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Biophysics of disordered nuclear receptors and their DNA binding regulation

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Citation

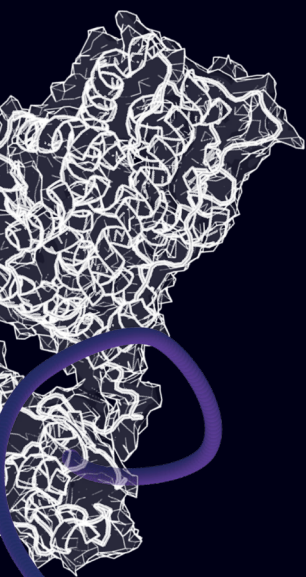
Heling, L. W. H. J. (2026, June 25). *Biophysics of disordered nuclear receptors and their DNA binding regulation*. Retrieved from <https://hdl.handle.net/1887/4306978>

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Chapter 1

**Beyond the Structure: the role of Intrinsic
Disorder in Nuclear Receptors**



Nuclear receptors comprise a superfamily of transcription factors (TFs) that are widely expressed throughout the body. They control a vast array of physiological processes, including metabolism, reproduction, inflammation, and cell proliferation^{1,2}. Since the cloning of the first nuclear receptor 40 years ago³, 48 members have been identified in humans^{4,5}. These proteins function as master regulators of gene expression, translating diverse stimuli into specific cellular responses. Consequently, dysregulation of nuclear receptor signalling pathways is implicated in a range of pathologies, from cancer and diabetes to chronic inflammatory conditions^{6–10}. Because of their central role in health and disease, nuclear receptors represent a highly valuable class of therapeutic targets.

Nearly all nuclear receptors share a common modular architecture, typically composed of five distinct domains (A–E)^{11,12} (**Figure 1A**). The central DNA-binding domain (DBD) contains 8 conserved cysteine residues that coordinate 2 zinc ions into finger motifs, necessary for the receptor's specific binding to DNA motifs called response elements (REs) configured as single, inverted or direct repeats^{7,11,12}. The first zinc finger contains the “P-box,” a motif that makes direct contact with the DNA major groove, thereby governing sequence recognition. The second finger contains the “D-box” motif, which is involved in receptor dimerisation on DNA. Sequence specificity has been linked to DBD dimerisation^{13,14}. ChIP-seq analyses have allowed for genome-wide identification of nuclear receptor binding sites, the cooperation between nuclear receptors and different TFs, and the genome wide effects of nuclear receptor activation on acetylation and methylation of histones markers¹⁵.

The ligand binding domain (LBD) of nuclear receptors is a complex allosteric signalling domain, typically composed of 12 α -helices and 4 β -sheets that fold into a three-layered sandwich, creating a hydrophobic ligand binding pocket (LBP) at the base of the domain which enables interactions with cholesterol, steroid hormones and with cellular metabolic products^{7,8}. Steroid ligand binding to the LBD is suggested to induce a conformational change, repositioning the C-terminal helix 12 to create a surface known as activation function 2 (AF-2)¹⁶. This surface is essential for protein–protein interactions (PPIs) with transcriptional co-regulators, which enhances nuclear receptor activity^{17,18}. Steroid ligand-induced folding of the LBD creates a distinct surface pocket known as binding function 3 (BF-3)¹⁹. This site is of particular interest as it is conserved among steroid hormone receptors, and mutations in residues lining this pocket are implicated in disease. BF-3 may serve as an alternative surface for PPIs, and several small molecule modulators have been reported to bind to this region¹⁹.

A short, flexible hinge region connects the DBD with the LBD⁷. Long considered merely a linker region, the hinge contains the nuclear localisation signal motifs shown to be essential regulators of nuclear translocation²⁰. Although poorly conserved across nuclear receptors, this region is rich in post-translational modifications (PTM) sites that influence receptor activity^{21–23}. Several mutations for diseases like steroid insensitive syndrome and

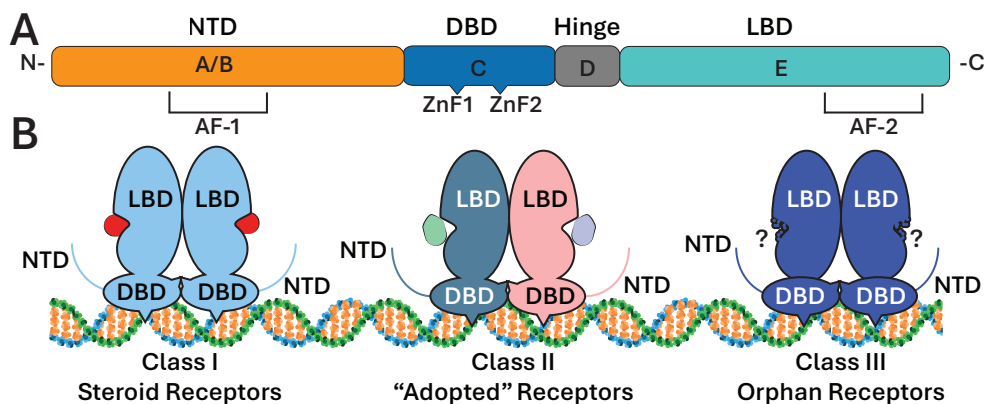


Figure 1. Canonical nuclear receptor domains and classification. (A) Cartoon of the linear protein structure of nuclear receptors, divided in 5 sections: A/B encodes the N-terminal domain (NTD) including the AF-1 region, C the DNA-binding domain (DBD) with its two zinc finger (Znf) domains, D the hinge region, and E the ligand-binding domain (LBD) with the AF-2 region. (B) Nuclear receptors can be divided in three classes based on their ligand and DNA binding characteristics. For class I receptors, the ligand binds with high affinity, while for class II this affinity is attenuated. Question marks next to the class III orphan receptor ligand binding pocket denote that physiological ligands are not known. The ligand binding pocket is lined with bulky amino acids, making ligand binding probably more difficult.

cancer are mapped to this region, further illustrating its functional role²³.

NRs are often classified into three or four major groups based on their ligand-binding, and DNA bound dimerisation characteristics⁸ (Figure 1B). Class I includes classical steroid hormone receptors like the androgen receptor (AR), estrogen receptor (ER) and glucocorticoid receptor (GR). Class II consists of “adopted” receptors originally thought to be orphans (i.e. no endogenous ligands had been identified) for which naturally occurring ligands have been found since, including the retinoid X receptor (RXR) and peroxisome proliferator-activated receptors (PPARs). The ligands for these receptors often have a lower reported affinity for their LBDs than class I nuclear receptor ligands⁷. Class III is made up of orphan nuclear receptors, for which no definitive endogenous ligands have yet been identified²⁴. For many of these orphans, such as the NR4A subfamily members, the LBD is packed with bulky amino acid side chains, which is thought to obstruct conventional ligand binding²⁴. As a result, it is suggested that the regulation of Class III (and IV) nuclear receptors depend primarily on the control of their expression, PTMs, and PPIs, rather than direct ligand activation²⁴.

The structures of the DBD and LBD domains of many nuclear receptors have been experimentally determined, albeit typically in complex with ligands or DNA. Some nuclear receptors are shown to be in a complex with molecular chaperones. These proteins (reviewed here²⁵) are key to cellular protein folding, control the conformational landscape and protect against aggregation. For steroid nuclear receptors, chaperones are essential

throughout the cellular lifetime²⁶, and consequently, the structure of the hormone-lacking (apo) state of the protein remains enigmatic. Furthermore, the presence of a variable, intrinsically disordered N-terminal transactivation domain (NTD) has hindered complete structural elucidation of nuclear receptors.

Intrinsically disordered protein regions - central to the functional characterisation of nuclear receptors

In 1894, Emil Fischer's lock-and-key model established the paradigm that protein function, specifically enzymatic activity, is governed by 3D structure. This structural conformation dictates the protein's ability to selectively bind a complementary substrate, thereby facilitating the catalytic process²⁷. Over the course of the 20th century, this lock-and-key model was further refined through experimental observations^{28–37}, incorporating configurational adaptability or induced fit theory. This model posits that substrate binding induces structural alignment within the active site to facilitate function^{38–40}. These two frameworks strongly supported the structure–function paradigm of protein, which became the leading focus in the field of structural biology^{30,37}. From the beginning, structural biology techniques (X-ray crystallography and later nuclear magnetic resonance (NMR) and (cryo-) electron microscopy) have captured regions of proteins with low electron density. These were considered to represent the absence of structure and, likely due to a lack of understanding, it remained largely ignored within the field of structural biology³⁰.

Advances in computational biology in the 1990s and early 2000s led to the development of protein structure prediction methods^{41–43}. This revealed that a large number of proteins in the eukaryotic proteome contained long natively unstructured/disordered^{4,43–47} stretches, over 30 amino acids long⁴⁸. Because these disordered sections often lie within highly functional proteins, focus on these regions has accelerated and led to new discoveries. Intrinsic disorder is encoded in the protein sequence, with an enrichment of amino acids that lead to low mean hydrophobicity and high net charge⁴⁹. This makes it possible to accurately predict intrinsic disorder from the amino acid sequence of a protein^{44,50}.

The conceptual shift from stable protein structures to dynamic conformational ensembles is fundamental for understanding the roles of these intrinsically disordered proteins (IDPs) and regions (IDRs). Despite lacking a fixed three-dimensional structure, IDRs facilitate key cellular processes, including signalling and regulatory pathways⁵¹. Their structural flexibility provides distinct functional advantages⁴⁴. They are required for mediating receptor-dependent transcription, facilitating intradomain interactions, and enabling allosteric regulation^{51–55}. The inherent disorder allows IDRs to engage in transient interactions with a wide array of binding partners, which can vary depending on cell type and cell-specific signalling cues^{56–58}. This adaptability is often achieved through a mechanism known as “coupled folding and binding,” where the disordered region undergoes a local

conformational transition, forming an α -helix for instance, upon interacting with its target^{53,55}. This process permits the burial of large intermolecular surface areas, even when the interacting domains are small, thus achieving high-affinity binding. The resulting protein complexes are sometimes described as “fuzzy,” as the IDR may retain significant disorder even in the bound state⁵⁹. This allows IDPs to adopt different structures, within the same motif, while binding to varying partners^{59–61}. Another characteristic feature in IDRs is the high density of PTM sites, which can alter the physicochemical and structural landscape, further increasing conformational and functional adaptability⁶². IDRs are frequently subjected to phosphorylation, acetylation, methylation, ubiquitination and SUMOylation, which affect the compactness⁶³, cause a switch from a disordered to an ordered state⁶⁴ or alter the phase separation qualities *in vitro*⁶⁵.

The combination of conformational plasticity, binding promiscuity and temporal regulation through PTMs explains the abundance of IDRs in TFs. Once described as “acid blobs and negative noodles”⁶⁶, the IDR of TFs are core elements regulating gene expression. Transcription relies on the spatiotemporal composition of a multitude of PPIs^{67–69}, which is uniquely suited for IDRs.

The NTD of nuclear receptors are classified as IDRs. In contrast to the generally stable, globular structures of the DBD and LBD, the NTD exhibits a high degree of structural plasticity. It is best described as a collection of conformations, comprising varying levels of unfolded, secondary and tertiary structures^{4,7}. The NTD is the least evolutionarily conserved domain of nuclear receptors across species and superfamily members, and plays an essential role in transcriptional activation and regulation, through its structural plasticity and propensity for PTMs. Given the central role of nuclear receptors in key biological pathways, the NTD is implicated in many pathologies^{10,21,70–72}. However, its elusive structural nature has complicated detailed conformational and functional characterisation^{7,8,10,71–74}. The androgen receptor and Nur77 are nuclear receptors implicated in severe pathologies, including castration-resistant prostate cancer and chronic inflammatory disease, that are driven by their large, disordered NTDs. Understanding the dynamic mechanisms of these domains is therefore a critical frontier for novel drug development.

Androgen receptor - allosteric hub defined by disorder

The Androgen Receptor (AR/NR3C4) is a 110 kDa, classic steroid hormone receptor, belonging to Class I of the nuclear receptor superfamily^{7,75} (**Figure 2**). Testosterone and dihydrotestosterone (DHT) hormones primarily regulate AR action which is important for male sexual development and reproductive health⁷⁶. Its dysregulation is linked to various disorders, including neuromuscular disease and cancer⁷⁵.

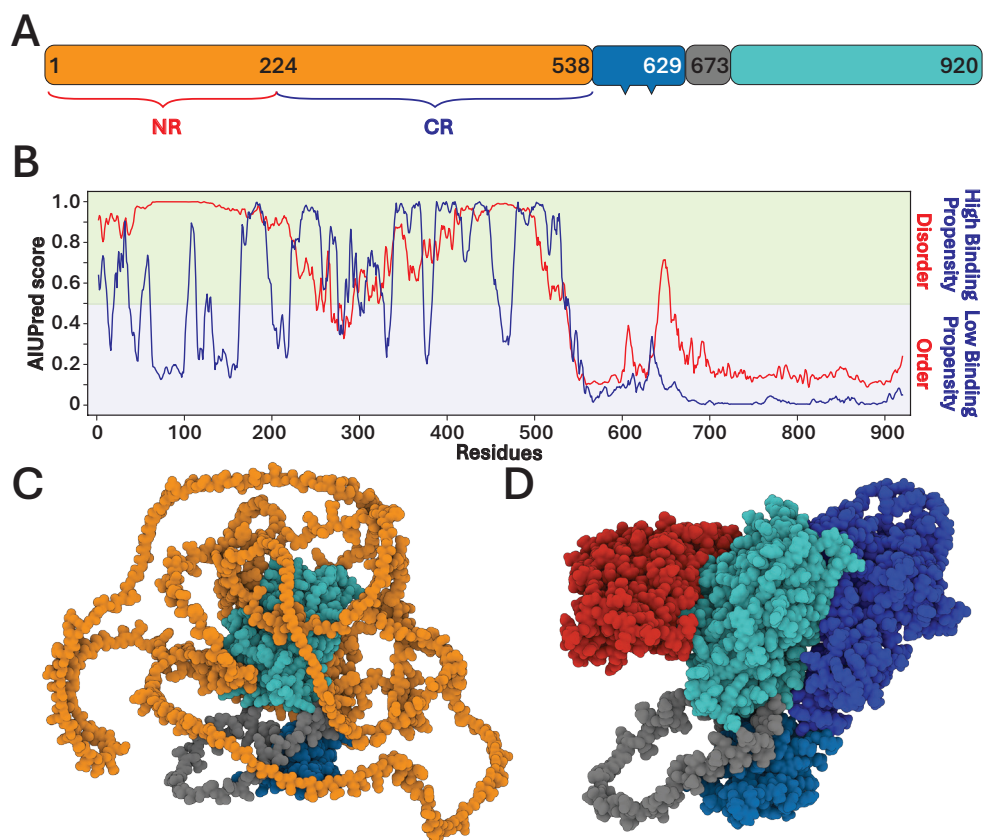


Figure 2. Structural and functional classification of disorder in the Androgen Receptor. (A) Cartoon of the linear protein structure of AR with the residue numbers depicting the borders of each region. The colours align with the linear structure in **Figure 1A**. (B) Plot from AIUPred^{99,100} (<https://aiupred.elte.hu/>), which predicts the likelihood of residues being more structurally ordered or disordered (in red), and which residues and regions have a propensity to be functional binding motifs (in blue, scores >0.5). (C) AlphaFold predicted model of the full-length AR protein (AF-P10275-F1-v4), colour coded according to the linear structure in panel A. The structural dynamics of the NTD cannot be captured by the algorithm and present as a characteristic ribbon around the more stable structures of the protein. (D) Full length AR model from Sheikhhassani et al. (2022)⁹⁸, which identified, using molecular dynamics simulations and topological analyses, that the NTD can form two highly dynamic regions, the NR (residues 1–224) and CR (residues 225–538), coloured in red and darkblue respectively. The NTD and full-length model showed remarkable agreement with experimental evidence.

The mechanism of AR activation is a multi-step, allosteric process⁷⁷. Upon ligand binding, the AR-LBD is thought to undergo conformational changes, which lead to the dissociation from chaperone and co-chaperone complexes⁷⁸, repositioning helix 12 to form the AF-2 pocket^{79,80}. This surface is an interaction site for co-activator proteins. A unique feature of AR is the preference of its AF-2 pocket to the ²³FQNLF²⁷ motif located within AR-

NTD⁸¹. This N/C (N-terminal to C-terminal) interdomain interaction, which is induced by androgen binding, is required for full AR activity⁸² and involves the FQNLF motif folding into an α -helix upon binding the LBD. Once activated, AR translocates to the nucleus and binds to specific DNA sequences known as androgen response elements (AREs) as a homodimer. Recent structural work has shown that this binding is asymmetric and cooperative, with the DNA itself acting as an allosteric effector to stabilise the dimer on the response element⁸³.

The AR-NTD, which comprises more than half of the protein sequence^{20,77} (**Figure 2A**), contains the AF-1 and is essential for transactivation^{74,82}. The AF-1 has further been dissected into two transactivation units (TAU-1 and TAU-5)^{84,85}. In addition, several linear motifs have been identified along the NTD sequence, the aforementioned FQNLF, the ¹⁷⁹LKDIL¹⁸³, and the ⁴³³WHTLF⁴³⁷⁷⁵. The NTD also contains binding sites for the TFIIIF transcription factor, the p160 coactivators and co-chaperone proteins^{86–90}, such as the fully conserved AR NTD signature sequence (ANTS)⁹¹ between residues 233 and 246. Several amino acid repeats, including the polymorphic polyglutamine (pQ; residues 58–80) and polyglycine (pG; residues 372–379) stretches, are also encoded within the NTD. The physiological roles of these polymorphic regions are unclear, but they have been proposed to provide inhibitory regulation on AR²⁰. These stretches are involved in disease. For instance, expansion of the pQ repeat beyond 38 residues is the mutation leading to spinal and bulbar muscular atrophy (SBMA; also known as Kennedy's disease), a neurodegenerative disease⁹², while the length of the pQ and pG stretch is inversely correlated with prostate cancer risk^{93,94}. The NTD is furthermore indispensable for the activity of LBD-truncated AR splice variants (AR-Vs), which are key drivers of castration-resistant prostate cancer (CRPC)⁹⁵. While the structural information of the DBD and LBD of AR have been experimentally characterised, structural information on the NTD has remained elusive, as a result of the high propensity for disorder (**Figure 2B**). State-of-the-art approaches based on artificial intelligence have also not been able to overcome this (**Figure 2C**). Given its key role in AR function and its structural plasticity, the AR-NTD has become a major focus for drug screening programs for CRPC⁷⁷. Small molecule inhibitors, such as EPI-001, target the AR-NTD, blocking its transactivation function and indicating a potential mechanism for overcoming therapy resistance⁹⁹.

Nur77 - Regulation through Interaction

The NR4A subfamily, which comprises Nur77 (NR4A1), Nurr1 (NR4A2) and NOR-1 (NR4A3) belongs to Class III of orphan nuclear receptors²⁴. While the DBDs have high sequence homology, the LBD and NTD are more divergent between the subfamily members, with 58–68% and <30% similarity respectively. Nur77 (**Figure 3**), also known as NGFI-B and TR3, is predominantly localised in the nucleus where it functions as a transcription factor, modulating gene expression through binding to specific DNA motifs.

Nur77 has an important role in immune regulation, influencing pro-inflammatory signalling pathways and cellular metabolism¹⁰⁰. It generally acts as an anti-inflammatory factor, with a protective role in chronic inflammatory diseases like atherosclerosis and inflammatory bowel disease. Its anti-inflammatory effects include negative crosstalk with the NF- κ B signalling pathway, where it can directly bind and trans-repress the p65 subunit¹⁰¹. Recent work has expanded Nur77's regulatory influence to immunometabolism and has identified it as a novel regulator of macrophage immunomechanics¹⁰². This study showed that Nur77-deficient macrophages exhibit altered actin networks, leading to a stiffer cellular phenotype, enhanced migration, and increased phagocytic activity. Transcriptomic analysis revealed that Nur77 directly regulates the expression of genes related to the cytoskeleton and cellular mechanics. This direct regulation of mechanobiology genes by Nur77 shows an additional mechanism through which it restrains inflammation by modulating the mechanical characteristics of macrophages¹⁰².

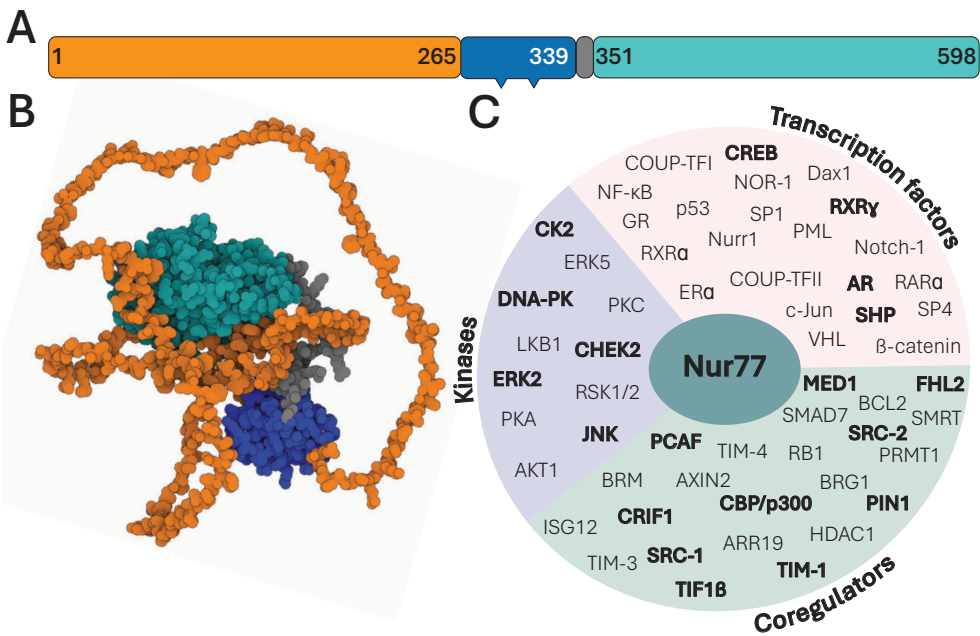


Figure 3. Nur77 activity is likely regulated through protein-protein interactions. (A) Cartoon of the linear protein structure of Nur77, with the residue numbers depicting the borders of each region. The hinge region is encoded by residue 340 to 350. The colours align with the linear structure in figure 1A. (B) AlphaFold predicted model of the full-length Nur77 protein (AF-P22736-F1-v4), colour coded according to the linear structure in panel A. (C) Transcription factors, coregulators and kinases identified to interact with Nur77. Proteins with bold names have been identified to interact through the NTD of Nur77. List of interactome taken from Kurakula et al. (2014)²⁴.

A defining characteristic of Nur77 is the absence of an identified endogenous activating ligand^{24,100}. This is likely because its LBD is packed with bulky hydrophobic amino acid sidechains, which obstruct conventional ligand binding. However, recent studies did

suggest potential ligands that may bind and regulate Nur77^{103–108}, although the molecular mechanisms behind the regulation are unclear, and the potential has not been consistently verified. The LBD of Nur77 is dispensable, while like AR, the NTD plays a major role in transactivation^{109,110}. Another unique characteristic of Nur77 is the ability to bind specific DNA motifs as a monomer or as a homodimer¹¹¹, or form activating heterodimers with retinoid X receptors (RXRs).

As the endogenous ligand for Nur77 remains unknown, the current consensus is that activity is likely regulated through expression levels, PTMs, and PPIs²⁴ (**Figure 3C**). Expression of Nur77 is transiently and rapidly induced by diverse cell-specific signals, like growth factors, cytokines, kinases and mechanical stress¹¹². Various other transcription factors and co-activators have been found in complex with Nur77, including NF- κ B, SP1, p300, Bcl-2, p53 and GR^{24,113–116}. These interactions can enhance or repress the transcriptional activity of Nur77, while vice versa, Nur77 has been found to induce or inhibit the activity of the interacting receptors^{24,117–119}.

Cell-type specific, it has been shown that Nur77's roles also include non-transcriptional functions. Nur77 interaction with Bcl-2 at the mitochondria induces apoptosis^{114,115}. These processes can be modulated by PTMs and PPIs. As most of the PPI and PTM sites lie within the NTD, this further establishes the critical importance of this domain.

Drugging the dynamics - tackling challenges for the therapeutic frontier

The characterisation of AR and Nur77 shows a need to move beyond static structural descriptions and to study their transient folding and dynamic molecular interactions. The evidence concerning the intrinsic disorder within their NTDs shows that conformational flexibility and dynamic transitions are active determinants of function and regulation. The complex interplay between the AR-NTD's intrinsic disorder and its implications in diseases exemplifies the effect of dynamic structural properties on biological function. For Nur77, the absence of a conventional ligand-binding pocket means that its activity is modulated by its transient PPIs and PTMs through the NTD. This inherent disorder in IDRs presents both challenges and opportunities for developing new therapeutic interventions^{70,120–122}.

Current therapeutic strategies targeting nuclear receptors often have limitations, including off-target effects and the emergence of resistance, like AR-Vs in prostate cancer^{7,84}. The NTDs of nuclear receptors, particularly, are emerging as promising drug targets despite their disordered nature. The challenge lies in the rational design of small molecules that can precisely modulate the transient conformational states and dynamic interactions of these IDRs. Traditional structure-based drug design is often not applicable to IDRs. Unlike the lock-and-key or induced-fit models applicable to globular domains, the

elusive structural nature of IDRs makes them a very challenging target for homology and functional classification. State-of-the-art AI approaches, like AlphaFold¹²³, have emerged as a prominent tool for predicting the 3D structures of proteins and protein complexes. However these fail to accurately capture the conformational dynamics of intrinsically disordered sequences. This has hindered drug development, and the general “druggability” of IDRs remains unclear¹²⁴. Despite the limited knowledge on the conformational ensemble of IDRs, several small molecules have been found to bind and interact with disordered sequences^{125–131}, and some were shown to affect biological activity^{128,129,131}. One of the most promising pharmacological compounds is the aforementioned EPI family, such as EPI-001⁹⁹, which targets the AR-NTD and has shown efficacy in blocking AR transactivation and mitigating resistance in prostate cancer¹³². This highlights the importance for a better understanding of these dynamical regions of nuclear receptors and important features within.

Overcoming this challenge requires new developments. In the last two decades, several tools have emerged. Computational approaches have been developed to readily identify regions and residues within IDPs as functional binding sites^{97,98}. Recently, a machine learning tool allowed for the identification of protein binders targeting disordered regions, and functionally modulating IDPs *in vitro*¹³³. Molecular dynamics (MD) simulations have emerged as an indispensable technology that can characterise the very dynamics that render the NTD elusive to classical approaches. By simulating the atomic motions of the protein over time, MD, coupled with complementary experimental assays such as NMR spectroscopy, can map the dynamic conformational landscape of IDPs, revealing the distribution of states it populates^{134–136}. NMR spectroscopy is continuously developed to characterise disorder and the functional atomistic details within¹³⁷ and has been used to localise sequences within IDPs which are stabilised by compounds through chemical shift perturbations⁹⁹.

Combining MD simulations with topological analysis allows for the identification of motifs within the conformational noise. Topology is a mathematical framework that is capable to detect invariant shapes within geometric dynamics. The field of protein topology was traditionally focussed on knotted conformations and ignored intrachain amino acid contacts^{138,139}. The emergence of circuit topology (CT, **Figure 4**) provides a complementary topological approach to knot theory which can fully characterise folded molecular chains^{140–144}. CT in its simplest form characterises a pair of intramolecular contacts as one of three configuration: series (S), parallel (P) and cross (X). A pair of contacts in the S configuration are independent from each other and can be distant, while P and X contacts indicate more entanglement of protein chain. The CT framework, which had already provided insights into molecular folding processes and the 3D genome architecture^{145–155}, was enhanced with a lifetime distribution parameter to create dynamic CT (dCT)—ideally suited to capture the transient nature of IDPs. MD simulations and dCT were first combined in our study on the full length wild-type (wt) AR-NTD⁹⁶. This revealed with

atomistic detail that AR-NTD form two highly dynamic disjoint regions, a N-terminal region (NR; residues 1–224) and C-terminal region (CR; 225–538) (**Figure 2D**). Beyond the structural observations from the MD simulation results, the topological analysis revealed stark differences between the two subregions. CR contacts showed more entanglement (as evidenced by higher P and X relations) and had longer lifetimes, indicating higher stability and compaction compared to the NR, which explored the topological space in what appeared a more random fashion⁹⁶. This study presented a novel and a protocol validated by experimental evidence for studying IDP and IDRs, as exemplified by the comparative study on the AR, ER and GR¹⁵⁶. Recently, a CT-based fold analysis approach was able to detect the emergence of disordered compact states in a study on the effects of phosphorylation on the AF1 core of GR¹⁵⁷.

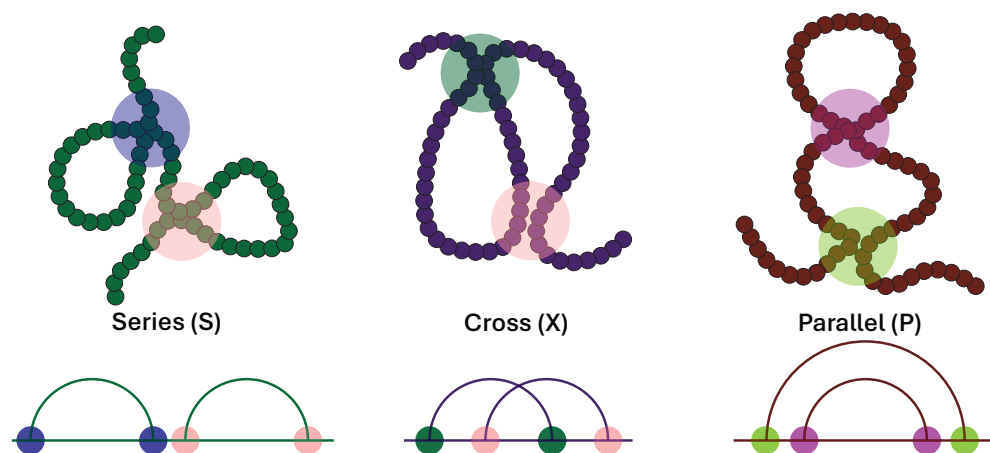


Figure 4. Circuit topology identifies relations between contacts. A pair of contacts can be in one of three relations, with varying levels of complexity. Two pairs in series relations are entirely independent of each other. When one half of the binding site is within another pair, this is described as a cross relation, while if one contact lies entirely within another contact the relations is described as parallel. Respective circuit diagrams below offer a simpler overview of these relations in linear space.

These approaches have provided new tools for the studying of IDPs and IDRs, offering frameworks for the experimental study of these highly dynamic and functionally important protein regions.

Scope of this thesis

Nuclear receptors are invaluable mediators of biological processes, and their dysregulation is central to numerous pathologies, including cancer and inflammatory diseases. Consequently, they have been a major focus of drug development. However, the efficacy of developments has been hindered by the limited knowledge on the conformational and allosteric dynamics of nuclear receptors. This thesis aims to gain a more comprehensive understanding of how the structural and functional dynamics mediate the Androgen Receptor and Nur77 nuclear receptors.

In **Chapter 2**, we look at effects of the disease-causing mutation, pQ expansion, on the folding dynamics of AR-NTD using extensive coarse-grained and all-atom molecular dynamics simulations, topological analyses and advanced docking approaches. We investigate how expansion of the pQ tract leads to misfolding of AR-NTD not seen in wt AR-NTD. We examine the consequences for the delicate balance of PPI and oligomerisation patterns, and how this disrupts critical interactions and regulatory mechanisms.

Our wt AR-NTD computational model suggested a distinct differential regulatory role for NR and CR on AR's ability to interact with DNA. In **Chapter 3** we utilise a combination of real-time single-molecule fluorescence microscopy and a thermophoretic approach to study the effects of these subregions on AR DBD-LBD (Δ NTD-AR) DNA binding capabilities.

Nur77 is an orphan receptor, meaning it does not have an (identified) endogenous ligand. The main consensus among researchers is that the activity of Nur77 is regulated through a plethora of external signals, including PTMs and PPIs. A critical interaction occurs with the glucocorticoid receptor (GR). These receptors are thought to engage in reciprocal repression, yet the molecular mechanism driving this cross-talk is unknown. **Chapter 4** demonstrates the DNA binding behaviours of Nur77, and how activated GR affects this process. Additionally, we look at the effects of dexamethasone, a common synthetic glucocorticoid, on Nur77's DNA interactions.

No endogenous ligands are known for Nur77, but some potential compounds have been identified to regulate Nur77's expression and/or activity. In **Chapter 5**, we examine cytosporone-B to determine its impact on DNA binding specificity and to propose a mechanistic model for its modulation of Nur77.

Finally, in **Chapter 6** the results of this thesis will be summarised and discussed within the wider scope of nuclear receptor and transcription factors and directions for future research into nuclear receptor structure and function will be proposed.

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