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Allosteric small molecule modulators of nuclear receptors

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ABSTRACT

Nuclear Receptors (NRs) are multi-domain proteins, whose natural regulation occurs via ligands for a classical, orthosteric, binding pocket and via intra- and inter-domain allosteric mechanisms. Allosteric modulation of NRs via synthetic small molecules has recently emerged as an interesting entry to address the need for small molecules targeting NRs in pathology, via novel modes of action and with beneficial profiles. In this review the general concept of allosteric modulation in drug discovery is first discussed, serving as a background and inspiration for NRs. Subsequently, the review focuses on examples of small molecules that allosterically modulate NRs, with a strong focus on structural information and the ligand binding domain. Recently discovered nanomolar potent allosteric site NR modulators are catapulting allosteric targeting of NRs to the center of attention. The obtained insights serve as a basis for recommendations for the next steps to take in allosteric small molecular targeting of NRs.

1. Introduction

1.1. Structural organization of nuclear receptors

The nuclear receptor (NR) superfamily consists of 48 members that are structurally related, but have functions in all kinds of physiological processes and, in connection with that, diseases. Their key role in these processes makes them a very attractive drug target. NRs have a conserved domain organization starting at the N-terminus with the highly variable N-terminal domain (NTD). For most NRs this domain contains a ligand-independent activation function (AF1). The NTD is intrinsically disordered (ID), but the interaction with binding partners can induce folding which can enhance transcriptional activity (Kumar and Litwack, 2009; Helsen and Claessens, 2014). The NTD is followed by the DNA binding domain (DBD) which uses its two zinc fingers to recognize specific hormone response elements (HREs). The affinity for specific HREs is dependent on the NR subtype and NR homo- or heterodimerization, or in some cases monomeric binding to extended HREs (Helsen et al., 2012a). The DBD is connected to the ligand binding domain (LBD) via a hinge region that typically contains a nuclear localization signal and undergoes posttranslational modifications (PTMs) (Haelens et al., 2007). The LBD is a highly conserved domain typically consisting of 12 helices which are organized in an antiparallel, globular arrangement (Bourguet et al., 1995). The classical "mouse-trap" model

states that when an agonistic ligand binds in the ligand binding pocket (LBP), helix 12 (H12) covers the pocket and creates a surface suitable for coactivator binding (Renaud et al., 1995). Nowadays, this model is under debate and an alternative "dynamic stabilization" model is evoked (Pissios et al., 2000). This concept states that the LBD in its apo form is in a partially molten state and can adopt a wide range of conformations. Agonist binding stabilizes the fold of H12 and the surface elements required for coactivator binding in the active conformation (Rastinejad et al., 2013). Inverse agonists, on the other hand, can increase corepressor recruitment which represses transcription in constitutively active NRs (Kojetin and Burris, 2013). Antagonists do not cause cofactor recruitment, but do prevent agonist binding and therefore passively repress transcription (Kojetin and Burris, 2013). C-terminal to the LBD, certain NRs have an additional F-domain that has a variable length and has functions ranging from interacting with other proteins to stabilizing the ligand bound conformations of the LBD (Patel and Skafar, 2015).
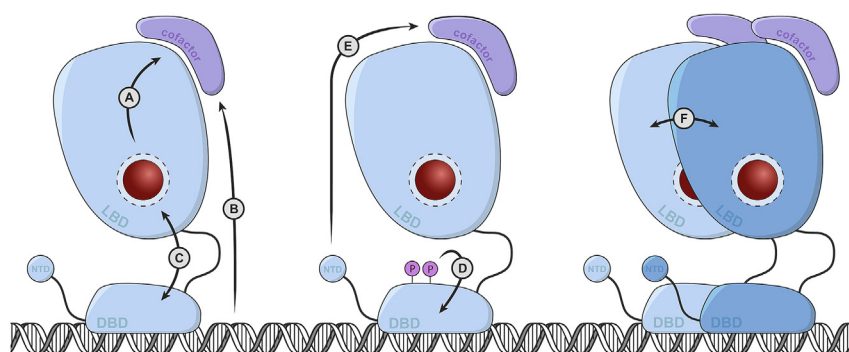
1.2. Inter-domain allosteric regulation of nuclear receptor activity

Up until a few years ago, NR structural information was only available for certain separate domains. This did give a lot of information on ligand- and DNA binding as well as on dimerization of separate LBDs or DBDs, but left a demand for information on the implications of

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Fig. 1. Conceptual modes of endogenous NR allosteric regulation. A) From the Ligand Binding Pocket to the cofactor binding site B) From the DNA to the cofactor binding site C) Between the DNA Binding Domain and the Ligand Binding Domain D) Via post-translational modifications within and over NR domains E) From the N-terminal domain to the cofactor binding site F) Between NR dimerization partners.

inter-domain communication (Rastinejad et al., 2013). Since 2008, multi-domain structures obtained via different scattering techniques started appearing (Chandra et al., 2008; Orlov et al., 2012; Rochel et al., 2011). These structures provided more insight into the interplay between the different NR domains. In particular, it became clear that the different domains within a NR act and communicate via modulatory, allosteric mechanisms. Allostery, in general, is the process where binding of an interaction partner, e.g. ligand or protein, at one site of a protein results in a functional change at another, topographically distinct, site (Motlagh et al., 2014; Nussinov & Tsai, 2013). This means that there is communication over a distance between the binding site, 'input', and the site of the biological response, 'output', via a conformational change in the protein structure (Changeux and Christopoulos, 2016). Different endogenic types of allosteric regulatory mechanisms have been described for NRs as conceptually summarized in Fig. 1.

First of all, and most obvious, the majority of NR ligands bind to the LBP, which causes a conformational change in the LBD and as a result regulates cofactor recruitment elsewhere on the LBD, outside of the LBP (Fig. 1A). (Hilser and Thompson, 2011) As an illustration, Folkertsma et al. showed that by clustering correlations between the ligand binding mode and peptide recruitment can be delineated (Folkertsma et al., 2007). This can be helpful to identify compounds which cause the anticipated cofactor recruitment (Folkertsma et al., 2007). Besides this prime NR allosteric mechanism, other and frequently more subtle allosteric mechanisms can be found in endogenous NR regulation.

The interplay of the specific HRE with the cofactor binding site of a NR is an allosteric mechanism via inter-domain communication (Fig. 1B). This interplay has been studied, amongst others, in the estrogen receptor (ER). Using a phage ELISA assay, cofactor recruitment in response to various estrogen response elements (EREs) was tested by Hall et al. (2002). They showed that the structure of the coactivator pocket is influenced by the type of ERE, pointing to the ERE as a regulator of biological activity (Hall et al., 2002). A similar example was found for the glucocorticoid receptor (GR). The GR binding sequence (GBS) affinity and transcriptional activity could not be directly correlated and it was found that the only structural difference in the DBD upon binding of different GBSs occurs in the "lever arm" loop that connects the DNA recognition helix and the dimerization domain (Meijsing et al., 2009). Helsen et al. proposed that these subtle differences are propagated through the full-length receptor (Helsen et al., 2012b). Molecular dynamics (MD) simulations of the GR DBD in its DNA bound and free state by Frank et al. gave an explanation of the allosteric behavior since conformational sampling of the lever arm is observed to be reduced by DNA binding and dimerization (Frank et al., 2018). The small number of distinct conformations the DNA-bound DBD can adopt, might provide recruitment of different co-regulators (Frank et al., 2018).

Several additional studies have been performed to obtain a better understanding of the allosteric communication between DBD and cofactor binding site, e.g. for the thyroid receptor (TR) (Putcha and

Fernandez, 2009), VDR-RXR heterodimer (Zhang et al., 2011) and PPARγ-RXRα complex (de Vera et al., 2017). In these studies, isothermal titration calorimetry (ITC) and hydrogen/deuterium exchange (HDX) techniques were used, showing an enhancement of cofactor recruitment upon binding of the receptor complex to its HRE.

The LBD and DBD can also allosterically influence each-others ligand affinity, as exemplified by Helsen et al. (2012b) (Fig. 1C). By overlaying the androgen receptor (AR) LBD and DBD crystal structures with the full-length heterodimeric PPARγ-RXRα crystal structure, residues in the LBD-DBD interaction surface were identified (Helsen et al., 2012b; Chandra et al., 2008). Residues from this surface that are known to correlate with pathologies were mutated and their effect on ligand and DNA binding was analyzed (Helsen et al., 2012b). Four of these mutations in the LBD led to reduced DNA binding and transactivation while not affecting ligand binding, conversely three DBD mutations reduced ligand binding and transactivation, but not DNA binding (Helsen et al., 2012b).

Another example of allosteric mechanisms in NR regulation is their mediation via post-translational modifications (PTMs) present at one site of the protein that can have various effects, including modifying cofactor interactions, changing cellular localization, regulating protein stability and influencing DNA binding at a different site (Fig. 1D). (Becares Salles et al., 2016) The most intensively studied PTMs on NRs are phosphorylation, acetylation, ubiquitinylation and SUMOylation. Examples of these are known for almost all NRs and their functioning, including allosteric modes, has been reviewed elsewhere (Becares Salles et al., 2016; Faus and Haendler, 2006; Anbalagan et al., 2012; Brunmeir and Xu, 2018).

The NR NTD has also been described to be involved in allosteric communication (Fig. 1E). Khan et al., for example, showed that when the TATA box binding protein (TBP) binds the GR NTD, this induces an increased helical content, as observed in circular dichroism, and the interactions with coactivators and transcriptional activity were, as a result, enhanced (Khan et al., 2011). Similar observations were found for the mineralocorticoid receptor (MR) and progesterone receptor (PR) which supports the notion that there is a common mechanism of regulation via the NTD, despite its poorly conserved sequence (Fischer et al., 2010; Kumar et al., 2013).

Homodimerization or heterodimerization with the retinoid X receptor (RXR) is a common phenomenon for NRs. These processes, similar to the binding of other protein partners, change the structural plasticity, and thereby the behavior of a NR (Jin and Li, 2010). This, in turn, can allosterically affect the recruitment and binding of ligands. As an example, the retinoic acid receptor β (RARβ) is known to be a monomer in solution, but upon binding of cofactors, it forms a homodimeric assembly (Venepally et al., 1997). Similar behavior was found for VDR and TR when bound to coactivators (Takeshita et al., 2000; Velasco et al., 2007). In order to dimerize, the receptor has to undergo a conformational change, which can lead to asymmetry in the dimer complex. For both RARβ and the estrogen receptor α (ERα) it was observed that in the homodimer complex, this asymmetry was altering

the binding mode of the ligand, as revealed for example via two different poses of the same ligand in each the two monomers (Delfosse et al., 2012; Billas and Moras, 2013). Furthermore, it has been reported that also in the absence of a ligand, the NR homodimer can be asymmetric. The crystal structure of the apo-PPARγ LBD contains two monomers in the asymmetric unit forming a homodimer, with only one of the monomers present in its active conformation (Nolte et al., 1998).

The above summary acts to illustrate that NR regulation involves several allosteric mechanisms. For a more detailed review on this subject the reader could consider an extensive review by Fernandez (2018). Despite these various endogenous allosteric mechanisms, the number of actual endogenic *molecules* known to act via allosteric pockets on the NR, is extremely limited. In this review, allosteric processes in NR modulation using small molecules will be discussed. Allosteric NR modulation by synthetic small molecules is a relatively new area of research, in contrast to the classic targeting of the orthosteric LBP (Moore et al., 2010). While extremely successful, targeting NRs via the orthosteric LBP also brings along certain limitations and challenges (Caboni and Lloyd, 2013). Selectivity issues can arise due to a high degree of similarity in the LBP between certain NRs, which can lead to side effects. Furthermore, mutations can occur in the LBP upon prolonged drug-treatment, which can lead to drug resistance or even agonist/antagonist switching (Caboni and Lloyd, 2013; Lu et al., 2014; Liu et al., 2016). Allosteric modulation of NRs with small molecules, via targeting binding sites beyond the orthosteric pocket, could therefore be a valuable alternative way of targeting NRs that could potentially overcome some of the described shortcomings of orthosteric ligands.

In this review, first the general concept of allosteric modulation in drug discovery is discussed, specifically focused on the different types of receptor classes, as a background setting and inspiration for future NR focused work. The body of the review will then focus on examples of small molecules that allosterically modulate the NR LBD. Finally, the obtained insights will be translated into recommendations for future research.

2. Small molecule allosteric modulation in drug discovery

2.1. Allosteric small molecule modulators and their potential in drug discovery

The vast majority of receptor classes possess endogenous ligands that bind to a defined site, typically termed the orthosteric binding site. Most drugs on the market are similarly targeting these orthosteric pockets and are in essence thus competing with the endogenous ligands (Moore et al., 2010; Caboni and Lloyd, 2013; Tice and Zheng, 2016). Allosteric ligands bind to sites on proteins that do not overlap with the orthosteric site and are therefore potentially resistant to orthosteric competition (van Westen et al., 2014). For multiple receptor classes, such as GPCRs and ligand-gated ion channels, but also for enzymes, a significant number of allosteric modulators have already been identified with considerable success (Foster and Conn, 2017; Taly et al., 2014; Changeux and Christopoulos, 2017; Thal et al., 2018; Gentry et al., 2015). Especially for GPCRs and proteins kinases, multiple allosteric ligands have been identified with some being approved by the FDA, such as Sensipar and Gleevec, and multiple drugs are undergoing clinical trials (Changeux and Christopoulos, 2017; Block et al., 2004; Buchdunger et al., 1996). For some of these proteins, allosteric modulation might be the sole option to selectively target them because of the evolutionarily conserved nature of their orthosteric site, such as the ATP binding site of kinases (Ghoreschi et al., 2009). Within the last two decades, an increasing number of connected publications can be observed, demonstrating the growth in interest and development of allosteric modulators (Fig. 2). During this time, multiple excellent review articles have been published for receptor classes that can be targeted by allosteric modulators (Changeux and Christopoulos, 2016; Foster and Conn, 2017; Taly et al., 2014; Thal et al., 2018; Gentry et al., 2015; Lisi
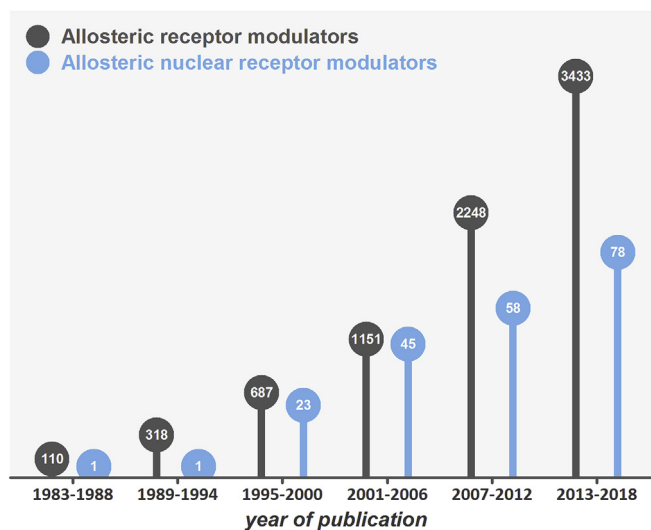
Fig. 2. Results of a SciFinder search showing the number of publications on 'allosteric receptor modulators' (shown in grey) between 1983 and 2018. The subset of these publications focusing on 'allosteric ligands for nuclear receptors' is shown in blue.

and Loria, 2017). NRs have similarly seen an increase in attention regarding the identification of allosteric ligands, but here the number of publications is still lagging strongly behind that of other receptor classes.

The rapid increase in the attention for allosteric modulators can be explained by several key advantages such ligands have in comparison with orthosteric ligands. First, allosteric sites show greater structural diversity compared to the more conserved orthosteric sites. This could thus lead to a higher degree of selectivity for closely related proteins (Lu et al., 2014; Dixon et al., 2016; Nussinov and Tsai, 2013). Second, as mentioned earlier, allosteric compounds are typically not in competition with the receptor's endogenous ligand. Therefore, allosteric modulators could eventually be used at lower concentrations resulting in reduced side effects. Furthermore, in the case of drug-resistant mutations in the orthosteric binding site, allosteric ligands can pose a promising alternative entry. In addition, since the binding of the allosteric ligand can potentially modulate the binding affinity and efficacy of the orthosteric ligand, a pathway is provided to fine-tune the physiological response of the both. Finally, the chemical structure properties of allosteric ligands are slightly more beneficial over orthosteric ones, as explored by Westen et al. (van Westen et al. (2014)) Most importantly here, allosteric modulators appear to be more rigid and they adhere slightly better to Lipinski's rule of 5 which gives a higher potential for good oral drug uptake (van Westen et al., 2014). Despite these favorable characteristics, the challenge of discovering allosteric drugs is a highly relevant current limitation, since allosteric sites are often not identified or lack specific assay formats for target ligand screening (Nussinov and Tsai, 2013). At present, the majority of potential drug molecules are identified using high-throughput screening (HTS), for which the binding affinity of the ligand is often a hallmark to define the quality of the hit. The orthosteric site can, however, be expected to be dominant in this aspect, limiting the identification of ligands with allosteric binding modes. In addition, especially allosteric ligands for GPCRs are notorious to have a flat 'SAR', meaning that minor modifications on the modulator scaffold lead to a complete loss of activity, further complicating the discovery and optimization of these ligands (Conn et al., 2012).

The different modes-of-action of allosteric modulators described in literature, as well as the different assay techniques developed for the identification of such allosteric ligands, can serve as inspiration for allosteric targeting of NRs. A compact summary of these concepts,
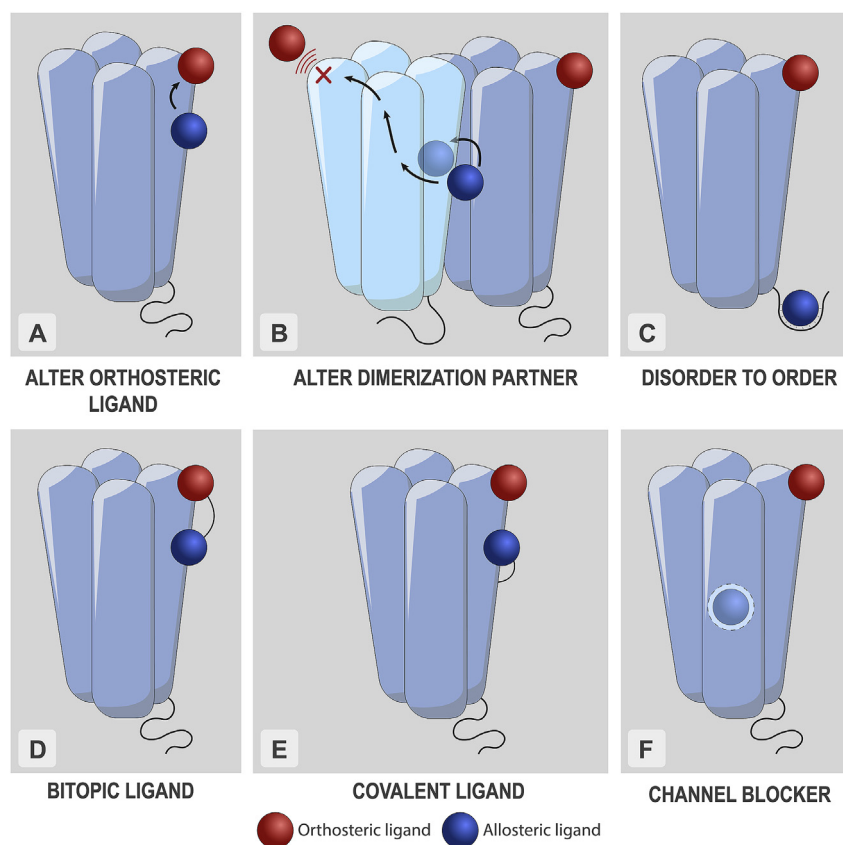
Fig. 3. Schematic representation of six different modes of allosteric modulation of receptor activity.

illustrated with selected highlights, as described for other receptor classes is therefore discussed below.

2.2. Mechanisms of action of allosteric receptor modulators

The mechanisms by which allosteric modulators exert their function have been investigated in molecular detail, especially using crystal- and solution-based structural data. Allosteric modulators can induce an altered physiological response in multiple ways, because their binding changes the preference of populating certain protein conformations, alone or in consort with an endogenous ligand for the orthosteric site (Fig. 3). The section below is geared mostly to membrane proteins and specifically GPCRs as the studies of these proteins serve as important illustrative examples for the much less investigated small molecule NR allosteric modulation.

The majority of the allosteric ligands present in literature bind to pockets in an area surrounding the canonical binding site. This, in turn, influences the binding affinity and efficacy of the orthosteric ligand (Fig. 3A). (Changeux and Christopoulos, 2017; Kenakin, 2014) Benzodiazepines are a group of very successful modulators that bind to the Gamma-aminobutyric acid A (GABA$_A$) receptor on the extracellular domain (ECD) (Lu et al., 2014; Smith and Olsen, 2000). Binding induces a significant conformational shift in the protein, leading to a greatly enhanced affinity for the natural ligand GABA (Williams and Akabas, 2000). Nevertheless, an allosteric molecule does not have to be in close proximity of the orthosteric site to alter the binding kinetics of the endogenous ligand. Multiple examples are known in literature where allosteric ligands bind to a distant site while still exerting an influence on the endogenous ligand (Changeux and Christopoulos, 2017; Smerdon et al., 1994; Horn and Shoichet, 2004; Oikonomakos et al., 2000). This arises from the fact that allosterically induced conformational shifts can be at one part of the protein, but can also change the conformation of the entire protein (Oikonomakos et al., 2000; Pargellis

et al., 2002).

The dimerization behavior of a protein can also be altered by allosteric ligands (Fig. 3B). Allosteric modulation of receptor tyrosine kinases (RTKs) primarily involves monoclonal antibodies targeting the ECD (Cho et al., 2003; Franklin et al., 2004; Hicklin et al., 2001). The function of the RTK HER2/neu, which is involved in breast cancer, can be inhibited by antibodies using specifically two approaches. Trastuzumab binds to subdomain IV of the HER2/neu RTK thereby inhibiting its activation. Pertuzumab, on the other hand, interacts with subdomain II, which inhibits the activation by blocking dimerization (Cho et al., 2003; Fendly et al., 1990). Also, for the insulin RTK antibodies were identified that influence dimerization behavior (Bhaskar et al., 2012; Corbin et al., 2014). Very recently, the covalent modulator JZ-5209 was identified to bind to the β-Glucocerebrosidase (GCase). This small molecule covalently binds to a lysine residue which is present at the dimerization interface of the GCase, enhancing dimerization and exposing the active site (Zheng et al., 2018).

Intrinsically disordered (ID) protein domains have also come to the forefront for allosteric regulation with small molecules (Fig. 3C). The absence of a defined secondary structure in these domains makes them devoid of a classical binding pocket (Dunker et al., 1998; Wright and Dyson, 2015). Nevertheless, small molecules and macromolecules have been shown to be capable of binding to these regions and of subsequently inducing a defined secondary structure (Motlagh et al., 2012, 2014). The protein tyrosine phosphatase 1B is a therapeutic target for both diabetes and obesity. This protein contains an ID domain at its C-terminus which is located close to the catalytic domain. The small molecule MSI-1436 binds to this ID site with high affinity, thereby locking the protein into an inactive conformation and reducing the phosphatase activity (Smith et al., 2017).

Bitopic ligands, also known as dualsteric ligands, are allosteric modulators in which the orthosteric and allosteric pharmacophore are covalently linked, allowing them to bind to both pockets

simultaneously (Fig. 3D). Apart from ensuring a stronger binding of the bitopic ligand to the receptor, the additional allosteric pharmacophore can lead to an improved selectivity and a nuance in receptor activation (Catterall et al., 2007; Lane et al., 2013; Mohr et al., 2010). The bitopic ligand THRX160209 targets the muscarinic M₂ acetylcholine receptor, which is a member of the GPCR protein family. For this receptor, the orthosteric site, located in the narrow cavity formed by the seven transmembrane helices, and the allosteric site, located in the ECD, are in close proximity of each other. By combining both pharmacophores, the binding affinity was enhanced with several orders of magnitude. Interestingly, THRX160209 showed to have a superior specificity for the M₂ subtype over other four very homologous subtypes (Steinfeld et al., 2007). A similar mode of action is observed for the bitopic ligand SB269652, which binds to both D2 and D3 dopamine receptors with high affinity. In the D₂R dimer, SB269652 binds to one monomer which reduces the binding of the orthosteric agonists and inverse agonists in the other monomer (Lane et al., 2014).

Certain receptor proteins contain allosteric pockets featuring a re-active group that can be addressed with a covalent ligand (Fig. 3E). From a therapeutic point-of-view, such reactive groups are especially relevant when they represent mutated residues correlated to disease development. An example of a covalent modulator is the previously discussed compound JZ-5209, which enhances the dimerization of GCase (Zheng et al., 2018). In addition, the GTPase K-Ras can be tar-geted mutant specifically, exploiting the oncogenic G12C mutation. This mutation diminishes the GTP hydrolysis and is closely located to both the nucleotide and the effector binding pocket. By covalently linking compounds to this cysteine residue, the compounds allosteri-cally control the GTP affinity and the normal function of the enzyme can be restored. Since these compounds make use of a mutated residue, the natural protein is not affected (Ostrem et al., 2013).

A final example of allosteric modulation relates to ion channels, responsible for the ion exchange over the cell membrane and of parti-cular relevance in the central nervous system. In general, ion channels are either activated by ligand binding to the extracellular domain, in the case of ligand-gated ion channels, or through a change in membrane potential, in the case of voltage-gated ion channels (Waszkielewicz et al., 2013; Niemeyer et al., 2001). Allosteric ligands and toxins tar-geting this class of receptors can bind in the channel of the protein, thereby physically blocking the ion influx (Fig. 3F). These so-called "channel blockers" are predominantly found as the active substance in animal venoms (Catterall and Swanson, 2015; Cestèle and Catterall, 2000). In recent years, these venoms were used as an inspiration to synthesize synthetic ligands and peptides that target these channels, some of which are used in the treatment of heart diseases and cancer (Catterall and Swanson, 2015; Buchanan and McCloskey, 2016).

2.3. Techniques for the identification of allosteric ligands

The increase in focus on the modulation of proteins by allosteric ligands comes with a concurrent demand for novel screening techniques that both aid in the identification of allosteric pockets and in the dis-covery of small molecules specifically targeting these pockets. Some of the most promising techniques and examples, including computational approaches, fragment soaking, tethering and phage display, are shortly summarized mentioned here to act as a potential framework to be translated to NRs.

Crystal structures of ligand-bound proteins provide high-resolution information about the binding mode of the ligand. The resulting static picture can be further refined with techniques providing dynamic in-formation (Carvalho et al., 2010). Over the last decades, numerous computational methods have been developed to provide a better un-derstanding of the dynamic behavior of the protein, which is of high relevance for the identification of allosteric sites (Huang et al., 2013, 2015; Lu et al., 2018; Greener and Sternberg, 2018; Lu and Zhang, 2017). The computer-aided drug design allows to help understand how

allosteric modulators exert their function (Macalino et al., 2015). Making use of molecular dynamics, ligands could successfully be identified that allosterically bind to the β2-adrenergic class of GPCRs. Also, a hidden allosteric site in the oncogenic K-Ras4B protein was found using molecular dynamics by sampling a conformation of the protein which was not observed in the crystal structure (Lu et al., 2018).

Fragment-based drug discovery is achieving a strong impact for discovering allosteric binding sites, for example via X-ray crystal-lography based fragment soaking approaches (Kuo, 2011). Typically, cocktails of 5–10 fragments are soaked into a crystal of the protein of interest (Kuo, 2011; Bauman et al., 2014). When one or more fragments bind to the protein the additional electron density directly reveals the location of the fragment's binding pocket and the structure of the fragment. As an example, for the HIV-1 reverse transcriptase enzyme, three new binding sites were found that also proved to be inhibitory in enzymatic assays (Bauman et al., 2014). Similarly, for tyrosine phos-phatase 1B 11 binding sites outside the active site have been elucidated (Keedy et al., 2018).

Tethering is not only a technique applied to discover covalent drug binding or orthosteric drugs, but it could also be considered as a technique to identify ligands for allosteric pockets. Different tethering strategies have been developed, of which for example disulfide te-thering is well-known. This technique involves the formation of a re-versible disulfide bond via the linking of a thiol-containing fragment to a, potentially genetically introduced, cysteine residue in a protein (Erlanson et al., 2004; Hardy, 2008). At equilibrium, the mixture will consist predominantly of the protein bound to the fragment with the highest binding affinity. By use of the covalent bond formation between the fragment and the protein, the affinity of the fragment for the target protein is amplified, which can be useful for detection at lower con-centrations. The direction of the ligands to a specific site makes the approach particularly useful in case of a known allosteric site for which novel allosteric ligands should be identified.

Phage display methods have been used occasionally in the search for allosteric receptor modulators. The technique involves the expres-sion of a random library of peptides on the coat proteins of bacter-iophages (Pande et al., 2010). By physically linking the peptide and its genetic material, novel ligands with desired properties can be easily identified via this approach. A recent study by Tipps et al. uses a combination of phage display technology and standard electrophysical testing for the discovery of novel allosteric modulators for the glycine receptor, a member of the superfamily of ligand-gated ion channels (Tipps et al., 2010). The unbiased nature of the phage display technique provides potential for the identification of allosteric modulators for targets of which specific allosteric pockets have not yet been identified.

3. Nuclear receptor allosteric modulators

NRs, like other receptors, have been targeted on allosteric sites. At the moment, the most studied allosteric modulators include those that target the activation function 2 (AF-2) cofactor binding site, the DBD or DNA response elements, modulate the receptor dimerization, modulate PTMs or target alternative sites in the LBD. These different classes will be discussed briefly, with the main focus on the alternative pockets in the LBD. In the context of this review, allosteric compounds are con-sidered as compounds that bind at a different site than the orthosteric site and lead to stabilization of other NR conformations, and not only inhibit, for example, DNA or coactivator binding.

3.1. Approaches for allosteric modulation of NRs on and beyond the ligand binding domain

NRs are typically activated by the binding of agonistic ligands, re-sulting in the recruitment of specific cofactors leading to a certain biological response via enhanced gene transcription. One type of
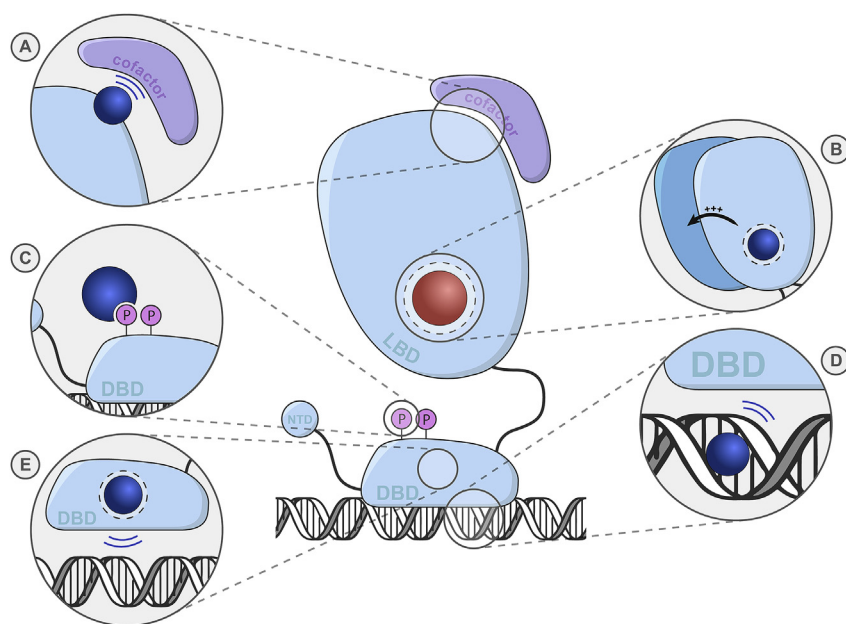
Fig. 4. Different modes of actions for small molecule-based allosteric NR modulation outside of the ligand binding domain. A) Via binding to the cofactor binding site. B) Via compounds modulating the NR dimerization. C) Via the recognition of post-translational modifications. D) Via modulation of the DNA response element. E) Via binding to the DNA binding domain.

alternative NR modulation is, therefore, via the direct inhibition of cofactor binding at the AF-2 site (Fig. 4A). (Wang et al., 2006) Many types of AF-2 site inhibitors have been described, such as constrained peptides, peptide mimetics and small molecule inhibitors, all typically mimicking the helical LXXLL cofactor binding motif (Moore et al., 2010; Caboni and Lloyd, 2013; Tice and Zheng, 2016). This form of direct inhibition of NR-protein interactions is described by other reviews in this issue and therefore not further elaborated here.

Interactions between the NR DBD and the DNA are crucial for the expression of the NR target genes. Targeting the DBD is, therefore, another potential strategy for NR modulation (Moore et al., 2010; Caboni and Lloyd, 2013). The first option is the blocking of specific HREs at the DNA, inhibiting the binding of the NR DBD (Fig. 4D). The most common examples of this approach are hairpin polyamides that non-covalently bind to the minor groove of DNA and thereby disrupt the interaction with the NRs such as ERα and AR (Caboni and Lloyd, 2013; Gearhart et al., 2005; Nickols and Dervan, 2007). The second strategy is via ligands that target the zinc fingers of the DBD (Fig. 4E). Zinc fingers play an important role in stabilizing the DBD, meaning that disrupting these zinc fingers results in a destabilization of the DBD leading to a reduction of DNA binding (Caboni and Lloyd, 2013; Whittal et al., 2000). Most modulators are oxidizing agents that focus on disrupting the Zn-S interaction; they displace the zinc ion in the zinc finger and cause disulfide bond formation of the cysteines that normally coordinate the zinc ions via electrostatic interactions (Caboni and Lloyd, 2013). The most challenging issue for designing such modulators is to achieve selectivity over other NR members since the DBD is the most highly conserved domain in this class of proteins (Caboni and Lloyd, 2013). Targeting the DBD and HRE interactions will also be discussed in detail elsewhere in this issue.

NR homo- and heterodimerzation also provide entries for allosteric modulation via small molecules (Fig. 4B). Through its ability to form heterodimeric assemblies, RXR can modulate and activate multiple NR partners such as PPAR, LXR and Nurr1. In general, RXR dimerization partners can be arranged into two classes, being permissive and non-permissive heterodimer partners. For permissive heterodimers, the sole presence of the RXR ligand is enough to make the heterodimer transcriptionally active. If the partner's ligand is also present, this results in a stronger activation. In contrast, for non-permissive heterodimers, the partner's ligand is required for activation, since they are not responsive to rexinoids on their own. However, like permissive heterodimers, the presence of an RXR ligand does enhance the biological response

(Forman et al., 1995; Kurokawa et al., 1994). The structural mechanism by which RXR transduces its signal to its dimer partner has been further elucidated recently (Kojetin et al., 2015). As an example of the fine interplay of RXR heterodimer regulation, in a Parkinson's disease mouse model, treatment with the RXR agonist bexarotene enhanced the clearance of β-amyloids, improving the neural function. This is believed to be caused by the activation of the permissive RXR-PPAR and RXR-LXR heterodimers (Cramer et al., 2012). Furthermore, it has been shown that the type of RXR ligand can have a substantial effect on the dimerization behavior of RXR and therefore on the transactivation to its dimer partner (Scheepstra et al., 2017; McFarland et al., 2013; Giner et al., 2015). The RXR ligand LG101506 was shown to selectively activate RXR-PPAR heterodimers over other heterodimers e.g. RXR-RAR, RXR-LXR and RXR-FXR. Selective activation of RXR-PPAR has been shown to be beneficial in the treatment of type 2 diabetes by improving insulin sensitivity while simultaneously keeping the triglyceride levels consistent (Leibowitz et al., 2006; Michellys et al., 2003). Lastly, in a recent study, the effect of minor modifications on RXR ligand scaffolds on influencing the heterodimerization of RXR with the orphan NR Nurr1 was analyzed. Interestingly, ligands that introduced steric bulk towards either H7 or H11 of RXR significantly inhibited the heterodimer formation. It is postulated that this behavior is observed because of the presence of Nurr1 H12 making contact with H7 and H11 of RXR at the dimerization interface (Spathis et al., 2017).

Several ligands are known that allosterically modulate NRs by acting at or via PTMs (Fig. 4C). For PPARγ, for example, phosphorylation of S273 was found to decrease insulin sensitivity by upregulating a specific set of target genes without fully activating the receptor (Choi et al., 2010). When compounds with minimal agonistic activity bind the PPARγ LBP, there is inhibition of S273 phosphorylation without causing PPARγ activation associated side-effects (Choi et al., 2011). A similar modulatory effect might be achievable for other NRs since ligand-induced PTMs are common. In the case of the farnesoid X receptor (FXR), agonist-activated SUMOylation at K277, which is necessary for the transrepression of pro-inflammatory genes, is inhibited by acetylation at K217 in obese individuals (Kim et al., 2015). Targeting this acetylation site is postulated as an approach to improve hepatic inflammation and glucose tolerance (Kim et al., 2015). A different approach to target a PTM using small-molecules to alter NR activity was shown for ERα, for which a phosphorylation site in the F-domain was identified which interacts with the hub-protein 14-3-3 (De Vries-van Leeuwen et al., 2013). This interaction can be stabilized by the small
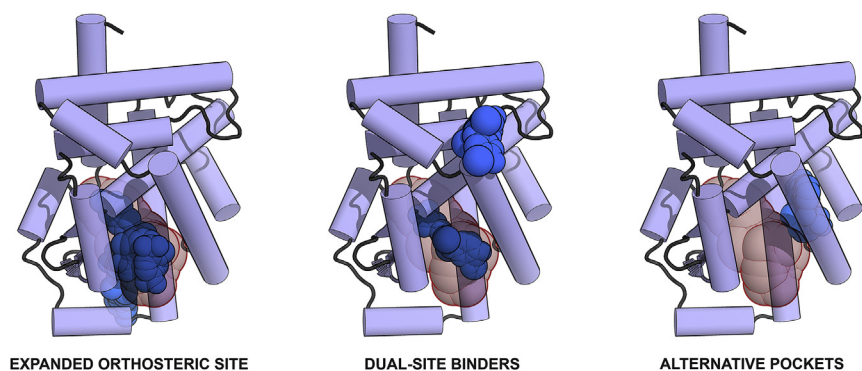
Fig. 5. Three conceptual modes of allosteric binding sites in the NR LBD. The orthosteric LBP is displayed in red and the ligands are shown in blue. The expanded orthosteric site binders address the classical LBP, but also extend into novel pockets, unique for a specific NR. The dual-site binders bind twice to the LBD, typically one time in the LBP and a second time to an allosteric site. The alternative pockets modulators bind exclusively to an allosteric pocket.

EXPANDED ORTHOSTERIC SITE   **DUAL-SITE BINDERS**   **ALTERNATIVE POCKETS**

molecule Fusicoccin, which reduces ERα dimerization, resulting in inhibition of downstream gene expression (De Vries-van Leeuwen et al., 2013). Such PTMs beyond the LBD might provide an advantage over ligand-induced resistance mutations in the LBD (Zwart et al., 2007).

3.2. Allosteric pockets on and in the NR LBD

The major part of this review is dedicated to compounds that bind to allosteric sites within the LBD, which we have categorized into three sub-groups, depending on their binding location (Fig. 5). The first group, targeting expanded orthosteric sites, binds (part of) the orthosteric LBP and subsequently extends from there into additional pockets that are unique for a certain NR. The compounds in the second group, the dual site binders, bind the LBD in a two to one stoichiometry where typically one of the two ligands binds in the LBP and the second ligand binds a surface exposed site elsewhere on the LBD. The third group of compounds addresses pockets fully outside of the orthosteric LBP, which we will term alternative pockets.

3.2.1. Expanded orthosteric site

The size and plasticity of the orthosteric pocket greatly varies between different NRs, ranging from barely existing to as big as 1600 Å^3 (Gallastegui et al., 2015). In the past, there has been structural evidence that ligands, especially fatty acid analogs, can bind to the orthosteric LBP of various NRs with a different binding mode. Therefore, NRs can recognize the same ligand but binding can lead to another biological response (Egea et al., 2002; Itoh et al., 2008). In this section, we explore ligands that take it one step further by binding to the orthosteric LBP and extending out to neighboring pockets connected to the classical LBP. These ligands are often found in NRs that contain a larger orthosteric pocket like PPAR and FXR. Apart from inducing possible alternative protein conformations, the targeting of these 'novel' pockets potentially allows for enhancing NR subtype selectivity. Below an overview is provided of examples of ligands acting via the expanded orthosteric site mechanisms, with a focus on those allosteric ligands for which structural information is available.

3.2.1.1. FXR. The farnesoid X receptor (FXR) contains an extra binding site connected to the orthosteric LBP. FXR is a NR that regulates lipid, glucose and amino acid metabolism (Massafra and van Mil, 2018). The extra binding site was initially found by a molecular modeling study which docked E/Z-guggulsterone in a new pocket defined by both the loop between H1 and H2, and H3, H5, and H8 (Fig. 6A). (Meyer et al., 2005) The location of this pocket, defined as the S2 site, was later confirmed via HDX-MS studies, but no crystallographic data is currently available (Yang et al., 2014). When bile acids, the endogenous ligands of FXR, bind to the receptor the carboxylic acid moiety of the side chain points towards the back of the LBD close to the loop between H1 and H2, and H3 and H5 (Fig. 6A). (Mi et al., 2003; Pellicciari et al., 2006) This is of particular interest, since for other NRs that bind cholesterol metabolites this part of the ligand points in the direction of H12. When

overlaying the crystal structure of FXR co-crystallized with chenodeoxycholic acid (CDCA), the most potent endogenous ligand, with the docked pose of E/Z-guggulsterone it can be appreciated that the carboxylic acid tail points towards the S2 site (Pellicciari et al., 2006). Pellicciari et al. used this information to design a ligand library with the carboxylic acid position of CDCA exchanged with chemically diverse groups (Pellicciari et al., 2006). Modification of this part of the ligand scaffold yields compounds that expand out of the classical LBP which leads to effects ranging from antagonism to partial and full agonism. This research was continued by further exploring the bile acid scaffold, leading to the discovery of molecules with a high efficacy and affinity (Gioiello et al., 2011; Pellicciari et al., 2016). This approach of gaining selectivity by targeting the unique properties of a single NR exemplifies the utility of targeting allosteric pockets. In addition to the S2 site, it has been shown that the orthosteric LBP of FXR can expand by shifting H2 and H6 outwards, generating additional space in the LBP. Therefore, FXR can accommodate larger sized compounds such as ivermectin that alters the cofactor binding pattern of FXR by allosterically enhancing the flexibility of the cofactor binding site (Fig. 6B) (Jin et al., 2013).

3.2.1.2. LRH-1. The binding of liver receptor homolog-1 (LRH-1) to phospholipids is also an example of NR modulation via addressing an expanded orthosteric site. Tuning the activity of LRH-1 can be beneficial in multiple types of cancer and metabolic diseases (Lazarus et al., 2012). In a study by Musille et al., the crystal structure of LRH-1 with phospholipid dilauroylphophatidylcholine (DLPC) was solved at a resolution of 1.8 Å, revealing the binding site of this phospholipid (Fig. 6C). (Musille et al., 2012) It could be clearly observed that DLPC is located near the β-sheet-H6 region and makes contacts with residues of H3, H5, H6 and H7. In this binding mode the compound occupies not only the LBP, but also extends out of the canonical pocket. In a second study, crystallography and molecular modeling were combined to identify an allosteric network connecting the novel extended binding pocket with the AF-2 site via the orthosteric LBP, elucidating an allosteric mechanism for the activation of LRH-1 by phospholipids such as DLPC (Musille et al., 2016). A highly similar binding mode is observed in the other member of the steroidogenic factor-like subfamily, the steroidogenic factor-1 (SF-1) (Sablin et al., 2009). Blind and coworkers confirmed via biochemical and structural analysis that phospholipid PIP$_3$ stabilizes the previously determined critical H2-3 and H11-12 loops and thereby enhances coactivator recruitment (Sablin et al., 2009; Blind et al., 2014). Finally, a related example is the glucocorticoid deacylcortivazol (DAC) which binds to an extension of the LBP located at the top half of the LBD of GR, thereby effectively doubling the size of the binding pocket (Suino-Powell et al., 2008).

3.2.1.3. PPARγ. A NR that is well-known for having one of the largest orthosteric binding-pockets is PPARγ. The PPARγ pocket is composed of three sub-pockets which have a combined volume of > 1200 Å^3 (Brust
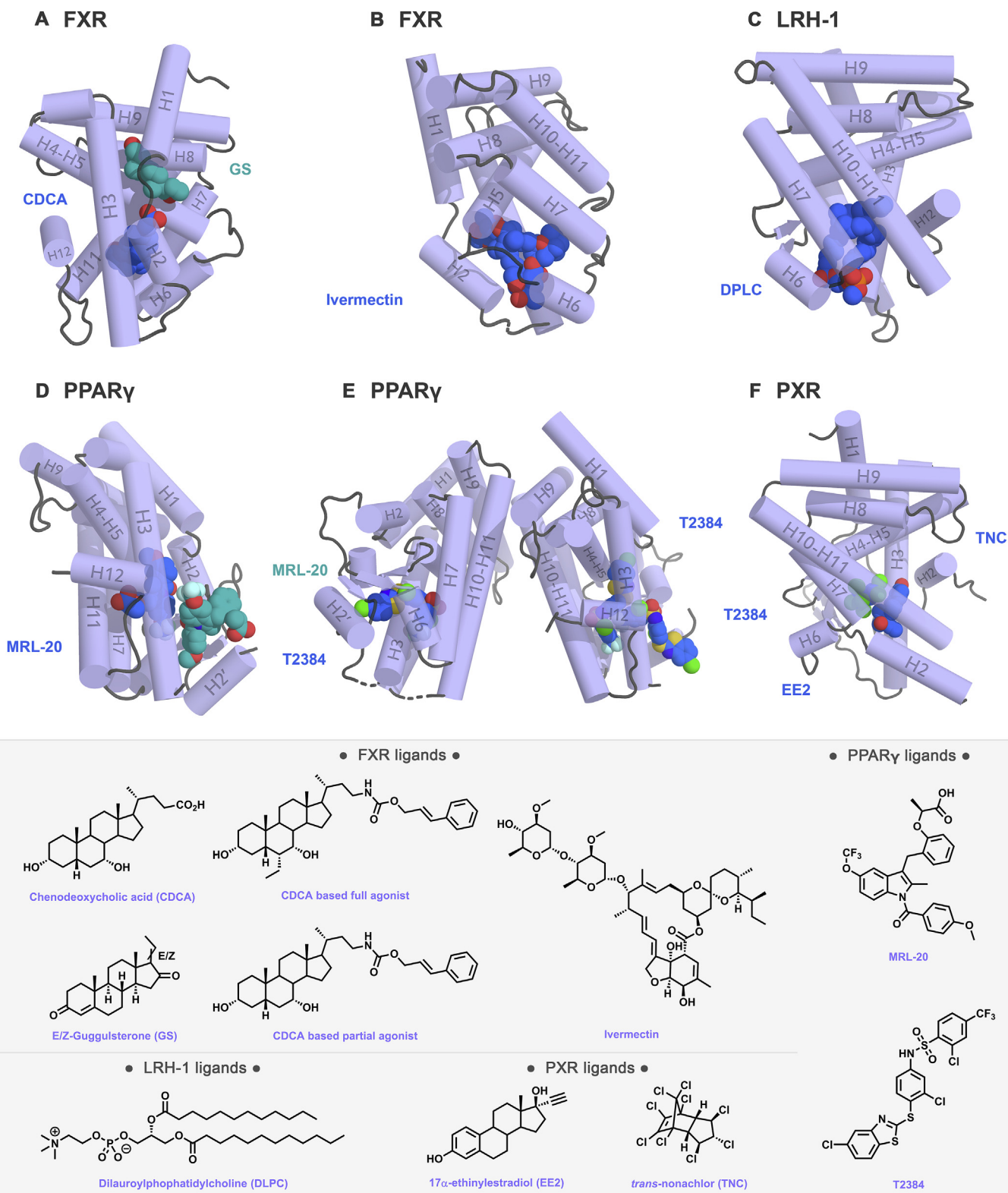
Fig. 6. Overview of allosteric ligands binding to an expanded orthosteric site and their corresponding crystal structures. A) FXR with CDCA in blue (PDB: 1OSV). GS (shown in teal) is positioned at the predicted binding site. B) FXR with ivermectin (PDB: 4WVD). C) LRH-1 with DPLC (PDB: 1YUC). D) PPARγ with MRL-20 (blue) (PDB: 2Q59). The second MRL-20 (in teal) is positioned at the predicted binding site. E) PPARγ with T2384 (PDB: 3K8S). F) PXR with EE2 and TNC (PDB: 4X1G).

et al., 2017). PPARγ plays an important role in energy homeostasis and inflammation and therefore modulating its activity can be beneficial in e.g. obesity and diabetes (Varga et al., 2011). Hughes et al. discovered that at high concentrations the agonist MRL-20 still increased the interaction between PPARγ and coactivator when the orthosteric site

was blocked using a small covalent modifier (Hughes et al., 2014). Using NMR and TR-FRET they confirmed that MRL-20 binds with high affinity to the orthosteric site and with lower affinity in an adjacent pocket, in both cases causing agonist behavior (Hughes et al., 2014). The agonistic behavior of the first binding site is caused by direct

stabilization of H12 and the AF-2 surface, whereas the second binding site likely stabilizes H3 which facilitates interactions with the loop between H11 and H12 and with that causes stabilization of the co-activator binding region (Hughes et al., 2014). Covalent modifiers with different sizes and chemical properties were used to define the binding site of MRL-20 more precisely (Fig. 6D). (Brust et al., 2017) Another example where one compound is able to bind two connected sites at the same time in the PPARγ LBD is the flexible ligand T2384 (Li et al., 2008). In the crystal structure, T2384 is both observed in a U-shape in the canonical LBP and in a S-shape in the canonical LBP in combination with an extra molecule in the alternate site (Fig. 6E). (Li et al., 2008) By making mutant proteins that lack one of the two binding sites, it was elucidated that the combined antagonist/agonist behavior that is observed for this compound in the native protein is caused by an agonistic effect of binding in the orthosteric pocket and an antagonistic effect of binding in the connected allosteric pocket (Li et al., 2008). These binding-modes were confirmed with NMR where it was concluded that both the U and S compound-conformation in the orthosteric pocket exist when adding one equivalent of the compound and the alternate binding site gets populated when adding more than one equivalent (Hughes et al., 2016). In addition to the instances where one compound binds to both the orthosteric and a connected alternate site, there are also a couple of examples where a synthetic ligand and a naturally occurring lipid together occupy the two pockets of PPARγ (Jang et al., 2017; Shang et al., 2018).

3.2.1.4. PXR. Finally, the pregnane X receptor (PXR) also has a unique way in which its orthosteric binding pocket can be expanded (Delfosse et al., 2015). PXR is known for binding structurally diverse ligands and with that serving as a hydrophobic toxin sensor (Kliewer et al., 2002). Both 17α-ethinylestradiol (EE2), which is used as the active component in contraceptive pills, and *trans*-nonachlor (TNC), a pesticide, are poor agonists for PXR. However, when these compounds are combined they trigger PXR activation almost as efficient as SR12813 which is a well-characterized PXR full agonist (Fig. 6F). (Delfosse et al., 2015; Jones et al., 2000) This cooperative effect has been studied both by functional assays in cells and by biochemical assays that focus on compound binding, isothermal titration calorimetry, and coactivator recruitment, fluorescence anisotropy (Delfosse et al., 2015). In the crystal structures of PXR with EE2 with and without TNC, it can be appreciated that TNC occupies a portion of the LBP that is only accessible when EE2 is bound (Delfosse et al., 2015). This observation is in agreement with other findings in which LBPs can adjust their size to adopt different-sized compounds (i.e. (Färnegårdh et al., 2003)), but it is the first time that such an effect occurs for cooperative binding of supramolecular interacting compounds (Delfosse et al., 2015).

3.2.2. Dual site binding

In addition to the canonical LBP, ligands were also found that can bind both to the orthosteric site and to another, surface exposed, site on the LBD (Fig. 7). Although the function of the binding to these secondary binding sites is frequently not fully understood yet, some of these ligands were shown to have the capacity to modulate the NRs in a non-traditional way. A number of crystal structures of NRs have been reported with an additional ligand present in the hydrophobic groove of the coactivator binding site e.g. hydroxytamoxifen in complex with ERβ (Wang et al., 2006). Generally, this ligand binding to the AF-2 site directly prevents coactivators from binding. For the scope of this review, these examples were not included here and we will primarily focus on the other secondary sites which modulate the receptor via allosteric mechanisms.

3.2.2.1. VDR. The vitamin D receptor (VDR), in addition to its binding to the endogenous ligand vitamin D, also acts as a sensor for the toxic secondary bile acid lithocholic acid (LCA) (Makishima et al., 2002). LCA acts as an agonist for VDR which, in turn, facilitates the clearance

of LCA metabolites. A recent crystal structure of zebrafish VDR LBD co-crystallized with LCA showed LCA to bind both in the orthosteric pocket as well as to a surface exposed site near the cofactor binding site (between L1-3, H2' and H3) (Fig. 7). A combination of biological, computational and structural analysis showed that both sites have to be occupied by LCA for it to act as a VDR agonist. The LCA molecule at the secondary site does not interact with H12 directly but is in close proximity of the coactivator. Comparison of the B-factors of the crystal structures of VDR bound to either one or two LCA molecules showed that the presence of both LCA molecules leads to a stabilization of H12, the loop between H11 and H12 and the cofactor. Therefore, binding of a ligand to this site might reveal a mechanism of ligands to induce coactivator selectivity (Belorusova et al., 2014).

3.2.2.2. PPARα. The potent and PPARα-selective agonist WY-14643 (also known as pirinixic acid) was shown to inhibit NF-κB activity and decrease anti-inflammatory responses. In addition to binding to the orthosteric pocket, WY-14643 binds to an additional binding site in a pocket between H2', H3 and the loop between H11-H12 (Fig. 7). Using mutational studies, it was shown that binding of the second ligand significantly enhances the activation of PPARα. Because the second ligand is not in contact with H12 directly, the increased activation was further investigated using MD. In this simulation, WY-14643 stabilized the otherwise flexible Ω-loop (between H2 and H3) which, in turn, indirectly stabilized H12 (Bernardes et al., 2013). This indirect modulation of H12 via another part of the protein has already been reported earlier in literature for this receptor class. Orthosteric ligands of PPARγ have been shown to stabilize the Ω-loop and β-sheet region in PPARγ, both leading to the stabilization of H12 (Puhl et al., 2012; Waku et al., 2009a, 2009b; Bruning et al., 2007).

3.2.2.3. TRα. In 2011, HDX studies were used to show that the natural ligand of the thyroid receptor (TR), tri-iodothyronine (T_3), was likely to also bind in a region of the protein between H9, H10 and H11, located at the opposite site of the AF-2 (Figueira et al., 2011). Three years later, the crystal structure was solved of TRα with T_3 bound to the orthosteric pocket and either T_3 or T_4 located at the earlier predicted position (Fig. 7). (Souza et al., 2014) The same group also showed that in an earlier solved crystal structure of the TRβ-selective ligand GC-24 in complex with human TRβ, an additional GC-24 molecule was similarly present in this region. The undefined electron density was initially presumed to be an artifact (Souza et al., 2014; Borngraeber et al., 2003). Interestingly, the position of this binding site is the same as the predicted binding site of the F-domain of TRα. It is, therefore, hypothesized that the secondary binding site suppresses the activation of TRα when there is a high concentration of ligand present by preventing recruitment of certain cofactors (Souza et al., 2014). In addition, T_3 also binds to other NRs such as AR and ER (Bona et al., 1979). Interestingly, when AR was co-crystallized with both 5-alpha-dihydrotestosterone and T_3, another allosteric pocket was found for T_3 at the other side of H9 compared to the TRα structure (Tanenbaum et al., 1998).

3.2.3. Alternative pockets in the LBD

The two previously mentioned classes of LBD allosteric sites are either an expansion of the orthosteric site or a secondary binding site for an orthosteric ligand (dual site binding). The third class, discussed below, deals with allosteric pockets on the LBD fully independent from the orthosteric pocket in terms of the type of ligand or spatial overlap. Such allosteric sites are known and structurally described for RORγt, Nurr1, Nur77, RXR, and AR (Fig. 8). Of course, it should be noted that nevertheless, the actual ligand binding to the orthosteric and allosteric pockets could potentially still influence each other, because of allosteric effects.

3.2.3.1. RORγt. Retinoic acid related orphan receptor γ t (RORγt) is a
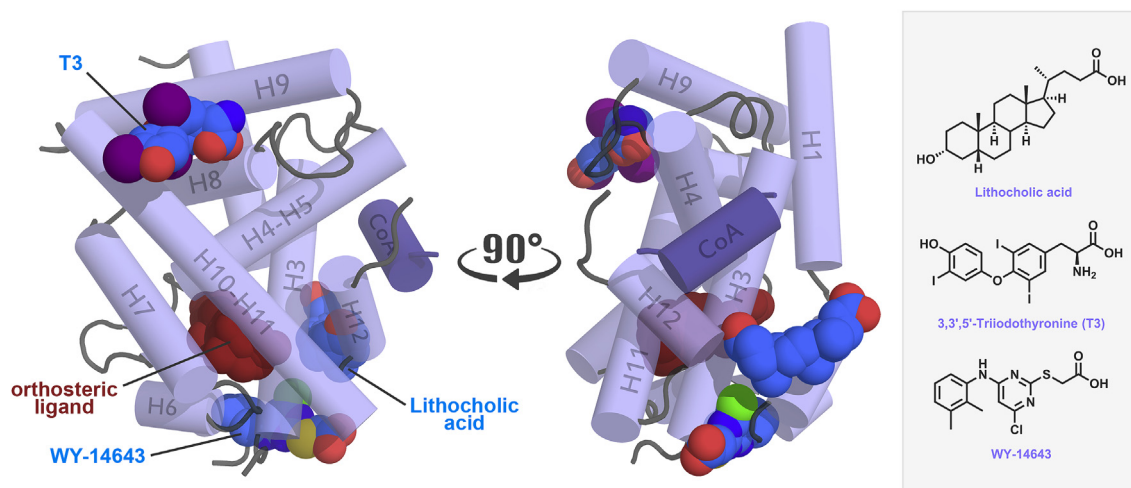
Fig. 7. Overview of small molecule ligands binding both to the orthosteric site of NRs and to an allosteric site, located at another structural element of the LBD of the NR. The protein structure of VDR bound to lithocholic acid (LCA) was used as a reference structure, showing the orthosteric ligand in red (PDB: 4Q0A). The structures of PPARα bound to WY-14643 (PDB: 4BCR) and TRα bound to T$_3$ (PDB: 4LNW) were superimposed on this structure to visualize the relative positions of the allosteric ligands.

key regulator protein for the development of T cells into Th17 cells, which results in tissue inflammation by the excretion of pro-inflammatory cytokines such as IL-17a (Ivanov et al., 2006; Korn et al., 2009; Yang et al., 2008). Inhibition of RORγt has, therefore, been found to be a promising strategy in the treatment of autoimmune diseases by diminishing the inflammatory response (Isono et al., 2014). Many synthetic small molecule inverse agonists for RORγt have been identified in literature, most of them targeting the orthosteric LBP (Fauber and Magnuson, 2014; Kamenecka et al., 2013). However, a few years ago a novel acyl-indazole series of RORγt modulators was disclosed by the company Merck/MSD containing a different chemotype in comparison with the already known modulators (Karstens et al., 2012). Scheepstra et al. identified that these ligands were binding at an alternative binding pocket in the LBD of RORγt (Scheepstra et al., 2015). For four of these acyl-indazole ligands (MRL-871, MRL-299, MRL-367, MRL-673), a high resolution crystal structure has been elucidated, in which it can be clearly observed that these modulators bind in an allosteric binding site, distal to the orthosteric binding pocket, at the position where H12 is normally located in its agonistic conformation. This allosteric binding site is shown illustratively for MRL-871 in Fig. 8A. The allosteric pocket is formed by H4, H5, H11 and the repositioned H12, which has a unique conformation by folding back over the ligand. In this conformation, the recruitment of coactivators to the AF-2 is prevented. Via this binding mode, the allosteric ligands are functionally acting as inverse agonists with a similar effect as regular orthosteric inhibitors, but in contrast, not in competition with the orthosteric ligands. The acyl-indazole ligands interact with the allosteric pocket mainly via hydrophobic interactions and additionally via H-bonding between the carboxylic acid moiety of the ligand and the Q329 side chain (H3) as well as the main chain amide of A497 and F498 (H12) of the protein. In addition to structural studies, the allosteric modulators were also tested in time-resolved fluorescence resonance energy transfer (TR-FRET) coactivator recruitment assays and cellular assays, which showed IC$_{50}$ values in the nanomolar range. This means that these compounds can be considered as NR allosteric compounds with a potency that reaches clinical demands.

With the discovery of these RORγt allosteric ligands, follow-up research on these compounds has been performed by many companies and research groups, most extensively by Genentech, Glenmark Pharmaceuticals and Ouvry et al.. Genentech performed an extensive SAR study around the MRL series of compounds, varying the indazole

core and also making changes to the carboxylic acid and 3-acylarene moiety (Fauber et al., 2015). They specifically attempted to improve the selectivity for RORγt over other NRs, since a remarkable cross-reactivity with PPARγ was observed for the MRL derivatives (Scheepstra et al., 2015). From this study, GNE-0946 and GNE-6468 (Fig. 8) were identified as highly potent compounds in both biochemical and cellular assays, with improved selectivity and physiochemical properties. Glenmark Pharmaceuticals performed a scaffold hopping approach with MRL-871 as a starting point, resulting in a compound with a thiophenopyrazole core (Fig. 8) as one of the best hits (Gege, 2015; Chaudari et al., 2015). The compounds were tested in biochemical TR-FRET assays, where one of the compounds showed an IC$_{50}$ value of less than 50 nM. Finally, Ouvry et al. mainly focused on improving the metabolic stability of MRL by replacing the heterocyclic amide linkage, since related compounds have shown in vivo instability due to hydrolytic cleavage (Ouvry et al., 2016). For this purpose, different linkers were incorporated on a reverse indazole scaffold, which was derived from one of the MRL analogs, and the library was screened in a RORγt/Gal4 cell-based assay. Incorporation of an ether linker, compound 27 (Fig. 8), resulted in the most potent ligand. Furthermore, the ligand was also tested in phototoxicity assays where it turned out to have a phototoxic irritancy factor above the toxic level, which could not be improved by some further structural changes. Interestingly, the crystal structure of RORγt with phenoxyindazole 27 was elucidated which revealed binding to the same allosteric pocket as MRL-871 in a similar orientation (Ouvry et al., 2016).

3.2.3.2. Nur. Nurr1 and Nur77, two closely related orphan NRs belonging to the NR4A family, are transcription factors that are involved in many physiological functions, including innate immune cell differentiation, brain functions and metabolism (Kurakula et al., 2014). In contrast to most other NRs, Nurr1 and Nur77 have long been considered to lack a canonical LBP, since the X-ray crystal structures of the LBD show no physical space in the region where other NRs typically bind their ligands (de Vera et al., 2016, 2019; Windshugel, 2018). However, recently de Vera et al. and Windshugel showed, using comprehensive NMR spectroscopy, HDX-MS studies and MD simulations, that the LBP of Nurr1 is dynamic and that the pocket allows binding of unsaturated fatty acids by expanding from the collapsed conformation (de Vera et al., 2016, 2019; Windshugel, 2018). A recent study indicates that Nurr1 can also be allosterically modulated via an alternative binding pocket, at a similar position on
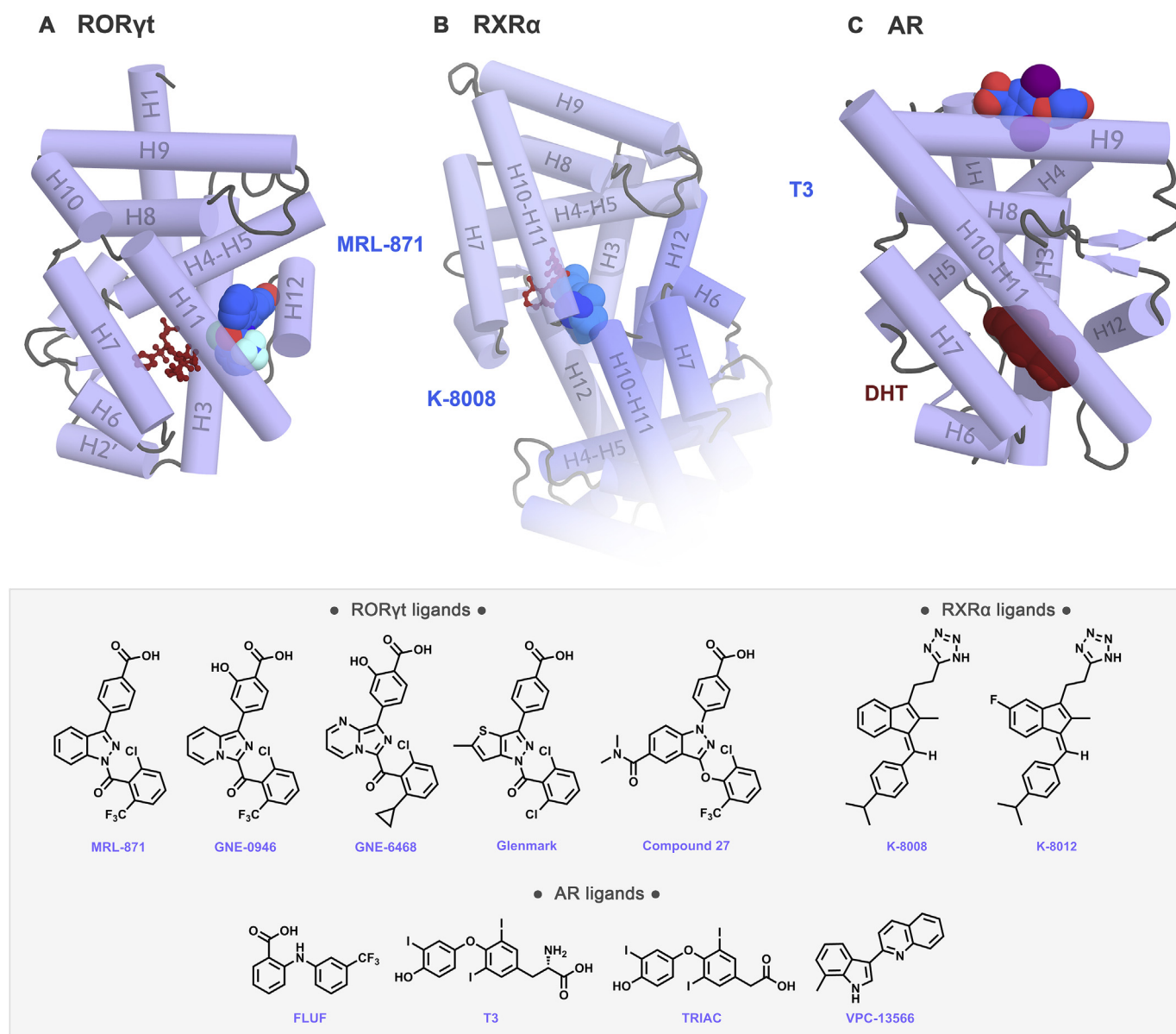
Fig. 8. Overview of NRs targeted via alternative allosteric pockets. A) RORγt with MRL-871 (overlay with the protein with T0901317 (PDB: 4YPQ & 4NB6)). B) RXRα with K-8008 (overlay with the protein with 9-cis retinoic acid (PDB: 4N8R & 1FBY)). C) AR with T₃ and DHT (PDB: 2PIT). The orthosteric ligands were shown in red (the licorice representation was used if the ligand was used as a reference compound in the orthosteric site, a spherical representation is used if the ligand is actually present in the crystal structure) and the allosteric ligands in blue. Also, a selection of reported RORγt, RXRα, and AR allosteric ligands is shown.

the LBD as discovered for RORγt. Two crystal structures were solved showing two different cyclopentenone prostaglandins (cyPGs) covalently bound to C566 of Nurr1. Binding of the molecule resulted in a significant shift of H12 compared to the apo structure. In an *in vitro* luciferase reporter assay, cyPGs enhanced the transcriptional activity of Nurr1 in HEK293T cells (Yoon et al., 2018).

Recent crystallography studies indicate that also Nur77 can bind ligands at alternate sites. Wu et al. identified the cytosporone B analogue TMPA to bind Nur77 at two surface exposed sites compromised by either H4, H11 and H12 or H1, H5, H7 and H8 (Zhan et al., 2012). Via mutational and functional studies it was discovered that the first site, which has similarities to the allosteric pocket in RORγt, is important for mutually exclusive TMPA binding but also liver kinase B1 (LKB1) interaction (Zhan et al., 2012). Nur77 also plays a suppressing role in lipopolysaccharide (LPS)-induced sepsis by associating with p65 and blocking its binding to κB (Li et al., 2015). This interaction is, however, diminished by p38α phosphorylation of T27 and T143 in the

N-terminal domain of Nur77 (Li et al., 2015). Binding of PDNPA, which resembles cytosporone B even closer, to the same site where TMPA and LKB1 can bind, blocks binding of p38α to Nur77 resulting in reduced LPS-induced inflammation (Li et al., 2015). Via similar methods, the same group identified the trihydroxyphenyl derivative THPN that, instead of disrupting a protein-protein interaction, creates a binding interface for NIP3-like protein X (Nix) (Wang et al., 2014). THPN binds in a surface exposed cavity between H5, H7, H8, H9 and H10 of Nur77 (Wang et al., 2014). After THPN binding, Nur77 translocates to the mitochondria through binding to the mitochondrial outer membrane protein Nix which, on its turn, starts a cascade leading to autophagy (Wang et al., 2014). By combining THPN with Akt2 inhibitor administration, this approach can be expanded to other cancer types where Nur77 phosphorylation inhibits THPN-induced mitochondrial targeting (Wang et al., 2015).

3.2.3.3. RXR. A secondary binding site on the retinoid X receptor alpha

(RXRα) was discovered a few years ago by Chen et al. In their study, two Sulindac-derived analogs, K-8008 and K-8012 (Fig. 8), were identified as novel RXRα allosteric antagonists, inhibiting coactivator recruitment with IC_{50} values around 10 μM (Chen et al., 2014). These ligands were identified as inhibitors of the interaction between RXRα and the p85a subunit of the phosphatidylinositol-3-OH kinase (PI3K), resulting in a decrease in the activation of the TNFα-activated PI3K/Akt pathway and therefore led to induction of apoptosis (Chen et al., 2014; Zhang et al., 2015). The X-ray crystal structures of the compounds in complex with the RXRα LBD revealed a homo-tetrameric structure of RXRα LBDs, with one ligand binding to each dimer partner (Fig. 8B). (Chen et al., 2014) In this structure, the ligands bind at a novel site, distinct from the orthosteric LBP. This hydrophobic binding site is located at the surface of the LBD, near the dimer-dimer interface of the tetramer. Specifically, it is formed by H3, H5 and H10/H11, nearby the entry of the canonical LBP, but not overlapping with it. Ligands binding to the tetrameric structure may help to stabilize the tetramer, which might explain the diminished interaction of RXRα with p85a. In additional experiments, the therapeutic relevance of targeting RXRα with the ligands was shown, since they were able to induce apoptosis *in vitro* and also inhibit tumor growth in animals without apparent toxicity. Thus, despite their relatively weak affinity for RXRα, the compounds could be clinically relevant for developing RXRα modulators in cancer therapies.

3.2.3.4. AR. The androgen receptor (AR) is an important regulator for the growth and function of the prostate gland, meaning that overstimulation of the receptor can lead to prostate cancer (Biron and Bédard, 2016). Therefore, AR inhibition is a relevant strategy for the treatment of this type of cancer. Currently, most AR antagonists act by binding to the canonical LBP, but often resistance to these antiandrogen drugs is developed over time in cancer patients (Biron and Bédard, 2016). Therefore, there is a high demand for alternative strategies to target AR.

Around a decade ago, an allosteric binding site in the AR was discovered, termed binding function-3 (BF-3). This hydrophobic binding site has a size that is comparable to the AF-2 site and is located at the junction of H1, the loop of H3-5 and H9, adjacent to AF-2 (Fig. 8C). (Biron and Bédard, 2016) Estebanez-Perpina et al. used a fluorescence polarization-based assay in combination with a crystallographic screening approach, in which a large library screening was performed and three inhibitors were identified; flufenamic acid (FLUF), T_3, which was already discussed as a TRα dual site binder, and triiodothyroacetic acid (TRIAC) (Fig. 8). (Biron and Bédard, 2016; Estebanez-Perpina et al., 2007) These compounds were shown to preferentially interact with the BF-3 site (IC_{50} values ~50 μM in FP assays) and to have some minor cross-talk to the AF-2 site. A number of follow-up studies were performed by Cherkasov et al. (Biron and Bédard, 2016; Lack et al., 2013; Munuganti et al., 2013, 2014; Ban et al., 2014), specifically focused on improving the affinity and selectivity for the BF-3 site, while also inhibiting prostate cancer cell growth. Recently, VPC-13566 (Fig. 8) was found as a highly potent (IC_{50} = 50 nM) and selective compound, also inducing a significant inhibition of cancer cell growth (Lallous et al., 2016). Although the BF-3 site is an allosteric one, the conformational change induced in the AR protein appears to be limited. Recently it was found that the way in which the BF-3 site targeted compounds regulate AR activity is via the inhibition of the binding of co-chaperones (Bag-1L (Jehle et al., 2014), FKBP52 (Stope and Burchardt, 2012) and SGTA (Lallous et al., 2016)), which normally interact with the AR LBD via the BF-3 site.

4. Conclusion & perspective

NRs are a promising target in pathology because of their regulating role within the human genome. The primary focus of NR targeting has classically been via the orthosteric LBP. Small-molecule modulators binding to this part of the protein have yielded great successes, nevertheless issues such as cross-reactivity, selectivity, and drug resistance remain major challenges. In recent years, allosteric modulation of protein receptors has gained attention, with drugs with allosteric modes of action on the market for GPCRs and kinases, and multiple drugs undergoing clinical trials. The first allosteric ligands for NRs were shown to have low binding affinities towards their target. But, recently, nanomolar potent allosteric site modulators have been discovered for several NRs, bringing allosteric targeting of NRs also to the center of attention.

The LBD is presently the most promising NR domain for allosteric targeting due to the high affinities and the functional effects of the allosteric compounds that have been found. In addition, there is considerable structural information available for this domain, making *de novo* drug synthesis, computational guided studies, and medicinal chemistry more straightforward. Nevertheless, the scarcity of full-length structures and the absence thereof in the presence of allosteric ligands, still limits a concise delineation of the allosteric effects on the multi-domain protein level for these new kind of compounds.

Allosteric modulation via nuclear receptor dimerization, mainly via the LBD, has delivered interesting entries for drug development that could be further investigated. To date, the mechanism by which the activation of specific dimers is controlled is still rather poorly understood. The structural diversity of the amino acid residues present at the dimerization interface between NRs is significant. Therefore, the possibility arises to generate synthetic ligands that can selectively bind to the dimerization interface to either stabilize or inhibit specific homo- and heterodimer formation. These synthetic ligands can serve as interesting approaches to target specific pathways, but also to fundamentally understand the importance of the protein dimers.

Although most success in NR allosteric targeting has been on the LBD, other strategies such as allosteric modulation via PTMs also show high potential and have already yielded some successes. The intrinsically disordered nature of the NTD of NRs together with the fact that this domain contains multiple sites for PTMs makes this domain of NRs of particular interest for allosteric modulation via addressing the PTMs (Simons et al., 2014; Hill et al., 2012). Allosteric modulators which either enhance or abolish these PTMs selectively, can lead to an improved understanding on the modulatory role of these PTMs and can potentially be interesting for drug discovery.

Reflecting on the allosteric mechanisms found for other receptor classes, it becomes clear that NRs possibly also harbor analogous sites with the potential to be similarly allosterically modulated. To date, the vast majority of these approaches, such as bitopic ligands and covalent attaching, have remained unexplored for NRs while the potential for drug discovery can be substantial. Allosteric sites that are in close proximity of the canonical LBP can be targeted by bitopic ligands to more accurately force a desired biological response. Additionally, for NRs with an expanded orthosteric site, the orthosteric ligand can be combined with pharmacophores that occupy directly neighboring pockets. This will ultimately potentially result in a modulation of efficacy, enhanced affinity, and selectivity of the ligand for the specific NR. These type of ligands can, therefore, reduce the cross-reactivity of ligands to other NRs, which remains a serious problem in NR research (Moras and Gronemeyer, 1998). Also covalent attachment of molecules is an interesting approach to target NRs. Covalent modulators have the potential to more selectively target NRs using a lower dose, and perhaps also specifically target NR mutants.

A major challenge for the field is the actual discovery of such allosteric ligands, which is in part due to the high potency of orthosteric ligands, biasing screening campaigns. Several entries could be explored in order to identify allosteric modulators more easily. (1) By blocking the orthosteric site with a transcriptionally active or silent covalent modulator, NRs can subsequently only be modulated using an allosteric ligand, which can then be detected by conventional screening assays. (2) By (temporary) covalent tethering of compounds at a specific,

allosteric site of the protein. (3) By exploiting the orthosteric ligand as entry for generation of bitopic ligands for both expanded LBPs and pockets that are in close proximity. (4) Besides the above mentioned specific techniques and targets, also more diverse and directed screening methods, such as those based on computational approaches, fragment-based screening, and phage display could be further optimized or developed towards allosteric site targeting.

NRs have been shown to harbor multiple allosteric sites that can potentially be used for drug discovery. A number of promising examples of small molecule allosteric NR targeting concepts have recently been reported, illustrating the high potential of this concept. Inspiration for novel modes of allosteric NR modulation can be found in other receptor classes or, alternatively, via novel chemical concepts such as tethering, bitopic ligands and fragment based screening. The future for allosteric small molecule modulators of Nuclear Receptors is highly promising.

Acknowledgements

References

Anbalagan, M., Huderson, B., Murphy, L., Rowan, B.G., 2012. Post-translational modifications of nuclear receptors and human disease. Nucl. Recept. Signal. 10, e001.

Ban, F., et al., 2014. Discovery of 1 H-indole-2-carboxamides as novel inhibitors of the androgen receptor binding function 3 (BF3). J. Med. Chem. 57, 6867–6872.

Bauman, J.D., et al., 2014. Detecting allosteric sites of HIV-1 reverse transcriptase by X-Ray crystallographic fragment screening. J. Med. Chem. 56, 2738–2746.

Becares Salles, N., Gage, M.C., Pineda-Torra, I., 2016. Post-translational modifications of lipid-activated nuclear receptors: focus on metabolism. Endocrinology 158, 213–225.

Belorusova, A.Y., et al., 2014. Structural insights into the molecular mechanism of vitamin D receptor activation by lithocholic acid involving a new mode of ligand recognition. J. Med. Chem. 57, 4710–4719.

Bernardes, A., et al., 2013. Molecular mechanism of peroxisome proliferator-activated receptor α activation by WY14643: a new mode of ligand recognition and receptor stabilization. J. Mol. Biol. 425, 2878–2893.

Bhaskar, V., et al., 2012. A fully human, allosteric monoclonal antibody that activates the insulin receptor and improves glycemic control. Diabetes 61, 1263–1271.

Billas, I., Moras, D., 2013. Allosteric controls of nuclear receptor function in the regulation of transcription. J. Mol. Biol. 425, 2317–2329.

Biron, E., Bédard, F., 2016. Recent progress in the development of protein-protein interaction inhibitors targeting androgen receptor-coactivator binding in prostate cancer. J. Steroid Biochem. Mol. Biol. 161, 36–44.

Blind, R.D., et al., 2014. The signaling phospholipid PIP3 creates a new interaction surface on the nuclear receptor SF-1. Proc. Natl. Acad. Sci. Unit. States Am. 111, 15054–15059.

Block, G.A., et al., 2004. Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. N. Engl. J. Med. 350, 1516–1525.

Bona, C., Hooghe, R., Cazenave, P.A., Leguérn, C., Paul, W.E., 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. J. Exp. Med. 149, 815–823.

Borngraeber, S., et al., 2003. Ligand selectivity by seeking hydrophobicity in thyroid hormone receptor. Proc. Natl. Acad. Sci. U.S.A. 100, 15358–15363.

Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H., Moras, D., 1995. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-α. Nature 375, 377–382.

Bruning, J.B., et al., 2007. Partial agonists activate PPARγ using a helix 12 independent mechanism. Structure 15, 1258–1271.

Brunmeir, R., Xu, F., 2018. Functional regulation of PPARs through post-translational modifications. Int. J. Mol. Sci. 19 e1738.

Brust, R., et al., 2017. Modification of the orthosteric PPARγ covalent antagonist scaffold yields an improved dual-site allosteric ligand. ACS Chem. Biol. 12, 969–978.

Buchanan, P.J., McCloskey, K.D., 2016. CaV channels and cancer: canonical functions indicate benefits of repurposed drugs as cancer therapeutics. Eur. Biophys. J. 45, 621–633.

Buchdunger, E., et al., 1996. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res. 56, 100–104.

Caboni, L., Lloyd, D.G., 2013. Beyond the ligand-binding pocket: targeting alternate sites in nuclear receptors. Med. Res. Rev. 33, 1081–1118.

Carvalho, A.L., Trincão, J., Romão, M.J., 2010. X-ray crystallography in drug discovery. Methods Mol. Biol. 572, 31–56.

Catterall, W.A., Swanson, T.M., 2015. Structural basis for pharmacology of voltage-gated sodium and calcium channels. Mol. Pharmacol. 88, 141–150.

Catterall, W.A., et al., 2007. Voltage-gated ion channels and gating modifier toxins.

Toxicon 49, 124–141.

Cestèle, S., Catterall, W.A., 2000. Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. Biochimie 82, 883–892.

Chandra, V., et al., 2008. Structure of the intact PPAR-γ–RXR-α nuclear receptor complex on DNA. Nature 456, 350–356.

Changeux, J.P., Christopoulos, A., 2016. Allosteric modulation as a unifying mechanism for receptor function and regulation. Cell 166, 1084–1102.

Changeux, J.-P., Christopoulos, A., 2017. Allosteric modulation as a unifying mechanism for receptor function and regulation. Diabetes Obes. Metab. 19, 4–21.

Chaudari, S.S., et al., 2015. Bicyclic heterocyclic compounds as ROR gamma modulators. PCT Int. Appl. WO 2015/008234.

Chen, L., et al., 2014. Sulindac-derived RXRalpha modulators inhibit cancer cell growth by binding to a novel site. Chem. Biol. 21, 596–607.

Cho, H.-S., et al., 2003. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. Nature 421, 756–760.

Choi, J.H., et al., 2010. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARγ by Cdk5. Nature 466, 451–456.

Choi, J.H., et al., 2011. Antidiabetic actions of a non-agonist PPARγ ligand blocking Cdk5-mediated phosphorylation. Nature 477, 477–481.

Conn, P.J., Kuduk, S.D., Doller, D., 2012. Drug design strategies for GPCR allosteric modulators. Annu. Rep. Med. Chem. 47, 441–457.

Corbin, J.A., et al., 2014. Improved Glucose Metabolism In Vitro and In Vivo by an Allosteric Monoclonal Antibody That Increases Insulin Receptor Binding Affinity. PLoS One 9, e88684.

Cramer, P.E., et al., 2012. ApoE-directed therapeutics rapidly clear -amyloid and reverse deficits in AD mouse models. Science 335, 1503–1506.

Delfosse, V., et al., 2012. Structural and mechanistic insights into bisphenols action provide guidelines for risk assessment and discovery of bisphenol A substitutes. Proc. Natl. Acad. Sci. U.S.A. 109, 14930–14935.

Delfosse, V., et al., 2015. Synergistic activation of human pregnane X receptor by binary cocktails of pharmaceutical and environmental compounds. Nat. Commun. 6, 8089.

Dixon, A.S., et al., 2016. NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. ACS Chem. Biol. 11, 400–408.

Dunker, A.K., et al., 1998. Protein disorder and the evolution of molecular recognition: theory, predictions and observations. Pac. Symp. Biocomput. 473–484.

Egea, P.F., Mitschler, A., Moras, D., 2002. Molecular recognition of agonist ligands by RXRs. Mol. Endocrinol. 16, 987–997.

Erlanson, D.A., Wells, J.A., Braisted, A.C., 2004. Tethering: fragment-based discovery. Annu. Rev. Biophys. Biomol. Struct. 33, 199–223.

Estebanez-Perpina, E., et al., 2007. A surface on the androgen receptor that allosterically regulates coactivator binding. Proc. Natl. Acad. Sci. Unit. States Am. 104, 16074–16079.

Färnegårdh, M., et al., 2003. The three-dimensional structure of the liver X receptor beta reveals a flexible ligand-binding pocket that can accommodate fundamentally different ligands. J. Biol. Chem. 278, 38821–38828.

Fauber, B.P., Magnuson, S., 2014. Modulators of the nuclear receptor retinoic acid receptor-related orphan receptor-gamma (RORgamma or RORc). J. Med. Chem. 57, 5871–5892.

Fauber, B.P., et al., 2015. Discovery of imidazo[1,5-a]pyridines and -pyrimidines as potent and selective RORc inverse agonists. Bioorg. Med. Chem. Lett 25, 2907–2912.

Faus, H., Haendler, B., 2006. Post-translational modifications of steroid receptors. Biomed. Pharmacother. 60, 520–528.

Fendly, B.M., et al., 1990. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. Cancer Res. 50, 1550–1558.

Fernandez, E.J., 2018. Allosteric pathways in nuclear receptors - potential targets for drug design. Pharmacol. Ther. 183, 152–159.

Figueira, A.C.M., et al., 2011. Analysis of agonist and antagonist effects on thyroid hormone receptor conformation by hydrogen/deuterium exchange. Mol. Endocrinol. 25, 15–31.

Fischer, K., Kelly, S.M., Watt, K., Price, N.C., McEwan, I.J., 2010. Conformation of the mineralocorticoid receptor N-terminal domain: evidence for induced and stable structure. Mol. Endocrinol. 24, 1935–1948.

Folkertsma, S., et al., 2007. The use of in vitro peptide binding profiles and in silico ligand-receptor interaction profiles to describe ligand-induced conformations of the retinoid X receptor α ligand-binding domain. Mol. Endocrinol. 21, 30–48.

Forman, B.M., Umesono, K., Chen, J., Evans, R.M., 1995. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell 81, 541–550.

Foster, D.J., Conn, P.J., 2017. Allosteric modulation of GPCRs: new insights and potential utility for treatment of schizophrenia and other CNS disorders. Neuron 94, 431–446.

Frank, F., Okafor, C.D., Ortlund, E.A., 2018. The first crystal structure of a DNA-free nuclear receptor DNA binding domain sheds light on DNA-driven allostery in the glucocorticoid receptor. Sci. Rep. 8, 13497.

Franklin, M.C., et al., 2004. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. Cancer Cell 5, 317–328.

Gallastegui, N., Mackinnon, J.A.G., Fletterick, R.J., Estébanez-Perpiñá, E., 2015. Advances in our structural understanding of orphan nuclear receptors. Trends Biochem. Sci. 40, 25–35.

Gearhart, M.D., et al., 2005. Inhibition of DNA binding by human estrogen-related receptor 2 and estrogen receptor α with minor groove binding polyamides. Biochemistry 44, 4196–4203.

Gege, C., 2015. Retinoid-related orphan receptor γ t modulators: comparison of Glenmark's me-too patent application (WO2015008234) with the originator application from Merck Sharp and Dohme (WO2012106995). Expert Opin. Ther. Pat. 25, 1215–1221.

Gentry, P.R., Sexton, P.M., Christopoulos, A., 2015. Novel allosteric modulators of G protein-coupled receptors. J. Biol. Chem. 290, 19478–19488.

Ghoreschi, K., Laurence, A., O'Shea, J.J., 2009. Selectivity and therapeutic inhibition of kinases: to be or not to be? Nat. Immunol. 10, 356–360.

Giner, X.C., Cotnoir-White, D., Mader, S., Lévesque, D., 2015. Selective ligand activity at Nur/retinoid X receptor complexes revealed by dimer-specific bioluminescence resonance energy transfer-based sensors. FASEB J. 29, 4256–4267.

Gioiello, A., et al., 2011. Extending SAR of bile acids as FXR ligands: discovery of 23-N-(carbocinnamyloxy)-3α,7α-dihydroxy-6α-ethyl-24-nor-5β-cholan-23-amine. Bioorg. Med. Chem. 19, 2650–2658.

Greener, J.G., Sternberg, M.J., 2018. Structure-based prediction of protein allostery. Curr. Opin. Struct. Biol. 50, 1–8.

Haelens, A., Tanner, T., Denayer, S., Callewaert, L., Claessens, F., 2007. The hinge region regulates DNA binding, nuclear translocation, and transactivation of the androgen receptor. Cancer Res. 67, 4514–4523.

Hall, J.M., McDonnell, D.P., Korach, K.S., 2002. Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements. Mol. Endocrinol. 16, 469–486.

Hardy, J.A., 2008. A link means a lot: disulfide tethering in structure-based drug design. Comput. Struct. Approaches to Drug Discov. Ligand-Protein Interact. 318–347.

Helsen, C., Claessens, F., 2014. Looking at nuclear receptors from a new angle. Mol. Cell. Endocrinol. 382, 97–106.

Helsen, C., et al., 2012a. Structural basis for nuclear hormone receptor DNA binding. Mol. Cell. Endocrinol. 348, 411–417.

Helsen, C., et al., 2012b. Evidence for DNA-binding domain–ligand-binding domain communications in the androgen receptor. Mol. Cell Biol. 32, 3033–3043.

Hicklin, D.J., et al., 2001. Monoclonal antibody strategies to block angiogenesis. Drug Discov. Today 6, 517–528.

Hill, K.K., Roemer, S.C., Churchill, M.E.A., Edwards, D.P., 2012. Structural and functional analysis of domains of the progesterone receptor. Mol. Cell. Endocrinol. 348, 418–429.

Hilser, V.J., Thompson, E.B., 2011. Structural dynamics, intrinsic disorder, and allostery in nuclear receptors as transcription factors. J. Biol. Chem. 286, 39675–39682.

Horn, J.R., Shoichet, B.K., 2004. Allosteric inhibition through core disruption. J. Mol. Biol. 336, 1283–1291.

Huang, W., et al., 2013. Allosite: a method for predicting allosteric sites. Bioinformatics 29, 2357–2359.

Huang, W., et al., 2015. ASBench: benchmarking sets for allosteric discovery. Bioinformatics 31, 2598–2600.

Hughes, T.S., et al., 2014. An alternate binding site for PPARγ ligands. Nat. Commun. 5, 3571.

Hughes, T.S., et al., 2016. Probing the complex binding modes of the PPARγ partial agonist 2-chloro- *N* -(3-chloro-4-((5-chlorobenzo[*d*]thiazol-2-yl)thio)phenyl)-4-(trifluoromethyl)benzenesulfonamide (T2384) to orthosteric and allosteric sites with NMR spectroscopy. J. Med. Chem. 59, 10335–10341.

Isono, F., Fujita-Sato, S., Ito, S., 2014. Inhibiting RORγt/Th17 axis for autoimmune disorders. Drug Discov. Today 19, 1205–1211.

Itoh, T., et al., 2008. Structural basis for the activation of PPARgamma by oxidized fatty acids. Nat. Struct. Mol. Biol. 15, 924–931.

Ivanov, I.I., et al., 2006. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121–1133.

Jang, J.Y., et al., 2017. Structural basis for differential activities of enantiomeric PPARγ agonists: binding of S35 to the alternate site. Biochim. Biophys. Acta Protein Proteonomics 1865, 674–681.

Jehle, K., et al., 2014. Coregulator control of androgen receptor action by a novel nuclear receptor-binding motif. J. Biol. Chem. 289, 8839–8851.

Jin, L., Li, Y., 2010. Structural and functional insights into nuclear receptor signaling. Adv. Drug Deliv. Rev. 62, 1218–1226.

Jin, L., et al., 2013. The antiparasitic drug ivermectin is a novel FXR ligand that regulates metabolism. Nat. Commun. 4, 1937.

Jones, S.A., et al., 2000. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. Mol. Endocrinol. 14, 27–39.

Kamenecka, T.M., Lyda, B., Chang, M.R., Griffin, P.R., 2013. Synthetic modulators of the retinoic acid receptor-related orphan receptors. MedChemComm 4, 764–776.

Karstens, W.F.J., et al., 2012. RORgammaT inhibitors, PCT Int. Appl. WO 2012/106995.

Keedy, D.A., et al., 2018. An expanded allosteric network in PTP1B by multitemperature crystallography, fragment screening, and covalent tethering. Elife 7, 1–36.

Kenakin, T.P., 2014. Allosteric modulation. In: A Pharmacology Primer. Techniques for More Effect and Strategic Drug Discovery. Academic Press, pp. 155–180.

Khan, S.H., Ling, J., Kumar, R., 2011. TBP Binding-Induced Folding of the Glucocorticoid Receptor AF1 Domain Facilitates Its Interaction with Steroid Receptor Coactivator-1. PLoS One 6, e21939.

Kim, D.-H., et al., 2015. A dysregulated acetyl/SUMO switch of FXR promotes hepatic inflammation in obesity. EMBO J. 34, 184–199.

Kliewer, S.A., Goodwin, B., Willson, T.M., 2002. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr. Rev. 23, 687–702.

Kojetin, D.J., Burris, T.P., 2013. Small molecule modulation of nuclear receptor conformational dynamics: implications for function and drug discovery. Mol. Pharmacol. 83, 1–8.

Kojetin, D.J., et al., 2015. Structural mechanism for signal transduction in RXR nuclear receptor heterodimers. Nat. Commun. 6, 8013.

Korn, T., Bettelli, E., Oukka, M., Kuchroo, V.K., 2009. IL-17 and Th17 cells. Annu. Rev. Immunol. 27, 485–517.

Kumar, R., Litwack, G., 2009. Structural and functional relationships of the steroid hormone receptors' N-terminal transactivation domain. Steroids 74, 877–883.

Kumar, R., et al., 2013. Regulation of the structurally dynamic N-terminal domain of progesterone receptor by protein-induced folding. J. Biol. Chem. 288, 30285–30299.

Kuo, L.C., 2011. Fragment-based drug design: tools, practical approaches, and examples. In: Methods in Enzymology, vol. 493 Academic Press xxi-xxii.

Kurakula, K., Koenis, D.S., van Tiel, C.M., de Vries, C.J.M., 2014. NR4A nuclear receptors are orphans but not lonesome. Biochim. Biophys. Acta Mol. Cell Res. 1843, 2543–2555.

Kurokawa, R., et al., 1994. Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. Nature 371, 528–531.

Lack, N.A., et al., 2013. Targeting the Binding Function 3 (BF3) site of the human androgen receptor through virtual screening. J. Med. Chem. 54, 8563–8573.

Lallous, N., et al., 2016. Targeting binding function-3 of the androgen receptor blocks its Co-chaperone interactions, nuclear translocation, and activation. Mol. Canc. Therapeut. 15, 2936–2945.

Lane, J.R., Sexton, P.M., Christopoulos, A., 2013. Bridging the gap: bitopic ligands of G-protein-coupled receptors. Trends Pharmacol. Sci. 34, 59–66.

Lane, J.R., et al., 2014. A new mechanism of allostery in a G protein–coupled receptor dimer. Nat. Chem. Biol. 10, 745–752.

Lazarus, K.A., Wijayakumara, D., Chand, A.L., Simpson, E.R., Clyne, C.D., 2012. Therapeutic potential of liver receptor homolog-1 modulators. J. Steroid Biochem. Mol. Biol. 130, 138–146.

Leibowitz, M.D., et al., 2006. Biological characterization of a heterodimer-selective retinoid X receptor modulator: potential benefits for the treatment of type 2 diabetes. Endocrinology 147, 1044–1053.

Li, Y., et al., 2008. T2384, a novel antidiabetic agent with unique peroxisome proliferator-activated receptor gamma binding properties. J. Biol. Chem. 283, 9168–9176.

Li, L., et al., 2015. Impeding the interaction between Nur77 and p38 reduces LPS-induced inflammation. Nat. Chem. Biol. 11, 339–346.

Lisi, G.P., Loria, J.P., 2017. Allostery in enzyme catalysis. Curr. Opin. Struct. Biol. 47, 123–130.

Liu, H., Han, R., Li, J., Liu, H., Zheng, L., 2016. Molecular mechanism of R-bicalutamide switching from androgen receptor antagonist to agonist induced by amino acid mutations using molecular dynamics simulations and free energy calculation. J. Comput. Aided Mol. Des. 30, 1189–1200.

Lu, S., Zhang, J., 2017. Allosteric modulators. In: Comprehensive Medicinal Chemistry III, vol. 2. Elsevier, pp. 276–296.

Lu, S., Li, S., Zhang, J., 2014. Harnessing allostery: a novel approach to drug discovery. Med. Res. Rev. 34, 1242–1285.

Lu, S., Ji, M., Ni, D., Zhang, J., 2018. Discovery of hidden allosteric sites as novel targets for allosteric drug design. Drug Discov. Today 23, 359–365.

Macalino, S.J.Y., Gosu, V., Hong, S., Choi, S., 2015. Role of computer-aided drug design in modern drug discovery. Arch Pharm. Res. 38, 1686–1701.

Makishima, M., et al., 2002. Vitamin D receptor as an intestinal bile acid sensor. Science 296, 1313–1316.

Massafra, V., van Mil, S.W.C., 2018. Farnesoid X receptor: a "homeostat" for hepatic nutrient metabolism. Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1864, 45–59.

McFarland, K., et al., 2013. Low dose bexarotene treatment rescues dopamine neurons and restores behavioral function in models of Parkinson's disease. ACS Chem. Neurosci. 4, 1430–1438.

Meijsing, S.H., et al., 2009. DNA binding site sequence directs glucocorticoid receptor structure and activity. Science 324, 407–410.

Meyer, U., Costantino, G., Macchiarulo, A., Pelliccari, R., 2005. Is antagonism of E/Z-guggulsterone at the farnesoid X receptor mediated by a noncanonical binding site? A molecular modeling study. J. Med. Chem. 48, 6948–6955.

Mi, L.-Z., et al., 2003. Structural basis for bile acid binding and activation of the nuclear receptor FXR. Mol. Cell 11, 1093–1100.

Michellys, P., et al., 2003. Design, synthesis, and Structure−Activity relationship studies of novel 6,7-Locked-[7-(2-alkoxy-3,5-dialkylbenzene)-3-methylocta]-2,4,6-trienoic acids. J. Med. Chem. 46, 4087–4103.

Mohr, K., et al., 2010. Rational design of dualsteric GPCR ligands: quests and promise. Br. J. Pharmacol. 159, 997–1008.

Moore, T.W., Mayne, C.G., Katzenellenbogen, J.A., 2010. Minireview: not picking pockets: nuclear receptor alternate-site modulators (NRAMs). Mol. Endocrinol. 24, 683–695.

Moras, D., Gronemeyer, H., 1998. The nuclear receptor ligand-binding domain: structure and function. Curr. Opin. Cell Biol. 10, 384–391.

Motlagh, H.N., Li, J., Thompson, E.B., Hilser, V.J., 2012. Interplay between allostery and intrinsic disorder in an ensemble. Biochem. Soc. Trans. 40, 975–980.

Motlagh, H.N., Wrabl, J.O., Li, J., Hilser, V.J., 2014. The ensemble nature of allostery. Nature 508, 331–339.

Munuganti, R.S.N., et al., 2013. Targeting the binding function 3 (BF3) site of the androgen receptor through virtual screening. 2. Development of 2-((2-phenoxyethyl) thio)-1H-benzimidazole derivatives. J. Med. Chem. 56, 1136–1148.

Munuganti, R.S.N., et al., 2014. Identification of a potent antiandrogen that targets the BF3 site of the androgen receptor and inhibits enzalutamide-resistant prostate cancer. Chem. Biol. 21, 1476–1485.

Musille, P.M., et al., 2012. Antidiabetic phospholipid – nuclear receptor complex reveals the mechanism for phospholipid driven gene regulation. Nat. Struct. Mol. Biol. 19 532-S2.

Musille, P.M., Kossmann, B.R., Kohn, J.A., 2016. Ivanov, I. & Ortlund, E. A. Unexpected allosteric network contributes to LRH-1 co-regulator selectivity. J. Biol. Chem. 291, 1411–1426.

Nickols, N.G., Dervan, P.B., 2007. Suppression of androgen receptor-mediated gene expression by a sequence-specific DNA-binding polyamide. Proc. Natl. Acad. Sci. U.S.A. 104, 10418–10423.

Niemeyer, B.A., et al., 2001. Ion channels in health and disease. 83rd boehringer ingelheim fonds international titisee conference. EMBO Rep. 2, 568–573.

Nolte, R.T., et al., 1998. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. Nature 395, 137–143.

Nussinov, R., Tsai, C.-J., 2013. Allostery in disease and in drug discovery. Cell 153, 293–305.

Oikonomakos, N.G., Skamnaki, V.T., Tsitsanou, K.E., Gavalas, N.G., Johnson, L.N., 2000. A new allosteric site in glycogen phosphorylase b as a target for drug interactions. Structure 8, 575–584.

Orlov, I., Rochel, N., Moras, D., Klaholz, B.P., 2012. Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA. EMBO J 31, 291–300.

Ostrem, J.M., Peters, U., Sos, M.L., Wells, J.A., Shokat, K.M., 2013. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature 503, 548–551.

Ouvry, G., et al., 2016. Discovery of phenoxyindazoles and phenylthioindazoles as RORγ inverse agonists. Bioorg. Med. Chem. Lett 26, 5802–5808.

Pande, J., Szewczyk, M.M., Grover, A.K., 2010. Phage display: concept, innovations, applications and future. Biotechnol. Adv. 28, 849–858.

Pargellis, C., et al., 2002. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. Nat. Struct. Biol. 9, 268–272.

Patel, S.R., Skafar, D.F., 2015. Modulation of nuclear receptor activity by the F domain. Mol. Cell. Endocrinol. 418, 298–305.

Pellicciari, R., et al., 2006. Back door modulation of the farnesoid X Receptor: design, synthesis, and biological evaluation of a series of side chain modified. Chenodeoxycholic Acid Derivatives 49, 4208–4215.

Pellicciari, R., et al., 2016. Discovery of 3α,7α,11β-Trihydroxy-6α-ethyl-5β-cholan-24-oic acid (TC-100), a novel bile acid as potent and highly selective FXR agonist for enterohepatic disorders. J. Med. Chem. 59, 9201–9214.

Pissios, P., Tzameli, I., Kushner, P.J., Moore, D.D., 2000. Dynamic stabilization of nuclear receptor ligand binding domains by hormone or corepressor binding. Mol. Cell 6, 245–253.

Puhl, A.C., et al., 2012. Mode of peroxisome proliferator-activated receptor γ activation by luteolin. Mol. Pharmacol. 81, 788–799.

Putcha, B.-D.K., Fernandez, E.J., 2009. Direct interdomain interactions can mediate allosterism in the thyroid receptor. J. Biol. Chem. 284, 22517–22524.

Rastinejad, F., Huang, P., Chandra, V., Khorasanizadeh, S., 2013. Understanding nuclear receptor form and function using structural biology. J. Mol. Endocrinol. 51, T1–T21.

Renaud, J.P., et al., 1995. Crystal structure of the RAR-γ ligand-binding domain bound to all-trans retinoic acid. Nature 378, 681–689.

Rochel, N., et al., 2011. Common architecture of nuclear receptor heterodimers on DNA direct repeat elements with different spacings. Nat. Struct. Mol. Biol. 18, 564–570.

Sablin, E.P., et al., 2009. Structure of SF-1 bound by different phospholipids: evidence for regulatory ligands. Mol. Endocrinol. 23, 25–34.

Scheepstra, M., et al., 2015. Identification of an allosteric binding site for RORγt inhibition. Nat. Commun. 6, 8833.

Scheepstra, M., et al., 2017. Ligand dependent switch from RXR homo- to RXR-NURR1 heterodimerization. ACS Chem. Neurosci. 8, 2065–2077.

Shang, J., et al., 2018. Cooperative cobinding of synthetic and natural ligands to the nuclear receptor PPARγ. Elife 7.

Simons, S.S., Edwards, D.P., Kumar, R., Kumar, R., 2014. Minireview: dynamic structures of nuclear hormone receptors: new promises and challenges. Mol. Endocrinol. 28, 173–182.

Smerdon, S.J., et al., 1994. Structure of the binding site for nonnucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. U.S.A. 91, 3911–3915.

Smith, G.B., Olsen, R.W., 2000. Deduction of amino acid residues in the GABAA receptor α subunits photoaffinity labeled with the benzodiazepine flunitrazepam. Neuropharmacology 39, 55–64.

Smith, A.M., et al., 2017. The protein tyrosine phosphatase 1B inhibitor MSI-1436 stimulates regeneration of heart and multiple other tissues. npj Regen. Med. 2, 4.

Souza, P.C.T., et al., 2014. Identification of a new hormone-binding site on the surface of thyroid hormone receptor. Mol. Endocrinol. 28, 534–545.

Spathis, A.D., et al., 2017. Nurr1:RXRα heterodimer activation as monotherapy for Parkinson's disease. Proc. Natl. Acad. Sci. U.S.A. 114, 3999–4004.

Steinfeld, T., Mammen, M., Smith, J.A.M., Wilson, R.D., Jasper, J.R., 2007. A novel multivalent ligand that bridges the allosteric and orthosteric binding sites of the M2 muscarinic receptor. Mol. Pharmacol. 72, 291–302.

Stope, M.B., Burchardt, M., 2012. Re: targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. Eur. Urol. 62, 931–932.

Suino-Powell, K., et al., 2008. Doubling the size of the glucocorticoid receptor ligand binding pocket by deacylcortivazol. Mol. Cell Biol. 28, 1915–1923.

Takeshita, A., Ozawa, Y., Chin, W.W., 2000. Nuclear receptor coactivators facilitate vitamin D receptor homodimer action on direct repeat hormone response elements. Endocrinology 141 1281–1281.

Taly, A., Hénin, J., Changeux, J.-P., Cecchini, M., 2014. Allosteric regulation of pentameric ligand-gated ion channels: an emerging mechanistic perspective. Channels 8, 350–360.

Tanenbaum, D.M., et al., 1998. Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. Proc. Natl. Acad. Sci. Unit. States Am. 95, 5998–6003.

Thal, D.M., Glukhova, A., Sexton, P.M., Christopoulos, A., 2018. Structural insights into G-protein-coupled receptor allostery. Nature 559, 45–53.

Tice, C.M., Zheng, Y.J., 2016. Non-canonical modulators of nuclear receptors. Bioorg. Med. Chem. Lett 26, 4157–4164.

Tipps, M.E., Lawshe, J.E., Ellington, A.D., Mihic, S.J., 2010. Identification of novel specific allosteric modulators of the glycine receptor using phage display. J. Biol. Chem. 285, 22840–22845.

Varga, T., Czimmerer, Z., Nagy, L., 2011. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. Biochim. Biophys. Acta 1812, 1007–1022.

Velasco, L.F.R., et al., 2007. Thyroid hormone response element organization dictates the composition of active receptor. J. Biol. Chem. 282, 12458–12466.

Venepally, P., Reddy, L.G., Sani, B.P., 1997. Analysis of homo- and heterodimerization of retinoid receptors in solution. Arch. Biochem. Biophys. 343, 234–242.

de Vera, I.M.S., et al., 2016. Identification of a binding site for unsaturated fatty acids in the orphan nuclear receptor Nurr1. ACS Chem. Biol. 11, 1795–1799.

de Vera, I.M.S., et al., 2017. Synergistic regulation of coregulator/nuclear receptor interaction by ligand and DNA. Structure 25 1506–1518.e4.

de Vera, I.M.S., et al., 2019. Defining a canonical ligand-binding pocket in the orphan nuclear receptor Nurr1. Structure 27 66–77.e5.

De Vries-van Leeuwen, I.J., et al., 2013. Interaction of 14-3-3 proteins with the Estrogen Receptor Alpha F domain provides a drug target interface. Proc. Natl. Acad. Sci. Unit. States Am. 110, 8894–8899.

Waku, T., Shiraki, T., Oyama, T., Morikawa, K., 2009a. Atomic structure of mutant PPARγ LBD complexed with 15d-PGJ $_2$: novel modulation mechanism of PPARγ/RXRα function by covalently bound ligands. FEBS Lett. 583, 320–324.

Waku, T., et al., 2009b. Structural insight into PPARγ activation through covalent modification with endogenous fatty acids. J. Mol. Biol. 385, 188–199.

Wang, Y., et al., 2006. A second binding site for hydroxytamoxifen within the coactivator-binding groove of estrogen receptor beta. Proc. Natl. Acad. Sci. Unit. States Am. 103, 9908–9911.

Wang, W., et al., 2014. Orphan nuclear receptor TR3 acts in autophagic cell death via mitochondrial signaling pathway. Nat. Chem. Biol. 10, 133–140.

Wang, W., et al., 2015. Induction of autophagic death in cancer cells by agonizing TR3 and attenuating Akt2 activity. Chem. Biol. 22, 1040–1051.

Waszkielewicz, A.M., et al., 2013. Ion channels as drug targets in central nervous system disorders. Curr. Med. Chem. 20, 1241–1285.

van Westen, G.J.P., Gaulton, A., Overington, J.P., 2014. Chemical, target, and bioactive properties of allosteric modulation. PLoS Comput. Biol. 10, e1003559.

Whittal, R.M., et al., 2000. Preferential oxidation of zinc finger 2 in estrogen receptor DNA-binding domain prevents dimerization and, hence, DNA binding. Biochemistry 39, 8406–8417.

Williams, D.B., Akabas, M.H., 2000. Benzodiazepines induce a conformational change in the region of the gamma-aminobutyric acid type A receptor alpha(1)-subunit M3 membrane-spanning segment. Mol. Pharmacol. 58, 1129–1136.

Windshugel, B., 2018. Structural insights into ligand-binding pocket formation in Nurr1 by molecular dynamics simulations. J. Biomol. Struct. Dyn. 1–21.

Wright, P.E., Dyson, H.J., 2015. Intrinsically disordered proteins in cellular signalling and regulation. Nat. Rev. Mol. Cell Biol. 16, 18–29.

Yang, X.O., et al., 2008. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity 28, 29–39.

Yang, L., Broderick, D., Jiang, Y., Hsu, V., Maier, C.S., 2014. Conformational dynamics of human FXR-LBD ligand interactions studied by hydrogen/deuterium exchange mass spectrometry: insights into the antagonism of the hypolipidemic agent Z-guggulsterone. Biochim. Biophys. Acta Protein Proteonomics 1844, 1684–1693.

Yoon, H.S., et al., 2018. Co-crystals of Nurr1-LBD in complex with a cyclopentenone prostaglandin and modulators of Nurr1, PCT Int. Appl. WO 2018/056905.

Zhan, Y., et al., 2012. The orphan nuclear receptor Nur77 regulates LKB1 localization and activates AMPK. Nat. Chem. Biol. 8, 897–904.

Zhang, J., et al., 2011. DNA binding alters coactivator interaction surfaces of the intact VDR-RXR complex. Nat. Struct. Mol. Biol. 18, 556–563.

Zhang, X., Zhou, H., Su, Y., 2015. Targeting truncated RXRα for cancer therapy. Acta Biochim. Biophys. Sin. 48, 49–59.

Zheng, J., et al., 2018. β-Glucocerebrosidase modulators promote dimerization of β-glucocerebrosidase and reveal an allosteric binding site. J. Am. Chem. Soc. 140, 5914–5924.

Zwart, W., et al., 2007. PKA-induced resistance to tamoxifen is associated with an altered orientation of ERalpha towards co-activator SRC-1. EMBO J. 26, 3534–3544.