High-Throughput Nanobody Design:

Integrating Machine Learning with Rapid Cell-Free Expression

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Outline

1. Ordaos Technology & Strategy
   → Lab-in-the-loop AI-derived binders against disease-specific targets
   → Nanobodies and their Advantages
   → Cell-Free Expression (CFE)

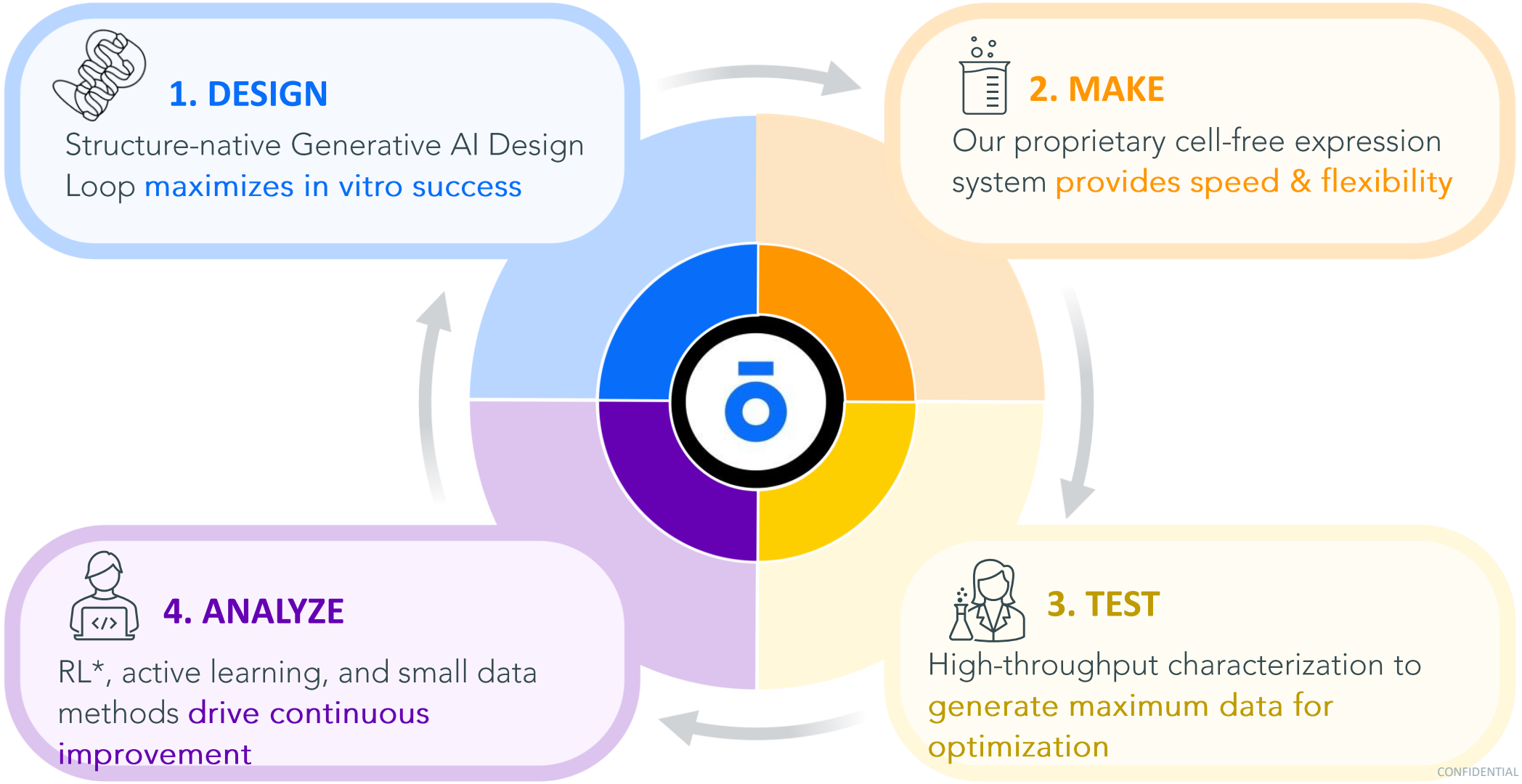
2. Rapid Screening
   → Carterra LSA® platform:
      - High-throughput screening with correlated crude/pure binding data
      - Epitope binning: Evaluating binding/competition profiles
   → Wet Lab QC

3. *In Vitro Evaluations*
   → Cell binding assays: Confirm binding in biologically relevant contexts

4. Engineering Function
   → Strategic multimerization: Bispecifics/multivalents to bridge targets and drive activity

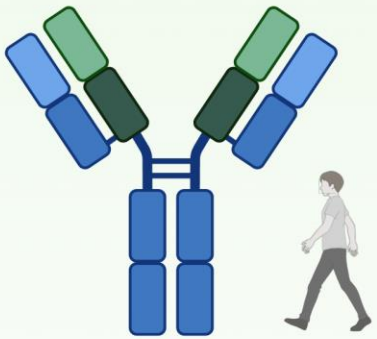
Ordaōs Reimagines Biologics Discovery As Biologics *Design*

Lab-in-the-loop Genai-designed Mini-proteins & Single Domain Antibodies

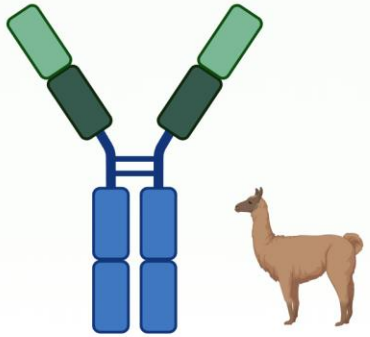


Ordaōs provides Nanobody and De Novo Protein Solutions

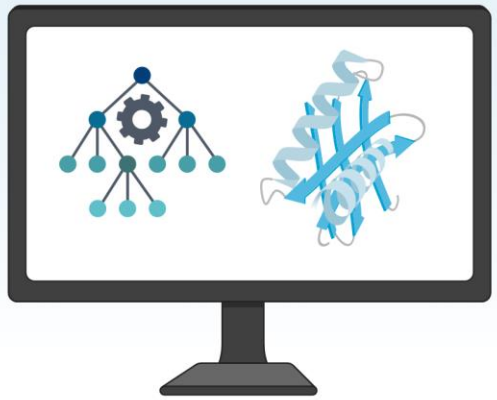
Ai-driven Small Protein Solutions For Next-generation Biologics



Conventional IgG
(~150 kDa)



Camelid Hc-only Antibody
(HcAb)
(~90 kDa)



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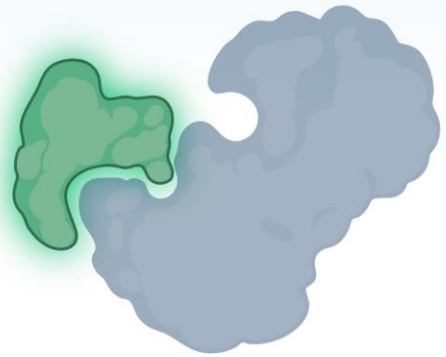
Machine learning
Algorithms

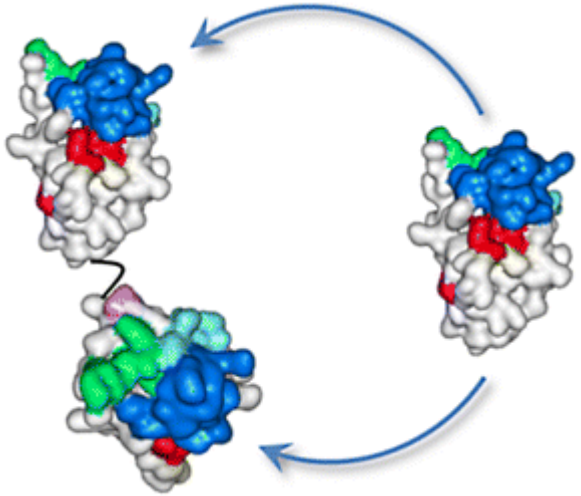
VHH (Nanobody)
(~15 kDa)



De Novo Mini Protein
(4-18 kDa)

Nanobody Therapeutics: Key Advantages at a Glance

Biophysical Features Enabling Novel Therapeutic Modalities

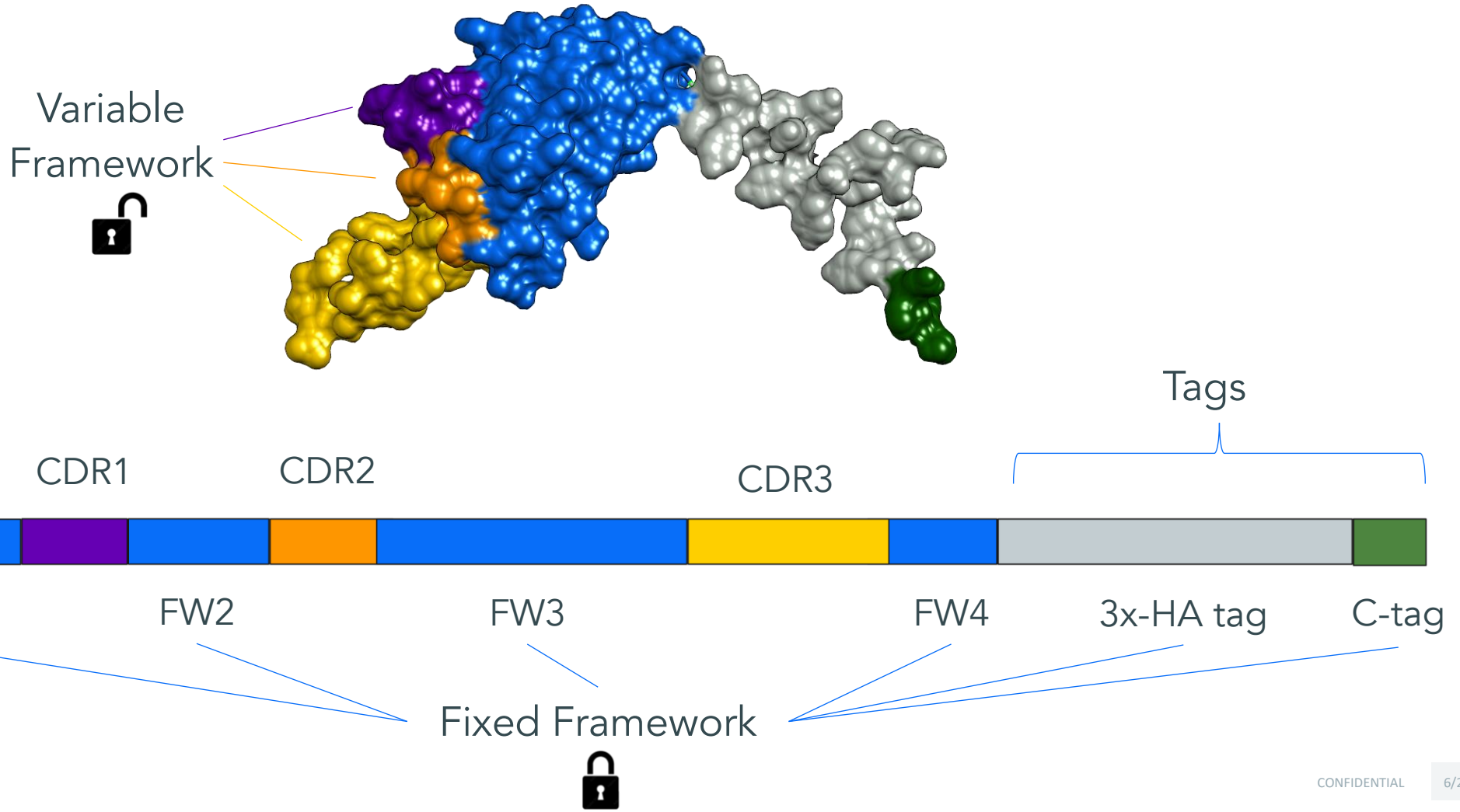


Caplacizumab (Cablivi)
Bivalent (homodimeric) Nanobody

- **Genetic simplicity** → A single cDNA (≈ 400 bp) encodes the complete binding unit. No need to co-express and correctly pair heavy + light chains.
- **Very small size (~15 kDa)** → superior tissue penetration (including blood-brain barrier) and the ability to reach smaller epitopes
- **High physicochemical stability** → withstands extreme pH, temperature, freeze-thaws; rapidly refolds, enabling room-temperature storage
- **Low-cost, high-yield microbial production** → expresses efficiently in E. coli, yeast, and CFE (no need for chaperones/expensive mammalian cell culture)
- **Modular engineering** → simple genetic fusions create multivalent, multi-specific molecules, e.g. bi-specific formats, albumin-binding formats to improve half-life
- **Reduced immunogenic risk** → humanized VHHs resemble human VH3 domains and lack an Fc region, lowering possibility for immune side effects
- **Regulatory proof-of-concept** → FDA-approved Caplacizumab shows nanobodies can meet safety, efficacy and manufacturing standards

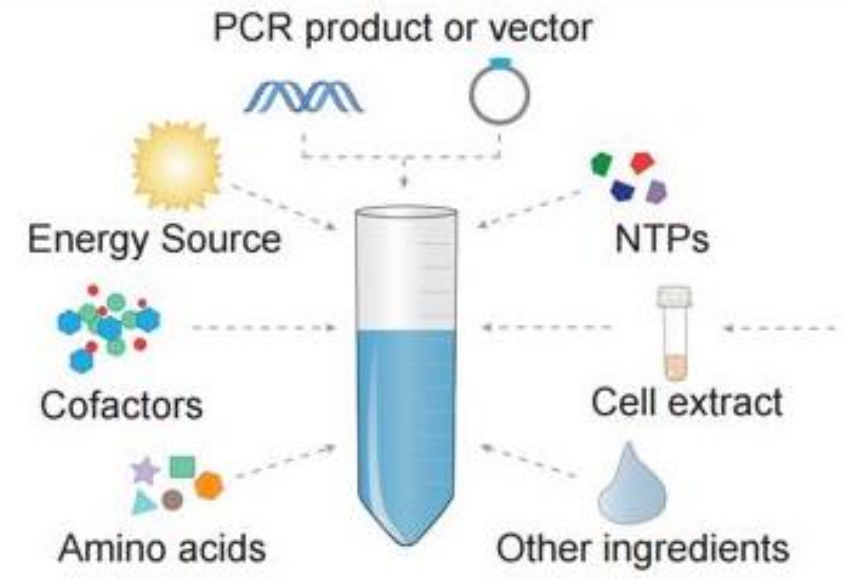
Constant Scaffold with ML-Driven CDR Diversification

Backbone And Tags Stay Fixed While Machine-learning Guides Sequence Variation Only Within CDR1-3



Ordaōs Proprietary Cell-Free Expression Platform

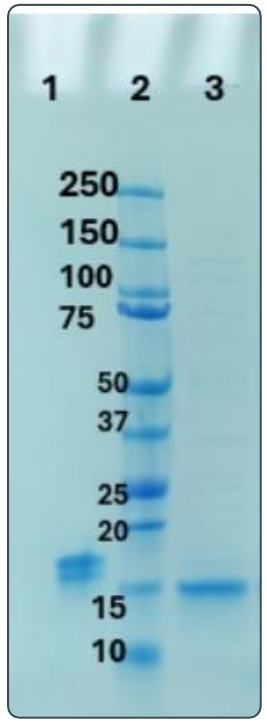
From −80 °C Freezer Stocks To Functional Protein In < 2 hours



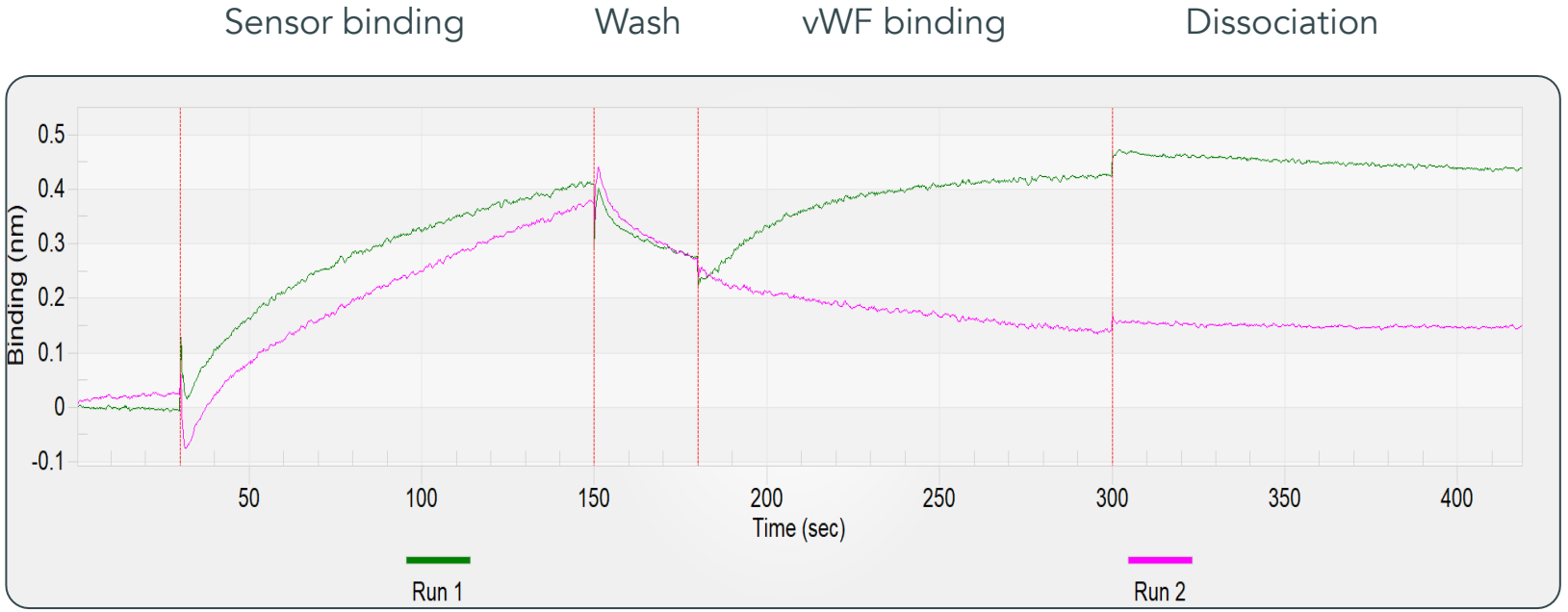
- **Rapid Protein Generation** → 60–90 min expression run; complete freezer-to-protein cycle in under two hours.
- **Cost-Efficient & In-House Controlled** → reduces per-sample cost and gives greater flexibility to optimize reaction conditions.
- **Engineered E. coli Lysate** → Mechanically lysed e. coli strain engineered for high soluble yield.
- **Minimal Reagent needed** → 20 µL-scale reactions (ng production) produce enough protein for Carterra screens.
- **Flexible DNA template** → Accepts both linear DNA or plasmids.
- **Handles "Difficult" Targets** → Toxic, membrane, or aggregation-prone proteins expressed without the constraints of live-cell growth.
- **Easily Scalable & Automatable** → 2–50 µL reactions in 96- or 384-well plates; compatible with liquid-handling robots.

Cell-Free-Expressed Caplacizumab Binds vWF with Native Affinity

Purified CFE Product Yields Correctly Folded, Active, Disulfide-bonded VHH



Lane 1: Mammalian produced VHH
Lane 2: Ladder
Lane 3: CFE produced VHH; C-tag Purified



vWF in green BSA in pink

- Functional CFE Nanobodies → CFE-produced Caplacizumab (nanobody monomer), binds to von Willebrand factor (vWF), demonstrating the capability of our CFE system to produce properly folded, disulfide-formed, functional nanobodies

Our fully integrated RL-guided GenAI tailors proteins to disease specific-targets

Reinforcement Learning-Based Model Update

IN SILICO DESIGN ENGINE

Broad Design

Structure-Native *de novo* Generation

Optimization

Alanine Scan-Guided Positional Selection

LLM* Mutation

Inpainting

Prep

Structure Quality Assessment

Complex Docking

MD Simulation

Evaluation

Ab initio Physio-Chemical Property Characterization

Foundation Model-Based Metric Evaluation

Graph-Based Structure, Interaction Evaluation

IN VITRO WETLAB

Characterization

- Target Binding
- Expression Yield
- Protein Purity
- Thermostability
- Aggregation
- Target Epitope
- Specificity
- Immunogenicity

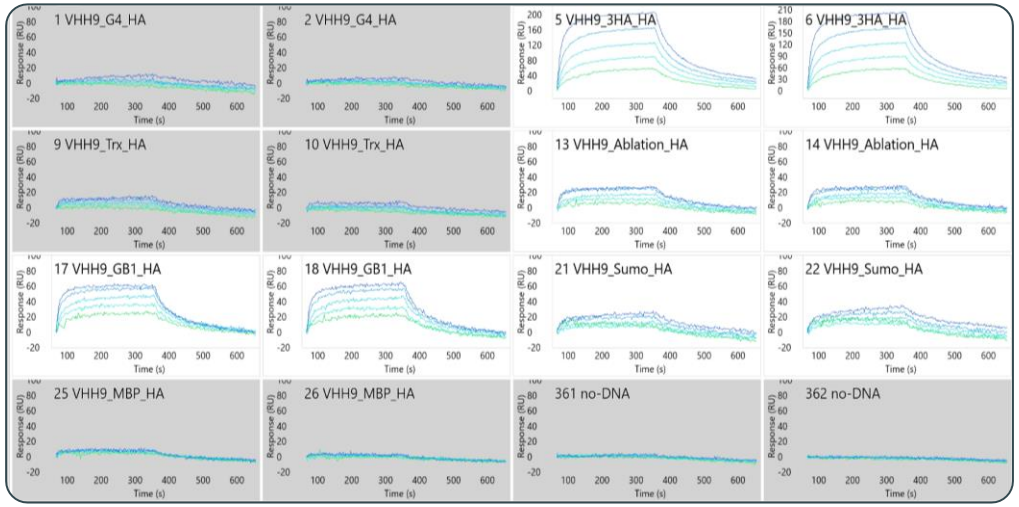
Heuristic Search

Small-Data Optimized Model Update

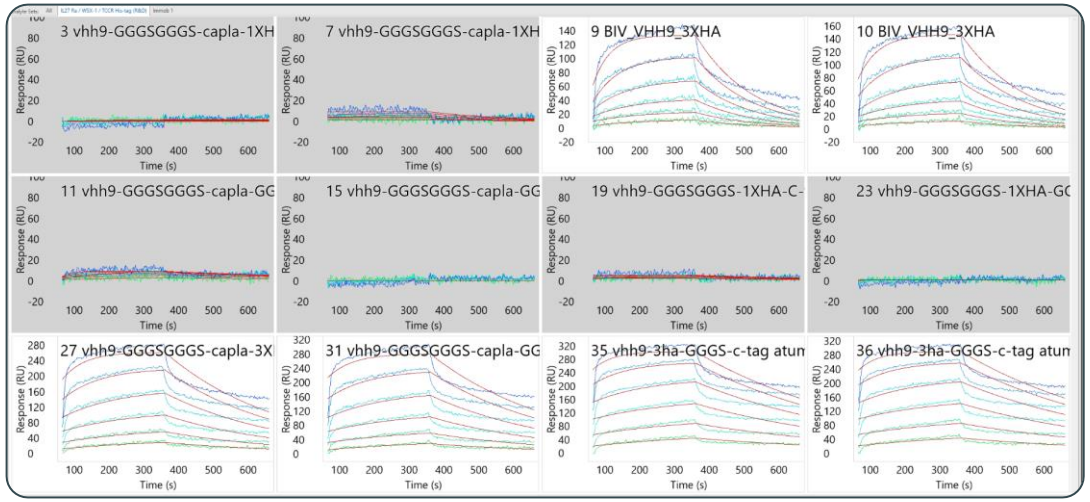
*Large Language Model

Optimizing Linker and Tag Strategies for Enhanced SPR Performance and Purification

Maximizing SPR Retention and Purification Efficiency through High-Affinity Tag



Changing type of spacer/linker

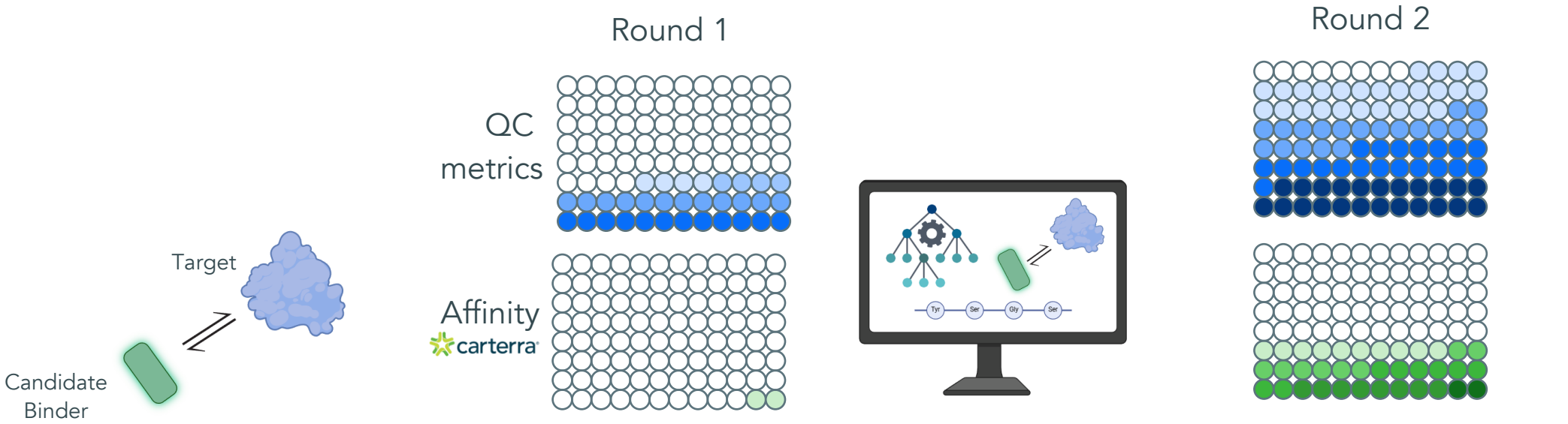


Changing order and length of linker

- **Linker Optimization** → Discovered that the 3xHA tag outperforms the 1xHA tag in terms of SPR performance.
  - Achieved picomolar capture affinity for our Nanobodies with 3xHA tag
  - Excellent retention on SPR chip, even remains after glycine pH 2.0 regeneration.
- **Tag Order Doesn't Impact Performance** → Modulating tag order had minimal effect on retention and affinity.
- **C-Tag for Purification** → Used for protein purification, also providing high specificity and affinity with our method. We may pivot this tag for capture as well, as the anti-Ctag VHH is extremely stable/regeneratable

Iterative ML-Driven Binder Optimization

Iteration Strategy: Computational design, rapid screening, and ML feedback in a closed loop to Refine binders

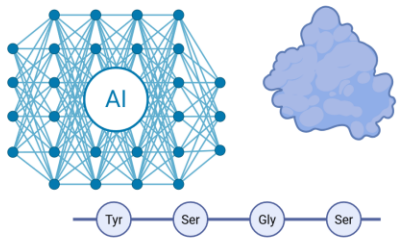


Ordaōs Utilizes Crude CFE Screening with Carterra LSA

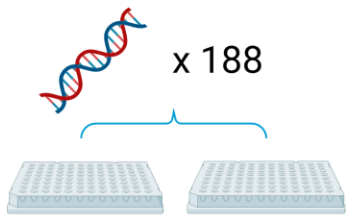
Shorter Iteration Time Means More Data And Faster Improvement

Variable
(hours-days)

Step 1: AI Protein Design

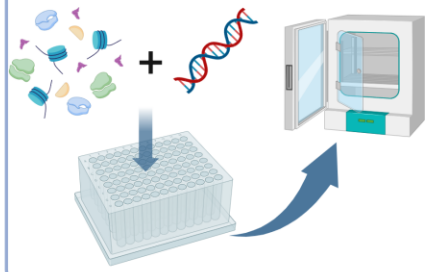


Step 2: CRO DNA Synthesis



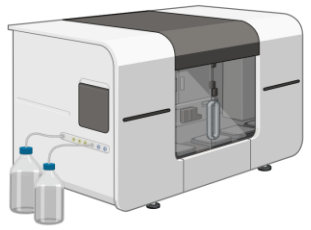
Linear DNA made
In ~3 days
Biggest bottleneck is DNA production

Step 3: Cell-free Expression



~2 hours
for prep & incubation

Step 4: Screen with Crude on Carterra



6-hour* Carterra LSA run

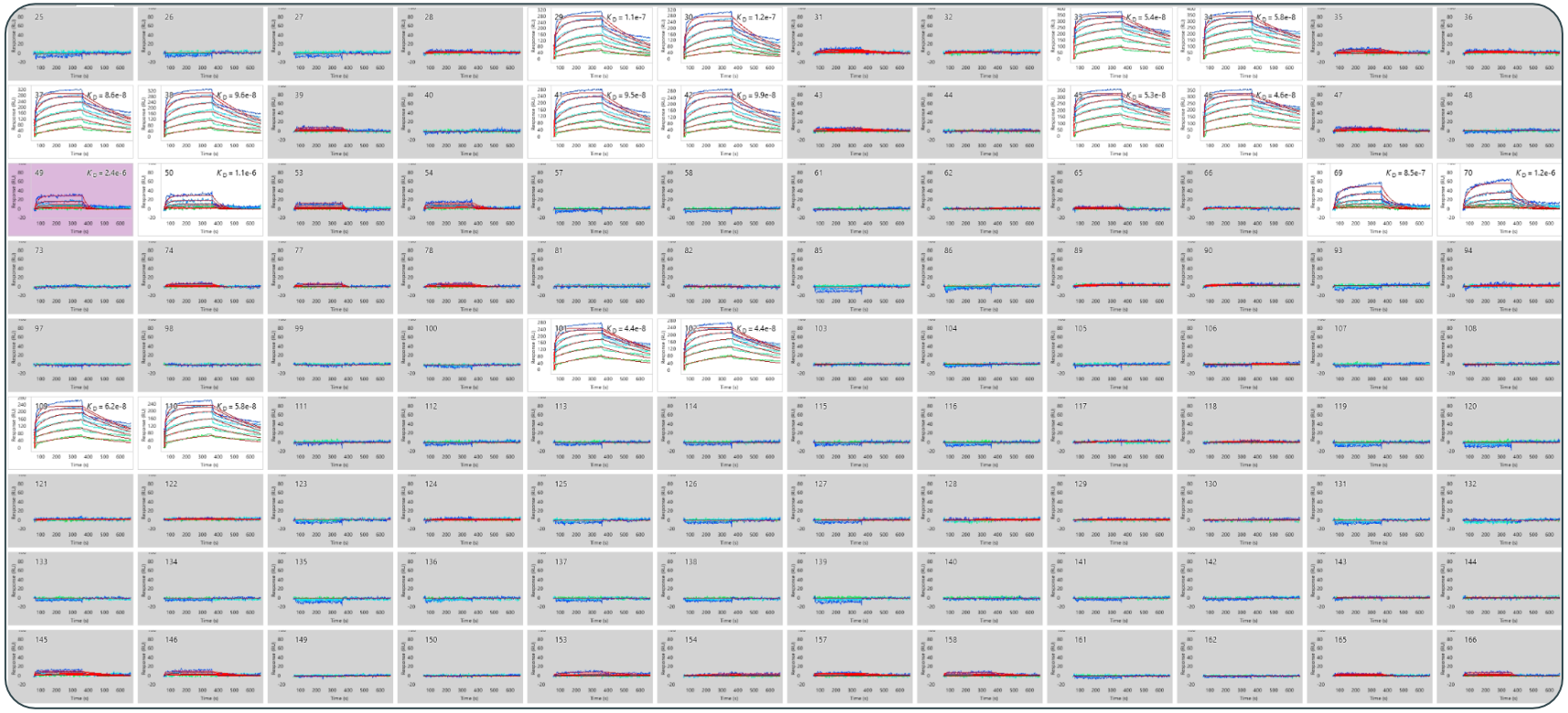
*or longer if testing multiple targets

Day 1

Day 4

High-Quality Binder Screening on Carterra LSA with µL-Scale CFE Lysate

High-Throughput Assessment of Candidates is possible with Carterra LSA

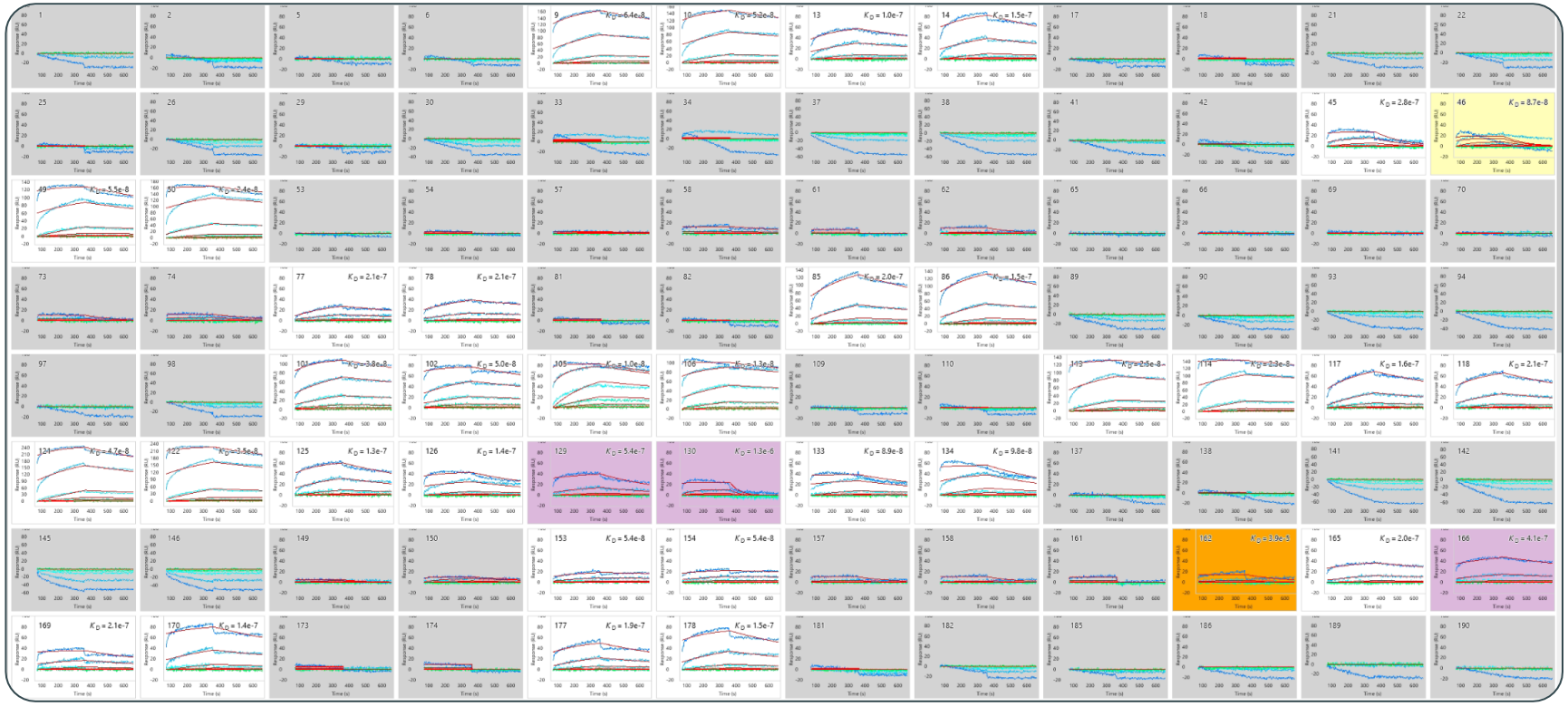


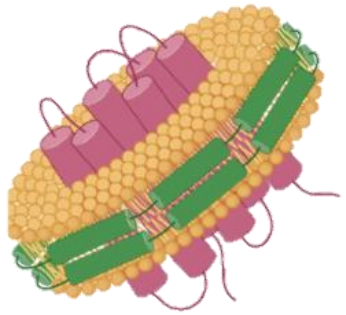
- Crude binding done with 20 µL CFE material and 1 µL DNA
- 2 wells per plate used for negative and positive control
- Duplicates performed for all samples enabling 188 candidates tested per run

Nanodiscs: GPR Binder Screening on Carterra LSA with µL-Scale CFE Lysate

Binder screening against difficult membrane-bound GPCR targets

carterra

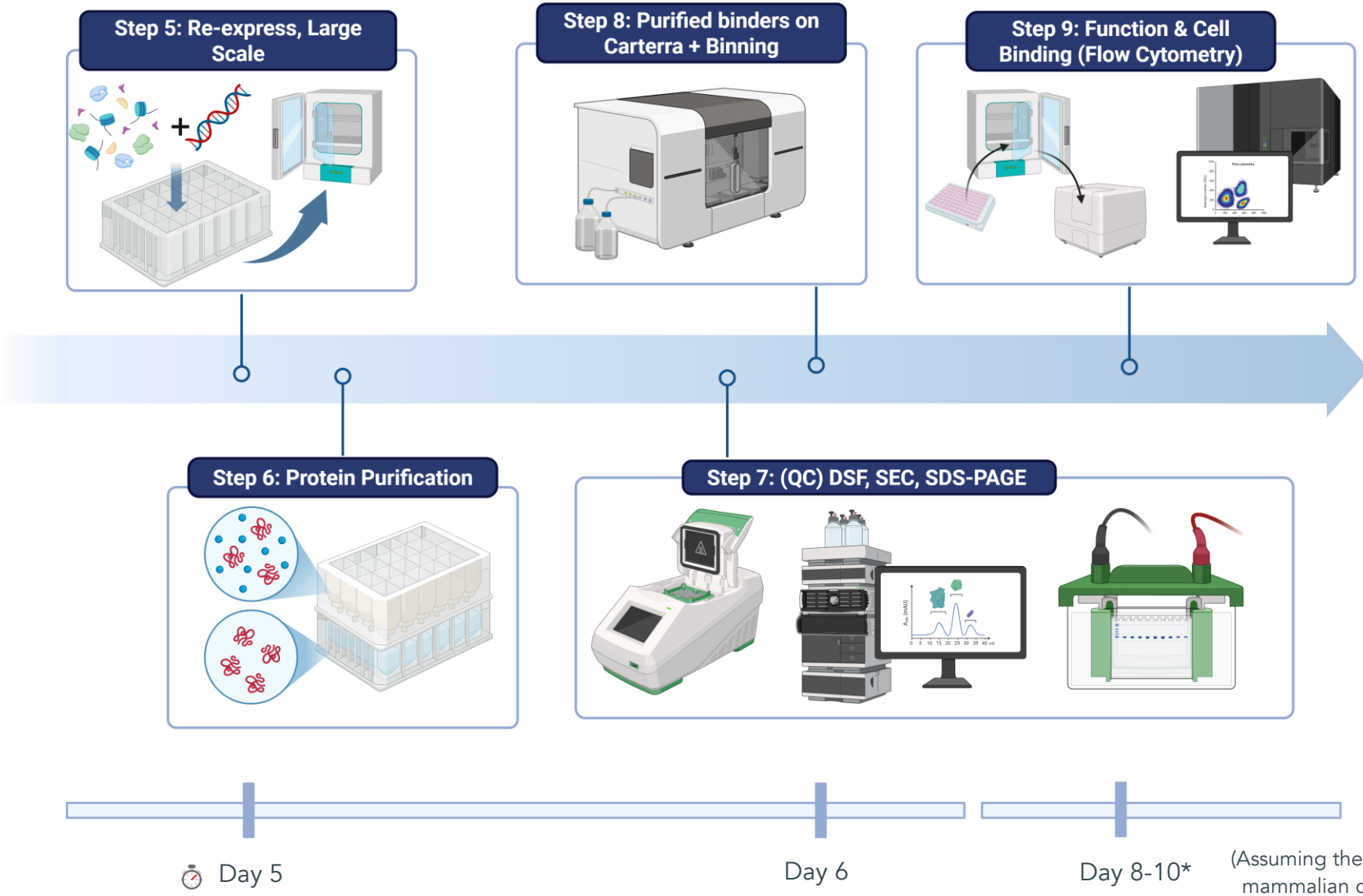




- **Nanodisc prep** → GPCRs reconstituted in-house or sourced commercially, preserving native conformation for SPR.
- **Crude lysate screen** → µL-scale CFE lysate identifies high-affinity, GPCR-specific binders

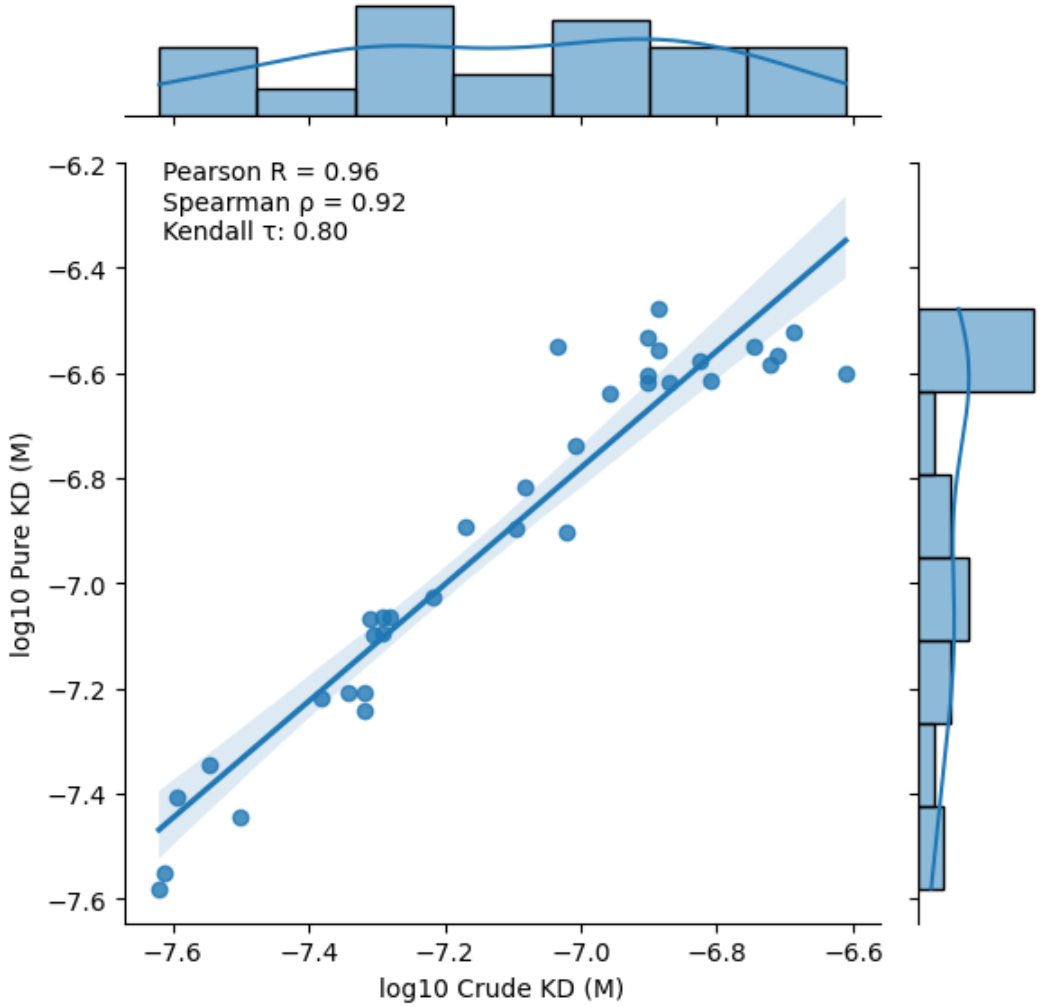
Ordaōs generates validated proteins from AI designs in days

SHORTER ITERATION TIME MEANS MORE DATA AND FASTER IMPROVEMENT



Crude Lysate Screening Accurately Predicts Purified Protein Binding

Comparable Kinetics Observed With Both Crude And Purified Formats

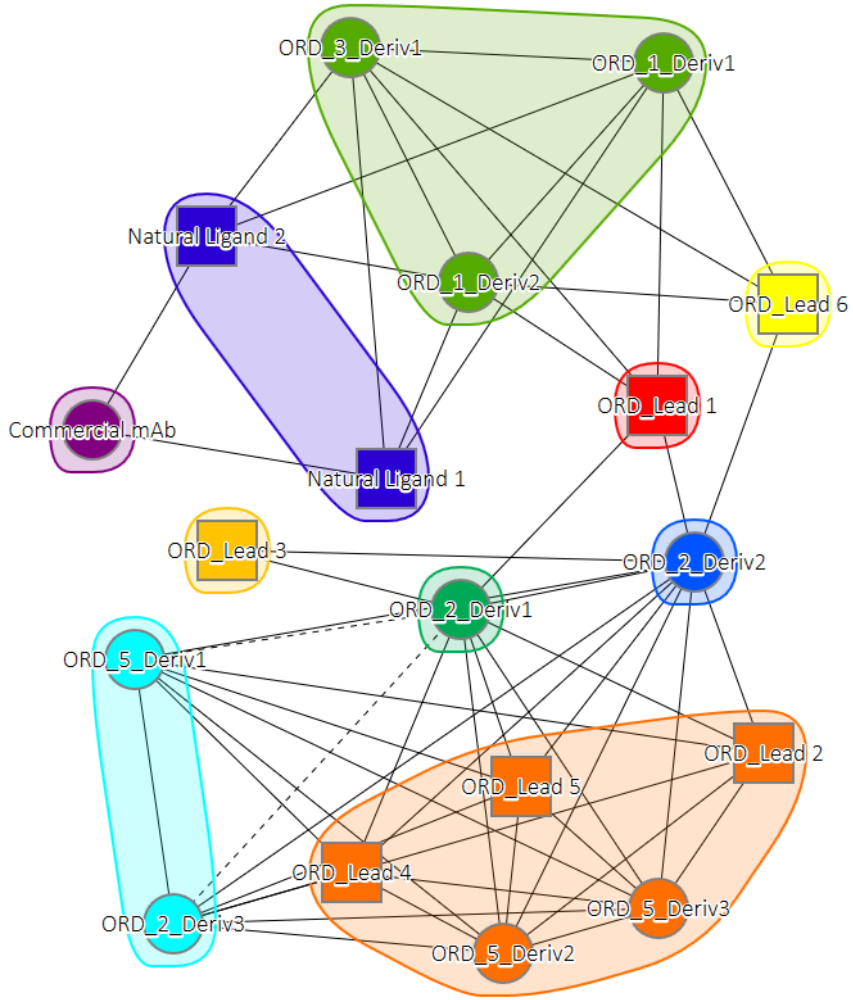


Carterra LSA Crude Lysate vs Purified Binding Affinity

- **Strong agreement** → (R = 0.96; ρ = 0.92) between KD measured in CFE crude lysate and the same binders after purification via c-tag
  - High correlation means we can rank candidates directly from crude reactions, then purify only the top hits for confirmation
  - Validates crude-lysate SPR as a reliable, high-throughput strategy for our ML-driven optimization loop
- **Saves time and materials** → Cuts days and consumables out of each design cycle while maintaining data quality

Epitope Binning to Characterize Nanobody Binding Profiles

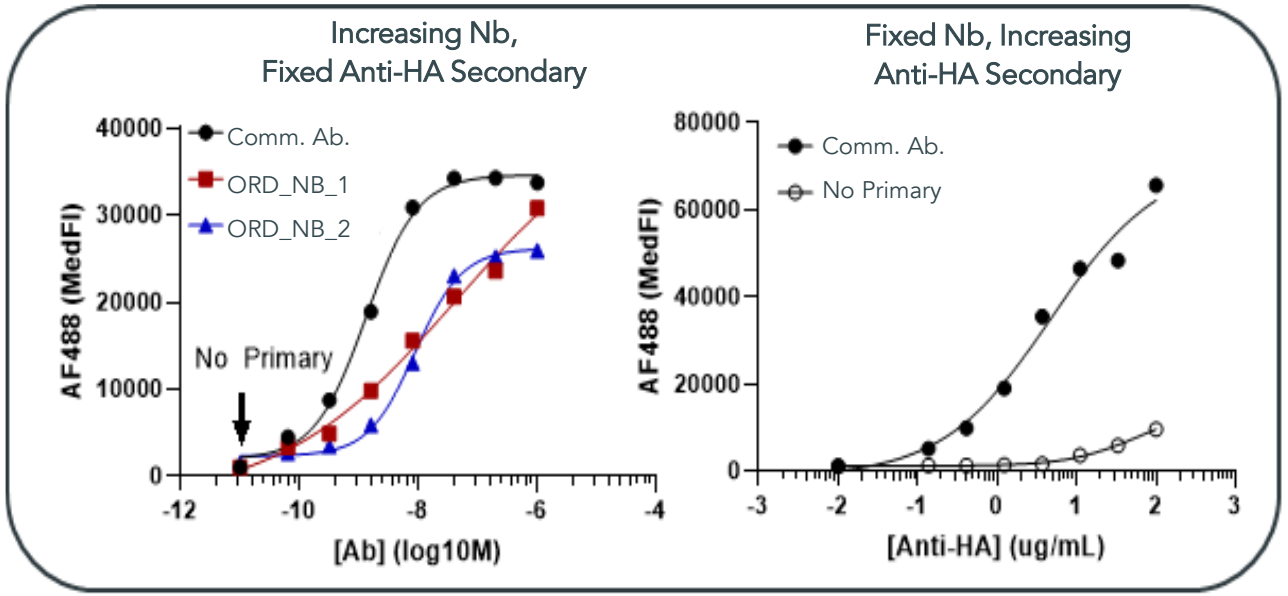
Assessing Epitope Overlap and Competition with Natural Ligands and Commercial Antibodies

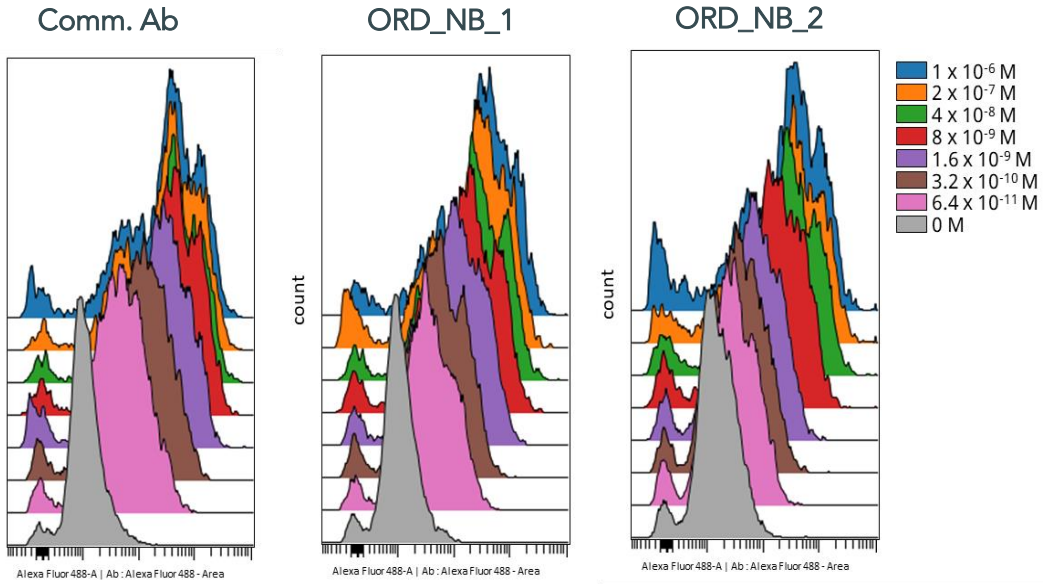


- **Binning** → Carterra LSA groups nanobodies into distinct epitope communities based on competition profiles
  - We can also understand potential steric clashes between nanobody candidates that compete but are known to have distinct epitopes based on HDX data
- **Natural Ligand & Commercial Benchmark** → With epitope binning, we can see whether our nanobodies compete/share epitopes with commercial antibodies as well as the target's natural ligand
- **Candidate Selection** → Informs rational combinations and bispecific designs based on epitope relationships.

Cell Binding Assessment of Purified Nanobody Candidates

Flow Cytometry to Confirm Target Engagement on Cells



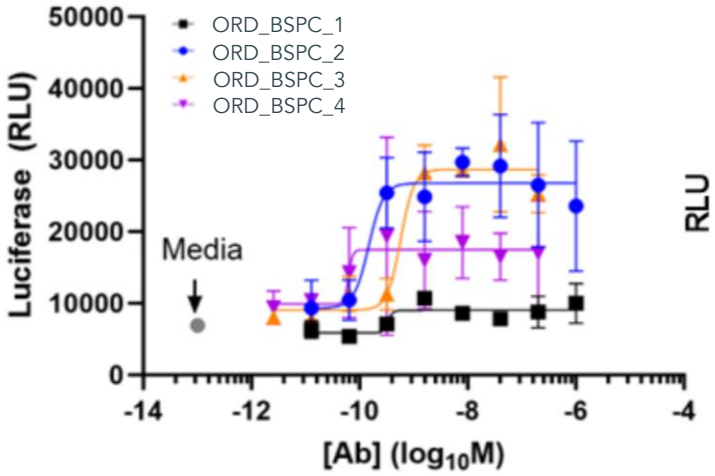


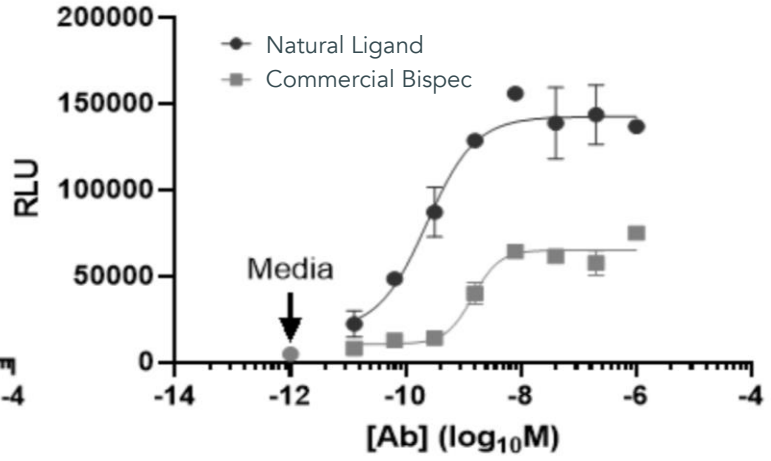
- **Validated & Purified Binders** → Among binders that bind and pass QC thresholds, candidates advance to cell-binding assays to determine whether binding is also seen *in vitro*

Strategic Multimerization to make Functional Molecules

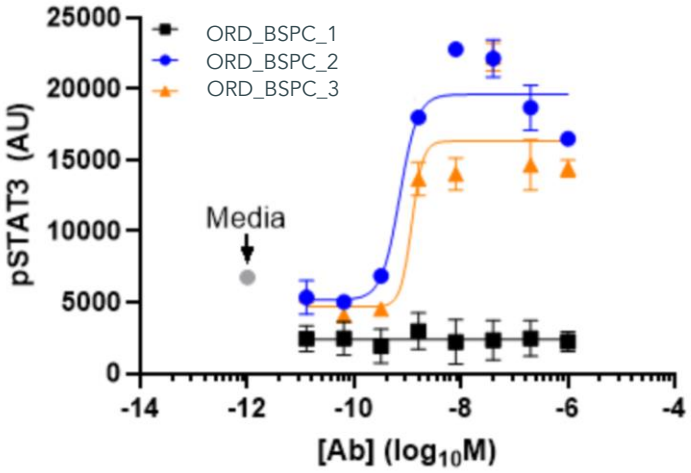
Bispecific Reformatting and Avidity Optimization Yield Clinically Relevant Functional Responses

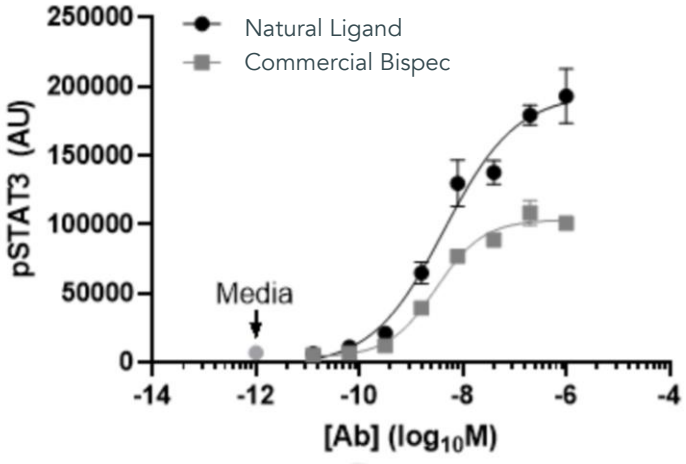
Luciferase Functional Assay



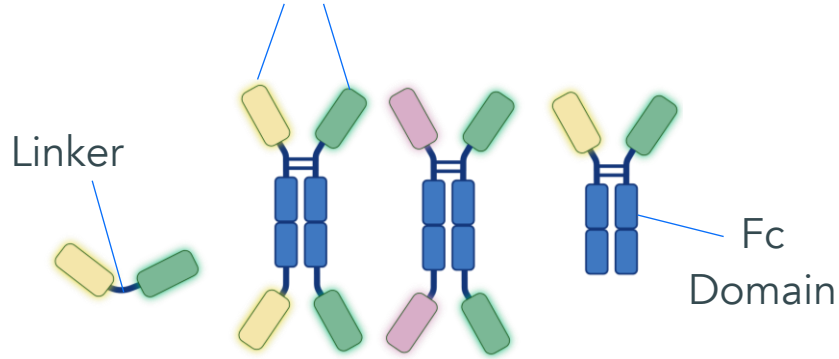


AlphaLISA Functional Assay





Nanobodies against different protein targets



- **Multivalency Enables Function** → In cases where bridging of multiple proteins is required for function, we engineer multivalent molecules
  - Nanobodies are selected with kinetics matched to cell surface target density
  - Linker configurations guided by in silico modeling

Summary

- ML-Guided Binder Design → Rapid generation of high-affinity candidates against disease targets.
- High-Throughput Cell-Free Expression → Produce and screen crude lysates at µL-scale
- Carterra LSA-Powered Screening → Identify hits with crude-to-pure correlation and map epitope diversity via binning
- QC → Evaluate stability, producibility, aggregability of binders.
- In Vitro Binding Validation → Determine cell-binding activity and prioritize candidates with therapeutic potential.
- Functional Engineering → Optimize via multimerization or fusion strategies to enhance avidity and mechanism-specific activity.

Thank you! Let's Connect!

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https://ordaos.bio/

Human-enabled
Machine-driven
De novo designed mini-proteins to help drug hunters deliver life-saving treatments.