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Development of G Protein Peptidomimetics to Stabilize Active State G Protein-Coupled Receptors

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Introduction

G protein-coupled receptors are cell-surface proteins that have been of long-standing interest because of their involvement in various physiological processes. To date, the pharmaceutical industry has more effectively exploited GPCRs than any other target class. However, the vast majority of this large class of targets remains therapeutically untapped [1]. Targeting GPCRs for drug discovery is still challenging because of their intrinsic dynamics and instability when extracted from the cell membrane, making them difficult to isolate and purify for use in screening assays. In past years, Confobodies (Cb, single-chain camelid antibody) were discovered to stabilize GPCRs in their active signaling state and they played an important role in the search for orthosteric GPCR agonist ligands [2,3]. The Confobody strategy is an attractive technology, that enables agonist drug discovery by looking for small molecule fragments that selectively bind to the confobody-constrained signaling conformer of a GPCR. Although Cbs have been recognized to be of extreme value in the search for novel therapeutics and in structural biology, the significant costs and time required for the discovery and development of specific Cbs has sparked our interest to develop a new, potentially more generic, technology to stabilize GPCRs to use in drug discovery. To overcome the drawbacks associated to Cbs, we developed an approach to mimic the G protein, an endogenous allosteric modulator of GPCRs, *via* a peptide mimicry approach [4]. Based on a rational design strategy a set of peptidomimetic ligands, stabilizing the active state conformation of the β_2 adrenergic receptor (β_2AR), was developed [5,6]. Therefore, a previously identified epitope of the G_s protein, the α_5 helix (F^{376}NDCRDIIQRMHLRQYELL^{394}), that is responsible for most interaction with the β_2AR [7], was chosen as template to design the peptidomimetics (Figure 1).

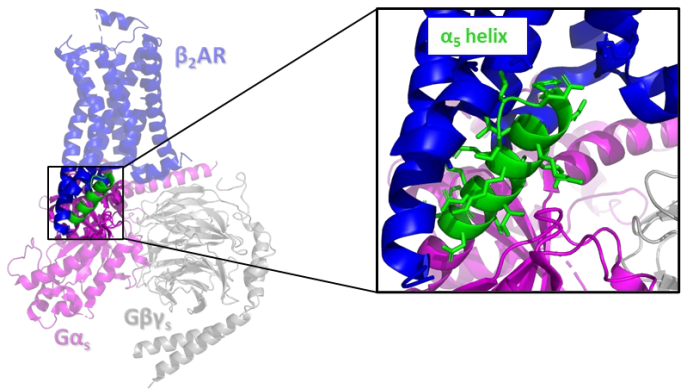

Fig. 1. Crystal structure of the β_2AR bound to the heterotrimeric G_s protein from protein data bank entry (PDB): 3SN6. Zoom on the interaction of the α_5 helix with the receptor.

Results and Discussion

The peptidomimetics were prepared using the Fmoc-based solid phase peptide synthesis (SPPS) and stabilized using 'stapling' strategies to improve binding to the targeted receptor (Figure 2). This was realized by varying the length, the type and the position of the staple that covalently bridges one or

two helical loops, inserted on one side of the helix, where no critical native contacts are made with the receptor. The α-helicity of the stapled peptides was evaluated using CD spectroscopy and the best results were obtained for the peptidomimetics with a triazole bridge near the *N*-terminus, generated through a copper-catalyzed azide-alkyne cycloaddition (**SBL-CM-12,** Ac-FN$_c$**[Pra**CRD**Azk]**IQRMH LRQYELL-OH). In addition, a trilysine (KKK) motif was introduced at the *N*-terminus to ensure solubility of the peptidomimetic. Simultaneously, the functionality of the peptidomimetic was screened using the radioligand displacement assay, to quantify the stabilization of the receptor in the active state conformation. Since the four last *C*-terminal amino acids of the key helix were suggested to be crucial residues for interaction with the receptor, point mutations were introduced in the lead peptide, **SBL- CM-12**. Unexpectedly, the substitution of the penultimate leucine (L^{393}) by a cyclohexylalanine residue (Cha), to give **SBL-CM-51**, resulted in an extraordinary affinity increase of the agonist for the stabilized receptor (Figure 2), comparable to Cb80, a well-studied allosteric modulator of β_2AR [2,5,6].

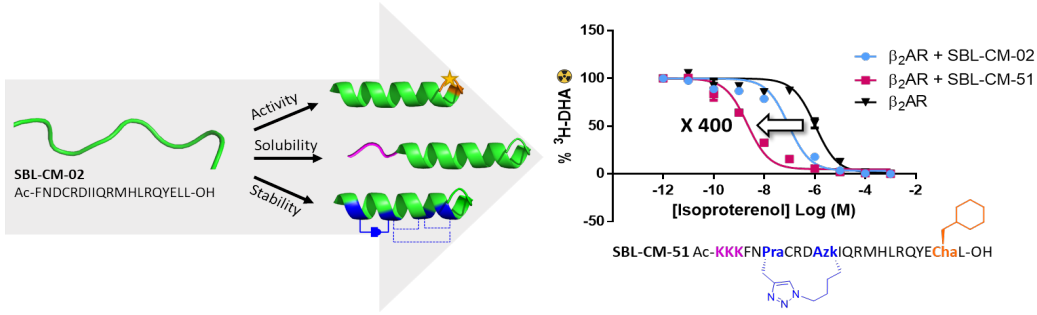

Fig. 2. Design of G_s-subunit peptidomimetics to stabilize β_2AR in the active signaling conformation. (standard amino acid one letter coding used; Azk: azidolysine; Cha: cyclohexylalanine; Pra: propargylglycine).

Next, the genericity of the identified G_s peptidomimetics was explored by testing other GPCRs, that signal through G_s [5,6]. Selected peptide mimetics were screened to evaluate their ability to bind and stabilize an active conformation of the dopamine 1 receptor (D1R) and similarly to β_2AR, the highest affinity shift was obtained for the stapled peptidomimetic containing the Cha point mutation (**SBL- CM-51**). The best-performing peptidomimetic **SBL-CM-51** was used next for fragment-based screenings on conformationally constrained receptors, to challenge the efficiency of the peptidomimetics to pick up fragments, for further design of agonist therapeutics. For both receptors, β_2AR and D1R, potential agonist fragments were identified and these could be optimized to design novel conformational-selective drugs targeting GPCRs [5,6].

Acknowledgments

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References

1. Hauser, A.S., et al. *Nature Reviews Drug Discovery* **16**, 829-842 (2017), https://doi.org/10.1038/nrd.2017.178
2. Rasmussen, S.G., et al. *Nature* **469**, 175-180 (2011), https://doi.org/10.1038/nature09648
3. Pardon, E., et al. *Angewandte Chemie International Edition* **57**, 5292-5295 (2018), https://doi.org/10.1002/anie.201712581
4. Mannes, M., et al. *Trends in Pharmacological Sciences* **43**, 406-423 (2022), https://doi.org/10.1016/j.tips.2021.10.011
5. Mannes, M., et al. *Angewandte Chemie International Edition* **60**, 10247-10254 (2021), https://doi.org/10.1002/anie.202100180
6. Mannes, M., Martin, C., and Ballet, S. EP4015529 (2022).
7. Rasmussen, S.G., et al. *Nature* **477**, 549-555 (2011), https://doi.org/10.1038/nature10361