Universiteit
Leiden
The Netherlands

Biophysics of disordered nuclear receptors and their DNA binding regulation

Heling, L.W.H.J.

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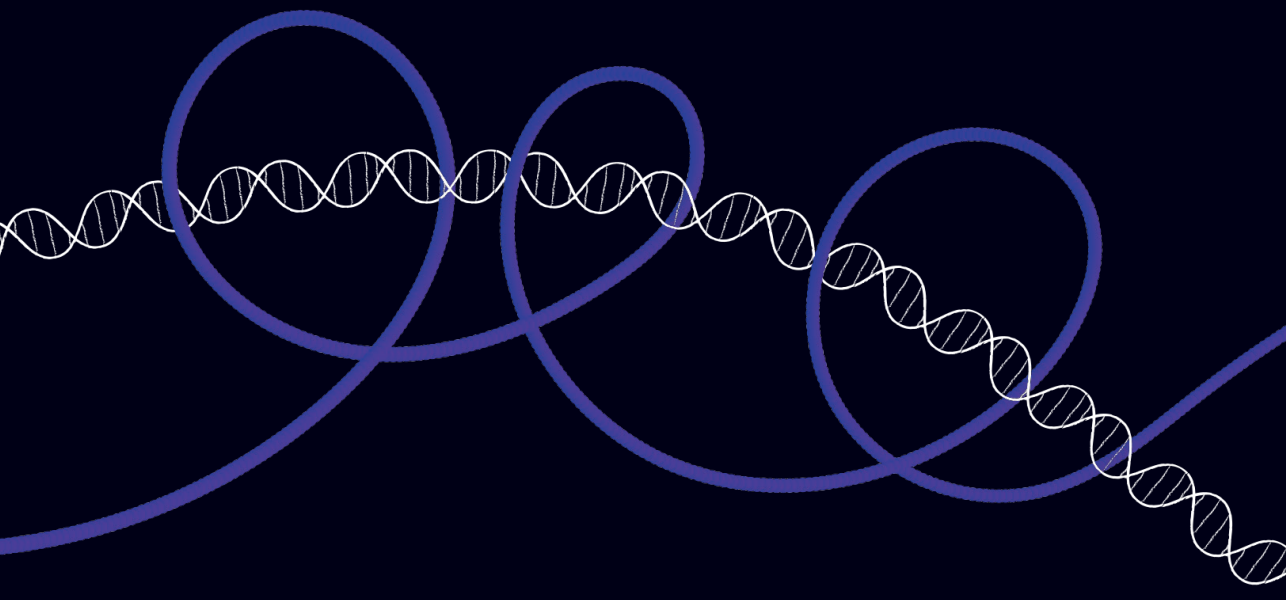

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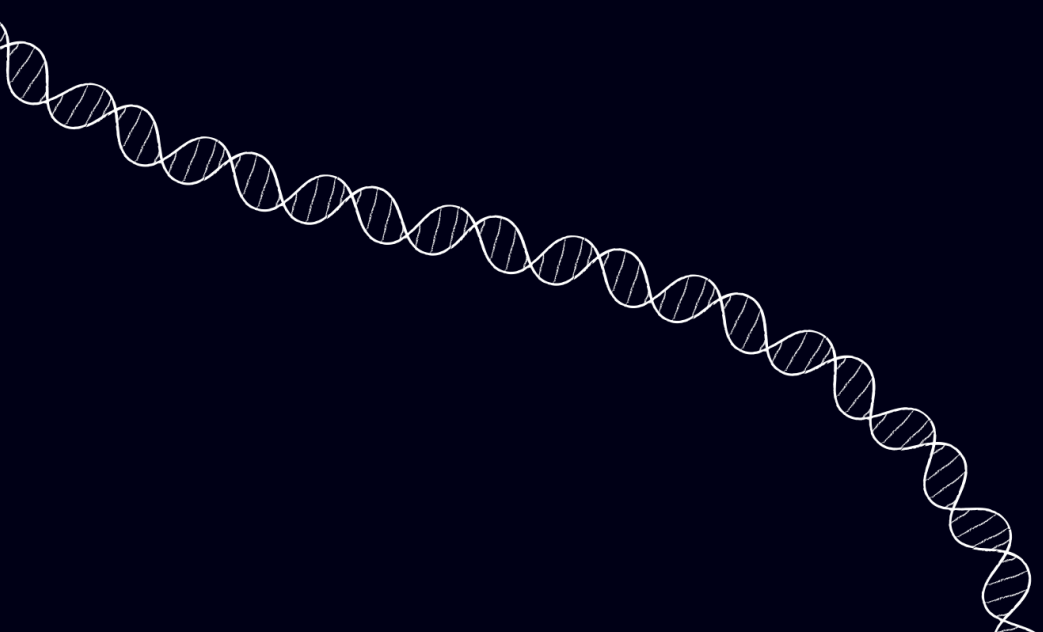

English summary

For decades, the structure–function paradigm has been the bedrock of molecular biology, asserting that a protein's three-dimensional shape dictates its role. This view was challenged by the discovery of intrinsically disordered proteins (IDPs) and regions (IDRs)—vast, flexible domains that, lacking a stable structure, were initially dismissed as mere linkers. Far from being passive, it is now clear that this structural plasticity is a key to function. In **Chapter 1**, we explore the knowledge gap by reviewing the established principles of intrinsic disorder within the context of the nuclear receptor superfamily, establishing the central theme: that the inherent flexibility of the N-terminal domain (NTD) is a fundamental regulatory mechanism. Building on this foundation, the subsequent chapters will demonstrate this principle by combining advanced molecular modelling with single-molecule biophysics through the lens of two key nuclear receptors: the Androgen Receptor (AR) and the orphan receptor Nur77.

Chapter 2 applies these concepts to a critical disease model, presenting the first detailed molecular model of the polyglutamine (pQ) expansion within the AR-NTD, the mutation responsible for Spinal Bulbar Muscular Atrophy (SBMA). Our findings reveal that the pQ expansion induces a dynamic misfolding of the NTD, which alters its global structure and disrupts the delicate balance of its interactions. This work provides the first atomistic-level explanation for how a mutation in a disordered region can alter oligomerisation patterns and lead to toxic protein aggregation, linking the receptor's structural dynamics directly to its pathological gain- and loss-of-function.

The discovery that the AR-NTD comprises distinct dynamic subregions naturally raised the question of whether they possess unique functions. **Chapter 3** addresses this directly, investigating how these subregions — the N-terminal region (NR) and C-terminal region (CR) — allosterically regulate the receptor's ability to bind DNA. Using single-molecule techniques to visualise these interactions in real-time, we reveal a sophisticated regulatory interplay. Our findings demonstrate that these two disordered regions function as a modular control system with differential and synergistic effects: the CR acts as an inhibitor, reducing AR's binding affinity for DNA, while the NR functions as a kinetic modulator, accelerating the receptor's dissociation rate, thereby collectively fine-tuning the receptor's interaction with DNA.

Having established the principles of IDR-mediated regulation in a well-studied receptor, the thesis then broadens its scope to test if these principles apply to more enigmatic cases. **Chapter 4** explores the regulatory crosstalk between the orphan receptor Nur77 and the Glucocorticoid Receptor (GR). Our single-molecule assays reveal that the GR protein directly stabilises Nur77 on DNA, increasing its residence time. Strikingly, we also discovered that dexamethasone, a common synthetic glucocorticoid, directly disrupts Nur77's ability to bind DNA, independent of GR. This study uncovers a dual mechanism

of regulation—one mediated by the GR protein and another directly by the steroid ligand itself—adding a new layer of complexity to their antagonistic relationship.

The unexpected finding that a small molecule could directly impact Nur77 prompted the investigation in **Chapter 5**, which examines how Cytosporone B (Csn-B), a compound known to bind Nur77, modulates its DNA-binding activity. Our analysis reveals that Csn-B acts as an allosteric modulator that fundamentally reprograms Nur77's DNA search mechanism. It enhances Nur77's specificity for its dimeric DNA motif while reducing its affinity for non-specific DNA and monomeric motifs. This suggests a mechanistic model where a ligand induces a conformational switch in an orphan receptor that favors a specific oligomeric state and, consequently, a specific DNA motif.

Finally, **Chapter 6** summarises the work in this thesis and bridges the gap between the precise, reductionist models of the preceding chapters and the complex, crowded environment of the living cell. It discusses how the fundamental biophysical properties uncovered in this thesis—the dynamic misfolding, the allosteric modulation, the altered kinetics—provide a crucial foundation for understanding an exciting new frontier in cell biology: biomolecular condensates. This chapter posits that the IDR-driven interactions and the modulation of DNA residence times observed here are likely key factors in the formation and regulation of the transcriptional hubs where gene expression is orchestrated. It frames the thesis not as an endpoint, but as a vital starting point, providing the molecular-level data necessary to begin deciphering the complex interplay of forces that govern gene regulation within these dynamic compartments.