Gq protein peptidomimetics as allosteric modulators of the ghrelin receptor

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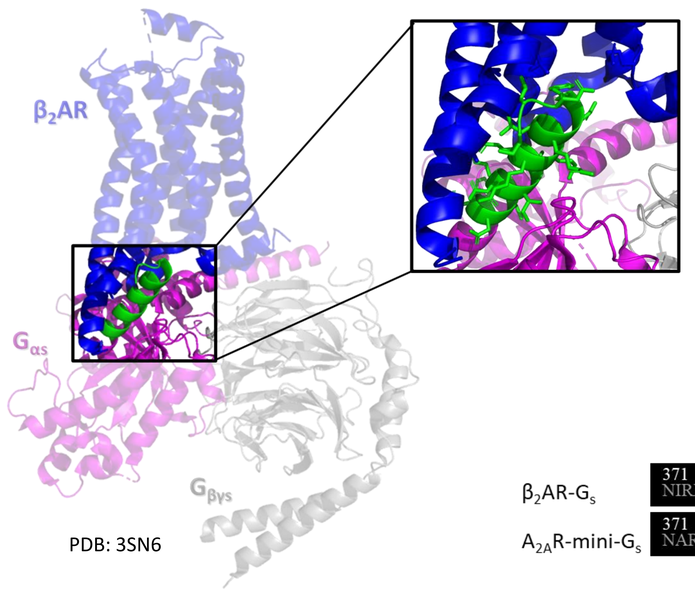
INTRODUCTION

G protein-coupled receptors (GPCRs) represent an important family of membrane receptors that play a central role in modern medicine. Unfortunately, it is quite challenging to gain insights into the structure and functioning of GPCRs due to their **intrinsic dynamics** and **high instability** when extracted from the cell membrane.

In past years, **Nanobodies** (Nbs) were discovered to lock GPCRs in active signaling states and these have been recognized to be of extreme value in the search of specific orthosteric GPCR agonist ligands.[1] However, to overcome the limitations associated to Nbs, in terms of development costs for the discovery and purification, a set of **peptidomimetics** stabilizing the active state conformation of G protein-coupled receptors, was developed.[2,3] Herein, a peptide mimicry approach was used to **mimic the G protein**, an endogenous allosteric modulator of GPCRs, by synthesizing rigidified variants of the helical *C*-terminal Gα protein epitope (α_5 helix), which interacts with the GPCR.[4]

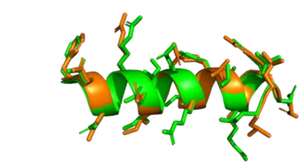
DESIGN OF G_s PROTEIN PEPTIDOMIMETICS



α_5 helix

- Highly conserved
- Major interaction with GPCR

→ Key epitope for (generic) G_s mimetics



	371	376	381	386	391
β_2AR-G_s	N	I	R	R	V
A_{2A}R-mini-G_s	N	A	R	R	I

F	N	D	C	R	D
F	N	D	C	R	D

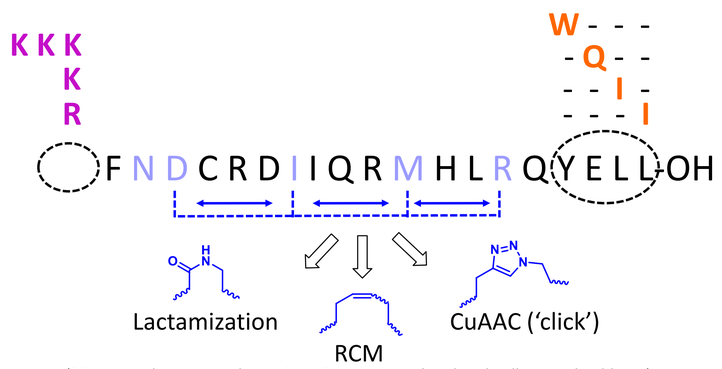
I	Q	R	M	H	L
I	Q	R	M	H	L

R	Q	Y	E	L	L
R	Q	Y	E	L	L

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF G_s PROTEIN PEPTIDOMIMETICS

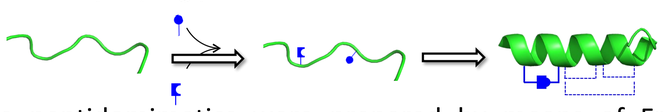
***N*-terminus modification**
-> To improve solubility

***C*-terminus modification**
-> To improve affinity for receptor



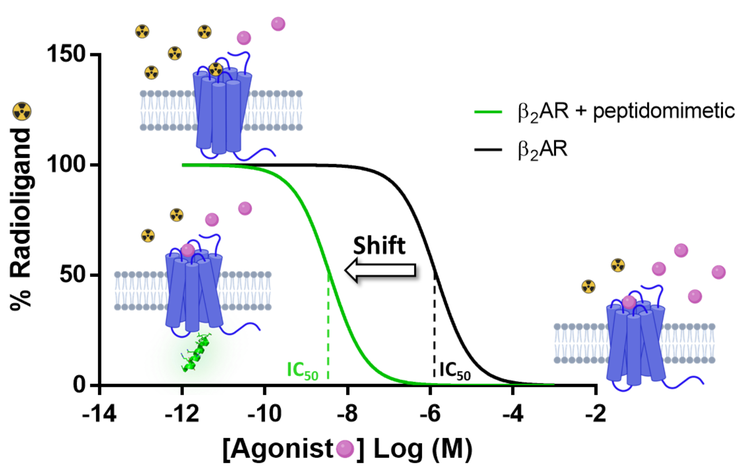
(RCM: ring-closing metathesis; CuAAC: copper-catalyzed azide-alkyne cycloaddition)

Cyclization ('stapling')

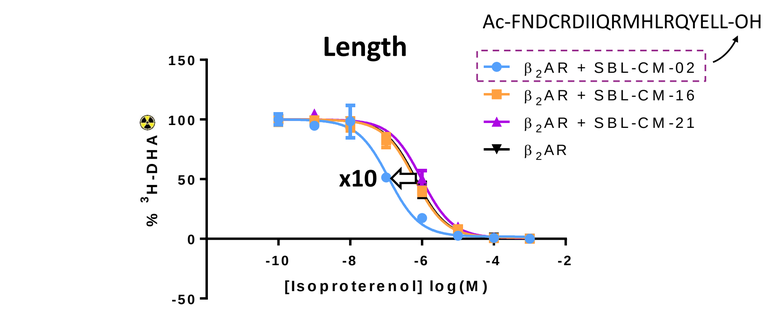


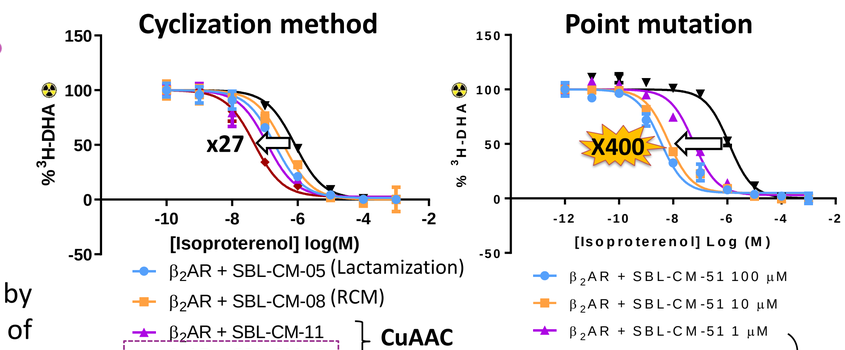
The peptidomimetics were prepared by means of Fmoc-based solid phase peptide synthesis (**SPPS**) and the α-helical conformation was stabilized using '**stapling**' strategies. Based on the X-ray crystal structure of the β_2AR in complex with the G_s protein, **point mutations** were introduced at the *C*-terminus.[5]

Radioligand displacement assay (RLA) on β_2AR



Optimization cycles of peptidomimetics were guided by radioligand binding assays, that quantify the stabilization of the receptor in active conformation.

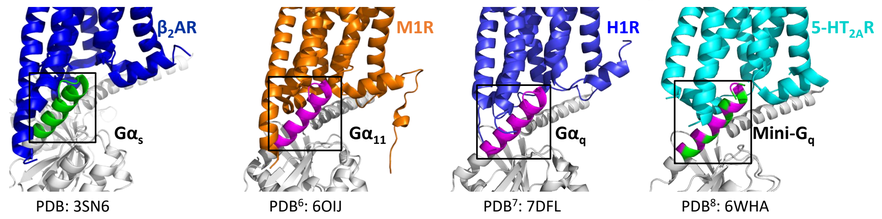




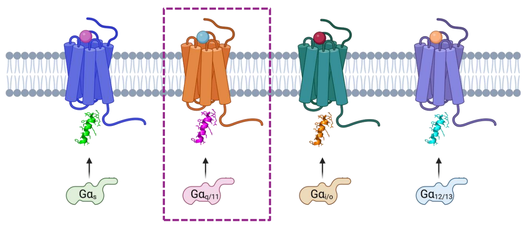
(Azk: Azidolysine; Cha: Cyclohexylalanine; Pra: Propargylglycine)

EXPLORE OTHER G PROTEIN FAMILIES

Crystal structure GPCR-G_s | **Cryo-EM structure GPCR-$G_{q/11}$**



PDB: 3SN6 | PDB[6]: 6OIJ | PDB[7]: 7DFL | PDB[8]: 6WHA



⇨ **Insert best modifications of G_s peptide into (mini-)$G_{q/11}$ peptides**[9,10]

SBL-CM-51 (G_s peptide)
Ac-**KKK**FN$_c$[**Pra**CRD**Azk**]IQRMHLRQYE**Cha**L-OH

$G_{q/11}$	Mini-G_q
SBL-GQ-05 Ac-**KKK**FA$_c$[**Pra**VKD**Azk**]ILQLNLKEYNLV-OH	**SBL-GQ-11** Ac-**KKK**FN$_c$[**Pra**CKD**Azk**]ILQMNLREYNLV-OH
SBL-GQ-06 Ac-**KKK**FA$_c$[**Pra**VKD**Azk**]ILQNLKEYN**Cha**V-OH	**SBL-GQ-12** Ac-**KKK**FN$_c$[**Pra**CKD**Azk**]ILQMNLREYN**Cha**V-OH

CONCLUSIONS

Proof-of-concept: G_s peptidomimetics

- Stabilize active state of GPCR
- Generic toolset for G_s-coupled receptors
- ✓ Validation on Dopamine 1 receptor (D1R)
- Identification of agonist-like fragments by fragment-based screening

⇨ **Same methodology used to develop G_q peptidomimetics**

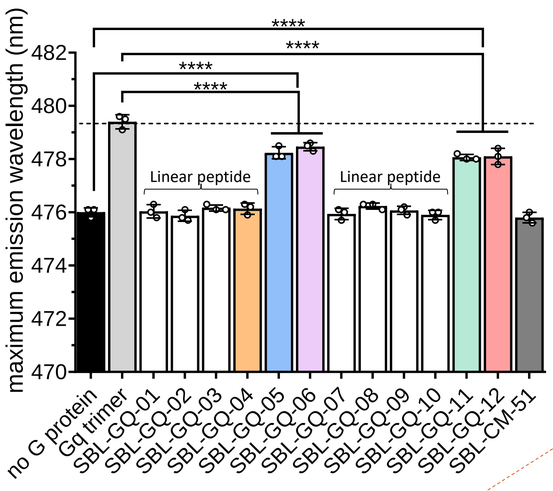
PERSPECTIVES

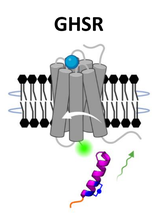
- Explore other G protein epitopes ($G_{i/o}$, $G_{12/13}$)
  - -> Drug discovery
  - -> Further unveil GPCR:G protein coupling
- Investigate cell permeability of the G protein peptidomimetics

PHARMACOLOGICAL EVALUATION OF G_q PEPTIDOMIMETICS

Bimane fluorescence assay

⇨ Detect conformational changes associated with receptor activation



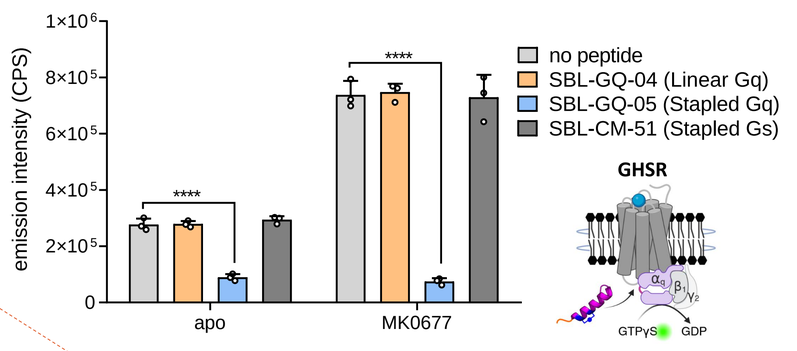


- Increase in maximum emission wavelength with stapled G_q peptides
- No change with linear G_q peptides

-> Staple required to affect receptor conformation

BODIPY-GTPγS assay

⇨ Investigate competition with G_q protein (measure GTP uptake)



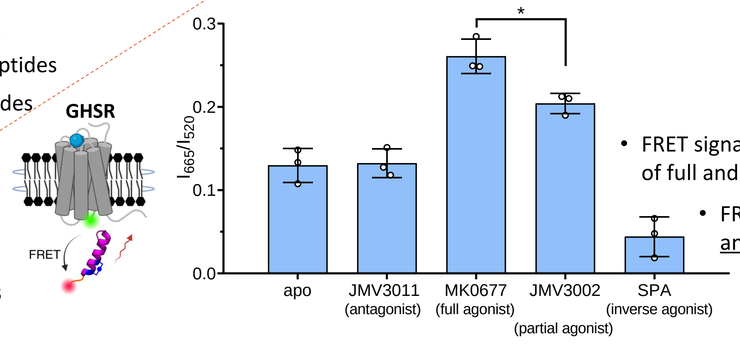
Receptor activation: GDP-to-GTP exchange

- SBL-GQ-05 compete with receptor-catalyzed G protein activation
  - Linear G_q peptide and stapled G_s peptide do not affect GTPγS binding

-> Specificity of the constrained *C*-terminal helix of Gα$_q$

FRET-based assay

⇨ Measure direct binding to receptor



- FRET signal increased in presence of full and partial agonist
  - FRET signal not affected by neutral antagonist
    - FRET signal completely abolished in presence of inverse agonist

REFERENCES

1. Rasmussen, S. G. *et al. Nature* **2011**, *469*, 175.
2. Mannes, M. *et al. Angew. Chem. Int. Ed.* **2021**, *60*, 10247.
3. Mannes, M. *et al.* International Patent Application No. PCT/EP2021/086733.
4. Mannes, M. *et al. Trends Pharmacol. Sci.* **2022**, *43*, 406.
5. Rasmussen, S. G. *et al. Nature* **2011**, *477*, 549.
6. Maeda, S. *et al. Science* **2019**, *364*, 552.
7. Xia, R. *et al. Nat. Commun.* **2021**, *12*, 1.
8. Kim, K. *et al. Cell* **2020**, *182*, 1574.
9. Mannes, M. *et al.* European Patent Application No. 22180492, **2022**
10. Mannes, M. *et al. Science Signaling* (Under revision)

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