Form Follows **Function**

Innophore

Enzyme & Drug Discovery

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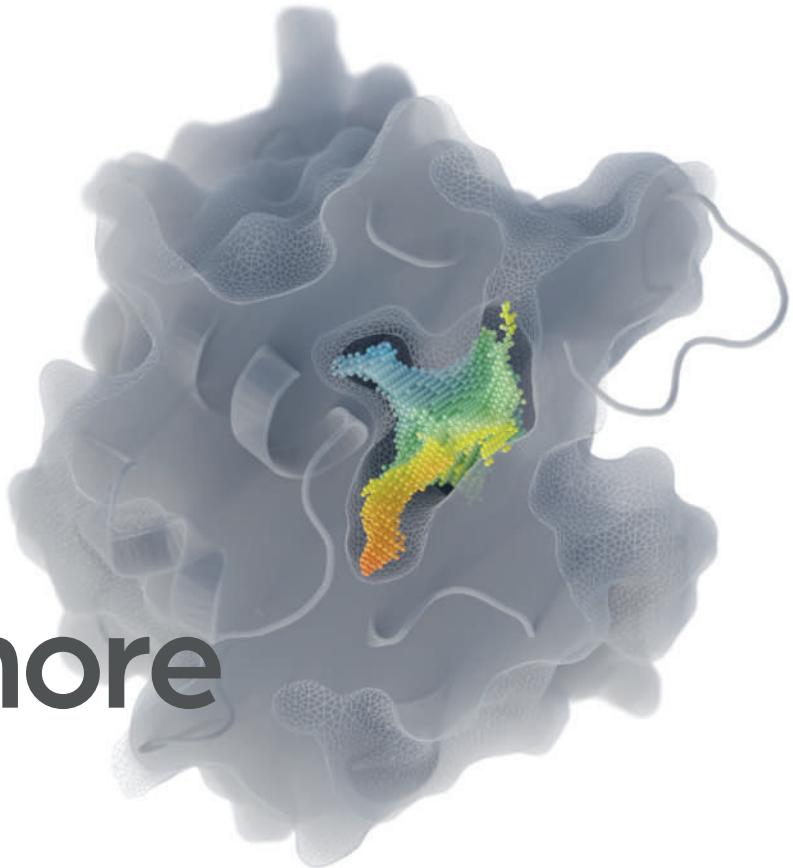


Innophore

ABOUT **INNOPHORE**

Innophore was founded in 2018 as a spin-off of the University of Graz and the Austrian Center of Industrial Biotechnology (acib). Since the start, the team has grown steadily, and we are continuously improving our technology. Interdisciplinarity is key to our success. Together we combine a broad expertise in the fields of molecular biology, biotechnology, (bio)chemistry, physics, machine learning, deep learning (AI) and computer sciences.

OUR **GOAL**

We are dedicated to identifying and developing high-value industrial and therapeutic enzymes by exploring the physico-chemical properties and structures of protein cavities. Our innovative Catalophore technology transforms the discovery and optimization of proteins and drugs. By streamlining this process, we foster innovation and effectively address the challenges posed by traditional sequence- or structure-based methods. Our versatile approach is tailored to tackle the complexities of biotechnology and pharmaceutical development, paving the way for advancements in these fields.

HIGH **PERFORMANCE** AND **BIG DATA**

High-performance supercomputing is our daily business. Digital experiments on our platform perform a wide range of tasks, including point cloud generation (for cavity detection), point cloud matching, homology modeling, and molecular docking. These experiments are run highly parallelized on our computer clusters. Extending our computational capabilities, we can also deploy all experiments to the cloud *via* Amazon Web Services (AWS) and Google Cloud. We mine, integrate, and standardize publicly as well as non-publicly available data sources such as PDB, PISA, UniProt, Pfam, and others, ensuring our platform operates with high-quality, comprehensive datasets.

CavitOmiX

Catalophore™ Cavity

WE SEE THE WORLD IN **3D POINT CLOUDS**

Point clouds are structurally independent representations of properties within a cavity or on a surface, where each point encodes a specific property at a precise location. We use point clouds as a means to decouple structural information from functional characteristics. They also serve as search templates to identify proteins with similar binding features. By generating point clouds, we produce simplified yet distinctive, machine-readable datasets that can be efficiently processed through our database. Millions of these point clouds are available for comparison, enabling rapid detection of similarities and functional matches. This forms the foundation of Catalophores, the art and science of understanding binding and interaction sites in biomolecules. Our technology allows us to bypass many of the traditional bottlenecks encountered in conventional approaches to protein and cavity analysis.

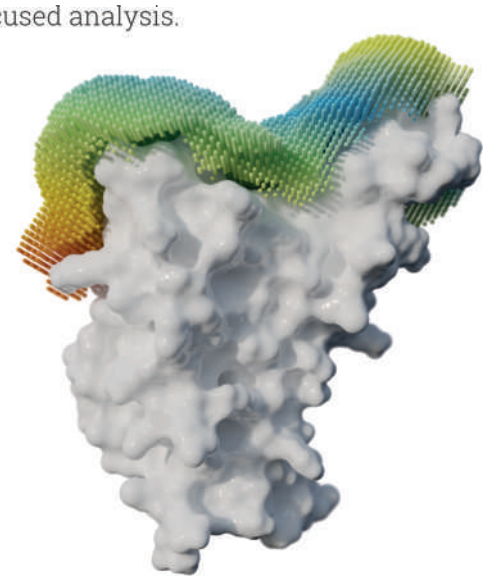
STANDARD IS NOT ENOUGH

Conventional methods often rely on a protein's sequence or overall structure as a search template, while overlooking the functional environment. By intentionally disregarding the surrounding protein shell of an active site, or the region where binding occurs, we shift the focus from structure to function. This functional-centric approach enables the discovery of proteins that traditional, structure-based search methods would likely miss. Our platform supports advanced applications such as next-level homology modeling, *in silico* protein engineering, docking, *in silico* protein modification, and MD simulations, pushing the boundaries of protein research and design.

Catalophore™ Halo

CAVITIES AND BEYOND

Halos provide a detailed representation of the surface environment and can be used to identify potential binding partners—such as proteins, ligands, peptides, or other surface interactions. This surface-level insight enables more accurate predictions of molecular interactions, expanding the utility of our platform beyond cavity-focused analysis.



WE **OFFER**

RaaS
Full CRO service – Idea to product
Using the Catalophore software and sub-contractors

SaaS
CavitOmiX and Copilots

(Shared-) **IPR**
Partnerships with shared-IPR
Internal IPR research

RaaS

WORKING WITH US – A COLLABORATIVE AND EFFICIENT PROCESS

We provide comprehensive bioinformatics Contract Research Organization (CRO) services designed to accelerate your research and development with remarkable speed and precision. Our streamlined approach and innovative Catalophore technology empower you to achieve faster, data-driven results without compromising quality. From the start, we collaborate closely with you to understand your unique needs and deliver a tailored project plan that maximizes efficiency. Using Innophore's advanced bioinformatics capabilities, we transform complex data into actionable insights swiftly, helping you make informed decisions sooner. Throughout the project, you'll enjoy transparent communication, clear milestones, and real-time access to our CavitOmiX platform, keeping you connected, informed, and in control every step of the way.
By partnering with us, you benefit from:

» Rapid turnaround times that accelerate your research cycle
» Our Catalophore technology for in-depth, high-throughput bioinformatics analyses
» Data-driven insights that improve accuracy and reduce uncertainty
» End-to-end project support tailored to your goals and timelines

SaaS

NEXT-GEN SOFTWARE FOR **ENZYME AND DRUG DISCOVERY**

We offer a Software-as-a-Service (SaaS) platform that provides rapid, scalable, and secure access to its proprietary enzyme and protein discovery technologies.
Through the cloud-based CavitOmiX platform, users can analyze molecular structures, identify active sites, and screen large compound libraries with ease. Complementing this, our online copilots are ready-to-use tools powered by extensive datasets, designed to simplify and accelerate scientific workflows. Tailored for pharmaceutical, biotech, and industrial partners, Innophore's SaaS model is built to speed up R&D processes, lower costs, and open new ways in enzyme engineering and drug discovery.

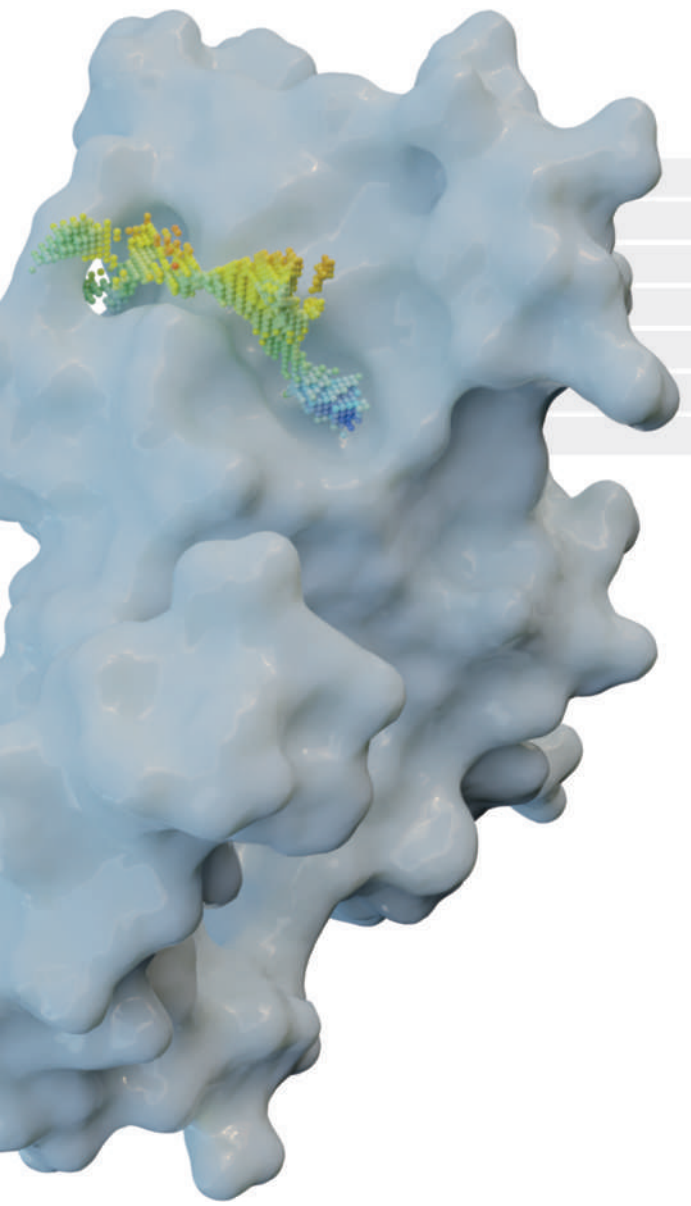
Whether you choose SaaS or RaaS, you get:

» **Private & Secure** – Your own dedicated workspace, backed by industry-leading security. Your data stays yours.
» **Full Access & Ownership** – Keep 100% of your IP and results, with complete access to the CavitOmiX Pro platform for viewing, analysis, and sharing.

With SaaS, you also enjoy:

» **All-Inclusive Computing** – Powerful infrastructure ready to run your projects, no setup needed.
» **Expert Support** – Technical and scientific guidance from our in-house specialists.
» **Seamless Data Management** – Upload, store, and organize your data with ease.

OUR EXPERTISE IN **BIOTECHNOLOGY**



Optimisation in the fields of...

We are involved in several projects where we optimize enzymes for diverse applications, tailoring their performance to meet specific industrial and research needs.

- Activity
- Stability (pH, solvent, temperature)
- Co-factor dependency
- Selectivity (stereo- and regioselectivity)
- Product specificity
- Solubility
- Inhibition reduction

ENZYME TRACK RECORD

Our team has a strong track record of working with a wide range of enzyme classes, backed by both theoretical knowledge and practical experience.

- » Peroxidases/peroxygenases
- » CYP P450s
- » Proteases
- » Flavoproteins
- » Methyltransferases
- » KREDs
- » IREDs/RedAms
- » Nitrilases
- » Glycosyl transferases
- » Transaminases
- » Cyclases
- » (De)hydratases
- » Epoxide hydrolases
- » Transferases
- » Lipases
- » Esterases
- » Oxygenases
- » Decarboxylases
- » Ligases
- » Kinases
- » Polymerases
- » Phosphatases
- » Haloperoxidases
- » ...

OUR EXPERTISE IN **PHARMA**

1. Drug Repurposing

» Identified FDA-approved drugs for SARS-CoV-2 MPro inhibition
» Identified Chikungunya nsP3 inhibitors
» Repurposed allergy medication for novel uses

2. Off-Target & Safety Profiling

» Modelled the most comprehensive screenable human proteome and their binding site Catalophores
» Screened 467 human proteins for off-target interactions
» Predicted host-pathogen cross-reactivity (Chikungunya nsP3, Tuberculosis lipase)

3. Protein-Protein Interaction Discovery

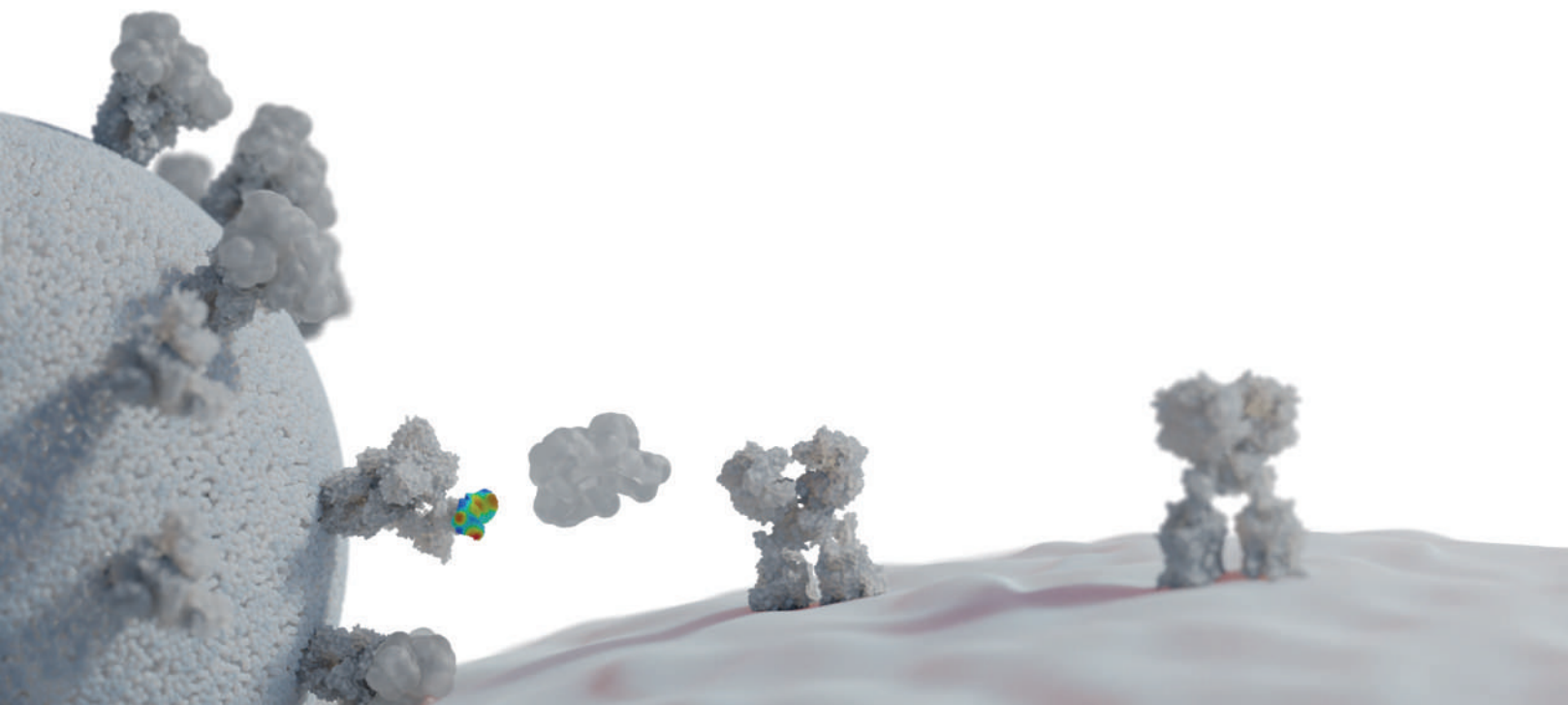
» Predicted NGFR and CD3e binding interfaces for immune modulation
» Characterised TIMP-1 interactions for oncology applications

4. Peptide & Protein Therapeutics Design

» Optimised antibody binding sites (Herceptin derivatives)
» Developed GPX4 inhibitors (neurodegeneration/oncology)
» Designed ACE2 decoys to block SARS-CoV-2 spike

5. Viral Evolution & Variant Tracking during Viral Outbreaks

» Monitored mutation landscapes for SARS-CoV-2, RSV, Influenza, Monkeypox, Chikungunya, Dengue, Zika
» Assessed implications of emerging mutations on protein-interaction level



DISCOVER WHAT'S POSSIBLE WITH INNOPHORE

FAST-TRACK RESEARCH PROJECTS

Our short-term projects, designed to last up to 5 working days, offer a proactive and effective approach to addressing specific scientific questions, evaluating feasibility, or enhancing ongoing research efforts. These fast-track studies prioritize both speed and scientific integrity, making them an excellent choice for decision support, early-stage discoveries, or generating crucial preliminary data. Led by expert scientists, each project culminates in a final meeting summarizing the findings. Additionally, when relevant, sequences and structural models can be easily accessed through our CavitOmiX platform.

Explore Our Range of Short Projects

1. Protein Structure Modeling
   » Utilize homology modeling and AI-based structure prediction.
   » Optionally, enhance your results with loop remodeling or molecular simulation studies.

2. Molecular Docking Studies
   » Conduct high-throughput *in silico* binding predictions between ligands and proteins.
   » Implement flexible docking that considers the selective flexibility of residues.
   » Optionally, refine your findings with pose rescoring using various scoring functions.

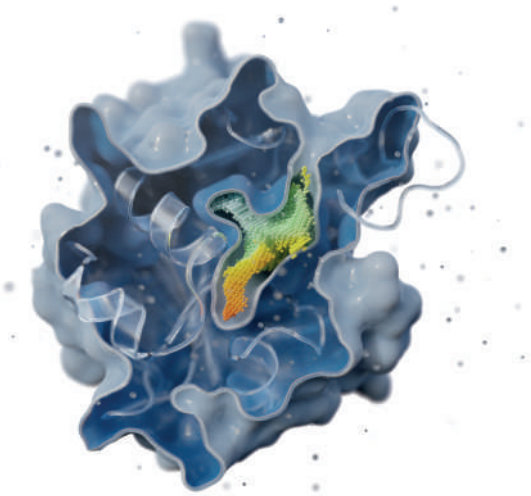
3. Binding Site and Cavity Analysis
   » Identify and characterize protein pockets.
   » Match high-interest pockets against our expansive databases, featuring over 1 million cavities from experimentally solved protein structures.

4. Drug Repurposing
   » Leverage cavity-based screening of over 200,000 ligand-binding sites in CavitOmiX Pro.
   » Identify potential binders through multidimensional binding-site similarity.
   » Gain deeper insights *via* molecular docking of these identified compounds.

5. Off- and On-Target Screening
   » Engage in multi-tool (AI-guided and physics-based) screening to discover alternative binding partners within over 800,000 human binding sites.
   » Initiate your exploration from the drug-target site: no chemical structures are necessary!

6. Surface Exploration - Halo Scan
   » Uncover potential binding sites on protein surfaces for small molecules, peptides, or protein partners.

7. Phylogenetic and Variant Analysis
   » Conduct phylogenetic profiling of protein families or viral proteins.
   » Analyze the dynamics of mutations within protein sequences.
   » Model the structures of protein variants and receive an in-depth comparison of their functional sites with Innophore's multidimensional point-cloud matching.



THE CavitOmiX COPILOT

SOFTWARE | DRUG DISCOVERY AND TARGET PROFILING

Accelerating **On- and Off-Target Discovery** With CavitOmiX Copilot

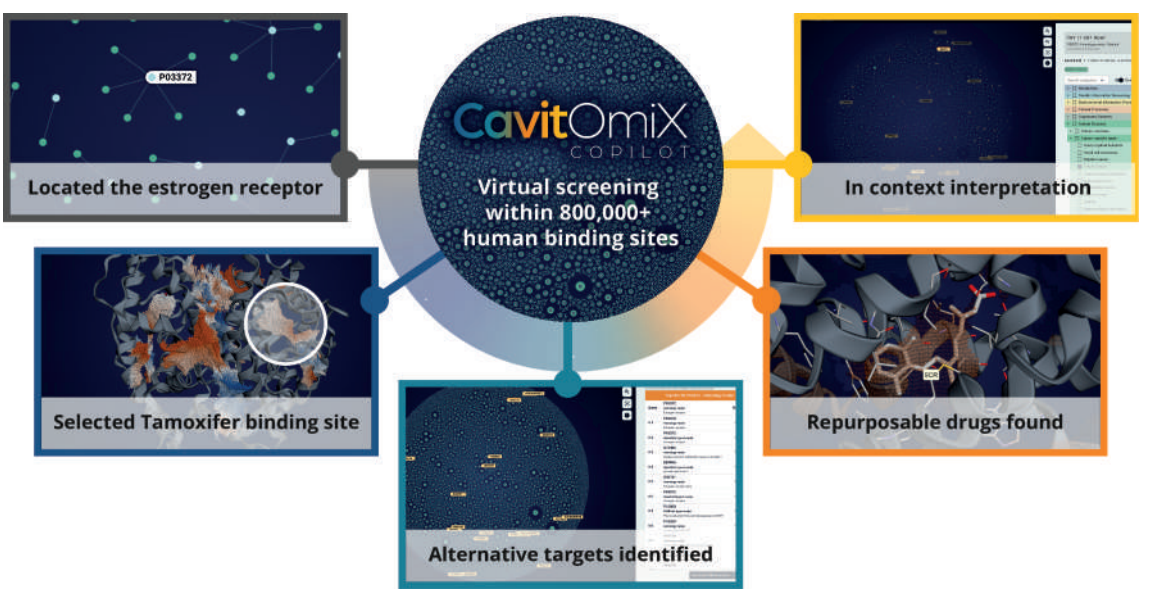
CavitOmiX Copilot empowers pharmaceutical innovators to rapidly uncover new therapeutic opportunities and minimize off-target risks. Our advanced web-based platform is screening over 800,000 human protein binding sites in seconds, revealing alternative protein targets and potential small-molecule binders. We have effectively leveraged our web-based application, CavitOmiX Copilot, to explore potential off-targets for anti-cancer drugs, including tamoxifen, which specifically targets the estrogen receptor (UniProt ID: P03372). By focusing on the drug-binding site of the estrogen receptor, we initiated a comprehensive similarity screening of over 800,000 human protein cavities, represented by our innovative multidimensional Catalophores. Within just a few seconds, the platform generated a ranked list of similar binding sites within the human proteome.

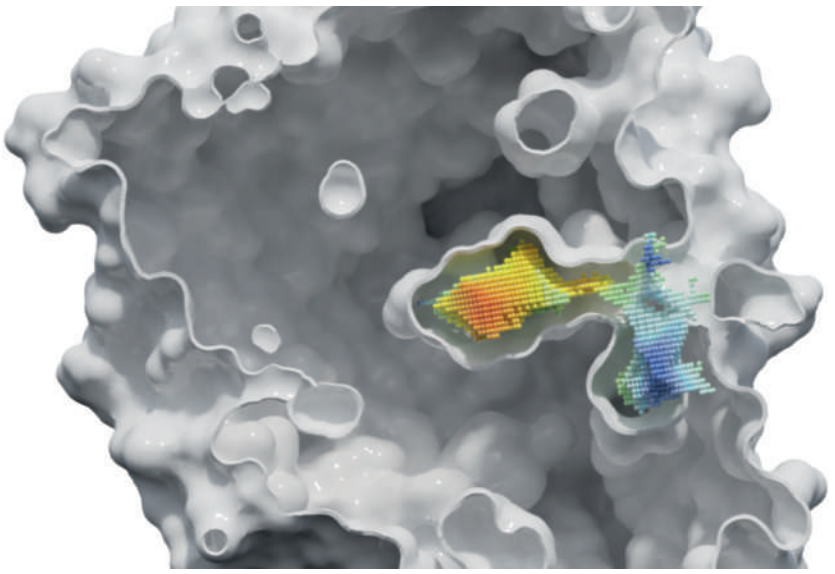
We identified the nuclear receptor RXRα (UniProt ID: Q15406) as the leading candidate, which is known to bind alitretinoin. The notable similarity between their binding sites indicates a potential interaction between alitretinoin and the estrogen receptor. This finding is particularly promising, as preclinical studies have shown that the combination of tamoxifen with retinoids, such as alitretinoin, can work synergistically to inhibit breast cancer cell growth by promoting apoptosis.

Furthermore, our investigations into integrated ligand-target databases have revealed a range of compounds that could be repurposed for therapeutic use. Some of the newly identified protein targets, which have not been previously linked to cancer or recognized as druggable, open up exciting new avenues for future research and development in cancer therapy

Start exploring!

https://copilot.cavitomix.bio/explore

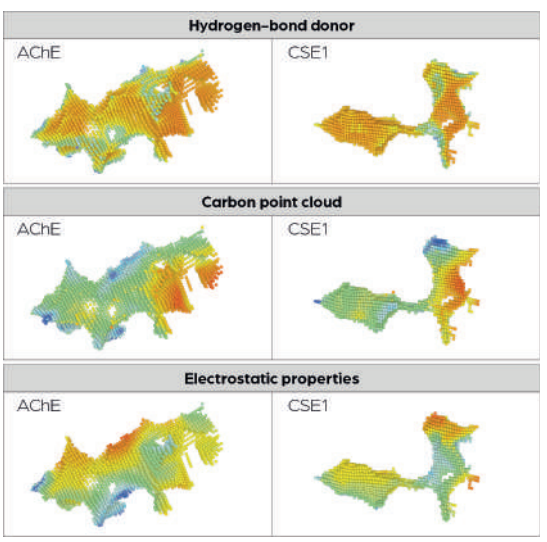




CASE STUDY | PROTEIN-LIGAND INTERACTIONS & DRUG DESIGN

Off-Target Identification

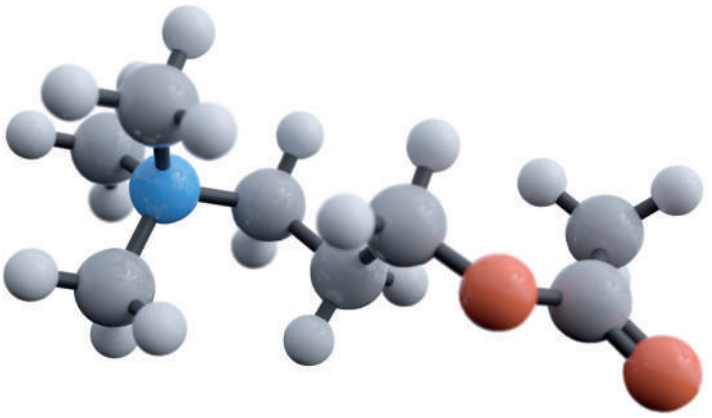
CavitOmiX enables the sensitive detection of potential off-target interactions early in the drug development process. Our approach leverages a proprietary library of over 800,000 Catalophores, representing the binding-site cavities of nearly all proteins in the human body. This extensive resource allows for comprehensive and systematic off-target screening. By operating ligand-agnostically and without relying on sequence or fold similarity, our technology is uniquely positioned to uncover off-targets across diverse protein families, including those that might be missed by conventional methods.



To demonstrate the effectiveness of our platform, we evaluated its performance on 500 known drug targets, each with experimentally validated off-targets across a broad range of protein classes. Remarkably, on average, 36% of known off-targets were identified among the top 10 matches, underscoring the power of our method to detect functionally relevant protein similarities.

A specific example highlighting this capability is our analysis of acetylcholinesterase (AChE), a key therapeutic target in Alzheimer's disease. Using our human Catalophore database, we performed a cavity-matching experiment and identified human carboxylesterase 1 (CES1)—a known off-target of AChE inhibitors—among the top-ranked results. This finding was achieved despite significant geometric differences between their binding cavities, demonstrating the platform's ability to detect subtle yet functionally meaningful similarities. This example illustrates the strong potential of CavitOmiX to deepen our understanding of off-target interactions and significantly improve the safety and efficacy of drug development.

Hetmann, M., Langner, C., Durmaz, V., Cespugli, M., Köchl, K., Krassnigg, A., Blaschitz, K., Groiss, S., Loibner, M., Ruau, D., Zatloukal, K., Gruber, K., Steinkellner, G. & Gruber, C. C. *Sci. Rep.* **13**, 11783 (2023).
https://doi.org/10.1038/s41598-023-39071-z



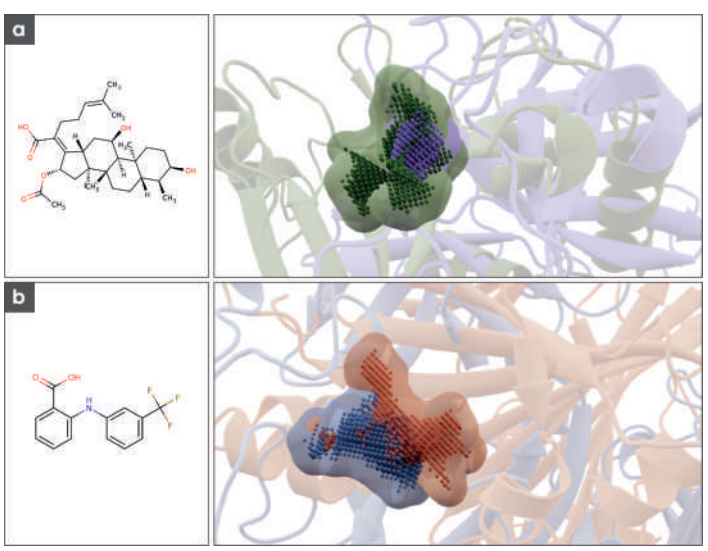
CASE STUDY | PROTEIN-LIGAND INTERACTIONS & DRUG DESIGN

Drug Repurposing: Cavity Screening Beyond the Human Proteome

The CavitOmiX platform presents an exciting opportunity to expand drug repurposing efforts beyond traditional off-target screening within the human proteome. Its core strength lies in the ability to analyze ligand-binding cavities, independent of sequence or structural characteristics, facilitating systematic drug repurposing by examining cavities in a variety of organisms.

In our research, we focused on two pivotal proteins involved in SARS-CoV-2 infection: the viral main protease M^{pro} and the human protease TMPRSS2. By calculating Cataloophores that reflect different dynamic states of their druggable active sites, we were able to screen these against ligand-binding cavities from the Protein Data Bank (PDB). This diverse dataset comprises proteins from various organisms, allowing us to identify small molecules that bind to analogous protein pockets, even when the proteins themselves lack structural or sequence similarity.

Following extensive computational screening, we selected ten FDA-approved drugs for validation in wet-lab infection assays. Notably, fusidic acid and flufenamic acid were found to effectively inhibit viral replication in cell-based assays. The source proteins associated with these ligands showed no sequence or structural resemblance to either viral or host targets, underscoring the unique capabilities of our cavity-driven, ligand-agnostic methodology. This innovative approach not only enhances the precision of drug repurposing but also opens up new avenues for rapid response to emerging viral threats, offering significant potential for future therapeutic development.



(**a**) The purple structure and cavity represent TMPRSS2, while the green corresponds to chloramphenicol acetyltransferase.

(**b**) The blue structure and cavity represent M^{pro}, while the red corresponds to the transcription factor TEAD2.

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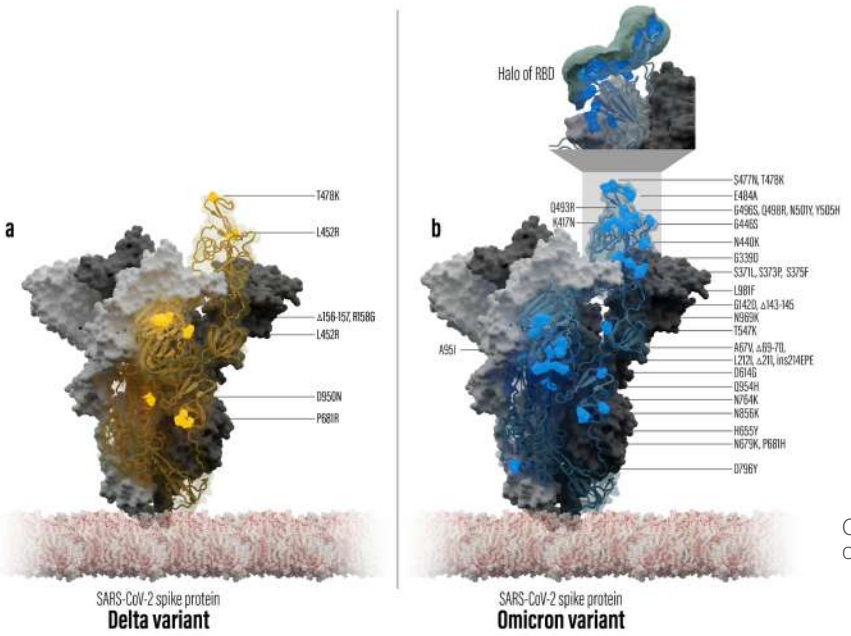


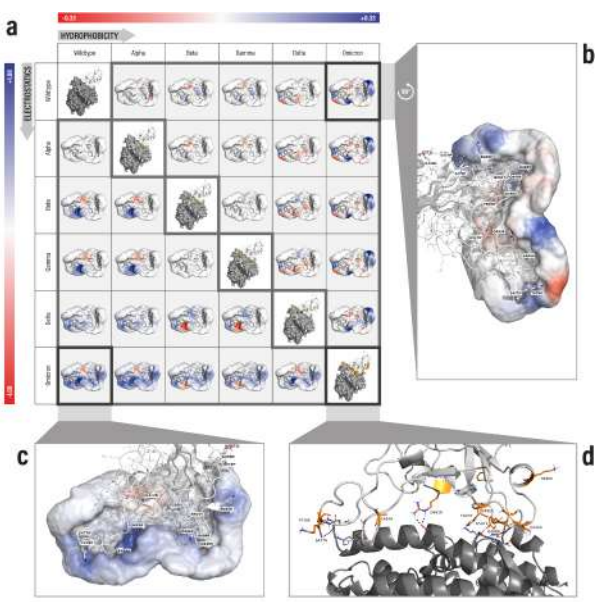
Comparison of mutation sites of Delta and Omicron variants.

CASE STUDY | PROTEIN-PROTEIN INTERACTION

Binding **Affinity**

Understanding and optimizing protein-protein interactions (PPI) is critical for advancing therapeutic design, particularly in areas like antibody engineering or PPI inhibitor design. We applied our Halo technology to explore the influence of amino acid exchanges on the affinity of the SARS-CoV-2 spike receptor-binding domain (RBD) for the human receptor, angiotensin-converting enzyme 2 (hACE2). We applied our Halo technology to explore the influence of amino acid exchanges on the affinity of the SARS-CoV-2 spike receptor-binding domain (RBD) for the human receptor, hACE2. In this process, we developed an empirical scoring function (ESF), closely related to the linear interaction energy (LIE) method, which draws on experimental binding data and MD simulations. Our analysis focuses on the effects of various amino acid substitutions. We thoroughly examined the Halos for both the wild-type and different RBD variants. When our comparisons revealed significant changes, we integrated these variants into our ESF-based MD modeling pipeline to predict their effect on binding affinity.

This approach effectively provided early structural insights and binding affinity estimates, allowing us to anticipate findings before experimental complex structures and binding data of an emerging SARS-CoV-2 variant became available.



Pairwise spike RBD variant comparisons with Halo point clouds.

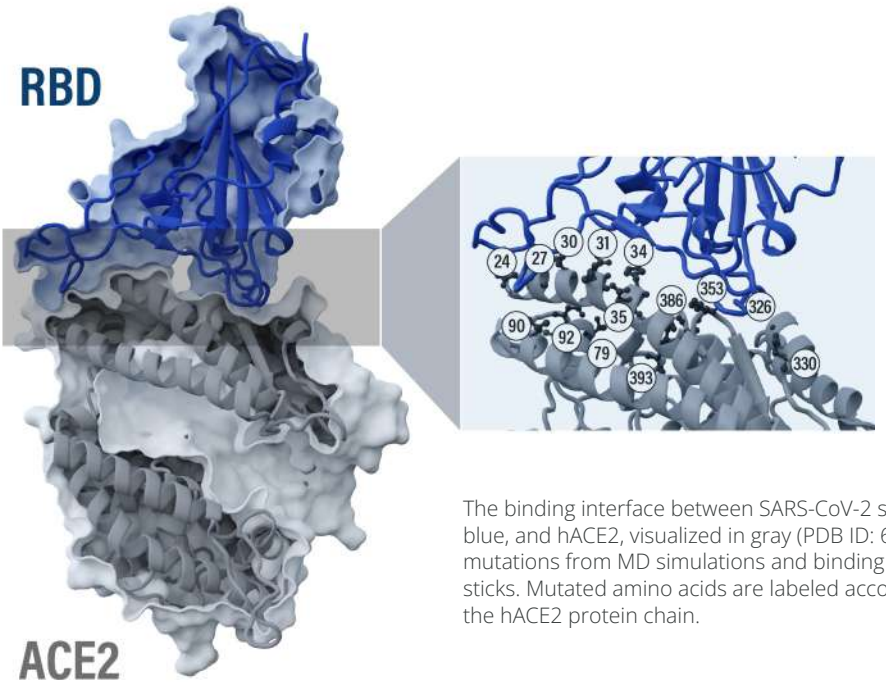
a: The difference between the Halo field values of two variants are shown; upper triangle: hydrophobicity differences; lower triangle: electrostatics differences; diagonal: binding interfaces of spike RBD variants in complex with hACE2.

b: Hydrophobicity difference field between wild-type (WT) and Omicron RBD.

c: Electrostatic difference field between wild type and Omicron RBD.

d: Omicron RBD–hACE2 binding interface showing an additional hydrogen bond between Arg493 (RBD) and Glu35 (hACE2).

Durmaz, V., Köchl, K., Krassnigg, A., Parigger, L., Hetmann, M., Singh, A., Nutz, D., Korsunsky, A., Kahler, U., König, C., Chang, L., Krebs, M., Bassetto, R., Pavkov-Keller, T., Resch, V., Gruber, K., Steinkellner, G. & Gruber, C. C. *Sci. Rep*. **12**, 14534 (2022). https://doi.org/10.1038/s41598-022-18507-y

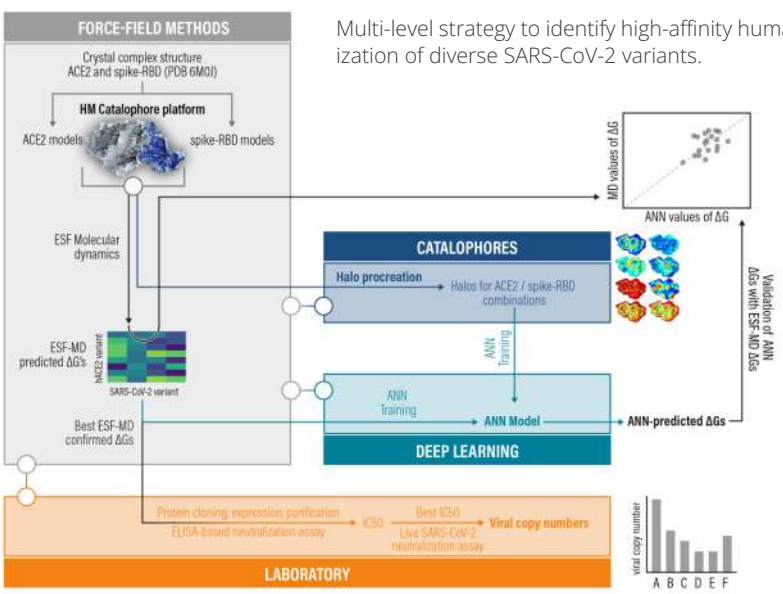


The binding interface between SARS-CoV-2 spike RBD, shown in dark blue, and hACE2, visualized in gray (PDB ID: 6M0J). Selection of hACE2 mutations from MD simulations and binding affinity assays are shown as sticks. Mutated amino acids are labeled according to their position in the hACE2 protein chain.

CASE STUDY | PROTEIN-PROTEIN INTERACTION

Evaluating and Enhancing **Protein-Protein Binding Affinity**

The Catalophore Halo technology, combined with extensive MD simulations, was used to investigate how amino acid changes affect the affinity of the SARS-CoV-2 spike receptor-binding domain (RBD) for the human receptor hACE2. This approach also aimed to design high-affinity variants of hACE2 as potential therapeutic decoys. A deep learning model, trained on the resulting data, enables the rapid prediction of binding affinities between different SARS-CoV-2 spike variants and hACE2 variants. Several promising hACE2 variants have been validated *in vitro* and have shown significantly enhanced activity against SARS-CoV-2 infections compared to the wild-type hACE2. This strategy is broadly applicable to various protein-protein interactions, streamlining the protein design process and reducing the number of samples needed for experimental testing.



Multi-level strategy to identify high-affinity human ACE2 (hACE2) variants for neutralization of diverse SARS-CoV-2 variants.

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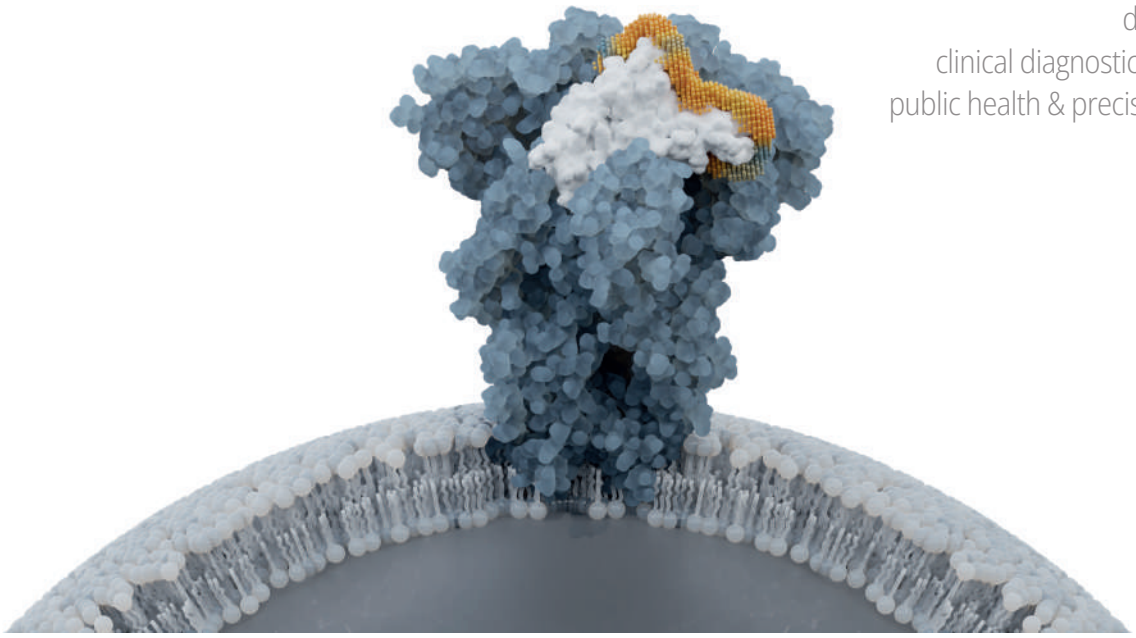
Köchl, K., Schopper, T., Durmaz, V., Parigger, L., Singh, A., Krassnigg, A., Cespugli, M., Wu, W., Yang, X., Zhang, Y., Wang, W. W.-S., Selluski, C., Zhao, T., Zhang, X., Bai, C., Lin, L., Hu, Y., Xie, Z., Zhang, Z., Yan, J., Zatloukal, K., Gruber, K., Steinkellner, G. & Gruber, C. C. *Sci. Rep.* **13**, 774 (2023). https://doi.org/10.1038/s41598-023-27636-x

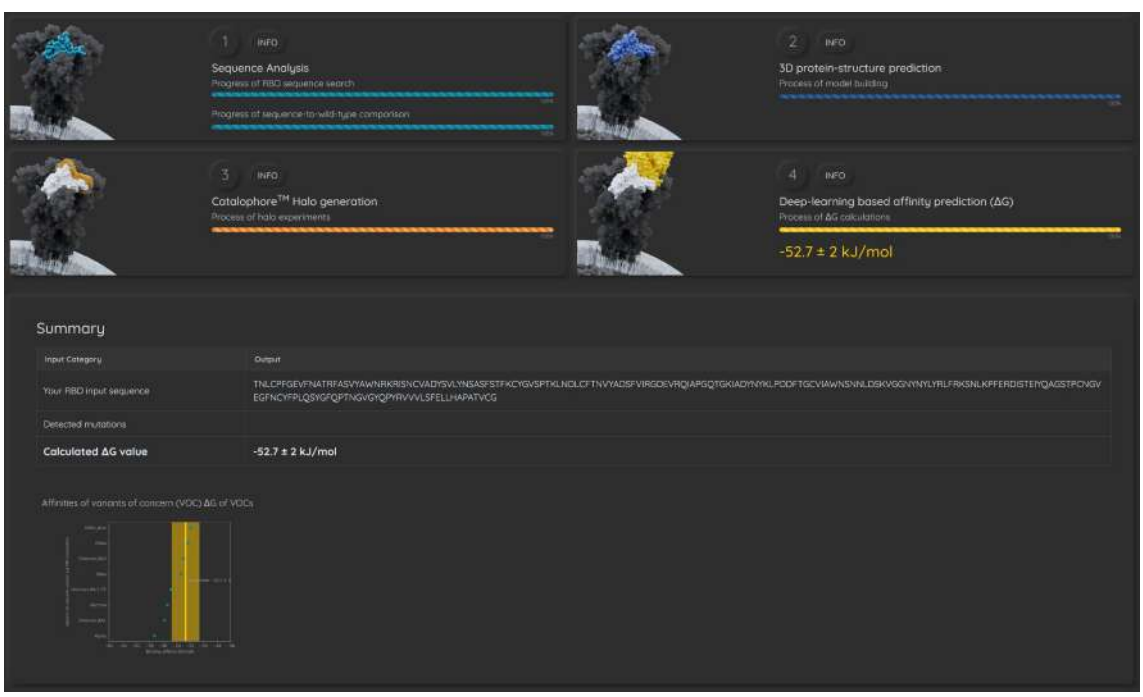


CASE STUDY | VIRUS WATCH

Virus Watch - AI-based SARS-CoV-2 Affinity Prediction

In partnership with Amazon Web Services (AWS) and as part of the global Diagnostic Development Initiative (DDI) program, we have successfully developed an innovative and user-friendly web application that predicts the binding affinity between the SARS-CoV-2 receptor-binding domain (RBD) and the human angiotensin-converting enzyme 2 (hACE2). Our approach utilizes advanced structural and model data, harnessing the power of our Catalophore point-cloud technology to forecast emerging SARS-CoV-2 variants. This application serves a vital role in supporting diagnostics and drug development by identifying potentially concerning or novel infectious variants. By providing meaningful predictions, we aim to enhance confidence and preparedness in addressing new variants of the pathogen.

To explore the app and learn more, please visit: **https://covid.virus.watch**



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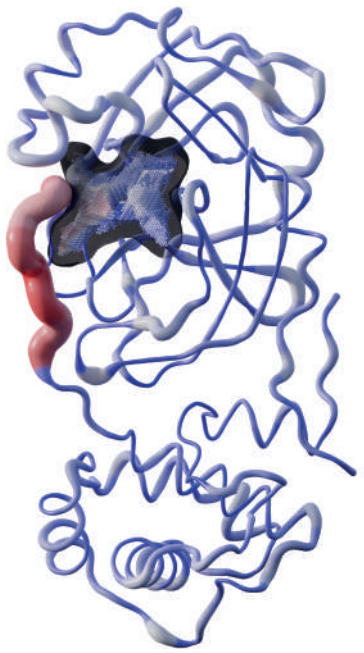


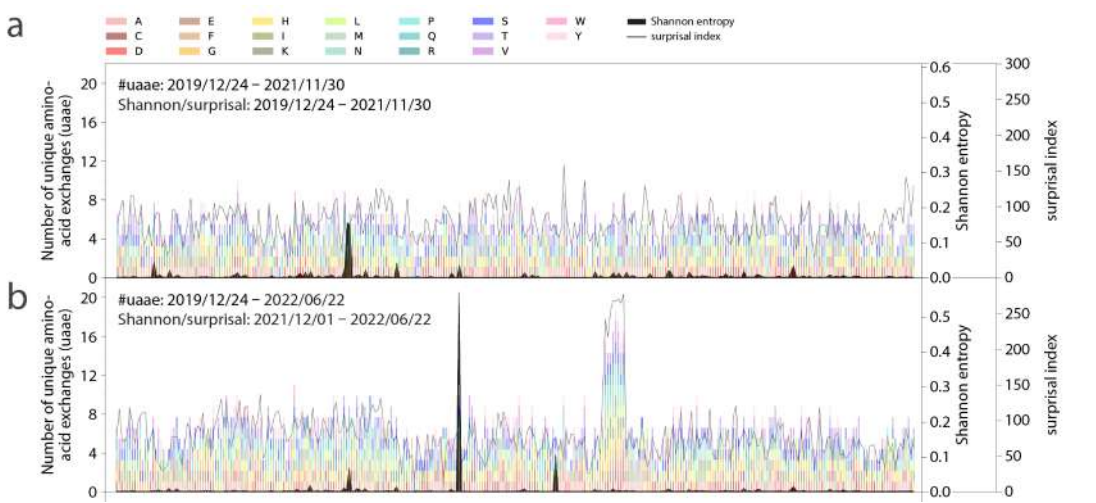
Structural representation of mutation dynamics showing unique amino-acid exchanges along the protein sequence, including genomes sampled until June 2022. Thickness of the ribbon corresponds to number of mutations present at given position.

CASE STUDY | DISEASE MONITORING

Tracking **Mutational Dynamics**

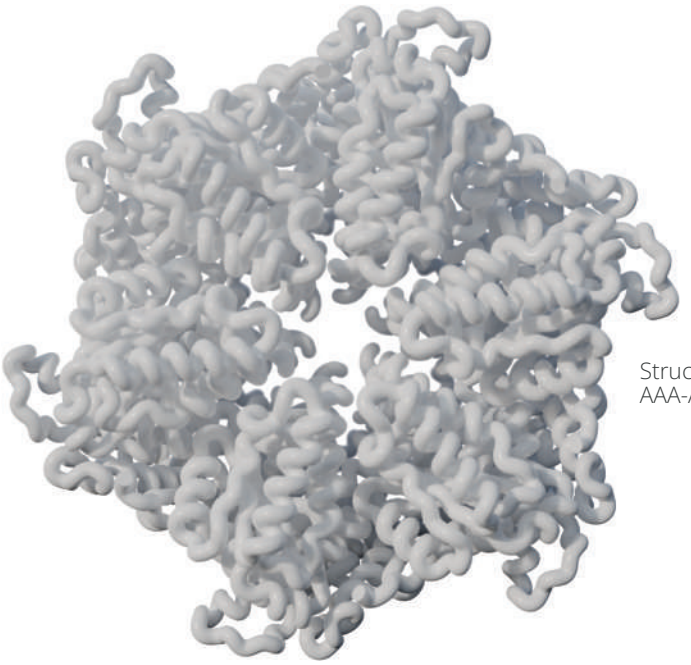
Understanding how amino acid mutations affect functionally critical protein regions is essential for anticipating drug resistance, improving therapeutic strategies, and guiding surveillance efforts. Catalophore-based cavity analysis enables precise tracking of mutational changes within binding sites and other key functional regions.

During the Covid-19 pandemic, our analysis of SARS-CoV-2 genomic data revealed an accelerated accumulation of mutations near the active site of the main protease (M^{pro}) shortly after the first antiviral targeting this site was granted emergency use authorization in December 2021. Our findings underscore the importance of monitoring mutational dynamics in drug-targeted regions, where selective pressure can rapidly drive amino acid substitutions that may affect therapeutic efficacy.



Mutation dynamics in the M^{pro} amino acid sequence over two different time periods.

Parigger, L., Krassnigg, A., Schopper, T., Singh, A., Tappler, K., Köchl, K., Hetmann, M., Gruber, K., Steinkellner, G. & Gruber, C. C. *Front. Med.* **9**, 1061142 (2022). https://doi.org/10.3389/fmed.2022.1061142



Structure of hexameric AAA-ATPase Drg1 (AFG2).

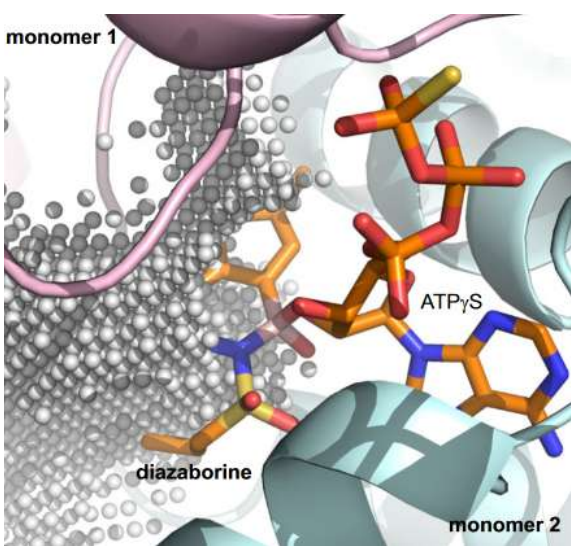
CASE STUDY | PROTEIN-LIGAND INTERACTIONS & DRUG DESIGN

Drug **Resistance**

The hexameric AAA-ATPase Drg1 plays a pivotal role in ribosome biogenesis in eukaryotes by facilitating the release of the shuttling maturation factor Rlp24. Diazaborine, a known inhibitor, disrupts this release. To enhance our understanding of the inhibition mechanism, we determined the cryo-EM structure of the Drg1 hexamer in complex with diazaborine, providing a valuable framework for future investigations.

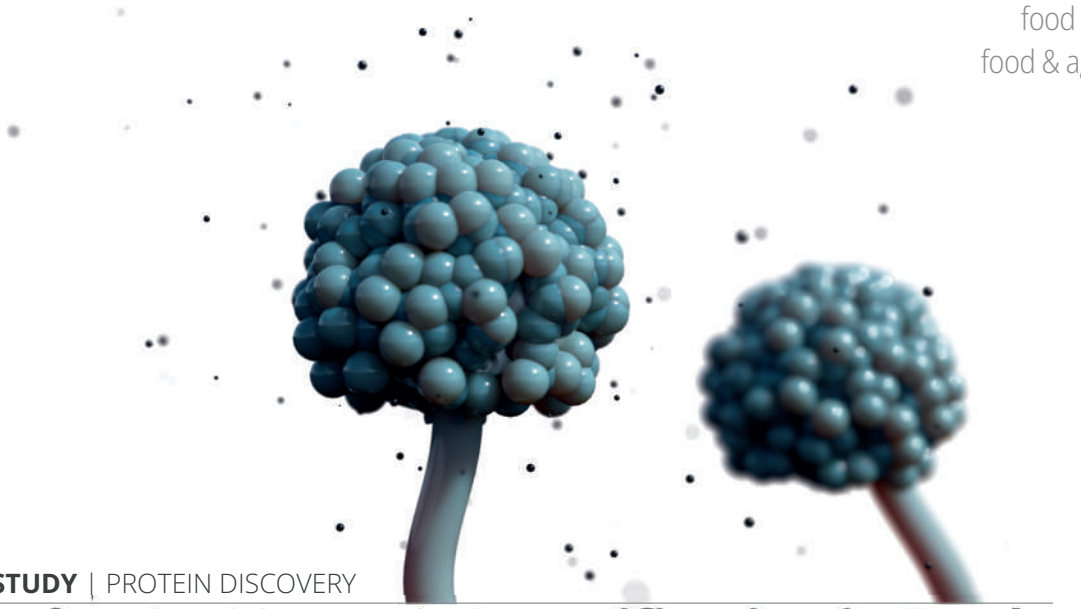
Furthermore, we performed a comprehensive cavity analysis using our Catalophore technology to evaluate the available space for inhibitor binding in proximity to ADP molecules within the homology model. This analysis not only sheds light on the binding interactions but also identifies potential avenues for developing diazaborine resistance.

Through this approach, we discovered resistance-causing mutations near the D2 nucleotide-binding pocket, highlighting the spatial incompatibility between diazaborine and the binding site. This insight opens up new possibilities for designing more effective inhibitors.



Cavity analysis of the binding pocket of the model. The cavity near ADP is represented as light gray spheres. Larger parts of the diazaborine molecule not covered by the point cloud indicate that the inhibitor is incompatible with the available space in the active site of Drg1.

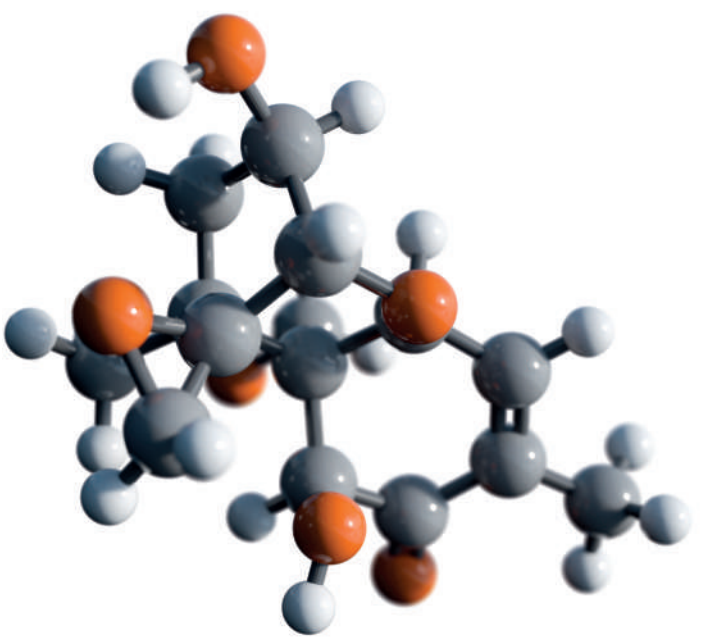
Prattes, M., Grishkovskaya, I., Hodirnau, V. V., Rossler, I., Klein, I., Hetzmannseder, C., Zisser, G., Gruber, C. C., Gruber, K., Haselbach, D. & Bergler, H. *Nat. Commun.* **12**, 3483 (2021). https://doi.org/10.1038/s41467-021-23854-x



CASE STUDY | PROTEIN DISCOVERY

Transforming Mycotoxin **Detoxification in Food and Feed Safety**

A critical opportunity in enhancing food and feed safety lies in the effective conversion as well as detoxification of both established and emerging mycotoxins, as well as various harmful secondary metabolites produced by fungi. These substances pose significant health and economic challenges. Traditional approaches to discovering enzymes for mycotoxin conversion and detoxification tend to rely heavily on sequence or structural similarities. This often results in overlooking innovative or potentially more effective biocatalysts.

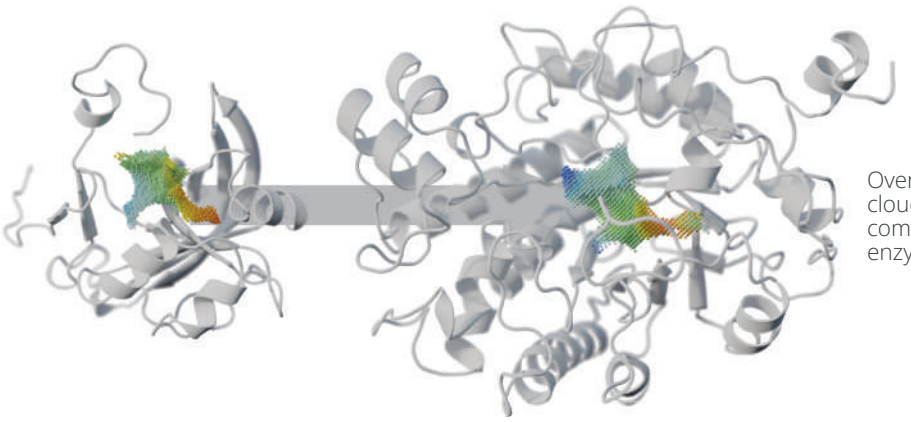


Structure of the mycotoxin DON (deoxynivaleol).

Innophore's Catalophore technology offers a promising solution by analyzing the three-dimensional structures and chemical properties of enzyme active sites. This sophisticated approach enables the identification of new enzymes that can transform or neutralize a wide variety of (also emerging) mycotoxins and secondary metabolites, regardless of their overall protein similarities to previously known enzymes.

By utilizing advanced *in silico* screening, the company has successfully discovered and validated enzymes that effectively break down toxins, such as aflatoxins and deoxynivalenol (DON). These promising candidates exhibit enhanced stability and activity under various processing conditions. This cutting-edge approach not only expedites the research and development timeline and reduces costs but also broadens the company's innovation pipeline and enhances its operational flexibility.

Overall, the transformative impact of the Catalophore technology on enzyme discovery empowers the development of effective solutions for detoxifying food and feed, paving the way for a safer food supply.

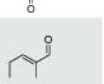
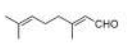


Overall structure and point cloud of search template (left) compared to newly identified enzyme (right).

CASE STUDY | BIOCATALYSIS IN DRUG DEVELOPMENT & MANUFACTURING

Unexpected **Enzymatic Activity**

Almost a decade ago, we made significant strides in structural bioinformatics by developing a novel method for predicting the catalytic activities of enzymes based on the three-dimensional arrangements of functional groups in their active sites, which we have termed Catalophores. As part of our proof of concept, we successfully identified two enzymes with predicted promiscuous ene reductase activity, specifically targeting the reduction of activated C=C double bonds, and we compared their activities to those of established ene reductases. Despite completely different amino acid sequences (sequence identity of 9%), overall structures and protein folds, high-resolution crystal structures revealed comparable binding modes for known substrates and ligands of the Old Yellow Enzyme (a well-characterized flavin-dependent ene reductase), along with similar catalytic activities. Additionally, we were able to identify enzymes that displayed switched enantioselectivity, highlighting the potential of our method to uncover unique enzyme characteristics and functionalities.

	*Ph*ENR		*Tt*ENR	
	c (%)	e.e.(%)	c (%)	e.e.(%)
	17	-	3	-
	28	87 (S)	81	4 (S)
	>99	15 (S)	45	12 (S)
	19	96 (R)	<1	n.d.
	77	62 (S)	86	77 (S)

Selected substrates used to verify the ene reductase activity of the newly found enzymes (PhENR = ene reductase from *Pyrococcus horikoshii*, TtENR = ene reductase from *Thermus thermophilus*).

c, conversion; *e.e.*, enantiomeric excess; n.d., not determined

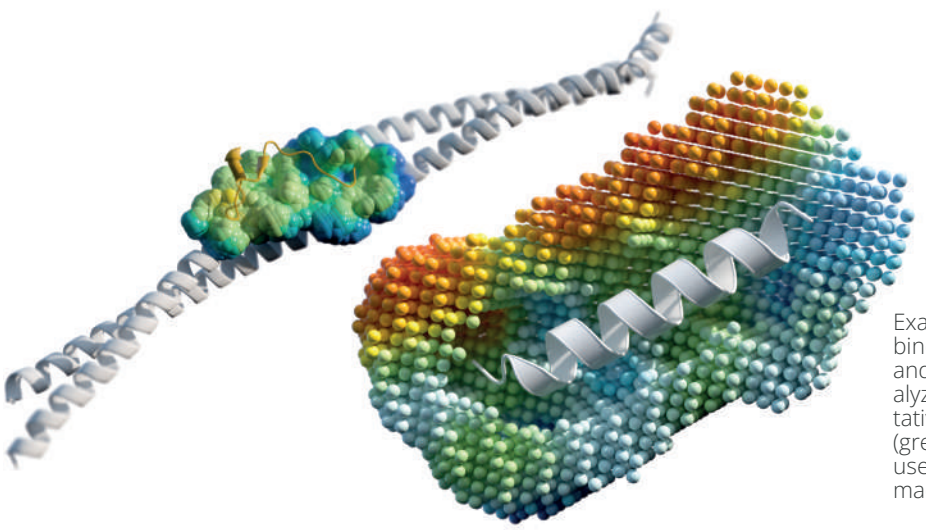
Steinkellner, G., Gruber, C. C., Pavkov-Keller, T., Binter, A., Steiner, K., Winkler, C., Lyskowski, A., Schwamberger, O., Oberer, M., Schwab, H., Faber, K., Macheroux, P. & Gruber, K. *Nat. Commun.* **5**, 4150 (2014).
https://doi.org/10.1038/ncomms5150

CASE STUDY | STRUCTURE-BASED LIGAND DESIGN

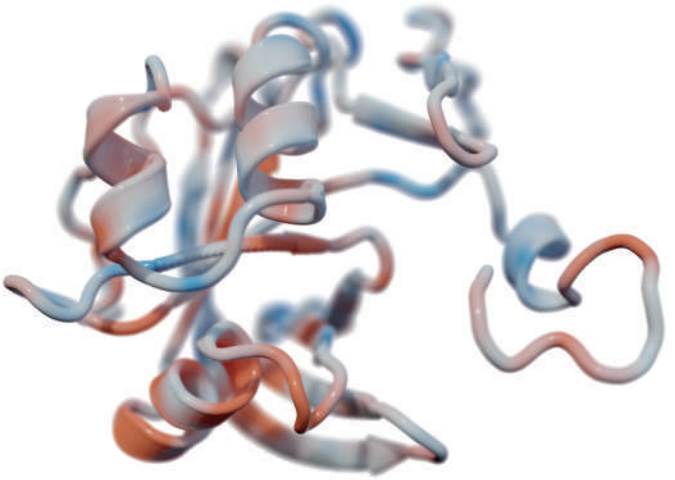
Synthetic **Peptides**

Targeted interactions with biological materials are essential for functional peptide design. We focused on developing peptides that selectively bind to specific proteins and lipids. We started with the modeling of the relevant protein complexes and lipid layers and created a diverse library of peptides, comprising protein fragments, engineered binders sourced from existing literature, and innovative *de novo* peptide designs. We developed structural models for thousands of peptides and used physico-chemical properties to identify the most promising candidates, leveraging a Halo point cloud-based machine learning workflow. This was followed by large-scale MD simulations to comprehensively assess the interaction behaviors of the peptides with the biological materials and estimate their binding affinities. As a result, we were able to curate a focused set of candidates for experimental testing.

Our peptide design workflow showcases significant potential across various sectors, including pharmaceuticals, diagnostics, cosmetics, and the food industry. This research not only deepens our understanding of peptide interactions but also lays the groundwork for innovative solutions in these pivotal fields.



Example peptide structure (yellow) binding to the protein target (gray) and Halo at their interface used to analyze the binding site (left). Representative structural model of a peptide (grey) with its corresponding Halo, as used in the Halo point cloud-based machine learning workflow (right).

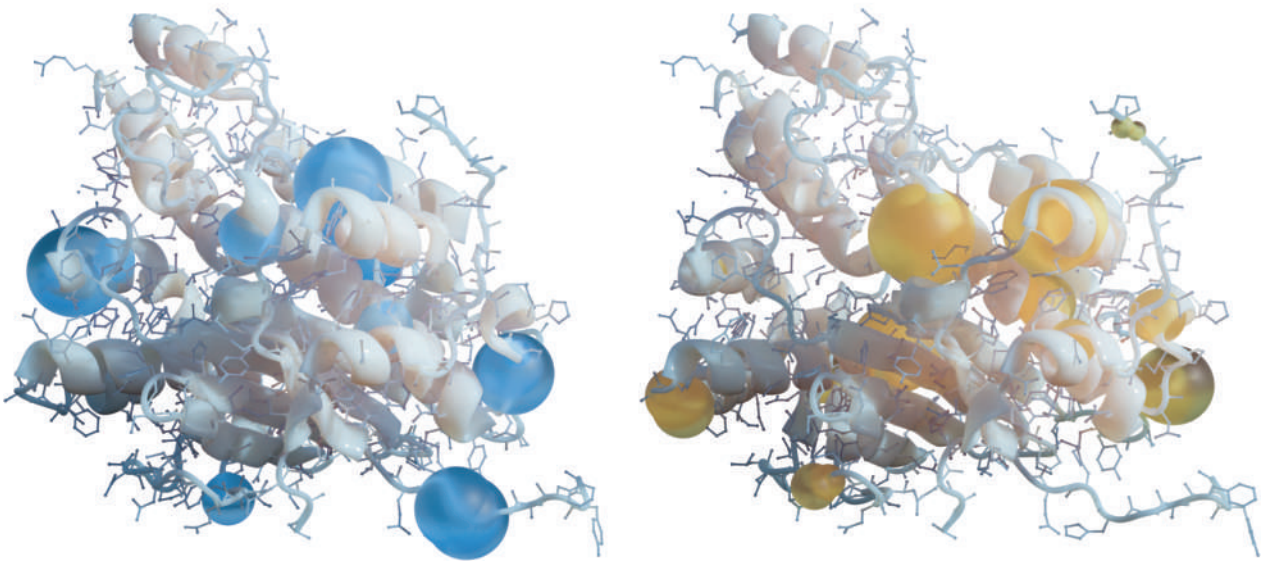


CASE STUDY | PROTEIN ENGINEERING

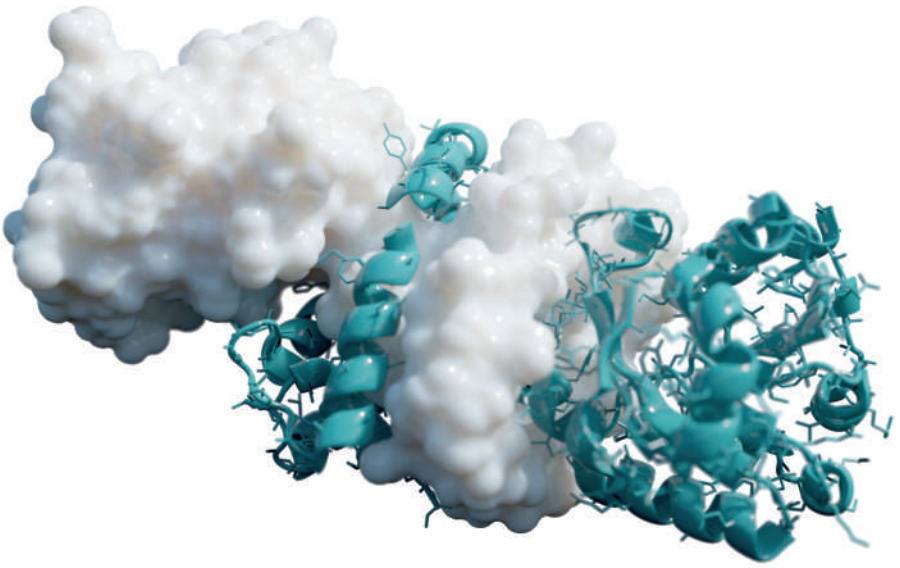
Engineering Highly **Active, Robust, and Selective Enzymes**

We conducted a series of enzyme engineering projects focused on redox and hydrolytic enzymes, aiming to develop highly active and process-stable enzymes while minimizing the generation of undesired side products. Utilizing the Catalophore technology alongside evolutionary conservation analysis, strategic rational mutagenesis, computational protein design workflows leveraging state-of-the-art tools such as Rosetta or ProteinMPNN, ancestral sequence reconstruction, and deep learning-guided predictions, we executed structure-based design campaigns. These campaigns targeted stability-enhancing motifs and key residues that are crucial for selectivity and catalytic performance.

The resultant enzyme variants exhibited significant enhancements: redox enzymes displayed increased product conversion and selectivity, along with a marked reduction in side product formation. Concurrently, hydrolytic enzymes demonstrated substantial improvements in both activity and stability. These findings underscore how our methodology can facilitate the optimization of enzymes. The approaches employed can be readily adapted for application across a diverse range of enzyme classes.



Positions targeted for enzyme engineering based on predictions from the stability workflow. Blue spheres (left picture) indicate proposed locations for introducing new salt bridges, while yellow spheres (right picture) indicate proposed sites for introducing disulfide bridges to enhance stability.



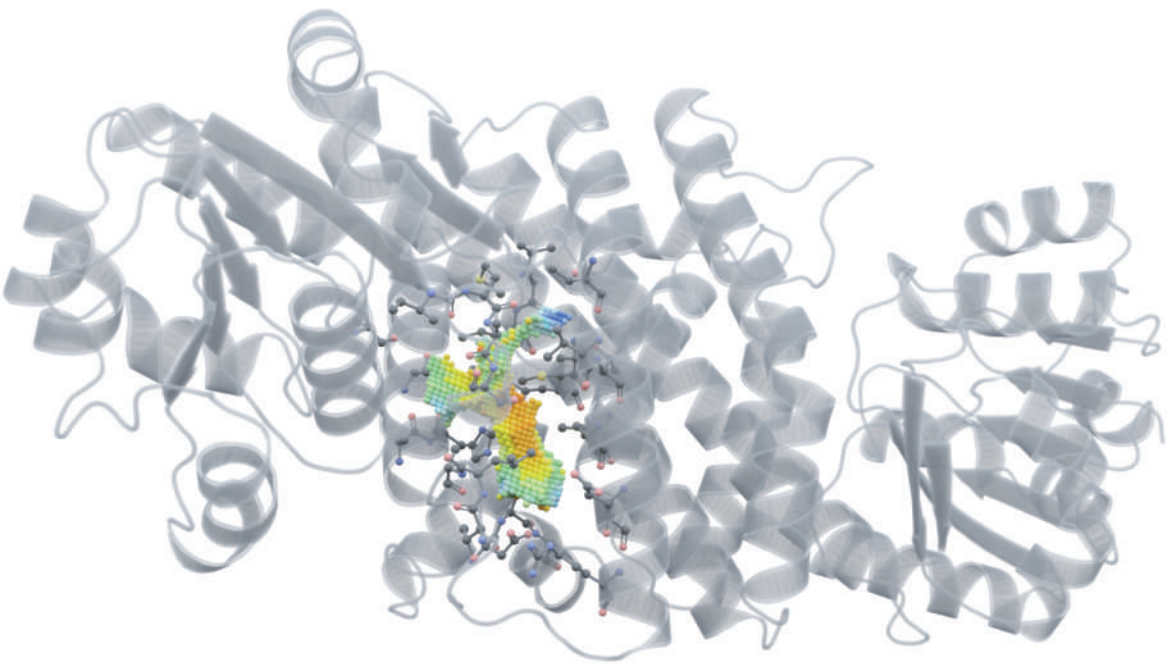
SOFTWARE | PROTEIN DISCOVERY

IRED Copilot - Simple and Rapid Enzyme Search

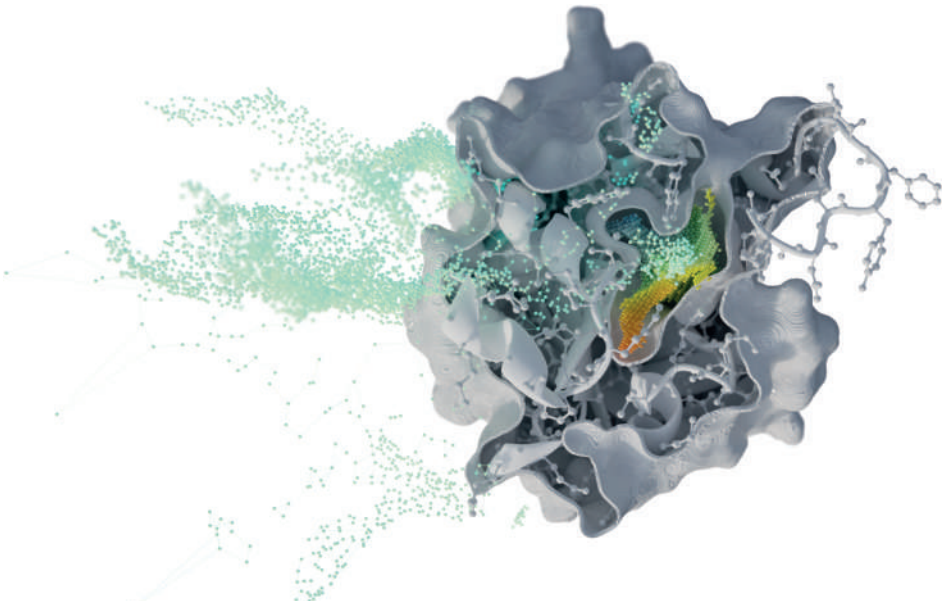
When a leading pharmaceutical research team set out to develop a novel chiral amine for a next-generation drug, they chose Innophore's IRED Copilot to accelerate their enzyme discovery process. By simply entering their target substrate into our AI-powered platform, they rapidly explored a curated database of over 10,000 natural IREDs/RedAms, identifying the most promising enzyme candidates in just minutes. With detailed binding site insights powered by Catalophore point clouds, the team confidently selected enzymes with optimal active site properties, ensuring the highest likelihood of success. Downloadable protein structures and annotated cavities were seamlessly integrated into their computational workflows, accelerating modeling and design. Automated, print-ready reports made it easy to share results and plan next steps, keeping the project on track and stakeholders informed.

Start exploring!

https://ired-copilot.cavitomix.bio/explore



Active site of an IRED visualized through Catalophore electrostatic point clouds, with key active site residues aligned for structural and electrostatic comparison.



OUR BIOINFORMATICS **TOOLS & TECHNOLOGY**

We leverage cutting-edge computational tools in combination with expert insights at every stage of our projects. By incorporating experimental feedback through our "lab in the loop" approach, we continuously strive to ensure that our data-driven results are effectively grounded in real-world applications. This collaborative methodology fosters innovation and enhances the reliability of our findings.

Technology track record:

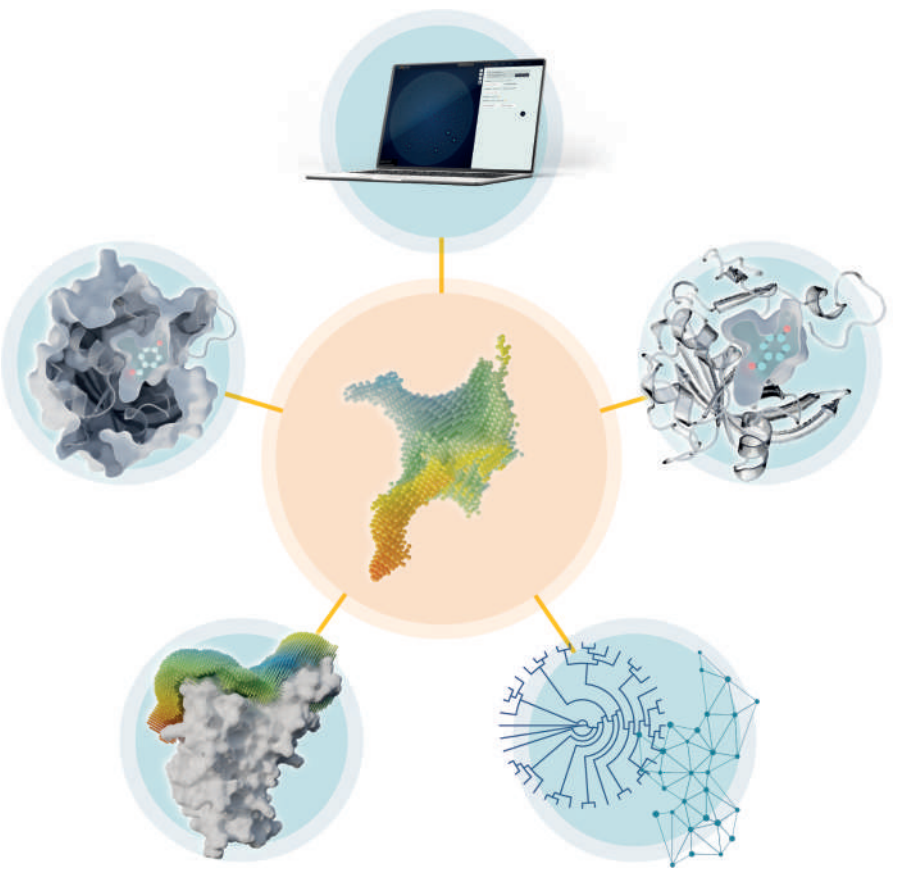
- » CavitOmiX platform
- » CavitOmiX Copilots
- » In-house stability workflow
- » In-house sequence analysis tools (phylogeny, clustering, consensus-driven evolution)
- » In-house DeepSeek-R1
- » Rosetta Suite
- » YASARA Structure
- » GROMACS
- » RFDiffusion
- » ProteinMPNN and LigandMPNN
- » AutoDock Vina / Gnina
- » Protein folding tools (Boltz-1, Colabfold, ESMFold, YASARA homology modelling)
- » State-of-the-art offline sequence analysis tools (e.g. BLAST, MAFFT, MMSeqs2, GRASP, EpHod)
- » Typical Python libraries (e.g., Biopython, RDKit, MDTraj, Pytorch, LightningAI, SKlearn, XGBoost)
- » C++, Flask, React for high-performance applications and modern frameworks

Tools and datasets include:

- » 98% of the experimental structures from RCSB PDB
- » 100% of the biological assemblies from PDBe and PISA
- » AlphaFold protein structure data
- » About 4 million cavities and variants thereof
- » With and without cofactors and metal ions
- » Shaped around ligands (enclosing the molecule)
- » High-throughput distributed automatic comparative modeling
- » Tailored *in silico* mutant libraries
- » Synthetic proteins including non-canonical amino acids
- » Non-public sequence data

READY TO GET **STARTED**?

Contact us to explore how a short project can address your current research question or open new directions. We're happy to discuss feasibility, tailor the scope to your specific needs, and ensure a quick turnaround.



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