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Production, characterization, and optimization of engineered enzymes for application in artificial photosynthetic systems

The increasing exploitation of fossil fuels for the production of energy is closely related to increased CO₂ release into the atmosphere, with climate change as main consequence

[1]. Photosynthesis has been the subject of inspiration for the development of systems able to convert solar energy into chemical energy. Artificial photosynthesis (AP) might allow the production of not-polluting gases (O₂ and H₂) through light-driven water splitting [2].

The main focus of this project is the development and optimization of biological catalysts for their application in AP. Specific enzymes, such as Ir-substituted carbonic anhydrase [3], Ni-Fe and Fe-Fe hydrogenase [4], and Ni-Fe carbon monoxide dehydrogenase [5], have been selected, and will be investigated and modified to improve their chemical and physical properties for integration in AP systems.

To develop this project, the synthetic genes encoding for selected proteins of interest, including human carbonic anhydrase, were cloned in expression plasmids. The enzymes, produced in *E. coli*, are purified by combining different chromatographic techniques, including affinity and size exclusion. The structure and function of these enzymes will be characterized and investigated by combining various techniques, including structural studies by means of X-ray crystallography.

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