Pharmacological Research 197 (2023) 106971

Contents lists available at ScienceDirect

Pharmacological Research

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

Intramolecular activity regulation of adhesion GPCRs in light of recent structural and evolutionary information

Gunnar Kleinau [a], Amal Hassan Ali [a], Franziska Wiechert [b], Michal Szczepek [a], Andrea Schmidt [a], Christian M.T. Spahn [b], Ines Liebscher [c], Torsten Schöneberg [c,d,*], Patrick Scheerer [a,e,**]

[a] Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Medical Physics and Biophysics, Group Structural Biology of Cellular Signaling, Charitéplatz 1, D-10117 Berlin, Germany
[b] Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Medical Physics and Biophysics, Berlin, Germany
[c] Rudolf Schönheimer Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, Johannisallee 30, 04103 Leipzig, Germany
[d] School of Medicine, University of Global Health Equity (UGHE), Kigali, Rwanda
[e] DZHK (German Centre for Cardiovascular Research), Partner Site Berlin, Berlin, Germany

ARTICLE INFO

Keywords:
G protein-coupled receptor
Adhesion GPCR
AGPCR
Tethered agonist
Stachel sequence
Intramolecular agonist

ABSTRACT

The class B2 of GPCRs known as adhesion G protein-coupled receptors (aGPCRs) has come under increasing academic and nonacademic research focus over the past decade due to their physiological importance as mechano-sensors in cell-cell and cell-matrix contexts. A major advance in understanding signal transduction of aGPCRs was achieved by the identification of the so-called *Stachel* sequence, which acts as an intramolecular agonist at the interface between the N terminus (Nt) and the seven-transmembrane helix domain (7TMD). Distinct extracellular signals received by the Nt are integrated at the *Stachel* into structural changes of the 7TMD towards an active state conformation. Until recently, little information was available on how the activation process of aGPCRs is realized at the molecular level. In the past three years several structures of the 7TMD plus the *Stachel* in complex with G proteins have been determined, which provide new insights into the architecture and molecular function of this receptor class. Herein, we review this structural information to extract common and distinct aGPCR features with particular focus on the *Stachel* binding site within the 7TMD. Our analysis extends the current view of aGPCR activation and exposes similarities and differences not only between diverse aGPCR members, but also compared to other GPCR classes.

Abbreviations: 7TMD, seven-transmembrane helix domain; aa, amino acid; β-subunit, C-terminal fragment (CTF) including the *Stachel* sequence; βT-120CT, C-terminal fragment (CTF) including the *Stachel* sequence, mostly without the intracellular C terminus; BCM, beclomethasone; CD55, complement decay-accelerating factor; CTF, C-terminal fragment; cryo-EM, cryo-electron microscopy; dnGs, dominant-negative Gs; ECD, extracellular domain; ECR, extracellular region; ECRΔ1, extracellular region without EGF-repeats; EGF, epidermal growth factor-like domain; FL, full-length receptor; FL-AA, full-length receptor with a double alanine mutation in the GPS sequence at cleavage relevant residues; FLRT3, fibronectin leucine-rich transmembrane protein 3; FN3, fibronectin type III domain; EGF, endothelial growth factor-like domain; EL, extracellular loop; GAIN, G protein-coupled receptor autoproteolysis-inducing domain; GPCR, G protein-coupled receptor; GPS, G protein-coupled receptor proteolysis site; H8, helix 8; IL, intracellular loop; HormR, hormone receptor domain; Ig, immunoglobulin domain; IP15, IS(4-MeF) GILLDLSRTSLP (4-MeF indicates 4-methylphenyl-alanine); LPC, lysophosphatidylcholine; LRRD, leucine-rich repeat domain; Nt, N terminus; NTF, N-terminal fragment; PLL, Pentraxin/Laminin/neurexin/sex-hormone-binding-globulin-Like; SCR, short consensus repeat; *Sf*9, *Spodoptera frugiperda*; *Stachel*, intramolecular agonistic sequence; TA, tethered agonist (*Stachel* sequence); TEN2, teneurin-2; XRD, protein X-ray crystallography.

* Correspondence to: Rudolf Schönheimer Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, University of Leipzig, Johannisallee 30, D-04103 Leipzig, Germany.

** Correspondence to: Institute of Medical Physics and Biophysics, Group Structural Biology of Cellular Signaling, Charité – Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany.

E-mail addresses: schoberg@medizin.uni-leipzig.de (T. Schöneberg), patrick.scheerer@charite.de (P. Scheerer).

https://doi.org/10.1016/j.phrs.2023.106971
Received 27 September 2023; Received in revised form 25 October 2023; Accepted 25 October 2023
Available online 30 October 2023

1. Introduction

Adhesion GPCRs (aGPCR, class B2) together with the secretin-like receptors (class B1) constitute the class B of GPCRs [1]. Previous studies have identified aGPCR sequences in both Protostomia and Deuterostomia [2,3], indicating an evolutionary age as old as Bilateria (> 680 million years) [4]. Prototypes of aGPCRs but not of secretin-like GPCRs have been found in unicellular eukaryotes, indicating that aGPCRs are probably the most ancient receptors among class B GPCRs [3,5]. New insights from recent phylogenetic studies suggest that secretin-like receptors evolved from ancient aGPCRs [2,3,5,6]. To date, 33 intact genes and several pseudogenes of aGPCRs have been identified in the human genome [7,8]. Based on specific features in their sequences, these receptors were classified into nine distinct groups [7] (this classification is also used here to enable comparison with previous publications (suppl. Tab. S1)), although recent cluster analysis of hundreds of vertebrate aGPCR ortholog sequences has refined this hierarchical grouping, providing a more consistent classification [5].

Several aGPCRs have been shown to play key roles in numerous physiological processes, such as cell proliferation and cell communication. aGPCRs are involved in transducing mechanical forces [9,10] of cell–cell or cell–matrix interactions [11–13]. Subsequently, they can also participate in various pathogenic conditions, including cancer development [14–16].

Adhesion GPCRs have the typical GPCR architecture with an extracellular N terminus (Nt), a seven-transmembrane helix domain (7TMD) and a cytosolic C terminus (Ct) (Fig. 1). However, in contrast to most rhodopsin-like (class A) GPCRs, they have long Nt's, often consisting of multiple domains. Although commonly used, the Nt of an aGPCR should not be referred to as an "extracellular domain" (ECD), as it is not a single domain but rather composed of multiple domains. Within the Nt, most aGPCRs contain a **G**PCR-**A**utoproteolysis **IN**ducing (GAIN) domain,

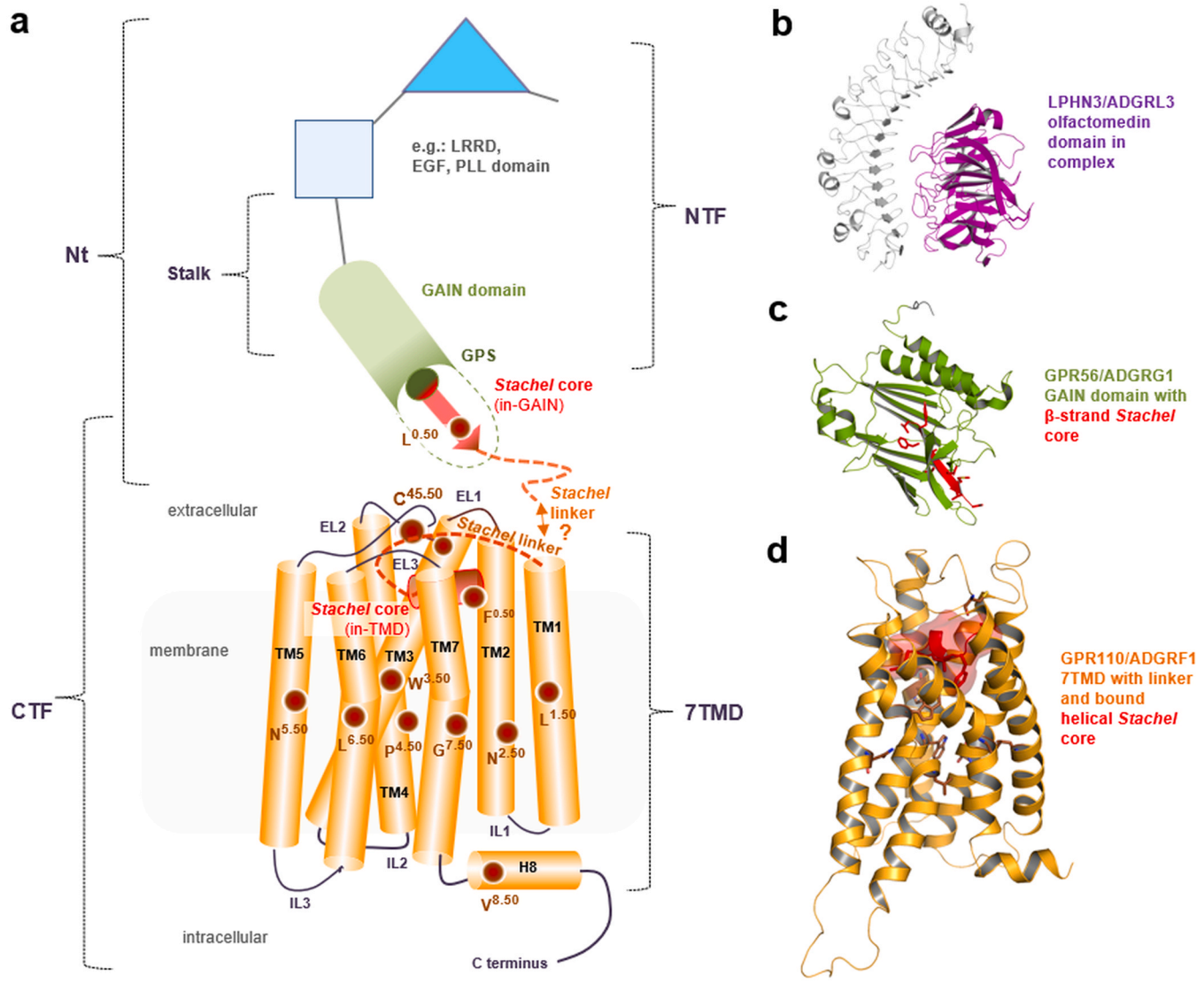

Fig. 1. Structural architecture of aGPCRs. (a) The components constituting a prototypical aGPCR are presented. The large extracellular N terminus (Nt) can be subdivided into distinct domains and interconnecting regions (e.g., the Stalk between the GAIN domain and other domains of the Nt). The region interconnecting the GAIN and the 7TMD is called *Stachel* sequence and serves as an intramolecular agonist. The *Stachel* sequence can be subdivided into a *Stachel* core and a *Stachel* linker. For the *Stachel* core sequence, two distinct spatial positions and secondary structures in different aGPCRs were determined by structural studies, either inside the isolated GAIN domain (β-strand) or bound within the 7TMD (helical). The orientation of the *Stachel* linker in a full-length receptor is currently unknown (indicated by "?"). The most highly conserved amino acid positions in the 7TMD of class B GPCRs are highlighted as red circles, according to the unifying numbering scheme for class B GPCRs (see below). (b–d) Representative structures of aGPCR domains are shown (b = PDB ID 5cmn, c = PDB ID 5kvm, d = PDB ID 7wu5). All structural representations were generated using the PyMol Molecular Graphics System Version 2.5.5 (Schrödinger, LLC, New York, NY).

which can include a "**G** protein-coupled receptor (GPCR) **P**roteolysis **S**ite" (GPS) with an auto-cleavable HXS/T motif [17,18]. The GAIN domain catalyzes autoproteolytic cleavage at the GPS, which separates the N-terminal fragment (NTF) from the remaining C-terminal fragment (CTF) of the aGPCR (Fig. 1a). It is important to note that the frequently used term "subunit" for the NTF (α-subunit) or CTF (β-subunit) is misleading, because subunits are usually derived from individual genes (e.g., α-, β- and γ-subunits of a G protein, α- and β-subunit of the hemoglobin), which is not the case in aGPCRs.

In addition to the GAIN domain, the Nt can contain multiple other domains, such as epidermal growth factor (EGF) -like domain, leucine-rich repeat domain (LRRD), immunoglobulin-like (Ig) domain, and/or pentraxin-like domains, some of which are proposed to be involved in cell-cell or cell-matrix adhesion, homophilic multimerization, or hormone binding (Fig. 1a–c). The sequence connecting extracellular domains and the GPS was initially referred to as the *Stalk* sequence [19]. Following the discovery of the GAIN domain, the *Stalk* is defined as the region between the GAIN and other extracellular domains [17]. Thus, the *Stalk* sequence should not be confused with the intramolecular agonistic sequence, called *Stachel* sequence, which is located C-terminally of the GPS [20,21] (Fig. 1). In general, several structural-functional features of aGPCRs are inconsistently defined with different terms being used for potentially identical features, such as `ECR` (extracellular region) and `ECD` (extracellular domain) (Table 1; specific terms and abbreviations for structural parts are taken from corresponding publications). These and the other examples given below indicate that a unified terminology of the aGPCR architecture would be both useful (Fig. 1a, suppl. Fig. S1), and feasible, given that there are a reasonable number of aGPCR structures (Table 1) and sequences [5] available. As in many emerging fields, the terminology of members, structural components and domains, specific features, numbering systems and functions evolves historically and depends on the current state of knowledge. However, accurate terminology is necessary to synchronize contributions from different disciplines and needs to be adapted specifically when a taxonomy has been disproven or terminology is ambiguous or potentially misleading. Based on the current knowledge, we tried here to clarify ambiguities and incorrect terminologies, but also keep with established wording, even when some naming is a matter of semantics.

The CTF includes the 7TMD plus the extracellular *Stachel* core (Fig. 1d) and the interconnecting *Stachel* linker sequence of varying length (12–20 amino acid residues). The *Stachel* was experimentally identified by peptide studies [20,21]. It features mainly hydrophobic amino acid residues as a core sequence and also known as "tethered agonist" (TA). However, this term is inaccurate as the *Stachel* sequence is a transcribed and translated part of the receptor polypeptide rather than a "tethered" component, such as retinal, which is covalently bound to a lysine in opsins via a Schiff base [22]. Therefore, a more precise term to

Table 1

Overview of published aGPCRs structures. For eight different aGPCRs 26 7TMD structures with or without the *Stachel* ligand were determined so far (July 2023), representing four out of nine aGPCR groups. The background-coloring scheme is according to the individual groups of the receptors provided in the suppl. Tab. S1. For seven different aGPCRs eleven structures of entire N termini or single domains solved by protein X-ray crystallography (XRD) (in one case by cryo-EM) were published. The overall resolutions and protein specifications are provided as described in the original publications (might differ from data provided in the PDB [92]). "*" – information according to the original publication; "§" - linker GSGENLYFQSGSSSSGWRGGHV; "#" *Stachel* is structurally visible in the 7TMD; Abbreviations are explained in the "Abbreviations" section [81-91].

Group	Receptor name	PDB ID	Species	Expression system	External ligand	Complex binder/ Interaction protein	Overall resolution (Å)*	Method	Protein specifications and modifications *	Year of publication	Ref.
Transmembrane domain											
I	LPHN3/ ADGRL3	7sf7 #	human	*Sf*9 insect cells		mini-G13	2.90	cryo-EM	TA peptide, 7TMD (residues 842-1138)	2022	(47)
		7wy5 #	mouse	*Sf*9 insect cells		Gq	2.75	cryo-EM	7TMD (residues 923-1229)	2022	(81)
		7wy8 #	mouse	*Sf*9 insect cells		Gs	2.8	cryo-EM	7TMD (residues 923-1229)		
		7x10 #	mouse	*Sf*9 insect cells		G12	2.93	cryo-EM	7TMD (residues 923-1229)		
		7wyb #	mouse	*Sf*9 insect cells		Gi	2.97	cryo-EM	7TMD (residues 923-1229)		
V	GPR133/ ADGRD1	7ept #	human	*Sf*9 insect cells		Gs	3.00	cryo-EM	CTF (residues 545-874)	2022	(43)
		7wu2 #	human	high-five insect cells		mini-Gs	2.8	cryo-EM	7TMD (truncated NTF M1-L544; truncated C-term S828-V874)	2022	(48)
VI	GPR110/ ADGRF1	7wu3 #	human	High Five™ insect cells		mini-Gs	3.10	cryo-EM	7TMD (truncated NTF 1-250; truncated C-term 861-910)	2022	(48)
		7wu4 #	human	High Five™ insect cells	LPC	mini-Gi	3.40	cryo-EM	7TMD (truncated NTF 1-R50; truncated C-term 861-910)		
		7wu5 #	human	High Five™ insect cells	LPC	mini-Gi	3.00	cryo-EM	FL-AA (truncated NTF M1-R250; truncated C-term 861-E910; substitution H565A/T567A)		
		7wxu #	human	*Sf*9 insect cells		Gq	2.85	cryo-EM	CTF (residues 567-873), LgBiT fusion	2022	(82)
		7wxw #	human	*Sf*9 insect cells		Gs	2.84	cryo-EM	CTF (residues 567-873), LgBiT fusion		
		7x2v #	human	*Sf*9 insect cells		Gi	3.09	cryo-EM	CTF (residues 567-873), LgBiT fusion		
		7wz7	human	*Sf*9 insect cells		G12	2.8	cryo-EM	CTF (residues 567-873), LgBiT fusion		
		7wy0 #	human	*Sf*9 insect cells		G13	2.66	cryo-EM	CTF (residues 567-873), LgBiT fusion		
		8G2Y #	human	Expi293F	LPC	mini-$G_{s/q}$	3.44	cryo-EM	CTF	2023	(83)
VIII	GPR112/ ADGRG4	7wuj #	human	*Sf*9 insect cells		Gs	3.30	cryo- EM	β-subunit (residues 2722–3080)	2022	(49)
	GPR64/ ADGRG2	7wui	mouse	*Sf*9 insect cells	IP15	Gs	3.10	cryo-EM	FL-AA (signal-peptide removed; C-term from GPR120; substitution H597A/T599A)	2022	
		7wuq #	mouse	*Sf*9 insect cells		Gs	2.90	cryo-EM	β-subunit (residues 597-1009)	2022	
		7xkd	human	*Sf*9 insect cells	DHEA	Gs	2.40	cryo-EM	β-subunit	2022	(84)
		7xke	human	*Sf*9 insect cells	DHEA	mini-Gs	2.90	cryo-EM	FL-AA (truncated C-term)		
		7xkf	human	*Sf*9 insect cells	DHEA	Gs	2.40	cryo-EM	β-subunit		
	GPR114/ ADGRG5	7eq1 #	human	*Sf*9 insect cells		Gs	3.3	cryo-EM	CTF (residues 227-528)	2022	(43)
	GPR56/ ADGRG1	7sf8 #	human	*Sf*9 insect cells		mini-G13	2.70	cryo-EM	TA peptide plus 7TMD (residues 383-687)	2022	(47)
	GPR97/ ADGRG3	7d76	human	*Sf*9 insect cells	BCM	mini-Go with palmitoylation	3.1	cryo-EM	FL-AA (residues 21-549; substitution H248A/T250A)	2021	(50)
		7d77	human	*Sf*9 insect cells	Cortisol	mini-Go with palmitoylation	2.9	cryo-EM	FL-AA (residues 21-549; substitution H248A/T250A)	2021	
Entire or partial extracellular N terminus											
I	LPHN3/ ADGRL3	6vhh	human	High Five™ insect cells		ECRΔ1 of human TEN2 (T727-R2648)	2.97	cryo-EM	Full-length ECD (residues 21-866)	2020	(85)
		5cmn	human	insect cells		FLRT3 LRR domain (residues 29-357)	3.60	XRD	olfactomedin domain (residues 132-392)	2015	(86)
		4rmk	mouse	HEK293 GNTI-			1.6	XRD	olfactomedin domain	2015	(87)
		4rml	mouse	HEK293 GNTI-			1.6	XRD	olfactomedin domain		
		4yeb	mouse	HEK293 GNTI-		FLRT3 LRR domain	3.2	XRD	olfactomedin domain		
	LPHN1/ ADGRL1	4dlq	rat	High Five™ insect cells			1.85	XRD	GAIN and HormR (residues 460-849)	2012	(17)
II	CD97/ ADGRE5	7do4	human	HEK293S GnTI-			3.19	XRD	EGF domain (21-165) linked by 24 aa § to CD55 SCR domain (35-285)	2021	(88)
VII	BAI3/ ADGRB3	4dlo	human	High Five™ insect cells			2.30	XRD	GAIN and HormR (residues 460-849)	2012	(17)
VIII	GPR126/ ADGRG6	6v55	zebrafish	High Five™ insect cells			2.38	XRD	ECR (residues 38-853)	2020	(89)
	GPR56/ ADGRG1	5kvm	mouse	High Five™ insect cells		α5 monobody	2.45	XRD	entire ECR	2016	(90)
	GPR97/ ADGRG3	7qu8	human	HEK293S			3.37	XRD	entire ECR	2023	(91)

define the *Stachel* sequence would be "intramolecular agonist". Groundbreaking new aGPCR structures were published over the past 3 years (Table 1) [23,24] providing deep insights into the structural organization of the *Stachel* sequence and its interplay with the 7TMD. Furthermore, these structural data should be discussed in light of both previous and recent functional data (e.g., [7, 15, 20, 25–27]) and also taking aGPCR evolution into account by comparing orthologous sequences. This review analyzes and summarizes insights from these structures with a focus on the binding mode of the *Stachel* and associated mechanisms involved in intramolecular aGPCR signaling.

2. A common *Stachel* and extended unifying residue numbering system for class B GPCRs

2.1. Numbering scheme in the 7TMD and in helix 8

A unified numbering system to denote corresponding amino acid residues within aGPCRs is of fundamental importance for experimentally addressing and discussing structural and mechanistic questions. Such a numbering system was introduced many years ago for rhodopsin-like GPCRs (class A) and is based on structurally conserved residues in the transmembrane helices (TM) [28]. In this numbering system the most conserved residue of each TM is assigned as the number X.50 (where X is the TM number), and all other residues in that part are numbered according to their relative position compared to the 'X' position.

Recent in-depth analyses of sequence conservation within the class B GPCRs enabled the development of a comprehensive TM numbering system [5], which finally unified the 7TMDs of secretin-like (class B1) and aGPCRs (class B2) [5] (Fig. 1a, suppl. Fig. S1). This numbering system differs significantly from the previously proposed secretin-like receptor (class B1) numbering system [29], which is also often applied to aGPCRs even though there are significant differences in the conservation of amino acid residues in the TM's between secretin-like GPCRs and aGPCRs [5].

Here, we used the recent unifying numbering system of the whole class B GPCRs and, furthermore, implemented a loop numbering system as has been previously suggested for the extracellular loop 2 (ECL2) [30]. A highly conserved cysteine in this loop is the reference (Fig. 1a, suppl. Fig. S1), which forms a disulfide bridge to another highly conserved cysteine residue located in the N-terminal part of TM3. The ECL2 connects TMs 4 and 5, therefore, this cysteine position is termed $C^{45.50}$, which is indicative of the explicit loop and the conserved cysteine residue. The numbering of all other amino acids within this loop are relative to the cysteine. In the intracellularly located helix 8, which has been described as a structural key for mechano-sensitivity of GPCRs [31], we identified a valine as being the most conserved residue, and is thus termed $V^{8.50}$ (Fig. 1a, suppl. Fig. S1).

2.2. A common Stachel numbering

In contrast to class A GPCRs, where sequences and structures of the N termini often lack high conservation even between orthologs, aGPCRs have conserved domains or motifs in their Nt's, such as the GPS and the *Stachel* core (suppl. Fig. S1). Unfortunately, conservation analysis of the GAIN domain revealed low conservation over the entire domain, even within orthologous sequences. In particular, the N-terminal part of the GAIN domain is less conserved, while conservation is higher in the C-terminal part containing the *Stachel* region (see suppl. Material files: Movie S1 and Movie S2 (legends are provided in the suppl. Information); sequences by FASTA files: Suppl_Alignment_Human_ADGR_orthologs_GAIN and Suppl_Alignment_vertebrate_ADGRL1_orthologs_GAIN). Due to this lower conservation, a general numbering assignment of GAIN domain residues was not feasible, although some studies have taken the first position after the GPS as a reference [21,32], despite the fact that many aGPCRs (one third of aGPCR members) do not have this cleavage motif [27,33] (suppl. Tab. S2, suppl. Fig. S1). Because the *Stachel* sequence, which is of high functional importance in many aGPCRs, is still not consistently numbered, we re-analyzed the conservation of the *Stachel* sequence in over 4900 vertebrate aGPCR orthologues (suppl. Fig. S2). A leucine, six amino acid residues downstream of the usual cleavage site, is the most conserved residue found in 94% of all vertebrate aGPCRs examined. Therefore, we propose this position as the first reference position for the *Stachel* sequence – numbered as $L^{0.50}$ (Figs. 1–2). All residues in the *Stachel* or adjacent can be numbered relative to this conserved position. The second most conserved position in the *Stachel* is a phenylalanine ($F^{0.47}$) located in close proximity to the GPS motif. Our proposed numbering system is supported by the solved CTF structures (Fig. 2) showing that the conserved region between $T^{0.45}$ and $M^{0.51}$ (suppl. Fig. S2) has an identical helical fold bound within a very defined structural region of the 7TMD (Fig. 2c). The high sequence and structural conservation as well as its similar localization in the CTF-*Stachel* structures support the definition of the *Stachel* core (positions 0.45–0.51) as the most activation-relevant element. Thus, the physicochemical properties of the *Stachel* core sequence can be condensed to X-X-φ$^{0.47}$-X-φ-φ$^{0.50}$-φ (φ indicates a hydrophobic and X a variable residue). We refer to this numbering system throughout the review and term the new numbering system as "*class B GPCR numbering 3.0*".

Supplementary material related to this article can be found online at doi:10.1016/j.phrs.2023.106971.

3. Structure and binding modes of the intramolecular *Stachel* ligand

3.1. "In-GAIN" and "in-7TMD" bound Stachel core structures

As of July 2023, structures of 11 extracellular domains or complete N termini of aGPCRs were published (Table 1) with resolutions ranging between 1.6 and 3.6 Å. Several of these partial aGPCR structures provided first important insights into, e.g., the GAIN domain, where the *Stachel* core sequence was resolved as a β-strand and positioned along the C-terminal β-sheet of the GAIN domain (Fig. 3a). It should be noted that the GAIN domain structures are retrieved from partial N termini, which are soluble and not membrane-bound. Moreover, the *Stachel* sequence only contains the *Stachel* core but not the *Stachel* linker. In contrast, several CTF structures in complex with their cognate G protein solved by cryo-electron microscopy (cyro-EM) localize the *Stachel* core sequence as an α-helical intramolecular ligand embedded in the extracellularly oriented part of the 7TMD (Figs. 2c, 3b). This difference between 7TMD- and GAIN-bound *Stachel* raises the question of how the *Stachel* is transferred from an inactive in-GAIN bound to an active in-7TMD bound state? Currently, the localization of the *Stachel* in a full-length aGPCR is not known for either an inactive or activated conformation, making it difficult to answer this fundamental question of aGPCR activation. CTFs of many aGPCRs with their complete *Stachel* sequence are naturally translated proteins that are generated by promoters within introns upstream of the GPS or by alternative splicing [26]. Most interestingly, all vertebrate aGPCR genes, except ADGRF members, have intron-exon boundaries within or in close proximity to the GPS coding sequence, suggesting that aGPCR genes evolved in a modular fashion fusing the 7TMD/CTF with different N-terminal domains. Therefore, one should consider CTF structures as physiological protein species of aGPCR genes [26].

To explain the observed differences in the secondary structure of the *Stachel* core one can consider the different environments provided by the GAIN domain and the 7TMD, but also differences in the primary sequence found in the *Stachel* core. Interestingly, the sequence of the *Stachel* core shows a tendency to favor a helical fold when it contains an $F^{0.47}$ and a GPS sequence (Fig. 3d). In contrast, *Stachel* core sequences which do not contain a phenylalanine residue at position 0.47 have a preference for a β-strand conformation, regardless of a GPS motif (Fig. 3d). This suggests that $F^{0.47}$ may have a key role in forming a

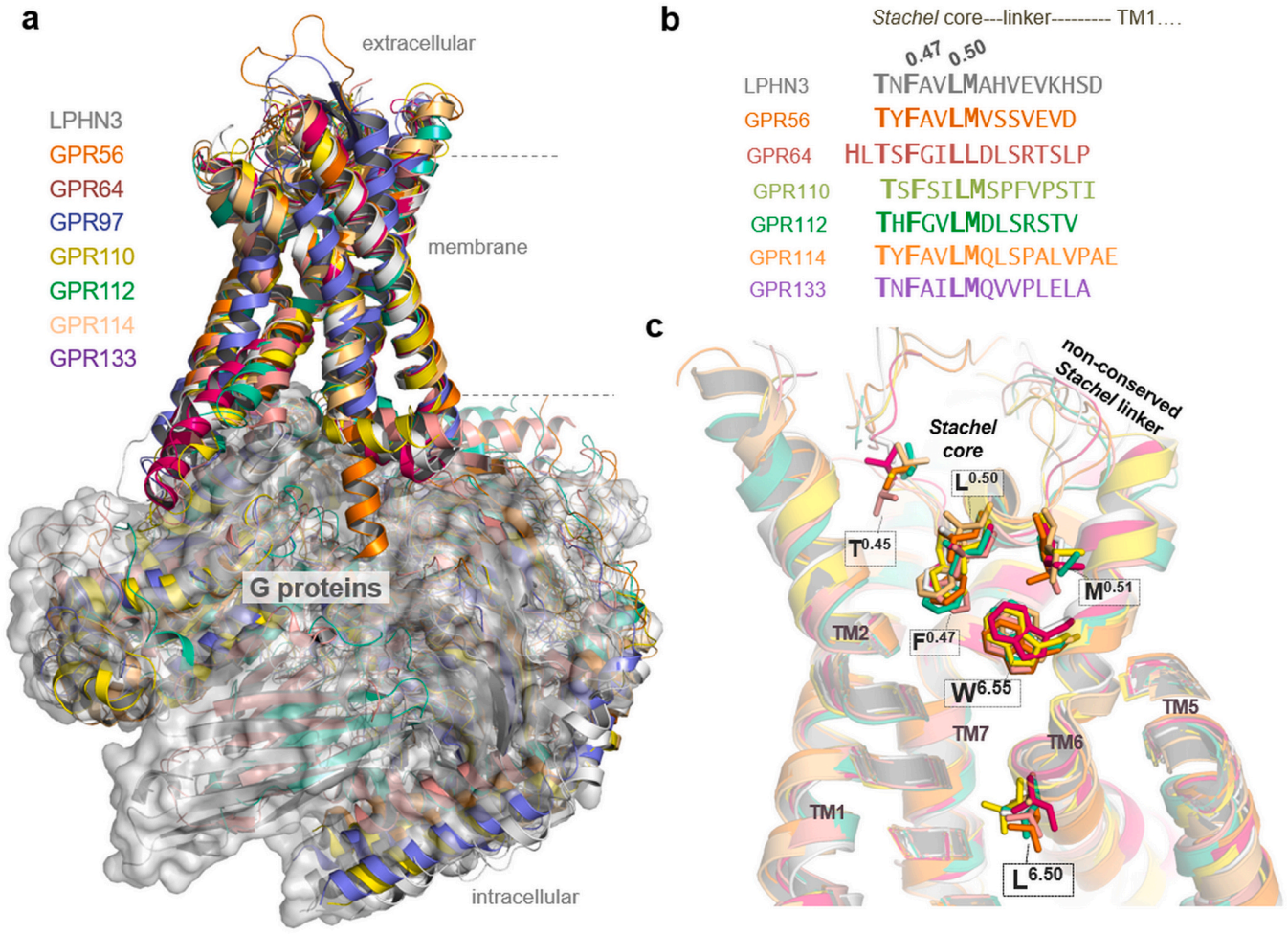

Fig. 2. Superimposed CTF/G-protein complex structures in an active state conformation. (a) The superimposed structures of 8 different aGPCR CTF/G protein complexes in an active state conformation show high overlap in the membrane spanning region, with some exceptions, e.g., the extended TM7 of GPR114/ADGRG5 and the straight TM6 of GPR97/ADGRG3. (b) The *Stachel* sequences contain several conserved residues (bold enlarged letters) (see also suppl. Figs. S1, S2). Only the GPR64 structure has a motif ("HL") prior to $T^{0.45}$. The phenylalanine residue proximal to the GPS is numbered as $F^{0.47}$ according to the new aGPCR numbering system (see main text). The *Stachel* linker between the *Stachel* core and TM1 is not conserved among aGPCRs, neither in length nor in its amino acid composition. (c) The most conserved *Stachel* core residues (as sticks) are superimposed highlighting their conserved binding mode between the transmembrane helices and ECLs. $W^{6.55}$ in TM6 of the 7TMD has main interactions with $F^{0.47}$, $L^{0.50}$ and $M^{0.51}$. In contrast to the *Stachel* core, the *Stachel* linker is not conserved in sequence and structure.

helical secondary structure of the *Stachel* core in an active 7TMD-bound state and, thereby, to stabilize an active conformation. One could speculate that the energetically unfavorable β-strand conformation in the inactive "in-GAIN" state supports the *Stachel* release from the GAIN domain following an extracellular signal (e.g., mechanical traction, ligand binding). However, this raises the question as to how the *Stachel* core of GPR124/ADGRA2, GPR125/ADGRA3, EMR1/ADGRE1, and VLGR1/ADGRV1, all lacking a phenylalanine residue at position 0.47, are released and fold into the 7TMD in an active state.

The position of the *Stachel* core in the determined CTF structures shows strong overlap with the common ligand binding region in class A GPCRs [34–36]. The evolutionary success of class A GPCRs, reflected in their enormous gene expansion, diversification and adaptation to a huge variety of different ligands (such as light, hormones, ions, peptides, proteins, metabolites, amino acids [37]), is thought to be linked to a binding site for external stimuli inside the relatively stable scaffold of the 7TMD. This feature enables signal initiation by diverse chemical ligand moieties in new receptor variants at activation-related trigger points [38]. In contrast, sensing of the different external stimuli by aGPCRs occurs, according to current knowledge, at the large N-terminal part, accompanied by a multistep activation mechanism following a common intramolecular pathway: "Extracellular stimulus → *Stachel* exposition/release → 7TMD rearrangement → G-protein activation". Herein, the *Stachel* core sequence is a rather conserved intramolecular and orthosteric agonist that integrates the various extracellular signals into a conformational 7TMD rearrangement, as proposed for other GPCRs with long N termini such as glycoprotein hormone receptors [39, 40].

3.2. The GPS cleavage motif

With regard to the potential activation mechanisms, one must consider the fact that the GPS cleavage motif is missing or is not conserved in about 36% of the aGPCR members, as evident from sequence analyses of over 4900 aGPCR orthologs (suppl. Tab. S2). In fact, the number of non-cleaved aGPCRs is probably higher, as it has been shown that some aGPCRs with an intact GPS do not undergo cleavage (e.g., GPR114/ADGRG5, BAI2/ADGRB2) [27,41]. However, there are conflicting results for mouse and human GPR114/ADGRG5 [27,42]. Support for a missing autocleavage comes from evolutionary data. For instance, many BAI2/ADGRB2 orthologs, unlike their human variant, lack an HXS/T motif and have different amino acid residues (K, R, Q, Y) instead of histidine, suggesting that these receptors generally do not need to be cleaved for receptor activation. However,

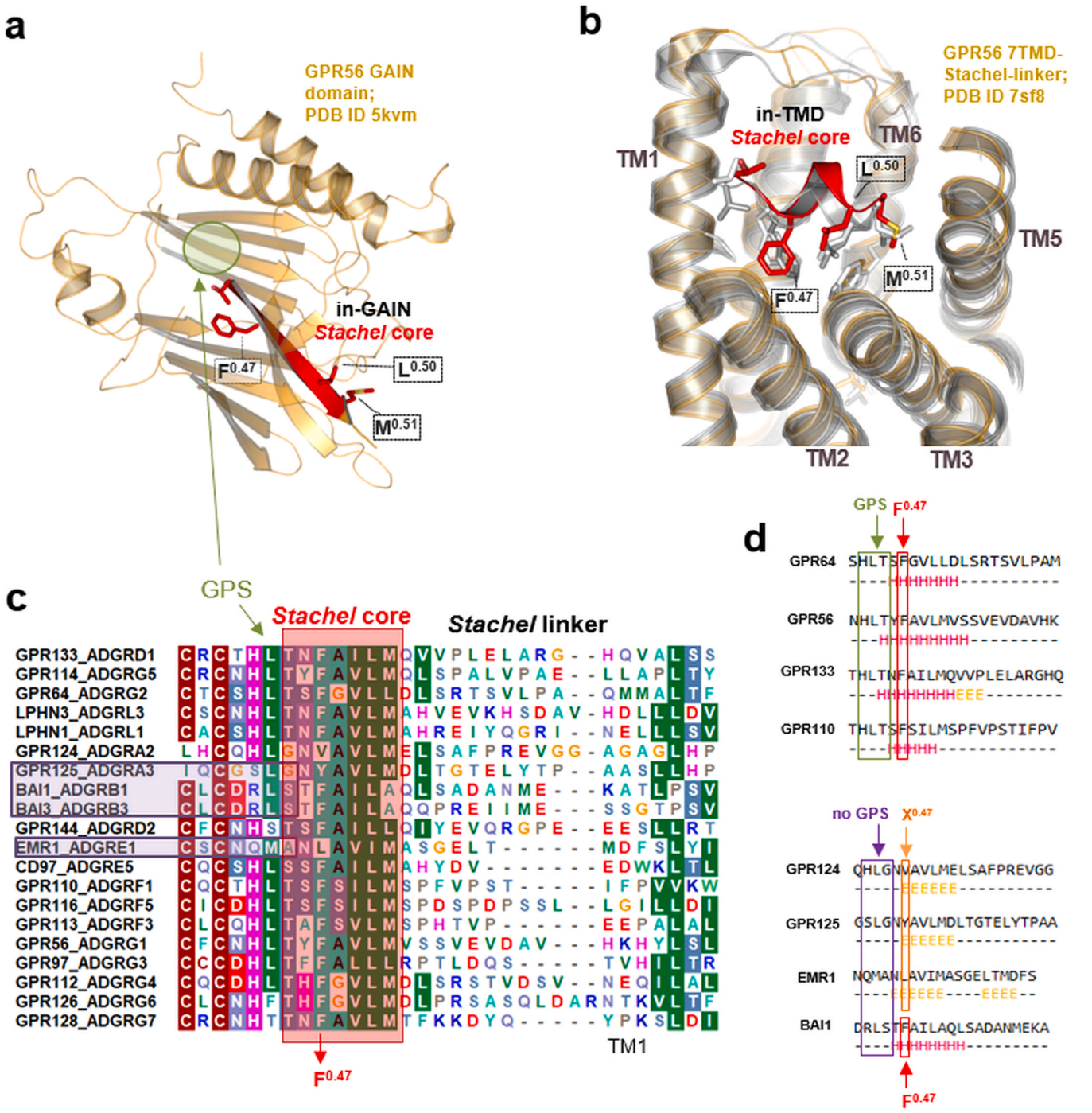

Fig. 3. The *Stachel* core sequence in different conformations and binding modes. (a) In the GAIN domain of GPR56/ADGRG1 the *Stachel* core sequence is a β-strand forming part of a β-sheet. The localization of the cleavage site is highlighted (green circle). (b) In contrast, in the active state structure of the ADGRG1/G protein complex, the *Stachel* core sequence forms an α-helical conformation and is positioned between the transmembrane helices and extracellular loops. (c) The alignment of *Stachel* sequences from 20 different human aGPCRs representing seven out of nine groups, reveals that neither the GPS nor the *Stachel* core sequence (red box) are fully conserved among aGPCRs. (d) Prediction of the *Stachel* sequence capacities to form specific secondary structures (Software: https://www.compbio.dundee.ac.uk/jpred [93]). Examples investigated are from aGPCRs either with or without a GPS sequence (blue transparent box in (2d), but also aGPCRs with variations in the *Stachel* sequence, specifically at the conserved position 0.47 (phenylalanine, the numbering scheme is explained in the text). H – α-helix tendency, E – β-strand tendency.

GPR114/ADGRG5 requires the *Stachel* for activation [27], suggesting a *Stachel*-mediated activation mechanism that is independent from cleavage, at least in the mouse orthologue. In this case, the *Stachel* may be pre-bound in the 7TMD of a full-length structure, or alternatively, the *Stachel* can be exposed from the in-GAIN domain bound state without cleavage. Furthermore, cleavage-deficient GPR133 or dCirl $H^{-2}A$ mutants still exhibit *Stachel*-dependent mechano-activation [10,43]. Interestingly, GPR133$H^{-2}R$ mutants eliminate this activation type [44], which indicates that mutational approaches to dissect the role of cleavage on *Stachel*-mediated activation can be confounded by unknown effects induced by the specific amino acids used as substitutes. Moreover, it should be considered that the GPS is directly flanked by two

conserved disulfide bridges of the GAIN domain (suppl. Figs. S1, S3). Thus, for aGPCRs lacking cleavage capacity in the GPS and assuming a similar fold of the GAIN domain with the *Stachel* core, it would be intriguing to explain an "out-of GAIN domain" exposition of the *Stachel* toward the 7TMD during activation. The GAIN domain-stabilizing disulfide bridges would probably not provide the required structural flexibility to allow for a transition into the 7TMD and, thereby, functional interaction of *Stachel* core residues in the orthosteric 7TMD binding pocket. Consequently, aGPCRs with a non-cleavable GPS would require either larger GAIN domain rearrangements (yellow marked in suppl. Fig. S3), or the entire GAIN domain fold is arranged differently in those aGPCRs allowing for *Stachel* exposition into the 7TMD. Therefore, a common mechanism for *Stachel*-mediated activation of all aGPCRs cannot be proposed yet. Moreover, there are several aGPCRs where experiments failed to show a functional *Stachel* or the lack or low sequence homology makes the existence of a functional *Stachel* highly unlikely (e.g., GPR123/ADGRA1, CELSR1/ADGRC1, CELSR3/ADGRC3, GPR111/ADGRF2, GPR115/ADGRF4,) [45,46].

3.3. The Stachel-7TMD binding sites

3.3.1. Insights from determined structures

There are currently 26 published cryo-EM structures of the CTF of 8 different aGPCRs in an active state conformation with a G protein bound (resolutions ranging between 2.7 and 3.4 Å) (Table 1). These receptors are mostly arranged as complexes of the 7TMD plus the *Stachel* as intramolecular agonist or with a peptide derived from the *Stachel* sequence. However, as yet no full-length aGPCR structure has been determined because the extracellular parts of full-length receptors appear to be highly flexible and stabilizing binding partners and antibodies are currently missing [47–50]. The CTF structures in the active state include representatives of four of the nine aGPCR groups (Table 1), displaying a high degree of structural similarity in the membrane region across these groups (Fig. 2a). Although GPR97/ADGRG3 shows very specific features in the TM5 and TM6 (discussed below), the activated 7TMD structures (backbone) are similar between the different aGPCRs.

Given the high amino acid conservation of the *Stachel* core sequences even between different aGPCR groups (suppl. Fig. S2), cross-activation of different aGPCRs with various *Stachel*-derived peptides can be expected from the cryo-EM structures and has already been experimentally observed [45]. However, the orthosteric *Stachel* core/7TMD binding site (direct interactions) and the extended shell (surrounding amino acid residues at a distance for potential contacts) are composed of a variable number of amino acid residues (suppl. Fig. S4, suppl. Tab. S3). By analyzing and comparing 4 determined *Stachel* core/7TMD structures (suppl. Tab. S3), we note that ~25 partially corresponding 7TMD positions participate in the *Stachel* core binding, although only four conserved 7TMD positions in TM1 (1.39), TM2 (2.62), ECL2 (45.51), and TM6 (6.55) mediate common interactions with three highly conserved hydrophobic-aromatic positions in the *Stachel* core sequences ($F^{0.47}$, $L^{0.50}$, $M^{0.51}$) (suppl. Fig. S4). $L^{0.50}$ in the *Stachel* sequence has two proposed interactions with conserved residues in the 7TMD, namely $L/I/F^{2.62}$ and $W^{45.51}$, making this leucine a key player in the interaction between *Stachel* and the 7TMD. Mutagenesis studies of different aGPCRs have shown that the most conserved $F^{0.47}$ and $L^{0.50}$ in the *Stachel* core are obligatory for signaling capacity [20,32,43,51] fitting well to the *Stachel*-CTF contacts observed in an active state conformation (Fig. 2c). Other conserved *Stachel* core residues interact with $W^{6.55}$ of TM6 (Fig. 2), which can be considered a common anchor between the *Stachel* core ligand and the 7TMD for stabilizing an active state conformation. Moreover, less-conserved amino acid residues within the *Stachel* sequence (e.g., positions 0.46 and 0.48) also participate in *Stachel* core/7TMD interactions (suppl. Tab. S3). They contribute to some flexibility in the 7TMD/*Stachel* core binding pocket, which may cause differences in receptor specificity and signaling regulation for particular aGPCRs. This has also been observed in other GPCRs, as for example the interplay between opsin and the ligand retinal [52,53]. In opsins the general binding mode of this ligand is highly conserved and the signal transduction of the activated agonist (11-*cis* to *all-trans* isomerization of the retinal) depends on which wavelength of light is absorbed in the retinal [54,55]. The wavelength specificities of opsins are determined by differences in the amino acid residues forming direct contact to the retinal or constituting the binding site shell of the ligand [56]. In aGPCRs the binding mode of the *Stachel* is also conserved, but differences in the *Stachel*-7TMD interactions (suppl. Tab. S3) may enable or hinder e.g., ligand promiscuity of *Stachel* sequences derived from different aGPCRs [45], regulate basal signaling activity, or determine receptor/G protein-subtype preferences.

3.3.2. The Stachel linker

In contrast to the *Stachel* core, the *Stachel* linker length (between 12 and 19 amino acids) or sequence composition is not conserved among aGPCRs. In the active state CTF structures the *Stachel* linker is located in spatial proximity to non-conserved and more flexibly adapted regions of the TM6 and ECL3 (Fig. 4). At least two functions can be assumed for the *Stachel* linker: *(i)* it ensures the 1:1 ligand-7TMD stoichiometry within a defined space and mobility, and *(ii)* its non-conserved properties are required to participate in the individual transition from the in-GAIN bound to the in-7TMD bound state of the *Stachel* ligand. It has already been proposed that the specificity of *Stachel* binding for ADGRF4/GPR115 and ADGRF5/GPR116 is associated with amino acid residues in the linker [45]. However, *Stachel*-peptide binding and activity studies have shown that *Stachel* core action at the 7TMD is not dependent on entire linker sequences [21,57,58].

3.3.3. Conservation of Stachel binding sites among the 7TMD of aGPCRs

To address the question whether the *Stachel* core binding site is conserved among the majority of aGPCRs or not, we examined similarities and variations in the putative binding sites of 20 different human aGPCRs by comparing the residues identified as *Stachel* binding sites in four solved aGPCR CTF structures (suppl. Tab. S3) with corresponding residues of other human aGPCRs. Using the amino acids of the *Stachel* binding sites from GPR64/ADGRG2, GPR114/ADGRG5, LPNH3/ADGRL3, and GPR133/ADGRD1 (suppl. Fig. S4) as templates and exploring corresponding residues in other human aGPCRs by a phylogenetic analysis, it becomes clear that, except of a few conserved amino acid residues (suppl. Tabl. S3), the assumed *Stachel* binding sites in the 7TMDs show an overall low conservation of the participating amino acid residues between the different aGPCR groups.

However, there is a high degree of similarity in the binding sites of groups E and L (suppl. Figs. S5, S6). Based on their close phylogenetic relationship it was previously suggested that these groups should be merged into one group [5]. Moreover, in contrast to the phylogenetic analyses of the entire CTF, the groups B and D cluster close together, when comparing their putative binding sites (suppl. Figs. S5, S6). This result may point towards an overlapping binding mode of *Stachel* core sequences of these aGPCR groups. The differences in the positioning of groups B and D in the phylogenetic trees (binding pockets versus complete CTFs) might be explained by low power of the sequence number and length used in the analyses or that the function of the CTF developed divergently between group B and D, but keeping the binding mode of the *Stachel* conserved. Future analyses involving additional CTF structures and orthologous sequences may shed more light on possible divergent evolution of intramolecular components in aGPCRs.

3.4. Exceptional structural properties of GPR97/ADGRG3 indicates certain flexibility in the 7TMD

The GPR97/ADGRG3 is the only determined active state CTF structure without the *Stachel* as a bound internal agonist (PDB ID's 7d76, 7d77 [50]), even though this receptor has a GPS motif and a typical *Stachel* core sequence with the highly conserved residues (Fig. 3c).

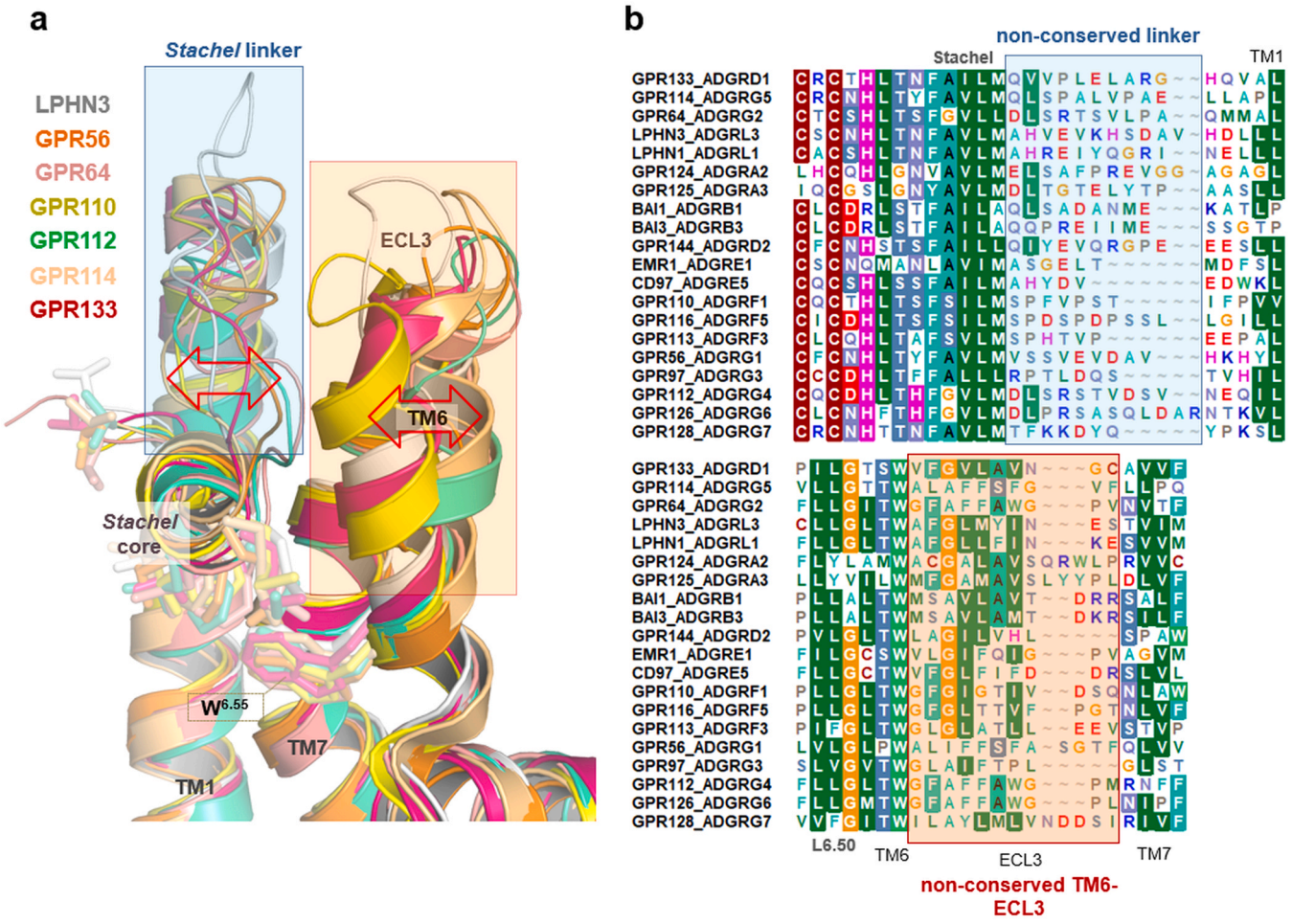

Fig. 4. Interactions between the non-conserved *Stachel* linker region and the 7TMD. (a) Superimposed aGPCR CTF structures with a specific focus on the *Stachel* linker and the 7TMD which, in contrast to the conserved *Stachel* core binding mode is rather non-conserved. (b) Alignment of human aGPCR sequences of the *Stachel* linker and the interacting TM6-ECL3 transition. The interaction analyses of the *Stachel* linker region with the TM6-ECL3 does not support conserved interactions (suppl. Tab. S3). Several other non-conserved receptor parts can be involved in *Stachel* contacts depending on the individual aGPCR, e.g., the GPR114/ADGRG5 with an exceptionally long ECL2 and contacts between the linker and this loop (PDB ID 7eq1, [43]).

Glucocorticoids were applied in the sample preparation for structure determination, likely displacing *Stachel* core binding close to the conserved activation trigger position $W^{6.55}$ (in almost 100% of all vertebrate aGPCR) and the highly conserved $G^{6.52}$ in TM6. A strong helix-kink-supported movement of TM6 during receptor activation is well-known as a crucial step in the activation mechanism of class A and class B GPCRs. This movement opens a crevice at the intracellular site mandatory for full binding of the G protein or arrestin [59,60]. TM6 of the GPR97/ADGRG3 structures has a unique feature compared to other CTF structures (Fig. 5a). Here, TM6 is not kinked, although a kink-supporting glycine in TM6 is also present (suppl. Figs. S1, S5). At position $G^{6.52}$ in TM6, directly adjacent to the conserved $W^{6.55}$, three amino acid residues of aGPCRs interact with each other by hydrogen bonds, as observed in class A GPCRs (called "PIF-motif" [61] or in variants a "MIF-motif"[62]). $N^{5.50}$, which is highly conserved among class B GPCRs, is the key amino acid residue mediating essential contacts between TM3-TM5 and TM6. This asparagine stabilizes the active state conformations via hydrogen bonds with positions in TM3 and TM6 (e.g., $W^{6.55}$ and $G^{6.52}$), but in GPR97/ADGRG3 a glycine is found instead at this corresponding TM5-position. In consequence, the TM6-kink stabilizing "triple-interaction" between TM3–5–6 is absent in GPR97/ADGRG3, which likely leads to unique structural properties (non-kinked TM6) and ligand binding capacities.

4. Intramolecular ligands - specific or common in GPCRs?

Intramolecular agonists for aGPCRs are generally accepted as structural components acting as signaling activators [20]. The question arises whether such structural and functional features are unique to this class or whether they are also present in other GPCRs?

Increasing data on the relevance of the N termini during activation of e.g. several class A GPCRs is already well known (reviews [39,63,64]). Agonistic intramolecular ligands have been demonstrated or hypothesized for e.g., protease-activated receptors (PARs) [65], the melanocortin receptor type 4 (MC4R) [66], and GPR83 [67]. Other GPCRs in which the N terminus has a regulatory function for signal transmission are the sphingosine-1-phosphate receptor 2 (S1P2R) [68], the yeast Ste2p [69] and the $\alpha 1_D$-adrenergic receptor (ADRA1D) [70]. During the activation process intramolecular agonists change their conformation and interactions to distinct receptor components, and subsequently the conformational equilibrium of the receptor into an active state.

For a few GPCRs, intramolecular antagonists or inverse agonists silencing the receptor in a resting state are reported. Here, an external stimulus (ligand binding) converts an intramolecular antagonist into an agonist or removes antagonistic self-restrictions (e.g., relaxin receptor LGR7 [71,72]). In glycoprotein hormone receptors (GPHRs) [40, 73–77], an intramolecular inverse agonist (constituted by various extracellular fragments) can be converted into an intramolecular agonist

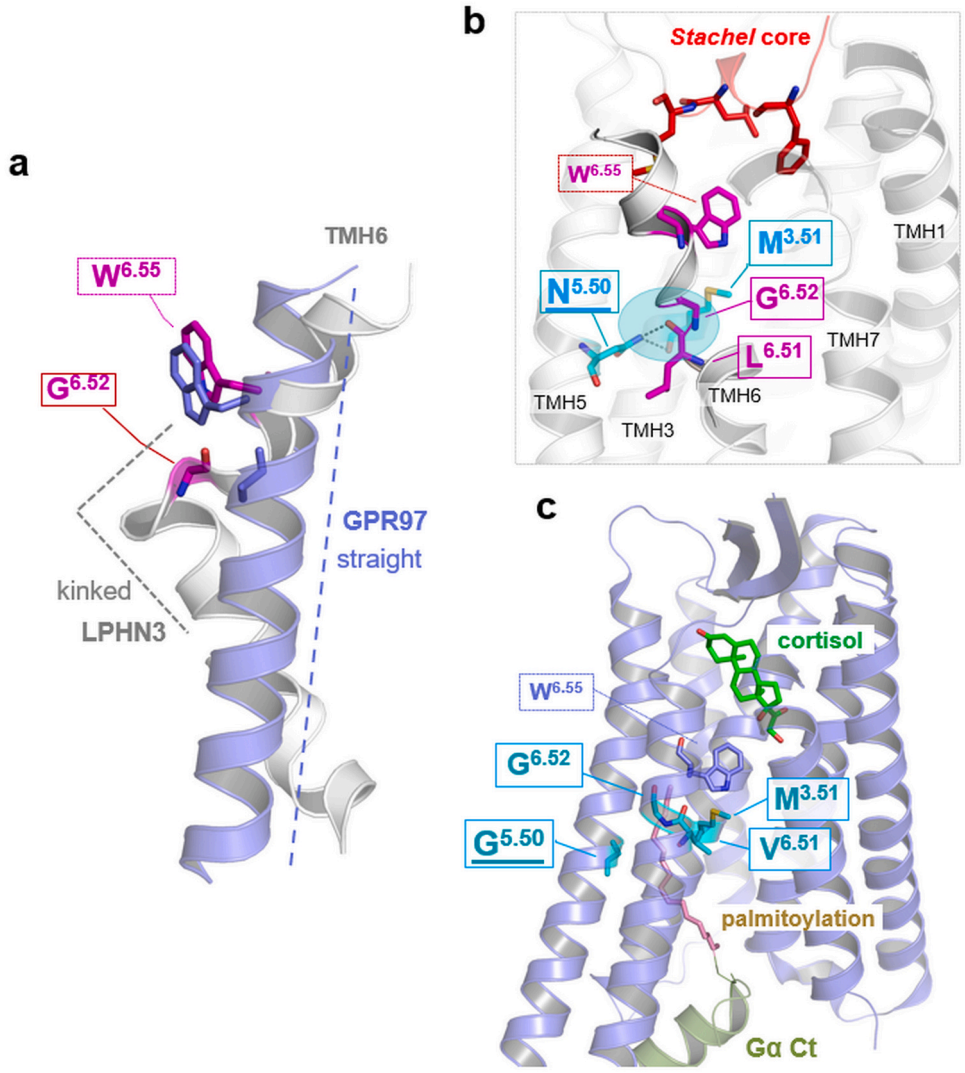

Fig. 5. Comparison between TM6 of LPNH3/ADGRL3 with the specific conformation of the non-prototypical TM6 in GPR97/ADGRG3. (a) The superimposed active state TM6 conformations of LPNH3/ADGRL3 and GPR97/ADGRG3 are different, being either kinked (LPNH3), as known from all other determined aGPCR CTF structures, or straight (GPR97). (b) In contrast to class A GPCRs with a proline-supported kink in TM6, aGPCRs have a glycine at their kinked helix (suppl. Fig. S1), which leads to a weakened regular helix by a missing side chain hydrogen bond interaction [94]. (c) The TM5 of GPR97 is characterized by a glycine at position 5.50, which cannot stabilize the arrangement between TM3, TM5 and TM6 in an active state structure by hydrogen bonds.

by mutations, antibody or glycoprotein hormone binding [73,75,78,79]. Of note, rhodopsin, a so-called "prototypical" class A GPCR, is fully functional only with the "tethered" (light-sensitive) small molecule ligand retinal covalently bound via a Schiff base [22], but this “tethered inverse agonism” is not comparable to intramolecular ligands that are part of the receptor itself.

In GPCRs with intramolecular ligands the definition of allosteric and orthosteric binding sites, or causal signaling triggers (primary, secondary) is more complex than for receptors without. If the intramolecular ligand has an effect on the respective receptor and is also involved in stimulation by external ligands, the intramolecular ligand should be considered as the primary ligand that occupies the orthosteric binding site. All other stimuli requiring the primary ligand should be seen as co-ligands or as positive or negative allosteric modulators (PAMs, NAMs).

Finally, intramolecular ligands enable specific functional features as a fast-signaling process, regulation of basal activity, or the convergent integration of chemical (e.g., soluble ligands) and physical (e.g., mechanical forces) signals. The fact that many GPCRs function via an intramolecular ligand highlights this mechanism as evolutionarily successful, and might be found to be of relevance also in so far orphan receptors, as it was shown previously for GPR61 with intramolecular agonistic activity (constitutive activity) of the ECL2 [80].

5. Concluding remarks and future perspectives

The new aGPCR structures determined in the past years provide deep molecular insights into different receptor parts, including complexes with external factors. One of the most exciting findings of recent years is the conserved binding mode and structural conformation of the *Stachel* core bound in the activated 7TMD, underscoring previous functional findings of an agonistic effect by *Stachel* peptides. The binding sites of the *Stachel* core sequences in each aGPCR group encrypt specificity, but some key interactions between the *Stachel* core and the 7TMD are conserved among all receptors, which are likely responsible for anchoring the α-helical *Stachel* core and stabilizing the 7TMD in an active state. However, no full-length aGPCR structure has yet been determined. This also explains why various questions about the structural basis of intramolecular signal transduction within aGPCRs are still open. Interrelated to this, the relevance and mechanism of extracellular cleavage for receptor activation has not been comprehensively

elucidated. In this context, the transition of the *Stachel* core from the observed in-GAIN domain- to the in-7TMD-bound state is still controversial and requires intensified structural investigation.

Acknowledgement/fundings

This work was supported by the Deutsche Forschungsgemeinschaft (DFG) (German Research Foundation) through SFB1423, Project-ID 421152132, subprojects A01 (to P.S.), A05 (to T.S., C.M.T. S. and P. S.), B05 (to I.L.), C04 (to T.S.); through Germany's Excellence Strategy – EXC 2008 – 390540038 – UniSysCat (Research Unit E) (to A.S, G.K and P.S.); by the Minerva Fellowship Programme (funded by the German Federal Ministry for Education and Research) (to A.H.A.), by the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 956314 [ALLODD] (to P.S.) and by the COST (European Cooperation in Science and Technology) Action CA18240 (Adher´nRise).

CRediT authorship contribution statement

Gunnar Kleinau: Conceptualization, Investigation, Visualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Amal Hassan Ali:** Visualization, Investigation, Writing – review & editing. **Franziska Wiechert:** Visualization, Formal analysis, Investigation, Writing – review & editing. **Michal Szczepek:** Investigation, Writing – review & editing. **Andrea Schmidt:** Investigation, Writing – review & editing. **Christian M.T. Spahn:** Investigation, Writing – review & editing. **Ines Liebscher:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Torsten Schöneberg:** Conceptualization, Visualization, Investigation, Supervision, Formal analysis Resources, Funding acquisition, Writing – original draft, Writing – review & editing, Project administration. **Patrick Scheerer:** Conceptualization, Visualization, Investigation, Supervision, Formal analysis, Resources, Funding acquisition, Writing – original draft, Writing – review & editing, Project administration.

Declaration of Competing Interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2023.106971.

References

[1] S.P.H. Alexander, A. Christopoulos, A.P. Davenport, E. Kelly, A. Mathie, J.A. Peters, E.L. Veale, J.F. Armstrong, E. Faccenda, S.D. Harding, A.J. Pawson, J.L. Sharman, C. Southan, J.A. Davies, C. Collaborators, The concise guide to pharmacology 2019/20: G protein-coupled receptors, Br. J. Pharmacol. 176 (Suppl 1) (2019) S21–S141.

[2] N. Scholz, T. Langenhan, T. Schoneberg, Revisiting the classification of adhesion GPCRs, Ann. N. Y. Acad. Sci. 1456 (1) (2019) 80–95.

[3] K.J. Nordstrom, M.C. Lagerstrom, L.M. Waller, R. Fredriksson, H.B. Schioth, The Secretin GPCRs descended from the family of Adhesion GPCRs, Mol. Biol. Evol. 26 (1) (2009) 71–84.

[4] M. Dohrmann, G. Worheide, Dating early animal evolution using phylogenomic data, Sci. Rep. 7 (1) (2017), 3599.

[5] A. Wittlake, S. Promel, T. Schoneberg, The evolutionary history of vertebrate adhesion GPCRs and its implication on their classification, Int J. Mol. Sci. 22 (21) (2021).

[6] A. Krishnan, R. Dnyansagar, M.S. Almen, M.J. Williams, R. Fredriksson, N. Manoj, H.B. Schioth, The GPCR repertoire in the demosponge Amphimedon queenslandica: insights into the GPCR system at the early divergence of animals, BMC Evol. Biol. 14 (2014), 270.

[7] J. Hamann, G. Aust, D. Arac, F.B. Engel, C. Formstone, R. Fredriksson, R.A. Hall, B. L. Harty, C. Kirchhoff, B. Knapp, A. Krishnan, I. Liebscher, H.H. Lin, D.C. Martinelli, K.R. Monk, M.C. Peeters, X. Piao, S. Promel, T. Schoneberg, T.W. Schwartz, K. Singer, M. Stacey, Y.A. Ushkaryov, M. Vallon, U. Wolfrum, M.W. Wright, L. Xu, T. Langenhan, H.B. Schioth, International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors, Pharmacol. Rev. 67 (2) (2015) 338–367.

[8] T.K. Bjarnadottir, R. Fredriksson, P.J. Hoglund, D.E. Gloriam, M.C. Lagerstrom, H. B. Schioth, The human and mouse repertoire of the adhesion family of G-protein-coupled receptors, Genomics 84 (1) (2004) 23–33.

[9] S.C. Petersen, R. Luo, I. Liebscher, S. Giera, S.J. Jeong, A. Mogha, M. Ghidinelli, M. L. Feltri, T. Schoneberg, X. Piao, K.R. Monk, The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211, Neuron 85 (4) (2015) 755–769.

[10] N. Scholz, C. Guan, M. Nieberler, A. Grotemeyer, I. Maiellaro, S. Gao, S. Beck, M. Pawlak, M. Sauer, E. Asan, S. Rothemund, J. Winkler, S. Promel, G. Nagel, T. Langenhan, R.J. Kittel, Mechano-dependent signaling by Latrophilin/CIRL quenches cAMP in proprioceptive neurons, Elife 6 (2017).

[11] H.A. Dunn, C. Orlandi, K.A. Martemyanov, Beyond the ligand: extracellular and transcellular G protein-coupled receptor complexes in physiology and pharmacology, Pharm. Rev. 71 (4) (2019) 503–519.

[12] H.H. Lin, K.F. Ng, T.C. Chen, W.Y. Tseng, Ligands and beyond: mechanosensitive adhesion GPCRs, Pharmaceuticals 15 (2) (2022).

[13] C. Wilde, J. Mitgau, T. Suchy, T. Schoneberg, I. Liebscher, Translating the force-mechano-sensing GPCRs, Am. J. Physiol. Cell Physiol. 322 (6) (2022) C1047–C1060.

[14] T. Fukuzawa, J. Ishida, A. Kato, T. Ichinose, D.M. Ariestanti, T. Takahashi, K. Ito, J. Abe, T. Suzuki, S. Wakana, A. Fukamizu, N. Nakamura, S. Hirose, Lung surfactant levels are regulated by Ig-Hepta/GPR116 by monitoring surfactant protein D, PLoS One 8 (7) (2013), e69451.

[15] T. Lala, R.A. Hall, Adhesion G protein-coupled receptors: structure, signaling, physiology, and pathophysiology, Physiol. Rev. 102 (4) (2022) 1587–1624.

[16] T. Schoneberg, I. Liebscher, Mutations in G protein-coupled receptors: mechanisms, pathophysiology and potential therapeutic approaches, Pharm. Rev. 73 (1) (2021) 89–119.

[17] D. Arac, A.A. Boucard, M.F. Bolliger, J. Nguyen, S.M. Soltis, T.C. Sudhof, A. T. Brunger, A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis, EMBO J. 31 (6) (2012) 1364–1378.

[18] H.H. Lin, G.W. Chang, J.Q. Davies, M. Stacey, J. Harris, S. Gordon, Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif, J. Biol. Chem. 279 (30) (2004) 31823–31832.

[19] G.W. Chang, M. Stacey, M.J. Kwakkenbos, J. Hamann, S. Gordon, H.H. Lin, Proteolytic cleavage of the EMR2 receptor requires both the extracellular stalk and the GPS motif, FEBS Lett. 547 (1–3) (2003) 145–150.

[20] I. Liebscher, J. Schon, S.C. Petersen, L. Fischer, N. Auerbach, L.M. Demberg, A. Mogha, M. Coster, K.U. Simon, S. Rothemund, K.R. Monk, T. Schoneberg, A tethered agonist within the ectodomain activates the adhesion G protein-coupled receptors GPR126 and GPR133, Cell Rep. 9 (6) (2014) 2018–2026.

[21] H.M. Stoveken, A.G. Hajduczok, L. Xu, G.G. Tall, Adhesion G protein-coupled receptors are activated by exposure of a cryptic tethered agonist, Proc. Natl. Acad. Sci. USA 112 (19) (2015) 6194–6199.

[22] K.P. Hofmann, P. Scheerer, P.W. Hildebrand, H.W. Choe, J.H. Park, M. Heck, O. P. Ernst, A G protein-coupled receptor at work: the rhodopsin model, Trends Biochem. Sci. 34 (11) (2009) 540–552.

[23] C. Gupta, T.F. Bernadyn, G.G. Tall, Structural clarity is brought to adhesion G protein-coupled receptor tethered agonism, Basic Clin. Pharmacol. Toxicol. (2022).

[24] I. Liebscher, T. Schoneberg, D. Thor, Stachel-mediated activation of adhesion G protein-coupled receptors: insights from cryo-EM studies, Signal Transduct. Target Ther. 7 (1) (2022), 227.

[25] J.M. Einspahr, D.G. Tilley, Pathophysiological impact of the adhesion G protein-coupled receptor family, Am. J. Physiol. Cell Physiol. 323 (2) (2022) C640–C647.

[26] A.B. Knierim, J. Rothe, M.V. Cakir, V. Lede, C. Wilde, I. Liebscher, D. Thor, T. Schoneberg, Genetic basis of functional variability in adhesion G protein-coupled receptors, Sci. Rep. 9 (1) (2019), 11036.

[27] C. Wilde, L. Fischer, V. Lede, J. Kirchberger, S. Rothemund, T. Schoneberg, I. Liebscher, The constitutive activity of the adhesion GPCR GPR114/ADGRG5 is mediated by its tethered agonist, FASEB J. 30 (2) (2016) 666–673.

[28] J.A. Ballesteros, H. Weinstein, Integrated methods for the construction of three-dimensional models and computational probing of structure-function relations in G protein-coupled receptors, Methods Neurosci. 25 (1995) 366–428.

[29] D. Wootten, J. Simms, L.J. Miller, A. Christopoulos, P.M. Sexton, Polar transmembrane interactions drive formation of ligand-specific and signal pathway-biased family B G protein-coupled receptor conformations, Proc. Natl. Acad. Sci. USA 110 (13) (2013) 5211–5216.

[30] C. de Graaf, N. Foata, O. Engkvist, D. Rognan, Molecular modeling of the second extracellular loop of G-protein coupled receptors and its implication on structure-based virtual screening, Proteins 71 (2) (2008) 599–620.

[31] S. Erdogmus, U. Storch, L. Danner, J. Becker, M. Winter, N. Ziegler, A. Wirth, S. Offermanns, C. Hoffmann, T. Gudermann, Y.S.M. Mederos, Helix 8 is the essential structural motif of mechanosensitive GPCRs, Nat. Commun. 10 (1) (2019), 5784.

[32] Y. Sun, D. Zhang, M.L. Ma, H. Lin, Y. Song, J. Wang, C. Ma, K. Yu, W. An, S. Guo, D. He, Z. Yang, P. Xiao, G. Hou, X. Yu, J.P. Sun, Optimization of a peptide ligand for the adhesion GPCR ADGRG2 provides a potent tool to explore receptor biology, J. Biol. Chem. 296 (2021), 100174.

[33] S. Promel, H. Waller-Evans, J. Dixon, D. Zahn, W.H. Colledge, J. Doran, M. B. Carlton, J. Grosse, T. Schoneberg, A.P. Russ, T. Langenhan, Characterization and functional study of a cluster of four highly conserved orphan adhesion-GPCR in mouse, Dev. Dyn. 241 (10) (2012) 1591–1602.

[34] D.E. Gloriam, S.M. Foord, F.E. Blaney, S.L. Garland, Definition of the G protein-coupled receptor transmembrane bundle binding pocket and calculation of receptor similarities for drug design, J. Med. Chem. 52 (14) (2009) 4429–4442.
[35] J.D. Wichard, A. Ter Laak, G. Krause, N. Heinrich, R. Kuhne, G. Kleinau, Chemogenomic analysis of G-protein coupled receptors and their ligands deciphers locks and keys governing diverse aspects of signalling, PLoS One 6 (2) (2011), e16811.
[36] R.P. Bywater, Location and nature of the residues important for ligand recognition in G-protein coupled receptors, J. Mol. Recognit. 18 (1) (2005) 60–72.
[37] L.E. Limbird, The receptor concept: a continuing evolution, Mol. Interv. 4 (6) (2004) 326–336.
[38] M.C. Lagerstrom, H.B. Schioth, Structural diversity of G protein-coupled receptors and significance for drug discovery, Nat. Rev. Drug Discov. 7 (4) (2008) 339–357.
[39] T. Schoneberg, G. Kleinau, A. Bruser, What are they waiting for?-Tethered agonism in G protein-coupled receptors. Pharmacol. Res. 108 (2016) 9–15.
[40] A. Bruser, A. Schulz, S. Rothemund, A. Ricken, D. Calebiro, G. Kleinau, T. Schoneberg, The activation mechanism of glycoprotein hormone receptors with implications in the cause and therapy of endocrine diseases, J. Biol. Chem. 291 (2) (2016) 508–520.
[41] F. Pohl, F. Seufert, Y.K. Chung, D. Volke, R. Hoffmann, T. Schöneberg, T. Langenhan, P.W. Hildebrand, N. Sträter, Structural basis of GAIN domain autoproteolysis and cleavage-resistance in the adhesion G-protein coupled receptors, 2023.
[42] T.F. Bernadyn, A. Vizurraga, R. Adhikari, F. Kwarcinski, G.G. Tall, GPR114/ADGRG5 is activated by its tethered-peptide-agonist because it is a cleaved adhesion GPCR, J. Biol. Chem. (2023), 105223.
[43] Y.Q. Ping, P. Xiao, F. Yang, R.J. Zhao, S.C. Guo, X. Yan, X. Wu, C. Zhang, Y. Lu, F. Zhao, F. Zhou, Y.T. Xi, W. Yin, F.Z. Liu, D.F. He, D.L. Zhang, Z.L. Zhu, Y. Jiang, L. Du, S.Q. Feng, T. Schoneberg, I. Liebscher, Xu HE, J.P. Sun, Structural basis for the tethered peptide activation of adhesion GPCRs, Nature 604 (7907) (2022) 763–770.
[44] G. Stephan, J.D. Frenster, I. Liebscher, D.G. Placantonakis, Activation of the adhesion G protein-coupled receptor GPR133 by antibodies targeting its N-terminus, J. Biol. Chem. 298 (6) (2022), 101949.
[45] L.M. Demberg, J. Winkler, C. Wilde, K.U. Simon, J. Schon, S. Rothemund, T. Schoneberg, S. Promel, I. Liebscher, Activation of Adhesion G Protein-coupled Receptors: agonist specificity of stachel sequence-derived peptides, J. Biol. Chem. 292 (11) (2017) 4383–4394.
[46] D.L.H. Bui, A. Roach, J. Li, S.J. Bandekar, E. Orput, R. Raghavan, D. Arac, R. C. Sando, The adhesion GPCRs CELSR1-3 and LPHN3 engage G proteins via distinct activation mechanisms, Cell Rep. 42 (6) (2023), 112552.
[47] X. Barros-Alvarez, R.M. Nwokonko, A. Vizurraga, D. Matzov, F. He, M. M. Papasergi-Scott, M.J. Robertson, O. Panova, E.H. Yardeni, A.B. Seven, F. E. Kwarcinski, H. Su, M.C. Peroto, J.G. Meyerowitz, M. Shalev-Benami, G.G. Tall, G. Skiniotis, The tethered peptide activation mechanism of adhesion GPCRs, Nature 604 (7907) (2022) 757–762.
[48] X. Qu, N. Qiu, M. Wang, B. Zhang, J. Du, Z. Zhong, W. Xu, X. Chu, L. Ma, C. Yi, S. Han, W. Shui, Q. Zhao, B. Wu, Structural basis of tethered agonism of the adhesion GPCRs ADGRD1 and ADGRF1, Nature 604 (7907) (2022) 779–785.
[49] P. Xiao, S. Guo, X. Wen, Q.T. He, H. Lin, S.M. Huang, L. Gou, C. Zhang, Z. Yang, Y. N. Zhong, C.C. Yang, Y. Li, Z. Gong, X.N. Tao, Z.S. Yang, Y. Lu, S.L. Li, J.Y. He, C. Wang, L. Zhang, L. Kong, J.P. Sun, X. Yu, Tethered peptide activation mechanism of the adhesion GPCRs ADGRG2 and ADGRG4, Nature 604 (7907) (2022) 771–778.
[50] Y.Q. Ping, C. Mao, P. Xiao, R.J. Zhao, Y. Jiang, Z. Yang, W.T. An, D.D. Shen, F. Yang, H. Zhang, C. Qu, Q. Shen, C. Tian, Z.J. Li, S. Li, G.Y. Wang, X. Tao, X. Wen, Y.N. Zhong, J. Yang, F. Yi, X. Yu, H.E. Xu, Y. Zhang, J.P. Sun, Structures of the glucocorticoid-bound adhesion receptor GPR97-Go complex, Nature 589 (7843) (2021) 620–626.
[51] J.P. Bridges, C. Safina, B. Pirard, K. Brown, A. Filuta, R. Panchanathan, R. Bouhelal, N. Reymann, S. Patel, K. Seuwen, W.E. Miller, M.G. Ludwig, Regulation of pulmonary surfactant by the adhesion GPCR GPR116/ADGRF5 requires a tethered agonist-mediated activation mechanism, Elife 11 (2022).
[52] K.L. Carleton, T.C. Spady, R.H. Cote, Rod and cone opsin families differ in spectral tuning domains but not signal transducing domains as judged by saturated evolutionary trace analysis, J. Mol. Evol. 61 (1) (2005) 75–89.
[53] S. Yokoyama, Evolution of dim-light and color vision pigments, Annu. Rev. Genom. Hum. Genet. 9 (2008) 259–282.
[54] H.W. Choe, Y.J. Kim, J.H. Park, T. Morizumi, E.F. Pai, N. Krauss, K.P. Hofmann, P. Scheerer, O.P. Ernst, Crystal structure of metarhodopsin II, Nature 471 (7340) (2011) 651–655.
[55] K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto, M. Miyano, Crystal structure of rhodopsin: a G protein-coupled receptor, Science 289 (5480) (2000) 739–745.
[56] J.F.D. Hagen, N.S. Roberts, R.J., Jr Johnston, The evolutionary history and spectral tuning of vertebrate visual opsins, Dev. Biol. 493 (2023) 40–66.
[57] L.M. Demberg, S. Rothemund, T. Schoneberg, I. Liebscher, Identification of the tethered peptide agonist of the adhesion G protein-coupled receptor GPR64/ADGRG2, Biochem. Biophys. Res. Commun. 464 (3) (2015) 743–747.
[58] I. Liebscher, T. Schoneberg, Tethered agonism: a common activation mechanism of adhesion GPCRs, Handb. Exp. Pharmacol. 234 (2016) 111–125.
[59] P. Scheerer, J.H. Park, P.W. Hildebrand, Y.J. Kim, N. Krauss, H.W. Choe, K. P. Hofmann, O.P. Ernst, Crystal structure of opsin in its G-protein-interacting conformation, Nature 455 (7212) (2008) 497–502.
[60] M. Szczepek, F. Beyriere, K.P. Hofmann, M. Elgeti, R. Kazmin, A. Rose, F.J. Bartl, D. von Stetten, M. Heck, M.E. Sommer, P.W. Hildebrand, P. Scheerer, Crystal structure of a common GPCR-binding interface for G protein and arrestin, Nat. Commun. 5 (2014), 4801.
[61] B.K. Kobilka, Structural insights into adrenergic receptor function and pharmacology, Trends Pharmacol. Sci. 32 (4) (2011) 213–218.
[62] N.A. Heyder, G. Kleinau, D. Speck, A. Schmidt, S. Paisdzior, M. Szczepek, B. Bauer, A. Koch, M. Gallandi, D. Kwiatkowski, J. Burger, T. Mielke, A.G. Beck-Sickinger, P. W. Hildebrand, C.M.T. Spahn, D. Hilger, M. Schacherl, H. Biebermann, T. Hilal, P. Kuhnen, B.K. Kobilka, P. Scheerer, Structures of active melanocortin-4 receptor-Gs-protein complexes with NDP-alpha-MSH and setmelanotide, Cell Res. 31 (11) (2021) 1176–1189.
[63] J.L. Coleman, T. Ngo, N.J. Smith, The G protein-coupled receptor N-terminus and receptor signalling: N-tering a new era, Cell Signal 33 (2017) 1–9.
[64] L.H. Zhao, Y. Yin, D. Yang, B. Liu, L. Hou, X. Wang, K. Pal, Y. Jiang, Y. Feng, X. Cai, A. Dai, M. Liu, M.W. Wang, K. Melcher, H.E. Xu, Differential requirement of the extracellular domain in activation of class B G protein-coupled receptors, J. Biol. Chem. 291 (29) (2016) 15119–15130.
[65] S.R. Coughlin, Protease-activated receptors in hemostasis, thrombosis and vascular biology, J. Thromb. Haemost. 3 (8) (2005) 1800–1814.
[66] S. Srinivasan, C. Lubrano-Berthelier, C. Govaerts, F. Picard, P. Santiago, B. R. Conklin, C. Vaisse, Constitutive activity of the melanocortin-4 receptor is maintained by its N-terminal domain and plays a role in energy homeostasis in humans, J. Clin. Invest. 114 (8) (2004) 1158–1164.
[67] A. Muller, J.C. Berkmann, P. Scheerer, H. Biebermann, G. Kleinau, Insights into basal signaling regulation, oligomerization, and structural organization of the human G-protein coupled receptor 83, PLoS One 11 (12) (2016), e0168260.
[68] A. El Buri, D.R. Adams, D. Smith, R.J. Tate, M. Mullin, S. Pyne, N.J. Pyne, The sphingosine 1-phosphate receptor 2 is shed in exosomes from breast cancer cells and is N-terminally processed to a short constitutively active form that promotes extracellular signal regulated kinase activation and DNA synthesis in fibroblasts, Oncotarget 9 (50) (2018) 29453–29467.
[69] M.S. Uddin, F. Naider, J.M. Becker, Dynamic roles for the N-terminus of the yeast G protein-coupled receptor Ste2p, Biochim. Biophys. Acta Biomembr. 1859 (10) (2017) 2058–2067.
[70] T.S. Kountz, K.S. Lee, S. Aggarwal-Howarth, E. Curran, J.M. Park, D.A. Harris, A. Stewart, J. Hendrickson, N.D. Camp, A. Wolf-Yadlin, E.H. Wang, J.D. Scott, C. Hague, Endogenous N-terminal domain cleavage modulates alpha1D-adrenergic receptor pharmacodynamics, J. Biol. Chem. 291 (35) (2016) 18210–18221.
[71] A. Sethi, S. Bruell, N. Patil, M.A. Hossain, D.J. Scott, E.J. Petrie, R.A. Bathgate, P. R. Gooley, The complex binding mode of the peptide hormone H2 relaxin to its receptor RXFP1, Nat. Commun. 7 (2016), 11344.
[72] S.C. Erlandson, S. Rawson, J. Osei-Owusu, K.P. Brock, X. Liu, J.A. Paulo, J. Mintseris, S.P. Gygi, D.S. Marks, X. Cong, A.C. Kruse, The relaxin receptor RXFP1 signals through a mechanism of autoinhibition, Nat. Chem. Biol. 19 (8) (2023) 1013–1021.
[73] V. Vlaeminck-Guillem, S.C. Ho, P. Rodien, G. Vassart, S. Costagliola, Activation of the cAMP pathway by the TSH receptor involves switching of the ectodomain from a tethered inverse agonist to an agonist, Mol. Endocrinol. 16 (4) (2002) 736–746.
[74] J. Duan, P. Xu, H. Zhang, X. Luan, J. Yang, X. He, C. Mao, D.D. Shen, Y. Ji, X. Cheng, H. Jiang, Y. Jiang, S. Zhang, Y. Zhang, H.E. Xu, Mechanism of hormone and allosteric agonist mediated activation of follicle stimulating hormone receptor, Nat. Commun. 14 (1) (2023), 519.
[75] J. Duan, P. Xu, X. Luan, Y. Ji, X. He, N. Song, Q. Yuan, Y. Jin, X. Cheng, H. Jiang, J. Zheng, S. Zhang, Y. Jiang, H.E. Xu, Hormone- and antibody-mediated activation of the thyrotropin receptor, Nature 609 (7928) (2022) 854–859.
[76] G. Kleinau, G. Krause, Thyrotropin and homologous glycoprotein hormone receptors: structural and functional aspects of extracellular signaling mechanisms, Endocr. Rev. 30 (2) (2009) 133–151.
[77] A. Schulze, G. Kleinau, S. Neumann, P. Scheerer, T. Schoneberg, A. Bruser, The intramolecular agonist is obligate for activation of glycoprotein hormone receptors, FASEB J. 34 (8) (2020) 11243–11256.
[78] B. Faust, C.B. Billesbolle, C.M. Suomivuori, I. Singh, K. Zhang, N. Hoppe, A.F. M. Pinto, J.K. Diedrich, Y. Muftuoglu, M.W. Szkudlinski, A. Saghatelian, R.O. Dror, Y. Cheng, A. Manglik, Autoantibody mimicry of hormone action at the thyrotropin receptor, Nature 609 (7928) (2022) 846–853.
[79] G. Kleinau, C.L. Worth, A. Kreuchwig, H. Biebermann, P. Marcinkowski, P. Scheerer, G. Krause, Structural-functional features of the thyrotropin receptor: a class A G-protein-coupled receptor at work, Front Endocrinol. 8 (2017), 86.
[80] Y. Nie, Z. Qiu, S. Chen, Z. Chen, X. Song, Y. Ma, N. Huang, J.G. Cyster, S. Zheng, Specific binding of GPR174 by endogenous lysophosphatidylserine leads to high constitutive G(s) signaling, Nat. Commun. 14 (1) (2023), 5901.
[81] Y. Qian, Z. Ma, C. Liu, X. Li, X. Zhu, N. Wang, Z. Xu, R. Xia, J. Liang, Y. Duan, H. Yin, Y. Xiong, A. Zhang, C. Guo, Z. Chen, Z. Huang, Y. He, Structural insights into adhesion GPCR ADGRL3 activation and G(q), G(s), G(i), and G(12) coupling. Mol. Cell 82 (22) (2022) 4340–4352, e4346.
[82] X. Zhu, Y. Qian, X. Li, Z. Xu, R. Xia, N. Wang, J. Liang, H. Yin, A. Zhang, C. Guo, G. Wang, Y. He, Structural basis of adhesion GPCR GPR110 activation by stalk peptide and G-proteins coupling, Nat. Commun. 13 (1) (2022), 5513.
[83] D.T.D. Jones, A.N. Dates, S.D. Rawson, M.M. Burruss, C.H. Lipper, S.C. Blacklow, Tethered agonist activated ADGRF1 structure and signalling analysis reveal basis for G protein coupling, Nat. Commun. 14 (1) (2023), 2490.
[84] H. Lin, P. Xiao, R.Q. Bu, S. Guo, Z. Yang, D. Yuan, Z.L. Zhu, C.X. Zhang, Q.T. He, C. Zhang, Y.Q. Ping, R.J. Zhao, C.S. Ma, C.H. Liu, X.N. Zhang, D. Jiang, S. Huang, Y.T. Xi, D.L. Zhang, C.Y. Xue, B.S. Yang, J.Y. Li, H.C. Lin, X.H. Zeng, H. Zhao, W.

M. Xu, F. Yi, Z. Liu, J.P. Sun, X. Yu, Structures of the ADGRG2-G(s) complex in apo and ligand-bound forms. Nat. Chem. Biol. 18 (11) (2022) 1196–1203.
[85] J. Li, Y. Xie, S. Cornelius, X. Jiang, R. Sando, S.P. Kordon, M. Pan, K. Leon, T. C. Sudhof, M. Zhao, D. Arac, Alternative splicing controls teneurin-latrophilin interaction and synapse specificity by a shape-shifting mechanism, Nat. Commun. 11 (1) (2020), 2140.
[86] Y.C. Lu, O.V. Nazarko, R., 3rd Sando, G.S. Salzman, N.S. Li, T.C. Sudhof, D. Arac, Structural basis of latrophilin-FLRT-UNC5 interaction in cell adhesion, Structure 23 (9) (2015) 1678–1691.
[87] F.M. Ranaivoson, Q. Liu, F. Martini, F. Bergami, S. von Daake, S. Li, D. Lee, B. Demeler, W.A. Hendrickson, D. Comoletti, Structural and mechanistic insights into the latrophilin3-FLRT3 complex that mediates glutamatergic synapse development, Structure 23 (9) (2015) 1665–1677.
[88] M. Niu, S. Xu, J. Yang, D. Yao, N. Li, J. Yan, G. Zhong, G. Song, Structural basis for CD97 recognition of the decay-accelerating factor CD55 suggests mechanosensitive activation of adhesion GPCRs, J. Biol. Chem. 296 (2021), 100776.
[89] K. Leon, R.L. Cunningham, J.A. Riback, E. Feldman, J. Li, T.R. Sosnick, M. Zhao, K. R. Monk, D. Arac, Structural basis for adhesion G protein-coupled receptor Gpr126 function, Nat. Commun. 11 (1) (2020), 194.
[90] G.S. Salzman, S.D. Ackerman, C. Ding, A. Koide, K. Leon, R. Luo, H.M. Stoveken, C. G. Fernandez, G.G. Tall, X. Piao, K.R. Monk, S. Koide, D. Arac, Structural basis for regulation of GPR56/ADGRG1 by its alternatively spliced extracellular domains, Neuron 91 (6) (2016) 1292–1304.
[91] T.Y. Chu, C. Zheng-Gerard, K.Y. Huang, Y.C. Chang, Y.W. Chen, I. KY, Y.L. Lo, N. Y. Chiang, H.Y. Chen, M. Stacey, S. Gordon, W.Y. Tseng, C.Y. Sun, Y.M. Wu, Y. S. Pan, C.H. Huang, C.Y. Lin, T.C. Chen, K. El Omari, M. Antonelou, S. R. Henderson, A. Salama, E. Seiradake, H.H. Lin, GPR97 triggers inflammatory processes in human neutrophils via a macromolecular complex upstream of PAR2 activation, Nat. Commun. 13 (1) (2022), 6385.
[92] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I. N. Shindyalov, P.E. Bourne, The protein data bank, Nucleic Acids Res. 28 (1) (2000) 235–242.
[93] A. Drozdetskiy, C. Cole, J. Procter, G.J. Barton, JPred4: a protein secondary structure prediction server, Nucleic Acids Res. 43 (W1) (2015) W389–W394.
[94] S. Yohannan, S. Faham, D. Yang, J.P. Whitelegge, J.U. Bowie, The evolution of transmembrane helix kinks and the structural diversity of G protein-coupled receptors, Proc. Natl. Acad. Sci. USA 101 (4) (2004) 959–963.