Project summary

Nature has developed mechanisms for reversible enzyme (de)activation to maintain overall enzymatic homeostasis, controlling thousands of proteins in a cell exquisitely with a handful of chemistries available (e.g., oxidation, reduction, phosphorylation, ubiquitination, etc.). This project aims to develop new strategies to modulate protein catalytic activities through dynamic combinatorial chemistry.

Ph.D. training program

The integrative training of a Ph.D. as a researcher requires several elements: A doctorate program, training given at the host laboratory while developing the actual research project, and complementary training. The training plan proposed for the Ph.D. student tries to integrate all these elements:

Regarding **Doctorate Program**, the Ph.D. student will join the Doctorate Program in Advanced Chemistry at the Universidad Complutense de Madrid. The Doctorate Committee keeps track of the student training every year by following the up-load information at the UCM platform (RAPI): (i) the activities carried out within the Ph.D. program; (ii) the activities carried out outside the Ph.D. Program; and (iii) their research plan indicating the yearly progress of the specific activities and up-sating the tasks for the upcoming year. These activities must be approved by Thesis Director, the Tutor, and the Doctorate Committee before registration in the following academic term. Within the Doctorate Program, workshops and PhDay are organized annually with keynote lectures by prestigious researchers to instruct Ph.D. students in advanced topics.

The **research project** proposed here will allow the Ph.D. student to be trained in Chemical Biology and become a dynamic covalent chemistry expert with an **interdisciplinary perspective**. He/she will learn various essential techniques in drug discovery with extensive impact on modern Science in both the pharmaceutical industry and academic environments. attendance at national and international conferences, participation in specific workshops (*e.g.*, protein-ligand analytical techniques training, etc.), and short stays (usually a 3-month) at research centres of excellence abroad are key ingredients of his/her education.

Additionally, CSIC offers **complementary multidisciplinary educational opportunities** that should converge towards a high-quality training environment: courses (ethics, Good Scientific Practice, entrepreneurship, women in science, oral communication skills, workplace safety), workshops (CSIC Ph.D. Day, "Yo Investigo. Yo Soy CSIC" contest), dissemination activities to the general public (Science Week, European Night of Researchers, talks, and workshops for High school students).

Summary Lab activities and results

Perez's research group uses dynamic combinatorial libraries (DCLs) of exchangeable chemical compounds (building blocks), where proteins direct the synthesis of the best ligands *in situ* (Protein-directed Dynamic Combinatorial Chemistry, P-D DCC) to find protein modulators. Recently, we reported the first activator of protein-protein interaction (NCS-1/Ric8a) that regenerated synapsis (*Nat. Commun.* **2019**), leading to the recovery of memory and psychomotor activity in animal models with Alzheimer's' disease. Besides, we found a small molecule that acted as a catalyst for the hydrazone exchange and worked effectively below room temperature. We have studied the hydrazone formation and exchange reaction mechanism in-depth and, for the first time, analyzed the two steps independently in *Org. Biomol. Chem.* **2021**, leading to interesting mechanistic conclusions. In another example, Perez's lab mimicked Nature's strategy and, by reactivating a reversible chemical system, reached 18-fold acceleration over the uncatalyzed thiol-disulfide exchange reaction, discovering the first allosteric modulator of glucose oxidase and providing a new tool to fold misfolded proteins even under acidic conditions (*Nat. Commun.* **2021**). This research has proven that it is possible to find protein activators using dynamic chemical systems and that these chemical networks can mimic functions encountered in Nature.

Selected publications

-Canal-Martín, A.; Pérez-Fernández, R. *Nat. Commun.* **2021**, *12*, 163.
-Canal-Martín, A.; Navo, C.D.; Saez, E.; Molero, D.; Jiménez-Osés, G.; **Pérez-Fernández, R.** Nucleophilic catalysis of *p*-substituted aniline derivatives in acylhydrazone formation and exchange. *Org. Biomol. Chem.,* **2021**, *19*, 7202.
-Canal-Martín, A.; Pérez-Fernández, Ruth. *ACS Omega* **2020**, *5*, 26307−26315.
-Canal-Martín, A.; Sastre, J.; Sanchez, M.J.; Canales, A.; Baldominos, S.; Pascual, N.; Martínez- González, L.; Molero, D.; Fernández-Valle, M. Martín-Santamaría, S.; Sáiz, A.; Mansilla, A.; Cañada, F. J.; Jiménez-Barbero, J.; Martínez, A.; Pérez-Fernández, R. *Nat. Commun.* **2019**, *10 (1),* 2798.

Research line related to the project

The PhD student will develop the project in the Protein-directed dynamic chemical systems research line.