University of Trieste
Department of Chemical and Pharmaceutical Sciences

Doctorate in Chemistry

2020

Research Projects

Project for position MD/1 CHIM/06

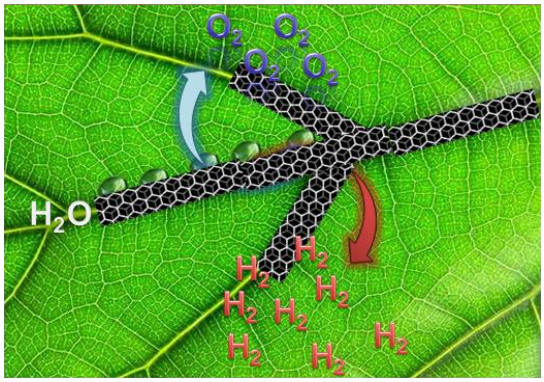
Organic Chemistry for Multiredox Catalysis

Supervisor: Prof. Maurizio Prato Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: prato@units.it

This project aims at the synthesis of hybrid nanosystems that can perform relevant redox catalytic processes. Target applications include water splitting and CO_2 reduction.

Energy- and sustainability-related research plays a major role in shaping our future. Ideally, it would be desirable to harvest clean energy, e.g. solar, and be able to valorize waste products, such as CO_2. Our group has longstanding expertise in the use of organic chemistry and carbon nanomaterials to address these societal challenges.[1] Our philosophy is to employ organic synthesis to engineer hybrid nanostructures that address specific needs. In the coming years, we plan to advance the state of the art concerning water splitting and carbon dioxide reduction.

For water splitting, we have demonstrated the feasibility of combining light-harvesting chromophores with oxygen evolution catalysts, such as polyoxometalates.[1b] The next step will be to engineer tridimensional networks that optimize charge transport and water accessibility, towards the development of an artificial "off-leaf" transposition of photosynthesis.



enable light harvesting and photoredox catalysis. Examples include water splitting and

On the other hand, we are eager to employ carbon nanodots and other carbon nanostructures such as carbon nanohorns for the photochemical reduction of CO_2 and the electrochemical production of H_2O_2.[3] Critical for the successful outcome of these projects will be the chemical functionalization nanomaterials. Overall, the PhD student will be engaged in a variety of topics having the common underlying principle of using organic chemistry as a tool to tackle challenges central to our society.

The PhD student will design synthesize, characterize, and test the target systems. The obtained materials will be analyzed with state-of-the-art spectroscopic methods, including nuclear magnetic resonance (NMR), optical spectroscopies, atomic force and transition electron microscopies (AFM, TEM), and infrared spectroscopy (IR). Our group has also a strong track record of fruitful collaborations, that involve also the use of X-ray facilities at the synchrotron (Trieste) or CIC biomaGUNE (San Sebastian, Spain). The performances of the materials will be tested in the framework of established collaborations. On top of this, for the optimal development of collaborative projects, it is likely for PhD students to perform a research stay abroad.

Our group is a lively cluster committed to interdisciplinarity and the student will be exposed also to several aspects of carbon nanotechnology, that represent the core expertise of the group. Typically, the PhD work starts from an ongoing project. Then, the individual interests and attitudes of the student come into play and shape the development of his path into research.

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[3] (a) "Design, synthesis, and functionalization strategies of tailored carbon nanodots", F. Arcudi, L. Đorđević, M. Prato, *Acc. Chem. Res.* **2019**, 52, 2070; (b) "The Rise of Hydrogen Peroxide as the Main Product by Metal-Free Catalysis in Oxygen Reductions", M. Melchionna, P. Fornasiero, M. Prato, *Adv. Mater*. **2019**, 31, 1802920.

Project for position MD/2 CHIM/06

Dynamic supramolecular systems with *time-control* for smart applications

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Life is composed of supramolecular systems that are dynamic and adaptive to the environment.[1] Living systems evolve over time to allow life processes to occur. In particular, proteins exert the most fascinating roles, being able to control 1) cellular movement (through the cytoskeleton); 2) molecular cargo entry into, and exit out of, cells (through membrane channels and receptors); 3) biochemical pathways for the synthesis of bioactive molecules (through enzymatic cascades), and so on. However, proteins display a structural complexity that risks denaturation, with subsequent loss of function, upon change of experimental conditions.

These shortcomings could be overcome by using instead *minimalistic peptides* made of just a few amino acids that share great chemical diversity just as the building blocks of proteins. Upon design, they can self-organize hierarchically into systems that span from the nano- to the macroscopic scale – thus forming nanostructured materials that we can see by eye.[2] Importantly, they are more robust than proteins, and can mimic enzymatic activity when assembled into a supramolecular structure, similarly to a folded protein with hydrophobic pockets for reactions to occur.[2b] The function can be switched on/off with assembly/disassembly, to give as waste simply water and biodegradable molecules that are environmentally-friendly. These findings open thus the way to smart systems that can change over time, and that could be coupled to others by means of orthogonal chemistry for advanced functions, such as selective chemical separation from a mixture.[3]

Inclusion of amino acids with different functional groups can be exploited for a variety of chemical modifications, such as metal coordination, redox reactions, phosphorylation, and so on, potentially also in a reversible manner. These processes could in fact act as triggers to introduce changes in the system and make it *adaptive* to experimental conditions. This ability, coupled to chemical reactions that yield metastable products, opens the way to the design of complex, multifunctional systems that can evolve over time and adapt to the environment. For instance, fine control over kinetics can yield systems with time-control,[4] *i.e.*, at the desired time they self-organize into a functional material of *controlled lifetime*, and then disassemble into either the starting material (to allow for repetitive cycles), or into a different product (that could even trigger another reaction). In other words, multi-component supramolecular systems will be designed to achieve a *"reaction-clock"* (Fig. 1) to recapitulate fine levels of complexity displayed by elements that compose living systems.

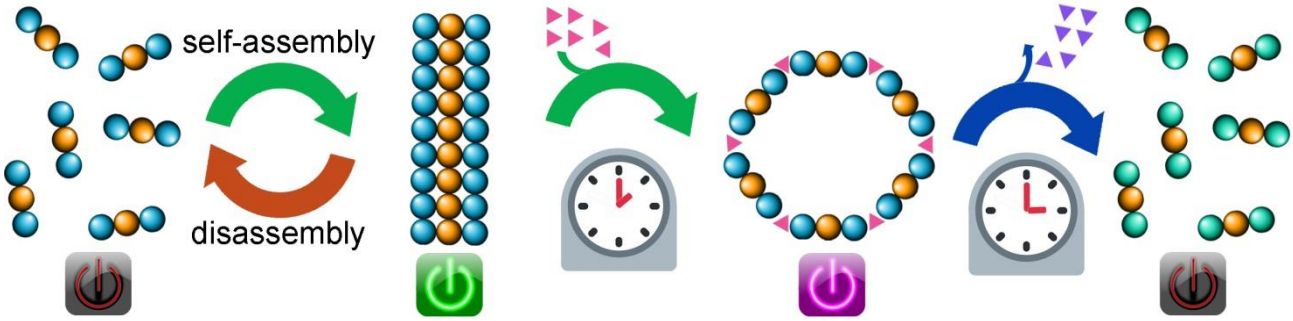

Fig. 1. Self-assembly of tripeptides can be a reversible process (*left*).[2] Addition of a chemical reactant (*pink triangle*) can lead to structural a rearrangement to change the function of the system (*pink switch*). If the latter is a metastable product, its conversion into a final product will switch off the system. Kinetics control the *"reaction-clock"*.

References:

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Project for position MD/3 CERIC@ELETTRA

Structural and functional analysis of helicases involved in genome maintenance

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Co-supervisors:

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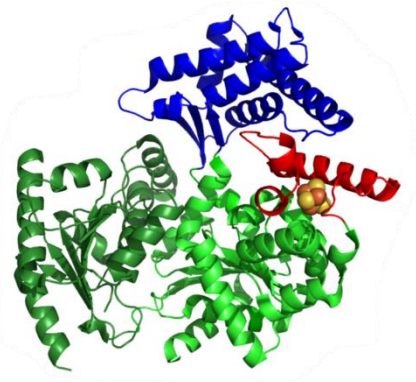
Rita De Zorzi, Department of Chemical and Pharmaceutical Sciences, University of Trieste, rdezorzi@units.it

Helicases are ubiquitous enzymes required in DNA replication, repair, recombination, chromatin maintenance and genome homeostasis, and are essential players in preserving the integrity and stability of the genetic information in all living organisms. Due to their importance, they are often involved in *genetic diseases* and *cancer development*. We are actively working on a subset of human helicases with a specificity towards unusual nucleic acid structures, such as triple helices, Holliday junctions, D- and R-loops and G-quadruplexes. These include the RecQ4 helicase and helicases belonging to the FeS helicase family (characterized by the presence of iron-sulfur clusters), including DDX11, RTEL1 and FANCJ, all of which have important medical implications in genetic diseases and cancer development.



We aim to study the *structural aspects of some of these helicases*, exploiting a combination of macromolecular crystallography and cryo-electron microscopy. Structural studies will be coupled to biochemistry and functional/cellular analysis, to understand the exact role of these proteins within cells. Due to the intrinsic uncertainty of the likelihood of obtaining well-diffracting crystals and/or good micrographs for single particle cryoEM analyses, we choose to start with multiple proteins, with the aim of focussing on a single helicase during the course of the PhD project, depending on the initial results.

The project will be based in the laboratory of S. Onesti (http://www.elettra.trieste.it/labs/structural-biology), who has a long-standing expertise in the structural and functional analysis of helicases, together with S. Glatt, who has worked on the structure of human RecQ4 and proteins containing FeS clusters, and with R. De Zorzi, who has expertises in both crystallography and cryoEM. The selected student will spend some of his time at the newly established cryoEM facility in Krakow; in the framework of the CERIC consortium, the project will also involve collaborations with the SAXS beamline (Elettra-Graz), the NMR centre in Ljubljana and the NanoInnovation laboratory at Elettra. There will be a strong synergy with existing projects in the lab on the structure and function of helicases:: in particular the student will benefit from the thriving training programme implemented by the MSCA AntiHelix project (https://www.elettra.eu/AntiHelix).

References:

Molecular and Cellular Functions of the Warsaw Breakage Syndrome DNA Helicase DDX11. Pisani F.M., Napolitano E., Napolitano L.M.R. and Onesti S. (2018). *Genes* 9, 564.

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Project for position MD/4 CERIC@ELETTRA

Integrated Structural Analysis of Human USPs, a Novel Family of Druggable Targets

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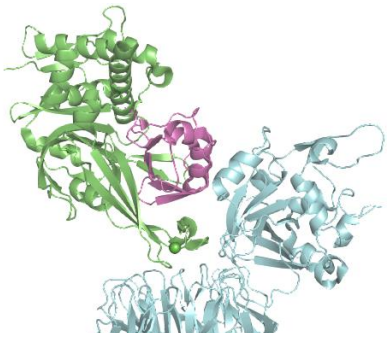
Co-Supervisor: Prof. Federico Berti Department of Chemical and Pharmaceutical Sciences, University of Trieste, email: fberti@units.it

The process of ubiquitination/deubiquitination is tightly regulated in the cell, and deubiquitynating enzymes (DUBs) which catalyse the removal of ubiquitin chains from substrate proteins, have the role of editing and correcting the ubiquitin code [1]. The human genome encodes 99 DUBs that are clustered in 7 DUBs families, of which the Ubiquitin-Specific Proteases family (USP) is the most represented one. USPs act in different cellular districts and are deregulated in life threathening diseases [2]. In cancer they play a dual role as tumour suppressors and oncogenes and have been proposed as valuable therapeutic targets. The scientific community and the pharma have grown interest in this protein family, starting various drug discovery initiatives. Despite the proven druggability of USPs, only few potent compounds have been developed so far. The slow development is also ascribed to the limited number of high-resolution structures available. Indeed, USPs are multidomain systems, with flexible regions and regulatory partners, that need to be studied by a combination of different techniques. This motivate a focalized effort to obtain 3D structures using an intergrated structural biology approach.

In the group of P.Storici at the Elettra Synchrotron (www.elettra.eu/PEOPLE/index.php?n=PaolaStorici.HomePage) we are studing a number of USPs involved in cancer development [3], and we are now interested in understanding the functional/structural mechanism of USP1 and the active USP1/UAF1 that has an important role in DNA repair mechanisms, and is involved in aplastic anemia, multiple myeloma, breast cancer and brain tumors [4].



USP12-UAF1-Ub complex (1L8W)

The PhD project aim will be to clarify the structural details of USP1 alone, of USP1/UAF1 complex and of some homologues in order to help the rational design of effective USPs inhibitors that can become promising drug candidates. The strategy will be to apply an integrated structural biology approach combining NMR, MX, SAXS and Cryo-EM tools, exploiting the partner facilities of the CERIC-ERIC Consortium (https://www.ceric-eric.eu) to reconstruct the 3D structure of the functional USP1/UAF1 active system to support the development of new inhibitors. The project is also in collaboration with the medicinal chemistry group of F. Berti from University of Trieste that will develop USP's small molecule inhibitors. The selected student will be based in the laboratory of P. Storici at Elettra but will also have the opportunity to spend some time at the CERIC partners facilities [i.e. XRD2 beam line (Elettra-Trieste, IT), SAXS beamline (Elettra-Trieste, IT; TU-Graz, AU), NMR centre (Ljubljana – SLO), Cryo-EM facility (Krakow, PL)] getting in contact with stimulating and multidisciplinary environments.

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1) Ann.Rev.Biochem. 81, 203-229; 2017; doi.org/10.1146/annurev-biochem-060310-170328
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Project for position MD/5 CRO Aviano

Development of novel therapeutic strategies with CAR-T cells by integrated chemical/biomedical approaches

Supervisor: Dott. Giuseppe Toffoli, CRO of Aviano, gtoffoli@cro.it
Co-supervisor: Dr.ssa Stephanie Federico, Department of Chemical and Pharmaceutical Sciences, University of Trieste: sfederico@units.it

The novel strategies for the development of efficient anti-cancer therapies require multidisciplinary approaches integrating chemical and biomedical strategies. The multisciplinary approach is mandatory with the novel cell therapies in cancer patients.
Adoptive cell therapies using chimeric antigen redirected (CAR)-T cells, represents one of the most promising immunotherapeutic approaches in cancerpatients. CAR-T cells have shownsignificant results for the treatment of hematological malignancies, and the use of CAR-T treatment in solid tumors appears to be promising. Several factors have a pivotal role in CAR-T cell therapy in solid tumors. They includethe identification of useful tumor associated antigens (TAA) to be employed as effective therapeutic targets and the identification of resistance biochemical mechanisms. These resistance mechanisms could depend both onthe antigen loss during the treatment of the tumor clone and onimmunosuppressive mechanisms due to the tumor/ tumor microenvironment/ immune system crosstalk.[1] In this context, several studies demonstrated a role of adenosine in favoring the tumor immune escape. For this reason, both adenosine receptor antagonists and ecto-5'-nucleotidase (CD73) inhibitors have been suggested as immune checkpoint inhibitors.[2]

At the Experimental and Clinical Pharmacology Unit of Aviano CRO, several studies are ongoing with the aim of developing novel therapeutic strategies employing CAR-T cells to target proteoglycans expressed on the cell surface of solid tumor cells. A key element in the context of the CAR-T design and development is represented by the capability of the single chain variable fragment of the CAR-T to efficiently bind the proteoglycan molecules on the tumor cell surface.[3] This single chain variable fragment is produced from the action of a relative antibody able to recognize a specific epitope of the proteoglycan. In this context, several experiments will be planned to bio-molecularly characterize the antibody/proteoglycan interaction. This characterization will encompass the affinity between antigen and receptor with analytical methodologies employing Surface Plasmon Resonance (SPR), as well as the biochemical modulation processes with the aim of ameliorating the selectivity of the single chain variable fragment to tumor cells and of limiting the onset of resistance mechanisms.

The multidisciplinary approach that connects chemical, pharmaceutical and biological aspects will define the set-up of innovative strategies to optimize the efficacy of CAR-T cells targeting proteoglycans on the surface of tumor cells. In particular, the project will investigate the biochemical features involved in specific immune-related mechanisms. Moreover, it will define molecules useful to enhance the cytotoxic activity of CAR-T cells. On this regard, part of the work will be the design, synthesis and study of new dual adenosine receptor/CD73 inhibitors, taking advantage of the expertise present at the Department of Chemical and Pharmaceutical Sciences at the University of Trieste.

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3. Dal Bo M. et al. *DrugResistanceUpdates*, 2020: 51, 100702.

Project for position M/6 CHIM/01

Analytical chemistry methodologies applied to ancient numismatic research

Supervisor: Prof. Gianpiero Adami, Department of Chemical and Pharmaceutical Sciences, University of Trieste
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Analytical chemistry involves many innovative fields of science and technology. In cultural heritage science, it includes studies using historical, conservative, chemical, biological and physical methods. The research approach can therefore be defined as interdisciplinary. Chemistry was first applied to the conservation field in the 18th century and current analytical chemistry plays a crucial role in the characterization of artistic and cultural heritage and in the support of archaeometric studies, identifying causes and mechanisms of degradation, developing and evaluating the performance of both materials and restoration methods [1].

Non-exhaustive examples of advanced analytical methods, essential to this field, are: electron microscopy, scanning probe microscopy, ion beam, mass spectrometry (MS), laser, X-ray (including synchrotron radiation, SR), electrochemistry, chromatography (both HPLC and GC). In addition, to minimize the number and quantity of samples collected, it is necessary to combine non-destructive techniques with micro-destructive ones [2].

Many of these techniques have been applied for the analysis of ancient coins, mainly for the determination of their chemical composition [3]. The analysis of late antique solidi (IV-V century A.D.) from gold coin hoards in the Balkan area is a clear example of the interdisciplinary nature of analytical chemistry, as it has allowed the collaboration of the research group of Prof. G. Adami with the Department of Humanities (University of Trieste), the *Elettra Sincrotrone Trieste* and the Department of Chemistry (University of Torino). Combining laboratory techniques (ED-μXRF, ICP-AES, ICP-MS) with SR techniques (ESCA Microscopy beamline and XRF beamline), it is possible to precisely evaluate the purity of gold, to quantify the presence of trace elements (i.e. indicating the geographical location of the deposits or the purification processes) and to distinguish the composition of the alloy from that of subsequent deposits due to erosive processes [4]. Moreover the study of patinas is often used for authentication as described in a recent work on bronze coins found in the area of Monte Cesén (Treviso, Italy) [5].

This doctorate project is based on the use of several analytical techniques on ancient coins and will shed light on: 1) the major metal content, linked to the socioeconomic situation at the time of coining; 2) the metal purification processes; 3) the presence of elements linked to external contaminations, revealing the fate of the coins during the centuries. An additional aim is to investigate and implement the multiple applications of analytical chemistry from the previously described studies. The PhD student will learn how to collect and analyze data and how to work in a multi-disciplinary team involving chemists, physicists, and historians.

References

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University of Trieste
Department of Chemical and Pharmaceutical Sciences

Doctorate in Chemistry

2020

Research projects (in alphabetical order)

for the free fellowship

Position M/7

Project 1 for position M/7 CHIM/03

Novel heteroleptic metallacycles of porphyrins for supramolecular architectures

Supervisor: Prof. Enzo Alessio, Department of Chemical and Pharmaceutical Sciences, UniTS
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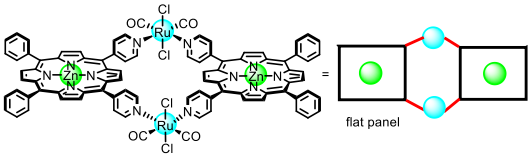
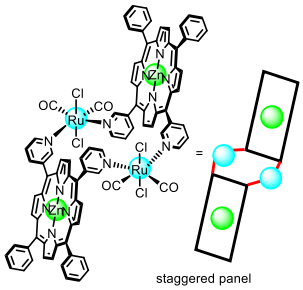
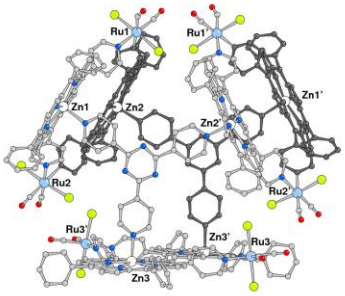
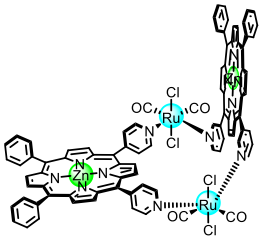
In the past we described the stepwise preparation and characterization of metal-mediated multiporphyrin 3D assemblies.[1] Aside from their structural beauty, often evidenced by spectacular X-ray structures, such assemblies find interest in host-guest chemistry, photocatalysis and – in particular – artificial photosynthesis. The first step was the preparation of homoleptic 2+2 metallacycles, in which two equal *cis*-dipyridylporphyrins (*cis*DPyP) are connected by two equal and symmetrical Ru(II) fragments. Depending on the nature of the porphyrin, the metallacycle is flat (4′-*cis*DPyP, left) or staggered (3′-*cis*DPyP, right). The insertion of Zn into the porphyrins transforms the metallacycles into *molecular panels* with two axial connecting points to be used for *higher order self-assembly*. For example the supramolecular prism, whose X-ray structure is shown in the figure, was obtained by treatment of a tritopic linker with the flat 2-point panel. More recently we prepared heteroleptic metallacycles featuring stereoisomeric Ru(II) connectors.[2]

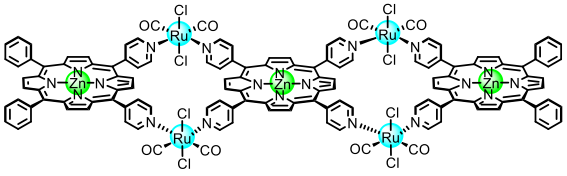






This ambitious project is aimed at preparing **unprecedented heteroleptic metallacycles** that, while keeping the usual symmetrical Ru(II) connecting unit, contain different porphyrins both in terms of number of pyridyl rings (2 or 4) and/or in the orientation of the connecting bonds (4′ or 3′). The new heteroleptic metallacycles will thus feature a larger number of porphyrins and/or new shapes. Examples are the angular 2+2 metallacycle, with one 4′-*cis*DPyP and one 3′-*cis*DPyP (left), or the extended flat tris-porphyrin metallacycle with two peripheral 4′-*cis*DPyP units and one central tetrapyridyl (4′-TPyP) unit (right). The larger panels, featuring three axial connecting points, will allow us to build more stable assemblies.





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 zincated derivatives (panels) will be treated with appropriate linkers for the construction of higher-order assemblies. 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.

References

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Project 2 for position M/7 CHIM/12

Methodological developments and field studies for an extended physical-chemical and genetic characterization of bioaerosol in Friuli Venezia Giulia: particulate matter as an underestimated factor in disease dynamics

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co-Supervisor: Alberto Pallavicini, email: pallavic@units.it

Atmospheric particulate matter derives from natural and anthropogenic processes, has composition and size distribution [1] 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 [2], even if WHO is still not considering aiborne route as a relevant way of contagion. Standardized methods for ambient bioaerosol sampling and analyses are not available, even if literature is growing on the subject [3]. An interdisciplinary group at the University of Trieste has searched and identified by RT PCR, viral RNA on PM collected in the Po Valley during the COVID-19 outbreack [4]. Bioaerosol components (bacteria, viruses) and variability are still largely unknown; extended microbiome characterization by shotgun sequencing analysis on particulate matter extracts [5] can provide insight on environmental factors governing the spread of airborne diseases (as common influenza). Relevant aspect to be accounted for are also the molecular evidences of antibiotic resistance in bioaerosol [6]. During the research project, the student will operate in a multidisciplinary team of environmental chemists, genetists and biostatisticians, setting bioaerosol sampling and analysis protocols, 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/7 CHIM/02

A quantum approach for ultrafast spectroscopies: role of correlation and of the circular dichroism in the electron dynamics of molecules and clusters

Supervisor: Dr. Emanuele Coccia, Department of Chemical and Pharmaceutical Sciences, UniTS
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The 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) which can be understood semi-classically as a sequence of three steps [2]: 1) electron ionization in a strong infrared field, 2) electron acceleration due to the laser field, and 3) electron recombination with the parent ion. During the recombination, coherent XUV and soft X-ray radiation, i.e. HHG, are emitted. The many-electron dynamics implicated in the HHG process can be quite complex. 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: i) the role of electron correlation, specifically the intereference effects between different excited states during the time propagation of the wavepacket. This can be accomplished by an accurate analysis of the various components of the wavepacket [4]. Thymine and uracil [5] will be studied; ii) the role of circular dichroism when a chiral molecule is irradiated by a circularly polarized laser field, as experimentally shown for limonene [6]; iii) circular dichroism in chiral metallic structures, as coiled nanowires, will be also studied, 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 [7]) and quantum-chemistry level, and a real time propagation of TDSE. 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/7 CHIM/08

Graphene Quantum Dots: bio- and electrochemical applications

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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, 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 explore and will be devoted to ameliorate the preparation of new GQDs derivatives, especially in combination with other specific dyes to be used for biological purposes and in electrochromic devices.

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Project 5 for position M/7 CHIM/03

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

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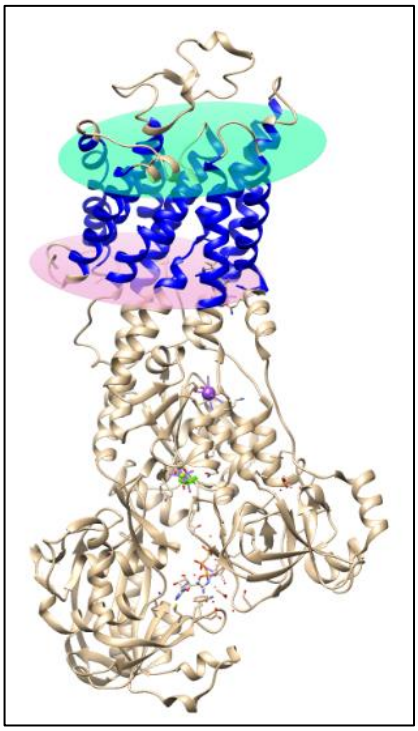
In biological systems, vesicle trafficking is one of the main means of exchange of materials between the cell and the external environment, and between different cell compartments. Vesicle formation relies on a change in membrane curvature that contributes to recruiting coating proteins on the membrane 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 are involved in a wide range of serious diseases, from hepatic cholestasis to diabetes, cancer and Alzheimer's disease.

P4-ATPases are intergral 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 similar to their close relatives, the P-ATPases, a large class of proteins involved in active transport of ions. However, specific aspects of the P4-ATPase structure are involved in recognition and translocation of their large, amphipathic substrates. Recently, two pivotal studies determined the structures of a human and a yeast P-IV ATPases using cryo-Electron Microscopy (cryo-EM) on single particles [1-2], contributing to our understaining 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, namely the protein Neo1. Preliminary results show the stability of the protein 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, an emerging technique in structural biology.

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

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Project 6 for position M/7 CHIM/06

Semisynthetic natural products derivatives for Animal health

Supervisor: Cristina Forzato, Department of Chemical and Pharmaceutical Sciences, UniTS
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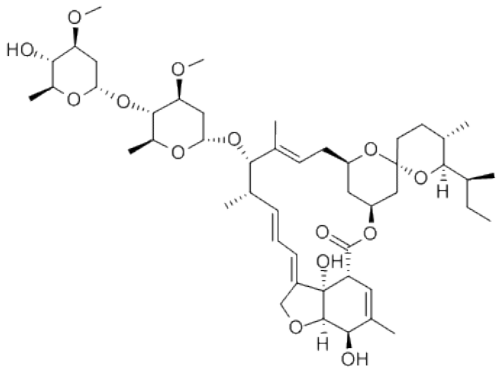
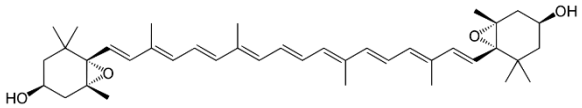
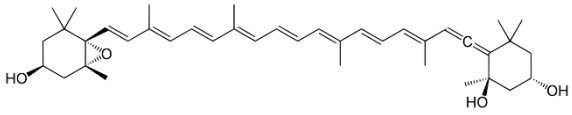
The project will focus on **helminthiases**,[1] a condition regarding animal health, since it is spreading among the EU territory and it is connected with the climate change and the diffusion of vectors.
Helminthiases are treated with three broad-spectrum drugs, the only reliable way to control them in livestock, but resistance is spreading due to erroneous use and/or abuse, causing economic losses, and greatly impairing animal health and welfare. 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 anthelmintics 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, reducing pollution and residues in the animal products, thus protecting the consumers.
In this doctorate new anthelmintics will be developed starting from commercially available natural products that will be modified to obtain substrate analogues fitting the binding site of the quinol-fumarate reductase. Its substrate is related to carotenoids, and inhibitors could be found among semisynthetic derivatives of violaxanthine or neoxanthine. Modification of the well known ivermectin will be also considered as this macrolactone contains several interesting structural moieties that could be modified to improve its activity. For the new compounds against helminthiasis, a nematicidal screening will be performed in collaboration with the University of Vienna using the model organism *C. elegans*. The evaluation on *C. elegans* will be complemented by the University of Padova by testing of the novel compounds against the main helminths affecting horses and sheep, including parasite populations previously detected as resistant to current drugs.



Ivermectin



Violaxanthine



neoxanthine

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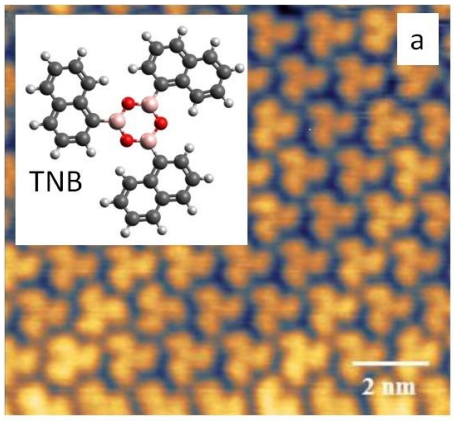
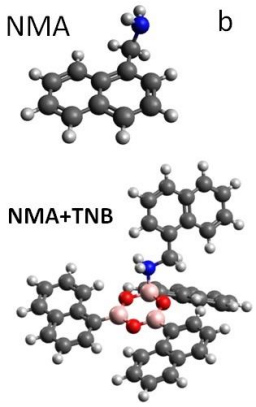
Project 7 for position M/7 CHIM/02

Computational core electron spectroscopies of gas phase molecules and their 2D covalent frameworks on metallic surfaces

Supervisor: Prof. Giovanna Fronzoni (DSCF, email: fronzoni@units.it),
Co-supervisor: Prof. Daniele Toffoli (DSCF, email: toffoli@units.it)

The focus of the project is the investigation of the adsorption and assembly of medium-sized organic molecules on metallic surfaces. NEXAFS (Near Edge X-ray Absorption Fine Structure) is a powerful tool to investigate the geometry of molecules adsorbed on surfaces [1]. Density Functional Theory (DFT) and its time-dependent generalization (TD-DFT) will be used to accurately simulate core spectroscopies such as XPS and NEXAFS of gas phase molecules and their 2D polymerization products on the surfaces of interest. The computational protocol consists in *i)* an accurate modelling of the surface/adsorbate interface with periodic boundary conditions and plane-wave basis sets (by using solid-state programs such as QUANTUM ESPRESSO [2]) followed by *ii)* the generation of a suitable set of clusters (cut from the periodic structure) from which angularly resolved NEXAFS spectra are calculated with techniques of quantum chemistry and localized basis sets, implemented in the ADF suite [3]. A new method to compute vibrationally resolved NEXAFS spectra of gas phase molecules will be developed within the framework of DFT theory during the three years spanned by the project.
Systems of current interest are boroxine-based 2D structures on metallic surfaces [4]. These 2D COFs (Covalent Organic Frameworks) can selectively host other molecules by shape matching and drive the formation of complex interfaces. The possibility to exploit the chemical affinity between the boroxine group (as a monolayer of trinaphthylboroxine molecules, TNB, Figure 1a) (Lewis acid) and a methylamine-terminated molecule, namely the naphtylmethylamine (NMA, Figure 1b) (Lewis base) will be considered, as an alternative route in the guest-host recognition scheme. These systems will be studied in close collaboration with experimentalists working at the ALOISA beamline of the ELETTRA Synchrotron Lab of Trieste.





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Project 8 for position M/7 CHIM/03

Functional supramolecular polymers for self-diagnostic composites

Supervisor: Prof. Silvano Geremia, Department of Chemical and Pharmaceutical Sciences, UniTS
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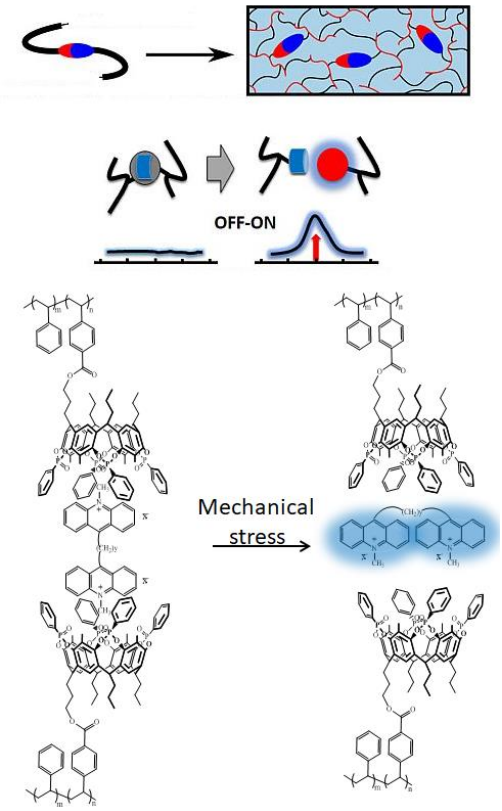
This financed PRIN-2017 (Progetti di Ricerca di rilevante Interesse Nazionale) project aims at introducing self-diagnostic properties into polymers and carbon fiber reinforced composites. The challenge is to produce fluorescence signals directly linked to the stress-driven breaking of the weak bonds 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 9 for position M/7 CHIM/03

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

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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).[3,4] for instance, a Sn^{IV}(N-acetyl-L-tyrosinato)$_2$-porphyrin conjugate (**1**) was found to generate a surprisingly long-lived radical pair state, by visible light excitation and in the presence of pyrrolidine (Figure). The single crystal X-ray structure of **1**, determined at the XRD1 ELETTRA synchrotron light source, evidences 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.

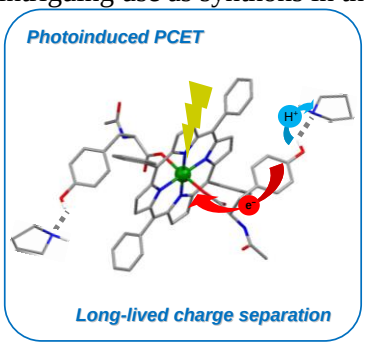

Figure. X-ray structure of **1** (solvent molecules and hydrogens, except for those of the OH groups, are omitted for clarity) and Schematic depiction of the (concerted) PCET process in the presence of pyrrolidine, mediated by H-bonding between the aa -OH residues and the base. Color code: H, white; C, gray; N, blue; O, red; Sn, green.

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 10 for position M/7 CHIM/06

New synthetic routes for fluorinated acyl succinates and paraconic acids

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Organofluorine compounds are widely used in various areas of chemistry, including agrochemistry, materials science, and medicinal chemistry. It is well known that the presence of fluorine atoms or fluorine-containing motifs in organic molecules alters their physical and chemical properties, such as their electronic nature, conformation, lipophilicity, and stability, and it can also affect their metabolism [1]. In a medicinal chemistry context, the improved binding affinities and biological activities of fluorinated compounds have prompted organic chemists to develop new synthetic strategies for the selective incorporation of fluorine into organic compounds.

The project is mainly focused on the synthesis of a new class of fluorinated acyl succinates and fluorinated 2-alkyl paraconic acids [2-4] with biological activity.

For this purpose, in the crucial steps of the synthesis, catalytic strategies including transition-metal catalyzed reactions and enzymatic resolution procedures will be developed.

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Project 11 for position M/7 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, UniTS
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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 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, McGill University-Montreal, 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.

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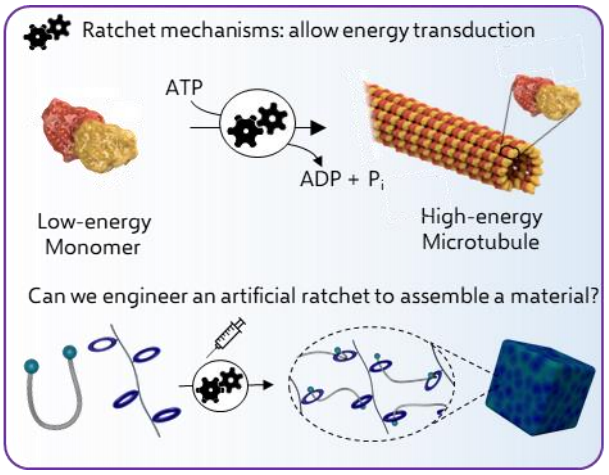
Project 12 for position M/7 CHIM/06

Non-equilibrium self-assembling materials

Supervisor: Dr. Giulio Ragazzon, Department of Chemical and Pharmaceutical Sciences, UniTS, email: gragazzon@units.it
Co-supervisor: Prof. Maurizio Prato

This project aims at the development of supramolecular materials that self-assemble thanks to the operation of molecular machines. This process affords materials having a time-correlated memory.

A fundamental characteristic of biological entities is the ability to exploit energy sources to perform nanoscale tasks that would not occur spontaneously. Examples are the directional stepping of kinesin and the non-equilibrium assembly of microtubules, that require ATP (adenosine triphosphate) consumption. Such endergonic operations rely on mechanisms called ratchets.[1] So far these mechanisms have only been engineered into the most advanced molecular machines: can we use molecular ratchets to assemble innovative materials?[2]



Non-equilibrium materials: biological systems can harvest chemical energy and use it to assemble high-energy structures. Analogous strategies will be implemented in artificial systems.

This project will re-engineer molecular ratchets based on pseudotoraxanes, extending their scope.[3] Bifunctional threads will be combined with multifunctional hosts, leading to supramolecular polymerization, crosslinking and gelation. The new materials will be used to develop soft actuators having time-correlated memory.

The PhD student will synthesize, characterize, and operate the target systems. The synthesis and operation of model systems will be studied by nuclear magnetic resonance (NMR). The obtained materials will be analyzed with state-of-the-art methods, including rheological measurements, optical spectroscopies, and atomic force microscopy (AFM). Our group has also a strong track record of fruitful collaborations, that involve also the use of X-ray facilities at the synchrotron (Trieste) or CIC biomaGUNE (San Sebastian, Spain). For the optimal development of collaborative projects, it is likely for PhD students to perform a research stay abroad.

The student will be fully integrated into the group of Prof. Prato, thus experiencing a lively and stimulating environment for the professional development of new members. Our group is committed to interdisciplinarity and the student will be exposed to other fields related to carbon nanomaterials, that represent the group core expertise. Typically, the PhD work starts from an ongoing project. Then, the individual interests and attitudes of the student come into play and shape the development of his path into research.

References:

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Project 13 for position M/7 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 interest in this research is twofold: on one hand to get insight on the molecular basis of recognition and transport, and on the other hand to get control of the 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, 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.

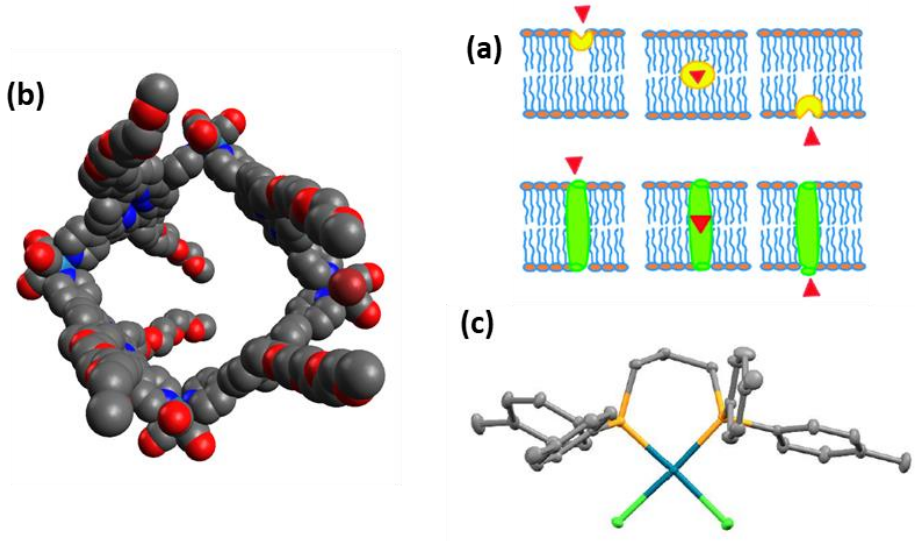

Figure: (a) schematic representation of a carrier (top) and a channel ionophore (bottom); (b) a channel forming porphyrin metallacycle; (c) a Pd(II)-diphosphine complex acting as a carrier for chloride.

References:

[1] M. Tosolini, P. Pengo, P. Tecilla, *Curr. Med. Chem.*, **2018**, *25*, 3560-3576.
[2] F. De Riccardis, I. Izzo, D. Montesarchio, P. Tecilla, *Acc. Chem. Res.* **2013**, *46*, 2781–2790
[3] D. Milano, B. Benedetti, M. Boccalon, A. Brugnara, E. Iengo, P. Tecilla, *Chem. Commun.* **2014**, *50*, 9157–9160.