Title	**Molecular engineering of enzymes for biocatalysis and bioremediation**
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Project description:

The proposed research activity, focused on the study of enzymes, targets the specific fields of biocatalysis and bioremediation, and will predictably proceed through the following step: (*i*) in silico identification and recombinant production of new enzymes; (*ii*) their biochemical and structural characterization (crystallization or modeling); (*iii*) bioinformatics analysis and engineering of the proteins (site-specific mutagenesis / rational design / directed evolution); (*iv*) biocatalytic characterization, with identification of the new/desired substrate repertoire. Activity tests will be conducted either in vitro using purified enzymes or in whole-cell configurations, depending on the application and context of the research.

Chemical industry is increasingly interested in biocatalysis to assist synthesis of biobricks or molecules with high added value. Enzymes can help in overcoming critical steps in organic synthesis by furnishing high chemo-, regio- and enantio-specificity and selectivity. The widespread application of biocatalysts has however to face the limited number of simultaneously synthetically useful and commercially available enzymes. Basic research is needed to uncover new enzymes and to improve their stability and substrate repertoire, as well as their capability of withstanding the harsh conditions often required in industrial reactors (non-aqueous solvents or extreme salinity, temperature, pH).

Pollution of freshwater by PFASs (PerFluoroAlkyl Substances) is currently an emergence in Veneto. It poses a significant environmental challenge, and bioremediation strategies offer promising approaches for mitigating their impact. Enzymatic degradation using laccases, peroxidases, and fluoroacetate dehalogenases, properly engineerd and expressed in suitable microbial strains like the cyanobacterium *Synechocystis*, holds potential for remediation efforts.

Representative recent publications:

- Robescu, et al. Asymmetric Proton Transfer Catalysis by Stereocomplementary Old Yellow Enzymes for C═C Bond Isomerization Reaction. ACS Catalysis 12.12 (2022): 7396-7405.
- Marchetto, et al. Bioremediation of per-and poly-fluoroalkyl substances (PFAS) by *Synechocystis* sp. PCC 6803: A chassis for a synthetic biology approach. Life 11.12 (2021): 1300.
- Robescu, et al. From the Amelioration of a NADP+-dependent Formate Dehydrogenase to the Discovery of a New Enzyme: Round Trip from Theory to Practice. ChemCatChem 12.9 (2020): 2478-2487.

International collaborations:

- Bruno Miroux, Laboratory of Physical and Chemical Biology of Membrane Proteins UMR 7099, Institute of Physico-Chemical Biology, Paris, France – University Paris Diderot
- Marco Fraaije, Molecular Enzymology Group, University of Groningen, Groningen, The Netherlands;
- Melanie Hall, Department of Chemistry, University of Graz, Austria.