



Structural considerations of vitamin D signaling

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Crystal structures represent the static picture in the life of a molecule giving a sneak preview what it might be in reality. Hence, it is very hard to extrapolate from these photos toward dynamic processes such as transcriptional regulation. Mechanistically VDR may be considered as molecular machine able to perform ligand-, DNA- and protein recognition, and interaction in a multi-task manner. Taking this into account the functional net effect will be the combination of all these processes. The long awaited answer to explain the differences in physiological effects for various ligands was one of the biggest disappointment that crystal structures provided since no substantial distinction could be made for the conformation of the active VDR-ligand complexes. This may have come from the limitation on the complexity of the available ligand-VDR structures. The recent studies with full length VDR-RXR α showed somewhat more comprehensive perspective for the 3D organization and possible function of the VDR-RXR α -cofactor complex. In addition to *in vitro* approaches, also computational tools had been introduced with the aim to get understanding on the mechanic and dynamic properties of the VDR complexes with some success. Using these methods and based on measurable descriptors such as pocket size and positions of side chains it is possible to note subtle differences between the structures. The meaning of these differences has not been fully understood yet but the possibility of a “butterfly effect” may have more extreme consequences in terms of VDR signaling. In this review, the three functional aspects (ligand-, DNA- and protein recognition, and binding) will be discussed with respect to available data as well as possible implication and questions that may be important to address in the future.

Keywords: VDR, crystal structure, molecular dynamics, molecular mechanism, cofactors, response elements, ligand-binding

INTRODUCTION

One way of understanding life at molecular level is to obtain the three-dimensional (3D) structures of the molecules. Such structural views represent a static picture in the life of a molecule giving a sneak preview what it might be in reality. For the understanding of the functional implication of vitamin D (VD) signaling it is also important to look at various structural complexes of the vitamin D receptor (VDR), which may outline its possible dynamics and mechanics. VDR is able to perform the ligand-, DNA- and protein recognition, and interaction in a multi-task manner thus can be viewed as molecular machine which will regulate gene expression with the combination/sum of all these particular functions.

Before the year 2000 people in the VD field were only guessing how may the 3D structure of VDR look like. Some implications

were coming from already known crystal structures e.g., receptors for retinoids such as retinoid X receptor (RXR) (Bourguet et al., 1995) and retinoic acid receptor (RAR) (Renaud et al., 1995). Not until exactly 14 years ago the structure of VDR-1 α ,25-dihydroxyvitamin D₃ (1,25D₃) complex has been solved (Rochel et al., 2000) and a long journey started in understanding the binding of various VDR analogs and the structure-based analog design. Within the first 5 years more structures have been solved (Tocchini-Valentini et al., 2001, 2004; Eelen et al., 2005) but the long awaited answer to explain the differences in physiological effects for various ligands was one of the biggest disappointment that crystal structures provided since no substantial distinction could be made for the conformation of the active VDR-ligand complexes.

Compared to the beginning of the last decade a huge number of X-ray crystal structures are available for VDR. In detail, there are VDR LBDs from three different species *H. sapiens* (34), *R. norvegicus* (40) and *D. rerio* (13) and four DBD-DNA complexes from *H. sapiens*. The basic information about these complexes is summarized in **Table 1**. This data makes also possible to analyze orthologous molecules with reflection to functional and structural differences. However, to understand this aspect well it would be beneficial to have more data from numerous organisms. In this review evolutionary aspects and species-specific difference will be not discussed in depth. More space will be given to

Abbreviations: 1,25D₃, 1 α , 25-dihydroxyvitamin D₃; 3D, three-dimensional; 3Klca, 3-keto lithocholic acid; 9-*cis* RA, 9-*cis* retinoic acid; aa, amino acids; cryo-EM, cryo-electron microscope; *Cyp27b1*, 25-hydroxyvitamin D₃ 1 α -hydroxylase; *D. rerio*, Danio rerio; DBD, DNA-binding domain; DNA, deoxyribonucleic acid; DR, direct repeat; DRIP, vitamin-D-receptor interacting protein; h, human; *H. sapiens*, *Homo sapiens*; HDX, H/D exchange; LBD, ligand-binding domain; LBP, ligand-binding pocket; LCA, lithocholic acid; MD, molecular dynamics; OH, hydroxyl; *P. marinus*, *Petromyzon marinus*; PXR, pregnane X receptor; RAR, Retinoic acid receptor; r, rat; *R. norvegicus*, *Rattus norvegicus*; RE, response element; RID, receptor interaction domain; RXR, retinoid X receptor; SAXS, short angle X-ray scattering; TR, thyroid hormone receptor; VD, vitamin D; VDR, vitamin D receptor; VDRE, VD response element; z, zebrafish.

Table 1 | List of crystal structure data available for LBD and DBD of VDR (source: www.pdb.org).

Ortholog	PDBID ^a	References	Ligand name	Notes	PDBID ^a	References	Ligand name	Notes
H. sapiens LBDs (34)	1DB1	Rochel et al., 2000	1,25D ₃		3CS6	Hourai et al., 2008	AMCR277B	
	1IE8	Tocchini-Valentini et al., 2001	KH1060		3M7R	Rochel et al., 2010	1,25D ₃	H305Q mutant
	1IE9		MC1288		3B0T	b	Maxacalcitol	
	1S0Z	Tocchini-Valentini et al., 2004	EB1089		3OGT	b	(1S,3R,5Z,7E,14beta,17alpha,20S)-20-[5-(1-hydroxy-1-methylethyl)furan-2-yl]-9,10-secopregna-5,7,10-triene-1,3-diol	
	1S19		MC903		3P8X	Verlinden et al., 2011	20(17→18)-abeo-1α,25-dihydroxy-22-homo-21-norvitamin D ₃	
	1TXI	Eelen et al., 2005	TX522		3AUQ	Sawada et al., 2011	14-epi-2α-methyl-19-norvitamin D ₃	
	2HAM	Hourai et al., 2006		2α-propyl-1,25D ₃	3AUR		14-epi-2β-methyl-19-norvitamin D ₃	
	2HAR			2α-(3-hydroxy-1-propoxy)-1,25D ₃	3AX8	Shindo et al., 2011	15α-methoxy-1,25D ₃	
	2HAS			2α-(1-propoxy)-1,25D ₃	3KPZ	Rochel and Moras, 2012	ZK203278	
	2HB7			2α(3-hydroxy-1-propyl)-1,25D ₃	3AZ1	Kashiwagi et al., 2011	(4-[3-(4-[(2R)-2-hydroxy-3,3-dimethylbutyl]oxy)-3-methylphenyl]pentan-3-yl)-2-methylphenoxy)acetic acid	
	2HB8			2α-methyl-1,25D ₃	3AZ2		5-(4-[3-(4-[(2R)-2-hydroxy-3,3-dimethylbutyl]oxy)-3-methylphenyl]pentan-3-yl)-2-methylphenoxy)pentanoic acid	

(Continued)

Table 1 | Continued

Ortholog	PDBID ^a	References	Ligand name	Notes	PDBID ^a	References	Ligand name	Notes
3A2I	3A2I	Kakuda et al., 2010	TEI-9647 hVDR_H305F		3AZ3		(4S)-4-hydroxy-5-[4-(3-(4-(3S)-3-hydroxy-4,4-dimethylpentyl)-3-methylphenyl)pentan-3-yl]-2-methylphenoxy]pentanoic acid	
3A2J	3A2J		TEI-9647 hVDR_H305F/H397F		3TKC	Fraga et al., 2012	(1S,3R,5Z,7E,14beta,17alpha,20S)-20-[5-(1-hydroxy-1-methylethyl)furan-2-yl]-9,10-secopregnane-5,7,10-triene-1,3-diol	
3A3Z	3A3Z	Antony et al., 2010	2 α -methyl-AMCR277A(C23S)		3VHW	Saito et al., 2013	4MP	
3A40	3A40		2 α -methyl-AMCR277B(C23F)		3W0A	b	(4S)-4-hydroxy-5-[2-methyl-4-(3-(3-methyl-4-(4,4-trifluoro-3-hydroxy-3-(trifluoromethyl)but-1-yn-1-yl)phenyl)pentan-3-yl)phenoxy]pentanoic acid	
3A78	3A78	Molnár et al., 2011	1,25-3-epi-D ₃		3W0C		(4S)-4-hydroxy-5-[2-methyl-4-(3-(3-methyl-4-[(1E)-4,4-trifluoro-3-hydroxy-3-(trifluoromethyl)but-1-en-1-yl]phenyl)pentan-3-yl)phenoxy]pentanoic acid	
3CS4	3CS4	Hourai et al., 2008	AMCR277A		3W0Y		[3-fluoro-2'-methyl-4'-(3- β -methyl-4-(1E)-4,4-trifluoro-3-hydroxy-3-(trifluoromethyl)but-1-en-1-yl]phenyl)pentan-3-yl]biphenyl-4-yl]acetic acid	

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Table 1 | Continued

Ortholog	PDBID ^a	References	Ligand name	Notes	PDBID ^a	References	Ligand name	Notes
H. sapiens DBDs (4)	1KB2	Shaffer and Gewirth, 2002	mouse osteopontin DR3		1KB6	Shaffer and Gewirth, 2002	rat osteocalcin DR3	
	1KB4	Shaffer and Gewirth, 2002	Canonical DR3		1YNW	Shaffer and Gewirth, 2004	Canonical DR3	+ RXR α DBD
R. norvegicus LBDs (40)	1RKJ	Vanhook et al., 2004	2-methylene-19-nor-(20S)-1,25D ₃	+ DRIP205 (NR2) ^c	3VRU	Yoshimoto et al., 2012	2-Methylidene-19,24-dinor-1 α ,25D ₃	+ DRIP205 (NR2) ^c
	1RK3		1,25D ₃	+ DRIP205 (NR2) ^c	3VRV		2-Methylidene-26,27-dimethyl-19,24-dinor-1 α ,25D ₃	+ DRIP205 (NR2) ^c
	1RKG		1 α -hydroxy-2-methylene-19-nor-(20S)-bismopregnacaliferol	+ DRIP205 (NR2) ^c	3VRW		2S-Butyl-2-methylidene-26,27-dimethyl-19,24-dinor-1 α ,25D ₃	+ DRIP205 (NR2) ^c
	1RKH		2 α -methylene-19-nor-1,25D ₃	+ DRIP205 (NR2) ^c	3VT3	Nakabayashi et al., 2013	1,25D ₃	+ DRIP205 (NR2) ^c R270L mutant
	2O4J	Vanhook et al., 2007	17Z-1,25-17(20)-dehydro-2-methylene-19-nor-D ₃	+ DRIP205 (NR2) ^c	3VT4		(1R,2Z,3R,5E,7E)-17-(1S)-1-[(2-ethyl-2-hydroxybutyl)sulfonyl]ethyl-2-(2-hydroxyethylidene)-9,10-secostra-5,7,16-triene-1,3-diol	+ DRIP205 (NR2) ^c R270L mutant
	2O4R		17E-1,25-17(20)-dehydro-2-methylene-19-nor-D ₃	+ DRIP205 (NR2) ^c	3VT5		(1R,2E,3R,5Z,7E)-17-(1S)-1-[(2-ethyl-2-hydroxybutyl)sulfonyl]ethyl-2-(2-hydroxyethylidene)-9,10-secostra-5,7,16-triene-1,3-diol	+ DRIP205 (NR2) ^c R270L mutant
	2ZFX	Kakuda et al., 2008	YR301	+ DRIP205 (NR2) ^c	3VT7		1,25D ₃	+ DRIP205 (NR2) ^c W282R mutant
	2ZL9	Shimizu et al., 2008	(20S)-1,25-2 β -(2-hydroxyethoxy)-16-ene-22-thia-26,27-dimethyl-19,24-dinor-D ₃	+ DRIP205 (NR2) ^c	3VT8		(1R,3R,7E,9beta,17beta)-9-butyl-17-[(2R)-6-hydroxy-6-methylheptan-2-yl]-9,10-secostra-5,7-diene-1,3-diol	+ DRIP205 (NR2) ^c W282R mutant

(Continued)

Table 1 | Continued

Ortholog	PDBID ^a	References	Ligand name	Notes	PDBID ^a	References	Ligand name	Notes
ZZLA			(20R)-1,25-2 β -(2-hydroxy ethoxy)-16-ene-22-thia-26,27-dimethyl-19,24-dinor-D ₃	+ DRIP205 (NR2) ^c	3VT9		(1R,2Z,3R,5E,7E,9beta)-2-(2-hydroxyethylidene)-17-[(2R)-6-hydroxy-6-methylheptan-2-yl]-9-(prop-2-en-1-yl)-9,10-secoestra-5,7-diene-1,3-diol	+ DRIP205 (NR2) ^c W282R mutant
ZZLC		Shimizu et al., 2008	1,25D ₃	+ DRIP205 (NR2) ^c	3VTB	^b	(1R,3R,7E,17beta)-17-[(2R,6S)-6-hydroxy-6-[(3S,5S,7S)-tricyclo[3.3.1.1~3,7~]dec-1-yl]hex-4-yn-2-yl]-2-methylidene-9,10-secoestra-5,7-diene-1,3-diol	+ DRIP205 (NR2) ^c
ZZMH		Nakabayashi et al., 2008	(25R)-25-adamantyl-1,25-2-methylene-22,23-didehydro-19,26,27-trinor-20-epi-D ₃	+ DRIP205 (NR2) ^c	3VTC		(1R,3R,7E,17beta)-17-[(2R,6R)-6-hydroxy-7-[(3S,5S,7S)-tricyclo[3.3.1.1~3,7~]dec-1-yl]hept-4-yn-2-yl]-2-methylidene-9,10-secoestra-5,7-diene-1,3-diol	+ DRIP205 (NR2) ^c
ZZMI			(24R)-24-adamantyl-1,24-2-methylene-22,23-didehydro-19,25,26,27-tetranor-20-epi-D ₃	+ DRIP205 (NR2) ^c	3VTD		(1R,3R,7E,17beta)-17-[(2R,6S)-6-hydroxy-7-[(3S,5S,7S)-tricyclo[3.3.1.1~3,7~]dec-1-yl]hept-4-yn-2-yl]-2-methylidene-9,10-secoestra-5,7-diene-1,3-diol	+ DRIP205 (NR2) ^c
ZZMJ			26-adamantyl-1,25R-2-methylene-22,23-didehydro-19,27-dinor-20-epi-D ₃	+ DRIP205 (NR2) ^c	3W5P	Masuno et al., 2013	lithocholic acid	+ DRIP205 (NR2) ^c
ZZXM		Inaba et al., 2009	(22S)-butyl-1,24-24,25,26-trinor-D ₃	+ DRIP205 (NR2) ^c	3W5Q		3-keto lithocholic acid	+ DRIP205 (NR2) ^c

(Continued)

Table 1 | Continued

Ortholog	PDBID ^a	References	Ligand name	Notes	PDBID ^a	References	Ligand name	Notes
	2ZXN		20S(2S)-butyl-1,24-24,25,26-trinor-D ₃	+ DRIP205 (NR2) ^c	3W5R		lithocholic acid acetate	+ DRIP205 (NR2) ^c
	3A2H	Kakuda et al., 2010	TEI-9647	+ DRIP205 (NR2) ^c	3W5T		lithocholic acid propionate	+ DRIP205 (NR2) ^c
	3AFR	Inaba et al., 2010	22S-Butyl-1a,24R-dihydroxyvitamin D ₃	+ DRIP205 (NR2) ^c	3W0G	Asano et al., 2013	(2S)-3-(4-[(2-[(2R)-2-hydroxy-3,3-dimethylbutylloxy]phenyl)propan-2-yl]phenoxy)propane-1,2-diol	+ DRIP205 (NR2) ^c
	3VJS	Fujii et al., 2011	1-(2-[(S)-2,4-Dihydroxybutoxy]ethyl)-12-(5-ethyl-5-hydroxyheptyl)-1,12-dicarba-closo-dodecaborane	+ DRIP205 (NR2) ^c	3W0H		(2S)-3-(4-[(4-[(2R)-2-hydroxy-3,3-dimethylbutylloxy]phenyl)heptan-4-yl]phenoxy)propane-1,2-diol	+ DRIP205 (NR2) ^c
	3VJT		1-(2-[(R)-2,4-Dihydroxybutoxy]ethyl)-12-(5-ethyl-5-hydroxyheptyl)-1,12-dicarba-closo-dodecaborane	+ DRIP205 (NR2) ^c	3W0I		(2S)-3-(4-[(3-(4-[(2R)-2-hydroxy-3,3-dimethylbutylloxy]phenyl)pentan-3-yl]phenoxy)propane-1,2-diol	+ DRIP205 (NR2) ^c
	3VRT	Yoshimoto et al., 2012	2-Methylidene-19,25,26,27-tetranor-1 α ,24-D ₃	+ DRIP205 (NR2) ^c	3W0J		(2S)-3-(4-[(2-(4-[(2R)-2-hydroxy-3,3-dimethylbutylloxy]-3-methylphenyl)propan-2-yl]-2-methylphenoxy)propane-1,2-diol	+ DRIP205 (NR2) ^c
D. rerio LBDS (19)	2HBH	Rochel et al., 2007	21-nor-20(22),23-diyne-1,25D ₃	+ SRC1 (NR2) ^d	4G20	Ciesielski et al., 2012	CD4849	+ SRC1 (NR2) ^d
	2HC4	Ciesielski et al., 2007	1,25D ₃	+ SRC1 (NR2) ^d	4G21		CD4742	+ SRC1 (NR2) ^d
	2HCD		Gemini	+ SRC1 (NR2) ^d	4G2H		CD4528	+ SRC1 (NR2) ^d

(Continued)

Table 1 | Continued

Ortholog	PDBID ^a	References	Ligand name	Notes	PDBID ^a	References	Ligand name	Notes
3DR1		Eelen et al., 2008	CD578	+ SRC1 (NR2) ^d	4FHH	Fischer et al., 2012	N-hydroxy-2-(4-[3-(4-[(2S)-2-hydroxy-3,3-dimethylbutylloxy]-3-methylphenyl)pentan-3-yl]-2-methylphenoxy)acetamide	+ SRC1 (NR2) ^d
3O1D		Huet et al., 2011	Gemini-0072	+ SRC1 (NR2) ^d	4FHI		N-hydroxy-2-(4-[3-(4-[(2R)-2-hydroxy-3,3-dimethylbutylloxy]-3-methylphenyl)pentan-3-yl]-2-methylphenoxy)acetamide	+ SRC1 (NR2) ^d
3O1E			Gemini-0097	+ SRC1 (NR2) ^d	4IA1	Maehr et al., 2013	1,25-Dihydroxy-21-(3-hydroxy-3-trideuteriomethyl)-4,4-tri-deuteriobut-1-yl)-19-nor-cholecalciferol	+ SRC1 (NR2) ^d
4G1D		Ciesielski et al., 2012	CD4720	+ SRC1 (NR2) ^d	4IA2		1,25-Dihydroxy-21-(3-hydroxy-3-trideuteriomethyl)-4,4-tri-deuteriobut-1-yl)-cholecalciferol	+ SRC1 (NR2) ^d
4G1Y			CD3938	+ SRC1 (NR2) ^d	4IA3		1 α ,25-Dihydroxy-21-(3-hydroxy-3-methyl-but-1-yl)-hexadeuterio-cholecalciferol	+ SRC1 (NR2) ^d
4G2I			CD4528	+ SRC1 (NR2) ^d	4IA7		21-nor-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol, 20-(4-hydroxy-4-methylpentyl), (1A,3B,5Z,7E)	+ SRC1 (NR2) ^d
4G1Z			CD4802	+ SRC1 (NR2) ^d				

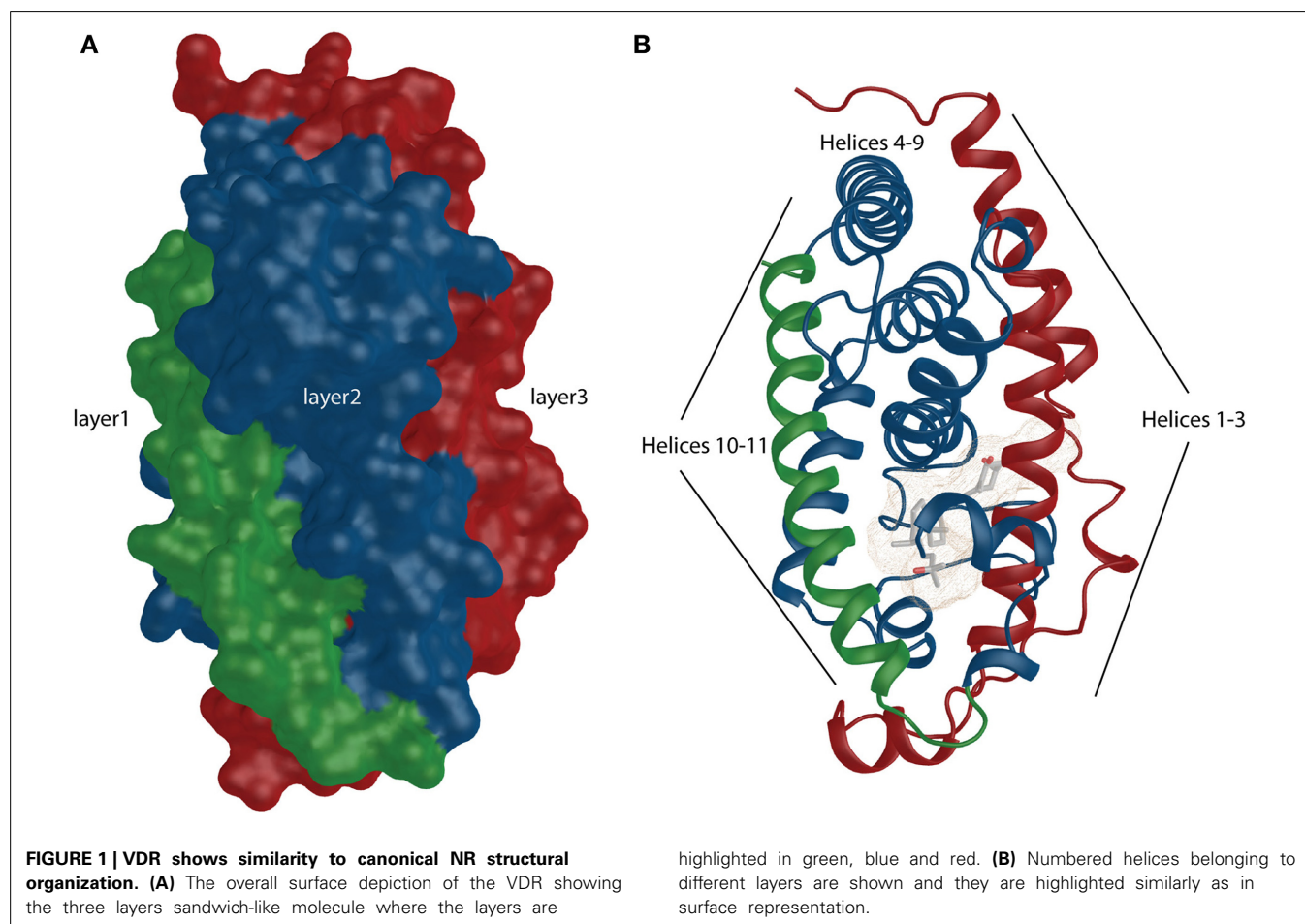
^a Protein Data Bank identifier.^b Unpublished structure.^c Synthetic peptide corresponding to NR2 Box of DRIP205/TRAP220/MED1.^d Synthetic peptide corresponding to NR2 Box of SRC1.

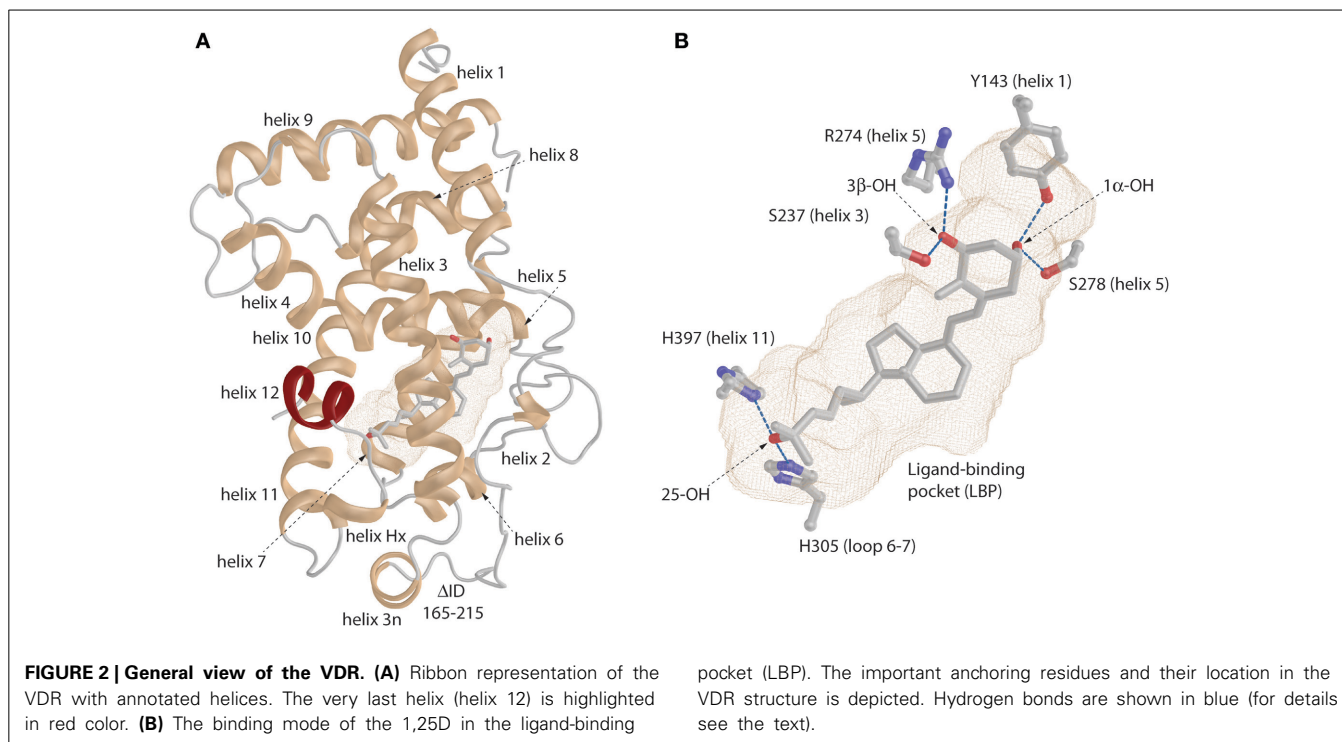
general domain organization, binding mode of natural ligands and recognition of DNA by VDR. Some data coming from molecular dynamics (MD) simulations will be also discussed since this approach represents a compromise in obtaining 3D structural models and have been proven to be well aligned with the wet lab data. At last, recent data from small-angle X-ray scattering (SAXS), cryo-electron microscopy (cryo-EM) and H/D exchange (HDX) experiments will be discussed with some perspectives highlighted.

WHAT DO VDR STRUCTURES TELL US?

From functional and structural organization point of view VDR is formed by DNA-binding domain (DBD:24–89 aa; domain C), ligand-binding domain (LBD:126–427 aa; domain E), a connective hinge (domain D) between them and a short A/B domain located at the N-terminus. Compare to RXR it completely lacks the F domain, which is the very last part of the LBD after helix 12. The LBD is formed by a three layer anti-parallel α -helical sandwich (Figures 1A,B highlighted in green, blue and red). Based of the particular structure it contains all together 11–13 α -helices (Li et al., 2003) (Figure 2A). The internal structure of the LBDs of the respective nuclear receptors (NRs) shows a high similarity with specialized diversity based on functional properties of the particular receptor. In VDR, the LBD is responsible for active ligand recognition and interaction with partnering proteins

such as coregulators, and RXR to form the functionally active RXR-VDR heterodimer. In particular, helices 3, 4, and 10–12 are involve in the interaction with protein partners. Interestingly, to date all solved VDR crystal structures show very ubiquitous and conserved organization of the of overall structural fold not reflecting the divergent nature of the bound natural or synthetic ligands. What are in fact the differences in structures that reflect various physiological effects of the particular ligands? Allegedly there will not be a simple answer to this question since we may face the limitation on the complexity of the available ligand-VDR structures or have to allow the possibility that the subtle differences between the structures may cause a “butterfly effect” that have more extreme consequences in terms of VDR signaling than initially thought. By all means there are important differences in the metabolism of various synthetic ligands and a possible unique coactivator recruitment may also play its role. However, none of these possibilities can be fully explored using the available VDR crystal structures. Nevertheless, what we may agree on is that all VDR crystal structures show agonistic conformation, surprisingly even in case of antagonists, that is canonically represented by a closed conformation of the helix 12 providing a docking platform for the recruitment of coactivators. This may be due to the shifted equilibrium that drives VDR for closed helix 12 with minimal energy conformation. In addition, the VDR structures do tell us the binding mode, anchoring points and subtle changes





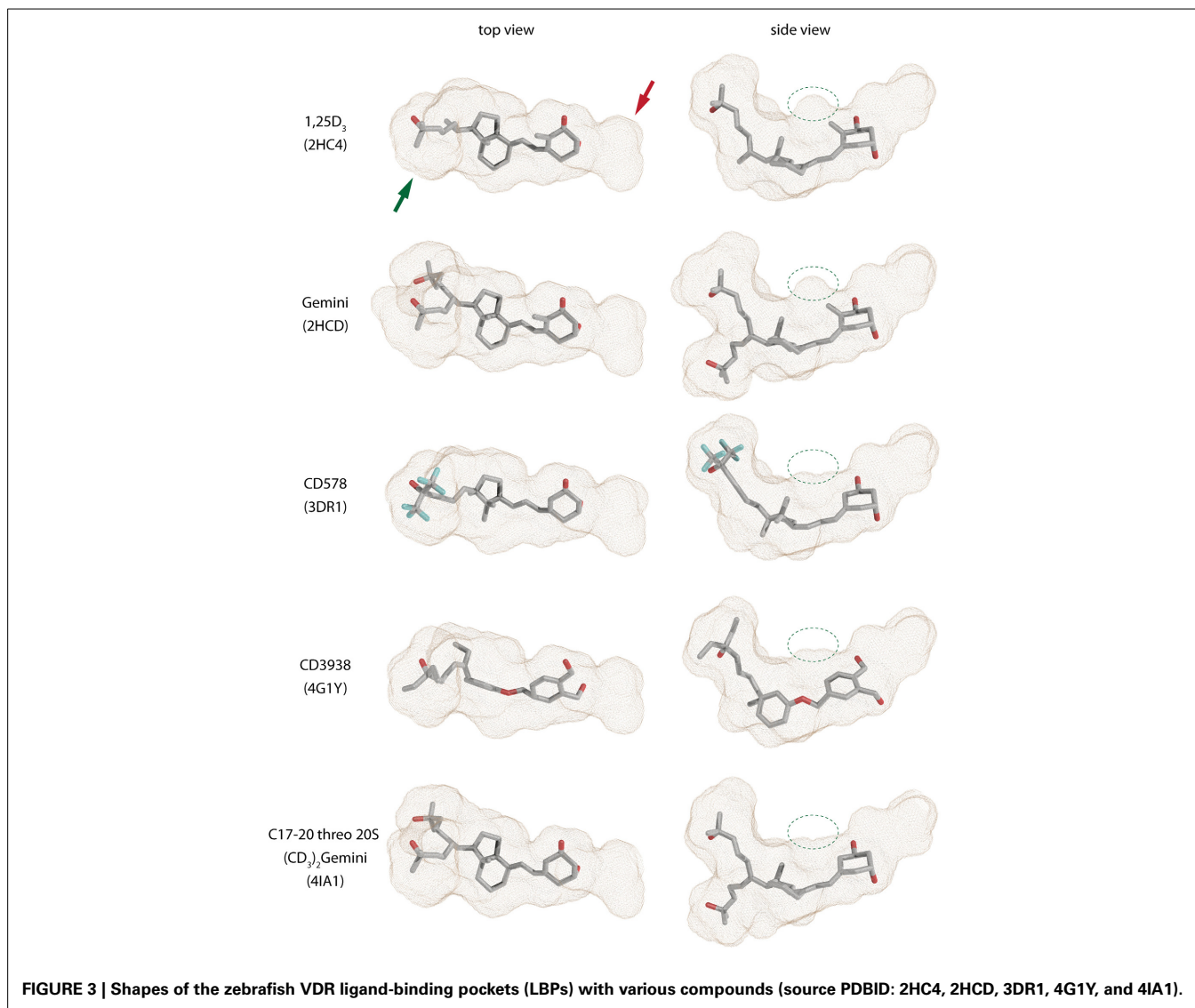
in the position of residues that may be effectively used for *de novo* design of superagonist such as AMCR277A (PDBID:3CS4) (Hourai et al., 2008). The frequently mentioned subtle changes that are characteristic for the ligand-binding pocket (LBP) may be further analyzed and can explain some of the binding differences between various ligands in correlation to their functional and biological properties.

THE INNER CIRCLE: LOOKING INTO THE VDR POCKETS

The “lower part” of the LBDs of all ligand-activated NRs contains a LBP which volume size range between 400 and 1400 Å³ (Figures 2A,B light-brown mesh) (Nagy and Schwabe, 2004). It is not quite different in case of VDR where LBP primarily serves for effective recognition of various natural ligands such as 1,25D₃ and its metabolites or bile acids. In addition, this is one of the most important parts to modulate VDR’s activity via various synthetic compounds. The VDR pocket can be placed in the middle range of the volume scale showing rather high dynamic plasticity toward various ligands.

The first VDR crystal structure confirmed the conserved contact or anchoring points for the interaction of VDR with 1,25D₃ (Rochel et al., 2000) (Figure 2B). The residues involved in the positioning of the 1,25D₃ in the LBP are Y143 (helix 1) and S278 (helix 5) that contact the 1,25D₃ 1α-hydroxyl (OH) group, S237 (helix 3) and R274 (helix 5) contact 3β-OH, and H305 (loop 6–7) and H397 (helix 11) interact with 25-OH. In most of the cases if these anchoring point are disrupted a decrease in the activation potential of the ligand can be observed. Besides these residues the interior surface of the VDR LBP is formed of about 40 mostly non-polar amino acids. An interesting approach is to look how the LBP is changed upon ligand-binding especially its volume or how much volume (%) is occupied by the ligand. A rather

straightforward example is the comparison of the 1,25D₃ and MC1288 LBPs. Although the volumes of these ligands are highly comparable 434 Å³ and 427 Å³ (probe radius 1.9 Å), respectively, the volumes of the LBP show slight differences 776 Å³ and 643 Å³ (same probe radius) leading to ratio of 56 and 66% of ligand occupancy of the LBP volume (Molnár et al., 2006). When a ligand occupancy is higher compared to 1,25D₃ then it increases the stability of VDR. This factor holds true for ligands that show high structural similarity and binding mode to 1,25D₃. It seems to some extent that the ratio of the ligand to LBP volume can be a good descriptor of the ligands’ activation potential. In addition, the actual shape of the cavity also reflects differences in the binding of various ligands which is illustrated in Figure 3. There are slight differences in the shapes of the LBP depending on the bound ligand. The red arrow shows the part where the shape is conserved well. Topologically this is the place where the 1,25D₃ A-ring is located e.g., helices 1, 3, and 5. The more plastic part is the one where the 1,25D₃ side chain is found with helices 6, 7, and 11. This region shows the highest variation between the ligand-bound structures and is indicated with green arrow Figure 3. In addition, small changes can be also detected for instance the part highlighted with green circle. Very interesting in the difference between the two Gemini structures 2HCD and 4IA1, where the largest difference for the two side chains is that “C17–20 threo 20S—Gemini” has the hydrogens in its methyl groups substituted with deuterium. Interestingly, in this case the pocket shows more compact shape in the region with the double side chains and the volume of the location highlighted with green circle is decreased, which may be due to modification of the A-ring. Another interesting question is the maximum volume to which the VDR LBP can be stretched. MD simulations showed that by docking a Gemini with fluorinated methyls groups, (CF₃)₂-Gemini, the LBP could



be expanded by 1/3 of the 1,25D₃ LBP volume. In proportion to this, the compound's volume in the pocket is also increased about 30%.

An appealing view opens up when a comparison of the VDR structure with one of its closest relative pregnane X receptor (PXR) is made (Watkins et al., 2001). As for VDR there are plenty of PXR crystal structures available and it can extend its LBP to very large volume ($\sim 1400 \text{ \AA}^3$) to accommodate various compounds. The published VDR structures lack the insertion domain ($\Delta 166\text{--}216$ aa), but even without it the LBP as discussed earlier is as large as $700\text{--}800 \text{ \AA}^3$ with MD simulations showing that it can expand beyond 1000 \AA^3 , which is already comparable to PXR's LBP. This may suggest that VDR is able to accommodate variety of other compounds in addition to 1,25D₃ such as LCA/3-keto-LCA (Makishima et al., 2002). The later have been crystallized with rVDR and will be discussed in this review. The indications for binding additional compounds besides 1,25D₃ are coming also from *P. marinus* (sea lamprey) where despite of lack of the

calcified skeleton and teeth it may serve as a xenobiotic activator for detoxification by regulating P450 enzymes (Whitfield et al., 2003; Krasowski et al., 2005). However, it is yet to be determined whether there are existing other ligands that bind to VDR. One part of the LBD, which may allow the binding of these compounds is the insertion domain. Although its clear functional role has not been identified and it seems that it is not directly required for the binding of the 1,25D₃ (Rochel et al., 2001), it may play some other roles. A mutation C190W was reported in patients that results in loss of 1,25D₃ binding (Malloy et al., 1999), though this may be due to the disruption of the VDR structure by introducing a large bulky tryptophan residue. Secondly, the homologous part of PXR (142–431 aa) especially the occurrence of the two β -strands and the associated coiled regions are responsible for the expandability of the PXR's LBP thus an analogous role cannot be out ruled in case of VDR as well. There has been also proposed that an alternative pocket, which can be considered as an enlargement of the original pocket, is formed in the VDR that

extends toward the helix 2/ β -sheet region of the LBD (Mizwicki et al., 2004). Especially this can be observed with covalently locked 1,25D₃-derived compounds such as 1,25(OH)₂-lumisterol, which has been showed by *in silico* docking studies.

Due to the space limitation this review cannot address and discuss all the VDR-ligand complexes, for some more details see text below or recent reviews (Carlberg and Molnár, 2012; Carlberg et al., 2012), but some of the structures with natural ligands will be discussed in more details. One of them is 3-epi-1 α ,25-dihydroxyvitamin D₃ (3-epi-1,25D₃) a 1,25D₃ metabolite that has been shown to exhibit tissue specific activities comparable to 1,25D₃ (Norman et al., 1993; Reddy et al., 2000). The structural analysis showed a binding mode very similar to that of 1,25D₃ with interesting compensation for the lacking S278-3 α -OH hydrogen bond for the epimer using water mediated contacts **Figure 4** (Molnár et al., 2011). Interestingly, the same water channel is present in the 1,25D₃ complex and was observed with other complexes as well (Tocchini-Valentini et al., 2001; Hourai et al., 2006).

A widely accepted fact is that precursor 25-hydroxyvitamin D₃ (25D₃) and its metabolite 24R,25-hydroxyvitamin D₃ (24,25D₃) does not possess significant biological activities. However, it has been shown that in *Cyp27b1*^{-/-} cells, that are unable to produce actively 1,25D₃, the VD signaling may be primary mediated via 25D₃ (Lou et al., 2010). It stays a matter of discussion that under physiological condition how much of the VD signaling is mediated via 1,25D₃ vs. 25D₃. 24,25D₃ shows only weak potency of influencing VD signaling at concentration 500nM although an enhancement for human osteoblast differentiation has been shown at concentration 1 μ M (van Driel et al., 2006). Docking and subsequent MD simulations have been done to see the

binding mode of these compounds and it has been confirmed that the position of residues mediating the anchoring hydrogen bonds are conserved with the exception of R274 which is located further than 3.5 Å from 24,25D₃ **Figure 5**. Another residue that is unable to make a binding contribution is S237, but its position in the pocket is maintained. The void created by the missing R274 increases the LBP and lowers the occupancy factor for this compound. The analysis of the simple binding mode confers the activity range from 1,25D₃ > 25D₃ > 24,25D₃. This also shows in general the importance of the 1 α -OH group for the potency of the VDR agonist.

Recently, the crystal structures for another group of natural ligands have been solved. From the identification of secondary bile acids as VDR agonist (Makishima et al., 2002) the interesting question remained how these compounds bind to VDR. From the structural data it is evident that the lithocholic acid (LCA) and 3-keto lithocholic acid (3kLCA) are located in the opposite orientation than 1,25D₃ (**Figure 6**). The 24-carboxyl group faces the β -turns of VDR, the β -region of the steroid backbone the helix 6–7/11 region and the A-ring is in the direction of helix 12. The S274 (hVDR S278) and Y143 hydrogen bonds are conserved

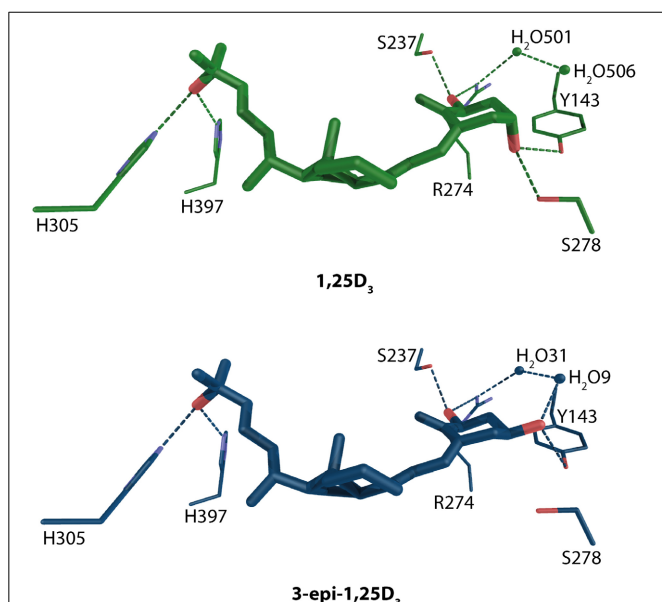


FIGURE 4 | Binding mode of 1 α , 25-dihydroxyvitamin D₃ and 3-epi-1 α ,25-dihydroxyvitamin D₃ in the LBP of human VDR (source PDBID: 1DB1 and 3A78). 1,25D₃ and 3-epi-1,25D₃ are highlighted in green and blue color, respectively. The hydrogen bonds are in green and blue dashed lines.

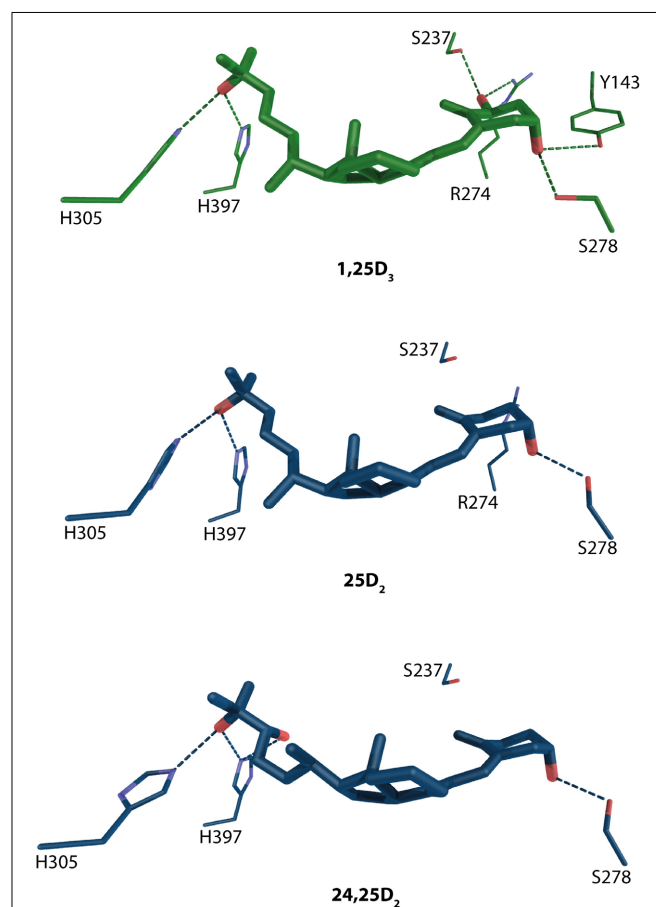
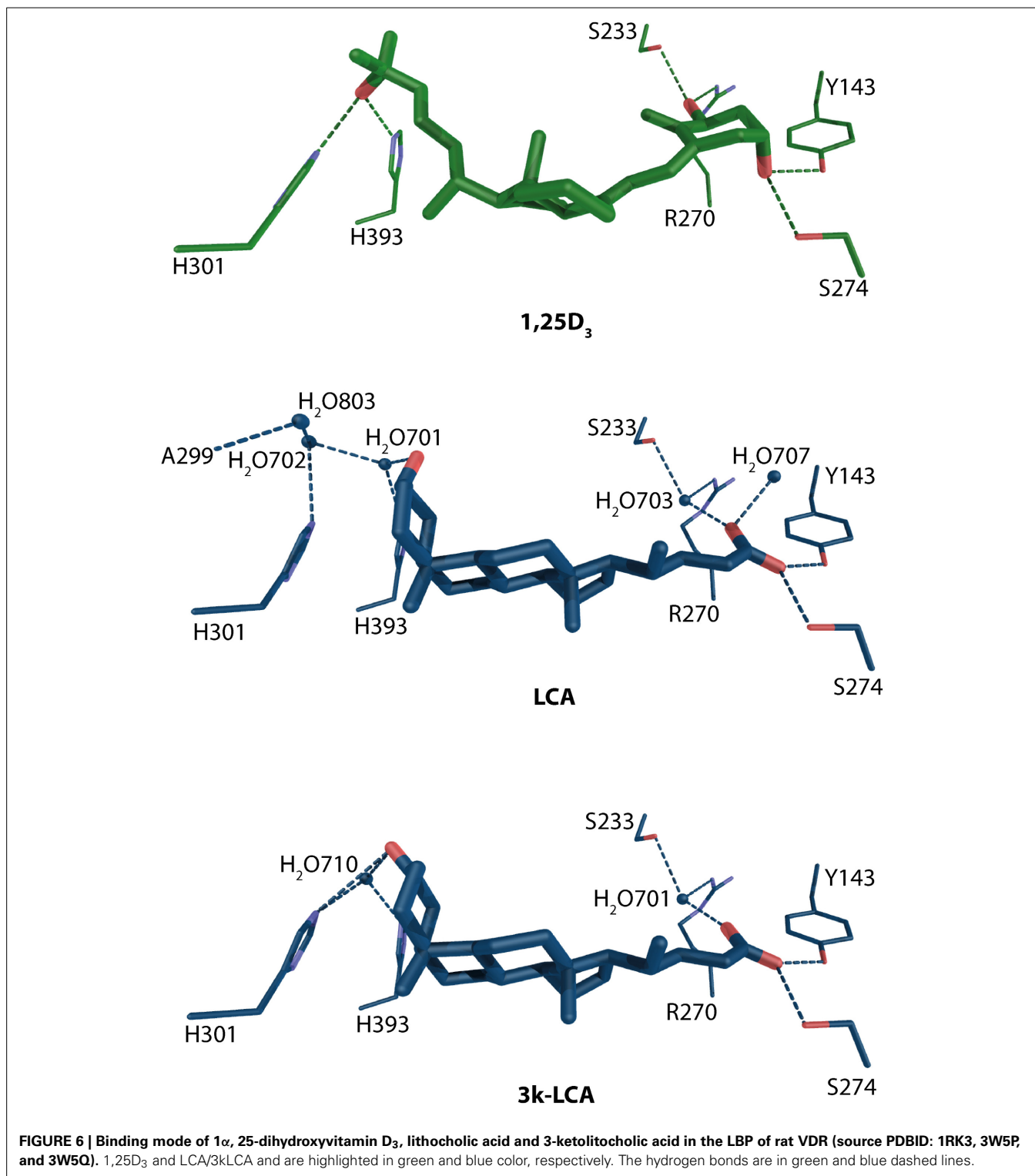


FIGURE 5 | Binding mode of 1 α , 25-dihydroxyvitamin D₃, 25-hydroxyvitamin D₃ and its metabolite 24R,25-hydroxyvitamin D₃ in the LBP of human VDR (source MD simulation Lou et al., 2010). 1,25D₃ and 25D₃/24,25D₃ and are highlighted in green and blue color, respectively. The hydrogen bonds are in green and blue dashed lines.



in all structures (Figure 6). The difference for this part of the ligand-binding comes from the water mediated contacts with both R270 (hVDR R274) and S233 (hVDR S237). These contacts seem to be weaker compare to 1,25D₃ but not so weak as in case of 25D₃ and 24,25D₃, where in fact they are missing due to lack of 1 α -OH group. The opposite part of the bile acids shows

also weaker hydrogen bonding network than for the previously discussed VD metabolites including 1,25D₃. The structural comparison between the two bile acids shows a less complex, more straightforward binding mode for 3kLCA with only one water molecule involved. Whereas, for LCA there are no direct contacts with H301 (hVDR H305) and H393 (hVDR H397). All these

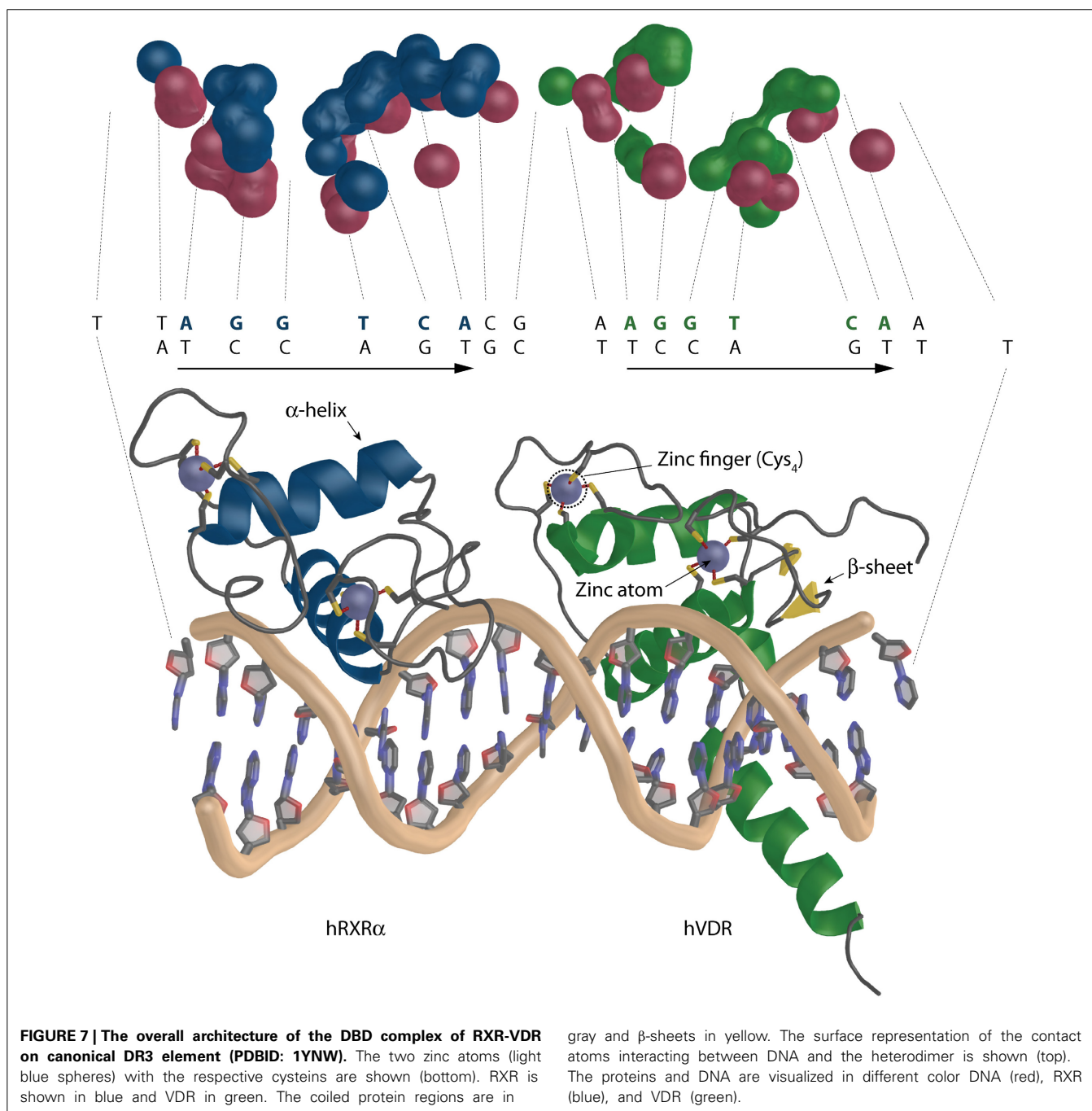
contacts are mediated through waters. This also may explain the lower VDR activation potential of 3kLCA compared to LCA.

It would seem that the evolutionary design of VDR reached by recognizing 1,25D₃ is its perfection. All the anchoring points show effective hydrogen bonding and by looking at the limited data for natural ligands any deviation from 1,25D₃ will result in the use of alternative bridging contacts such as water molecules. This shows a rather limited adaptability of VDR in effectively recognizing its natural ligands, yet larger than we envisioned a decade ago. One must keep in mind that the activation of VDR with one or another metabolite will largely depend on the local cellular concentration

of this compounds leading for instance for favored activation via bile acids instead of classical 1,25D₃ binding.

DNA-RECOGNITION AND BINDING

VDR belongs to the class of zinc finger transcriptional factors with DBD that consists of a highly conserved 66 aa residue core (Khorasanizadeh and Rastinejad, 2001) and an adjacent C-terminal extension. The conserved core has two zinc fingers where one contains four cysteine residues per atom of zinc (Figure 7). This feature allows VDR to effectively recognize and bind hormone response elements (REs) - termed VDREs. VDREs are



typically made up of two hexameric half-sites whose consensus sequence is 5'-RGKTCA-3', where R = A or G and K = G or T. The half-sites may be arranged in various orientation most commonly forming a direct repeat with three neutral base pairs separating the half-sites (DR3) (Umesono et al., 1991). The unliganded VDR can occupy its REs also as a homodimer (Carlberg et al., 1993). Upon binding of ligand, VDR interacts with RXR and forms a heterodimeric complex that binds to VDREs with 5'-prime bound RXR.

The data for structural view on VDR-DNA recognition is scarce. To date we have only four structures published where three represent VDR homodimers on direct repeat 3 (DR3) from mouse osteopontin (mSPP) (CACGGTTCACGAGGTCA), rat osteocalcin promoter (rOC)(CACGGGTGAATGAGGACA) and a canonical DR3 element (cDR3) (CACAGGTACGAAGGTCA) (Table 1) (Shaffer and Gewirth, 2002). The last structure represents the DBDs of the heterodimeric RXR-VDR on canonical DR3 element (cDR3) (TTAGGTCACGAAGGTCAA) (Table 1) (Shaffer and Gewirth, 2004). The 66 to 70 aa of the DBD are structurally coordinated by two zinc atoms that create a structure (Figure 7), in which one short α -helix is interacting directly with the major groove of the DNA (Hård et al., 1990; Lee et al., 1993). The VDR homodimers show asymmetric head to tail arrangements. The experimentally determined range of affinities of DR3s used for crystallographic studies are mSPP > cDR3 > rOC with mSPP supporting both VDR homodimer and RXR-VDR heterodimer binding, cDR3 requiring 10x higher VDR homodimer protein levels and weak heterodimer binding, whereas rOC is unable to bind VDR homodimers and has a very weak heterodimer binding ability (Freedman and Towers, 1991; Nishikawa et al., 1993; Toell et al., 2000). The change at third position, a purine to pyrimidine, of the consensus half sites AGGTCA allows the additional water mediated hydrogen contact of E42 with the DNA, which increase the stability of the mSPP-VDR complex. In case of rOC the reason for diminished VDR homodimer binding is because there is a G at position five of the upstream half-site GGGTGA, where supposedly RXR is bound in case of heterodimer binding. In high affinity half-sites in this location there is a C in the first strand but a complementary G in the second DNA strand. The interaction, which involves hydrogen bonds is between the R50 of VDR and the G from the second complementary strand. In rOC upstream half-site instead of G there is a C in the second strand, which is not a hydrogen bond acceptor thus R50 cannot interact with it. There is also some agreement between the strength of the homodimer binding and a sum of all existing DNA-VDR contacts within 3.5 Å calculated with the *ncont* program of the CCP4 suite (Winn et al., 2011). The results show 88, 85, and 83 contacts for rOC, cDR3, and mSPP, respectively. Interesting is also the interacting surface ratio between the two VDR homodimer molecules in percentage (5'upstream: 3'downstream) 38.52:61.48% for rOC, 46.21: 53.8% for the cDR3 and a reversed ratio of 57.3:42.7% for mSPP. This would suggest that for strong VDR homodimer binding there is a more contribution from the upstream half-site, but a clear conclusion cannot be reached based on this limited data set. In the RXR-VDR crystal structure there are two asymmetric units where the full complex shows an orientation RXR to VDR for unit 2 and

a reversed orientation VDR to RXR for unit 1 with RXR bound on the downstream part of the VDRE. This may be due to the stabilization contacts between the adjacent VDR molecules where the hinge of one VDR molecule is stabilized with the DBD of the second VDR molecule. The general organization of the unit 2 is depicted also on the Figure 7 bottom. The two zinc atoms (light blue color) with the respective cysteines are visualized (the second heterodimer unit is missing from the representation). The overall number of contacts is only 65, which is much lower than in case of VDR homodimers discussed earlier. This indicates that the binding of the RXR-DBD heterodimer to cDR3s is not optimal and for an effective binding a certain point of VDRE degeneration is needed. Moreover, the contribution of the monomers to the binding is rather interesting. The ratio between interacting monomeric surfaces is 56.22:44.26% (RXR-VDR) suggesting a higher contribution of RXR to the binding Figure 7 top (see also the H/D exchange experiments discussed below). This is most likely due to the reverse orientation of the RXR-VDR on the cDR3. The surfaces of the interacting atoms are visualized in different color DNA (red), RXR (blue) and VDR (green). It is to be noted that compared to DNA-protein interaction there are hardly any interactions between the protein monomers. None for RXR-VDR, for VDR homodimers there are two for mSPP and rOC, and five on cDR3. This is quite in agreement with full length receptor studies which suggest that most of the heterodimerisation is contributed from hinges and LBDs.

THE OUTSIDE SHELL: PARTNERING AND THE COMPLEX VIEW

VDR INTERACTING PROTEIN PARTNERS

For VDR to function effectively as a regulator of transcription it is inevitable to interact with various protein partners. They show high structural and functional diversities ranging from enzymes, co-integrators and cofactors to components of distinct transduction signaling pathways. A comprehensive list of these partners with the accompanying citations are listed in Table 2.

One of the first complex identified using co-immunoprecipitation from mammalian cells was the VDR interacting protein DRIP complex, which is recruited to VDR in a completely ligand-dependent manner (Rachez et al., 1999). Many of the its components were shared with the earlier identified thyroid hormone receptor (TR) interacting protein complex TRAP (Fondell et al., 1996). It has been no surprise that majority of the interacting proteins can be related directly to transcriptional regulation such as subunits of the mediator complex MED1, 4, 6, 7, 12, 16, 17, and 23 (Rachez et al., 1999) or cofactors such as coactivators NCOA1-3 (Hong et al., 1997; Castillo et al., 1999; Molnár et al., 2005), NCOA6 (Mahajan and Samuels, 2000), ARA54 (Ting et al., 2005), SKIP (Baudino et al., 1998), RBP2 (Chan and Hong, 2001), SRB7 (Ito et al., 1999) and corepressors Alien (Polly et al., 2000), NCOR1 and 2 (Tagami et al., 1998; Kim et al., 2009), SIN3A (Fujiki et al., 2005), LCOR (Fernandes et al., 2003). Others show more selective properties functioning as coactivators and corepressors depending on the particular conditions such as RIP140 (Albers et al., 2005) or TIF1 α (Thénot et al., 1997). Some are implicated in cellular processes such as cell cycle regulation CDK7 (Nevado et al., 2004), RAP46 (Guzey et al., 2000), DNA repair

Table 2 | List of VDR interacting proteins.

Name	Gene symbol/alternative name	Role	References
Alien	ALIEN	Transcriptional corepressor	Polly et al., 2000
Androgen receptor-associated protein 54	ARA54*	Transcriptional coactivator	Ting et al., 2005
Androgen receptor-associated protein 70	ARA70	Transcriptional coactivator implicated in cancer	Ting et al., 2005
Brahma-related gene 1	BRG1/SMARCA4	ATPase subunit of the SWI/SNF complex	Fujiki et al., 2005
CREB-binding protein	CBP	Transcriptional cointegrator	Castillo et al., 1999
Cyclin D3	CCD3	Subunits of the cyclin-dependent kinases	Jian et al., 2005
Cyclin-dependent kinase 7	CDK7/hMo15	Component of the TFIIH transcription complex	Nevado et al., 2004
CXXC finger 5	CXXC5	Cell cycle regulation	Marshall et al., 2012
E1A binding protein p300	p300	Transcriptional cointegrator	Kim et al., 2005
Fas-activated serine/threonine kinase	FASTK	Involvement in splicing	Marshall et al., 2012
Feline Gardner-Rasheed sarcoma viral oncogene homolog	FRG	Signal transduction (protein tyrosine kinase)	Ellison et al., 2005
General transcription factor IIB	TFIIB	Subunit of the basal transcription machinery	Nevado et al., 2004
Hairless	HR	Transcriptional corepressor	Hsieh et al., 2003
High mobility group nucleosomal binding domain 3	HMGN3/TRIP7	Possible chromatin modifier	Albers et al., 2005
Histone deacetylase 2	HDAC2	Histone modifier	Fujiki et al., 2005
Ligand-dependent NR corepressor	LCOR	Transcriptional corepressor	Fernandes et al., 2003
Mediator complex subunit 1	MED1/TRAP220/RIP205/PPARBP	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 4	MED4/DRIP36/p34	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 6	MED6/DRIP33	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 7	MED7/DRIP34	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 12	MED12/DRIP240/ARC240/TRAP230	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 16	MED16/DRIP92/TRAP95	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 17	MED16/DRIP77/TRAP80	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mediator complex subunit 23	MED23/DRIP130/CRSP130	Transcriptional regulation/part of the mediator	Rachez et al., 1999
Mothers against decapentaplegic homolog 3	SMAD3	Transcriptional coactivator	Yanagisawa et al., 1999
Myosin light chain 3	MYL3	Regulatory light chain of myosin	Marshall et al., 2012
NR coactivator 1	NCOA1/SRC1	p160 family coactivator	Castillo et al., 1999
NR coactivator 2	NCOA2/TIF2/GRIP1	p160 family coactivator	Hong et al., 1997)
NR coactivator 3	NCOA3/RAC3/SRC3/AIB1	p160 family coactivator	Molnár et al., 2005
NR coactivator 6	NCOA6/PRIP/ASC2	Transcriptional coactivator	Mahajan and Samuels, 2000
NR corepressor 1	NCOR1	Transcriptional corepressor	Tagami et al., 1998
NR corepressor 2	NCOR2/SMRT/TRAC2	Transcriptional corepressor	Kim et al., 2009
NR subfamily 0, group B, member 2	NR0B2 (SHP)	Negative transcriptional regulator	Albers et al., 2005
NR subfamily 4, group A, member 1	NR4A1 (NGFIB)	Expression genes during liver regeneration	Marshall et al., 2012
p53	PT53	Tumor suppression	Stambolsky et al., 2010

(Continued)

Table 2 | Continued

Name	Gene symbol/alternative name	Role	References
Receptor-associated protein 46	RAP46/BAG1	Regulation of cell growth in response to stress	Guzey et al., 2000
Receptor-interacting protein 140	RIP140/NRIP1	Coregulator with selective properties	Albers et al., 2005
Retinoblastoma 1	RB	NR coregulator/tumor suppressor	Chan and Hong, 2001
Retinoblastoma-binding protein 2	RBP2	Transcriptional coactivator	Chan and Hong, 2001
Retinoid X receptor α	RXR α	Heterodimeric VDR partner	Liu et al., 2000
Retinoid X receptor β	RXR β	Heterodimeric VDR partner	Rachez et al., 1999
Retinoid X receptor γ	RXR γ	Heterodimeric VDR partner	Albers et al., 2005
Protooncogene c jun	JUN	Transcriptional factor	Towers et al., 1999
SIN3 homolog A, transcriptional regulator (yeast)	SIN3A	Transcriptional corepressor/cointergrator	Fujiki et al., 2005
SKI interacting protein	SKIP/SNW1/NCoA-62	Transcriptional coactivator	Baudino et al., 1998
Suppressor of RNA polymerase B 7	SRB7	Transcriptional coactivator	Ito et al., 1999
Thymine-DNA glycosylase	TDG	Coregulator/base excision repair	Chen et al., 2003
Thyroid receptor-interacting protein 1	TRIP1/SUG1/PSMC5	CAD (Conserved ATPase domain) protein	Masuyama and Hiramatsu, 2004
Transcriptional intermediary factor 1	TIF1 α /CCCP	Coregulator with selective properties	Thénot et al., 1997
Tropomyosin	TPM2	Possible role in receptor internalization	Marshall et al., 2012
Vitamin D receptor-interacting protein (100kD)	DRIP100/ARC100/TRAP100	VDR associated DRIP complex	Rachez et al., 1999
Vitamin D receptor-interacting protein (150kD)	DRIP150/ARC150/TRAP170	VDR associated DRIP complex	Rachez et al., 1999
Vitamin D receptor-interacting repressor	VDIR/TCF3/ITF1	Negative regulator of the CYP27B1	Kim et al., 2007
Williams syndrome transcription factor	WSTF/BAZ1B	Recruitment of unliganded VDR to target promoters	Fujiki et al., 2005
Xin actin-binding repeat containing protein 1	XIRP1	Protects actin filaments from depolymerization	Marshall et al., 2012

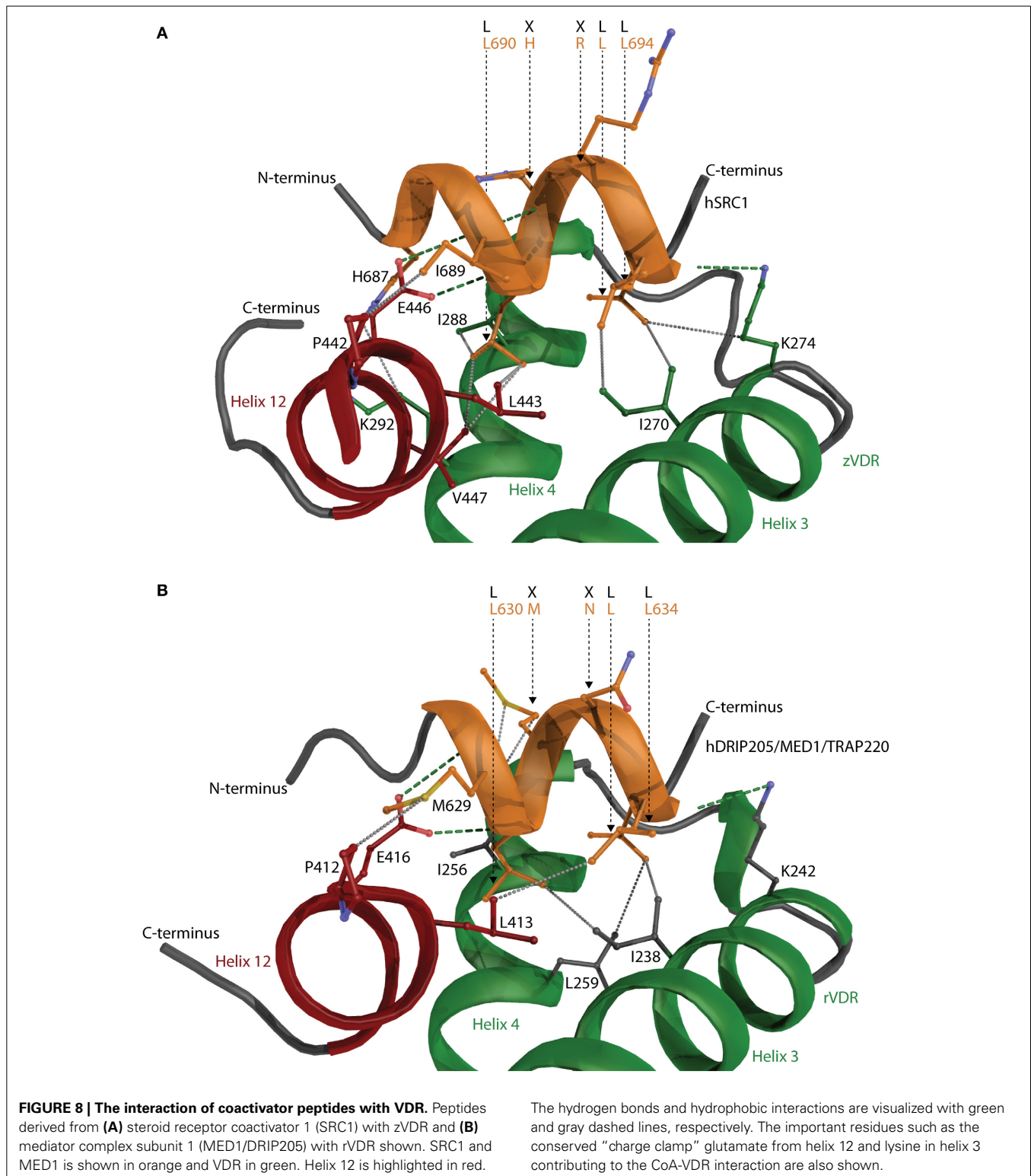
*no direct physical interaction but has positive effect on VDR transactivation.

TDG (Chen et al., 2003) or signaling cascade FRG (Ellison et al., 2005). Interesting is the interaction and thus possible crosstalk with other NRs such as SHP (Albers et al., 2005), which lacks DBD and has corepressor-like behavior, and NGFIB, which has been shown to have a role during liver regeneration (Marshall et al., 2012). Some of the newly identified protein partners that may implicate VDR's involvements in new processes are XIRP1 that protects actin filaments from depolymerization or MYL3 a regulatory light chain of myosin (Marshall et al., 2012).

STRUCTURAL DATA OF SRC1 AND MED1/DRIP205 INTERACTION WITH VDR

There is a big limitation in obtaining large transcriptional complexes, which is firstly due to the transient nature of the complex where VDR serves as a docking and acquiring platform bringing other proteins that either act as chromatin modifiers, parts of the mediator, of various cofactors and bridging factors to the close proximity of the functional VDREs. The complex may be assembled for a short moment to initiate and/or repress the transcription thereafter fulfilling this action it falls apart. The second reason might be that many of the interacting proteins such as

cofactors show a high degree of disorder. The crystallization of unfolded proteins is very tricky and many times even impossible. The intrinsic disorder of a VDR interacting proteins is an expected structural property since e.g., members of the p160 general coactivator family have to adopt and interact with many various transcription factors. Thus structural data from only short interacting peptides derived from steroid receptor coactivator 1 (SRC1) with zVDR (**Figure 8A**) and mediator complex subunit 1 (MED1/DRIP205) with rVDR are available showing only the core interaction between VDR and the LXXLL motif of coactivators (**Figure 8B**). Both peptides interact in a very similar fashion. The α -helix of the peptide is oriented with its N-terminus toward helix 12. The two peptides interact through their LXXLL motifs, LHRLI in SRC1 and LMNLL in MED1, and most of the interaction is contributed from hydrophobic contacts of coactivator's leucine residues with the hydrophobic core from VDR helices 3, 4, and 12. The anchoring points of the short α -helix are based on the interaction with the "charge clamp" consisting of the conserved glutamate in helix 12 and lysine in helix 3, and the backbone amides of the coactivator peptide. The similar interaction of the two LXXLL motifs raises the question on how the



specificity is achieved in the interaction. The situation is complicated with the fact that some of the coactivators have more than one interaction motif such as SRC1 has five of them that are similar or related to LXXLL motifs, but so far only three of them were reported/studied in detail.

SAXS, CRYO-EM, AND H/D EXCHANGE STUDIES WITH FULL LENGTH RXR-VDR COMPLEXES

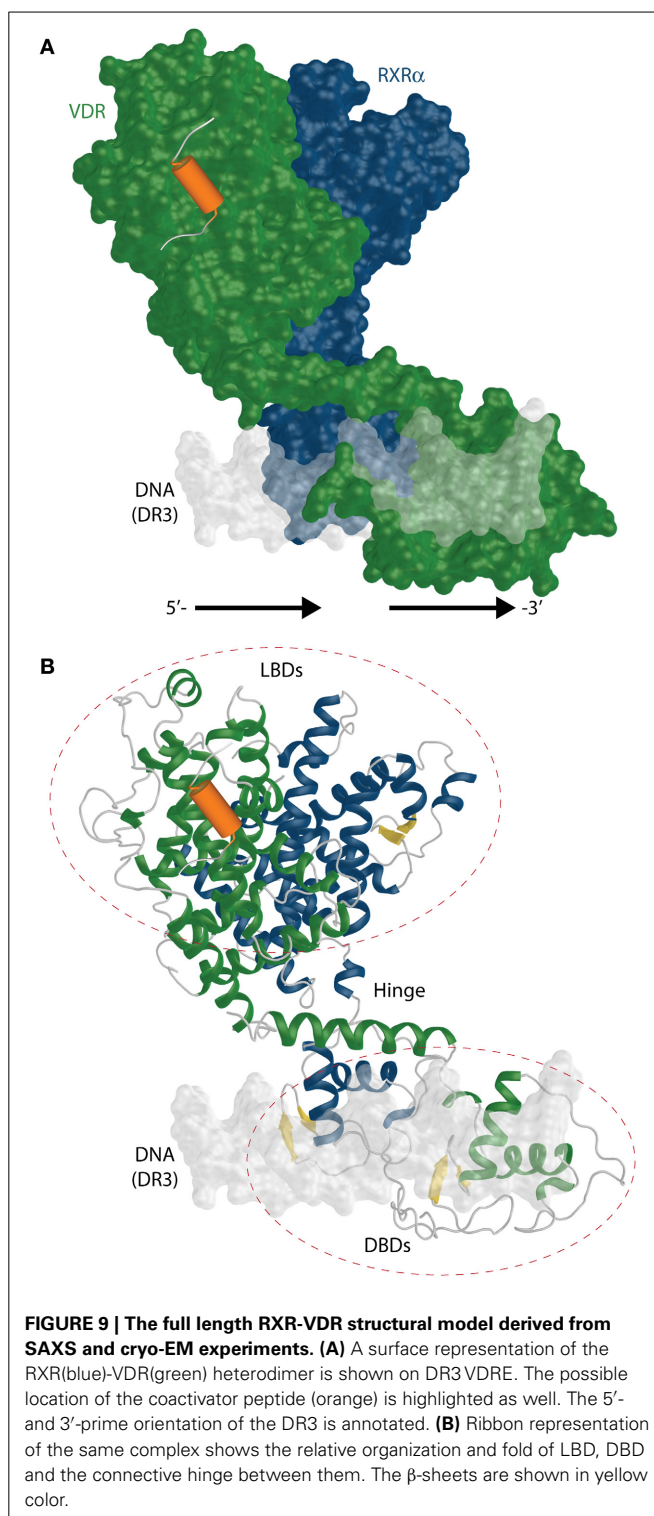
The recent studies with VDR complexes in solution that employed the use of modern techniques such as SAXS, cryo-EM, HDX with full length VDR-RXR α showed somewhat more comprehensive

perspective for the 3D organization and possible function of the VDR-RXR α -cofactor complex. The SAXS (Rochel et al., 2011) and cryo-EM (Orlov et al., 2012) derived model is shown in **Figure 9A**.

Upon binding to DNA from osteocalcin VDRE, the SAXS data derived RXR-VDR shows an elongated asymmetric open conformation with separate DBDs and LBDs and a well structured VDR hinge with VDR located downstream and RXR on upstream half sites (Rochel et al., 2011) (**Figure 9B**). On contrary the coiled structure of the RXR hinge allows its adaptability to different REs. The hinges play an important role in an open conformation. The hinges also underly one of the very important feature of the RXR-VDR complex, the dynamic character. The DBD binding to DR3 results in a rotation of the LBD dimers take around 90° with respect to the DNA (**Figure 9A**). The same study showed that coactivators MED1/DRIP205 and SRC1 have higher affinity to VDR compared to RXR. This points to the binding of only one molecule of coactivator through VDR, which is not supporting the “deck model” of binding (one LXXLL motif to VDR and the other to RXR) for these coactivators. Studies using mutants show the preferential binding of VDR to the second LXXLL motif of MED1/DRIP205 compared the weak recruitment of the first motif to RXR (Ren et al., 2000). However, both motifs are crucial for the effectivity of the NR activation complex *in vivo* (Malik et al., 2004). RXR may play a role in coactivator recruitment as well by associating to some other factors.

The cryo-EM studies of the RXR-VDR have a higher resolution than SAXS data and it is possible to obtain more precise electron densities for VDR A/B and hinge domains for both receptors (Orlov et al., 2012). The heterodimer takes an L-shape form on the DR3 with a proper orientation of RXR on the upstream and VDR on the downstream half site (similar to SAXS model). The complex supports also the asymmetric open architecture from SAXS data. Both LBDs are positioned above the 5' upstream half site as shown on **Figure 9A**. This result also emphasizes the importance of the hinges for the correct function of the complex. The main nature of the flexible hinge of RXR allows to contact the CTE helix with helix 1 and makes it possible to adopt differential spacing in DR REs. In addition, the coiled RXR hinge has to be long to reach DBD to its LBD which is located on the opposite side of the DR3 VDRE. The cryo-EM data points to one interesting feature, the potential in modulation of the DNA-binding using the 17 aa long A/B domain of VDR, which in fact interacts with the major groove of the DNA.

The H/D exchange (HDX) is a great tools to address the dynamic properties of the RXR-VDR-SRC1 complex (Zhang et al., 2011). The addition of RXR to VDR stabilizes region with helices 6–7, very similar to what is seen when some of agonists bind to VDR. As expected, upon 1,25D₃ binding in the VDR LBD the helices 1, 3, 5–7, and 11 (the actual region that forms the LBP) have been stabilized, but binding efficiency of RXR to VDR is not enhanced. For RXR the helices 7 and 10 are stabilized (increase of the heterodimerisation) and an allosteric communication has been shown for the helix 3 of RXR. The 9-*cis* RA binding in general stabilized RXR, but in contrast to the crystal structure, it increased the fluctuation in helix 12. This observation may also indicate that the crystal structure take the minimized



energy conformation of the complex, but in reality it is more dynamic. The allosteric communication in VDR upon 9-*cis* RA binding was seen in the helices 3, 5, and 7 that are adjacent from the heterodimerisation interface pointing to modulation of the complex upon only 9-*cis* RA binding. Surprisingly, the concurrent binding of 1,25D₃ and 9-*cis* RA has a destabilizing affect

on the VDR DBD. A stronger increase of the VDR binding has been observed compared to RXR DBD in the presence of cDR3 element and in the absence of ligands, pointing to the higher contribution of the VDR for DNA interaction. In addition, interesting allosteric stabilization were observed for the VDR hinge and for helices 7–8 and 9–10. Moreover, unexpectedly the helix 12 of VDR showed increased fluctuation. However, it should be noted that in this experimental setup a coactivator has been missing from the protein complex (see below). The binding to the natural VDRE from *CYP24A1* gene showed similar result except it seemed that 5'-AGGTCA-3' half-site was occupied by VDR and helix 12 of RXR was more stable. Unexpectedly, the stability of the heterodimer on *CYP24A1* VDRE was reduced although the binding affinities of the two VDREs are in the same magnitude. The interaction of the coactivator SRC1, that contained three LXXLL motifs, with the heterodimer bound to both 1,25D₃ and 9-*cis* RA increased the stability of VDR's helix 12 and helices 3 and 10–11 of RXR. Helices 3 and 4 of VDR cannot be further stabilized since they achieve maximal stabilization upon 1,25D₃ binding. For RXR the loop between helices 10 and 11 is important in the formation of the hydrophobic groove facilitating coactivator binding. Besides the classical charge clamp RXR contains the so called "aromatic clamp" consisting of residues in helices 3 (F437, F277) and 12 (F450) that is important for coactivator binding. As expected in the absence of both ligand no coactivator interaction was observed and the separate addition of the 1,25D₃ or 9-*cis* RA recruited the coactivator in a ligand-specific manner. Further HDX and cell based experiments showed that a simultaneous binding of the coactivator to both receptors is important and in the interaction with the RXR-VDR heterodimer only one SRC1 molecule is required (Zhang et al., 2011). This is in contrast to the SAXS derived model (Rochel et al., 2011). For the RXR-VDR-SRC1 complex the helix 12 of VDR has been stabilized upon addition of the DNA. In addition, HDX shows that the DNA-binding enhances the recruitment of SRC1 to RXR, thus the binding of the DNA stabilizes the recruitment of SRC1 to the whole heterodimer not just to the partner such as VDR.

Both, the SAXS and cryo-EM, studies highlighted the open architecture conformation in solution unlike it has been shown for the full length PPAR-RXR (Chandra et al., 2008). The recent crystal structure of the full length RXR α -LXR β on DR4 RE provides also a support for the open conformation of the NR heterodimer complex (Lou et al., 2014). It will have to be seen whether the closed PPAR-RXR complex on DR1 is an exception, although more plausible is the open conformation giving a rather high dynamic freedom for the NR heterodimer in the large transcriptional complex.

CONCLUSIONS

The main aim of this review was to collect and discuss structural data that is related to vitamin D signaling. The structural data for various isolated domains (LBD and DBD) show their organization on atomic level. This data is sufficient for understanding the particular ligand- and DNA-protein interactions but fails to provide spatial information on the mutual orientation of the domains of RXR-VDR on its natural promoters. It also fails to highlight inter-domain communication after DNA-,

ligand- or cofactor-binding. However, they are irreplaceable tools for structure-based drug design and mechanistic view of the VDR action. The dissemination of the information derived from structural data and *in silico* models may help to understand how VDR works in its natural settings and provides a space for the intervention in various diseases. The recent SAXS, Cryo-EM and H/D exchange studies with full length RXR-VDR complexes show more complex views on VDR function and provide first tools for the integration of structural information with genomic, epi-genomic, transcriptional and functional data.

FUTURE PERSPECTIVE

There are numerous scientific questions connected to VDR that may be interesting to answer on structural level. The list is not complete but here are some of them:

- Do the subtle differences between the various ligand-bound VDR complexes indeed represent the differences in the biological specificities and activities *in vivo*?
- Can the LBP related measurements and other descriptors be related to the potency of VDR ligands?
- Are there some additional natural compounds that are able to bind VDR and thus influence the metabolism and detoxification?
- What is the real role of the insertion domain in VDR?
- Is it possible to relate structurally the binding affinities of the RXR-VDR complexes with DNA?
- Are there other *in vivo* significant binding modes of VDR to the DNA such as monomers?
- What is the architecture of the RXR-VDR complex on non-DR3 VDREs and in indirect DNA interactions?
- How the recruitment specificity of the LXXLL containing proteins is regulated in the cell?
- How is the inter-domain communication precisely mediated upon ligand- DNA- and protein-VDR interaction?

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