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Extramembranous Regions in G Protein-Coupled Receptors: Cinderella in Receptor Biology?

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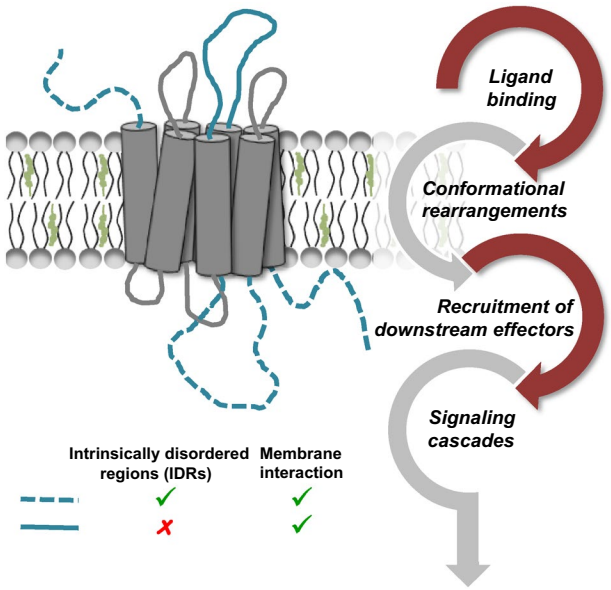
Abstract

G protein-coupled receptors (GPCRs) are the largest class of membrane proteins involved in signal transduction and are characterized by seven transmembrane domain architecture interconnected by extra- and intracellular loops. These loops, along with the N- and C-terminal domains, constitute the extramembranous regions in GPCRs. These regions, accounting for ~40% or more amino acid residues across different GPCR classes, are distinct from the conserved transmembrane domains in terms of nonconservation of sequence, diversity in length, and conformational heterogeneity. Due to technical challenges in exploring the molecular basis underlying the relation between structure, dynamics, and function in these regions, their contribution to GPCR organization and signaling remain underappreciated. Despite existing literature on the involvement of GPCR loops in numerous aspects of GPCR biology, the functional relevance of GPCR loops in the context of their inherent conformational heterogeneity and probable membrane interaction are not well understood. This review focuses on highlighting these aspects of GPCR extramembranous regions in the overall context of GPCR organization, dynamics, and biology. We envision that a judicious combination of insights obtained from structured transmembrane domains and disordered extramembranous regions in GPCRs would be crucial in arriving at a comprehensive understanding of GPCR structure, function, and dynamics, thereby leading to efficient drug discovery.

Graphical Abstract



Keywords GPCR · GPCR extramembranous regions · Conformational heterogeneity · Intrinsically disordered regions in GPCRs · Membrane interaction of GPCR loops

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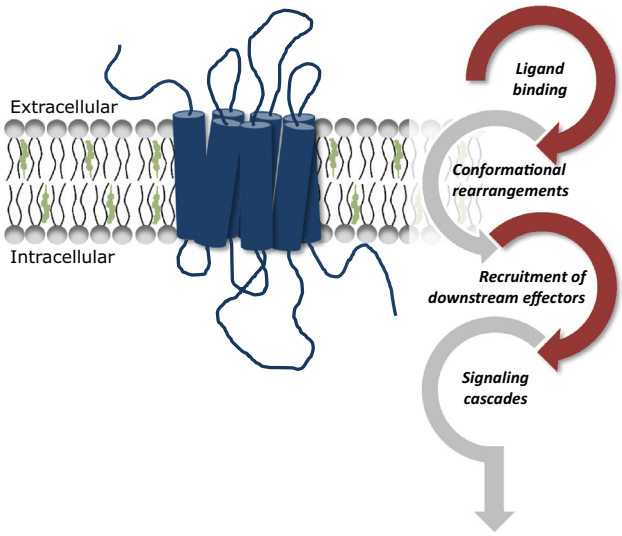

Fig. 1 A schematic representation of the molecular architecture of G protein-coupled receptors (GPCRs). The GPCR superfamily is the largest and most diverse group of proteins in mammals involved in signal transduction. Their basic architecture consists of seven transmembrane helices (shown as blue cylinders) covalently linked by extra- and intracellular loops (shown as blue lines). Phospholipids are depicted with gray headgroups and black acyl chains, and cholesterol, the predominant functionally relevant sterol in eukaryotes, is shown in green. GPCRs act as cellular nanotransducers that detect information, undergo conformational rearrangements, and trigger appropriate responses in the form of various signaling cascades depending on the effector molecules recruited in the cellular interior (these steps are shown by interlocking curly arrows). GPCRs have emerged as major drug targets due to the wide range of physiological responses mediated by these membrane receptors. These functionally diverse roles assumed by GPCRs are believed to partly originate from the diversity encoded in the GPCR extramembranous regions. These regions, characterized by a high degree of variability in sequence and length, serve as functional checkpoints with receptor-specific fingerprints. See text for more details

Molecular Architecture and Membrane Interaction of G Protein-Coupled Receptors

G protein-coupled receptors (GPCRs) are cellular nanomachines involved in signal transduction from the extracellular milieu to the cellular interior and constitute the largest class of integral membrane proteins in mammals (Pierce et al. 2002; Rosenbaum et al. 2009; Chattopadhyay 2014; Weis and Kobilka 2018). The GPCR superfamily consists of more than 800 members encoded by ~5% of the human genome (Zhang et al. 2006). GPCRs are characterized by a canonical seven transmembrane domain architecture, with extra- and intracellular loops acting as covalent interhelical linkers (Fig. 1). These receptors detect information (encoded by ligands such as neurotransmitters, hormones, peptides, odorants, and even photons) at the cell surface and undergo conformational rearrangements that trigger appropriate biochemical responses in the cellular interior. In mechanistic terms, GPCRs are allosteric proteins since ligand binding at the extracellular face (termed as the orthosteric site) triggers recruitment of downstream effectors (such as G-proteins) at the intracellular face, due to the presence of 'molecular switches' (conserved structural motifs) that induce concerted structural rearrangements in the transmembrane region (Filipek 2019). Information about GPCR activation pathways can be mapped to sequence and structural features that are characteristic of transmembrane domains (Weis and Kobilka 2018). As an immediate consequence of the range of physiological responses (such as neurotransmission, cellular growth and differentiation, and immune response) mediated by them, GPCRs have emerged as major drug targets across all clinical areas (Jacobson 2015; Hauser et al. 2017; Chan et al. 2019; Insel et al. 2019).

GPCRs are intimately associated with their immediate membrane microenvironment due to their multitransmembrane domain architecture. There is extensive literature (encompassing structural, biochemical, biophysical, and computational approaches) on the role of membrane lipids in GPCR biology. In particular, membrane cholesterol has been shown to be a crucial modulator of GPCR organization, dynamics, oligomerization, and function (Pucadyil and Chattopadhyay 2006; Paila and Chattopadhyay 2010; Oates and Watts 2011; Jafurulla and Chattopadhyay 2013; Chattopadhyay 2014; Sengupta and Chattopadhyay 2015; Gimpl 2016; Sengupta et al. 2018). The mechanism underlying such modulation could be via specific interactions of membrane cholesterol with GPCRs, or cholesterol-induced changes in global bilayer properties, or a combination of both (recently reviewed in Jafurulla et al. 2019). In addition, the paradigm of GPCR–lipid interaction has been enriched with emerging evidence on the influence of anionic phospholipids (Kimura et al. 2012; Dawaliby et al. 2016; Strohman et al. 2019) and sphingolipids (Jafurulla and Chattopadhyay 2015) in regulating GPCR structure and function. This has been complemented by reports on the modulation of membrane organization and function of GPCRs by global bilayer properties such as membrane viscosity (Pal et al. 2016), elasticity (Prasad et al. 2009), curvature (Brown 2012), and thickness (Alves et al. 2005; Rao et al. 2017).

Extramembranous Regions of GPCRs: Not Just *Extras*

Quantitative analyses of integral membrane protein sequence and structure indicate that more than 60% of amino acid residues in α-helical membrane proteins lie outside the transmembrane region (Ulmschneider and Sansom 2001). Conventionally, these loop regions have been considered to be spacers covalently linking helical domains in membrane

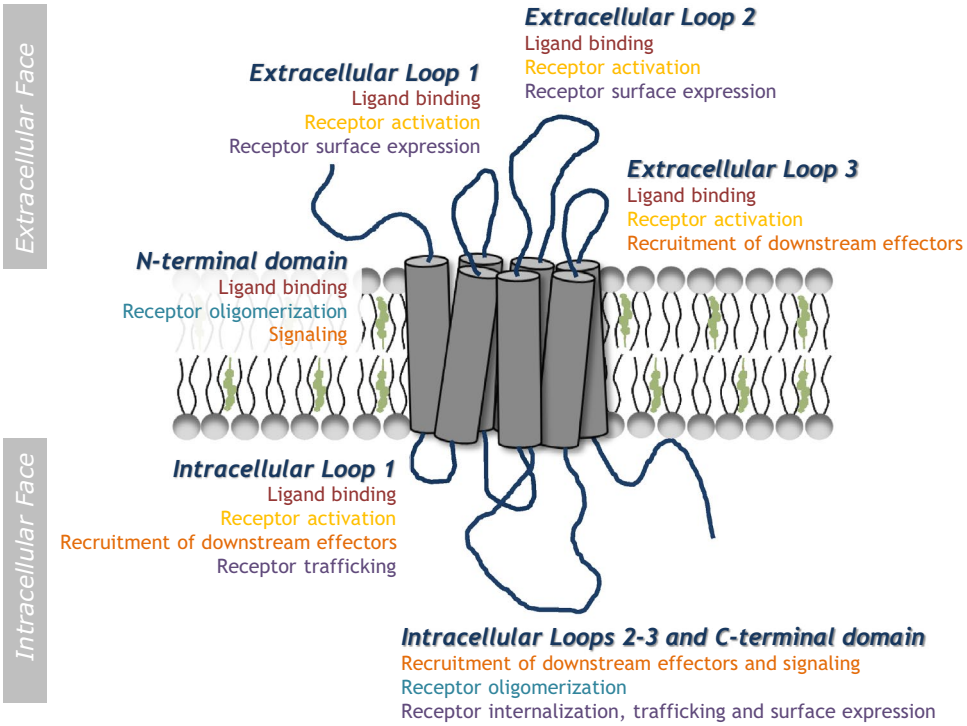

Fig. 2 Role of extramembranous regions in GPCR biology. GPCR extramembranous regions, which typically have ~40% amino acids in class A GPCRs, consist of the N-terminal domain; three extracellular loops: ECL1, ECL2, and ECL3; three intracellular loops: ICL1, ICL2, and ICL3; and the C-terminal domain. In sharp contrast to the seven transmembrane domain GPCR scaffold, these regions are characterized by substantial sequence diversity and length variability across receptor types and even subtypes. This translates to differential ligand binding at the extracellular face and stringently controlled recruitment of specific downstream effectors at the cytoplasmic face, thereby affecting almost every aspect of GPCR biology. The functionally diverse roles assumed by GPCRs in the context of cellular physiology originate, at least partially, from the diversity encoded in these extramembranous regions. Drug discovery approaches using subtle yet distinct receptor-specific differences in sequence or conformation of these regions remain largely unexplored and are envisioned to yield therapeutic interventions with minimal receptor crosstalk and side effects. See text and Table 1 for more details

proteins. However, the large fraction of amino acids in membrane protein loops raises the possibility of involvement of these regions in membrane protein structure and function. From a structural perspective, loops are known to influence the tertiary structure and stability of membrane proteins by constraining the distance between transmembrane helices (Tastan et al. 2009). In addition, the distribution of hydrophobic residues in loops (which is similar to soluble proteins) could be envisioned to result in a compact secondary structure due to preferential shielding of the hydrophobic residues from the polar aqueous microenvironment. These secondary structural elements, along with distance constraints originating from the length of loop regions, trigger and aid in the assembly of transmembrane helices into a defined supramolecular structure with functional consequences (Tastan et al. 2009).

GPCR extramembranous regions consist of three extracellular loops (ECL1-3), three intracellular loops (ICL1-3), and N- and C-termini (Fig. 2). The extramembranous regions of GPCRs are believed to lock the transmembrane domains in their basal state in the absence of ligands (Kobilka and Deupi 2007). These regions constitute ~40% of amino acid residues in class A GPCRs and more than 70% residues in class B and class C GPCRs (Venkatakrishnan et al. 2014). In sharp contrast to the presence of multiple highly conserved residues in transmembrane helical domains of GPCRs, the largely disordered loop regions are characterized by an immense diversity within and across GPCR classes (Karnik et al. 2003), both in terms of sequence and length. The N-terminus, C-terminus, and ICL3 exhibit the largest variability in length, while ECL1, ECL3, ICL1, and ICL2 display the highest conservation in length among GPCRs (Karnik et al. 2003; Unal and Karnik 2012). In addition, crystallographic analyses point to a greater diversity in sequence and secondary structure in the GPCR extracellular face and upper half of the transmembrane domains, relative to the lower half and the intracellular (cytoplasmic) loops (Katritch et al. 2012). Interestingly, the extramembranous regions characterized by highest length variability correspond to a pattern of

intrinsically disordered regions (IDRs) unique to GPCRs (Jaakola et al. 2005; Venkatakrishnan et al. 2014). The sequence diversity and length variability in GPCR loops allow differential ligand binding at the extracellular face and stringent recruitment of specific downstream effectors at the cytoplasmic face of these receptors. These unique features of GPCR loops affect almost every aspect of GPCR biology (see Fig. 2, Table 1). The functionally diverse roles assumed by GPCRs in the context of cellular physiology could therefore originate from the diversity encoded in the GPCR extramembranous regions. This is further highlighted by the fact that ~40% of point mutations that lead to altered GPCR function can be mapped to extramembranous regions (Karnik et al. 2003).

GPCR Extracellular Loops (ECLs)

The extracellular face of GPCRs consists of the N-terminal domain and the three loops ECL1-3. These loops collectively play an important role in the recognition of diverse ligands (Peeters et al. 2011b; Wheatley et al. 2012), due to the formation of receptor-specific compact structures held together by electrostatic salt bridges, hydrophobic contacts, and hydrogen bonds. The presence of a conserved disulfide bond between ECL2 and TM3, along with other interloop disulfide bonds specific to certain receptor subtypes, is known to impose conformational constraints on the receptor (Wheatley et al. 2012), leading to stable receptor conformations (Katritch et al. 2012). In fact, strategic insertion of cysteine residues resulting in the formation of additional disulfide bond(s) is a popular approach employed to generate thermostable receptor mutants amenable to crystallographic studies (Popov et al. 2018). The nature of forces governing the assembly of extracellular domains influences the packing geometry of GPCR transmembrane helices, which in turn may craft the ligand binding pocket (Karnik et al. 2003; Wheatley et al. 2012).

Apart from the recognition of diverse ligands by the extracellular face, ECLs could act as a 'gatekeeper' by tuning ligand accessibility to binding pockets due to the presence of multiple charged residues at conserved positions (Hawtin et al. 2006) and participate in activation and allosteric modulation of receptors (Peeters et al. 2011b; Unal and Karnik 2012; Wheatley et al. 2012). In addition, ECLs, especially the N-terminal domain, have been implicated in homo- (Romano et al. 1996) and hetero-oligomerization (Schwarz et al. 2000) of certain GPCRs, which may translate to differential signaling. Even though specific ECLs have been implicated in different aspects of GPCR biology (see Table 1, Fig. 2 for a representative list of the functional role assumed by ECLs in different receptors), the importance of ECLs stems from the strength of interaction between these loops and the factors governing such interactions (Peeters et al. 2011b; Wheatley et al. 2012).

Studies on chemokine CXC receptors (CXCRs), which belong to the peptide-binding GPCR family and are associated with diverse immune and inflammatory responses (Hughes and Nibbs 2018), have provided fundamental insights into the importance of N-terminal domains in GPCR biology. The N-terminal domain in these GPCRs has been reported to be an important structural determinant for ligand binding, receptor internalization, and signaling (Rajagopalan and Rajarathnam 2004, 2006; Prado et al. 2007). Since the CXCR N-terminal domain is implicated in differential binding to ligands of different classes (Rajagopalan and Rajarathnam 2004) or different oligomeric states (Ravindran et al. 2009), diversities in CXCR-mediated inflammatory and noninflammatory responses are believed to predominantly originate from the sequence, structure, and dynamics of the N-terminal domain in particular, and the receptor extracellular face in general (Kleist et al. 2016). This has led to therapeutic interventions that target CXCR N-terminal domains and associated interactions for a multitude of pathophysiological conditions ranging from pulmonary and autoimmune disorders to type 1 diabetes and cancer (Szpakowska et al. 2012).

Taken together, the extracellular face of GPCRs can be conceptualized as a 'funnel' that distills divergent receptor–ligand interactions into a unifying series of transmembrane conformational changes, which in turn, trigger appropriate cellular signaling cascades (Venkatakrishnan et al. 2016). The importance of ECLs in GPCR biology is highlighted by the fact that numerous diseases such as retinitis pigmentosa, nephrogenic diabetes insipidus, and hypo- and hyperthyroidism have been mapped to mutations at the extracellular face (Spiegel 1995; Schöneberg et al. 2004). Due to high sequence and structure variability among ECLs of related receptors (or receptor subtypes), drug discovery utilizing subtle receptor-specific differences in ECL conformation (or sequence) is envisioned to yield therapeutic interventions with minimal crosstalk and side effects.

GPCR Intracellular Loops (ICLs)

The intracellular face of GPCRs, consisting of the three ICLs and the C-terminal domain, forms the interface between GPCRs and their signalosomes, and facilitates spatiotemporally regulated coupling of conformational rearrangements in GPCR transmembrane domains to the appropriate cytosolic machinery. Recent high-resolution, time-resolved spectroscopic and molecular dynamics (MD) studies of GPCRs have revealed the formation of transient secondary structural elements at the intracellular face upon ligand binding and G-protein coupling (Dror et al. 2009; Du et al. 2019). A

Table 1 Functional aspects of extramembranous regions in GPCRs

Function	Representative examples
(a) Extracellular loops	
N-terminal domain	
Ligand binding	CB_1 (Sabatucci et al. 2018), CXCR1 and CXCR2 (Prado et al. 2007; Ravindran et al. 2009; Berkamp et al. 2017), NPYR (Zou et al. 2009), $V_{1a}R$ (Hawtin et al. 2000)
Receptor oligomerization	$GABA_B$ receptor (Schwarz et al. 2000), mGluR5 (Romano et al. 1996)
Signaling	CXCR1 and CXCR2 (Prado et al. 2007), $V_{1a}R$ (Hawtin et al. 2000)
Extracellular loop 1 (ECL1)	
Ligand binding	$S1P_4$ receptor (Pham et al. 2007), $V_{1a}R$ (Hawtin et al. 2006)
Receptor activation	Adesonine A_{2B} receptor (Peeters et al. 2011a), $V_{1a}R$ (Hawtin et al. 2006)
Receptor surface expression	$V_{1a}R$ (Hawtin et al. 2006)
Extracellular loop 2 (ECL2)	
Ligand binding	Adesonine A_1 and A_{2A} receptor (Nguyen et al. 2016; Glukhova et al. 2017; Cao et al. 2018), $5HT_{1B}R$, $5HT_{2B}R$, and $5HT_{2A}R$ (Wacker et al. 2013; Iglesias et al. 2017), $V_{1a}R$ (Hawtin et al. 2006; Conner et al. 2007)
Receptor activation	M_3 mAChR (Scarselli et al. 2007), $P2Y_1$ receptor (Hoffmann et al. 1999), $V_{1a}R$ (Conner et al. 2007)
Surface expression	$P2Y_1$ receptor (Hoffmann et al. 1999)
Extracellular loop 3 (ECL3)	
Ligand binding	AT_2R (Hines et al. 2001)
Recruitment of downstream effectors and activation	β_2-AR (Zhao et al. 1998), AT_2R (Hines et al. 2001)
(b) Intracellular loops	
Intracellular loop 1 (ICL1)	
Ligand binding	$S1P_1$ receptor (Valentine et al. 2011)
Recruitment of downstream effectors and activation	β_2-AR (Grisanti et al. 2018), FZD_4 receptor (Strakova et al. 2017), $S1P_1$ receptor (Valentine et al. 2011)
Receptor trafficking	α_{2B}-AR, α_{1B}-AR, and β_2-AR (Duvernay et al. 2009), AT_1 receptor (Duvernay et al. 2009)
Intracellular loop 2 (ICL2)	
Recruitment of downstream effectors and signaling	β_2-AR (Komolov et al. 2017), $GABA_B$ receptor (Havlickova et al. 2002), AT_{1A} receptor (Gáborik et al. 2003), D_3R (Sun et al. 2017), M_1 mAChR (Moro et al. 1993), $5HT_{1A}R$ and $5HT_{2A}R$ (Varrault et al. 1994; Kushwaha et al. 2006; Hall et al. 2012), $V_{1a}R$ (Liu and Wess 1996)
Receptor trafficking and plasma membrane expression	δ-OR (St-Louis et al. 2017)
Intracellular loop 3 (ICL3)	
Recruitment of downstream effectors and signaling	α_1-AR, α_2-AR, and β_2-AR (Kobilka et al. 1988; Cotecchia et al. 1990; Hausdorff et al. 1990; Cheung et al. 1991; Ikezu et al. 1992; DeGraff et al. 2002; Chakir et al. 2003; Komolov et al. 2017), $GABA_B$ receptor (Havlickova et al. 2002), M_1 mAChR (Jung et al. 2017), $5HT_{1A}R$ and $5HT_6R$ (Varrault et al. 1994; Malmberg and Strange 2000; Kohen et al. 2001; Turner et al. 2004), V_2R (Liu and Wess 1996)
Receptor oligomerization	D_2R and D_3R (Borroto-Escuela et al. 2010, O'Dowd et al. 2012; Bontempi et al. 2017), M_3 mAChR (Maggio et al. 1996), rhodopsin (Liang et al. 2003)
Receptor trafficking (or internalization) and plasma membrane expression	D_2R (Clayton et al. 2014), δ-OR (St-Louis et al. 2017), $5HT_6R$ (Brodsky et al. 2017)
C-terminal domain	
Recruitment of downstream effectors and signaling	β_2-AR (Chakir et al. 2003; Komolov et al. 2017), CB_1 (Eldeeb et al. 2019), mGluR2 (Bruno et al. 2012), rhodopsin (Kirchberg et al. 2011)
Receptor oligomerization	Adenosine A_{2A} receptor (Borroto-Escuela et al. 2010), β_2-AR (Parmar et al. 2017), $GABA_B$ receptor (Margeta-Mitrovic et al. 2000), δ-OR (Cvejic and Devi 1997), D_1R (O'Dowd et al. 2012)
Receptor trafficking (or internalization), transport and plasma membrane expression	α_{2B}-AR (Duvernay et al. 2004), $GABA_B$ receptor (Margeta-Mitrovic et al. 2000; Calver et al. 2001), AT_1R (Duvernay et al. 2004; Zhang and Wu 2019), δ-OR (Cvejic and Devi 1997)

5-HTR serotonin receptor, *AR* adrenergic receptor, *AT receptor* angiotensin II receptor, *CB* cannabinoid receptor, *DR* dopamine receptor, *FZD receptor* frizzled receptor, *GABA receptor* γ-aminobutyric acid receptor, *mAChR* muscarinic acetylcholine receptor, *mGluR* metabotropic glutamate receptor, *NPY receptor* neuropeptide Y receptor, *OR* opioid receptor, *P2Y receptor* purinergic receptor, *S1P receptor* sphingosine 1-phosphate receptor, *VR* vasopressin receptor

unique structural aspect of the GPCR intracellular face is a short helical domain (helix 8) in the C-terminal segment, which was first observed in the crystal structure of rhodopsin (Palczewski et al. 2000). This helix is now recognized as a key structural feature conserved in most GPCRs (Bruno et al. 2012). Interestingly, a recent bioinformatics study suggests that the nature of the conserved second residue in helix 8 may form the basis of G-protein specificity exhibited by different GPCR classes (Sato 2019).

The information transfer from the GPCR transmembrane helices to the cellular interior occurs predominantly via transient covalent modifications (such as phosphorylation) of specific residues in the ICLs and C-terminal domain. These covalent modifications lead to the formation of unique barcodes at the GPCR intracellular face (Liggett 2011; Yang et al. 2017) for recruitment of specific effectors characterized by cognate barcodes (Flock et al. 2017), resulting in distinct signaling cascades and receptor fate. Since phosphorylation occurs predominantly at residues in ICL3 and C-terminal domain, these regions are involved in receptor desensitization, internalization, recycling, and associated signaling events (Yang et al. 2017). Another level of complexity in GPCR signaling originates from the presence of receptor splice variants differing in the length of their IDRs, leading to diverse and complex responses to similar ligands via differential recruitment of signaling partners (Buljan et al. 2012). GPCR splice variants with differing ICL3 lengths, in particular, have been reported to exhibit differential ligand binding, desensitization, dimerization, and signaling (Giros et al. 1989; Usiello et al. 2000). In addition, the intracellular face has been implicated in homo- and hetero-oligomerization of GPCRs, with receptor-specific consequences for intracellular trafficking and plasma membrane expression due to the presence of endoplasmic reticulum retention/export motifs in this region (Milligan 2010). Emerging evidence points to nuclear transport of several GPCRs due to the presence of nuclear localization signals at the intracellular face, leading to distinct signaling pathways implicated in many cellular processes like transcription and cellular proliferation (Cattaneo et al. 2016). In spite of the multifaceted involvement of ICLs in GPCR biology (see Table 1, Fig. 2 for representative examples) and diseases associated with GPCR dysfunction (Schöneberg et al. 2004), these regions remain largely unexplored as drug targets, except for the pepducin class of lipopeptides. Pepducins, derived from cognate GPCR ICLs, target the receptor–effector interface in an allosteric manner and have emerged as a viable therapeutic strategy for a variety of diseases (Zhang et al. 2015).

GPCR Extramembranous Regions in Receptor Biology: Challenges and Emerging Paradigms

Structure–function relationship in GPCR extramembranous regions has been explored by introducing mutations at single or multiple residues, followed by biochemical characterization of the receptor. Important insights into structural components of GPCRs involved in coupling to downstream effectors have been acquired from mutagenesis studies in hybrid and chimeric receptors (Gudermann et al. 1997). More recently, x-ray crystallography (Ghosh et al. 2014) and cryo-electron microscopy (Safdari et al. 2018) approaches have been established as toolboxes of choice for exploring the structural correlates of GPCR biology. However, the availability of information about GPCR loops from crystallographic studies has been severely limited because the flexible ICL3 loop is either stabilized using a monoclonal antibody or replaced with T4 lysozyme (Ghosh et al. 2015) due to the inherent conformational flexibility of the loop poses a problem for x-ray crystallography, and the 'static' nature of crystallographic approaches. In addition, spectroscopic techniques such as fluorescence resonance energy transfer (FRET) (Kauk and Hoffmann 2018), electron spin resonance (ESR) (Manglik et al. 2015; Van Eps et al. 2015) and nuclear magnetic resonance (NMR) (Manglik et al. 2015; Bostock et al. 2019) have emerged as powerful tools for mapping receptor conformational dynamics to different facets of GPCR biology. Importantly, these spectroscopic techniques offer substantial adaptability to the inherent conformational dynamics of GPCR loop regions and are therefore envisioned to be instrumental in gaining fundamental insights into the functional relevance of GPCR extramembranous regions. However, establishing structure–dynamics–function relationships in intact GPCRs, in an appropriate membrane lipid milieu supporting receptor function, poses considerable challenge, predominantly due to technical difficulties associated with GPCR solubilization (Kalipatnapu and Chattopadhyay 2005; Chattopadhyay et al. 2015). Therefore, spectroscopic approaches such as solution NMR, circular dichroism, and fluorescence have mainly focused on exploring the structure, dynamics, and probable membrane interaction of peptides derived from or mimicking GPCR loops (Pham et al. 2007; Zou et al. 2009; Haldar et al. 2010; Chen et al. 2011; Chaudhuri et al. 2013; Berkamp et al. 2017; Pal et al. 2018).

Theoretical and computational approaches, such as homology modeling and MD simulations, lie at the other end of this spectrum and are capable of providing information on structure and dynamics of GPCR loops across various spatiotemporal scales of resolution (Sengupta et al. 2016, 2017). Although difficulties in assignment of GPCR loop

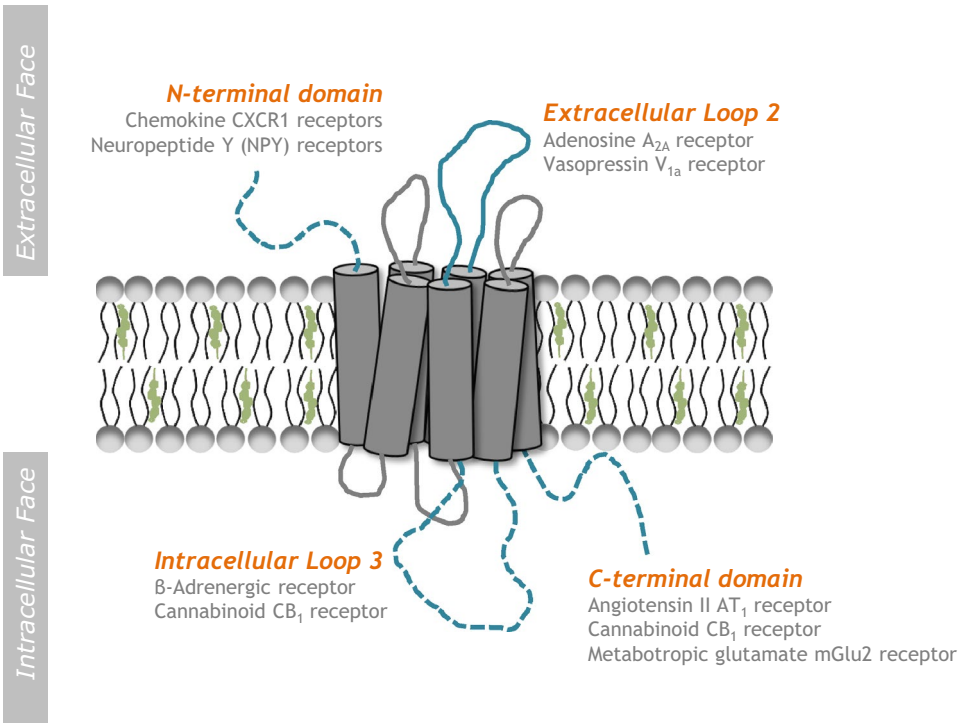

Fig. 3 Conformational heterogeneity and membrane interaction of GPCR extramembranous regions. GPCR extramembranous regions, particularly the N- and C-terminal domains and ICL3 (represented as dashed lines), show unique patterns of long intrinsically disordered regions (IDRs), which correspond to regions of sequence diversity and length variability. These IDRs are believed to expand the functional and regulatory repertoire of GPCRs by amplifying the conformational space available to the extra- and intracellular faces, leading to accelerated molecular recognition of cognates via dimensionality reduction mechanisms. Importantly, emerging literature suggests the involvement of these IDRs, along with ECL2 (shown in blue, with examples of receptors for which membrane interaction have been reported), in interaction with membranes. Since both soluble and intrinsically disordered proteins are known to adopt distinct structural conformations on interaction with membranes, encountering membrane lipids during sampling of the conformational landscape could bias these loop regions toward a specific conformational space. Emerging literature on the interplay of conformational dynamics and membrane interaction in GPCR extramembranous regions represent a novel paradigm shift in the regulation of GPCR biology by its immediate membrane microenvironment. However, current understanding of the fundamental principles linking conformational dynamics, heterogeneity, and membrane interactions of these IDRs to GPCR biology is limited. See text for more details

regions represent an early bottleneck in homology modeling due to low sequence conservation and inherent dynamics in loops (Soto et al. 2008), the growing number of high-resolution GPCR structures in recent years have resulted in a number of refined loop prediction algorithms (Goldfeld et al. 2012). Similarly, early attempts to simulate GPCRs with intact extramembranous regions faced problems due to unavailability of 'template' crystal structures with complete loops and technical challenges associated with very long convergence times of loop regions due to their conformational dynamics (Grossfield 2011, Sengupta et al. 2016). In other words, the inherent dynamics of GPCR loops, which impart functionality to these regions, makes it difficult to explore GPCR loop structure and dynamics. As such, a challenging aspect of contemporary GPCR research is to obtain a comprehensive understanding of the functional relevance of GPCR loops.

Conformational Heterogeneity in GPCR Extramembranous Regions

GPCR extramembranous regions show unique patterns of long IDRs (see Fig. 3 and its legend), which correspond to regions of sequence diversity and length variability (Jaakola et al. 2005). Although the extent of disorder varies among different GPCR classes and even between receptors of the same class (Venkatakrishnan et al. 2014), regions with the highest diversity in sequence and length such as N-terminal domain, ICL3, and C-terminal domain exhibit the highest predicted degree of intrinsic disorder (Jaakola et al. 2005). In keeping with trends predicted for transmembrane proteins (Bürgi et al. 2016), these IDRs are localized predominantly toward the cytoplasmic side. Interestingly, the amino acid composition of these regions is significantly different compared to that observed in other intrinsically disordered proteins (IDPs) (Jaakola et al. 2005). This is particularly valid in case of ICL3.

The prevalence of disordered regions should translate to adaptability to a wide array of interaction partners due to greater structural flexibility. This is relevant in the context of GPCR biology, since the extra- and intracellular faces are believed to function as converging and diverging checkpoints. In other words, diverse receptor–ligand interactions at the extracellular side merge to a unified set of transmembrane conformational rearrangements that trigger the formation of a multitude of receptor–effector complexes, thereby constituting the dynamic GPCR signalosome. This could account for the vast diversity in signaling pathways mediated by GPCRs.

The three IDRs (at N-terminal domain, ICL3, and C-terminal domain) are believed to expand the functional and regulatory repertoire of GPCRs by amplifying the conformational space available to the extra- and intracellular faces, leading to accelerated molecular recognition of cognates (ligands/effectors) via dimensionality reduction mechanisms such as the fly-casting mechanism (Shoemaker et al. 2000). This has been demonstrated in case of rhodopsin, where an increased capture radius of the unstructured ICL3 region catalyzes its G-protein coupling (Elgeti et al. 2013). Many IDPs are known to undergo local disorder-to-order transitions, concomitant with the binding step, in the proximity of their interaction partners (Dyson and Wright 2005). An important thermodynamic consequence of this coupled folding and binding mode is the formation of receptor–ligand or receptor–effector complexes with high specificity but low affinity, leading to a tradeoff between specificity and flexibility crucial for spatiotemporal control of GPCR signaling (Elgeti et al. 2013). In addition, IDRs could act as scaffolds for modulating the local concentration of GPCR signaling partners, thereby allowing the coordination and crosstalk of multiple cellular processes across spatiotemporal scales (Cumberworth et al. 2013).

The N-terminal domain of CXCR1, a class A GPCR, has been shown to exhibit substantial conformational dynamics (Park et al. 2011). We have shown that the conformational dynamics of the CXCR1 N-terminal domain peptide (Fig. 4a) is influenced by environmental factors such as proximity to membranes (Haldar et al. 2010; Kharche et al. 2018) and differential hydration (Chaudhuri et al. 2013), with consequences for ligand binding of the receptor (Rajagopalan and Rajarathnam 2004; Joseph et al. 2018). Conformational dynamics at the intracellular face of GPCRs has been reported to be distinctly different within GPCR classes (Bourque et al. 2017) and even in case of the same receptor bound to different ligands (Ghanouni et al. 2001). At the intracellular face, ICL3 is known to be largely unstructured (see Fig. 4b; Ulfers et al. 2002; Chen et al. 2011; Pal et al. 2018), with short secondary structural elements including α-helices and β-sheets distributed along the length (Huang et al. 2016) and at the two juxtamembranous ends (Varrault et al. 1994; Ulfers et al. 2002; Chen et al. 2011). These short structural stretches are believed to contain activator sequences important for coupling to G-proteins (Cheung et al. 1991; Hayataka et al. 1998; Ortiz et al. 2000) and calmodulin (Turner et al. 2004; Chen et al. 2011).

Interestingly, emerging literature suggests a role of the sole tryptophan residue in the serotonin$_{1A}$ receptor ICL3 (highlighted in yellow in Fig. 4b) in binding of this loop to calmodulin (Chen et al. 2011). We have previously reported that this tryptophan residue experiences a restricted microenvironment due to constraints induced by local secondary structural elements (Pal et al. 2018). This gives rise to the exciting possibility of exploring subtle conformational changes in GPCR ICL3 peptides through the microenvironment-sensitive spectroscopic window of intrinsic tryptophan fluorescence. The third IDR in GPCRs, the C-terminal domain, exhibits considerable conformational dynamics, particularly in helix 8, with consequences in receptor function (Kirchberg et al. 2011). Taken together, the conformational heterogeneity in GPCR extramembranous regions in general, and GPCR IDRs in particular, has important consequences for GPCR signaling and its modulation, and therefore assumes significance in the development of better therapeutics. However, present understanding of the fundamental principles linking conformational dynamics and heterogeneity to GPCR biology is still emerging and would require a judicious synthesis of insights obtained across receptor classes utilizing a variety of experimental and theoretical approaches.

Membrane Interaction of GPCR Extramembranous Regions

Extramembranous regions, constituting at least ~40% amino acid residues in GPCRs, are mostly unstructured with short stretches of α-helical and β-sheet secondary structural elements. As discussed above, this lack of stable secondary structure imparts greater flexibility and adaptability to the extramembranous regions, thereby enabling a rapid response from these regions to changes in their immediate microenvironment. These changes in microenvironment could be diffusing ligands at the extracellular face, subtle changes in transmembrane helices, and local concentration of signaling partners at the intracellular side. The induction of local secondary structural elements at appropriate hotspots in GPCR extramembranous regions could be coupled to the dimensionality reduction mechanism (Shoemaker et al. 2000) employed by these regions in searching for a conformational optimum for binding to ligands or downstream effectors. Since both soluble proteins and IDPs (Das and Eliezer 2019) are known to adopt distinct structural

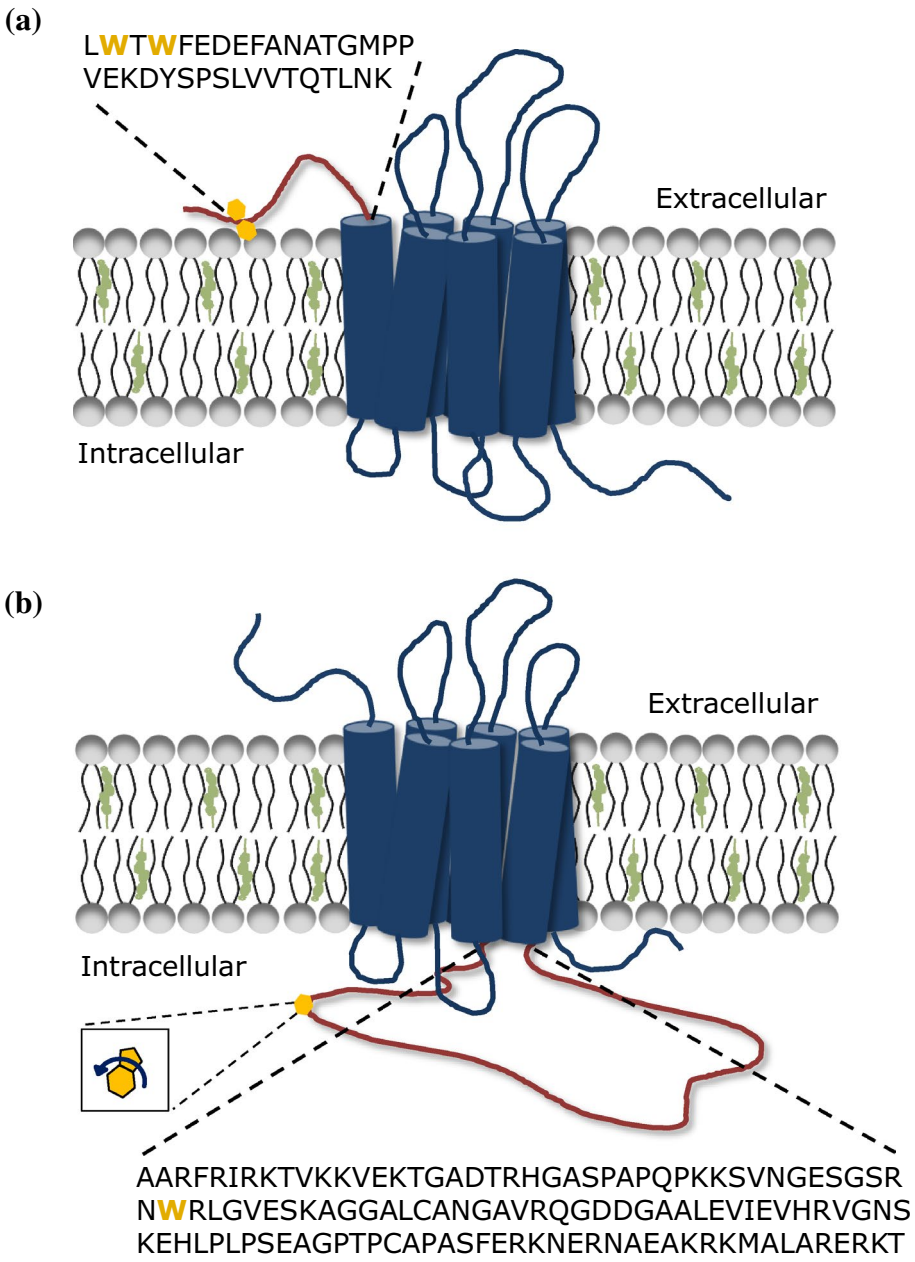

Fig. 4 Representative examples of conformational dynamics and membrane interaction of GPCR extramembranous regions: **a** Membrane interaction of the CXC chemokine receptor 1 (CXCR1) N-terminal domain. CXCR1, a member of the peptide-binding GPCR family, is associated with immune and inflammatory responses and represents an efficacious drug target for a multitude of pathophysiological conditions ranging from pulmonary and autoimmune disorders to type 1 diabetes and cancer. Interestingly, the N-terminal domain of CXCR1 (shown in maroon with its amino acid sequence and the two tryptophan residues highlighted) is crucial for imparting ligand binding specificity to the receptor. Importantly, the membrane interaction of the CXCR1 N-terminal domain is believed to regulate the conformational dynamics of this loop and influence its ligand binding properties. **b** Conformational dynamics of the serotonin$_{1A}$ receptor third intracellular loop (ICL3). The ICL3 segment (shown in maroon with its amino acid sequence and the sole tryptophan residue highlighted) connects transmembrane helices V and VI. The serotonin$_{1A}$ receptor ICL3 has been shown to be crucial for G-protein coupling and subsequent receptor activation. Mutations in this segment is known to switch the mode of G-protein coupling of the receptor from G_i to G_s in a ligand-dependent fashion. However, the role of ICL3 in modulating the cellular response to ligand-induced conformational changes in GPCR transmembrane domains remain largely underappreciated due to the replacement or stabilization of this region in high-resolution crystallographic studies of GPCRs. Adapted and modified from Pal et al. 2018. See text for more details

conformations on interaction with membranes, it is plausible that encountering membrane (lipids) during sampling of the conformational landscape would bias these loop regions toward a specific conformational space, with consequences for GPCR organization, dynamics, and signaling. This could be important in expanding the mechanistic framework for the otherwise well documented lipid regulation of GPCR function (Paila and Chattopadhyay 2010; Oates and Watts 2011; Chattopadhyay 2014; Jafurulla and Chattopadhyay 2015; Jafurulla et al. 2019; Strohman et al. 2019), as apparent from emerging literature on lipid-binding sites (Gimpl 2016) and/or collages of such sites (Fatakia et al. 2019) in GPCR extramembranous regions. Interestingly, most of the existing literature on membrane interactions of GPCR loops report the interaction of IDRs (N-terminal domain, ICL3, and C-terminal domain) in GPCRs with membranes.

Membrane interaction of the N-terminal domain of CXCRs (see Figs. 3, 4a) constitutes one of the well characterized systems (Haldar et al. 2010; Chaudhuri et al. 2013; Kharche et al. 2018), with distinct implications in ligand binding and signaling (Rajagopalan and Rajarathnam 2004; Prado et al. 2007; Joseph et al. 2018). Membrane interactions of the neuropeptide Y (NPY) receptor N-terminal domain (Zou et al. 2009) and the adenosine A_{2A} receptor ECL2 (Cao et al. 2018) have been implicated in chaperoning the respective ligands into the orthosteric binding pocket. In addition, cholesterol-mediated sphingolipid interaction with ECL1 in serotonin$_{1A}$ receptors (Prasanna et al. 2016), and cholesterol-specificity in class F GPCRs (Byrne et al. 2016) have been reported to involve the extracellular face of these receptors, thereby highlighting long- and short-range membrane interactions of GPCR extracellular loops. Membrane interaction of ECL2 in the neurohypophysial peptide GPCR subfamily (e.g., vasopressin receptors) has been reported (Hawtin et al. 2006).

At the intracellular face, membrane interaction appears to be mediated predominantly via the C-terminal domain (see Fig. 3) across several class A GPCRs (Mozsolits et al. 2002; Xie and Chen 2005; Bruno et al. 2012), with the membrane interacting residues acting as a sensor for anionic lipids (Mozsolits et al. 2002) and cholesterol (Bruno et al. 2012). In contrast to class A GPCRs where helix 8 anchors to membranes due to the presence of palmitoylation sites (Goddard and Watts 2012), the membrane interaction of helix 8 in class B GPCRs is mediated through a tryptophan residue (Conner et al. 2008) due to the absence of palmitoylation sites. Importantly, tryptophan residues in membrane proteins are known to act as membrane anchors and influence membrane protein function (Kelkar and Chattopadhyay 2006). The juxtamembranous ends of the β-adrenergic receptor ICL3 (Cheung et al. 1991) and a central hydrophobic patch in the cannabinoid CB_1 receptor ICL3 (Ulfers et al. 2002) have been implicated in membrane interaction. Proximity to the membrane microenvironment could also be reflected in changes in structure, organization, and dynamics of GPCR-interacting proteins including peptide ligands (Sankararamakrishnan 2006) and downstream effectors (Casas et al. 2017), thereby broadening the role of membranes in modulating GPCR-mediated signaling.

Conclusions and Emerging Avenues

GPCR extramembranous regions account for ~40% or more amino acid residues across various GPCR classes and are implicated in several aspects of GPCR biology, including receptor oligomerization, trafficking, and signaling. However, the lacunae in contemporary GPCR research lies in understanding the functional relevance of GPCR loops in the context of their intrinsic conformational heterogeneity and probable membrane interaction. This has led to a scenario where the GPCR extramembranous regions, despite serving as functional checkpoints with receptor-specific fingerprints, have remained largely unexplored in terms of their therapeutic potential. In addition, a comprehensive understanding of the crosstalk between structured transmembrane domains and disordered extramembranous regions in GPCRs is envisioned to result in novel bioengineering approaches (Airan et al. 2009, Mansouri et al. 2019), where cellular signaling can be precisely controlled by harnessing various components of the GPCR signaling hub. We believe that an intelligent synthesis of insights from structured transmembrane domains and disordered extramembranous regions in GPCRs would result in a comprehensive understanding of GPCR structure, function, and dynamics, thereby enhancing our ability to design better therapeutic strategies to combat diseases related to malfunctioning of GPCRs.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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