

RESEARCH HIGHLIGHT



Orchestrating NTSR1 signaling from the interface

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Biased allosteric modulators provide great therapeutic potential by selectively directing signal bias in the presence of endogenous ligand under (patho)physiological conditions. In a recent *Cell Research* paper, Sun et al. revealed the structural mechanisms underlying the biased allosteric modulation exerted by SBI-553 directly at the neurotensin receptor 1- β -arrestin1 interface.

Upon activation, G protein-coupled receptors (GPCRs) elicit diverse downstream signaling cascades by coupling to different heterotrimeric G proteins. These signals are spatially and temporally regulated through the coupling to the non-G protein effectors such as GPCR protein kinases (GRKs) and arrestins. Biased signaling is a phenomenon in which GPCR is stabilized in a certain conformation that preferentially activates certain pathways over the others.¹ This concept has attracted significant attention in GPCR drug discovery due to the potential for selectively activating the beneficial signaling pathways while minimizing activation of those associated with side effects. Acting on less-conserved allosteric sites, allosteric modulators show advantages in finely tuning the actions of orthosteric ligands selectively. Biased allosteric modulators (BAMs) therefore represent a promising class of GPCR ligands for developing safer and more effective therapeutics.²

Through high-throughput screening and medicinal chemistry optimization, SBI-553 was discovered as a small-molecule BAM that selectively modulates neurotensin receptor 1 (NTSR1). It enhances NTSR1-mediated β -arrestin1/2 (β Arr1/2) recruitment while hindering the activation of G_q protein in the presence of neurotensin (NTS).^{3–5} Given its high oral bioavailability, brain-penetrant property and interesting BAM activity, SBI-553 represents a promising candidate for developing safe therapeutics targeting NTSR1 for anti-psychostimulant effects.^{3–5} It is therefore crucial to understand the molecular mechanisms of this pathway-selective allosteric modulation. Using cryo-electron microscopy (cryo-EM), previous studies have elucidated the structures of NTS–NTSR1 in complex with GRK2⁶/ G_o protein⁵ with and without SBI-553 bound, as well as the NTS–NTSR1– β Arr complexes.^{7,8} In a recent study, Sun et al.⁹ resolved the final piece of the puzzle by elucidating the structure of the full-length NTSR1 with β Arr1 bound in the presence of both SBI-553 and NTS, revealing a new engagement configuration of the GPCR– β Arr1 complex.

To determine a high-resolution structure, highly stable and homogenous complex samples are essential. Sun et al. employed a chemical protein synthesis strategy to obtain full-length NTSR1 with six phosphorylated residues at its C-terminus for β Arr1 binding. They first identified and validated the phosphorylation sites essential for high-affinity β Arr1 binding via fluorescence

polarization assay. Next, the authors synthesized the hexa-phosphorylated C-terminal tail (C-tail) of NTSR1 with microwave-assisted solid phase peptide synthesis. This phosphorylated C-tail and NTSR1 transmembrane domain were fused with the segments from the split Cfa DnaE intein. The engineered split intein segments directed both parts of the NTSR1 to join, followed by *trans*-splicing reaction removing the intein itself, leading to the generation of full-length phosphorylated NTSR1. This method ensured a uniform and precisely controlled phosphorylation pattern of GPCR, compared to the traditional way which relies on the action of GRK.

The authors then assembled this phosphorylated NTSR1 with constitutively active β Arr1 and NTS_{8–13}, with or without SBI-553, for structural determination using cryo-EM. A fragment antigen-binding region Fab30 and phosphatidylinositol-4,5-bisphosphate were also added to further enhance the stability of the complexes. With these optimizations, Sun et al. successfully determined the cryo-EM structure of the active NTSR1– β Arr1 complex at a higher resolution than all the previously reported GPCR– β Arr1 structures.

Consistent with the previously reported NTSR1– β Arr1 structures,^{7,8} NTSR1 interacts with β Arr1 through both ‘core engagement’ and ‘tail engagement’, where its intracellular transmembrane cavity interacts with the central crest of β Arr1 and its phosphorylated C-tail engages with the N-terminal lobe of β Arr1. These two primary interfaces were found in the complexes regardless of the presence of SBI-553. Although the binding of SBI-553 in the intracellular cavity slightly pushes down the finger loop in the central crest of the β Arr1, the interaction between NTSR1 and β Arr1 is not disrupted. SBI-553 directly interacts with the hydrophobic intracellular cavity of NTSR1 via its pendant phenyl ring and engages the finger loop of β Arr1 through its quinazoline group, bridging and stabilizing the interactions between NTSR1 and β Arr1.

In addition to the conventional core engagement configuration, Sun and colleagues discovered a new ‘loop engagement’ configuration that is only observed in the SBI-553-bound complex but not in the SBI-553-unbound complex (Fig. 1a). In this ‘loop engagement’ complex, both intracellular loop 1 (ICL1) and ICL3 of NTSR1 are more extended. The quinazoline group interacting with the finger loop of β Arr1 in the conventional ‘core engagement’, now forms hydrophobic interactions with the ICL3 of NTSR1. This, in turn, bent the loop towards the intracellular cavity and stabilized it, which enables the ICL3 to interact with the finger, middle, gate and lariat loops in the central crest of β Arr1. In addition, the more extended ICL1 of the NTSR1 forms strong interactions with the lariat loop of β Arr1. The interactions between these key residues were confirmed using mutagenesis study. Other than these additional interfaces, Sun et al. found that the

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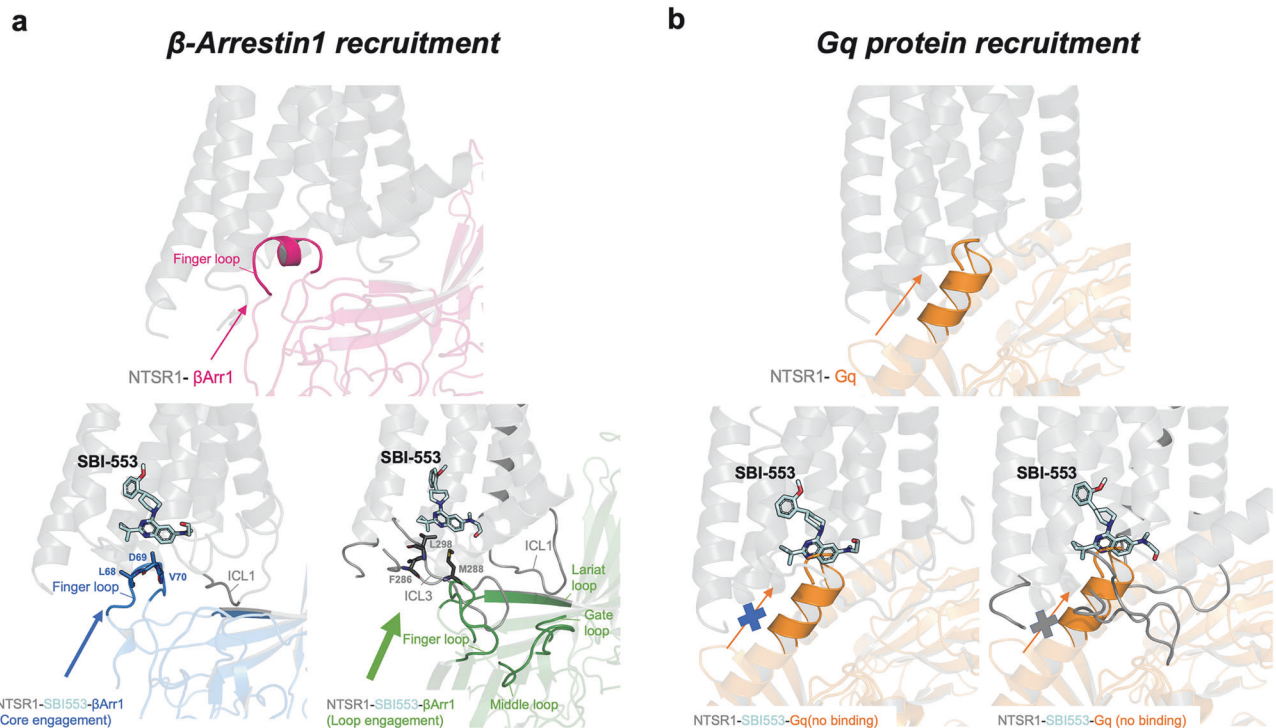


Fig. 1 Structural mechanisms of the biased allosteric modulation exerted by SBI-553 at NTSR1. **a, b** BAM SBI-553 potentiates β Arr1 recruitment (**a**) and prevents G_q protein recruitment (**b**) by binding to the intracellular cavity of active NTSR1. **a** Structural model of NTSR1- β Arr1 complexes in the absence of SBI-553 (top, PDB: 6UP7⁸) and in the presence of SBI-553 (bottom left, PDB: 8ZYU; bottom right, PDB: 8ZYT⁹). SBI-553 could either directly bind to the finger loop of β Arr1 to facilitate the interactions (core engagement) or orientate the ICL3 of NTSR1 to form extensive interactions with the loops in β Arr1 (loop engagement). The bottom part of NTSR1 is shown in light gray cartoon with the loops interacting with β Arr1 labeled in dark gray. β Arr1 in SBI553-unbound/core engagement/loop engagement configuration is labeled in pink/blue/green cartoon, respectively, with the key interacting loops highlighted. Critical residues interacting with SBI-553 are shown in sticks. **b** Structural model of the active NTSR1- G_q complex (top, PDB: 8FMZ³). Structural overlay of the active NTSR1- G_q complex with the SBI-553-NTSR1 from the SBI-553-NTSR1- β Arr1 core engagement complex (bottom left) or from the SBI-553-NTSR1- β Arr1 loop engagement complex (bottom right). The structural overlay suggests that SBI-553 occupies the binding site of the C-terminus of G_q $\alpha 5$ helix (orange helix). In addition, active NTSR1 with ICL3 bent and stabilized by SBI-553 could not bind to G_q protein due to strong steric clashes.

engagement of the phosphorylated C-tail of NTSR1 with β Arr1 is still maintained. Using PDBePISA, the authors quantified the total interaction area, revealing that the 'loop engagement' configuration exhibits stronger interactions between NTSR1 and β Arr1 than the 'core engagement' configuration. Importantly, Sun et al. suggested that the orientation of these ICLs stabilized by SBI-553 only fits well with the loops in β Arr1 but creates steric hinderance for G protein binding based on the overlayed structural models (Fig. 1b).

In summary, both SBI-553-bound NTSR1 configurations favor β Arr1 binding over the other transducers, explaining the BAM activity of SBI-553 from an elegant structural perspective. Superimposition of the arrestin complex structures with previously reported structures of GRK/ G_{oA} complexes reveals a strikingly conserved NTSR1 receptor core with only changes in ICL1 and ICL3 as discussed earlier. Additionally, the pose of SBI-553 quinazoline moiety is also well conserved in these structures with only minor displacement of the pendant phenyl moiety. The flexibility of its binding pose within the hydrophobic cavity provides great potential for future drug discovery. Together, these structural insights could further guide the design and development of BAMs, for precisely tuning biased signaling directly from the receptor-transducer interface.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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