Molecular-dynamics study of horseradish peroxidase embedded in cubic phases

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Cubic phases are interesting objects obtained by mixing water and certain lipids in suitable proportions, where the lipid bilayer self-organizes according to minimal surfaces creating labyrinthine water channels [1] (Fig. 1). These objects form spontaneously e.g. during milk digestion, and are being widely studied due to their many applications, including biosensors based on nanoconfined enzymatic reactions [2], which can potentially operate even at subzero temperature [3].

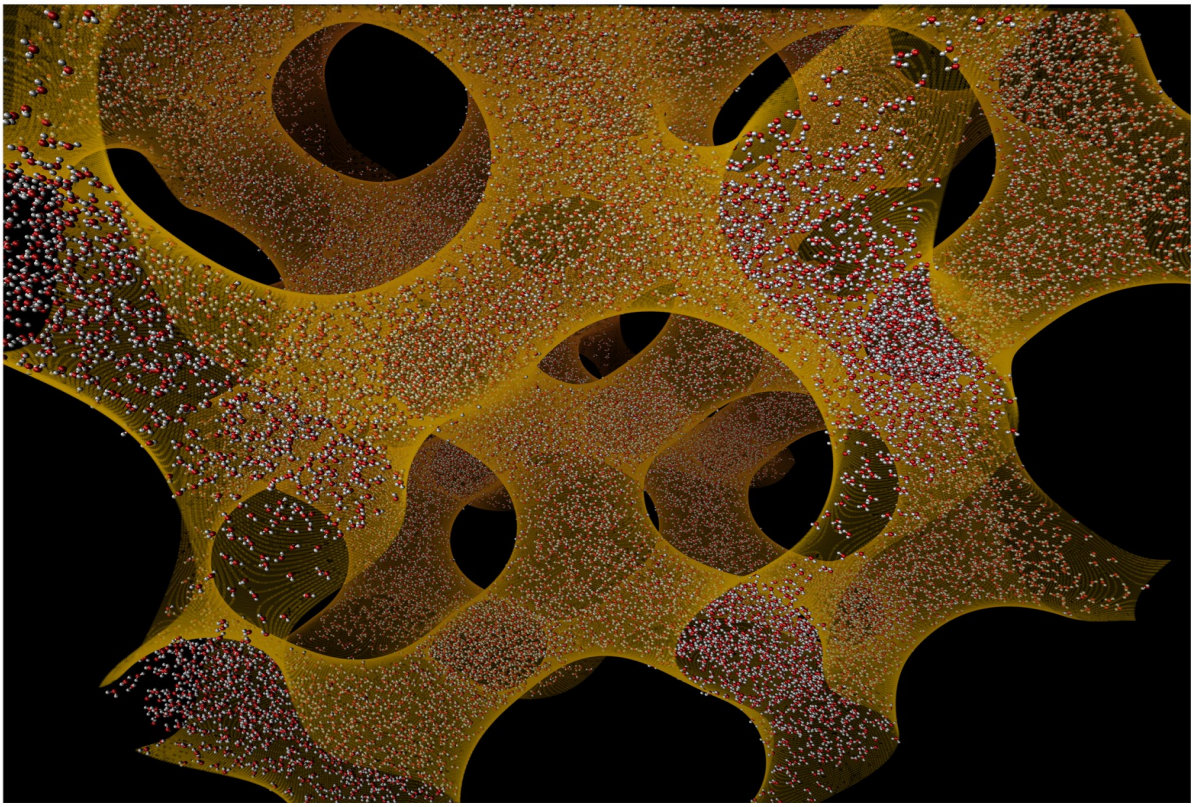

Figure 1: *Representative image of the geometry of a cubic phase (Pn3m symmetry). The lipid-water interface is depicted in orange, while representative water molecules (density not in scale) in the water channels are depicted as white and red beads.*

Experimental assays have proposed the use of cubic phases as hosts for enzymatic reactions, where the low mobility of the enzyme induced by the confinement is exploited for reusability of the enzyme itself [4]. Particularly, horseradish peroxidase is widely employed as a model system, due to its high level of characterization. Intriguingly, the experiments show that the kinetics of the enzymatic reaction within a cubic phase is strongly different than in bulk water [4]. Possible

microscopic mechanisms behind this qualitative change rely on the impact of the intricate geometry of the water channels on transport of reactants and products, the exotic properties of nanoconfined water or the different accessibility to the active center of the enzyme due to the small size of the channels.

From an experimental perspective, there is no way to ascertain to which extent each mechanism is affecting the outcome of the reaction. In this project, we will tackle this fascinating problem by means of molecular dynamics simulations, where the horseradish peroxidase and the cubic phase are simulated with atomistic accuracy. We will address how the enzyme adjusts to the nanoconfinining environment, thus understanding the accessibility of the active center and the change in the solvation water with respect to the bulk case. This will provide a novel source of precious information to better understand the kinetics of enzymatic reactions under confinement, thus aiding the development of future experimental assays.

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[3] Y. Yao, T. Zhou, R. Färber, U. Grossner, G. Floudas and R. Mezzenga, *Nature Nanotechnology* **16**:802-810 (2021).
[4] W. Sun, J. J. Vallooran, A. Zabara and R. Mezzenga, *Nanoscale* **6**:6853 (2014)