

Novel and more robust
fungal peroxidases as
industrial biocatalysts



2011 - 2013

Grant agreement n°: KBBE-2010-4-265397

OBJECTIVES

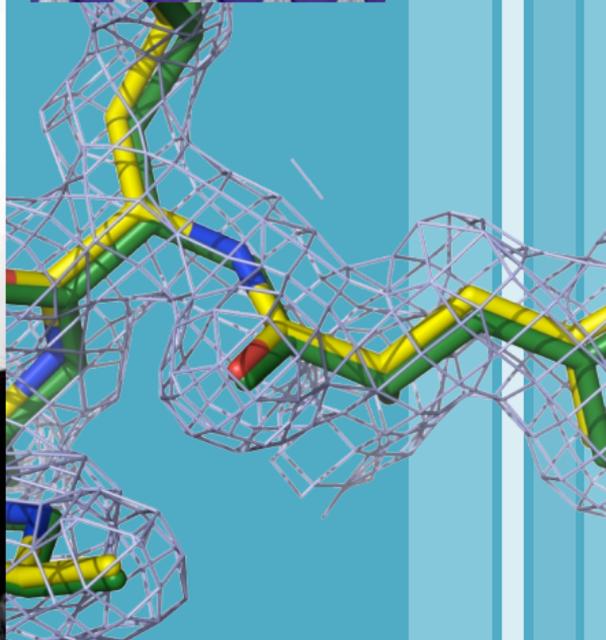
1 Search for peroxidases with novel properties of interest (such as self-sufficient monooxygenation and oxidation of chemically-inaccessible compounds) by **screening microbial cultures** and the exponentially-increasing **genomic resources**.

2 Structural-functional characterization of selected peroxidases to understand the bases of their catalytic properties, and to engineer them by a **rational design** based on the above information using site-directed mutagenesis and other techniques.

3 To perform directed evolution, and related tools, as a **non-rational alternative** with the aim of solving some of the drawbacks that cannot be rationally addressed because a direct structure-function relationship cannot be established.

4 To optimize strategies for the **production** of peroxidases adapted to the needs of gene **screening** and rational or non-rational **design**, as well as the use of **industrial hosts** for large-scale production of the selected enzyme candidates.

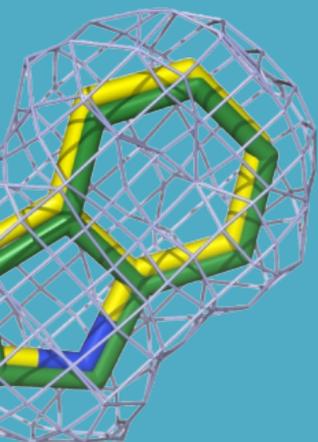
5 Detailed **chemical analysis** of reaction products from the new peroxidases to evaluate their industrial interest in **biodegradation** and **oxyfunctionalization** reactions using both aromatic and lipophilic substrates.



Enzymes catalyzing redox reactions

(**oxidoreductases**) represent an environmentally friendly **alternative to harsh chemicals** in industrial processes that include oxidative transformations for **production of chemicals** and other value-added products with large markets in developed and emerging economies.

**Peroxicats**
peroxidases as biocatalysts



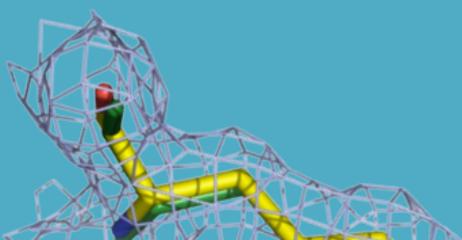
Fungi and other **microorganisms**

provide the wider and more easily **exploitable source** for oxidative enzymes.

However, the **penetration** of microbial oxidoreductases in the chemical markets is **still low** despite the **recent** discovery of very **promising** enzymes.

The use of these enzymes as biocatalysts requires **tuning** their **catalytic** and **operational** properties (a type of manipulation that is possible nowadays using protein engineering tools).

In addition to microbial screening, the huge amount of **genomic resources** available nowadays, and to be generated during the course of the project, will be **exploited** in the search for new fungal peroxidase/ peroxygenases.



Some of the main issues

presently limiting the industrial application of peroxidases will be addressed, such as:

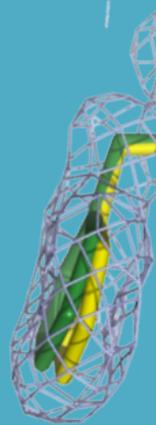
- Their suicide **inactivation** by H₂O₂
- Low functional **expression**
- Limited **oxygen transfer** potential

Moreover, the **catalytic properties** of the most interesting enzymes will be **modulated** to adapt them to the industrial processes.

A combination of **rational** and **non-rational** design will be used, based on directed mutagenesis, and random mutagenesis (together with high-throughput screening), respectively.

The reaction mechanisms and industrial interest of the selected peroxidases/ peroxygenases will be studied using **modern analytical techniques**, including two-dimensional NMR, that are able to provide information on the modifications produced on both simple and complex substrates.

In this way novel and robust peroxidases/ peroxygenases will be obtained with high potential both in **bulk chemistry**, e.g. for hydrocarbon **oxyfunctionalization** and **oxidation** of recalcitrant compounds, and **fine chemistry**, e.g. substituting costly hydroxylation reactions in the pharmaceutical sector.





Peroxocats

peroxidases as biocatalysts

[Peroxidases as biocatalysts]. Novel and more robust fungal peroxidases as industrial biocatalysts.

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More information: www.peroxocats.org