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Spotlight

De-Nterm-ining GLP1R signaling bias

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Biased agonism has emerged as a promising strategy for improving incretin therapeutics, yet the structural determinants of signaling bias remain incompletely understood. Building on their recent parathyroid hormone 1 receptor–β-arrestin structure, Zhao and colleagues identify extracellular loop 3 as a conformational switch controlling glucagon-like peptide-1 receptor transducer selectivity, enabling rational design of biased agonists.

Glucagon-like peptide-1 receptor (GLP1R) agonists, including liraglutide and semaglutide, have transformed the treatment of type 2 diabetes and obesity. GLP1R is expressed in key metabolic tissues, including pancreatic islets and the central nervous system, where its activation enhances glucose-dependent insulin secretion and reduces food intake [1]. As a class B G protein-coupled receptor (GPCR), GLP1R primarily signals through Gαs to stimulate cyclic AMP (cAMP) production. Receptor activation also promotes phosphorylation by GPCR kinases and recruitment of β-arrestins, which regulate receptor trafficking, desensitization, and intracellular signaling [1].

Increasing evidence suggests that not all GLP1R agonists engage signaling pathways equally. Early studies demonstrated that endogenous and therapeutic GLP1R agonists, including GLP1, oxyntomodulin, and exendin-4, can stabilize distinct receptor states, resulting in divergent signaling and trafficking responses [2]. Seminal pharmacological and structural studies identified the extracellular receptor surface as a key determinant of signaling bias, while cryo-electron microscopy structures revealed the structural basis by which biased agonists favor particular signaling outcomes [2,3]. These observations were subsequently reinforced by studies of N-terminally modified agonists, including exendin-P5, which displayed enhanced G-protein selectivity and reduced β-arrestin recruitment [3]. Despite reduced acute signaling efficacy, these ligands produced prolonged antihyperglycemic and weight-lowering effects, establishing signaling bias as a potentially exploitable feature of GLP1R pharmacology [4].

Recently, Zhao and colleagues reported the first class B GPCR–β-arrestin structure using the related parathyroid hormone 1 receptor (PTH1R), identifying reciprocal extracellular loop 3 (ECL3) conformations associated with β-arrestin and G-protein engagement [5] (Figure 1A). Whereas β-arrestin coupling was associated with inward movement of ECL3 and the extracellular end of transmembrane helix 5 (TM5), the G-protein-bound state retained ECL3 in an outward position. Guided by this reciprocal arrangement, they engineered PTH analogs bearing enlarged N-terminal side chains that sterically hindered ECL3 inward movement, generating G-protein-biased agonists. Whether this ECL3-based design principle could be translated to GLP1R was the question addressed next.

In a recent issue of *Cell Reports*, the same group identifies an unexpected structural feature associated with GLP1R signaling bias: outward displacement of ECL3 [6]. To identify structural determinants of signaling bias, the authors compared GLP1R–G_s complexes bound to GLP1, oxyntomodulin, and exendin-P5, representing balanced, β-arrestin-biased, and G-protein-biased agonists, respectively. A striking pattern emerged. Only the G-protein-biased agonist exendin-P5 induced an outward displacement of ECL3, located between transmembrane helices 6 and 7 (TM6 and TM7), away from the ligand-binding pocket. By contrast, GLP1- and oxyntomodulin-bound structures retained ECL3 in a position closer to the peptide ligand. These findings led the authors to propose ECL3 as an extracellular switch linking ligand engagement to transducer selectivity (Figure 1B), extending to GLP1R the ECL3-based mechanism of G protein versus β-arrestin coupling recently identified at PTH1R [5].

Having identified ECL3 as a candidate extracellular switch, Zhao and colleagues next asked whether signaling bias could be rationally designed. Guided by interactions between ECL3 and the peptide N-terminal region, and building on the steric design strategy established at PTH1R, they generated a panel of GLP1 analogs incorporating N-terminal acetylation or bulky amino acid substitutions at positions 7 and 11 (Figure 1C). The strategy proved successful: acetylated GLP1 and a GLP1 analog containing a histidine-to-tryptophan substitution at position 7 (H7W; GLP1-W7) displayed pronounced G-protein bias, with substantially reduced recruitment of both β-arrestin isoforms. Importantly, this design principle also translated to semaglutide. N-terminal acetylation of semaglutide similarly reduced β-arrestin engagement, although the effect was more selective for β-arrestin1 over β-arrestin2, suggesting that the consequences of N-terminal modification may depend on the broader ligand scaffold. Consistent with their signaling profile, Ac-GLP1 and Ac-semaglutide exhibited altered receptor trafficking dynamics and more sustained cAMP responses. These changes translated into enhanced glucose lowering *in vivo*, although the effects were context-dependent. While Ac-GLP1 showed improved acute glucose control relative to native GLP1, the most pronounced effects were observed with Ac-semaglutide, which maintained superior glucose-lowering efficacy several days after administration in lean mice and mice with

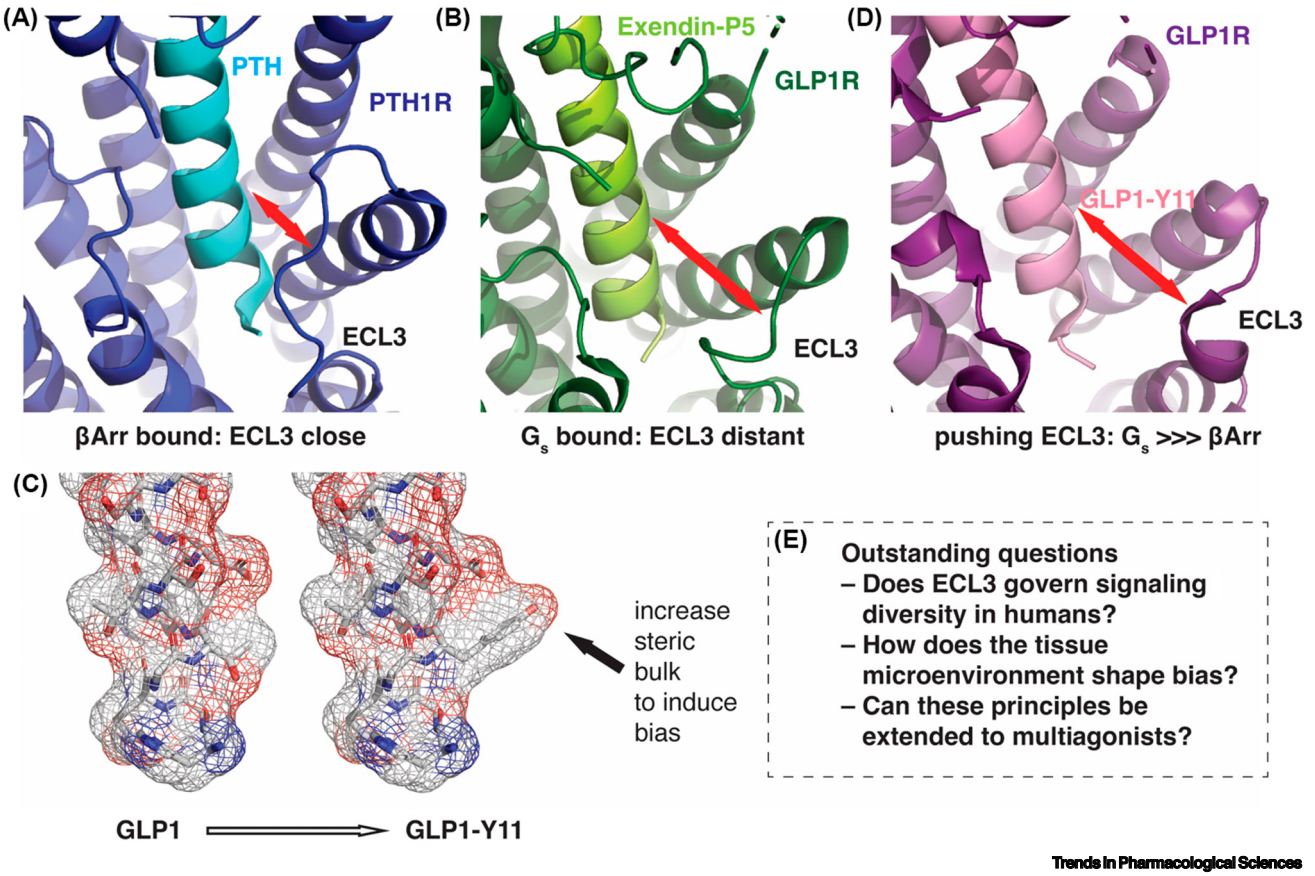


Figure 1. ECL3 as a structural switch controlling GLP1R signaling bias. (A) The inward ECL3 conformation associated with β-arrestin engagement is inferred from the core-engaged PTH1R–β-arrestin1 structures (PDB: 9LXR and 9LXP). (B) Structure of GLP1R bound to G_s-biased exendin-P5 (PDB: 6B3J), showing ECL3 displaced away from the peptide ligand. (C) Space-filling representations of GLP1 and GLP1-Y11 illustrating the increase in steric bulk introduced by the T11Y substitution. (D) Conceptual model of GLP1R bound to GLP1-Y11, informed by the GLP1R:exendin-P5 structure (PDB: 6B3J), in which ECL3 is displaced away from the ligand. Zhao and colleagues propose that this conformational change favors G protein over β-arrestin recruitment. (E) Outstanding questions for future studies. βArr: β-arrestin; ECL3: extracellular loop 3; GLP1R: glucagon-like peptide-1 receptor; PTH1R: parathyroid hormone 1 receptor; PTH: parathyroid hormone; GLP1-Y11: GLP1 analog containing a threonine-to-tyrosine substitution at position 11.

diet-induced obesity. Together, these findings link ECL3 displacement to signaling bias and altered receptor dynamics, but the extent to which this also contributes to prolonged glycemic control remains an important question.

Finally, the authors brought the story full circle by solving the structure of GLP1R bound to a GLP1 analog containing a threonine-to-tyrosine substitution at position 11 (T11Y; GLP1-Y11), the ligand in the series displaying the greatest signaling bias. As predicted, GLP1-Y11 drove a marked outward displacement of ECL3 (Figure 1D). This movement was associated with weaker interactions between the peptide and residues within the TM7–ECL3 interface, reducing stabilization of the loop and allowing greater conformational freedom. From a drug discovery perspective, the implications are considerable: subtle increases in steric bulk within the peptide N-terminal region may be sufficient to reshape receptor conformations and determine signaling outcomes. That the same N-terminal steric principle is observed at two class B receptors, PTH1R and GLP1R, argues that it may be a transferable design rule rather than a receptor-specific quirk. Whether such bias ultimately translates into superior clinical efficacy, however, remains less certain. Although biased agonists have demonstrated promising efficacy in preclinical systems [4], the contribution of signaling bias to therapeutic outcomes in humans remains difficult to disentangle from other pharmacological properties, including receptor potency, pharmacokinetics, and dual- and triple-agonist activity. Along these lines, it will be important to determine whether the principles uncovered by Zhao and colleagues extend beyond selective GLP1R agonists. The impact of analogous N-terminal modifications on dual- and triple-agonist pharmacology remains largely unexplored, particularly in an era where

receptor imbalance favoring glucose-dependent insulinotropic polypeptide receptor (GIPR) activation is viewed as a desirable therapeutic trait [7]. Understanding how signaling bias intersects with receptor selectivity may therefore help guide the design of future multiagonist therapeutics.

A broader challenge is determining how measurements of signaling bias relate to pharmacological responses *in vivo*. Zhao and colleagues take an important step in this direction by linking ECL3 displacement to altered receptor trafficking, prolonged cAMP signaling, and improved glucose-lowering efficacy in preclinical models. Nevertheless, bias is typically quantified in recombinant cell systems, whereas therapeutic peptides operate within a far more complex physiological milieu. Emerging evidence suggests that the spatial organization of GLP1R signaling, including receptor nanodomain formation, membrane localization, and pre-internalization, can itself influence downstream responses, highlighting the importance of cellular context in determining receptor function [8,9]. Thus, while Zhao and colleagues provide a compelling structural blueprint for engineering GLP1R bias, an equally important question is whether signaling bias represents a fixed property of a ligand or a feature shaped by the wider tissue microenvironment. A further step will be to determine whether this structural principle can be extended beyond engineered ligands. Testing naturally occurring GLP1R variants, alongside mutations within the ECL3 interface that are predicted to alter loop mobility, could establish whether ECL3 displacement is also a determinant of signaling diversity in humans. A GLP1R–β-arrestin structure would allow the arrestin-bound ECL3 conformation to be visualized directly, providing an important test of the mechanism inferred from PTH1R [5]. Such studies would complement emerging human genetic evidence that variation in GLP1R signaling and trafficking influences metabolic phenotypes [10], potentially providing a direct connection between receptor bias and clinical efficacy. Resolving these questions will determine whether engineering signaling bias becomes a core design principle for the next generation of incretin therapeutics (Figure 1E).

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used OpenAI's ChatGPT to improve the accessibility, grammar, and readability of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of interests

D.J.H. and J.B. have filed a patent on incretin receptor chemical probes (WO2024133236A3). D.J.H. and J.B. receive licensing revenue from Celtarys Research for the provision of incretin receptor chemical probes. D.J.H. has filed patents related to diabetes therapy (WO2024062254A1 and WO2025191276A1). D.J.H. receives research funding from Amgen Inc.

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