University of Trieste
Department of Chemical and Pharmaceutical Sciences

Doctorate in Chemistry

2022

Cycle 38

Research Projects PNNR (DM 351 & 352)

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The three positions are on predetermined topics

Project for position PNNR/1 **CHIM/03**

DM 351

Sn^{IV}-porphyrins as supramolecular building cores for self-assembling processes

Supervisor: Prof. Elisabetta Iengo (eiengo@units.it)

(Metallo)porphyrins are directly involved in **fundamental processes of living organisms**, such as oxygen transport (myoglobin/hemoglobin), plant and bacteria **photosynthesis** (antenna systems and reaction center), and **cellular metabolism** (enzymatic prosthetic groups such as cytochrome p450, cytochrome C oxidase, catalase). The high adaptability of these molecules is strictly related to their inherent structural characteristics. Porphyrins are flat heterocyclic macrocycle compounds, with an extended aromatic conjugation, and can accommodate at their inner tetrapyrrolic core a large variety of metal cations, belonging both to main groups and transition series (Fig. 1). The possibility to fine-tune their properties (*e.g.* physico-chemical, **optical, redox**), by either tailoring the macrocyclic skeleton at the periphery or by metal insertion, has made them ideal components of artificial complex systems for a variety of applications, such as molecular recognition,[1a] **photodynamic therapy** (***PDT***),[1b] **photocatalysis**,[1c] and **artificial photosynthesis**.[1c] Interestingly, adducts containing (metallo)porphyrins as chromophores may satisfy some crucial aspects for the development of a sustainable future: **activation by visisble light** and **possibility to operate in water**.

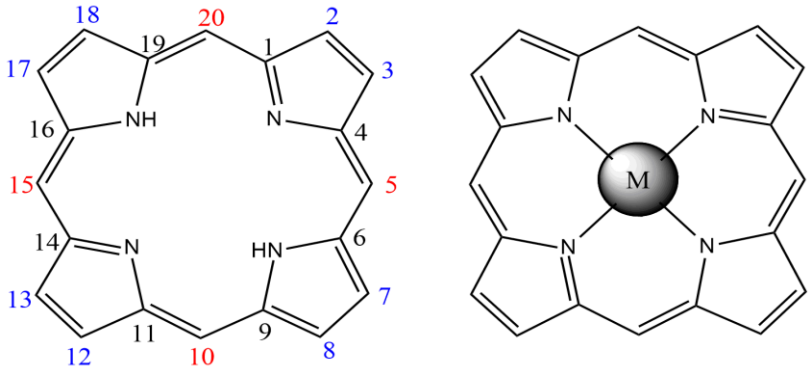
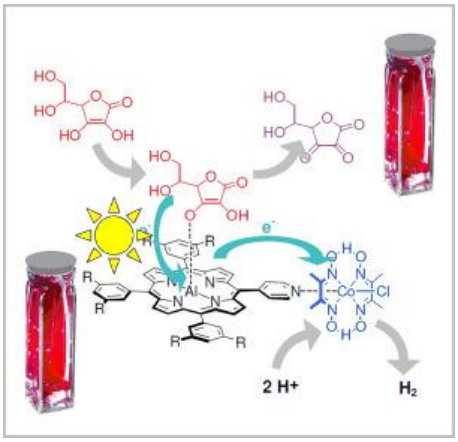


Fig. 1: Schematic structure of a porphyrin, left, and of a metalloporphyrin, right; M = Zn^{II}, $Ru^{II}(CO)$, $Al^{III}(OH)$, Co^{II}, $Sn^{IV}(OH)_2$, ..; in red and blue the peripheral position that can be tailored, *meso* and *β-pirrolyc*, respectively.

In the past we reported on Al^{III}- or Co^{II}-porphyrin conjugates as **photosensitizers** (***PS***) and catalysts for the photogenreation of by means of **visible light** and in **aqueous solution** (Fig. 2).[2] More recently, the characteristics of Sn^{IV}-porphyrin, planarity, six-coordination, robust and **quantitative** binding to oxyanions, ^{119}Sn NMR active nucleous and ease of reduction,[3] have attracted our interest.[4] We developed a series of **chiral** Sn^{IV}-porphyrin conjugates, differing for the substitution pattern at the macrocycle and bearing two L-amminiacids (aa) at metal axial positions. Two of those systems (Fig. 3) were found to generate a radical pair state in the presence of base, by visible light excitation and by *Proton Coupled Electron Transfer* (*PCET*),[4] a mechanism typical of natural electron transfer processes involving tyrosine residues. Moreover, The single crystal X-ray structures of both systems evidence ordered patterns of intermolecular H-bonds.



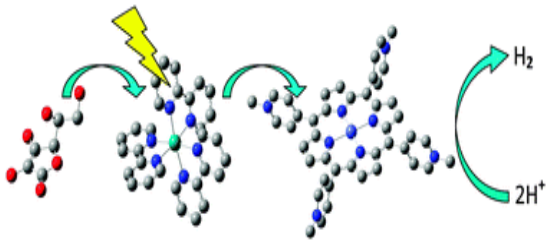


Fig. 2: AscorbicAcid/Al^{III}-porphyrin/Cobaloxime and AscorbicAcid/$[Ru(bipy)_3]^{2+}$/Co^{II}-porphyrin conjugates (left and right respectively) for the the photogeneration of H_2 by **visible light** and in aqueous solution.[2]

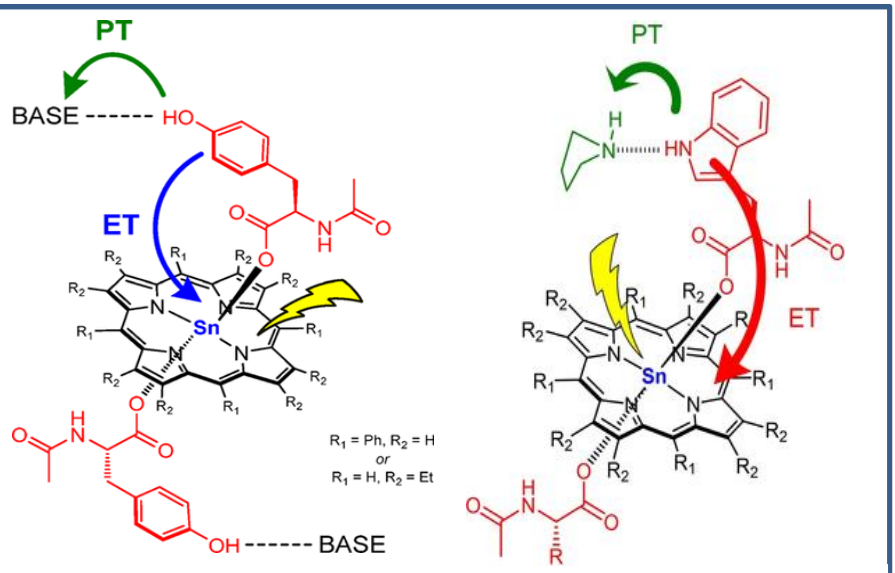


Fig. 3: Sn(L-tyrOH)$_2$- e Sn(L-trpNH)$_2$-porphyrin conjugates undergoing ***PCET*** by **visible light** excitation and in the presence of a base.[5]

The present project aims at further developing the study on these **biomimetic conjugates** for the **conversion of solar energy** and in particular it will address the preparation and characterization of a library of tin-porphyrin beraing short oligopeptides at the metal center, and terminating with either tyrosine (aa_n-tyrOH) or tryptophane (aa_n-trpNH), n ≥ 1 (Fig. 4). The library members will differ in the nature of the aa, aswell as in the distance of the terminal tyrOH/trpNH groups from the tin-porphyrin ***PS***. Inorganic, organic and supramolecular synthetic methodologies will be employed, alongside a variety of characterization techniques (in solution: ESI-MS spectrometry, NMR, UV-vis, emission and CD spectroscopies, ciclyc voltammetry; in the solid state: X-ray diffraction by means of the local ELETTRA synchrotron light source). The library members will tested with these objectives: *i)* capability to self-assemble in ordered discrete **multi*PS***-tyrOH/trpNH adducts *via* non-covalent iteractions (e.g. **hydrogen bonding**) between the lateral oligopeptide side-chains (Fig. 4); *ii)* response to **visible light** for the achievement of long-lived charge separation, in view of **sustainable** photocatalytic appplications (Fig. 5); *iii)* possibility to perform as **visible light** activated metallo-drugs in the field of ***PDT***. The last two objectives will be tackled also in collaboration with

other groups, and in particular during a six-month stay in the laboratories of Prof. S. Bonnet at the Leiden Institute of Chemistry (The Netherlands).

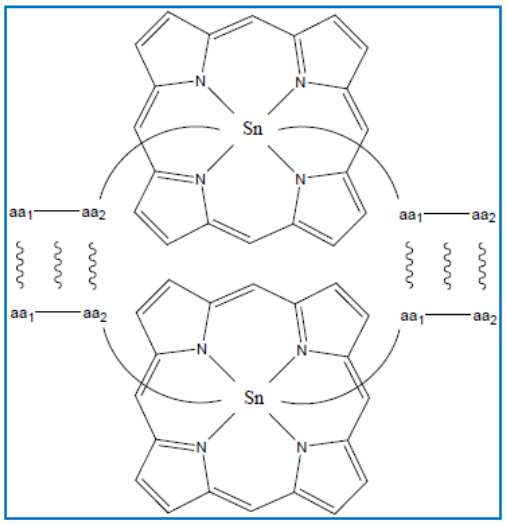


Fig. 4: Schematic representation of the self-assembling into in ordered dicrete **multi*PS***-tyrOH/trpNH adducts *via non covalent* interactions between the lateral oligopeptide side-chains ; aa_1 = tyrOH ortrpNH.

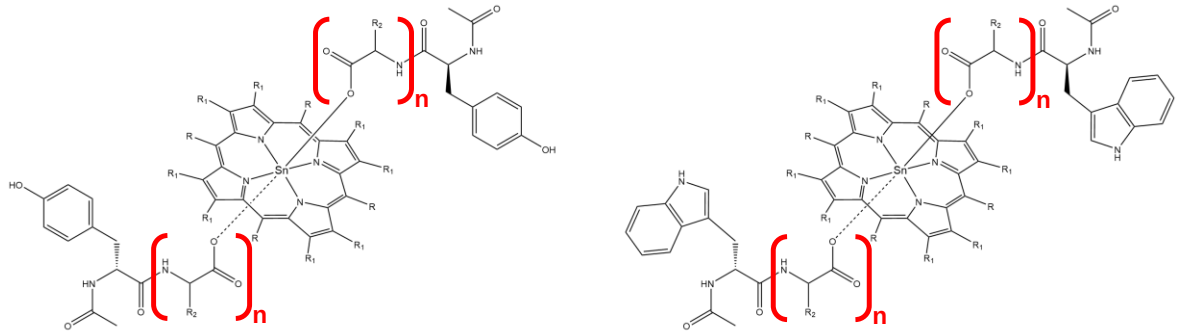


Fig. 5: Schematic representation of two libraries of Sn(aa_n-tyrOH)$_2$- and Sn(aa_n-trpNH)$_2$-porphyrin conjugates for the achievment of **biomimetic photinduced processes** by means of **visible light**.

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5. (a) M. Natali, A. Amati, N. Demitri, E. Iengo *Chem. Eur. J.* **2021**, 27, 7872; (b) M. Natali, A. Amati, S. Merchiori, B. Ventura, E. Iengo *J. Phys. Chem. C* **2020**, *124*, 8514; (c) M. Natali, A. Amati, N. Demitri, E. Iengo *Chem. Commun.* **2018**, *54*, 6148.

Project for position PNNR/2 **CHIM/03**

DM 352

Towards new metal-based radiopharmaceuticals for imaging and therapy

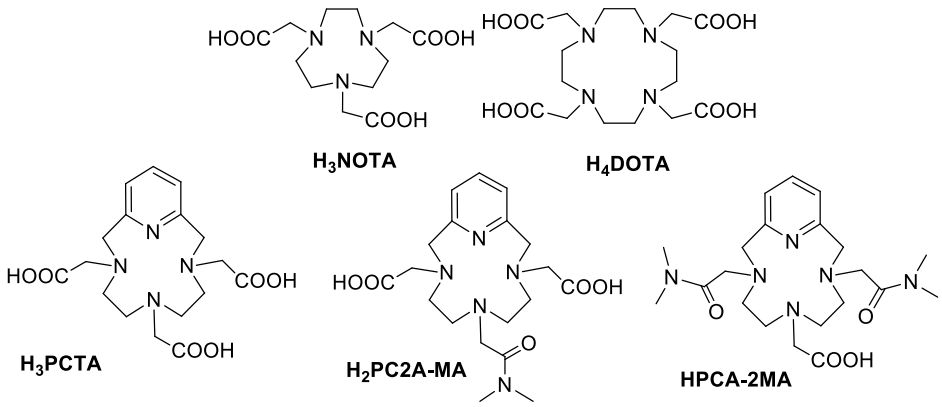
Supervisor: Prof. Enzo Alessio (alessi@units.it)
Company involved: Bracco Imaging SpA, Basovizza (TS), co-Supervisor at Bracco Imaging: Dr. Zsolt Baranyai (

^{68}Ga and ^{44}Sc for PET imaging

Positron Emission Tomography (PET) is one of the most sensitive medical imaging techniques, capable of visualizing different diseases and disorders and also metabolic processes. Acquisition of PET image requires the application of a β^+ emitter isotope, that must be placed in the diagnostically important region of the living system. Therefore, specific carrier molecules (targeting vectors) are generally labelled with β^+ emitters like ^{11}C, ^{15}O, ^{18}F, etc. ^{18}F has very good radiochemical properties. In particular ^{18}F -fluorodeoxyglucose (^{18}F-FDG) is commonly used for different applications like diagnosis, staging, and monitoring treatment of cancers. However, ^{18}F-FDG requires an elaborate synthetic procedure leading to a significant loss of activity and to the risk of radiation exposure of the operating staff during the labelling process.[1] For these reasons, in the last few years there has been active research for suitable tracers as an alternative to ^{18}F. Among the possible candidates such as ^{89}Zr,[3] ^{64}Cu,[4] ^{44}Sc[5] and ^{68}Ga[6], ^{68}Ga and ^{44}Sc are particularly attractive for preclinical and translational research, due to their availability from ^{68}Ge/^{68}Ga and ^{44}Ti/^{44}Sc generators with a sufficiently long shelf life. Both metals have cold isotopes on which the chemistry can be developed.[2]

For targeted clinical applications radioactive metal ions must be tightly complexed by the bifunctional chelating ligand. These complexes must be characterized by high thermodynamic stability, kinetic inertness and fast formation (even in highly diluted solutions). Moreover, the chelating ligand should be easily conjugated to a biological vector molecule (bifunctional ligands). In the last two decades, it has been demonstrated that the free or bioconjugated macrocyclic triaza- and tetraaza ligands such as NOTA and DOTA (Scheme 1) form thermodynamically stable and kinetically inert complexes with isotopes of ^{68}Ga and ^{44}Sc.[2] However, the efficient labeling of NOTA, DOTA and their derivative ligands with ^{68}Ga and ^{44}Sc isotopes requires a large excess of ligand (>1000 fold) and high temperature (95°C) which promote the denaturation of the biologically active protein bioconjugates.[3] PCTA is a tetradentate macrocycle similar to DOTA in which however one sp^3 N atom is replaced by an sp^2 pyridyl N atom (Scheme 1). The physico-chemical properties of several metal complexes formed with PCTA ligand have been reported, showing its remarkable affinity to lanthanides and transition metal ions. However, the effect of the pendant arm on the PCTA scaffold, used for the conjugation to the targeting molecules, on the physico-chemical properties of the metal-complexes have not been studied previously.

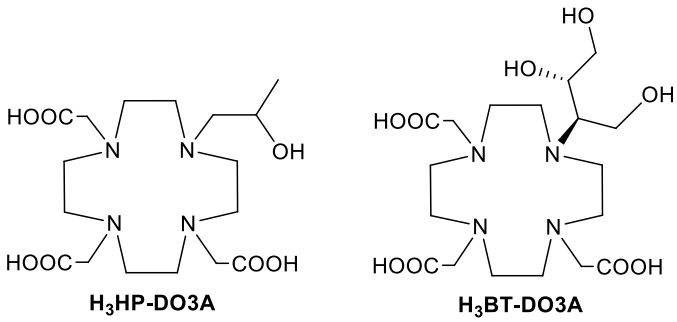
In this project several PCTA derivatives (Scheme 1) will be synthesized and their protonation behavior and complex-forming properties with Ga^{3+} and Sc^{3+} ions will be evaluated. Solid state structures will be determined by X-ray crystallography. The solution structures and dynamics of the Ga^{III} and Sc^{III}-complexes will be investigated by multinuclear NMR spectroscopy. The kinetic inertness of Ga^{III}- and Sc^{III} complexes will be investigated via the metal and ligand exchange reactions under near physiological conditions.



Scheme 1. Structure of NOTA, DOTA, PCTA and its derivatives

$^{212/213}$Bi for therapy:

Targeted α therapy (TAT) is currently being actively investigated as a potential treatment of cancer relying on α-emitting radionuclides (e.g. ^{225}Ac, ^{211}At, ^{223}Ra, $^{212/213}$Bi). α-particles can transfer high linear energy in a short range, allowing an internal radiotherapy confined in a limited area while reducing off-target damage. Among α-emitting radionuclides, ^{213}Bi ($t_{1/2}$ = 46 min) deserves a special attention since it is available through commercially benchtop generators (^{225}Ac/^{213}Bi) and its TAT conjugates have been proposed for the treatment of leukemias, lymphomas and micrometastatic neoplasms and in other cancers and diseases.[4] To date, derivatives of DTPA and DOTA have been used as ligands in the complexation of ^{213}Bi isotope.[2] Considering the rather short half-life of ^{213}Bi ($t_{1/2}$=45 min), the labeling of DOTA and its derivatives takes place at very harsh condition which is not compatible for some thermosensitive peptide conjugates. In order to obtain an efficient ^{213}Bi-based TAT agent, the physico-chemical properties of Bi^{III}-complexes must be characterized in details. However, the studies focusing on the equilibrium, formation and dissociation kinetics and structural properties of Bi^{III}-complexes are very rare.[5] In this work we plan the systematic studies on the Bi^{III} complexes of two DOTA derivatives with the alcoholic -OH group instead of carboxylate group (Scheme 2). The formation of Bi^{III} complexes will be investigated by UV-spectrophotometry in the presence of ligand excess in order to guarantee the pseudo-first-order kinetic condition. Solid state structures will be determined by X-ray crystallography. The kinetic inertness of the Bi^{III}-complexes will be investigated by following their transchelation reactions with UV-spectrophotometry. The solution structures and dynamics of the Bi^{III}-complexes will be investigated by multinuclear NMR spectroscopy.



Scheme 2. Structure of HP-DO3A and BT-DO3A ligands

References

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[5] D. Horváth, F. Travagin, N. Guidolin, F. Buonsanti, G. Tircsó, I. Tóth, F. Bruchertseifer, A. Morgenstern, J. Notni, G. B. Giovenzana, Z. Baranyai, *Inorg. Chem. Front.* **2021**, *8*, 3893–3904.

The successful candidate will spend at least 6 months at Bracco Imaging SpA, in particular in the branch located nearby the Elettra Sincrothron of Trieste, under the direct supervision of Dr. Zsolt Baranyai. In addition, the PhD student will spend 6 months in the group of Prof. Dr. Gyula Tircsó, Department of Physical-Chemistry, University of Debrecen, Hungary. During the stage, selected Ga^{III}, Sc^{III} and Bi^{III} -complexes characterized by high stability, kinetic inertness and fast-formation will be labelled with ^{68}Ga, ^{44}Sc and $^{205/206}Bi$ isotopes. Labeling procedures will be optimized based on the physico-chemical properties of the corresponding *cold* complexes.

Project for position PNNR/3 **CHIM/06**

DM 352

Development of photosynthetic protocells for low-cost production of solar fuels

Supervisor: Pierangelo Gobbo (https://gobbo-group.com)
Institution: Department of Chemical and Pharmaceutical Sciences, University of Trieste (Italy)
Industry involved: Enphos S.R.L. (https://www.enphos.com)

The increase in global energy demand and the climate change crisis is causing a progressive increase in global warming and deoxygenation of our atmosphere.[1] Therefore, it is of paramount importance to develop new technologies to simultaneously (i) reduce the global amount of CO_2 in our atmosphere, (ii) produce eco-sustainable fuels, and (iii) reoxygenate our planet.

In the past decade, micro-compartmentalized systems called "protocells" have been developed to study complex biological phenomena such as enzymatic metabolism, chemical signaling or photosynthesis under simple and controllable experimental conditions.[2,3] Recently, Dr. Gobbo, in collaboration with the University of Bristol (UK) and the University of Padua (Italy), has synthesized catalytic protocells capable of decomposing H_2O_2 into oxygen and water starting from poly(diallyldimethylammonium chloride) (PDDA) and a mixture of polyoxometalates: sodium phosphotungstate (PTA) and a synthetic catalyst called "Ru_4POM".[4] The research team has then demonstrated that the same catalytic protocells are capable of utilizing sunlight to oxidize water to protons and O_2 (**Figure 1**) when dispersed in a solution containing $Ru(bpy)_3Cl_2$ (a photosensitizer) and $Na_2S_2O_8$ (a sacrificial electron acceptor).

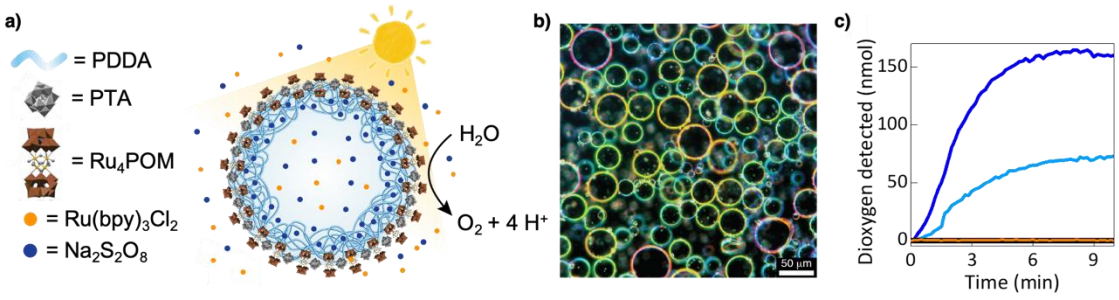

Figure 1: a) Schematic representation of a catalytic protocell and its photo-reactivity. **b)** Darkfield microscopy image of a population of protocells structured like described in **(a)**. **c)** Graph showing the time-dependent production of O2 for: a sample of photocatalytic protocells (dark blue plot); a sample of photocatalytic protocells that have been utilized a second time (light blue plot); control experiments carried out in the absence of light (black plot) or in the absence of the Ru_4POM catalyst (orange plot). Non-published preliminary results.

The project starts from these important preliminary results and aims to develop the first photosynthetic protocells capable of utilizing sunlight, water and CO_2 to autonomously and continuously synthesize O_2 and fuels at low costs. The student will develop these preliminary results into groundbreaking research following their interests and attitudes. Specifically, the student will explore the following possibilities:

1) Test different POM catalysts to lower the production costs of the protocells while maintaining high photocatalytic efficiency.

2) Substitute the $Ru_4POM/Ru(bpy)_3Cl_2/Na_2S_2O_8$ system with groundbreaking and more efficient antenna systems such as the "quantasome complex" of Ru_4POM and perylene bisimide recently reported in *Nature Chemistry*.[5]
3) Develop protocells with phototactic abilities (autonomous movement towards or away from a light source) to improve their photosynthetic efficiency.
4) Couple the photo-assisted water oxidation reaction to an enzymatic reaction through the use of cofactors such as NADH or methyl-viologen for the synthesis of eco-sustainable fuels such as methanol, ethanol, formaldehyde, methane, and formic acid (FA). In particular, FA is also an ideal precursor of hydrogen fuel and if we can implement protocells capable of autonomously and continuously producing FA from light, water and CO_2, we could also explore the possibility of exploring the final step, that is the reduction of FA to hydrogen gas.

Through this multidisciplinary project, the student will develop skills in synthetic chemistry, polymer chemistry, soft materials chemistry, photocatalysis and biochemistry. The protocells that will be developed and their reactivity will be characterized *via* advanced spectroscopic (NMR, UV-vis and fluorescence) and microscopy (brightfield, darkfield, fluorescence, and electron) methods.

To fully develop the project the student will work for six months at Enphos, an industry in northeast of Italy that develops eco-sustainable systems to produce solar fuels and hydrogen. The student will also work for six months in the research group of Prof. Giovanni Finazzi (Commissariat a l'Energie Atomique et Aux Energies Alternative, Grenoble, France), where they will learn important biology techniques that will be integrated into the project to develop phototactic protocells.

References:

[1] J. Huang *et al. Sci. Bull.* **2018**, *18*, 1180-1186.
[2] P. Gobbo* *et al. Front. Mol. Biosci.*, **2021**, *8*, 804717.
[3] C. Xu, *et al. Mater. Today* **2016**, *19*, 516-532.
[4] P. Gobbo, M. Bonchio, S. Mann, S. *et al.* Nat. Commun. **2020**, *11*, 41.
[5] M. Bonchio, M. Prato, *et al. Nat. Chem.* **2019**, *11*, 146-153.

Students interested in this project should contact Dr. Gobbo as soon as possible (pierangelo.gobbo@units.it). They should send their CV and explain (i) the reason for their interest in the project, and (ii) how their previous research experience could contribute to the project's success.