Biased Agonism and Polymorphic Variation at the GLP-1 Receptor: Implications for the Development of Personalised Therapeutics

El Eid, L., Reynolds, C. A., Tomas, A. & Ben Jones.

DOI 10.1016/j.phrs.2022.106411
ISSN 1043-6618
ESSN 1096-1186

Publisher: Elsevier

Pharmacological Research 184 (2022) 106411

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Biased agonism and polymorphic variation at the GLP-1 receptor: Implications for the development of personalised therapeutics

Liliane El Eid [a], Christopher A. Reynolds [b,c], Alejandra Tomas [a,*], Ben Jones [d,**]

[a] Section of Cell Biology and Functional Genomics, Imperial College London, London, United Kingdom
[b] Centre for Sport, Exercise and Life Sciences, Faculty of Health and Life Sciences, Coventry University, Alison Gingell Building, United Kingdom
[c] School of Life Sciences, University of Essex, Colchester, United Kingdom
[d] Section of Endocrinology and Investigative Medicine, Imperial College London, London, United Kingdom

ARTICLE INFO

Keywords:
GLP-1
Bias
Polymorphisms
Arrestin

ABSTRACT

Glucagon-like peptide-1 receptor (GLP-1R) is a well-studied incretin hormone receptor and target of several therapeutic drugs for type 2 diabetes (T2D), obesity and, more recently, cardiovascular disease. Some signalling pathways downstream of GLP-1R may be responsible for drug adverse effects such as nausea, while others mediate therapeutic outcomes of incretin-based T2D therapeutics. Understanding the interplay between different factors that alter signalling, trafficking, and receptor activity, including biased agonism, single nucleotide polymorphisms and structural modifications is key to develop the next-generation of personalised GLP-1R agonists. However, these interactions remain poorly described, especially for novel therapeutics such as dual and tri-agonists that target more than one incretin receptor. Comparison of GLP-1R structures in complex with G proteins and different peptide and non-peptide agonists has revealed novel insights into important agonist-residue interactions and networks crucial for receptor activation, recruitment of G proteins and engagement of specific signalling pathways. Here, we review the latest knowledge on GLP-1R structure and activation, providing structural evidence for biased agonism and delineating important networks associated with this phenomenon. We survey current biased agonists and multi-agonists at different stages of development, highlighting possible challenges in their translational potential. Lastly, we discuss findings related to non-synonymous genomic variants of *GLP1R* and the functional importance of specific residues involved in GLP-1R function. We propose that studies of GLP-1R polymorphisms, and specifically their effect on receptor dynamics and pharmacology in response to biased agonists, could have a significant impact in delineating precision medicine approaches and development of novel therapeutics.

1. Introduction

The glucagon-like peptide-1 receptor (GLP-1R), a transmembrane G protein-coupled receptor (GPCR) belonging to the class B/secretin family, mediates the physiological response to the incretin hormone glucagon-like peptide-1 (GLP-1), and is currently a major therapeutic target for metabolic disorders including type 2 diabetes (T2D) and obesity. GLP-1 is released by intestinal L-cells in response to meal ingestion and plays an important role in regulating postprandial glycemia. Class B GPCRs are structurally similar, characterised by a distinct large extracellular N-terminal domain (ECD) (~120 residues), seven transmembrane domains (TMDs) separated by three intracellular loops (ICL1–3), three extracellular loops (ECL1–3), and an intracellular C-terminal domain [1]. The human and rat GLP-1R were first cloned by the Thorens group from a pancreatic islet cDNA library in 1992, revealing a 463-residue receptor [2,3].

The main functions of the GLP-1R include potentiation of glucose-stimulated insulin secretion (GSIS), insulin biosynthesis, inhibition of glucagon secretion, slowing of gastrointestinal mobility, and appetite regulation [4]. Additional effects reported include maintenance of renal function [5], regulation of blood lipids [6,7], and reduction of circulating inflammatory factors such as TNF-α [8]. The glucoregulatory and weight-lowering effects of GLP-1R activation make it an attractive target for the treatment of people with diabetes or obesity, but native GLP-1 is

* Corresponding authors.
** Corresponding authors.
E-mail addresses: a.tomas-catala@imperial.ac.uk (A. Tomas), ben.jones@imperial.ac.uk (Ben Jones).

https://doi.org/10.1016/j.phrs.2022.106411
Received 3 July 2022; Received in revised form 16 August 2022; Accepted 18 August 2022
Available online 22 August 2022

unsuitable as a drug as it has a short half-life (~2 mins), being rapidly degraded and inactivated by the enzyme dipeptidyl peptidase 4 (DPP-4) in the circulation [9]. Hence, several peptide-based GLP-1R agonists (GLP-1RAs) have now been developed, with structural similarity to GLP-1, engineered for enhanced degradation resistance and prolonged pharmacokinetics.

Upon stimulation by endogenous or exogenous ligands, the GLP-1R activates Gαs leading to the accumulation of cyclic adenosine monophosphate (cAMP) and subsequent activation of pathways downstream of protein kinase A (PKA) and the guanyl nucleotide exchange factor EPAC2A [10–13]. It is generally believed that these pathways are the predominant signalling mediators of the insulinotropic effects of GLP-1 in pancreatic β-cells [11,12]. Beyond canonical cAMP-dependent signalling effects, the GLP-1R can couple to alternative G protein effectors such as Gαi/o and Gαq proteins, initiating signalling through other important pathways incorporating ERK1/2 and protein kinase B (PKB/Akt) [14–16]. Agonist binding to the GLP-1R also mediates the G protein receptor kinase (GRK)-mediated phosphorylation and rapid desensitisation of the active receptors, facilitating the coupling of β-arrestin 1 and β-arrestin 2 [17,18]. β-arrestins are typically believed to mediate receptor internalisation, but also act as scaffolds for G protein-independent signalling [18]. Moreover, β-arrestin 1 also promotes proliferation and inhibits apoptosis in β-cells [19,20]. A recent study investigating in vivo and *ex vivo* responses to GLP-1R pharmacological agonists (GLP-1RAs) in a β-cell specific β-arrestin 2 KO mouse model suggests that the absence of β-arrestin 2 leads to worse acute, but enhanced sustained pharmacological GLP-1R responses [21]. Contrary to the effect in other GPCRs, this study also revealed that GLP-1R receptor internalisation was not affected by the absence of β-arrestin 2, and instead other receptor trafficking mechanisms such as recycling and degradation were significantly altered [21]. Interestingly, the balance between how the active receptor engages with key intracellular effectors such as G proteins and β-arrestins can be agonist-specific; this phenomenon, known as "biased agonism", has attracted significant interest as it is hoped that it can be leveraged to optimise the pharmacodynamic profile of novel GLP-1R therapeutics [22].

GLP-1R signalling is further regulated by agonist-mediated trafficking of the receptor between different cellular compartments. For example, the magnitude of receptor responses to extracellular ligands is modulated by the endocytic removal of active receptors from the plasma membrane and recycling of receptors back to the cell surface for resensitisation. Moreover, recent studies have highlighted more complex aspects of spatiotemporal regulation of receptor signalling compared to simple regulation of receptor cell surface levels [14,23,24], in particular hitherto underappreciated endosomal signalling mechanisms which fine-tune the localisation of intracellular signals.

Advances in cryo-electron microscopy (cryo-EM) have assisted in the discovery of many active GLP-1R structures in complex with various agonists such as semaglutide, taspoglutide, the allosteric modulator compound 2, non-peptide agonists and GLP-1 [25–27]. These structures capture the subtle conformational changes promoted by differential ligand binding and provide a framework to understand mechanisms of GLP-1R activation. For example, recent cryo-EM structures of the GLP-1R complexed with orally deliverable non-peptide agonists identified novel binding pockets, unique activation profiles and active-state conformations induced by these agonists [28]. The latter study revealed the importance of structural differences involving water-mediated hydrogen networks that stabilise agonist binding, especially when correlated to functional data to show that oral small molecule non-peptide agonists such as danoglipron (PF-06882961) can closely resemble GLP-1 pharmacological properties [27]. Resolving GLP-1R structures at high resolution continues to aid our understanding of intricate phenomena such as biased signalling; for example, modest structural differences have already been revealed between GLP-1 and the biased agonist exendin-P5 bound to GLP-1R-G protein complexes [27].

Despite widespread adoption of GLP-1RAs in the clinic, evidence from clinical trials shows that clinical outcomes to GLP-1RAs are subject to inter-individual variability, both in terms of therapeutic and adverse events. For example, whilst GLP-1RAs are *on average* highly effective for weight loss, this disguises the observation that significant numbers of patients fail to lose a clinically important amount of weight [30]. Similarly, gastro-intestinal side effects affect ~30% of people taking GLP-1RAs, but some are significantly more affected than others and may need to discontinue treatment. Genomic variation in the *GLP1R* gene is one of several likely explanations for variance in GLP-1RA responses [31,32], and holds implications for personalisation of GLP-1RA therapy.

In this review, we first summarise the current knowledge of the structural basis of GLP-1R activation. We then describe current understanding of the interplay between GLP-1R trafficking and signalling, highlighting recent advances in the areas of spatiotemporal control and biased agonism. The latest biased, dual, or triple co-agonists in development are also addressed. Lastly, we discuss novel insights into the effect of non-synonymous single nucleotide *GLP1R* variants, which could potentially alter the receptor's response to endogenous and exogenous GLP-1RAs.

2. GLP-1R structure, binding and activation

2.1. GLP-1R structural review

Understanding the high-resolution structural details of GLP-1R activation, signalling and regulation is a key research focus for a number of academic groups and commercial organisations, as GLP-1R is a critical drug target for T2D, obesity, and cardiovascular disease. Obtaining high-resolution GLP-1R structures by X-ray crystallography is challenging, hence single particle cryo-EM has emerged as an attractive method to elucidate high-resolution signalling complexes of the activated GLP-1R with peptide agonists, non-peptide agonists, antagonists, allosteric modulators as well as heterotrimeric G proteins and auxiliary molecules such as cholesterol. In fact, twenty-one different GLP-1R:G protein structures complexed with different agonists have already been determined using cryo-EM and X-ray crystallography in just 5 years (Table 1). The first ~4 Å cryo-EM structure of the GLP-1R in complex with its endogenous agonist GLP-1 and heterotrimeric G protein was published in 2017 [35]. Since then, structures of various other GLP-1R:G protein complexes have been presented with other agonists such as danuglipron (an orally available small molecule GLP-1RA in Phase II trials for T2D and obesity) [27,126], the biased agonist exendin-P5 [29], peptide 19 (a GLP-1R/GIPR dual agonist) [33], compound 2 (a positive allosteric modulator) [26], and the FDA-approved agonist semaglutide [34].

The impact of determining the full active state structure of GLP-1R on understanding the molecular basis of GLP-1R signalling and pharmacology has been substantial. The structural elucidation of the active state GLP-1:GLP-1R:Gs complex with near atomic resolution revealed important residues involved in the orthosteric agonist binding site, and several critical interactions of the receptor with heterotrimeric G proteins, providing important insights into the molecular mechanisms and conformational changes occurring upon ligand binding, receptor activation and G protein engagement [35]. This structure, for instance, provides evidence for the exact mode of recruitment of the agonist by the N-terminal domain, followed by communication of peptide binding to the receptor TM core, and lastly opening of the helically unstable TM6 to create a cavity, and releasing residues to promote the engagement and activation of the Gαs protein [35].

Further structural information could be harnessed from comparing the active full-length GLP-1R structure with available structures of family A GPCRs such as the β2 adrenergic receptor (β2AR) to identify conserved G protein-binding motifs. In fact, superposition of the GLP-1:GLP-1R:Gαs complex with the active β2AR:Gs complex showed an almost identical G protein conformation, whereby β2AR forms a similar

Table 1
List of all available PDB structures with details including bound agonists, state, auxiliary molecules, structure length, and reference.

PDB ID	Ligand	Method	Receptor Length	State	Signalling Proteins	Auxiliary Molecules	Date	Reference
7X8S	WB4–24 (Boc5 analogue)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	06/22	Cong et al. (2022)
7X8R	Boc5 (orthosteric non-peptide GLP-1R agonist)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	06/22	Cong et al. (2022)
7RGP	Tirzepatide	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	NB35	04/22	Sun et al. (2022)
7VBH	Peptide 20 (GIRP/GLP-1R/GCGR tri-agonist)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	NB35	04/22	Zhao et al. (2022)
7LLL	Exendin-4	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	NB35	01/22	Wootten et al. (2022)
7LLY	Oxyntomodulin	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	NB35	01/22	Wootten et al. (2022)
7RTB	Peptide 19 (GLP-1R GIPR dual agonist)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35 AIB	10/21	Johnson et al. (2021)
7E14	LY3502970 or OWL833 (orally active non- peptide agonist)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, Cholesterol, HNO(N-tert-butyl-6,7-bis(chloranyl) quinoxalin-2-amine)	08/21	Cong et al. (2021)
7DUR	Compound 2 (positive ago-allosteric modulator)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, Cholesterol, HNO(N-tert-butyl-6,7-bis(chloranyl) quinoxalin-2-amine)	08/21	Cong et al. (2021)
7EVM	Compound 2	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, Cholesterol, HNO(N-tert-butyl-6,7-bis(chloranyl) quinoxalin-2-amine)	08/21	Cong et al. (2021)
7DUR	Compound 2	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, Cholesterol, HNO(N-tert-butyl-6,7-bis(chloranyl) quinoxalin-2-amine)	08/21	Cong et al. (2021)
7EVM	Compound 2	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, Cholesterol, HNO(N-tert-butyl-6,7-bis(chloranyl) quinoxalin-2-amine)	08/21	Cong et al. (2021)
7KI1	Taspoglutide	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, AIB	08/21	Zhang et al. (2021)
7KI0	Semaglutide	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, AIB, WF1	07/21	Zhang et al. (2021)
7DUQ	Compound 2	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35 Cholesterol, HNO	07/21	Cong et al. (2021)
7LCI	Danuglipron(PF-06882961) small molecule agonist	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	UK4	01/21	Zhang et al. (2021)
7LCJ	Danuglipron	Cryo-EM	Full	Active	None	UK4	01/21	Zhang et al. (2021)
7LCK	Danuglipron	Cryo-EM	Full	Active	None	UK4	01/21	Zhang et al. (2021)
6XOX	LY3502970 or V6G	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35, scFv16	11/20	Kawai et al. (2020)
6 × 18	GLP-1	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	09/20	Zhang et al. (2020)
6X1A	Danuglipron	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	09/20	Zhang et al. (2020)
6 × 19	CHU-128 (non-peptide agonist)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	09/20	Zhang et al. (2020)
7C2E	RGT1383 (small molecule full agonist)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	08/20	Ma et al. (2020)
6VCB	GLP-1 and LSN3160440/QW7 (positive allosteric modulator)	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	07/20	Bueno et al. (2020)
6ORV	TT-OAD2(non-peptide agonist) /N2V	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	1/20	Zhao et al. (2020)
6B3J	Exendin-P5	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	08/18	Liang et al. (2018)
5VAI	GLP-1	Cryo-EM	Full	Active	Gαs, Gβ1, Gγ2	Nb35	05/17	Zhang et al. (2017)
5NX2	Truncated peptide agonist	X-ray	Full	Intermediate	None	Chitin/ NAG, SOG	05/17	Jazayeri et al. (2017)
6LN2	PF-06372222 (Negative allosteric modulator)/97Y	X-ray	Full	Inactive	None	Fab7F38 light chain Fab7F38 heavy chain Zinc ion, NAG	03/20	Wu et al. (2020)
5VEW	PF-06372222	X-ray	Full	Inactive	None	Endolysin Chimera, oleic acid (OLA), OLC, T4-Lysozyme	05/17	Song et al. (2017)
5VEX	NNC0640 (Negative allosteric modulator)/97 V	X-ray	Full	Inactive	None	T4-Lysozyme, Endolysin Chimera	05/17	Song et al. (2017)
6KJV	PF-06372222	X-ray		Inactive	None	T4-Lysozyme, Endolysin Chimera OLC	11/19	Xu et al. (2019)

(*continued on next page*)

Table 1 (*continued*)

PDB ID	Ligand	Method	Receptor Length	State	Signalling Proteins	Auxiliary Molecules	Date	Reference
			TM domain, Thermal-stabilised M9					
6KK1	PF-06372222	X-ray	TM domain, Thermal-stabilised M8	Inactive	None	T4-Lysozyme, Endolysin Chimera	11/19	Xu et al. (2019)
6KK7	PF-06372222	X-ray	TM domain, Thermal-stabilised M6	Inactive	None	T4-Lysozyme, Endolysin Chimera	11/19	Xu et al. (2019)
5OTX	GLP-1 variant Ala8Cys/ Thr11Cys	X-ray	ECD	Active	None	None	07/18	Oddo et al. (2018)
5OTV	GLP-1 variant AlaCyc/ Thr11Hcs	X-ray	ECD	Active	None	None	07/18	Oddo et al. (2018)
5OTW	GLP-1 variant Ala8Hcs/ Thr11Cys	X-ray	ECD	Active	None	None	07/18	Oddo et al. (2018)
5OTT	Exendin-4 variant Gly2Hcs/ Thr5Hcs	X-ray	ECD	Active	None	None	07/18	Oddo et al. (2018)
5OTU	GLP-1 variant Ala8Hcs/ Thr11Hcs	X-ray	ECD	Active	None	None	07/18	Oddo et al. (2018)
6GB1	Peptide 11 (exendin-4 derived GLP-1 and GCG dual agonist)	X-ray	ECD	Active	None	Sulfate ion, AIB, HEZ(hexane-1,6-diol)	06/18	Schreuder and Liesum (2018)
4ZGM	Semaglutide backbone	X-ray	ECD	Active	None	8AIB, 34R-GLP-1(7-37)-OH, 32 M	09/15	Reedtz-Rung, S. (2015)
3IOL	Glucagon	X-ray	ECD	Active	None	10 M	10/09	Reedtz-Rung, S. (2010)
3C5T	Exendin-4	X-ray	ECD	Active	None	10 M	10/09	Reedtz-Rung, S. (2010)
3C59	Exendin-4	X-ray	ECD	Active	None	10 M	02/08	Reedtz-Rung, S. (2008)
5E94	None	X-ray	ECD	Inactive	None	Fab7F38 light chain Fab7F38 heavy chain	08/16	Soroka V. et al. (2016)

Gαs recognition cavity with equivalent non-polar residues [35]. On the other hand, there is a notable difference in peptide binding motifs, reflecting size and type differences in the mode of ligand activation of the receptor [35]. These similarities in G protein conformations of active class A and B GPCRs highlight the congruence in G protein activation pathways, enabling these receptors to signal via similar intracellular pathways despite binding diverse ligands.

Valuable insights into GLP-1R activation models have come from comparisons between inactive and ligand-bound active state structures. Differences in these states include conformational rearrangements, hydrogen-bond interactions, and residue orientations. The resolution of specific regions in a structure is an indicator of the degree of flexibility in the active structure upon peptide binding. For instance, the linker region between the ECD and the core in active Gαs protein-complexed GLP-1R structures is poorly resolved, suggesting high flexibility in this region and occurrence of conformational changes [39]. Moreover, there is increasing evidence that activation of both GLP-1R and the glucagon receptor (GCGR), but not other class B GPCRs such as CRF_1R, PAC1R and PTH1R, is dependent on close ECD-TMD interactions [37–39]. Additional mutagenesis studies have elucidated potential ECD binding sites, specifically residues in ECL1, 2 and 3 [37]. Yet, mutational studies in GCGR observe that the apo-state also exhibits distinct ECD-TM1-ECL3 interactions that auto-inhibit receptor activity under basal conditions [38,40]. Wu et al., 2020 provided increased understanding of the role of the ECD in GLP-1R activation, utilising the inactive human GLP-1R crystal structure, showing an ECD-TMD interface and interactions with ECL1 and 3 [41]. However, they argue that in the apo-state the ECD is dynamic, despite favouring a closed conformation stabilised by weak interactions with ECL1/3 [41].

Homology modelling of the receptor inactive state has also revealed that distinct residues and orientations play a role in maintaining inactive and quiescent conformations. In the inactive state, large aromatic residues in the ECL2 loop are oriented towards the binding pocket and this is stabilised by hydrogen bonding [39]. Activation of the receptor initiates reorganisation of the ECL2 and reorientation of these residues away from the binding pocket, facilitating direct contact with the peptide. Further molecular dynamic simulation studies also suggest that this aromatic residue cap acts as an energy barrier, preventing deeper entry of GLP-1 into the receptor [39].

Despite the recent improvements in cryo-EM technology, including in hardware and software, the resolution of cryo-EM is still low compared to X-ray structures [42]. The limited resolution of cryo-EM maps hinders GLP-1R structure modelling in some receptor regions, where the density is less-well defined, such as the cytoplasmic half of TM6 and the stalk between TM1 and ECD [26,33,41]. These are often omitted, limiting our understanding of interactions in these regions. Computational approaches may help to address these limitations by predicting the structure of these poorly resolved regions and producing more accurate models. AlphaFold, a novel machine learning approach based on neural networks recently developed by DeepMind, predicts protein structures from amino acid sequences with atomic accuracy [43]. Several structural biology studies have already trialled AlphaFold to aid in the interpretation of cryo-EM maps [44–46]. The proposed AlphaFold human GLP-1R structure is depicted in Fig. 2 [43], including missing regions and superimposition with the active GLP-1R:GLP-1:Gαs complex for comparison (PDB 6X18). However, it must be noted that the accuracy of these missing regions is low according to the per-residue confidence scores, so any inference based on the structure must be interpreted carefully.

2.2. GLP-1R activation and interactions

As with other secretin-like class B receptors, GLP-1R typically binds peptide ligands according to the 'two-domain' model. The N-terminal ECD, containing six conserved cysteine residues, is the peptide binding energy source for this binding mechanism [35,47]. First, the extended extracellular structure forms several interactions with the peptide agonist along a peptide binding cavity, triggering the activation of the core TMD by the N-terminal end of the peptide. The TMD is then responsible for initiating G protein engagement and activation [35].

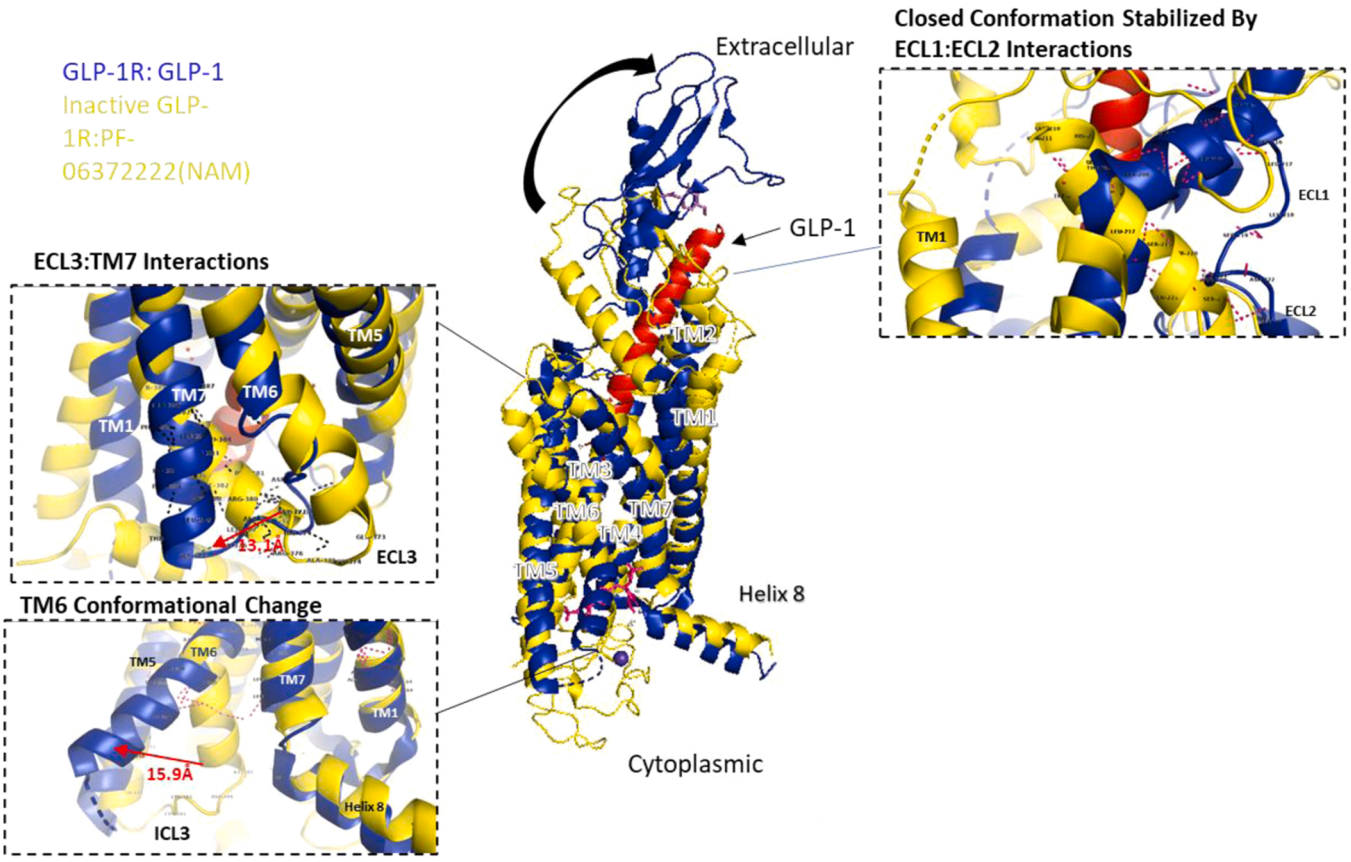

Fig. 1. Active vs inactive GLP-1R structures including major activation networks. GLP-1R:GLP-1 complex (PDB 6X18) in blue was superimposed over the inactive GLP-1R:PF06372222 NAM structure in yellow (PDB 6LN2). Alignment of structures was done using PyMOL(TM) Molecular Graphics System, Version 2.1.0.

Early studies in 2007 by Mann et al. were the first to investigate GLP-1R binding and peptide-receptor interactions utilising in vitro refolding, NMR structure analysis and mutation studies [47]. Two main receptor-peptide interactions were characterised, between the helical region of the peptide (for GLP-1 residues 24–22) and the receptor ECD, and between the peptide N-terminal region and the receptor TMD [47]. This was further confirmed by alanine scanning mutagenesis and modelling studies that also revealed the importance of TM7, ECL2 and ECL3, residues which form part of GLP-1's binding cavity and are crucial for its potency. Interestingly, the study identified differences in the binding mode of GLP-1 compared to the pharmacological agonist exendin-4; specifically, GLP-1 formed greater interactions with the receptor core domains, whereas exendin-4 exhibited greater affinity to the receptor ECD due to an extra C-terminal motif [47].

A 'two-domain' peptide agonist binding mechanism for the GLP-1R was further supported by the determination of the GLP-1:GLP-1R:Gαs complex structure in 2017 using cryo-EM and density mapping [35]. The active GLP-1R was compared to an inactive GCGR structure to elucidate the binding and activation mechanisms of the GLP-1R [35]. Two major GLP-1R conformations were identified: the inactive and the active conformation. In the inactive conformation, the peptide binding pocket is partially blocked by ECD helix residues (Thr27-Leu50) and motions between the ECD and the TMD, which are separated by a flexible linker sequence. This lack of direct interaction plays a role in the initial engagement of the peptide [35]. Furthermore, the inactive state is believed to involve hydrated residues on the open extracellular interface, with activation involving the displacement of water and reordering of constraining central hydrogen bonding networks [36,48]. Specific side chain orientations and hydrogen bonding networks with upstream polar residues such as Arg190 and Asn240, constrain and restrict the apo-state receptor and are highly conserved amongst other class B GPCRs such as the GCGR [36,48]. Akin to observations in the GCGR, activation of the receptor might involve the release of these interactions.

Whilst in the active conformation, the peptide binding pocket is fully accessible, this conformational state is linked to the inactive structure via low frequency conformational movements. Consistent with the 'two-domain' binding model, the receptor ECD was found to bind first to the C-terminal helical region of the ligand, triggering the formation of an extended helical turn by the N-terminus of GLP-1 in the core of the receptor within the open cavity of the TM bundle. Moreover, the GLP-1 peptide is anchored in its position via an extensive network of residue-peptide interactions, validated by extensive mutagenesis studies [33,36,48]. The GLP-1 binding pocket resides deep within the TM bundle, involving an extensive network of interactions comprising TMs 1/2/5/7, ECL1 and 2. The two main GLP-1 residues interacting with the TM core are His7 and Ala8, forming extensive hydrogen bonding and hydrophobic interactions with TMs 3/5 and 7, respectively [33,36,48]. Critical structural waters also anchor the peptide in place by forming a network with His7 on the peptide and TM5 on the receptor. Following peptide binding and interaction with the TM core, one of the major conformational events is the opening motion of cytoplasmic TM6 and its rearrangement due to breakage of polar interactions in the HETX motif (TM2–6–7-helix 8), forming a Gαs binding cavity [27,36].

Engagement of G proteins is mainly mediated by the ICLs in the GLP-1R. Yet, distinct TM interactions and orientations are linked with differential coupling to G proteins and inherent signal bias. For instance, the cytoplasmic region of GLP-1R, including the N-terminal side of ICL3, TM7 and Helix 8 interact with alpha 5 helix of the Gαs protein, whereas Gi/o coupling is mediated by the C-terminal side of ICL3 [35]. These regions are most likely targeted and modified by biased agonists to promote the preference of one G protein signalling pathway over the other.

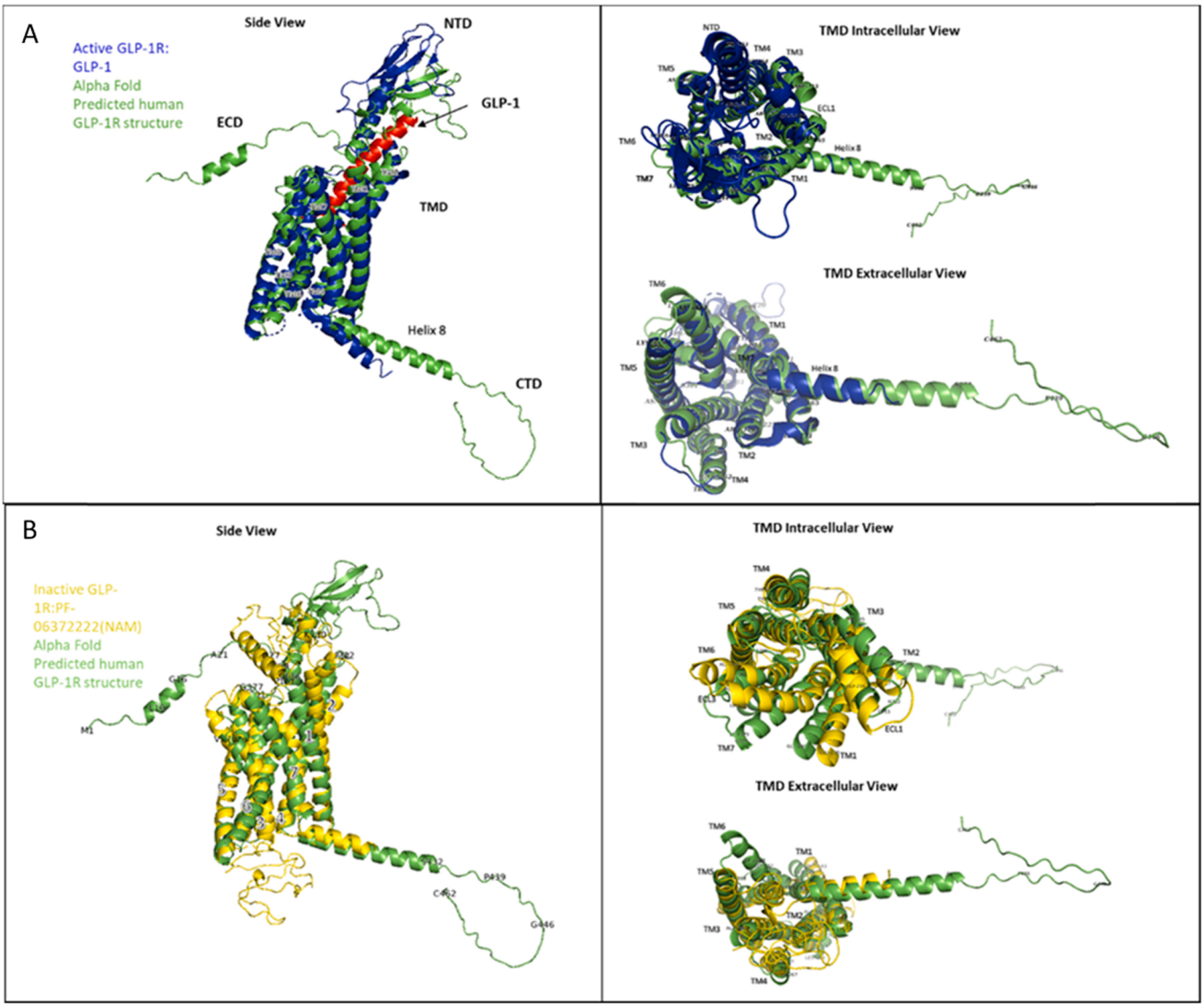

Fig. 2. A) Active GLP-1R:GLP-1 (PDB 6X18) complex (blue) superimposed with the AlphaFold Prediction of GLP-1R Apo-Structure (green). B) Inactive NAM-bound GLP-1R structure (PDB 6LN2) (yellow) superimposed with the AlphaFold predicted Structure of GLP-1R (green). Alignment of structures was done using PyMOL(TM) Molecular Graphics System, Version 2.1.0. Figures on the right-hand side depict the intracellular and extracellular view of the receptor complexes superimposed with the ECD and peptide removed to provide a clearer depiction of the TMDs.

Different agonists are likely to mediate differential intracellular signalling and trafficking via changes in receptor binding and activation, including distinct structural rearrangements and orientations. Although liraglutide, an acylated, long-acting GLP-1 analogue, was assumed to adopt a very similar binding mode to native GLP-1, *in silico* studies observed that, rather than occupying the length of the receptor binding site like GLP-1, the liraglutide side chain points inside the receptor without disturbing this binding site [49]. In parallel to this, semaglutide also exhibited slight differences in binding compared to its root peptide GLP-1 [35]. Despite adopting a similar binding mechanism, with the C-terminus bound to the ECD and the N-terminus penetrating into the TMD core and adopting highly conserved interactions, the substitution of Ala8 by aminoisobutyric acid (Aib) in semaglutide results in different hydrophobic interactions and water mediated hydrogen bonds with TM7 and TM3, respectively, compared to GLP-1 [27,36,57].

Non-peptide GLP-1RAs and allosteric modulators also exhibit unique binding mechanisms. For example, binding of the positive allosteric modulator, compound 2, involves a cavity formed by ECL1 and 2 near the TM5 and TM6 helices [26]. Specifically, compound 2 forms a co-valent bond with residue C347 triggering the outward movement of TM6, hydrophobic interactions between key aromatic residues and the downward shift of the N-terminal alpha helix of the ECD, facilitating peptide binding and the positive allosteric action modulated by compound 2 [14,26,27].

Conversely, the GLP-1R bound structure of LY3502970 (OWL833), an oral non-peptide GLP-1RA with a favourable pharmacokinetic profile being developed by Chugai/Eli Lilly, exhibits a unique binding pocket in the upper helical bundle of the receptor bound by residues in ECD, ECL2, and TM helices 1–3 and 7 domains [27,28] (Fig. 3). These distinct binding interactions lack connections with TM4–6 unlike GLP-1 which is bound by residues in all the TM segments [28]. Its distinctive binding mechanism may explain its partial agonism and biased signalling in favour of G protein activation. Another non-peptide agonist with a distinct binding mode and unique signalling properties compared to GLP-1 is TT-OAD2, yet it shows less pharmacological promise compared to OWL833 and Danuglipron [27,76]. Although the ECL2 in the TT-OAD2 bound GLP-1R adopts an active conformation promoting Gαs coupling, the ECD N-terminal helix and ECL3 interactions are scarce, like the inactive structure [27]. Another factor reducing TT-OAD's pharmacological potency is the fact that it can't form a polar network

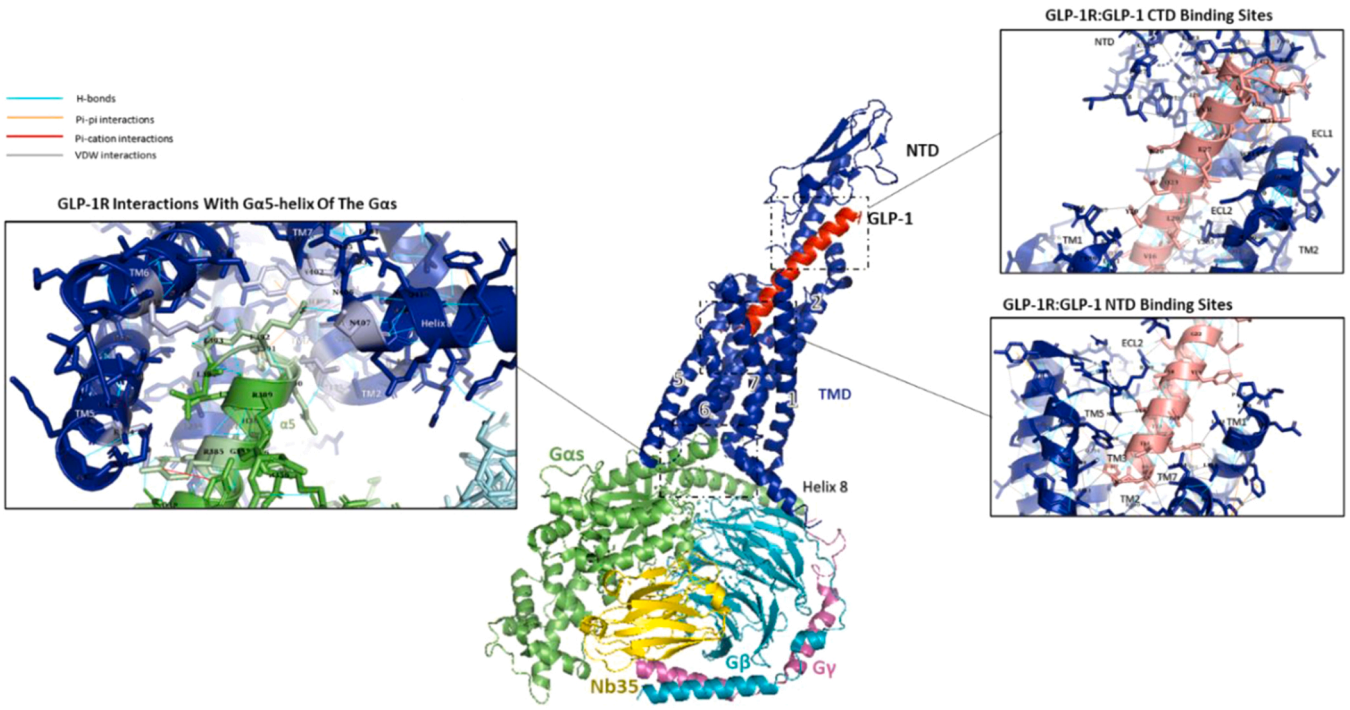

Fig. 3. Active GLP-1R:GLP-1:G protein complex (PDB 6X18) peptide-receptor interactions at three activation specific regions. 1) The GLP-1 C-terminal binding sites with the receptor N-terminal domain. 2) The GLP-1 N-terminal binding sites within the core of the receptor and TMDs. 3) G protein binding motifs in the cytoplasmic interface of the receptor. Colours are as follows: GLP-1R (blue), GLP-1 (red), Gαs (green), Gβ (cyan), Gγ (pink), and Nb35 (yellow). Figures were made using PyMOL (TM) Molecular Graphics System, Version 2.1.0. Interactions were identified using RING software.

constraining the TM1–2 interface, which has been shown to play a critical role in ligand potency of other non-peptide agonists like PF-0688291 [27]. PF-0688291 (Danuglipron) a non-peptide agonist developed by Pfizer in clinical trials for T2D therapy, exhibits a close pharmacological, signalling, and regulatory profile to the endogenous agonist GLP-1 at the GLP-1R [27,126]. Moreover, discovery of the Cryo-EM structures of this agonist bound to GLP-1R show substantial overlap with the GLP-1 bound structure. Whereby, PF-0688291 docks in a pocket buried deep within the receptor overlapping with GLP-1 residues G10-E21, thus sharing similar ligand-residue interactions with receptor in the ECD, TM1–3, ECL2–3 and TM7 [27]. Despite GLP-1 conferring more interactions with these domains, PF-0688291 extends deeper into the receptor core stabilizing similar conformational rearrangements and water networks in the receptor to GLP-1 [27].

More recently, two peptidomimetic non-peptide agonists of GLP-1R have been developed and extensively characterised, including Boc5 the first orthosteric non-peptide GLP-1R agonist and its analogue WB4–24 [127,128]. Although these agonists display poor solubility and low oral bioavailability hindering their therapeutic development, their peptidomimetic binding and signalling properties are useful for further pharmacological optimization of other non-peptide GLP-1R agonists [127]. Akin to OWL833, Boc5 also binds and interacts with the upper helical bundles of the receptor including the ECD, ECL2, TM1–3 and TM7 domains [28,127]. The cryo-EM structure of Boc5 bound GLP-1R reveals that one arm of this compound binds deeply into the peptide ligand accessible binding pocket partially overlapping GLP-1 residues, explaining the peptidomimetic agonism of the small molecule agonist [127]. Both non-peptide agonists elicited the similar activation associated conformational changes in the receptor relative to peptide-agonists like GLP-1. These include movement of ECD towards the ECL2 and the movement of the intracellular part of TM6 outwards [127]. However, unlike GLP-1, Boc5 and WB4–24 are unable to directly interact with residues in TM3 and 5 to activate the receptor, instead they induce similar conformations to those in the central polar network at the bottom of the orthosteric binding pocket [127]. Interestingly, phenyl groups in these two agonists adopted a similar V-shaped orientation in the TM1–2 and TM2–3 clefts to TT-OAD2 and OWL833 [27,28,127]. Yet, the phenyl group of WB4–24 is bulkier conferring distinct extra interactions with residues in TM1 and 7 establishing a unique binding mode that might explain improved potency compared to Boc5 and the other non-peptide agonists in vivo and in vitro [127]. Perhaps, studying these conformational changes and phenyl group interactions could help optimize the response of TT-OAD2 making it a more promising drug candidate.

3. Mechanisms of GLP-1R signalling and trafficking

3.1. G protein-dependent signalling

GLP-1R primarily couples to Gαs, inducing intracellular accumulation of cAMP and downstream protein kinase A signalling mediated by cAMP [10–13,23]. In turn, activation of the cAMP-PKA pathway and subsequent translocation of EPAC2A to the cytoplasmic membrane induces multiple pathways within β-cells resulting in promotion of β-cell proliferation, changes in gene expression, mobilisation of intracellular calcium and GSIS amplification [11–13].

Like many GPCRs, GLP-1R is pleiotropically coupled to additional Gα subtypes including Gαq and Gαi/o proteins, which contribute to intracellular calcium mobilisation, activation of ERK1/2 and Akt pathways, and phosphorylation of phosphoinositide-3-kinase (PI3K), amongst other readouts [14–16,23]. The Gαs- and Gαq-stimulated downstream signalling pathways converge on common targets regulating GSIS. Intriguingly, this fact has formed the basis of a possible molecular explanation for the “incretin bias” – characteristically seen in the early stages of T2D – whereby patients partially retain first-phase insulin release in response to GLP-1 but are resistant to the action of the other incretin glucose-dependent insulinotropic polypeptide (GIP) [50,51]. Important observations by Odouri et al. in K_{ATP} channel-defective mice (lacking the Kir6.2 channel component) revealed that GLP-1R, unlike GIPR, is able to compensate for the loss in Gαs signalling by engaging the

alternate Gαq signalling pathway to preserve GLP-1-mediated GSIS responses [52,53]. This signalling switch only occurs during diabetes pathogenesis, whereby K_{ATP} channel activity is defective despite elevated intracellular Ca^{2+} levels [52,53]. These findings raise many questions with regards to this apparent switch in Gα-subtype coupling, such as its precise molecular mechanism, whether it is a feature of human diabetes, and whether it would be possible to design biased GLP-1RAs or dual incretin receptor agonists that will allow recoupling to Gαs or reactivation of GIPR signalling in β-cells during T2D.

3.2. GLP-1R internalisation and intracellular trafficking

GLP-1R trafficking is a crucial regulator of signalling capacity and can be manipulated to enhance the pharmacological effects of GLP-1RAs. Trafficking mechanisms triggered by ligand activation include receptor internalisation by endocytosis, recycling to the plasma membrane, and lysosomal targeting for degradation. Receptor internalisation regulates signalling through reducing receptor expression at the plasma membrane. The GLP-1R is believed to be internalised by clathrin-dependent and possibly clathrin-independent endocytosis into a complex network of trafficking pathways, encompassing key subcellular compartments including early and recycling endosomes, multivesicular bodies and late endosomes, lysosomes, the Golgi apparatus, and the endoplasmic reticulum [52–54,68]. Surprisingly little is understood about the molecular basis of GLP-1R internalisation, and what has been reported is inconsistent. For example, there is evidence to support both clathrin-coated pits and caveolin-1-dependent mechanisms [54–56]. However, both mechanisms are dependent on dynamin, and as such inhibition of dynamin via a dominant negative form (dynamin-1 K44E), halts agonist-mediated GLP-1R internalisation in FlpIn-CHO cells as measured by BRET assays [57]. It is possible that receptor internalisation is context-dependent, utilising alternate pathways depending on the cell type or specific agonist. Residual internalisation through an alternative pathway may well be upregulated in the event of experimental inhibition of the "primary" pathway, making it difficult to experimentally validate the main endocytosis route employed by the receptor.

After reaching early (sorting) endosomes, the receptor is either sorted for recycling, promoting re-sensitisation through re-supply of an adequate pool of membrane GLP-1Rs, or towards late endosomes and lysosomes for degradation and signal termination [58]. In the conventional view of GPCR endocytosis, phosphorylation of the active receptor by intracellular GRKs mediates recruitment of β-arrestins to these phosphorylation sites, desensitising the receptor by uncoupling it from heterotrimeric G proteins [59] and facilitating interactions with clathrin adaptors; the receptor is then enveloped in clathrin-coated pits which eventually bud into vesicles and merge with the endocytic network.

3.3. β-arrestin-mediated signalling

β-arrestin recruitment also provides a scaffold for the receptor to engage alternative downstream effectors and signalling pathways [60]. Indeed, knockdown of β-arrestin 1 in rat INS-1 β-cells attenuated GLP-1 signalling and both glucose- and agonist-induced insulin secretion [61]. It has also been shown that, GLP-1R heterologously expressed in HEK293 cells co-localises with caveolin-1, the main component of caveolae, and was proposed to assemble signalling micro-complexes with β-arrestin 1 implicated in regulating receptor trafficking and ERK1/2 signalling [62–64]. Yet, β-arrestin 1 was found to be dispensable for agonist-induced GLP-1R internalisation, suggesting that it might act subsequently to receptor localisation to caveoli [61]. On the other hand, the absence of β-arrestin 2 was found to facilitate longer lasting agonist-induced cAMP signalling at the GLP-1R via significantly reducing receptor internalisation in CHO-K1 cells [65]. Despite decreased endocytosis and β-arrestin recruitment being more associated with prolonged cAMP signalling, depletion of β-arrestin 1 and 2 decreased ERK phosphorylation, which is associated with GLP-1R induced anti-apoptotic and proliferation effects in β-cells [61,65].

Moreover, depletion of β-arrestin 2 in mice on a high-fat diet led to better glucose control and insulin secretion in response to GLP-1RAs over prolonged periods of time, but attenuated acute cAMP signalling *ex vivo* [21]. Overall, it is becoming clear that β-arrestin-dependent signalling is more diverse, complex, and temporally specific than previously thought. Therefore, to fully harness the potential benefits of targeting β-arrestin signalling, it becomes critical to assess receptor-mediated β-arrestin conformational changes. Interestingly, recent studies have demonstrated that β-arrestins do in fact adopt two distinct conformations that determine their signalling activities: the 'core' conformation and the 'tail' conformation [66]. The core conformation involves a tight interaction between β-arrestin and the active receptor TM core, hindering G protein coupling and GPCR activity, whilst the 'tail' conformation involves looser interaction with the phosphorylated C-terminal tail of the active receptor, enabling G protein coupling, and also potentially allowing for continued cAMP and ERK signalling in endosomes [66,67]. One hypothesis used to explain how the tail conformation allows endosomal receptor signalling is via the formation of a GPCR:G protein:β-arrestin 'megaplex', whereby the G protein binds to the receptor TM core and the β-arrestin binds to the exposed C-terminal tail, allowing for G protein signalling to continue despite β-arrestin mediated internalisation [68,69]. To date, there are no GLP-1R:G-protein:β-arrestin megacomplexes identified and/or resolved [67,68]. Solving the structure for this putative megacomplex by cryo-EM would offer important insights about GLP-1R-activated β-arrestin conformations, providing a structural explanation for differential signalling pathways engaged in response to specific agonists.

3.4. Spatiotemporal compartmentalisation of signalling

There is increasing focus on the concept of spatiotemporal compartmentalisation of signalling [24]. The receptor's signalling profile is influenced not just by different ligands but also by the location (spatial) and duration (temporal) aspects of signalling, leading to the generation of differential ligand responses and physiological functions. Based on the classical receptor theory, there is a correlation between reduced cell surface expression of the GPCR by endocytosis and reduced receptor efficacy [70]. Yet, accumulating evidence suggests that, contrary to previous ideas, increased receptor internalisation might in fact not translate simply to signal termination, as persistent G protein signalling from intracellular locations may result in a more sustained response. Indeed, biased GLP-1RAs can exhibit distinct spatiotemporal profiles of signalling, which are dependent on GLP-1R internalisation [62,71]. The pERK1/2 biased agonists liraglutide and oxyntomodulin were able to induce both nuclear and cytosolic pERK1/2 activity, whereas GLP-1 and exendin-4 induced more sustained ERK signalling restricted to the nucleus [62]. Moreover, the effects of inhibiting receptor internalisation on signalling diverged in a compartment-, pathway- and agonist-specific manner: cytosolic cAMP was attenuated in cells with dyn-K44E-impaired internalisation in response to GLP-1, exendin-4 and oxyntomodulin, but not to liraglutide stimulation, which exhibited reduced cAMP in the plasma membrane instead. A role for GLP-1R localisation within membrane nanodomains, or lipid rafts, allowing receptor interaction with caveolin-1 and enhancing GLP-1 binding affinity has also been reported [54]. Studies monitoring long-term GPCR activity during the endosomal trafficking pathway using fluorescent biosensors reveal that some class B GPCRs such as the PTH receptor maintain an active conformational state at internalised endosomes promoting cAMP signalling [72]. Additionally, endosomal cAMP levels have been associated with regulation of cAMP-dependent transcriptional control. Indeed, endosomal trafficking of the GLP-1R and endosomal cAMP signalling were found to mediate insulin granule exocytosis in pancreatic β-cells [73]. Kuna et al. revealed that adenylate cyclase partly colocalised with the GLP-1R 5 min after internalisation, which became

extensive 30 mins post-internalisation, supporting persistent generation of cAMP signals from the endosome [73]. However, continuous monitoring GLP-1R activity and signalling over several hours, which is the time frame most relevant to the action of therapeutic GLP-1RA drugs, has not yet been performed, and is critical to understand the strength and duration of receptor activity and function.

4. Biased agonism of the GLP-1R

Increasingly, biased agonism has garnered interest in modern drug discovery for T2D and obesity, as fine-tuned GLP-1RAs have the potential to improve on existing therapies through, for instance, reducing or eliminating common side effects such as nausea, whilst maintaining or enhancing therapeutic effectiveness, e.g. through avoiding tachyphylaxis [22]. Investigating biased agonism could also explain the clinical outcomes observed in trials between different drugs with potentially biased profiles.

The now well-established pharmacological paradigm of 'biased agonism' or functional selectivity, originally roped by Jarpe et al., refers to the fundamental concept whereby a single GPCR can elicit functionally distinct signalling pathways and downstream effectors upon engagement with a specific ligand [74]. Different agonists can also induce distinct receptor internalisation and trafficking profiles, influencing engagement with these downstream signalling pathways. Studies have indicated that the utilisation of biased agonists to preferentially activate one pathway over another could increase the benefit-to-adverse effects ratio in the therapeutic management of diseases such as T2D and obesity [22]. For instance, exendin-4 is biased towards cAMP while the natural GLP-1R/GCGR co-agonist oxyntomodulin favours ERK1/2 signalling. Yet, both are more biased at recruiting β-arrestin compared to the endogenous peptide GLP-1. Moreover, Fletcher et al. assessed the influence of a panel of standard GLP-1RAs (GLP-1, exendin-4, liraglutide and oxyntomodulin) on downstream signalling pathways such as cAMP accumulation and ERK1/2 phosphorylation using live cell assays to additionally provide insights into kinetic differences. They found that exendin-4 and liraglutide reduced efficacy of cAMP response by half, except oxyntomodulin, which exhibited a significant attenuation in cAMP levels in comparison to GLP-1. Similarly, there was also a significant reduction in potency and efficacy of ERK1/2 phosphorylation in response to liraglutide and oxyntomodulin, and to a lower extent, exendin-4 compared to GLP-1. Recent studies reveal a difference in the effect of internalisation on signalling and insulinotropic effect depending on chronic versus acute administration of the agonist. For example, the chronic administration of the GLP-1 and exendin-4 derivatives, GLP-1-Val8 (Ala8 to Val substitution) and exendin-F1 (His1 to Phe), which both displayed reduced β-arrestin recruitment and impaired receptor internalisation, improved glucose tolerance and glycemic control in diabetic mice [75,76]. These observations suggest that chronic administration of these compounds might be leading to improved insulinotropic effects via a lower desensitisation of the receptor. This may also be explained by differences in intracellular trafficking of the receptor over prolonged periods of time.

Conventionally, physiologically important effects of GLP-1R activation such as insulin release are dependent on the Gαs signalling pathway, whereas β-arrestin recruitment is linked to desensitisation, down-regulation, and a decline in GLP-1RA efficacy. In parallel to this, recent studies describing GLP-1R biased agonists with reduced β-arrestin recruitment showed improved anti-diabetic effects in pre-clinical models [22,75,77–80]. The first compound designed specifically with biased agonism in mind is exendin-P5, which was identified via a high throughput autocrine-based screen of a large (>100 million) different peptides [81]. In vitro characterisation of P5 revealed a biased signalling profile, whereby P5 promoted similar G protein signalling, cAMP accumulation and Ca^{2+} mobilisation to exendin-4 and GLP-1, but significantly reduced β-arrestin recruitment [81]. In T2D mouse models, P5 was more effective than exendin-4 for lowering of blood glucose, although this was not explained by increases in insulin release, which was in fact attenuated with P5 [81]. This contrasts with other G protein-biased GLP-1RAs, exemplified by exendin-F1, which typically show time-dependent increases in insulin release through avoidance of β-cell GLP-1R desensitisation [22,75,77]. A recent study directly compared the signalling, trafficking and pharmacological profiles of P5 and exendin-F1 [75], showing that P5 shows predominantly "affinity--driven" bias, manifesting as variably reduced potency for different pathways, whereas exendin-F1 shows markedly reduced maximal responses for effector recruitment ("efficacy-driven" bias) [75], indicating that P5 and other biased GLP-1RAs may indeed achieve their anti-hyperglycaemic effects through distinct mechanisms. Indeed, it was proposed that insulin-independent mechanisms are involved in the P5 effects, potentially including alterations in adipogenesis, inflammatory pathways, or increased GIP/GIPR levels [81].

Whilst many studies have highlighted the importance of the peptide N-terminus in mediating bias effects, differences in the middle part of the peptide and its C-terminus are also important, particularly when considering peptide modifications such as installation of fatty acid moieties to enhance albumin binding and extended pharmacokinetics, e.g. for approved GLP-1RAs such as liraglutide and semaglutide [82–84]. Whilst close attention was paid to potential adverse effects on binding affinity and cAMP signalling of different fatty acids and linkers during the development process, this was prior to bias being recognised as a feature of GLP-1RA signalling [84]. Indeed, these fatty acid moieties have the potential to influence GLP-1R activity and signalling via altered interactions with the receptor structure, and thus could also promote biased agonism. Lucey et al., 2021 compared exendin-4 and "exendin-4-C16" (an exendin-4 analogue C-terminally attached to a fatty diacid) using high-content microscopy and novel complementation and proximity-based assays, revealing that addition of this fatty acid moiety results in reduced binding affinity and β-arrestin 2 recruitment [85]. Trafficking and subcellular compartmentalisation was also altered in response to exendin-4-C16, with reduced receptor internalisation, reduced clustering in membrane nanodomains (despite the molecule showing increased interaction with lipid bilayers) and reduced targeting to degradative late endosomal compartments [85]. Overall, this suggests that care must be taken in designing fatty acid-modified GLP-1RAs as the impact of these modification may extend beyond the canonical effects on pharmacokinetics. Additionally, it is conceivable that the physicochemical properties of different fatty acids might also influence the local distribution of acylated GLP-1RAs close to the plasma membrane, or influence receptor conformational rearrangements through concurrent affinity for nearby membrane regions whilst in the receptor-bound state. Some evidence for the latter was provided at the structural level for semaglutide, with the cryo-EM data suggesting the ligand is orientated with the lipid moiety extending towards the lipid bilayer [57].

5. Structural basis of GLP-1R biased signalling

Structural studies can provide important insights into the molecular and structural basis of biased agonism. It is fundamental to investigate conformational rearrangements and residue-residue interactions involved in receptor-ligand coupling and G protein engagement to predict ligand-specific differences in receptor activation and downstream signalling pathways. Specific receptor-ligand interactions are capable of stabilising distinct conformational assemblies which might promote the preference of one signalling pathway over another. A study by Wootten et al. using a combination of homology modelling, mutational analysis and molecular dynamics simulation elucidated key residues forming a central polar network involved in biased agonism [48]. They report that both residues involved in peptide mediated bias and general signalling bias are co-localised in the middle region of the helical bundle, with peptide specific residues facing the core of the bundle and general bias residues exhibiting a more peripheral localisation. Four key residues involved in peptide-specific bias were identified by

mutagenesis, Arg190, Asn240, Gln394 and His363, forming a polar network that plays a role in GLP-1- and exendin-4- but not oxyntomodulin-mediated cAMP signalling and ERK1/2 phosphorylation [48]. This highlights the fact that important residue interactions or networks are ligand-specific and can be targeted to amplify specific signalling pathways.

In parallel, another study compared the full GLP-1-activated GLP-1R structure with that in complex with the biased agonist exendin-P5. They report that exendin-P5 triggers similar conformational changes to GLP-1 in residues involved in receptor activation, such as the extracellular ends of TM1, 6 and 7 which move to accommodate peptide binding, and are likely conserved across all peptide complexes [27,29]. However, differences occur in the extent of movement in these regions, highlighting distinct activation modes. Moreover, structural studies superimposing different activated GLP-1R complexes aids in the identification of networks involved in distinct signalling pathway, such as the interface between TM1/TM7/ECL3 associated with Gαi/Gβγ/β-arrestin coupling to pERK, whilst reorganisation of TM5 and 6 is important for cAMP and intracellular calcium signalling [39]. Mutational and structural data suggest that agonist-specific interactions alter these interfaces, and so recruitment of downstream effectors. For instance, residues in ECL1 were found to be important for oxyntomodulin- but not exendin-4-mediated cAMP signalling, which requires ECL2 network instead [39].

Therefore, we can utilise a combination of structural, mutational and homology studies to identify distinct structural reorganisations and alterations in bias-associated networks to infer peptide-specific signalling pathways. For example, assessing the active semaglutide-bound GLP-1R structure revealed large movements in TM2 and ECL1 compared to GLP-1, with ECL1 playing an important role in cAMP maximal response, akin to oxyntomodulin [57]. Other more dramatic movements also occur in TMs1/6/7, altering directionality away from the core, hinting at an altered activation mode [57] (Fig. 3). Cryo-EM studies have also provided the structure of dual agonist-GLP-1R complexes, providing important insights into their mode of action in comparison with classical GLP-1RAs. Although peptide-19 (a novel dual GIPR/GLP-1R agonist) showed conserved N-terminal residues bound to the receptor core with GLP-1, it exhibited differences in the positions of TM4-ECL2-TM5 and so limited ECL2 interactions [33] (Fig. 3). Perhaps, like exendin-4, these ECL2 interactions may be important for peptide-19-mediated cAMP responses. Furthermore, peptide-19 exhibited similar conformations in ECL1, TM1 and TM7 to taspoglutide- and exendin-P5-bound GLP-1R [33], suggesting similar β-arrestin coupling and pERK activity to these peptides. Yet, there were marked distinctions in the conformations of the top of TM6 and ECL3 [33], which may indicate differences in G protein coupling and engagement.

Further structural analysis of the small non-peptide partial agonist OWL833 also shed light on potential structural interactions associated with biased signalling in non-peptide GLP-1R agonists [28]. Although OWL833 exhibits overlapping binding interactions to the GLP-1, it is more biased towards G protein activation over β-arrestin recruitment at the GLP-1R. This lack of detectable GLP-1R β-arrestin coupling is favourable as it enhances glucose and weight reducing effects of the compound. Structural studies of several agonist bound GLP-1R structures suggest that a close interaction between the extracellular portion of TM7 (particularly Arg380$^{7.35}$) and TM5 might be required for efficient β-arrestin recruitment [28]. As such, the position of residue Arg380$^{7.35}$ is shifted away from TM5 in OWL833, Ex-P5 and TT-OAD2 compared to the GLP-1 bound receptor structure which exhibits less bias toward the cAMP pathway and more β-arrestin coupling [27]. However, unlike TT-OAD2 which completely lacks interactions with Arg380 in TM7 stabilizing conformations of TM6-ECL3 and TM7, OWL833 does interact with other TM7 residues, explaining increased potency in stimulating the cAMP pathway in human GLP-1R expressing cell lines [27]. Bias associated structural and conformational observations may serve as a good indicator of pharmacological similarities or differences between GLP-1R agonists. This offers important insights into the molecular mechanisms of non-peptide agonist-mediated GLP-1R signalling suggesting that, despite their unique binding mode, they can still resemble other peptide GLP-1RAs such as exendin-P5 in terms of pharmacological outcomes.

Other increasingly interesting non-peptide GLP-1R agonists include Boc5, the cyclobutene derivative, and its analogue WB4–24 which elicit peptidomimetic responses upon GLP-1R activation. Impressively, Boc5 showed full agonism at the GLP-1R, eliciting Gs-mediated cAMP and insulin secretion responses in vitro and in vivo as GLP-1 [127]. Both agonists exhibit no detectable GLP-1R mediated β-arrestin recruitment, suggesting that glycemic and weight loss effects in vivo might be achieved by avoiding receptor desensitization [127]. In parallel to the other non-peptide agonists, conformational changes and distinct ligand-residue interactions could provide further insights into mechanisms underlying biased agonism. While one arm of the Boc5 compound inserts into an orthosteric peptidomimetic binding pocket, the other three arms extend into TM1–7, TM1–2 and TM2–3 cleft, forming similar interactions to those formed by the OWL833 bound receptor structure, but with subtle differences conferring signalling bias in TM7 [127]. Additionally, WB4–24 is bulkier promoting movement of the extracellular tips of TM2–3 and TM7 outwards compared to Boc5, TT-OAD2 and OWL-833 establishing differences in TM7 interactions and enhanced signalling potency [127]. This might also explain the reason for its more potent in vivo and in vitro activities compared to its parent molecule Boc5. Lastly, this also bolsters the crucial role of ligand-receptor interactions with TM7 in bias signalling.

6. Dual and triple incretin receptor agonists

The incretin hormone receptors (GLP-1R and GIPR) and the GCGR have similar structures, exert their effects through similar G protein-mediated mechanisms of action, and all have insulinotropic effects on pancreatic β-cells [86]. Interestingly though, these receptors exhibit differential localisation in tissues and tissue-specific characteristics, which likely explains the variation in their functions and mode of signalling [87], raising the possibility of synergistic effects that can be exploited to reduce adverse effects and increase drug tolerability [88, 105]. Indeed, there is a surge in interest in combining GLP-1R, GIPR and GCGR actions in novel therapeutic agents for metabolic diseases, typically as chimeric unimolecular dual- or tri-agonist peptides [87]. Several studies have reported superior outcomes of dual GIPR/GLP-1R or GLP-1R/GCGR and triple GIPR/GLP-1R/GCGR agonists over approved GLP-1RAs, and some have successfully entered clinical trials [22,87, 89–91]. For example, tirzepatide, a once-weekly dual GIPR/GLP-1R agonist, which showed unprecedented improvement in insulin sensitivity, weight loss and general metabolic health over currently approved agonists such as semaglutide in multiple clinical trials [92–95]. Tirzepatide has recently been approved by the FDA. Studies by Willard et al., in human GIPR/GLP-1R-expressing HEK cells showed that tirzepatide is an “imbalanced” dual agonist, favouring GIPR over GLP-1R activity, but also showing pronounced cAMP signalling bias over β-arrestin recruitment at the GLP-1R, associated with reduced GLP-1R internalisation [78]. Also relevant to the action of co-agonist molecules is the potential for receptor crosstalk to modulate signalling in ways not possible with mono-agonists; an example of this is shown by a recent study using BRET to reveal that co-expression of GLP-1R and GIPR enhanced GLP-1R-mediated cAMP accumulation whilst reducing β-arrestin 2 recruitment, yet had no effect on GIPR signalling [96]. The GIPR co-expression-linked reduction in β-arrestin 2 recruitment was also observed in cells treated with dual agonists such as peptide 19, peptide 18, tirzepatide, and a GLP-1R/GCGR/GIPR triagonist [91] reported to have a similar bias profile to the dual-agonists at the GLP-1R, with slightly increased Gαs activation and cAMP accumulation [91].

Considering the increased interest in these co-agonists, a recent study investigated the differences in spatiotemporal control of both the GLP-

1R and GIPR when stimulated with mono versus dual-agonists, such as tirzepatide (GIP-favoured agonism) and MAR709 (balanced agonism) [71]. The trafficking profile of MAR790 pointed to unique spatiotemporal pharmacology, as this ligand resulted in reduced internalisation and co-localisation in early endosomes and increased GLP-1R co-localisation to Rab11 + recycling endosomes in MIN6 β-cells, akin to the action of GLP-1 and semaglutide [71]. This suggests that either the most internalised receptors are targeted to the recycling endosomes for resensitisation, or that GLP-1R delivery to the plasma membrane is increased to replenish receptor supplies. This unique phenomenon can be used to further fine-tune agonist-induced cellular sensitisation of the receptor.

In addition to this, more evidence has shown that stimulation by tirzepatide (and to a lesser extent MAR709) resulted in markedly less GLP-1R internalisation and longer cell surface presence compared to the GLP-1R mono-agonists semaglutide and GLP-1 [71]. This is consistent with the effector recruitment profiles of tirzepatide and MAR709, which show, respectively, significantly reduced or totally absent GLP-1R-mediated β-arrestin 1/2 and Gαq recruitment, compared to the mono-agonists [71], suggesting that the two dual agonists, which differ in ratio of agonism to one receptor versus the other, also differ in G protein recruitment and receptor internalisation profiles.

From a structural perspective, tri-agonists and dual agonists seem to exhibit distinct binding conformations when bound to their different target receptors. Despite these peptides exhibiting common conformational features to the endogenous peptide binding-patterns (GIP, GCG or GLP-1), yet the three receptors still show conformational adaptability to stimulation by different agonists [97,98]. A recent study revealed the cryo-EM structures of dual agonist (GIPR and GLP-1R) tirzepatide and tri-agonist peptide 20 (GLP-1R-GIPR-GCGR) complexed with the GIPR-Gs, GLP-1R-Gs or GCGR-Gs for peptide-20 and compared this to each of these three receptors complexed to their corresponding endogenous agonists [98]. They showed that although the GIPR complex with tirzepatide and peptide-20 closely resembled that with the GIP agonist, there were differences in peptide binding compared to GIP [98], specifically in residue Arg 190 and nearby water-mediated polar interactions [97]. On the other hand, GIP and tirzepatide formed strong interactions with the TMD core compared to peptide-20 which was deficient in TMD contacts [98]. Although the tirzepatide and peptide-20 penetrated well into the TMD core in a highly similar orientation to GLP-1[98], suggesting a similar ligand recognition pattern another study showed differences in the mode by tirzepatide mediated GLP-1R activation [97]. Whereby, the tirzepatide-bound GLP-1R adopted a distinct conformation, akin to that found in the Ex-P5 and taspoglutide-bound GLP-1R complex, where the C-terminal segment of the receptor was tilted towards its ECL1 [97]. Hence, providing structural basis for similarities in pharmacological response between the biased agonists tirzpeatide and Ex-P5.

Lastly, by comparing the GCG-bound GCGR structure to the peptide 15 (GLP1R/GCGR dual agonist) and peptide 20 bound structure, a conserved ligand recognition pattern involving ECD, ECL1 and TM1 is highly conserved amongst the three peptides [98]. Observations of these cryo-EM structures also provide evidence that GIP and GLP-1 cannot form favourable contacts with the GCGR and so is unable to active it [98].

7. GLP-1R coding variants

Pharmacogenomics, defined as the study of variability in drug responses due to genomic variation, is a promising field directly linked to precision medicine. It is well known that, whilst GLP-1RAs are a highly effective class of agents for T2D treatment, some individuals benefit less or not at all, and some may experience a higher rate of detrimental side effects [99]. Diverse factors undoubtedly underlie inter-individual variability, with straightforward contributory factors such as adherence and achieved circulating drug levels likely to play a key role [100]. However, genomic variation in *GLP1R* and downstream genes involved in transducing the GLP-1R signal may be directly linked to therapeutic responses in a mechanistically resolvable manner. Beyond therapeutic relevance, studies of *GLP1R* variants can have a significant impact on pharmacology as they reveal residues important for ligand binding, signalling, and trafficking of the receptor. A growing body of literature has documented naturally occurring coding variation in the *GLP1R* locus, including nonsynonymous single nucleotide variants (SNVs) that are associated with specific clinical outcomes such as glucose levels, glycaemic responses, weight loss, T2D and cardiovascular risk [101–103] (Table 2) (Fig. 7).

However, dedicated experimental studies that confirm whether and how these SNVs modify the incretin effect or GLP-1RA responses are limited. An early study by Tokuyama et al., 2004 identified five missense variants in the *GLP1R* gene in a Japanese diabetic and non-diabetic cohort [104]. Only the Thr149Met mutation was detected in one T2D patient, associated with impaired insulin secretion, sensitivity and glycaemic response [104]. Likewise, functional assessment of this variant in COS-7 and HEK293 cells compared to the wild-type receptor revealed reduced binding affinity with GLP-1 and exendin-4, regardless of unchanged cell surface expression levels [105], thus this variant confers a loss of function. A more comprehensive investigation of ten SNVs and their corresponding effect on the three main GLP-1R-mediated signalling pathways (cAMP, pERK1/2, and intracellular calcium mobilisation) was conducted, and results demonstrated ligand-specific pathway effects [106]. The Thr149Met variant was again shown to have one of the most significant effects, with loss of peptide-induced responses across all three pathways [106]. The degree of loss of function was pathway-dependent, affecting cAMP accumulation and Ca^{2+} mobilisation more than pERK1/2 signalling. In contrast to this, the S333C mutation exhibited the opposite effect with preserved responses with all peptides tested [106] (Fig. 8).

It is interesting to note that the pharmacological impact of *GLP1R* variants may be ligand- and pathway-specific, with certain variants resulting in disproportionate loss/gain for certain signalling pathways over others. For instance, intracellular Ca^{2+} signalling was significantly reduced in response to exendin-4 but not in response to GLP-1 in the Ser333Cys variant [106]. Previous studies have addressed pharmacological effects arising from variants focused on changes in downstream signalling pathways. Fang et al., 2020 supported previous studies by showing that Thr149Met variant reduced mini-G_s and β-arrestin-2 recruitment, but also reported alterations in trafficking with lack of internalisation, faster recycling, and decreased degradation in response to exendin-4 [107]. Intriguingly, whilst Thr149Met is commonly assumed to be a loss-of-function variant, its pharmacological profile mimics that of the biased agonist exendin-F1, which is a low efficacy molecule that nevertheless possesses enhanced insulinotropism through avoidance of GLP-1R desensitisation. To understand their therapeutic relevance, *GLP1R* SNV effects need to be evaluated not only for their acute signalling profiles but also under conditions of sustained activation.

Recent genome wide association studies (GWAS) have identified novel GLP-1R coding variants associated with glycaemic traits such as fasting glucose, random glucose and T2D risk [101,103]. In particular, a *GLP1R* low-frequency missense variant (Ala316Thr; rs10305492, maf = 1.4%) was found associated with lower T2D risk, lower fasting glucose, altered insulin responses and lower cardiovascular risk [103]. More recently, the structural, pharmacological, and physiological effects of this missense variant were further investigated in concert with other *GLP1R* coding variants by leveraging population data from the UK Biobank and other large datasets along with experimental and computational approaches [101]. Specifically, molecular dynamics simulations of hGLP-1R bound to oxyntomodulin revealed that this single residue substitution in TM5 results in an alteration in the central hydrogen bonding network, orientation of TMs5/6 leading to a change in the conformation of ICL3 linking these two domains involved in G protein

Table 2
List of all SNPs and their coding consequences, allele frequency, associated clinical outcomes and experimental outcomes.

Variant ID	Coding Consequence	Allele Frequency	Associated Clinical Outcome	Reference for Clinical Associations	Experimental Traits	Reference for Experimental data
rs10305420	P7L	0.275335061	Impaired β cell insulin secretion and Increased β cell apoptosis	Yu, Wang, Liu and Cao, 2019	Retained cAMP agonism with compound 2 activation	C Koole et al. (2011)
			Reduced response to exenatide in overweight T2D patients	Daghlas et al., 2021	No change in OXM response by compound	
			Decreased glycated haemoglobin levels, Heart failure and left ventricle ejection fraction	Jensterle et al., 2015 B. McKinstry, et al. 2017	Attenuated allosteric enhancement of oxyntomodulin cAMP signalling with compound 2 Non-significant decrease in CSE	
			Poor response and weight loss in response to liraglutide 12-week treatment			
			NAFLD in the SHARE Scottish medical records			
rs2295006	R44H	0.001669647	Bone Mineral Density in postmenopausal women, Glycaemic traits	O, Fedoryak, 2017	Significantly enhanced oxyntomodulin response, Loss of Function in mini-Gs recruitment, non-significant reduction in endocytosis in response to exendin-4	C Koole et al. (2011) Lagou et al. (2021)
rs150253529	R48H	2.62892E-05	Colorectal cancer	Santarius et al., 2010	No change in surface expression, endocytosis or mini-Gs recruitment in response to exendin-4	Lagou et al. (2021)
rs774357734	D67N	2.3903E-05	Associated with Random Glucose	Lagou et al., 2021	Loss of function in mini-Gs recruitment, endocytosis and reduced surface expression	Lagou et al. (2021)
rs3765467	R131Q	0.016058001	Blood sugar levels	Qui X et al.,2020	Reduced peptide efficacy for GLP-1 and Exendin-4, attenuated allosteric enhancement of oxyntomodulin cAMP response with compound 2 no change in surface expression	C Koole et al. (2011)
			Increased Type 2 diabetes, Increased risk of Parkinson's Disease, Male lean, and adipose tissue mutations	Zhang et al., 2020 Li W et al., 2020	Unchanged mini-Gs recruitment, endocytosis, and cell surface expression in response to exendin-4	Lagou et al. (2021)
			Bone Mineral Density and Osteoporosis, Impaired β cell insulin secretion and Increased β cell apoptosis	Zeng Z et al.,2020		
			Response to DPP4 inhibitors in T2D patients	Nishiya Y et al., 2020		
			Nutrient-dependant decrease in insulin secretion	Dawed AY et al. 2016		
			Decreased standard deviation of plasma glucose after exenatide 5 ug treatment twice daily	C.H. Lin et al., 2015		
			Better responses to liraglutide	Jensterle et al., 2015		
			Exogenous GLP-1 responses	Sathananthan A et al. 2010		
rs147627784	G132R	6.57082E-06	Non-significant decrease in fasting glucose	Wessel et al., 2015	Unchanged mini-Gs recruitment, endocytosis, and cell surface expression	Lagou et al. (2021)
rs112198	T149M	1.50E-05	higher Type 2 Diabetes risk, found in patient with T2D, impairs insulin secretory response to GLP-1	D de Luis., et al., 2014	Significant reduction in cell surface expression and affinity to GLP-1, exendin-4 and oxyntomodulin, reduced potency of cAMP signalling for GLP-1, ex-4 and oxn, reduced intracellular Ca2 + response, response to orthosteric agonists can be rescued by compound 2	C Koole et al. (2011)
					significant reduction in exendin-4 induced mini-Gs and B-arrestin-2 recruitment, significantly reduced internalisation, reduced recycling and delayed degradation, Loss of Function	Fang et al. (2020), Lagou et al. (2021)
rs6923761	G168S	0.21897527	Smaller HbA1c reduction after gliptin therapy	Zeng Z et al.,2020	Significant reduction in surface expression, reduced efficacy to GLP1, exendin-4, attenuated allosteric enhancement in oxyntomodulin induced cAMP response	C Koole et al. (2011)
			Higher alcohol consumption in humans	Suchankova et al., 2015	Small RG lowering effect and subtle increases in function in response to exendin-4 and semaglutide.	Lagou et al. (2021)
			Increased weight loss and metabolic improvement in diabetic patients treated with liraglutide	D de Luis., et al., 2015		

(*continued on next page*)

Table 2 (*continued*)

Variant ID	Coding Consequence	Allele Frequency	Associated Clinical Outcome	Reference for Clinical Associations	Experimental Traits	Reference for Experimental data
			Decreased insulin secretion in non-diabetic patients after a 2 h GLP-1 infusion	Sathananthan A et al. 2010		
			Less weight loss after Biliopancreatic diversion surgery	D de Luis, D Pacheco, R Aller, O Izaola, 2014		
rs146340667	V194I	0.000282553	Associated with Random Glucose	Lagou et al., 2021	Increase in mini Gs recruitment, surface expression and endocytosis	Lagou et al. (2021)
rs140642887	A239T	0.000368373	Associated with Random Glucose	Lagou et al., 2021	Loss of function in mini-Gs recruitment, endocytosis, and reduced surface expression	Lagou et al. (2021)
rs185053350	S261A	6.57168E-06	Associated with Random Glucose	Lagou et al., 2021	No change endocytosis or mini-Gs recruitment in response to exendin-4	Lagou et al. (2021)
rs145619754	L268F	0.000151127	Non-significant reduction in fasting glucose	Wessel et al., 2015	No change endocytosis or mini-Gs recruitment in response to exendin-4	Lagou et al. (2021)
rs10305492	A316T	0.010692551	Impaired β cell insulin secretion and Increased β cell apoptosis, Reduced Fasting Glucose	Li W et al., 2020	significant reduction in surface expression, weak intracellular Ca2 + response, unchanged cAMP response with compound 2	C Koole et al. (2011)
			Reduced Type 2 Diabetes Risk	Wessel et al., 2015	Significant increase in mini-Gs recruitment and endocytosis in response to GLP1, OXM, GCG, Semaglutide and Tirzepatide but not exendin-4	Lagou et al. (2021)
			Early insulin secretion	Mahajan A. et al., 2015		
			High 2-h glucose	Chen J et al., 2021		
			Decreased HA1c levels	Scott et al.,2016		
			Reduced Cardiovascular Risk			
			Reward Learning and Anhedonia	Yapici-Eser et al., 2020		
rs10305493	S333C	4.60115E-05	lower T2D risk, lower fasting and random glucose, lower HbA1c		preserved peptide affinity and cAMP responses, reduced cAMP signal with compound 2, reduced efficacy of intracellular Ca2 + levels with exendin-4 reduced mini-Gs recruitment with Exendin-4	C Koole et al. (2011) Lagou et al. (2021)
rs202171972	D344E	8.54061E-05	Associated with Random Glucose	Lagou et al., 2021	No change endocytosis or mini-Gs recruitment in response to exendin-4	Lagou et al. (2021)
rs527991362	A375T	1.97163E-05	Associated with Random Glucose	Lagou et al., 2021	Reduction in exendin-4 mediated mini-Gs recruitment	Lagou et al. (2021)
rs61733062	R376Q	0.000519245	Non-significant decrease in fasting glucose	Wessel et al., 2015	Reduction in exendin-4 mediated endocytosis	Lagou et al. (2021)
rs146868158	R421W	0.000532502	Keratoconus, Random Glucose, Blood Glucose Homeostasis	J., Hardcastle et al., 2021 Lagou et al., 2021	Significantly reduced endocytosis and mini-Gs recruitment with all peptides GLP1, OXM, GCG, Exendin-4 Semaglutide and Tirzepatide	Lagou et al. (2021)
rs10305510	R421Q	0.005695645	Non-significant decrease in fasting glucose	Wessel et al., 2015	Reduced Ca2 + to GLP-1 and exendin-4, compound 2 mediated enhancement of oxyntomodulin cAMP response	C Koole et al. (2011)
					Significantly reduced endocytosis and mini-Gs recruitment with Exendin-4	Lagou et al. (2021)
rs201223341	T440A	0.000157741	Associated with Random Glucose	Lagou et al., 2021	No change endocytosis or mini-Gs recruitment in response to exendin-4	Lagou et al. (2021)
rs201672448	S445T	0.000499435	Significant increase in fasting glucose	Wessel et al., 2015	No change endocytosis or mini-Gs recruitment in response to exendin-4	Lagou et al. (2021)

engagement [101,103]. This is consistent with mini-Gs complementation assays revealing increased Gαs coupling for this variant in response to several ligands including GLP-1, glucagon, oxyntomodulin, semaglutide and tirzepatide in HEK293 cells [101]. Individuals with this variant had lower blood glucose levels, in line with its pharmacological characterisation. Of note, previous attempts to characterise this variant in vitro documented a marked reduction (75%) in cell surface expression compared to the wild-type, and greatly reduced intracellular calcium mobilisation in response to the endogenous ligand (GLP-1) and exendin-4 [106]. This explains the reduced early insulin response observed, yet chronic incretin responses might still be enhanced leading to an overall reduction in T2D risk. So far, in vitro experiments have used heterologous expression systems, and it will be important to evaluate how this and other *GLP1R* variants behave when expressed at endogenous levels in their native cellular environment and in vivo.

In the same study, the common (rs10305492, maf = 22%) *GLP1R* missense variant Gly168Ser showed a small lowering effect on blood glucose, along with a trend towards increased mini-Gs recruitment and GLP-1R endocytosis compared to the wild-type receptor stimulated with native GLP-1. Notably, a "tirzepatide-specific" gain-of-function pattern was observed for G168S not present with exendin-4 at the in vitro level [101]. Interestingly, carriers of this SNV exhibited lower BMI, glucose, triglycerides, leptin, and insulin levels [108]. This variant has also previously been associated with greater weight loss in diabetic patients treated with liraglutide [109], but less weight loss in patients who have undergone biliopancreatic surgery [110]. Glucose excursions after a mixed meal were suppressed in individuals with the Gly168Ser variant, but the added benefit of treatment with a DPP-4 inhibitor on the same readout was attenuated [111], and associated with smaller HbA1c improvements after sustained DPP-4 inhibitor treatment [112], and reduced insulin secretion levels in response to GLP-1 infusion in non-diabetic patients [113]. Taken together these findings suggest that Gly168Ser may be capable of enhancing endogenous GLP-1R action but this gain-of-function is lost or even reversed under conditions of elevated GLP-1 (e.g. as seen with DPP-4 treatment of bariatric surgery). In some ways, this resembles the paradoxical effects of the Glu354Gln common *GIPR* variant, for which gain-of-function when assessed acutely in vitro can tip over into increased desensitisation which leads to impaired GIPR signalling and adverse effects on bone and glycaemic health [114].

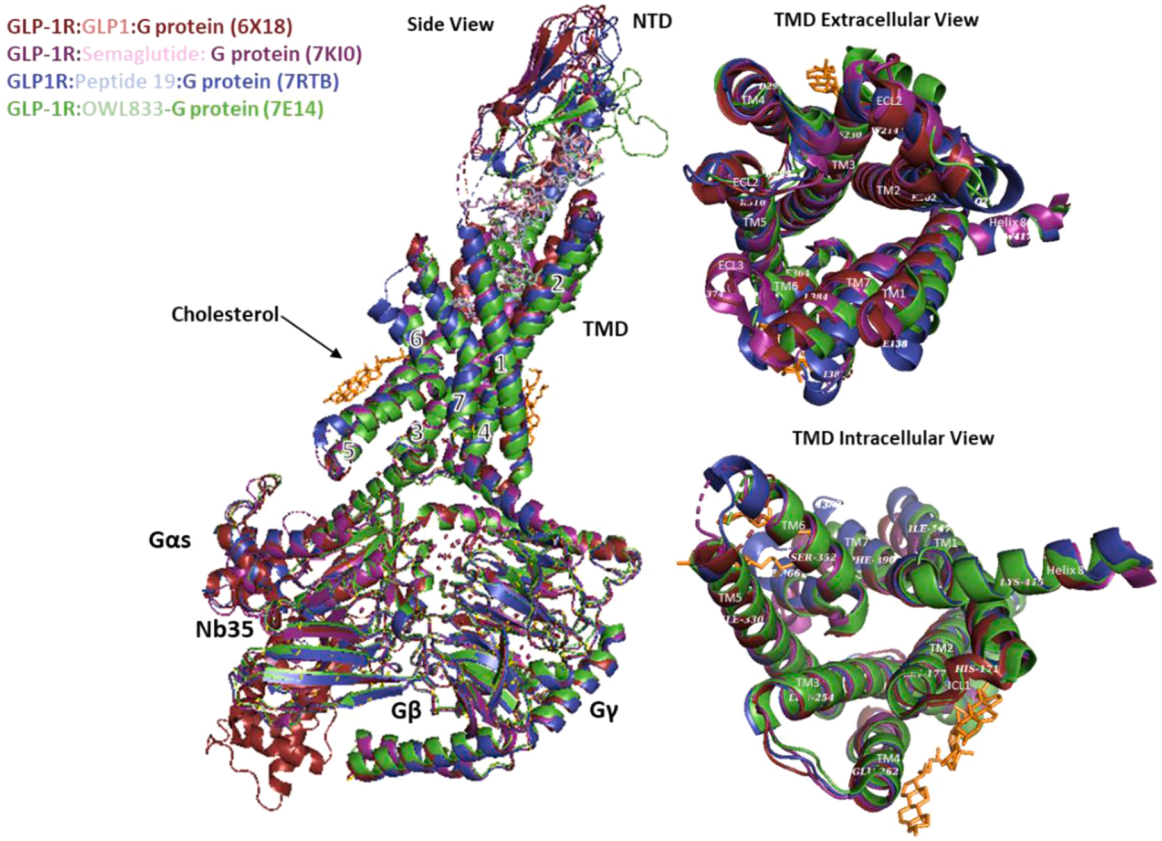

Fig. 4. Active GLP-1R: Agonist: G protein complexes for the four GLP-1RAs GLP1, semaglutide, peptide 19, and the non-peptide agonist OWL833. On the left is the side view of the full receptor structures superimposed using PYMOL. On the right are the extracellular and intracellular views of the receptor-agonist complexes superimposed with the ECD domain removed for better view of the TMD. Alignment of structures was done using PyMOL(TM) Molecular Graphics System, Version 2.1.0.

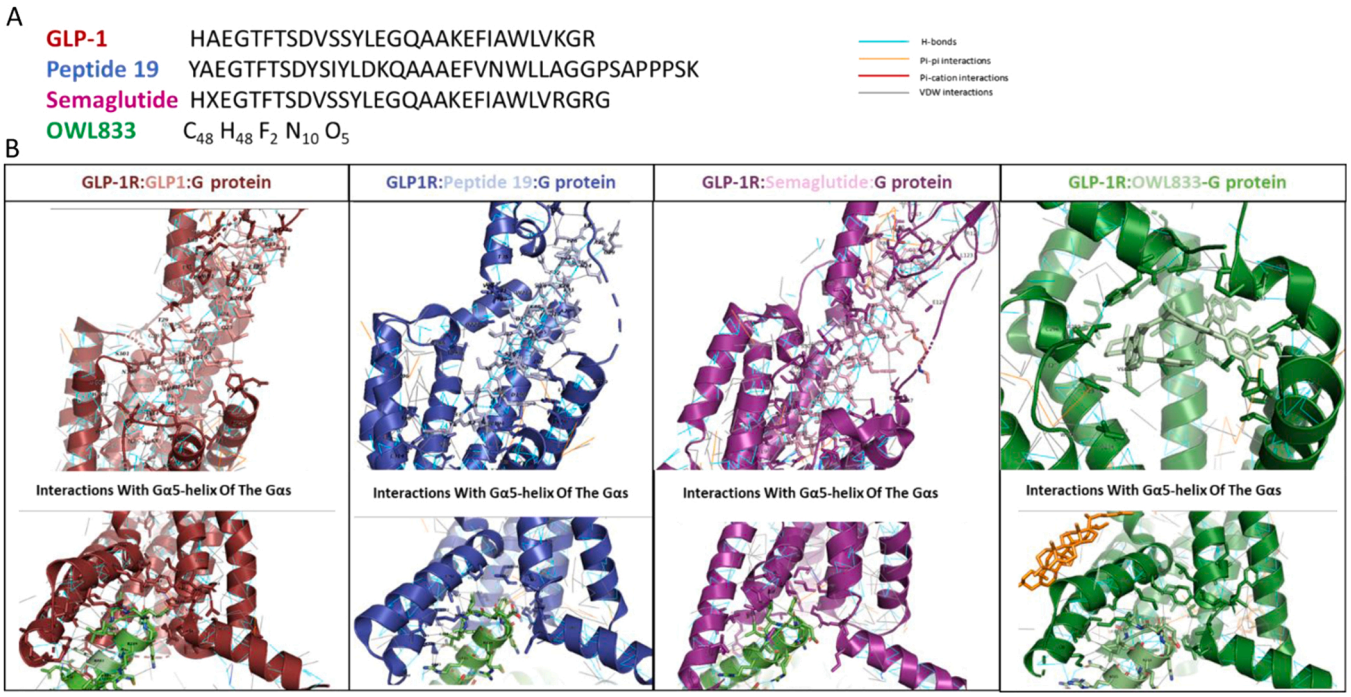

Fig. 5. A) Sequences and chemical composition of GLP-1RAs. B) Active GLP-1R:agonist:G protein complexes for the four GLP-1RAs GLP1 (6X18), semaglutide (7KI0), peptide 19 (7RTB), and the non-peptide agonist OWL833 (7E14), with focus on agonist binding site interactions and interactions with Gα5-helix of the Gαs protein. On the top are the structures with the agonist-receptor interactions and binding pocket zoomed in. On the bottom are the structures with the receptor Gαs protein binding sites highlighted. Figures were produced using PyMOL(TM) Molecular Graphics System, Version 2.1.0.

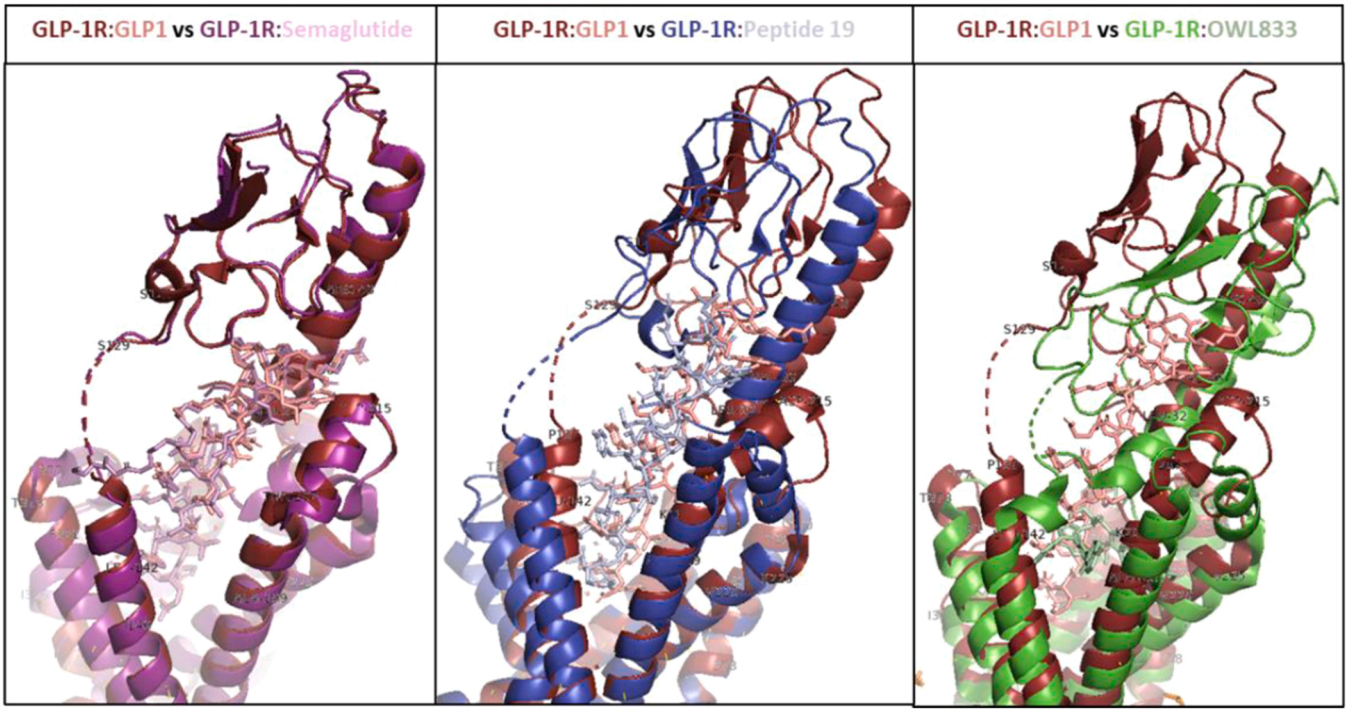

Fig. 6. Agonist binding pocket for active GLP-1R:GLP-1 complex superimposed with GLP-1R: semaglutide and GLP-1R:OWL833. The structure in brick red depicts GLP-1R:GLP-1, the structure in pink depicts GLP-1R:semaglutide, the structure in blue depicts GLP-1R:peptide 19, and the structure in green depicts GLP-1R:OWL83 (non-peptide agonist). Alignments and figures were produced using PyMOL(TM) Molecular Graphics System, Version 2.1.0.

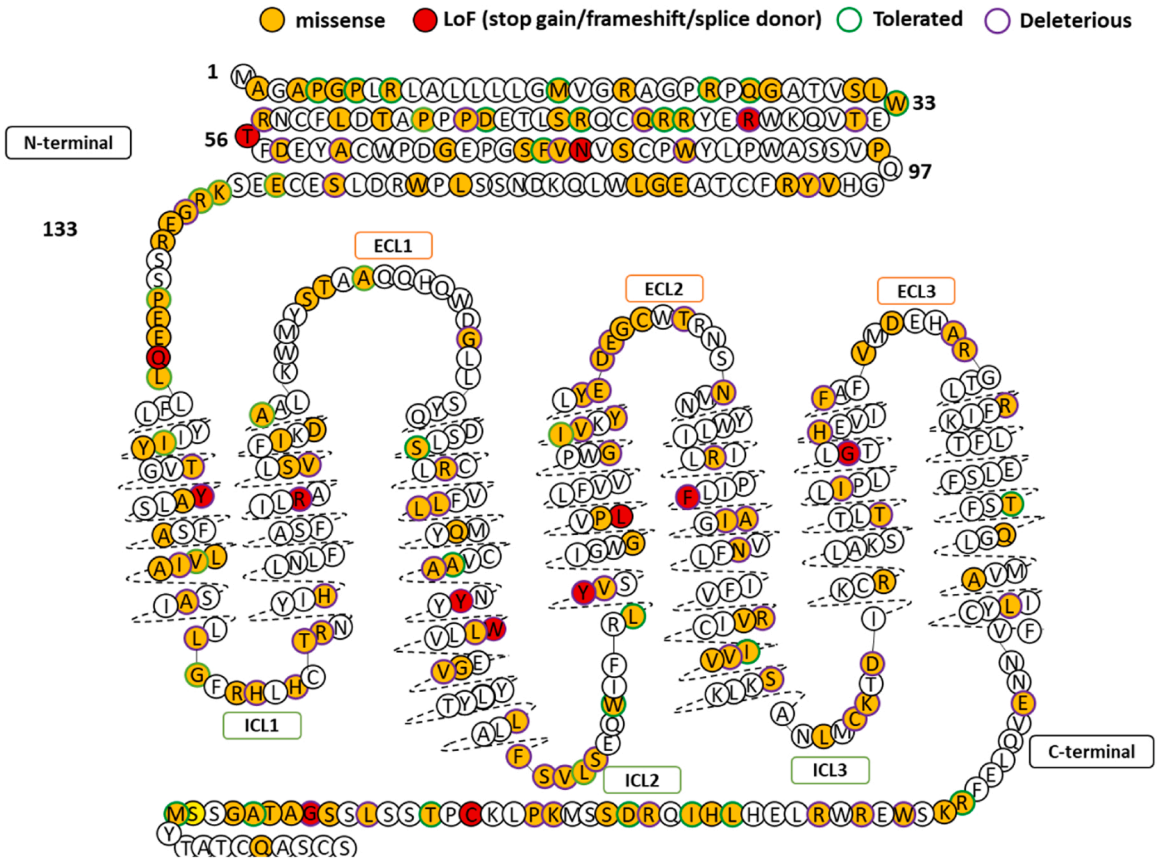

Fig. 7. Snake plot of the coding variants present in the GLP-1R protein sequence. Uniprot accession: GLP1R_HUMAN; generated with PowerPoint. Extracellular regions are shown at the top, cytoplasmic regions at the bottom, and transmembrane domains between in the middle. Missense mutations are displayed in yellow, loss-of-function including stop gain mutations, frameshifts, and splice donors in red. GLP-1R variants that are tolerated are depicted with a green outer outline while deleterious variants have a purple outline. Data for each mutation was acquired from GPCRdb.org and gnomAD v2.1.1 database.

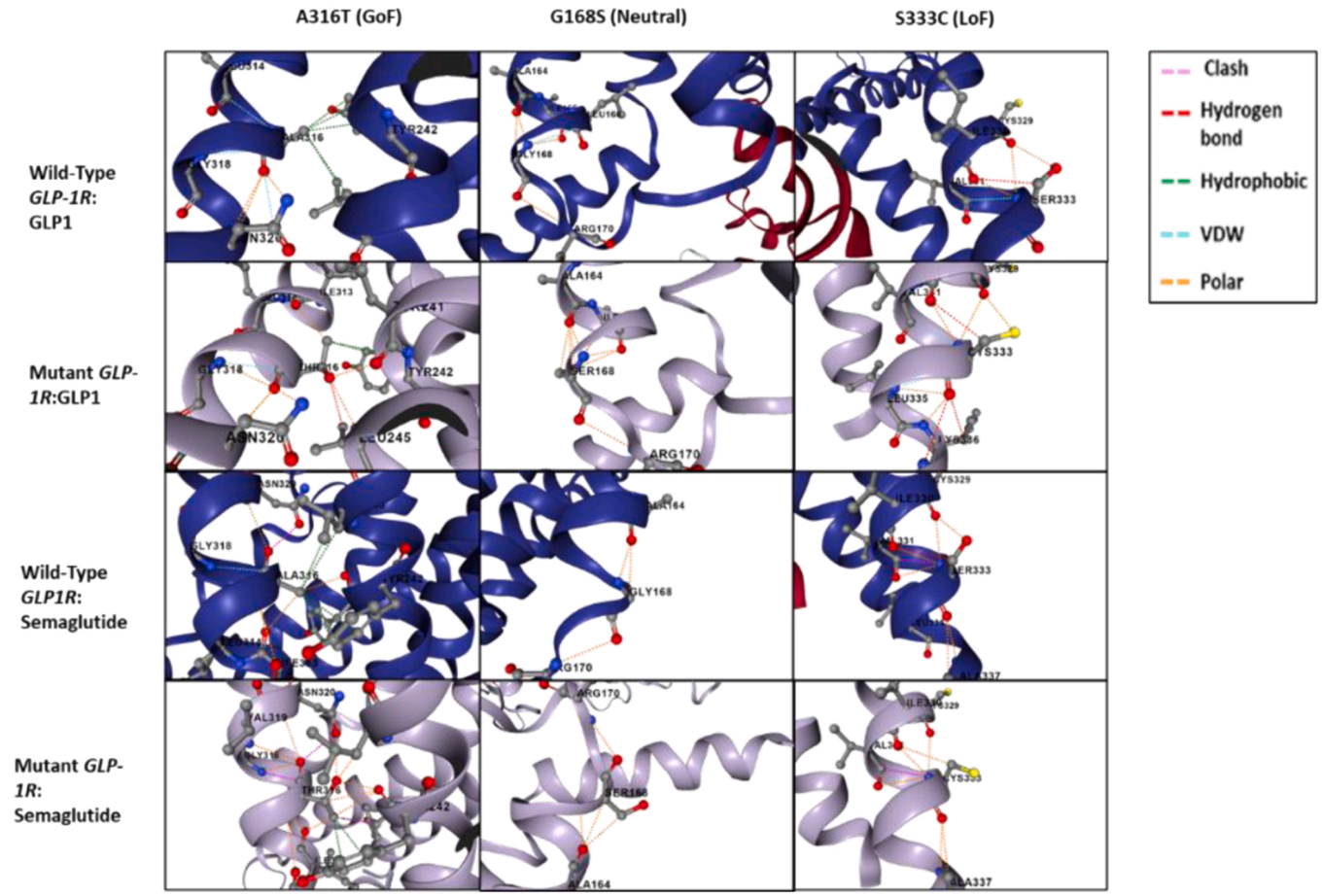

Fig. 8. Single nucleotide polymorphisms in the *GLP1R* gene alter residue-residue interactions within specific agonist-receptor complexes. Left column depicts residue-residue interactions of the Ala316 amino acid when bound to GLP-1 and semaglutide in the wild-type and mutant state. Middle column depicts residue-residue interactions of the Gly168 residue when bound to GLP-1 and semaglutide in the wild-type and mutant state. Last column depicts residue-residue interactions of the Ser333 amino acid when bound to GLP-1 and semaglutide in the wild-type and mutant state. Figures were produced using the mCSM-PPI2 server.

Comparing the structure of variant GLP-1Rs in complex with GLP-1 and other GLP-1 agonists such as semaglutide, to that of the wild-type receptor could provide novel insights into the distinct interactions made by the altered residue and the molecular mechanisms underlying biased agonism. Elucidating the role of domain-domain and residue-residue interactions in receptor function, G protein engagement and signalling, both in general and in a ligand-dependent manner, will help predict the effect of certain polymorphisms. For instance, the substitution of the non-polar alanine residue with the threonine residue in position 316 in the GLP-1R:GLP-1 complex results in a loss of hydrophobic interactions and a gain of polar interactions, with nearby residues such as Asn320 and Gly318 forming a polar-bonding network that might influence the binding affinity of positive allosteric modulators as these residues are part of their transmembrane binding pocket (Fig. 8) [115]. Interestingly, hydrophobic interactions involving the same residues are replaced by polar residues in the wild-type receptor structure when bound to semaglutide, which is further pronounced in the Ala316Thr mutant (Fig. 8). Similarly, when the glycine residue in position 168 is replaced by the polar residue serine in the GLP-1-bound receptor structure (Gly168Ser variant), there is a gain of polar interactions with nearby Ala164 and Arg170 residues residing in ICL1 which may form part of the Gαs:GLP-1R interface, specifically direct interactions with Gβ [35] (Fig. 8). Unlike the Ala316Thr variant, residue-residue interactions in the vicinity of the G168 position are not altered with binding to semaglutide, and only fewer polar interactions exist compared to the GLP-1-bound mutant (Fig. 8). Lastly, the Ser333 residue residing at the TM5/ICL3 junction gains polar, hydrogen and weak van der waals interactions with Leu335 and Lys336 in the GLP-1-bound structure (Fig. 8). Akin to the Ala316Thr mutant, the semaglutide-bound wild-type structure had similar interactions to the mutant GLP-1-bound structure, but unlike the Ala316Thr mutant, the Ser333Cys mutant bound to semaglutide had more conserved interactions with its wild-type counterpart (Fig. 8). This serine residue is a candidate phosphorylation site, hence this substitution for a cysteine will reduce phosphorylation on this site, possibly leading to altered downstream signalling. In addition to this, the TM5/ICL3 interface is involved in G protein coupling, and so this variant could also lead to altered G protein recruitment [1]. Overall, these structures clearly depict that residue-residue interactions are both ligand-dependant and can be altered by missense variants leading to amino acid substitutions.

Another complicating factor to be considered in *GLP1R* pharmacogenomics is the presence of multi-nucleotide variants, i.e., more than one variant in one individual. In addition to the likely co-occurrence of common variants such as Gly168Ser with other less common variants, analysis of the gnomAD database of *GLP1R* single nucleotide variants with MAF< 5% revealed two such variants, Arg176Lys (rs771324929) and Val287Asp (rs767188992) [116]. In the case of Val287Asp (rs767188992), this was found to occur in phase with the synonymous mutation Val287Val (rs772793425) altering the coded amino acid to glutamic acid [117], leading to alterations in the residue-peptide ligand interactions and structure of the receptor, culminating in subsequent differences in signalling, trafficking, and pharmacodynamics. A recent study investigated seven *GLP1R* SNPs in 152 T2DM patients, determining a genetic relationship between gastrointestinal adverse reactions

(GIARs) in response to liraglutide and these variants. These SNPs seemed to be in linkage disequilibrium, especially rs2254336 (intron 3 variant, MAF=55.75%), rs3675468 (Lys130Lys, MAF=20.63%), and rs3765467 (Arg131Gln, MAF=22.72%) [118]. This suggests that these variants are in association in this population of type 2 diabetes patients, hence could co-occur in an individual resulting in an additive effect. The variants rs2254336 and rs3765467 appear to be associated with liraglutide-dependent GIARs, so if these two variants were present in an individual this could result in a further increase in adverse effects [116]. More research is needed into the likelihood of *GLP1R* variants occurring in the same haplotype, linkage disequilibrium and association with clinical characteristics related to GLP-1R agonists. In addition to this, more studies should investigate not only missense *GLP1R* variants but also variants in intronic regions and their associations with pharmacological traits such as adverse effects and response to GLP-1R agonists.

8. Future directions

Overall, both genetic (e.g. target and effector SNVs) and ligand factors (biased agonism and spatiotemporal signalling) contribute to different GLP-1RA responses in individuals. It appears likely that these factors interact with each other, so that different GLP-1RAs will show differential therapeutic performance for different individuals. Increased knowledge at the molecular, pharmacological and clinical level is required to fully understand these phenomena to leverage therapeutic benefits. For example, molecular dynamics simulation studies and receptor-agonist modelling could be utilised to investigate and predict the effects of specific single nucleotide polymorphisms on receptor activation, signalling and trafficking. Improved analysis of *GLP1R* coding variant responses to different GLP-1R agonists should include studying their effects in their native environment, both in cellular and in vivo assays, as a majority of studies so far have used overexpression systems which may deviate from the normal situation. Whilst small studies have revealed the impact of certain *GLP1R* variants on responses to DPP4 inhibitors [119] responses and liraglutide [109], a large scale pharmacogenomic analysis of trials of modern GLP-1RAs is needed to fully understand their impact in clinical practice. This may be partly hindered by the fact that genomic datasets from clinical trials, when available, are traditionally in the form of SNP array data only, which has limited performance in identifying rare variants; full exome sequencing represents a significantly more powerful approach to delineate these effects and may be more realistic in the future due to reduced costs of this technology.

Although use of dual and tri-agonists to initiate synergistic effects on diabetes and metabolic diseases is a popular area of interest, the potential impact of biased signalling and/or target genomic variation provides a further level of complexity that may need to be considered. Ideally, future studies should integrate site directed mutagenesis, structural studies, and clinical data from patients with specific receptor variants to identify important favourable peptide recognition residues by each receptor. This will aid better development of agonists targeted to specific signalling pathways thus achieving optimal therapeutic benefits from combinatorial agonism. For example, a recent study determined cryo-EM structures of the dual agonist tirzepatide bound GIPR and GLP-1R, as well as the tri-agonist peptide-20 coupled to GLP-1R, GIPR, and GCGR [25]. They reveal that tirzepatide and peptide-20 exhibit highly similar recognition networks between the peptide and TM core compared to GLP-1, but the dual and tri-agonist form extra novel interactions with residues in the TM1-TM2 cleft [25].

Another important consideration for the design of multi-agonist T2D therapies is the optimum ratio of agonism between one receptor and the other; this will likely depend on the candidate metabolic endpoint targeted by the drug [87]. For example, it has been proposed that targeting GIPR agonism is a valid means to attenuate GLP-1RA-induced nausea, but the right ratio of GLP-1R versus GIPR activity needs to be found to achieve optimal insulinotropic actions and outcomes [105]. Lastly, molecular mechanisms underlying the basis of metabolic synergism in dual- and tri-agonists remain unclear. Hence, further work is required to understand the possible pathways by which synergism occurs and cross-talks with biased agonists to better exploit this phenomenon in therapeutic development.

The GLP-1R is expressed in several human and monkey tissues, including pancreatic islets (α-, β-, and δ-cells), CNS, heart (sinoatrial node mycocytes), lung, kidney (smooth muscle cells), and stomach (parietal cells) [120,121]. Determining tissue- and cell-specific expression levels of the GLP-1R and its downstream transducers is crucial to elucidate the specific role of agonist-induced GLP-1R signalling on tissue function, and conceivably may reduce adverse side effects through ligand-specific responses in extra-pancreatic tissues. Previously, investigating the specific presence of GLP-1R in distinct tissues has been hampered by the lack of sensitive and accurate antibodies against the receptor [122]. Richards et al. have overcome this issue by generating a transgenic mouse line expressing "humanised" Cre recombinase (iCre) under the control of the GLP-1R promoter, and they used this in vivo model to compare the expression of GLP-1R in the pancreas to that of other organs including the heart, kidney skeletal muscle and neurons [123]. This study confirmed the restriction of main GLP-1R expression to β-cells in the pancreas. They also revealed co-localisation of GLP-1R with αSMA in the arteries suggesting that GLP-1R could play a role in vascular smooth muscle cells that could explain reduced blood pressure and increased heart rate in T2D patients treated with liraglutide and exenatide [123]. Despite the benefits of this antibody-independent approach, antibodies are still needed to study receptor function such as receptor interactomes and downstream signalling. Detecting mRNA transcripts using techniques such as in-situ hybridisation remains the gold standard for identification of *GLP1R* tissue-specific expression, at least at the genetic level. Indeed, RNAscope has been recently used to demonstrate overlap of *GIPR* and *GLP1R* expression in mouse and human hypothalamus, specifically in the arcuate and dorsomedial hypothalamic nuclei, with *GIPR* solely expressed in periventricular cells in the ependymal regions [124]. This suggests that GLP-1R/GIPR dual agonist design will need to consider the different tissue-specific roles of these receptors in the pancreas versus the hypothalamus and possibly other regions as well. Moreover, tissue-specific alternate transcripts/splice variants might need to be considered while using these techniques by using different probes for each transcript/splice variant. Transcription of the *GLP1R* gene produces five different mRNA variants, and 4 of these are alternatively spliced, resulting in distinct isoforms [125]. There is a lack of data to characterise the expression of these variants in different tissues, and RNAScope multiplex fluorescence assays can be used via the design of variant specific probes to profile *GLP1R* mRNA variant expression in different tissues.

Hence, our understanding of GLP-1R and the complex factors that mediate its actions and responses to different agonists is far from complete, and further research, including into the compounding influence of biased agonism, polymorphisms, lipid interactions, and tissue-specific variation, is warranted to enhance the development of personalised therapeutics for metabolic disorders like T2D and obesity.

Declaration of Competing Interest

AT and BJ have received funding from Eli Lilly. AT has also received funding from Sun Pharmaceuticals.

Data Availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by a Diabetes UK-funded PhD Studentship to A.T. and L.E.E., as well as by MRC grant number MR/R010676/1 to A.

T. and B.J. and by UKRI COVID-19 Grant Extension Allocation (coA) to A.T. A.T. and B.J. also acknowledge support from the EFSD. A.T. acknowledges funding from the Commonwealth and the Integrated Biological Imaging Network (IBIN). B.J. acknowledges funding from Diabetes UK and the IPPRF scheme.

Declarations of interest

A.T. and B.J. have received grant funding from Sun Pharmaceuticals and Eli Lilly.

Author contributions

Main writing and figures generated by L.E.E. All the authors contributed to writing and editing the review.

References

[1] D. Donnelly, The structure and function of the glucagon-like peptide-1 receptor and its ligands, Br. J. Pharmacol. 166 (1) (2012) 27, https://doi.org/10.1111/J.1476-5381.2011.01687.X [Online] Wiley-Blackwell (Available from:) [Accessed: 15th August 2021].

[2] B. Thorens, A. Porret, L. Buhler, S.-P. Deng, P. Morel, C. Windmann, Cloning and functional expression of the human islet GLP-1 receptor. Demonstration that exendin-4 is an agonist and exendin-(9-39) an antagonist of the receptor, Diabetes 42 (11) (1993) 1678–1682, https://doi.org/10.2337/DIAB.42.11.1678 [Online] Diabetes (Available from:) [Accessed: 15th August 2021].

[3] B. Thorens, Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1, Proc. Natl. Acad. Sci. U. S. A. 89 (18) (1992) 8641–8645, https://doi.org/10.1073/PNAS.89.18.8641 [Online] Proc Natl Acad Sci U S A (Available from:) [Accessed: 15th August 2021].

[4] R.T. Mcgrath, S.J. Glastras, S.L. Hocking, I. Tjoeng, M. Krause, G.R. Fulcher, Central functions of glucagon-like peptide-1: roles in energy regulation and neuroprotection, Endocrinol. Metab. Level 3 (2) (2015) 1045, https://doi.org/10.4172/2157-7536.1000.152 [Online] (Available from:).

[5] Greco E.V., Russo G., Giandalia A., Viazzi F., Pontremoli R., Cosmo S.De GLP-1 Receptor agonists and kidney protection. Medicina. [Online] Multidisciplinary Digital Publishing Institute (MDPI); 2019;55(6). Available from: doi:10.3390/MEDICINA55060233 [Accessed: 29th October 2021].

[6] S. Farr, J. Taher, K. Adeli, Glucagon-like peptide-1 as a key regulator of lipid and lipoprotein metabolism in fasting and postprandial states. Cardiovascular & hematological disorders drug targets, Cardiovasc. Hematol. Disord. Drug Targets 14 (2) (2014) 126–136, https://doi.org/10.2174/1871529X14666140505125300 [Online] (Available from:) [Accessed: 29th October 2021].

[7] F. Sun, S. Wu, J. Wang, S. Guo, S. Chai, Z. Yang, L. Li, Y. Zhang, L. Ji, S. Zhan, Effect of glucagon-like peptide-1 receptor agonists on lipid profiles among type 2 diabetes: a systematic review and network meta-analysis, Clin. Ther. 37 (1) (2015) 225–241, https://doi.org/10.1016/J.CLINTHERA.2014.11.008, e8. [Online] (Available from:) [Accessed: 29th October 2021].

[8] Y.-S. Lee, H.-S. Jun, Anti-Inflammatory effects of GLP-1-based therapies beyond glucose control, Mediat. Inflamm. (2016) 2016, https://doi.org/10.1155/2016/3094642 [Online] (Available from:) [Accessed: 29th October 2021].

[9] J.J. Holst, C. Ørskov, The incretin approach for diabetes treatment: modulation of islet hormone release by GLP-1 agonism, Diabetes (2004) S197–S204, https://doi.org/10.2337/diabetes.53.suppl_3.S197 [Online] American Diabetes Association (Available from:) [Accessed: 2nd April 2021].

[10] T. Tsuboi, G.Da Silva Xavier, G.G. Holz, L.S. Jouaville, A.P. Thomas, G.A. Rutter, Glucagon-like peptide-1 mobilizes intracellular Ca2+ and stimulates mitochondrial ATP synthesis in pancreatic MIN6 beta-cells, Biochem. J. 369 (2) (2003) 287–299, https://doi.org/10.1042/BJ20021288 [Online] Portland Press (Available from:) [Accessed: 28th November 2021].

[11] G. Kang, J.W. Joseph, O.G. Chepurny, M. Monaco, M.B. Wheeler, J.L. Bos, et al., Epac-selective cAMP Analog 8-pCPT-2′-O-Me-cAMP as a stimulus for Ca2+-induced Ca2+ release and exocytosis in pancreatic β-cells*, J. Biol. Chem. 278 (10) (2003) 8279–8285, https://doi.org/10.1074/JBC.M211682200 [Online] Elsevier (Available from:) [Accessed: 28th November 2021].

[12] Dzhura I., Chepurny O.G., Leech C.A., Roe M.W., Dzhura E., Xu X., et al. Phospholipase C-ε links Epac2 activation to the potentiation of glucose-stimulated insulin secretion from mouse islets of Langerhans. ⟨https://doi.org/10.4161/isl.3.3.15507⟩. [Online] Taylor & Francis; 2011;3(3): 121–128. Available from: doi:10.4161/ISL.3.3.15507 [Accessed: 28th November 2021].

[13] Holz G.G. Epac: A New cAMP-binding protein in support of glucagon-like peptide-1 receptor-mediated signal transduction in the pancreatic β-cell. Diabetes. [Online] American Diabetes Association; 2004;53(1): 5–13. Available from: doi:10.2337/DIABETES.53.1.5 [Accessed: 28th November 2021].

[14] C. Koole, D. Wootten, J. Simms, C. Valant, R. Sridhar, O.L. Woodman, et al., Allosteric ligands of the glucagon-like peptide 1 receptor (GLP-1R) differentially modulate endogenous and exogenous peptide responses in a pathway-selective manner: implications for drug screening, Mol. Pharmacol. 78 (3) (2010) 456–465, https://doi.org/10.1124/MOL.110.065664 [Online] (Available from:) [Accessed: 28th November 2021].

[15] P.E. MacDonald, X. Wang, F. Xia, W. El-Kholy, E.D. Targonsky, R.G. Tsushima, et al., Antagonism of rat β-cell voltage-dependent K+ currents by exendin 4 requires dual activation of the cAMP/protein Kinase A and phosphatidylinositol 3-Kinase signaling pathways*, J. Biol. Chem. 278 (52) (2003) 52446–52453, https://doi.org/10.1074/JBC.M307612200 [Online] Elsevier (Available from:) [Accessed: 28th November 2021].

[16] C. Montrose-Rafizadeh, P. Avdonin, M.J. Garant, B.D. Rodgers, S. Kole, H. Yang, et al., Pancreatic glucagon-like peptide-1 receptor couples to multiple G proteins and activates mitogen-activated protein kinase pathways in Chinese hamster ovary cells, Endocrinology 140 (3) (1999) 1132–1140, https://doi.org/10.1210/ENDO.140.3.6550 [Online] (Available from:) [Accessed: 28th November 2021].

[17] A.C. Arcones, R. Vila-Bedmar, M. Mirasierra, M. Cruces-Sande, M. Vallejo, B. Jones, et al., GRK2 regulates GLP-1R-mediated early phase insulin secretion in vivo, BMC Biol. 19 (1) (2021) 1–16, https://doi.org/10.1186/S12915-021-00966-W [Online] BioMed Central (2021 19:1) (Available from:) [Accessed: 28th May 2022].

[18] Hager M.V., Johnson L.M., Wootten D., Sexton P.M., Gellman S.H. β-Arrestin-biased agonists of the GLP-1 receptor from β-amino acid residue incorporation into GLP-1 analogues. Journal of the American Chemical Society. [Online] NIH Public Access; 2016;138(45): 14970. Available from: doi:10.1021/JACS.6B08323 [Accessed: 28th May 2022].

[19] Sonoda N., Imamura T., Yoshizaki T., Babendure J.L., Lu J.C., Olefsky J.M. Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. Proceedings of the National Academy of Sciences of the United States of America. [Online] Proc Natl Acad Sci U S A; 2008;105(18): 6614–6619. Available from: doi:10.1073/PNAS.0710402105 [Accessed: 28th May 2022].

[20] J. Talbot, E. Joly, M. Prentki, J. Buteau, β-Arrestin1-mediated recruitment of c-Src underlies the proliferative action of glucagon-like peptide-1 in pancreatic β INS832/13 cells, Mol. Cell. Endocrinol. 364 (1–2) (2012) 65–70, https://doi.org/10.1016/J.MCE.2012.08.010 [Online] (Available from:) [Accessed: 28th May 2022].

[21] Bitsi S., Manchanda Y., ElEid L., Mohamed N., Hansen B., Suba K., et al. Divergent acute versus prolonged pharmacological GLP-1R responses in adult beta cell-selective β-arrestin 2 knockout mice. bioRxiv. [Online] Cold Spring Harbor Laboratory; 2022; 2022.04.21.489075. Available from: doi:10.1101/2022.04.21.489075 [Accessed: 31st July 2022].

[22] Jones B. The therapeutic potential of GLP-1 receptor biased agonism. British Journal of Pharmacology. [Online] John Wiley & Sons, Ltd; 2021; Available from: doi:10.1111/BPH.15497 [Accessed: 18th November 2021].

[23] M.M. Fletcher, M.L. Halls, A. Christopoulos, P.M. Sexton, D. Wootten, The complexity of signalling mediated by the glucagon-like peptide-1 receptor, Biochem. Soc. Trans. 44 (2) (2016) 582–588, https://doi.org/10.1042/BST20150244 [Online] Portland Press (Available from:) [Accessed: 28th November 2021].

[24] Y. Manchanda, S. Bitsi, Y. Kang, B. Jones, A. Tomas, Spatiotemporal control of GLP-1 receptor activity, Curr. Opin. Endocr. Metab. Res. 16 (2021) 19–27, https://doi.org/10.1016/J.COEMR.2020.07.003 [Online] Elsevier (Available from:) [Accessed: 28th November 2021].

[25] Zhao F., Zhou Q., Cong Z., Hang K., Zou X., Zhang C., et al. Structural basis for the therapeutic advantage of dual and triple agonists at the human GIP, GLP-1 or GCG receptors. bioRxiv. [Online] Cold Spring Harbor Laboratory; 2021; 2021.07.29.454286. Available from: doi:10.1101/2021.07.29.454286 [Accessed: 27th November 2021].

[26] Z. Cong, L.N. Chen, H. Ma, Q. Zhou, X. Zou, C. Ye, et al., Molecular insights into ago-allosteric modulation of the human glucagon-like peptide-1 receptor, Nat. Commun. 12 (1) (2021) 1–11, https://doi.org/10.1038/s41467-021-24058-z, 2021 12:1. [Online] Nature Publishing Group (Available from:) [Accessed: 18th November 2021].

[27] X. Zhang, M.J. Belousoff, P. Zhao, A.J. Kooistra, T.T. Truong, S.Y. Ang, et al., Differential GLP-1R binding and activation by peptide and non-peptide agonists, Mol. Cell. 80 (3) (2020) 485–500, https://doi.org/10.1016/j.molcel.2020.09.020 [Online] Cell Press (e7) (Available from:) [Accessed: 15th December 2020].

[28] T. Kawai, B. Sun, H. Yoshino, D. Feng, Y. Suzuki, M. Fukazawa, et al., Structural basis for GLP-1 receptor activation by LY3502970, an orally active nonpeptide agonist, Proc. Natl. Acad. Sci. U. S. A. 117 (47) (2020) 29959–29967, https://doi.org/10.1073/PNAS.2014879117/-/DCSUPPLEMENTAL [Online] National Academy of Sciences (Available from:) [Accessed: 18th November 2021].

[29] Liang Y.L., Khoshouei M., Glukhova A., Furness S.G.B., Zhao P., Clydesdale L., et al. Phase-plate cryo-EM structure of a biased agonist-bound human GLP-1 receptor–Gs complex. Nature 2018 555:7694. [Online] Nature Publishing Group; 2018;555(7694): 121–125. Available from: doi:10.1038/nature25773 [Accessed: 13th November 2021].

[30] Davies M., Færch L., Jeppesen O.K., Pakseresht A., Pedersen S.D., Perreault L., et al. Semaglutide 2·4 mg once a week in adults with overweight or obesity, and type 2 diabetes (STEP 2): a randomised, double-blind, double-dummy, placebo-controlled, phase 3 trial. The Lancet. [Online] Elsevier; 2021;397(10278): 971–984. Available from: doi:10.1016/S0140-6736(21)00213-0 [Accessed: 29th October 2021].

[31] D. Hinnen, Glucagon-like peptide 1 receptor agonists for type 2 diabetes, Diab. Spectr. 30 (3) (2017) 202–210, https://doi.org/10.2337/DS16-0026 [Online] American Diabetes Association (Available from:) [Accessed: 7th October 2021].

[32] T.D. Filippatos, T.V. Panagiotopoulou, M.S. Elisaf, Adverse effects of GLP-1 receptor agonists, Rev. Diab. Stud. RDS 11 (3) (2014) 202, https://doi.org/

10.1900/RDS.2014.11.202 [Online] Society for Biomedical Diabetes Research (Available from:) [Accessed: 7th October 2021].
[33] R.M. Johnson, X. Zhang, S.J. Piper, T.J. Nettleton, T.H. Vandekolk, C. J. Langmead, et al., Cryo-EM structure of the dual incretin receptor agonist, peptide-19, in complex with the glucagon-like peptide-1 receptor, Biochem. Biophys. Res. Commun. 578 (2021) 84–90, https://doi.org/10.1016/J.BBRC.2021.09.016 [Online] Academic Press (Available from:).
[34] X. Zhang, M.J. Belousoff, Y.L. Liang, R. Danev, P.M. Sexton, D. Wootten, Structure and dynamics of semaglutide- and taspoglutide-bound GLP-1R-Gs complexes, Cell Rep. 36 (2) (2021), 109374, https://doi.org/10.1016/J.CELREP.2021.109374/ATTACHMENT/8647B7BB-D966-4207-9712-0C82738E3189/MMC1.PDF [Online] Elsevier B.V. (Available from:) [Accessed: 31st July 2022].
[35] Y. Zhang, B. Sun, D. Feng, H. Hu, M. Chu, Q. Qu, et al., Cryo-EM structure of the activated GLP-1 receptor in complex with G protein, Nature. 546 (7657) (2017) 248, https://doi.org/10.1038/NATURE22394 [Online] NIH Public Access (Available from:) [Accessed: 30th November 2021].
[36] S. Lei, L. Clydesdale, A. Dai, X. Cai, Y. Feng, D. Yang, et al., Two distinct domains of the glucagon-like peptide-1 receptor control peptide-mediated biased agonism, J. Biol. Chem. 293 (24) (2018) 9370, https://doi.org/10.1074/JBC.RA118.003278 [Online] American Society for Biochemistry and Molecular Biology (Available from:) [Accessed: 13th November 2021].
[37] Y. Yin, X.E. Zhou, L. Hou, L.H. Zhao, B. Liu, G. Wang, et al., An intrinsic agonist mechanism for activation of glucagon-like peptide-1 receptor by its extracellular domain, Cell Discov. 2 (1) (2016) 1–18, https://doi.org/10.1038/celldisc.2016.42 [Online] Nature Publishing Group (2016 2:1) (Available from:) [Accessed: 17th November 2021].
[38] S. Mukund, Y. Shang, H.J. Clarke, A. Madjidi, J.E. Corn, L. Kates, et al., Inhibitory mechanism of an allosteric antibody targeting the glucagon receptor, J. Biol. Chem. 288 (50) (2013) 36168–36178, https://doi.org/10.1074/JBC.M113.496984/ATTACHMENT/0AE55E51-19A9-4160-BD7A-BB9360FD1A2F/MMC1.ZIP [Online] Elsevier (Available from:) [Accessed: 17th November 2021].
[39] L.H. Zhao, Y. Yin, D. Yang, B. Liu, L. Hou, X. Wang, et al., Differential requirement of the extracellular domain in activation of class B G protein-coupled receptors, J. Biol. Chem. 291 (29) (2016) 15119–15130, https://doi.org/10.1074/JBC.M116.726620 [Online] Elsevier (Available from:).
[40] C.M. Koth, J.M. Murray, S. Mukund, A. Madjidi, A. Minn, H.J. Clarke, et al., Molecular basis for negative regulation of the glucagon receptor, Proc. Natl. Acad. Sci. U. S. A. 109 (36) (2012) 14393–14398, https://doi.org/10.1073/PNAS.1206734109/-/DCSUPPLEMENTAL [Online] National Academy of Sciences (Available from:) [Accessed: 17th November 2021].
[41] F. Wu, L. Yang, K. Hang, M. Laursen, L. Wu, G.W. Han, et al., Full-length human GLP-1 receptor structure without orthosteric ligands, Nat. Commun. 11 (1) (2020) 1–10, https://doi.org/10.1038/s41467-020-14934-5 [Online] Nature Publishing Group (Available from:) [Accessed: 17th November 2021].
[42] J.H. Van Drie, L. Tong, Cryo-EM as a powerful tool for drug discovery, Bioorgan. Med. Chem. Lett. 30 (22) (2020), 127524, https://doi.org/10.1016/J.BMCL.2020.127524 [Online] Elsevier (Available from:) [Accessed: 18th November 2021].
[43] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, et al., Highly accurate protein structure prediction with AlphaFold, Nature 596 (7873) (2021) 583–589, https://doi.org/10.1038/s41586-021-03819-2 [Online] Nature Publishing Group (Available from:) [Accessed: 18th November 2021].
[44] M. Alquraishi, AlphaFold at CASP13, Bioinformatics 35 (22) (2019) 4862–4865, https://doi.org/10.1093/BIOINFORMATICS/BTZ422 (Available from:) [Accessed: 18th November 2021].
[45] F. Pinheiro, J. Santos, S. Ventura, AlphaFold and the amyloid landscape, J. Mol. Biol. 433 (20) (2021), https://doi.org/10.1016/J.JMB.2021.167059 [Online] (Available from:) [Accessed: 18th November 2021].
[46] Pereira J., Simpkin A.J., Hartmann M.D., Rigden D.J., Keegan R.M., Lupas A.N. High-accuracy protein structure prediction in CASP14. Proteins: Structure, Function, and Bioinformatics. [Online] John Wiley & Sons, Ltd; 2021; Available from: doi:10.1002/PROT.26171 [Accessed: 18th November 2021].
[47] R. Mann, N. Nasr, D. Hadden, J. Sinfield, F. Abidi, S. Al-Sabah, et al., Peptide binding at the GLP-1 receptor, Biochem. Soc. Trans. 35 (4) (2007) 713–716, https://doi.org/10.1042/BST0350713 [Online] Portland Press Ltd (Available from:) [Accessed: 18th November 2021].
[48] D. Wootten, C.A. Reynolds, K.J. Smith, J.C. Mobarec, C. Koole, E.E. Savage, et al., The extracellular surface of the GLP-1 receptor is a molecular trigger for biased agonism, Cell. 165 (7) (2016) 1632–1643, https://doi.org/10.1016/J.CELL.2016.05.023 [Online] Cell (Available from:) [Accessed: 30th November 2021].
[49] Maja Frimann T., Kyu Ko S., Harris P., Thostrup Bukrinski J., J Peters G.H., Maja Frimann Ã.T., et al. In-silico study of the interactions between acylated glucagon like-peptide-1 analogues and the native receptor. https://doi.org/10.1080/07391102.2022.2078409. [Online] Taylor & Francis; 2022; 1–15. Available from: doi:10.1080/07391102.2022.2078409 [Accessed: 28th May 2022].
[50] J.J. Holst, J. Gromada, Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans, Am. J. Physiol. Endocrinol. Metab. 287 (2) (2004), https://doi.org/10.1152/AJPENDO.00545.2003 [Online] (Available from:) [Accessed: 29th November 2021].
[51] D. Elahi, M. McAloon-Dyke, N.K. Fukagawa, G.S. Meneilly, A.L. Sclater, K. L. Minaker, et al., The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7-37) in normal and diabetic subjects, Regul. Pept. 51 (1) (1994) 63–74, https://doi.org/10.1016/0167-0115(94)90136-8 [Online] (Available from:) [Accessed: 29th November 2021].
[52] M.A. Hussain, E. Laimon-Thomson, S.M. Mustafa, A. Deck, B. Song, Detour ahead: incretin hormone signaling alters its intracellular path as β-cell failure progresses during diabetes, Front. Endocrinol. (2021) 12, https://doi.org/10.3389/FENDO.2021.665345 [Online] Frontiers Media SA (Available from:) [Accessed: 29th November 2021].
[53] O.S. Oduori, N. Murao, K. Shimomura, H. Takahashi, Q. Zhang, H. Dou, et al., Gs/Gq signaling switch in β cells defines incretin effectiveness in diabetes, J. Clin. Investig. 130 (12) (2020) 6639, https://doi.org/10.1172/JCI140046 [Online] American Society for Clinical Investigation (Available from:) [Accessed: 29th November 2021].
[54] T. Buenaventura, S. Bitsi, W.E. Laughlin, T. Burgoyne, Z. Lyu, A.I. Oqua, et al., Agonist-induced membrane nanodomain clustering drives GLP-1 receptor responses in pancreatic beta cells, PLoS Biol. 17 (8) (2019), e3000097, https://doi.org/10.1371/JOURNAL.PBIO.3000097 [Online] Public Library of Science (Available from:) [Accessed: 19th August 2021].
[55] A. Thompson, V. Kanamarlapudi, Agonist-induced internalisation of the glucagon-like peptide-1 receptor is mediated by the Gαq pathway, Biochem. Pharmacol. 93 (1) (2015) 72–84, https://doi.org/10.1016/J.BCP.2014.10.015 [Online] Elsevier (Available from:).
[56] P. Vazquez, I. Roncero, E. Blazquez, E. Alvarez, The cytoplasmic domain close to the transmembrane region of the glucagon-like peptide-1 receptor contains sequence elements that regulate agonist-dependent internalisation, J. Endocrinol. 186 (1) (2005) 221–231, https://doi.org/10.1677/JOE.1.06179 [Online] (Available from:) [Accessed: 19th August 2021].
[57] S.N. Roed, A.C. No, P. Wismann, H. Iversen, H. Bräuner-Osborne, S.M. Knudsen, et al., Functional consequences of glucagon-like peptide-1 receptor cross-talk and trafficking, J. Biol. Chem. 290 (2) (2015) 1233–1243, https://doi.org/10.1074/JBC.M114.592436 [Online] (Available from:) [Accessed: 30th November 2021].
[58] A. Marzook, A. Tomas, B. Jones, The interplay of glucagon-like peptide-1 receptor trafficking and signalling in pancreatic beta cells, Front. Endocrinol. (2021) 12, https://doi.org/10.3389/FENDO.2021.678055 [Online] Frontiers Media SA (Available from:) [Accessed: 25th September 2021].
[59] M.G.H. Scott, A. Benmerah, O. Muntaner, S. Marullo, Recruitment of activated G protein-coupled receptors to pre-existing clathrin-coated pits in living cells, J. Biol. Chem. 277 (5) (2002) 3552–3559, https://doi.org/10.1074/JBC.M106586200 [Online] Elsevier (Available from:) [Accessed: 19th August 2021].
[60] N.K. Smith, T.A. Hackett, A. Galli, C.R. Flynn, GLP-1: molecular mechanisms and outcomes of a complex signaling system, Neurochem. Int. 128 (2019) 94, https://doi.org/10.1016/J.NEUINT.2019.04.010 [Online] NIH Public Access (Available from:) [Accessed: 25th September 2021].
[61] Sonoda N., Imamura T., Yoshizaki T., Babendure J.L., Lu J.C., Olefsky J.M. Beta-Arrestin-1 mediates glucagon-like peptide-1 signaling to insulin secretion in cultured pancreatic beta cells. Proceedings of the National Academy of Sciences of the United States of America. [Online] Proc Natl Acad Sci U S A; 2008;105(18): 6614–6619. Available from: doi:10.1073/PNAS.0710402105 [Accessed: 29th November 2021].
[62] M.M. Fletcher, M.L. Halls, P. Zhao, L. Clydesdale, A. Christopoulos, P.M. Sexton, et al., Glucagon-like peptide-1 receptor internalisation controls spatiotemporal signalling mediated by biased agonists, Biochem. Pharmacol. 156 (2018) 406–419, https://doi.org/10.1016/J.BCP.2018.09.003 [Online] Biochem Pharmacol (Available from:) [Accessed: 29th November 2021].
[63] C.A. Syme, L. Zhang, A. Bisello, Caveolin-1 regulates cellular trafficking and function of the glucagon-like Peptide 1 receptor, Mol. Endocrinol. 20 (12) (2006) 3400–3411, https://doi.org/10.1210/ME.2006-0178 [Online] Mol Endocrinol (Available from:) [Accessed: 29th November 2021].
[64] A. Thompson, V. Kanamarlapudi, Agonist-induced internalisation of the glucagon-like peptide-1 receptor is mediated by the Gαq pathway, Biochem. Pharmacol. 93 (1) (2015) 72–84, https://doi.org/10.1016/J.BCP.2014.10.015 [Online] Biochem Pharmacol (Available from:) [Accessed: 29th November 2021].
[65] B. Jones, E.R. McGlone, Z. Fang, P. Pickford, I.R. Corrêa, A. Oishi, et al., Genetic and biased agonist-mediated reductions in β-Arrestin recruitment prolong cAMP signaling at glucagon family receptors, J. Biol. Chem. (2021) 296, https://doi.org/10.1074/JBC.RA120.016334/ATTACHMENT/26C0EF23-CBCA-4455-82D1-C4EBB7D2C7B1/MMC1.PDF [Online] American Society for Biochemistry and Molecular Biology Inc. (Available from:) [Accessed: 29th November 2021].
[66] A.K. Shukla, G.H. Westfield, K. Xiao, R.I. Reis, L.Y. Huang, P. Tripathi-Shukla, et al., Visualization of arrestin recruitment by a G-protein-coupled receptor, Nature 512 (7513) (2014) 218–222, https://doi.org/10.1038/NATURE13430 [Online] Nature (Available from:) [Accessed: 29th November 2021].
[67] P.Y. Jean-Charles, S. Kaur, S.K. Shenoy, GPCR signaling via β-arrestin-dependentmechanisms, J. Cardiovasc. Pharmacol. 70 (3) (2017) 142, https://doi.org/10.1097/FJC.0000000000000482 [Online] NIH Public Access (Available from:) [Accessed: 29th November 2021].
[68] A.H. Nguyen, A.R.B. Thomsen, T.J. Cahill, R. Huang, L.Y. Huang, T. Marcink, et al., Structure of an endosomal signaling GPCR–G protein–β-arrestin megacomplex, Nat. Struct. Mol. Biol. 26 (12) (2019) 1123–1131, https://doi.org/10.1038/s41594-019-0330-y [Online] Nature Publishing Group (Available from:) [Accessed: 28th May 2022].
[69] Thomsen A.R., Plouffe B., Cahill III T.J., Shukla A.K., Tarrasch J.T., Dosey A.M., et al. GPCR-G Protein-β-arrestin super-complex mediates sustained G protein signaling. 2016; Available from: doi:10.1016/j.cell.2016.07.004 [Accessed: 28th May 2022].
[70] R.B. Clark, B.J. Knoll, R. Barber, R.B. Clark, B.J. Knoll, R. Barber, et al., Partial agonists and G protein-coupled receptor desensitization, Trends Pharmacol. Sci. 20 (7) (1999) 279–286, https://doi.org/10.1016/S0165-6147(99)01351-6 [Online] Elsevier (Available from:) [Accessed: 19th June 2022].

[71] A. Novikoff, S.L. O'Brien, M. Bernecker, G. Grandl, M. Kleinert, P.J. Knerr, et al., Spatiotemporal GLP-1 and GIP receptor signaling and trafficking/recycling dynamics induced by selected receptor mono- and dual-agonists, Mol. Metab. (2021) 49, https://doi.org/10.1016/J.MOLMET.2021.101181 [Online] Mol Metab (Available from:) [Accessed: 19th August 2021].

[72] T.N. Feinstein, V.L. Wehbi, J.A. Ardura, D.S. Wheeler, S. Ferrandon, T.J. Gardella, et al., Retromer terminates the generation of cAMP by internalized PTH receptors, Nat. Chem. Biol. 7 (5) (2011) 278–284, https://doi.org/10.1038/nchembio.545 [Online] Nature Publishing Group (Available from:) [Accessed: 19th June 2022].

[73] R.S. Kuna, S.B. Girada, S. Asalla, J. Vallentyne, S. Maddika, J.T. Patterson, et al., Glucagon-like peptide-1 receptor-mediated endosomal cAMP generation promotes glucose-stimulated insulin secretion in pancreatic β-cells, Am. J. Physiol. Endocrinol. Metab. 305 (2) (2013) 161–170, https://doi.org/10.1152/AJPENDO.00551.2012/ASSET/IMAGES/LARGE/ZH10121368540008.JPEG [Online] American Physiological Society Bethesda, MD (Available from:) [Accessed: 30th November 2021].

[74] M.B. Jarpe, C. Knall, F.M. Mitchell, A.M. Buhl, E. Duzic, G.L. Johnson, [d-Arg1,d-Phe5,d-Trp7,9,Leu11]substance P acts as a biased agonist toward neuropeptide and chemokine receptors*, J. Biol. Chem. 273 (5) (1998) 3097–3104, https://doi.org/10.1074/JBC.273.5.3097 [Online] Elsevier (Available from:) [Accessed: 27th November 2021].

[75] A. Marzook, S. Chen, P. Pickford, M. Lucey, Y. Wang, I.R. Corrêa, et al., Evaluation of efficacy- versus affinity-driven agonism with biased GLP-1R ligands P5 and exendin-F1, Biochem. Pharmacol. (2021) 190, https://doi.org/10.1016/J.BCP.2021.114656 [Online] Biochem Pharmacol; (Available from:) [Accessed: 20th June 2022].

[76] W.J.C. Van Der Velden, F.X. Smit, C.B. Christiansen, T.C. Møller, G.M. Hjortø, O. Larsen, et al., GLP-1 Val8: a biased GLP-1R agonist with altered binding kinetics and impaired release of pancreatic hormones in rats, ACS Pharmacol. Transl. Sci. 4 (1) (2021) 296–313, https://doi.org/10.1021/ACSPTSCI.0C00193/SUPPL_FILE/PT0C00193_SI_001.PDF [Online] American Chemical Society (Available from:) [Accessed: 20th June 2022].

[77] B. Jones, T. Buenaventura, N. Kanda, P. Chabosseau, B.M. Owen, R. Scott, et al., Targeting GLP-1 receptor trafficking to improve agonist efficacy, Nat. Commun. 9 (1) (2018) 1–17, https://doi.org/10.1038/s41467-018-03941-2 [Online] Nature Publishing Group (Available from:) [Accessed: 27th November 2021].

[78] F.S. Willard, J.D. Douros, M.B.N. Gabe, A.D. Showalter, D.B. Wainscott, T. M. Suter, et al., Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist, JCI Insight 5 (17) (2020), https://doi.org/10.1172/JCI.INSIGHT.140532 [Online] American Society for Clinical Investigation (Available from:) [Accessed: 27th November 2021].

[79] P. Pickford, M. Lucey, R.M. Rujan, E.R. McGlone, S. Bitsi, F.B. Ashford, et al., Partial agonism improves the anti-hyperglycaemic efficacy of an oxyntomodulin-derived GLP-1R/GCGR co-agonist, Mol. Metab. 51 (2021), 101242, https://doi.org/10.1016/J.MOLMET.2021.101242 [Online] Elsevier (Available from:) [Accessed: 27th November 2021].

[80] N. Al-Zamel, S. Al-Sabah, Y. Luqmani, L. Adi, S. Chacko, T.D. Schneider, et al., A dual GLP-1/GIP receptor agonist does not antagonize glucagon at its receptor but may act as a biased agonist at the GLP-1 receptor, Int. J. Mol. Sci. 20 (14) (2019), https://doi.org/10.3390/IJMS20143532 [Online] (Available from:) [Accessed: 27th November 2021].

[81] H. Zhang, E. Sturchler, J. Zhu, A. Nieto, P.A. Cistrone, J. Xie, et al., Autocrine selection of a GLP-1R G-protein biased agonist with potent antidiabetic effects, Nat. Commun. 6 (1) (2015) 1–13, https://doi.org/10.1038/ncomms9918 [Online] Nature Publishing Group (Available from:) [Accessed: 26th November 2021].

[82] S. Havelund, A. Plum, U. Ribel, I. Jonassen, A. Vølund, J. Markussen, et al., The mechanism of protraction of insulin detemir, a long-acting, acylated analog of human insulin, Pharm. Res. 21 (8) (2004) 1498–1504, https://doi.org/10.1023/B:PHAM.0000036926.54824.37 [Online] (Available from:) [Accessed: 26th November 2021].

[83] D.K. Clodfelter, A.H. Pekar, D.M. Rebhun, K.A. Destrampe, H.A. Havel, S. R. Myers, et al., Effects of non-covalent self-association on the subcutaneous absorption of a therapeutic peptide, Pharm. Res. 15 (2) (1998) 254–262, https://doi.org/10.1023/A:1011918719017 [Online] (Available from:) [Accessed: 26th November 2021].

[84] L.B. Knudsen, J. Lau, The discovery and development of liraglutide and semaglutide, Front. Endocrinol. 10 (APR) (2019) 155, https://doi.org/10.3389/FENDO.2019.00155/BIBTEX [Online] Frontiers Media S.A. (Available from:) [Accessed: 26th November 2021].

[85] Lucey M., Ashik T., Marzook A., Wang Y., Goulding J., Oishi A., et al. Acylation of the incretin peptide exendin-4 directly impacts GLP-1 receptor signalling and trafficking. bioRxiv. [Online] Cold Spring Harbor Laboratory; 2021; 2021.04.01.438030. Available from: doi:10.1101/2021.04.01.438030 [Accessed: 26th September 2021].

[86] Y. Seino, M. Fukushima, D. Yabe, GIP and GLP-1, the two incretin hormones: similarities and differences, J. Diab. Investig. 1 (1–2) (2010) 8–23, https://doi.org/10.1111/J.2040-1124.2010.00022.X [Online] (Available from:) [Accessed: 25th June 2022].

[87] M.E. Capozzi, R.D. DiMarchi, M.H. Tschöp, B. Finan, J.E. Campbell, Targeting the incretin/glucagon system with triagonists to treat diabetes, Endocr. Rev. 39 (5) (2018) 719–738, https://doi.org/10.1210/ER.2018-00117 [Online] Oxford Academic (Available from:) [Accessed: 27th November 2021].

[88] T. Borner, C.E. Geisler, S.M. Fortin, R. Cosgrove, J. Alsina-Fernandez, M. Dogra, et al., GIP receptor agonism attenuates GLP-1 receptor agonist–induced nausea and emesis in preclinical models, Diabetes 70 (11) (2021) 2545–2553, https://doi.org/10.2337/DB21-0459 [Online] American Diabetes Association (Available from:) [Accessed: 27th November 2021].

[89] Lee Js Kim Jk, J. Choi, Jung Sy, Lee Sh, Choi Iy, et al., Novel combination of a long-acting GLP-1/GIP/glucagon triple agonist (HM15211) and once-weekly Basal Insulin (HM12460a) offers improved glucose lowering and weight loss in a diabetic animal model, Diabetes 67 (Supplement 1) (2018) 77-OR, https://doi.org/10.2337/DB18-77-OR [Online] American Diabetes Association (Available from:) [Accessed: 27th November 2021].

[90] T. Coskun, Moyers Js, Roell Wc, L. O'farrell, A. Regmi, X. Ruan, et al., 679-P: the novel GIP, GLP-1, and glucagon triple receptor agonist LY3437943 exhibits robust efficacy in preclinical models of obesity and diabetes, Diabetes 70 (Supplement 1) (2021) 679-P, https://doi.org/10.2337/DB21-679-P [Online] American Diabetes Association (Available from:) [Accessed: 27th November 2021].

[91] E. Yuliantie, S. Darbalaei, A. Dai, P. Zhao, D. Yang, P.M. Sexton, et al., Pharmacological characterization of mono-, dual- and tri-peptidic agonists at GIP and GLP-1 receptors, Biochem. Pharmacol. 177 (2020), 114001, https://doi.org/10.1016/J.BCP.2020.114001 [Online] Elsevier (Available from:) [Accessed: 27th November 2021].

[92] J.P. Frías, Tirzepatide: a glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) dual agonist in development for the treatment of type 2 diabetes, Exp. Rev. Endocrinol. Metab. 15 (6) (2020) 379–394, https://doi.org/10.1080/17446651.2020.1830759 [Online] Taylor and Francis Ltd (Available from:) [Accessed: 27th November 2021].

[93] M.K. Thomas, A. Nikooienejad, R. Bray, X. Cui, J. Wilson, K. Duffin, et al., Dual GIP and GLP-1 receptor agonist tirzepatide improves beta-cell function and insulin sensitivity in type 2 diabetes, J. Clin. Endocrinol. Metab. 106 (2) (2021) 388–396, https://doi.org/10.1210/clinem/dgaa863 [Online] Endocrine Society (Available from:) [Accessed: 27th November 2021].

[94] T. Coskun, K.W. Sloop, C. Loghin, J. Alsina-Fernandez, S. Urva, K.B. Bokvist, et al., LY3298176, a novel dual GIP and GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus: from discovery to clinical proof of concept, Mol. Metab. 18 (2018) 3–14, https://doi.org/10.1016/j.molmet.2018.09.009 [Online] Elsevier GmbH (Available from:) [Accessed: 27th November 2021].

[95] F.S. Willard, J.D. Douros, M.B.N. Gabe, A.D. Showalter, D.B. Wainscott, T. M. Suter, et al., Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist, JCI Insight 5 (17) (2020), https://doi.org/10.1172/JCI.INSIGHT.140532 [Online] (Available from:) [Accessed: 27th November 2021].

[96] Y.Zhe Wang, D.Hua Yang, M.Wei Wang, Signaling profiles in HEK 293T cells co-expressing GLP-1 and GIP receptors, Acta Pharmacol. Sin. (2021) 1–8, https://doi.org/10.1038/s41401-021-00758-6 [Online] Nature Publishing Group (Available from:) [Accessed: 27th November 2021].

[97] B. Sun, F.S. Willard, D. Feng, J. Alsina-Fernandez, Q. Chen, M. Vieth, et al., Structural determinants of dual incretin receptor agonism by tirzepatide, Proc. Natl. Acad. Sci. U. S. A. 119 (13) (2022), https://doi.org/10.1073/PNAS.2116506119/-/DCSUPPLEMENTAL [Online] National Academy of Sciences (Available from:) [Accessed: 27th June 2022].

[98] F. Zhao, Q. Zhou, Z. Cong, K. Hang, X. Zou, C. Zhang, et al., Structural insights into multiplexed pharmacological actions of tirzepatide and peptide 20 at the GIP, GLP-1 or glucagon receptors, Nat. Commun. 13 (1) (2022) 1–16, https://doi.org/10.1038/s41467-022-28683-0 [Online] Nature Publishing Group (Available from:) [Accessed: 27th June 2022].

[99] M.A. Nauck, D.R. Quast, J. Wefers, J.J. Meier, GLP-1 receptor agonists in the treatment of type 2 diabetes – state-of-the-art, Mol. Metab. 46 (2021), 101102, https://doi.org/10.1016/J.MOLMET.2020.101102 [Online] Elsevier (Available from:) [Accessed: 27th June 2022].

[100] R.V. Overgaard, C.L. Hertz, S.H. Ingwersen, A. Navarria, D.J. Drucker, Levels of circulating semaglutide determine reductions in HbA1c and body weight in people with type 2 diabetes, Cell Rep. Med. (2021) 2, https://doi.org/10.1016/j.xcrm.2021.100387 [Online] (Available from:) [Accessed: 28th May 2022].

[101] Lagou V., Jiang L., Ulrich A., Zudina L., González K.S.G., Balkhiyarova Z., et al. Random glucose GWAS in 493,036 individuals provides insights into diabetes pathophysiology, complications and treatment stratification. medRxiv. [Online] 2021;6: 82. Available from: doi:10.1101/2021.04.17.21255471 [Accessed: 28th November 2021].

[102] R.A. Scott, L.J. Scott, R. Mägi, L. Marullo, K.J. Gaulton, M. Kaakinen, et al., An expanded genome-wide association study of type 2 diabetes in Europeans, Diabetes. 66 (11) (2017) 2888–2902, https://doi.org/10.2337/DB16-1253/-/DC1 [Online] American Diabetes Association (Available from:) [Accessed: 28th November 2021].

[103] J. Wessel, A.Y. Chu, S.M. Willems, S. Wang, H. Yaghootkar, J.A. Brody, et al., Low-frequency and rare exome chip variants associate with fasting glucose and type 2 diabetes susceptibility, Nat. Commun. 6 (1) (2015) 1–16, https://doi.org/10.1038/ncomms6897 [Online] Nature Publishing Group (Available from:) [Accessed: 28th November 2021].

[104] Y. Tokuyama, K. Matsui, T. Egashira, O. Nozaki, T. Ishizuka, A. Kanatsuka, Five missense mutations in glucagon-like peptide 1 receptor gene in Japanese population, Diab. Res. Clin. Pract. 66 (1) (2004) 63–69, https://doi.org/10.1016/J.DIABRES.2004.02.004 [Online] (Available from:) [Accessed: 2nd December 2021].

[105] M. Beinborn, C.I. Worrall, E.W. McBride, A.S. Kopin, A human glucagon-like peptide-1 receptor polymorphism results in reduced agonist responsiveness, Regul. Pept. 130 (1–2) (2005) 1–6, https://doi.org/10.1016/J.REGPEP.2005.05.001 [Online] (Available from:) [Accessed: 27th November 2021].

[106] C. Koole, D. Wootten, J. Simms, C. Valant, L.J. Miller, A. Christopoulos, et al., Polymorphism and ligand dependent changes in human glucagon-like peptide-1 receptor (GLP-1R) function: allosteric rescue of loss of function mutation, Mol. Pharmacol. 80 (3) (2011) 486, https://doi.org/10.1124/MOL.111.072884 [Online] American Society for Pharmacology and Experimental Therapeutics (Available from:) [Accessed: 20th August 2021].

[107] Z. Fang, S. Chen, Y. Manchanda, S. Bitsi, P. Pickford, A. David, et al., Ligand-specific factors influencing GLP-1 receptor post-endocytic trafficking and degradation in pancreatic beta cells, Int. J. Mol. Sci. 21 (21) (2020) 8404, https://doi.org/10.3390/IJMS21218404 [Online] Multidisciplinary Digital Publishing Institute (Available from:) [Accessed: 27th November 2021].

[108] J. Michałowska, E. Miller-Kasprzak, P. Bogdański, Incretin hormones in obesity and related cardiometabolic disorders: the clinical perspective, Nutrients 13 (2) (2021) 1–32, https://doi.org/10.3390/NU13020351 [Online] Multidisciplinary Digital Publishing Institute (MDPI) (Available from:) [Accessed: 3rd December 2021].

[109] D.A. De Luis, G. Diaz Soto, O. Izaola, E. Romero, Evaluation of weight loss and metabolic changes in diabetic patients treated with liraglutide, effect of RS 6923761 gene variant of glucagon-like peptide 1 receptor, J. Diab. Complicat. 29 (4) (2015) 595–598, https://doi.org/10.1016/J.JDIACOMP.2015.02.010 [Online] Elsevier Inc (Available from:) [Accessed: 28th May 2022].

[110] D.A. De Luis, D. Pacheco, R. Aller, O. Izaolo, R. Bachiller, [Roles of rs 6923761 gene variant in glucagon-like peptide 1 receptor on weight, cardiovascular risk factor and serum adipokine levels in morbid obese patients], Nutr. Hosp. 29 (4) (2014) 889–893, https://doi.org/10.3305/NH.2014.29.4.7218 [Online] (Available from:) [Accessed: 19th August 2021].

[111] M. Mashayekhi, J.R. Wilson, S. Jafarian-Kerman, H. Nian, C. Yu, M.M. Shuey, et al., Association of a glucagon-like peptide-1 receptor gene variant with glucose response to a mixed meal, Diab. Obesity Metab. 23 (1) (2021) 281, https://doi.org/10.1111/DOM.14216 [Online] NIH Public Access (Available from:) [Accessed: 28th May 2022].

[112] A. Urgeová, M. Javorský, L. Klimčáková, J. Zidzik, J. Šalagovič, J.A. Hubáček, et al., Genetic variants associated with glycemic response to treatment with dipeptidylpeptidase 4 inhibitors, Pharmacogenomics 21 (5) (2020) 317–323, https://doi.org/10.2217/PGS-2019-0147 [Online] Future Medicine Ltd (Available from:) [Accessed: 28th May 2022].

[113] A. Sathananthan, C. Dalla Man, F. Micheletto, A.R. Zinsmeister, M. Camilleri, P. D. Giesler, et al., Common genetic variation in GLP1R and insulin secretion in response to exogenous GLP-1 in nondiabetic subjects: a pilot study, Diab. Care 33 (9) (2010) 2074, https://doi.org/10.2337/DC10-0200 [Online] American Diabetes Association (Available from:) [Accessed: 3rd December 2021].

[114] M.B.N. Gabe, W.J.C. van der Velden, S. Gadgaard, F.X. Smit, B. Hartmann, H. Bräuner-Osborne, et al., Enhanced agonist residence time, internalization rate and signalling of the GIP receptor variant [E354Q] facilitate receptor desensitization and long-term impairment of the GIP system, Basic Clin. Pharmacol. Toxicol. 126 (Suppl 6(Suppl 6)) (2020) 122–132, https://doi.org/10.1111/BCPT.13289 [Online] (Available from:) [Accessed: 28th May 2022].

[115] J. Wang, D. Yang, X. Cheng, L. Yang, Z. Wang, A. Dai, et al., Allosteric modulators enhancing GLP-1 Binding to GLP-1R via a transmembrane site, ACS Chem. Biol. (2021) 16, https://doi.org/10.1021/ACSCHEMBIO.1C00552/SUPPL_FILE/CB1C00552_SI_006.PDB [Online] American Chemical Society (Available from:) [Accessed: 3rd December 2021].

[116] GLP1R | gnomAD v2.1.1 | gnomAD. [Online] Available from: ⟨https://gnomad.broadinstitute.org/gene/ENSG00000112164?dataset=gnomad_r2_1⟩ [Accessed: 1st December 2021].

[117] Q. Zhou, W. Guo, A. Dai, X. Cai, M. Vass, C. de Graaf, et al., Discovery of novel allosteric modulators targeting an extra-helical binding site of GLP-1R using structure- and ligand-based virtual screening, Biomolecules. 11 (7) (2021), https://doi.org/10.3390/BIOM11070929 [Online] (Available from:) [Accessed: 1st December 2021].

[118] J. Long, Y. Liu, Y. Duan, Y. Li, G. Yang, Z. Ren, et al., Effect of GLP-1R rs2254336 and rs3765467 polymorphisms on gastrointestinal adverse reactions in type 2 diabetes patients treated with liraglutide, Eur. J. Clin. Pharmacol. 78 (4) (2022) 589–596, https://doi.org/10.1007/S00228-021-03225-7/TABLES/6 [Online] Springer Science and Business Media Deutschland GmbH (Available from:) [Accessed: 25th June 2022].

[119] M. Javorský, I. Gotthardová, L. Klimčáková, M. Kvapil, J. Židzik, Z. Schroner, et al., A missense variant in GLP1R gene is associated with the glycaemic response to treatment with gliptins, Diab. Obesity Metab. 18 (9) (2016) 941–944, https://doi.org/10.1111/DOM.12682 [Online] John Wiley & Sons, Ltd (Available from:) [Accessed: 19th August 2021].

[120] T.D. Müller, B. Finan, S.R. Bloom, D. D'Alessio, D.J. Drucker, P.R. Flatt, et al., Glucagon-like peptide 1 (GLP-1), Mol. Metab. 30 (2019) 72–130, https://doi.org/10.1016/J.MOLMET.2019.09.010 [Online] Elsevier (Available from:).

[121] C. Pyke, R.S. Heller, R.K. Kirk, C. Ørskov, S. Reedtz-Runge, P. Kaastrup, et al., GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody, Endocrinology 155 (4) (2014) 1280–1290, https://doi.org/10.1210/EN.2013-1934 [Online] Oxford Academic (Available from:) [Accessed: 7th October 2021].

[122] A. Aroor, R. Nistala, Tissue-specific expression of GLP1R in mice: is the problem of antibody nonspecificity solved? Diabetes 63 (4) (2014) 1182–1184, https://doi.org/10.2337/DB13-1937 [Online] American Diabetes Association (Available from:) [Accessed: 2nd December 2021].

[123] P. Richards, H.E. Parker, A.E. Adriaenssens, J.M. Hodgson, S.C. Cork, S. Trapp, et al., Identification and characterisation of glucagon-like peptide-1 receptor expressing cells using a new transgenic mouse model, Diabetes 63 (4) (2014) 1224, https://doi.org/10.2337/DB13-1440 [Online] Europe PMC Funders (Available from:) [Accessed: 2nd December 2021].

[124] A.E. Adriaenssens, E.K. Biggs, T. Darwish, J. Tadross, T. Sukthankar, M. Girish, et al., Glucose-dependent insulinotropic polypeptide receptor-expressing cells in the hypothalamus regulate food intake, Cell Metab. 30 (5) (2019) 987–996, https://doi.org/10.1016/J.CMET.2019.07.013 [Online] Cell Press (e6) (Available from:) [Accessed: 28th May 2022].

[125] Thierry-Mieg D., Thierry-Mieg J. AceView: a comprehensive cDNA-supported gene and transcripts annotation. Genome biology. [Online] BioMed Central; 2006;7 Suppl 1(1): 1–14. Available from: doi:10.1186/GB-2006-7-S1-S12/TABLES/2 [Accessed: 2nd December 2021].

[126] A.R. Saxena, D.N. Gorman, R.M. Esquejo, A. Bergman, K. Chidsey, C. Buckeridge, et al., Danuglipron (PF-06882961) in type 2 diabetes: a randomized, placebo-controlled, multiple ascending-dose phase 1 trial, Nat. Med. 27 (6) (2021) 1079–1087, https://doi.org/10.1038/s41591-021-01391-w [Online] (Available from:).

[127] Z. Cong, Q. Zhou, Y. Li, L.-N. Chen, Z.-C. Zhang, A. Liang, et al., Structural basis of peptidomimetic agonism revealed by small-molecule GLP-1R agonists Boc5 and WB4-24, Proc. Natl. Acad. Sci. (20) (2022) 119, https://doi.org/10.1073/pnas.2200155119 [Online] (Available from:).

[128] D. Chen, J. Liao, N. Li, C. Zhou, Q. Liu, G. Wang, et al., A nonpeptidic agonist of glucagon-like peptide 1 receptors with efficacy in diabetic db/db mice, Proc. Natl. Acad. Sci. U. S. A. 104 (3) (2007) 943–948, https://doi.org/10.1073/pnas.0610173104 [Online] (Available from:).