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# Allosteric Pathways in Nuclear Receptors – Potential Targets for Drug Design

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## Abstract

The nuclear receptor family of transcription factor proteins mediate endocrine function and play critical roles in development, physiology and pharmacology. Malfunctioning nuclear receptors are associated with several disease states. The functional activity of nuclear receptors is regulated by small molecular hormonal and synthetic molecules. Multiple sources of evidence have identified and distinguished between the different allosteric pathways initiated by ligands, DNA and cofactors such as co-activators and co-repressors. Also, these biophysical studies are attempting to determine how these pathways that regulate co-activator and DNA recognition can control gene transcription. Thus, there is a growing interest in determining the genome-scale impact of allostery in nuclear receptors. Today, it is accepted that a detailed understanding of the allosteric regulatory pathways within the nuclear receptor molecular complex will enable the development of efficient drug therapies in the long term.

### Keywords

allostery; nuclear receptor; transcription; drug design; endocrine; genome-scale

## 1. Introduction

Nuclear receptors (also nuclear hormone receptors and abbreviated as NR) are a family of transcription factors whose transcriptional activity is controlled by lipophilic hormone molecules and the ensuing recruitment of coactivator molecules (Evans, 1988; Evans & Mangelsdorf, 2014). There is a specific classification of nuclear receptors proposed by the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) (Alexander, et al., 2015; Auwerx, et al., 1999). It is based on a phylogenetic tree that connects all known nuclear receptor sequences. This nomenclature also accounts for the evolution of the two well-conserved domains of nuclear receptors (described below).

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## 2. Nuclear receptors, human disease and pharmacology

Nuclear receptors are widely expressed in all human tissue (Bookout, et al., 2006; Kumar, et al., 2013; McKenna & O'Malley,). For instance, the peroxisome proliferator-activated receptor (PPAR) and liver X receptor (LXR) isoforms are expressed in tissues as diverse as the epidermis (Schmuth, Jiang, Dubrac, Elias, & Feingold, 2008) and in adipose tissue (Michalik, et al., 2006). The estrogen receptor (ER) and PPAR are also expressed in neural tissue (Couse, Lindzey, Grandien, Gustafsson, & Korach, 1997; Cullingford, et al., 1998) and ER is also expressed within the reproductive tract, hypothalamus and the lungs (Couse, et al., 1997). The constitutive androstane receptor (CAR) whose activity is linked to xeno and endobiotic metabolism (Huang, et al., 2003; Sonoda, Rosenfeld, Xu, Evans, & Xie, 2003; Sueyoshi & Negishi, 2001; Wei, Zhang, Egan-Hafley, Liang, & Moore, 2000; Zhang, Huang, Chua, Wei, & Moore, 2002; Zhang, Huang, Qatanani, Evans, & Moore, 2004) is most abundantly expressed in the liver and intestine (Bertilsson, et al., 1998; Lamba, et al., 2004) and is also expressed in the testis, adrenal tissue and the brain (Lamba, et al., 2004). Multiple reviews on the many disease states associated with nuclear receptor malfunction already exist (McKenna & O'Malley). For instance, the PPARs are associated with diseases as diverse as diabetes (Cipolletta, et al., 2012) and Alzheimer’s (Moutinho & Landreth, 2017; Prakash & Kumar, 2014). Signaling through the glucocorticoid receptor (GR) has been linked with cardiovascular disease, psychiatric disorders and hyperglycemia, among many other ailments (Kadmiel & Cidlowski, 2013). Also, nuclear receptors have been linked to the progression of multiple cancers (McKenna & O'Malley; Tang, et al., 2011), by the fatty liver disease and liver tumors by the farnesoid X (FXR) (Neuschwander-Tetri, et al., 2015) and the constitutive androstane receptors (CAR) (Yamamoto, Moore, Goldsworthy, Negishi, & Maronpot, 2004).

Consequently, nuclear receptors are vital targets of therapeutic drugs (Alexander, et al., 2015; Burris, et al., 2013; Evans & Mangelsdorf, 2014; Moore, Collins, & Pearce, 2006; Safe, Jin, Hedrick, Reeder, & Lee, 2014). Multiple small-molecule scaffolds have been designed as pharmaceutical nuclear receptor ligands that function as agonists or antagonists. These include therapeutic drugs such as bicalutamide that bind to the androgen receptor (AR) and target prostate cancer (Blackledge, 1996), tamoxifen for ERs (that target breast cancer) (Ward, 1973), thiazolidinediones against PPARγ that target type II diabetes (Lehmann, et al., 1995) and corticosteroids such as dexamethasone which targets the GR when treating ailments associated with inflammation (Madretsma, Dijk, Tak, Wilson, & Zijlstra, 1996).

## 3. Nuclear receptor structural topology, assembly and signaling

Nuclear receptors have common modular structural features that include an N-terminal domain (A/B domain, Figure 1A). This A/B domain is of variable length and amino acid sequence and is critical for regulating transactivation (Dieken & Miesfeld, 1992; Kato, et al., 1995; O'Malley, et al., 1995; Tora, et al., 1989; Werman, et al., 1997). With a few exceptions, the A/B domain encompasses a ligand-independent transactivation function (AF1) domain (Tora, et al., 1989; Tsai & O'Malley, 1994). Following the A/B domain is a highly conserved DNA-binding domain (DBD) (C domain, Figure 1B) that binds

palindromic or direct repeat DNA sequences (six nucleotide segments of varied arrangements), or response elements (RE). A short 'hinge' sequence (D domain) connects the DBD to a C-terminal ligand-binding domain (LBD) (E & F domain, Figure 1A). Upon binding agonist-ligands the LBD undergoes conformational changes and recruits coactivator molecules to the ligand-dependent transactivation function (AF2) domain within the LBD (Suino, et al., 2004; Wright, et al., 2011; Wright, Vincent, & Fernandez, 2007; Xu, et al., 2004). Inverse agonists disrupt the 'active' AF2 conformation and the resulting LBD conformation functions as a docking site for co-repressors (Dussault, et al., 2002; Shan, et al., 2004).

These receptors function as monomers, homodimers and as heterodimers, most commonly in a bimolecular complex with the nuclear receptor, the retinoid X receptor (RXR) (Auwerx, et al., 1999; Evans & Mangelsdorf, 2014; Kliewer, Umesono, Noonan, Heyman, & Evans, 1992; D. D. Moore, et al., 2006). When activated, nuclear receptors bind specific DNA sequences called hormone response elements (HRE) which are usually labeled to signify the activity-initiating hormone. For instance, the estrogen response elements (ERE) are HREs that bind the estrogen hormone receptor (ER) (Klock, Strahle, & Schutz, 1987), the androgen response elements (ARE) bind the androgen hormone receptor (AR) (Cato, Henderson, & Ponta, 1987), glucocorticoid response elements (GRE) bind the glucocorticoid receptor (Klock, et al., 1987), and the thyroid hormone response elements (TRE) bind the thyroid hormone receptor (Figure 1B) (Umesono, Giguere, Glass, Rosenfeld, & Evans, 1988), among others (Evans, 1988; Olefsky, 2001). This DNA/nuclear receptor complex recruits and binds to transcriptional coactivator proteins such as the steroid receptor coactivators (SRC) (A. B. Johnson & O'Malley, 2012), TIF-2/GRIP-1/NcoA-2 (transcriptional intermediary factor 2/glucocorticoid receptor interacting protein 1/nuclear receptor coactivator 2) (Min, Kemper, & Kemper, 2002), peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1α) (Ding, Lichti, Kim, Gonzalez, & Staudinger, 2006; Shiraki, Sakai, Kanaya, & Jingami, 2003), Activating signal cointegrator-2 (ASC-2) (Choi, et al., 2005) and others (Arnold, Eichelbaum, & Burk, 2004). Coactivator recruitment can be accompanied by histone acetylation, recruitment of the RNA polymerase II complex and gene expression (Dasgupta, Lonard, & O'Malley, 2014; Glass & Rosenfeld, 2000; Kamei, et al., 1996; Rastinejad, Huang, Chandra, & Khorasanizadeh, 2013; Yao, Ku, Zhou, Scully, & Livingston, 1996). Within the nucleus, the transcriptional activity of nuclear receptors can be maintained in a repressed state by antagonists and inverse agonists (Weatherman, Fletterick, & Scanlan, 1999) which promote the recruitment of transcriptional co-repressors (Lonard & O'Malley, 2012) such as the silencing mediator of retinoid and thyroid-hormone receptors (SMRT) (J. D. Chen & Evans, 1995; Sande & Privalsky, 1996) and the nuclear receptor co-repressor (NCoR) (Figure 1C) (Horlein, et al., 1995; Seol, Mahon, Lee, & Moore, 1996).

## 4. Noncanonical nuclear receptor signaling

Several additional mechanisms can also control the action of nuclear receptors and alter target gene expression. For instance, RNA-seq studies on CAR in hepatocyte-like (HepaRG) cell lines have shown distinct ligand (CITCO)-dependent and ligand-independent (phenobarbital, PB, activated) gene expression profiles (Li, et al., 2015; Mutoh, et al., 2009). Likewise, diverse mechanisms can control the DNA-binding-site specificity of nuclear

receptors. Genome-scale studies with the estradiol (E2)-activated ER using ChIP-seq data in breast cancer tissue cell lines have been reported to bind multiple, non-overlapping ER-binding DNA sites (Welboren, Sweep, Span, & Stunnenberg, 2009; W. J. Welboren, et al., 2009). Also, different thiazolidinedione (TZDs)-agonists, the type 2 diabetes directed medications, that activate the PPARγ elicit overlapping but distinct *in vivo* gene expression profiles (Camp, et al., 2000; Lehmann, et al., 1995; Sears, et al., 2007). ChIP-seq studies using a border pattern-based motif recognition approach in multiple prostate cancer cell lines show that agonist dihydrotestosterone (DHT)-liganded human androgen receptor (AR) and antagonist bicalutamide and enzalutamide-liganded AR bind to distinctly different DNA ARE motifs (Z. Chen, et al., 2014). Furthermore, these motifs can be linked to distinct prostate cancer-relevant transcriptional outcomes.

Conversely, different DNA HRE sequences can also alternately activate or repress nuclear receptor transactivation. ‘Negative’ GREs are GR HREs that effectively repress transcription of agonist-liganded glucocorticoid (GR) (Surjit, et al., 2011). Indeed, these negative GREs promote the recruitment of transcriptional repression-associated SMRT and NCoR proteins to the negative GRE-GR(+agonist) molecular complex. Such negative HRE have also been reported to repress the thyroid receptors (TR) (Sharma, Thakran, Deng, Elam, & Park, 2013). By binding to TREs upstream, the agonist-ligand TRβ actively represses transcription of the secretory phospholipase A2 group IIa (PLA2g2a) gene. As with GR (Surjit, et al., 2011), the TRE-TR(+agonist) molecular complex also recruits co-repressor molecules SMRT and NCoR (Sharma, et al., 2013). Thus, noncanonical mechanisms can direct DNA-recognition for target gene selection by liganded nuclear receptors and a comprehensive analysis of these mechanisms will be essential to explain the overall *in vivo* significance of nuclear receptor ligands.

## 5. Allostery in biology

Allosteric coupling of distinct sites on proteins and DNA is fundamental to many biological processes (Monod, Changeux, & Jacob, 1963). Within nuclear receptors, allostery is increasingly recognized as a common regulatory process (Forman, Umesono, Chen, & Evans, 1995; Hilser & Thompson, 2011; Q. R. Johnson, Lindsay, Nellas, Fernandez, & Shen, 2015; Kojetin, et al., 2015; Mangelsdorf & Evans, 1995; Pavlin, Brunzelle, & Fernandez, 2014; Putcha, Wright, Brunzelle, & Fernandez, 2012; Shulman, Larson, Mangelsdorf, & Ranganathan, 2004; Wright, et al., 2011; Wright, et al., 2007). Structural and biophysical tools have shown that ligand binding and even minor perturbations (such as non-binding-site mutations) can be detected at distal regions of nuclear receptors. There are significant structural changes associated with allostery which are observed with crystallography (Osz, et al., 2012; Putcha, et al., 2012), hydrogen-deuterium exchange mass spectrometry (HDX MS) (Wright, et al., 2011) and NMR spectroscopy (Kojetin, et al., 2015).

## 6. Linking ligand and ligand through allostery

Since both receptors within nuclear receptor heterodimers can bind small-molecule agonist ligands in the simplest model for transactivation, agonist binding to either receptor can

generate comparable transcriptional levels of downstream genes (Figure 1B) (Evans, 1988; Forman & Samuels, 1990). Such model systems are exemplified by the PPAR:RXR; CAR:RXR and LXR:RXR heterodimers (Clark, et al., 2016; Shulman, et al., 2004; Wright, et al., 2011). Yet, there are other nuclear receptor heterodimers that exhibit transcriptional responses that are distinct from this model (Forman, et al., 1995; Shulman, et al., 2004). For instance, transactivation by RAR:RXR, VDR:RXR and TR:RXR only occurs in the presence of the RAR, VDR and TR agonists, respectively. However, when these agonists are applied exogenously in combination with the agonist for the heterodimeric partner RXR (9-*cis* retinoic acid, ***9c***), transactivation levels are either enhanced, unaffected or are repressed, respectively (Forman, et al., 1995; Kurokawa, et al., 1994; Putcha, et al., 2012; Shulman, et al., 2004; Yao, et al., 1993). Such ligand-ligand allostery has been observed to link ligand bound to one nuclear receptor subunit to ligand and co-activator binding to the associated dimeric partner as observed in the RAR:RXR (Schulman, Li, Schwabe, & Evans, 1997) and TR:RXR (Putcha, et al., 2012) heterodimeric complexes (Figure 2A). These allostery-initiated conformational changes are significantly large and have easily identifiable local conformational pathways that can be characterized through multiply-liganded crystal structure analyses of the RAR:RXR and VDR:RXR heterodimers (Rochel, et al., 2011). With the TR:RXR heterodimer complex, the corresponding conformational changes are less obvious (Putcha, et al., 2012). The '*frustrated fit*' mechanism is a recent approach to understanding how subtle conformational changes associated with ligand-ligand allostery are propagated throughout the molecular complex and across the heterodimer interface (Clark, et al., 2016; Q. R. Johnson, et al., 2015). The propagation of these allosteric signals can also be ligand-specific as observed in the murine CAR:RXR heterodimer with the agonists tcpobop and meclizine (Huang, Zhang, Wei, Schrader, & Moore, 2004; Wright, et al., 2011). Although these ligands elicit comparable transcriptional activity, studies with fluorescence spectroscopy and HDX MS show that they induce discrete conformational changes across the CAR:RXR dimer interface (Wright, et al., 2011).

The immediate molecular consequence of this ligand-ligand allostery results in unique nuclear receptor heterodimer:co-activator molecular stoichiometries: 1:2 nuclear receptor↔co-activator stoichiometries for CAR:RXR (Pavlin, et al., 2014) and RARβ (Osz, et al., 2012) and ER homodimers (Yi, et al., 2015), reflecting the ligand response of these receptor systems, the 1:1 nuclear receptor↔co-activator stoichiometries for RAR:RXR and VDR:RXR (Rochel, et al., 2011) and the 'phantom ligand effect' where binding of ligand to RXR within the RAR:RXR heterodimer results in a linked conformational change within RAR (Schulman, et al., 1997).

## 7. Linking ligand and DNA through allostery

Ligand-DNA allostery is a potent mechanism for ligand-dependent gene expression (Meijsing, Elbi, Luecke, Hager, & Yamamoto, 2007; Meijsing, et al., 2009). It has been shown that ligand binding can also affect the DNA-binding-site specificity of the nuclear receptor (ligand-DNA allostery) (Forman, et al., 1995) (Figure 3). For instance, the thyroid hormone, T3, has been reported to promote the binding of monomeric TRβ to TRE DNA (Ribeiro, Kushner, Apriletti, West, & Baxter, 1992). In these studies, T3 is also observed to increase the gel mobility of these TR(+T3) monomer-DNA complexes suggesting a ligand-

induced conformational change in the TR(+T3) molecular complex. Furthermore, T3 appears to subtly change the specificity of TR to different TREs. In nuclear receptors, the DBD and LBD have been shown to interact directly, and structural changes in the LBD can influence DNA binding (Chandra, et al., 2008; Putcha & Fernandez, 2009). Through biochemical studies Rastinejad *et al.* have shown that a single point mutation within the PPARγ LBD can reduce the DNA-binding affinity of the PPARγ:RXRα heterodimer (Chandra, et al., 2008). Likewise, point mutations within the androgen receptor (AR) LBD – associated with androgen insensitivity syndromes – decrease the DNA-binding affinity of the AR homodimer, while leaving intact the ligand-binding affinity (Helsen, et al., 2012). Additionally, distinct ligand-dependent recognition patterns of promoter DNA by CAR have been identified (Cherian, Lin, Wu, & Chen, 2015; Hosoda, et al., 2015; Li, et al., 2015; H. Wang, et al., 2003).

Ligand *identity* can also affect nuclear receptor target gene expression and DNA binding *in vivo*, further suggesting that ligand molecules directly influence the DNA binding-site *specificity* of nuclear receptors, and not just the binding-affinity for DNA. For instance, a recently identified human CAR inverse agonist (CAR inhibitor not PXR activator 1, CINPA1) is observed to induce the dissociation of CAR from the promoter when used alone (Cherian, et al., 2015). It is speculated that this LBD-targeted inverse agonist also functions through allostery to induce conformational changes within the DBD that decreases the CAR-CARE binding affinity. In other studies, when the two ERα ligands E2 and 4-hydroxytamoxifen, 4-OHT are used in concert with different EREs there are notable differences in the sensitivity of each ER(+ligand)-ERE molecular complex to digestion by the protease chymotrypsin (Klinge, Jernigan, Smith, Tyulmenkov, & Kulakosky, 2001). Thus, different ERE-ligand combinations appear to induce distinct conformations in ERα. It is also reported in the study above that transcriptional activity correlates both, with distinct ligand-ERE combinations and with the ER-ERE binding affinity (Klinge, et al., 2001). Additionally and as noted above, multiple genome scale studies have shown that the TZD-ligand activation of PPARγ can elicit overlapping but discrete patterns of promoter binding and target gene expression (Camp, et al., 2000; Sears, et al., 2007). Similarly, different GR ligands such as the arylpyrazole compounds prednisolone and dexamethasone can induce different gene expression patterns and lead to distinct GR ChIP-seq-defined GRE-binding patterns (J. C. Wang, et al., 2006). Also, comparative studies by Wang et al. on AR show how different ligands (bound to the LBD) can result in the switching of DNA ARE motifs by the receptor DBD (Z. Chen, et al., 2014).

## 8. Linking co-activator/co-repressor and DNA through allostery

Conversely, DNA HREs can also function as allosteric regulators of co-activator and co-repressor recruitment by nuclear receptors (Figure 4A **& 3B**) (Gronemeyer & Bourguet, 2009; Putcha & Fernandez, 2009). Biophysical studies using isothermal titration calorimetry (ITC) show significant differences in both binding affinity, $K_D$, and the thermodynamic binding parameters for SRC-derived LXXLL peptides by monomeric TR and TR:RXR heterodimers in the presence and absence of DNA TREs (Putcha & Fernandez, 2009). In separate studies, different DNA ERE sequences conferred distinct binding preferences for LXXLL-containing peptides to both ERα and ERβ (Hall, McDonnell, & Korach, 2002;

Wood, Likhite, Loven, & Nardulli, 2001). Also, while the LXXLL peptides derived from SRC3/ACTR are recruited equally well by ERβ to four different EREs, analogous peptides from the co-activators SRC1 and GRIP1 show distinct preferences for different EREs (Figure 4C). Studies on GR have shown that by knocking down expression levels of the GR co-activators Brahma and the co-activator-associated arginine methyltransferase 1 (CARM1) co-activators the specific sequence of the DNA GRE is able to regulate GR transcriptional activity by specifically altering the molecular composition of the transcriptional co-activator complex (Meijsing, et al., 2009).

Indeed, nuclear receptor structure is strongly affected by the presence and even sequence of the DNA response element, and this provides a mechanism for co-activator-DNA allosteric communication. The source of these differential interactions has been shown to derive from conformational changes within the DBD as specifically observed in structural analyses on GR bound to multiple GREs (Lefstin & Yamamoto, 1998; Meijsing, et al., 2009; Watson, et al., 2013). These studies by Yamamoto et al. show that the conformation of a 'lever arm' region in the DBD which is known to regulate the transcriptional activity of GR, is affected by the GRE DNA sequence. NMR studies further reveal that the specificity of interactions with GRE bases affects the conformation of distal regions of the GR DBD (Watson, et al., 2013). It is likely that analogous conformational changes within the DBD are propagated to the DBD-LBD interface and may explain the DNA-dependent interactions between the DBD and LBD within TR (Putcha & Fernandez, 2009). Likewise, the propagation of such DNA-induced conformational changes within the vitamin D receptor (RXR:VDR) heterodimer are manifested as fluctuating structural dynamics of the co-activator binding surfaces in the DNA-bound RXR:VDR which are initiated by distinct sequences of the DNA response element (Zhang, et al., 2011).

Remarkably, the base composition of the DNA HRE can also reverse the canonical role of ligands (Figure 4D). As noted above, GR can also mediate gene repression by recruiting transcriptional repressors to specific *negative* GREs that differ in sequence from canonical, activating GREs (Surjit, et al., 2011). Similar studies on the TR agonist-ligand triiodothyronine (T3) response element (TRE) within the promoter of the secretory phospholipase A2 group IIa (PLA2g2a) gene suggest that this promoter sequence functions as a negative regulator of T3 agonist-bound TRβ (Sharma, et al., 2013). Furthermore, when associated with the PLA2g2a promoter, the TRβ(+T3) complex actively recruits co-repressors to inhibit PLA2g2a expression.

## 9. Linking the N-terminal A/B domain and DNA through allostery

The N-terminal domain of nuclear receptors is the least understood (Figure 1A). This N-terminal A/B region is diverse in size, sequence and is conformationally malleable suggesting that this domain plays a role in conferring cell type and/or promoter specificity (Hill, Roemer, Churchill, & Edwards, 2012; Lavery & McEwan, 2005; Uversky, Oldfield, & Dunker, 2005; Warnmark, Treuter, Wright, & Gustafsson, 2003). Often, nuclear receptors differ most significantly in the amino-acid composition of the N-terminal A/B domain, implying that this region may play a significant role in mediating different effects of these receptors (Baniahmad, et al., 1993; Evans & Mangelsdorf, 2014; Hadzic, et al., 1995;

McEwan & Gustafsson, 1997; Tian, Mahajan, Wong, Habeos, & Samuels, 2006; Tomura, Lazar, Phyillaier, & Nikodem, 1995). The absence of 3-dimensional data on the atomic resolution structure of any nuclear receptor A/B domain has necessitated a broad reliance on the use of spectroscopy and other biophysical tools to infer conformational changes within this domain. Multiple lines of evidence suggest that the nuclear receptor A/B domains are flexible and can adopt distinct conformations through allostery initiated by DNA:DBD interactions (D. L. Bain, Franden, McManaman, Takimoto, & Horwitz, 2000; David L. Bain, Franden, McManaman, Takimoto, & Horwitz, 2001; Baskakov, et al., 1999; Brodie & McEwan, 2005; Connaghan-Jones, Heneghan, Miura, & Bain, 2007; Fernandez, Gahlot, Rodriguez, & Amburn, 2017; Kumar, Lee, Bolen, & Thompson, 2001; Kumar, et al., 2013; Lavery & McEwan, 2005; McEwan, Lavery, Fischer, & Watt, 2007; Reid, Kelly, Watt, Price, & McEwan, 2002; Simons, Edwards, & Kumar, 2014). A common observation is that the A/B domains in all nuclear receptors studied to date, the DNA-initiated allostery elicits a conformational change in the N-terminal A/B domain (Figure 5). This structural flexibility has been proposed to enable the A/B domains to achieve multiple inter-molecular interactions have also been observed for AR (Lavery & McEwan, 2008), GR (Ford, McEwan, Wright, & Gustafsson, 1997; Khan, et al., 2012), PR (Hill, et al., 2012) and TR (Fondell, Brunel, Hisatake, & Roeder, 1996; Fondell, Roy, & Roeder, 1993). Furthermore, the A/B domain observed to fine-tune DNA recognition is finely tuned by the domains flanking the DBD (C domain) (Fernandez, et al., 2017). Thus even subtle changes within these flanking domains (A/B or E/F domains) such as mutations (Helsen, et al., 2012) and molecular interactions with cellular factors (Putcha & Fernandez, 2009) or small-molecule ligands (Chandra, et al., 2008) can affect DNA binding.

## 10. Conclusions and Outlook

Although significant advances have been made in our understanding of how these transcription factors respond to specific stimuli and regulate gene expression, critical details remain uncharacterized, such as, (1) how these nuclear receptors with overlapping DNA-binding specificity control specific gene transcription, and (2) how the finely-tuned gene-specific recruitment of distinct coregulatory molecules is achieved. Detailed structural analyses on multiple nuclear receptors that include the PPAR:RXR heterodimer (Chandra, et al., 2008), AR (Helsen, et al., 2012) and the GR homodimers (Meijsing, et al., 2009; Watson, et al., 2013) have already identified a few residues that transmit allosteric conformational changes. Already, the allosteric ‘BF-3’ surface site on the AR LBD has been targeted by transcriptional-activity modulating small molecules (Estebanez-Perpina, et al., 2007). However, at the genomic level, the role of ligand-to-DNA, DNA-to-co-activator and ligand-to-ligand allostery remains less characterized. Evidence strongly suggests that DNA HRE sequences and the ligand conformation have considerable long-range effects on nuclear receptor structure. Allosteric communication between co-activator-DNA-binding sites provides a link between target genes and distinct co-activators; however, studies have been restricted to only a few DNA sequences which have limited our understanding of the role of allostery on a genomic scale. Yet, its impact and prevalence in nuclear receptor signaling is of potentially substantial importance in nuclear receptor biology and pharmacology. Such studies will establish a functional connection between the nuclear receptor ligand, co-

activators and DNA-binding sites. The genome-scale analysis of allosteric effects on gene transcription, combined with the proposed structural analysis of nuclear receptor-ligand interactions, will provide a unique opportunity for future drug-design studies and will explore how novel ligands will reshape nuclear receptor structure, HRE-DNA recognition and gene transcription. Also, these studies may inherently be collaborative to be performed in an iterative feed-back process where data from one study will inform towards designing more efficacious nuclear receptor modulators through rational ligand design. Comprehensive studies to predict residues that propagate intermolecular interactions across the heterodimer resulting in finely tuned activity states, novel high-throughput computational methods will need to be developed.

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## Abbreviations

| | |
|---|---|
| **9c** | 9-*cis* retinoic acid |
| **AF1 (or 2)** | activation function 1(or 2) |
| **AR** | androgen receptor |
| **CAR** | constitutive androstane receptor |
| **ChIP** | chromatin immunoprecipitation |
| **DBD** | DNA binding domain |
| **ER** | estrogen receptor |
| **ERE** | estrogen response elements |
| **GR** | glucocorticoid receptor |
| **HRE** | hormone response element |
| **LBD** | ligand binding domain |
| **LBP** | ligand binding pocket |
| **LXR** | liver X receptor |
| **NCoR** | nuclear receptor corepressor |
| **NR** | nuclear receptor |
| **PPARɣ** | Peroxisome proliferator-activated receptor ɣ |
| **RXR** | retinoid X receptor |
| **SMRT** | silencing mediator of retinoid and thyroid-hormone receptors |

| | |
|---|---|
| **SRC** | steroid receptor coactivator |
| **T3** | *tri*iodothyronine |
| **TR** | thyroid receptor |
| **TRE** | thyroid receptor response element |

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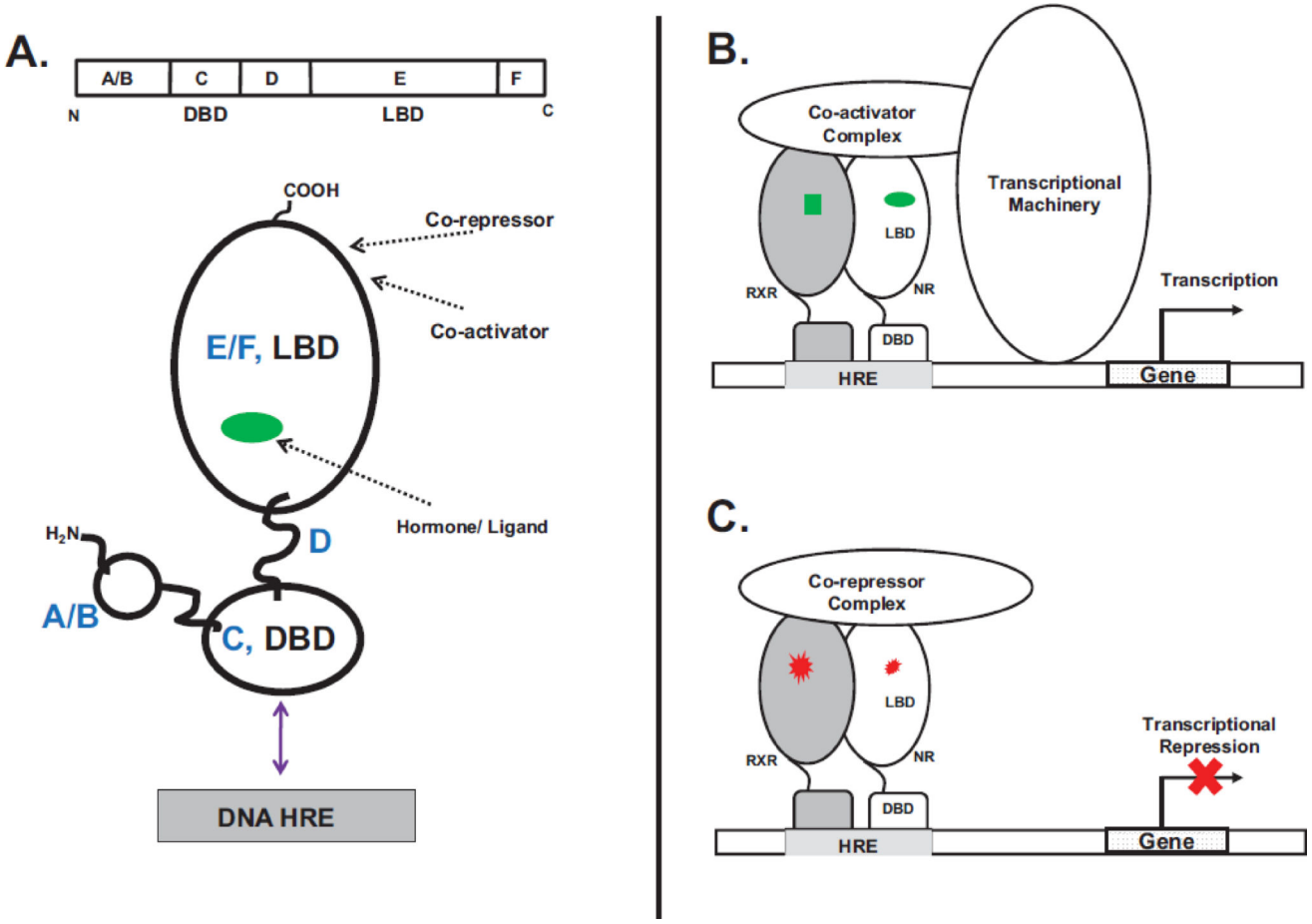


**Figure 1. Nuclear Receptor (NR) Mode of Action and Molecular Topology**

**A**. The nuclear receptor topology and functional organization consists of distinct N-terminal A/B, a DNA-binding (C, DBD), linker D and C-terminal ligand-binding (EF, LBD) domains. Arrows show locations of the binding sites for ligand, co-activators/co-repressors and the DNA HRE. **B**. Ligand agonists (green) interact with the receptor (heterodimer of nuclear receptor (NR):retinoid X receptor, (RXR)). Ligand binding is accompanied by the recruitment of co-activators and the basal transcriptional machinery. **C**. In the absence of agonists or when bound to antagonists (red) the nuclear receptor is maintained in an inactive transcriptional state by co-repressor molecules.

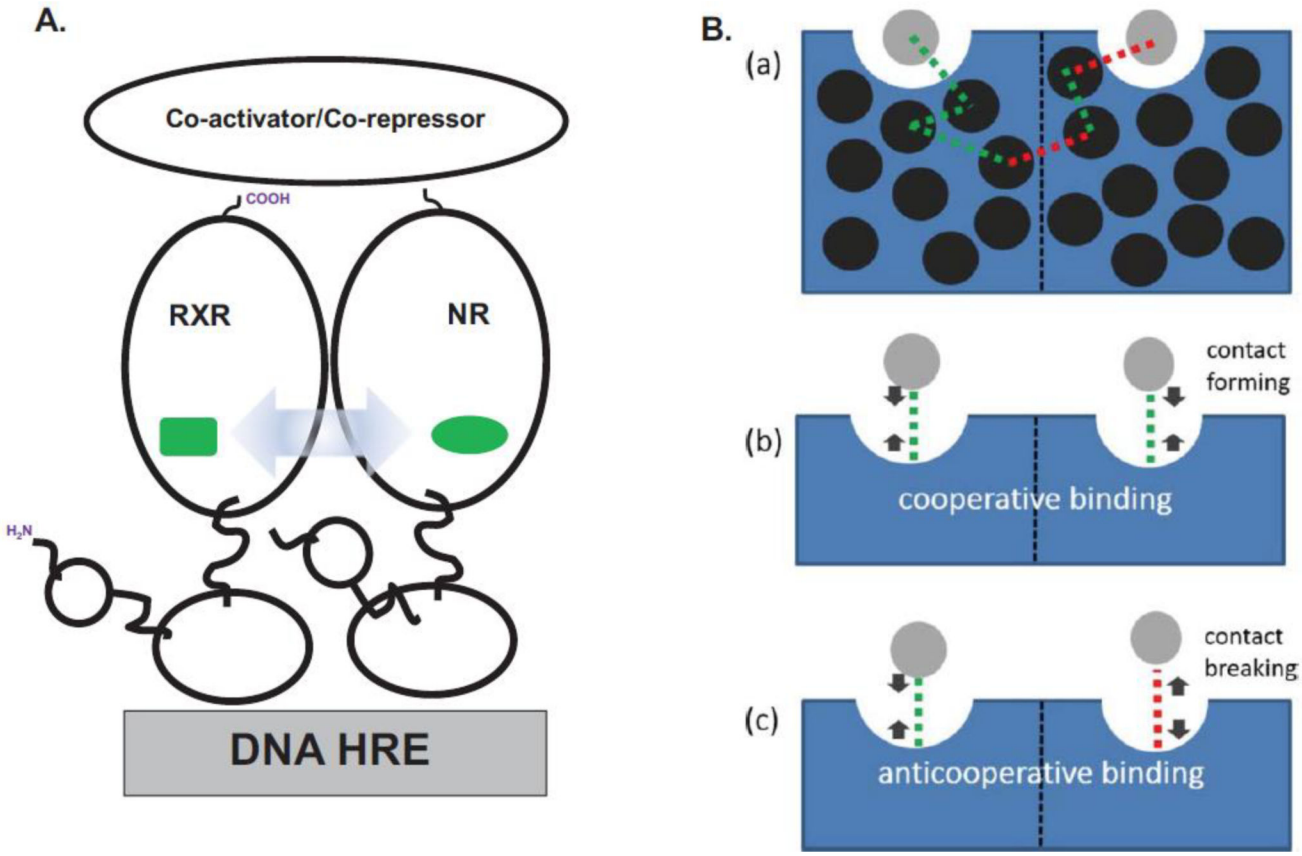


**Figure 2. Ligand-to-Ligand Allostery**

**A**. In nuclear receptor heterodimers, the conformational changes (blue arrow) induced within one receptor subunit upon binding its cognate ligand (green) are transmitted to the ligand-binding pocket of the second receptor (green). **B**. (**a**) The allosteric pathway from one ligand-binding pocket to the second includes the formation of both new interactions and the breaking of old interactions, as determined by the program CAMERRA (Q. R. Johnson, et al., 2015). (**b**) The result of these allosteric conformational changes are cooperative implying that the binding of ligand to one ligand-binding pocket can facilitate the binding of ligand to the second binding pocket in the nuclear receptor heterodimer. (**c**) Conversely, anticooperative binding occurs when the binding of ligand to one binding pocket diminishes the binding-affinity of the second binding pocket for ligand. Adapted with permission from (Clark, et al., 2016). Copyright (2016) American Chemical Society.

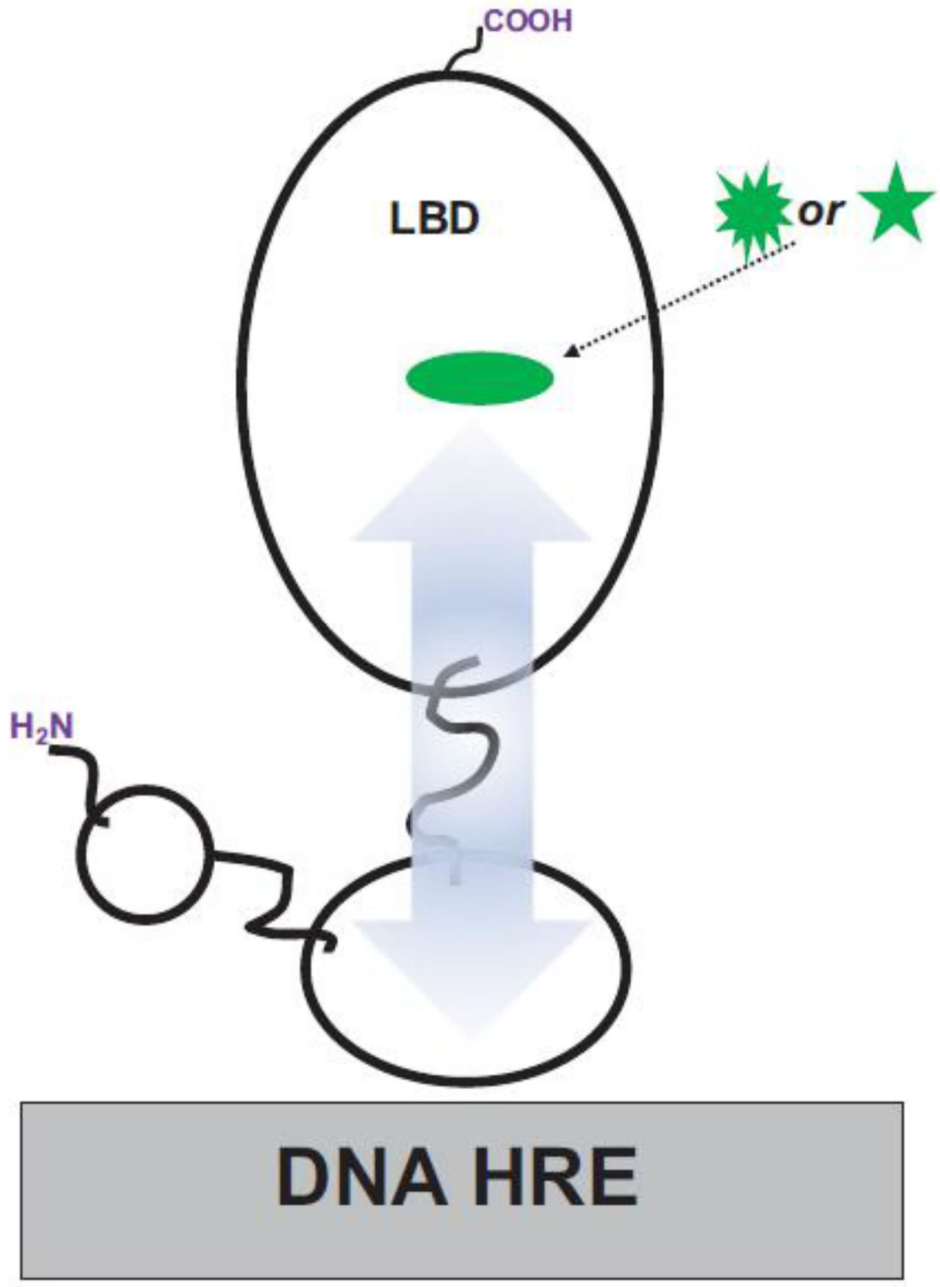


**Figure 3. Ligand-to-DNA Allostery**

Different conformational changes in the ligand-binding pocket induced by distinct ligands (green shapes) can lead to the binding of discrete DNA HRE sequences by the distal DBD through interdomain LBD↔DBD allosteric pathways (blue arrow).

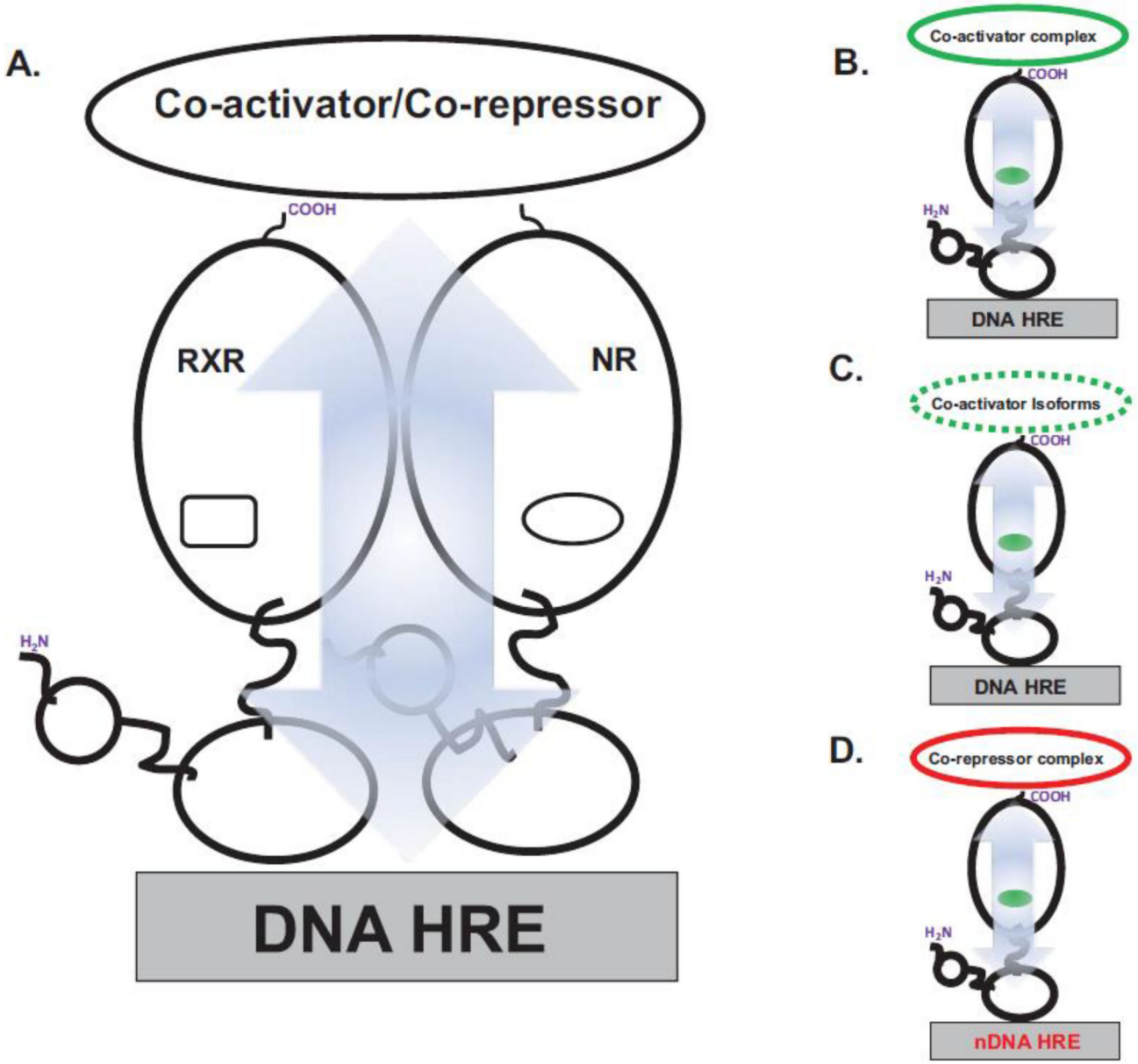


**Figure 4. DNA-to-Co-activator/Co-repressor Allostery**

**A**. Allosteric pathways (blue arrow) link the DNA HRE-binding and co-activator/co-repressor-binding sites. **B**. Binding of DNA HRE can impact the binding-affinity of co-activators and conversely, the presence of co-activator protein can change the binding of the DNA HRE. **C**. DNA HREs can also control the specificity for distinct co-activator isoforms (broken line). **D**. Negative DNA HREs (nDNA HRE) overturn the ligand-agonist paradigm. Thus, nDNA HREs can promote the recruitment of transcriptional silencing co-repressor molecules to nuclear receptors bound to their cognate agonists.

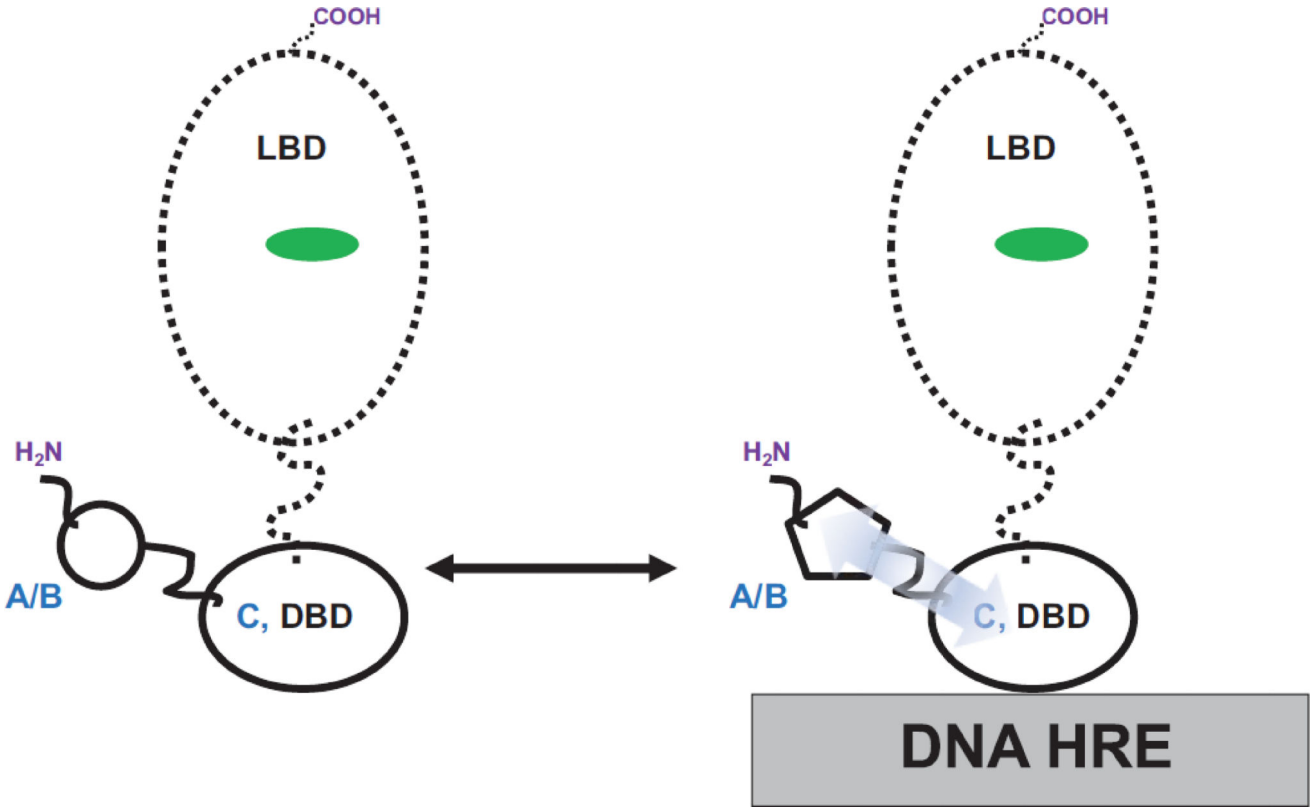


**Figure 5. A/B domain-to-DNA Allostery**

Biophysical studies with isolated A/B+DBD and DBD-only domain constructs indicate that the A/B domain and DBD also communicate through allosteric pathways (blue arrow). The A/B domain is shown to influence DNA HRE binding to the DBD and in turn, the DNA HRE induces observable conformational changes within the A/B domain (note the different shapes of the A/B domain sketches: left (DNA-free) to right (DNA-bound)).