UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.14

Proponent: Prof. Marina Lotti

Project Title: Properties and engineering of enzymes active at low temperatures

Scientific background and 'open issues'

Due to today's interest in eco-friendly and sustainable processes, the use of enzymes is of increasing relevance in various industrial sectors and reactions, for example food, pharmaceutical, detergents, as well as in the treatment of biomass and raw materials of different origin and composition. In this scenario, a major focus is on enzymes from extremophilic organisms, adapted to thrive in extreme conditions such as temperature, pH, salinity.

Organisms adapted to low temperatures (so-called psychrophiles) have developed a multitude of adaptive strategies, among these "cold-active enzymes" that allow psychrophilic organisms to maintain high metabolic activity at low temperatures and to survive in nutrient-poor environments such as the Arctic and Antarctic marine sediments.

Cold-active enzymes offer a unique tool to study temperature adaptation of proteins from a biochemical and thermodynamic perspective. Furthermore, these enzymes find application in reactions with thermolabile substrates, particularly in the food sector. The laboratory has long experience in cold-active enzymes and in recent years has focused on esterases and glycoside hydrolases.

The potential of biocatalysis, however, is not yet fully exploited, especially because most enzymes, and in particular psychrophilic ones, do not tolerate the conditions applied in the process. Stability is therefore a key parameter that can be implemented with different methods, including biodiversity extraction, rational mutagenesis and directed evolution. To make variant design and selection less experimentally challenging, computational enzyme engineering tools have been developed that can create and screen large virtual libraries or predict beneficial mutations.

Objectives

The project will target one of the enzymes identified (or to be identified) in the laboratory and aim to improve its properties through protein engineering.

Characteristics of interest are in particular stability, mainly to temperature but also to other stresses, for example salinity and pH and organic solvents, specificity, catalytic performances.

A very relevant goal in this field is to introduce new properties without losing the original ones, for example adding thermostability yet maintaining activity at low temperatures, in view of the production of robust and versatile biocatalysts. This kind of research opens

interesting windows into fundamental issues of protein science, for example the evolution and structural basis of enzyme biochemical properties.
In these studies, different strategies can be used also thanks to ongoing collaborations with expert groups in developing tools to limit experimental work related to protein engineering, allowing the identification of relevant protein regions via computational and structural methods.

Methodologies
To be optimized, enzymes must be available in recombinant form. Therefore, after selection of sequences of interest, synthetic genes are expressed in bacterial cells.
The design of enzyme variants is implemented based on comparison with more stable/performing proteins and/or structural data and/or through computational approaches in collaboration with partner laboratories (see below). Rational approaches can be complemented by "directed evolution" mutagenesis on the entire protein or of specific protein regions.
The variants are fully characterized regarding their activity, long-term stability, specificity and other relevant characteristics related to structure and conformation using circular dichroism, infrared spectroscopy and other biophysical methods.
Improved variants are tested in reactions of industrial interest under process conditions.

Collaboration / Co-tutoring opportunities
The group in involved in several collaboration, included the COST project **COZYME** (Establishing a pan-european network on computational redesign of enzymes). This project is built around three issues: 1. Improvement of generic enzyme properties such as stability and solubility; 2. Optimization of catalytic properties e.g. activity and stereoselectivity;3. Advancement of experimental approaches to generate and evaluate computational predictions. In this frame, co-tutoring opportunities can be found as well as with other Italian universities, according to the detail of the project.

Project's Sustainability & Mobility
The laboratory involved has deep expertise in cold-active enzymes and can support all experiments regarding expression, biochemistry, protein engineering, structural and functional issues. We collaborate with other groups in the Department on particular topics, and with colleagues from other Italian and international universities.

As part of the COST project, we are included in a very broad network of collaborations. The project is strongly focused on the training of young scientists. To date we have already collaborated with the University of Crete for the identification of extremophilic enzymes from halophilic organisms. Other possible internship locations are the universities of Brno (CZ), Lisbon (PT), Groningen (NL), Stuttgart (D). In Italy, we have active collaborations with the University of Padua (Prof. Fusco) on the processing of natural carbohydrates and with the State University of Milano (Prof. Nardini) for crystallographic characterization.

Recent research articles can be found in the following paragraph.

References

Louise Jane Gourlay L.J., Mangiagalli M., Moroni E., Lotti M.*, and Nardini M.*. Structural determinants of cold-activity and glucose tolerance of a family 1 glycoside hydrolase (GH1) from Antarctic *Marinomonas* sp. ef1. FEBS J., in press doi:10.1111/febs.17096

Marchetti A., Orlando M., Mangiagalli M. *, Lotti M. (2023) A cold-active esterase improves its mesophilic properties through Mn^{2+} binding. FEBS J 290(9): 2394-2411

Mangiagalli M. and Lotti M. (2021) Cold-active β-galactosidases: insight into cold adaption mechanisms and biotechnological exploitation. Mar. Drugs 2021, 19, 43. https://doi.org/10.3390/md19010043