Oligomerization of GPCRs involved in endocrine regulation

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Abstract

More than 800 different human membrane-spanning G-protein-coupled receptors (GPCRs) serve as signal transducers at biological barriers. These receptors are activated by a wide variety of ligands such as peptides, ions and hormones, and are able to activate a diverse set of intracellular signaling pathways. GPCRs are of central importance in endocrine regulation, which underpins the significance of comprehensively studying these receptors and interrelated systems. During the last decade, the capacity for multimerization of GPCRs was found to be a common and functionally relevant property. The interaction between GPCR monomers results in higher order complexes such as homomers (identical receptor subtype) or heteromers (different receptor subtypes), which may be present in a specific and dynamic monomer/oligomer equilibrium. It is widely accepted that the oligomerization of GPCRs is a mechanism for determining the fine-tuning and expansion of cellular processes by modification of ligand action, expression levels, and related signaling outcome. Accordingly, oligomerization provides exciting opportunities to optimize pharmacological treatment with respect to receptor target and tissue selectivity or for the development of diagnostic tools. On the other hand, GPCR heteromerization may be a potential reason for the undesired side effects of pharmacological interventions, faced with numerous and common mutual signaling modifications in heteromeric constellations. Finally, detailed deciphering of the physiological occurrence and relevance of specific GPCR/GPCR–ligand interactions poses a future challenge. This review will tackle the aspects of GPCR oligomerization with specific emphasis on family A GPCRs involved in endocrine regulation, whereby only a subset of these receptors will be discussed in detail.

Key Words

- G-protein-coupled receptors
- homomers
- heteromers
- homooligomerization
- heterooligomerization
- dimers
- oligomers
- endocrine regulation
- endocrine diseases
- signaling

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G-protein-coupled receptors

The importance of GPCRs

Physiological functions such as development, growth, behavior, learning, emotions, senses and aging are strongly dependent on the control of endocrine circuits. Hormonal signals exert their action via binding to nuclear receptors or membrane-bound receptors. The largest superfamily of membrane-bound receptors are G-protein-coupled receptors (GPCRs) (Kristiansen 2004), which are involved in the modulation of almost all physiological processes (Limbird 2004). The relevance of GPCRs is due to their fundamental role as information transducers (Raymond 1995, Wess 1998) by serving as hubs for signals transiting biological barriers (Rosenbaum *et al*. 2009).

Activation of GPCRs by different ligands, crosstalk of receptors within the membrane, and their interaction

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with cellular or extracellular proteins determine the subsequently induced intracellular signaling pathway. The overall result of GPCR activation are specific physiological responses (Schwartz *et al*. 2006) and are based on various mechanisms such as induction of gene expression (Ho *et al*. 2009) or ion channel regulation (Veldhuis *et al*. 2015). Due to their key role in tuning physiological processes, approximately 50% of approved drugs modulate GPCRs (Hopkins & Groom 2002, Tyndall & Sandilya 2005, Schlyer & Horuk 2006, Mason *et al*. 2012).

How do GPCRs function?

GPCRs are activated by a wide variety of ligands including small peptides, nucleotides, ions, amines, or even large glycoprotein hormones, and they activate various signaling pathways (Kristiansen 2004). Based on different conserved amino acid motifs, the superfamily of GPCRs is subdivided into several families, whereby family A, also known as family 1 or rhodopsin-like GPCRs, constitute the family with most different members (Fredriksson *et al*. 2003, Fredriksson & Schioth 2005). They share a common structural architecture of seven transmembrane helices (TMHs) that are connected by three intracellular loops (ICLs) and three extracellular loops (ECLs). This architecture is confirmed by the inspection of solved GPCR structures (reviewed in Kobilka & Schertler 2008, Hanson & Stevens 2009, Lodowski *et al*. 2009). As a result of advanced experimental methods (Tate & Schertler 2009), many GPCR crystal structures have been published in the last decade (Zhao & Wu 2012, Piscitelli *et al*. 2015) and are useful tools to improve pharmacological approaches directed to GPCRs (Carlsson *et al*. 2011, Kontoyianni & Liu 2012, Mason *et al*. 2012, Shoichet & Kobilka 2012).

Most of the endogenous and synthetic ligands of family A GPCRs are believed to bind within the transmembrane domain close to the second extracellular loop 2 (ECL2) (Surgand *et al*. 2006, Wichard *et al*. 2011), with the exception of glycoprotein hormone receptors (GPHRs) and leucine-rich repeat-containing G-protein-coupled receptors (LGRs) (Svendsen *et al*. 2008, Kleinau & Krause 2009, Svendsen *et al*. 2009, Kleinau *et al*. 2013). Based on a large amount of experimental data, a 'global toggle switching' mechanism is suggested to occur during ligand-induced activation (Schwartz *et al*. 2006, Smit *et al*. 2007). Correspondingly, activation is characterized by a spatial rearrangement of the TMHs relative to one another (Scheerer *et al*. 2008, 2009, Schertler 2008). This structural rearrangement is supported by amino acids acting as 'micro-switches' (Ahuja & Smith 2009, Hofmann *et al*. 2009, Nygaard *et al*. 2009). Different GPCR conformations are assumed to be related to different signaling activity states (Seifert & Wenzel-Seifert 2002, Kobilka & Deupi 2007, Deupi & Standfuss 2011). Due to the capability for activation of G-proteins and several G-protein-independent signaling pathways (e.g. via arrestin), GPCRs share common 'read-outs', even if they bind different ligands (reviewed in Galandrin *et al*. 2007, Oldham & Hamm 2008).

GPCR oligomerization as a regulatory mechanism in signaling

How can an oligomeric GPCR constellation be defined, and is there a relevance for oligomerization?

Organisation of GPCRs as oligomers is an interesting feature under structural and functional prospective. It has been reported for numerous GPCRs (Tadagaki *et al*. 2012a), also in native tissue (Albizu *et al*. 2010a, Bouvier 2001, Rozenfeld & Devi 2011). Dimerization is a general term used to describe a GPCR-x/GPCR-x (homomer) or GPCR-x/GPCR-y (heteromer) constellation. The term oligomerization is used for dimeric, tetrameric or higher order complexes between GPCR protomers (Fig. 1). Such GPCR–GPCR interrelations should be characterized by the following parameters (for further details, see also (Gomes *et al*. 2016)): (1) physical interactions (side-chain interactions); (2) direct mutual functional modulation; or (3) a particular spatial distance to one another. It could be hypothesized that a relevant oligomerization is dependent on a functional significance (Tadagaki *et al*. 2012a). In case of heterodimerization, GPCR expression in the same cell type (Waldhoer *et al*. 2005, Pin *et al*. 2007) and simultaneous occurrence (Gonzalez *et al*. 2012) are prerequisites. Oligomerization has been reported for several GPCR subfamilies such as for the family A, family B or taste receptors (Li *et al*. 2002, Harikumar *et al*. 2008, Ng *et al*. 2012, Ng & Chow 2015).

Which general functional role may GPCR oligomerization have in physiology? To date, the functional significance of both homo- and heteromerization on the molecular level is related to the determination, enlargement and fine-tuning of signaling options such as signal amplification or modification (see below for details). Moreover, the GPCR capacity to form heteromers particularly provides an opportunity for their respective ligands to act in a synchronized manner and thereby to balance or coordinate related cellular responses and biological processes (such as metabolism or reproduction). As a hypothetical example, one ligand activates a specific

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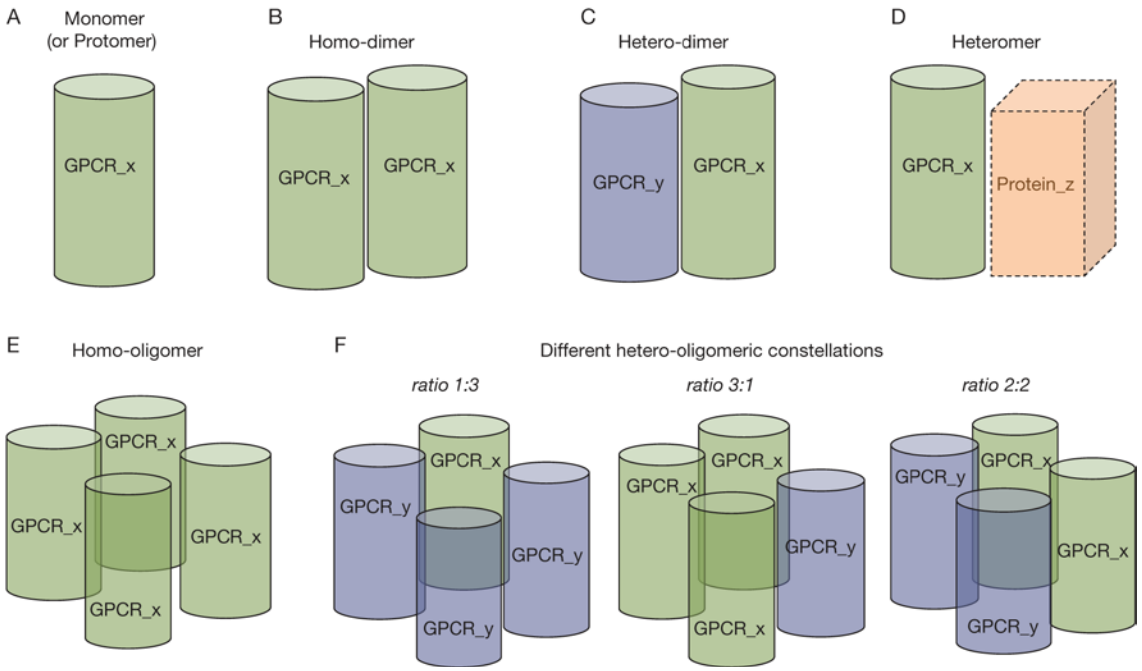

Figure 1
Putative constellations of oligomers. (A) A particular GPCR (GPCR_x) as a monomer (schematic cylinder indicates the entire receptor). (B) Two monomers of the same GPCR interacting as a homodimer. (C) Dimerization between GPCR_x with a different GPCR_y. (D) GPCR_x may potentially interact with a non-GPCR. (E) GPCR_x may also form homooligomers with different interfaces between the protomers. (F) Assuming tetrameric (or higher order) GPCR oligomers, complexes with different protomer ratios may be formed by GPCR_x and GPCR_y.

receptor, whereas the activity of the interacting heteromeric GPCR is simultaneously inhibited or downregulated by mutual effects, which is an effective and rapid system of counter-regulation. Notably, several GPCRs can interact with other GPCRs as oligomers, also in dependency on diverse cell types that also broadens the spectrum of a specific ligand to direct signaling in different associations with related physiological mechanisms. This general picture (explained in further detail below) becomes more complex in consideration of the frequently observed ligand property to interact with a multitude of diverse GPCR targets such as trace amines (Kleinau *et al*. 2011, Dinter *et al*. 2015*a*).

The involvement of GPCR oligomerization in the regulation of physiological processes, either as homo- or in heterooligomeric constellations, is strongly supported by previous and recent findings, e.g. for the α1-adrenergic receptor (α1AR), whereby heterodimerization of α1AR with the chemokine receptor 4 controls blood pressure regulation (Tripathi *et al*. 2015). Moreover, the trace amine-associated receptor 1 (TAAR1) interacts, for example, with the dopamine-2 receptor (D2R) and, in turn, modifies dopaminergic neurotransmission (Harmeier *et al*. 2015). Further examples include circadian-controlled heterodimers between the D4R and the β1- or α1B-adrenergic receptor, respectively, that are involved in the regulation of serotonin and melatonin syntheses (Gonzalez *et al*. 2012) and the interactome of melatonin receptors involved in circadian rhythm regulation (Benleulmi-Chaachoua *et al*. 2016).

What do we know about interactions between GPCR protomers constituting oligomeric interfaces?

Several GPCR-GPCR protomer interfaces have been reported under involvement of TMH4 (Carrillo *et al*. 2004, Hernanz-Falcon *et al*. 2004, Guo *et al*. 2005, 2008, Mancia *et al*. 2008), TMH1, and TMH5-6 (Hebert *et al*. 1996, McMillin *et al*. 2011, Yanagawa *et al*. 2011) or the extracellular N-terminal region (Uddin *et al*. 2012). The intermolecular interactions are constituted between single amino acids or between multitudes of side chains. Specific roles for such interactions or spatial distances between protomers are not defined as obligate for 'protomer interrelations' or cannot be determined as a standard, and may vary for different GPCRs. In conclusion, different putative interfaces and types of interactions for homo- and heterodimers can be assumed, whereby no universal interface property can be suggested. Several interfaces revealed by biophysical or biochemical methods have

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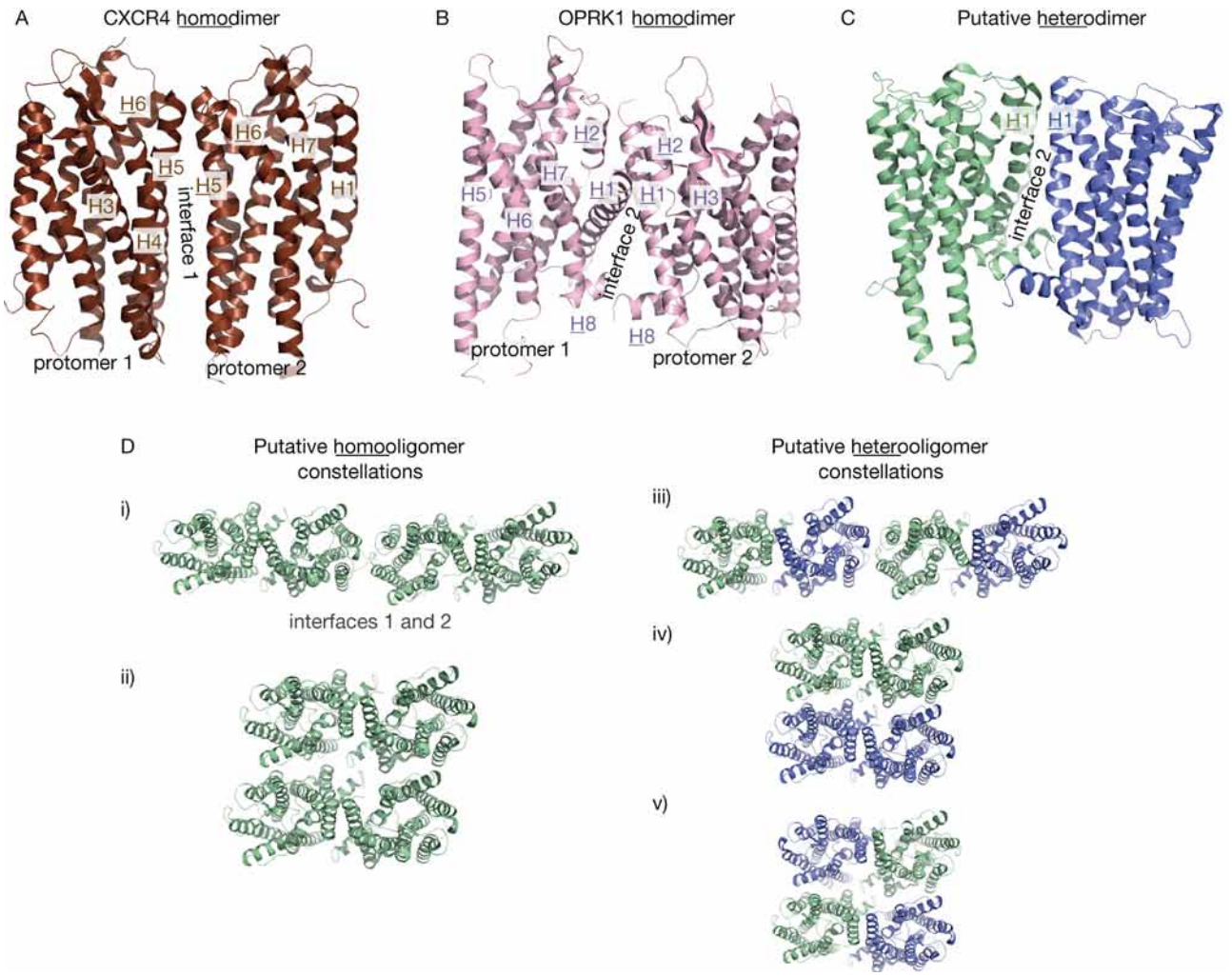

Figure 2
Observed GPCR homodimer interfaces and putative heterodimer constellations. Direct GPCR protomer interfaces are assumed to be characterized at the structural level by specific side-chain interactions or close spatial distances. Interfaces between the protomers have been found under experimental conditions for different GPCRs at the region from ICL2–TMH4 (Bakker *et al*. 2004, Guo *et al*. 2005, Mancia *et al*. 2008), TMH5–TMH6 (George *et al*. 1998, Yanagawa *et al*. 2011, Hu *et al*. 2013), and TMH4–TMH5 (Gorinski *et al*. 2012). Furthermore, several crystal structures of dimeric GPCR complexes were previously determined, such as those from the (A) chemokine receptor CXCR4 (Wu *et al*. 2010) or (B) the κ-opioid receptor (OPRK1 or KOR (Wu *et al*. 2012)). For OPRK1, as also for determined β-1AR and opsin structures, the protomer interface is located at TMH1–TMH2 and helix 8 (B), respectively. In line with biophysical data, dimer interfaces can be also observed between TMH5-6 (as in A). As a result, heteromeric states should also be characterized by these interfaces, but between diverse protomers (C) (protomers are colored differently for visual separation). For homomers, various scenarios of spatial protomer arrangements (a combination of dimers) can be speculated (D i and ii). The identical variety of combinations may also be assumed for heteromers, which finally includes heteromers of homomers (Ferre 2015) (D iii and v). The software PyMOL (Molecular Graphics System, Version 1.3 Schrödinger, LLC) was used for structural representation.

been confirmed by determined crystal structures of GPCR dimers (Fig. 2). Furthermore, the GPCR-GPCR interfaces are most likely to be of dynamic character (Hu *et al*. 2013). GPCRs were described to be potentially expressed as a mixture of monomers and homomers, and the two forms were found to interconvert dynamically in an equilibrium ((Hern *et al*. 2010, Kasai *et al*. 2011, Calebiro *et al*. 2013, Teichmann *et al*. 2014), reviewed in Lambert 2010).

Which functions or molecular properties of GPCRs are influenced by oligomerization?

Homo- and heterooligomerization is not a prerequisite for a general signaling capacity at family A GPCRs (Whorton *et al*. 2007), but it defines and widens the spectrum of fine-tuning options in signaling (White *et al*. 2007, Ciruela *et al*. 2010, Smith & Milligan 2010). Oligomerization can have a major influence on the

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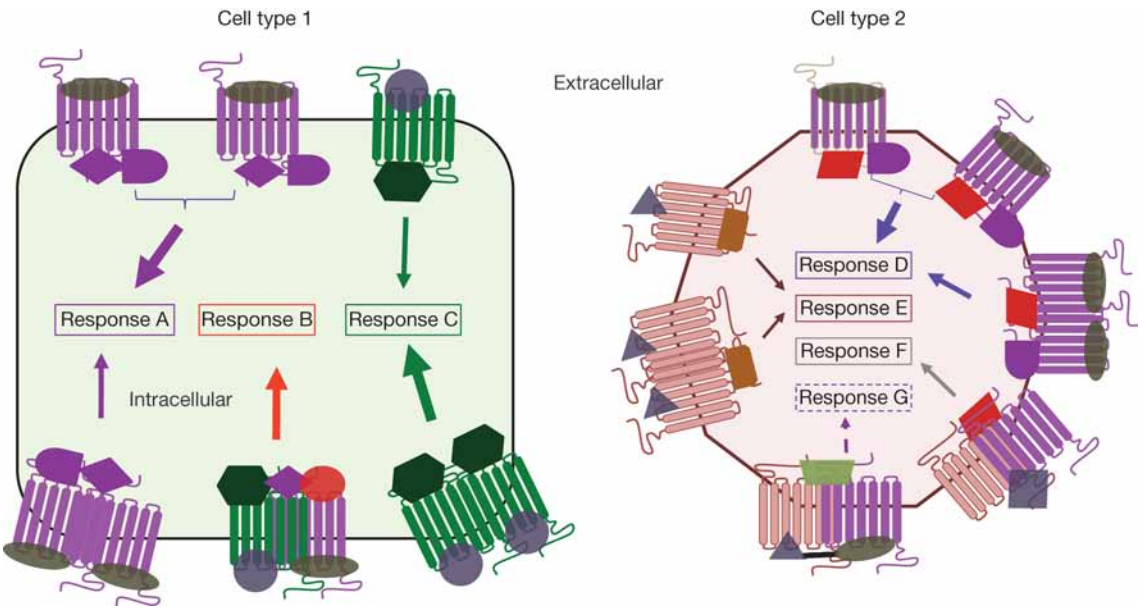

Figure 3
Schematic illustration of different signaling scenarios that potentially occur at GPCRs and GPCR oligomers in different cell types. This scheme illustrates potential scenarios that are feasible concerning receptor signaling and oligomerization in different cell types. Further diverse scenarios and levels of receptor organization are potentially probable but have not been implemented here (e.g. by heterotrimerization). Different intracellular signaling parameters in strength (arrows thin or bold) and constitution (response boxes A–G) are indicated. A particular receptor (e.g. purple) may activate diverse intracellular signaling pathways in different cell types, indicated as cell type 1 (response A) and cell type 2 (response D). In cell type 1, this receptor is capable of activating two different downstream signaling events after ligand binding. This receptor has different properties concerning binding and activation of intracellular effectors in cell type 1 as the set and amount of particular intracellular signaling determinants (e.g. G-protein subtypes or other downstream signaling molecules) differ. Moreover, in each cell type, a multitude of different GPCRs are expressed, indicated as additional green receptors in cell type 1 or of light beige color in cell type 2. These receptors activate different signaling pathways (responses B and E). In each cell type, orphan receptors with unknown ligand(s) may also occur and influence the system of interacting proteins (not shown). In cell type 1, homooligomerization of the receptors may lead to a lowered signaling (slim arrow) compared with receptor monomer signaling. Receptor oligomerization may lead to enhanced signaling as indicated for the green receptor in cell type 1 (bold arrow). Examples of such differences in signaling responses in dependency of oligomerization or monomerization have been reported in a previous study on the MC4R (Piechowski *et al.* 2013). Furthermore, there may be heterooligomerization of the receptors (as described for the MC4R), indicated by receptor pairs (mixed color). These interactions should lead to the activation of signaling pathways that are partially identical to each monomer, but may also result in the activation of other signaling events (response C or F). For homo- and heterooligomers, ligand binding properties are known to drastically differ to those of monomers (indicated in heterodimer cell type 2). Moreover, bivalent ligands (illustrated in cell type 2) combine particular ligands for the respective protomers, which may ultimately lead to alterations in the signaling properties of the complex (response G).

signaling properties of interacting protomers (Prinster *et al.* 2005) such as in ligand binding (Levoye *et al.* 2006*a*, Lohse 2010), G-protein coupling selectivity, and signal transduction mechanisms (reviewed in Bouvier 2001, George *et al.* 2002), or for cell surface expression (Uberti *et al.* 2005). Functional cross-regulation of G-protein activation may occur (Maurice *et al.* 2011*b*), despite the assembly of these GPCRs in heterooligomeric complexes (Nijmeijer *et al.* 2010).

Specifically, a particular GPCR in a specific cell type can function as a protomer, homomer, or in a heteromeric constellation (Fig. 3). Several functional scenarios of receptor property modifications dependent on oligomerization have been identified or have been suggested, as indicated in the following examples:

1. Constitution of homo- and/or heteromers:
   (i) The internalization of heteromers differs from that of homomers or monomers (Hanyaloglu *et al.* 2002, Kilpatrick *et al.* 2015).
   (ii) Signaling of the monomer is enhanced compared with the oligomer (Piechowski *et al.* 2013).
   (iii) Signaling of the monomer is weaker compared with the oligomer (Wilson *et al.* 2007, Magalhaes *et al.* 2010, Pellissier *et al.* 2011).
2. A homomer is capable of binding various ligands, and/or binding capacities differ compared with the monomer (Durroux 2005).
3. A heteromer, which may also be an orphan receptor (Levoye *et al.* 2006*b*), in a given cell type may:

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(i) activate the same signaling pathways as the protomer(s) (neutral heterooligomeric) (Prinster *et al*. 2005);
(ii) modulate signaling pathways (e.g. strength of signaling) (AbdAlla *et al*. 2000, Terrillon *et al*. 2004);
(iii) activate pathways that differ from both protomers (Hague *et al*. 2006, Rashid *et al*. 2007, Gomes *et al*. 2013);
(iv) bind ligand(s) that differ (e.g. also at allosteric binding sites) (Durroux 2005);
(v) bind artificial ligands that modify signaling pathways such as bivalent ligands (Shonberg *et al*. 2011, Mohr *et al*. 2013, Yuan *et al*. 2013).

How is oligomerization detected experimentally?

In the last two decades, various methods have been applied to detect GPCR–GPCR interactions (Table 1). Each of these techniques exhibits both advantages and disadvantages when compared with one another and with regard to their aim; however, this field is characterized by strong development and rapid advancement. Because of previous thorough reviews of methods for the detection of oligomers (e.g. Persani *et al*. 2007, Kaczor & Selent 2011, Bonomi & Persani 2013, Gomes *et al*. 2016), we will not describe the particular methods in detail, but will merely provide a brief overview of commonly used approaches. Currently applied techniques can be subdivided based on their methodological principles or experimental conditions as follows: (i) antibody-based and antibody-independent; (ii) dynamic and nondynamic systems; (iii) analyses of living, fixed or lysed cells; or (iv) in vitro cell culture or an in vivo model. Antibody-based techniques, such as co-immunoprecipitation, co-immunolocalization, sandwich-ELISA or the proximity ligation assay, primarily recognize epitope tags (Table 1). Due to the lack of specific GPCR antibodies, these techniques are often limited to in vitro cell culture overexpression conditions. Homo- or heteromer detection by antibody-based assays are mostly used with differentially epitope-tagged GPCRs and are effective in fixed or lysed cells (reviewed in e.g. Skieterska *et al*. 2013, Gomes *et al*. 2014). In the case of antibodies that exist for a specific GPCR or GPCR

Table 1 Methods that have been used to determine di- and oligomerization of GPCRs.

	Application			
	In vitro			
Method		In living cells	*In situ*	*In vivo*
Antibody-based				
Co-immunoprecipitation (Co-IP)	+		(+)	
Co-immunolocalization				
Sandwich-ELISA	+			
Proximity ligation assays (PLA)	+		(+)	
mostly antibody-independent				
Proximity biotinylation	+			
Resonance energy transfer (RET)	+	+	+	
FRET (F, fluorescence),				
e.g. via				
- fluorescent ligands or				
- based on SNAP- and CLIP-tag technology				
BRET (B, bioluminescence),				
SRET (S, sequential),				
TR-FRET (TR, time-resolved)				
e.g. via antibody-based HTRF				
Protein complementation assays (PCA)	+	+		
e.g. based on fluorescent proteins, enzymes, ubiquitin				
Photoactivated localization microscopy (PALM)	+	+		
Fluorescence cross-correlation spectroscopy (FCCS)	+	+		
Allosteric crosstalk	+	+	+	(+)
e.g. via radioligand binding				

Dimer detection using antibody-based and antibody-independent techniques. Antibody detection primarily requires tagging of GPCRs. Due to the availability of several primary GPCR antibodies and the possibility of tagged GPCR expression in animal models, *in situ* detection by antibody-based methods may be possible (indicated by bracketed plus symbol). The most promising technique used to detect GPCR–GPCR interactions in the physiological *in vivo* background is by analyzing allosteric crosstalk. The limitation of this method is the knowledge of adequate ligands, their detailed effects, and tissue specificity (also indicated as bracketed plus symbol).

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oligomer, expression and detection of labeled GPCRs are also possible in animal models.

Elaborate enhancement of methods and constantly refined techniques even allow for the detection of GPCR protomers and oligomers in living cells or tissue samples by some antibody-independent methods. Of these methods, the application of resonance energy transfer (RET) and protein complementation assay (PCA) (for review see e.g. Vidi & Watts 2009, Lohse *et al*. 2012, Ng *et al*. 2012, Bonomi & Persani 2013, Ciruela *et al*. 2014, van Unen *et al*. 2015) have been particularly used to detect GPCR homo- or heteromerization.

Moreover, photo-activated localization microscopy (PALM) and fluorescence fluctuation spectroscopy techniques (FCCS) have been developed (for review see Herrick-Davis *et al*. 2013, Hink & Postma 2013). These straightforward methods permit a quantified interaction monitoring of fluorescent-labeled GPCRs at a nanomolar concentration level or even as single molecules without a distance limit for interactions compared with RET.

The most promising methods to detect GPCR homo- and heteromerization in *in vivo* systems are via the analysis of allosteric crosstalk (Durroux 2005, Teitler & Klein 2012). This method is based on the idea that the dimerization process may lead to crosstalk between interacting GPCRs and may result in cooperative binding of ligands to these protomers. As a prerequisite, sufficient knowledge of the relevant GPCR-ligand interdependency (agonist, antagonist, inverse agonist effects) is required to analyze interactions with this method.

Oligomerization of receptors in endocrine regulation

Endocrine regulatory circuits consist of releasing hormones secreted from the hypothalamus. These hormones activate their cognate receptors on specialized pituitary cells, which subsequently lead to pituitary hormone release. The anterior pituitary hormones function at their end organs, which may be an endocrine gland. These hormones exert their action or further modulate end-organ hormone secretion, which is regulated in a negative feedback manner to the hypothalamus and the pituitary as it is known for the regulation of growth, thyroid function, fertility-reproduction and adrenal functions. Furthermore, many other endocrine functions such as energy metabolism or glucose homeostasis are tightly regulated by hormone/receptor feedback systems.

What is known about oligomerization of GPCRs involved in endocrine regulation?

Many receptors for releasing hormones and tropic hormones have been suggested to comprise of homo- or heterooligomers, whereby these findings are primarily based on in vitro studies. Although oligomerization of GPCRs is known for several of GPCR subfamilies (Young *et al*. 2007, Harikumar *et al*. 2008, Ng *et al*. 2012, Ng & Chow 2015), we will focus on several examples from the largest GPCR family, family A, to provide insights into linked aspects.

GPCRs of the pituitary-thyroid axis

In specific cells, the pituitary thyrotropin-releasing hormone (TRH) binds to the TRH receptor (TRHR) (Sun *et al*. 2003). In addition to TRHR expression in the pituitary, extrapituitary TRHR localization has also been found in various areas of the brain (Cao *et al*. 1998, Heuer *et al*. 1999, Bilek 2000). The TRHR may form homomers (Kroeger *et al*. 2001) in the absence of the ligand; however, ligand stimulation is suggested to further increase homooligomerization (Kroeger *et al*. 2001, Zhu *et al*. 2002). TRHR dimerization was found to affect trafficking but not signaling (Song & Hinkle 2005) and potentiated hormone-dependent receptor phosphorylation (Song *et al*. 2007).

There are two different subtypes of TRHR in rodents. These receptor subtypes are able to heterooligomerize, which has been found to change their functional properties in terms of β-arrestin recruitment (Hanyaloglu *et al*. 2002).

The secreted hormone thyrotropin (TSH), in turn, activates the thyroid gland via binding to the thyrotropin receptor (TSHR), which induces the production of thyroxine (T_4) and triiodothyronine (T_3). The TSHR has been previously reported to homooligomerize (Latif *et al*. 2001, Davies *et al*. 2002), which is most likely independent from TSH stimulation (Bonomi & Persani 2013; Urizar *et al*. 2005) or constitutive activation by mutation (Zoenen *et al*. 2012, Biebermann *et al*. 2012*b*). In regards to the TSHR, interaction between the receptor protomers occurs at the transmembrane bundle (Urizar *et al*. 2005), but an additional role of the extracellular domain on the oligomer constitution is proposed based on BRET experiments (Urizar *et al*. 2005). In the same study, further experimental evidence for negative cooperativity with respect to ligand binding was demonstrated and supports a functional relevance of homomers. This is of general

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importance to further understand endocrine regulation at a wide range of ligand (hormone) concentrations with the highest level of sensitivity occurring at lower hormone concentrations (Urizar *et al.* 2005). This is true for physiological TSH concentrations in serum that span one order of magnitude (Bianco *et al.* 2014). Moreover, a recent study leads to the observation that two TSH molecules bound at a TSHR homodimer are necessary to activate Gq (IP) (Allen *et al.* 2011). Further support for a functional role of TSHR oligomerization is provided by studies showing dominant negative effects by pathogenic mutations (Calebiro *et al.* 2005), which can be best explained by mutual interrelations between TSHR protomers.

The receptors of the hypothalamic-pituitary-gonadal (HPG) axis

The first step in the production and release of the gonadotropins lutropin (LH) and follitropin (FSH) is binding of gonadotropin-releasing hormone (GnRH) to its receptor (GnRHR) in the pituitary. Different studies revealed that the GnRHR is able to form microaggregates (oligomers) due to GnRH stimulation (Conn *et al.* 1982*a*,*b*, Cornea *et al.* 2001, Kroeger *et al.* 2001). Evidence of human GnRHR dimerization derives from mutant receptors that inhibit ligand binding and second messenger production of wild-type via a dominant negative effect (Brothers *et al.* 2004). Interestingly, in the protochordate Ciona intestinalis, which lacks a hypothalamus-pituitary-gonad axis, four different subtypes of GnRHR exist (reviewed in Satake *et al.* 2013). One of the receptor subtypes (R4) is an orphan receptor and modulates the functionality of a further particular GnRHR receptor subtype in terms of intracellular Ca2+ elevation and ERK phosphorylation (Sakai *et al.* 2008). Moreover, GnRHR-mediated cAMP signaling is decreased in the heterodimeric constellation between this orphan GnRHR and another paralog due to a shift from Gs to Gi signaling (Sakai *et al.* 2012). However, how GnRHR oligomerization influences the expression and secretion of the gonadotropins LH and FSH has not yet been thoroughly investigated (Satake *et al.* 2013).

The receptors for FSH (FSHR) and LH/CG (LHCGR) play a crucial role in reproductive physiology (Themmen & Huhtaniemi 2000). First indications for LHCGR oligomerization were achieved from studies where co-expression of binding-deficient (with full signaling capacity) and signaling-inhibited (with diminished binding capacity) receptor fragments partially restored ligand-induced signal generation (Osuga *et al.* 1997). This was the first indication of a functional interrelation between LHCGR monomers. For the FSHR, transactivation was found to most likely induce biased signaling in terms of the generation of only one of two potentially induced hormone signals, but not both simultaneously (Ji *et al.* 2004).

In 2004, constitutive and agonist-dependent self-association of the LHCGR was demonstrated (Tao *et al.* 2004, Fanelli 2007) as well as the negative effects of inactive LHCGR mutants on wild-type receptor signaling in oligomeric constellations (Tao *et al.* 2004). Moreover, the functional rescue of two loss-of-function mice LHCGR mutants, a ligand binding defective and a signaling defective that both suffer from infertility in the homozygous state, was proven (Rivero-Muller *et al.* 2010), which pointed to a physiological relevance of LHCGR oligomerization and receptor–protomer interplay. Recently, a sophisticated method of single molecule analysis has revealed contacts of interacting LHCGR variants (Jonas *et al.* 2015).

Furthermore, heteromerization of FSH and LHCGR co-expressed in *in vitro* cell systems have been observed (Feng *et al.* 2013, Mazurkiewicz *et al.* 2015). In granulosa cells, LHCGR and FSHR are co-expressed and LHCGR/FSHR interaction is speculated to play a role during granulosa cell differentiation.

Besides the classical form of endocrine feedback regulation comprising the hormone/receptor systems in the hypothalamus, pituitary, and end-organs, and their feedback regulation, many primary nonendocrine tissues also express and secrete hormones such as leptin and adiponectin (adipocytes), ghrelin (stomach), and PYY cholecystokinin (gut). Action of these hormones is tightly regulated and their receptors are reported to constitute and function as oligomers.

One prominent example is body weight maintenance. The most important pathway of weight regulation is the leptin–melanocortin pathway that communicates peripheral signals to the hypothalamus, where information is integrated to an orchestrated reaction resulting in the maintenance of a constant body weight (Cone 2005, Oswal & Yeo 2007, Biebermann *et al.* 2012*a*, Farooqi 2014). From the periphery, anorexigenic hormones, such as the adipocyte-derived leptin or the pancreatic β-cell-derived leptin, affect their receptors on neurons of the arcuate nucleus in the hypothalamus (ARC). As a result, the expression of orexigenic peptides, such as neuropeptide Y (NPY) and agouti-related peptide (AgRP), is repressed. Moreover, the expression of pro-opiomelanocortin (POMC) is enhanced, which gives rise to the expression of a

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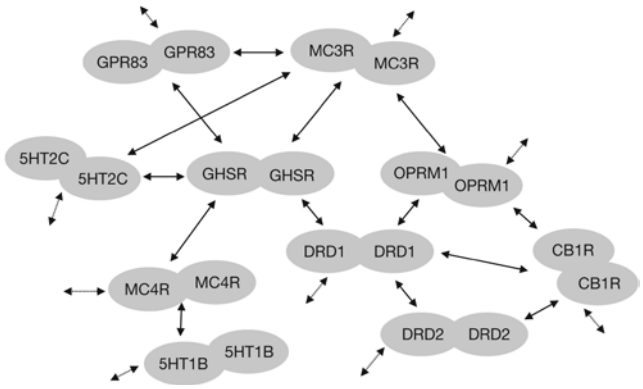

Figure 4
A complex of GPCR homo-/heterodimer interaction networks. Many members of the family A GPCRs have been reported to constitute homodimers and form heteromers (examples used here for this scheme (Pfeiffer *et al*. 2002, Ramsay *et al*. 2002, Mandrika *et al*. 2005, Ellis *et al*. 2006, Rios *et al*. 2006, Decaillot *et al*. 2008, Juhasz *et al*. 2008, Navarro *et al*. 2008, Vilardaga *et al*. 2008, Schellekens *et al*. 2013, Muller *et al*. 2013*b*)). Moreover, several of the so far analyzed subjects are able to heteromerize with different GPCRs such as the ghrelin receptor (GHSR, bold letters) (Jiang *et al*. 2006, Rediger *et al*. 2009, 2011, Schellekens *et al*. 2013). Consequently, the spectrum of potential interaction partners widens the putative modification of physiologically relevant signaling properties in dependency on the GPCR expression pattern in a particular cell type. For a comprehensive overview of available experiments and GPCR oligomer literature, see also the 'The G Protein Coupled Receptor-Oligomerization Knowledge Base Project' (Khelashvili *et al*. 2010). This scheme demonstrates a fragmented section of reported GPCR homo- and heteromers, whereby arrows indicate interactions between monomers (Fig. 2C) or homodimers (Ferre 2015) (potentially in constellations as suggested in Fig. 2D iii, iv and v). Dotted arrows indicate potentially more and/or thus far unknown partners. GHSR, ghrelin receptor; MC3R, melanocortin-3 receptor; 5HT2C, 5-hydroxytryptamine (serotonin) receptor 2C; 5HT1B, 5-hydroxytryptamine (serotonin) receptor 1B; GPR83, G-protein-coupled receptor 83; OPRM1, Mu opioid receptor; DRD1/DRD2, dopamine-1 and dopamine-2 receptors; Cb1R, cannabinoid receptor 1.

variety of additional peptides. Important peptides include α- and β-melanocyte-stimulating hormones (MSHs) that are capable of activating melanocortin 3 and 4 receptors (MC3R and MC4R) in the ARC and in the nucleus paraventricularis (PVN).

The MC4R, dominantly expressed in the PVN, is the most prominent GPCR in weight regulation (Cone 2005, Biebermann *et al*. 2012*a*) and appetite control (Garfield *et al*. 2015). Investigation of a naturally occurring heterozygous MC4R mutation in a patient suffering from severe early-onset obesity, a dominant-negative effect of the mutant on the wild type was detected, which can be best explained by homomerization of the MC4R and MC4R-MC4R variant (Biebermann *et al*. 2003). Further support for MC4R oligomerization has been provided by kinetic studies of ligand binding (Kopanchuk *et al*. 2006). The MC4R is also capable of interacting with other GPCRs that are expressed in the PVN (Fig. 4), although these results have only been indicated in *in vitro* studies (Rediger *et al*. 2009). *In vivo* studies confirming these preliminary results are lacking.

An interesting study on MC4R oligomerization revealed receptor determinants that are involved in the constitution of protomer interfaces. Inhibition of MC4R oligomerization could be achieved by domain substitution using the noninteracting cannabinoid-1 receptor (Piechowski *et al*. 2013). Substitutions of the MC4R ICL2 and adjacent regions of TMH3 and TMH4, with respective regions of the cannabinoid-1 receptor, lead to partial MC4R dimer dissociation. Most importantly, the signaling capacity was increased in monomeric MC4R variants, which indicates a link between receptor dimerization and the signaling capacity.

In the hypothalamus, the MC3R is expressed on POMC and AgRP/NPY neurons (Barsh *et al*. 2000, Rediger *et al*. 2011). The MC3R is known to be embedded in a network of GPCR interactions with e.g. the ghrelin receptor (GHSR) (Fig. 4). Interaction of MC3R with GHSR leads to the upregulation of α-MSH-stimulated signaling and the downregulation of basal- and ghrelin-induced GHSR signaling (Rediger *et al*. 2011). Both the GHSR and the MC3R have been reported to have further diverse GPCR interaction partners (Borroto-Escuela *et al*. 2014) and also interact with orphan GPCRs such as GPR83 (Muller *et al*. 2013*b*). This, in turn, makes predictions of any physiological/functional impact due to modifications at the MC3R, the MC4R, or further interacting partners difficult.

The GPR83 was recently identified as a new determinant involved in body weight regulation (Muller *et al*. 2013*b*). So far this orphan receptor is expressed e.g. in the thymus and hypothalamus (Harrigan *et al*. 1989, 1991, Brezillon *et al*. 2001). Most importantly, GPR83 has been previously found to be involved in the control of circulating adiponectin levels (De Moerlooze *et al*. 2000), and *Gpr83* expression is decreased in obese mice when compared with lean mice (Muller *et al*. 2013*b*). Moreover, GPR83 constitutes homodimers but also has the capacity for heteromerization, as has been demonstrated by heteromerization with the ghrelin receptor (Muller *et al*. 2013*a*). Interaction between both receptors leads to inhibition of the GHSR signaling capacity.

The signaling output of the previously mentioned GPCR interaction network (e.g. MC3R, GHSR and GPR83) is most likely crucial for the integration of all peripheral and central signals that are involved in energy metabolism. Future elucidation of combinatorial possibilities will be a matter of experimental confirmation and discussions (Fig. 4).

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Table 2 Examples of GPCR dimers involved in endocrine regulation.

Homomers	Heteromers	References
Weight and appetite regulation and/or metabolism		
MC3R-MC3R	MC3R-GHSR, NPY2R, MOR	(Rediger *et al*. 2011, 2012)
MC4R-MC4R	MC4R-5HTR1B, GPR7	(Biebermann *et al*. 2003, Tarnow *et al*. 2008, Rediger *et al*. 2009)
GPR83-GPR83	GPR83-GHSR, MC3R	(Muller *et al*. 2013*a*,*b*)
GHSR-GHSR	GHSR-MC3R, GPR83, SSTR5, DRD1/2, 5HTR2C	(Rediger *et al*. 2011, 2012, Kern *et al*. 2012, Park *et al*. 2012, Schellekens *et al*. 2013, Muller *et al*. 2013*b*)
GIPR-GIPR	GIPR-GLPR	(Schelshorn *et al*. 2012, Ng & Chow 2015)
TAAR1-TAAR1	TAAR1-ADRA2A, D2R	(Harmeier *et al*. 2015, Dinter *et al*. 2015*b*)
NPY2R-NPY2R	NPY2R-MC3R	(Dinger *et al*. 2003, Rediger *et al*. 2009)
NPY1R-NPY1R		(Dinger *et al*. 2003)
NPY5R-NPY5R		
Reproduction		
PROKR2-PROKR2		(Marsango *et al*. 2011)
FSHR-FSHR	FSHR-LHCGR	(Ji *et al*. 2002, 2004, Tao *et al*. 2004, Feng *et al*. 2013, Mazurkiewicz *et al*. 2015)
LHCGR-LHCGR	LHCGR-FSHR	(Ji *et al*. 2002, 2004, Tao *et al*. 2004, Feng *et al*. 2013, Mazurkiewicz *et al*. 2015)
GnRHR-GnRHR		(Cornea *et al*. 2001, Kroeger *et al*. 2001)

This overview represents examples of GPCRs that are involved in endocrine regulation, which have been reported to comprise homo- or heteromers. The examples in brackets are confirmed in their interaction but have not yet been functionally characterized.

Further examples of GPCRs involved in endocrine regulation, which have been reported to oligomerize, are the prokineticin-receptor 2 (PROKR2), the oxytocin receptor (OTR) (Table 2), or dopamine receptor (D2R). Several examples will be provided to pinpoint the complexity and common occurrence of oligomerization.

The OTR and vasopressin receptors (AVPRs: V1aR, V1BR, V2R) belong to a subclass of family A GPCRs. The OTR and OT are involved in memory and learning, stress regulation and anxiety, and aggressive, sexual and maternal behaviors (e.g. Argiolas 1992, Malik *et al*. 2012, Ebner *et al*. 2013). Moreover, the OTR is involved in thermoregulation and brain development (Gimpl & Fahrenholz 2001, Kasahara *et al*. 2013). In the periphery, the OTR is expressed in the uterus and in the myoepithelial cells of the mammary gland at the end of pregnancy, and plays an important role during labor and milk ejection in a positive feedback manner (Viero *et al*. 2010). Several diseases or pathogenic conditions are associated with the function of this receptor including autism and schizophrenia (Gimpl & Fahrenholz 2001). It was recently reported that OTR and vasopressin receptors form homo- and heterodimers *in vitro* and *in vivo* (Albizu *et al*. 2010*b*). Negative cooperative binding of oxytocin at the OTR was observed in *in vitro* systems, which points to a functional significance of dimerization (Albizu *et al*. 2006). It has been suggested that constitutive homodimerization and heterodimerization between the OTR and the V1aR and V2R is established early in the endoplasmatic reticulum (ER) and that ligand binding at the plasma membrane does not modify the dimerization state (Terrillon *et al*. 2003). Heterodimerization of V1aR and V2R vasopressin receptors determines the interaction with β-arrestin and their trafficking patterns (Terrillon *et al*. 2004). Of note, heteromerization may be of physiological relevance in tissues where the aforementioned receptors are co-expressed, such as heterodimers with the D2 dopamine receptor in the striatum (Romero-Fernandez *et al*. 2013). Heterodimerization was also reported for OTR and β-2 adrenergic receptors. This interaction modifies ERK1/2-activation allosterically in dependency of ligand treatment (agonists or antagonists) (Wrzal *et al*. 2012*a*,*b*). Interestingly, direct interactions between V1b and the corticotropin-releasing hormone receptor 1 were recently described (Murat *et al*. 2012). Via costimulation, this heterodimer mediates a synergistic catecholamine secretion compared with single ligand stimulation. Moreover, vasopressin potentiated CRH-induced cAMP accumulation and CRH potentiated AVD-induced inositol phosphate production. Vasopressin and CRH are both involved in the regulation of adrenocorticotropin and insulin release (Murat *et al*. 2012).

Four major dopaminergic pathways exist in the central nervous system (CNS). The nigrostriatal system is critical for movement and the mesolimbic and mesocortical neurons play a major role in cognitive functions including feeding, affect, reward, sleep, attention, memory and

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learning (Perreault *et al*. 2014). Dopamine receptors have been suggested to be divided into two classes related to their signaling capacities (Beaulieu & Gainetdinov 2011): D1-class receptors (D1 and D5 subtypes) activate the adenylyl cyclase and increase cAMP levels; and D2-class receptors (D2, D3 and D4 subtypes) inactivate the adenylyl cyclase. Regions where the D2R is expressed include the striatum, nucleus accumbens, olfactory tubercle and hippocampus (Beaulieu & Gainetdinov 2011). The D2R comprises homomers (Guo *et al*. 2003, 2005) and a multitude of heteromers (Perreault *et al*. 2014), e.g. with the D1R, D4R, D5R, adenosine A2 receptor (A2R), serotonin 2A receptor (5HT-2AR), OTR, and the histamine H3 receptor (H3R). A functional relevance for the D1R/D2R heterodimer has not yet been confirmed (Frederick *et al*. 2015). Recently, *in vivo* relevance for GHSR/D1R interrelation was found to be involved in hippocampal behavior and memory (Kern *et al*. 2015). Dopaminergic pathways play a critical role in diseases such as Parkinson's disease (nigrostriatal system), schizophrenia, and addiction (mesolimbic and mesocortical system) (Li & Ma 2013). In schizophrenia, cortical D2Rs may be hypersensitive to dopamine (Seeman *et al*. 2005, 2007) or present in increased amounts in some regions.

The prokineticin system is related to several physiological and pathological conditions (Maldonado-Perez *et al*. 2007, Negri *et al*. 2007, Zhou *et al*. 2012). Knockout mice models for both ligand and receptor revealed the role of PROK2 signaling in olfactory bulb morphogenesis and sexual maturation, and suggested PROK2 and PROKR2 as strong candidate genes for human GnRH deficiency (Ng *et al*. 2005, Matsumoto *et al*. 2006). The PROKR2 is involved in the pathogenesis of hypogonadotropic hypogonadism (Dode *et al*. 2006, Bonomi *et al*. 2012). Pathogenic germline PROKR2 mutations associated with central hypogonadism have been identified to cause modulation of distinct intracellular pathways (biased signaling modulation) (Libri *et al*. 2014, Sbai *et al*. 2014), whereby the PROKR2 wild type activates different G-protein subtypes (Chen *et al*. 2005). *PROKR2* variants have been further described in patients with idiopathic combined pituitary hormone deficits (CPHD) including gonadotropin deficiency (Raivio *et al*. 2012, Reynaud *et al*. 2012). The PROKR2 also forms constitutive homomers *in vivo* (Marsango *et al*. 2011), and specific protomer–protomer interfaces for the oligomer have been suggested (Sposini *et al*. 2015). Future investigation of the relationship between oligomerization and pathogenic biased signaling modulation at this receptor is of interest.

GPCRs in interaction with non-GPCR proteins

In addition to the above-described interactions between GPCRs in homomeric or heteromeric constellations, GPCRs are also able to associate with proteins that are capable of modifying signaling properties. They may impact trafficking or act as allosteric modulators (Maurice *et al*. 2011*a*). Furthermore, GPCRs also act as scaffolding proteins that link the receptor to downstream effectors (Walther & Ferguson 2015). Interacting proteins may interfere with the receptor either on the intracellular site or on the transmembrane spanning proteins and interact with the transmembrane helices (Kristiansen 2004).

Intracellular interacting proteins

Many interacting proteins exist, modifying the efficacy or mode of signaling and localization of the GPCRs (reviewed in Ritter & Hall 2009, Maurice *et al*. 2011a, Walther & Ferguson 2015). Examples of intracellular proteins can be subdivided into arrestin, PDZ (postsynaptic density 95/disc large/zona occludens-1)-containing proteins, and non-PDZ-containing proteins (Walther & Ferguson 2015), which are a large group of scaffolding proteins modifying GPCR functions. PDZ-containing proteins are a diverse group of proteins such as the Na+–H+ exchange regulatory factor (NHERF) (Dunn & Ferguson 2015), which changes signaling properties from Gs/adenylyl cyclase activation of the parathyroid hormone receptor (PTHR) to Gq/11 phospholipase C signaling (Ardura & Friedman 2011). For β-adrenergic receptor 2 (ADRB2), NHERF1 acts as a trigger for receptor resensitization, whereas in the absence of NHERF1, ADRB2 is degraded (Hall *et al*. 1998). A further example for non-PDZ-containing proteins that modulate GPCR function is the adaptor protein 14-3-3tau, which decreases FSH-induced cAMP accumulation (Cohen *et al*. 2004). Moreover, lysosomal degradation for the δ-opioid receptor occurs in the presence of an additional type of interacting protein, the GPCR-associated sorting protein 1 (GASP1), whereas in the absence of GASP1, the receptor is resensitized (Whistler *et al*. 2002).

Interacting transmembrane proteins

Identification of receptor activity modifying proteins (RAMPs), the single transmembrane-spanning proteins, underline a new mode of the functional regulation of GPCRs. It was initially identified as an obligatory factor for calcitonin gene-related peptide (CGRP) to activate its receptor, the calcitonin receptor-like receptor

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(CL-R) (McLatchie *et al*. 1998). Different members of RAMP modify ligand specificity such as RAMP1 associated with CL-R to facilitate CGRP binding; however, association with RAMP2 to CL-R enables adrenomedullin to bind to CL-R (McLatchie *et al*. 1998). Since then, different members of the RAMP family have been identified. All RAMPs interact with CL-R, the calcitonin and the VPAC1 receptor; however, other GPCRs, such as glucagon or the PTHR, function together with distinct RAMPs (Hay *et al*. 2006).

The importance of interacting proteins for GPCR function is further evidenced by various examples. Previously, investigation of the functional aspects of the ACTH receptor (MC2R, melanocortin 2 receptor) *in vitro* was inconceivable as cell surface expression in different cell types was impossible. This changed with the identification of the melanocortin receptor-associated protein (MRAP) (Metherell *et al*. 2005), a single transmembrane-spanning protein that escorts the MC2R to the cell surface. Other melanocortin receptors (MC1R, MC3R, MC4R and MC5R) are able to traffic to the cell surface in the absence of interacting proteins; however, melanocortin receptor-associated protein 2 (MRAP2), which is primarily expressed in the hypothalamus, has been identified to influence the function of these MCRs (Cooray & Clark 2011, Jackson *et al*. 2015). Moreover, prokineticin receptor-1 (PROKR1) is the first non-melanocortin GPCR that is regulated by MRAP2 (Chaly *et al*. 2016). This suggests a new pathway by which MRAP2 regulates energy homeostasis by inhibition of PROKR1.

Existence of direct interactions between GPCRs/ion channels and GPCRs/transporters

Direct protein–protein interactions have also been reported for GPCRs and ion channels such as KIR channels or voltage-gated calcium channel Cav 2.2 that form a macromolecular ion channel/GPCR signaling complex (Doupnik 2008, Benleulmi-Chaachoua *et al*. 2016). In turn, this leads to the idea of the possibility for a direct interaction and functional association between GPCRs and further transmembrane-spanning proteins. Channel types such as Ca2+ ion transporting transient receptor potential channels (TRPs) (Veldhuis *et al*. 2015) or substrate transporters, such as members of the major facilitator superfamily (MFS) (Law *et al*. 2008), are known to be closely linked to the function of GPCRs or vice versa. Specific signals from GPCRs located on sensory neurons converge on members of the TRP family, leading to channel sensitization and activation, which amplify e.g. pain, itch and neurogenic inflammation (Veldhuis *et al*. 2015). On the other hand, several GPCR ligands are transported out of the cell by members of the MFS with 12-transmembrane helices (e.g. dopamine transporter). Furthermore, transporter substrates, such as glucose for cell metabolism, are transported into the cell by MFS members (e.g. glucose transporter (GLUT)). There are few examples that support the notion of a direct GPCR/channel (Yekkirala 2013) or GPCR/transporter interactions, e.g. the observed interplay between the ADRB2, GLUT4 and melatonin 1 receptor/Cav 2.2 (Dehvari *et al*. 2012, Benleulmi-Chaachoua *et al*. 2016), or modulation of monoamine transporters by common biogenic amines via the TAAR1 (Xie *et al*. 2008). Altogether, this raises the possibility that an association between different proteins and GPCRs should be of importance due to a fast mutual or synergistic influence on functional properties. This hypothesis requires further and solid confirmation and validation.

GPCR oligomerization in endocrine dysfunctions

Is there a direct link between GPCR oligomerization and pathophysiology?

Malfunctions of approximately 100 GPCRs are associated with various human diseases (Hutchings *et al*. 2010) including cancer, viral infections, inflammation, infertility, and metabolic and neurological disorders (Seifert & Wenzel-Seifert 2002, Dorsam & Gutkind 2007, Garcia-Jimenez & Santisteban 2007, Schoneberg *et al*. 2004, Vassart & Costagliola 2011). Almost 20% of tumors harbor mutations in GPCRs and approximately 10% of cancer-related mutations have been identified in G-proteins (O'Hayre *et al*. 2013). An overview of the diverse roles of G-protein-coupled receptors (GPCRs) in the pathophysiology of various human diseases, subdivided into associations with obesity, diabetes, cardiovascular diseases, allergies or cancers, can be found in the excellent review by Heng and coworkers (2013). The pathogenic triggers at GPCRs vary from hormonal dysregulation, virus infections, mutations, or interactions with autoantibodies. Interestingly, it was recently reported that 365 human GPCRs are potential drug targets (Garland 2013).

As described in the numerous examples above, oligomerization is a common property for GPCRs (Ferre & Franco 2010) and can also be addressed to receptors

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involved in endocrine regulation such as the TSHR, LHCGR or MC4R (Table 2). Secondly, oligomerization is of functional relevance, although it has/could not have been rendered more precisely for all the GPCRs described here so far. By combining these two facts, that the correct function of GPCRs correlates with a specific protomer/protomer arrangement, makes it very likely that a dysfunction of GPCR signaling can also be due to modified oligomeric properties, e.g. by mutants in the protomer interfaces or in the ligand binding sites.

Despite the huge and increasing amount of *in vitro* findings and potentially artificial data, the relevance of GPCR oligomerization *in vivo* has been confirmed by several examples (Overton & Blumer 2000) including cases of pathophysiological importance (Tadagaki *et al.* 2011, 2012*b*, Tschische *et al.* 2011, Kern *et al.* 2015). GPCRs, in relation to neurophysiological processes, are one of the best studied subjects in this field (Gomes *et al.* 2000, 2013, Prinster *et al.* 2005, Fuxe *et al.* 2012). For example, a serotonin/glutamate receptor complex implicated in psychosis has been identified in previous studies and a role for GHSR in modulation of the D1R function in memory and behavior (Gonzalez-Maeso *et al.* 2008, Kern *et al.* 2015). Heteromerization between the dopamine receptor subtypes, D1R and D2R, are likely to be implicated in depression (Rashid *et al.* 2007). The D1R–D2R heterodimer was detected at higher levels in the postmortem striatum of patients than in normal subjects (Pei *et al.* 2010). Furthermore, the pathophysiological relevance of oligomerization was indicated in a specific case for angiotensin receptor II (AT1R) and bradykinin receptor-2 (B2R). The AT1R–B2R heterodimer is supposed to be functionally correlated with preeclampsia, as this heteromer was observed to be more abundant on platelets from pre-eclamptic women than on platelets from normotensive pregnant women (AbdAlla *et al.* 2001). Moreover, for the GABAB receptors, signaling activity is inhibited due to ligand-induced monomerization of the obligatory dimers, which has an impact on neuropathic pain sensitization (Laffray *et al.* 2012).

For GPCRs involved in endocrine regulation, which has omni-relevance in physiology and medicine, the elucidation of heteromeric forms have only recently started to become systematic (e.g. Rediger *et al.* 2009), with the motivation to investigate the entire 'interactome' and 'signalosome' of particular receptors (Table 2). The homomeric arrangement, even in an oligomer–monomer equilibrium (Lambert 2010), defines e.g. the LHCGR or TSHR *per se* as complexes. To date, there have been no difficulties in adequately combining or incorporating this property into current and previous mechanistical explanations of activation or inactivation, with few exceptions (Zoenen *et al.* 2012).

In the GPCRs described here, one particular feature potentially highlights a direct link between oligomerization and loss-of-function phenotypes: the dominant negative effect of reported heterozygote mutants or distinct receptor splice variants. Such dominant negative effects have been observed for numerous GPCRs such as rhodopsin (Kurada & O'Tousa 1995), AVPR2 (Zhu & Wess 1998), CCR5 (Benkirane *et al.* 1997), LHCGR (Tao *et al.* 2004), TSHR (Calebiro *et al.* 2005), MC1R (Beaumont *et al.* 2005), GnRHR (Grosse *et al.* 1997, Brothers *et al.* 2004), MC4R (Biebermann *et al.* 2003, Tarnow *et al.* 2008), or the prostacyclin receptor (Ibrahim *et al.* 2010). Dominant negative AT2R receptor oligomers induce G-protein arrest and symptoms of neurodegeneration (AbdAlla *et al.* 2009). For the aforementioned examples, it is generally assumed that the negative effect of receptor variants is transferred to the wild-type receptor by multimerization with the modified and nonfunctional receptor variant.

For the GHSR, MC3R, MC4R, PROKR2 or GPHRs, the occurrence of loss-of- or gain-of-function mutants in patients with hypo- or hyperfunction have been described (Themmen & Huhtaniemi 2000, Schoneberg *et al.* 2004, Tao 2006, 2010, Vassart & Costagliola 2011, Rediger *et al.* 2012, Troppmann *et al.* 2013, Libri *et al.* 2014, Vassart & Kleinau 2014). In particular, the mutations may cause different mechanisms at the receptor on the molecular level (excellently reviewed in Vassart & Costagliola 2011). In case of gain-of-function, constitutively activating mutations (CAMs) or substitutions leading to a loss of ligand selectivity have been described. The interrelated combination of both mechanisms caused by one single side-chain substitution such as that reported for the FSHR (Smits *et al.* 2003) is also feasible. Inactivating mutants accompanied by a loss-of-function may influence many individual or linked parameters at the receptor or ligand such as decreased expression level or loss of ligand binding capacity. In TSHR, pathogenic mutations lead to diseased conditions such as congenital hypothyroidism or nonautoimmune hyperthyroidism, whereas in the LHCGR, male-limited precocious puberty (by CAMs) or hypogonadism may be associated with inactivating variants, although rare. CAMs of the TSHR have been proven on their impact on oligomerization and a direct modification of oligomeric states has not yet been confirmed (Zoenen *et al.* 2012, Biebermann *et al.* 2012*b*).

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This does not exclude the possibility that natural mutations in other receptors may indeed modulate oligomerization. Moreover, the opportunity that inactivating mutations, or a specific entity, interferes with a proper oligomer function should be considered and systematically proven in future studies.

Open aspects and future perspectives

Oligomerization is widely accepted to be a pivotal aspect of GPCR function regarding transport, signal transduction, signaling regulation and pharmacology. A certain number of examples for relevant oligomers have been reported and will further increase with the use of advanced methods and by directed *in vivo* searches for GPCR oligomers. Therefore, it is necessary to take GPCR oligomers into consideration in comprehensively understanding their physiological roles, and to explain interrelated processes such as pathogenic dysfunctions and clinical disorders, and in endocrine regulation. This perspective opens fascinating avenues for potential directed and selective pharmacological interventions. The incorporation of oligomeric receptor models into strategies for GPCR drug discovery and the targeting of homo- or heteromers are both challenging and exciting for the identification of new perspectives on the mechanisms of established or new therapeutic agents. Therefore, studying GPCR oligomerization and related structural–functional consequences, including the physiological relevance, is of importance. This includes the identification of the particular GPCR interaction partners (interactome analyses), which is dependent on physiological parameters such as cell type or developmental stage.

A further major effort in future studies is the dissection of functional effects induced by either monomers or oligomers. This requires forced monomerization and oligomerization of GPCRs, which is currently difficult to achieve. Only a comparison of the properties of monomers, homodimers, and those of wild-type receptor 'mixtures' present in a monomer/oligomer equilibrium will lead to definitive answers for the functionality of the different assembly states (Song *et al*. 2007, Lambert 2010, Teichmann *et al*. 2012, 2014, Calebiro *et al*. 2013). Of specific note, at the cell surface with a high number of expressed GPCRs (likely to range between 10 and 100s, e.g. Regard *et al*. 2008) or many other transmembrane spanning proteins such as transporters, it must be considered that many different heteromers may indeed exist. Unfortunately, current measurement methods do not simultaneously detect quantitative numbers of homomers, heteromers and monomers. This leaves open the possibility of multiheteromers and multiple equilibriums for different oligomers, expecting diverse oligomers of a particular GPCR at the same time point. Finally, these questions must be tackled in future studies, in line with the importance of elucidating proteins expressed at specific cell types and the further development of methods to study functional oligomers in *in vivo* systems.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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