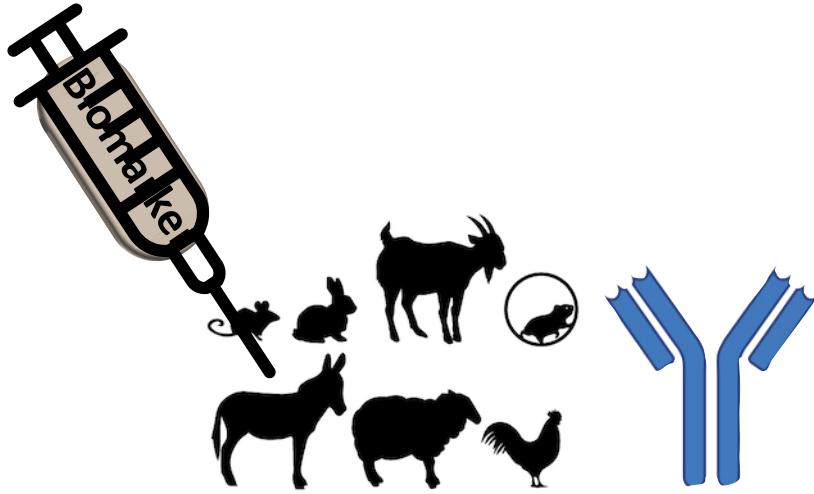


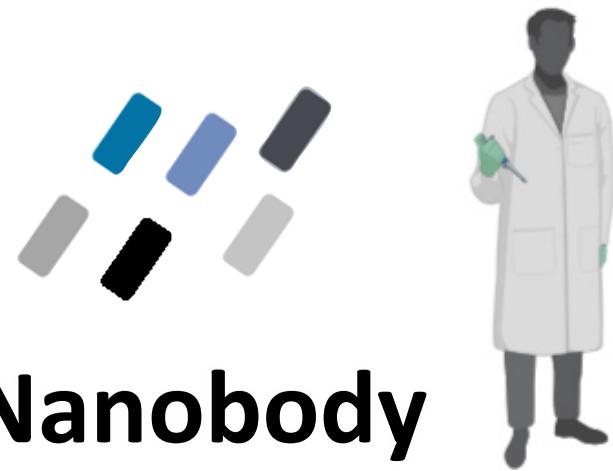
AI-assisted Nanobody design

Marco Orlando – 25/09/2025

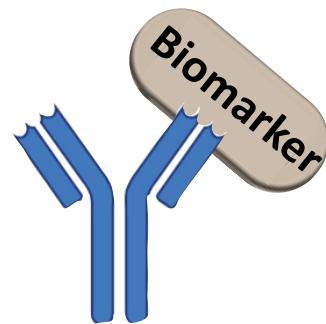
Antibody and Nanobody binders can be obtained using experimental screenings



IgG antibody



Nanobody

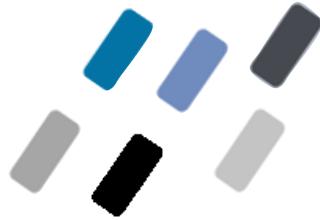


**There are ISSUES for BINDER development
to CHALLENGING BIOMARKERS, which are:**

Non immunogenic

Poorly soluble

Computational binder design can relieve these limitations



Nanobody



Generic binder



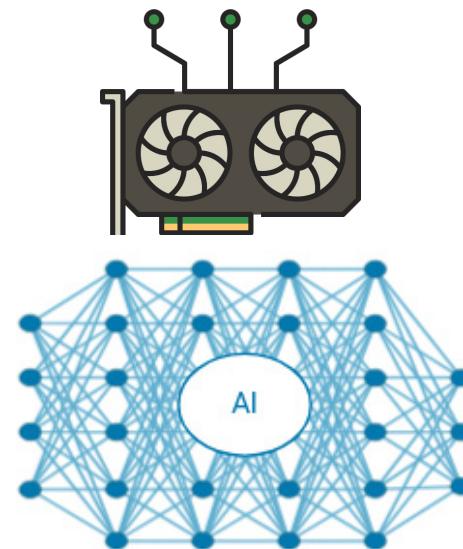
Now we are in the era of AI-centered development of algorithms and computing



Nanobody



Generic binder



AI proved to be effective in design of vaccines & venom-antidote proteins

<https://www.nature.com/articles/d43747-023-00051-x>

Using AI to create a vaccine revolution

Clinical stage company Evaxion Biotech is using artificial intelligence (AI) to simulate the immune system and create predictive models to identify novel targets for vaccines against bacterial and viral diseases and immunotherapies for cancer.

nature <https://www.nature.com/articles/s41586-024-08393-x>

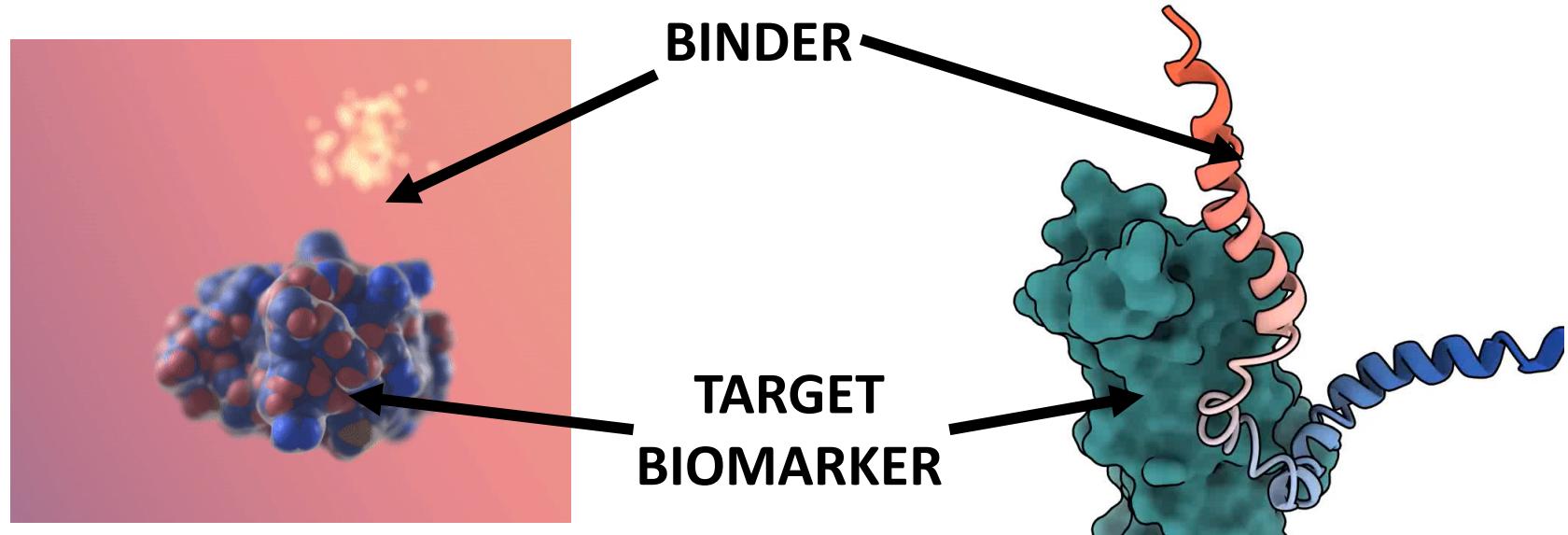
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De novo designed proteins neutralize lethal snake venom toxins

AI-assisted BINDER design is having a really a fast development rate



RosettaFold Diffusion+MPNN

(April 2023)

1 – 5% success rate

(< 100 nM affinity)

Few minutes per design

BindCraft

(November 2024)

5 – 50% success rate

(< 100 nM affinity)

10 - 15 minutes per design

TWO CATEGORIES

AI-based score trained to generate vectors that «guide» binder generation across the learnt folded protein universe («foldome»)

MINIMIZE DIFFERENCE FROM PREDICTION AND TRAINING DATA («foldome») IS THE TARGET

Handcrafted score that «guides» binder generation by traversing the sequence space for binders hallucination

MINIMIZE THE SCORE IS THE TARGET, IT IS AGNOSTIC WITH RESPECT TO TRAINING DATA

TWO CATEGORIES

AI-based score trained to generate vectors that «guide» binder generation across the learnt folded protein universe («foldome»)

DIFFUSION/FLOW-MATCHING

Handcrafted score that «guides» binder generation by traversing the sequence space for binders hallucination

GRADIENT DESCENT/BAYESIAN OPTIMIZATION

TWO CATEGORIES

AI-based score trained to generate vectors that «guide» binder generation across the learnt folded protein universe («foldome»)

DIFFUSION/FLOW-MATCHING

Try to interpolate the «foldome» in search for the target binder according to *a priori* constraints and/or conditions

Handcrafted score that «guides» binder generation by traversing the sequence space for binders hallucination

GRADIENT DESCENT/BAYESIAN OPTIMIZATION

Try to find minima of an interpretable score function that embeds those *a priori* constraints and AI-pretrained models

TWO CATEGORIES

AI-based score trained to generate vectors that «guide» binder generation across the learnt folded protein universe («foldome»)

DIFFUSION/FLOW-MATCHING

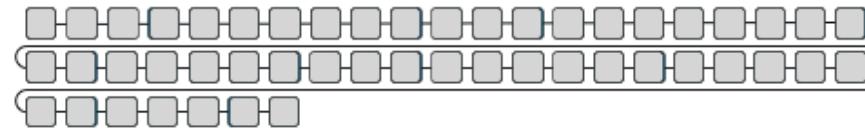
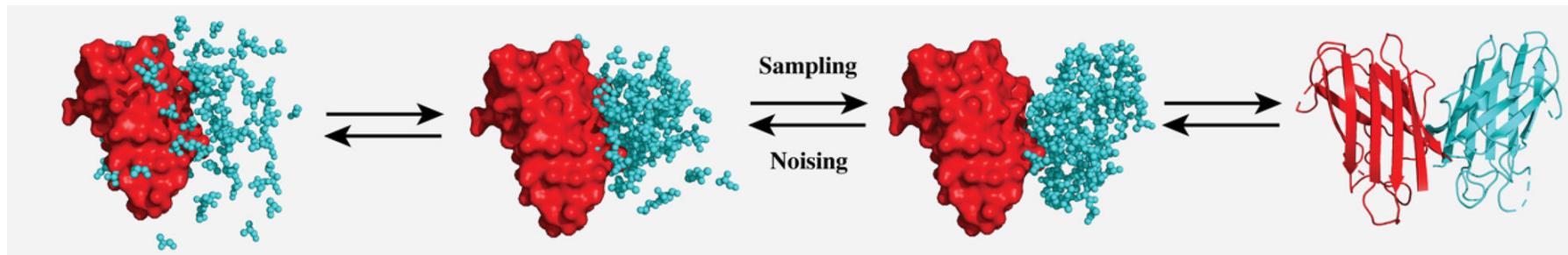
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GRADIENT DESCENT/BAYESIAN OPTIMIZATION

Try to find minima of an interpretable score function that embeds those *a priori* constraints and AI-pretrained models

DIFFUSION MODELS HAVE BEEN THE MOST DIFFUSED SINCE 2022



- 1) [Structure] iterations + Sequence | Final Structure

- 2) [Sequence + Structure | Sequence] iterations

HANDS ON WITH NANOBODIES

RFAntibody + ProteinMPNN + CDRs refinement

<https://github.com/margio91/De-novo-nanobody-re-design>

De Novo Nanobody Design

This repository contains the computational workflow for the *de novo* (re)design of a nanobody to specific target protein of interest. The pipeline is developed to use Google Colab resources and it is adequate for people with little knowledge on protein *de novo* design tools and limited structural biology background. It is actually made for demonstration and teaching, while being not adequate to scale for a production-level *in silico* screening.

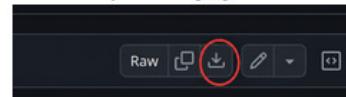
This material has been used during the **Nanobody Workshop (22-26 Sep 2025)**.

- Event Link: <https://indico.ijs.si/event/2966/>

Overview

The workshop starts from the 3D structure of two input PDBs, one of the target and one of the scaffold nanobody. The steps are performed using Google Colab notebooks, provided in this repository.

Note on File Access: The links to files in this README (like `7z1b.pdb`) are relative paths. If you click on them in a browser while logged into GitHub with access to this repository, you will see the file's content. To use the file, you will



need to manually click the "Download raw file" button → top-right button on the file viewer page.

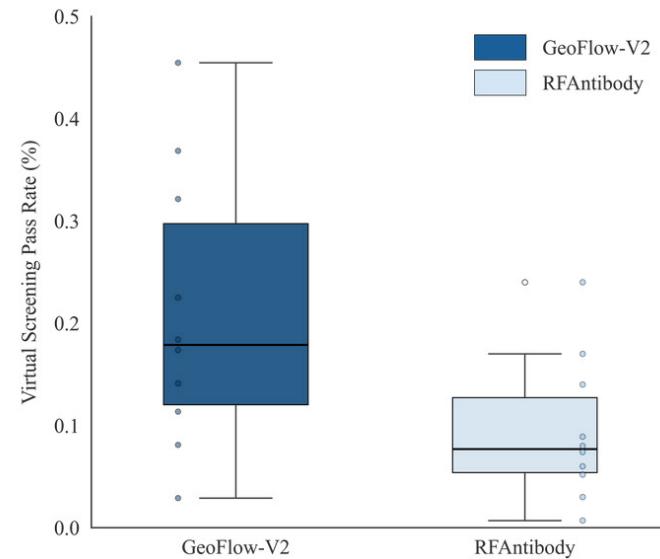
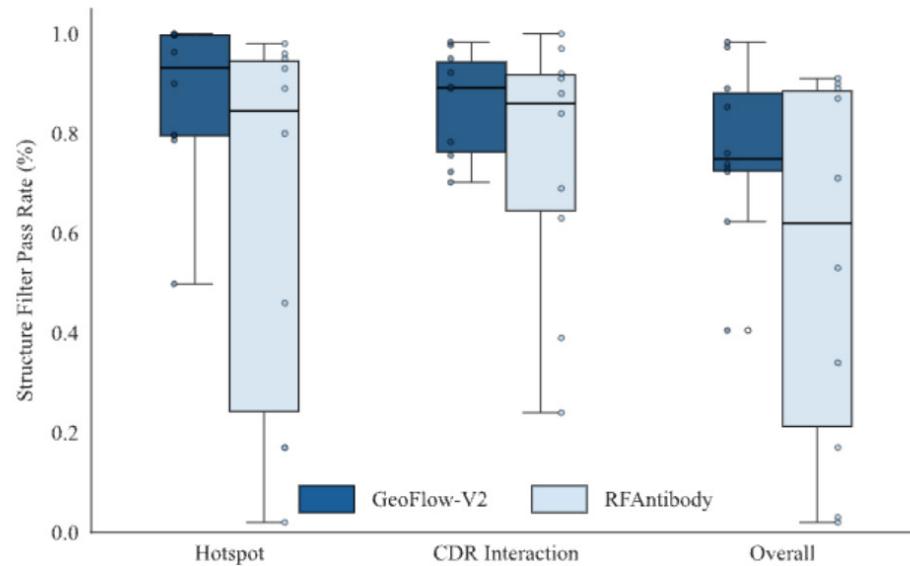
THE FIELD IS FAST MOVING!

ALL-ATOM DESIGN MODELS, BRINGING ALL THE WORKFLOW STEPS TOGHETER IN A SINGLE SAMPLING TRAJECTORY

GeoFlow-V2: A Unified Atomic Diffusion Model for Protein Structure Prediction and De Novo Design

BioGeometry Team

doi: <https://doi.org/10.1101/2025.05.06.652551>



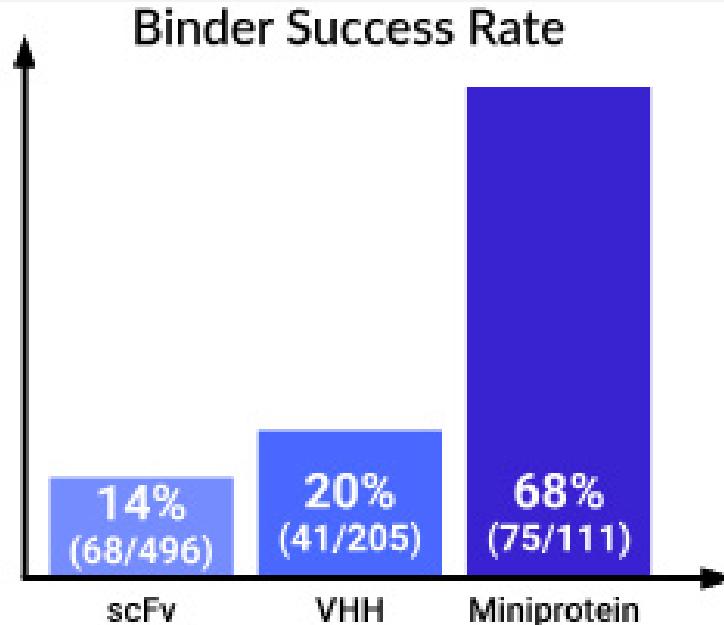
THE FIELD IS FAST MOVING!

ALL-ATOM DESIGN MODELS, BRINGING ALL THE WORKFLOW STEPS
TOGHETER IN A SINGLE SAMPLING TRAJECTORY

 Chai Discovery

Zero-shot antibody design in a 24-well plate

Chai Discovery Team



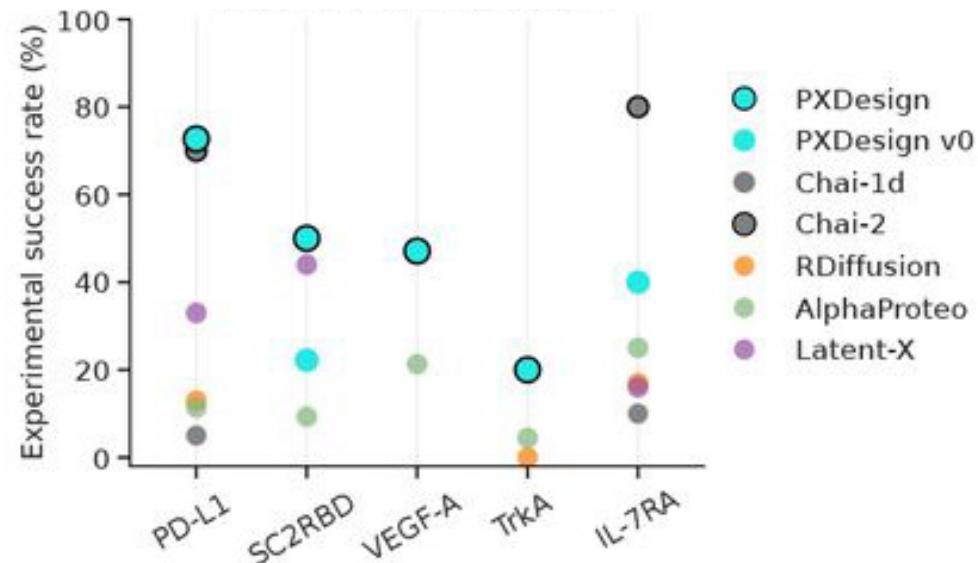
THE FIELD IS FAST MOVING!

ALL-ATOM DESIGN MODELS, BRINGING ALL THE WORKFLOW STEPS TOGHETER IN A SINGLE SAMPLING TRAJECTORY

PXDesign: Fast, Modular, and Accurate De Novo Design of Protein Binders

Milong Ren,  Jinyuan Sun, Jiaqi Guan,  Cong Liu,  Chengyue Gong,  Yuzhe Wang, Lan Wang, Qixu Cai,  Xinshi Chen, Wenzhi Xiao, Protenix Team

doi: <https://doi.org/10.1101/2025.08.15.670450>



THE FIELD IS FAST MOVING!

**HYBRID AI-GENERATIVE, BUT SCORE-RANKING MODELS,
CAPABLE TO DIRECTLY GENERATE BINDERS WITH HIGH
PROBABILITY TO HAVE THE DESIRED PROPERTIES**

(e.g., + binding affinity, - immunogenicity, ...)

**A synergistic generative-ranking framework for tailored design of therapeutic
single-domain antibodies**

Yu Kong, Jiale Shi, Fandi Wu, Ting Zhao, Rubo Wang, Xiaoyi Zhu, Qingyuan Xu, Yidong Song, Quanxiao Li, Yulu Wang, Xingyu Gao,  Yuedong Yang, Yanling Wu, Zhenlin Yang,  Jianhua Yao, Tianlei Ying

doi: <https://doi.org/10.1101/2025.05.09.653014>

<https://doi.org/10.1101/2025.05.09.653014>