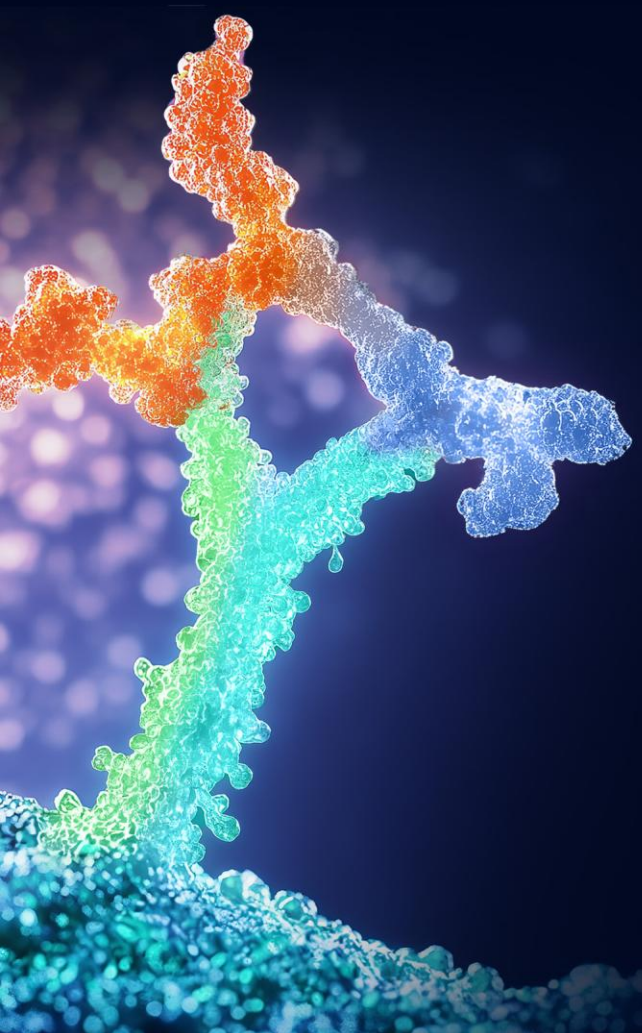
Membrane Protein Targets Reengineered for Soluble Expression

Alex Taguchi

The Trust Gap Between In-Silico Promise and R&D Reality



Generative AI

In silico antibody & antigen design

Trust Gap

R&D Costs

>$100M & >10 years to FDA approval[1]

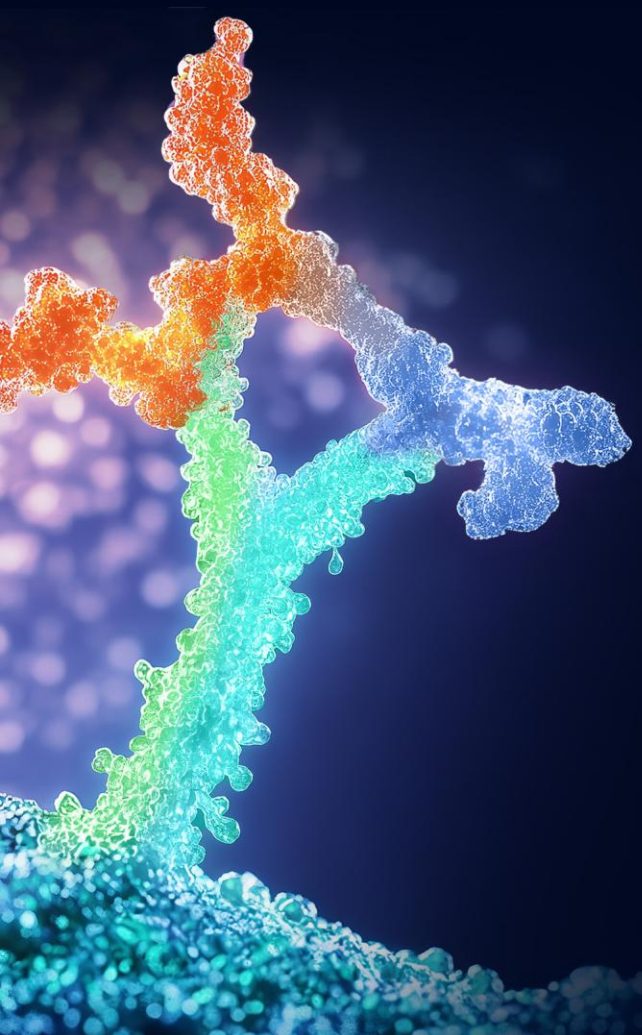
Is AI the right tool for my problem?

Which model(s) should I use?

How do I trust the model output(s)?

[1]*Wouters, O. et al., JAMA (2020)*

The Trust Gap Between In-Silico Promise and R&D Reality



Generative AI

In silico antibody & antigen design

Trust Gap

R&D Costs

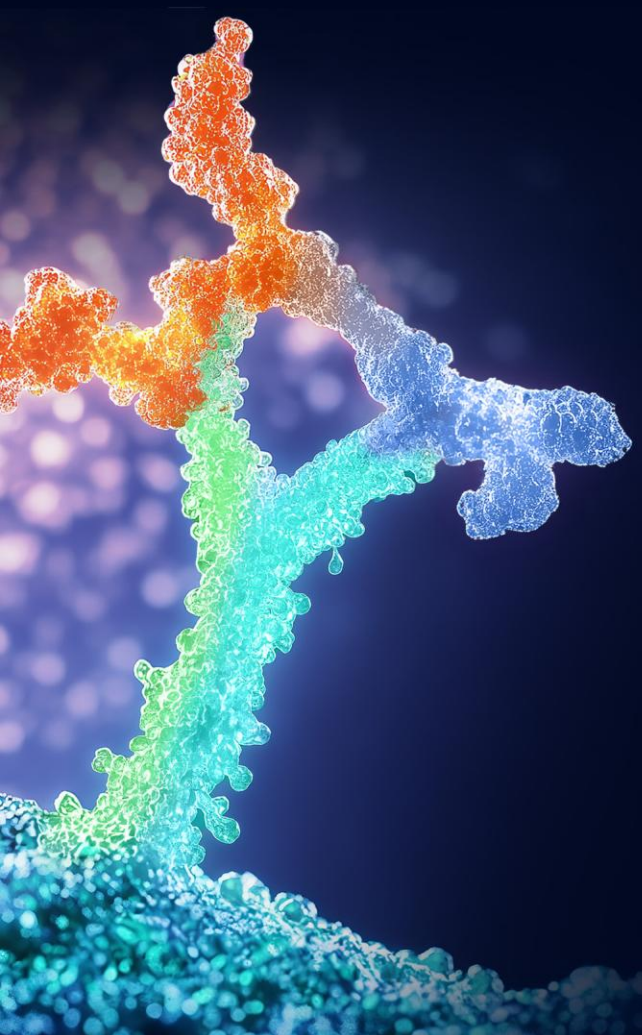
>$100M & >10 years to FDA approval[1]

iBio Engine

Integrated structural and functional validation

[1]Wouters, O. et al., JAMA (2020)

Antibody Discovery to Development Candidate in as Little as 7 Months

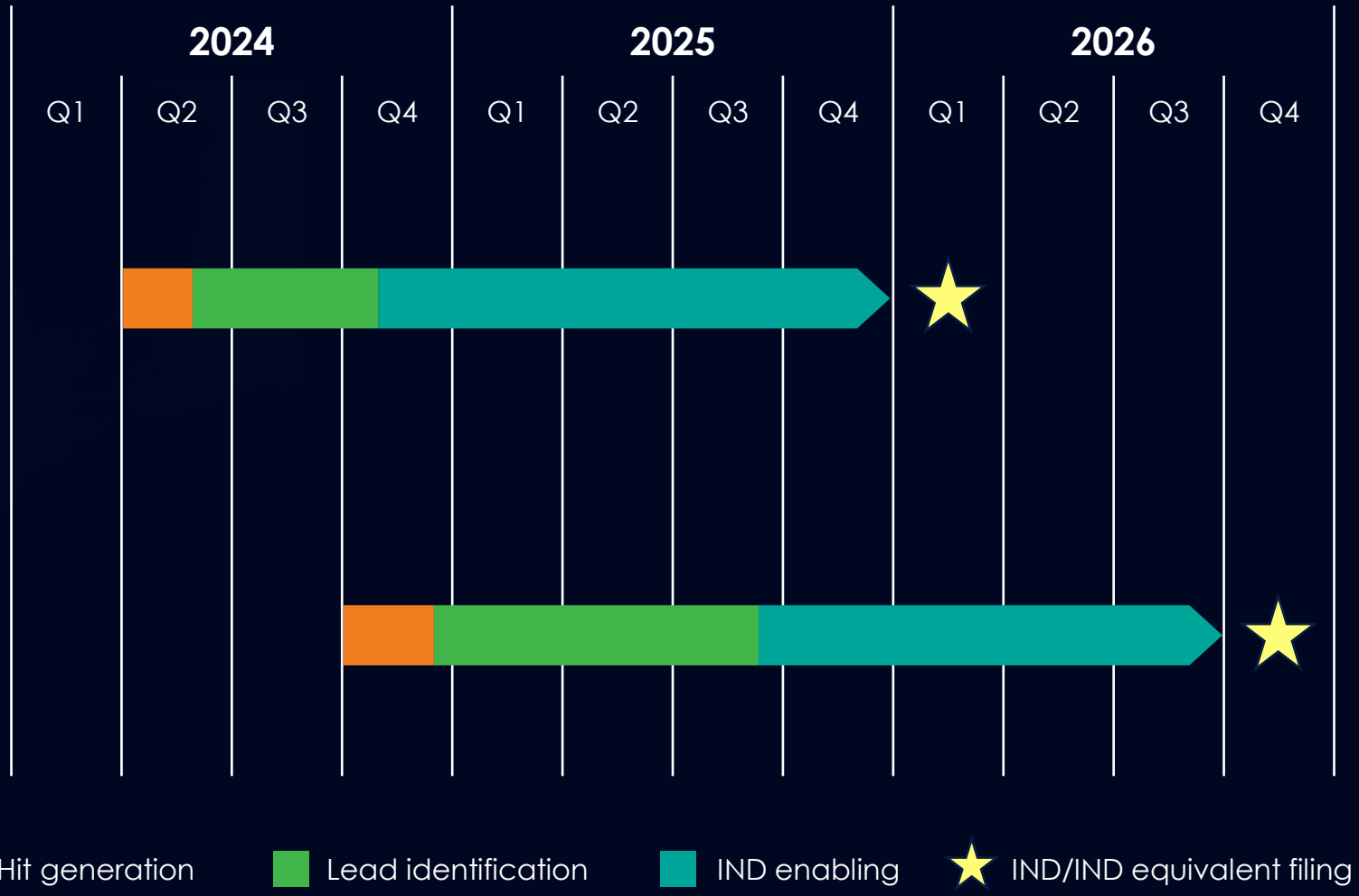


IBIO-600

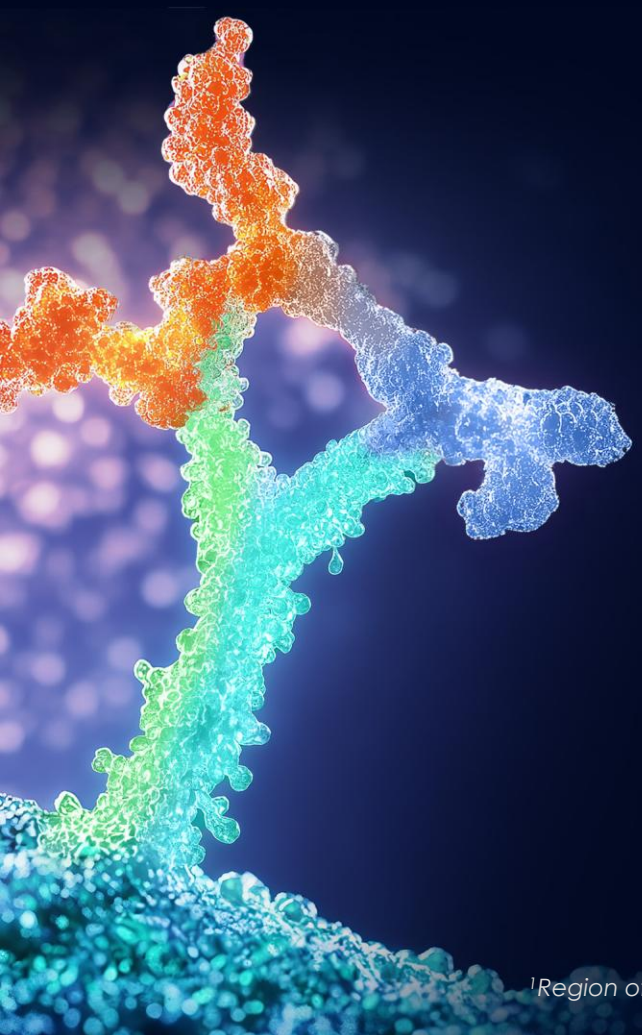
Long-acting myostatin antibody

IBIO-610

Potential first-in-class Activin E antibody

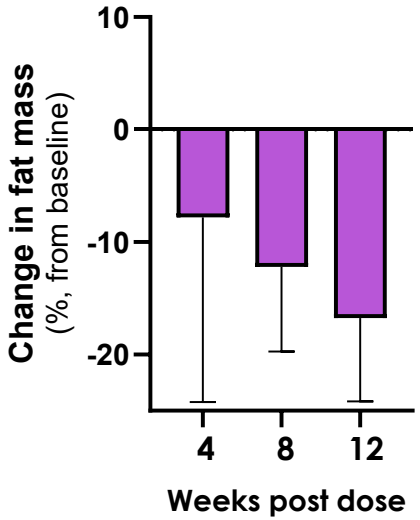


IBIO-600: Long-Acting Myostatin Antibody



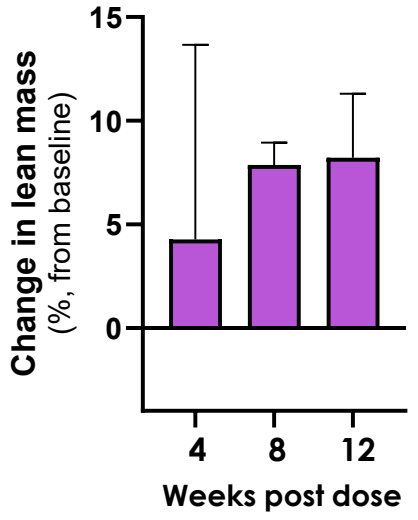
Fat Mass Decrease[1]

Single 5 mg/kg Dose in NHPs

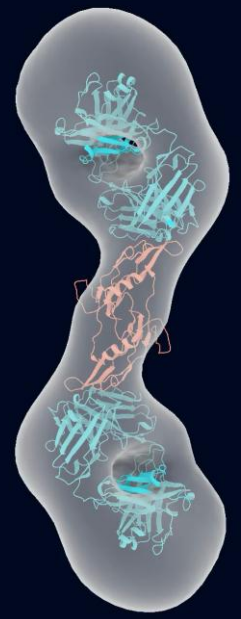


Lean Mass Increase[1]

Single 5 mg/kg Dose in NHPs



Epitope Mapping



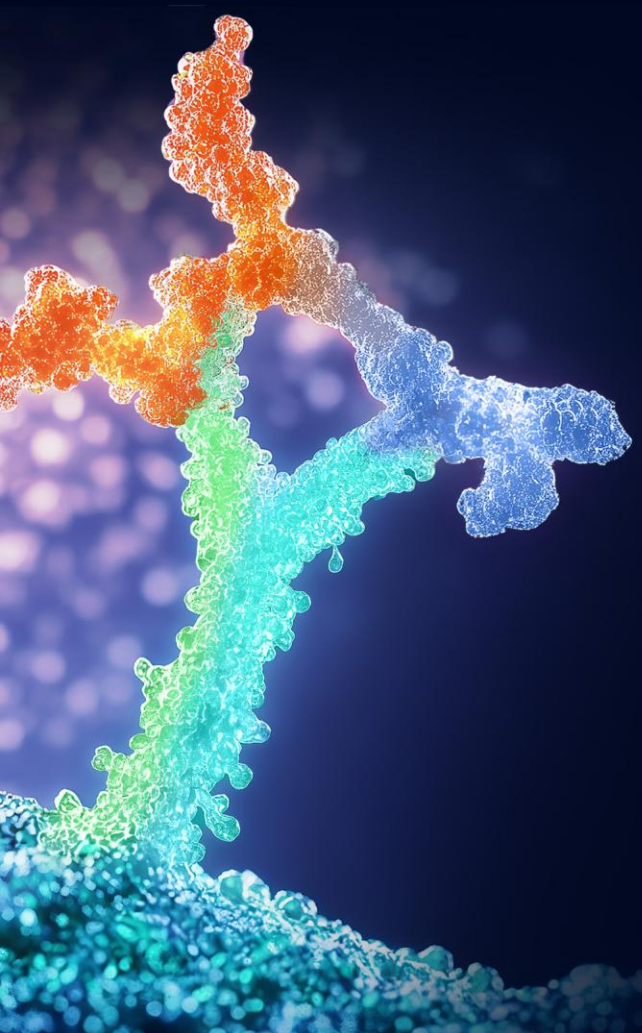
Dose	NHP Half-Life (measured)	Human Half-Life (predicted)[2,3]
5 mg/kg, I.V.	52.4 days	74-147 days

Predicted iBio-600 Myostatin Complex

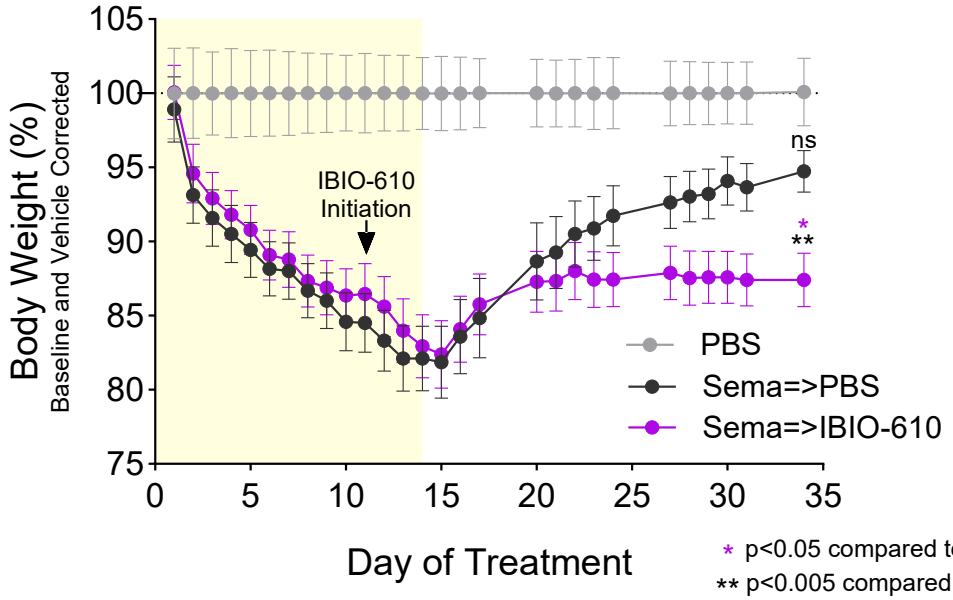
Experimental Electron Density Map

[1]Region of interest (ROI) DEXA focused on gluteal and thigh region, [2]Nakamura, G. et al. Biol. Pharm. Bull. (2020), [3]Haraya, K. & Tachibana, T. BioDrugs (2023)

IBIO-610: Potential First-in-Class Activin E Antibody



Significant Prevention of Weight Regain in DIO Mice



Study Design

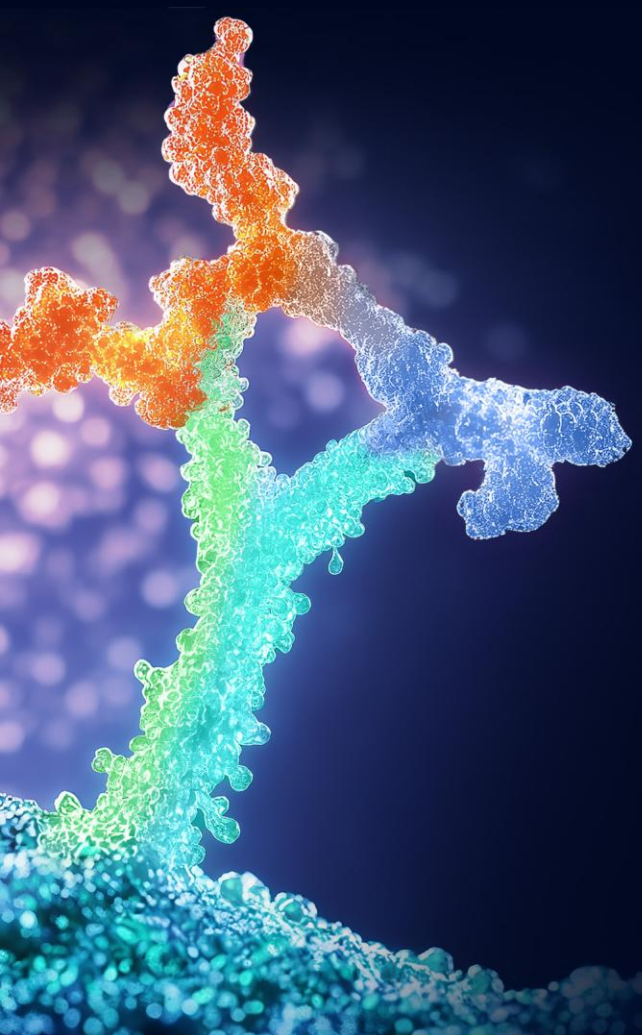
Two weeks of Semaglutide injections followed by IBIO-610 maintenance dosing starting on day 11

Dose	NHP Half-Life (measured)	Human Half-Life (predicted)[1,2]
10 mg/kg, I.V.	33.2 days	47-100 days

Epitope mapping completed; not shown for IP protection

[1]Nakamura, G. et al. Biol. Pharm. Bull. (2020), [2]Haraya, K. & Tachibana, T. BioDrugs (2023)

End-to-End Antibody Discovery Platform Accelerates Path to IND



1 **Engineered Epitope Steering***

AI-engineered antigens steer discovery to intended epitopes

2 **Human Antibody Library**

Naïve antibody library in clinically validated framework

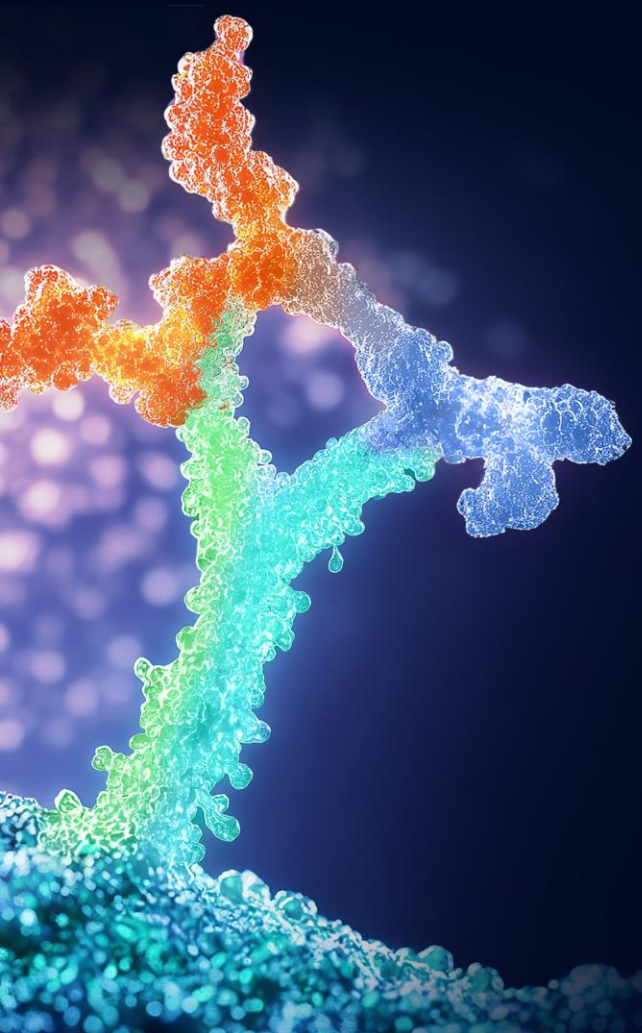
3 **StableHu™ Antibody Optimizer**

Whole molecule optimization with mammalian display platform

On-epitope, Developable Clinical Candidate

** U.S. Patent No. 11,545,238 (issued January 3, 2023)*

End-to-End Antibody Discovery Platform Accelerates Path to IND



1

Engineered Epitope Steering*

AI-engineered antigens steer discovery to intended epitopes

2

Human Antibody Library

Naïve antibody library in clinically validated framework

3

StableHu™ Antibody Optimizer

Whole molecule optimization with mammalian display platform

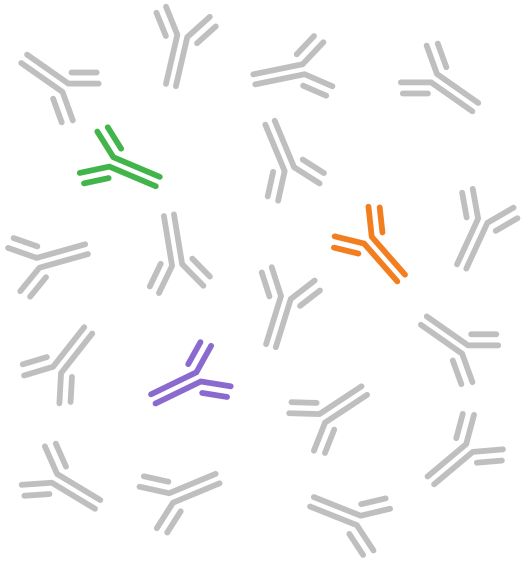
On-epitope, Developable Clinical Candidate

** U.S. Patent No. 11,545,238 (issued January 3, 2023)*

Engineered Epitopes Enable Rapid Discovery of On-Epitope Antibodies

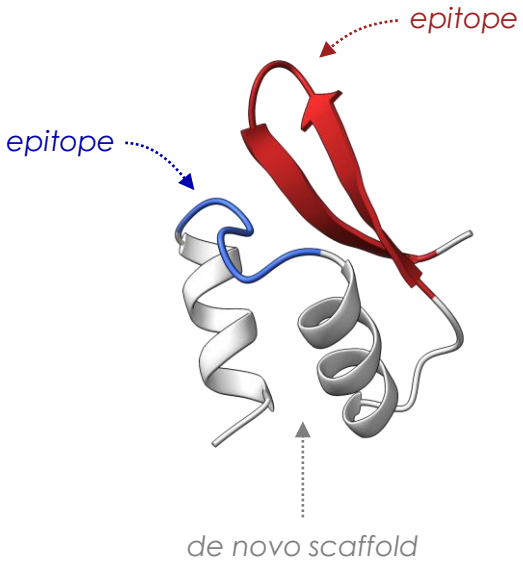
1

Naïve in vitro or in vivo antibody library



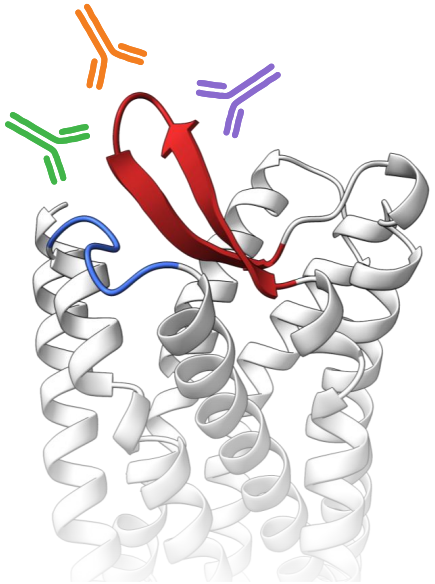
2

Focus library with engineered epitopes



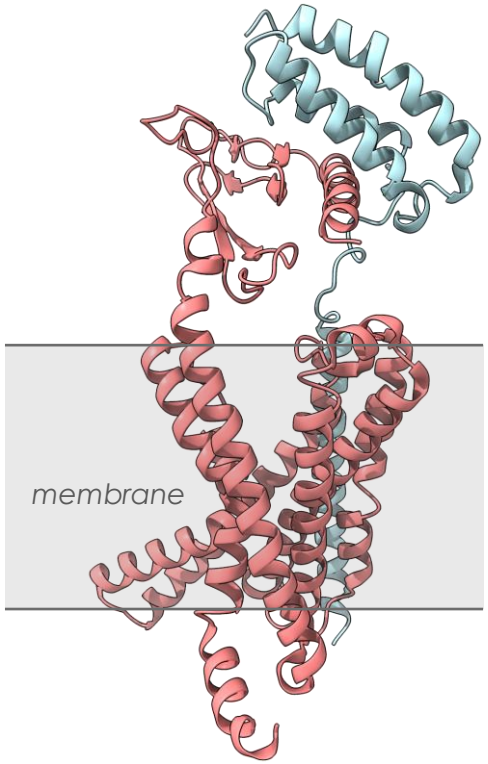
3

Efficient discovery of epitope-specific antibodies

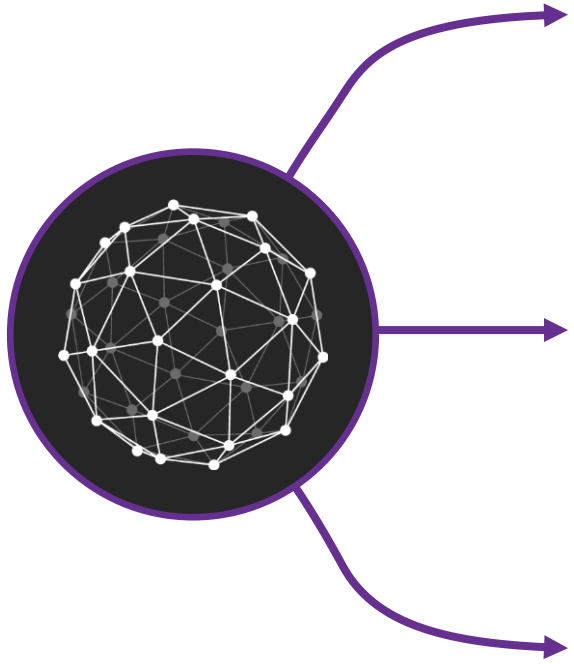


Engineered Epitopes Are Tailor-Made Solutions for Your Target of Interest

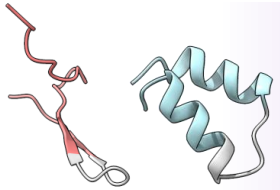
Target of Interest



Generative AI

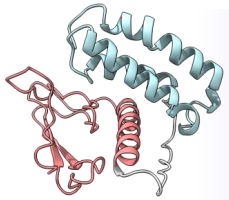


Use Cases



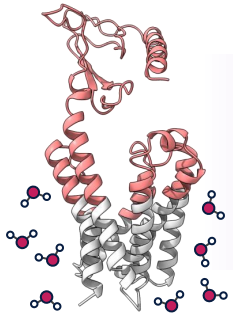
Epitopes of Interest

Design scaffold supporting native epitope structure



Protein Complexes

Stabilize junctional and/or discontinuous epitopes



Membrane Proteins

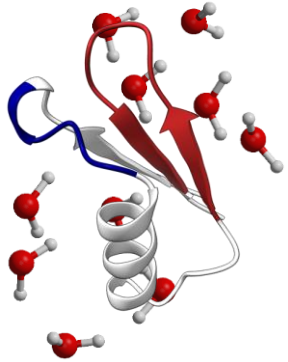
Solubilize transmembrane domains

Antigen Designs are Optimized for Antibody Drug Discovery

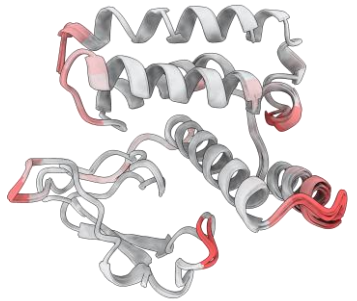
Water Solubility

Epitope

Scaffold



Structural Stability

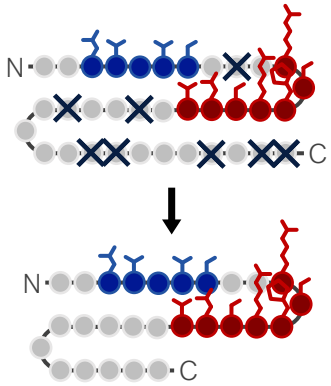


Predicted Flexibility

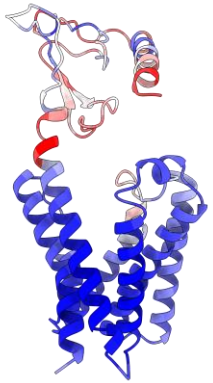
Scaffold Minimization

Epitope

Scaffold

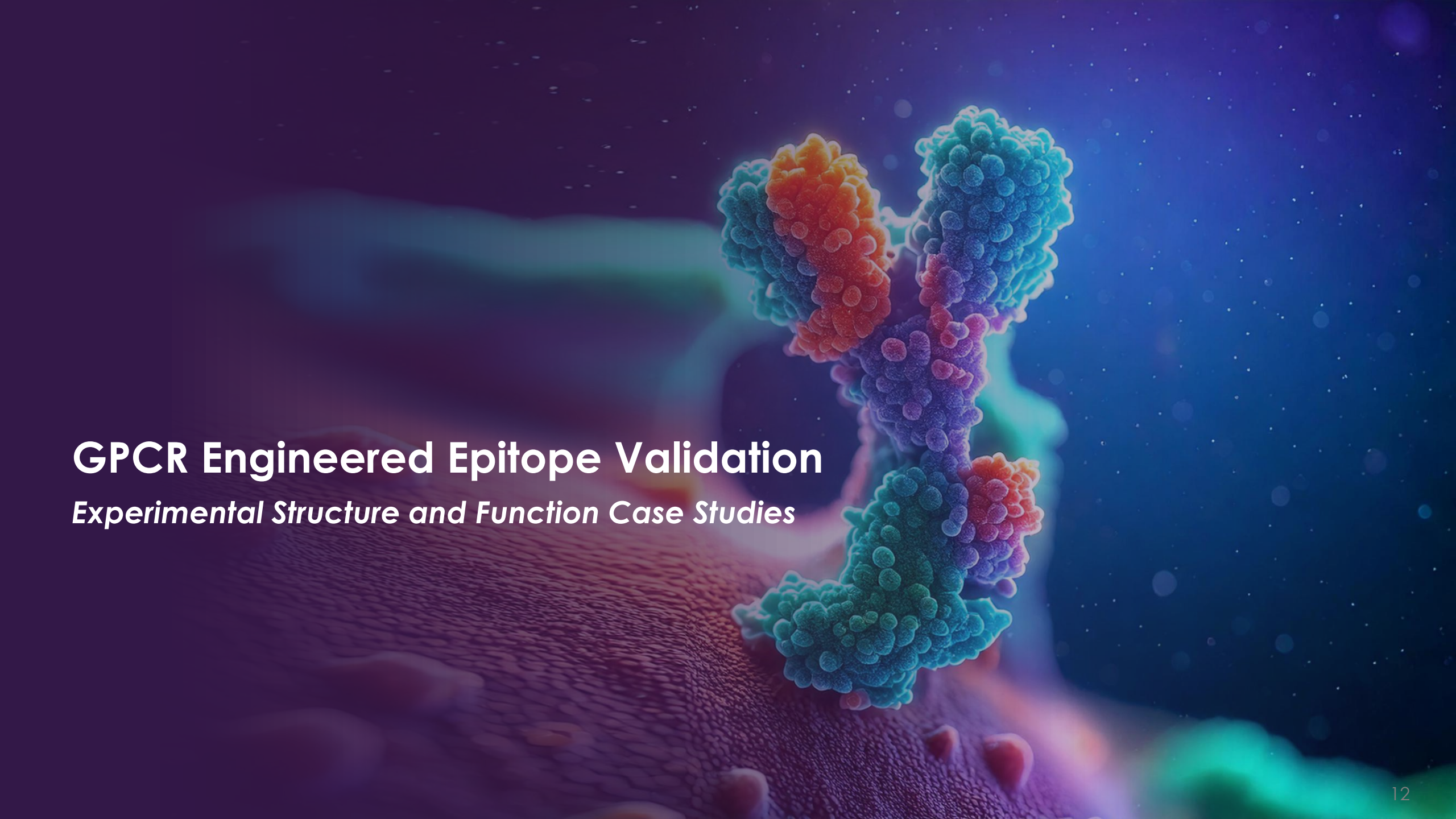


Low Scaffold Immunogenicity



Predicted Immunogenicity[1]

[1]Ponomarenko , J. et al. BMC Bioinformatics (2008)

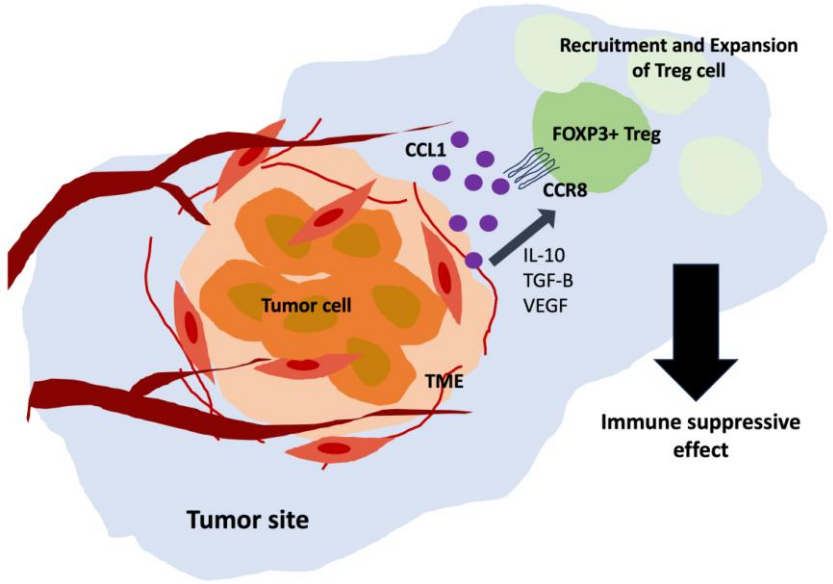


GPCR Engineered Epitope Validation

Experimental Structure and Function Case Studies

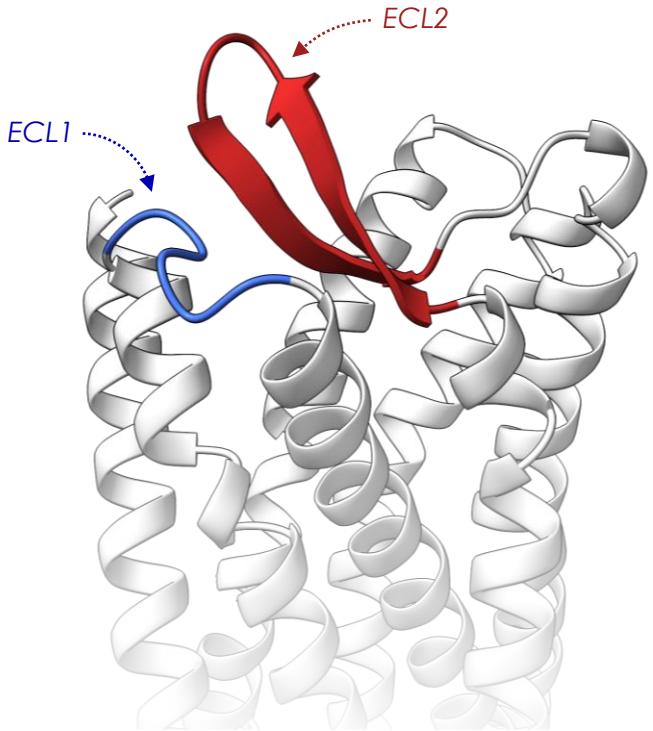
Case Study #1: CCR8

CCR8 is an Emerging Immuno-Oncology Target



CCR8 is upregulated on regulatory T cells (Tregs) within the tumor microenvironment (TME), where it promotes Treg recruitment, expansion, and immunosuppression

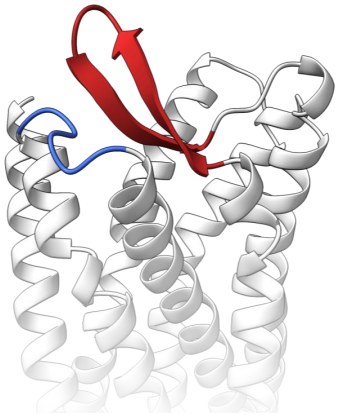
Epitope Targeted for Treg Depletion



NMR Structure Validates Engineered Epitope Design

Input Target

CCR8

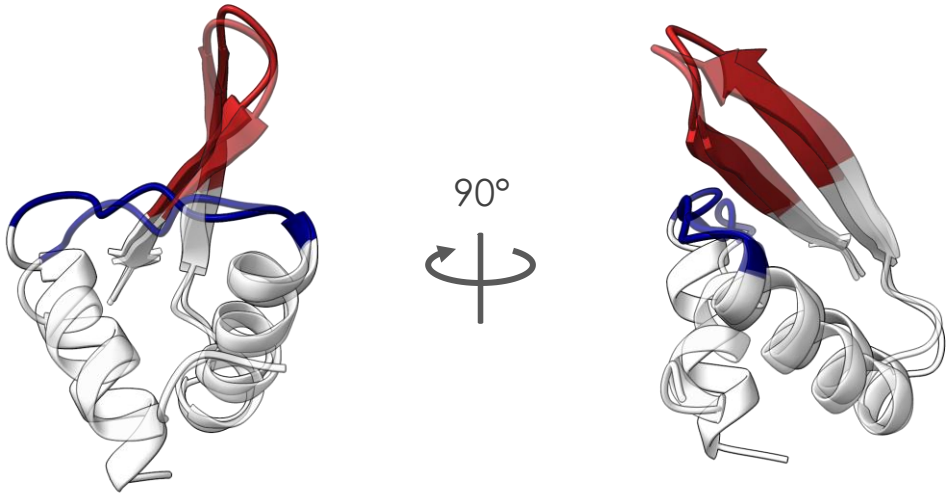


AI Protein Design

Discontinuous Epitope Scaffold Design

Engineered Epitope

Experimental NMR structure (transparent overlay) aligns well with generative AI design*

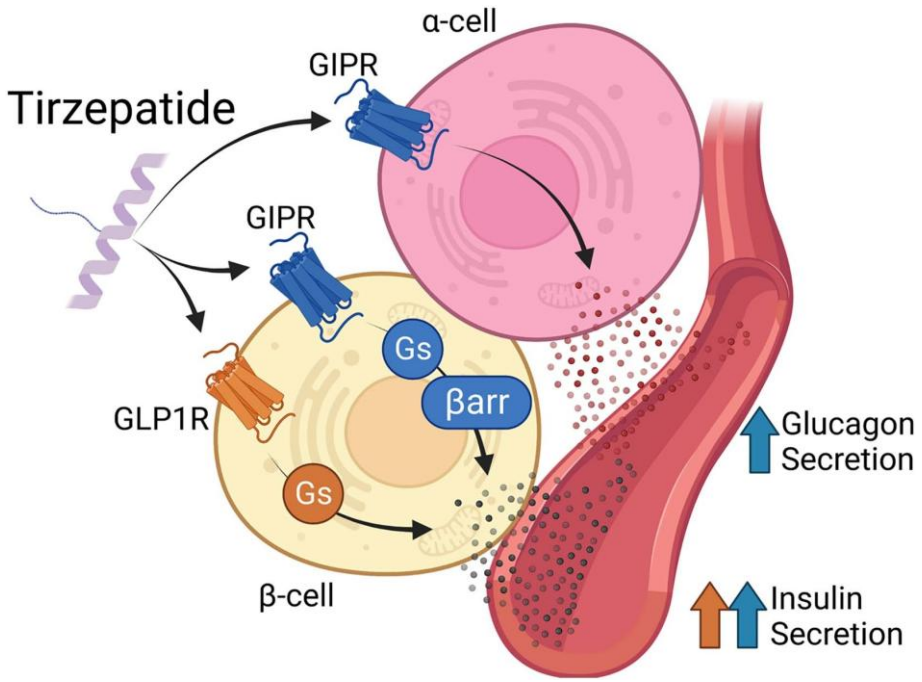


Backbone RMSD = 1.6 Å for epitope residues

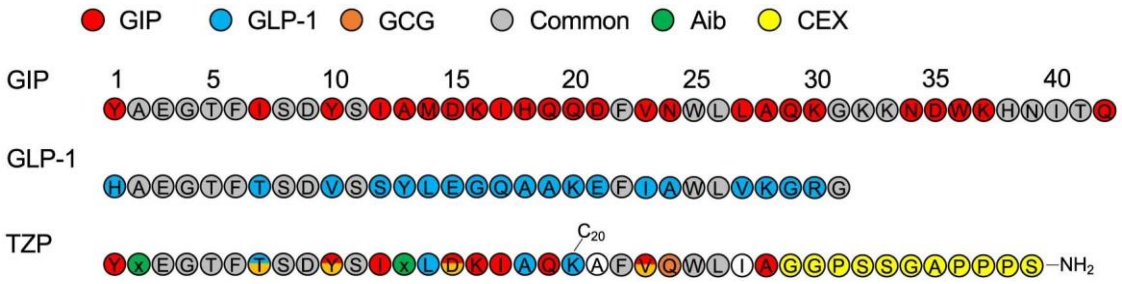
* *Unpublished collaboration with Arizona State University (2021)*

Case Study #2: GIPR

GIPR is a Target of Weight Loss Drug Zepbound® (Tirzepatide)



Challenge: Design Soluble GIPR that Specifically Binds GIP and not GLP-1

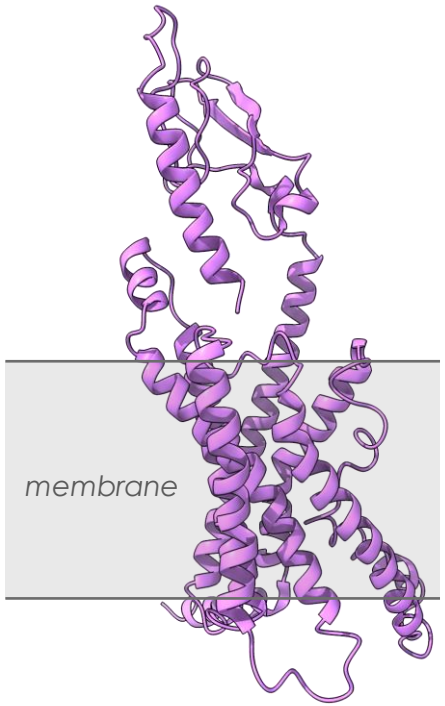


GIPR and GLP-1R are structural homologs whose natural ligands GIP and GLP-1, respectively, share high sequence identity at the N-terminus

GIPR is Solubilized by Reengineering the Transmembrane Domain

Input Target

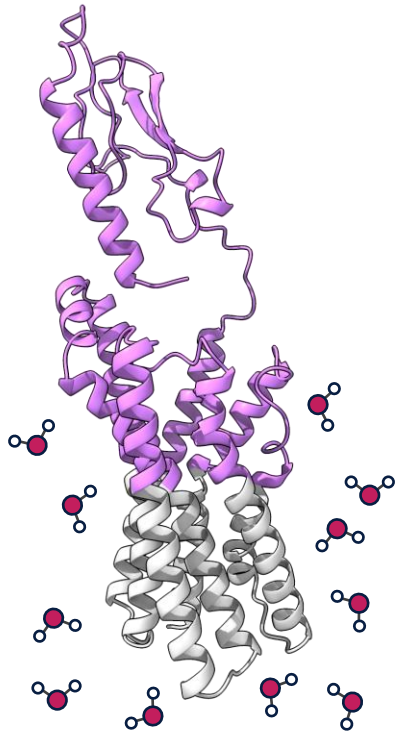
GIPR



Generative AI

Solubilization of transmembrane domain

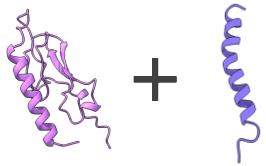
Soluble GPCR

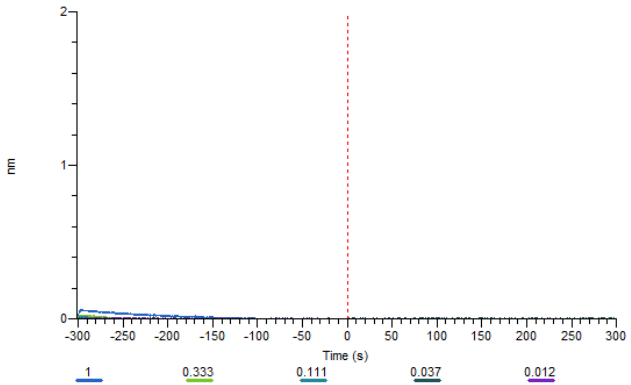


- ✓ *Native-like extracellular region*
- ✓ *Water solubility*
- ✓ *High Stability*
- ✓ *High Expression*
- ✓ *PTMs by expression in human cells*

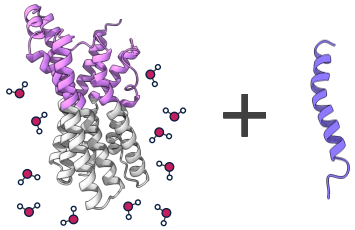
Both Extracellular and Transmembrane Domains are Required for GIP Binding

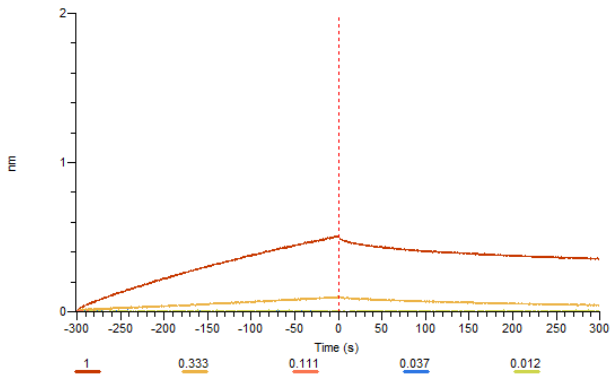
Extracellular Domain (ECD) Binding to GIP



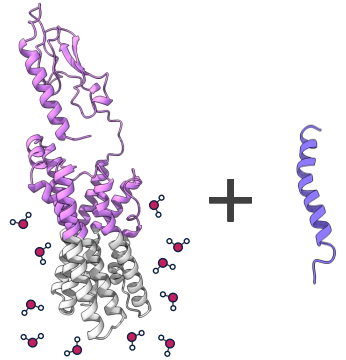


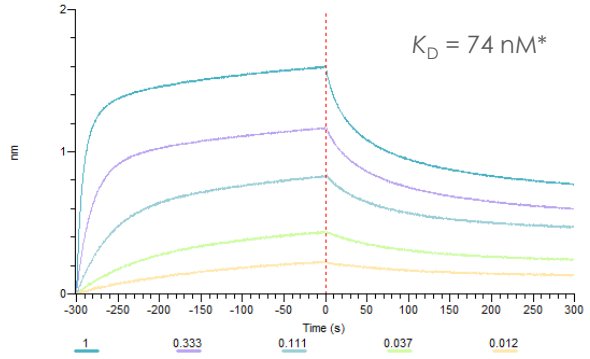
Transmembrane Domain (TMD) Binding to GIP





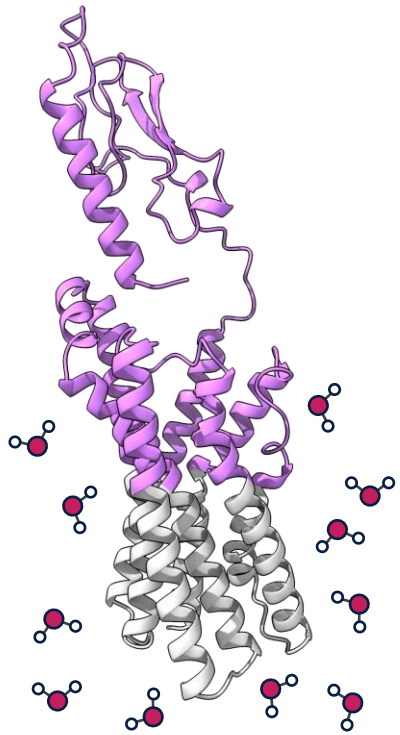
ECD + TMD Binding to GIP





K_D calculated from avidity binding kinetics

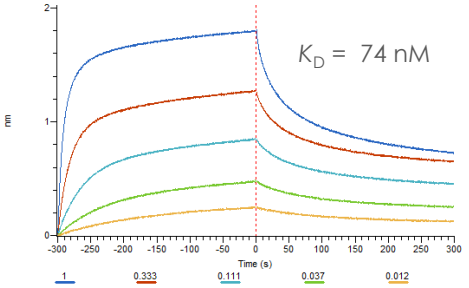
Soluble GIPR Binding is Specific to its Natural Ligand and Therapeutic Drugs



Avidity Ligand Binding Kinetics

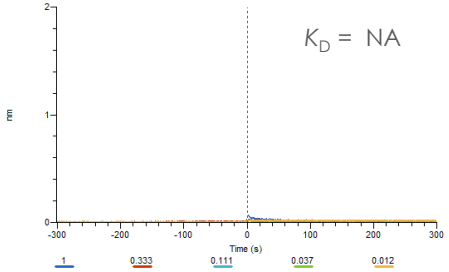
GIP

Natural Ligand



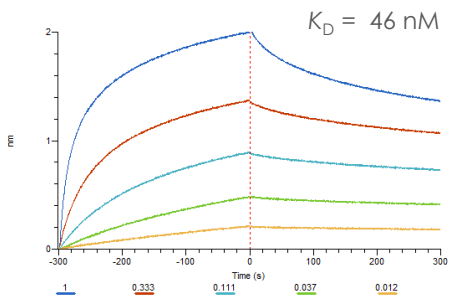
GLP-1

Negative Control



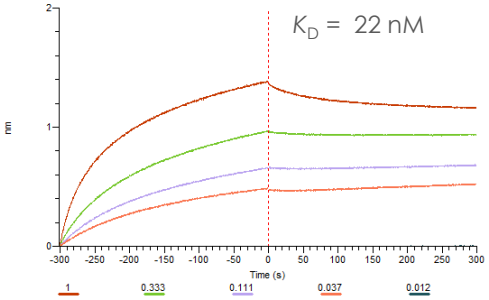
Zepbound®

Dual Agonist Therapeutic Drug



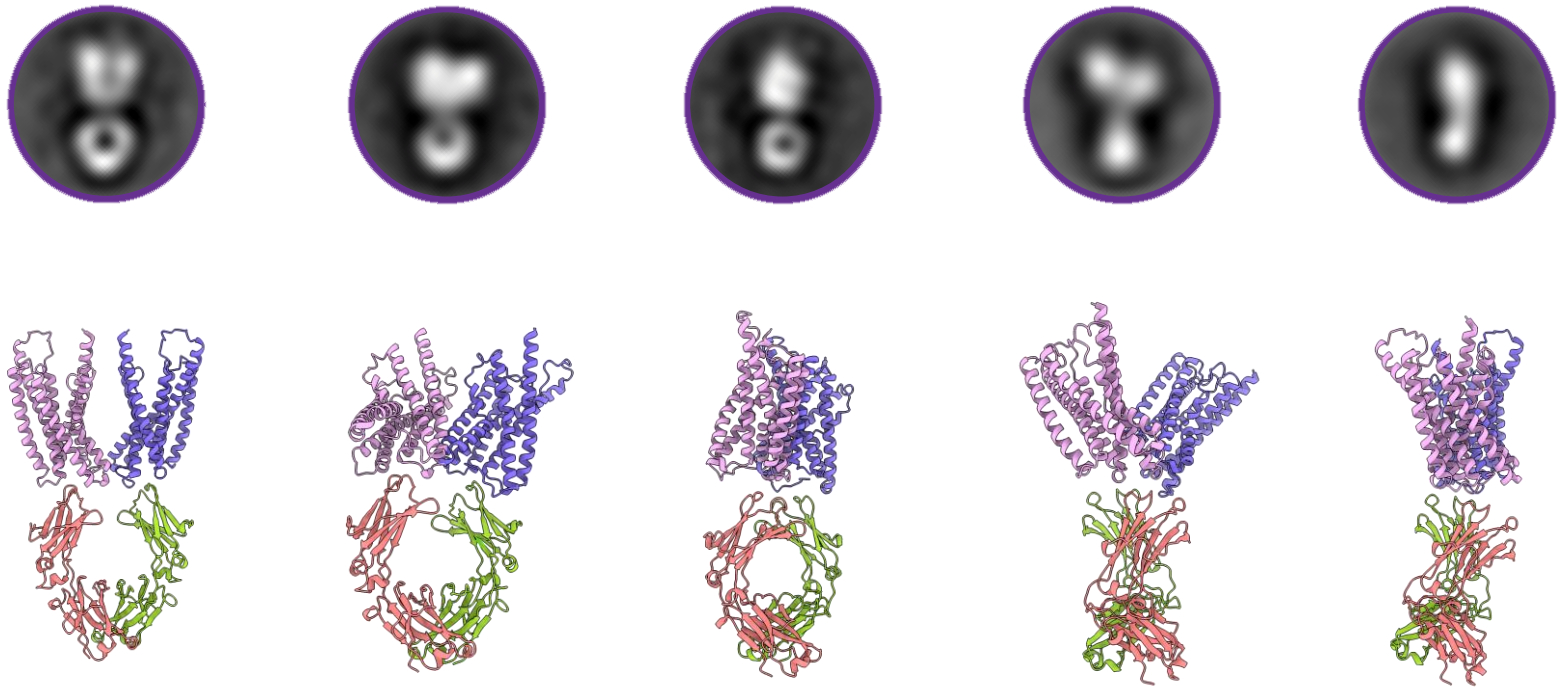
Retatrutide

Triple agonist



Negative Stain Electron Microscopy Supports Soluble Transmembrane Design

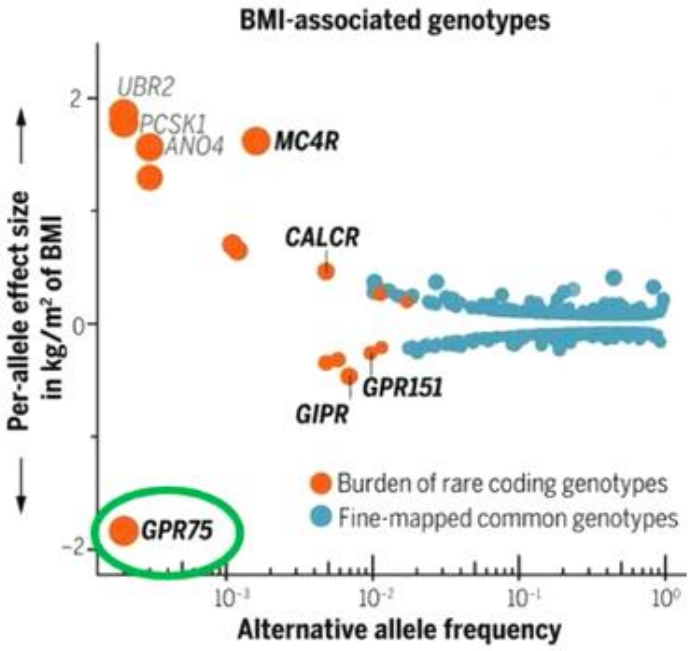
Negative Stain Electron Microscopy

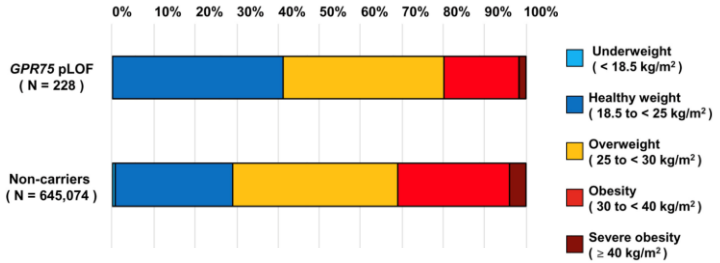


- Soluble GIPR expressed as Fc fusion
- 2D class averages agree with soluble transmembrane design
- Flexibility observed at the Fc hinge region

Case Study #3: GPR75

GPR75 is a Genetically Validated Obesity Target





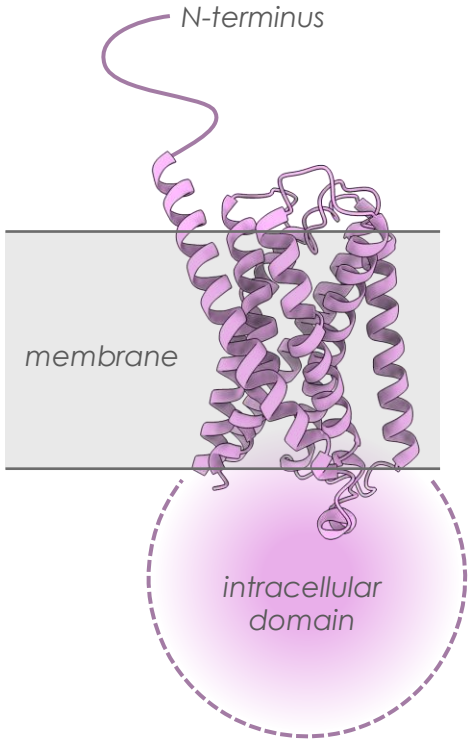
Challenges

- No experimental structures available at the time of this study
- 20-HETE binds the transmembrane domain
- CCL5 binds extracellularly, but the binding site is unknown

Soluble GPCR Designed from Sequence Alone

Input Target

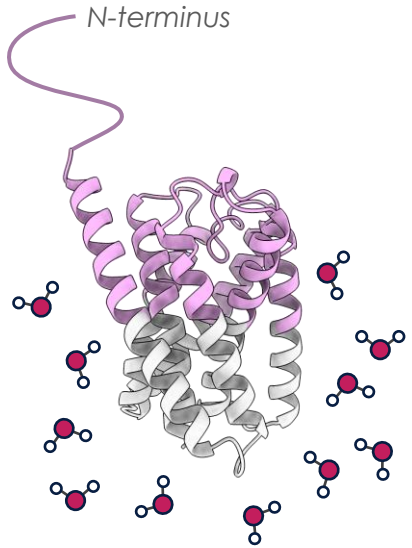
GPR75 (AlphaFold)



Generative AI

Solubilization of transmembrane domain

Soluble GPCR



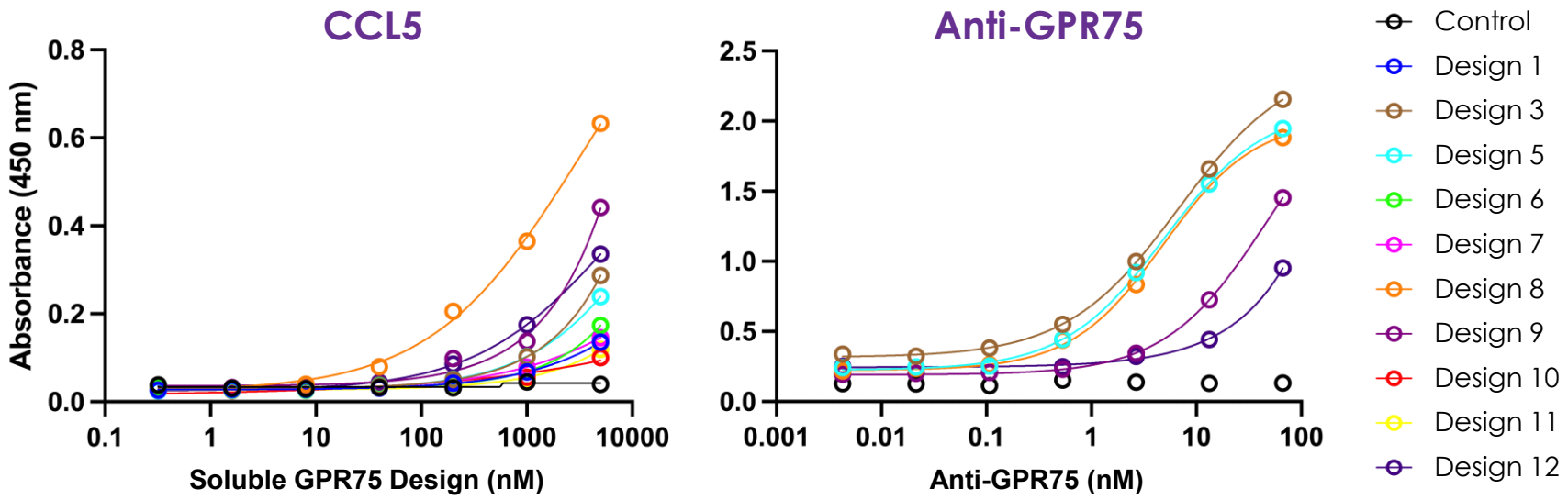
Soluble GPCR designs generated from sequence information alone

Removal of ~250 residue intracellular domain

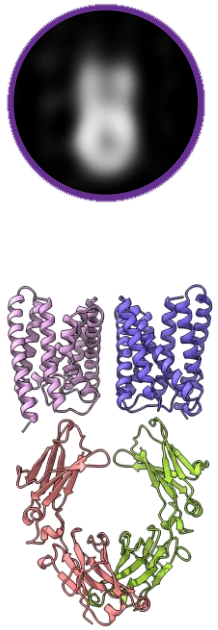
12 designs expressed and purified to test for binding to CCL5

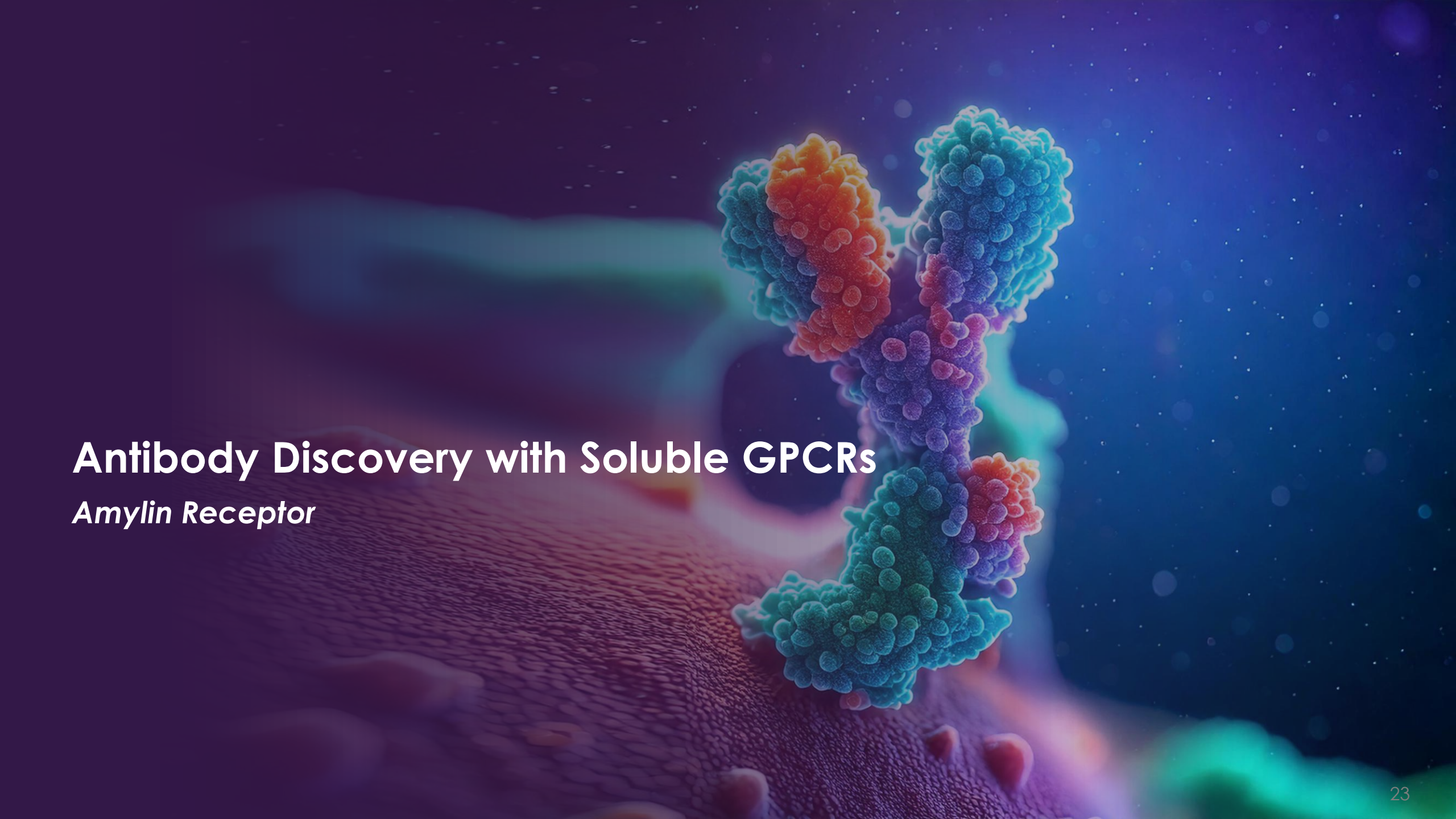
Solubilized GPR75 Binds CCL5 and Commercial Antibody

ELISA Binding



Negative Stain EM





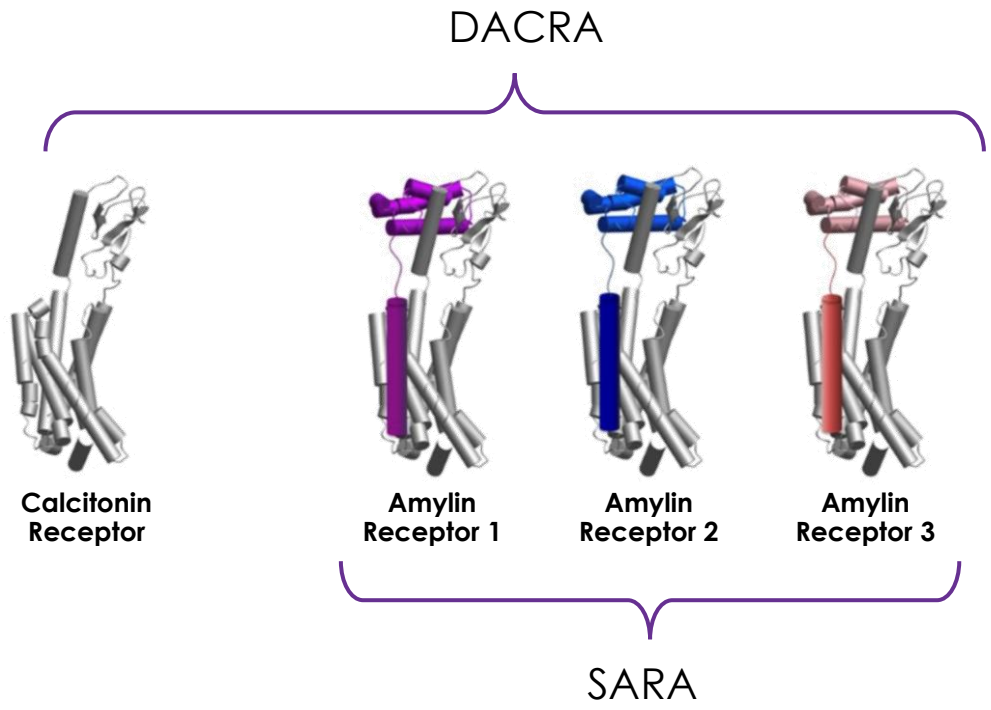
Antibody Discovery with Soluble GPCRs

Amylin Receptor

Amylin Receptor Agonism
A Complementary Pathway to GLP-1–Based Obesity Treatments

Why Target Amylin?

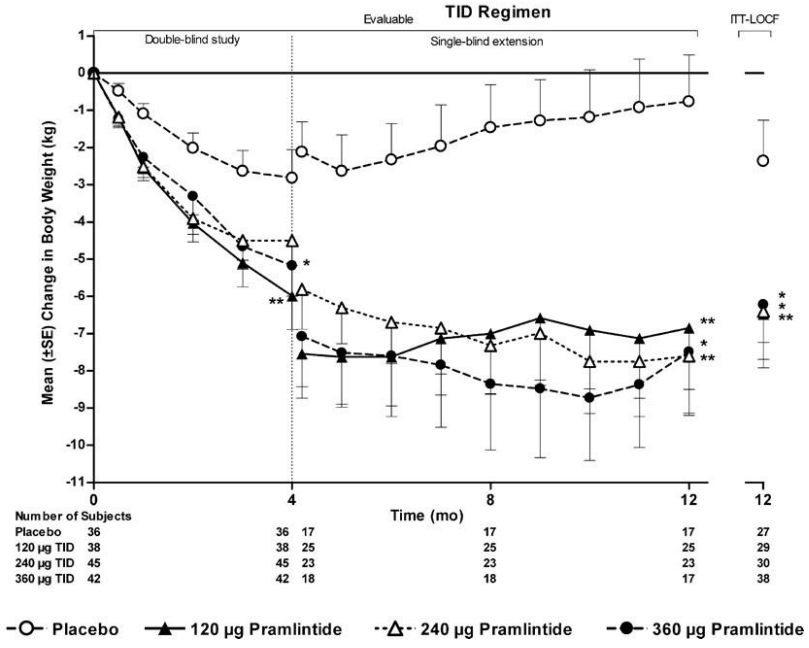
- **Validated metabolic hormone** that promotes satiety and slows gastric emptying
- Clinical studies with amylin analogs confirm efficacy in weight loss, but **peptide-based approaches may be sub-optimal** (dosing, tolerability, manufacturability)
- Antibody therapeutics could provide a differentiated profile, with **potential for longer duration of action, greater receptor specificity, and reduced side effects**



Selective Amylin Receptor Agonists (SARA) may more precisely target obesity intervention than Dual Amylin and Calcitonin Receptor Agonists (DACRA)

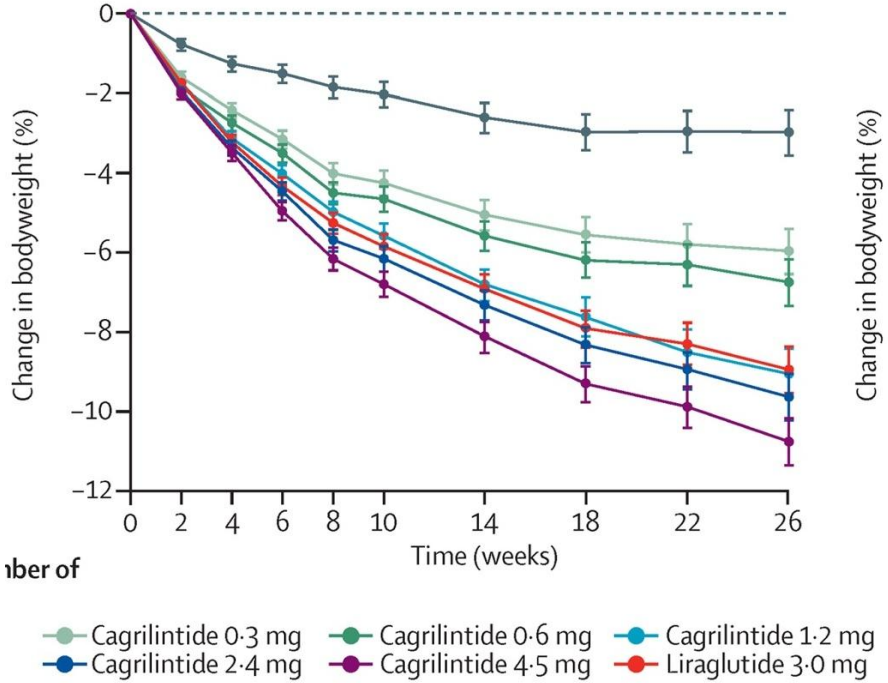
Peptide Drugs are Efficacious but Require Frequent Dosing

Weight Loss on FDA Approved Pramlintide



Limitation – requires multiple doses per day

Weight Loss on Long-Acting Cagrilintide



Limitation – still requires weekly dosing

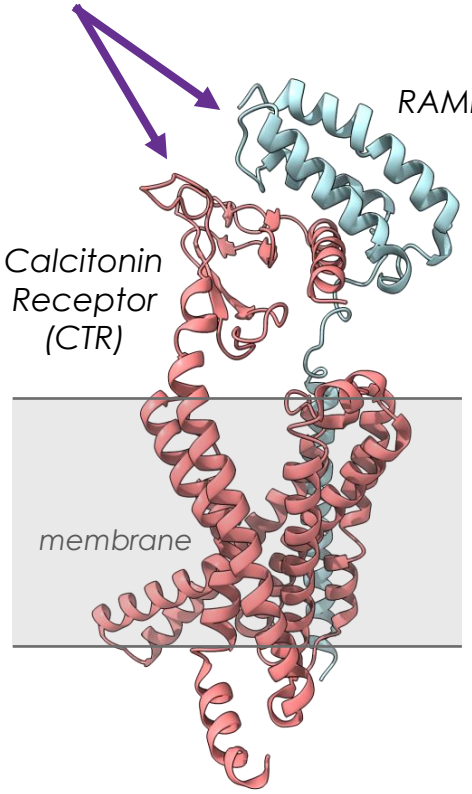
Goal: Improve Therapeutic Specificity and Longevity with Antibody Fusion

SARA

Combinations specific to amylin receptors 1, 2, and 3

DACRA

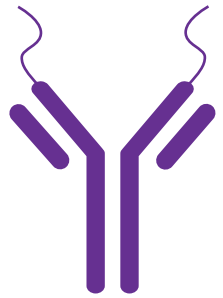
Calcitonin receptor and amylin receptors 1, 2, and 3



RAMP

Combinations of amylin receptors 1, 2, and 3 and other off-target proteins associated with RAMP

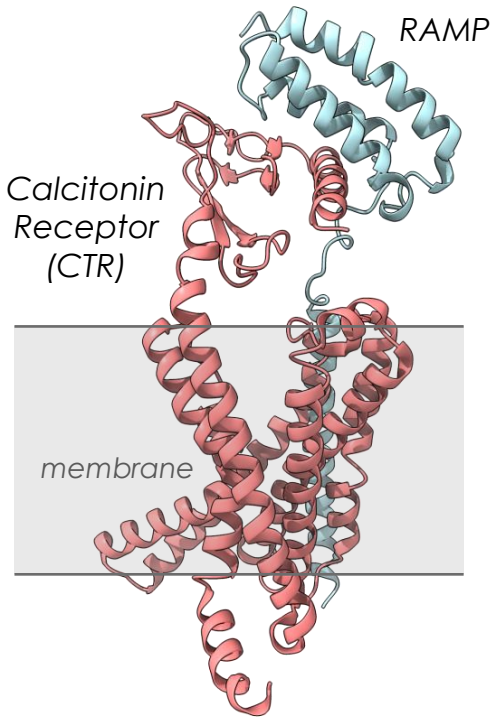
Synthetic Amylin + Antibody Fusion



Full Spectrum of Epitopes Designed for Amylin Receptor

Input Target

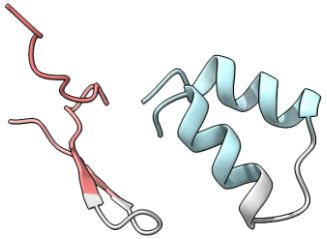
Amylin Receptor



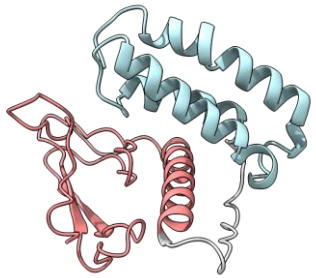
Generative AI

Epitope Designs

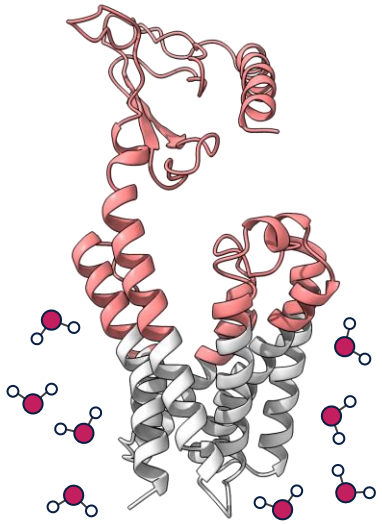
Individual Epitopes



CTR-RAMP Junction

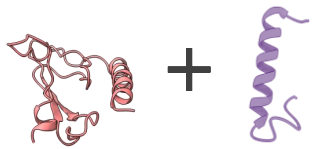


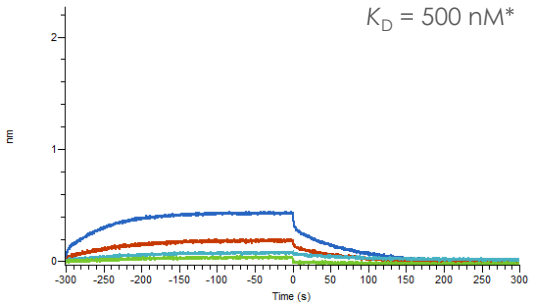
Soluble CTR



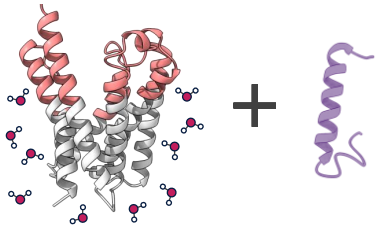
CTR Solubilization Validated by Salmon Calcitonin Binding

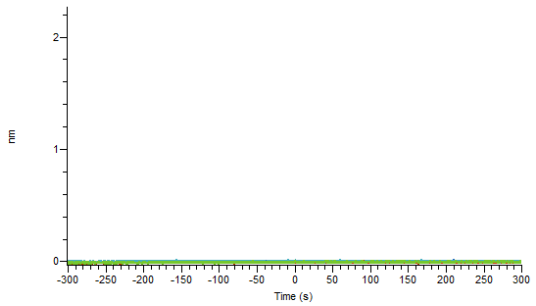
Extracellular Domain (ECD) Binding to Salmon Calcitonin



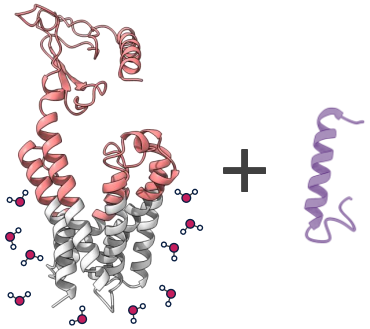


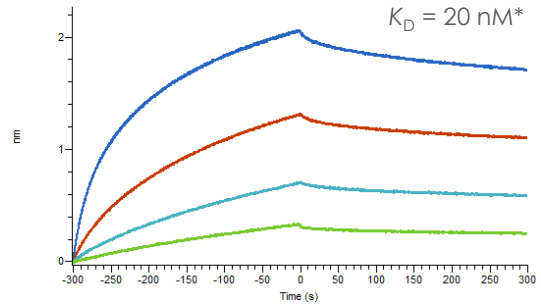
Transmembrane Domain (TMD) Binding to Salmon Calcitonin





ECD + TMD Binding to Salmon Calcitonin

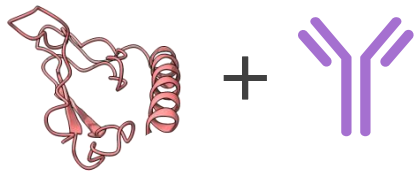


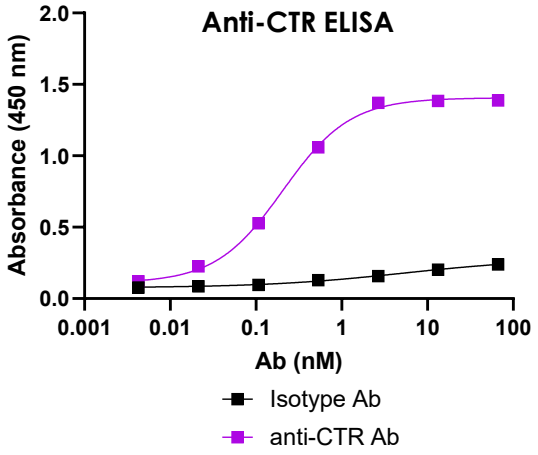


K_D calculated from avidity binding kinetics

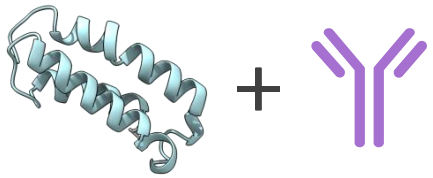
Amylin Junctional Epitope Design Retains Anti-CTR Antibody Binding

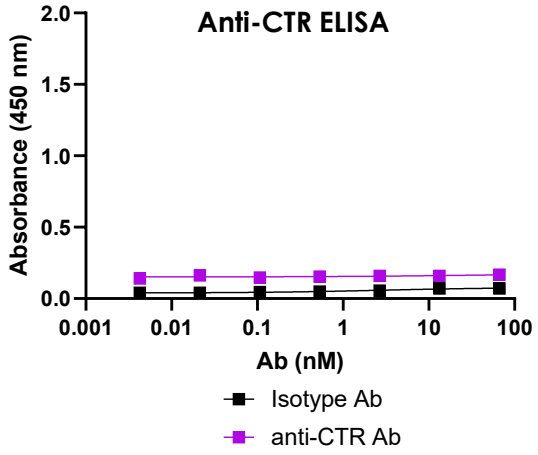
CTR ECD



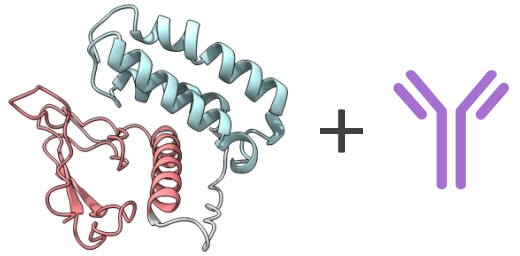


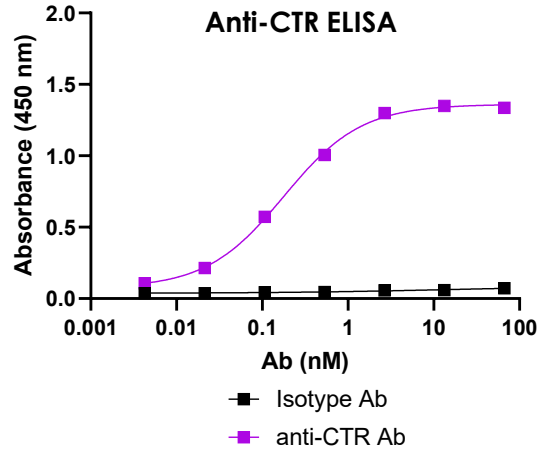
RAMP





Amylin ECD

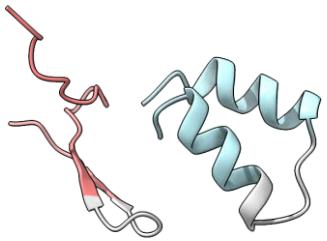




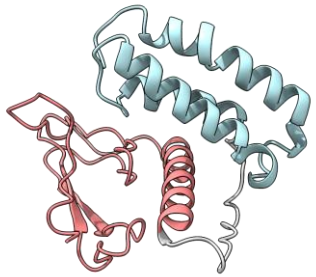
Epitope Designs are used for In Vivo and In Vitro Discovery

Epitope Designs

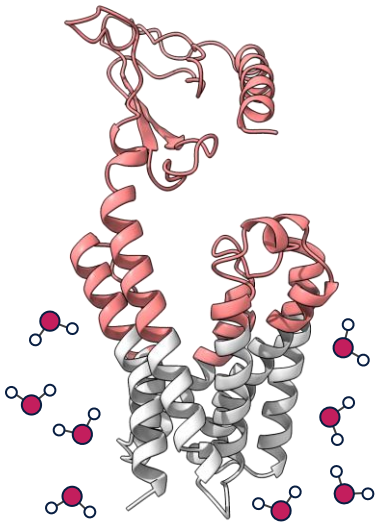
Individual Epitopes



CTR-RAMP Junction

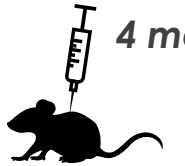


Soluble CTR



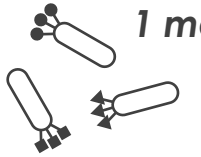
In Vivo/In Vitro Selection

Immunizations



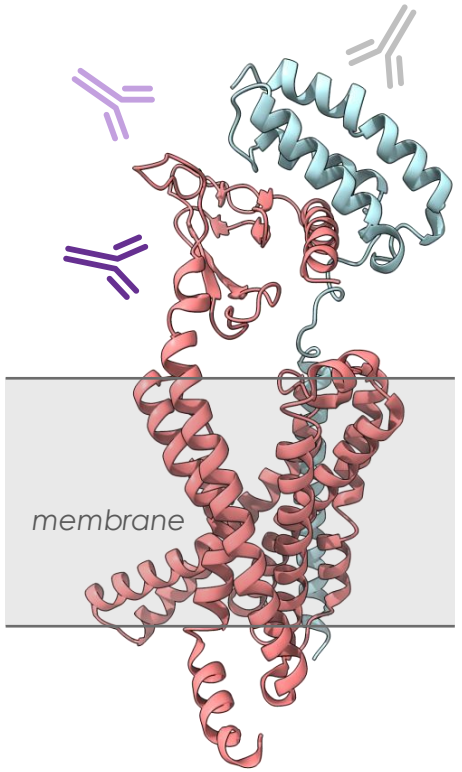
4 months

Phage panning



1 month

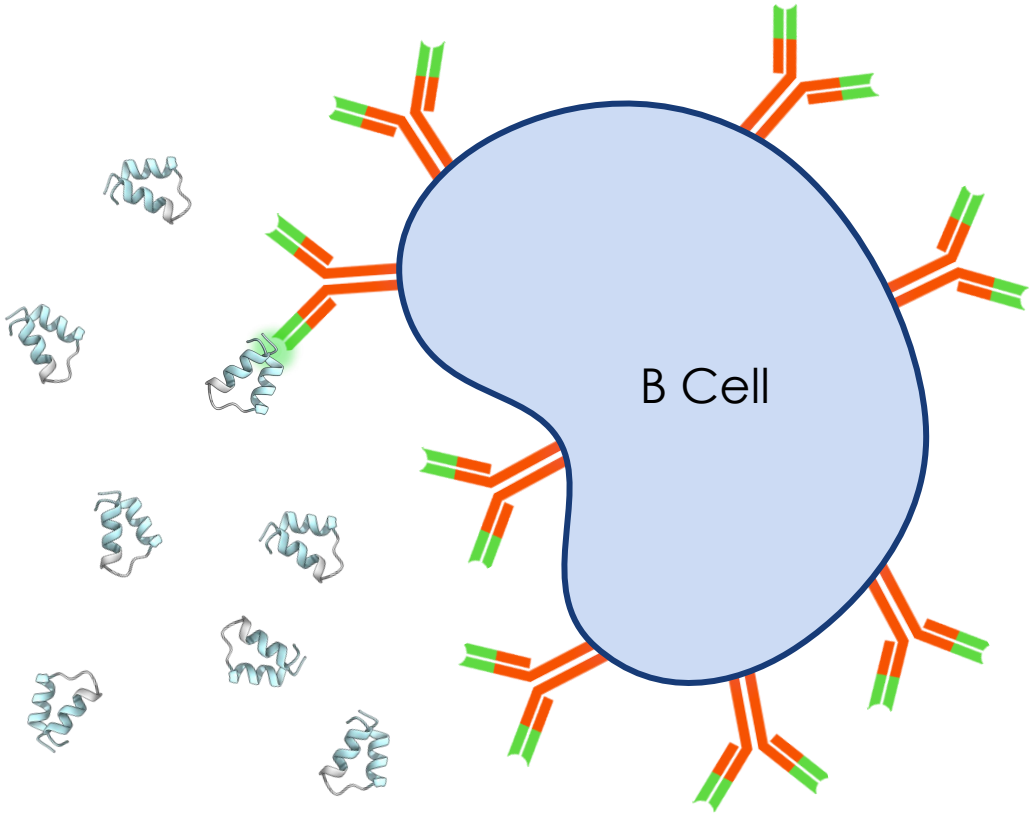
Antibody Discovery



Nanoparticle Display System Enhances Immune Response for Immunizations

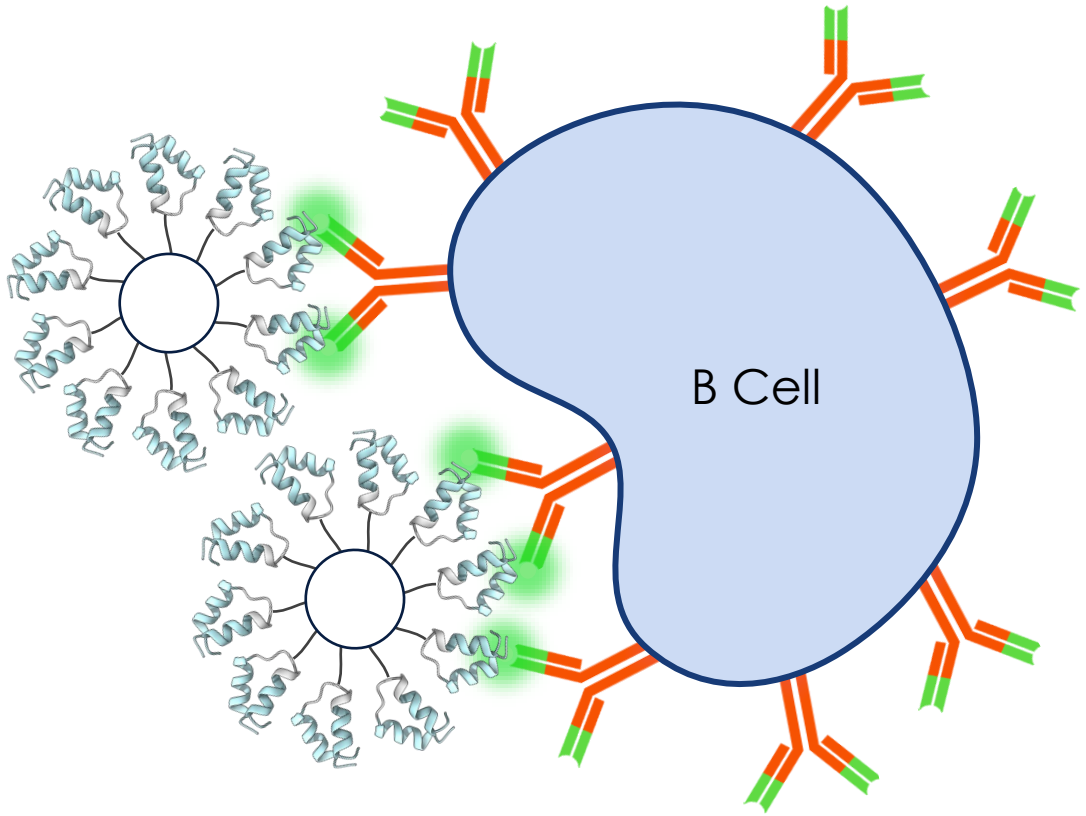
Engineered Epitope Immunization

Weak B cell activation



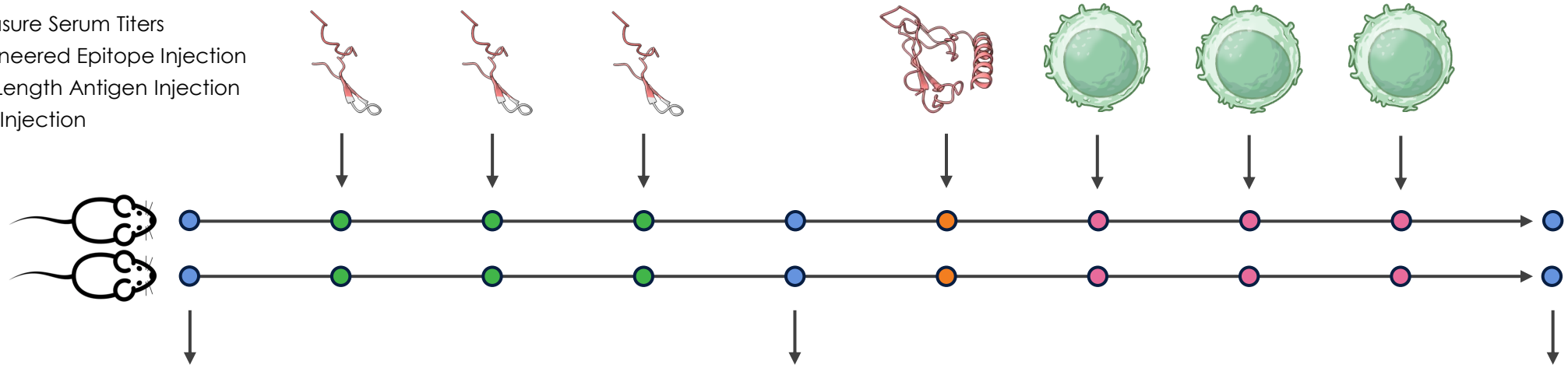
Nanoparticle Immunization

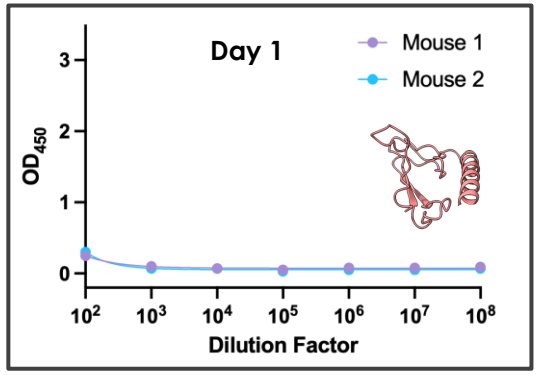
Strong B cell activation

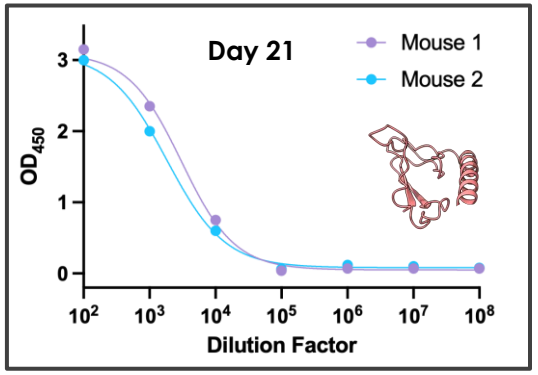


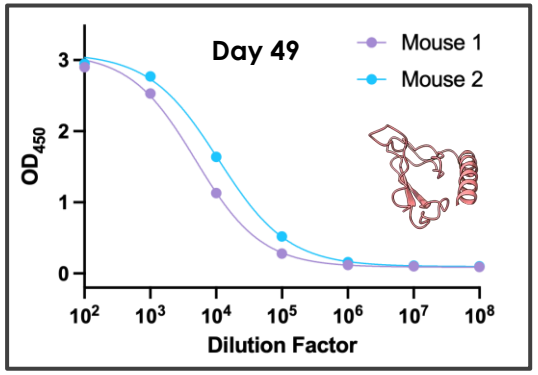
Engineered Epitopes Prime the Immune System for an Epitope-Focused Response

- Measure Serum Titers
- Engineered Epitope Injection
- Full-Length Antigen Injection
- Cell Injection



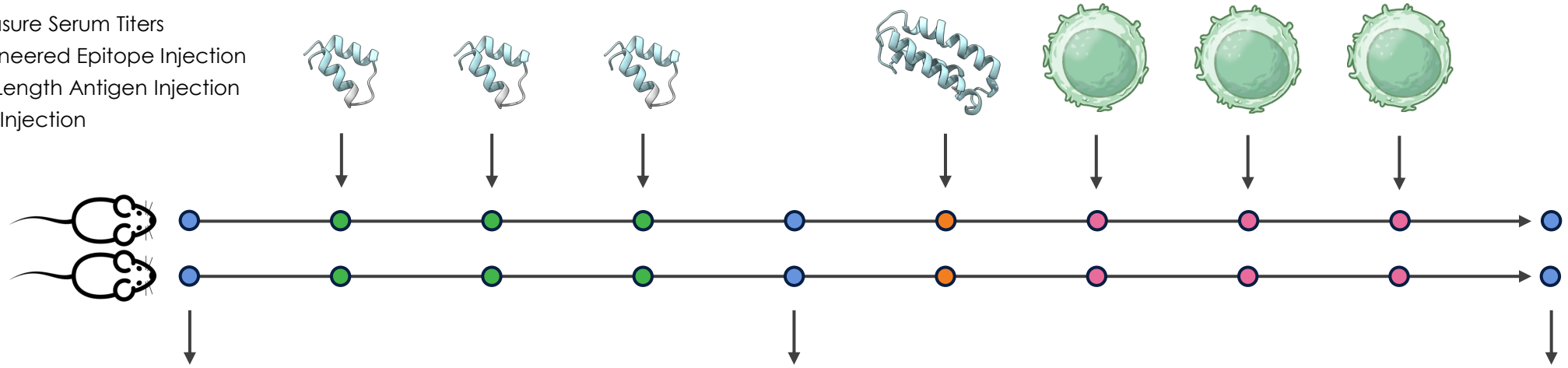


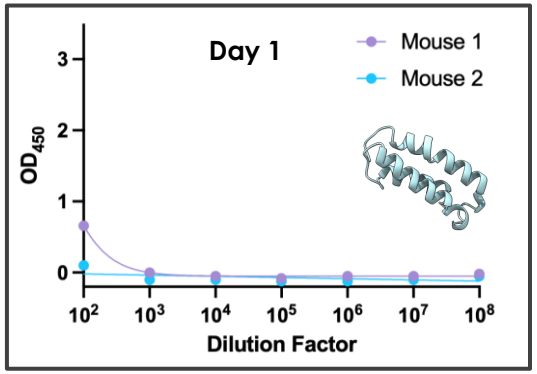


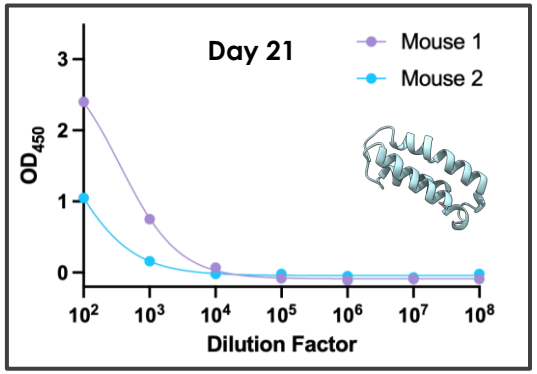


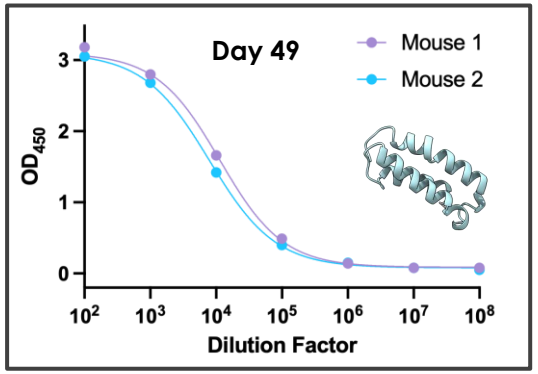
Engineered Epitopes Prime the Immune System for an Epitope-Focused Response

- Measure Serum Titers
- Engineered Epitope Injection
- Full-Length Antigen Injection
- Cell Injection



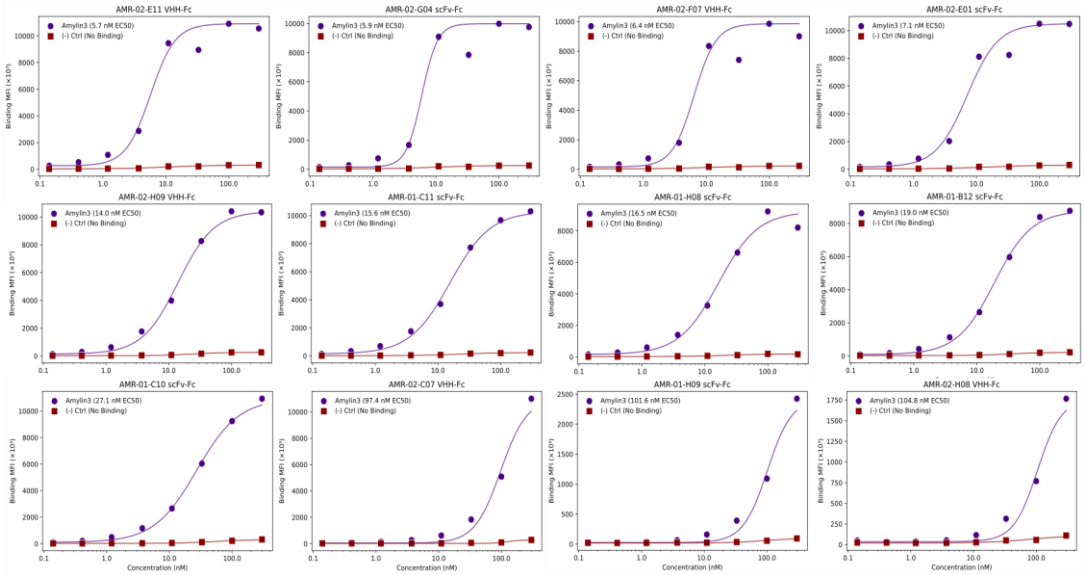






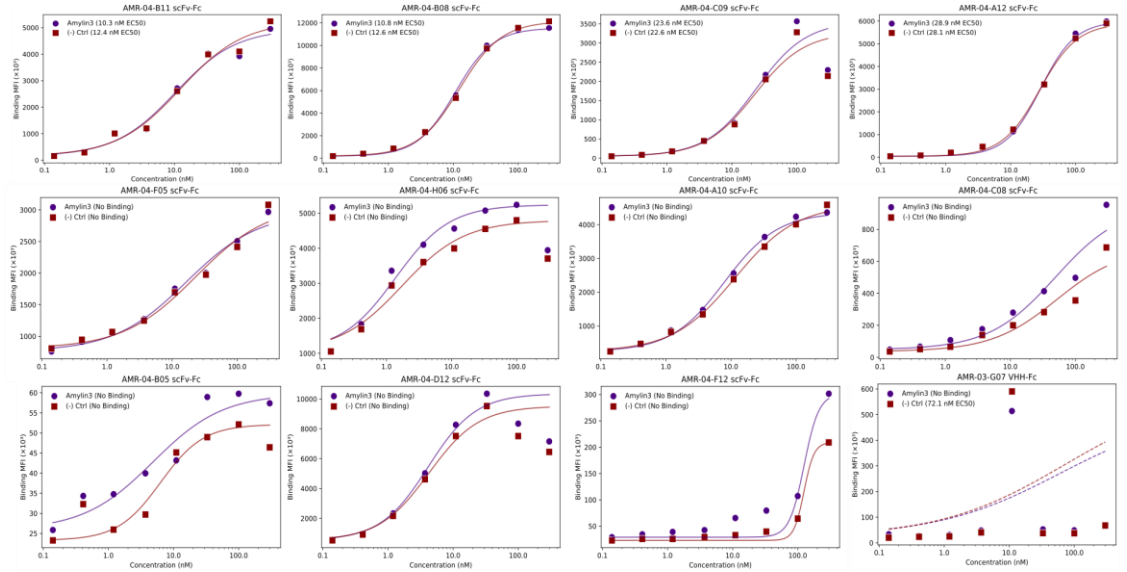
Phage Panning Against Engineered Epitopes Translates to Cell Binder Hits

Engineered Epitope Steered Strategies



Amylin Receptor Cell-Only Strategies

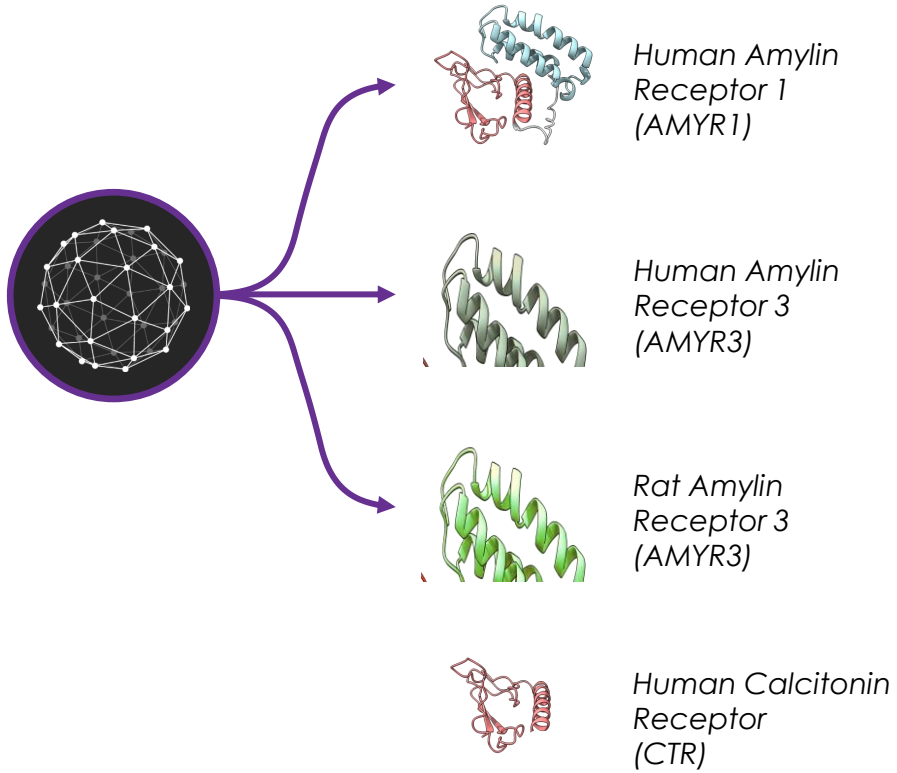
(No Specific Binders Found)



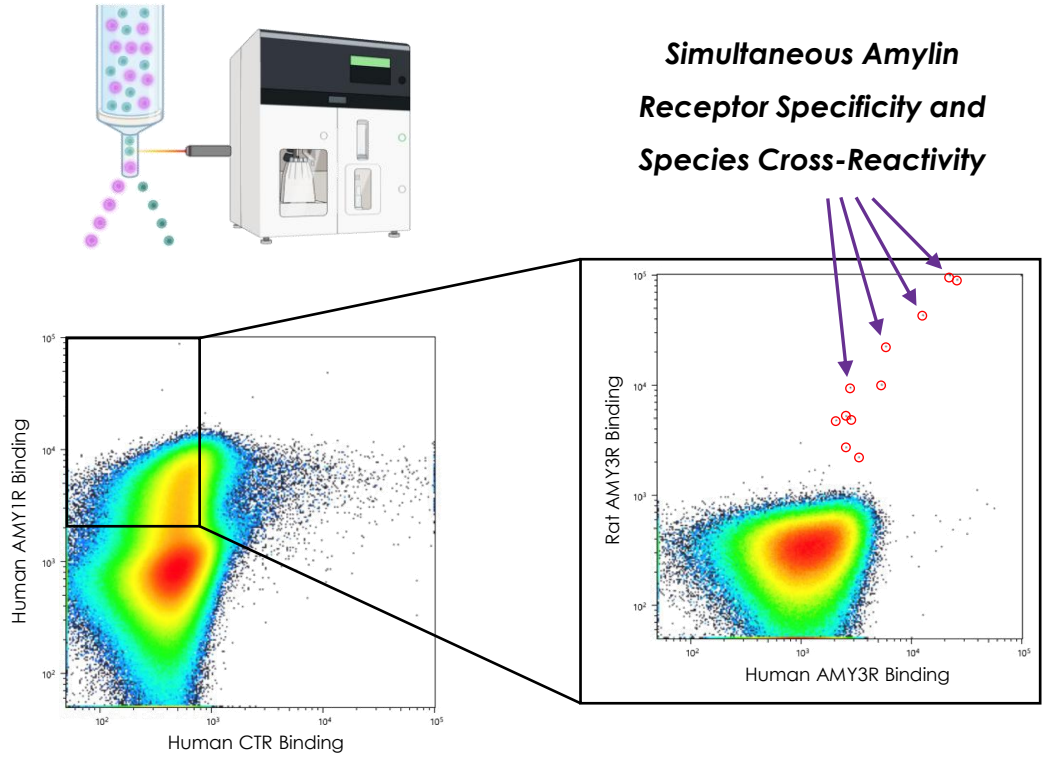
- Amylin3 Receptor Cell Binding
- Negative Control Cell Binding

Specificity and Cross-Reactivity in a Single Experiment

Species Cross-Reactive Designs

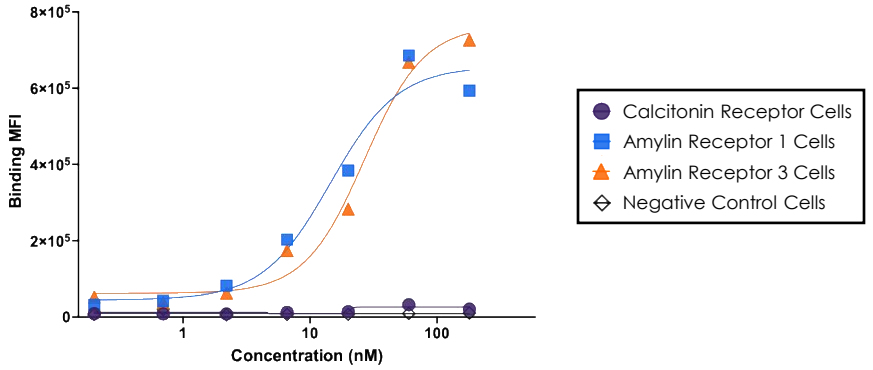


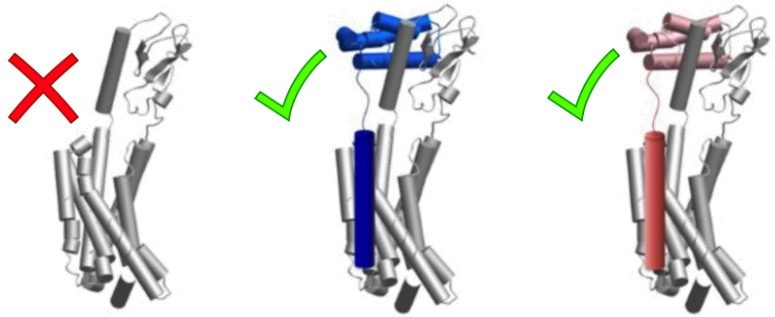
4-Dimensional Mammalian Display Sorting



Cell Binding and Agonism is Exquisitely Selective for Amylin Receptor

Cell Binding





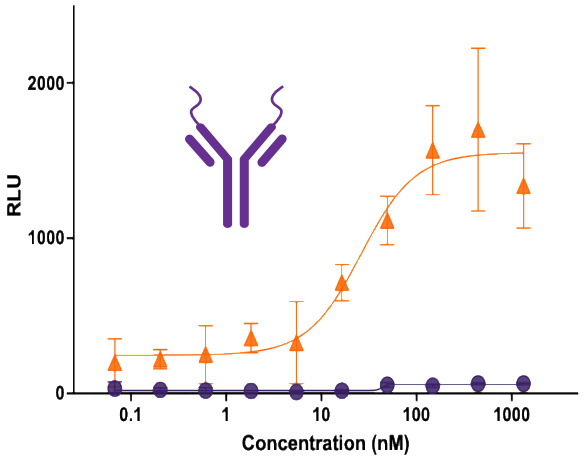
Calcitonin Receptor

Amylin Receptor 1

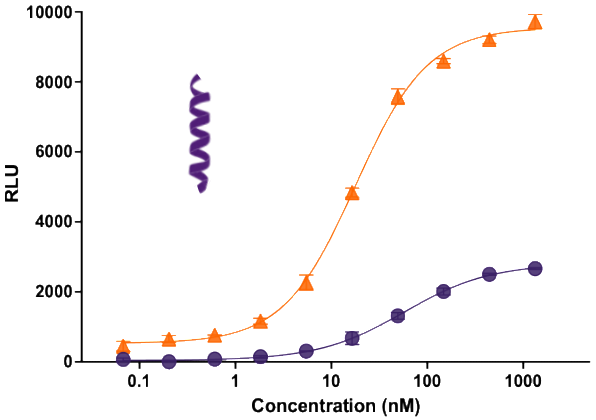
Amylin Receptor 3

Agonism Assay

Antibody Peptide Fusion

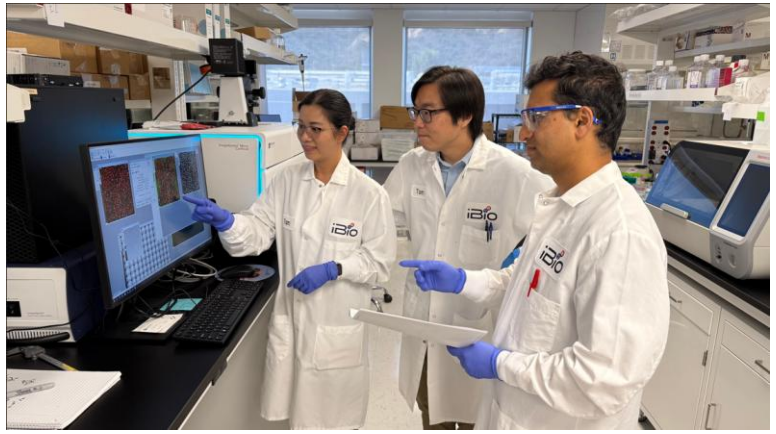


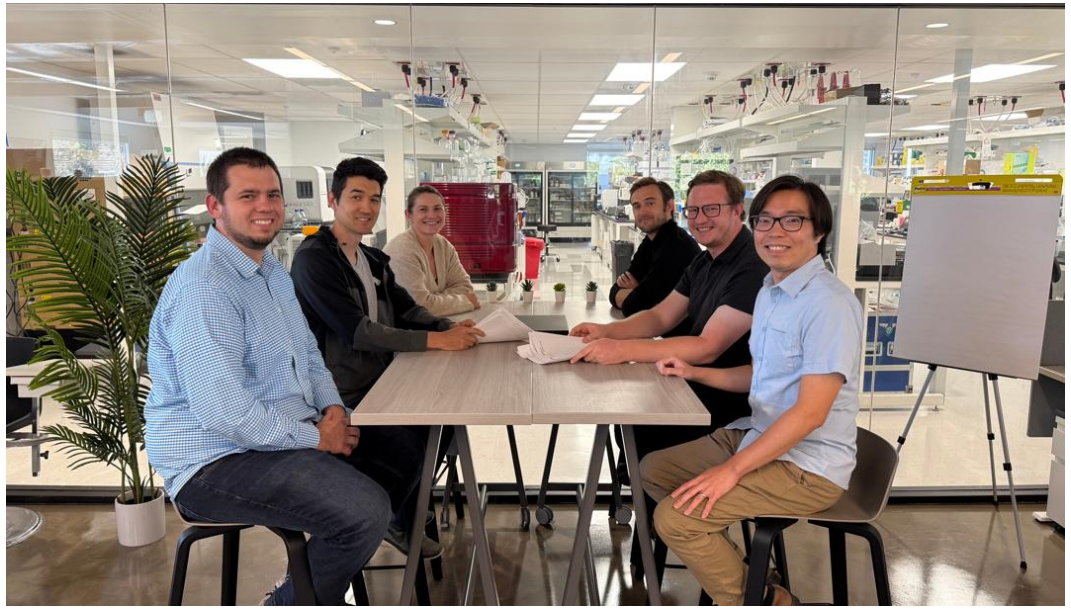
Cagrilintide



Calcitonin Receptor Cells
Amylin Receptor 3 Cells

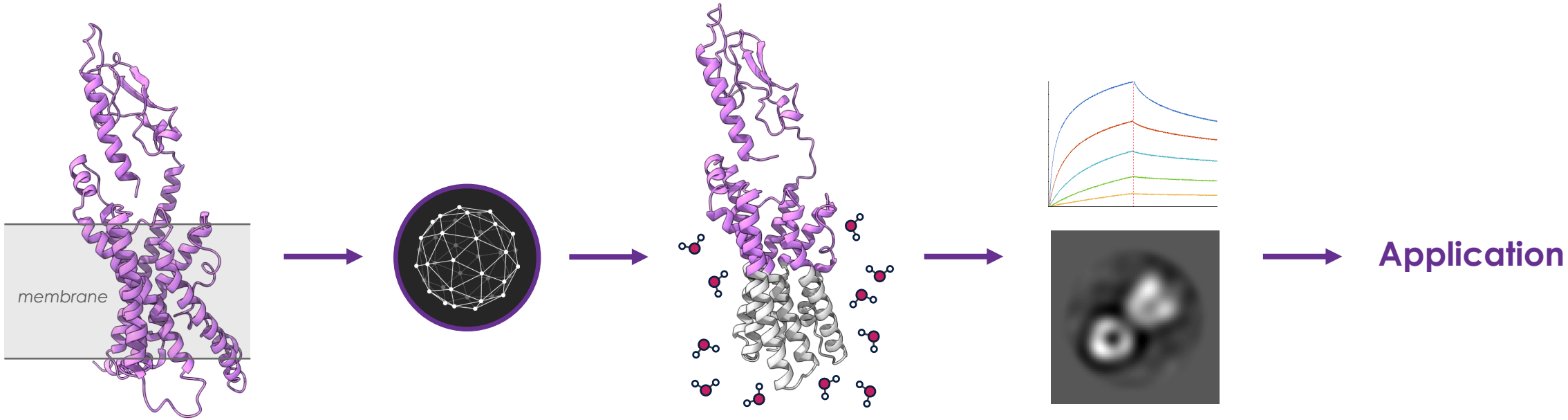
Acknowledgements





We Love Hard Problems

Have a problem you think could be addressed with GPCR solubilization?



We are open to collaboration! alex.taguchi@ibioinc.com