Leveraging antibodies and chemistry to interrogate cell surface receptor function

Shivani Sachdev[1], Brendan Creemer[1], Thomas J. Gardella[2], Ross W. Cheloha[1]
[1]Chemical Biology in Signaling Section, Laboratory of Bioorganic Chemistry, NIH, Bethesda, MD, United States
[2]Massachusetts General Hospital Endocrine Unit and Harvard Medical School, Boston, MA, United States
Email: shivani.sachdev@nih.gov

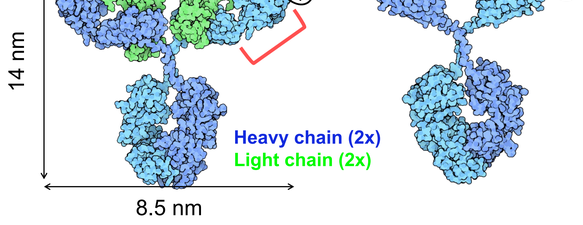
National Institute of Diabetes and Digestive and Kidney Diseases

Introduction

As one of the most successful set of targets for therapeutic development, GPCRs are activated by a diverse array of ligands to induce signaling through multiple distinct pathways, yielding varied biological and physiological responses. One such GPCR, the parathyroid hormone receptor-1 (PTHR1) is a validated target for the treatment of osteoporosis. Activated PTHR1 signal through multiple pathways. The pharmacological concept of biased signaling describes the ability of select ligands to preferentially activate one receptor-mediated signaling pathway over others. The development of biased ligands holds significant implications for the development of drugs with fewer side effects. Antibodies (Abs) are useful tools for targeting cell surface proteins; however, developing signaling competent GPCR-targeted Abs remains challenging and is often unsuccessful. Camelid single-domain antibodies (or nanobodies, Nbs) have been shown to target epitopes in GPCRs not accessible to conventional Abs. However, it remains difficult to identify Nbs that can directly activate GPCR signaling. To overcome this challenge, we developed a unique methodology in our lab to link PTHR1-binding Nbs with synthetic ligands that directly modulate GPCR function.

Tools and methods

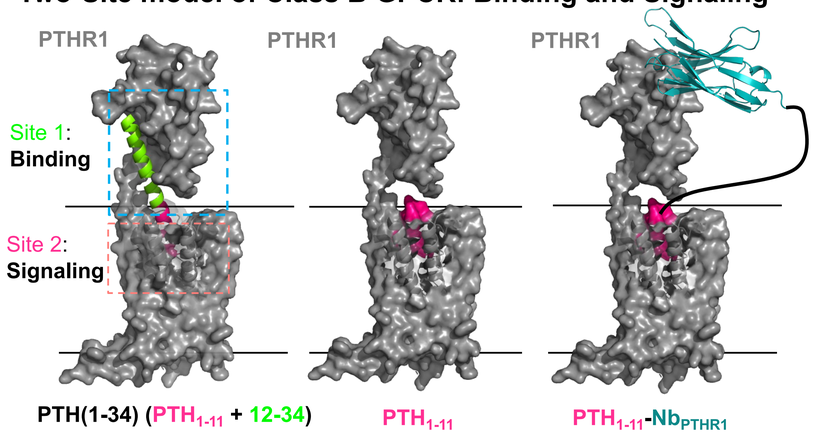
Single domain antibodies (nanobody/VHH)



Conventional antibody (IgG) features
- Gold standard for recognition of biomolecules
- Expressed in specialized cell lines or harvested from animal sera ($$)

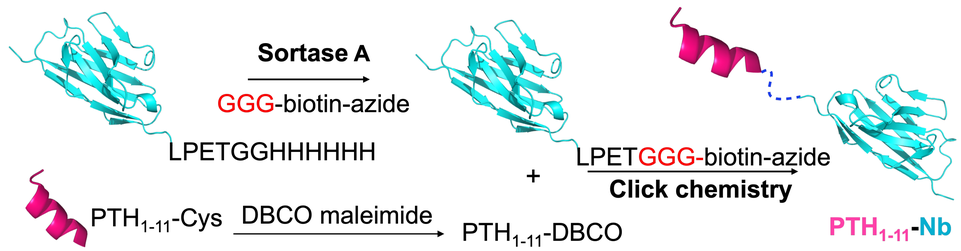
Nanobody (Nb) features
- Production by recombinant (bacterial) expression
- Straightforward protein engineering

Two-Site model of Class B GPCR: Binding and Signaling



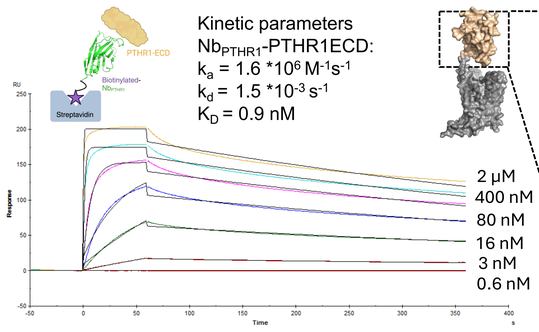
Results

1. Synthetic scheme for preparation of Nb-ligand conjugates

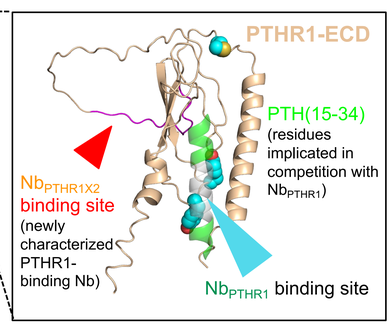


Homogenous preparation of Nb-ligand conjugates through a combination of recombinant Nb expression, enzymatic protein labeling, solid-phase peptide synthesis, and chemoselective click chemistry approach.

2. Characterization of the binding site of Nbs to PTHR1

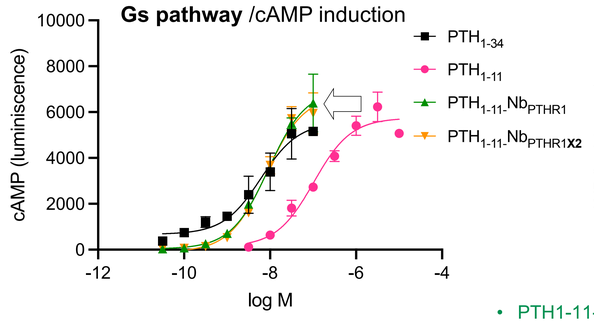


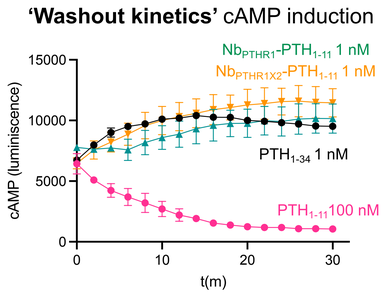
- **Assessment of Nb_{PTHR1} binding to PTHR1 extracellular domain** (ECD) using surface plasmon resonance demonstrated high affinity binding (K_d ~ 1 nM) with slow dissociation rates.

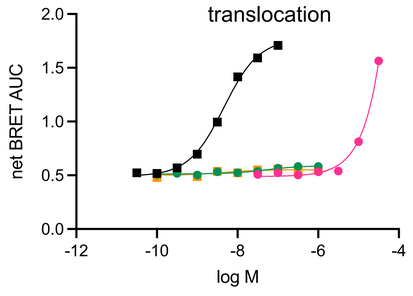


- A series of binding experiments revealed that **Nb_{PTHR1} partially shares its binding site with the PTH ligand** (PTH_{1-34}) while the newly characterized $Nb_{PTHR1X2}$ binds at a distinct site on PTHR1-ECD

3. Pharmacological characterization of Nb-ligand conjugates at PTHR1





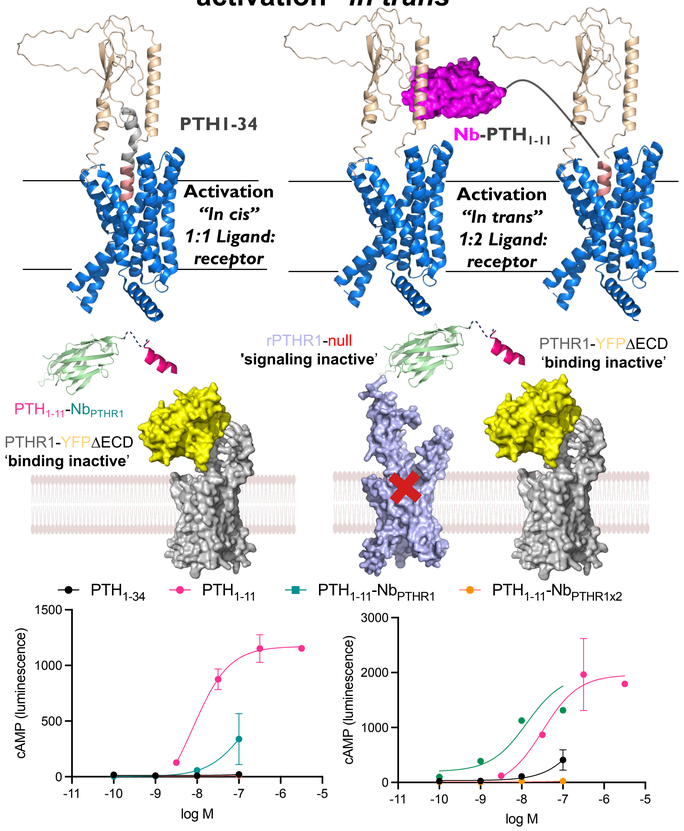


- PTH1-11-Nb conjugates **induced robust activation of Gαs** pathway without recruitment of β-arrestin (biased signaling).
- PTH1-11-Nb conjugates is **most highly biased agonist** of PTHR1 ligand reported to date!!!

PTHR1 EC50 (±SEM) Ligands	Gαs (cAMP)	cAMP Washout AUC	β-arrestin 2 recruitment
PTH_{1-34}	0.1 (0.06)	5.4 (1.7)	62 (12)
PTH_{1-11}	26 (2.6)	342 (116)	12300 (940)
PTH_{1-11}-Nb_{PTHR1}	3 (0.5)	4 (0.6)	-
PTH_{1-11}-$Nb_{PTHR1X2}$	5 (1.5)	2.3 (1)	-

*AUC area under curve

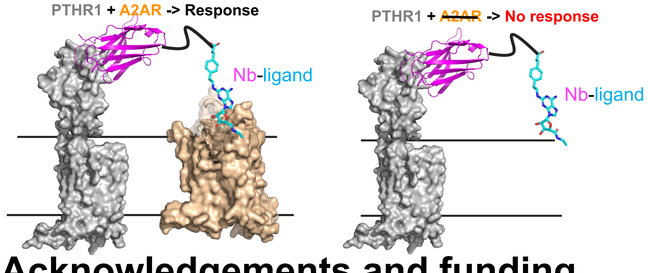
4. Putative mechanisms leading to biased agonism: activation "*in trans*"



'Activation in trans' by Nb-ligand conjugates was tested in a system expressing two PTHR1 constructs: a **N-terminally truncated PTHR1 that poorly binds Nb_{PTHR1}** but remains signaling competent, and a **full-length rat PTHR1 with mutations** in the transmembrane region that renders it **signaling inactive.**

Conclusion and future directions

Linking Nbs to peptide GPCR ligands provides:
- Modular semi-synthetic GPCR ligands
- Improved receptor affinity and potency
- Pathway selective signaling (biased agonism)
- New mechanism of receptor activation ("*in trans*")
- Using the platform to target receptor heterotrimeric complexes



Acknowledgements and funding

NIH Intramural Research Program
Our Research Changes Lives
HARVARD MEDICAL SCHOOL

Manuscript under revision in *Nature Communications;* Preprint available: Highly Biased agonism for GPCR ligands via nanobody tethering 10.1101/2023.10.10.561766