HDX reveals the conformational dynamics of DNA sequence specific VDR co-activator interactions

Jie Zheng¹, Mi Ra Chang¹, Ryan E. Stites², Yong Wang², John B. Bruning³, Bruce D. Pascal¹, Scott J. Novick¹, Ruben D. Garcia-Ordonez¹, Keith R. Stayrook², Michael J. Chalmers², Jeffrey A. Dodge² & Patrick R. Griffin¹

The vitamin D receptor/retinoid X receptor-α heterodimer (VDRRXRα) regulates bone mineralization via transcriptional control of osteocalcin (BGLAP) gene and is the receptor for 1α,25-dihydroxyvitamin D₃ (1,25D₃). However, supra-physiological levels of 1,25D₃ activates the calcium-regulating gene TRPV6 leading to hypercalcemia. An approach to attenuate this adverse effect is to develop selective VDR modulators (VDRMs) that differentially activate BGLAP but not TRPV6. Here we present structural insight for the action of a VDRM compared with agonists by employing hydrogen/deuterium exchange. Agonist binding directs crosstalk between co-receptors upon DNA binding, stabilizing the activation function 2 (AF2) surfaces of both receptors driving steroid receptor co-activator-1 (SRC1) interaction. In contrast, AF2 of VDR within VDRM:BGLAP bound heterodimer is more vulnerable for large stabilization upon SRC1 interaction compared with VDRM:TRPV6 bound heterodimer. These results reveal that the combination of ligand structure and DNA sequence tailor the transcriptional activity of VDR toward specific target genes.

¹Department of Molecular Medicine, The Scripps Research Institute, Jupiter, FL 33458, USA. ²Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46225, USA. ³The University of Adelaide, Institute for Photonics & Advanced Sensing (IPAS), School of Biological Sciences, University of Adelaide, Adelaide SA 5005, Australia. Correspondence and requests for materials should be addressed to P.R.G. (email: pgriffin@scripps.edu)
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alcium ion metabolism and homeostasis is co-operatively governed by the intestine, kidney, and bone to ensure physiological bone mineralization, an important process of laying down calcium phosphate on bone matrix. The vitamin D receptor (VDR), a member of the nuclear receptor (NR) superfamily, orchestrates calcium homeostasis and bone mineralization through transcriptional control of VDR target genes in various tissues. VDR is activated by the full agonist secosteroid hormone 1,25D3 (an active metabolite of vitamin D3) resulting in increased expression of the osteoblast hormone osteocalcin (bone gamma-carboxyglutamatic acid-containing protein, BGLAP), a protein essential for bone formation. As such, 1,25D3 is used to treat osteoporosis, a metabolic disease that manifests clinically as decreased bone mass density. Although 1,25D3 is essential for normal osteoclast formation, hyperactivation of VDR by this secosteroid hormone results in untoward effects including hypercalcemia and hypercalciuria derived from increased calcium absorptions in the intestine. The association of 1,25D3 treatment with hypercalcemia is in part related to VDR-mediated expression of the epithelial Ca2+ channel TRPV6 (transient receptor potential family type 6). This channel mediates transepithelial calcium transport, and it is a major VDR target gene activated by 1,25D3 action in intestine. Therefore, safer vitamin D therapies for treatment of osteoporosis are in high demand that drive bone mineralization but are devoid of hypercalcemia effects.

One approach has been the development of non-calcemic VDR modulators known as VDRMs. These compounds are designed to differentially activate VDR target genes. To help facilitate the design and discovery of such compounds, an understanding of the complexity of the structural and molecular mechanisms of VDRM action should be considered. This includes knowledge of the conformational changes within the VDRRXR heterodimeric receptor complex that are induced by ligand binding and interaction with co-regulatory proteins, as well as allosteric crosstalk upon binding specific regions thought to regulate VDR target genes.

VDR is a ligand-dependent transcription factor that forms an obligate heterodimer with retinoid X receptor α (RXRα). Like other NRs, VDR and RXR comprise four major functional domains. The N-terminal domain (NTD) is structurally disordered and contains the activation function 1 (AF1) motif which facilitates ligand-independent activation of the receptor. Adjacent to the NTD is the two-zinc finger-containing DNA binding domain (DBD) and a flexible hinge domain links the DBD to the ligand-binding domain (LBD). The LBD consists of three β sheets and 12 α helices, a canonical structure adopted by most NRs. Helices 3, 5, and 12 of the structured LBD serve as the ligand-dependent activation function 2 (AF2) surface for complementary binding of co-regulator proteins (e.g., the p160 family of steroid receptor co-activators such as SRC1). Agonist binding in the hydrophobic pocket of LBD facilitates AF2 to adopt a conformational rearrangement that facilitates high affinity docking of co-regulatory proteins possessing conserved helical nuclear receptor box (NR box) motifs (5’-LXXLL-3’) that dock into the AF2 surface. These co-regulatory proteins either contain chromatin remodeling activity or are scaffolds to tether remodeling enzymes to the receptor complex. Agonists of the receptor drive interaction with co-activator proteins with histone acetyl transferase activity to relax chromatin, whereas antagonists drive interaction with co-repressor proteins with histone deacetylase activity to condense chromatin.

NRs modulate transcriptional output by binding to specific DNA sequences (response elements) within the promoter and distal regions of their target genes, and recruiting chromatin remodeling enzymes to either facilitate binding or block binding of RNA polymerase II. Upon ligand binding, VDRRXRα translocates and associates with genomic DNA. The VDRRXR heterodimer binds with high affinity to short DNA sequence motifs known as VDR binding sequences (VBSs), which are typically hexameric direct repeats of 5’-AGGTCA-3’ separated by three base-pair spacers, a motif referred to as DR314–16. Within the receptor complex, the DBDs of both VDR and RXR are linked and the VDR hinge form the major structural determinants involved in VBS recognition. Previously it was shown that DNA sequences induce unique conformations within NRs, suggesting that DNA is not only a scaffold but is a regulatory ligand for the receptor complex, the DBDs of both VDR and RXR

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Results

Structure of VDRMs and their BGLAP and TRPV6 activation.

Compounds were tested for their hypercalcemia liabilities in BGLAP and TRPV6 qPCR gene activation assays. The chemical structures of the ligands used in these studies are presented in Table 1 and these include the endogenous full agonist 1,25D3, two synthetic full agonists (Cmpd1 and Cmpd2), and one VDRM (Cmpd3), defined as a compound that displays a unique activation profile as compared with agonists. Unlike 1,25D3, Cmpds 1, 2, and 3 are non-secosteroidal tool molecules that are highly optimized toward understanding functional selectivity of bone vs. calcium effects in cells. The natural agonist 1,25D3 exhibited potent hypercalcemia effects and enhanced activation profiles in both BGLAP (EC50 = 4.10 nM, 100% Max. Stim.) and TRPV6 (EC50 = 9.81 nM, 100% Max. Stim.) qPCR based assays. The maximum fold change over DMSO for cells treated with 1,25D3 was set to 100% maximum stimulation for both BGLAP and TRPV6, fold change for other compounds were normalized to the maximum stimulation value for 1,25D3 for each gene respectively. EC50 values are the average from the replicate runs. Cmpd1 was the most potent in the BGLAP activation assay (EC50 = 1.44 nM, 94.2% Max. Stim.) and was also able to effectively activate TRPV6 gene expression with an EC50 of 63 nM (80.3% Max. Stim.). Cmpds 1, 2, and 3 exhibited dissociated activation profiles with an EC50 of >10 μM (17.4% Max. Stim.) and 698 nM (75.9% Max. Stim.) in TRPV6

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than 85 and 90% sequence coverage was obtained for full-length individual amino acid values using a residue averaging approach. HDX Workbench was used to map the HDX data to the pymol structure model displayed in Supplementary Fig. 2c. Differential HDX was performed to investigate conformational perturbations within the intact VDRRXRα heterodimer induced upon binding to natural agonist, synthetic agonists and a non-calcemic VDRM.Regions within the receptor complex that exhibit a decrease in solvent exchange as compared to apo-complex are inferred to have been stabilized by the binding event. Likewise, regions within the receptor complex that exhibit increased exchange as compared with apo-complex are inferred to have been destabilized by the binding event. Binding of intact heterodimer to different ligands stabilized regions of the receptor corresponding to the consensus ligand binding sites of VDR and dimer interface within RXRα (RXRα: aa347–353 and aa419–430) (Fig. 2a–d and Supplementary Fig. 1b, d) and column (i), (ii), (iii), and (iv)). VDR residue regions—aa134–150 (H1), aa225–233 (H3), aa273–279 (H5), aa310–316 (H7), aa390–403 (H11) and aa411–419 (H12)—exhibited significantly reduced HDX kinetics upon binding to natural ligand 1,25D3 (Fig. 2a and Supplementary Fig. 1b, d, column (i)). Consistent with the structure of 1,25D3 bound VDR LBD (PDB: 1DB1), residues within these α-helices—Tyr143 (H1), Ser237 (H3), Arg274 (H5), Ser278 (H7), Trp286 (S1) and His305 (loop between H6 and H7)—form a hydrophobic ligand-binding pocket (LBP) within the VDR LBD fold for accommodation of 1,25D3. Furthermore, binding of Cmpd3 to heterodimer resulted in lower threshold of protection against deuterium exchange at residue regions—aa234–244 (H3), aa309–316 (H7), and aa390–403 (H11) (Fig. 2d and Supplementary Fig. 1b, d (column iv)). Despite being a weaker binder, interaction with this non-calcemic ligand afforded a similar extent of protection at VDR H12 compared to that of 1,25D3. Furthermore, binding of synthetic agonist Cmpd1 or Cmpd2 presented even higher magnitude of protection against solvent exchange at aforementioned regions compared to 1,25D3, suggesting that synthetic agonists associate with stronger hydrogen bonding networks within VDR LBP (Fig. 2b, c and Supplementary Fig. 1b, d column (ii) and column (iii)). Furthermore, binding of Cmpd3 resulted in protection to solvent exchange in the VDR hinge domain/C-terminal Extension (CTE) (aa93–99 and aa99–108) and RXRα residues 271–293 (H3), and 439–450 (H11) that were not present for natural or synthetic agonists, suggesting a distinct ligand binding property for this selective modulator (Fig. 2d and Supplementary Fig. 1b, d column (iv)). Remote from VDR LBP, the hinge domain could be possibly stabilized either through direct binding with ligand itself or allosteric intra-molecular...
interactions with VDR LBD. Interestingly, DNA binding could also disrupt the hydrogen bonding activities of VDR hinge domain\(^9\)\(^,\)\(^28\). It is hence possible that ligand binding could influence DNA binding activity by directly impacting the conformation of DNA-interacting hinge domain. Furthermore, binding of all VDR ligands led to allosteric destabilization or increased solvent exchange at residues aa82–89 derived from VDR DBD, indicating that ligand binding could induce allosteric intramolecular crosstalk and potentially modulate DBD function (Fig. 2a–d and Supplementary Fig. 1b, d column (i), (ii), (iii), and (iv)).

**LBD and hinge are functionally integrated with the DBD.** Next, we introduced oligonucleotides representative of two hypothetical yet distinct VBSs to investigate how DNA binding events affect conformational dynamics of VDRRXRa heterodimer when it is bound with the natural agonist 1,25D\(_3\). One synthetic oligo (DR3) represents an idealized direct repeat DR3 VBS (5′-CGTAGGTCAATCAGGTCACGTCGT-3′) containing two consensus half-sites 5′-AGGTCA-3′ separated by three base pairs, whereas the other oligo (DR3 half-site) contains only one consensus half-site (5′-CTAGCTCCCCAGGTCAGGACCGCGCAGG-3′). Interaction of the VDRRXRa heterodimer with either of the two hypothetical VBSs resulted in protection to solvent exchange in DBD regions of both receptor and coreceptor (Fig. 3a, b and Supplementary Fig. 1b, d column (v) and (vi)). Specifically, VDR residues aa17–25 containing zinc-coordinating residue Cys24 exhibited a twofold protection with DR3 half-site compared to DR3 VBS, suggesting perhaps that interaction with DR3 half-site VBS requires an alternative conformation conferred at the zinc finger (Supplementary Fig. 1b column (v) and column (vi)). Interestingly, VDR hinge/CTE domain afforded a higher magnitude of protection to solvent exchange upon binding of DR3 when compared to binding to DR3 half-site VBS (Supplementary Fig. 1b column (v) and column (vi)). VDR hinge/CTE domain, which contains positively charged residues Arg102, Lys103, and Arg103, might form stronger electrostatic interactions with negatively charged phosphate backbone of the DR3 oligo compared with that of DR3 half-site oligo. CTE-DNA interactions have also been observed in PPAR\(_\gamma\)RXRa and Rev-Erb structures with their CTEs inserted into the minor groove of DNA\(^27\),\(^29\). Unlike VDR, RXRa DBD (aa137–158 and aa169–178) presented analogous HDX profiles upon binding to DR3 half-site and DR3 VBSs (Supplementary Fig. 1b column (v) and column (vi)).
Fig. 1d column (v) and column (vi)). Such stabilization of the heterodimer could be either derived from protein-DNA interaction or heteromerization between the co-receptor’s DBDs.

The most striking differences in HDX kinetics upon binding the different VBSs was within the VDR LBD. The binding of DR3 half-site VBS resulted in substantial protection to solvent exchange in regions constituting the AF2 surface (aa300–308 (H5), aa391–403 (H11), and aa411–419 (H12)), as well as other regions within the VDR LBD; e.g., aa136–150 (H1), aa206–218 (loop) and aa245–259 (H4) (Fig. 3b and Supplementary Fig. 1b, d column (vi)). Specifically, VDR residues aa109–133 residing on the C-terminus of hinge/CTE and N-terminus of helix 1 (H1) underwent partial cooperative unfolding event (EX1 kinetics as revealed by detection of two distinct deuterated ion distributions for the same peptide) upon heterodimer binding to DR3 half-site VBS (Supplementary Fig. 2d). For EX1 kinetics the refolding rate of the corresponding protein region is slower than the solvent exchange rate of amide hydrogens in the unfolded region, thus these amide hydrogens exchange simultaneously. This situation gives rise to a distinct MS signature, specifically bimodal mass distributions, with the lower mass envelop corresponding to molecules that have not yet exchanged (not yet unfolded) and the higher mass envelop corresponding to molecules that have undergone solvent exchange (molecules that have unfolded).

Under native state conditions, proteins exhibit EX2 kinetics wherein only a single MS envelop is observed over time (the refolding rate is faster than the rate of solvent exchange). However, the occurrence of EX1 cooperative unfolding behavior in native state proteins have been shown to provide important clues to intermediate conformational states of protein.

The occurrence of EX1 behavior indicates that the hinge/H1 region of VDR is structurally heterogeneous in solution when 1,25D3-heterodimer is bound to DR3 half-site VBS, perhaps a mixture of molecules in either an open or closed conformation. These two distinct conformations might differentially orient the LBD with respect to the DBD. Unlike that observed upon binding DR3 half-site VBS, little conformational change was observed in VDR LBD when bound to DR3 (Fig. 3a, b and Supplementary Fig. 1b, d column (v) and (vi)), and the hinge/H1 region of VDR adopted a homogeneous conformation as only EX2 kinetics were observed (Supplementary Fig. 2d). In the previously reported cryo-EM structure of VDRRXRα heterodimer bound to DR3 VBS, Pro122, which resides on the VDR hinge, was described as the "kink residue" between the C-terminal hinge helix and helix H1 of the LBD playing an important role in dictating the orientation of the LBD, resulting in an open LBD dimer architecture facing away from DBD. It is thus reasonable to propose that the DBD/hinge domains of VDR confer target VBS sequence specificity and selectively transmit allosteric signals to alter the dynamics of the LBD. In contrast, the LBD of RXRα assumed similar HDX kinetics upon binding to either of the two VBSs (Fig. 3a, b and Supplementary Fig. 1d column (v) and (vi)). The RXRα dimer interface, aa420–430 (H10), showed reduced HDX, suggesting that ligand and DNA binding sequentially stabilize the VDRRXRα heterodimer, and that VDR heterodimerization with RXRα likely enhances binding affinity for DNA.

Importantly, as shown in Supplementary Fig. 2e, the ligand-free heterodimer binds to these two different VBSs with similar affinity as determined using an electrophoretic mobility shift assay (EMSA). This observation further supports that the sequence of the VBS, and not its affinity for heterodimer, correlates with alterations of AF2 dynamics within VDR that impact intra-molecular signaling and target gene transactivation.

To further investigate whether compounds with distinct chemical structures induce unique conformations within the heterodimer upon DNA binding, parallel HDX experiments were conducted in the presence of synthetic agonists to probe conformational dynamics of VDRRXRα heterodimer with and without DR3 and DR3 half-site VBSs, respectively. The synthetic agonists, Cmpd1 and Cmpd2, induced a greater magnitude of protection to solvent exchange across the DBD and hinge domain of VDR upon binding to DR3 VBS resulting in larger ΔΔHDX.
between the two VBS binding datasets (ΔΔHDX = ΔHDX_heterodimer/agonist ± DR3 − ΔHDX_heterodimer/agonist ± DR3 half-site). For instance, Cmpd1 and Cmpd2 induced a 21 and 19% ΔΔHDX (p < 0.001) in the VDR hinge region (aa97–108) whereas only 4% ΔΔHDX was observed when bound with 1,25D3 (Fig. 3c and Supplementary Fig. 1b, column (vii), (viii), (ix), and (x)). However, the most striking difference was observed within a region of the VDR LBD that possessed insensitive to the VBS sequence (Fig. 3c–f). In this region, binding of synthetic agonists and DNA resulted in protection to solvent exchange in LBD regions spanning H1, loop, H3, H5, H9, H11, and H12 (Fig. 3c–f and Supplementary Fig. 1b column (vii), (viii), (ix), and (x)). This was a dramatic contrast to that observed for 1,25D3-bound heterodimer upon binding DR3 and DR3 half-site VBSs, which each induced unique HDX signatures within the VDR LBD. In addition, the VDR hinge/H1 peptide (aa109–133) underwent partial unfolding (as revealed by observing EX1 kinetics) when heterodimer was bound to synthetic agonists and DNA (Supplementary Fig. 2d). It is possible that the presence of synthetic agonists and DNA drive structural rearrangement in VDR hinge/H1 to affect hydrogen bonding networks within the LBD. Such a scenario may suggest that transduction of signal from the receptors’ DBD to its LBD could be regulated not only by specific VBS sequence but also by the specific ligand in the LBP. Therefore, at least for VDR, the DBD appears to be functionally integrated with LBD in response to the specific sequence of a VBS within a target gene promoter, as well as the exact chemical structure of the ligand.

Interaction with synthetic agonists and VBSs drove unique HDX perturbation patterns in regions involved in RXRα hinge domain as well as its AF2 surface. On the RXRα side of the heterodimer, reduced solvent exchange was observed within the DBD (aa137–158, aa169–178), the dimer interface (aa419–430), hinge–H11 (aa220–237) region, and the loop region between H8 and H9 domain (aa376–390), which is oriented within the AF2 region (Fig. 3c–f and Supplementary Fig. 1d column (vii), (viii), (ix), and (x)). Previously it has been shown that the hinge domain of NRs plays a critical role in maintaining the activity of NRs28. The presence of Cmpd2 and DNA afforded additional protection to solvent exchange in H12 AF2 helix (aa452–462) of RXRα (Fig. 3e, f and Supplementary Fig. 1d column (ix) and (x)). To further examine whether these distinct HDX signatures are specific to heterodimer but not the RXRα co-receptor, we performed HDX studies of RXRα alone in the presence and absence of ligand and DNA. The binding of Cmpd1 to RXRα alone preserved apo-like dynamics suggesting that there are no detectable conformational changes of RXRα upon ligand binding (Supplementary Fig. 1d column (xxvi)). Sequential interaction with DR3 VBS resulted in protection to solvent exchange only in RXRα DBD (Supplementary Fig. 1d column (xxvii)). The absence

![Fig. 3 Comparisons of DR3 and DR3 half-site binding effects on natural agonist- and synthetic-agonist- bound heterodimer complexes. Schematic representations illustrate differential experiments of VDRRXRα: ligand complex versus VDRRXRα:DNA complex (on the left). Consolidated differential HDX data are mapped onto the VDRRXRα heterodimer structure model in ribbon when the complex is bound to different ligands and DNA VBS (on the right). Conformational changes of 1,25D3-bound heterodimer upon binding of a DR3 (Supplementary Fig. 1b, d, column (vi)) and b DR3 half-site (Supplementary Fig. 1b, d, column (vi)). Conformational changes of Cmpd1 bound heterodimer upon binding of c DR3 (Supplementary Fig. 1b, d, column (vi)) and d DR3 half-site (Supplementary Fig. 1b, d, column (vi)). Conformational changes of Cmpd2 bound heterodimer upon binding of e DR3 (Supplementary Fig. 1b, d, column (vi)) and f DR3 half-site (Supplementary Fig. 1b, d, column (vi)). Percentages of deuterium differences are coded as Fig. 1. Dark gray, no statistically significant changes between compared conditions; light gray, regions that have no sequence coverage and include proline residue that has no amide hydrogen exchange activity; purple, 1,25D3 ligand; black, DNA VBSs.
of VDR abrogated those unique HDX perturbations in RXRα. Upon heterodimerization with VDR, the dynamic nature of RXRα in the synthetic agonist bound state differs from that of 1,25D3 upon interacting with DNA. Therefore, these results suggest that ligand and DNA could both coordinate both intra-molecular and inter-molecular allosteric communications within a NR heterodimer complex.

**Agonists stabilize AF2 of both receptors upon SRC1 binding.**

The AF2 region of NRs serves as the binding surface for recruitment of transcriptional co- regulatory proteins that contain an α-helical nuclear receptor binding motif referred to as an NR box. To test the hypothesis that the alterations in the stability of the heterodimer driven by ligand and DNA binding could modulate co-activator interaction, we sequentially probed the conformational ensembles of heterodimer complex in the presence and absence of the receptor interaction domain (RID) of the p160 NR co-activator steroid co-activator 1 (SRC1 RID). The RID of SRC1 contains three LXXLL NR box motifs. In the presence of 1,25D3, interaction with SRC1 RID resulted in ~21 and ~16% reduction in solvent exchange (p < 0.001) within H12 (aa411–419) of VDR when the 1,25D3-bound heterodimer was in the presence of DR3 and DR3 half-site VBSs, respectively (Fig. 4a, b and Supplementary Fig. 1b column (xi) and (xii)). In contrast to that observed on the VDR side of the 1,25D3-bound heterodimer, the RXRα AF2 surface was insensitive to the presence of the SRC1 RID, further suggesting ligand dependency for SRC1 recruitment to the heterodimer is associated with exclusively the VDR AF2 surface, and upon binding forms a compact protein complex when bound to either DR3 and DR3 half-site VBSs. These HDX data are consistent with observations made using SAXS. In this study, it was concluded that the NR heterodimers bind only one molecule of SRC1 RID and does so through the RXRα partner. Binding of SRC1 RID to either Cmpd1-heterodimer-DR3 or Cmpd1-heterodimer-DR3 half-site complex resulted in nearly indistinguishable HDX patterns within H12 (aa412–419) of VDR with ~11% decrease in solvent exchange (Fig. 4c, d and Supplementary Fig. 1b, column (xiii and (xiv)). Similar effects on HDX kinetics were observed for both Cmpd2-heterodimer-VBS complexes where interaction with SRC1 RID resulted in decreased exchange of ~20% for H12, ~9% for H3, and ~6% for H1 (Fig. 4e, f and Supplementary Fig. 1b, column (xv) and (xvi)). In contrast to that observed with 1,25D3, in addition to stabilization of VDR AF2, binding of SRC1 RID stabilized regions of RXRα AF2 (aa211–279 (H3), aa438–449 (H11–12), and aa439–450 (H11–12) (Fig. 4c–f and Supplementary Fig. 1d column (xiii), (xiv), (xv), and (xvi)). Previously, it has been shown that residues Phe277 (H3), Phe437 (H11), and Phe450 (H12) are important for formation of a stable AF2 surface facilitating co-activator binding to RXRα. In the absence of VDR, RXRα alone was not capable of binding to SRC1 RID (Supplementary Fig. 1d column (xviii)). Combined, these data suggest that synthetic agonist and DNA binding to the VDNRXRs heterodimer results in distinct VDR and RXR orientations, which facilitate co-activator interaction and that synthetic agonists, but not 1,25D3, modulate inter-allosteric communications within VDNRXRs heterodimer to fine tune transcriptional activity.

**Cmpd3 vs. 1,25D3 liganded VDNRXRs on BGLAP and TRPV6 VBSs.**

HDX was employed to probe the structural dynamics and activation mechanism of 1,25D3 and the non-calcemic modulator Cmpd3 liganded heterodimer bound to oligonucleotides representative of VBSs linked to the VDR target genes BGLAP and TRPV6. The sequences of the oligonucleotides used were derived from VBSs reported in BGLAP and TRPV6, each containing two direct repeats separated by a three base-pair gap, a motif of a family of closely related VBSs. For BGLAP two distinct oligos were used. The first was representative of a VBS located at 457 b upstream from the transcription start site of BGLAP from rattus (5′-CTAGGTGAAAGACCAT-3′), a VBS used in the previously reported SAXS study. A second oligo was representative of a VBS located at 510 b upstream of start site of human BGLAP gene (5′-GGTGACTCAGGGGGTGACGGGGCCA-3′). For TRPV6, an oligo representative of a VBS located 4.3 kb upstream from the transcription start site of human TRPV6 gene (5′-AAAGCGGGTGAAGGCTCAAAAAGGCA-3′) was used. Binding of 1,25D3 liganded heterodimer to TRPV6 VBS resulted in higher protection to solvent exchange in both the DBD and hinge domains of VDR as compared to that observed upon interaction with the BGLAP VBSs, suggesting that the TRPV6 VBS makes more interactions with these domains than does the BGLAP VBS (Fig. 5a, b and Supplementary Fig. 1b, column (xvii) and (xviii)). In contrast to that observed with 1,25D3, the heterodimer complex forms an elongated conformation when bound to BGLAP VBSs. We then analyzed the protein complex bound to the human BGLAP VBS and obtained similar results to that with the rat VBS (Supplementary Fig. 1b, column (xvii)). Similarly, binding of 1,25D3 liganded heterodimer to TRPV6 VBSs displayed minor alterations in the dynamics of the VDR LBD (Fig. 5b, i and Supplementary Fig. 1b, column (xviii)). Additionally, RXRα exhibited reduced deuterium exchange only in its DBD and dimer interface upon binding to BGLAP and TRPV6 VBSs (Fig. 5a, b and Supplementary Fig. 1b, column (xvii) and (xviii)). Combined, these results further support that in the presence of the natural ligand 1,25D3, the heterodimer forms an open conformation upon binding to either BGLAP or TRPV6 VBSs regardless of their specific nucleotide sequence.

HDX analysis of ligand binding of VDRM Cmpd3 already revealed that it processes lower potency to perturb VDR LBP dynamics compared to that of 1,25D3 (Fig. 2d and Supplementary Fig. 1b column (iv)). The presence of Cmpd3 and TRPV6 VBSs resulted in further stabilization of the DBD and hinge domains of VDR as compared to the presence of the BGLAP VBSs, as shown by higher extent of protection to solvent exchange within these regions (Fig. 5c, d and Supplementary Fig. 1b column (xix) and (xx)). Unlike the effects of 1,25D3, the binding of BGLAP and TRPV6 VBSs to the VDRM-bound heterodimer induced different degrees of protection to solvent exchange within the VDR LBD (Fig. 5c, d and Supplementary Fig. 1b column (xix) and (xx)). Nearly identical HDX profiles were observed for the protein-ligand complex bound to either the rat BGLAP VBS or the human BGLAP VBS (Supplementary Fig. 1b, column (xix*)). A further comparison of the HDX data revealed that a larger surface area of VDR LBD demonstrated increased protection to solvent exchange in the presence of the TRPV6 VBS when compared with the presence of the BGLAP VBSs (Fig. 5c, d and Supplementary Fig. 1b column (xix) and (xx)). Additionally, RXRα exhibited very similar protection to exchange in its DBD and dimer interface upon binding to either BGLAP or TRPV6 VBSs (Fig. 5c, d and Supplementary Fig. 1b column (xix) and (xx)). Also, no statistically significant inter-molecular communication was observed between the co-receptors. These data demonstrate that
Cmpd3, a non-calcemic modulator, perturbs the dynamics of the heterodimer in an alternative mechanism from the natural ligand 1,25D3 in the presence of the BGLAP and TRPV6 VBSs.

To test whether these observed differences in structural dynamics could influence receptors binding to SRC1, we performed sequential HDX studies to probe the heterodimer in the presence of RID of the co-activator. Addition of the SRC1 RID to the 1,25D3-heterodimer-BGLAP complex resulted in increased stabilization of the VDR AF2 surface, with minimal differences as compared to that observed for the SRC1 RID:1,25D3-heterodimer-TRPV6 complex. HDX analysis of both complexes detects similar decrease in solvent exchange within the Cmpd3-heterodimer-BGLAP VBS and Cmpd3-heterodimer-TRPV6 VBS revealed differential interaction of the SRC1 RID with the VDR AF2 surface. There was nearly a twofold higher magnitude of protection to solvent exchange in the former complex (~ 20%) when compared to the latter (~ 9%) (Fig. 5g, h, and j and Supplementary Fig. 1b column (xxiii) and (xxiv)). This difference in stabilization of VDR AF2 would suggest a difference in af

Fig. 4 Ligand dependency of SRC1 RID binding to the VDRRXRα heterodimer AF2 surface. Schematic representations illustrate differential experiments of VDRRXRα ligand: DNA complex verse VDRRXRα: ligand: SRC1 RID complex (on the left). Differential HDX data are mapped onto the surface of VDRRXRα heterodimer structure model in the presence and absence of SRC1 RID (on the right). a Conformational changes of VDRRXRα: 1,25D3: DR3 complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xi)). b Conformational changes of VDRRXRα: 1,25D3: DR3 half-site complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xii)). c Conformational changes of VDRRXRα: Cmpd1: DR3 complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xiii)). d Conformational changes of VDRRXRα: Cmpd1: DR3 half-site complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xiv)). e Conformational changes of VDRRXRα: Cmpd2: DR3 complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xv)). f Conformational changes of VDRRXRα: Cmpd2: DR3 half-site complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xvi)). Percentages of deuterium differences are coded as Fig. 1. Dark gray, no statistically significant changes between compared conditions; light gray, regions that have no sequence coverage and include proline residue that has no amide hydrogen exchange activity; black, DNA VBSs.
perturbs heterodimer dynamics in an alternative mechanism from 1,25D3 at BGLAP and TRPV6 VBSs.

**Discussion**

VDR controls expression of a gene program regulating calcium hemostasis. Despite its beneficial effects for treatment of osteoporosis, usage of the VDR agonist 1,25D3 is limited by dose-dependent hypercalcemia\(^2, 39, 40\). To improve the therapeutic index of VDR ligands, efforts have focused on the development of 1,25D3 analogs and synthetic non-secosteroid ligands that exhibit a lower threshold for hypercalcemia. Such dissociated VDR ligands, called VDRMs, are positive on BGLAP expression and neutral on TRPV6 expression. Understanding the structural mechanism that affords this dissociated profile would greatly facilitate drug development. To address this, we performed a detailed comparative biophysical study with 1,25D3, two agonists Cmpds1–2, and a non-calcemic VDRM Cmpd3. These molecules have distinct chemical structures and are distinguished based on their hypercalcemia liability profile in a TRPV6 gene activation assay. To understand the conformational dynamics induced by these ligands upon binding VDR, differential HDX studies were performed on the VDRRXR heterodimer in the presence and absence of VBSs.

**Fig. 5** Activations of VDRRXR heterodimer with cognate VBS BGLAP and TRPV6 by selective modulator Cmpd3 versus natural ligand 1,25D3. Schematic representations illustrate differential experiments of ligand bound VDRRXR heterodimer with respect to DNA and SRC1 RID binding (on the left). Differential consolidation HDX data are mapped onto the VDRRXR heterodimer structure model when the complex is bound to different ligands and DNA (shown in ribbon a–d), and SRC1 RID (shown in surface e–h) (on the right). Conformational changes of 1,25D3-bound heterodimer upon binding of a BGLAP VBS (Supplementary Fig. 1b, d, column (xviii)) and b TRPV6 VBS (Supplementary Fig. 1b, d, column (xvii)). Conformational changes of selective modulator Cmpd3 bound heterodimer upon binding of c BGLAP VBS (Supplementary Fig. 1b, d, column (xxi)) and d TRPV6 VBS (Supplementary Fig. 1b, d, column (xxi)). Conformational changes of VDRRXR: 1,25D3: BGLAP complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xii)). Conformational changes of VDRRXR: 1,25D3: TRPV6 complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xxii)). Conformational changes of VDRRXR: Cmpd3: BGLAP complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xxii)). Conformational changes of VDRRXR: Cmpd3: TRPV6 complex upon SRC1 RID binding (Supplementary Fig. 1b, d, column (xxi)). Percentages of deuterium differences are coded as Fig. 1. Dark gray, no statistically significant changes between compared conditions; light gray, regions that have no sequence coverage and include proline residue that has no amide hydrogen exchange activity; black, DNA VBSs; purple, 1,25D3 ligand; pink, Cmpd3. Differential deuterium uptake plots of peptide aa411–419 from VDR H12 when heterodimer is bound to 1,25D3, DNA and SRC1 RID. j Differential deuterium uptake plots of peptide aa411–419 from VDR H12 when heterodimer is bound to Cmpd3, DNA, and SRC1 RID. The data are plotted as percent deuterium uptake versus time on a logarithmic scale.
absence of these ligands, VBSs including DR3, DR3 half-site, BGLAP and TRPV6, and the RID of the co-activator protein SRC1. These studies provide snapshots of distinct conformational ensembles of VDRRXRα heterodimer in solution and allow multiple comparisons of sequential binding events (e.g., ligand, DNA, and co-activator protein) to probe alterations in the dynamics of the heterodimer to drive molecular interactions with co-regulators. While these HDX data do not provide specific orientations of each structural element within the heterodimer complex, they yield unique insight into local and global conformational fluctuations within the protein complex that could not be inferred from static structures.

Differential HDX analysis reveals unique intra-molecular and inter-molecular communications between heterodimer LBDs and DBDs in response to various VDR ligands and DNA VBSs. For instance, ligand binding to the LBD modulates dynamics of the remote zinc fingers within the DBD and DNA binding in turn enables allosteric perturbations impacting LBD dynamics. Although it is not known how the receptor hinge domain interacts with the DBD and LBD, the HDX results support a structural model where the hinge plays a critical role in determining the structural arrangement of co-receptor’s LBDs to DBDs. Various combinations of ligands and DNA VBSs stabilize the hinge of VDR to different extents, suggesting that it adopts unique conformations depending on the ligand and the specific sequence of the VBS. In the presence of 1,25D3, binding of DR3 half-site but not DR3 VBS alters receptor dynamics in the remote LBD. This contrasts with what is observed with synthetic agonist action wherein the binding of either DR3 or DR3 half-site VBSs drive analogous perturbations in LBD of VDR. Interestingly, a region spanning the hinge and H1 of the VDR LBD undergoes partial cooperative unfolding (E2X1 kinetics) correlating with allosteric stabilizations observed in LBD suggesting the VDR hinge and H1 regions possess inherent flexibility enabling signals to be transmitted from the DBD to the LBD.

Interestingly, the presence of synthetic agonist and DNA drive long-range stabilization of hinge-H1 and H8–H9 loop region (part of the RXRα AF2 surface) in RXRα. Despite exhibiting

![Fig. 6](image-url)
relatively modest stabilization, the altered flexibility in these regions of RXRα may result in a unique orientation of the RXRα LBD relative to its dimer partner. This in turn would sequentially impact the positioning of co-activator upon interaction with the heterodimer perhaps impacting co-activator to DNA distance. In the context of the heterodimer, SRC1 RID binding drives stabilization of the AF2 surface in both VDR and RXRα. In contrast, binding of ligand and DNA to RXRα homodimer abrogates the perturbation dynamics of the RXRα LBD. This observation would suggest a potential role for VDR in mediating inter-receptor crosstalk within the heterodimer. Inter-molecular interactions would provide a direct mechanism for transducing information between the co-receptors VDR to RXRα.

As shown in Fig. 6, the results presented here suggest two distinct activation modes mediated by non-steroidal synthetic agonists and a VDRM (Cmpd3) as compared to that observed with the natural ligand 1,25D3. The binding of VDRM to heterodimer leads to an unique stabilization of the VDR hinge that is not detected upon binding natural ligand or synthetic agonists, suggesting that ligand itself could have a direct effect on DNA binding. VDRM binding afforded larger protection to solvent exchange within the LBP of VDR as compared to that observed upon binding 1,25D3. These differences in LBP dynamics that are initiated by ligand-binding properties likely give rise to the differences in DNA recognition properties and potency for SRC1 interaction observed for Cmpd3 when compared to 1,25D3. Binding of either BGLAP or TRPV6 VBS to 1,25D3-bound heterodimer fails to perturb the dynamics of the LBDs and results in an open AF2 conformation susceptible for co-activator binding. Whereas binding of TRPV6 but not BGLAP VBS to Cmpd3 liganded heterodimer specifically alters the stability of AF2 in VDR blunting the receptor’s ability to interact with SRC1. It is important to note that the BGLAP and TRPV6 VBS used in these studies are putative VDR response elements (VDRs) yet to be validated as bona fide VDRs in vivo. Taken together, these results suggest that the conformational flexibility of VDRRXRα heterodimer allows the core complex to adopt different conformations depending on the ligand bound, specific sequence of the VBS and co-regulatory protein binding, providing a common mechanism for ligand-dependency of NR activation on various target genes. The model presented may provide a framework for the design of improved VDRMs for the treatment of osteoporosis.

Methods
Reagents. Full-length WT His-hVDR (Δ151–190) and WT Flag-h RXRα were expressed in a baculovirus system and purified by Ni-NTASec or Flag-SEC, respectively. His-hSRC1 R1D (627–786) variant 1 (NM_003743) was expressed in E. coli (BL21 DE3) and purified using His-Trap (GE Healthcare). The final protein buffer was 50 mM NaCl, 10% glycerol and 2 mM DTT. The purity of each protein was >95% and was verified using SDS-PAGE, western blot and MALDI MS. The DR3 VBS 5′-CGTGGATCATGGCAGCGGGTCGTG-3′, DR3 half-site VBS 5′-CGTCGGATCATGGCAGCGGGTCG-3′, BGLAP VBS 5′-CGTCGGATCATGGCAGCGGGTCG-3′, and BGLAP VBS 5′-CGTGGATCATGGCAGCGGGTCG-3′ were selected for HDX-MS analysis: 10 μM of the apo protein was mixed with 1:10 molar excess of ligand and incubated for 2 h at 4°C for complex formation before subjecting them to HDX-MS analysis. For the differential HDX experiments, 5 μl of either the apo- or the liganded protein complex with DNA and SRC1 RID were mixed with 20 μl buffer containing HDX buffer, Tris-HCl (pH 8.0; 50 mM, 10% glycerol and 2 mM DTT). The mixture was incubated at 4°C for 2 h, 10, 30, 60, 300, 900 s, or 3,600 s. Following on-exchange, unwanted forward-exchange or back-exchange was minimized and the protein was denatured by the addition of 25 μl of a quencher solution (1% v/v TFA in 3 M urea and 50 mM TCEP). Samples were then immediately passed through an immobilized pep column (prepared in-house) at 50 μl/min -1 (0.1% v/v TFA, 15°C) and the resulting peptides were trapped and desalted on a 1.0 mm x 10 mm C18 trap column (Hypersil Gold, Thermo Fisher, Grand Island, NY) for 6 min. Sample handling and peptide separation were conducted at 4°C. The eluted peptides were then subjected to electrospray ionization directly coupled to a high resolution Orbitrap mass spectrometer (LTQ Orbitrap XL with ETD, Q Exactive, or Exactive, Thermo Fisher Scientific, San Jose, CA). Each HDX experiment was carried out in triplicate with an overlap of 10% of the peptide set. The HDX data is determined by an unpaired student’s t test (unpaired two-tailed t test at 4°C) and for fragment ions ±0.6 Da. Oxidation to methionine was selected for variable modification. Pepsin was used for digestion and no specific enzyme was selected in the Mascot search. The Mascot search was also performed against a decoy (reverse) sequence and false positives were ruled out if they did not pass a 1% false discovery rate. The MS/MS spectra of all the peptide ions from the Mascot search were further manually inspected and only the unique charged ions with the highest Mascot score were included in HDX product set.

HDX-MS analysis: 10 μM of the apo protein was mixed with 1:10 molar excess of ligand and incubated for 2 h at 4°C for complex formation before subjecting them to HDX-MS analysis.

Molecular docking. All docking simulations were carried out in ICM Pro (Molsoft) using the Docking module. The crystal structures of the VDR LBD (5V39) with waters and ligands removed was used as the receptor. The ICM Pro software implements an algorithm of a biased probability Monte Carlo (BPMC) procedure (PMID: 8289329). Cmpd 1–2 were docked and the lowest energy poses were used for interpretation. Figures were generated in PyMol (Schrödinger).

HDX-MS. Solution-phase amide HDX experiments were carried out with a fully automated system (CCT HTS PAL, LEAP Technologies, Carrboro, NC; housed inside a 4°C cabinet) as follows.

Peptide Identification: Peptides were identified using tandem MS (MS/MS) experiments performed with either a LTQ Orbitrap XL with ETD or a Q Exactive (Thermo Fisher Scientific, San Jose, CA) over a 70-min gradient. Product ion spectra were acquired in a data-dependent mode and the five most abundant ions were selected for the product ion analysis per scan event. The MS/MS *.raw data files were converted to .mgf files and then submitted to Mascot (version 2.3 Matrix Science, London, UK) for peptide identification. The maximum number of missed cleavages was set at 4 with the mass tolerance for precursor ions ±0.6 Da and for fragment ions ± 8 p.p.m. Oxidation to methionine was selected for variable modification. Pepsin was used for digestion and no specific enzyme was selected in the Mascot search during the search. Peptides included in the peptide set used for HDX detection had a Mascot score of 20 or greater. The MS/MS Mascot search was also performed against a decoy (reverse) sequence and false positives were ruled out if they did not pass a 1% false discovery rate. The MS/MS spectra of all the peptide ions from the Mascot search were further manually inspected and only the unique charged ions with the highest Mascot score were included in HDX product set.

HDX-MS analysis: 10 μM of the apo protein was mixed with 1:10 molar excess of ligand and incubated for 2 h at 4°C for complex formation before subjecting them to HDX-MS analysis. For the differential HDX experiments, 5 μl of either the apo- or the liganded protein complex with DNA and SRC1 RID were mixed with 20 μl buffer containing HDX buffer, Tris-HCl (pH 8.0; 50 mM, 10% glycerol and 2 mM DTT). The mixture was incubated at 4°C for 0 s, 10, 30, 60, 300, 900 s, or 3,600 s. Following on-exchange, unwanted forward-exchange or back-exchange was minimized and the protein was denatured by the addition of 25 μl of a quencher solution (1% v/v TFA in 3 M urea and 50 mM TCEP). Samples were then immediately passed through an immobilized pep column (prepared in-house) at 50 μl/min -1 (0.1% v/v TFA, 15°C) and the resulting peptides were trapped and desalted on a 1.0 mm x 10 mm C18 trap column (Hypersil Gold, Thermo Fisher, Grand Island, NY) for 6 min. Sample handling and peptide separation were conducted at 4°C. The eluted peptides were then subjected to electrospray ionization directly coupled to a high resolution Orbitrap mass spectrometer (LTQ Orbitrap XL with ETD, Q Exactive, or Exactive, Thermo Fisher Scientific, San Jose, CA). Each HDX experiment was carried out in triplicate with an overlap of 10% of the peptide set. The HDX data is determined by an unpaired student’s t test (unpaired two-tailed t test at 4°C) and for fragment ions ±0.6 Da. Oxidation to methionine was selected for variable modification. Pepsin was used for digestion and no specific enzyme was selected in the Mascot search during the search. Peptides included in the peptide set used for HDX detection had a Mascot score of 20 or greater. The MS/MS Mascot search was also performed against a decoy (reverse) sequence and false positives were ruled out if they did not pass a 1% false discovery rate. The MS/MS spectra of all the peptide ions from the Mascot search were further manually inspected and only the unique charged ions with the highest Mascot score were included in HDX product set. Data Rendering: the HDX data from all overlapping peptides were consolidated to individual amino acid values using a residue averaging approach. Briefly, for each residue, the deuterium incorporation values and peptide lengths from all overlapping peptides were assembled. A weighting function was applied in which shorter peptide sequences were weighted less and longer sequences were weighted less. Each of the weighted deuterium incorporation values were then averaged incorporating this weighting function to produce a single value for each amino acid.
acid. The initial two residues of each peptide, as well as prolines, were omitted from the calculations. This approach is similar to that previously described.

Data Statistics: Deuterium uptake for each peptide is calculated as the average of % D for all on-exchange time points and the difference in average %D values between the apo and ligand bound samples is presented as a heat map with a color code given at the bottom of the figure (warm colors for deprotection and cool colors for protection). Peptides are colored by the software automatically to display significant differences, determined either by a > 5% difference (less or more protection) in average deuterium uptake between the two states, or by using the results of unpaired t-tests at each time point (p-value < 0.05 for any two time points or a p-value < 0.01 for any single time point). Peptides with non-significant change between the two states are colored gray. The exchange at the first two residues for any given peptide is not colored. Each peptide bar in the heat map view displays the average Δ %D values, associated standard deviation, and the charge state. Additionally, overlapping peptides with a similar protection trend covering the same region are used to rule out data ambiguity.

Homology modeling. A structural model of the VDR (aa 18–455)/RXRα (aa 135–462) heterodimer bound to the DR3 VBS DNA fragment was created using Molsfit ICM Pro software. Amino Acids 201–223 of RXRα and 166–216 of VDR could not be modeled due to the lack of available homology models containing these amino acid residues. The heterodimer was created in the “open” conformation and a partial cryo-EM model (generous gift from Professor Bruno Klaholz and Dino Moras) was used to guide the creation of the model. Loops that could not be modeled from the cryo-EM data were added (mostly in the hinge regions). Proteins and hydrophobic tags were added to all amino acids and both orientations. The added loops were subject to loop modeling to improve clashing using the ICM-Pro loop modeling utility. The final structure was subjected to minimization, regularization, and annealing in ICM Pro.

BGLAP and TRPV6 activation assays. Human osteosarcoma cells MG-63, (CRL-1427, American Type Culture Collection (ATCC), Manassas, VA), were maintained in EMEM (ATCC TCC-30-2003) + 10% FBS in 5% CO₂. For bone gamma-carboxyglutamate protein (BGLAP) expression analysis MG-63 cells, suspended in EMEM (ATCC TCC-30-2003) + 5% charcoal dextran treated FBS, were seeded onto 96-well tissue culture treated plates at 25,000 cells per well. After an overnight incubation the medium was removed and serial dilutions of VDRM concentrations ranging from 10⁻⁰μM to 2nm in EMEM (ATCC TCC-30-2003) + 5% charcoal dextran treated FBS were added. Following a 24 h incubation the medium was removed and the plates were sealed and stored at -80 °C for later processing. Quantigen 2.0 Assay Kit (Affymetrix QS0009) was used to quantitate BGLAP and the Housekeeping gene GUSB mRNA levels. Following the kit instructions, lysis buffer was added to the frozen cells and incubated briefly at 55 °C. A volume of 20μl of BGLAP probeset (Affymetrix SA-10875-01) or 18 S (Affymetrix SA-10026) in control solutions was added to all samples. The added loops were subject to loop modeling to improve clashing using the ICM-Pro loop modeling utility. The final structure was subjected to minimization, regularization, and annealing in ICM Pro.

References

1. Fleet, J. C. & Schoch, R. D. Molecular mechanisms for regulation of intestinal calcium absorption by vitamin D and other factors. Crit. Rev. Clin. Lab. Sci. 47, 181–195 (2010).
2. Christakos, S., Dhawan, P., Verstuyf, A., Verlinden, L. & Carmeliet, G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiol. Rev. 96, 365–408 (2016).
3. Bocelet, P. & Chatterjee, A. Antiproliferative role of vitamin D and its analogs—a brief overview. Mol. Cell Biochem. 253, 247–254 (2003).
4. van de Peppel, J. & van Leeuwen, J. P. Vitamin D and gene networks in human osteoblasts. Front. Physiol. 5, 137 (2014).
5. Burris, T. P. et al. Nuclear receptors and their selective pharmacologic modulators. Pharmacol. Rev. 65, 710–778 (2013).
6. Manola, M. R., Zella, L. A., Nerent, R. D. & Pike, J. W. Characterizing early events associated with the activation of target genes by 1,25-dihydroxyvitamin D3 in mouse kidney and intestine in vivo. J. Biol. Chem. 282, 22344–22352 (2007).
7. Hoenderop, J. G. et al. Molecular identification of the apiac Ca²⁺ channel in 1, 25-dihydroxyvitamin D₃-responsive epithelium. J. Biol. Chem. 274, 8375–8378 (1999).
8. Orlov, I., Rochel, N., Moras, D. & Klaholz, B. Structure of the full human VDR/RXR nuclear receptor heterodimer complex with its DR3 target DNA. EMBO J. 31, 291–300 (2012).
9. Zhang, J. et al. DNA binding alters coactivator interaction surfaces of the intact VDR/RXR complex. Nat. Struct. Mol. Biol. 18, 556–563 (2011).
10. Wang, L. Y., Zhang, Y. Q., Chen, M. D., Liu, C. B. & Wu, J. P. Relationship of structure and function of DNA-binding domain in vitamin D receptor. Molecules 20, 12389–12399 (2015).
11. Kucharova, S. & Farkas, R. Hormone nuclear receptors and their ligands: role in programmed cell death (review). Endocr. Regul. 36, 37–60 (2002).
12. Rochel, N., Wurtz, J. M., Mitschler, A., Klaholz, B. & Moras, D. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. Mol. Cell 5, 173–179 (2000).
13. Herrey, D. M., Kalkoven, E., Hoare, S. & Parker, M. G. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387, 733–736 (1997).
14. Shaffer, P. L. & Gewirth, D. T. Structural basis of VDR-DNA interactions on direct repeat response elements. EMBO J. 21, 2242–2252 (2002).
15. Molnar, F. Structural considerations of vitamin D signaling. Front. Physiol. 5, 191 (2014).
16. Burris, T. P. et al. Nuclear receptors and their selective pharmacological modulators. Pharmacol. Rev. 65, 710–778 (2013).
17. Shaffer, P. L. & Gewirth, D. T. Structural analysis of RXR-VDR interactions on DR3 DNA. J. Steroid Biochem. Mol. Biol. 89–90, 215–219 (2004).
18. Rastinejad, F., Perlmann, T., Evans, R. M. & Sigler, P. B. Structural determinants of nuclear receptor assembly on DNA direct repeats. Nature 375, 203–211 (1995).
19. Meijering, S. H. et al. DNA binding site sequence directs glucocorticoid receptor structure and activity. Science 324, 407–410 (2009).
20. Lefstin, J. A. & Yamamoto, K. R. Allosteric effects of DNA on transcriptional regulators. Nature 392, 885–888 (1998).
21. Chalmers, M. J. et al. Probing protein ligand interactions by automated hydrogen/deuterium exchange mass spectrometry. Anal. Chem. 78, 1005–1014 (2006).
22. Dessing, J. et al. High-resolution HDX-MS reveals distinct mechanisms of RNA recognition and activation by RIG-I and MDA5. Nucleic Acids Res. 43, 1216–1230 (2015).
23. Chalmers, M. J., Busby, S. A., Pascal, B. D., West, G. M. & Griffin, P. R. Differential hydrogen/deuterium exchange mass spectrometry analysis of protein-ligand interactions. Expert Rev. Proteomics. 8, 43–59 (2011).
24. Bruning, J. B. et al. Partial agonists activate PPARgamma using a helix 12 modulator. Pharmacol. Rev. 66, 219–255 (2014).
25. Musille, P. M. et al. Antidiabetic phospholipid-nuclear receptor complex reveals the mechanism for phospholipid-driven gene regulation. Nat. Struct. Mol. Biol. 19, 532–537, S1–2 (2012).
26. Keppel, T. R. & Weiss, D. D. Mapping residual structure in intrinsically disordered proteins at residue resolution using millisecond hydrogen/deuterium exchange and residue averaging. J. Am. Soc. Mass Spectrom. 26, 547–554 (2015).
27. Chandra, V. et al. Structure of the intact PPAR-gamma-RXR- nuclear receptor complex on DNA. Nature 456, 350–356 (2008).
28. Miyamoto, T. et al. The role of hinge domain in heterodimerization and specific DNA recognition by nuclear receptors. Mol. Cell Endocrinol. 181, 229–238 (2001).
29. Zhao, Q., Khorasanizadeh, S., Miyoshi, Y., Lazar, M. A. & Rastinejad, F. Structural elements of an orphan nuclear receptor-DNA complex. Mol. Cell 1, 849–861 (1998).
30. Ferraro, D. M., Lazo, N. & Robertson, A. D. EX1 hydrogen exchange and protein folding. Biochemistry 43, 587–594 (2004).
31. Fang, J., Engen, J. R. & Beuning, P. J. Escherichia coli processivity clamp beta from DNA polymerase III is dynamic in solution. Biochemistry 50, 5958–5968 (2011).
32. Yang, B., Stjepanovic, G., Shen, Q., Martin, A. & Hurley, J. H. Vps4 disassembles an ESCRT-III filament by global unfolding and processive translocation. Nat. Struct. Mol. Biol. 22, 492–498 (2015).
33. Trelle, M. B., Madsen, J. B., Andreasen, P. A. & Jorgensen, T. J. Local transient unfolding of native state P4-1 associated with serpin metastability. Angew. Chem. Int. Ed. Engl. 53, 9751–9754 (2014).
34. Shaffer, P. L., McDonnell, D. P. & Gewirth, D. T. Characterization of transcriptional activation and DNA-binding functions in the hinge region of the vitamin D receptor. Biochemistry 44, 2678–2685 (2005).
35. Goswami, D. et al. Time window expansion for HDX analysis of an intrinsically disordered protein. J. Am. Soc. Mass Spectrom. 24, 1584–1592 (2013).
36. Rochel, N. et al. Common architecture of nuclear receptor heterodimers on DNA direct repeat elements with different spacings. Nat. Struct. Mol. Biol. 18, 564–570 (2011).
37. Meyer, M. B., Watanuki, M., Kim, S., Shevde, N. K. & Pike, J. W. The human transient receptor potential vanilloid type 6 distal promoter contains multiple vitamin D receptor binding sites that mediate activation by 1,25-dihydroxyvitamin D3 in intestinal cells. Mol. Endocrinol. 20, 1447–1461 (2006).
38. Ozono, K., Liao, J., Kerner, S. A., Scott, R. A. & Pike, J. W. The vitamin D-responsive element in the human osteocalcin gene. Association with a nuclear proto-oncogene enhancer. J. Biol. Chem. 265, 21881–21888 (1990).
39. Nagpal, S., Na, S. & Rathnachalam, R. Noncanonical actions of vitamin D receptor ligands. Endocr. Rev. 26, 662–687 (2005).
40. Liao, J., Ozono, K., Sone, T., McDonnell, D. P. & Pike, J. W. Vitamin D receptor interaction with specific DNA requires a nuclear protein and 1,25-dihydroxyvitamin D3. Proc. Natl Acad. Sci. USA 87, 9751–9755 (1990).
41. Murshudov, G. N., Vagin, A. A. & Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. Acta. Crystallogr. D Biol. Crystallogr. 53, 240–255 (1997).
42. Bricogne, G. Direct phase determination by entropy maximization and likelihood ranking: status report and perspectives. Acta. Crystallogr. D Biol. Crystallogr. 49, 37–60 (1993).
43. Emsley, P., Lohkamp, R., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta. Crystallogr. D Biol. Crystallogr. 66, 486–501 (2010).
44. Zhang, Z. & Smith, D. L. Determination of amide hydrogen exchange by mass spectrometry: a new tool for protein structure elucidation. Protein Sci. 2, 522–531 (1993).
45. Pascal, B. D. et al. HDX workbench: software for the analysis of H/D exchange MS data. J. Am. Soc. Mass Spectrom. 23, 1512–1521 (2012).
46. Keppel, T. R. & Weiss, D. D. Mapping residual structure in intrinsically disordered proteins at residue resolution using millisecond hydrogen/deuterium exchange and residue averaging. J. Am. Soc. Mass Spectrom. 26, 547–554 (2015).

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Author contributions
J.Z., R.E.S., M.I.C., J.A.D., and P.R.G. conceived of the project and designed the research. J.Z., M.R.C., R.E.S., Y.W., S.J.N., R.D.G.-O. conducted the research. J.Z., M.R.C., R.E.S., M.J.C., J.A.D., and P.R.G. wrote the paper with contributions from all authors.

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