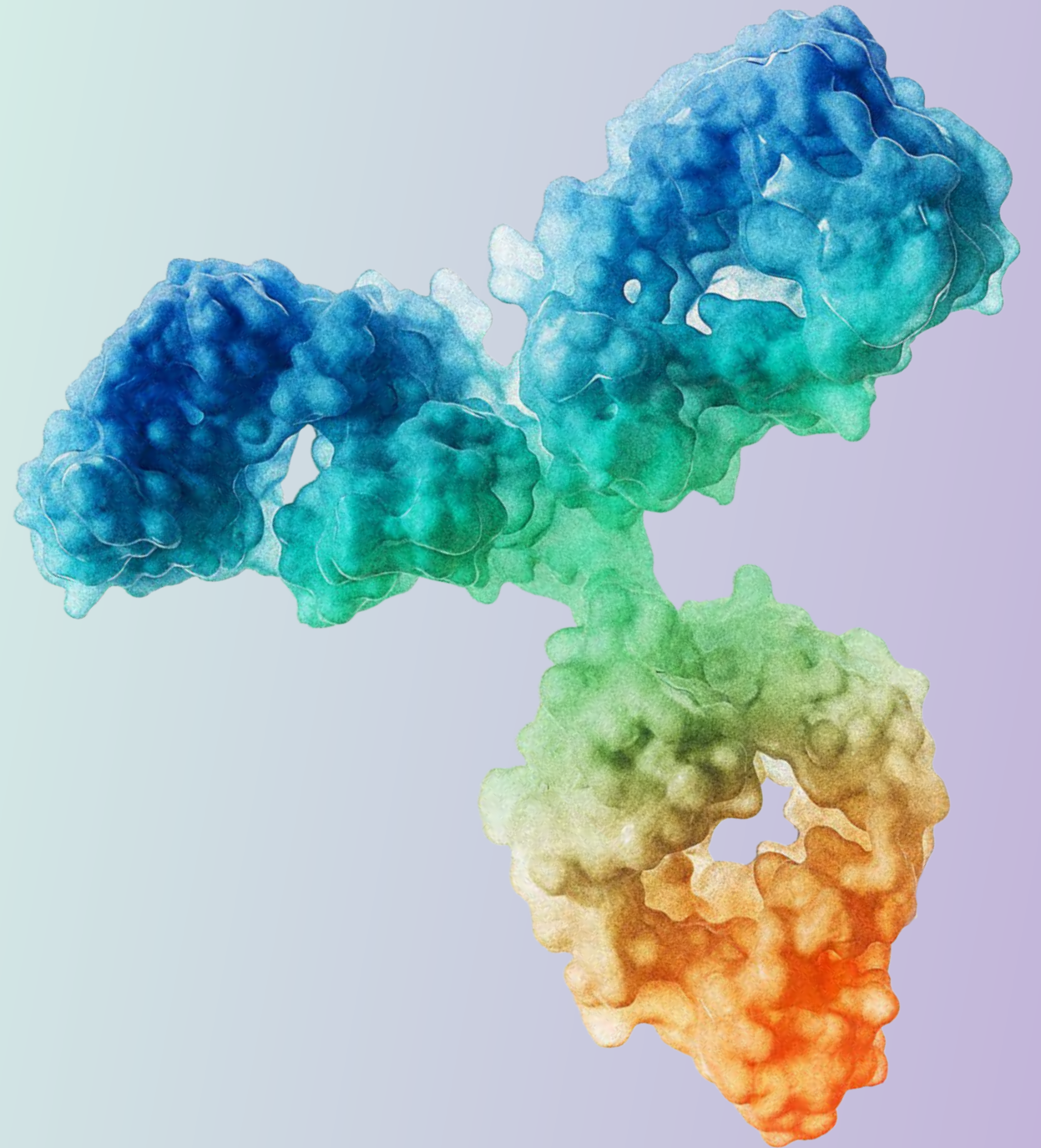


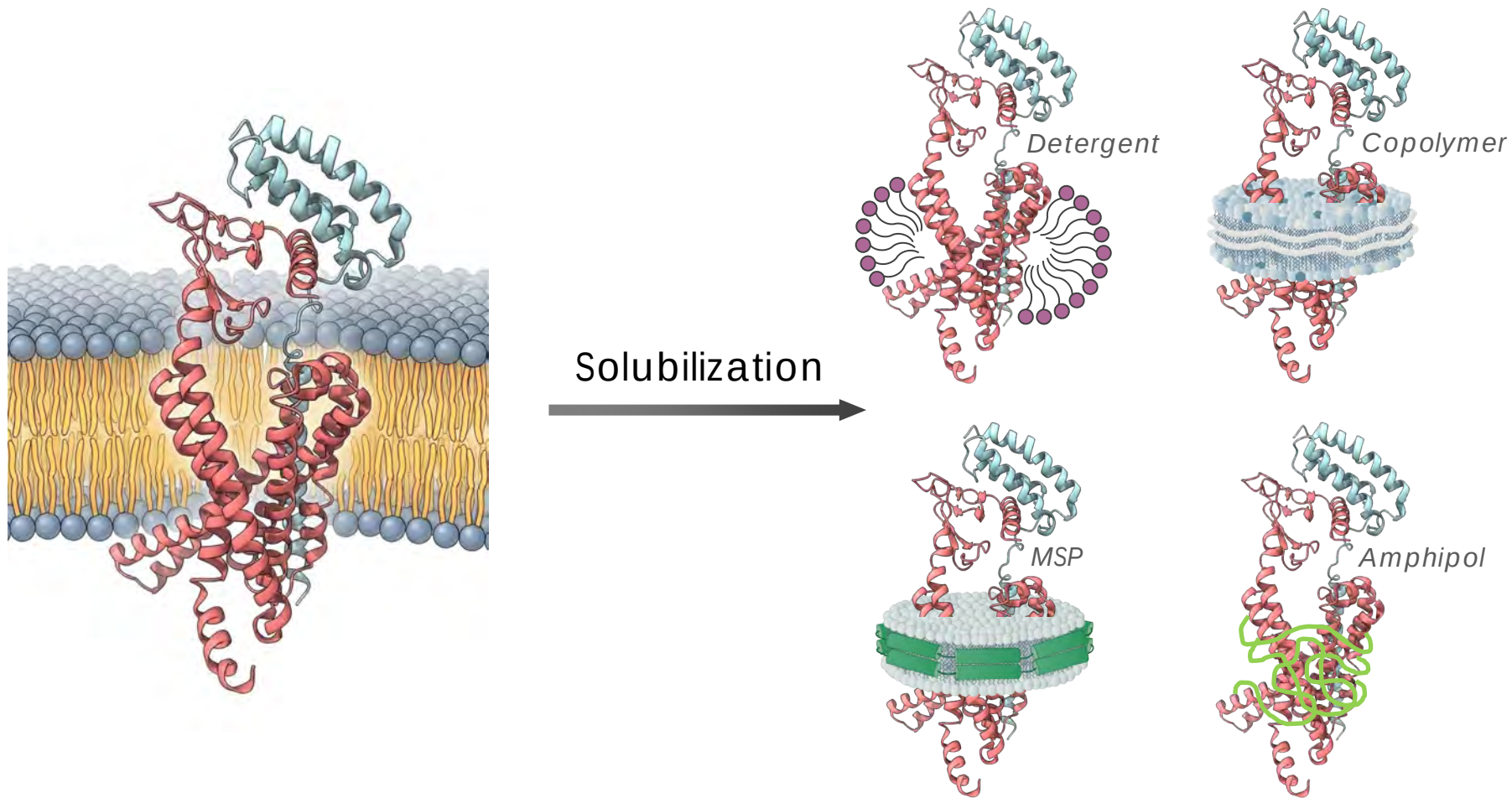
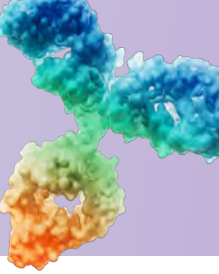


Generative AI-Guided Selections for GPCR Antibody Discovery

Alex Taguchi



GPCR Paradox: Prime Targets, Tough Antigens



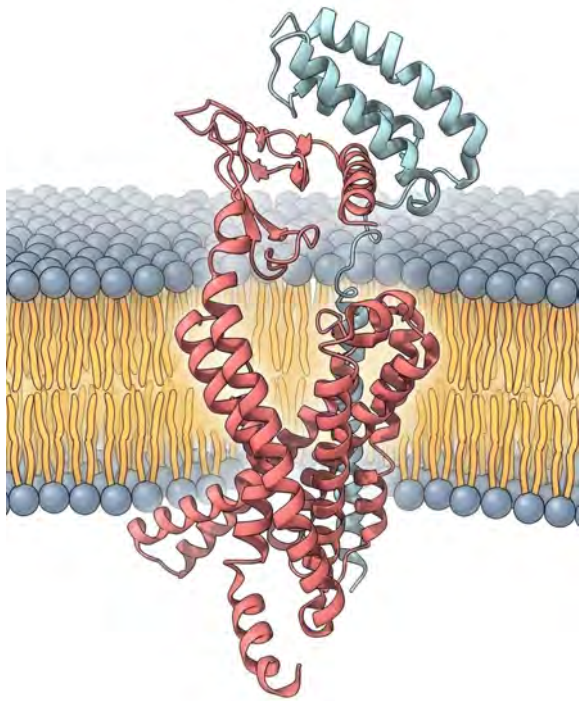
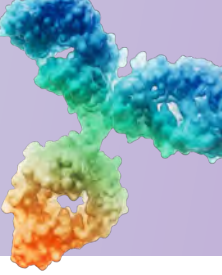
Benefits

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

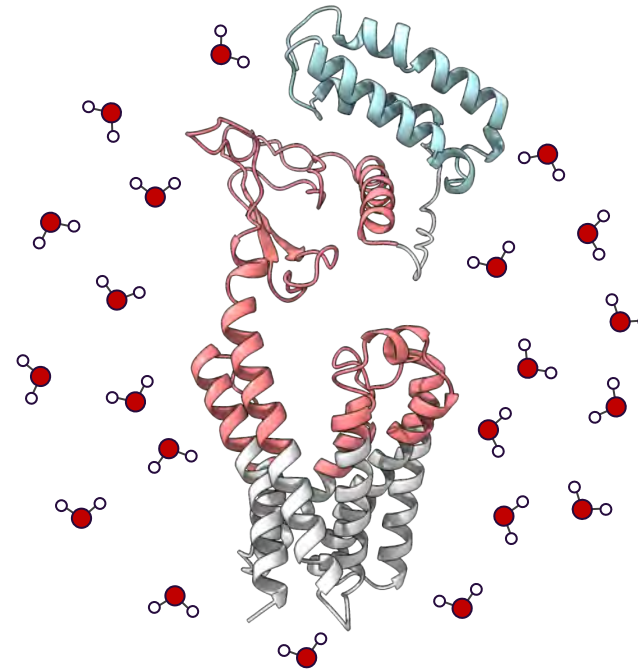
Complications

- ✓ *Time consuming*
- ✓ *Stability issues*
- ✓ *Heterogeneity*

AI-Designed Soluble GPCR Analogs for Antibody Drug Discovery



Protein Design



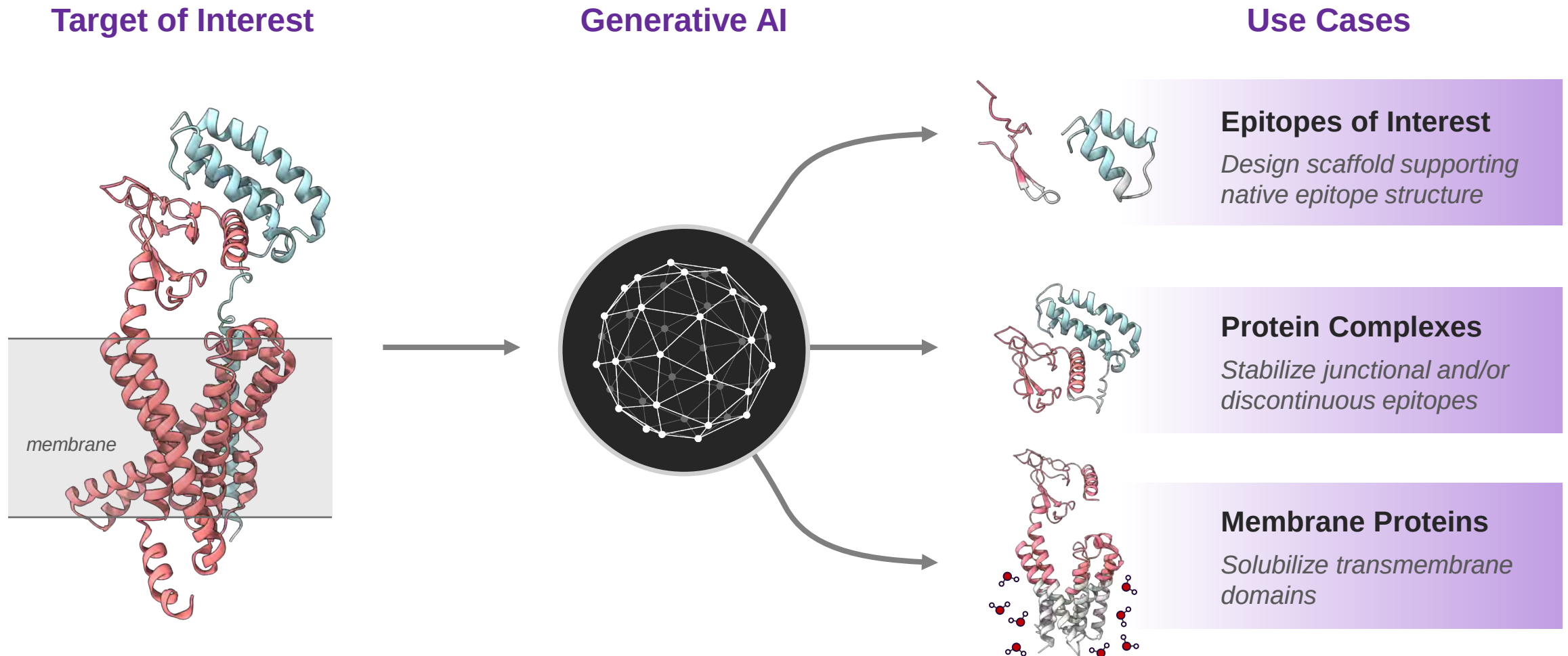
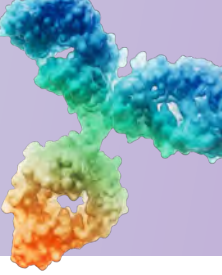
Benefits

- ✓ *Soluble*
- ✓ *Stable*
- ✓ *Scalable production*
- ✓ *Screening ready*

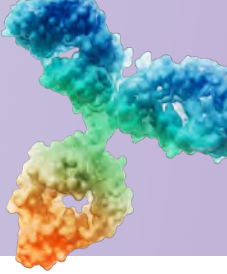
Complications

- ✓ *Potentially loses native-like structure*

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

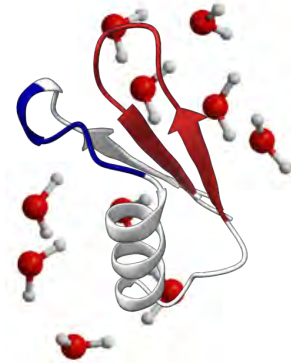


Antigen Designs are Optimized for Antibody Drug Discovery



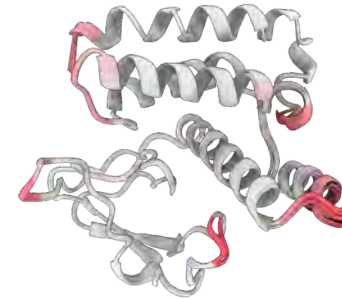
Water Solubility

 Epitope
 Scaffold



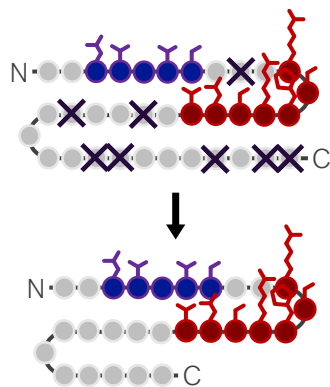
Structural Stability

Predicted Flexibility



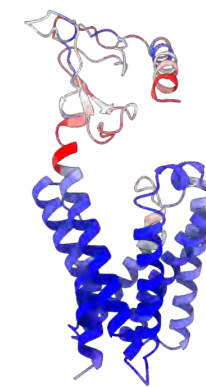
Scaffold Minimization

 Epitope
 Scaffold



Low Scaffold Immunogenicity

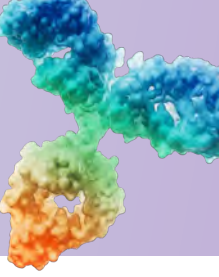
Predicted Immunogenicity¹



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



Engineered Epitopes Enable Rapid Discovery of On-Epitope Antibodies



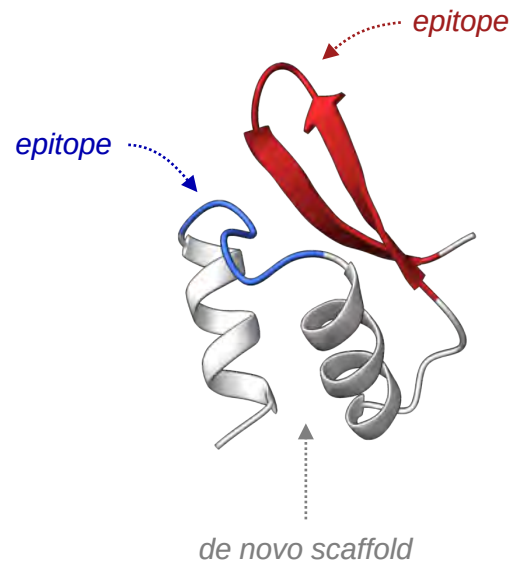
1

Naïve in vitro or in vivo
antibody library



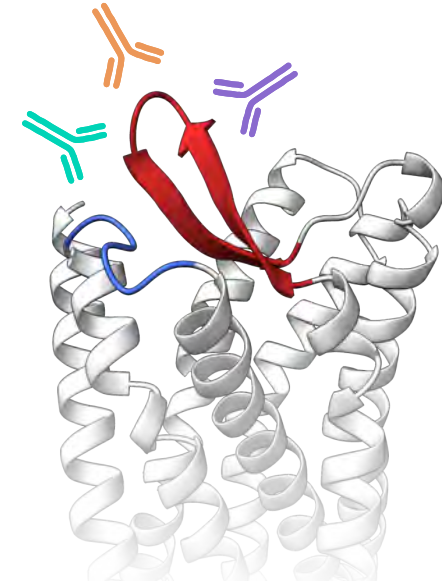
2

Focus library with
engineered epitopes

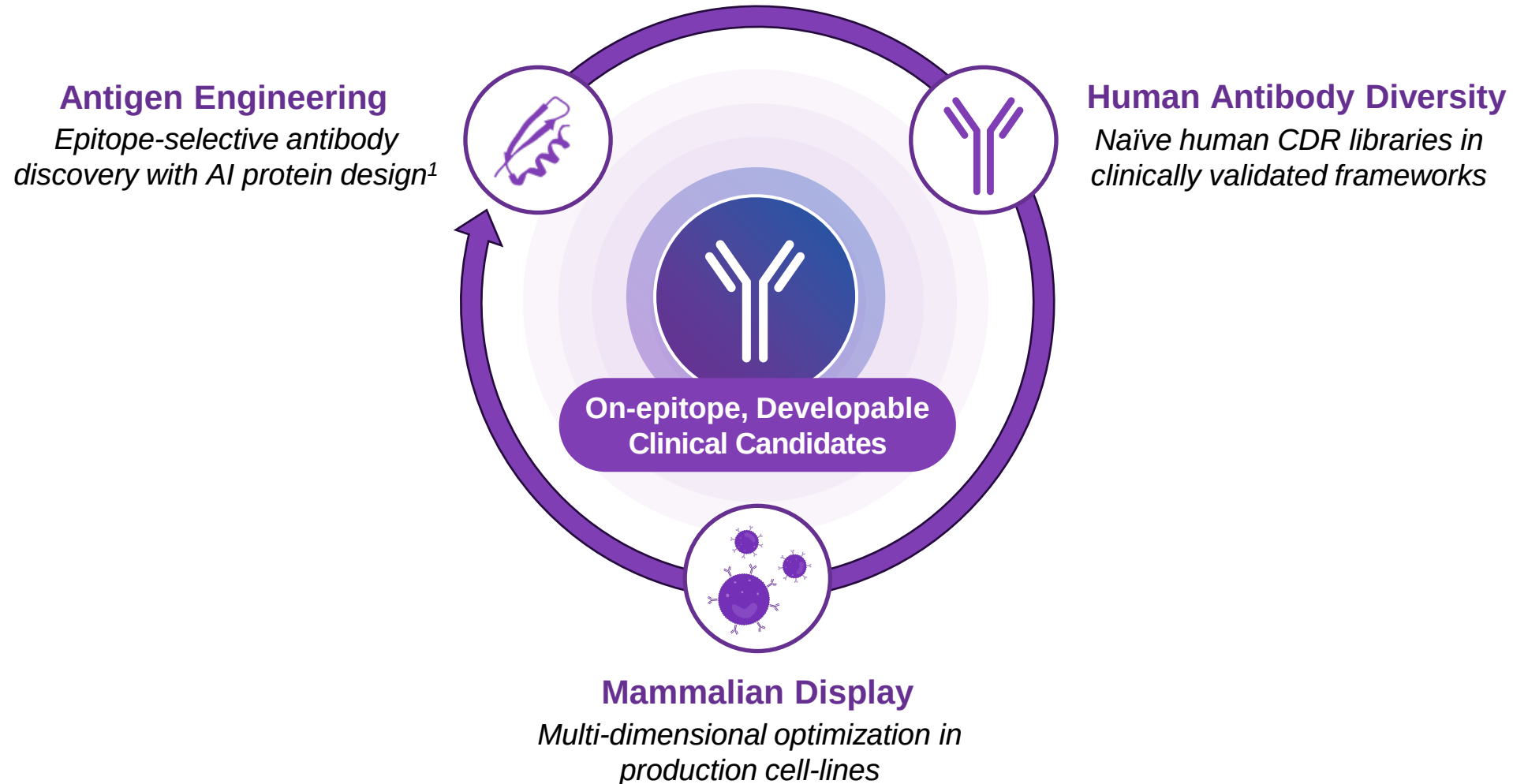
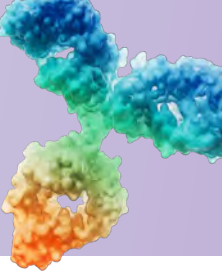


3

Efficient discovery of
epitope-specific antibodies



Closed-Loop Discovery Process Enables Translatability Assessment of Antigen Designs

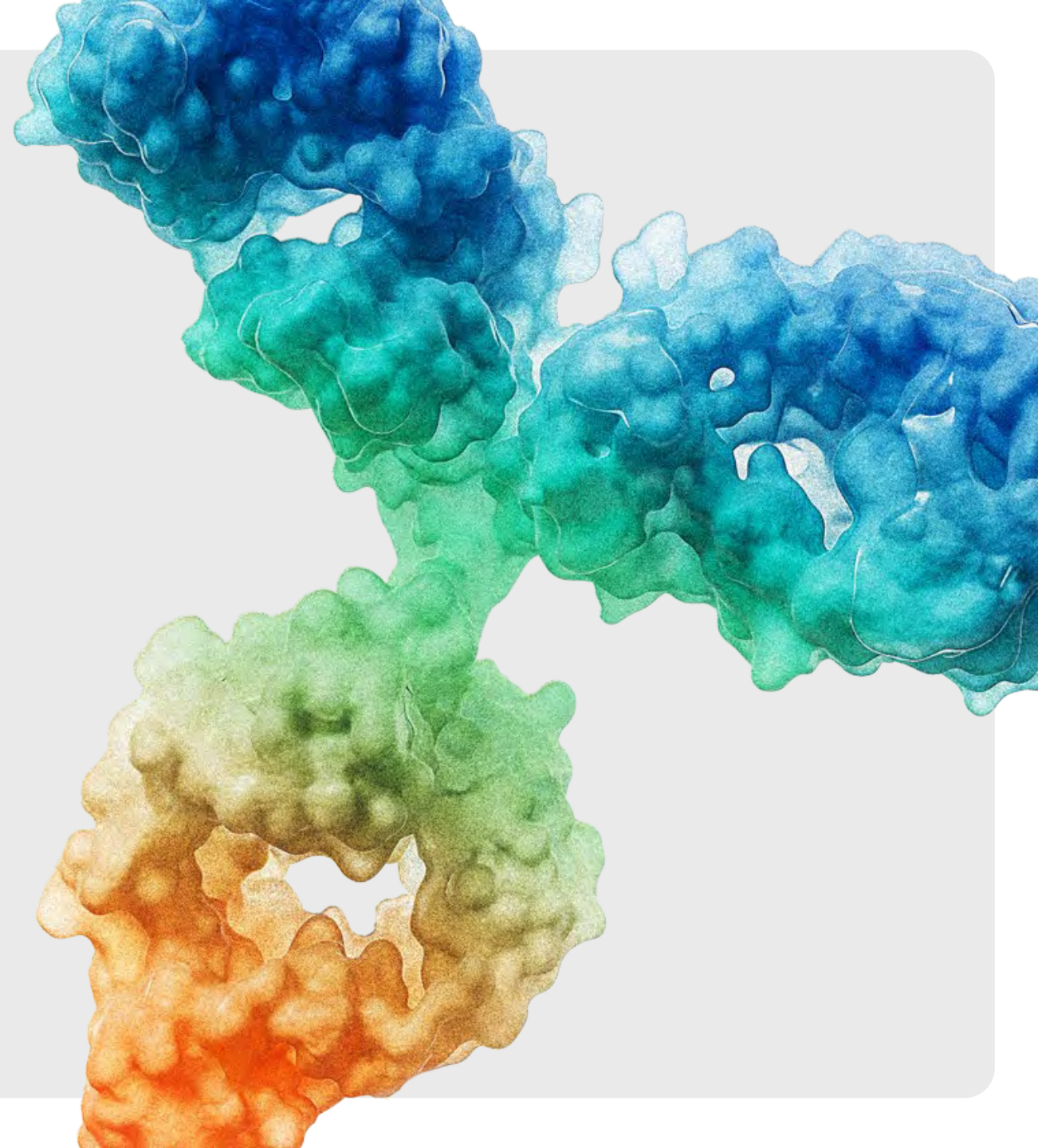


¹U.S. Patent No. 11,545,238 (issued January 3, 2023)

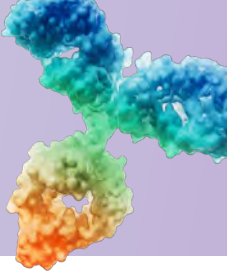


GPCR Engineered Antigen Validation

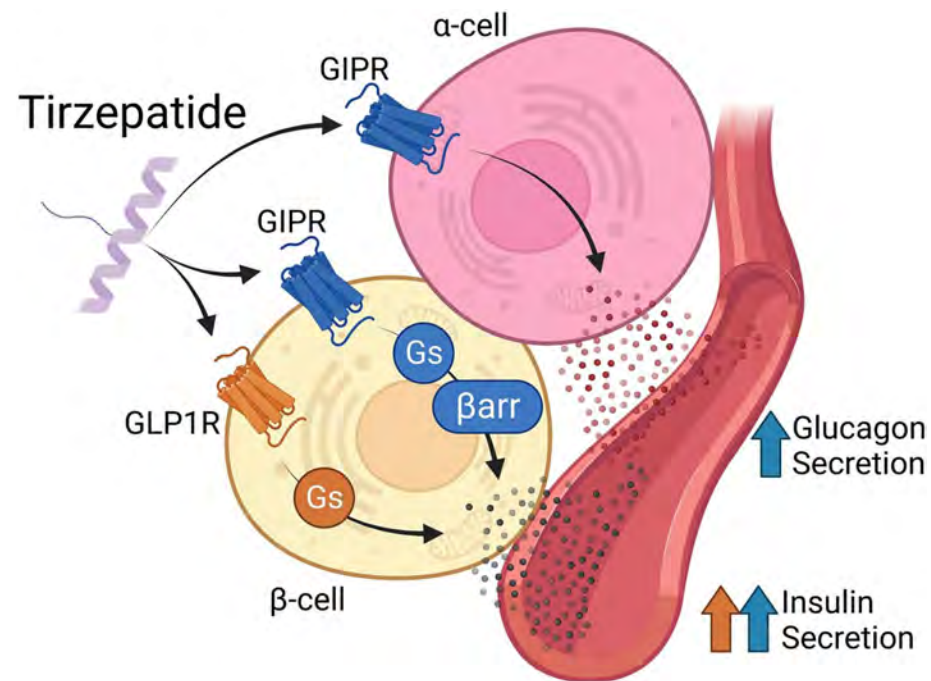
*Experimental Structure and
Function Case Studies*



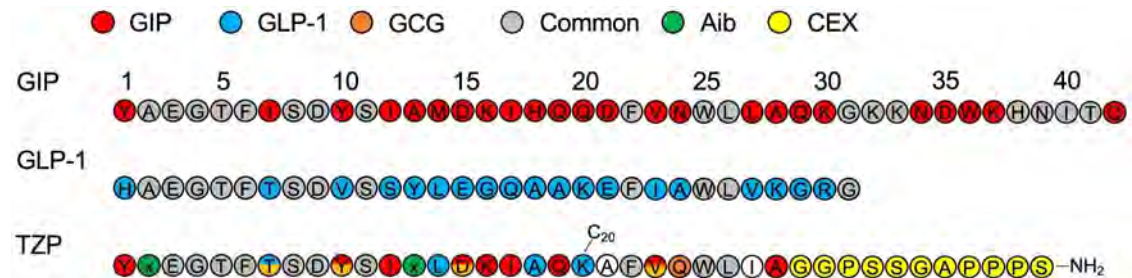
Case Study #1: Glucose-dependent Insulinotropic Polypeptide Receptor (GIPR)



GIPR is a Target of Weight Loss Drug Tirzepatide

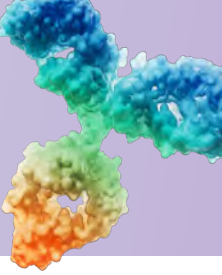


Challenge: Design Soluble GIPR that Specifically Binds GIP and not GLP-1



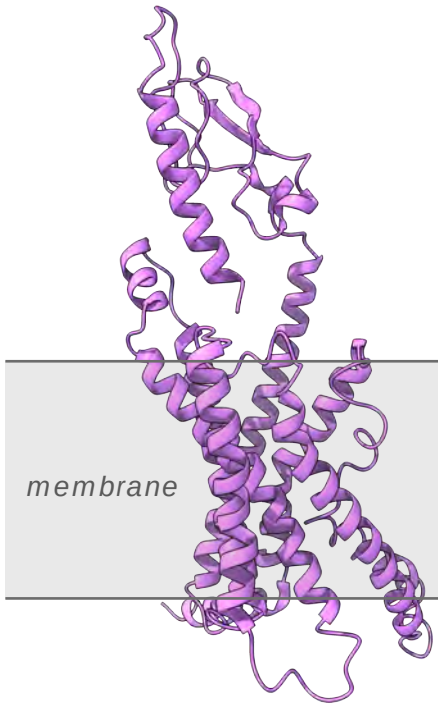
GIPR and GLP-1R are structural homologs whose natural ligands GIP and GLP-1, respectively, share high sequence identity at the N-terminus

GIPR is Solubilized by Reengineering the Transmembrane Domain



Input Target

GIPR

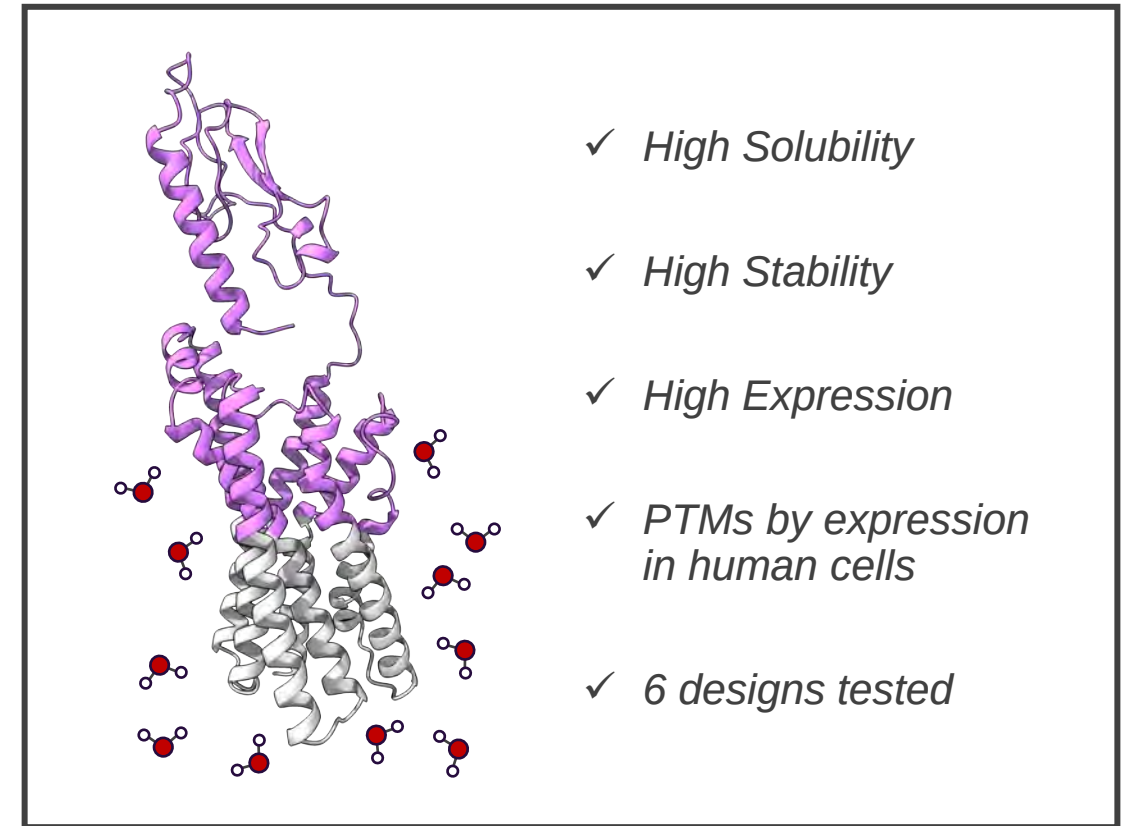


Generative AI

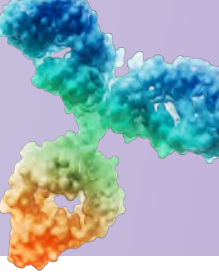
*Solubilization of
transmembrane domain*



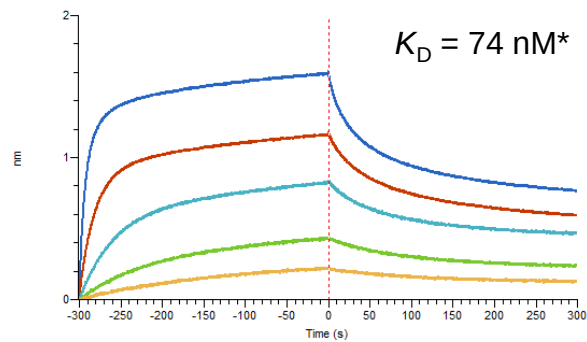
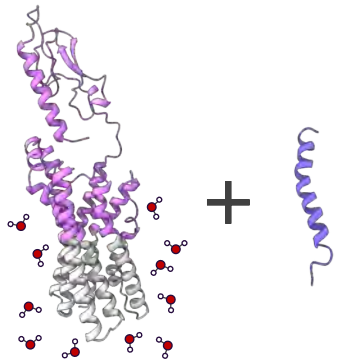
Soluble GPCR



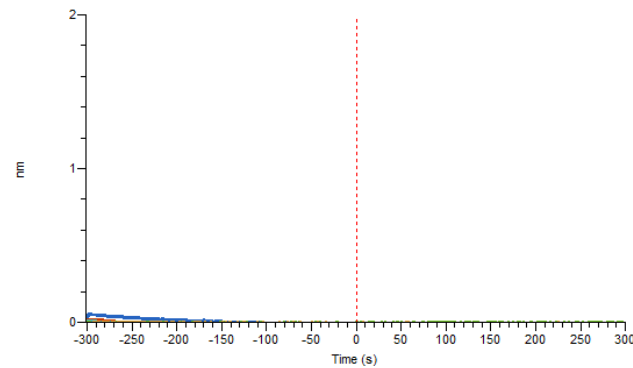
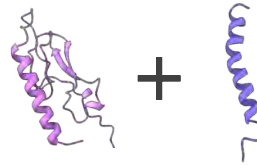
Soluble GIPR Binds its Native Ligand GIP



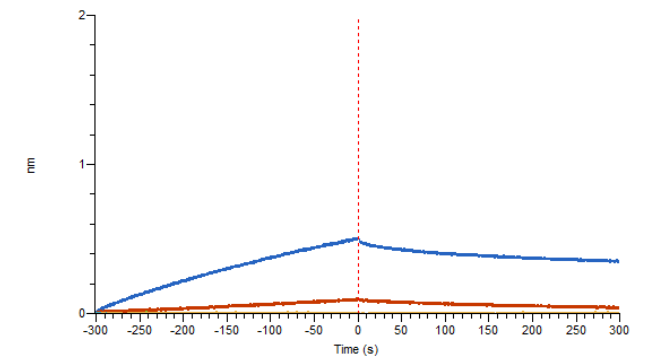
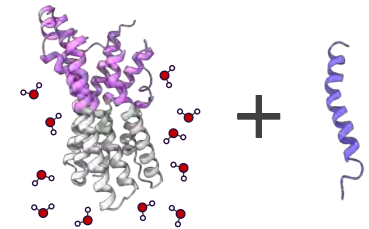
Soluble GIPR Binding to GIP



Extracellular Domain Binding to GIP



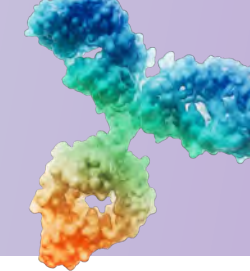
Transmembrane Domain Binding to GIP



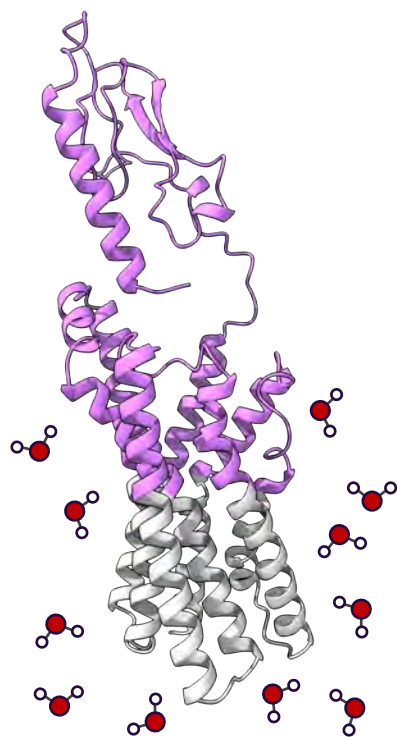
* K_D calculated from avidity binding kinetics; Data on File



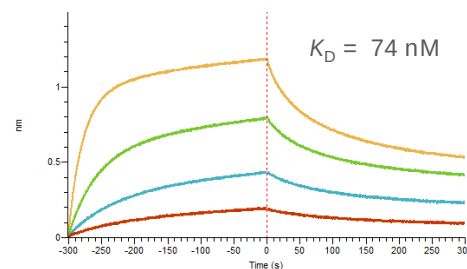
Soluble GIPR Binding is Specific to its Natural Ligand and Therapeutic Drugs



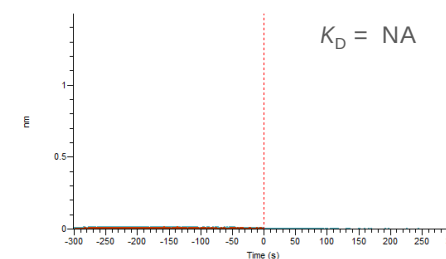
Avidity Ligand Binding Kinetics



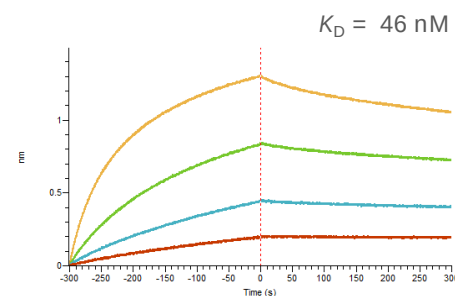
GIP
Natural Ligand



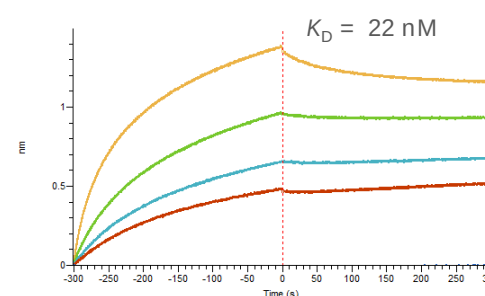
GLP-1
Negative Control



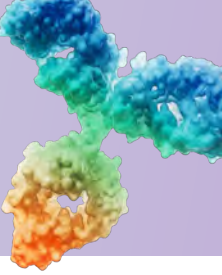
Tirzepatide
Dual Agonist Therapeutic Drug



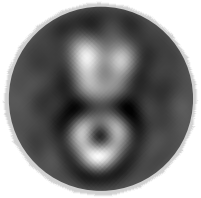
Retatrutide
Triple agonist



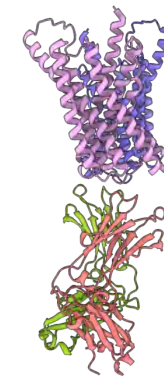
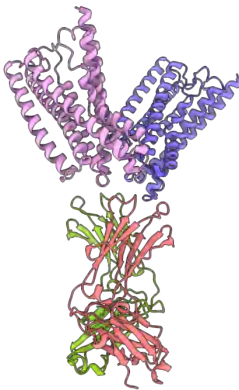
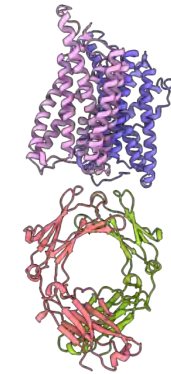
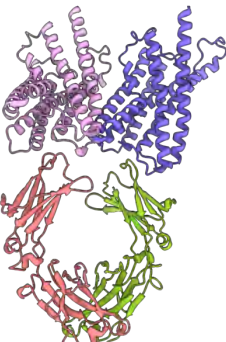
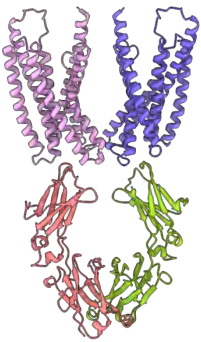
Negative Stain Electron Microscopy Supports Soluble Transmembrane Structure



Negative Stain Electron Microscopy



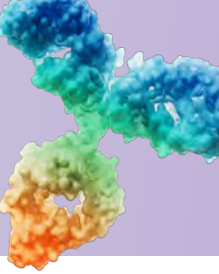
*Soluble GPCR expressed
as Fc fusion*



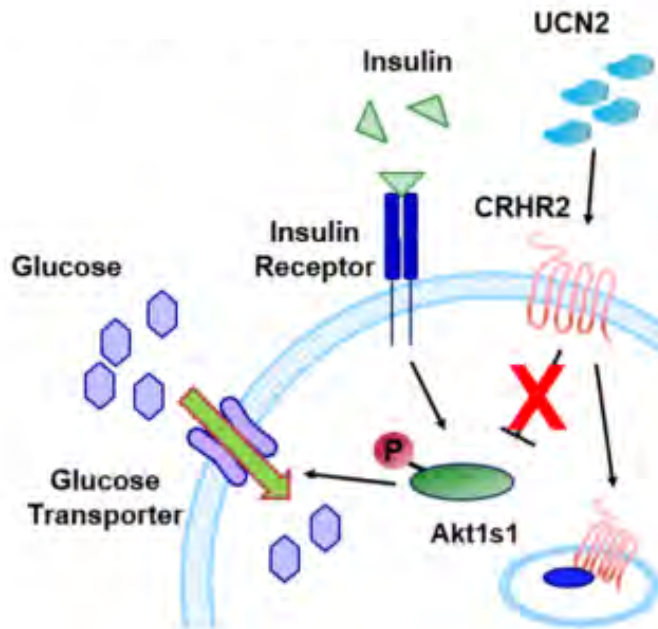
*Imaging supports soluble
transmembrane design*

*Flexibility observed at the
Fc hinge region*

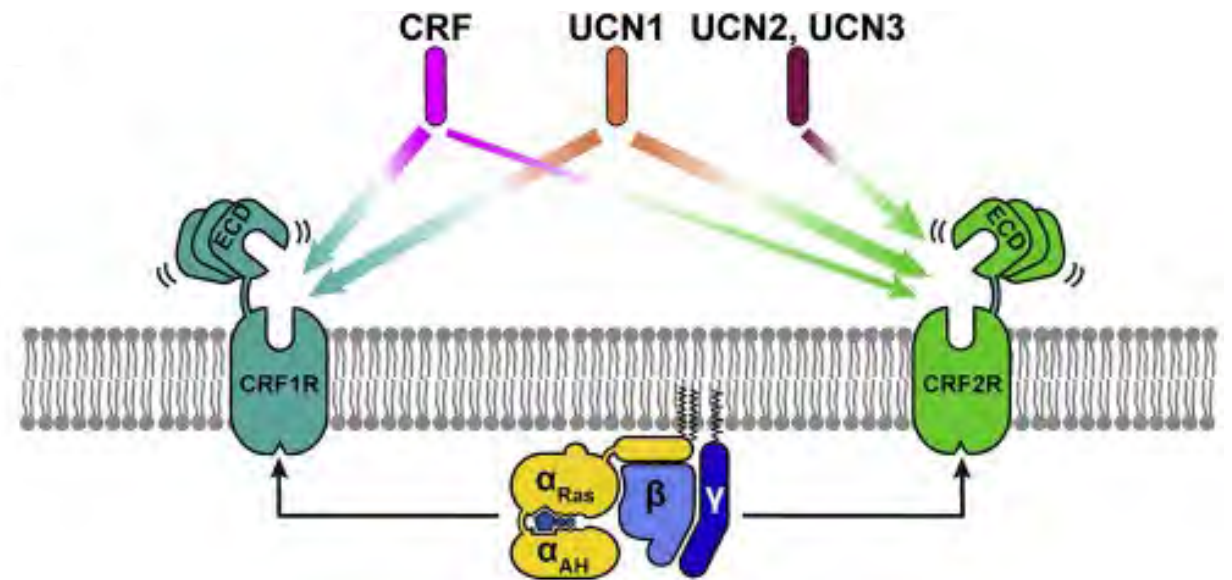
Case Study #2: Corticotropin-Releasing Factor Receptor 2 (CRF2R)



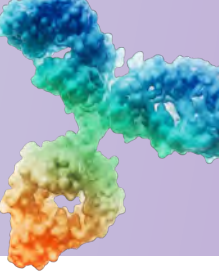
CRF2R is a Weight Loss Target Using UCN2 Analogs



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

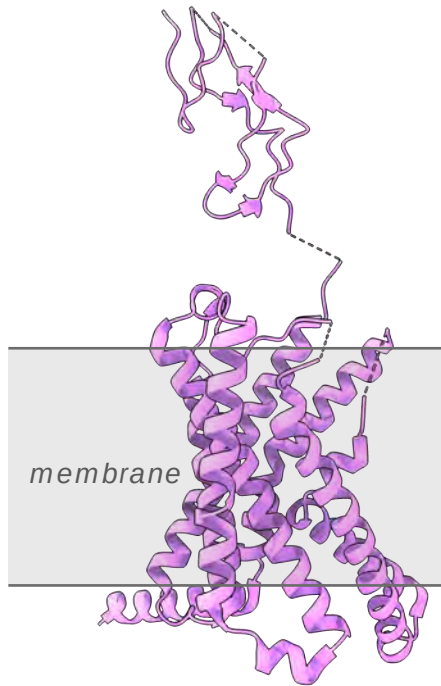


CRF2R is Solubilized by Reengineering the Transmembrane Domain



Input Target

CRF2R

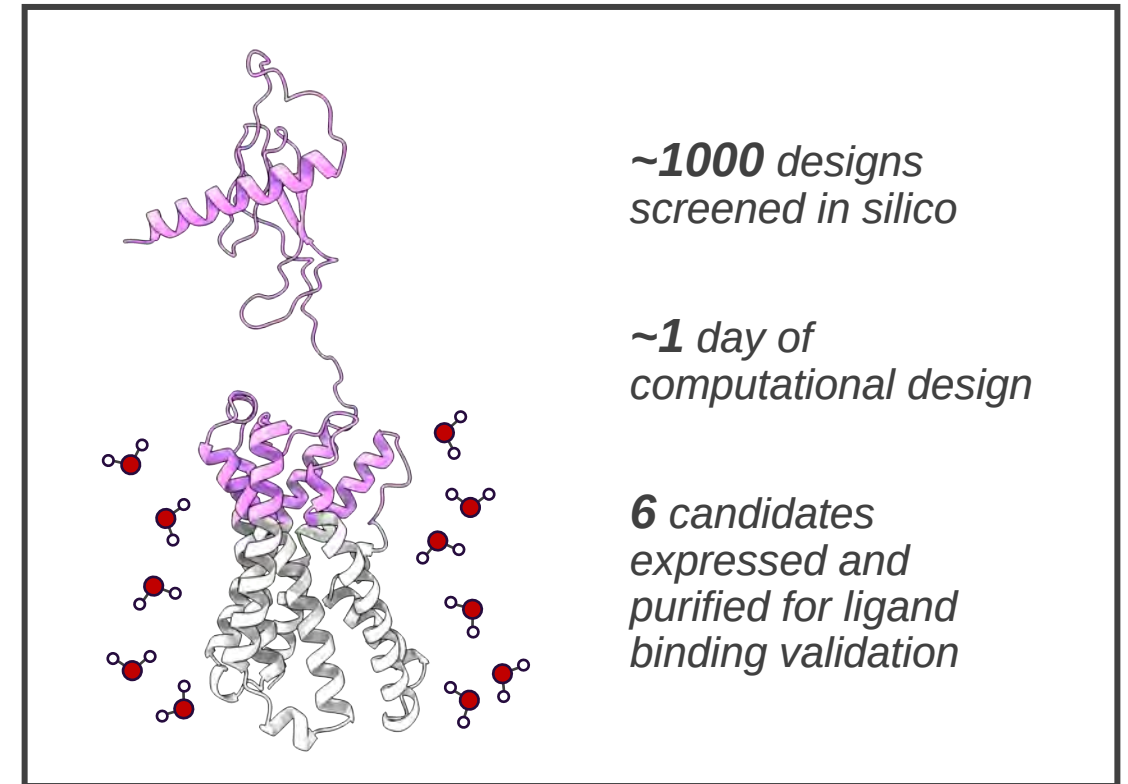


Generative AI

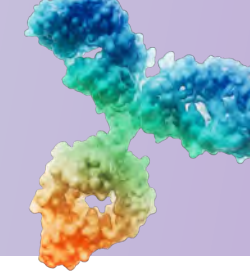
*Solubilization of
transmembrane domain*



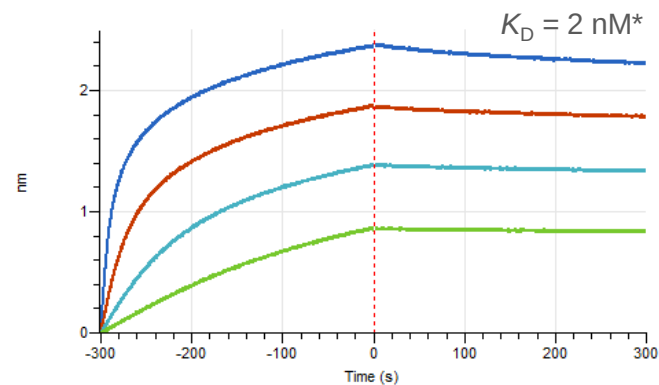
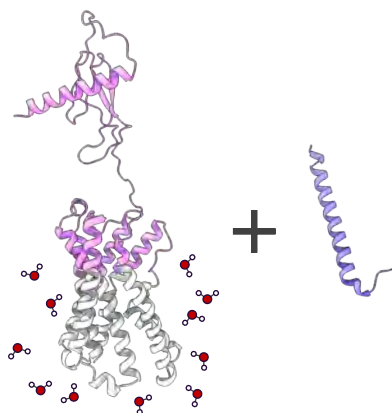
Soluble GPCR



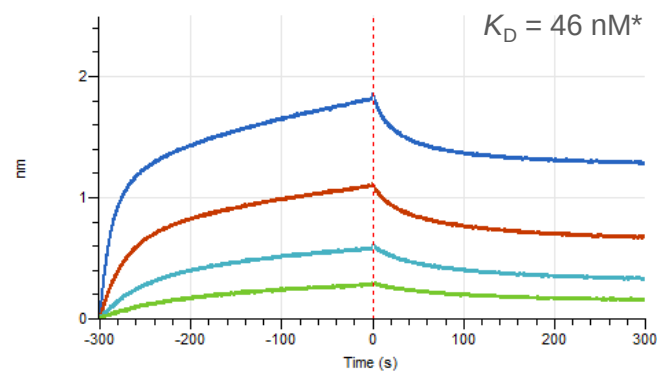
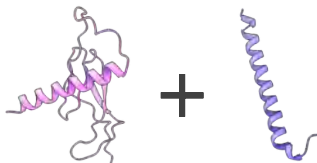
Soluble CRF2R Binds its Native Ligand UCN2



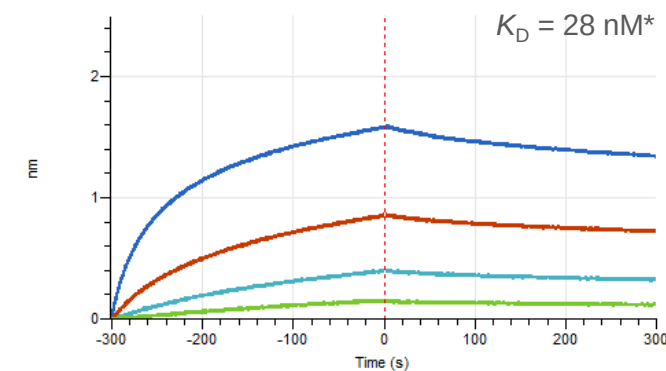
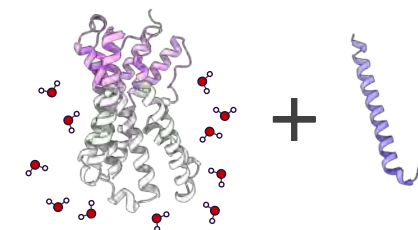
Soluble CRF2R Binding to UCN2



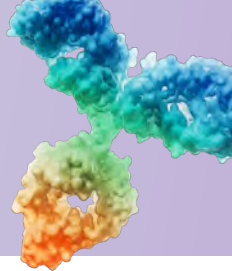
Extracellular Domain Binding to UCN2



Transmembrane Domain Binding to UCN2

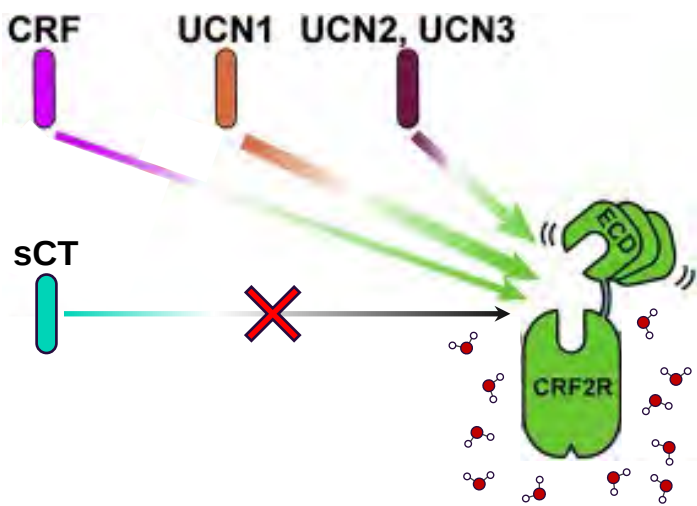


Soluble CRF2R Selectively Binds UCN1-3 and CRF

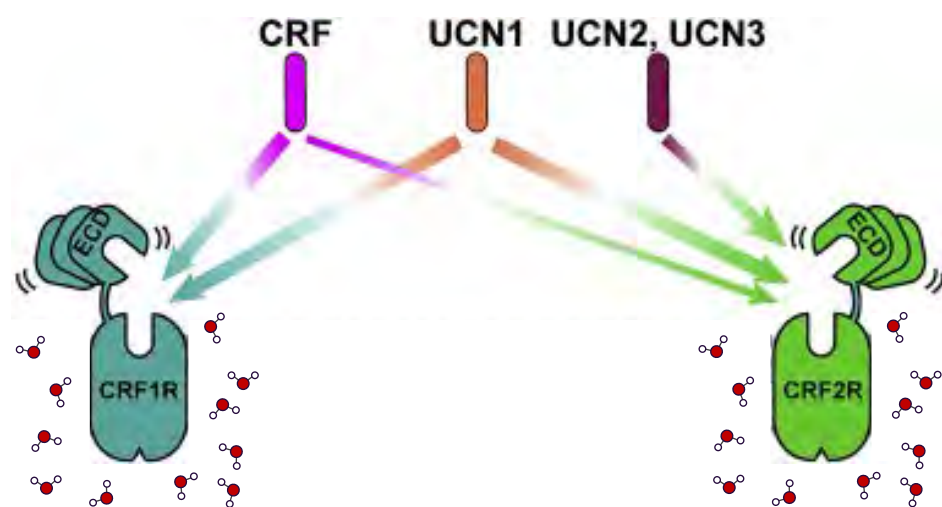
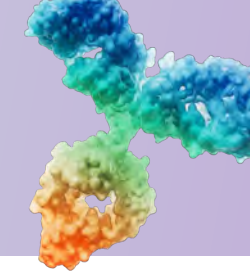


Binding Selectivity (Apparent K_D in nM)*

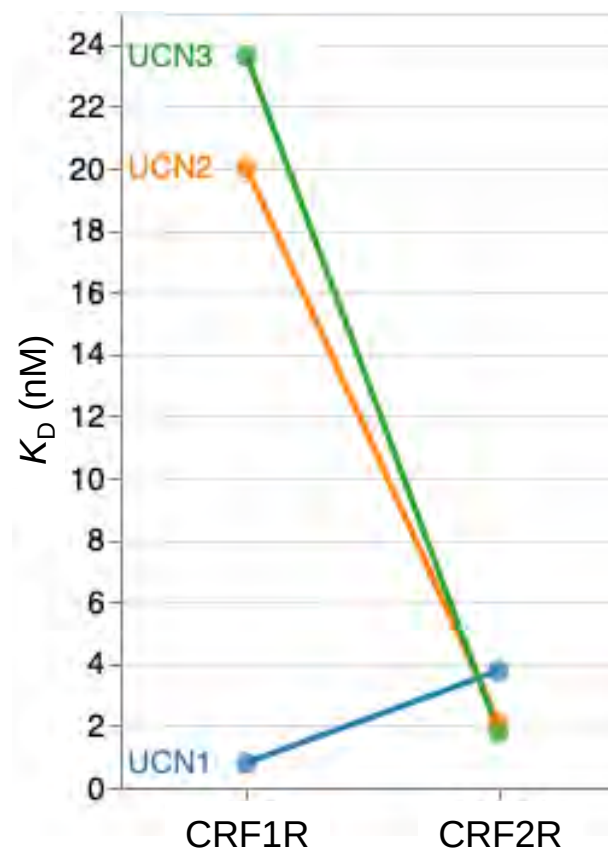
	UCN1	UCN2	UCN3	CRF	sCT
Soluble CRF2R	4	2	2	10	NB
Extracellular Domain	22	46	46	31	NB
Transmembrane Domain	NB	28	36	78	NB



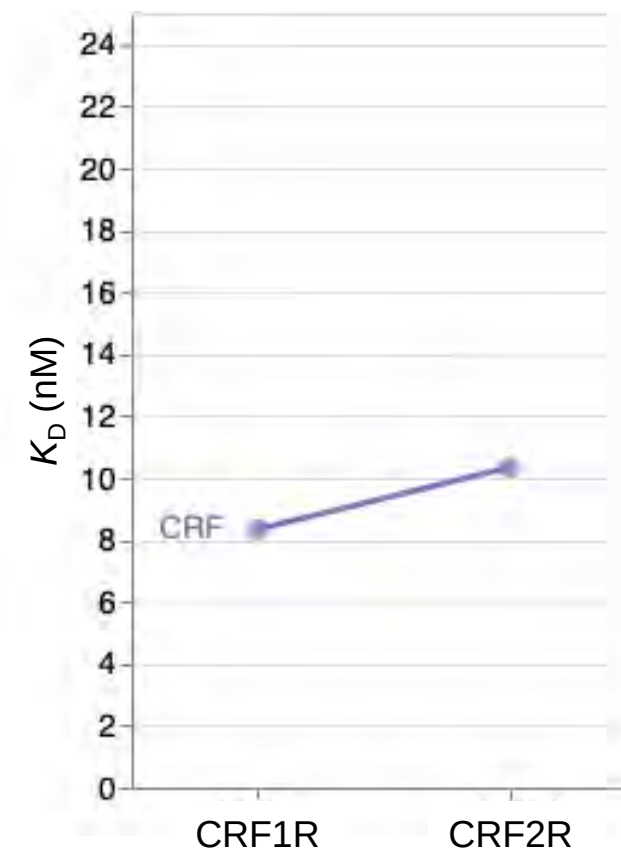
Natural Ligand Specificity is Preserved for Soluble CRF1R and CRF2R Designs



UCN Binding



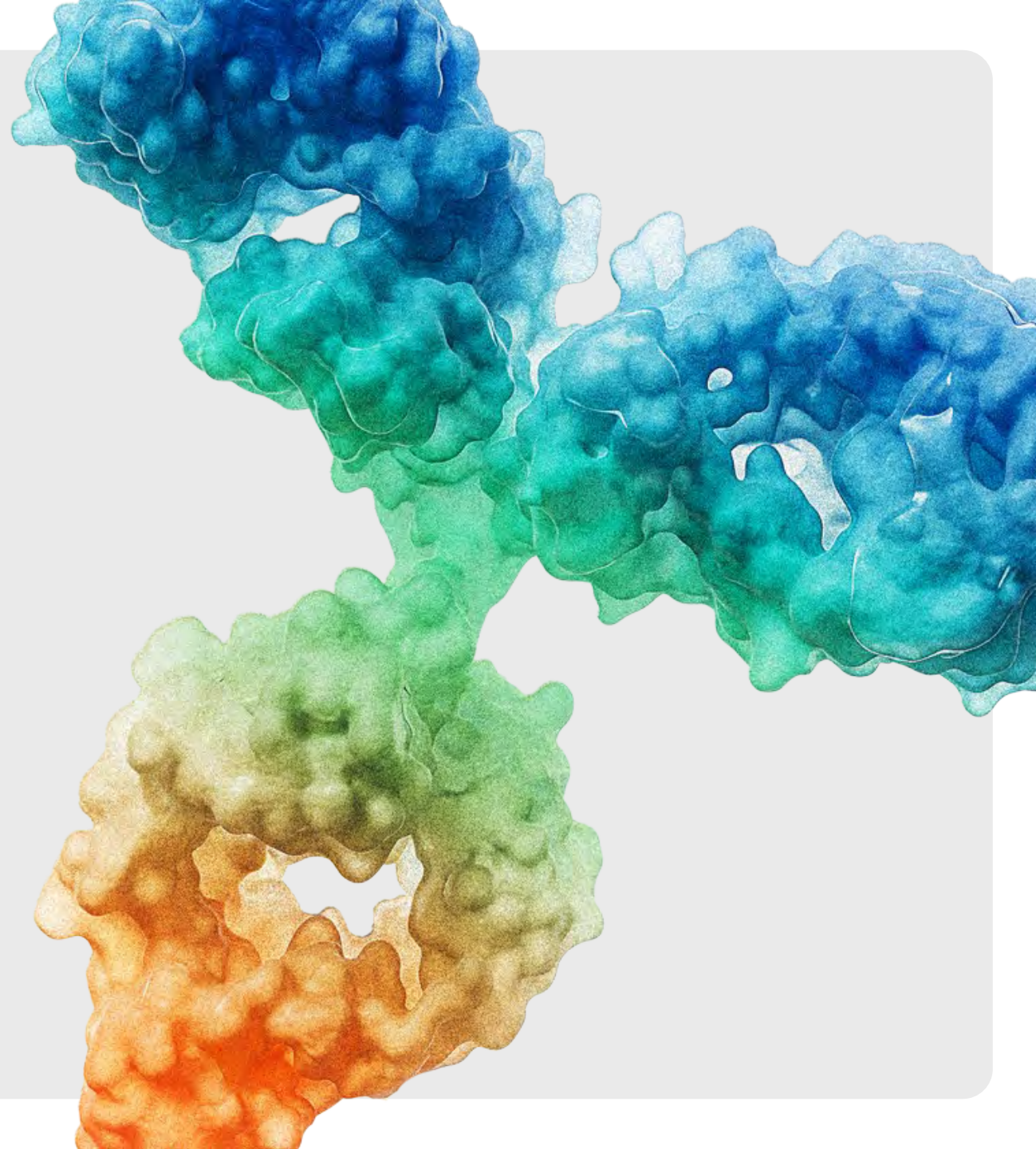
CRF Binding





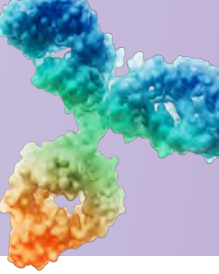
Antibody Discovery with GPCR Engineered Antigens

Amylin Receptor



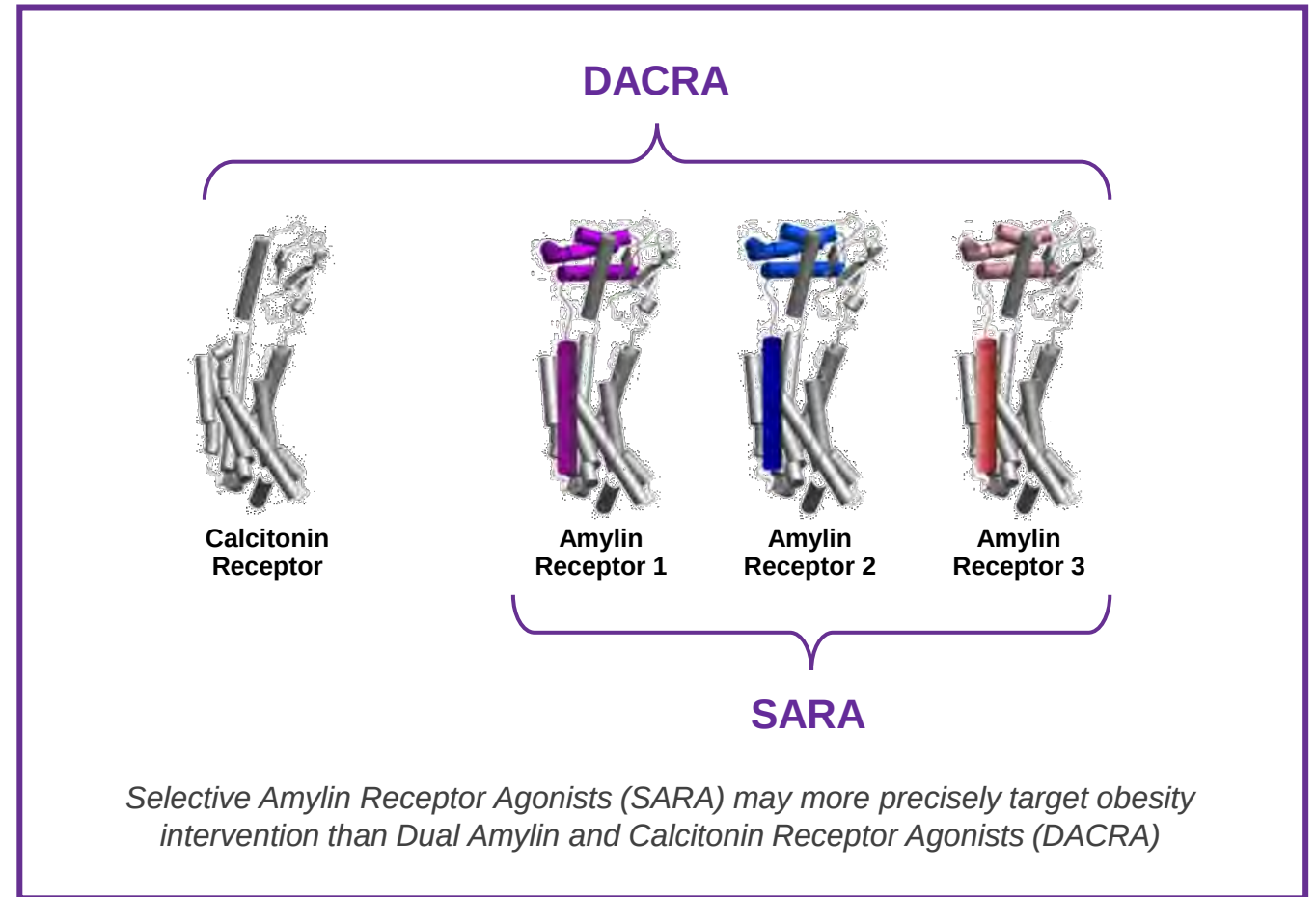
Amylin Receptor Agonism

A Complementary Pathway to GLP-1–Based Obesity Treatments

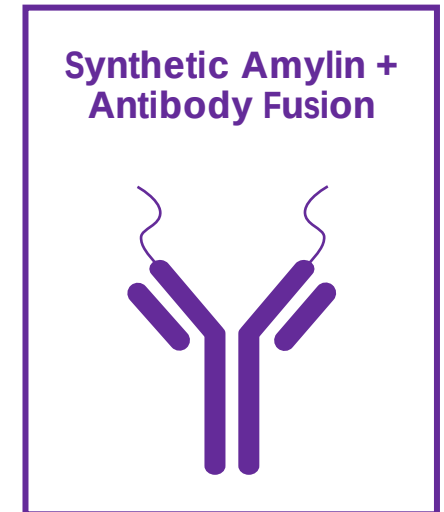
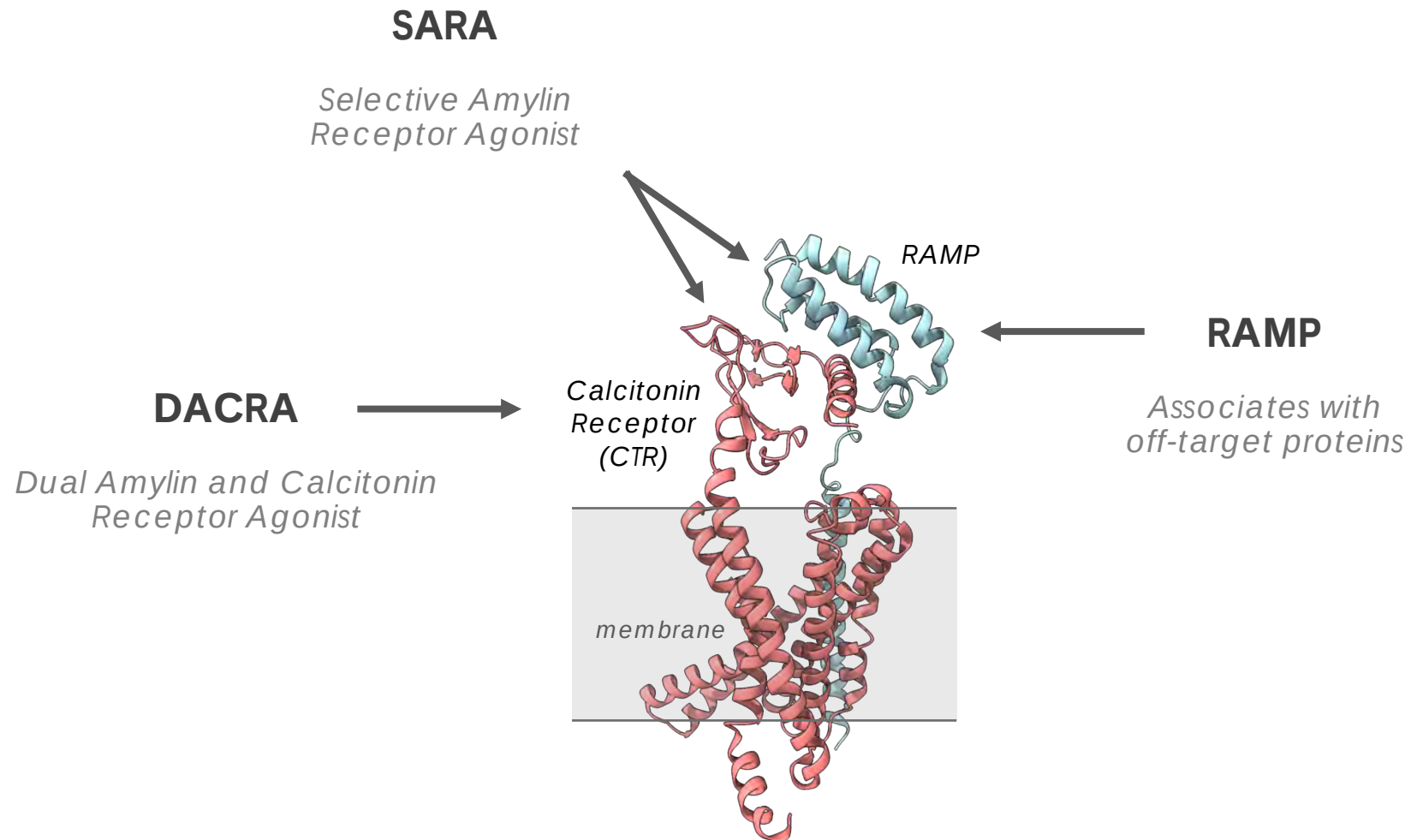
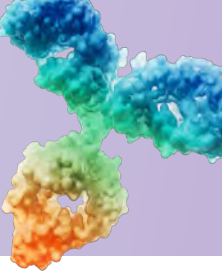


Why Target Amylin?

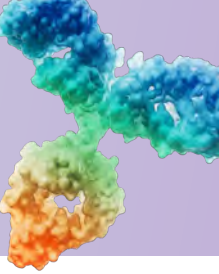
- **Validated metabolic hormone** that promotes satiety and slows gastric emptying
- Clinical studies with amylin analogs confirm efficacy in weight loss, but **peptide-based approaches may be sub-optimal** (dosing, tolerability, manufacturability)
- Antibody therapeutics could provide a differentiated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



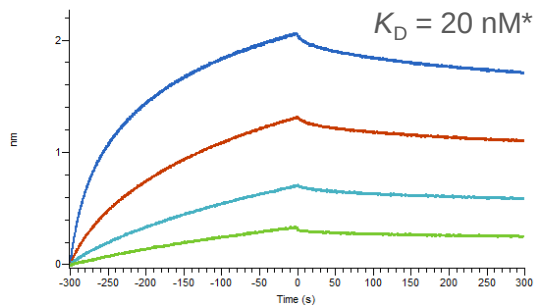
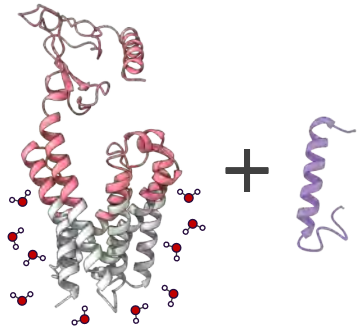
Goal: Improve Therapeutic Specificity and Longevity with Antibody Fusion



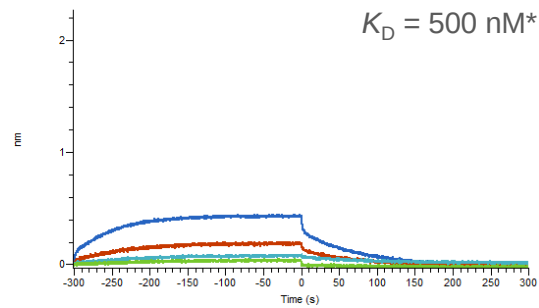
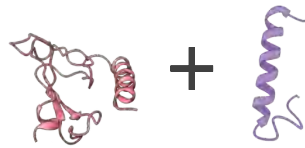
CTR Solubilization Validated by Salmon Calcitonin Binding



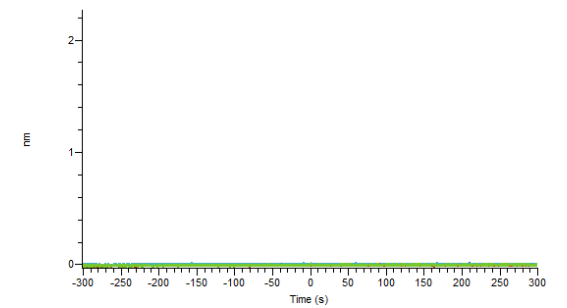
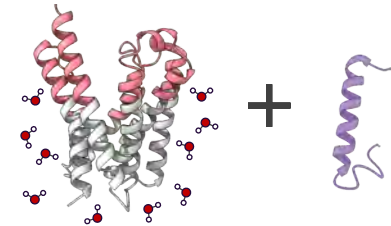
Soluble Receptor Binding to sCT



Extracellular Domain Binding to sCT



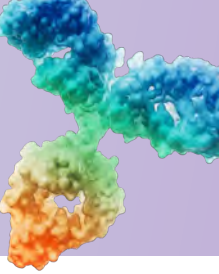
Transmembrane Domain Binding to sCT



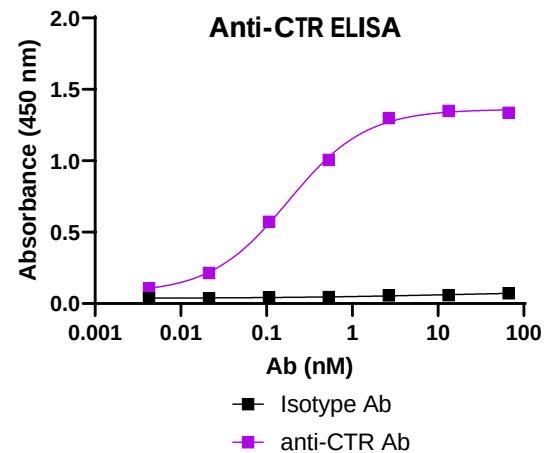
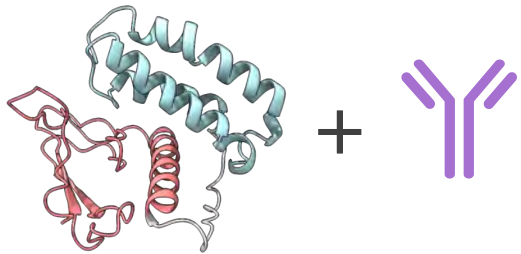
* K_D calculated from avidity binding kinetics; Data on File



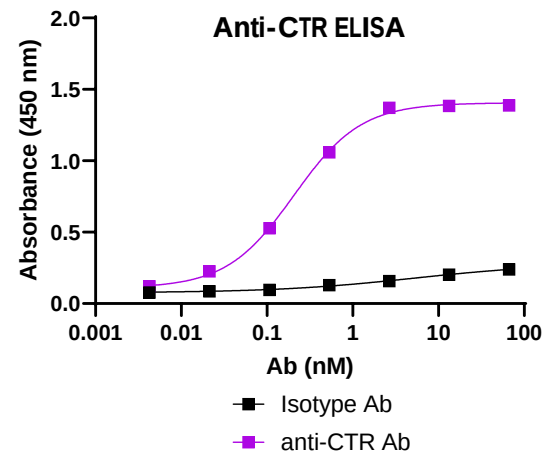
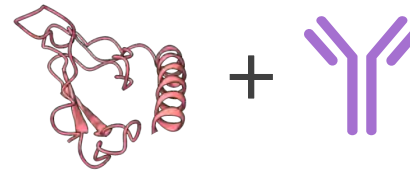
Amylin Junctional Epitope Design Retains Anti-CTR Antibody Binding



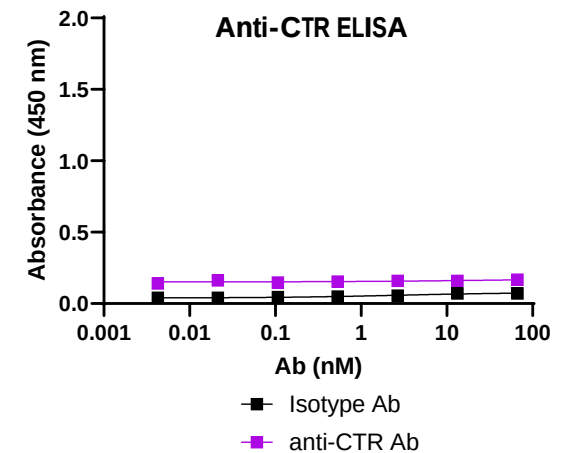
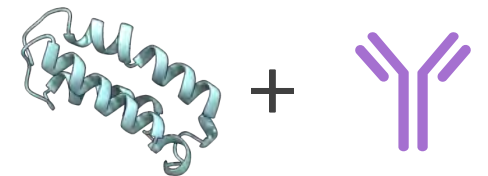
Amylin ECD



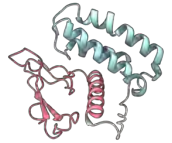
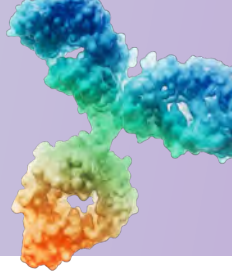
CTR ECD



RAMP



Phage Panning with Engineered Epitopes Generates Cell Binders

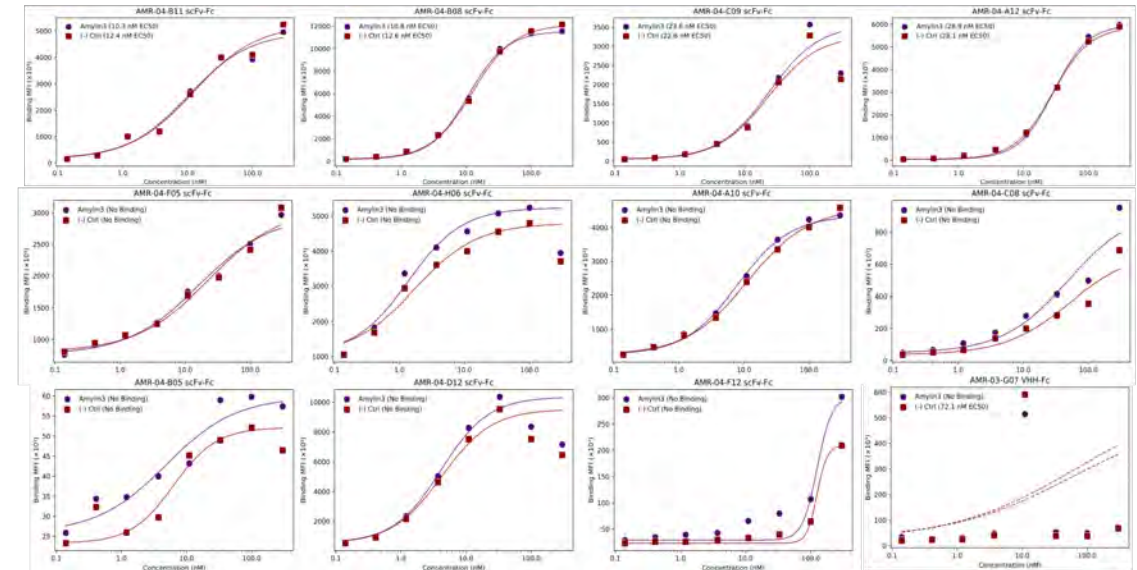
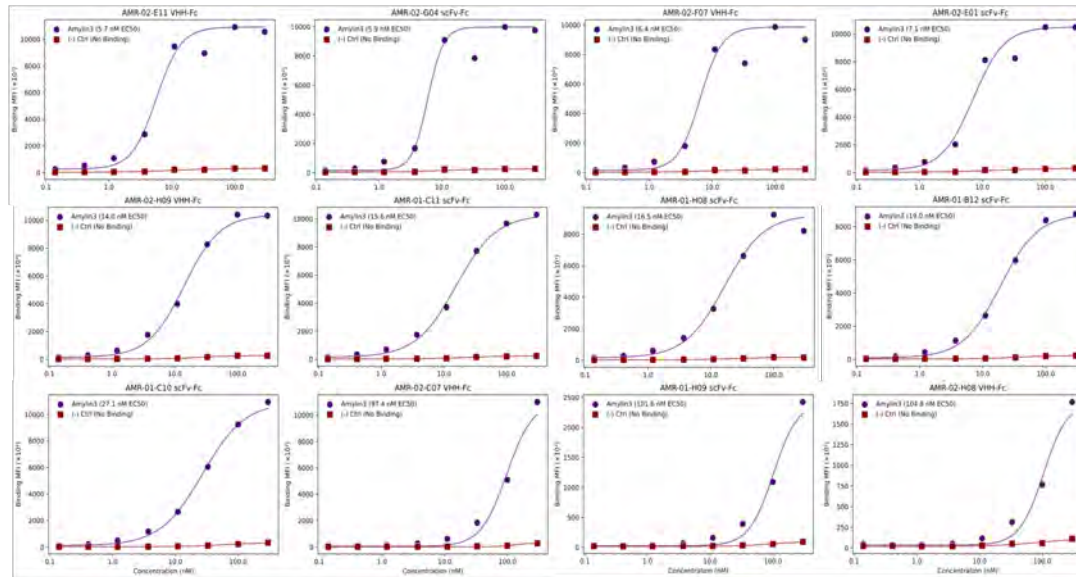


Engineered Epitope Steered Strategies



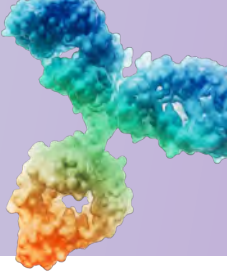
Amylin Receptor Cell-Only Strategies

(No Specific Binders Found)

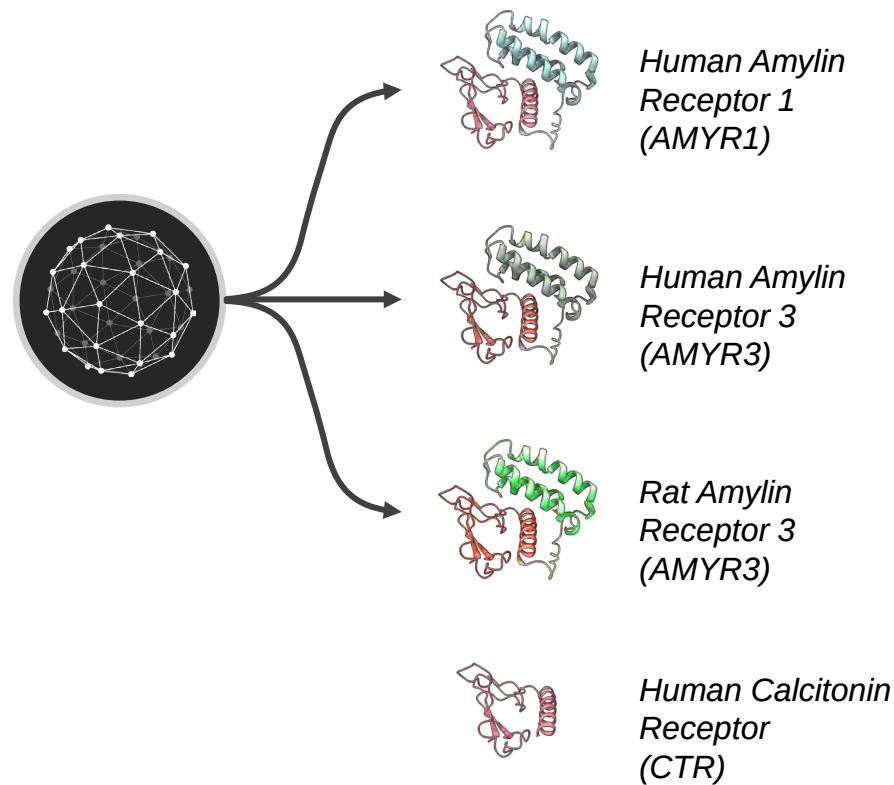


- Amylin3 Receptor Cell Binding
- Negative Control Cell Binding

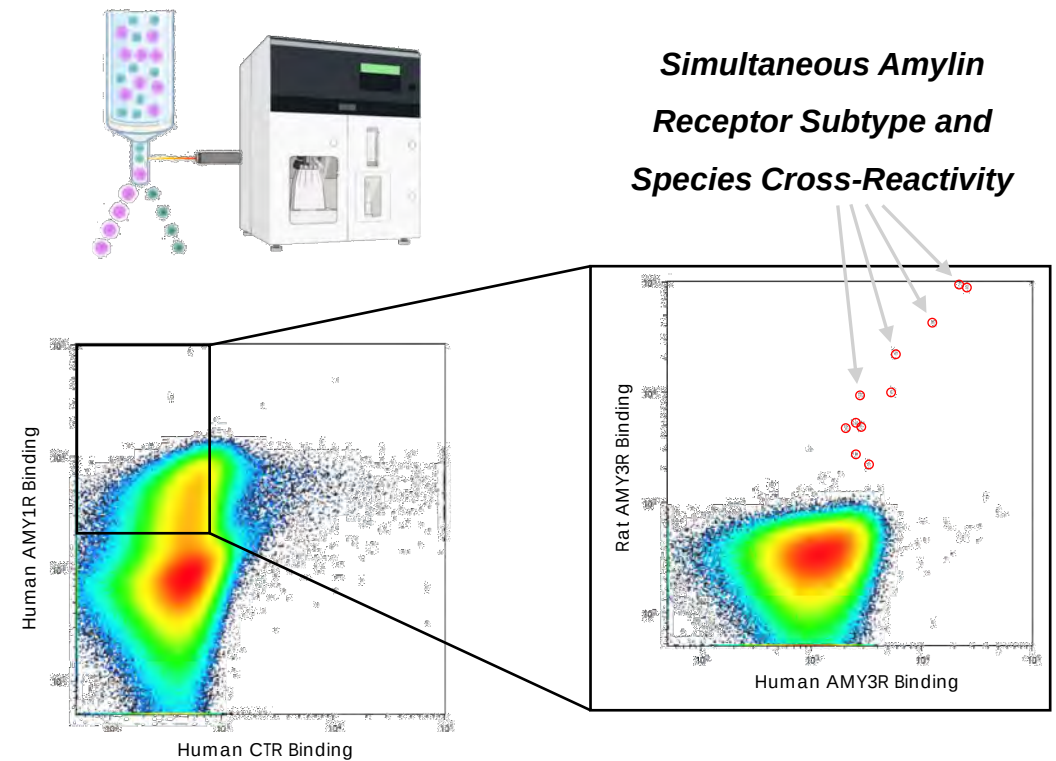
Combining Engineered Epitopes with Mammalian Display Generates Cross-Reactive Hits



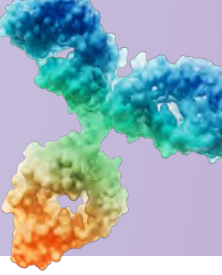
Subtype and Species Cross-Reactive Designs



4-Dimensional Mammalian Display Sorting



Engineered Epitope Selections Generate Native-Receptor Cell Binders



Single Epitope Strategies

No Binders
>3X
Background

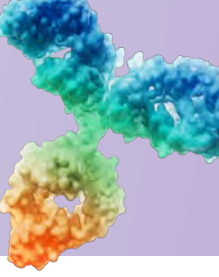


Strategy

Multi-Epitope Strategy (SARA)

No Binding

Engineered Epitope Binding Translates to Native-Receptor Cell Binding



AMYR Cell Binding vs SPR Epitope K_D

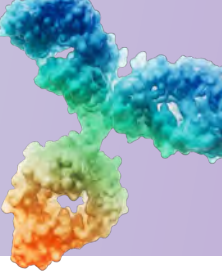
AMY1R

AMY3R

No Binding

No Binding

Selections Yield Four Distinct Receptor-Binding Profiles



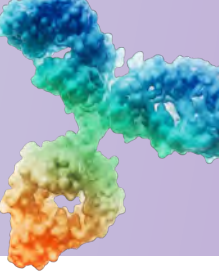
CTR & AMYR

AMY1R & AMY3R

AMY1R Only

AMY3R Only

Peptide-Antibody Fusions Deliver Exquisitely Selective Agonism



AMY3R

AMY1R

CTR

Acknowledgements

