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Non-canonical dimerization of the androgen receptor and other nuclear receptors: Implications for human disease

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Abstract

Nuclear receptors are transcription factors that play critical roles in development, homeostasis and metabolism in all multicellular organisms. An important family of nuclear receptors comprises those members that respond to steroid hormones, and which is subdivided in turn into estrogen receptor (ER) isoforms α and β (NR3A1 and A2, respectively), and a second subfamily of so called oxosteroid receptors. The latter includes the androgen receptor (AR/NR3C4), the glucocorticoid receptor (GR/NR3C1), the mineralocorticoid receptor (MR/NR3C2), and the progesterone receptor (PR/NR3C3). Here we review recent advances in our understanding of the structure-and-function relationship of steroid nuclear receptors, and discuss their implications for the etiology of human diseases. We focus in particular on the role played by AR dysregulation in both prostate cancer (PCa) and androgen insensitivity syndromes (AIS), but also discuss conditions linked to mutations of the *GR* gene as well as those in a non-steroidal receptor, the thyroid hormone receptor (TR). Finally, we explore how these recent results might be exploited for the development of novel and selective therapeutic strategies.

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Introduction

Nuclear receptors (NRs) form a superfamily of related transcription factors that play essential roles in multicellular organisms through coordination of pivotal signaling networks (Evans and Mangelsdorf 2014). One important family of NRs groups together those members that respond to steroid hormones, accordingly termed steroid family. In vertebrates, the steroid NRs include estrogen receptor (ER) isoforms ERα (NR3A1) and ERβ (NR3A2) together with the androgen receptor (AR/NR3C4), the glucocorticoid receptor (GR/NR3C1), the mineralocorticoid receptor (MR/NR3C2), and the progesterone receptor (PR/NR3C3). Phylogenetic studies show that AR, GR, MR and PR comprise a subfamily of so called oxosteroid NRs, which markedly differ from both ER isoforms (Bledsoe *et al.* 2002, Evans and Mangelsdorf 2014, Gallastegui *et al.* 2015, Zennaro and Fernandes-Rosa 2017, Katzenellenbogen *et al.* 2018). This phylogenetic separation is also reflected at the level of tertiary and quaternary structures, as we will discuss below.

We note that some members of other subfamilies of NRs specifically respond to steroid hormones, for instance the vitamin D3 receptor (VDR/NR1I1), the bile acid receptor (FXR/NR1H4), oxysterol receptors α and β (LXRα/NR1H3 and LXRβ/NR1H2, respectively) and the retinoid-related orphan receptors α, β and γ (RORα/NR1F1, RORβ/NR1F2, and RORγ/NR1F3, respectively). However, for space reasons we will focus in the current review almost exclusively on the members of the sequence and structurally related “classical” steroid family of NRs, most notably on the AR.

Similar to other NRs, the members of the steroid family display a marked modular structure: a variable N-terminal region called the N-terminal domain (NTD) is followed by a highly conserved DNA-binding domain (DBD) comprised of two zinc finger motifs, and a C-terminal ligand-binding domain (LBD) that contains the internal ligand-binding pocket (LBP) (Jenster *et al.* 1995, McEwan 2012a, Gallastegui *et al.* 2015, Tien and Sadar 2018, Weikum *et al.* 2018, Fuentes-Prior *et al.* 2019, Veras Ribeiro Filho *et al.* 2019). DBD and LBD modules are separated by a poorly conserved interdomain linker called 'hinge', which harbors a nuclear localization signal (NLS). (See Fig. 1 for a schematic representation of the domain organization in steroid NRs). All the NR-comprising domains are proposed drug targets, but the LBD remains still the main module for therapeutic intervention and, in spite of intense efforts, no molecule targeting other receptor domains has advanced into late clinical trials (Sadar 2011, Dalal *et al.* 2014, Nadal *et al.* 2017, Veras Ribeiro Filho *et al.* 2019). Over the years, several physiologically relevant protein partners of NRs have been reported to selectively fine-tune their actions in various tissues. These factors, collectively termed coregulators, can either enhance or inhibit transcription of target genes, and are therefore known as coactivators or corepressors, respectively (Hermanson *et al.* 2002, Perissi and Rosenfeld 2005, Dasgupta *et al.* 2014). These NR coregulators underlie the tissue-selective actions of NR ligands and are also emerging therapeutic targets *per se*, although they have proved hard to target so far (Yi *et al.* 2015, Wang *et al.* 2016, Ruggero *et al.* 2018). All NR domains have been involved in coregulator recruitment and macromolecular complex formation, although the atomic details still remain elusive (Dasgupta *et al.* 2014, Foley and Mitsiades 2016, Giudici *et al.* 2016, Lempiäinen *et al.* 2017).

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Although the NTDs are variable in length and intrinsically disordered (i.e., isolated NTDs lack stable secondary (2D) and/or tertiary (3D) structures under physiological conditions), the results of different biochemical and biophysical studies suggest that they undergo at least partial folding ("helicity induction") to modulate the formation of competent transcription activation complexes around the NRs (Kumar *et al.* 2004, McEwan *et al.* 2007, De Mol *et al.* 2016, De Mol *et al.* 2018). This is particularly relevant for a polypeptide stretch termed activation function-1 (AF-1; residues 142-485 in the human AR), which is a hormone-independent transactivation function (Fig. 1). An important result of early investigations on the role of the NTD was the realization of at least transient contacts with the C-terminal LBD moiety. These intra- or intermolecular amino/carboxy terminal (N-C) interactions play key roles in regulating the functions of some steroid receptors, both by stabilizing the overall protein structure and by modulating interactions with DNA and coregulators, thus ultimately controlling gene expression (Langley *et al.* 1998, He and Wilson 2002, Bai *et al.* 2005). On the LBD, a solvent-exposed pocket responsible for recognizing both AF-1 and coregulators has been extensively studied, which is called activation function 2 (AF-2) or ligand-dependent coactivator-binding site (Fig. 1, Fig. 2A). At least in the case of the AR, this is in addition to a nearby pocket that contributes to allosterically regulate receptor functions, and which has been termed binding function-3 (BF-3) (Estébanez-Perpiñá *et al.* 2005,

Estébanez-Perpiñá *et al.* 2007b, Buzón *et al.* 2012, Ravindranathan *et al.* 2013, Badders *et al.* 2018).[1] (Fig. 2B)

In the following section, we will briefly discuss recently presented results on the structure-and-function relationship of the AR, but will refer to and compare with other steroid NRs when appropriate.

The "life cycle" of the androgen receptor – From chaperone-bound monomer to multimers on chromatin

Newly synthesized AR is present in the cytoplasm associated with major molecular chaperones (Prescott and Coetzee 2006, Centenera *et al.* 2013a, Guy *et al.* 2015, Foley and Mitsiades 2016, Kita *et al.* 2017). AR first binds to the heat shock protein 70 (Hsp70)–Hsp40 complex, which is followed by binding of Hsp90 and co-chaperones and subsequent dissociation of Hsp70–Hsp40. Hsp90 binding ensures that the AR retains a high-affinity conformation for the androgenic hormones, testosterone and its more potent derivative, dihydrotestosterone (DHT) (Hernández *et al.* 2002, Azad *et al.* 2015). Another chaperone, Hsp27, is regulated by cell stress and prevents aggregation and degradation of the AR, besides promoting nuclear trafficking of the receptor and its binding to DNA (Zoubeidi *et al.* 2007).

Binding of androgens in the cytoplasm induces AR dissociation from the chaperone complex, likely accompanied by significant conformational changes, nuclear translocation of the receptor and its dimerization, which is a critical step

[1] A note of caution must be added here, as most of the structural and functional evidence comes from studies performed using short peptides derived from known coregulators, which might not correctly reproduce interactions found in multiprotein complexes *in vivo*.

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within this signaling pathway (Centenera *et al.* 2008, van Royen *et al.* 2012, Nadal *et al.* 2017). Pioneer studies suggested that AR oligomerization precedes binding to chromatin, and demonstrated that DNA binding was redox-dependent (Kokontis and Liao 1998). More recently, AR dimerization has been shown to enhance chromatin interaction and remodeling to modulate gene expression (Jin *et al.* 2013). However, it must be stressed that the exact cycle of AR monomer-to-oligomer transition in the cell has not been precisely elucidated (Schaufele *et al.* 2005, Centenera *et al.* 2008, van Royen *et al.* 2012, Nadal *et al.* 2017). It is usually assumed that this transition follows exactly the same steps as in related NRs, although data supporting this assumption is incomplete, or simply lacking.

Recent studies of steroid NR trafficking in living cells have dramatically advanced our understanding of this process, forcing a paradigm shift in the field. In particular, careful quantitation of GR, AR and PR dynamics at the single-molecule level have unexpectedly revealed that the dimeric forms of these receptors are intermediate states towards their biologically active, tetrameric arrangements ('dimers of dimers') (Presman *et al.* 2016, Paakinaho *et al.* 2017, Presman *et al.* 2017, Presman and Hager 2017). Further, it has been established that the regulatory complexes assemble around steroid NRs undergo a rapid exchange in the time scale of seconds (the "hit-and-run" model; refs. (Voss *et al.* 2011, Sung *et al.* 2014, Swinstead *et al.* 2016, Presman and Hager 2017). However, detailed information regarding the activities of these signaling complexes in normal and in tumor cells is still missing. The intricate links between NR oligomeric state, affinity for ligands (hormones), binding cooperativity and recruitment to specific chromatin sites remain to be worked out (Fuentes-Prior *et al.* 2019).

157 ***Quaternary structure of the AR-LBD: Functional implications***

158 Several AR features have complicated elucidation of the physiologically relevant
159 tertiary and quaternary structures of full-length AR (FL-AR) and other steroid
160 receptors, and it is still a matter of debate e.g. whether the biological units are
161 dimers or 'dimers of dimers' (see above and refs. (Presman *et al.* 2016, Nadal *et*
162 *al.* 2017, Presman *et al.* 2017, Presman and Hager 2017)). In addition to the
163 conformational flexibility that is a prerequisite for the functional versatility of the
164 individual AR modules, they are known to interact with each other to precisely
165 regulate the NR functions in a context-dependent manner. Further, it is worth
166 noting that post-translational modifications (PTMs) profoundly modulate AR
167 actions, both under physiological and pathological conditions (Faus and Haendler
168 2006, Gioeli and Paschal 2012, Treviño and Weigel 2013, van der Steen *et al.*
169 2013, Koryakina *et al.* 2014).

170 The intrinsically disordered nature of the long NTD and its unique
171 interactions with the LBD (the 'N/C contacts') raised over the years the issue of
172 whether FL-AR dimerizes in a parallel, "head-to-head" manner, or alternatively in
173 an anti-parallel, "head-to-tail" configuration (Langley *et al.* 1995, Langley *et al.*
174 1998, Schaufele *et al.* 2005, Centenera *et al.* 2008, Minges *et al.* 2013). On the
175 other hand, the crystal structure of rat AR-DBD revealed a head-to-head
176 homodimer bound to its cognate DNA (Shaffer *et al.* 2004), in an arrangement
177 quite similar to that adopted by DBD dimers of GR (Luisi *et al.* 1991) and ER
178 (Schwabe *et al.* 1993). In this conformation, the C-terminal residues of both
179 monomers, corresponding to L627 in the human receptor, are located far away
180 (about 50 Å). However, the length of the G628-E669 linker does not allow for an
181 accurate prediction of the likely arrangement of LBD monomers in a hypothetical

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dimer. In fact, limited and contradictory information in support of AR-LBD dimerization had been presented over the years, with some authors even suggesting that the LBD-LBD interactions may not significantly contribute to receptor oligomerization (Schaufele *et al.* 2005, Centenera *et al.* 2008, van Royen *et al.* 2012, Nadal *et al.* 2017).

The long standing questions of whether the AR-LBD domain dimerized and the relative arrangement of monomers have been answered with the resolution of the crystal structure of human AR-LBD bound to DHT and a peptide derived from the coregulator, ubiquitin-activating enzyme 3 (UBA3) (Nadal *et al.* 2017). All the AR-LBD structures previously solved by us and others had captured essentially the same conformation of monomeric AR-LBD (Matias *et al.* 2000, Sack *et al.* 2001, He *et al.* 2004, Estébanez-Perpiñá *et al.* 2005, Bohl *et al.* 2007, Estebanez-Perpina *et al.* 2007). In striking contrast, our recent crystal structure features four independent, helically arranged LBD molecules (Nadal *et al.* 2017) (Fig. 2B). Two of these LBD monomers are arranged in a symmetrical 'core dimer', while the other two peripheral partners are associated in a less compact manner to the BF-3 sites of each of these monomers (Fig. 2B). The core AR-LBD dimer exhibits a head-to-head arrangement and displays the corresponding coactivator binding sites (AF-2 pockets) facing opposite directions, thus able to independently interact with coregulators (Fig. 2A).

Helices 5 (H5) from both AR-LBD moieties occupy the center of the dimerization interface (Fig. 3A), which is thus topologically distinct from the 'canonical', H10-11-centered arrangements identified, among others, in ER homodimers and in RXR-mediated heterodimers (Fig. 3C) (Wurtz *et al.* 1996, Brzozowski *et al.* 1997, Bourguet *et al.* 2000, Chandra *et al.* 2008,

Khorasanizadeh and Rastinejad 2016, Chandra *et al.* 2017, Nadal *et al.* 2017, Fuentes-Prior *et al.* 2019). The H5-centered dimerization mode of human AR-LBD has been confirmed in solution and in cells (Nadal *et al.* 2017). This non-canonical dimeric conformation might be shared by other NRs, not only of the oxosteroid class (Fuentes-Prior *et al.* 2019). In fact, a topologically equivalent dimer of GR-LBD had been previously reported (Bledsoe *et al.* 2002) (Fig. 3B), although its biological relevance has been questioned upon careful comparison of currently available GR structures, and alternative GR dimeric conformations have been suggested (Bianchetti *et al.* 2018). Clearly, further experimental work is needed to fully clarify the physiologically relevant conformation(s) of the GR and other members of the oxosteroid subfamily to integrate structural, biochemical and cellular studies (Fig. 3) (Bledsoe *et al.* 2002, Kauppi *et al.* 2003, Robertson *et al.* 2013a, Robertson *et al.* 2013b, Presman *et al.* 2016, Paakinaho *et al.* 2017, Presman *et al.* 2017, Presman and Hager 2017, Weikum *et al.* 2017, Wilkinson *et al.* 2018).

Although specific functions have been commonly ascribed to the different NR domains (e.g., DNA and hormone binding), seminal X-ray crystallography studies of non-steroidal NRs indicate that DBD, hinge and LBD are more intricately interconnected than initially thought, both structurally and functionally ((Chandra *et al.* 2008, Chandra *et al.* 2013, Lou *et al.* 2014, Chandra *et al.* 2017), reviewed in (Rastinejad *et al.* 2015, Fuentes-Prior *et al.* 2019)). Regarding the AR and other steroid receptors, current knowledge of their inter-domain interactions derives mostly from biochemical and mutagenesis studies. A recent analysis of multidomain ER by small-angle X-ray scattering (SAXS), however, suggests an important cross-talk between DBD and LBD moieties (Huang *et al.*

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2018). These findings, at the light of the results of Rastinejad and coworkers with non-steroidal NRs, point to important DBD-linker-LBD interactions *in vivo* ((Chandra *et al.* 2008, Chandra *et al.* 2013, Chandra *et al.* 2017), reviewed in (Fuentes-Prior *et al.* 2019)). This is in addition to the well-known intra- or intermolecular N-C interactions (see above), which restrict the possible DBD/LBD relative orientations (Fig. 4B).

Allosteric modulation of AR activity

NRs are allosteric proteins *par excellence* (Kuriyan 2004, del Sol *et al.* 2006, McEwan 2012b, Mackinnon *et al.* 2014), and the AR is no exception in this regard (Estebanez-Perpina *et al.* 2007, Buzón *et al.* 2012, Grosdidier *et al.* 2012). Indeed, FL-AR functions as an allosteric switch alternating between inactive, chaperone-bound / ligand-free states, and active, hormone- and coactivator-bound conformations. Ligand binding and the exchange of chaperones by coactivators are allosterically coupled, but the sequence of molecular events and detailed conformational changes associated are only partially understood (Hur *et al.* 2004, Estébanez-Perpiñá *et al.* 2005). In particular, hormone binding to the LBP has been shown to trigger allosteric remodeling of the AF-2 and BF-3 interaction surfaces. In this manner, occupancy of the LBP by ligands regulates the dynamics and stability of surfaces that recognize coregulators, and is thus allosterically coupled to the recruitment of these proteins to the AR (Estébanez-Perpiñá *et al.* 2005, Estebanez-Perpina *et al.* 2007). Albeit a recent proteomics study have identified the essentially overlapping, agonist-specific interactomes of both AR and GR (Lempiäinen *et al.* 2017), the detailed molecular determinants of NR binding to a large number of coregulators are far from being well understood.

Noteworthy, in addition to allosteric rearrangements elicited by hormone binding, the target DNA sequences to which NRs bind also induce important remodeling of the receptor structure (Fuentes-Prior *et al.* 2019). Most impressively, cognate DNA binding sequences of the GR have been shown to function as true allosteric modulators, which are capable of affecting conformation and regulate receptor activity (Meijsing *et al.* 2009, Love *et al.* 2017, Weikum *et al.* 2017, Frank *et al.* 2018). Thus, a change in a single base pair in the GR binding site resulted in up to 10-fold higher affinity in the activation of a glucocorticoid response element (GRE) reporter gene in response to ligand. More recently, it has been reported that nucleotides directly flanking the core-binding site not only modulate the 3D structure of this site, but also that of the GR-DBD, and even the quaternary conformation of the dimeric receptor (Schöne *et al.* 2016). This implies that GR activity can be modulated by both sequence composition and DNA shape to achieve fine-tuning of the GR structure and activity downstream of binding. These features are likely to be shared by other members of the oxosteroid class of NRs, at the light of the almost identical sequences of DBDs and relative conservation of interdomain hinges. This flanking effect could explain how NRs predicted to recognize similar binding motifs show distinct DNA-binding preferences *in vivo*. It is feasible that while two related NRs can bind to the same DNA motif, the competent conformation required for an optimal transcriptional response is only achieved through specific flanking sequences. In particular, we assume that AR-DBD binding to its cognate DNA sequences will trigger intra- and interdomain conformational changes that would affect the overall quaternary structure of the receptor, including its hormone-binding domain.

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The precise molecular mechanisms underlying allosteric transitions in FL-AR, including the equilibrium between different conformational states and the impact of ligand binding to allosteric modulatory sites (e.g., AF-2, BF-3) on receptor oligomerization, remain to be worked out (Fig. 2A) (Nadal *et al.* 2017). In multi-domain AR, both intra- and inter-domain allostery may occur simultaneously (Fernandez *et al.* 2017, Fernandez 2018). The first may take place within either the NTD, DBD or LBD moieties, while inter-domain signal transduction appears to be essential in coordinating AR functions (e.g., through the well-studied N-C contacts, but also upon as of yet uncharacterized DBD-LBD and LBD-DNA interactions). Similar considerations might apply to other steroid NRs.

Dysregulation of steroid receptors and human disease

Given the essential roles of steroid hormones and their cognate NRs in development, homeostasis and metabolism, it is not surprising that dysregulation of their activities is directly responsible for a number of important human conditions (Huang *et al.* 2010, Evans and Mangelsdorf 2014, Luo *et al.* 2018). For instance, the ER is a key driver of 70% of breast cancer subtypes that require estrogen hormones for progression, and different point mutations in the *ER* genes are linked to acquired resistance to commonly used estrogen-blocking drugs such as tamoxifen (Kojetin *et al.* 2008, Katzenellenbogen *et al.* 2018, Nasrazadani *et al.* 2018, Reinert *et al.* 2018). Regarding oxosteroid receptors, single-residue mutations in *GR* and *MR* genes have mainly been associated to loss-of-function phenotypes (Zennaro and Fernandes-Rosa 2017). However, the most conspicuous association between a nuclear receptor and human disease links the AR to several biomedical conditions, as we briefly discuss below.

Genetic bases of drug resistance in prostate cancer

AR is encoded by a ubiquitously expressed gene located in the X-chromosome at Xq11-12, and is particularly important in prostate development and homeostasis (Lubahn *et al.* 1988). When dysregulated, however, AR activity is central to the onset, development, and progression to metastasis of prostate cancer (PCa), the most common cancer diagnosed in males worldwide (Matias *et al.* 2000, Gottlieb *et al.* 2004, Knudsen and Penning 2010, Arora and Barbieri 2018, Centenera *et al.* 2018, Cioni *et al.* 2018, Li *et al.* 2018, Luo *et al.* 2018, Nevedomskaya *et al.* 2018, Paschalis *et al.* 2018). In addition, *AR* mutations are linked to disorders of male sexual differentiation and development termed androgen insensitivity syndromes (AIS) (Hughes *et al.* 2012, Mongan *et al.* 2015, Gibson *et al.* 2018), and to the rare adult-onset hereditary neurodegenerative disorder known as spinal and bulbar muscular atrophy (SBMA or Kennedy's disease; OMIM #313200) (Spada *et al.* 1991, Badders *et al.* 2018, Cortes and La Spada 2018, Lieberman 2018, Pennuto and Rinaldi 2018). Finally, AR malfunction is also associated to androgenic alopecia or loss of scalp hair and skin malignancies (Ellis *et al.* 2001, Clocchiatti *et al.* 2018).

Several altered genetic and epigenetic mechanisms contribute to the development of metastatic and drug-resistant PCa (Knudsen and Penning 2010, Shen and Abate-Shen 2010, Grasso *et al.* 2012, Robinson *et al.* 2015, Pritchard *et al.* 2016, Baumgart and Haendler 2017, Armenia *et al.* 2018, Cotter and Rubin 2018, Karthaus and Sawyers 2018, Li *et al.* 2018, Quigley *et al.* 2018, Ruggero *et al.* 2018). For example, tandem duplications of an upstream enhancer of the *AR* are found in up to 87% of metastatic castration-resistant PCa (mCRPC) cases, compared to <2% of primary prostate cancers (Takeda *et al.* 2018,

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Viswanathan *et al.* 2018, Wu *et al.* 2018). In addition, it has been recently reported that deletion of the gene encoding the chromatin remodeler, chromatin helicase DNA-binding protein (CHD1), redistributes the AR cistrome in a manner that favors expression of PCa-specific oncogenic pathways (Augello *et al.* 2019). Not surprisingly, *CHD1* loss had been previously reported as one of the most common and deleterious genetic alterations in PCa (Grasso *et al.* 2012, Huang *et al.* 2012, Rodrigues *et al.* 2015, Zhao *et al.* 2017) and an early event in cancer development (Wedge *et al.* 2018).

Further, *AR* gene duplications and AR-mediated chromosomal rearrangements are common events. Among them, about half of the patients with PCa feature fusions of the AR-regulated gene, *TMPRSS2*, with different fragments of the *ETS-related gene* (*ERG*), a member of the ETS family of transcription factors. The presence of these *TMPRSS2:ERG* fusions defines the predominant molecular subtype of PCa (Yu *et al.* 2010, Park *et al.* 2014, Reig *et al.* 2016, Berglund *et al.* 2018, Stelloo *et al.* 2018). The encoded ERG truncated variants are resistant to degradation and transform the AR from a factor promoting lineage-specific differentiation of the prostate to one that potentiates de-differentiation into a stem cell-like state (Yu *et al.* 2010). However, *TMPRSS2:ERG* fusions alone do not appear to be transforming (Casey *et al.* 2012), and the precise mechanism(s) by which they contribute to PCa initiation and/or progression are still a matter of debate. For instance, it has been reported that these fusions synergize with deletion of the tumor suppressor, PTEN, to promote prostatic intraepithelial neoplasia (PIN) (Carver *et al.* 2009, King *et al.* 2009, Casey *et al.* 2012). In this regard, and also connecting to the work by Augello and coworkers cited above, it has been proposed that ETS factors alter

the AR cistrome to prime the prostate epithelium to respond to aberrant signals such as *PTEN* loss, thus ultimately resulting in prostate-specific transformation (Chen *et al.* 2013). Carver and coworkers also observed that two genes strongly associated with cell migration, ADAMTS1 and CXCR4, were upregulated upon ERG overexpression (Carver *et al.* 2009). Besides, overexpression of MMP9 and plexin B correlates with the presence of the fusion in samples of PCa patients, and has been linked to migration and invasion of prostate cancer cells (Liu *et al.* 2017).

On the other hand, several studies have described the synthesis *in vivo* of alternatively spliced transcripts encoding truncated AR isoforms. The identification of these constitutively active AR variants (AR-Vs), which lack portions or the entire hormone-sensitive LBD, has added an unanticipated level of complexity to the AR signaling pathways (Dehm and Tindall 2011, Centenera *et al.* 2013b, Ho and Dehm 2017). Many of these truncated AR-Vs seem to support androgen-independent expression of AR-target genes, and therefore androgen-independent growth of PCa cells (Dehm and Tindall 2011). However, the androgen-independency of AR-Vs does not imply that expressing cells are totally independent of the presence of androgenic hormones. The (patho)physiological implications of the coexistence of full-length AR with one or several AR-Vs in the same cell are still a matter of intense debate. The likely formation of AR-Vs (hetero)dimers with FL-AR, either through DBD-mediated or N-C interactions may still result in hormone-dependent cell growth leading to PCa progression (Liu *et al.* 2014, Uo *et al.* 2017). Further, potential differences in the interactomes of the different AR-Vs in comparison with that of the full-length receptor have not been explored so far.

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The AR-LBD dimerization interface is a hot spot of disease-linked mutations

Inspection of the 3D structure of the AR-LBD homodimer immediately revealed that a large number of hitherto unexplained mutations of solvent-exposed residues identified in PCa (Fig. 5A) and AIS patients cluster at the dimerization interface ((Nadal *et al.* 2017); see also Fig. 5B). We stress that these mutations might have additional consequences for AR protein structure and function, such as impaired interaction with chaperones in the monomeric state (see above). Accordingly, the situation *in vivo* does not fit a simplistic dichotomy of gain-of-function mutations causing PCa, while loss-of-function variants of the *AR* gene are linked to AIS (Hay and McEwan 2012).

Nevertheless, there are several correlations between e.g. the nature of the mutant residue and disease severity, which strongly point to impaired homodimer formation as more likely cause of the observed phenotype. This is in particular the case of repeatedly identified mutations that affect residues such as F755, N757, V758, N759, R761, and P767 (Fig. 4A, Fig. 5A and 5C). Most notably, the mutant Y764C, which has been found both in PCa and AIS, and residue F755 that has been found conservatively replaced by either leucine, in some patients with partial AIS, or by another aliphatic residue, valine, in cases of complete androgen insensitivity. The side chain of F755 makes important Van der Waals interactions with P802 from the second monomer (Fig. 4A), and modeling experiments indicate that a leucine residue at position 755 might still interact with this proline, supporting homodimer formation. However, a less bulky valine would not be able to contact P802, with concomitantly impaired dimerization. A second example of strong genotype-phenotype correlation is given by mutant p.V758I identified in PCa patients. Again, a fully conservative replacement of an aliphatic

residue leading to PCa is most likely reflects the higher stability of the homodimer formed by mutant AR-LBD molecules, as the bulkier I758 side chain fills better the intermonomer space. These findings, among others, lend extraordinary support to the (patho)physiological relevance of the AR homodimer interface, and suggest that this area might be targeted for pharmacological intervention (see below).

NR3C1/GR mutations linked to either glucocorticoid-resistance or hypersensitivity syndromes

Glucocorticoids (GCs) are major regulators of many physiological functions, and thus contribute substantially to tissue homeostasis. Dysfunction of GC-mediated actions underlies two human pathologic conditions, Cushing syndrome and Addison's disease, which are due to GC excess or deficiency, respectively. On the other hand, alterations in GR function (either due to mutations or polymorphisms of the encoding gene, *NR3C1*, or to other molecular changes along the GR signaling pathway) result in impaired tissue-specific sensitivity to GCs, which may manifest as GC resistance or hypersensitivity, both of which are associated with significant morbidity (Nicolaides and Charmandari 2015, Nicolaides *et al.* 2015a, Nicolaides *et al.* 2015b, Wilkinson *et al.* 2018).

For instance, in primary generalized GC resistance (PGGR or Chrousos syndrome, MIM #615962), *NR3C1* heterozygous variants (point mutations, insertions or deletions) result in impaired GR function and thus decreased sensitivity to GCs in all organs, although with a high degree of variability among different tissues . Alterations in the GR function include dysregulation of the GC-mediated negative feedback mechanisms, which results in compensatory activation of the hypothalamic–pituitary–adrenal (HPA) axis. In turn, HPA

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overactivation leads to hypersecretion of ACTH and cortisol (highly variable, ranging from severe symptoms to subclinical hypercortisolism), and may result in increased production of other adrenal steroids such as androgens and mineralocorticoids. Patients affected by this syndrome are thus commonly diagnosed by clinical signs of mineralocorticoid and/or androgen excess (hypertension due to hyperaldosteronism and/or hyperandrogenic signs), rather than those associated with GC deficiency (Kino and Chrousos 2001, Kino *et al.* 2002, Nicolaides *et al.* 2010, Charmandari *et al.* 2013, Nicolaides and Charmandari 2015, Nicolaides *et al.* 2015a, Nicolaides *et al.* 2015b, Wilkinson *et al.* 2018).

So far, 26 mutations have been described in the human *NR3C1* gene, most of which are missense mutations and affect the GR-LBD (Vitellius *et al.* 2016, Nicolaides and Charmandari 2017, Vitellius *et al.* 2018) (Fig. 5B). Functional studies indicate that most *NR3C1* mutations impair several steps of GC signaling including hormone recognition, GR nuclear translocation, DNA binding, and interactions with the coactivator, GRIP1, which ultimately leads to reduced or absent GR-dependent transactivation. Structural studies have contributed to a better understanding of how conformational changes in the receptor cause GC resistance, although a systematic analysis of all reported mutants is lacking. Computer simulations suggest that the primary cause of GC resistance in Chrousos syndrome is an overall destabilization of the LBD, as the result of single amino acid replacements that affect LBP, AF-2, or both (Hurt *et al.* 2016). The possible role of impaired receptor multimerization on this syndrome, however, has not been explored so far.

In addition, it has been recently reported that *NR3C1* mutations also cause another pathological condition called incidentaloma (incidentally discovered bilateral adrenal hyperplasia), with clinical features similar to those of Chrousos syndrome such as asymptomatic hypercortisolism and/or hypertension. Five novel heterozygous *NR3C1* mutations that cause impaired GR signaling were identified, which represents a relatively high prevalence of 5% in the analyzed cohort. These findings suggest that the overall prevalence of *NR3C1* mutations may have been previously underestimated, and advise to perform *GR* mutation screening in patients with adrenal incidentalomas (Vitellius *et al.* 2016, Vitellius *et al.* 2018). Although most of the identified NR3C1 mutations mapped at the GR-LBD, other (p.R477S, p.R477C, p.R477H, and p.Y478C) were located at the C-terminal end of the second zinc finger, implicated in the dimerization of the receptor.

By contrast, in the rare syndrome known as primary generalized glucocorticoid hypersensitivity (PGGH), patients exhibit clinical signs of metabolic syndrome without hypercortisolism, due to compensatory hypoactivation of the HPA axis (Nicolaides and Charmandari 2017). This disease mostly correlates with activating *NR3C1* polymorphisms while to date, a single patient harboring a point mutation in the NTD (p.D401H) exhibited clinical symptoms of GC hypersensitivity including obesity, type 2 diabetes, and hypertension (Charmandari *et al.* 2008).

Further, loss-of-function mutations in the *NR3C2*/*MR* gene are responsible for renal pseudohypoaldosteronism type 1 (MIM #177735), a rare disease of mineralocorticoid resistance characterized by sodium and potassium imbalances that manifests at birth with weight loss and dehydration despite elevated plasma

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levels of aldosterone. Conversely, an activating *MR* mutation that reshapes its LBP (p.S810L) has been associated with a severe form of inherited hypertension (Kino and Chrousos 2001, Zennaro and Fernandes-Rosa 2017). Finally, *PR* mutations are less frequent but they have been associated to an increased risk of breast, endometrial and colon cancer (Agoulnik *et al.* 2004).

Impact of mutations affecting the non-canonical dimerization interface in other non-steroidal NRs

We finish this section on the linkage between dysregulation of NR activities and pathologic conditions by presenting the case of a non-steroidal NR that has also been shown to dimerize in an AR-like, non-canonical conformation, the thyroid receptor (TR; refs. (Estébanez-Perpiñá *et al.* 2007a, Jouravel *et al.* 2009) and Fig. 5D). Vertebrates possess actually two TR isoforms, termed TRα (NR1A1) and TRβ (NR1A2), and which are encoded by two independent genes, *THRA* and *THRB*, respectively. In spite of their close structural similarity (70% sequence identity), these two isoforms differ strongly both in expression profile. TRα is ubiquitously expressed, while TRβ is mainly expressed in liver, pituitary, inner ear and some brain areas (Chatonnet *et al.* 2013), but in particular because they regulate different sets of genes. TRs respond to iodinated biomolecules known as thyroid hormones (TH), mostly to the active form 3,3',5-triiodo-L-thyronine (T3), which is generated in turn from the prohormone, L-thyroxine (T4) (Holzer *et al.* 2017).

Since thyroid hormones have fundamental functions in development, growth and metabolic homeostasis, alterations in TR structure affect these functions at many different levels. This is in particular the case of generalized thyroid hormone resistance (GTHR), a syndrome characterized by elevated

serum TH but impaired action of these hormones within the hypotalamic-pituitary-thyroid axis and variable tissue hyposensitivity to them (Huber *et al.* 2003a, Huber *et al.* 2003b, Onigata and Szinnai 2014). GTHR is linked to mutations located in the LBD of TRβ (Weiss *et al.* 1993, Adams *et al.* 1994). Similar to the results discussed above for GR, most of these mutations result in a lower affinity for T3 or a defective interaction with TR coregulators. Whether are least some of these variants would impair dimer formation has not been experimentally confirmed. However, inspection of the 3D structure of the TRβ homodimer (Estébanez-Perpiñá *et al.* 2007a, Jouravel *et al.* 2009) immediately reveals that some of the reported missense mutations actually map to the homodimer interface, and are therefore likely to disrupt receptor homodimerization (Fig. 5D). Particularly interesting mutations in this regard affect arginine residues at positions 320 (p.R320C, p.R320H) and 338 (p.R338W, p.R338L) in TRβ, as mutations of the topologically equivalent residues in several steroid receptors result in either AIS (p.R753P, p.R753Q in AR), GC resistance (p.R611L in GR) or estrogen insensitivity (p.R394H in ER) (see Table 1) (Weiss *et al.* 1993).

In summary, residues responsible for non-canonical homodimer formation in NRs play a critical functional role, leading to various pathological conditions when dimerization is disrupted. A list of mutations that are likely to interfere with non-canonical homodimer formation of some NRs and their associated conditions is given in Table 1.

Implications for the development of novel therapeutic strategies

All steroid NRs are major therapeutic targets to treat several endocrine-related diseases (Moore *et al.* 2006, Evans and Mangelsdorf 2014, Carroll 2016, Nasrazadani *et al.* 2018). Most notably, the dependence of PCa tumors on the

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AR protein and its cognate endogenous hormones is the basis for its pharmacological exploitation as drug target. Indeed, inhibition of AR functions by means of ligand depletion and/or the use of AR antagonists (antiandrogens) is the first line of therapeutic intervention against PCa (Chen *et al.* 2008, Mohler *et al.* 2012, Lorente *et al.* 2015, Aggarwal *et al.* 2017, Ponnusamy *et al.* 2017, Narayanan *et al.* 2018, Nevedomskaya *et al.* 2018). However, recurrent resistant and incurable tumors arise as a result of inappropriately restored AR function associated with various genetic and epigenetic accidents. In particular, emergence of point mutations as a response to antiandrogen therapy is a quite common event, which may result in antiandrogens acting as agonists rather than antagonists with severe clinical consequences (Knudsen and Penning 2010, Balbas *et al.* 2013, Joseph *et al.* 2013, Schrecengost and Knudsen 2013, Lorente *et al.* 2015, Watson *et al.* 2015, Jernberg *et al.* 2017, Giacinti *et al.* 2018). The identification of constitutively active, truncated splice AR variants (AR-Vs) lacking parts or the entire LBD also poses important pharmacological challenges (Dehm and Tindall 2011, Centenera *et al.* 2013b, Ho and Dehm 2017, Paschalis *et al.* 2018). This is in addition to the cross-reactivity of therapeutic steroidal androgens with other, highly related steroid NRs (i.e., GR) in PCa patients, which derives in unwanted side effects that pose additional limits to their clinical use (Arora *et al.* 2013, Karamouzis *et al.* 2016, Narayanan *et al.* 2016). Altogether, there is an unmet need for the development of novel therapeutic strategies including tissue-selective AR modulators (SARMs; (Estébanez-Perpiñá *et al.* 2007b, Dalton 2017, Narayanan *et al.* 2018)) that may overcome the problems encountered by currently used drugs.

555 Most therapeutic strategies to date have focused on the development of
556 small-molecule compounds that compete with receptor binding to natural
557 hormones (so called “LBP-focused strategies”). However, the recent advances in
558 the structure and function of NRs discussed above have largely expanded the
559 space for pharmacological intervention. First, we mention that AR chaperones
560 had been proposed as potential therapeutic targets against PCa and, after initial
561 failures, heat shock proteins, and in particular Hsp90, are currently considered
562 viable targets to treat PCa (Eskew *et al.* 2011, Centenera *et al.* 2012, Centenera
563 *et al.* 2013a, He *et al.* 2013). Indeed, inhibitors of Hsp90 C-terminal domain have
564 been recently shown to inhibit growth of various PCa cell lines without inducing
565 expression of other chaperones (Armstrong *et al.* 2016).

566 Novel strategies that directly target the NR include blocking receptor
567 interactions with specific coactivators (Azad *et al.* 2015, Guy *et al.* 2015, Foley
568 and Mitsiades 2016), directly targeting NR binding to cognate DNA sequences or
569 interfering with DBD dimerization (Dalal *et al.* 2017), but also modulating ligand-
570 independent functions (reviewed in (Caboni and Lloyd 2013)). Also along these
571 lines, the AR-NTD has been explored as drug target for AR function control, in
572 particular for management of its ligand-independent truncated variants (De Mol
573 *et al.* 2016, Imamura and Sadar 2016, Kumar *et al.* 2016, Monaghan and
574 McEwan 2016).

575 It is believed that conformational changes elicited upon ligand binding are
576 ultimately responsible for the different activity profiles exhibited by therapeutic
577 compounds in different cell types, similar to what has been shown for other NRs
578 (Srinivasan *et al.* 2013, Nwachukwu *et al.* 2016). Given that these signals might
579 propagate through the whole multi-domain protein, controlling NR functions by

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targeting novel unexplored sites is an attractive but challenging alternative. Most importantly, apart from the classical modulators targeting the LBP site, synthetic allosteric modulators are currently under investigation, thus substantially broadening the chemical space for novel antiandrogens (Buzón *et al.* 2012). In this manner, the AR LBP shall no longer be seen as the only pocket suitable for pharmacological intervention, but all NR domains and their protein-protein interaction sites are envisioned as potential drug targets (Estébanez-Perpiñá *et al.* 2007b, Buzón *et al.* 2012, Nadal *et al.* 2017, Badders *et al.* 2018). In this regard, we recall that both AF-2 and BF-3 sites are druggable, and are ideal candidates for off-LBP strategies (Estébanez-Perpiñá *et al.* 2007b, Buzón *et al.* 2012, Ravindranathan *et al.* 2013, Badders *et al.* 2018). Most notably, AF2-targeting compounds have been recently validated as potent androgen-sparing strategies for SBMA therapy (Badders *et al.* 2018). Last but not least, interfering with the non-canonical dimerization interfaces of the AR and other oxosteroid receptors (Nadal *et al.* 2017, Fuentes-Prior *et al.* 2019) offers hitherto unforeseen possibilities for the regulation of NR activities, and thus for the development of novel pharmaceutical drugs.

Declaration of interest

The authors declare that there is no conflict of interests.

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Figure 1 legend

Schematic representation of the domain organization in steroid receptors. Note the common modular protein structure of four main elements: the N-terminal domain (NTD), which contains the activating function 1 (AF1), the DNA-binding domain (DBD), a hinge region (H), and the actual ligand binding domain (LBD), which contains the activating function 2 (AF2) assembled by residues from helices H3, H4, H5 and H12. The so called “F domain”, located at the C-terminal end of the receptor, folds back and covers part of the globular LBD in all oxosteroid receptors, but not in ERα/β, whose F domains differ strongly from the one well-conserved in oxosteroid receptors. Other NRs such as the thyroid receptor (TR) lack an F domain altogether. Note also that the NTDs are highly variable in sequence and length. The AR-NTD can be further dissected into two partly overlapping transcription-activation units, TAU1 comprising residues 142-485 and TAU5 including residues 360-528 (Jenster *et al.* 1995, Hilser *et al.* 2012). Similarly, studies of translational isoforms with variable lengths of the GR-NTD have also found two coupled but thermodynamically distinct regions (Brinkmann *et al.* 1989, Michigami *et al.* 1999, Li *et al.* 2012). At least in the case of these two oxosteroid receptors, the main determinants for transactivation map to their highly similar AF-1 sites (46% sequence identity; (Kumar *et al.* 2004, McEwan *et al.* 2007, De Mol *et al.* 2018)).

637 **Figure 2 legend**

638 Functional surfaces of the androgen receptor. (A) Surface view of the AR-LBD
639 dimer structure (derived from PDB 5JJM) highlighting the BF3 pocket (pink
640 surface) with a small molecule ([4-(4-hydroxy-3-iodo-phenoxy)-3,5-diiodo-
641 phenyl]-acetic acid) bound, an UBA3-derived peptide bound to the AF2 pocket,
642 and the hormone (dihydrotestosterone, DHT) occupying to the ligand-binding
643 pocket (LBP). Note that the AR-LBD core dimer is closely packed; it buries a quite
644 extensive interface of ≈1,000 $Å^2$ of solvent exposed surface from each AR-LBD
645 partner. (B) View of the AR-LBD crystal structure highlighting the four
646 independent AR-LBD molecules found in the asymmetric unit. Notice that the two
647 middle AR-LBD monomers (colored grey) form a symmetrical core dimer, while
648 the two peripheral molecules (colored light pink) are associated through the BF-
649 3 grooves (highlighted in pink).

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651 **Figure 3 legend**

652 Crystal structures of homodimeric ligand-binding domains from various nuclear
653 receptors. (A) Overall structure of the AR-LBD core dimer (PDB 5JJM). The two
654 monomers are depicted as cartoons showing helices in blue and loops in pink;
655 helices H5, H9, H10-11 and H12 are marked. Note that the AR-LBD mainly
656 dimerizes through helices H5 of both monomers. The bound hormone
657 (dihydrotestosterone, DHT) and the UBA3 peptide are shown as pink spheres
658 and as a magenta cartoon, respectively. (B) Overall structure of the first reported
659 GR-LBD dimer (PDB 1M2Z). The two monomers are depicted as cartoons
660 showing helices in blue and loops in pink; helices H5, H9, and H10-11 are
661 highlighted. The bound hormone (dexamethasone, DEX) and the coactivator
662 (TIF2) peptide are shown as pink spheres and as a blue cartoon, respectively.
663 Note the topological equivalence to the AR-LBD dimer depicted in panel A, in
664 spite of less intimate contacts at the intermonomer interface. (C) Overall structure
665 of the ER-LBD dimer (PDB 1ERE). The two monomers are depicted as cartoons
666 showing helices in pink and loops in red; helices H9 and H10-11 are indicated.
667 Note that the ER-LBD dimerizes according to the 'canonical' mechanism, i.e.
668 through helices H10-11 of each monomer. The hormone (17beta-estradiol, EST)
669 is shown as blue spheres. (D) Overall structure of the TR-LBD dimer (PDB 2PIN).
670 The two monomers are shown as cartoons with helices in green and loops in
671 pink; helices H9, H10-11 and H12 are labeled. The ligand ([4-(4-hydroxy-3-iodo-
672 phenoxy)-3,5-diiodo-phenyl]-acetic acid, 4HY) is shown as pink spheres. Dashed
673 lines indicate parts of the structure that were not visible in the electron density
674 maps.

676 **Figure 4 legend**

677 Proximity of functionally relevant areas in non-canonical dimers. (A) Close-up of
678 the AR-LBD dimerization interface. Monomers are colored light pink and blue,
679 respectively, and the DHT molecules are shown as color-coded Van-der-Waals
680 spheres. Residues are shown as color-coded sticks (oxygen, red; nitrogen, blue;
681 carbon, yellow or brown) and labeled. Note that most residues making important
682 contributions to this interface possess hydrophobic/aromatic side chains (e.g.,
683 V685, W752, F755, V758, Y764, and P802). The location of residue R753 is also
684 noteworthy, as its side chain contacts the DHT molecule bound inside the internal
685 ligand-binding pocket (LBP) from its "own" monomer, while its main chain atoms
686 form part of the dimer interface, and in particular interact with N757 from the
687 second monomer. In this manner, hormone binding and receptor dimerization
688 appear to be intimately coupled in the AR. (B) Close-up of the AR-LBD surface
689 (as seen in PDB 1XOW), highlighting the proximity of the AF-2 pocket (colored
690 blue; the bound NH_2-terminal peptide of the receptor is colored cyan) to the LBP
691 (with bound methyltrienolone/R1881 colored magenta).

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693 **Figure 5 legend**

694 Mutations that affect the non-canonical, AR-like dimerization interface are linked
695 to various conditions. The LBD dimers of all four analyzed receptors are
696 represented as grey cartoons. (A, B) AR-LBD (PDB 5JJM) with the side chains
697 of all mutated interface residues shown with all their non-hydrogen atoms as
698 sticks and surface, colored red for mutations reported in PCa patients (A), or blue
699 for those linked to AIS (B). Mutations have been retrieved from the Androgen
700 Receptor Gene Mutations Database (http://androgendb.mcgill.ca/). (C) GR-LBD
701 (PDB 1M2Z) with the side chains of all mutated interface residues shown with all
702 their non-hydrogen atoms as sticks and surface, colored pink to highlight those
703 residues linked to glucocorticoid hormone resistance. Some of these residues
704 have been associated with primary generalized Chrousos syndrome. (D) Cartoon
705 representation of the TR-LBD dimer (grey, PDB 2PIN) with the side chains of all
706 mutated interface residues shown with all their non-hydrogen atoms as sticks and
707 surface, colored green to highlight those residues associated with thyroid
708 hormone resistance.

709

710

711

Table 1 legend

Mutations within the non-canonical dimerization interface are linked to various human conditions. Residues from the non-canonical dimerization interface of the AR-LBD were aligned to those of the corresponding domains in GR, ER and TR. Missense mutations that affect these topologically equivalent residues are given, along with the associated disease. Note in particular that several mutations affect topologically equivalent residues in AR-LBD and TR-LBD. Note also that replacement of the highly conserved residue R753 of AR-LBD, which corresponds to R611 of GR-LBD, R394 of ER-LBD and R320 of TR-LBD, causes hormone resistance in all receptors.

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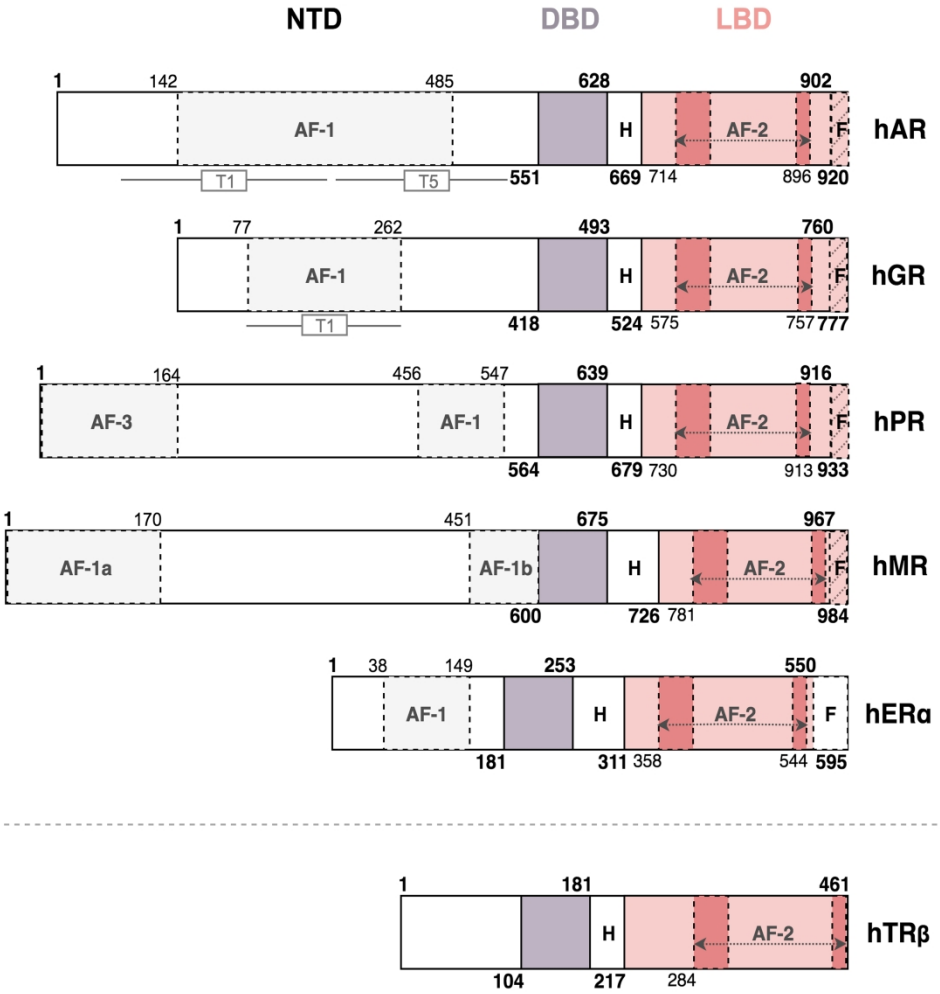

Figure 1

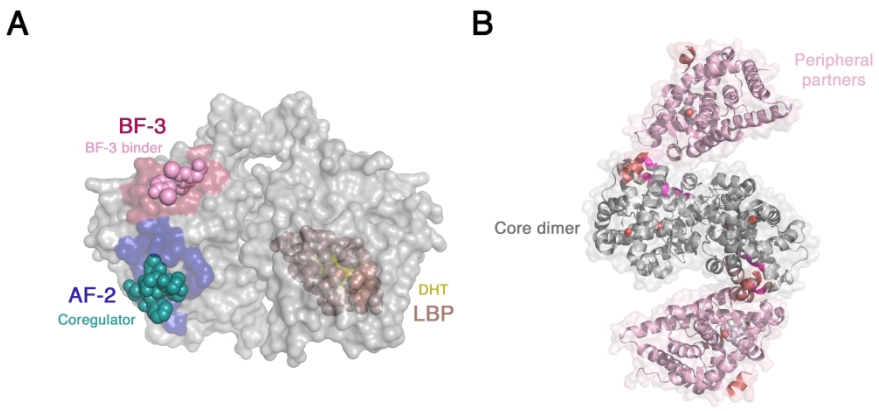

Figure 2

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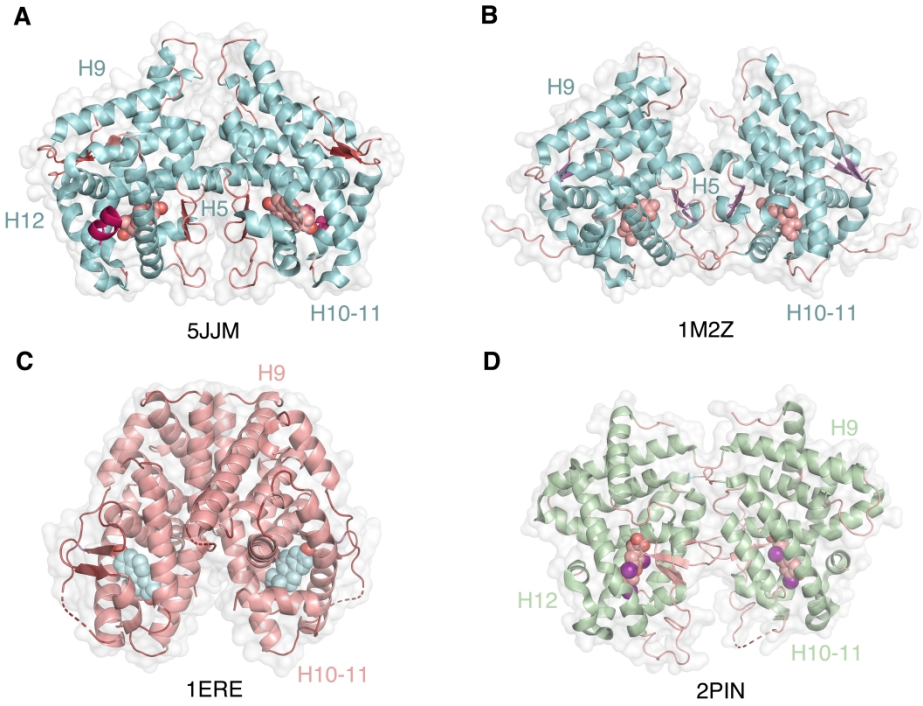

Figure 3

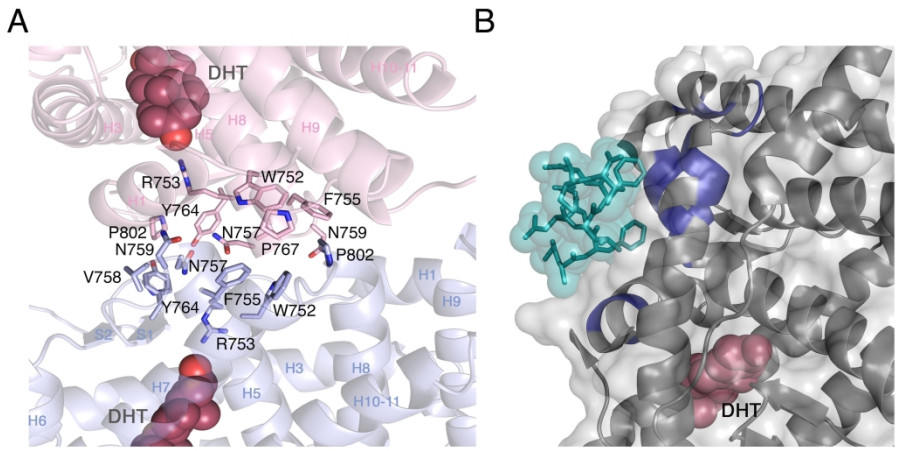

Figure 4

149x75mm (600 x 600 DPI)

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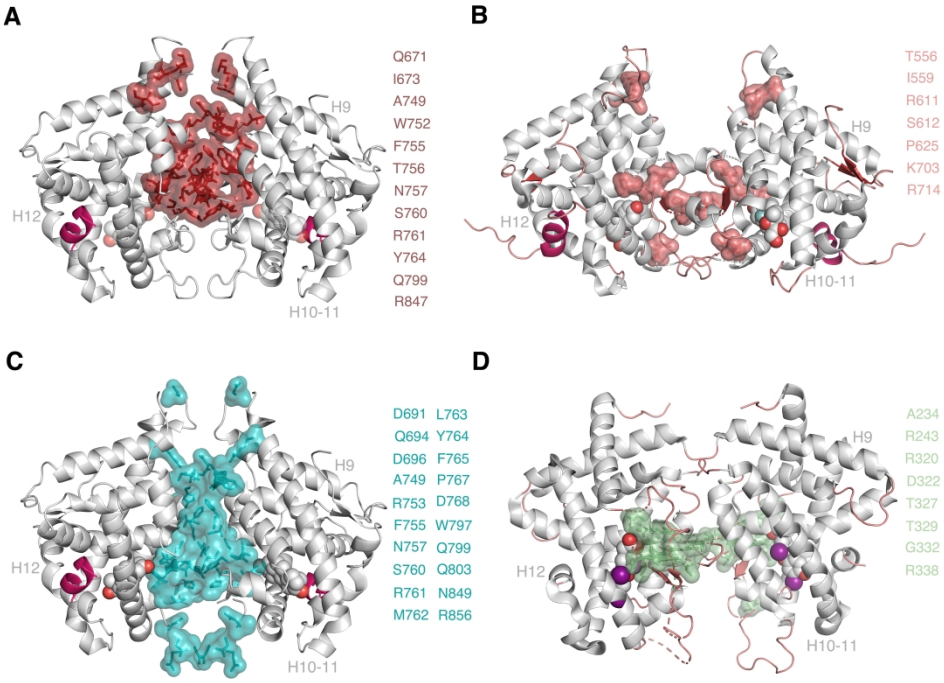

Figure 5

ANDROGEN RECEPTOR		GLUCOCORTICOID RECEPTOR		ESTROGEN RECEPTOR		THYROID RECEPTOR	
Residue	Disease	Residue	Disease	Residue	Disease	Residue	Disease
Q671R	PCa						
I673T	PCa						
V685I	CAIS	V543		I326		A234T	THR (Behr and Loos 1992)
C687R	PAIS	Y545		Y328		R243Q R243W	THR (J Pohlenz et al. 1996),(Yagi et al. 1997)
D691E **D691V**	PAIS CAIS	D549		X		K244	
D696H **D696N** **D696V** **D696Y**	CAIS CAIS/PAIS/M CAIS CAIS	D554		F337		I250 I250T	THR (Asadi et al. 2008)
A749T **A749D**	PCa PAIS						
W752R	PCa	W610		W393		V319	
R753P **R753Q**	CAIS CAIS	R611L	GCR (Schmidt et al. 2006)	R394H	Estrogen insensitivity (Bernard et al. 2017)	R320C R320H	THR (Weiss, Weinberg, and Refetoff 1993) (Cugini et al. 1992)
F755L **F755S** **F755V**	PCa PAIS/MAIS CAIS	Y613		M396		D322H	THR (Cheng et al. 1994)
T756A	PCa	R614		E397		L328S	THR (Grace, Buzard, and Weintraub 1995)
N757D **N757S**	PCa PAIS/MAIS	Q615		X		E324	
S760F **S760P** **S760Y**	CAIS PCa CAIS	A618		H398		P323	
R761? **R761S**	PCa PAIS	N619		G400		P323/D324	
M762T	PAIS	L620		K401		E326	
L763F	CAIS	L621		L402		T327P T327A	THR (Borck et al. 2009)

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							(Asadi et al. 2008)
Y764C **Y764H**	PCa/PAIS CAIS	C622		L403		T329N T329I	THR (Sarkissian et al. 1999) (P. and J. 2013)
F765L	CAIS	F623		F404		L330S	THR (Pohlenz 1997)
P767A **P767S**	CAIS CAIS	P625	(Bledsoe et al. 2002)	P406		G332R G332E	THR (Parrilla et al. 1991) (Adams et al. 1994)
D768E **D768Y**	CAIS CAIS	N626		N407		E333D	THR (Maraninchi et al. 2006)
E773		E631		R412		**R338W** **R338L**	THR (Sasaki, S et al 1995) THR (Menzaghi et al 1999)
W797del	CAIS	H655		R437		S362	
Q799E	PCa/PAIS/M	Q657		N439		N364	
Q803R	PAIS	E661		E443		T368	
R847G	PCa	E705		G494		X	
N849K	CAIS			T496		H210	
R856C **R856H**	CAIS CAIS/PAIS/M	R714Q	Chrousos Syndrome (Nader et al. 2010)	R503W	BCa (Jeselsohn et al. 2014)		

GCR= Glucocorticoid resistance

THR= Thyroid hormone resistance