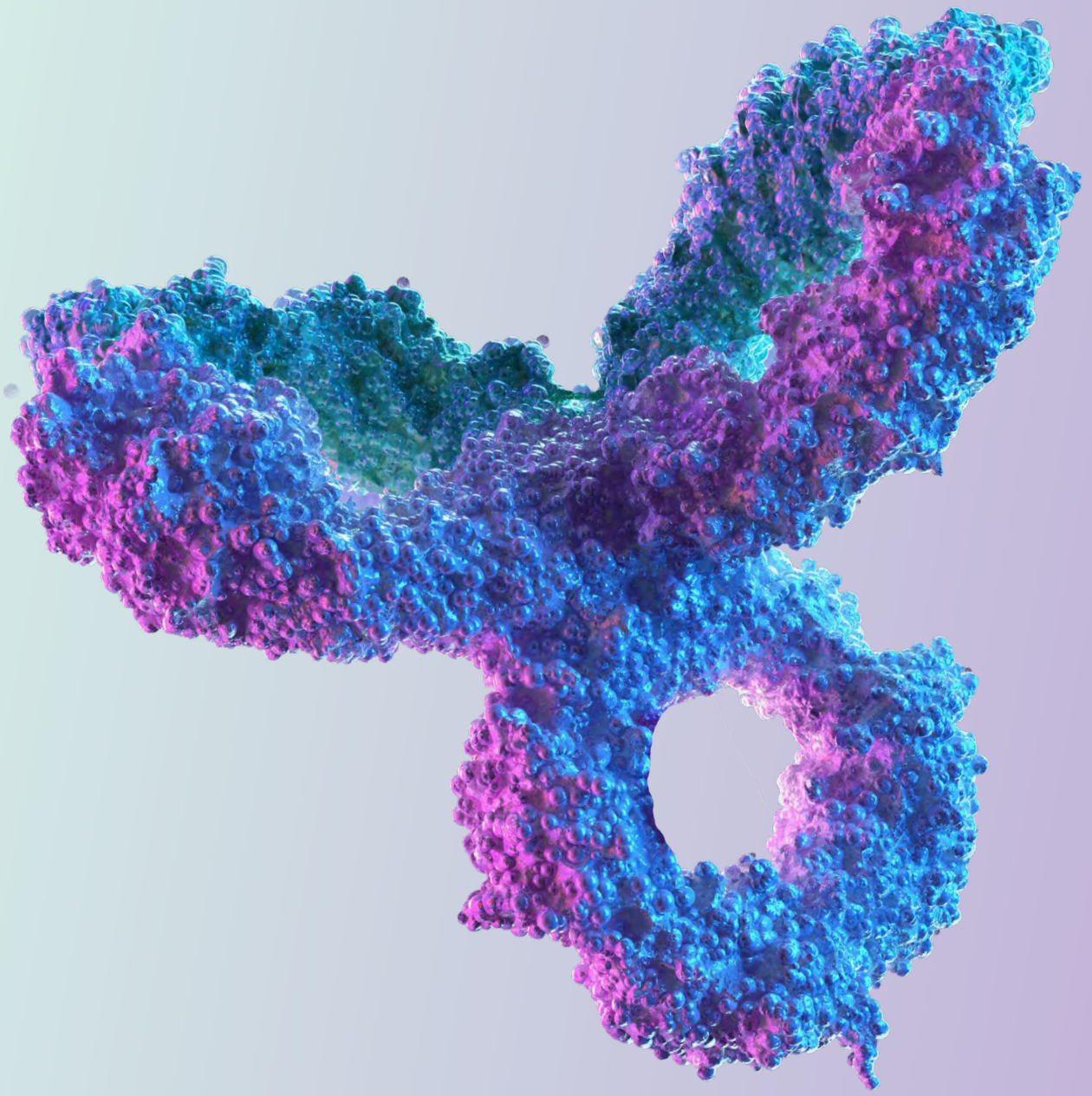
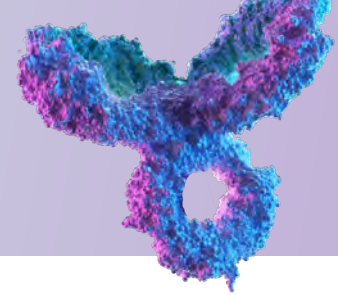
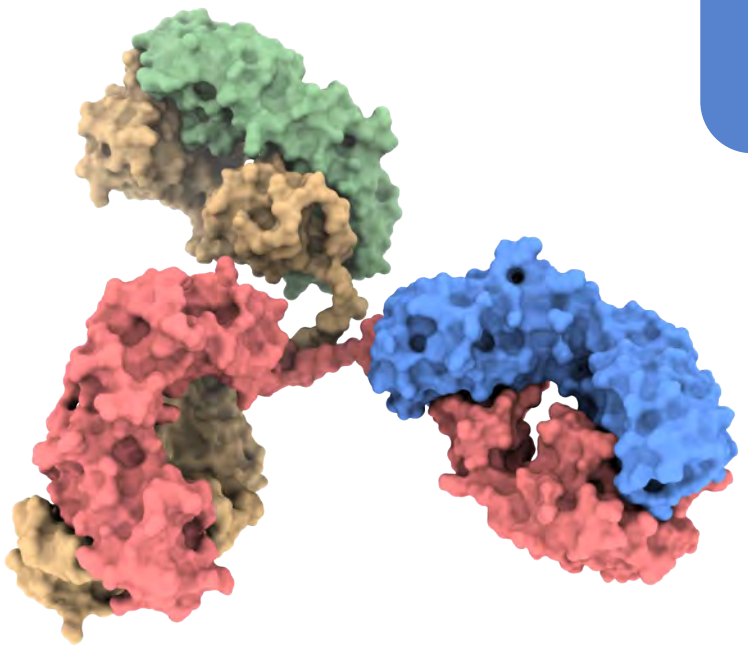
Generative Design of Soluble GPCRs for Drug Discovery



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]

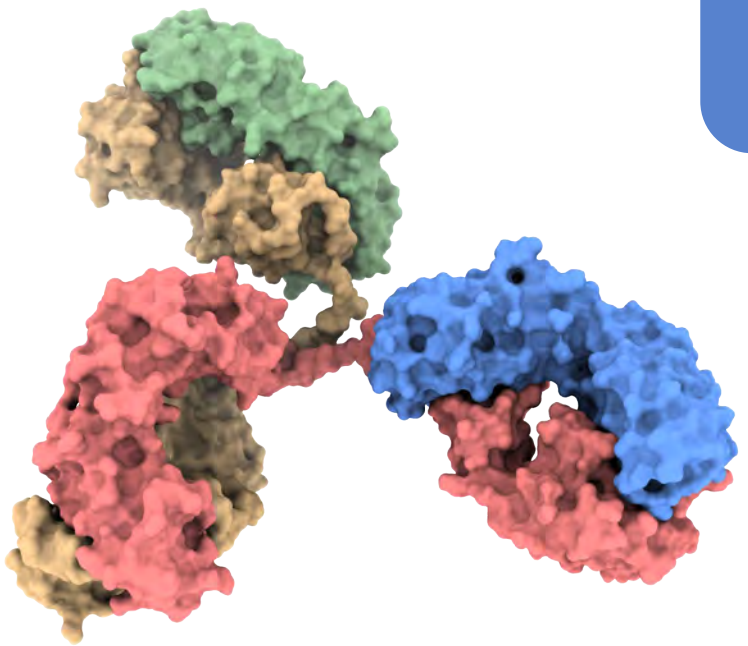
Is AI the right tool for my problem?

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

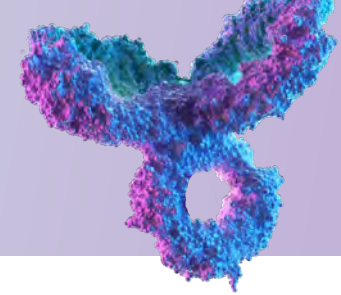
>$100M & >10 years to FDA approval[1]

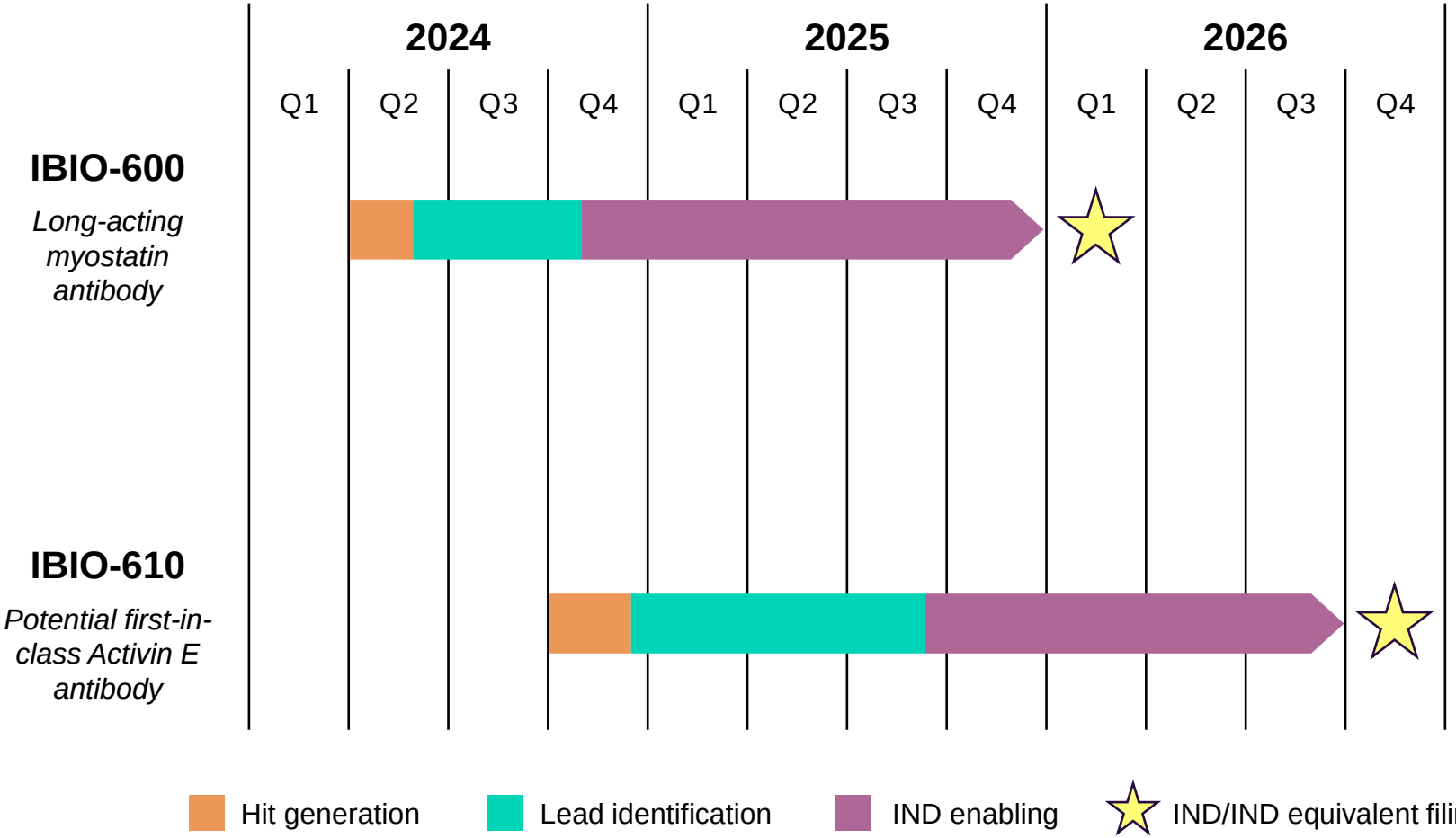
iBio Engine

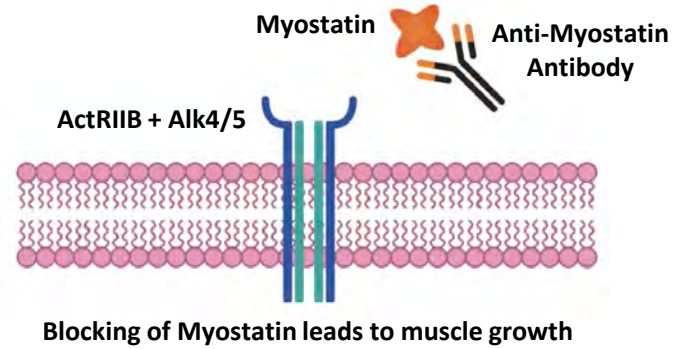
Integrated structural and functional validation

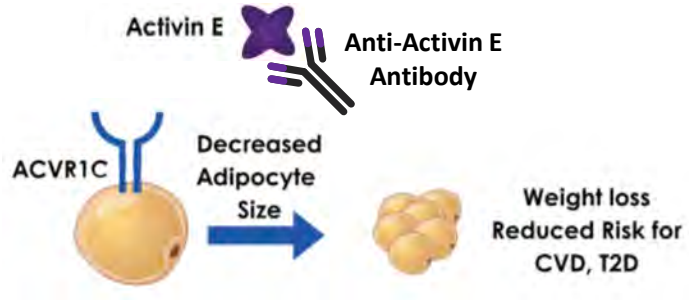
[1]Wouters, O. et al., JAMA (2020)

Antibody Discovery to Development Candidate in as Little as 7 Months



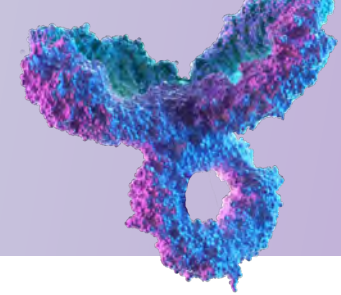






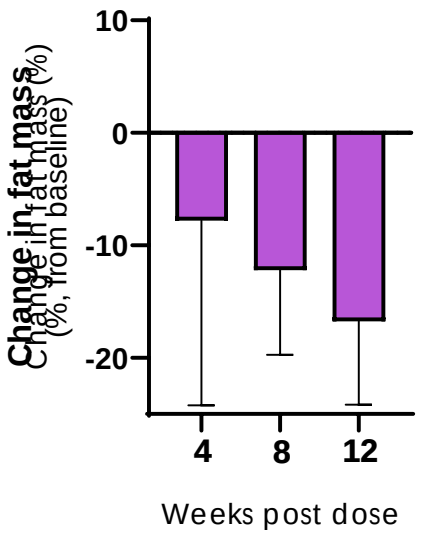
Akbari, P. et al. Nat. Commun. (2022), Deaton, A. M. et al. Nat. Commun. (2022), Schuelke M. et. al. N. Engl. J. Med. (2004), Deng, B. et. al. Nutrition and Metabolism (2017)
CVD: Cardiovascular Disease, T2D: Type 2 Diabetes

IBIO-600: Long-Acting Myostatin Antibody



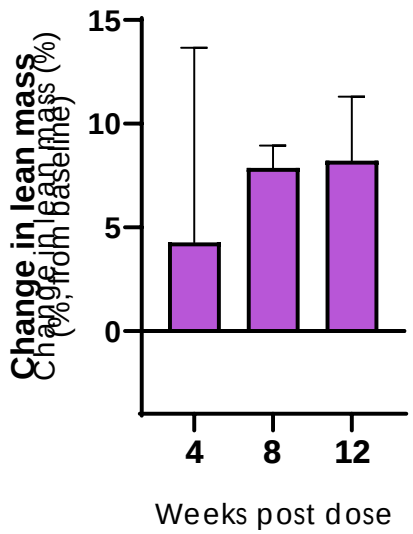
Fat Mass Decrease[1]

Single 5 mg/kg I.V. Dose in NHPs

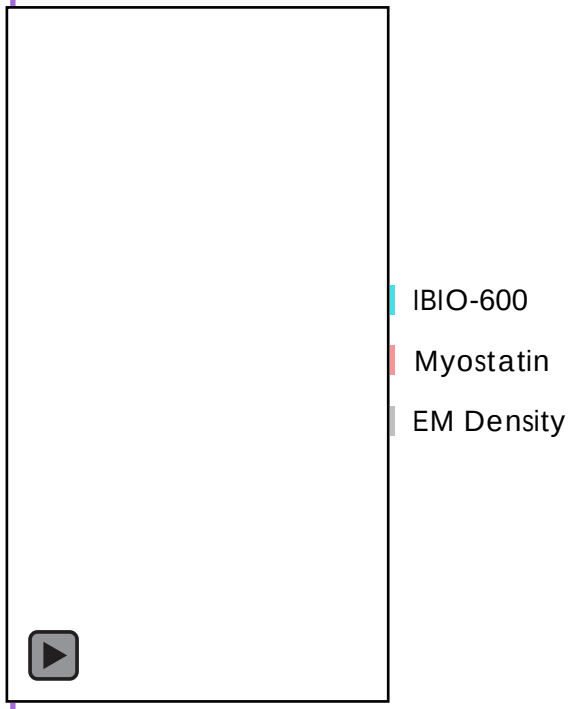


Lean Mass Increase[1]

Single 5 mg/kg I.V. Dose in NHPs



Molecular Modeling



NHP Half-Life (measured)

52.4 days

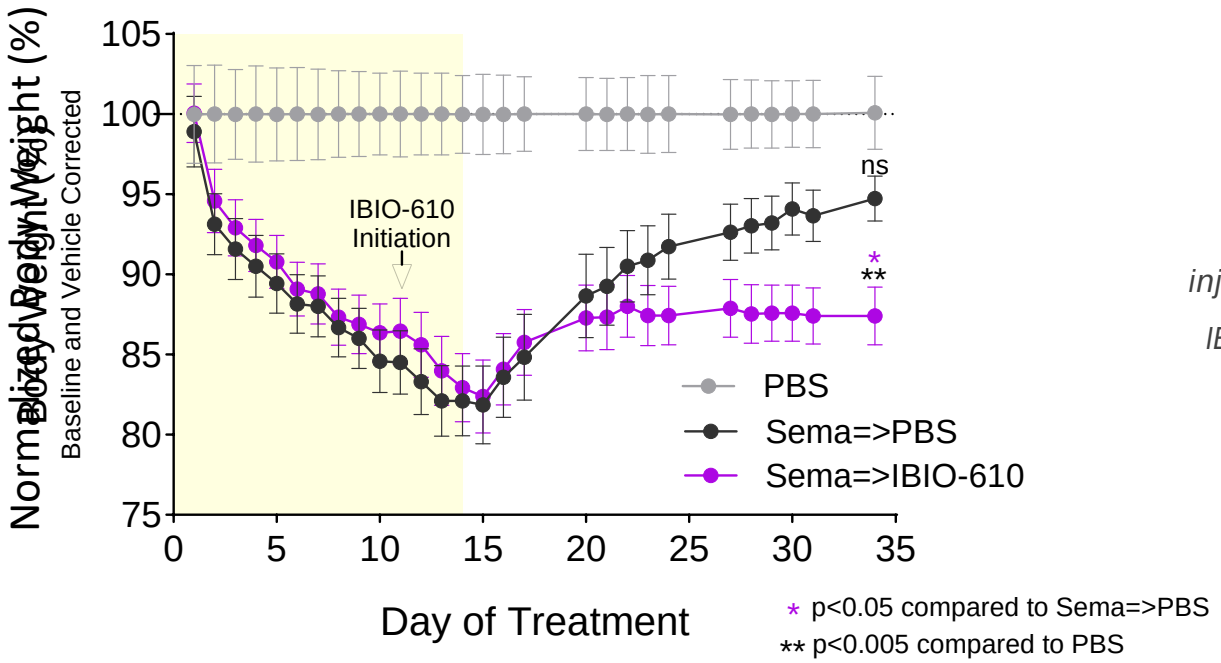
Human Half-Life (predicted)[2,3]

74-147 days

[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)
NHP: Nonhuman Primate, EM: Electron Microscopy

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

Significant Prevention of Weight Regain in DIO Mice



* p<0.05 compared to Sema=>PBS
** p<0.005 compared to PBS

Study Design

Two weeks of Semaglutide injections followed by 10 mg/kg IBIO-610 maintenance dosing starting on day 11

NHP Half-Life (measured)

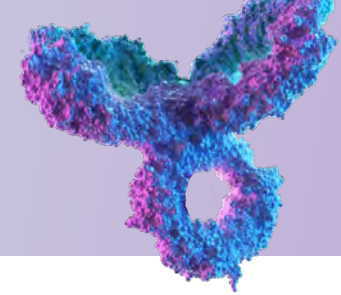
33.2 days

Human Half-Life (predicted)[1,2]

47-100 days

[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 **Antigen Engineering**

Epitope-selective antibody discovery with AI protein design[1]

2 **Human Antibody Library**

Naïve human CDR library in clinically validated framework

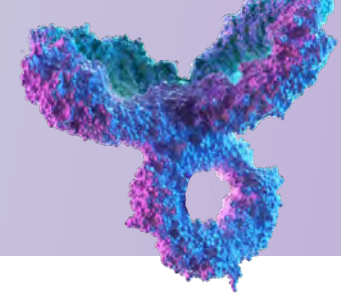
3 **Mammalian Selection**

Developability optimization in production cell-line

On-epitope, Developable Clinical Candidate

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

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



1 **Antigen Engineering**

Epitope-selective antibody discovery with AI protein design[1]

2 **Human Antibody Library**

Naïve human CDR library in clinically validated framework

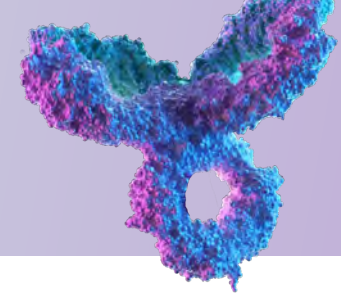
3 **Mammalian Selection**

Developability optimization in production cell-line

On-epitope, Developable Clinical Candidate

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

Engineered Epitopes Enable Rapid Discovery of On-Epitope Antibodies



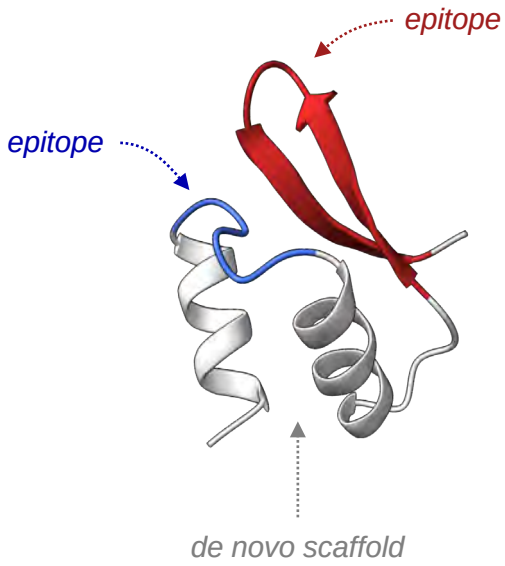
1

Naïve in vitro or in vivo antibody library



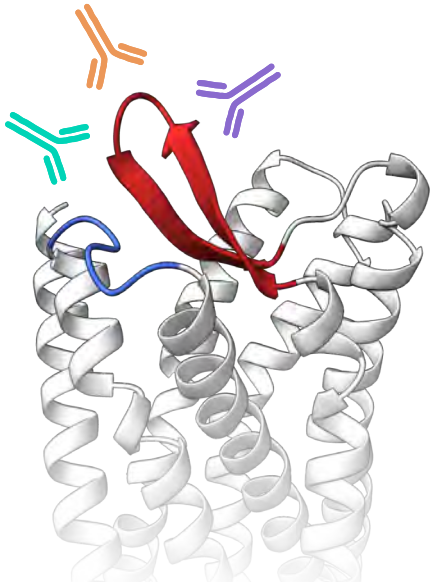
2

Focus library with engineered epitopes

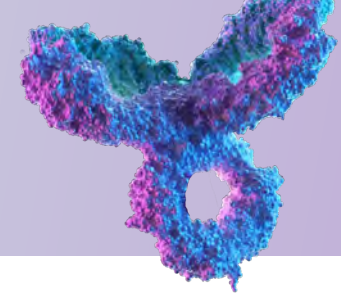


3

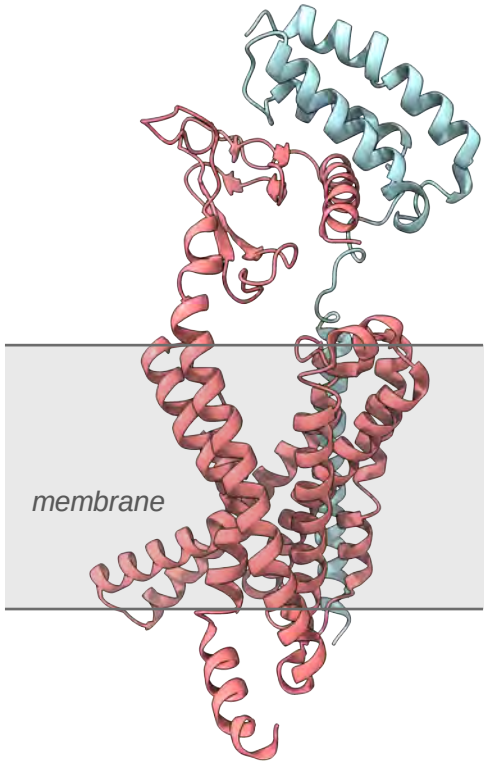
Efficient discovery of epitope-specific antibodies



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



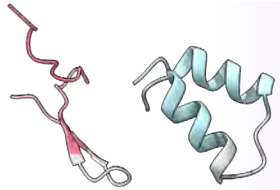
Target of Interest



Generative AI

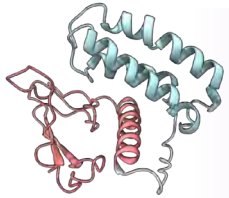


Use Cases



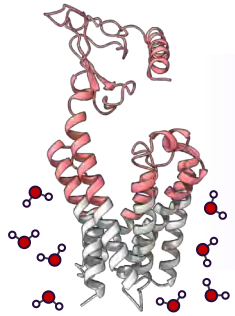
Epitopes of Interest

Design scaffold supporting native epitope structure



Protein Complexes

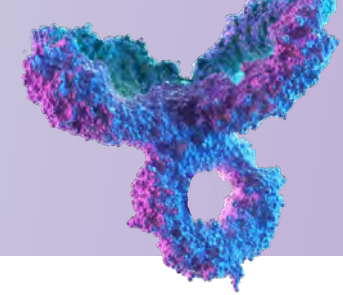
Stabilize junctional and/or discontinuous epitopes



Membrane Proteins

Solubilize transmembrane domains

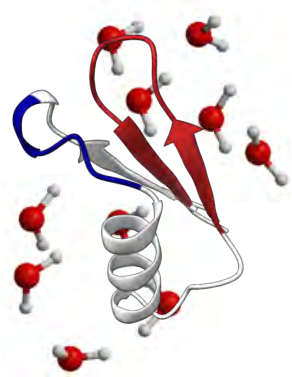
Antigen Designs are Optimized for Antibody Drug Discovery



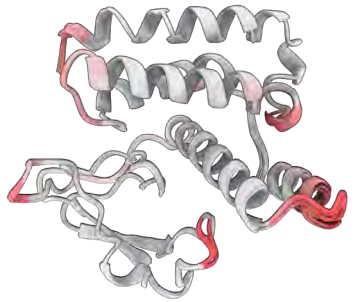
Water Solubility

Epitope

Scaffold



Structural Stability



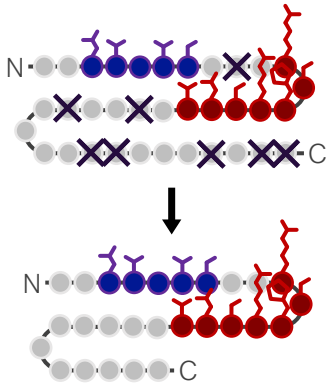
Predicted Flexibility



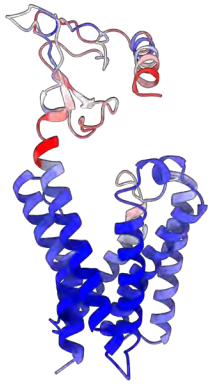
Scaffold Minimization

Epitope

Scaffold



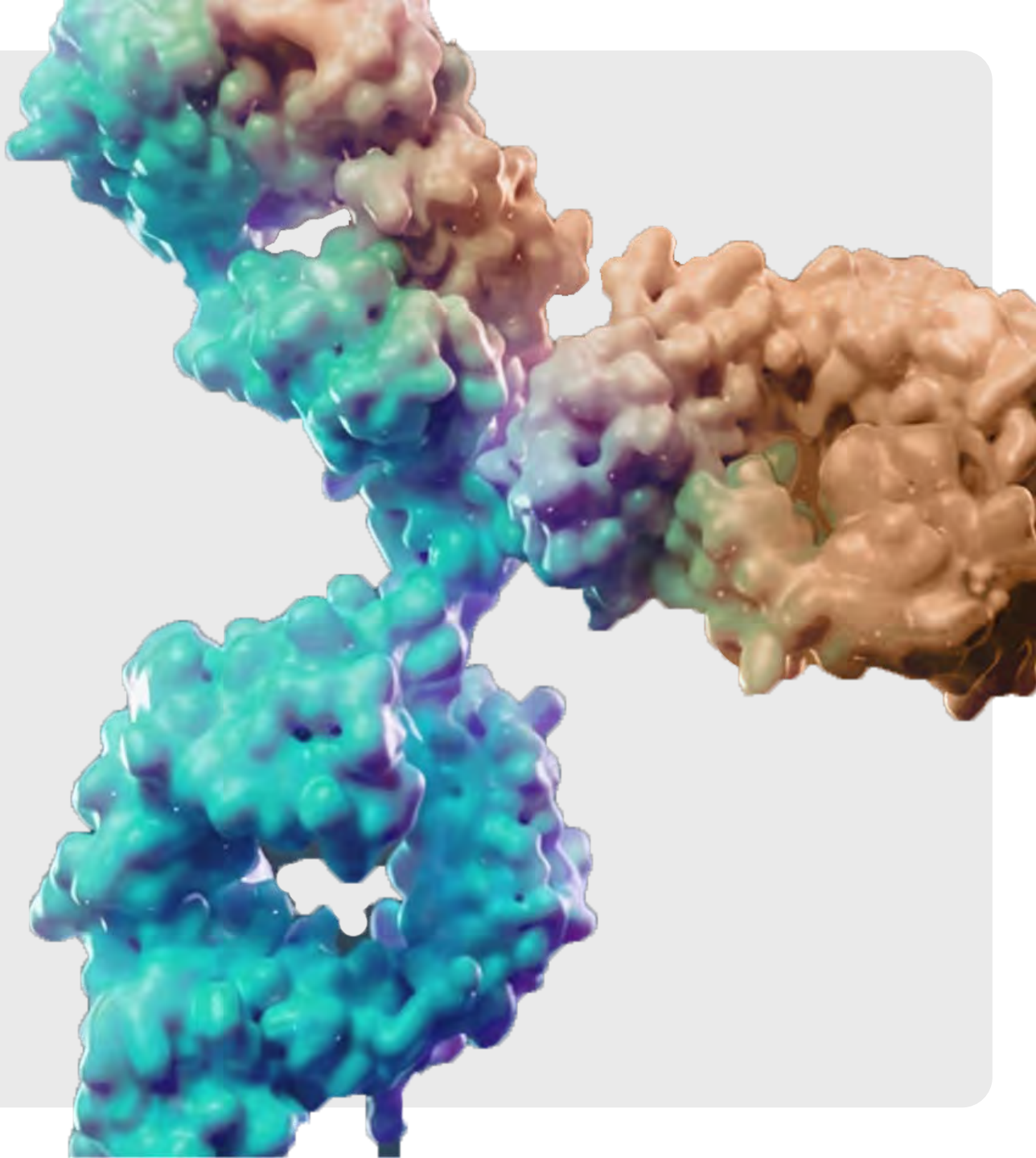
Low Scaffold Immunogenicity



Predicted Immunogenicity[1]

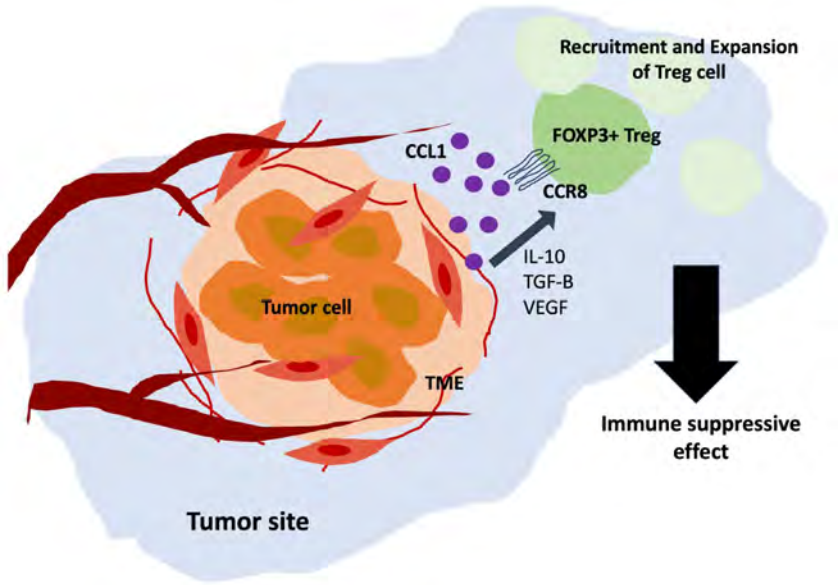
GPCR Engineered Epitope Validation

Experimental Structure and Function Case Studies



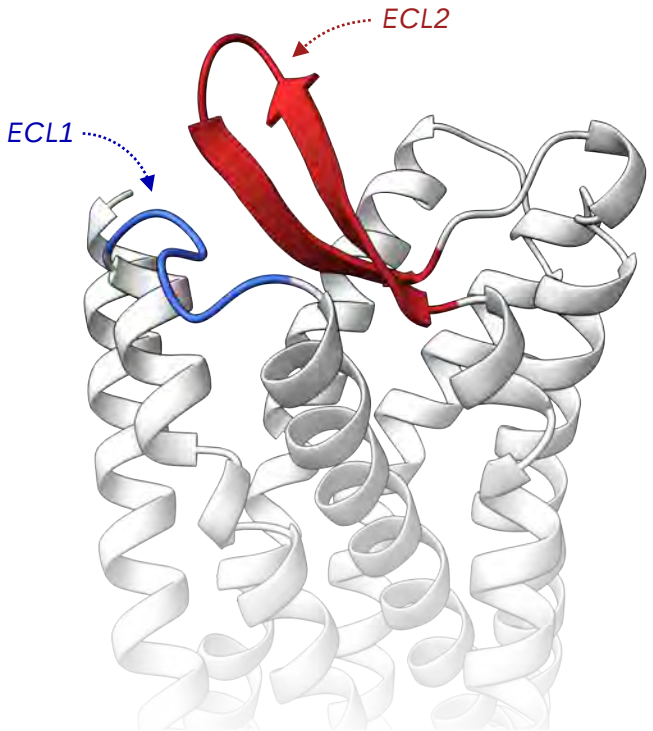
Case Study #1: CCR8

CCR8 is an Emerging Immuno-Oncology Target

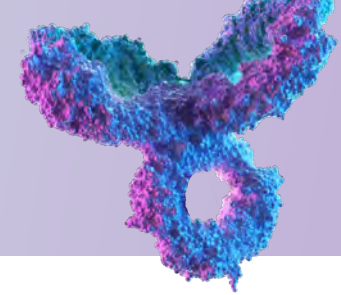


CCR8 is upregulated on regulatory T cells (Tregs) within the tumor microenvironment (TME), where it promotes Treg recruitment, expansion, and immunosuppression

Epitope Targeted for Treg Depletion

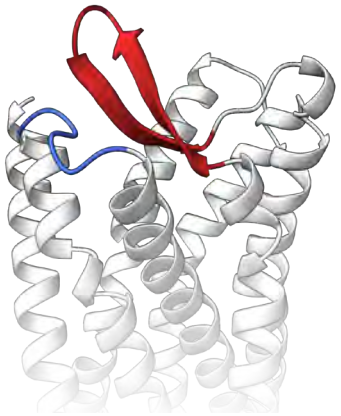


NMR Structure Validates Engineered Epitope Design



Input Target

CCR8



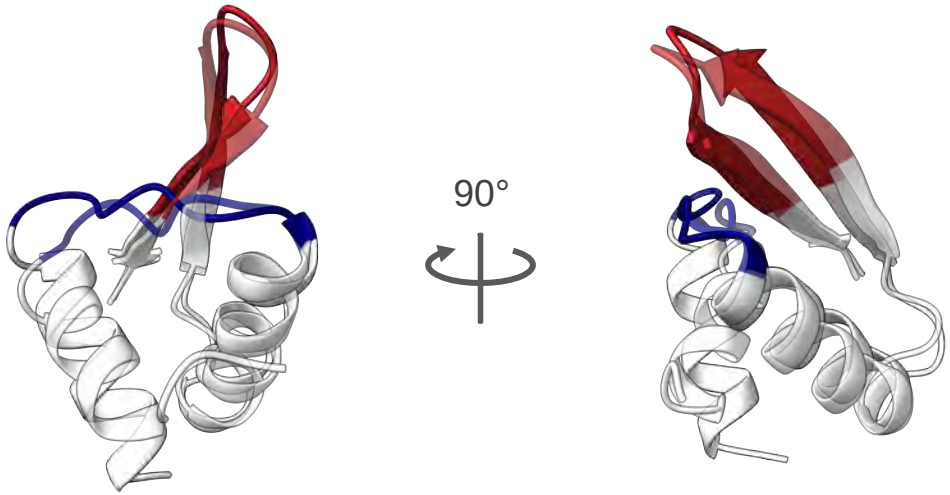
AI Protein Design

Discontinuous Epitope Scaffold Design



Engineered Epitope

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

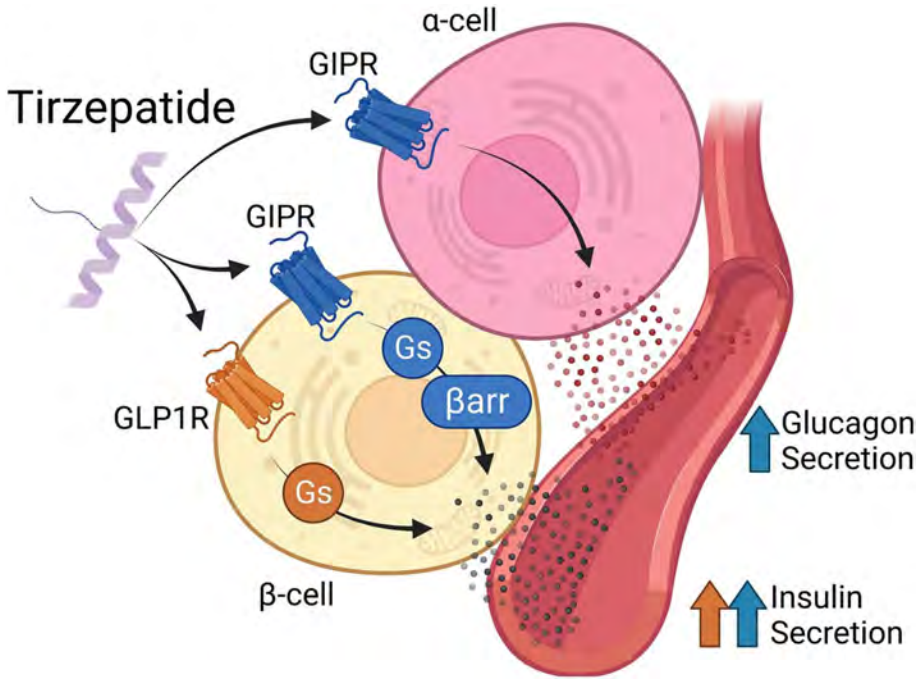


Backbone RMSD = 1.6 Å for epitope residues

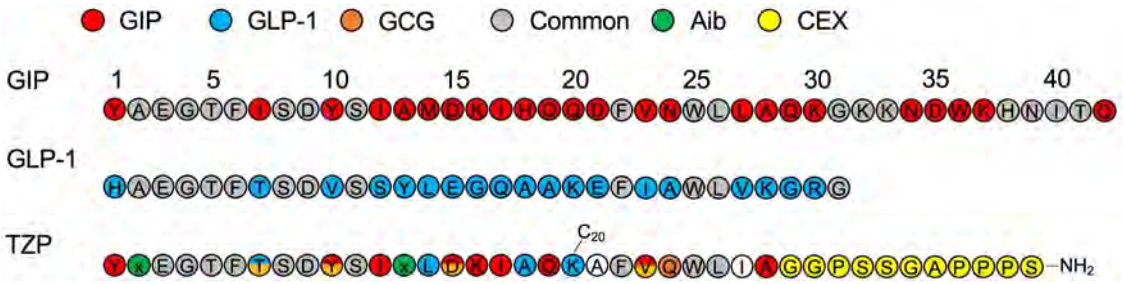
* *Unpublished collaboration with Arizona State University (2021)*

Case Study #2: GIPR

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

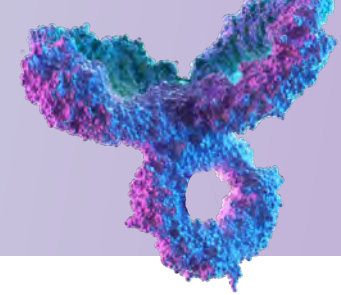


Challenge: Design Soluble GIPR that Specifically Binds GIP and not GLP-1



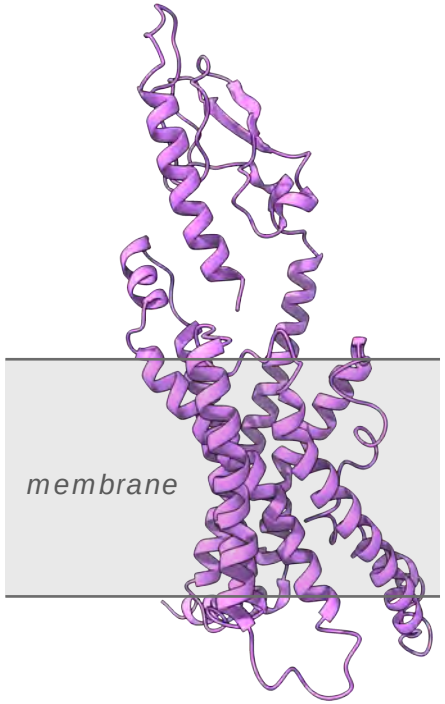
GIPR and GLP-1R are structural homologs whose natural ligands GIP and GLP-1, respectively, share high sequence identity at the N-terminus

GIPR is Solubilized by Reengineering the Transmembrane Domain



Input Target

GIPR

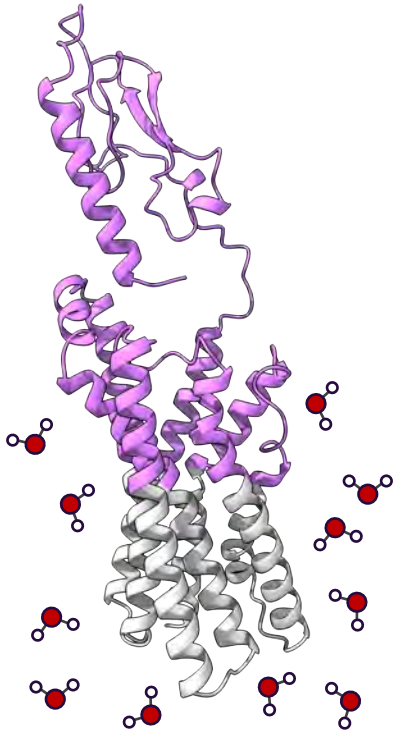


Generative AI

Solubilization of transmembrane domain

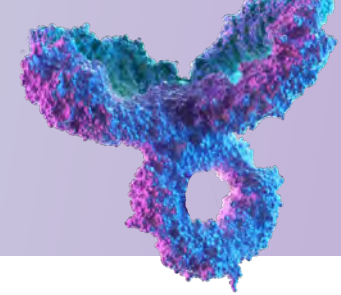


Soluble GPCR



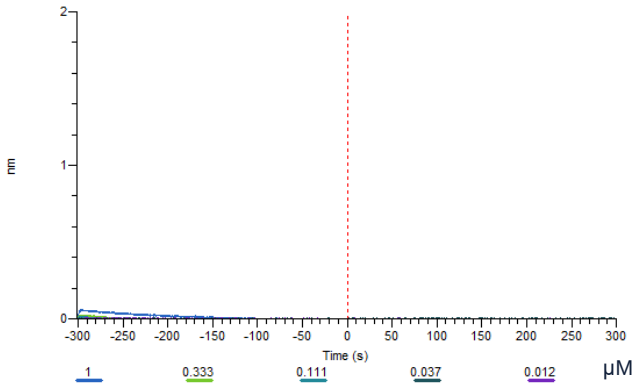
- ✓ *Native-like extracellular region*
- ✓ *Water solubility*
- ✓ *High Stability*
- ✓ *High Expression*
- ✓ *PTMs by expression in human cells*

Both Extracellular and Transmembrane Domains Required for GIP Binding

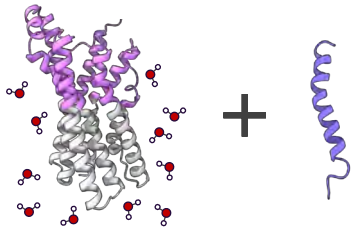


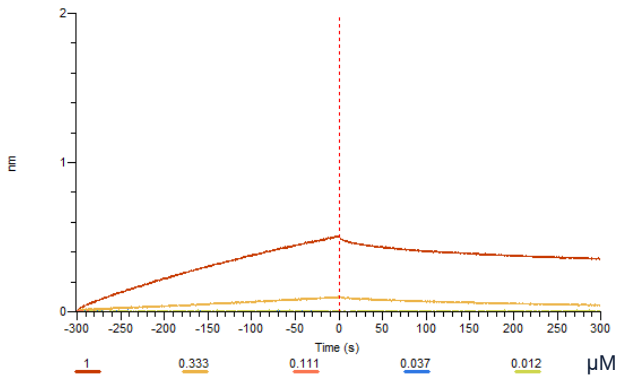
Extracellular Domain (ECD) Binding to GIP



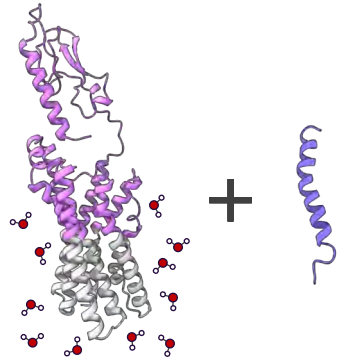


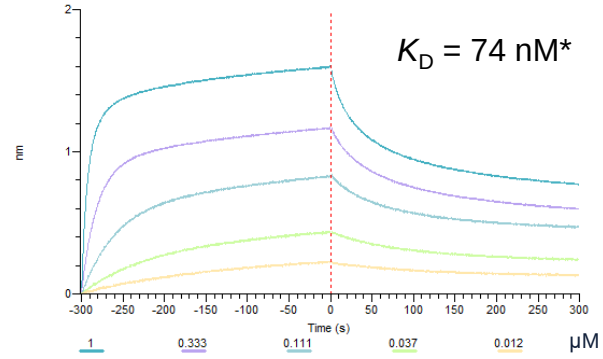
Transmembrane Domain (TMD) Binding to GIP





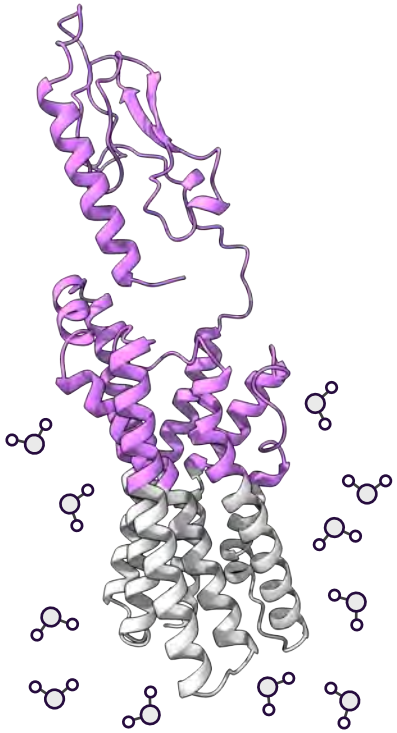
ECD + TMD Binding to GIP





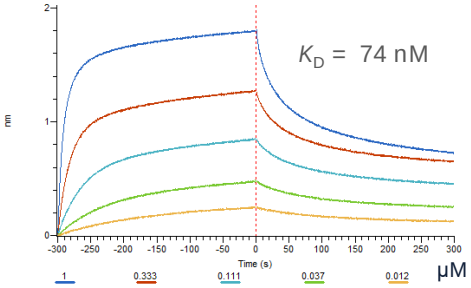
K_D calculated from avidity binding kinetics

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

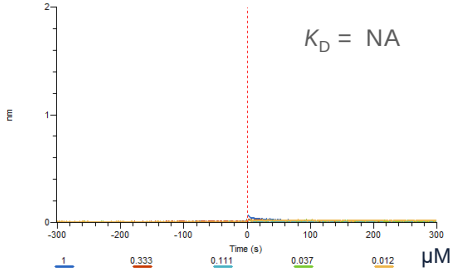


Avidity Ligand Binding Kinetics

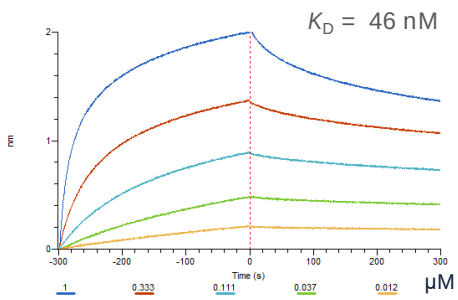
GIP
Natural Ligand



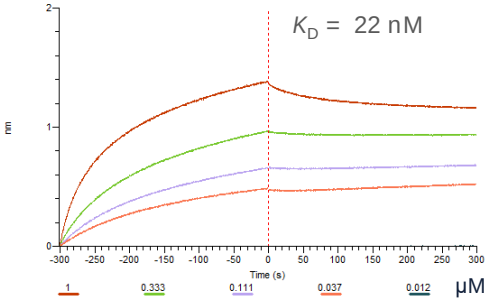
GLP-1
Negative Control



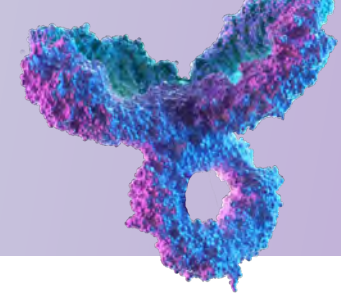
Zepbound®
Dual Agonist Therapeutic Drug



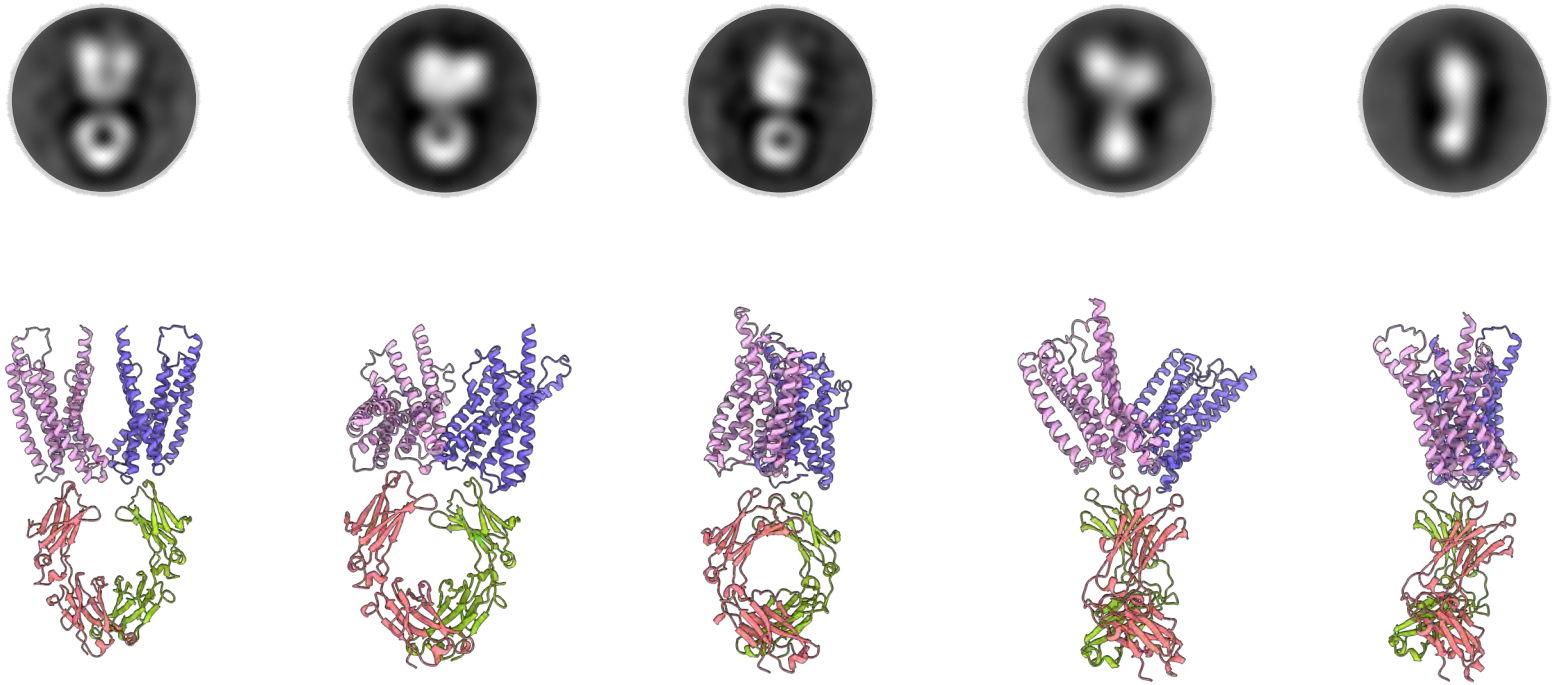
Retatrutide
Triple agonist



Negative Stain Electron Microscopy Supports Soluble Transmembrane Design

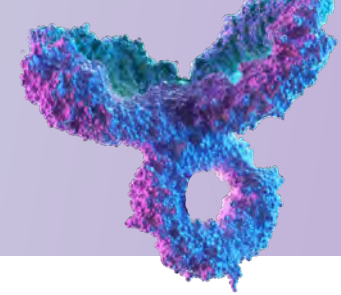


Negative Stain Electron Microscopy

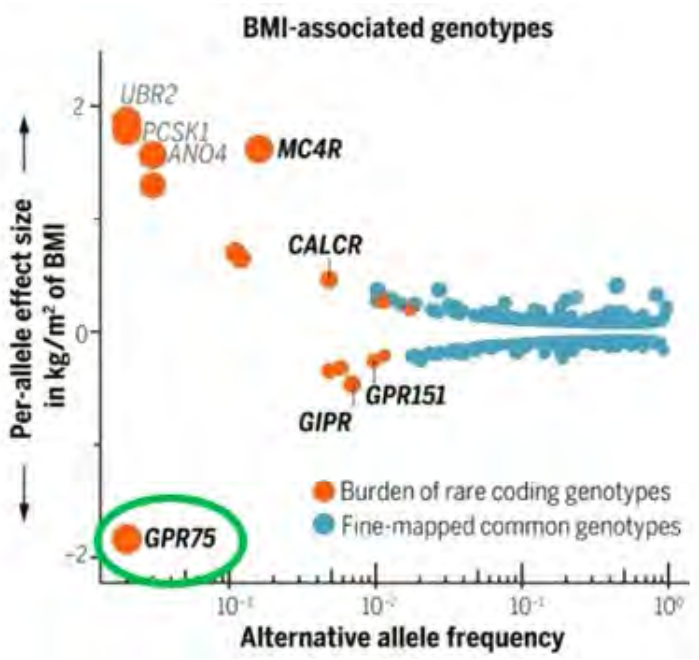


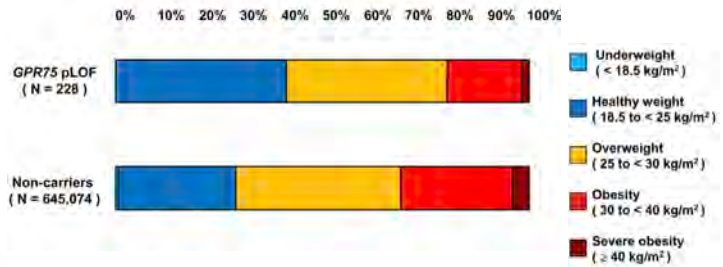
- Soluble GIPR expressed as Fc fusion
- 2D class averages agree with soluble transmembrane design
- Flexibility observed at the Fc hinge region

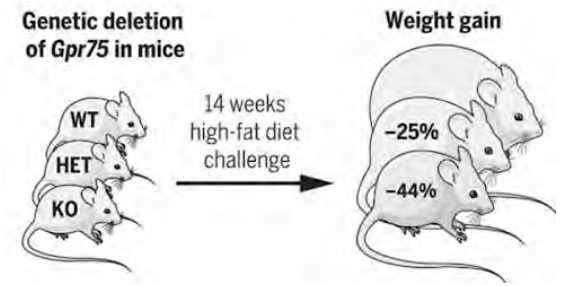
Case Study #3: GPR75



GPR75 is a Genetically Validated Obesity Target



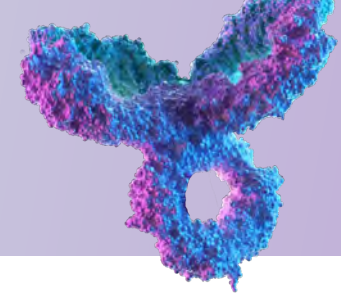




Challenges

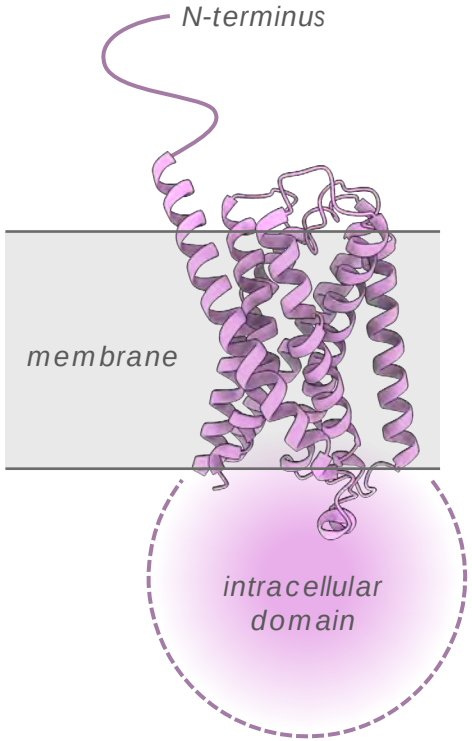
- No experimental structures available at the time of this study
- 20-HETE binds the transmembrane domain
- CCL5 binds extracellularly, but the binding site is unknown

Soluble GPCR Designed from Sequence Alone



Input Target

GPR75 (AlphaFold)

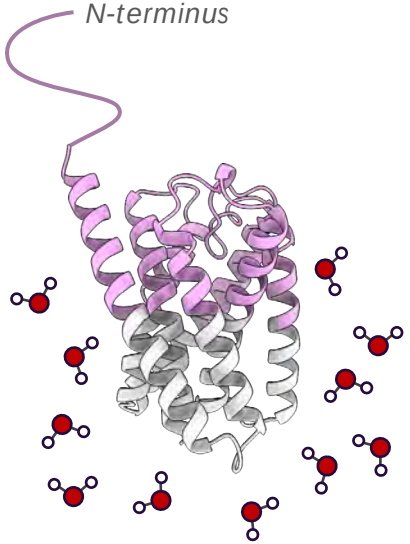


Generative AI

Solubilization of transmembrane domain



Soluble GPCR



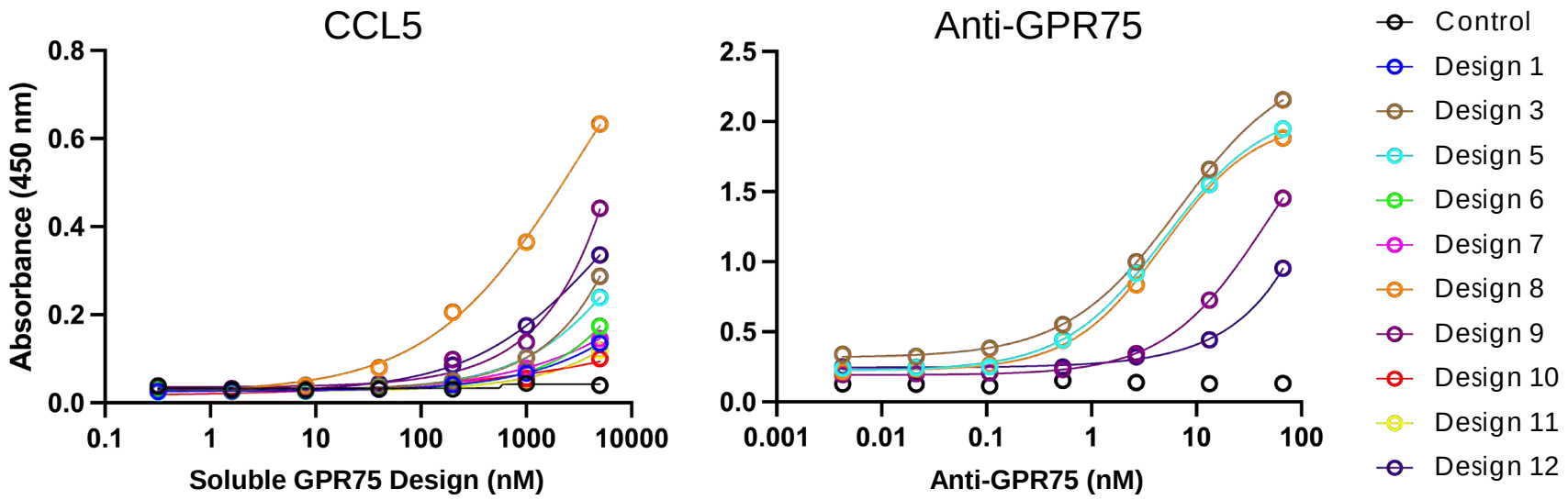
Soluble GPCR designs generated from sequence information alone

Removal of ~250 residue intracellular domain

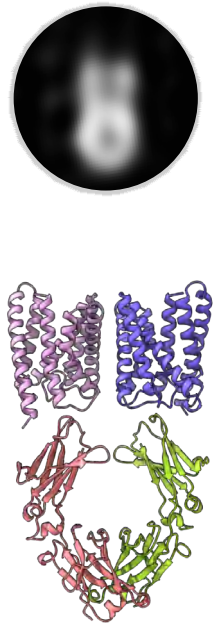
12 designs expressed and purified to test for binding to CCL5

Solubilized GPR75 Binds CCL5 and Commercial Antibody

ELISA Binding

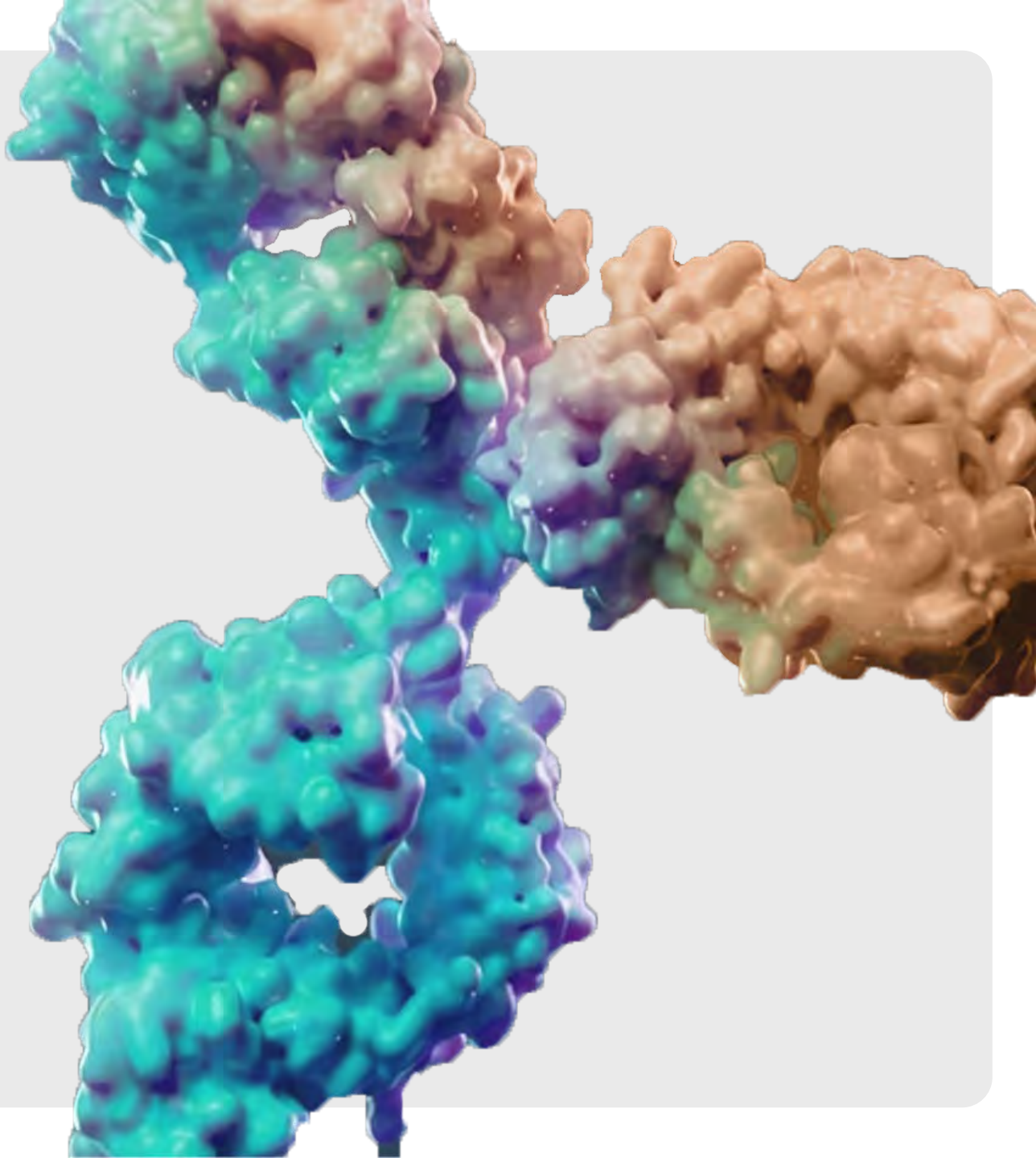


Negative Stain EM



Antibody Discovery with Soluble GPCRs

Amylin Receptor

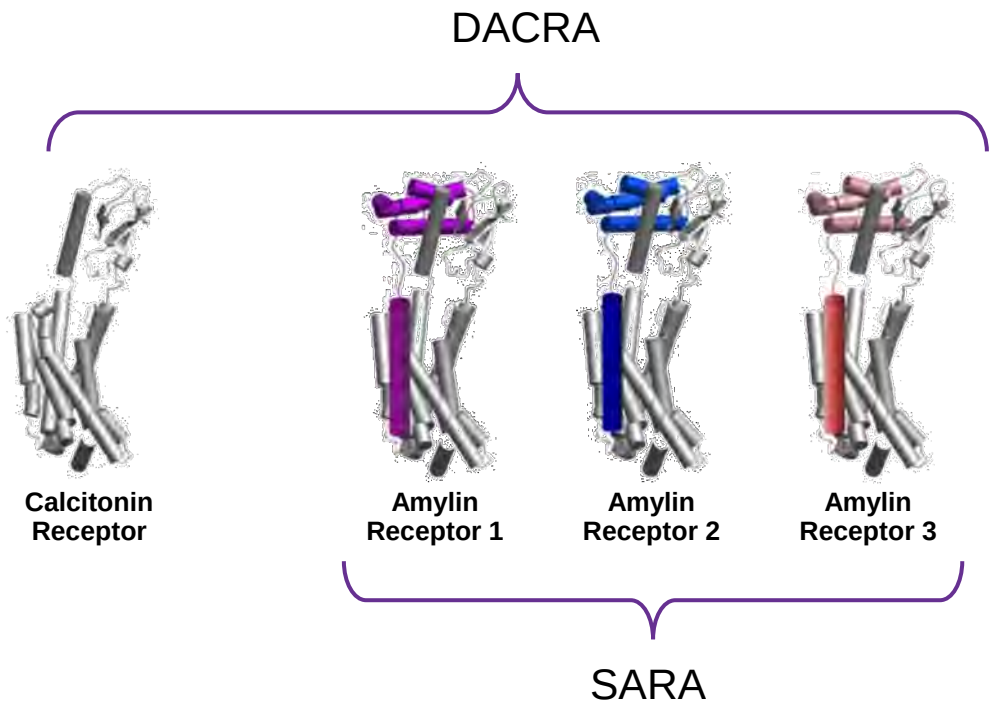


Amylin Receptor Agonism

A Complementary Pathway to GLP-1–Based Obesity Treatments

Why Target Amylin?

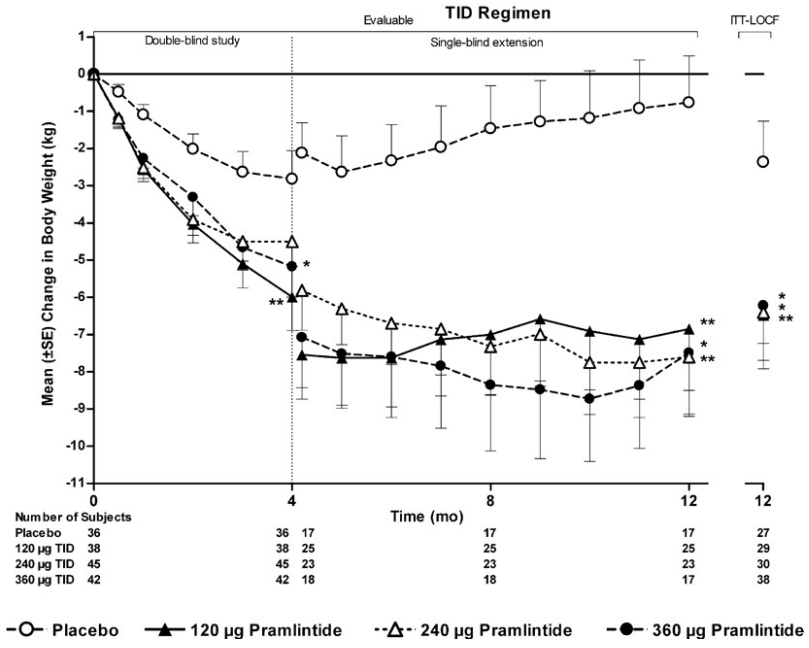
- **Validated metabolic hormone** that promotes satiety and slows gastric emptying
- Clinical studies with amylin analogs confirm efficacy in weight loss, but **peptide-based approaches may be sub-optimal** (dosing, tolerability, manufacturability)
- Antibody therapeutics could provide a differentiated 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)

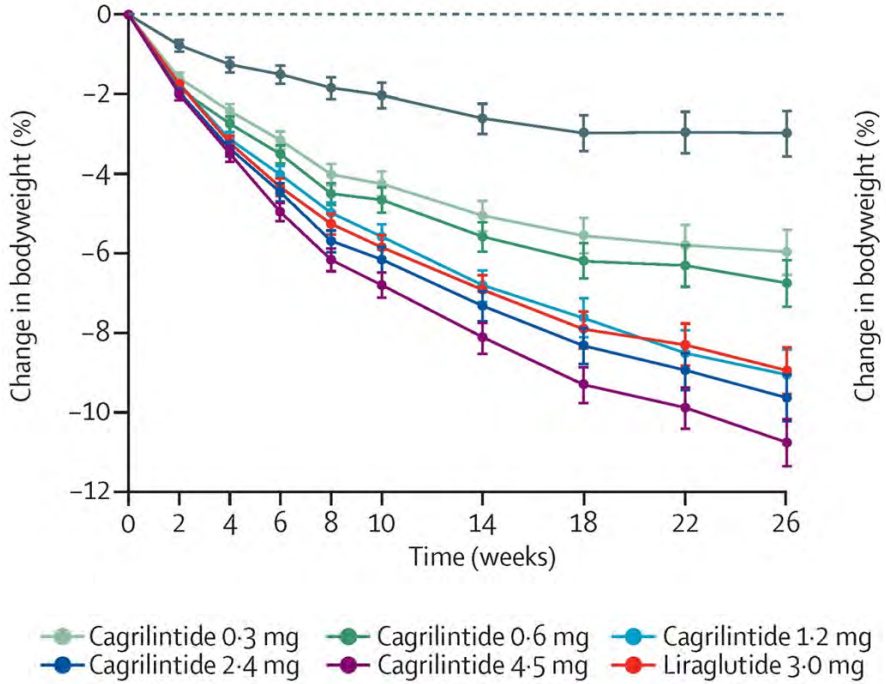
Peptide Drugs are Efficacious but Require Frequent Dosing

Weight Loss on FDA Approved Pramlintide



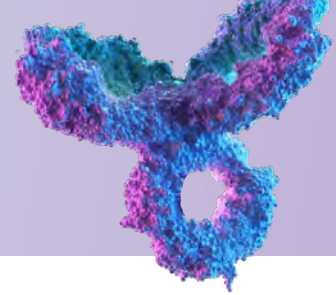
Limitation – requires <u>multiple doses per day</u>

Weight Loss on Long-Acting Cagrilintide



Limitation – still requires <u>weekly dosing</u>

Goal: Improve Therapeutic Specificity and Longevity with Antibody Fusion

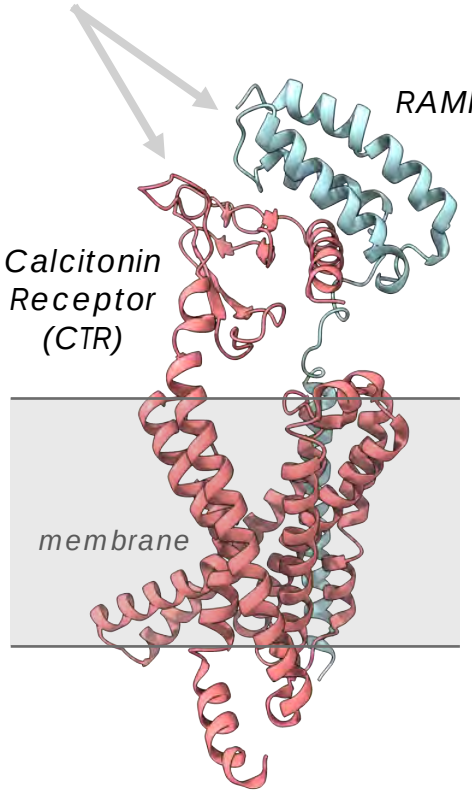


SARA

Combinations specific to amylin receptors 1, 2, and 3

DACRA

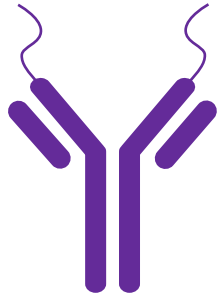
Calcitonin receptor and amylin receptors 1, 2, and 3



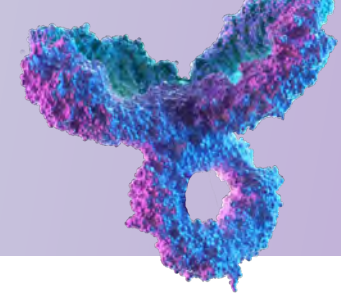
RAMP

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

Synthetic Amylin + Antibody Fusion

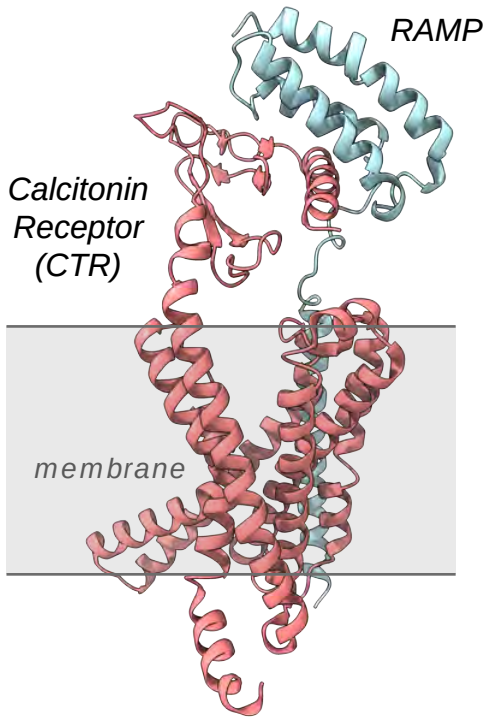


Full Spectrum of Epitopes Designed for Amylin Receptor



Input Target

Amylin Receptor

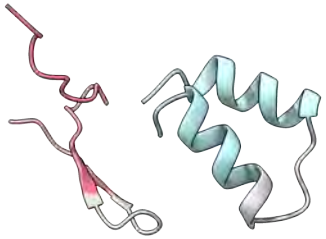


Generative AI

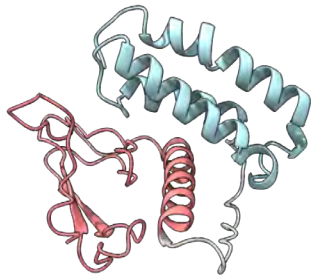


Epitope Designs

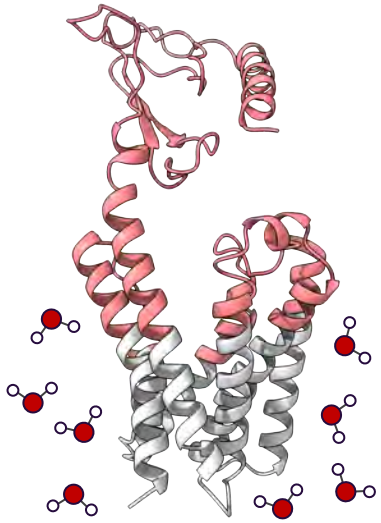
Individual Epitopes



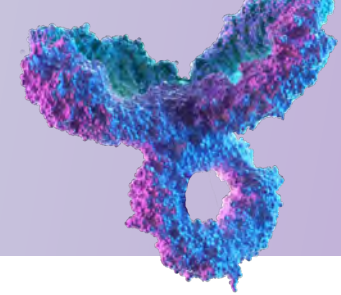
CTR-RAMP Junction



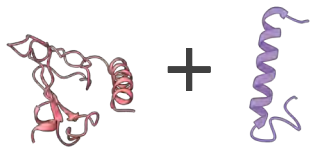
Soluble CTR

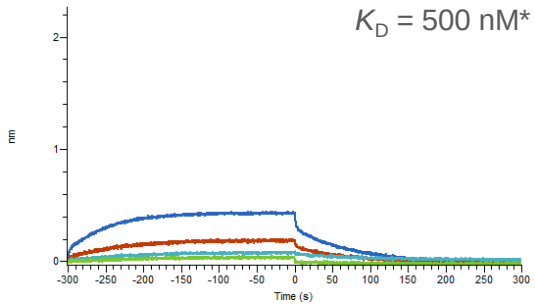


CTR Solubilization Validated by Salmon Calcitonin Binding

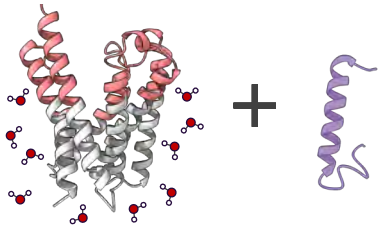


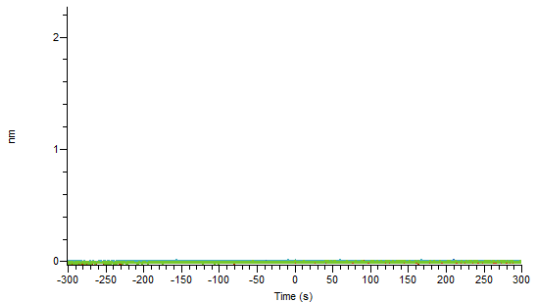
Extracellular Domain (ECD) Binding to Salmon Calcitonin



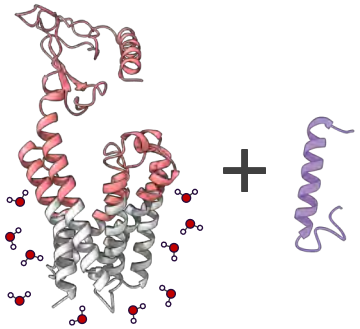


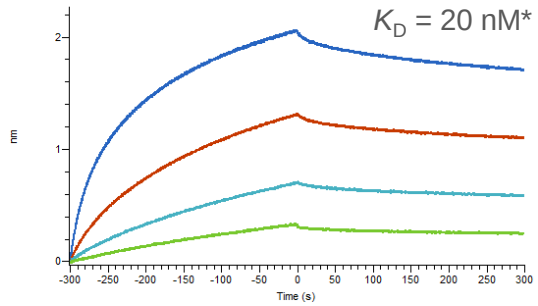
Transmembrane Domain (TMD) Binding to Salmon Calcitonin





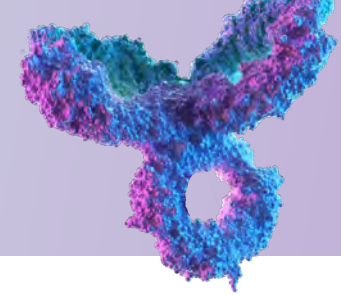
ECD + TMD Binding to Salmon Calcitonin



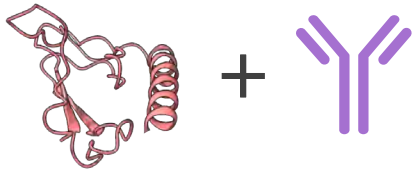


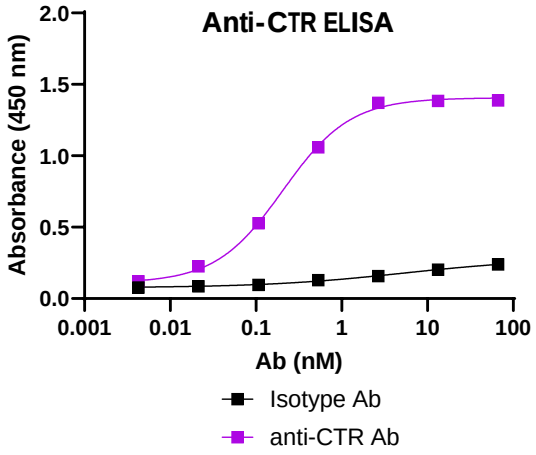
K_D calculated from avidity binding kinetics

Amylin Junctional Epitope Design Retains Anti-CTR Antibody Binding

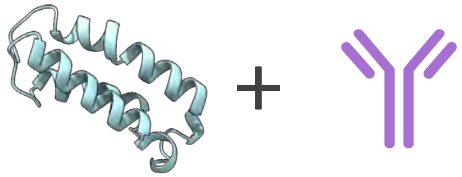


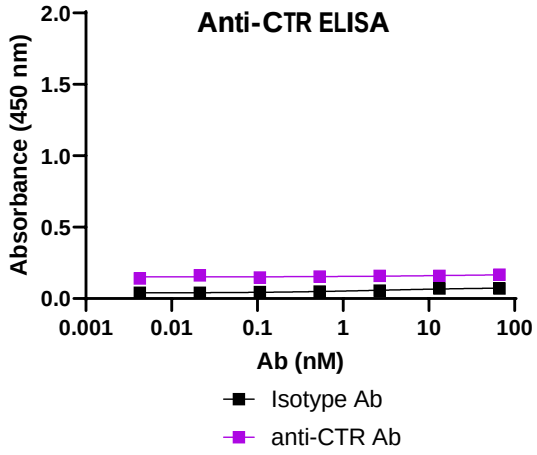
CTR ECD



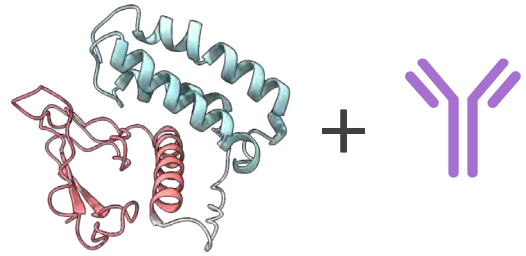


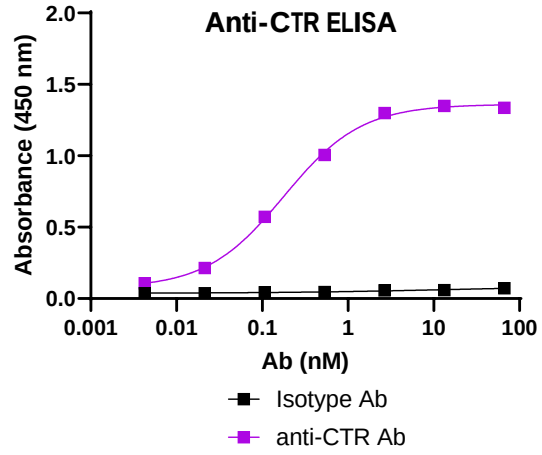
RAMP



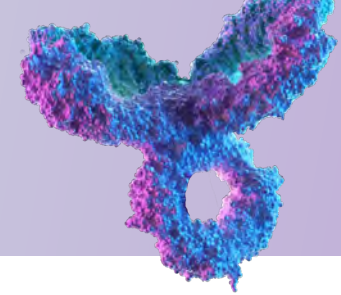


Amylin ECD



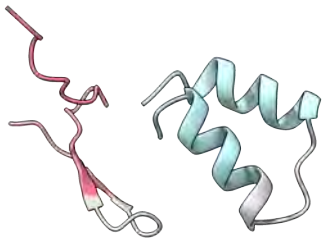


Epitope Designs Enable Both In Vivo and In Vitro Discovery

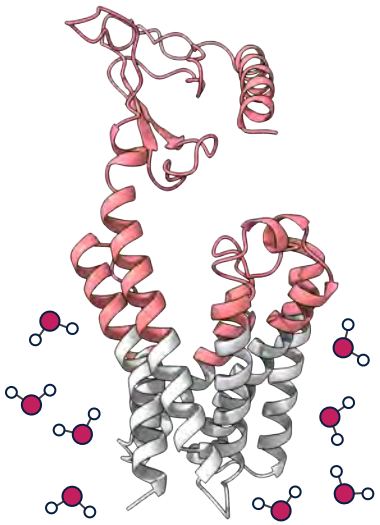


Epitope Designs

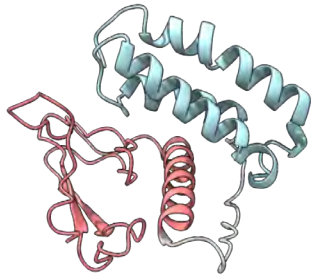
Individual Epitopes



Soluble CTR



CTR-RAMP Junction



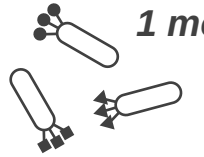
In Vivo/In Vitro Selection

Immunizations



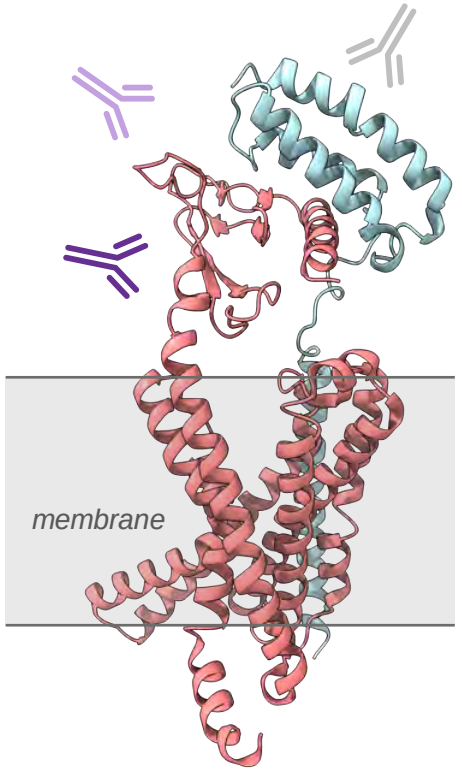
4 months

Phage panning

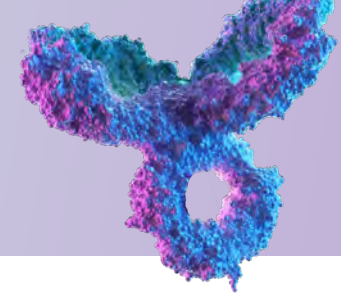


1 month

Antibody Discovery

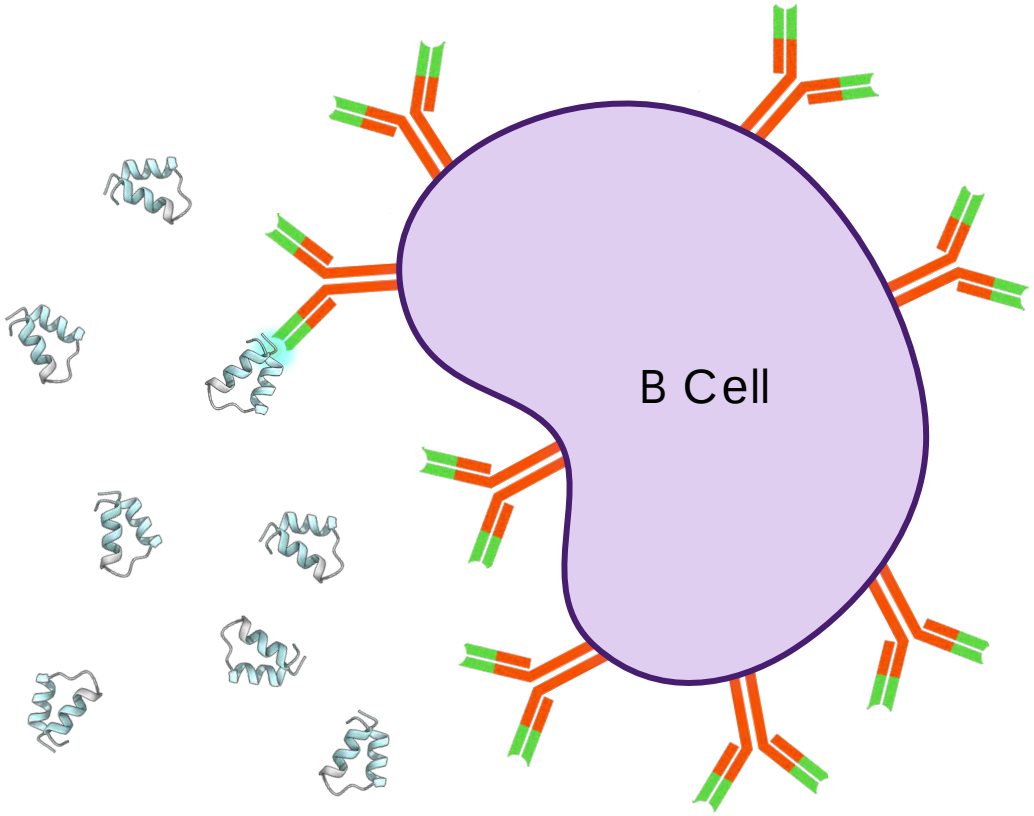


Nanoparticle Display System Enhances Immune Response for Immunizations



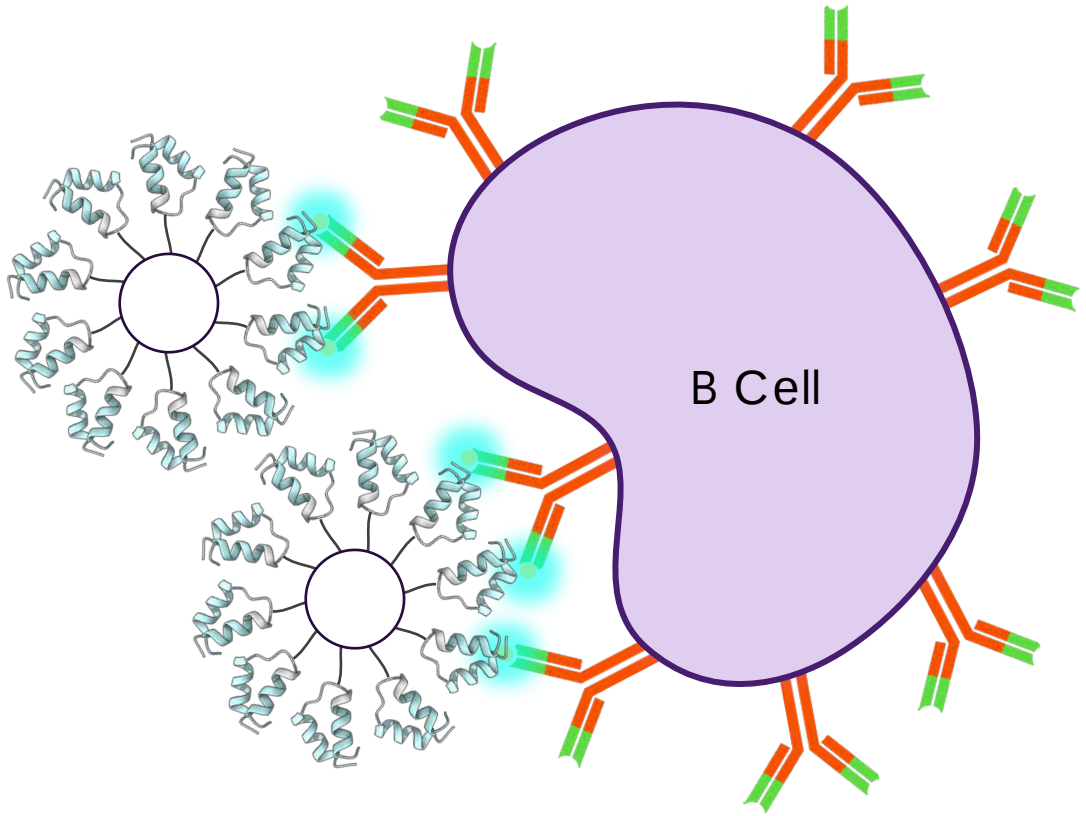
Engineered Epitope Immunization

Weak B cell activation

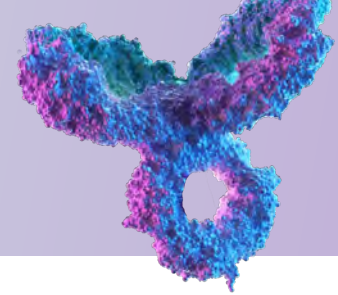


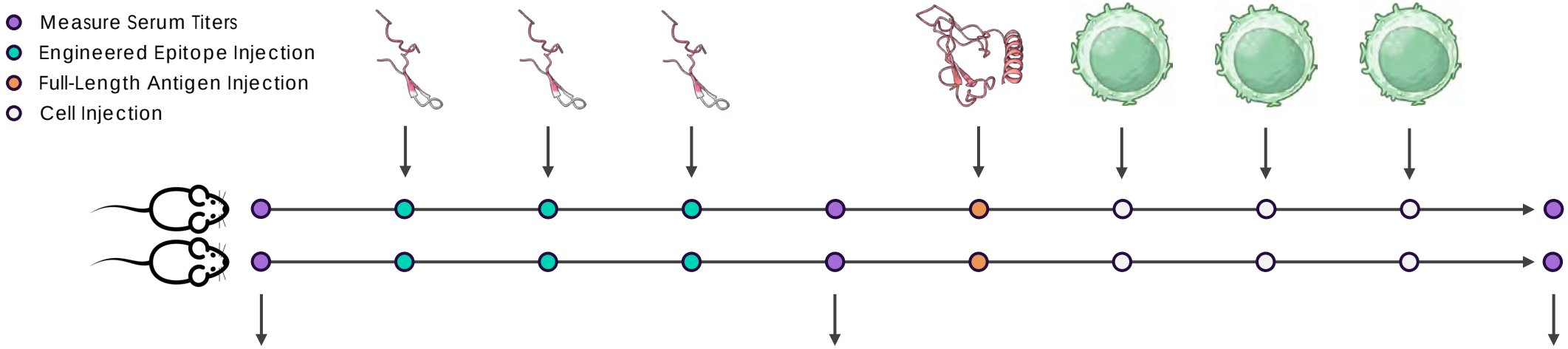
Nanoparticle Immunization

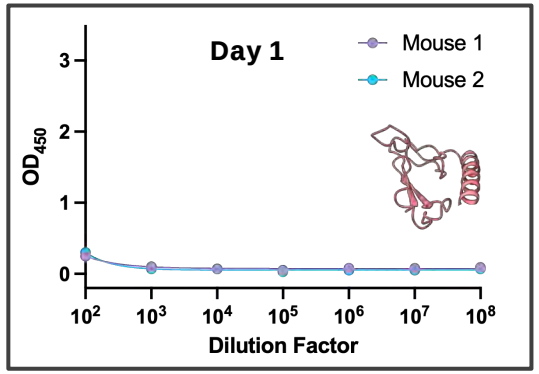
Strong B cell activation

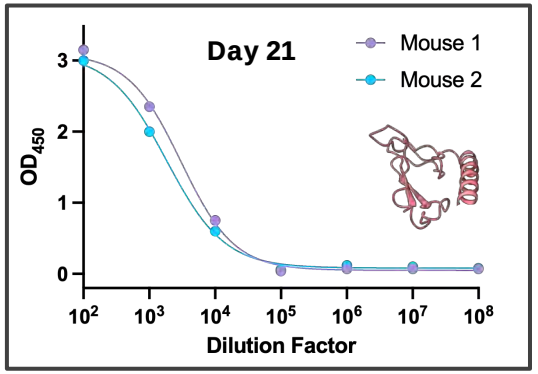


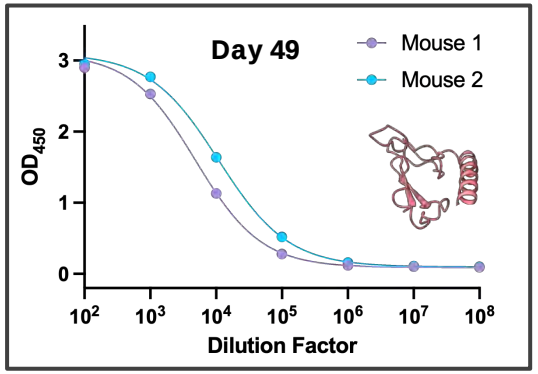
Engineered Epitopes Prime Immune System for an Epitope-Focused Response



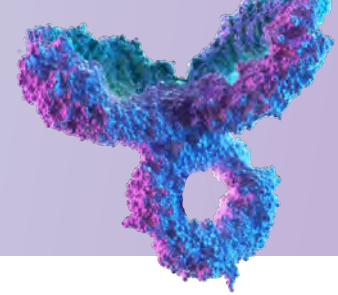


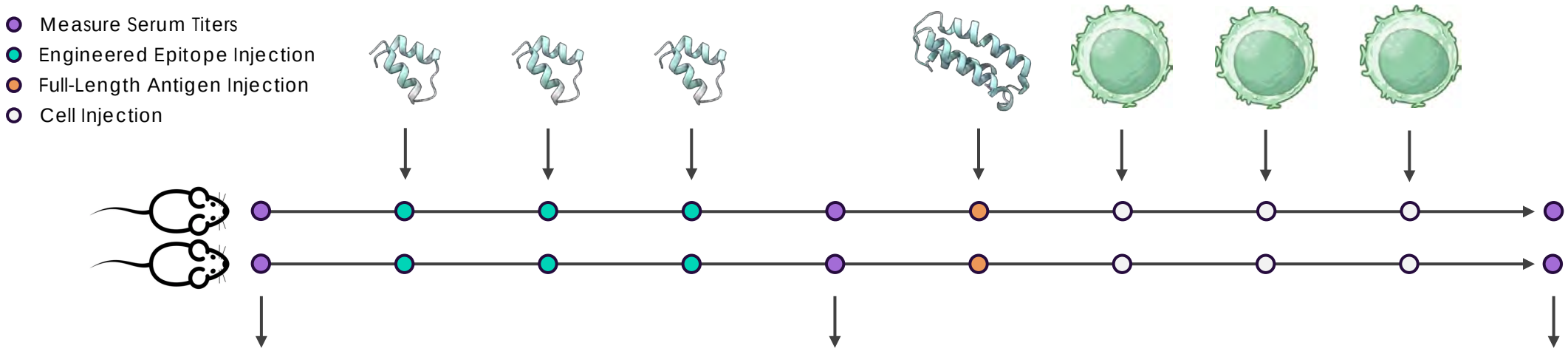


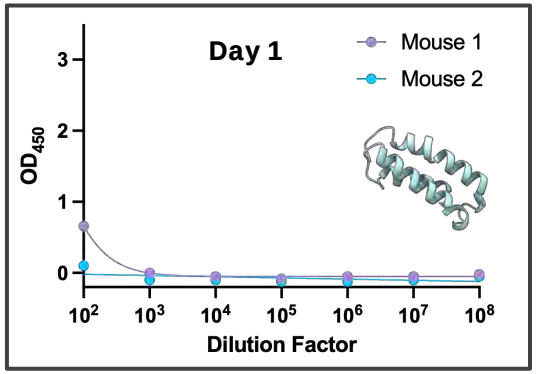


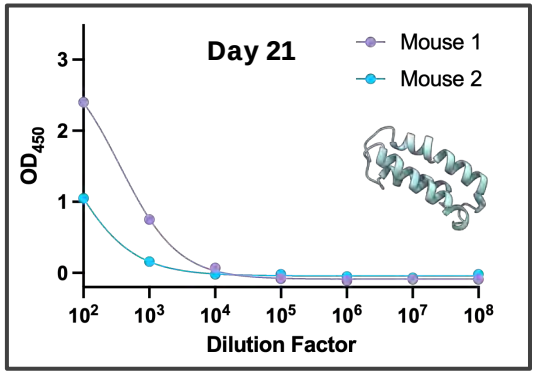


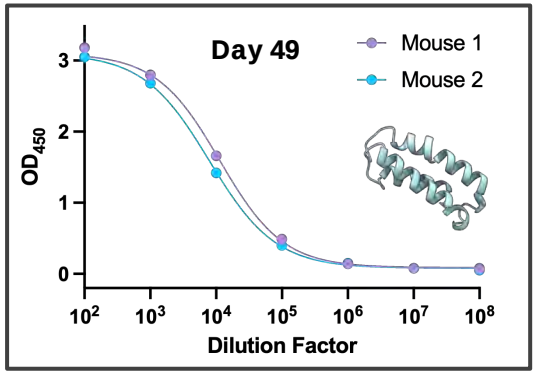
Engineered Epitopes Prime Immune System for an Epitope-Focused Response



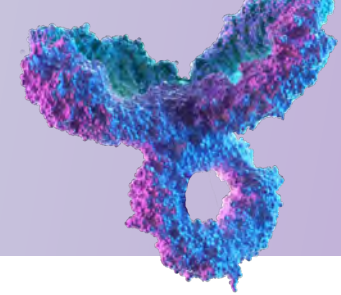




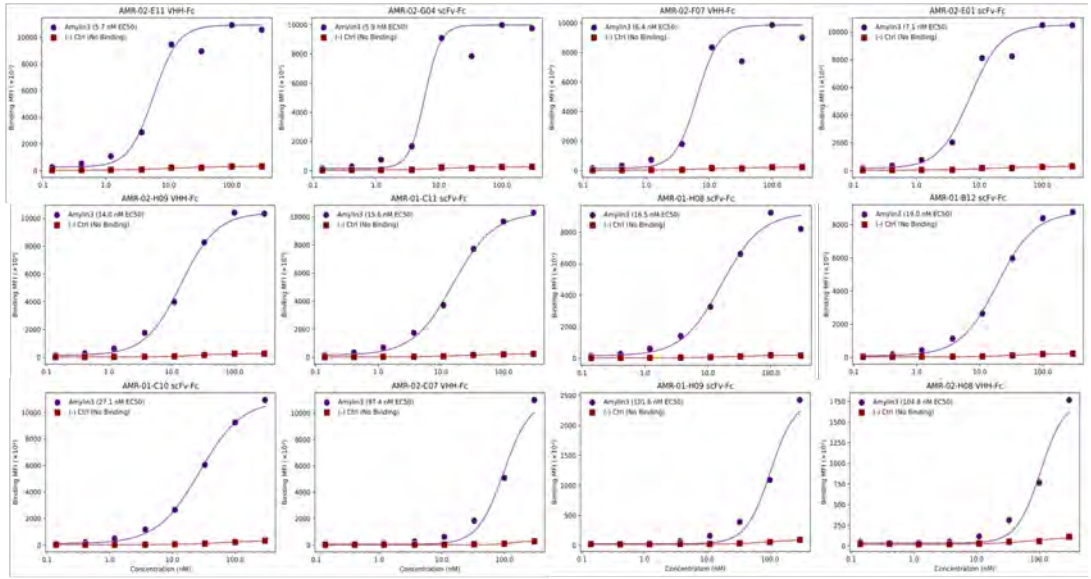




Phage Panning Against Engineered Epitopes Translates to Cell Binder Hits

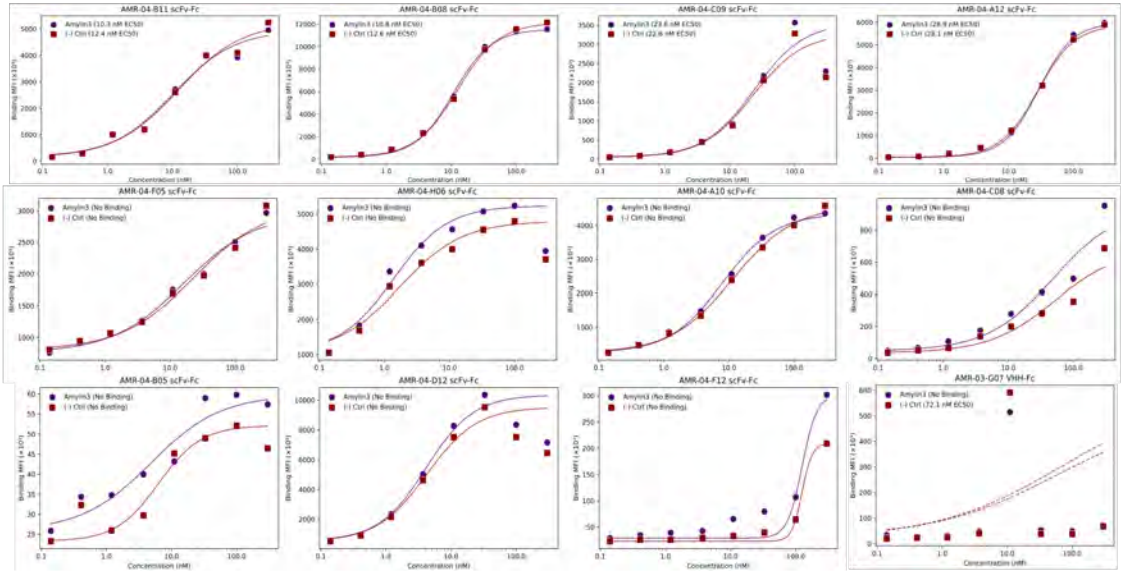


Engineered Epitope Steered Strategies



Amylin Receptor Cell-Only Strategies

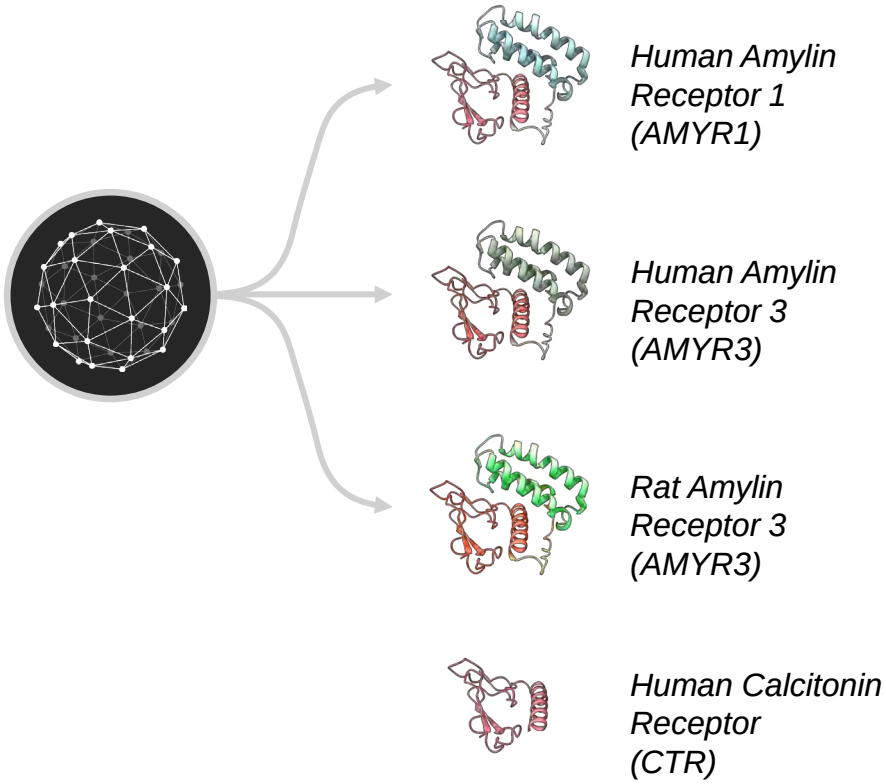
(No Specific Binders Found)



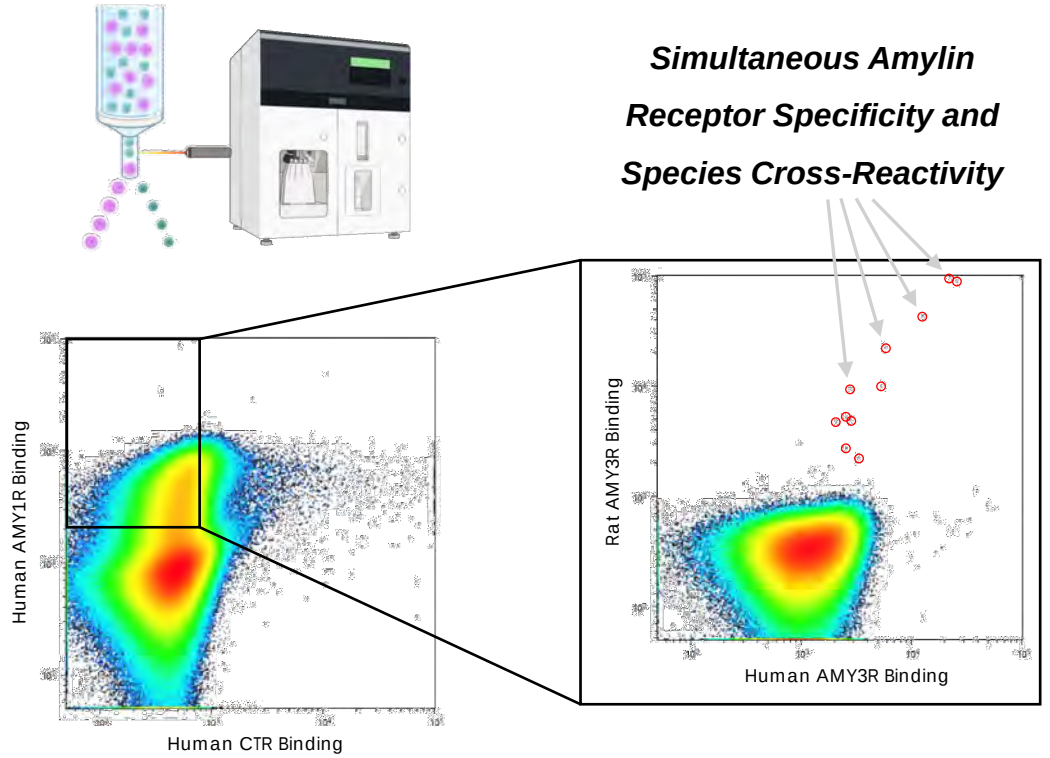
- Amylin3 Receptor Cell Binding
- Negative Control Cell Binding

Multi-Specificity and Cross-Reactivity in a Single Experiment

Species Cross-Reactive Designs



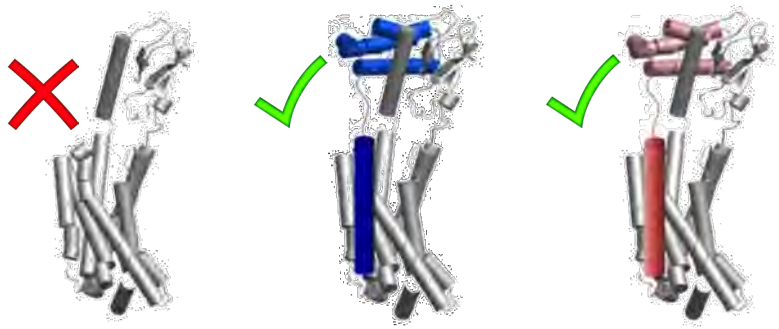
4-Dimensional Mammalian Display Sorting



Cell Binding and Agonism are Amylin Receptor Selective

Cell Binding

Calcitonin Receptor Cells
Amylin Receptor 1 Cells
Amylin Receptor 3 Cells
Negative Control Cells



Calcitonin Receptor | **Amylin Receptor 1** | **Amylin Receptor 3**

Agonism Assay

Antibody Peptide Fusion | ***Cagrilintide***





Calcitonin Receptor Cells
Amylin Receptor 3 Cells