including parasite populations previously detected as resistant to current drugs.



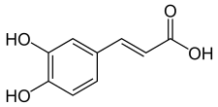
Oleanolic acid



Hesperetin



Glycyrrethinic acid



Caffeic acid

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Project 8 for position M/6 CHIM/06

Machine learning at the service of rational design and synthesis of biodegradable polymers

Supervisor: Prof. Lucia Gardossi, Department of Chemical and Pharmaceutical Sciences, UniTS
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Digitalization, artificial intelligence and machine learning are suitable instruments for supporting chemistry in the eco-design of new benign chemicals enabling circularity. Such interdisciplinary strategies can respond to the urgency of mitigating the environmental impact of plastics, fostering the synthesis of new bio-based monomers and polymers deriving from renewable resources.[1] Research aiming at developing the next generation of polyesters and polyamides must address not only sustainability but also pursue competitiveness in terms of superior technological and, on that respect, biocatalysis enables the synthesis of structured, functionalized and biodegradable polyesters through highly selective and benign synthetic processes.[2] The present project aims at implementing material's circularity through eco-design, biodegradation and upcyling of polymers deriving from renewable feedstock. The circular approach will be optimized with the aid of computational methods (e.g. molecular modelling, bioinformatics)[3,4] integrated within the frame of machine learnining algortithms.[5] The candidate will acquire both experimental (biocatalysis, polymer chemistry) and computational expertise, during a three year project based on multidisciplinary activities. The objectives of the thesis will be implemented in collaboration with international academic institutions (the group is currently involved in three on-going European projects). Moreover, some international leading companies in the fields of artificial intelligence and bio-based polymers will contribute to the project. The major objectives of the project will be the following: a) in silico screening of enzymes and mutants able to hydrolyze polyesters and polyamides; b) in silico rational design of new biobased poly-esters and –amides endowed with advanced technological properties (e.g. highly hydrophylic) prone to be biodegraded selectively; c) chemo-enzymatic synthesis of the designed polymers and experimental validation of their properties (first generation polymers) to collect data necessary for the construction of scoring functions; d) integration of computational models (i.e. polymer structures and enzymes) within machine learning algorithms for multi-objective optimization; e) validation of the authomatic work-flows for the in silico design of polymers prone to be biodegraded by selected enzymes. (synthesis of second generation polymers).

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Project 9 for position M/6 CHIM/03

Functional supramolecular polymers for self-diagnostic composites

Supervisor: Prof. Silvano Geremia, Department of Chemical and Pharmaceutical Sciences, UniTS
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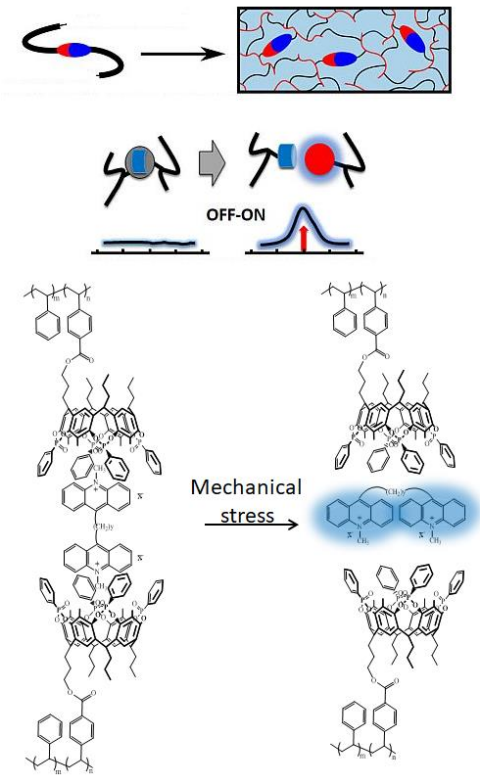
This financed PRIN-2017 (Progetti di Ricerca di rilevante Interesse Nazionale) project aims at introducing self-diagnostic properties into polymers and carbon fiber reinforced composites. The challenge is to produce fluorescence signals directly linked to the stress-driven breaking of the weak bonds in host-guest complexes, leading to the visualization of emerging mechanical stress in the polymer matrix of the composite. The ultimate goal is to provide enabling technologies to transform polymers into smart materials, to meet the rising demand of safety-related, non-destructive tests in structural composites. Molecular recognition has been chosen as an operating tool in the form of supramolecular cross-linking among complementary host-guest units embedded in the polymeric chains. The guests are selected luminophores, which are quenched in the complex and emitting in the free form. The hosts are calixarenes, cavitands and cucurbiturils, whose molecular recognition properties are well defined and predictable. The implementation of the project requires: specific, resilient and stimuli responsive host-guest systems; their insertion into polymers as weak cross-linking units; a molecular level understanding of number, distribution and connectivity of the host-guest interactions in the polymer matrix; testing protocols for the resulting self-diagnostic properties. The team is composed of 5 Research units: University of Parma (E. Dalcanale), University of Bologna (C. Gualandi), University of Messina (G. Gattuso), University of Pisa (A. Pucci) and University of Trieste (S. Geremia). The structural characterization of number, distribution and density of host-guest crosslinking in self-diagnostic polymers is essential for a precise structural description of the system with predictive value. Small scale oligomers of the desired polymeric systems with narrow molecular weight distribution will be prepared to facilitate the crystallization. The micro-scale crystallization experiments will be performed at Trieste on a small scale (0.1 microliter) to reduce the material demand and to permit many variations of conditions. Crystals of supramolecular polymers have generally small dimensions and the use of synchrotron radiation is mandatory to obtain diffraction data at atomic resolution. In the absence of single crystals, synchrotron powder X-ray diffraction data and Rietveld structure refinements will be used to examine the 3D arrangement. Small-angle X-ray scattering (SAXS) and Wide-angle X-ray scattering (WAXS) measurements *using synchrotron* radiation will also be performed to measure the electrospun nanofibers.



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Project 10 for position M/6 CHIM/03

Novel functional interpretations of Sn^{IV}-porphyrin metal scaffolds.

Supervisor: Prof. Elisabetta Iengo, Department of Chemical and Pharmaceutical Sciences, UniTS
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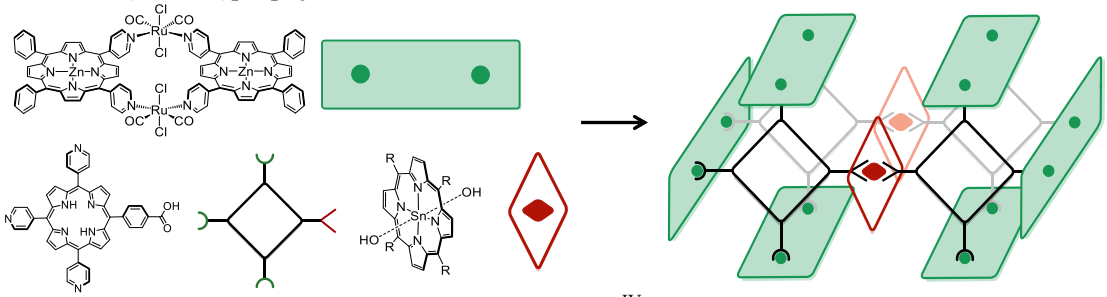
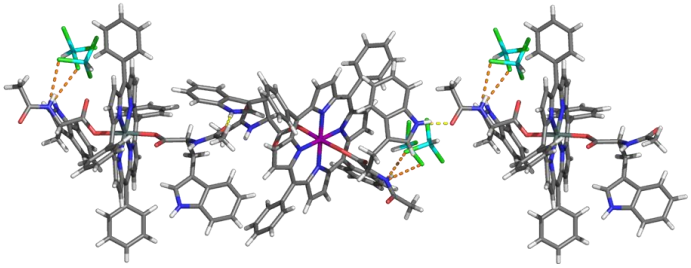
The properties and structural characteristics of Sn^{IV}-porphyrin – planarity, six-coordination, robust binding to oxyanions, tin NMR active nucleous, tunable opto-electronic features (i.e. absorption in the visible region, luminescence, and ease of reduction) – make them intriguing metal scaffolds for the construction of light-responsive supramolecular assemblies or materials, and attracted our interest.[1] We recently survied and established their possible use, in combination with Zn^{II}-porphyin metallacycles and *meso*-pyridyl/benzoic-porphyrins, for the metal-mediated assembling of 3D discrete hollow supramolecular structures, featuring different kinds of (metallo)porphyrin elements.[2]



In parallel, we initiated a fruitful investigation on Sn^{IV}-porphyrin/amino acids conjugates as novel biomimetic candidates for photoinduced proton-coupled electron-transfer (PCET).[3-5] For instance, Sn^{IV}(L-tyrosinato)$_2$-porphyrin and Sn^{IV}(L-tryptophanato)$_2$-porphyrin were found to generate a radical pair state, by visible light excitation and in the presence of pyrrolidine. The single crystal X-ray structures of both systems evidence ordered patterns of intermolecular H-bonds. Notably, reports on the preparation and characterization of tin-porphyrin derivatives featuring chiral aa as axial ligands are extremely limited, and their intriguing use as synthons in the supramolecular realm is yet to come.



More in particular, the project will address: i) preparation of tin-porphyrin/amino acids conjugates for the achievement of long-lived charge separation by Proton-Coupled-Electron-Transfer; ii) desing and assembling of multi-porphyrin 3D multiporphyrin containers for molecular recognition. The combinatorial flexibility granted by the metald-mediated approach should promote the obtainment of a common library of Sn^{IV}-porphyrin metal scaffolds. Inorganic, organic and supramolecular synthetic methodologies will be employed alongside a variety of characterization techniques (in solution: ESI-MS spectrometry, multinuclear advanced NMR, UV-vis, emission and CD spectroscopies; in the solid state: single crystal X-ray diffraction by means of the local ELETTRA synchrotron light source). Photochemical and receptor properties of selected assemblies will be addressed by additonal techniques, also in collaboration with other research groups. A six month stay abroad will be strongly recommended, in order to expand and differentiate the PhD fellow skills, research methodologies, as well as working and social environments.

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Project 11 for position M/6 CHIM/08

Selective inhibition of protein kinase CK1δ

Supervisor: Prof. Teresa Gianferrara, Department of Chemical and Pharmaceutical Sciences, UniTS, email: gianfer@units.it

The casein kinase CK1 is a class of monomeric, constitutively active Ser/Thr-protein kinases which includes in humans seven different isoforms, CK1α, CK1γ1, CK1γ2, CK1γ3, CK1δ, CK1ε and the less well described α-like, that share high homology in their kinase domains (53–98%).[1] Alterations of CK1 homoeostasis have been related to several neurodegenerative diseases,[2] and several studies outlined that CK1δ is involved in the pathogenesis of Alzheimer's and Parkinson's diseaeses, amyotrophic lateral sclerosis (ALS), sleep disorders, as well as playing an important role in cancer development and metastasis spreading.[3] Thus, CK1δ has emerged as an important drug target for therapeutic applications. The development of an inhibitor selective only towards this isoform is the biggest challenge of the research, since the isoforms are highly conserved, especially in their catalityc domain and, in addition, they exist as different splice variants mediating different activities but structurally very similar. The most interesting inhibitors developed as pharmacological tool and having the desired CK1δ isoform-specific inhibition profile are based on the pyrazolopyrimidine moiety, adenine scaffold or are benzothiazolyl-phenyl acetamide derivatives.[4] Very recently, also [1,2,4]triazolo[1,5-*c*]pyrimidines (TP) and [1,2,4]triazolo[1,5-*a*][1,3,5]triazines (TT) derivatives have been identified as new promising compounds.[5] A preliminary structure-activity relationship (SAR) study by Grieco et al. highlighted that TP and TT have superimposable structure and SAR.[5] Substitutions on all positions have been investigated: 2,5,7 and 8 for the TP scaffold and 2,5, and 7 for the TT one. SAR for these compounds revealed that a free amino group is required at the 5 position of the TP (and at the correspondent position 7 in the TT ring), while an opportunely substituted phenyl ring at the 2 position can tune kinase inhibitory activity. In particular, the insertion of hydroxy groups at the 3 and 5 positions gave optimal interactions in the catalytic binding cleft. The most potent compounds of the two series are reported in Figure 1. Docking studies performed using one of the available crystallographic structures of CK1δ (PDB ID: 4HNF) highlighted possible interactions with key residues of these compounds in the ATP binding pocket of kinase,[5] thus suggesting that a more potent inhibition could be obtained by a fine optimization of the structure.

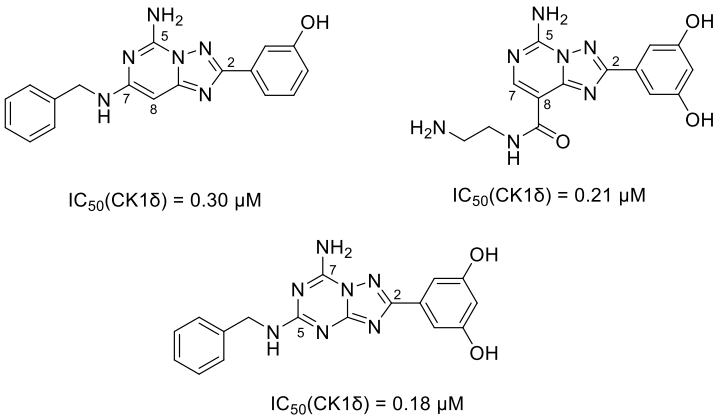

Figure 1.

In this project, new series of TP and TT compounds will be developed, investigating especially the 8 and 5 positions of the TP and TT scaffold, respectively. Furthermore, small differences in the catalytic pocket of the highly homologues isoforms δ and ε (*i.e.* residue 55, Ile *vs* Phe)[6] could be exploited to tune the selectivity for CK1δ, with the aid of computational studies performed in collaboration with Prof. Stefano Moro (Unversity of Padua).

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Project 12 for position M/6 ING-IND/24

Rheological and Low Field NMR characterization of biomedical gels

Supervisor: Prof. Mario Grassi, Department of Engineering and Architecture, UniTS, email: mario.grassi@dia.units.it

Typically, gels are constituted by a three-dimensional (polymeric) network able to entrap a large amount of liquid (usually water) that cannot induce network solubilisation thanks to the presence of chemical or physical junction zones, named crosslinks, among polymeric chains. Interestingly, despite the huge volume fraction of water hosted in the network (up to 99.5%), the mechanical properties of hydrogels are more similar to those of solids rather than those of liquids. This is the reason why they mechanically resemble natural living tissues much better than any other type of synthetic biomaterials. In the last years, hydrogels have been used in numerous applications, such as tissue engineering, regenerative medicine, scaffolds for cell growth and proliferation and drug delivery [1].

This project is focussed on the characterisation of two particular hydrogel kinds, namely the sputum of patients affected by Chronic Obstructive Pulmonary Disease (*COPD*) such as cystic fibrosis (*CF*), and hydrogels used as substrate for cells growth. Indeed, *COPD* implies the production of viscous mucoid secretions in the airways due to the pathological increase of proteins, mucin and biological polymers that give origin to a three-dimensional polymeric network pervading the whole mucus and impairing the muco-ciliary clearance that, in turn, promotes inflammation and bacterial infection [2, 3]. In order to monitor the clinical conditions of the patients and to improve the efficacy of the drugs usually used in therapy (such as, mucolytics, anti-inflammatory and antibiotics), it is of paramount importance knowing the nano-structure of the sputum network. Indeed, it is well-known that drug diffusivity inside a gel-like system depends on the mesh size distribution of the network. Previous researches [2, 3] confirm that the synergic combination of rheology and low field NMR (0.47 T) is very helpful at this purpose.

It is well known that the mechanical properties of gels substrate can influence the response of cells [4]. For example, the adhesion and morphology of chondrocytes is dependent on the stiffness of the alginate gels and the proliferation, apoptosis, and differentiation of pre-osteoblasts depends on alginate gel stiffness. In addition, the differentiation of fibroblasts to myofibroblasts requires a stiff matrix (Young modulus > 20 kPa) and cell respond to the mechanical properties of the extracellular matrix. Polyacrylamide gels with varying stiffness showed that freshly isolated rat Hepatic Stem Cells (HSC) on stiff supports (12 kPa) acquire a myofibroblastic phenotype while they remain quiescent on soft (0.4 kPa) supports. This range corresponds, respectively, to the stiffness of a normal rat liver (0.3–0.6 kPa) and a cirrhotic rat liver (3–12 kPa). As rheology is devoted to study the relation between stress and deformation, it is clear that it can be extremely useful in determining the mechanical properties of gels that are complex systems showing both viscous and elastic properties. The combination with Low Field NMR can give the opportunity to explain the macroscopic gels properties in the light of their nanostructure.

This project will be performed in collaboration with prof. Gabriele Grassi (DSV, UNITS), prof. Marco Confalonieri (Head of Pneumology – Cattinara Hospital TS), Dr. Massimo Maschio (IRCCS Ospedale infantile Burlo Garofolo, TS), Dr. Gianni Morana (Ospedale Ca' Foncello, TV), prof. Pierluigi Ciet (Erasmus MC Hospital, Rotterdam) and prof. Hai Nhung Truong (Laboratory of Stem cell Research and Application, University of Science, Ho Chi Minh City, Viet Nam).

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Project 13 for position M/6 CHIM/06

Modular synthesis of doped nanographenes

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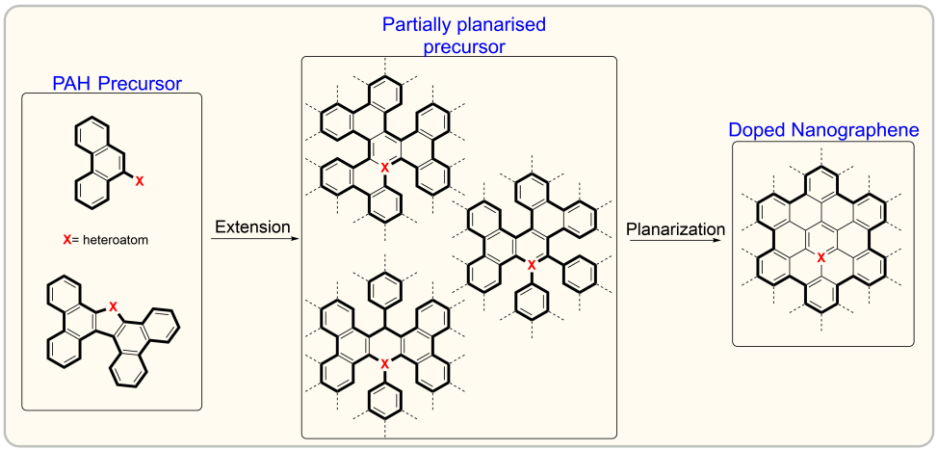
This project aims at the design of new synthetic strategies exploiting polyaromatic building blocks resulting in precisely doped nanographenes with a wide range of potential applications from light emitting devices to catalysis.

The synthesis of precisely doped nanographene molecules is one of the most attractive topics in organic chemistry and can help to unravel important theoretical aspects connected to crucial properties of materials. Consequently, many different approaches have been developed to prepare nanographenes presenting precise size, edge shape, and dopant atoms.[1,2] The introduction of heteroatoms in an aromatic scaffold has proved to be highly successful in tailoring the material properties resulting in enhanced emissions, hole/electron mobility, self assembly, and stability.[3] For these reasons, in our group we are particularly interested in developing new synthetic pathways able to generate extended nanographene molecules efficiently. In particular, we are currently investigating the synthesis of nitrogen doped nanographenes along with the potential use of extended dibenzofurans as versatile building blocks for the preparation of extended doped polyaromatics.



Scheme 1. Synthetic strategy for extension and planarization of PAH building blocks generating precisely doped nanographenes.

With great potential for future development, this project represents a great opportunity to extend the student knowledge in different sectors of organic chemistry. The designed nanographenes will be characterized by extensive use of nuclear magnetic resonance (NMR) and the opto electronic properties will be investigated by UV-Vis spectroscopy, cyclic voltammetry and more specific techniques depending on target applications.

As a result, the PhD student will become expert in the synthesis and characterization of polyaromatics, developing new valuable synthetic skills.

The multidisciplinarity of our group will be a unique chance of interacting with different fields of chemistry and nanomaterials, generating valuable opportunities for collaborations and training in different instruments and techniques. All together this will allow the PhD student to grow as an independent scientist able to contribute with ideas and important skills to the group, fostering the progression of his/her career.

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Project 14 for position M/6 CHIM/06

Artificial Ionophores

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Artificial ionophores are synthetic molecules able to promote the transport of ions and/or polar molecules across a biological membrane thus mimicking the action of natural occurring ion channels and carriers. The reaserach in this field is aimed to get insight on the molecular basis of recognition and transport and on the exploitation of this properties to control biomedical relevant processes. For example, several genetic diseases, the most known being cystic fibrosis, involve chloride channel impairments and current therapeutic leads comprise artificial ionophores able to restore the chloride transport process [1].

Ion transport across phospholipid membrane is a typical supramolecular function involving dynamic recognition of the substrate during the whole translocation process. Therefore, the design of artificial ionophores requires a careful balance of several factors from binding affinity to lipophilicity. We have been involved for some time in the design of artificial ionophores developing amphipathic molecules based on steroid, calixarene, porphyrin and other organic scaffolds [2]. More recently we have started a research program aimed to investigate the ability of metal complexes, in particular Pd(II) complexes (Figure 1), to act as molecular carrier of chloride and other biological relevant anions [3]. Within the project the candidate will identify and synthesize new ionophores and will study their ionophoric activity on model membranes with particular regard to the definition of the structure/activity correlation in order to investigate the mechanism of action and optimize the carrier efficiency. The best found ionophores will be tested for biological activity in a collaborative work.

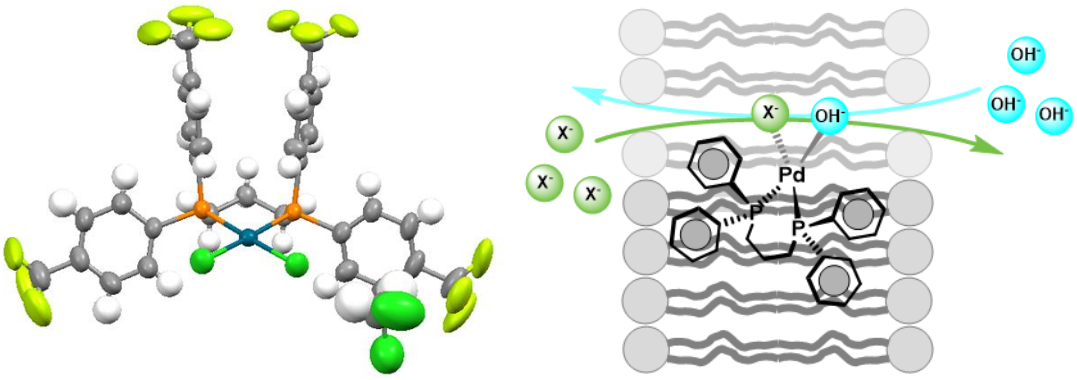

Figure 1: Left: X-Ray structure of a diphosphine-Pd(II) complex acting as chloride carrier across phospholipid membranes; Right: mechanism of ion exchange promoted by the Pd(II) complex.

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Project 15 for position M/6 CHIM/08

Synthesis of new σR and GluN2b modulators as neuroprotective agents

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Sigma receptor (σR) are a class of non-opiod receptor that binds diverse classes of psychotropic drugs and are subdivided into two subtypes named sigma-1 (σ1R) and sigma-2 (σ2R). These receptors are widely distributed and both subtypes are involved in several pathologies. The σ1Rs are involved in neuroprotective and antiamnesic activity [1], modulation of opioid analgesia [2] and drug addiction [3]. Similarly, σ1 antagonisst seem to be effective against the negative manifestations of schizophrenia, without producing extrapyramidal side effects [4,5]. The σ2R subtype has been recently purified and identified as transmembrane protein-97 (TMEM97) [6] and are overexpressed in many tumours. On the other hand, the N-methyl-D-aspartate receptors (NMDARs) are glutamate-responsive ion-channel receptors and likewise, for σRs, the NMDARs play key roles in synaptic transmission, synaptic plasticity, neuronal development, learning, memory and other physiological and pathological processes [7, 8]. Hence, antagonists of NMDAR (and, in particular, of the GluN2 subunits) are of interest as potential neuroprotective drugs [9] to treat several CNS disorders. After several years of dealing with σR ligands, our aim is to continuing with the discovery of new chemical entities gifted with a pan-affinity towards σ1/GluN2b receptors in order to develop new neuroprotective drugs useful for several neurodegenerative disorders aforementioned. Furthermore, the crystallographic structures of both receptors, σ1 and GluN2b are known, therefore our intention is to develop a new pharmacophore model for the GluN2b subunit, being its known ligands (i.e. ifenprodil) gifted with low selectivity for this receptor. Finally, a recent *in vivo* mechanical allodynia assay developed by our coworkers [10], will be used to define the antagonism profile of the new synthesized compounds.

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