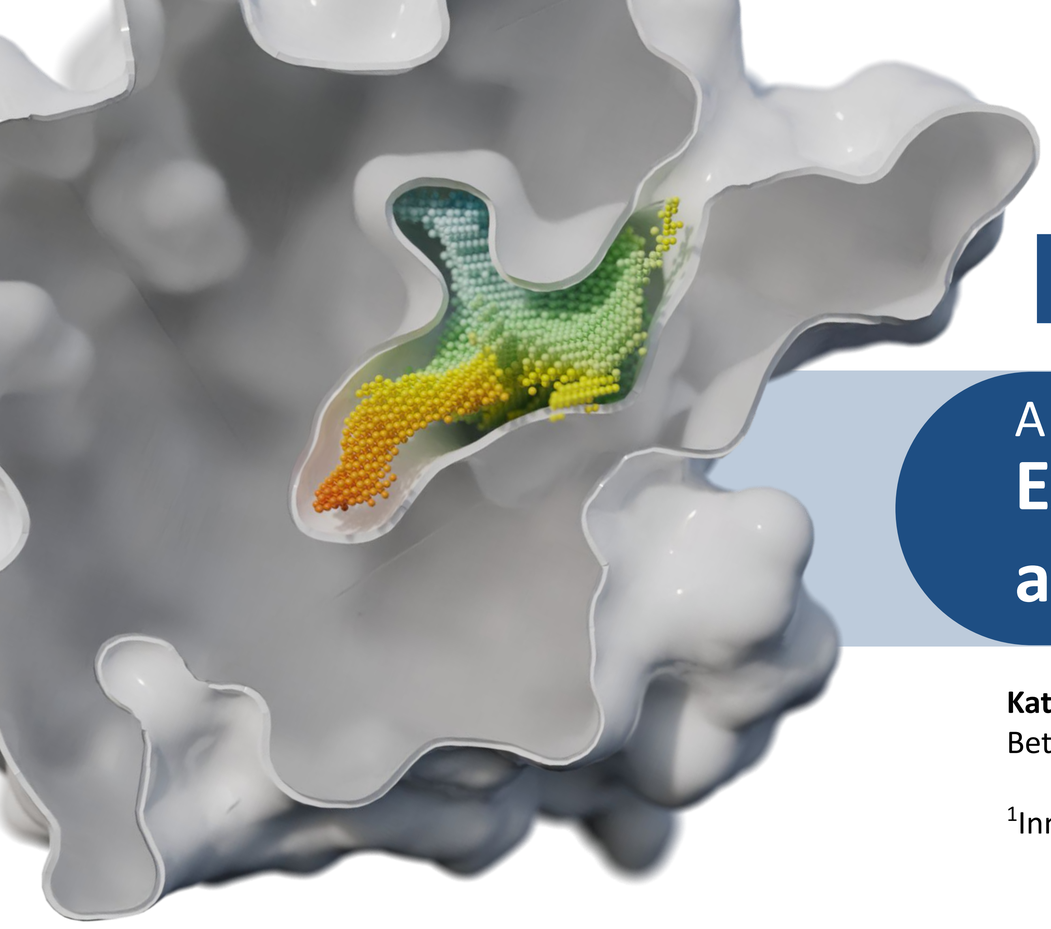

Innophore

A new way of searching

Exploring the mutational landscape of enzymatic active sites using 3D point clouds

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Catalophores: 3D point clouds **mapping enzymatic active sites**

The *catalophore* technology focuses on analyzing the binding sites, referred to as active sites or cavities, within enzyme structures.[2] These active sites are mapped using the patented 3D point-cloud technology, encompassing up to 19 physico-chemical properties, such as electrostatics, hydrophobicity, accessibility, hydrogen bonding potential, elasticity, among others (Fig. 1). The resulting *catalophores* serve as search templates to mine structural and sequence databases, identifying potential enzyme candidates suitable for industrial processes, biochemical reactions, and therapeutic applications across various scientific disciplines.[1]
Further, we systematically examine enzymatic active sites through our *catalophore*-driven deep learning approach, which serves to complement traditional methodologies.

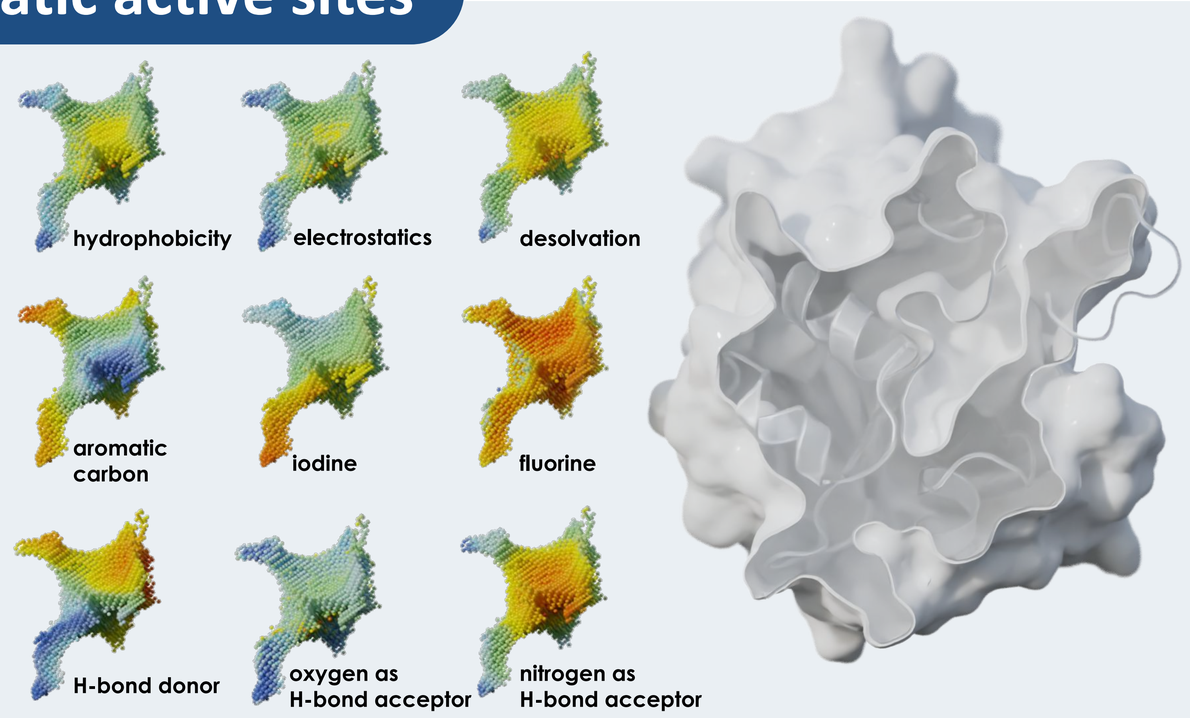

Figure 1: The physico-chemical properties inside a cavity are represented by *catalophore* point clouds.

Deep learning-based sampling of **active site cavities**

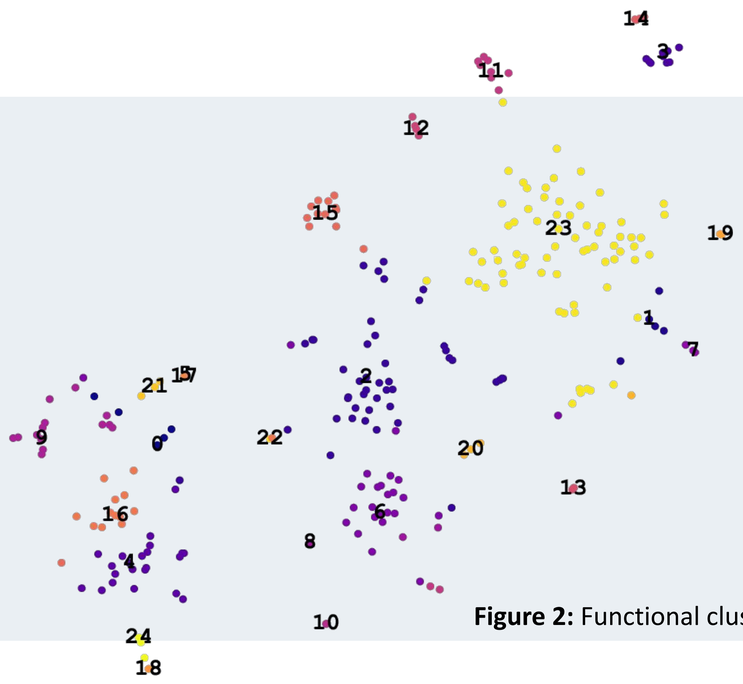

Figure 2: Functional clustering of active site *catalophores*.

To systematically explore the mutational landscape of an enzyme's active site, we developed an optimal sampling strategy leveraging *catalophore* point clouds, as demonstrated here using a hydrolase. We fine-tuned a deep learning model, trained to embed *catalophore* point clouds into a high-dimensional convex metric space, on the enzyme's sequence space. We generated all possible non-disruptive active-site single mutants of the enzyme and subsequently calculated their respective point clouds. A functional clustering was performed on the embedding space, allowing us to select 27 cluster representatives as candidates for further experimental testing (Fig. 2).

Enhancing **enzyme efficiency** while refining **training data**

Product accumulation and specific activity were assessed for the set of mutants representing different clusters. The sampling strategy revealed a diverse spectrum of hydrolase variants, exhibiting significant variability in product formations and specific activities (Fig. 3). Notably, the top variants doubled the specific activity and improved product formation rates by 50%, underscoring the effectiveness of our approach. Furthermore, this strategy established an optimal training set, thereby amplifying the effectiveness for future iterations by minimizing experimental costs and providing a robust baseline for predicting the performance of additional variants.

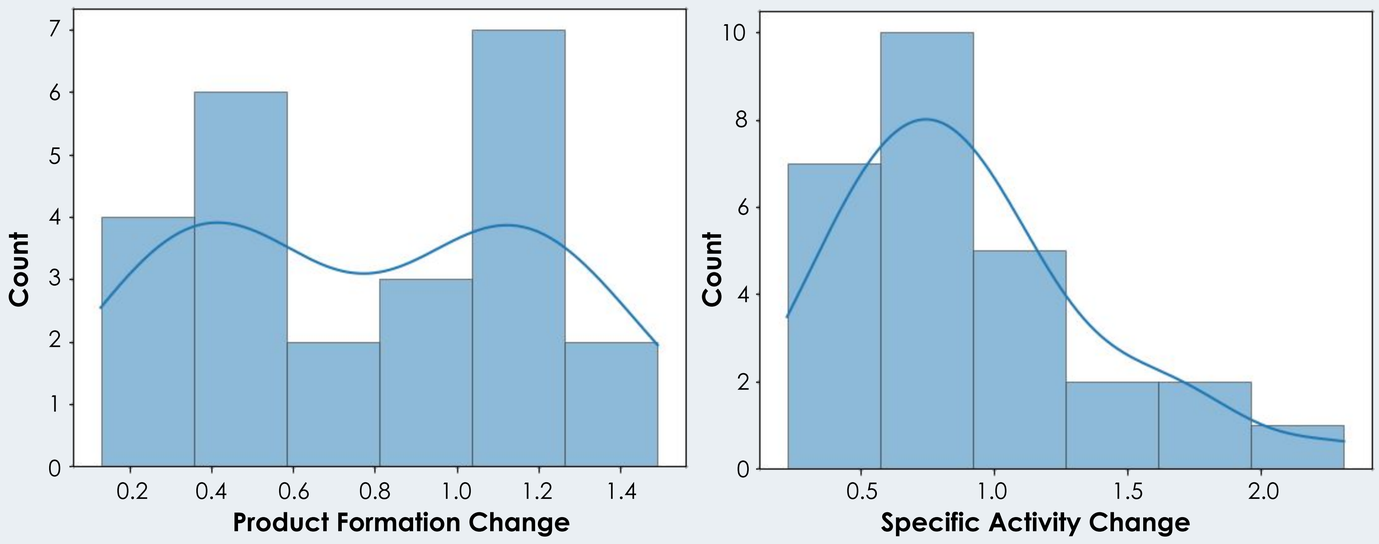

Figure 3: Changes in product formation and specific activity of mutants compared to the wild-type enzyme.

References

[1] Gruber, C. C., Steinkellner, G & Gruber, K. (2020) Determining novel enzymatic functionalities using three-dimensional point clouds representing physiochemical properties of protein cavities (WO Patent No. 2014/080005A1) World Intellectual Property Organization.
[2] Steinkellner, G., Gruber, C. C., Pavkov-Keller, T., Binter, A., Steiner, K., Winkler, C., Lyskowski, A., Schwamberger, O., Oberer, M., Schwab, H., Faber, K., Macheroux, P., Gruber, K. Identification of promiscuous ene-reductase activity by mining structural databases using active site constellations. (2014) Nature Communications, 5, art. no. 4150