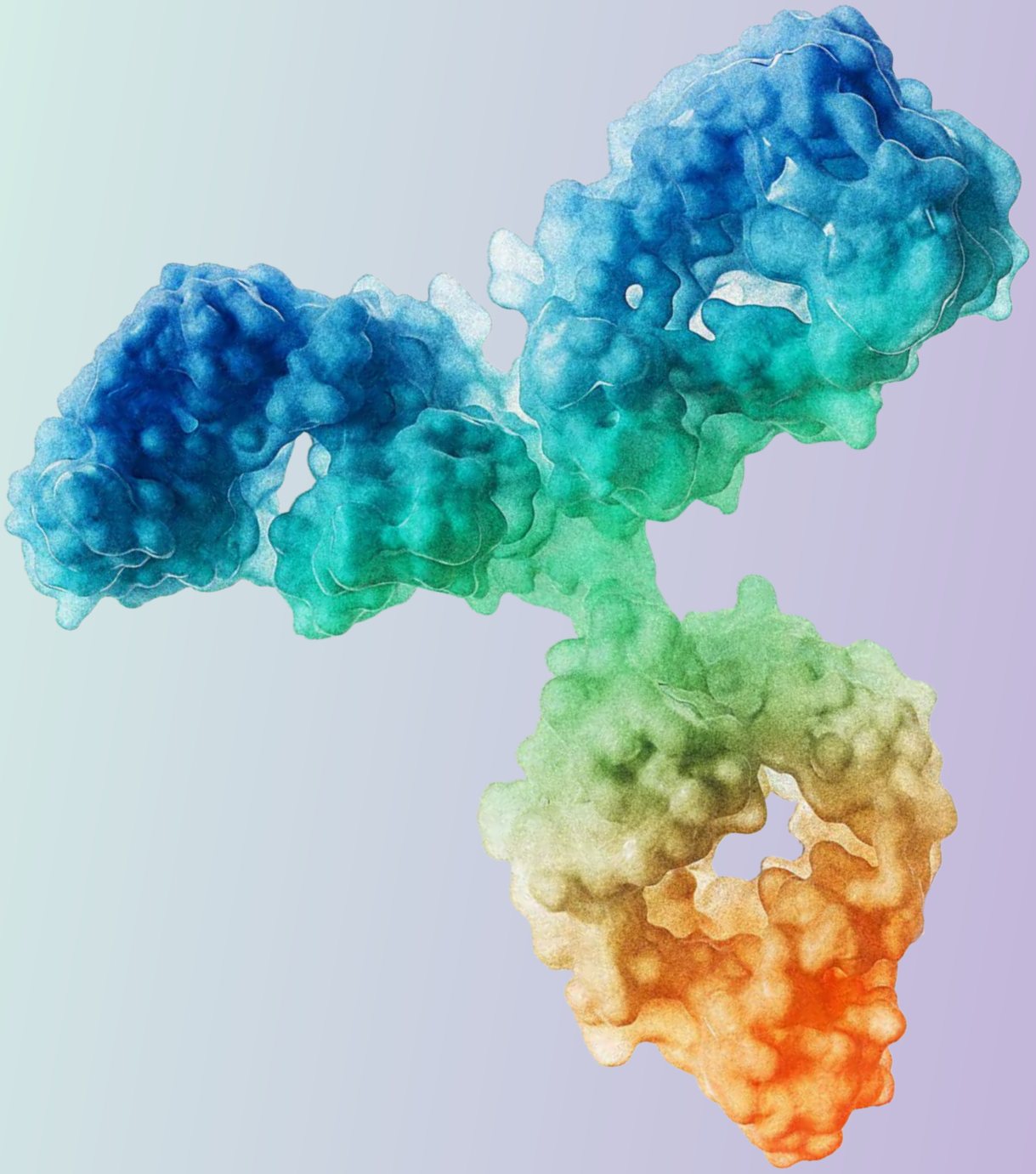
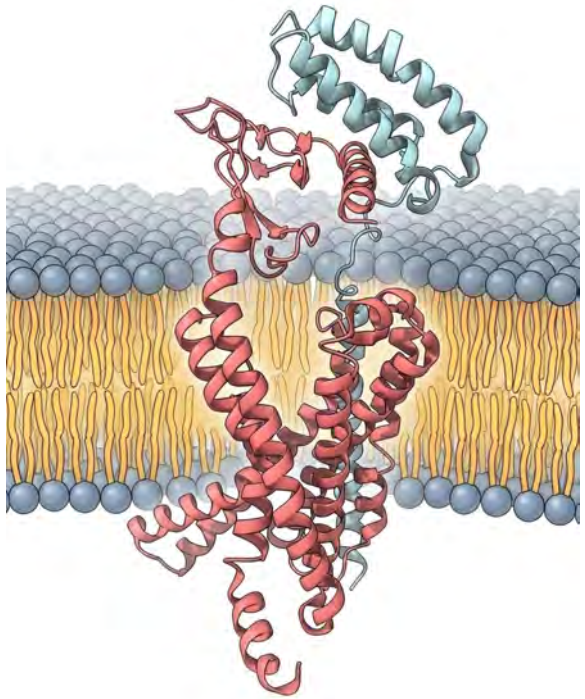
Engineered Scaffolds for Soluble GPCR Expression

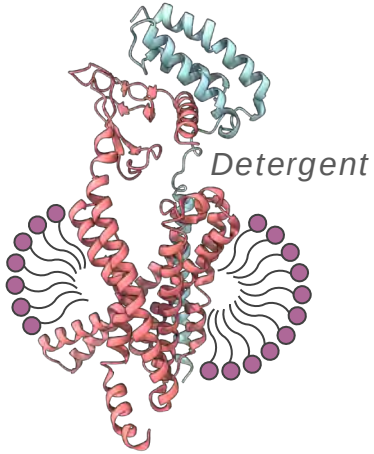


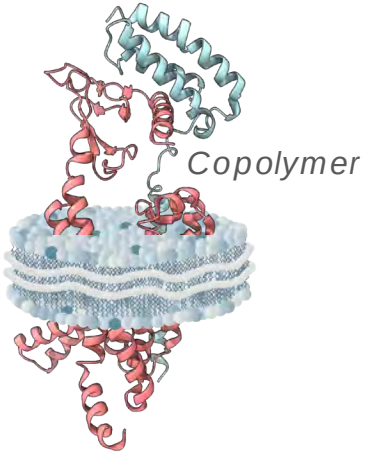
Alex Taguchi

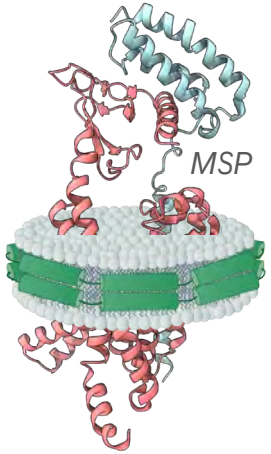
GPCR Paradox: Prime Targets, Tough Antigens to Produce

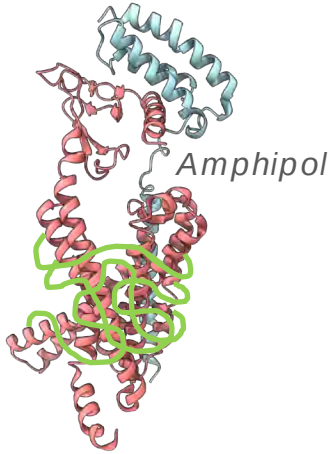


Solubilization









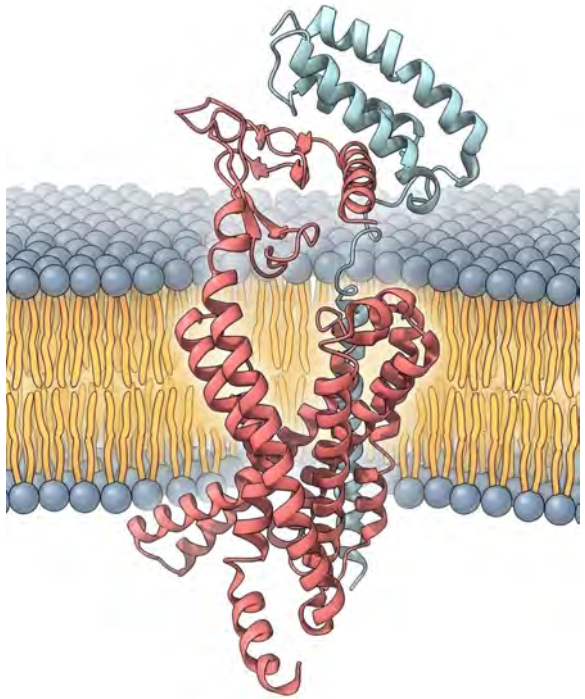
Benefits

- ✓ *Native sequence*
- ✓ *Native-like structure*

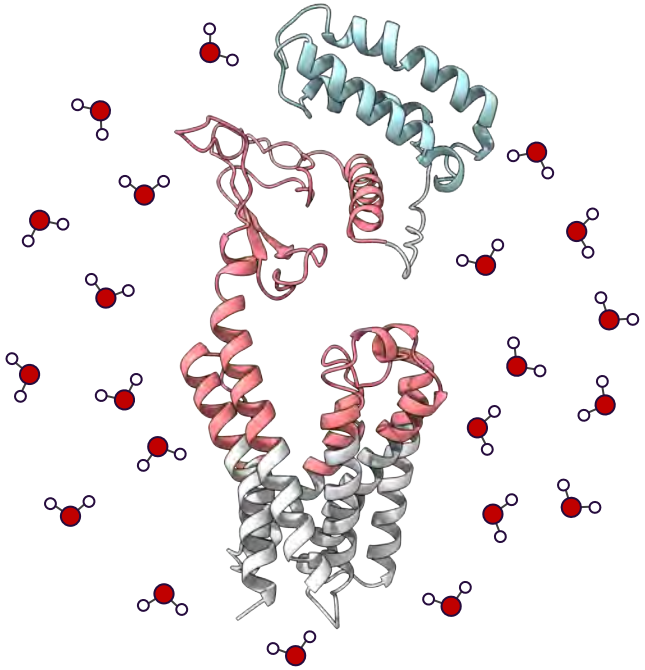
Complications

- ✓ *Time consuming*
- ✓ *Costly*
- ✓ *Stability issues*

GPCR Paradox: Prime Targets, Tough Antigens to Produce



Protein Design



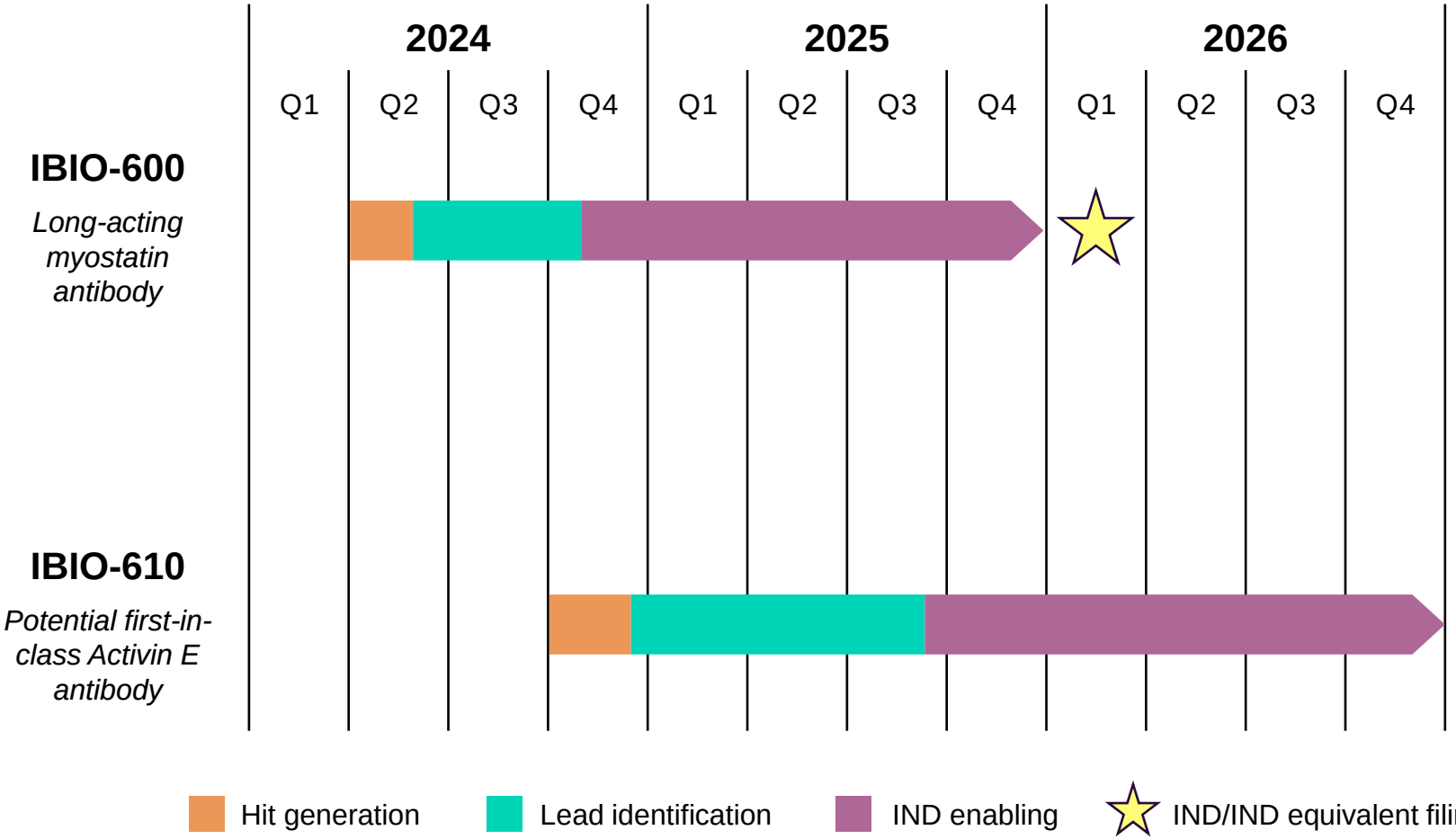
Benefits

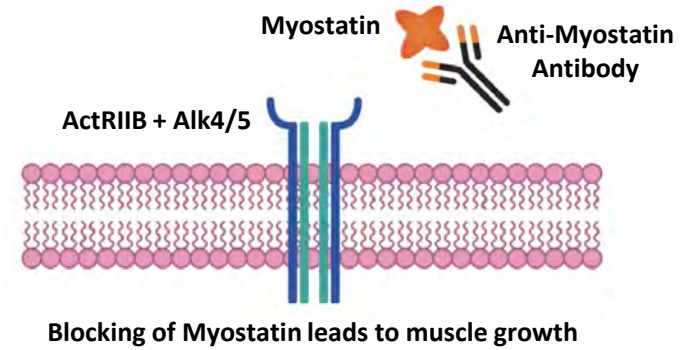
- ✓ *Soluble*
- ✓ *Stabile*
- ✓ *Fast to produce*
- ✓ *Cost effective*

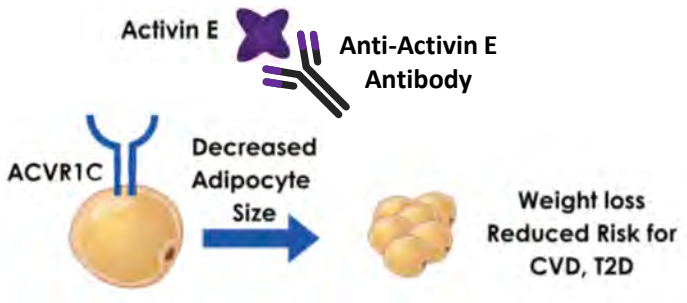
Complications

- ✓ *Potentially loses native-like structure*

Antibody Discovery to Development Candidate in as Little as 7 Months

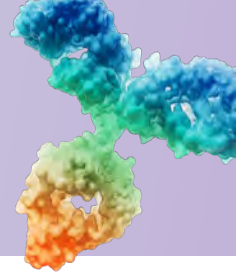






Akbari, P. et al. Nat. Commun. (2022), Deaton, A. M. et al. Nat. Commun. (2022), Schuelke M. et. al. N. Engl. J. Med. (2004), Deng, B. et. al. Nutrition and Metabolism (2017)
CVD: Cardiovascular Disease, T2D: Type 2 Diabetes

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



1 **Antigen Engineering**

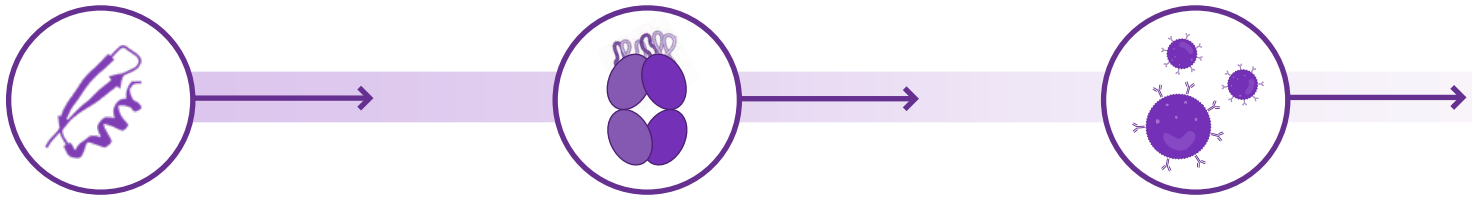
Epitope-selective antibody discovery with AI protein design[1]

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**

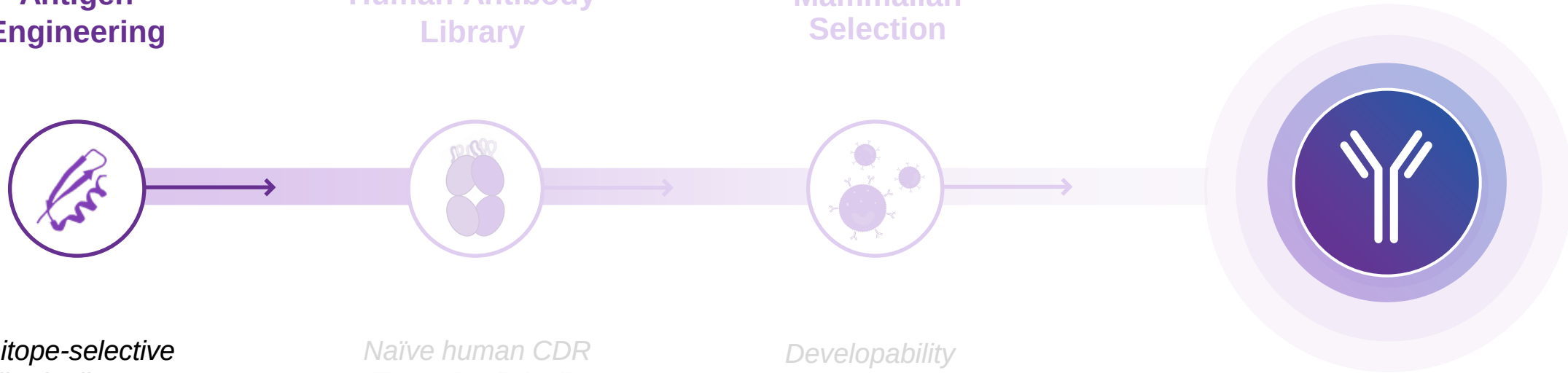
Epitope-selective antibody discovery with AI protein design[1]

2 **Human Antibody Library**

Naïve human CDR library in clinically validated framework

3 **Mammalian Selection**

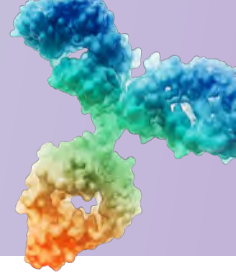
Developability optimization in production cell-line



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



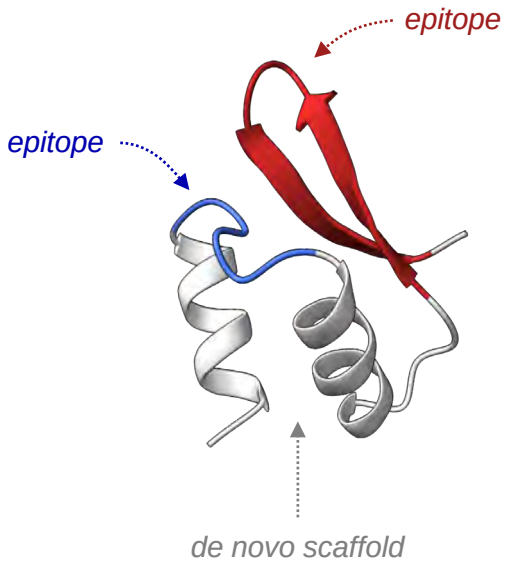
1

Naïve in vitro or in vivo antibody library



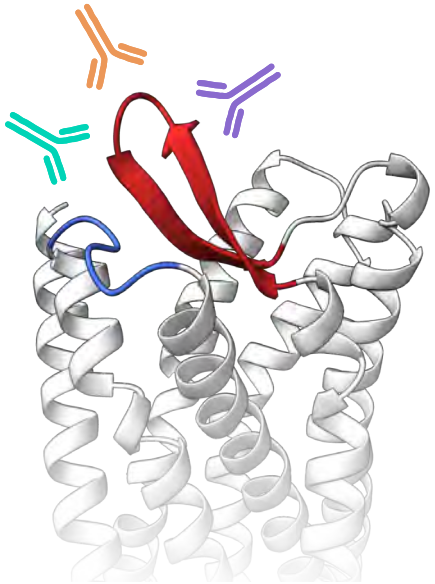
2

Focus library with engineered epitopes

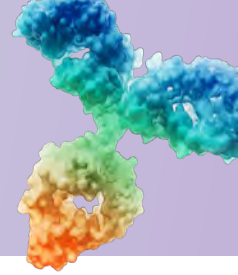


3

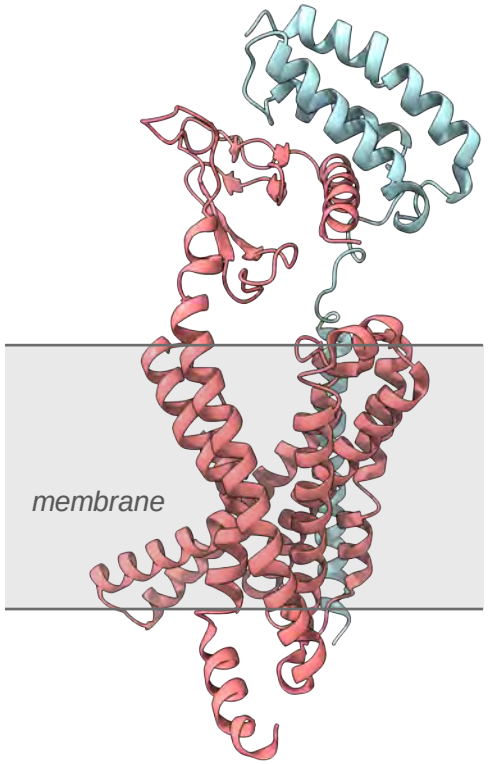
Efficient discovery of epitope-specific antibodies



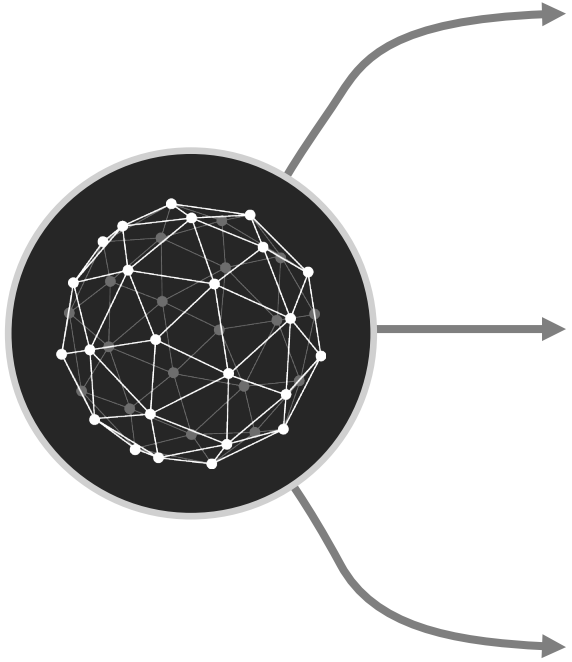
Engineered Epitopes Are Tailor-Made Solutions for a Target of Interest



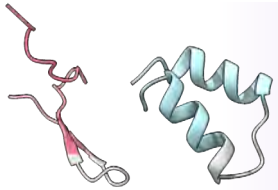
Target of Interest



Generative AI

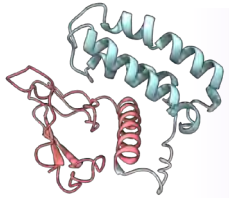


Use Cases



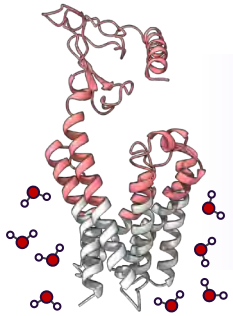
Epitopes of Interest

Design scaffold supporting native epitope structure



Protein Complexes

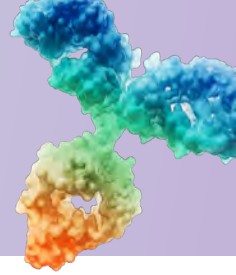
Stabilize junctional and/or discontinuous epitopes



Membrane Proteins

Solubilize transmembrane domains

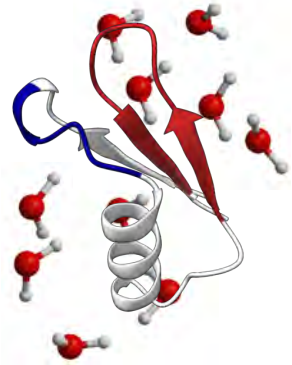
Antigen Designs are Optimized for Antibody Drug Discovery



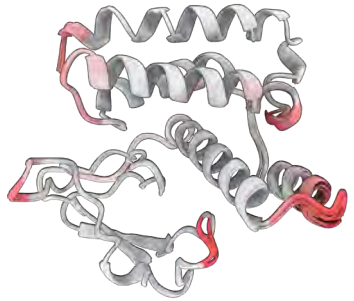
Water Solubility

Epitope

Scaffold



Structural Stability



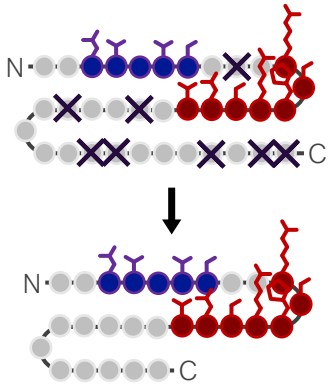
Predicted Flexibility



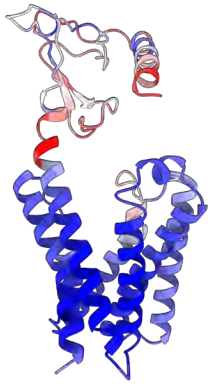
Scaffold Minimization

Epitope

Scaffold



Low Scaffold Immunogenicity

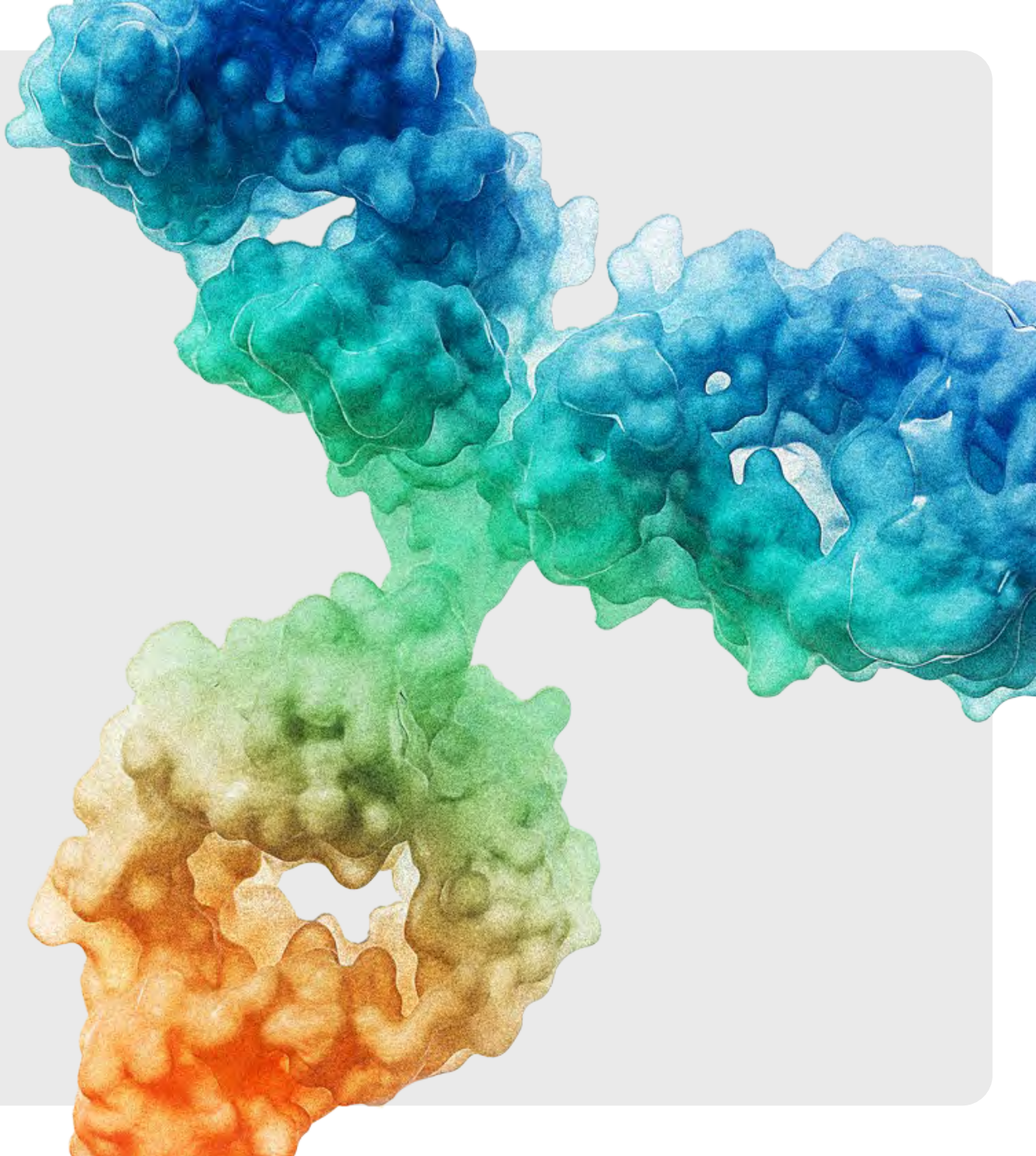


Predicted Immunogenicity[1]

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

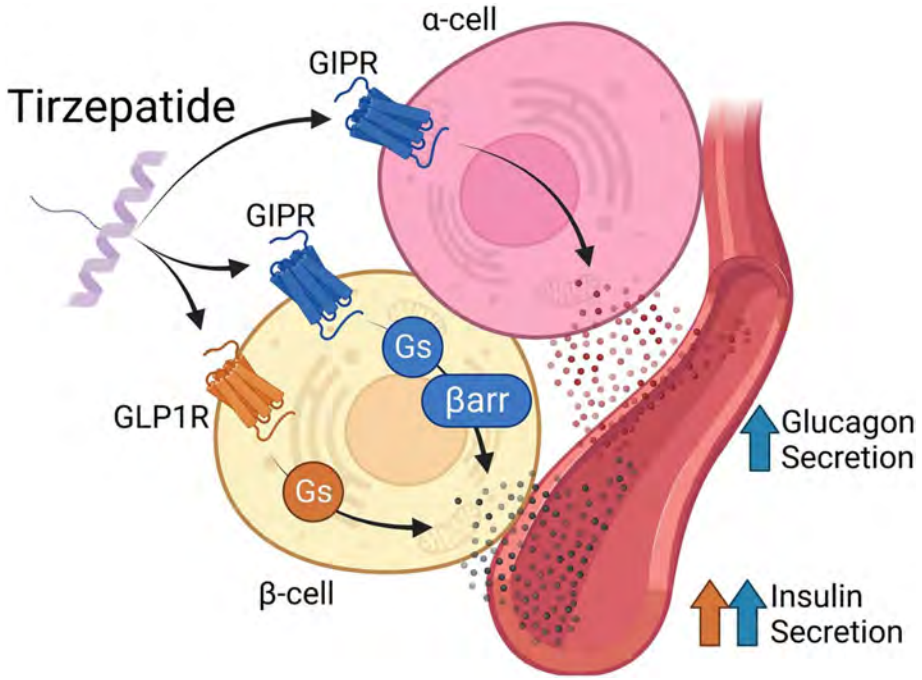
Soluble GPCR Validation

Experimental Structure and Function Case Studies

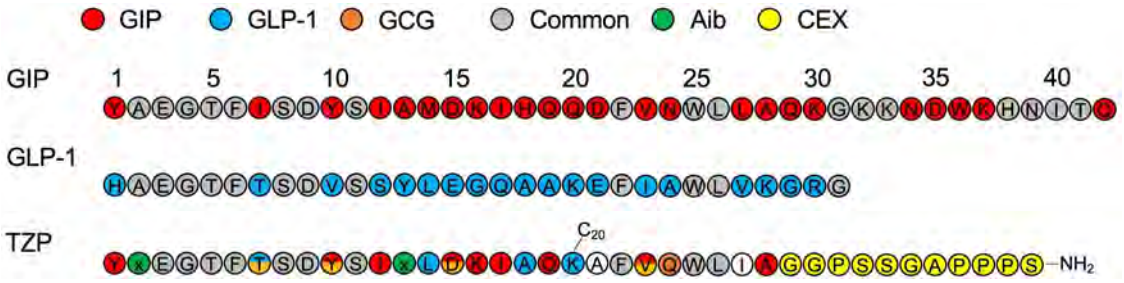


Case Study #1: GIPR

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

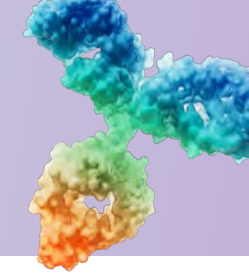


Challenge: Design Soluble GIPR that Specifically Binds GIP and not GLP-1



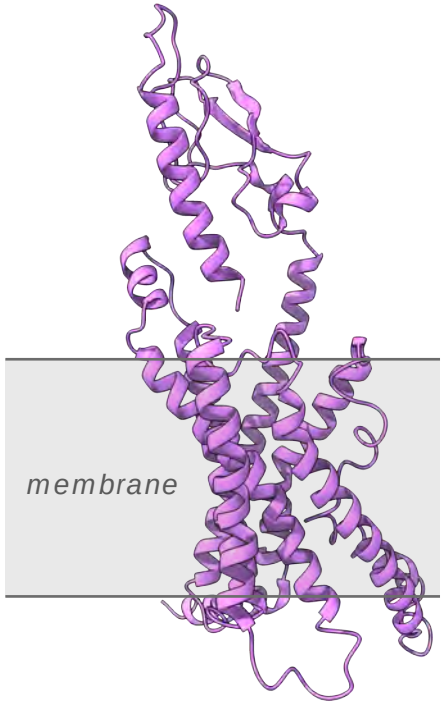
GIPR and GLP-1R are structural homologs whose natural ligands GIP and GLP-1, respectively, share high sequence identity at the N-terminus

GIPR is Solubilized by Reengineering the Transmembrane Domain



Input Target

GIPR

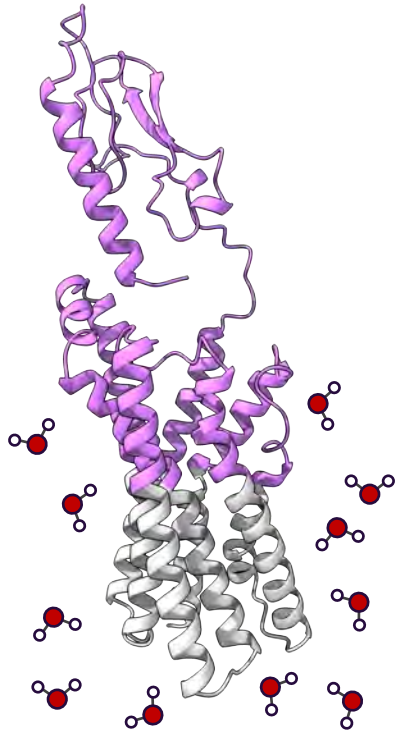


Generative AI

Solubilization of transmembrane domain



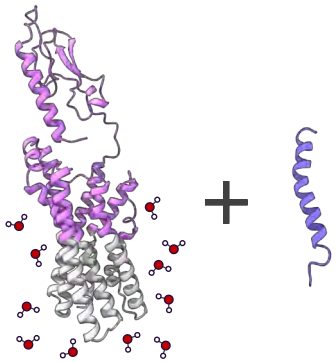
Soluble GPCR

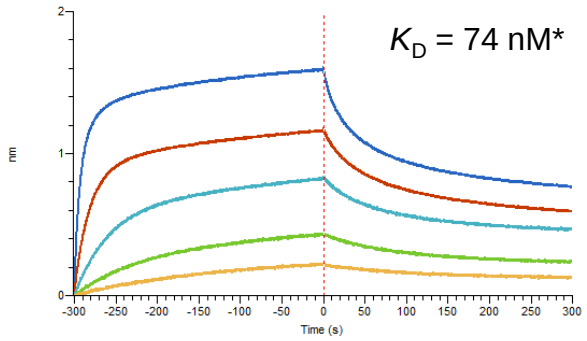


- ✓ *High Solubility*
- ✓ *High Stability*
- ✓ *High Expression*
- ✓ *PTMs by expression in human cells*
- ✓ *6 designs tested*

Soluble GIPR Binds its Native Ligand GIP

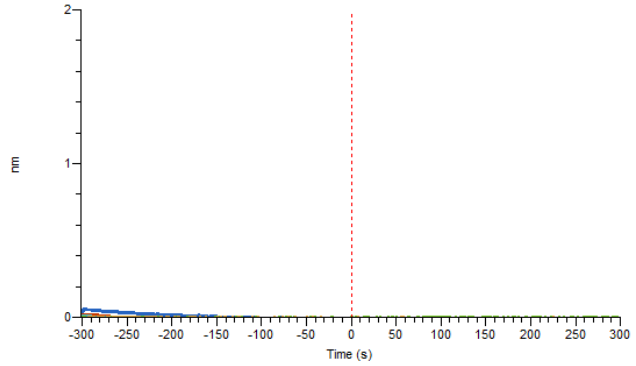
Soluble GIPR Binding to GIP



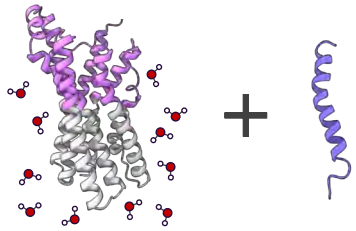


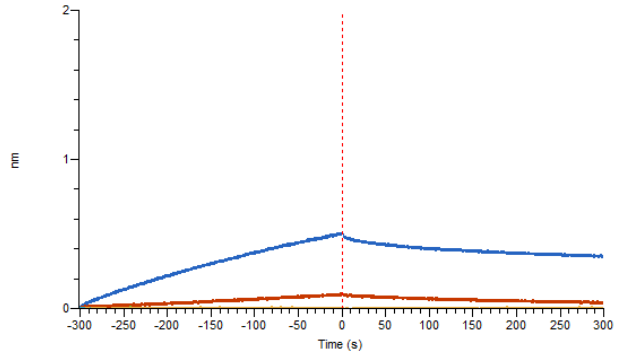
Extracellular Domain Binding to GIP





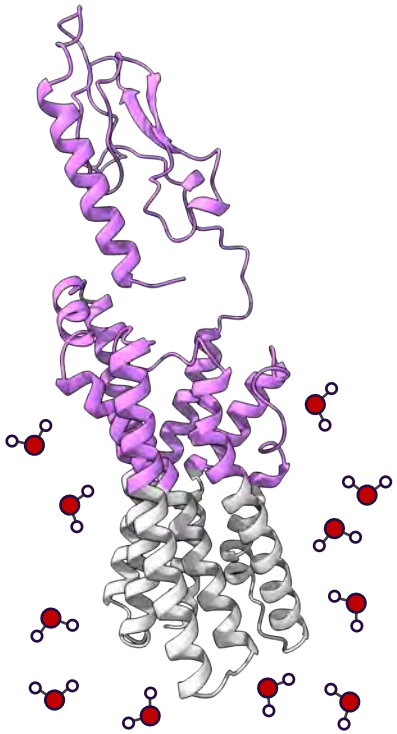
Transmembrane Domain Binding to GIP





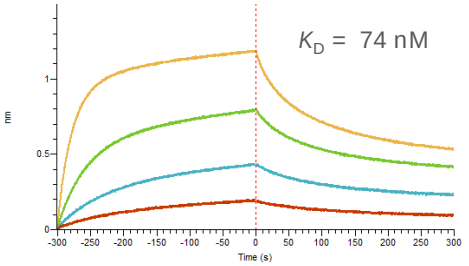
K_D calculated from avidity binding kinetics

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

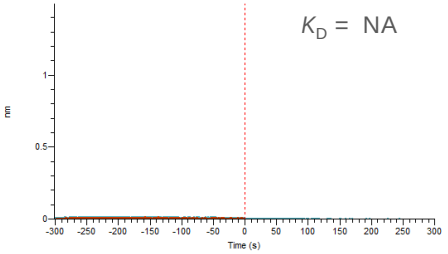


Avidity Ligand Binding Kinetics

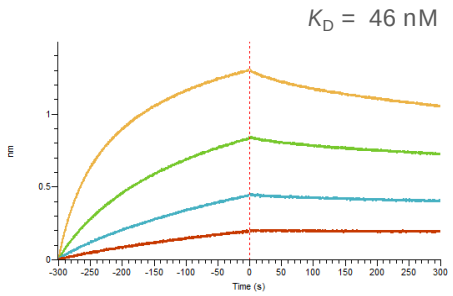
GIP
Natural Ligand



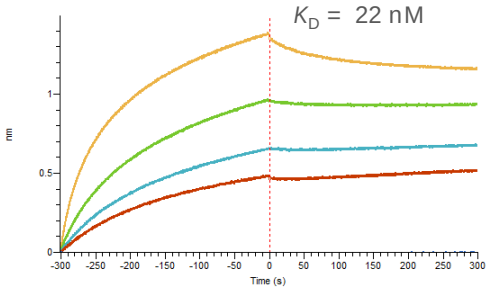
GLP-1
Negative Control



Zepbound®
Dual Agonist Therapeutic Drug

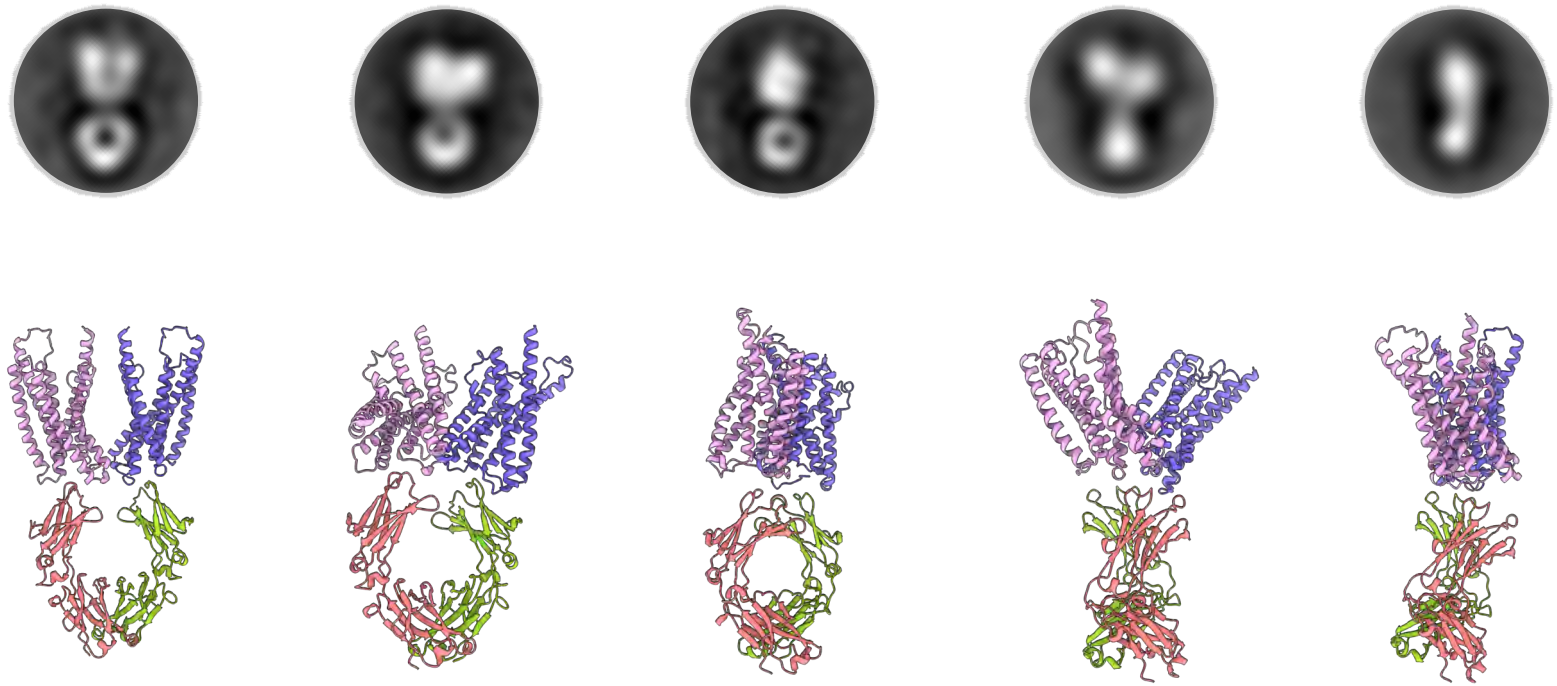


Retatrutide
Triple agonist



Negative Stain Electron Microscopy Supports Soluble Transmembrane Structure

Negative Stain Electron Microscopy



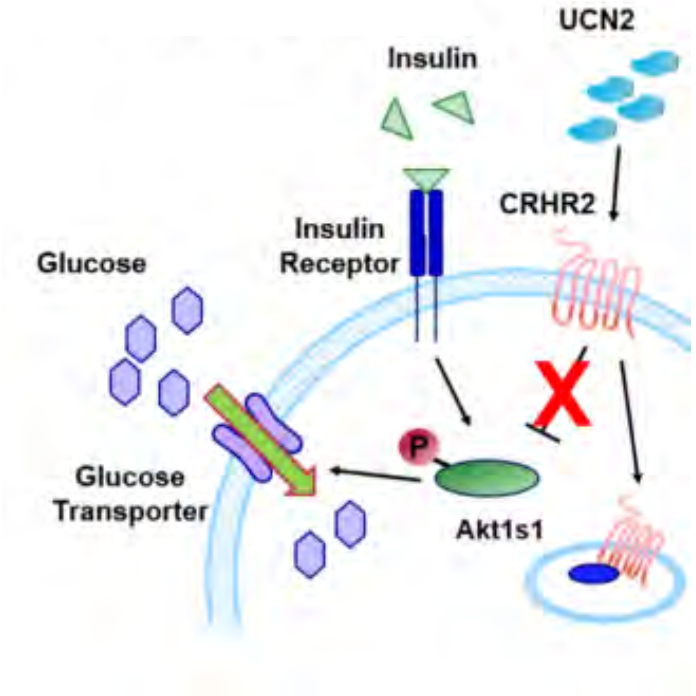
Soluble GIPR expressed as Fc fusion

Imaging supports soluble transmembrane design

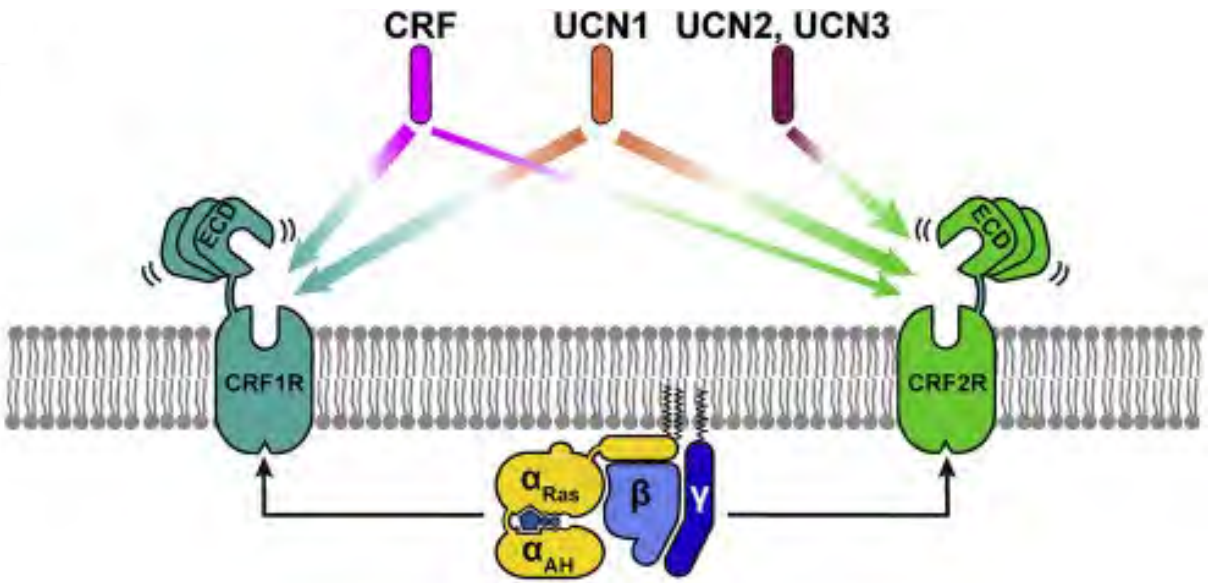
Flexibility observed at the Fc hinge region

Case Study #2: CRF2R

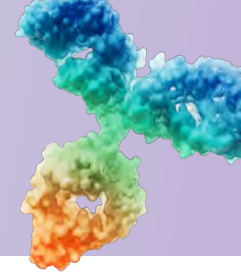
CRF2R is a Weight Loss Target Using UCN2 Analogs



Challenge: Design Soluble CRF2R that Binds UCN1-3 and CRF

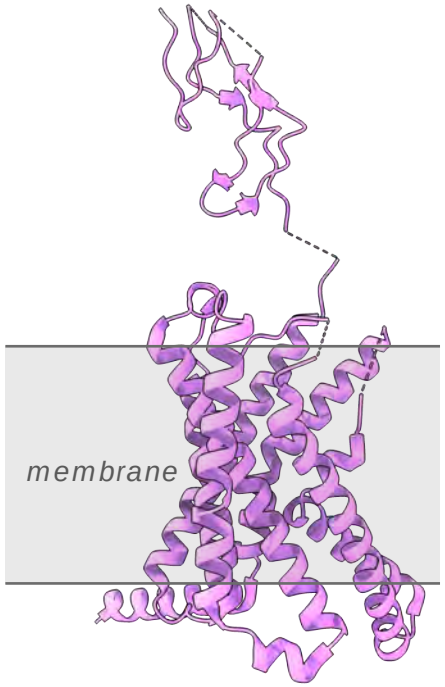


CRF2R is Solubilized by Reengineering the Transmembrane Domain



Input Target

CRF2R

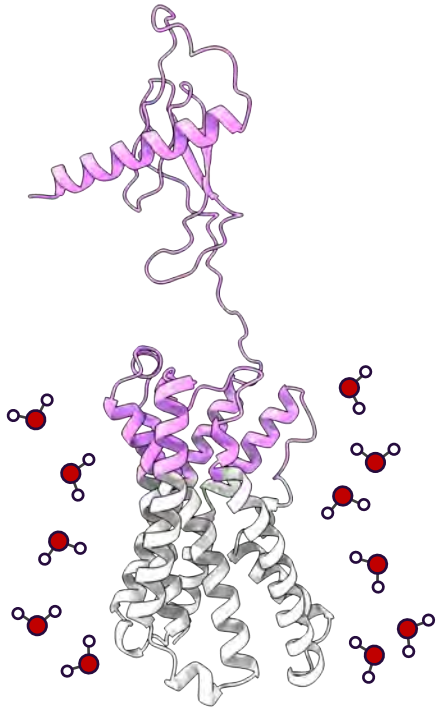


Generative AI

Solubilization of transmembrane domain



Soluble GPCR



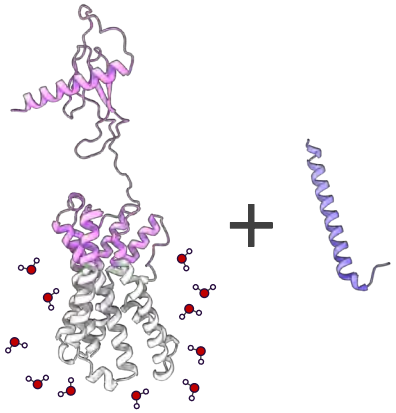
***~1000** designs screened in silico*

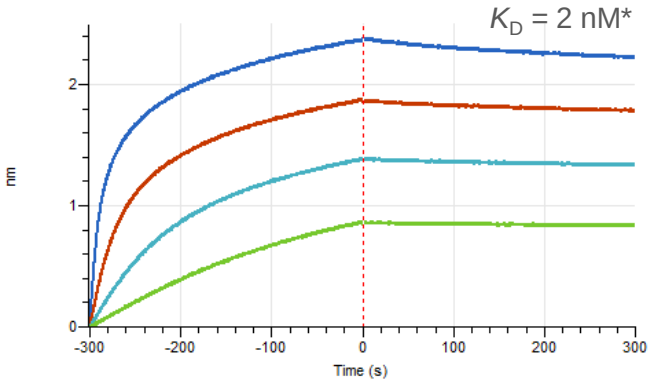
***~1** day of computational design*

***6** candidates expressed and purified for ligand binding validation*

Soluble CRF2R Binds its Native Ligand UCN2

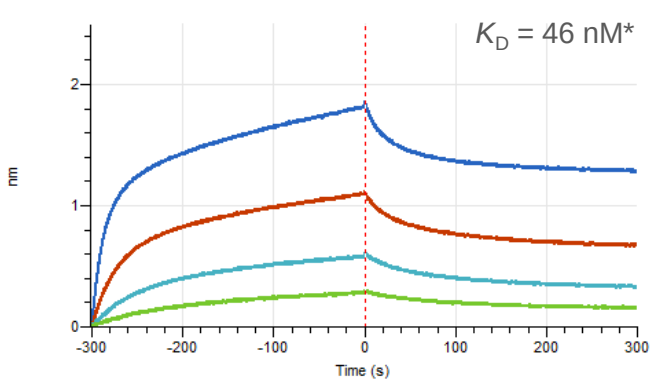
Soluble CRF2R Binding to UCN2



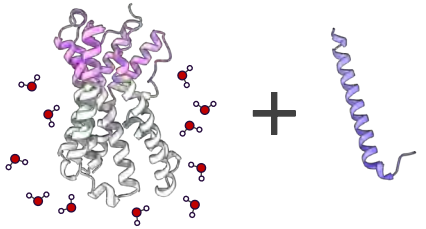


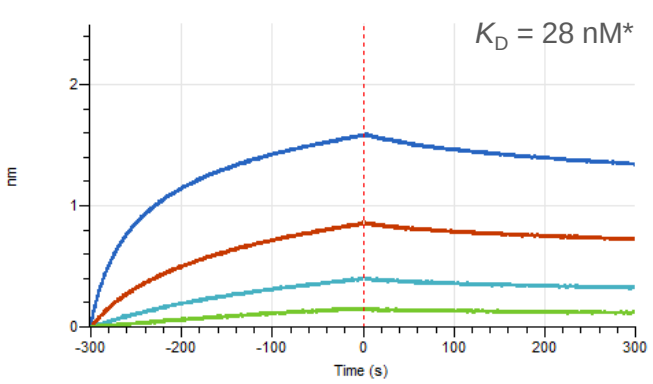
Extracellular Domain Binding to UCN2





Transmembrane Domain Binding to UCN2



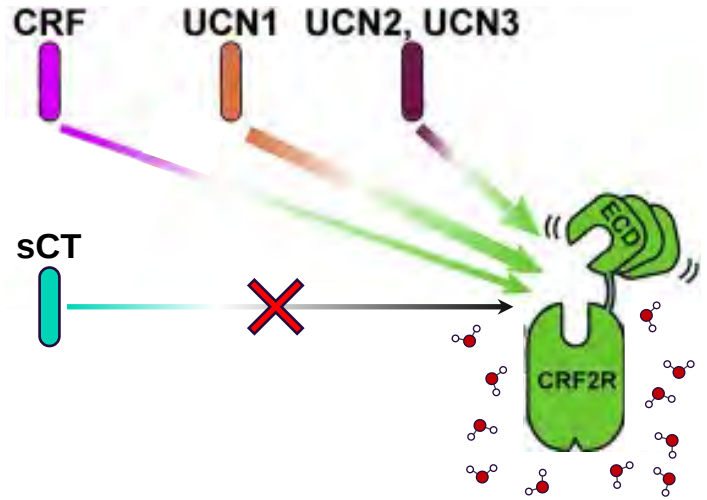


K_D calculated from avidity binding kinetics

Soluble CRF2R Selectively Binds UCN1-3 and CRF

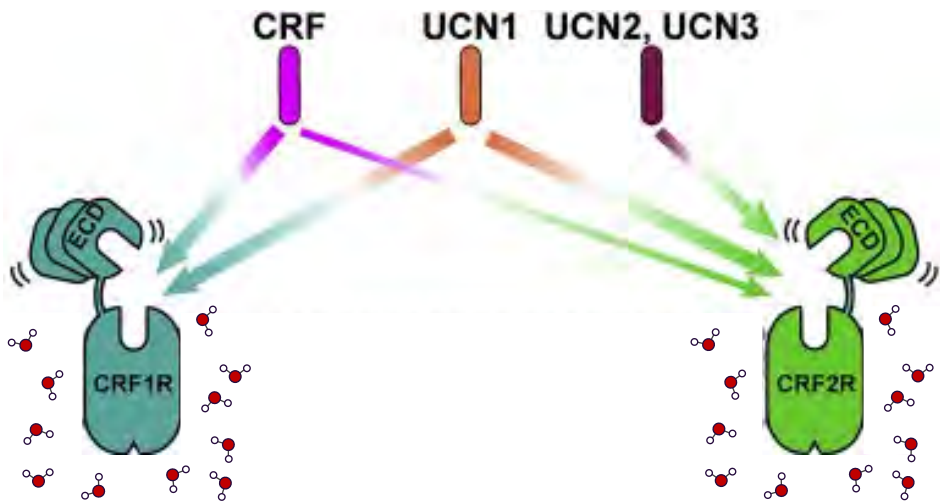
Binding Selectivity (Apparent K_D in nM)*

	UCN1	*UCN2*	*UCN3*	*CRF*	*sCT*
Soluble CRF2R	4	2	2	10	NB
Extracellular Domain	NB	46	46	31	NB
Transmembrane Domain	22	28	36	78	NB

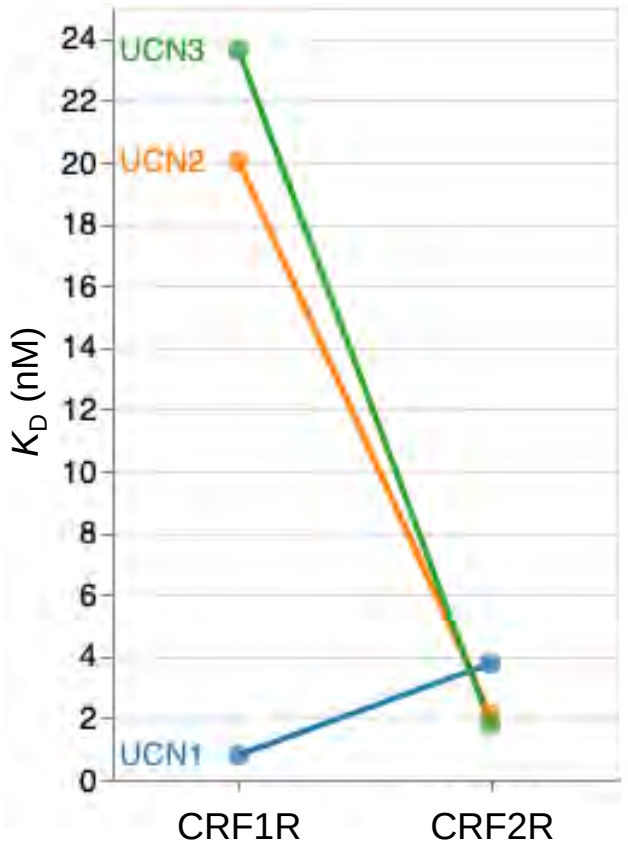


K_D calculated from avidity binding kinetics

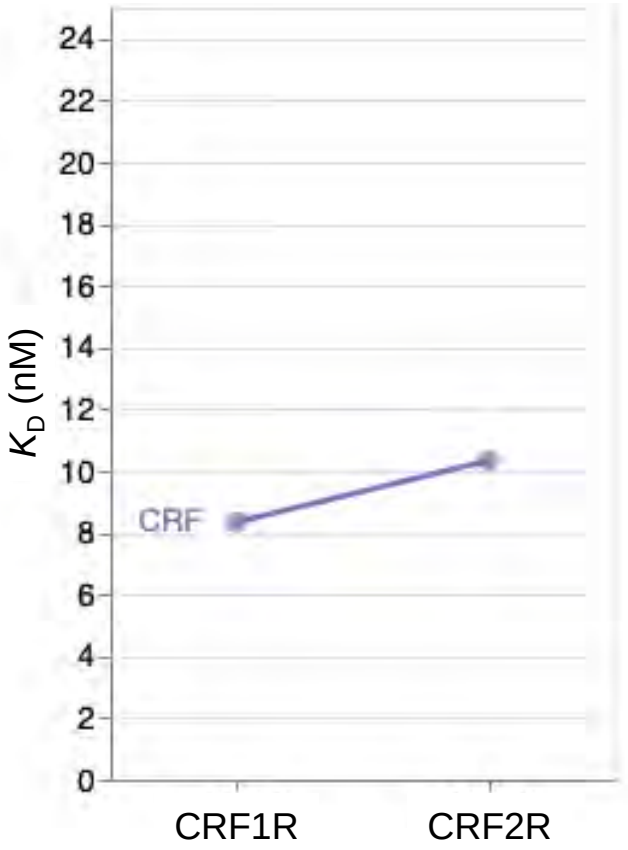
Natural Ligand Specificity is Preserved for Soluble CRF1R and CRF2R Designs



UCN Binding



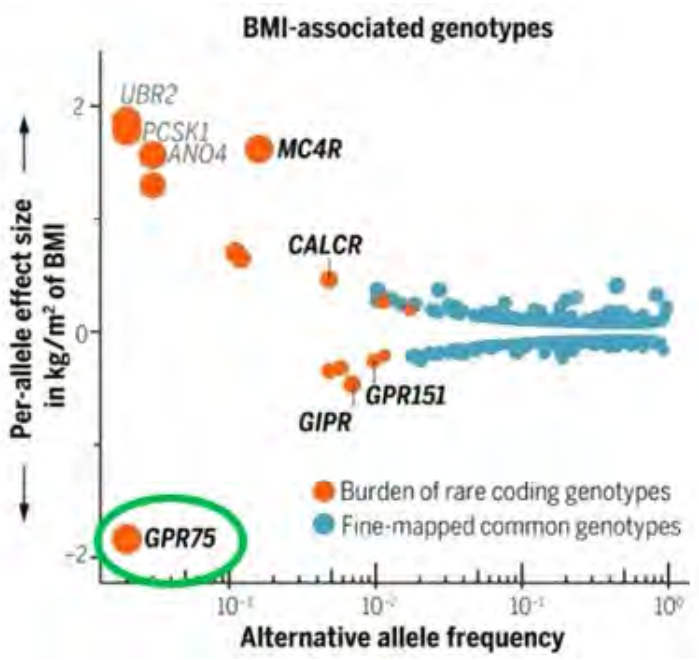
CRF Binding

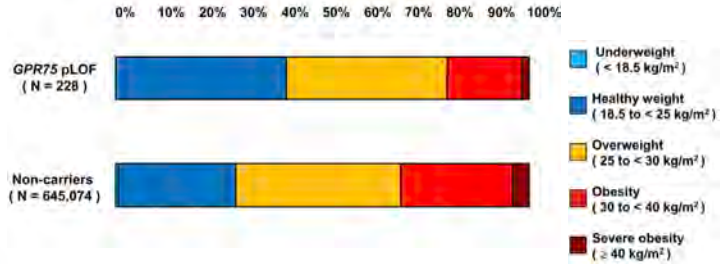


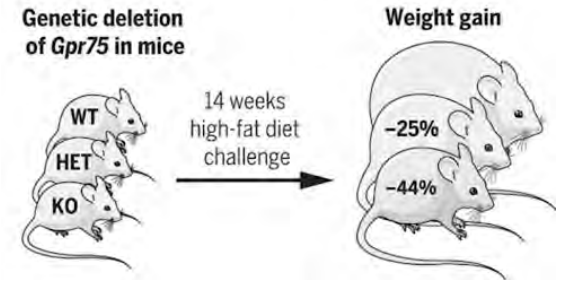
K_D calculated from avidity binding kinetics

Case Study #3: GPR75

GPR75 is a Genetically Validated Obesity Target







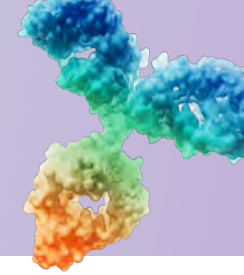
Challenges

No experimental structures available at the time of this study

20-HETE binds the transmembrane domain

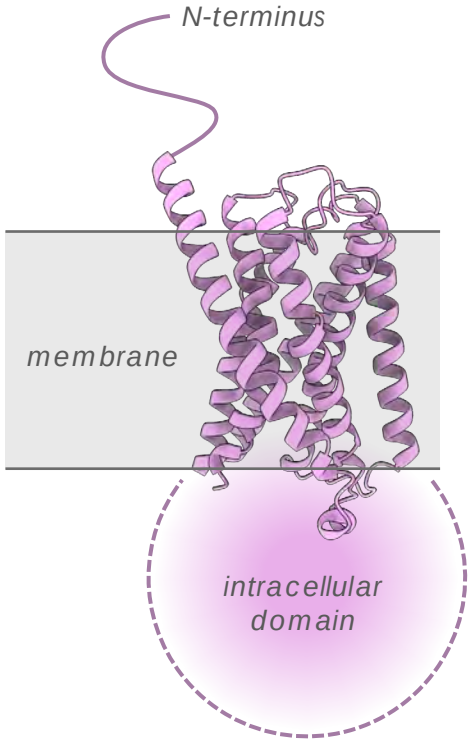
CCL5 binds extracellularly, but the binding site is unknown

Soluble GPCR Designed from Sequence Alone



Input Target

GPR75 (AlphaFold)

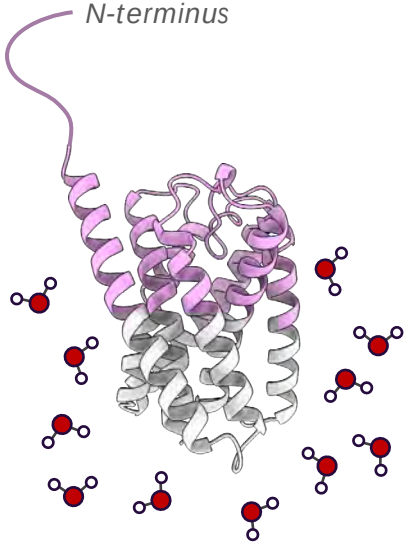


Generative AI

Solubilization of transmembrane domain



Soluble GPCR



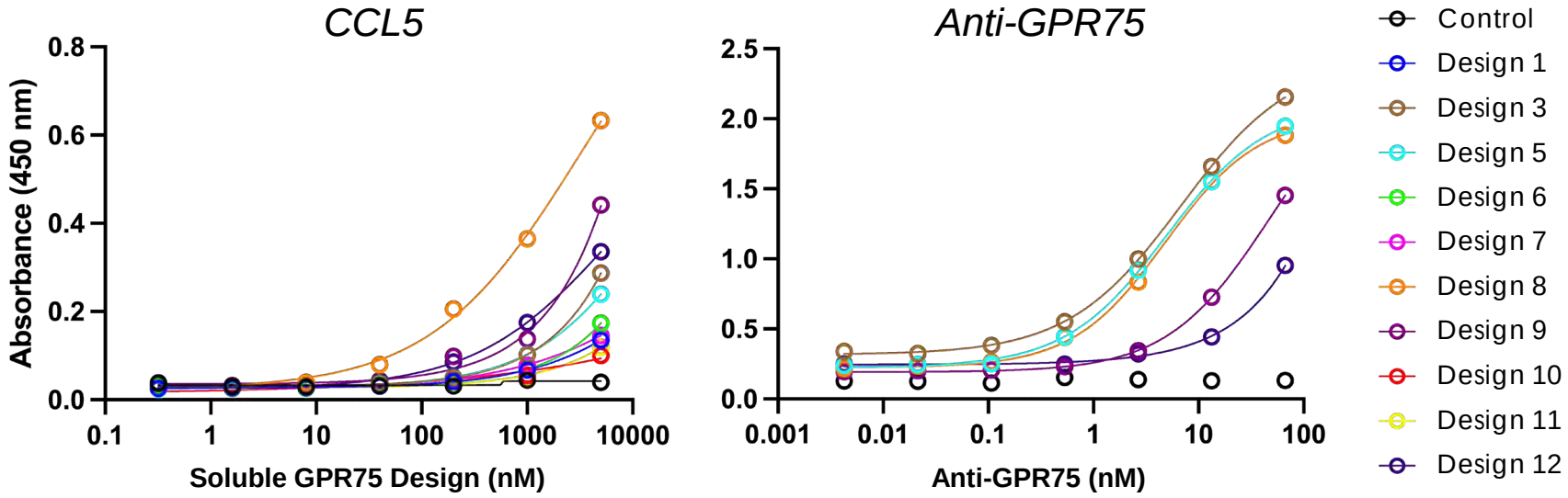
Soluble GPCR designs generated from sequence alone

Removal of ~250 residue intracellular domain

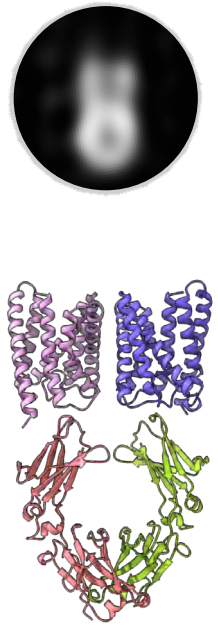
12 designs expressed and purified to test for binding to CCL5

Solubilized GPR75 Binds CCL5 and Commercial Antibody

ELISA Binding

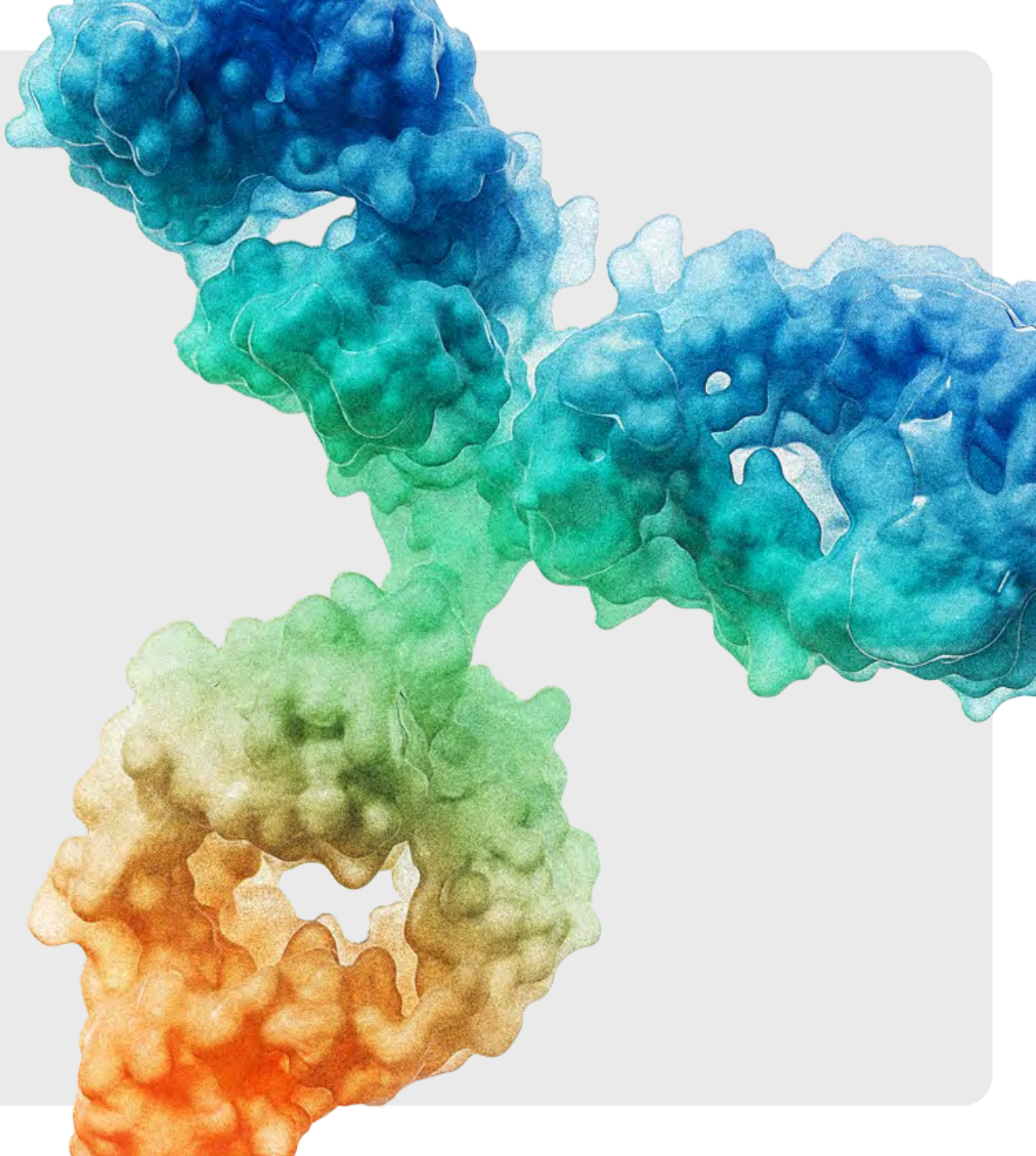


Negative Stain EM



Antibody Discovery with Soluble GPCRs

Amylin Receptor

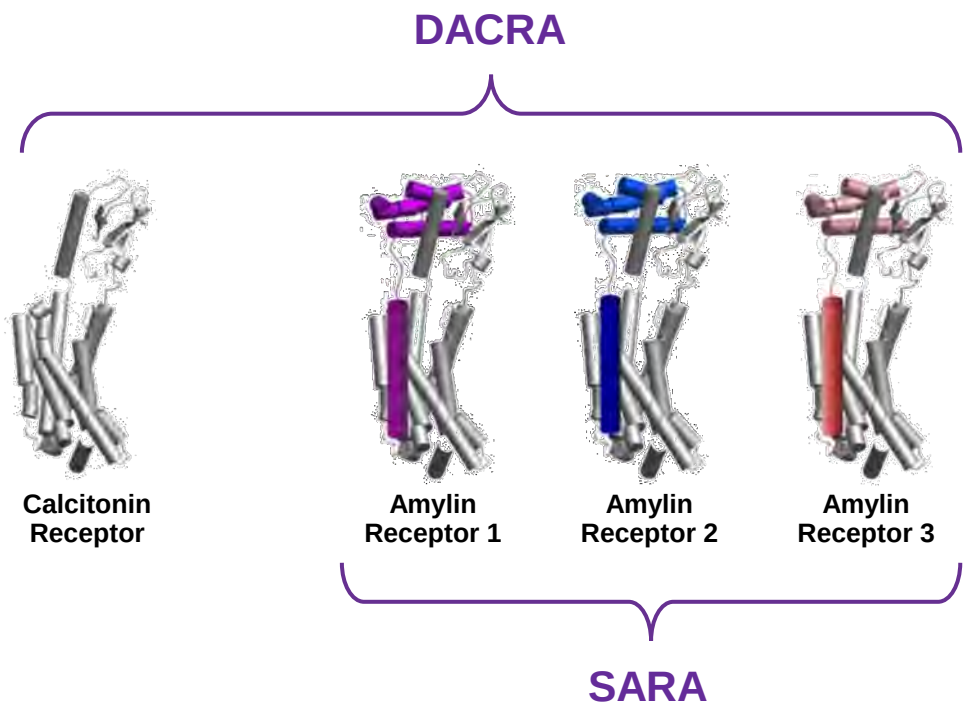


Amylin Receptor Agonism

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)

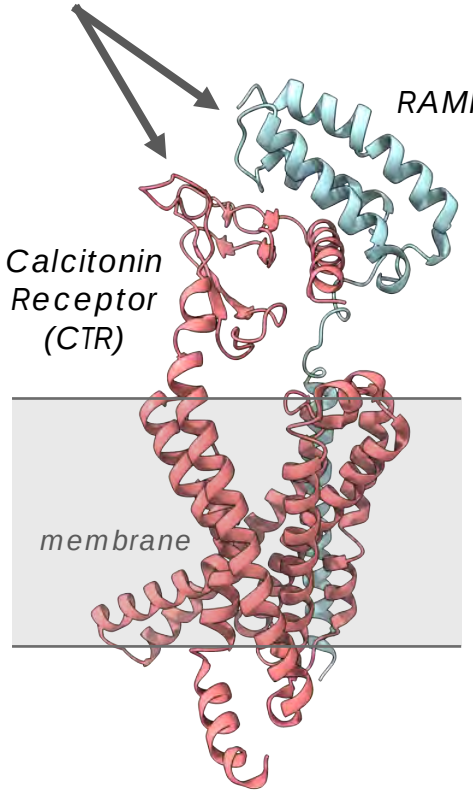
Goal: Improve Therapeutic Specificity and Longevity with Antibody Fusion

SARA

Selective Amylin Receptor Agonist

DACRA

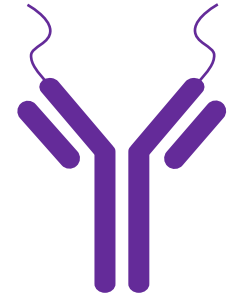
Dual Amylin and Calcitonin Receptor Agonist



RAMP

Associates with off-target proteins

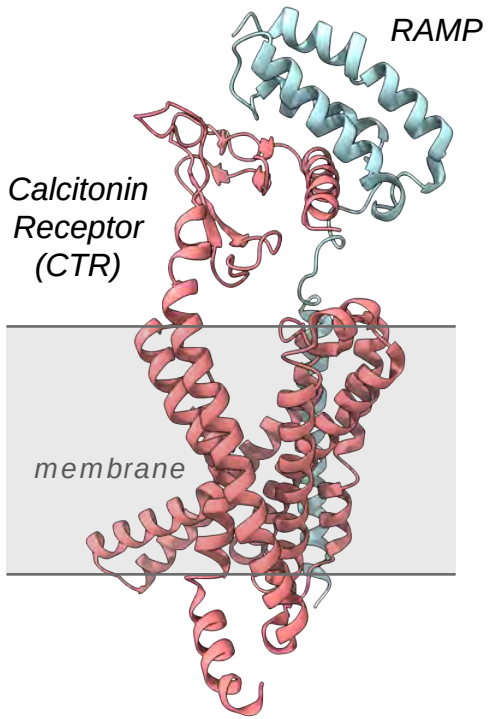
Synthetic Amylin + Antibody Fusion



Full Spectrum of Epitopes Designed for Amylin Receptor

Input Target

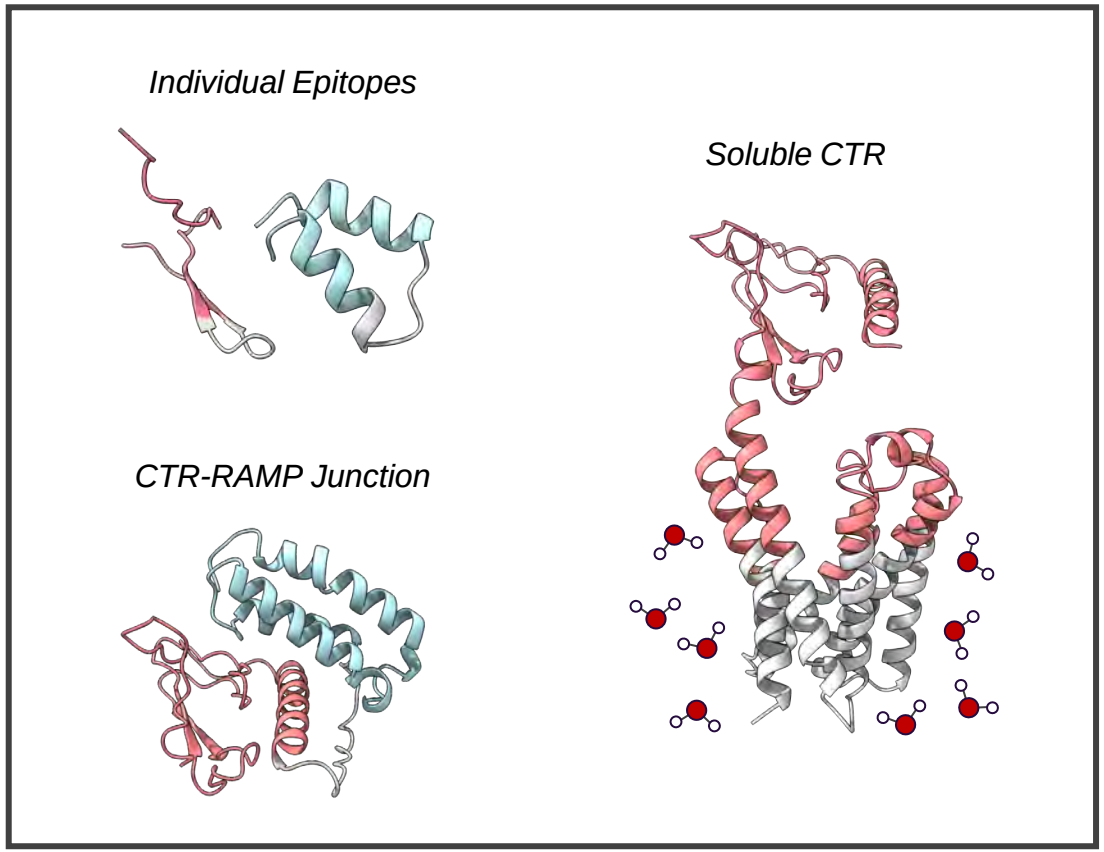
Amylin Receptor



Generative AI

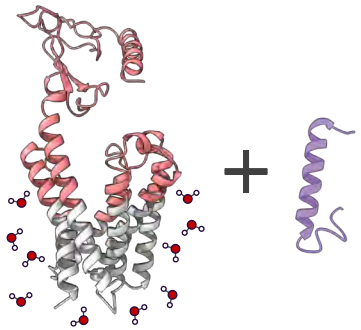


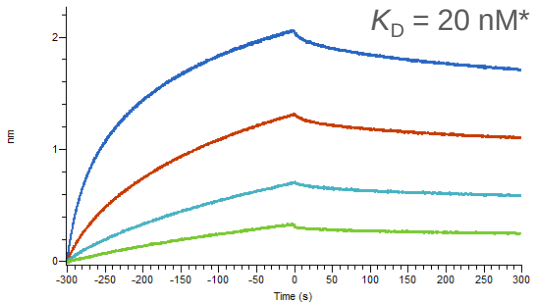
Epitope Designs



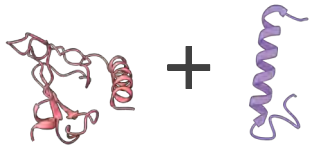
CTR Solubilization Validated by Salmon Calcitonin Binding

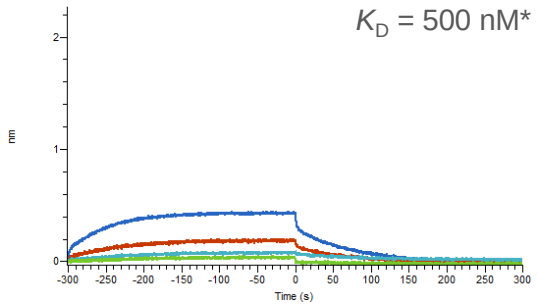
Soluble Receptor Binding to sCT



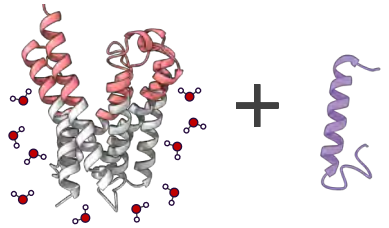


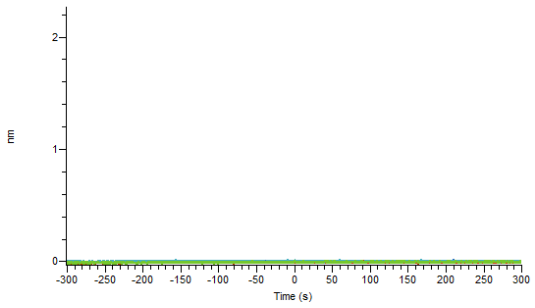
Extracellular Domain Binding to sCT





Transmembrane Domain Binding to sCT

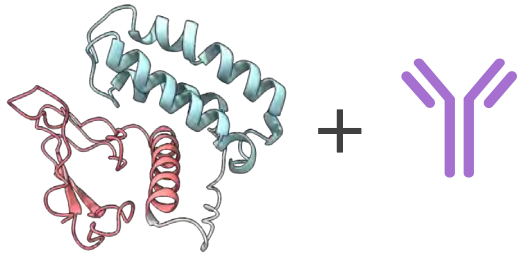


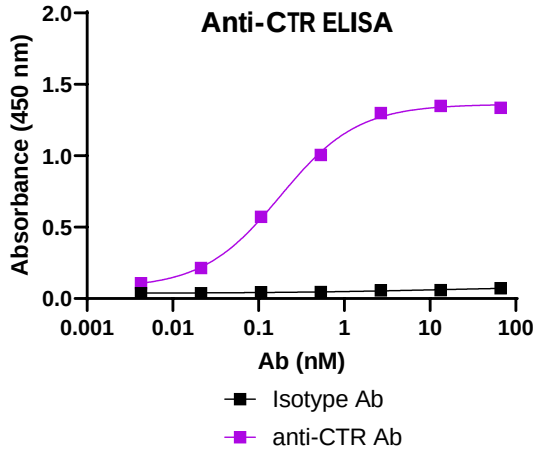


K_D calculated from avidity binding kinetics

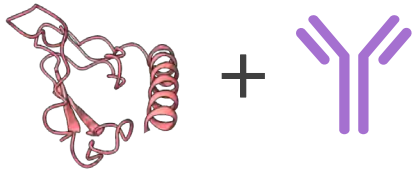
Amylin Junctional Epitope Design Retains Anti-CTR Antibody Binding

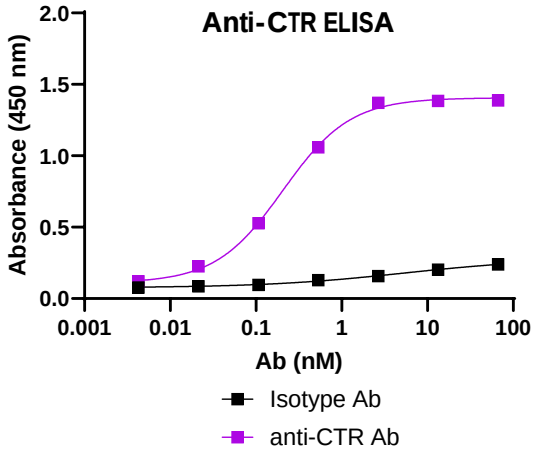
Amylin ECD



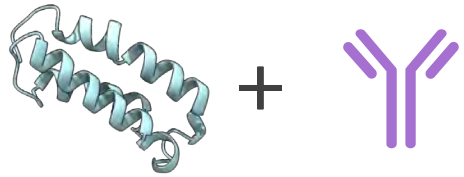


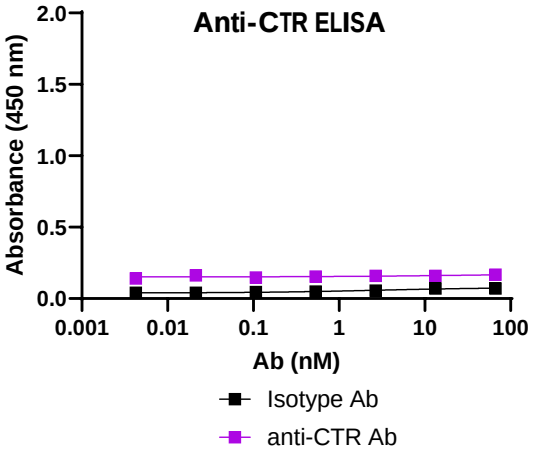
CTR ECD





RAMP

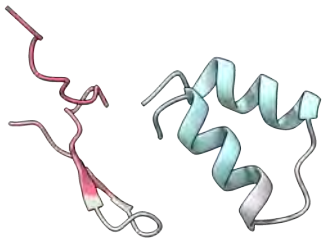




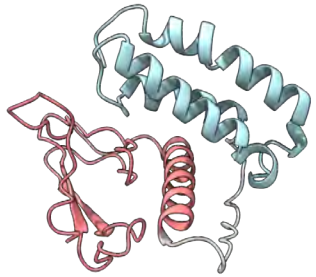
Epitope Designs Enable Both In Vivo and In Vitro Discovery

Epitope Designs

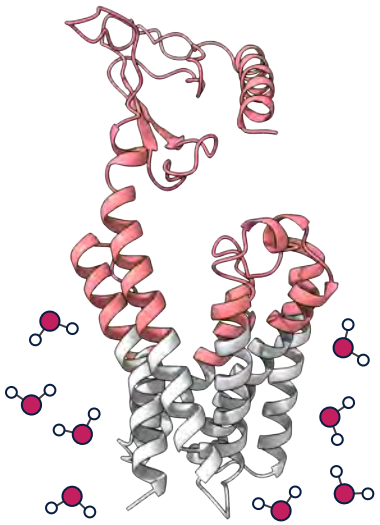
Individual Epitopes



CTR-RAMP Junction



Soluble CTR



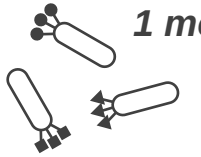
In Vivo/In Vitro Selection

Immunizations



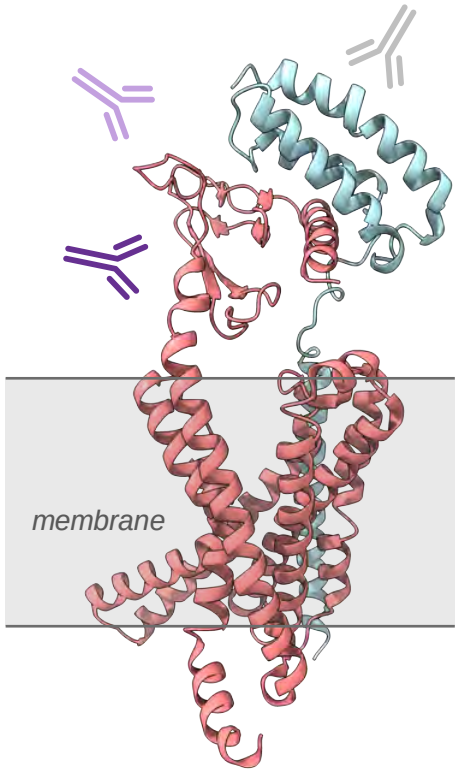
4 months

Phase panning



1 month

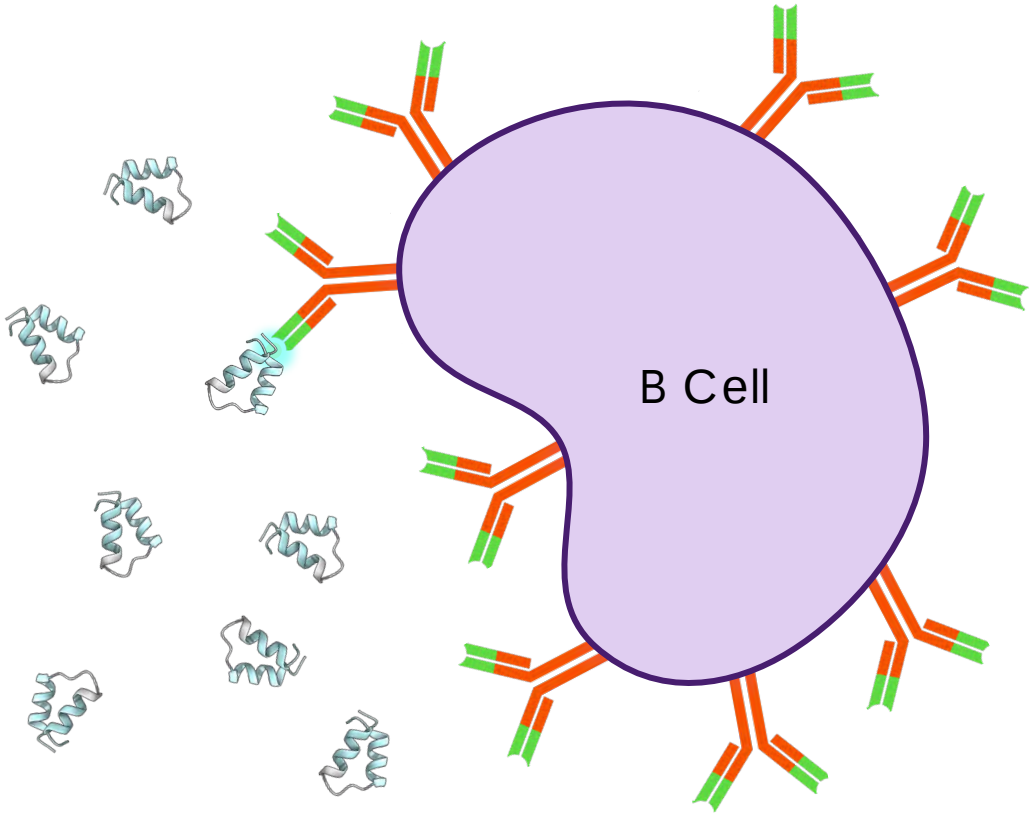
Antibody Discovery



Nanoparticle Display System Enhances Immune Response for Immunizations

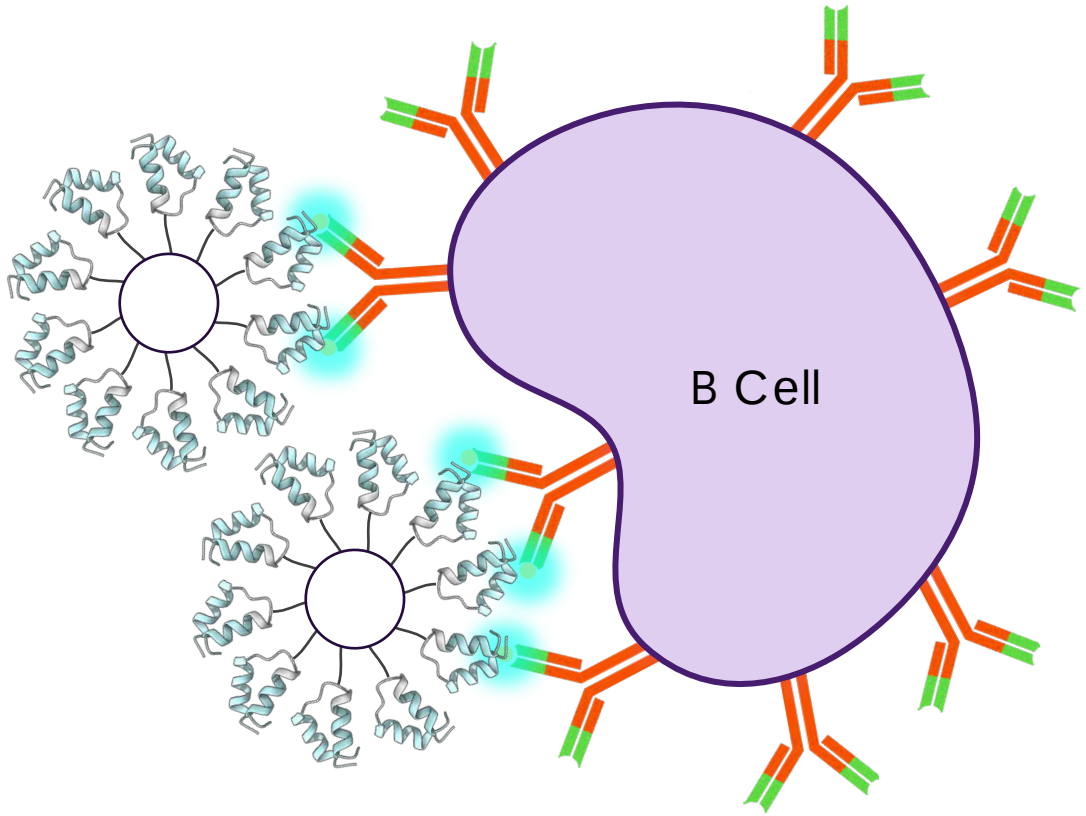
Engineered Epitope Immunization

Weak B cell activation

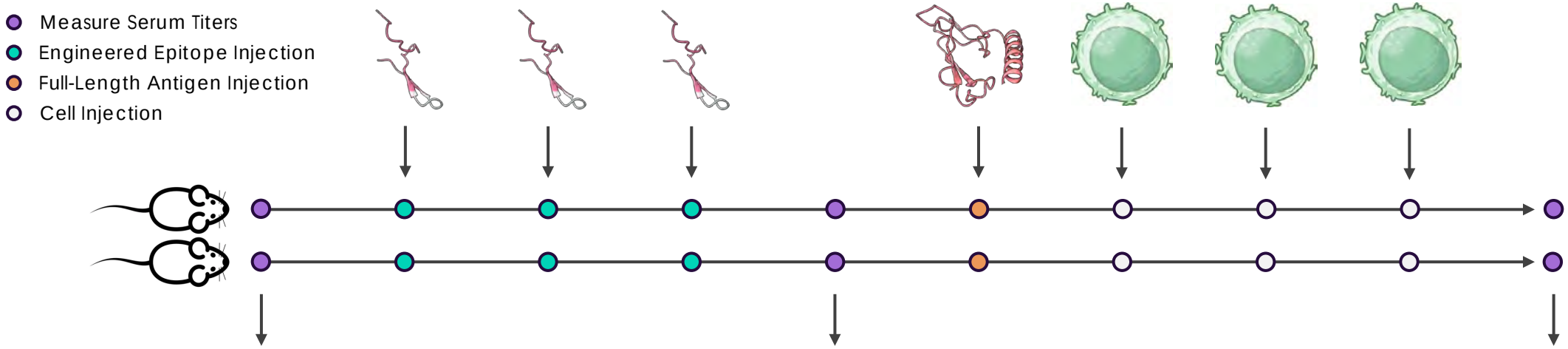


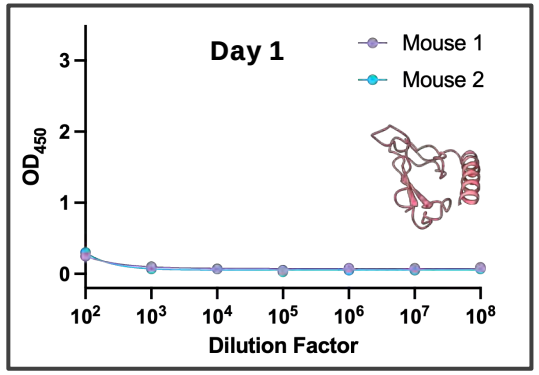
Nanoparticle Immunization

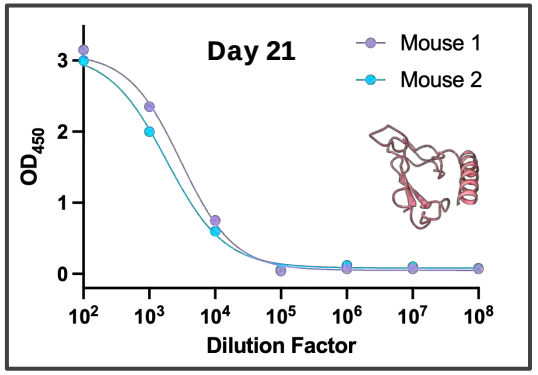
Strong B cell activation

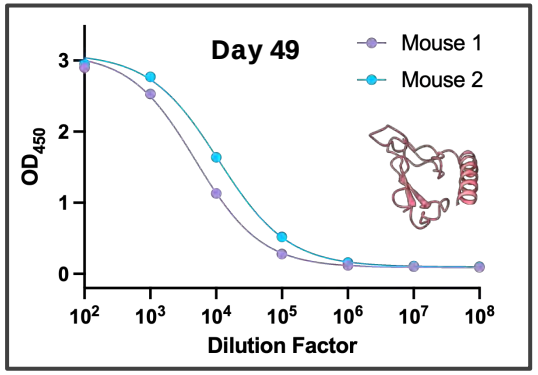


Engineered Epitopes Prime Immune System for an Epitope-Focused Response

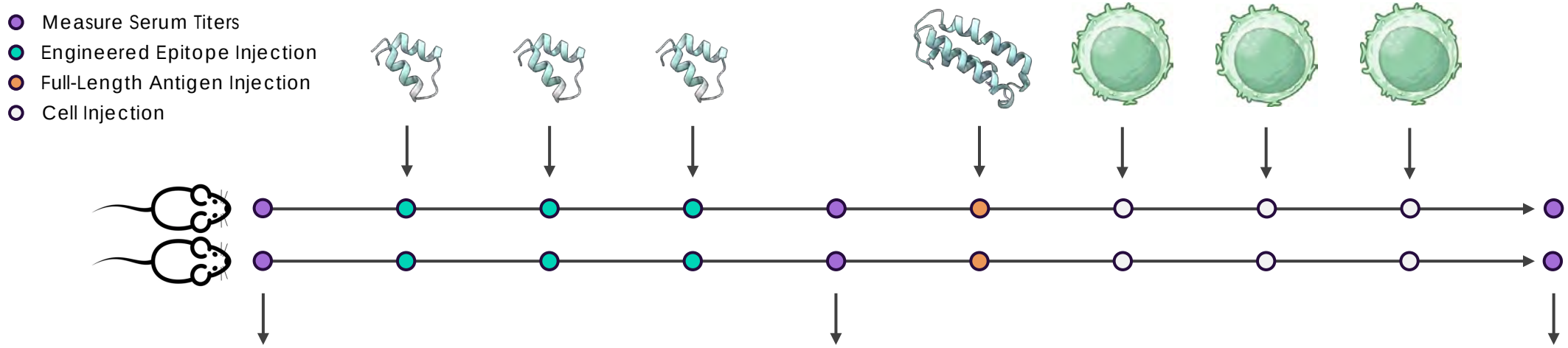


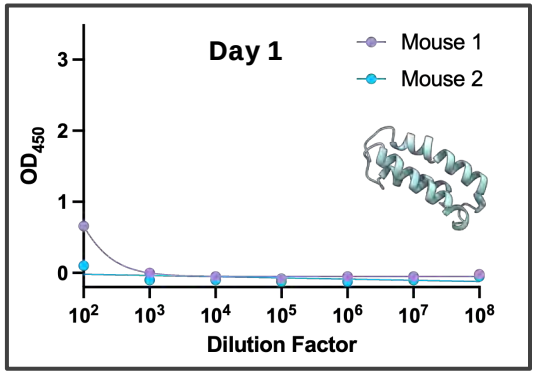


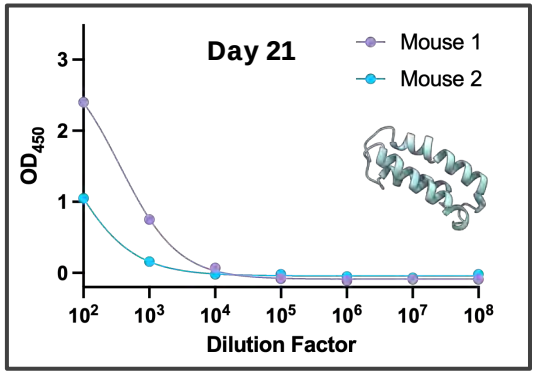


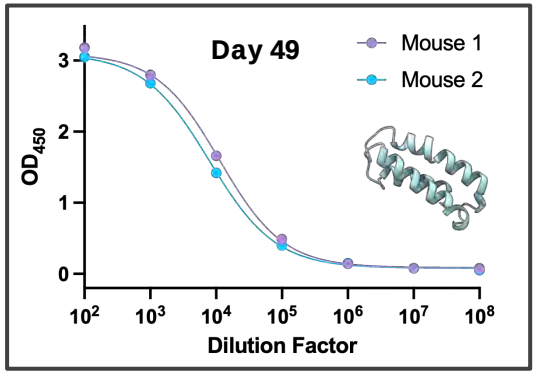


Engineered Epitopes Prime Immune System for an Epitope-Focused Response



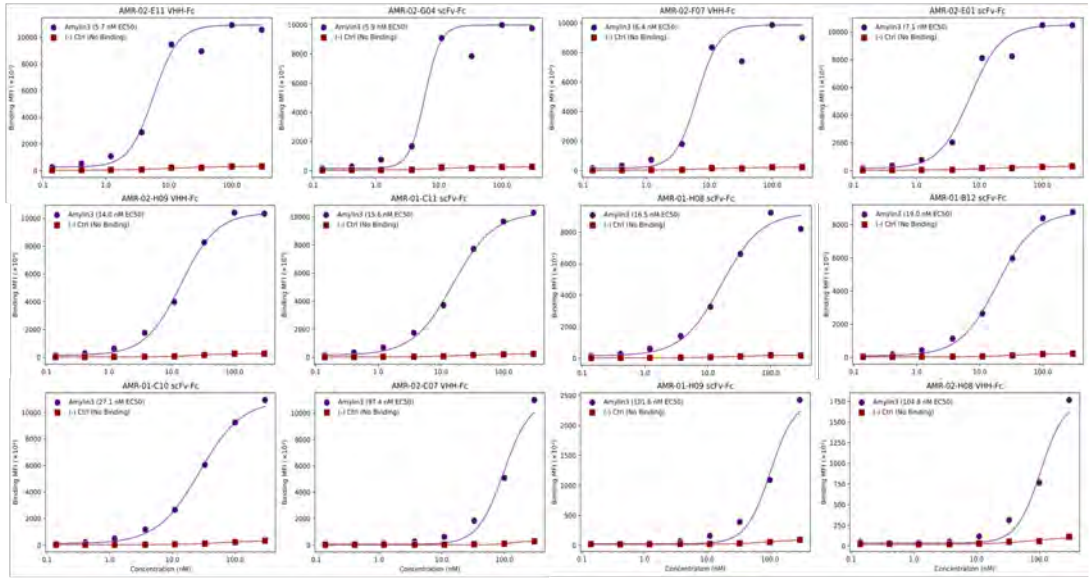






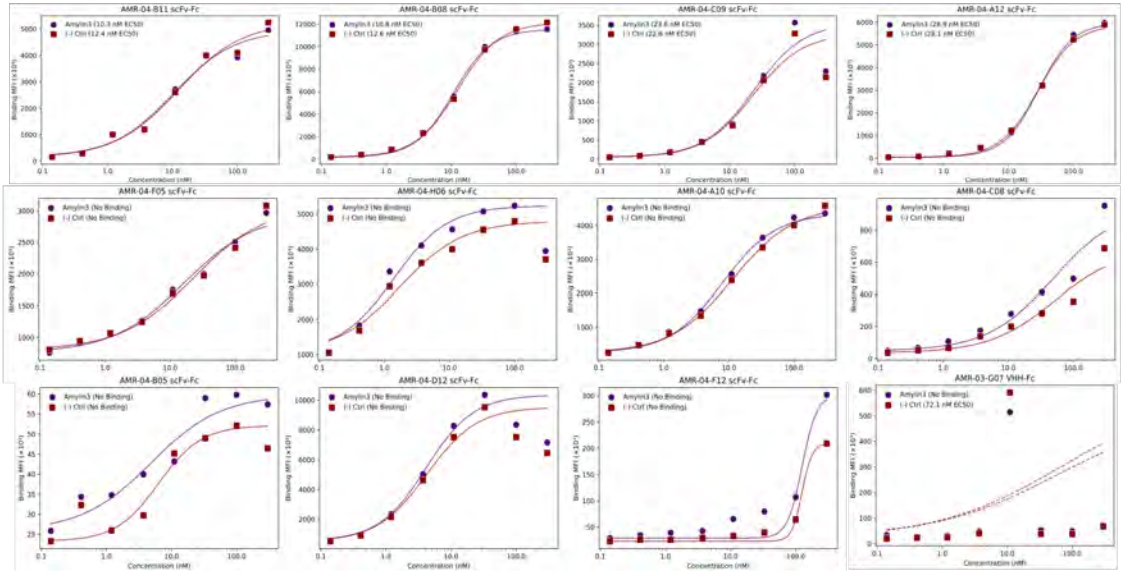
Phage Panning Against Engineered Epitopes Translates to Cell Binder Hits

Engineered Epitope Steered Strategies



Amylin Receptor Cell-Only Strategies

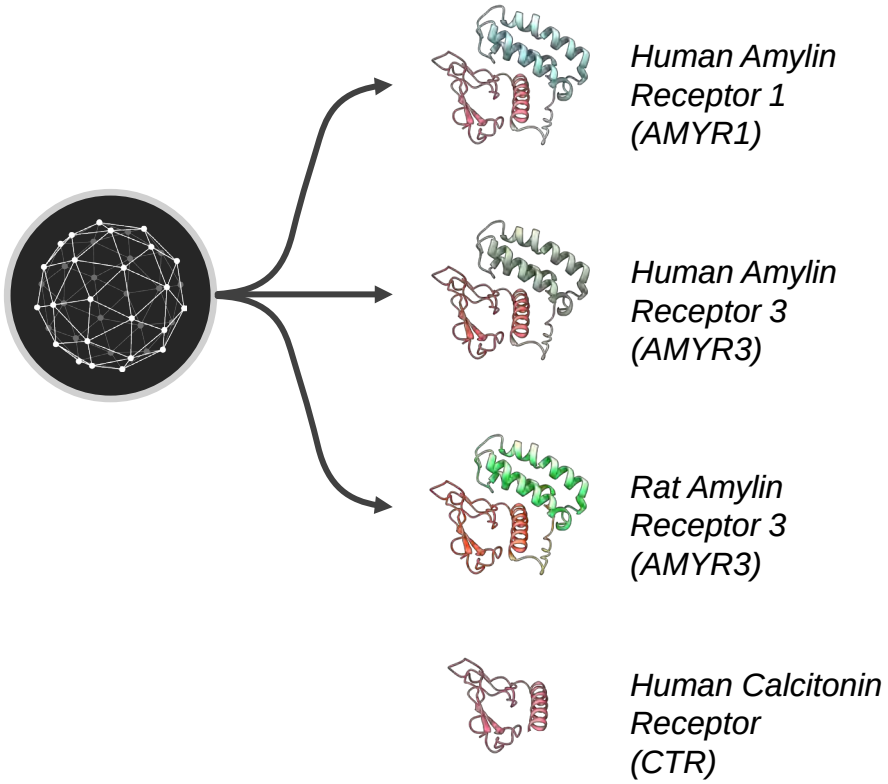
(No Specific Binders Found)



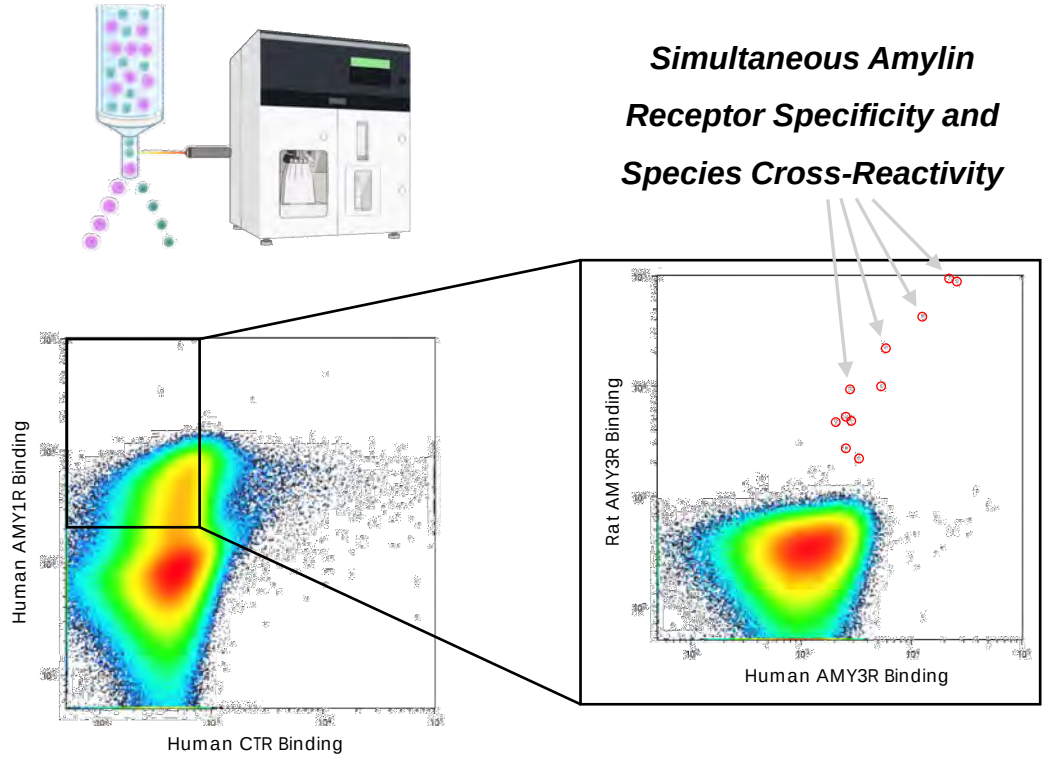
- Amylin3 Receptor Cell Binding
- Negative Control Cell Binding

Multi-Specificity and Cross-Reactivity in a Single Experiment

Species Cross-Reactive Designs



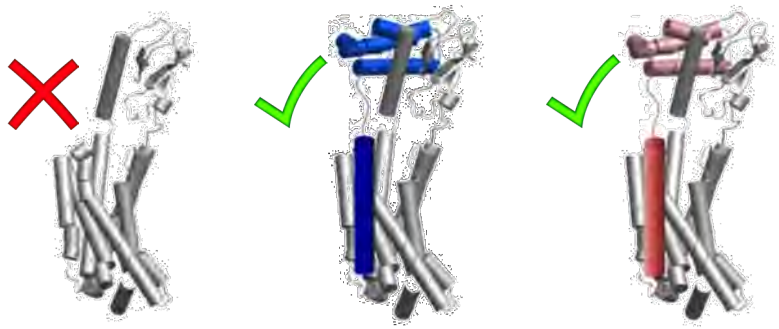
4-Dimensional Mammalian Display Sorting



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



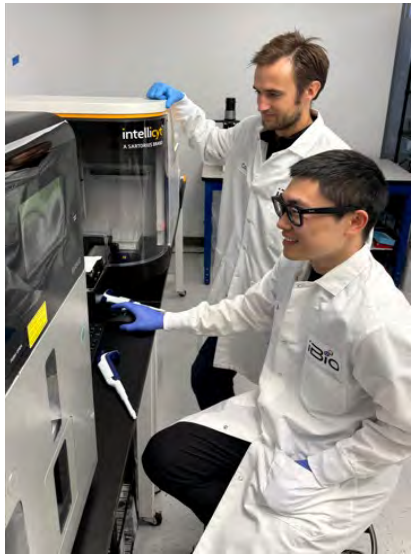
Cagrilintide

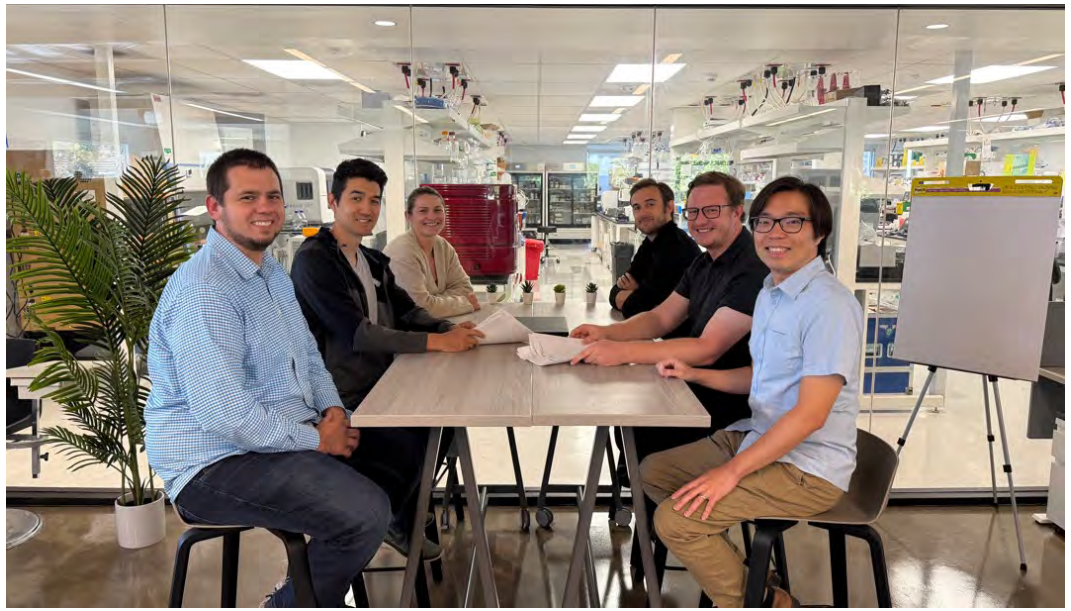


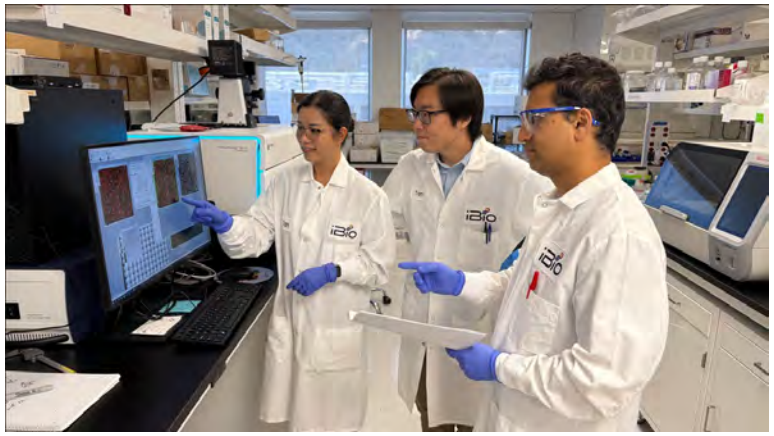
Calcitonin Receptor Cells
Amylin Receptor 3 Cells

Acknowledgements



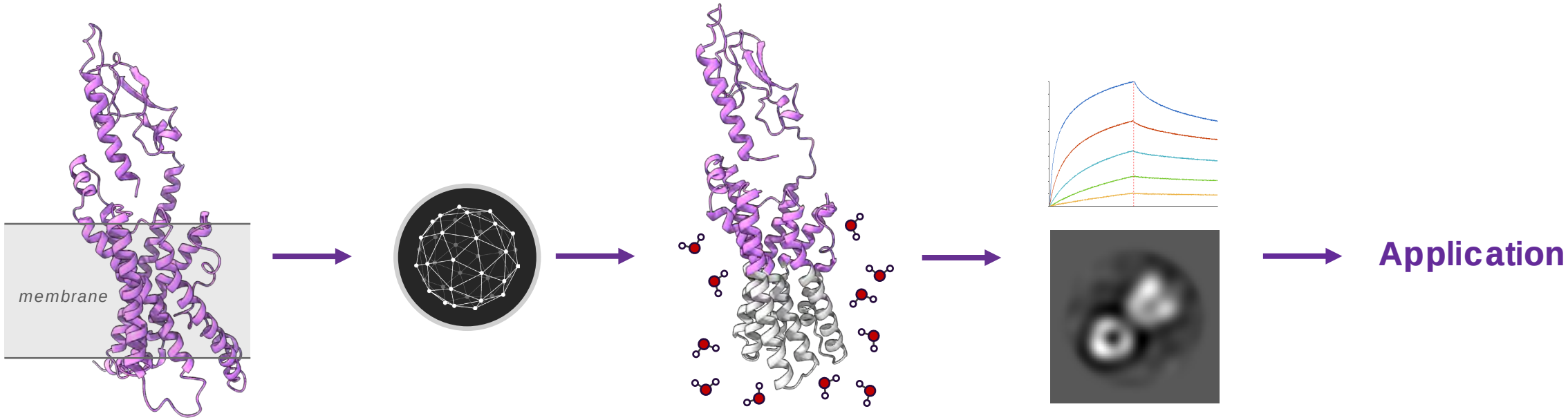






We Love Hard Problems

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



We are open to collaboration!

alex.taguchi@ibioinc.com