Bitopic Nanobody-ligand Conjugates as Tools to Characterize Consequences of Dual-engagement of GPCRs

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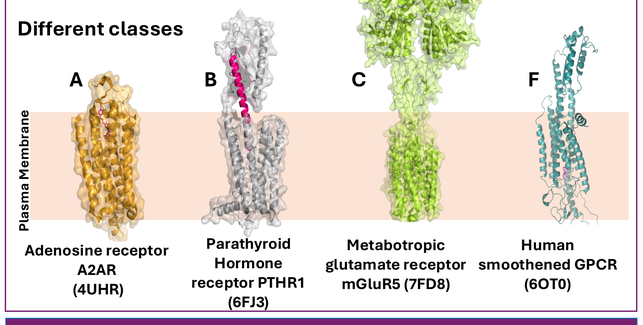
Background

- G protein-coupled receptors (GPCRs), regulates a plethora of physiological processes and are attractive targets for drug development.
- Approximately 35% of currently marketed therapeutics target GPCRs.
- Co-expression of multiple distinct GPCRs can modulate the downstream signaling responses which demands new tool to interrogate this complex process.
- Bitopic ligands that bind to two distinct receptors may enable study of dual engagement of GPCR.

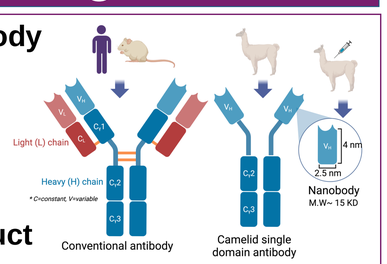


Objectives

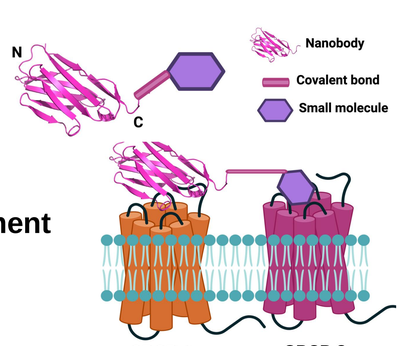
- Develop antibody fragment (Nanobody; Nb)-small molecule ligand as bitopic conjugate.
- Evaluate activity of Nb-ligand conjugate in pharmacological assays of G-protein activation and β-arrestin recruitment.
- Assess consequences of dual targeting of GPCR pairs.

Design

Source of Nanobody



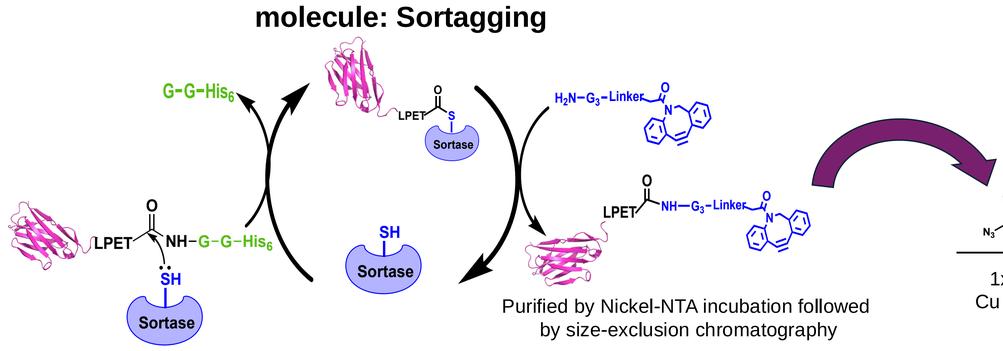
Nb-ligand construct



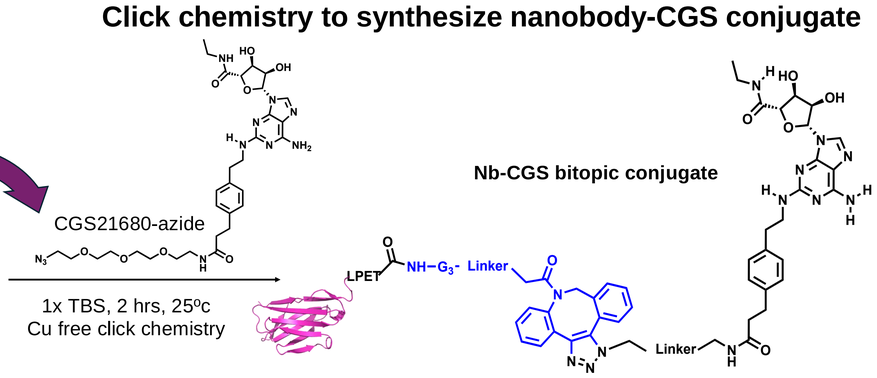
Dual engagement of GPCR

Method and Results

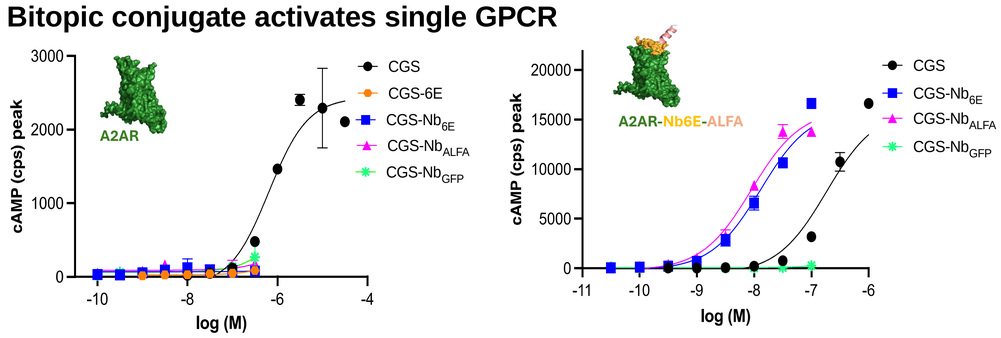
Chemoenzymatic reaction to conjugate nanobody to small molecule: Sortagging



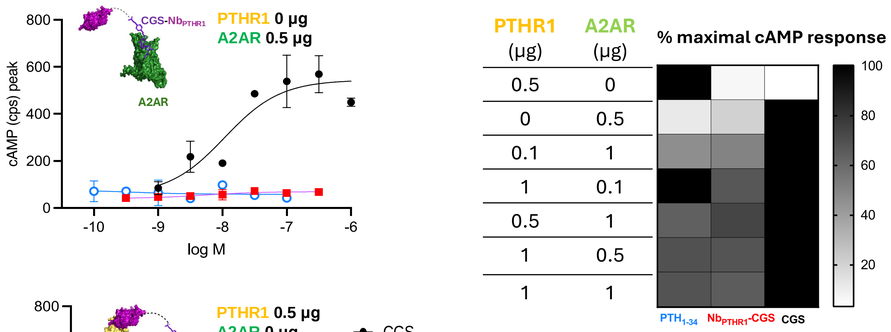
Click chemistry to synthesize nanobody-CGS conjugate



Bitopic conjugate activates single GPCR

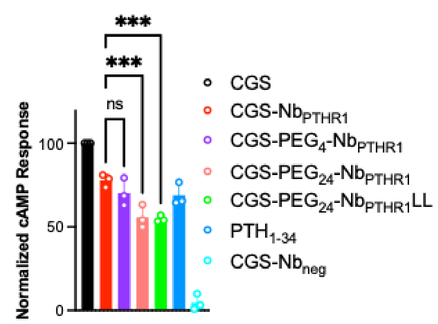


Logic-gated mechanism for coexpressed Class A (A2AR) and Class B (PTHR1) GPCR



PTHR1 (µg)	A2AR (µg)
0.5	0
0	0.5
0.1	1
1	0.1
0.5	1
1	0.5
1	1

Different plasmid ratio signifies the logic-gated mechanism



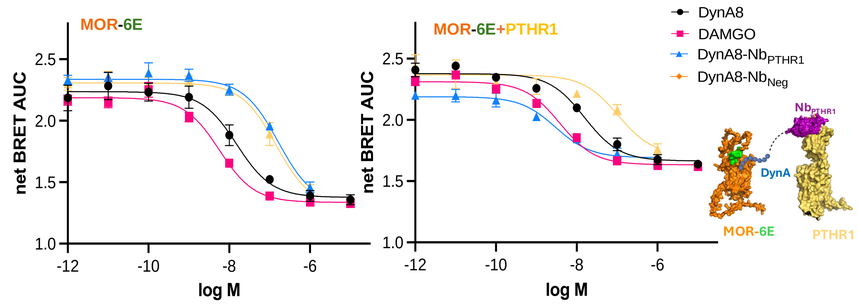
Decrease in max cAMP response with increased linker length

CGS21680 is agonist for A2AR; PTH1-34 is PTHR1 agonist; DAMGO and Dynorphin (DynA8) are orthosteric opioid receptor ligands

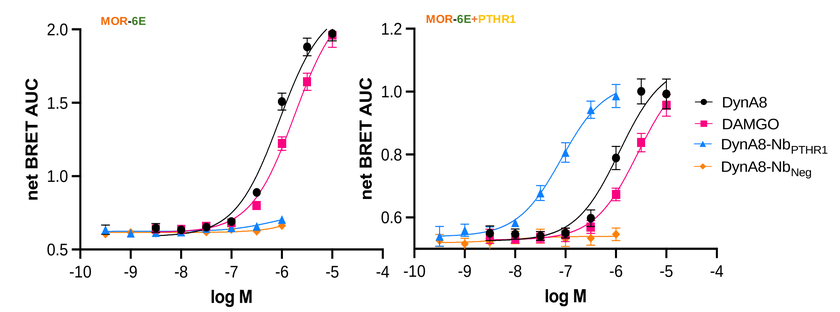
Logic-gated mechanism applies for other coexpressed receptor combinations

Single receptor	Coexpressed receptor
CGS-Nb_{GLP1R} EC_{50}±SEM= >10000	CGS-Nb_{GLP1R} EC_{50}±SEM= 34±19
GLP1R	GLP1R÷A2AR
CGS-Nb_{6E} EC_{50}±SEM= >10000	CGS-Nb_{6E} EC_{50}±SEM= 43±31
MOR-6E	MOR-6E÷A2AR

Enhanced Gαi activity showed by bitopic Nb-peptide conjugate in coexpressed receptor

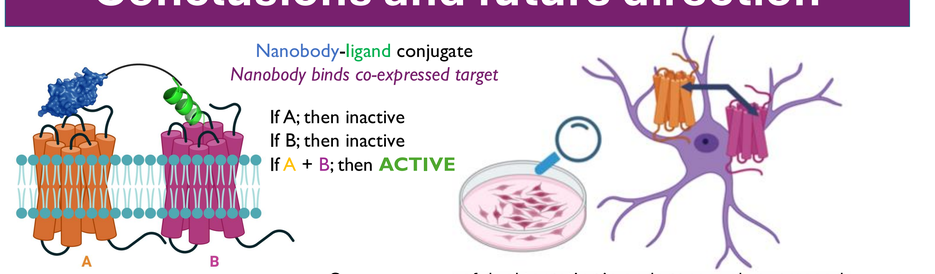


Augmented potential in β-arrestin recruitment by bitopic Nb-peptide conjugate in coexpressed receptor



$DynA8\text{-}Nb_{PTHR1}$ showed > 10-fold higher β-arrestin 2 recruitment than DAMGO or DynA8

Conclusions and future direction



References and Acknowledgement

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