It’s giving dEVA: An Algorithm for Structure-Based Enzyme Design

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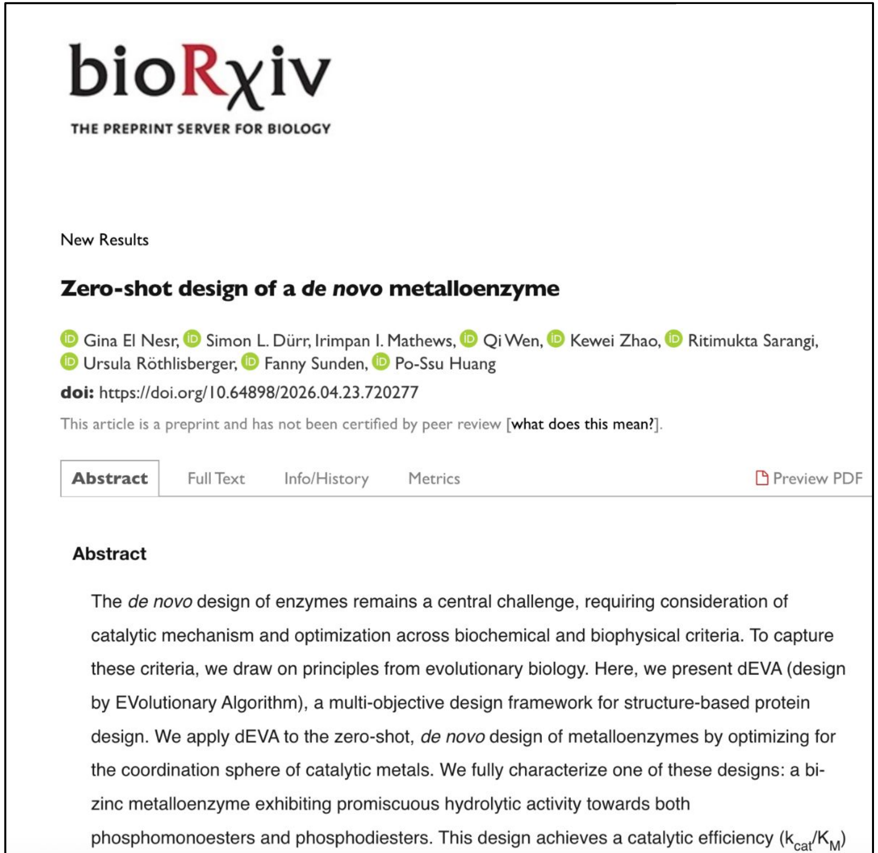
Zero-shot design of a de novo metalloenzyme

1. Foundational tools and challenges in *de novo* enzyme design
2. Design of *de novo* metalloproteins
3. Training data curation and model sensitivity
4. Design of *de novo* metalloenzymes
5. Conclusions
6. Strengths and Weaknesses
7. Future Directions



New Results

Zero-shot design of a *de novo* metalloenzyme

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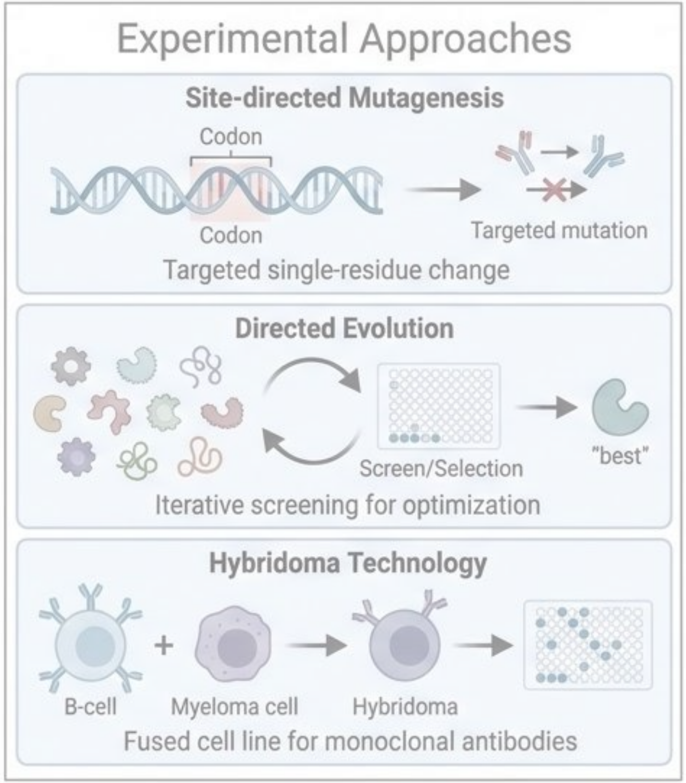
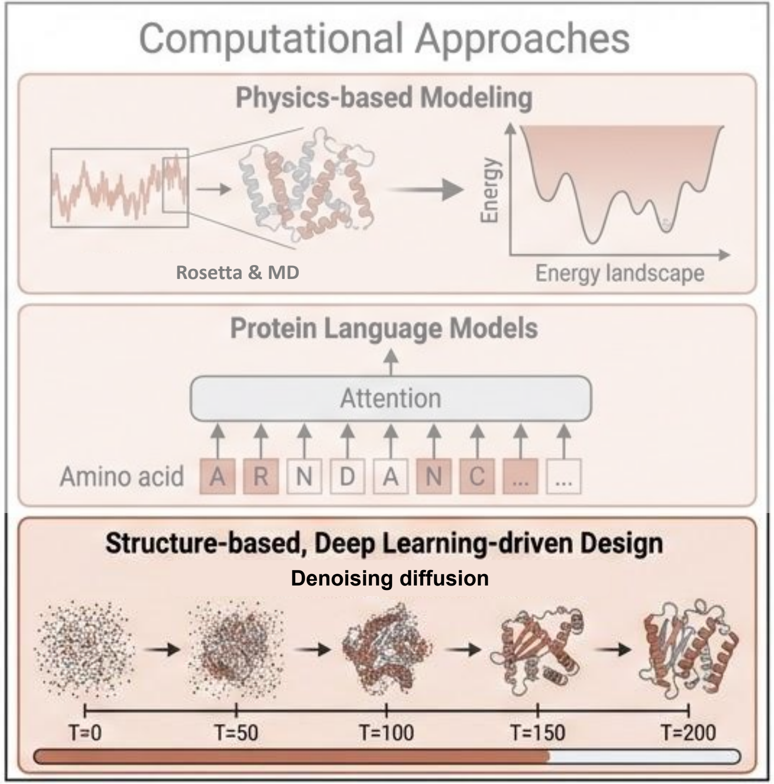
Abstract

The *de novo* design of enzymes remains a central challenge, requiring consideration of catalytic mechanism and optimization across biochemical and biophysical criteria. To capture these criteria, we draw on principles from evolutionary biology. Here, we present dEVA (design by EVolutionary Algorithm), a multi-objective design framework for structure-based protein design. We apply dEVA to the zero-shot, *de novo* design of metalloenzymes by optimizing for the coordination sphere of catalytic metals. We fully characterize one of these designs: a bi-zinc metalloenzyme exhibiting promiscuous hydrolytic activity towards both phosphomonoesters and phosphodiesters. This design achieves a catalytic efficiency (k_{cat}/K_M)

Protein engineering and design come in many flavors

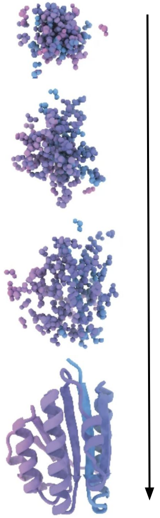




Notable deep learning tools for metalloenzyme design

Backbone Design

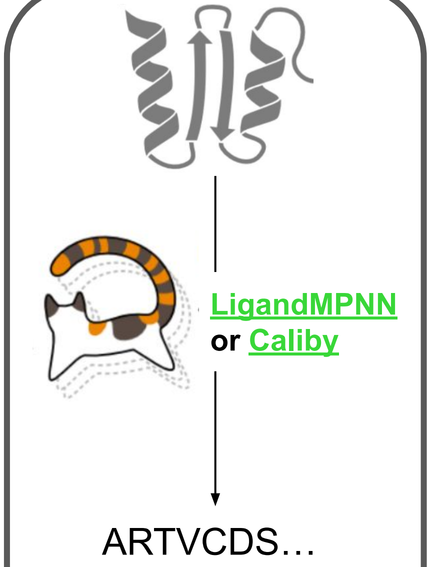
Protpardelle



Generate protein backbones for a given target or function

Chu *et al*, **PNAS**, 2024

Sequence Design



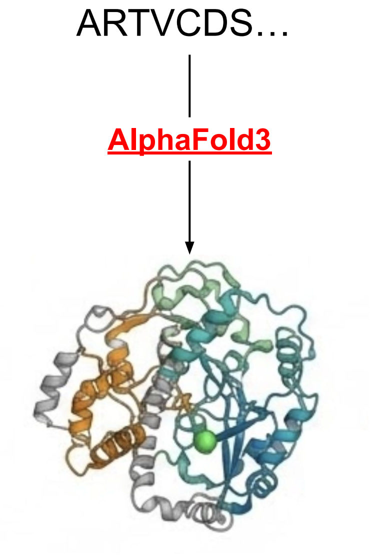
LigandMPNN or **Caliby**

ARTVCDS…

Predict a sequence with the potential to fold into a given backbone

Dauparas *et al*, **Nat Methods**, 2025
Shuai *et al*, **bioRxiv**, 2025

Structure Prediction



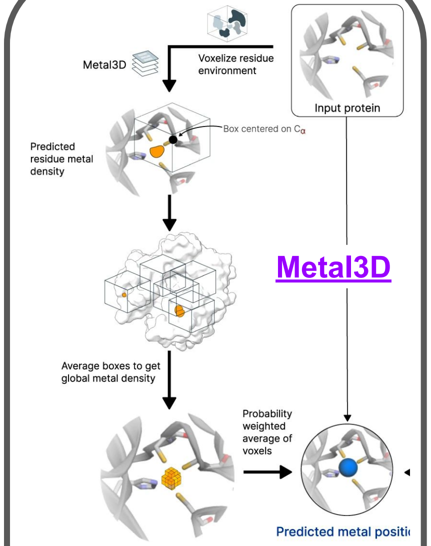
ARTVCDS…

AlphaFold3

Predict the structure of a given protein sequence

Abramson *et al*, **Nature**, 2024

Metal-specific



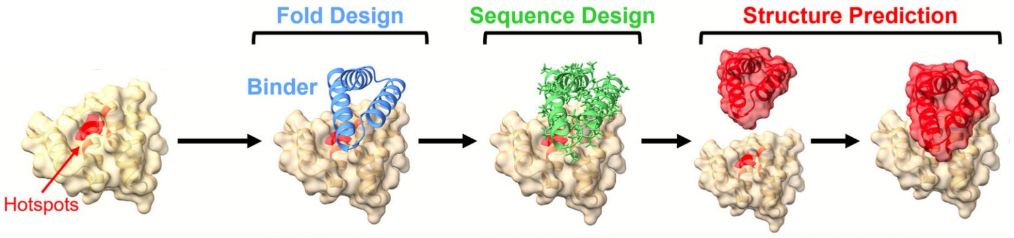
Metal3D

Predict the location of metal ions for a given protein structure

Dürr *et al*, **Nat Commun**, 2023

Structure-based approaches to *de novo* protein design

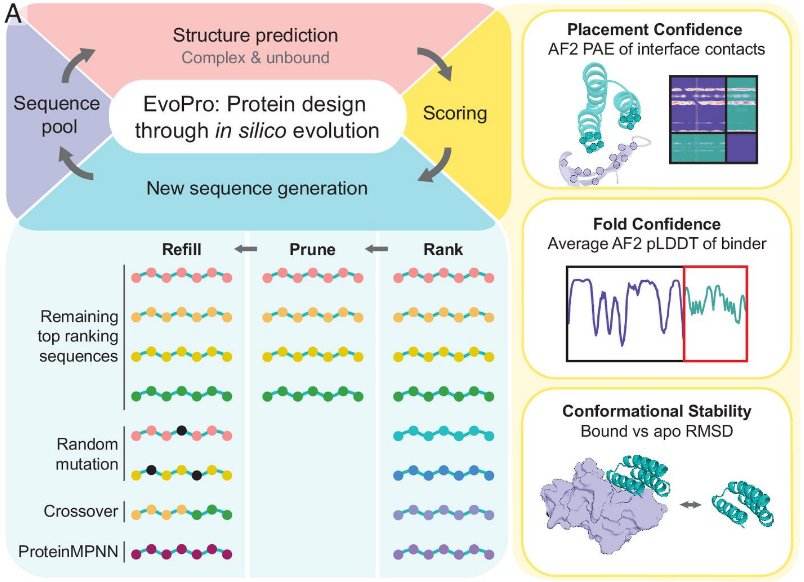
Parallelized, Generation-based



Silke *et al*, **Protein Sci**, 2026

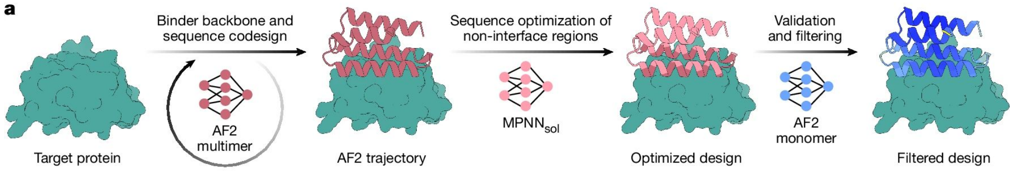
Iterative, Optimization-based

EvoPro



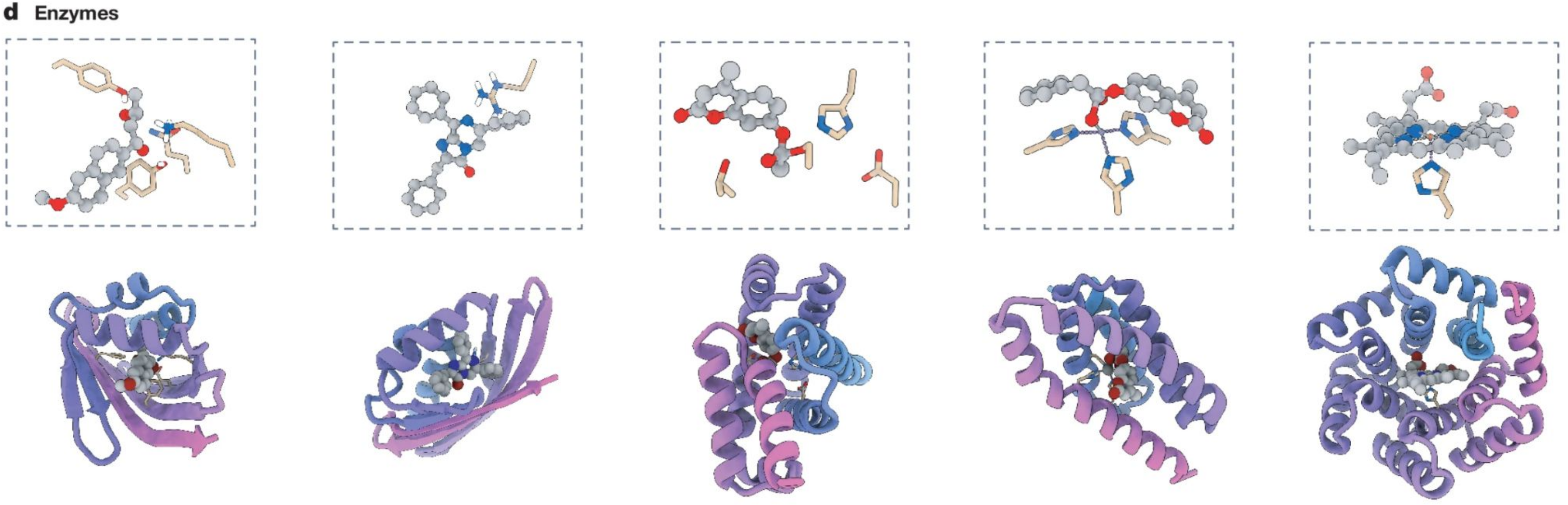
Goudy *et al*, **PNAS**, 2023

BindCraft



Pacesa *et al*, **Nature**, 2025

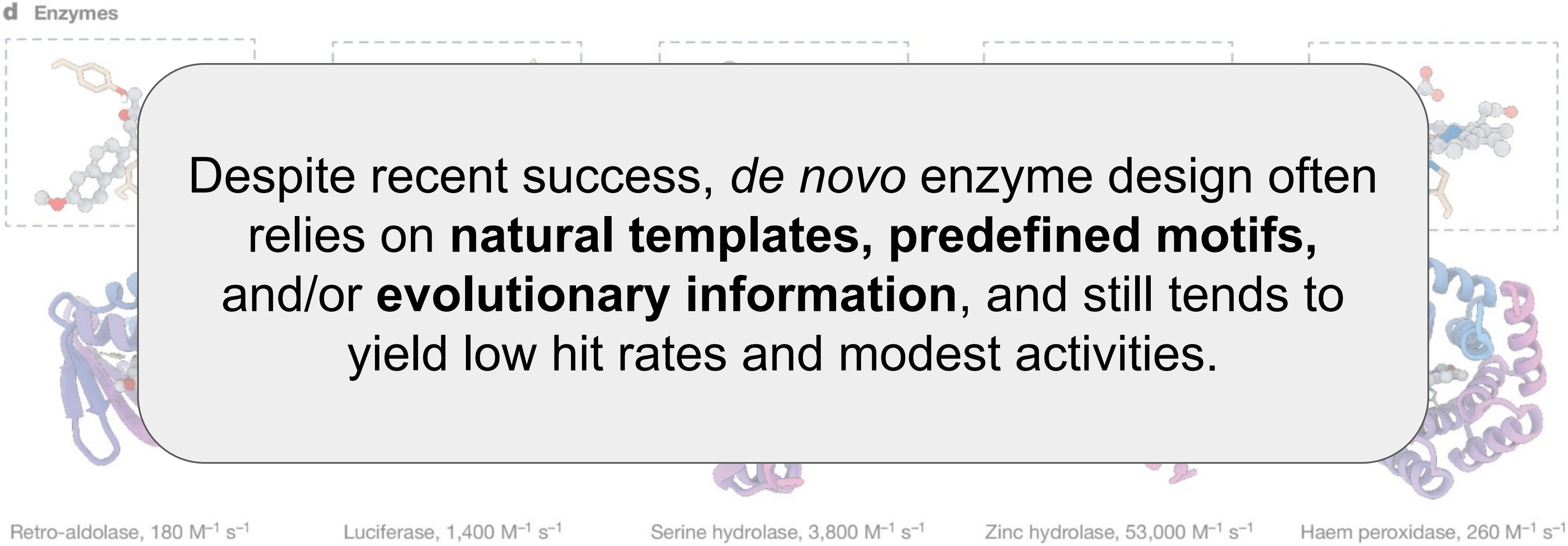
Previous success stories in *de novo* enzyme design



Retro-aldolase, 180 M^{-1} s^{-1} Luciferase, 1,400 M^{-1} s^{-1} Serine hydrolase, 3,800 M^{-1} s^{-1} Zinc hydrolase, 53,000 M^{-1} s^{-1} Haem peroxidase, 260 M^{-1} s^{-1}

Yang *et al*, **Nature**, 2026

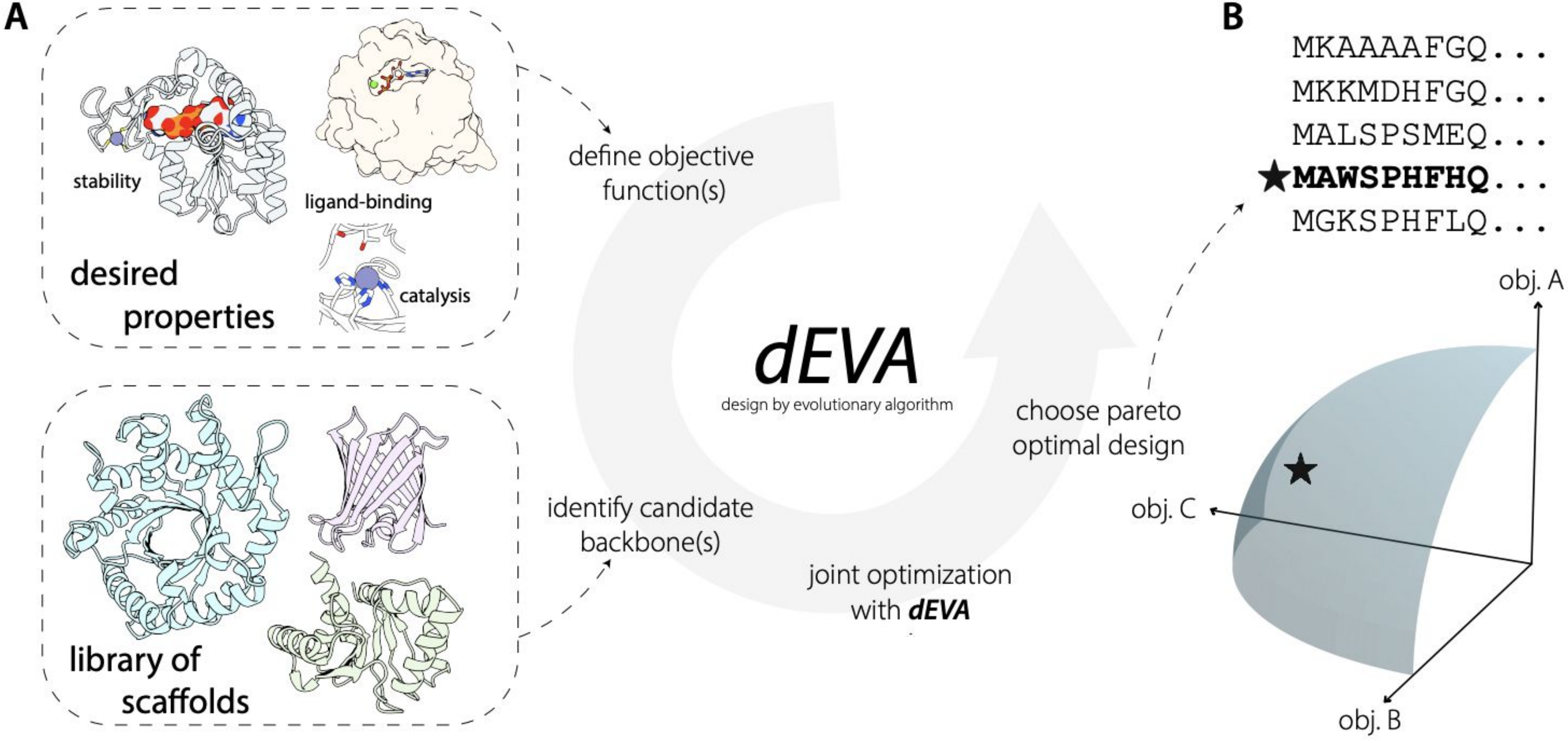
Previous success stories in *de novo* enzyme design



Despite recent success, *de novo* enzyme design often relies on **natural templates, predefined motifs,** and/or **evolutionary information**, and still tends to yield low hit rates and modest activities.

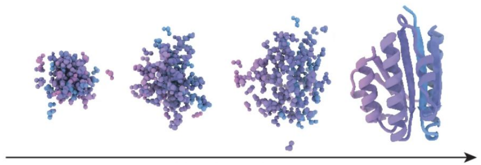
Yang *et al*, **Nature**, 2026

dEVA: a multi-objective framework for protein design

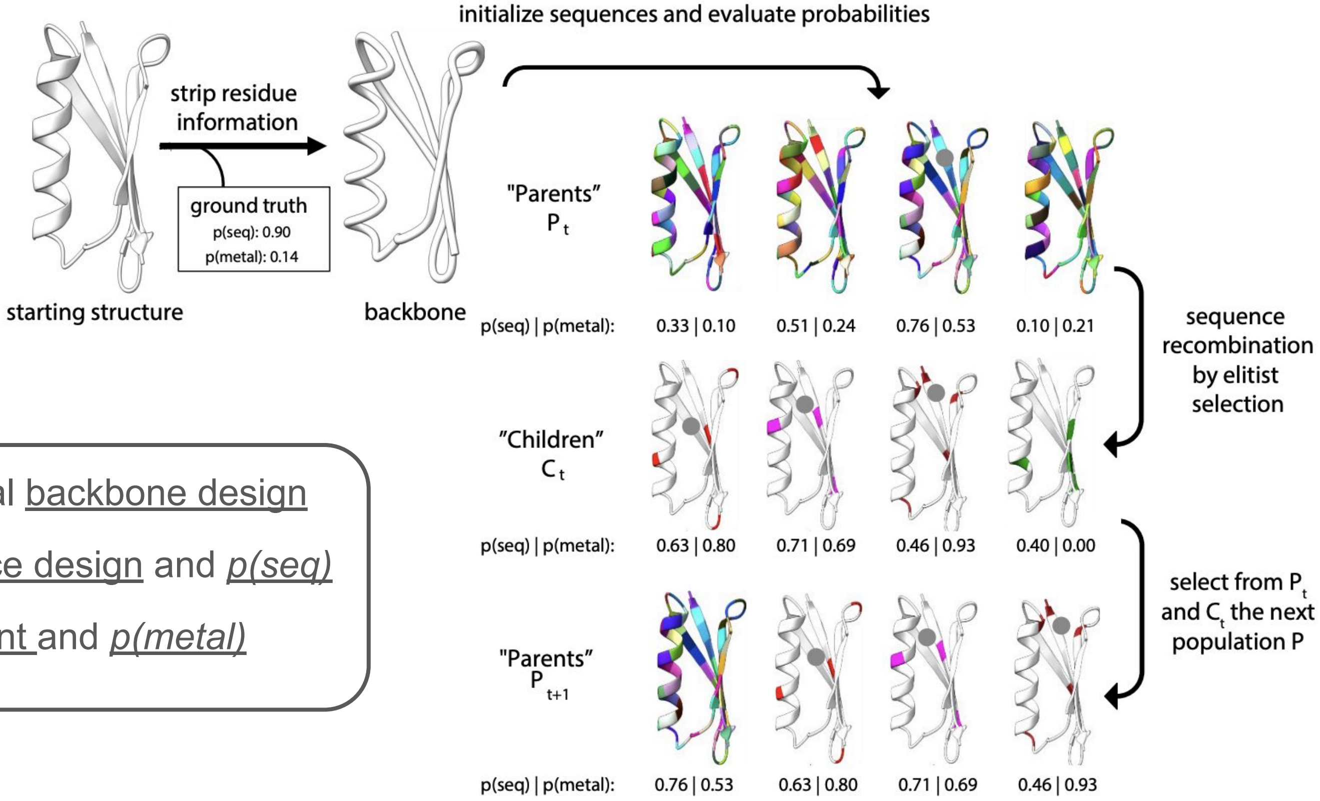


```
MKAAAAFGQ...
MKKMDHFGQ...
MALSPSMEQ...
★MAWSPHFHQ...
MGKSPHFLQ...
```

dEVA optimizes the designs via an evolutionary algorithm



Protpardelle

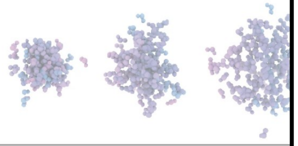


Propardelle for non-helical backbone design

LigandMPNN for sequence design and *p(seq)*

Metal3D for Zn^{2+} placement and *p(metal)*

dEVA optimizes the designs via an evolutionary algorithm

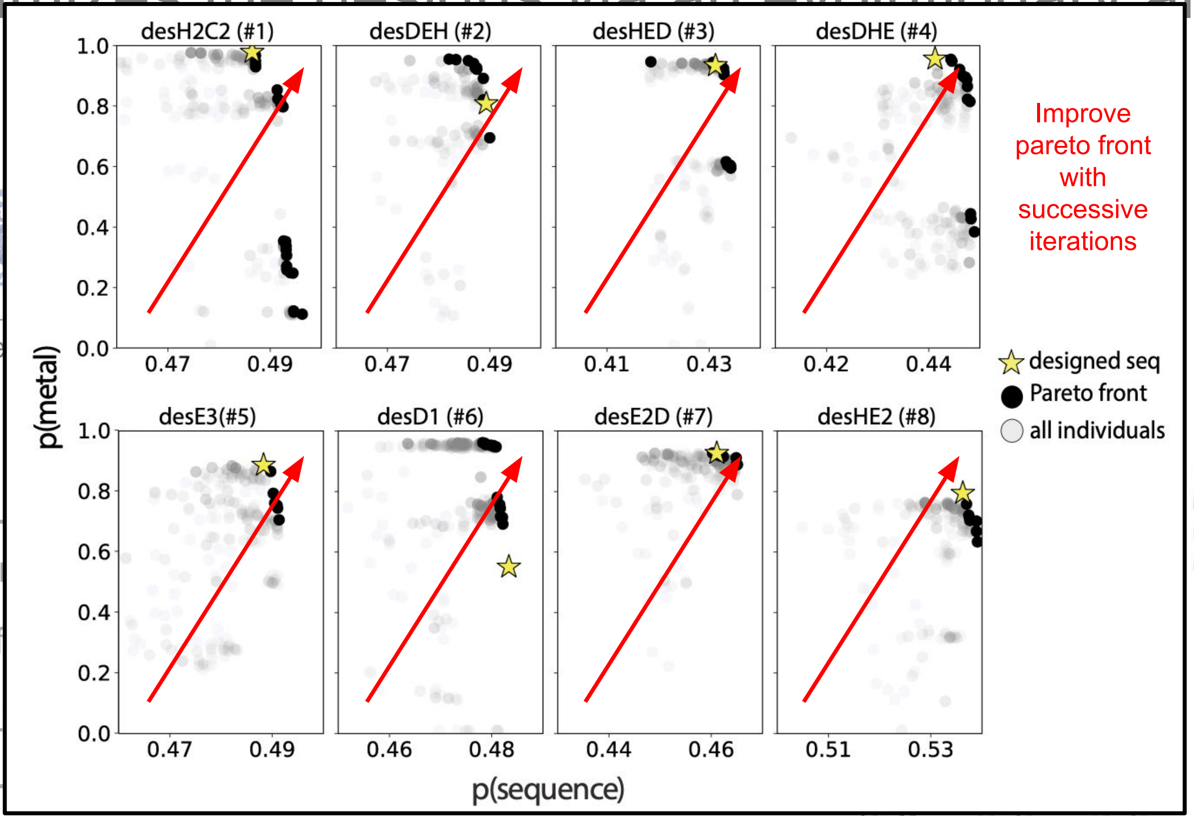


Protpardelle

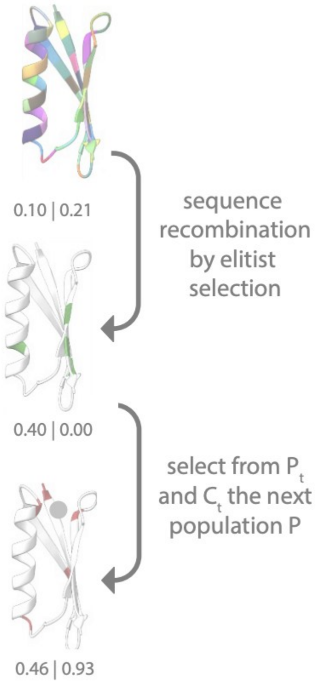
Propardelle fo

LigandMPNN f

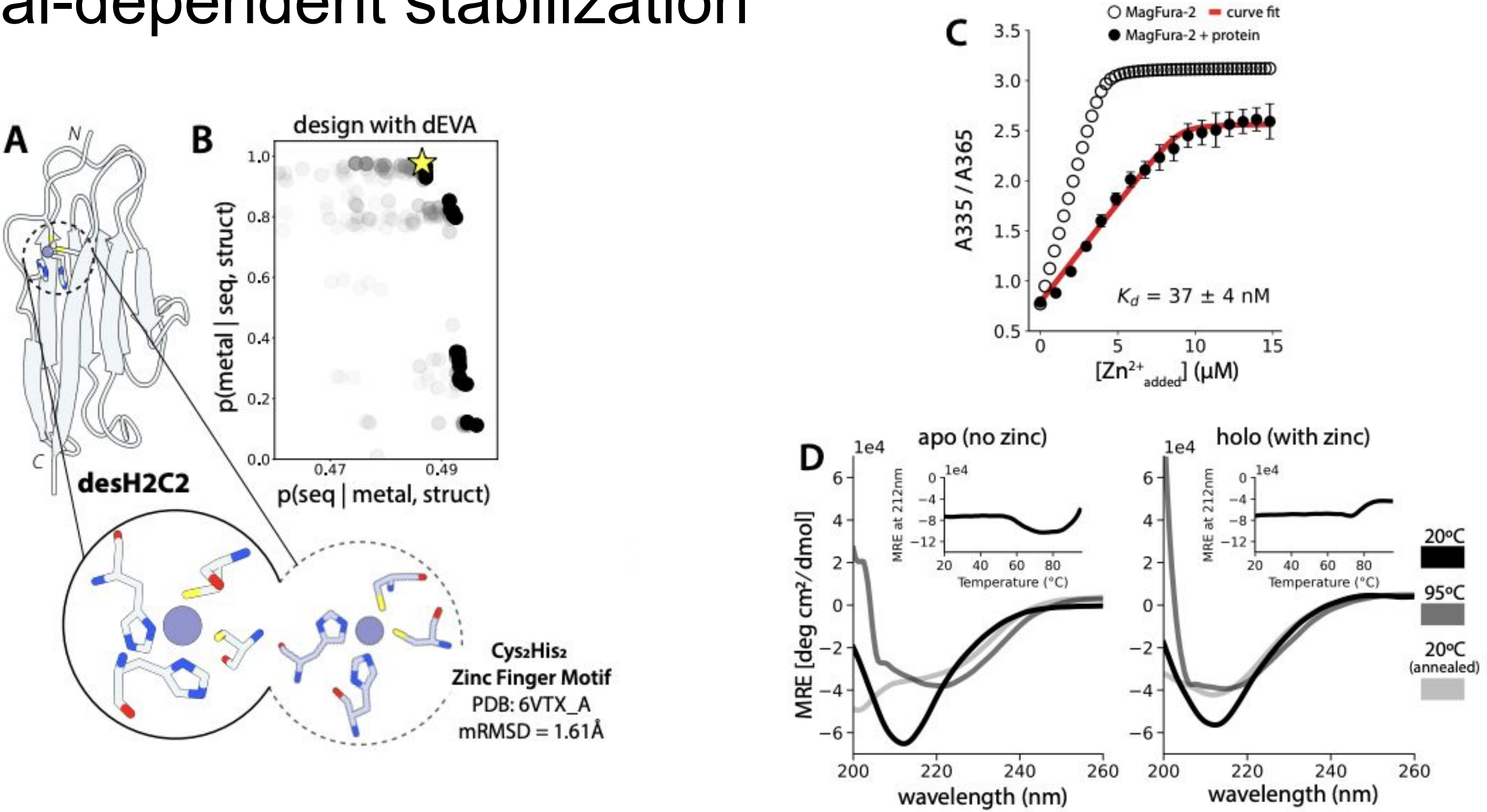
Metal3D for Zn



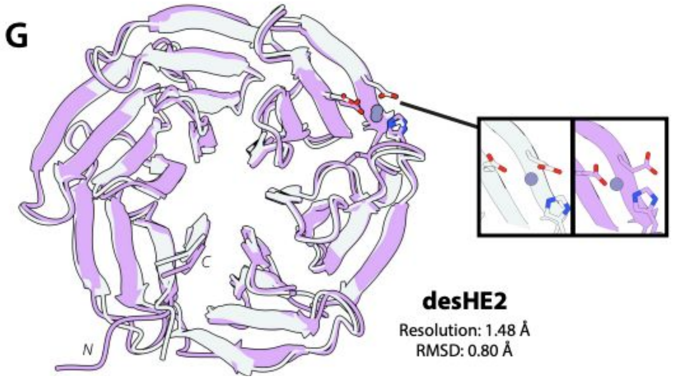
Improve pareto front with successive iterations

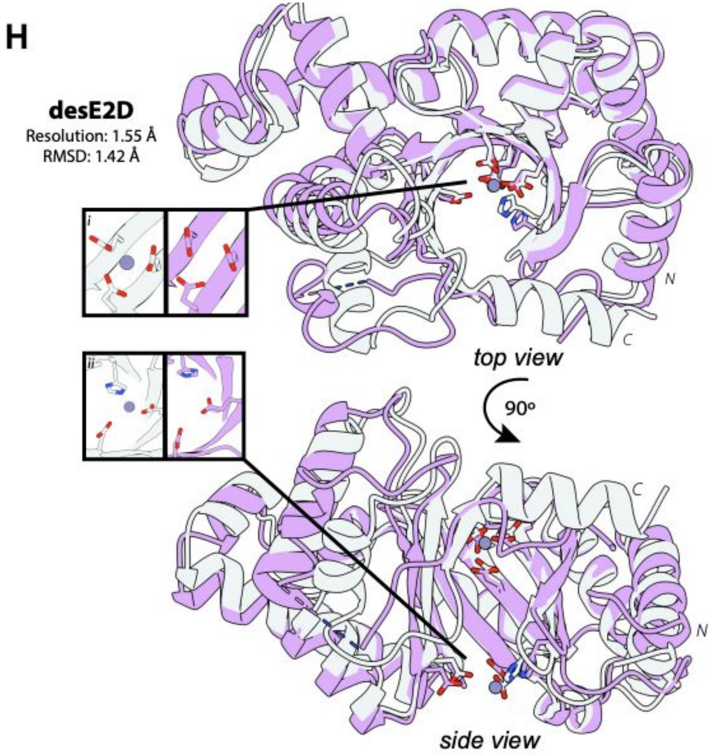


desH2C2 achieves top binding affinity and demonstrates metal-dependent stabilization

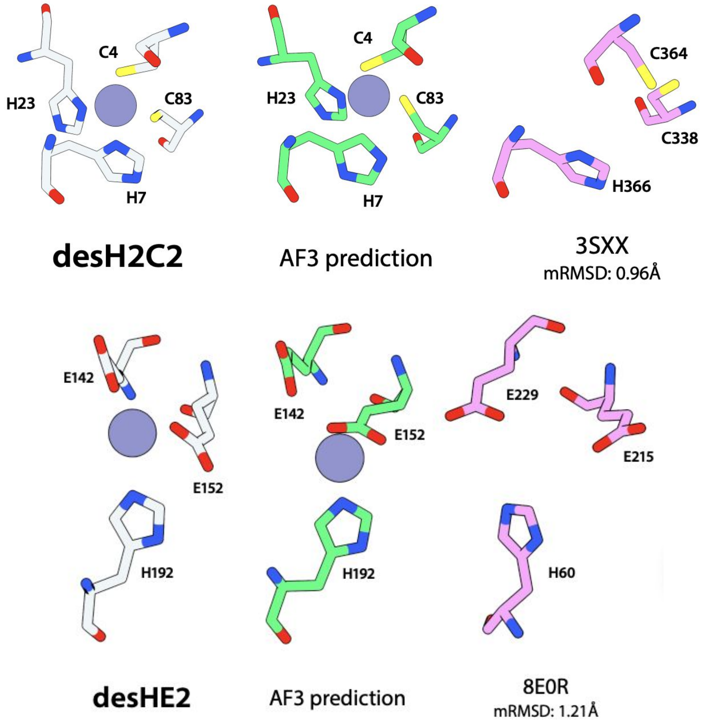


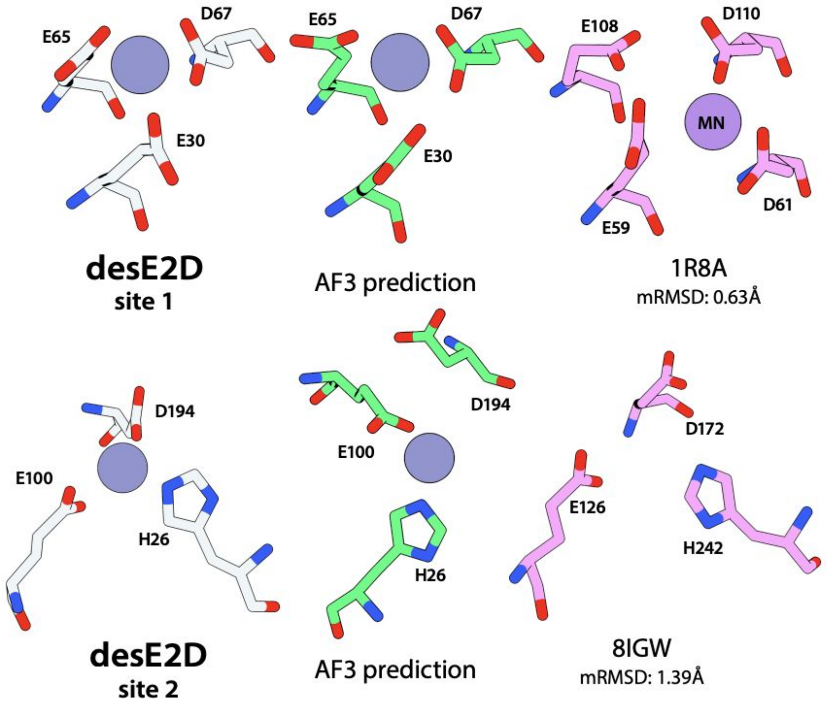
X-ray crystallography confirms dEVA can design functional sequences for diverse backbones



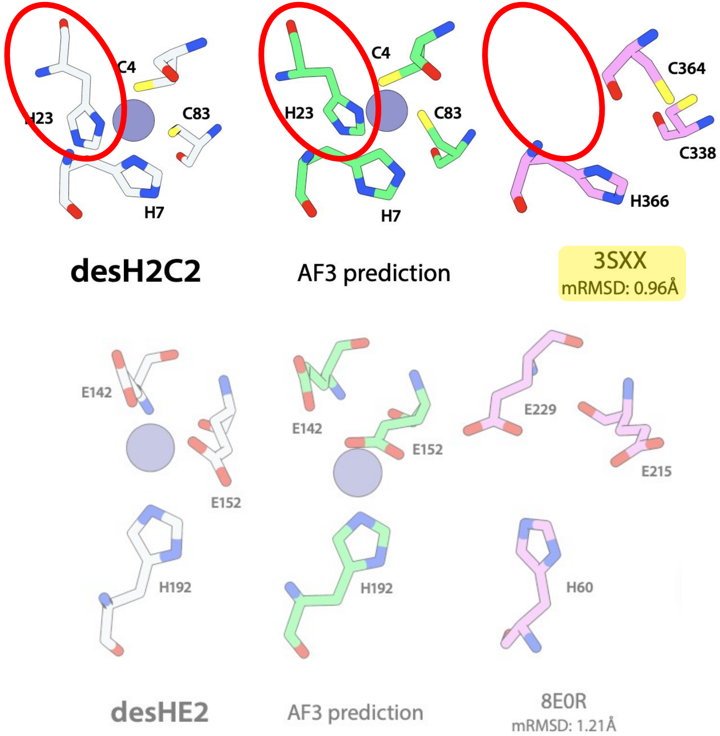


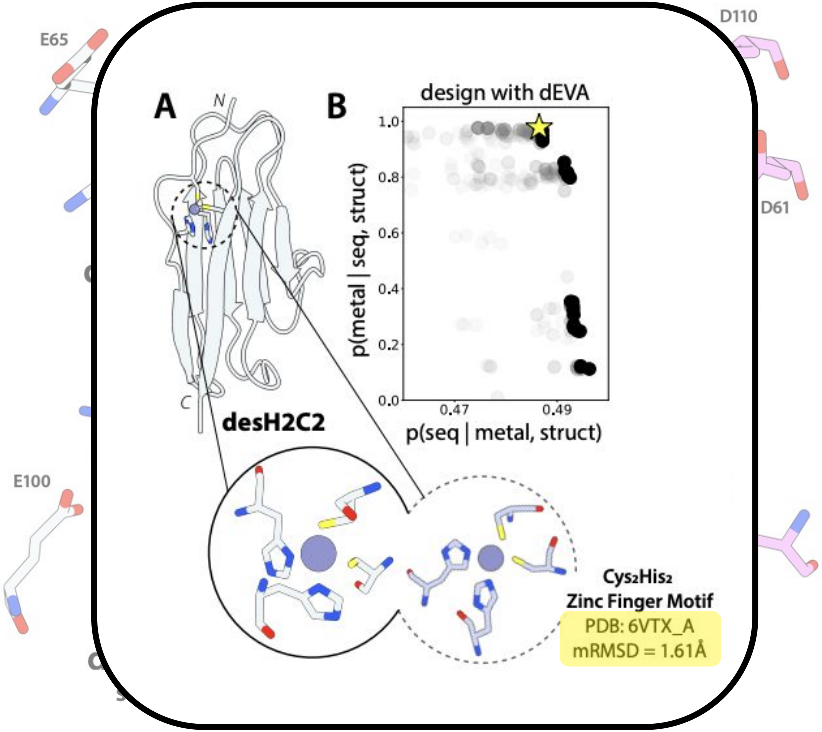
dEVA produces native-like, yet distinct, binding sites



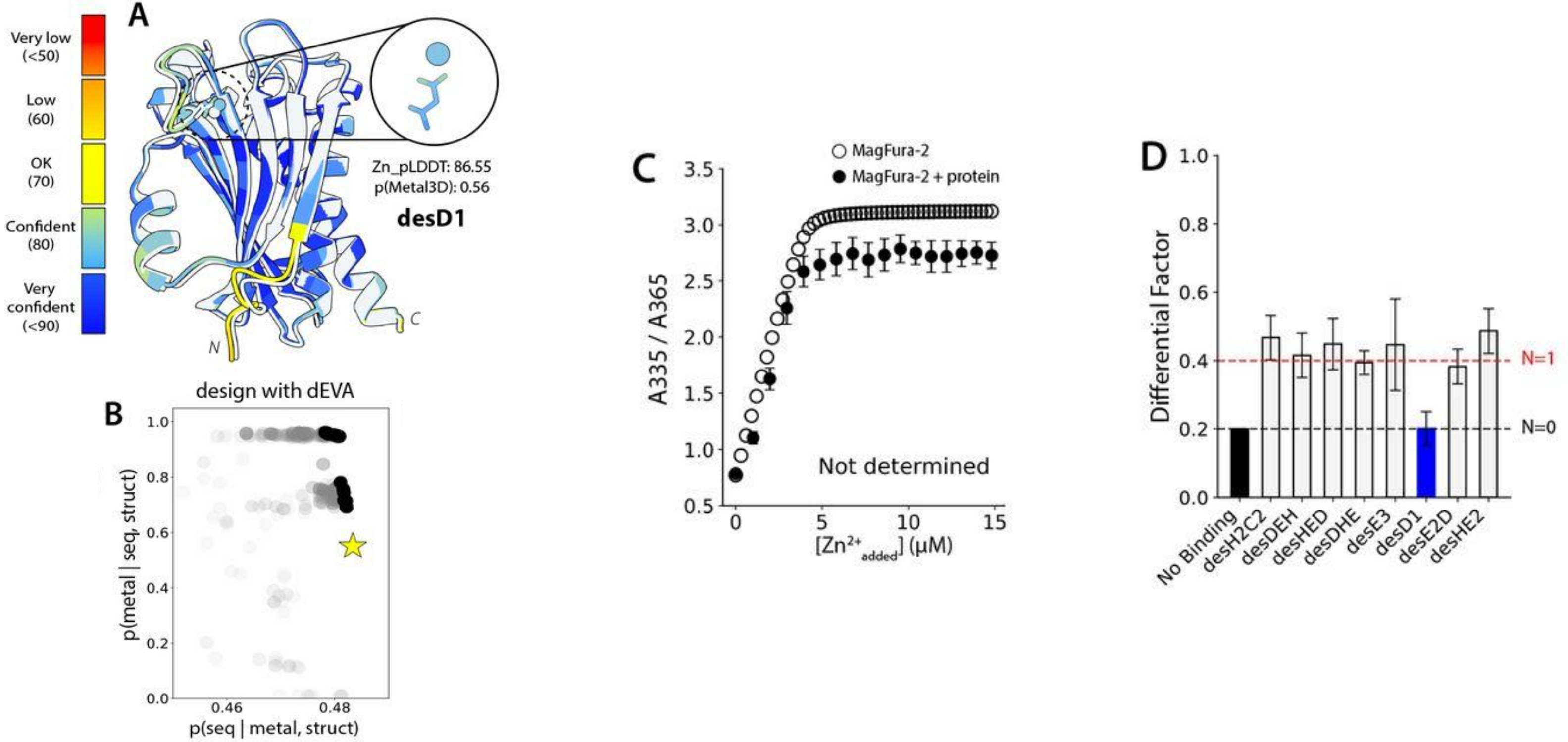


dEVA produces native-like, yet distinct, binding sites

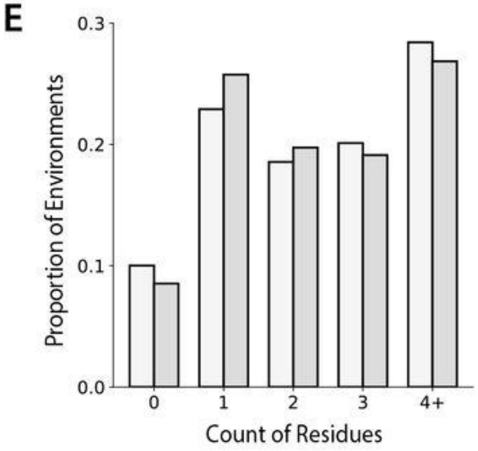


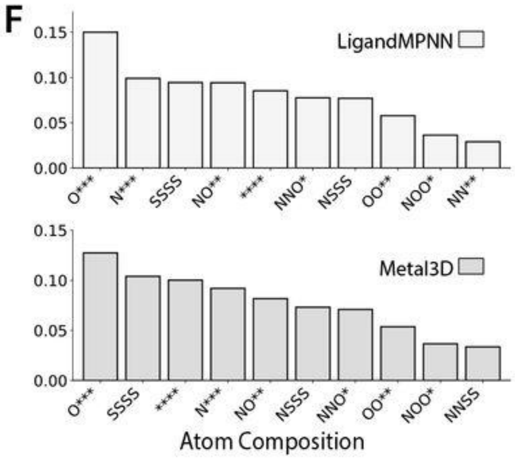


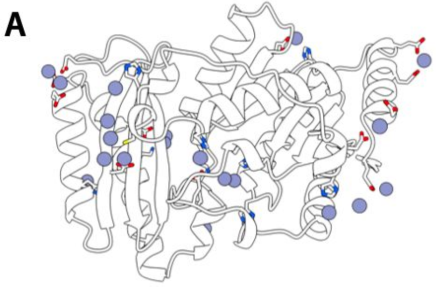
Negative control metalloprotein desD1 had a probable structure, but lacked experimental function



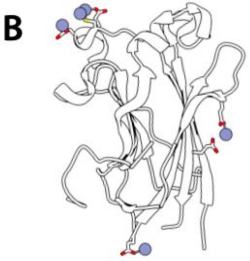
Analysis of training data for Metal3D showed >50% of coordination sites contained two or less residues





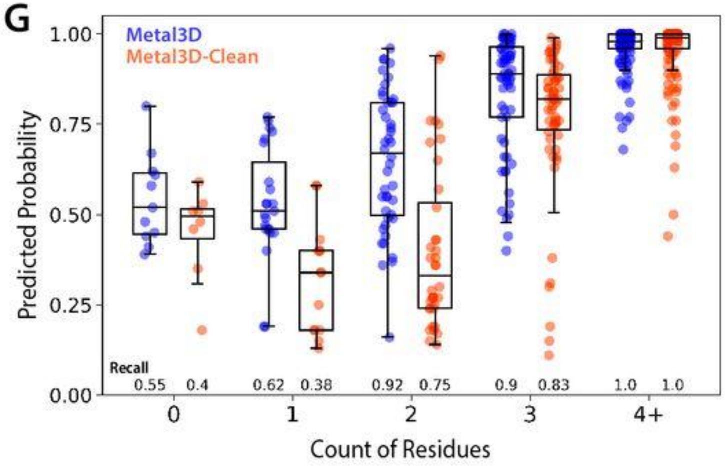


PDB: 2EJC_A

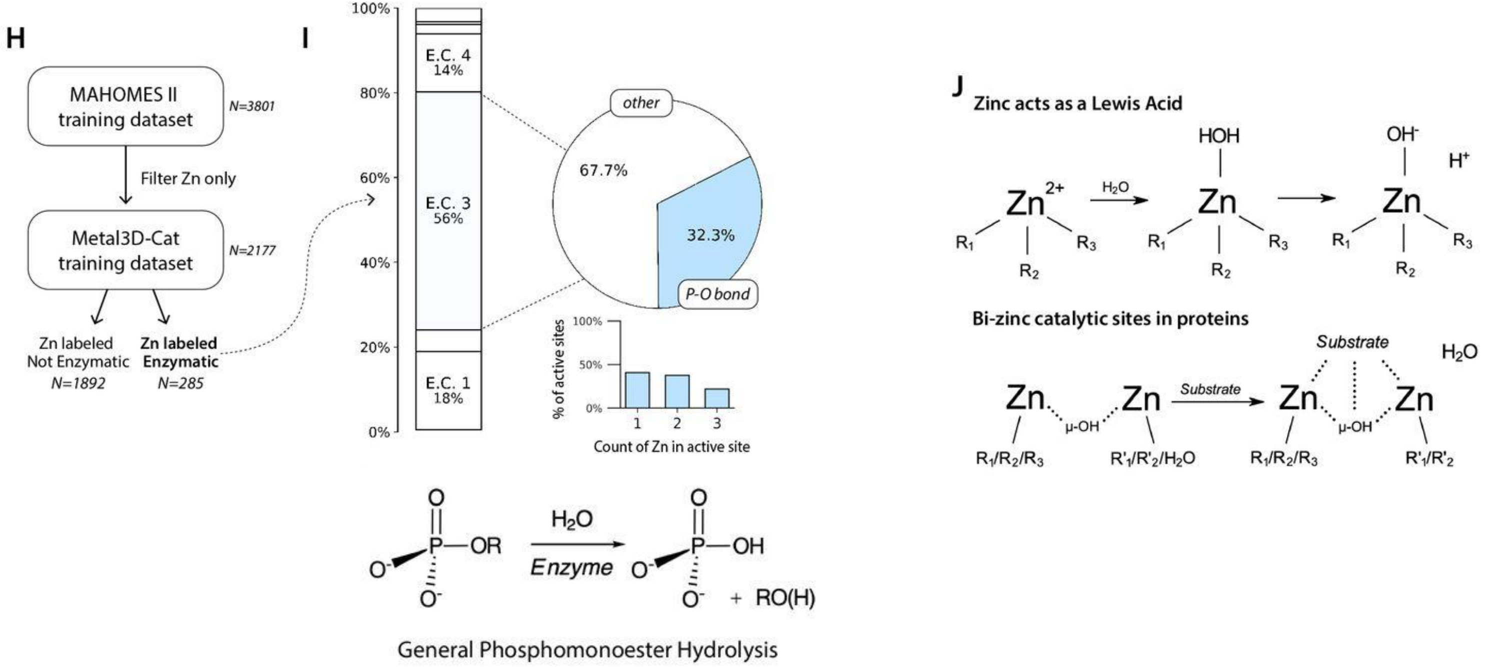


PDB: 3IVB_A

Exclusion of sites with less than three residues shifted the types of coordination sites predicted

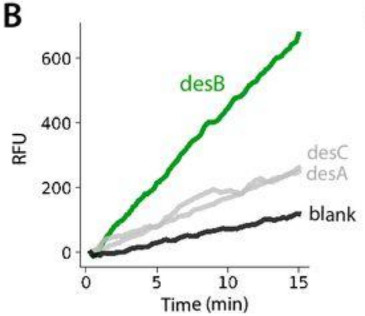


Improved Metal3D selectivity was then trained on catalytic zincs to model first- and second- shell environments

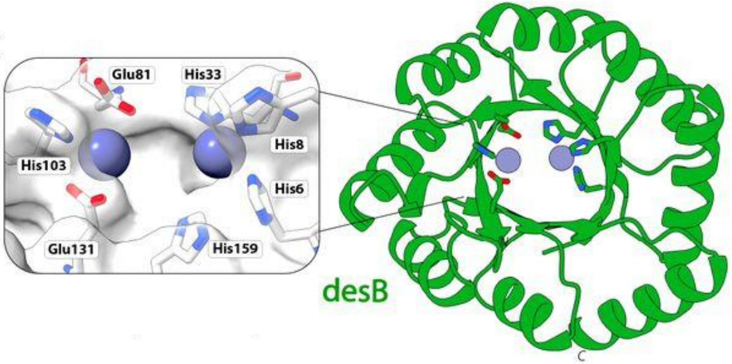


dEVA generated three beta-barrel structures with novel, catalytically active bi-nuclear zinc active sites

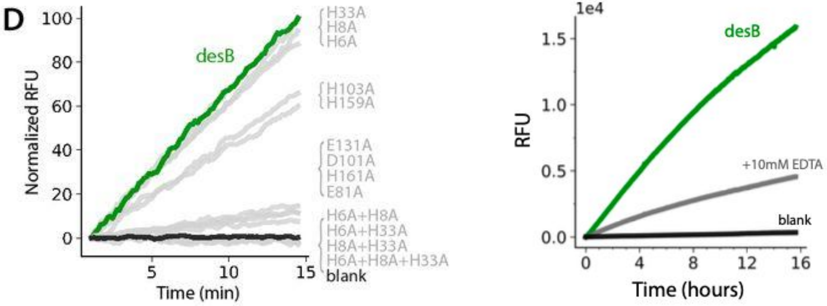
B



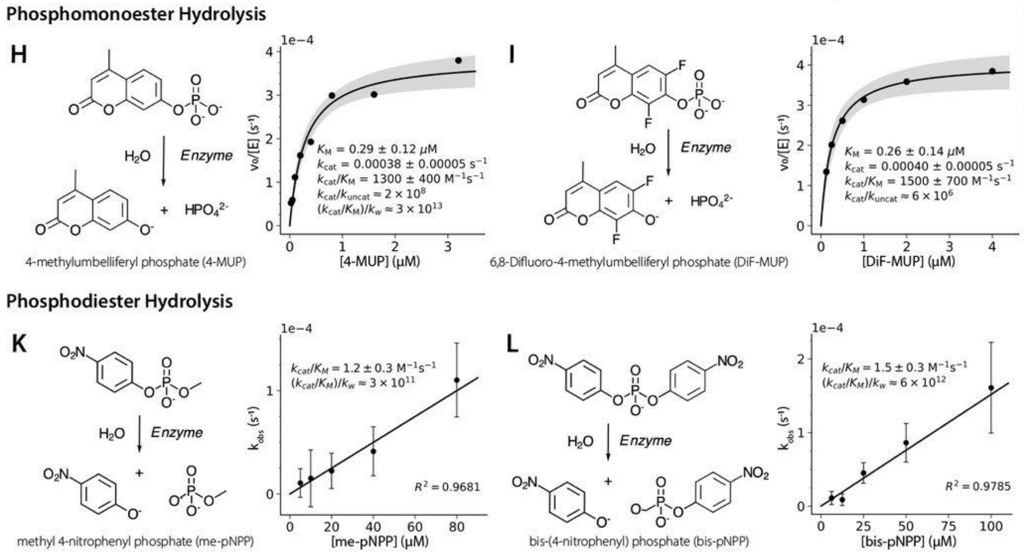
C

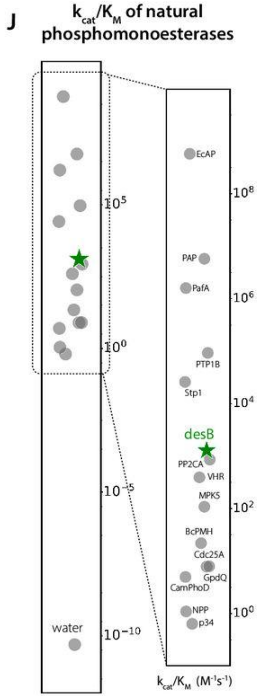


D



DesB is capable of catalyzing energetically demanding phosphate-oxygen bonds comparable to existing enzymes





Summary

- Developed protocol for multi-objective optimization and used it to generate metalloproteins with affinity for zinc.
- Improved coordination chemistry by considering the biophysical properties of the datasets that they were training on.
- Improved model was used to generate a bi-nuclear catalytically active zinc site with novel coordination ligands

Strengths vs Weaknesses

- Logical stepping in experimental complexity (Protein then enzyme)
- Generation of novel enzymatic coordination chemistries
- Ability to introduce enzymatic active sites into any structure

- Rationale for design/filtering decisions were not clear
- Limited by need for pre-existing annotated datasets
- Metalloenzyme scaffold ultimately resembled existing enzymes
- New-to-nature functions would not be achievable

Future Directions

- Expanding the filters on the types of structures used to predict metalloenzymes
- Attempting to generate an enzyme with a specific function rather than training on a group of functions
- Explore other types of metal coordination and enzymatic function for zinc
- Show generalizability across coordinating metals and enzyme types