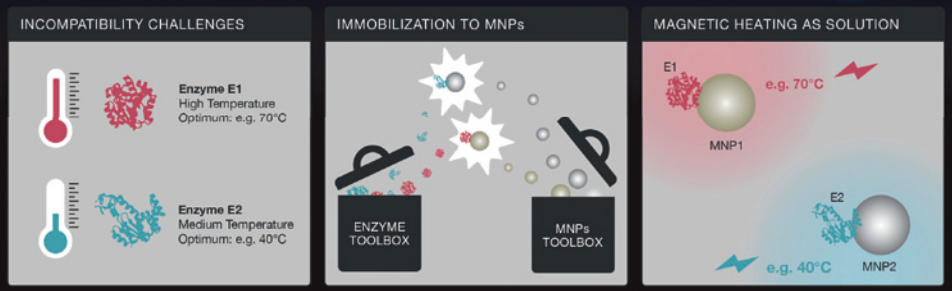
INCOMPATIBILITY CHALLENGES
One-pot multi-enzymatic processes require the selection of suitable enzymes. To coordinate the optimal temperature of all enzymes as well as to avoid negative impact of temperature on product- and cofactor stability is crucial. Further, to make sure the process unfolds its full potential, cross reactivity should be circumvented by on demand activation/inactivation of the enzyme(s). Unfortunately, the development of efficient, perfectly orchestrated and regulated cell-free metabolic systems is still an unmet need.

IMMOBILIZATION TO MNPs
To exert functional control over different enzymes, HOTZYMES uses magnetic heating on a molecular scale. Enzymes are immobilised on magnetic nanoparticles (MNPs), which are exposed to an alternating magnetic field (AMF). This triggers magnetic energy to be transferred as heat, which results in the creation of high temperature gradients at the location of the enzymes with respect to the bulk. This should allow an unprecedented temporal control over each enzyme activity whilst the global temperature of the reaction bulk is not increased.



Catalysing change in the chemical industry?

The current methods of producing pharmaceuticals and biocommodities are relatively inefficient and energy-intensive, leading researchers to investigate alternatives. Researchers in the HOTZYMES project are investigating the potential of using magnetic nanoparticles to heat up enzymes and catalyse reactions more efficiently, as **Dr. Valeria Grazú** explains.

The majority of the reactions used in the chemical industry to produce pharmaceuticals and biocommodities are accelerated by inorganic catalysts. While these catalysts speed up reactions, helping to reduce the costs of the eventual chemical products, there are also some disadvantages to this approach, as Dr. Valeria Grazú explains. "Usually pharmaceuticals and biocommodities are produced via a cascade of chemical reactions that occur sequentially. Since inorganic catalysts are not specific, using them to catalyse these reactions often generates unwanted by-products. This is a drawback of the existing approaches to industrial biotransformation, as these by-products have to be taken out by means of complex downstream processes, which decreases the yield of the product of interest and increases production costs," explains Dr. Grazú. "A further problem with inorganic catalysts is that they need very high temperatures in order to work effectively, which requires a high input of energy."

Hotzymes project

A more sustainable alternative approach could involve using enzyme cascades in a single pot to catalyse these reactions more efficiently, a topic that Dr. Grazú and her colleagues in the HOTZYMES project are exploring. Biological catalysts work at lower temperatures and are much more selective than inorganic catalysts, yet there are challenges to overcome in terms of using them in industry. "There are not many examples of enzymatic cascades being used in industry, because it's difficult to get biological catalysts, designed by nature, to work effectively in very specific non-natural environments," outlines Dr. Grazú. In nature, enzymes work together and catalyse chemical reactions in cells, yet Dr. Grazú says it is difficult to use these catalysts effectively in industrial settings. "From an industrial point of view, there is a process that could be improved. So we want to catalyse certain reactions, using the right enzymes for each step. But we may find that they are not all effective in the same conditions. Ideally we would like to combine the enzymes so that they work efficiently together in one pot," she says.

This goal is a central part of the HOTZYMES project's overall agenda, with researchers working to develop more efficient enzymatic cascades. The idea here is that the enzymes will catalyse a cascade of chemical reactions in the same vessel, with magnetic nanoparticles used to generate the ideal temperatures for each specific reaction. "Magnetic nanoparticles are very small and are superparamagnetic, which means that they don't have magnetic memory. So if they become magnetised they act like magnets, but when you remove the field, they lose their memory and re-disperse," explains Dr. Grazú. These magnetic nanoparticles are able to generate heat when placed in an alternating magnetic field. "That heat is very localised. Heat is generated very close to the surface of the magnetic nanoparticles," continues Dr. Grazú. "The idea in the project is to try to regulate the temperature of these nanoparticles in order to achieve a selective control of enzymes needed to carry out biotransformations of interest."

The project brings together partners from both academia and the commercial sector in pursuit of this goal, with researchers working on several different areas of technology. This includes the synthesis of the magnetic nanoparticles and the production of bioreactors for biocatalysis, which are central to the prospects of this approach being used in industry in the longer-term. "There are several challenges that we need to face in order to transform this scientific curiosity into a practicable technology," says Dr. Grazú. Magnetic heating is not an entirely new concept and is already applied in a number of areas, for example in cancer therapy, but the demands of industry are very different. "One of our project partners in the consortium (nanoScale Biomagnetics) has a lot of experience in the development of devices for the magnetic heating of nanoparticles. Their focus in this project is not on therapeutic applications, but industrial applications," outlines Dr. Grazú.

Industrial interest

A number of different processes of industrial interest have been selected for attention in the project, some of which lead to the production of active pharmaceutical ingredients. Some of these processes can't be carried out in the traditional way, because they are inefficient or too expensive for example, so Dr. Grazú says the project's research could open up new possibilities. "With one pot enzyme cascades and concurrent reactions, there is the opportunity to produce new chemicals that could be of interest, which at the moment cannot be produced efficiently," she explains. The goal at this stage is not to develop a market-ready product however, but rather to explore the feasibility of the idea. "The project is quite exploratory at this stage, but it could provide the basis to go further in future, if we have good results," says Dr. Grazú. "It's like the first step with a risky idea that could generate a breakthrough if successful. The next step then would be to go to the market."

The project's work could have a significant impact on the commercial sector in the long-term however, both helping companies produce existing chemicals more efficiently, and also opening up wider potential in production. While the project is still at a fairly early stage, Dr. Grazú says the results so far are encouraging. "We have just finished the first year of the project, but we have had very promising results so far," she says. Over the remainder of the funding term, Dr. Grazú and her colleagues will be working to both improve the technology and also address fundamental scientific questions. "This is a high-risk project, and there are a lot of basic science questions that we need to answer in order to achieve a technological breakthrough," she continues. "We are essentially pursuing research in two directions at the same time – from the basic point of view, and from the technological point of view. This is essential if we are to achieve a breakthrough."

Heat is generated **very close to the surface of the magnetic nanoparticles.** The idea of the project is to try to **regulate the temperature** of these nanoparticles in order that they could **heat different enzymes** needed to carry out **biotransformations** of industrial interest.

HOTZYMES

The Development of efficient Enzymatic Cascades in well-coordinated One-Pot-Systems

Project Objectives

HOTZYMES aims to enhance multi-enzymatic processes for the biotechnological production of pharmaceuticals and biocommodities. To enable optimal temperature conditions for each reaction in a multi-step-scheme, HOTZYMES couples enzymes to magnetic nanoparticles that are controllable at nano-scale locally using magnetic heating. Also, a new generation of magnetic bioreactors for biocatalysis will be designed.

Project Funding

The EU-project HOTZYMES is funded in the frame of the H2020-FETOPEN-2018-2020 call. It consists of a multidisciplinary consortium of 7 partners from four European countries, who implement the project with a budget of 3 million Euro.

Project Partners

• For details of project partners, please visit: ***https://www.hotzymes.eu/consortium/***

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Dr. Valeria Grazú is a senior researcher at CSIC, a position she has held since 2016. She has deep experience in the biofunctionalization of nanostructured materials and has worked with a wide variety of biomolecules (proteins, enzymes, DNA, sugars, peptides, etc), looking at their use in biotechnological and biomedical applications.

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