Structural insights into the heterodimeric complex of the nuclear receptors FXR and RXR

Weili Zheng, Yi Lu, Siyu Tian, Fengge Ma, Yijuan Wei, Shuanghuang Xu, and Yong Li

From the State Key Laboratory of Cellular Stress Biology, Innovation Center for Cell Signaling Network, School of Life Sciences, Xiamen University, Fujian 361005, China

Edited by Wolfgang Peti

Farnesoid X receptor (FXR) is a member of the family of ligand-activated nuclear receptors. FXR plays critical roles in maintaining many metabolic pathways, including bile acid regulation and glucose and lipid homeostasis, and forms a heterodimeric complex with the retinoid X receptor (RXR). Despite the important roles of the FXR/RXR heterodimerization in human physiology, the molecular basis underlying the FXR/RXR interaction is still uncertain in the absence of a complex structure. Here, we report the heterodimeric structure of FXR and RXR in the presence of an FXR agonist (WAY-362450), RXR agonist (9-cis-retinoic acid), and a peptide derived from a steroid receptor coactivator (SRC2), revealing both unique and conserved modes for FXR heterodimerization. We found that the dimerization with RXR induced allosteric conformational changes on the coactivator-binding site of FXR. These changes enhanced the transcriptional activity of FXR by promoting the coactivator binding, thus suggesting a structural basis for the functional permissiveness of the FXR/RXR heterodimer complex. Furthermore, sequence analyses together with functional mutagenesis studies indicated that the helix H10 largely responsible for the dimerization is highly conserved and also critical for the FXR transcriptional activity. Our findings highlight the important roles of RXR heterodimerization in the nuclear receptor signaling, providing a potential framework to develop pharmaceutical agents in treating FXR/RXR-related diseases.

Farnesoid X receptor (FXR), also known as bile acid receptor, is a member of nuclear receptor family of ligand-activated receptors, one of the largest groups of transcription factors essential for a broad aspect of human physiology (1–3). Highly expressed in the liver, intestine, kidney, and adrenals, FXR is responsible for the regulation of the specific target genes involved in biological processes including bile acids, glucose, and lipid homeostasis and thereby has become an important drug target for the treatment of many FXR-mediated diseases. Treatment of FXR agonists has shown beneficial roles in regulating glucose homeostasis and insulin sensitivity (4, 5). Activation of FXR also reduces the triglyceride level in liver tissues, suggesting the therapeutic effects of FXR agonists in nonalcoholic fatty liver disease (6, 7).

With a highly conserved DNA-binding domain and a moderately conserved ligand-binding domain (LBD), FXR regulates gene expression through ligand binding in the LBD by recruiting or releasing specific coregulators, including coactivators such as the steroid receptor coactivator (SRC) family and corepressors such as NCoR (nuclear corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors), a conserved mechanism for all nuclear receptors (8, 9). Importantly, the LBD contains an activation function-2 (AF2) motif, which usually is the helix H12, whose conformation is critical for the nuclear receptors to interact with coregulators.

In addition to the coregulator recruitment, the activity of FXR is also modulated by the heterodimerization with the retinoid X receptor (RXR), receptors for the vitamin A metabolite 9-cis-retinoic acid (9-cis-RA), thus providing an additional level of regulation of the FXR signaling pathways (10). As such, FXR transduces signals as a heterodimeric complex with RXR by binding to the regulatory promoter region of its target genes. In addition to FXR, RXRs are also promiscuous partners of heterodimeric associations with other members of the nuclear receptor superfamily. RXR ligands transcriptionally activate independently on their own in the “permissive” subclass of heterodimers, whereas the partner agonists are required to mediate the activity of “nonpermissive” subclass of heterodimers (11, 12). The “permissive” heterodimer partners include nuclear receptors FXR, the peroxisome proliferator-activated receptors (PPARs) and liver X receptor (LXR). The examples of “nonpermissive” partners are nuclear receptors retinoic acid receptor, vitamin D receptor, and thyroid-hormone receptor.

Crystal structures of the many heterodimers structures, such as PPAR/RXR (13), retinoic acid receptor/RXR (14), LXR/RXR (15), PXR/RXR (16), constitutive androstane receptor (CAR)/RXR (17, 18), and thyroid-hormone receptor/RXR (19, 20) heterodimers, have revealed a common mode of dimerization...
**Heterodimerization of FXR and RXR**

Figure 1. The heterodimerization with RXR is important for FXR ligand binding and coactivator recruitment. *a*, dose-response curves of the FXR/RXR heterodimer and the FXR LBD to recruit coactivator SRC1 motifs in response to WAY-362450 measured by AlphaScreen assays. The EC$_{50}$ of WAY-362450 are 3.9 nM for FXR and 2.9 nM for FXR/RXR heterodimer to recruit SRC1, respectively. *b*, binding affinities of the SRC2 motif to the FXR/RXR heterodimer and the FXR LBD were determined by peptide competition assay in the presence of WAY-362450. The IC$_{50}$ for SRC2 peptide to inhibit the biotin-SRC1 binding into FXR is 1118 nM, and that for FXR/RXR heterodimer is 410 nM. The data are the means ± S.D. of three independent experiments.

**Results**

**Characterization of the FXR/RXR heterodimer**

Human FXR LBD with an N-terminal 6×His tag was expressed either in the presence or in the absence of an untagged RXRα LBD. To determine the functional activity of the purified proteins, we used an amplified luminescence proximity homogenous assay (AlphaScreen, PerkinElmer) to measure the interaction of the FXR monomer or FXR/RXR heterodimer with a peptide containing the second LXXLL motif from the coactivator SRC1, respectively. The FXR agonist WAY-362450 promoted the binding of a SRC1 coactivator motif to FXR LBD in a dose-dependent manner (Fig. 1a), with an EC$_{50}$ of ~3.9 nM, which is consistent with the highly potent nature of the WAY-362450 to FXR (21). Interestingly, the potency of the FXR ligand was enhanced for the FXR/RXR heterodimer compared with that for FXR alone, suggesting that RXRα increases the ligand-binding affinity of FXR. Similarly, the presence of the RXRα moiety in the heterodimer further increased FXR’s affinity for the SRC2 coactivator motif (Fig. 1b). These results on the purified LBDs of FXR and RXR are consistent with the permissive nature of the FXR/RXR heterodimer, highlighting the importance of the RXR heterodimerization for FXR ligand binding and coactivator recruitment.

**Structural determination of the FXR/RXR heterodimer**

To determine the molecular basis of the specific interaction and the permissive function of the FXR/RXR heterodimeric complex, we crystallized the FXR/RXR heterodimer in the presence of WAY-362450, 9-cis-RA, and a peptide derived from the coactivator SRC2. The structure was solved with two FXR/RXR heterodimer complexes in each asymmetry unit (Table S1; PDB code 5Z12). Fig. 2 shows two views of the overall dimeric arrangement of the FXR/RXR heterodimer, with each LBD adopting a helical sandwich fold that is conserved across the nuclear receptor family. Similar to other RXR heterodimers, the dimer interface is mainly composed of H10, as well as a few contacts with helices H7, H9, and the loops between helices H8 and H9, from both receptors. The interaction between FXR and RXR is thus stabilized by a combination of hydrophobic and polar interactions between the two receptors (Table 1).

Because PPARγ and LXRα share the highest sequence similarity with FXR in their LBDs as heterodimeric nuclear receptors, with 23.3 and 32.4% sequence identity in their LBDs, respectively (Table 2), we performed both sequence and structural comparisons of the heterodimer complexes of these three nuclear receptors (Fig. S1 and Fig. 3). Structural alignment of FXR/RXR with PPARγ/RXR (Fig. 3a; PDB code 1FM9) and LXRα/RXR (22) (Fig. 3b; PDB code 3FAL) revealed conserved dimeric arrangements with RMSDs of 0.422 and 0.469 Å, respectively. Despite the high similarity, the configuration of FXR/RXR interface displayed three distinct structural features (Fig. 3c). First, helices H7 and H10 of FXR shifted inwards, with closer contacts with RXR than those from PPARγ and LXRα, respectively. Second, the shifted helices H7 and H10 of FXR may result in the slightly larger heterodimer interface for FXR/RXR, which is ~1048 Å$^2$, whereas the interfaces are 980 and 538 Å$^2$ for LXR/RXR and PPARγ/RXR, respectively. Third, the helix H10 of FXR is shorted by two turns of α-helix, although within the LBDs, wherein the conserved dimer interface is largely comprised of the N-terminal halves of helix H10 from each receptor, as well as unique features specific for each dimeric complex. Despite rich structural knowledge for RXR heterodimerization among nuclear receptors and also the importance of FXR in drug discovery, the molecular basis that determines FXR/RXR interaction is still uncertain in the absence of a complex structure. In fact, the FXR/RXR heterodimer remains the last unsolved structure among all heterodimeric nuclear receptors.

In an effort to gain insights into the molecular mechanism of the FXR/RXR heterodimerization, we determined the crystal structure of the liganded FXR/RXR heterodimer in complex with coactivator peptides. The crystal structure shows both conserved dimerization interface for all heterodimeric nuclear receptors and also unique structural features specific for FXR/RXR. The structural analysis further reveals an allosteric mechanism through which RXR binding stabilizes the active conformation of FXR, leading to the enhanced FXR transactivation, a permissive nature of the FXR/RXR heterodimer. Our findings may help to understand how heterodimerization modulates FXR signaling and provide a framework for the rational design of high affinity ligands targeted against the FXR/RXR complex for use in the treatment of FXR-mediated diseases.
this difference on the helix H10 is not located in the dimer interface with RXR and thereby will not directly influence the heterodimerization of RXR with FXR, PPAR or LXR.

**Conformational changes of FXR induced by RXR heterodimerization**

The RXR heterodimerization is critical for the FXR activity. Superposition of the FXR/RXR heterodimer structure with the FXR monomer structure revealed the molecular basis for the increased coactivator binding potency (Fig. 1) by RXR heterodimerization (Fig. 4). Despite the high conservation of two FXR domains from the monomer structure and RXR heterodimer structure, respectively, with an RMSD of 0.677 Å (Fig. 4a), the dimerization with RXR induced allosteric conformational changes on the coactivator-binding site of FXR. Compared with the FXR in the monomer form, the active conformation of FXR AF2 of the heterodimer form is shifted inward (Fig. 4b), thereby stabilizing the microenvironment of the coactivator-binding site, likely resulting in the improved coactivator binding with FXR, which is a common mechanism for receptor coactivator binding.

**Table 1**

Interactions in the FXR/RXRα dimer interface

*Table 2*

The sequences of helix H10 involved in the dimer interface are strongly conserved among different nuclear receptors

The sequence identity was summarized, wherein the sequences of LBDs and helix H10 from FXR, PPARγ, and LXRα were aligned, respectively.

| Sequence identity (%) | LBD | H10 in the dimer interface |
|-----------------------|-----|---------------------------|
| FXR/PPARγ             | 23.3| 42.1                      |
| FXR/LXRα              | 32.4| 36.8                      |
| PPARγ/LXRα            | 23  | 31.6                      |
for nuclear receptors to recruit coactivators. This structural difference provides the structural mechanisms for the improved ligand-mediated coactivator recruitment and transactivation of FXR upon the dimerization with RXR, a permissive nature of the FXR/RXR heterodimer.

**Functional correlation of the FXR/RXR heterodimerization**

To validate the roles of RXR heterodimerization in FXR transactivation, we mutated several key residues on the heterodimer interface of RXR and tested the transcriptional activity of these mutated RXR in cell-based reporter assays using full-length FXR and the EcRE reporter (Fig. 5a and Table 1). The mutations of D379L and R426L/S427L were designed to disrupt the salt bridges and hydrogen bonds that stabilize the interaction between FXR and RXR. P423W mutation was designed to destabilize the heterodimer interface by changing the corresponding residue to a tryptophan with the bulky side chain. As shown in Fig. 5b, the addition of RXR further enhanced the FXR transactivation, either with or without ligand 9-cis-RA. Interestingly, all the mutations that were predicted to negatively impact the binding between FXR and RXR also substantially reduced the FXR transcriptional activity by either GW4064 alone or together with 9-cis-RA (Fig. 5b), further affirming the critical roles of RXR heterodimerization in the FXR transactivation and signaling.

**Conservation of RXR heterodimerization through helix H10**

Given the importance of RXR heterodimerization in the activity of heterodimeric nuclear receptors, we further analyzed the sequence conservation of helix H10, which lies at the core of dimer interface. Despite the conservation of FXR with PPARγ (23.3%) and LXRα (32.4%) in their LBDs, the sequence identities of helix H10 in the dimer interface are even higher than those of LBDs (Table 2), suggesting the conserved mode of RXR heterodimerization among different heterodimeric nuclear receptors. To further investigate the coevolution of FXR/RXR interface among different species, we selected five model animals for this study with the sequences of LBDs and helix H10 of FXR aligned, respectively (Fig. S2). The summary of sequence homology (percentage of sequence identity) revealed that helix H10 shares a higher sequence identity compared with the LBD (Table 3). The conservation of helix H10 of FXR was further validated by the ConSurf evolutionary conservation analysis (Fig. S3) (23). Our results thus suggest that the mode of RXR heterodimerization is also conserved in evolution and highlighting the critical roles of RXR heterodimers in nuclear receptor signaling.

**Discussion**

Nuclear receptors are important transcriptional factors with both differential and conserved mechanisms in regulating their...
activity. Because the distinct functional profiles of nuclear receptors in response to the binding of various ligands are largely determined by the selective use of transcriptional coregulators, which is regulated by the conformation of helix H12 of nuclear receptors (24, 25), the ligand-binding pockets and dynamic binding interfaces between the nuclear receptors and coregulators have been the focus of drug discovery studies. However, several alternative mechanisms also exist in regulating the activity of nuclear receptors. RXR heterodimerization is critical for the nuclear receptor signaling by regulating either the recognition and binding affinity of nuclear receptors on target genes, coregulator binding, and the transcriptional activity via permissive and nonpermissive mechanisms (26). As such, the elucidation of structural mechanisms underlying the RXR heterodimerization will be of great importance in understanding the functional regulation of nuclear receptors in various biological processes, which can also provide novel design strategy for the drug development with selective modulator activity.

Given the important roles of FXR in drug discovery, there have been intense studies on the therapeutic potentials of FXR ligands. Although many lead compounds have been discovered and some are under evaluation at various clinical stages (27, 28), the clinical use of FXR modulators has been tempered by side effects such as severe pruritus and liver injury. Therefore, a new drug-design strategy will be of great use by developing novel agents in modulating FXR activity allosteric pathways without directly targeting FXR ligand-binding pocket (29).

RXR plays a vital role in FXR regulation of downstream genes by forming heterodimer with FXR and predominantly binding inverted repeats spaced by one nucleotide (IR-1) (30). As a permissive partner for FXR/RXR heterodimer, the integration of RXR signals with FXR, which can produce either additive or synergistic effects. For example, the combination of RXR ligand acyclic retinoid and FXR ligand GW4064 synergistically inhibited the growth of HLE cells by inducing apoptosis (31). RXRα promote FXR recruit the methyl transferase PRMT1 by forming a heterodimer with FXR, which is important to bile acid homeostasis (32). However, the therapeutic potential of RXR ligands in the FXR signaling still remains poorly developed, partly because of the lack of structural characterization of the heterodimeric complex of FXR and RXR. Interestingly, our structural analysis revealed conformational changes of the FXR coregulatory-binding site induced by heterodimerization with RXR, leading to a more stabilized AF2 for improving the recruitment of coactivator (Fig. 4b). The importance of the allostERIC connection between dimerization and coactivator binding has also been validated by the reporter assays on the cell-based dimerization mutants in Fig. 5b. Our structure thus revealed a molecular mechanism for the critical role of RXR dimerization in FXR transactivation, which has also been observed on other heterodimeric nuclear receptors like PXR (16). As such, RXR ligands offer a promising strategy in fine-tuning FXR-mediated processes.

The FXR/RXR structure in this study, together with many other heterodimers structures solved, have enabled the integration of nuclear receptor signals transduced across the heterodimer interface. Structural analysis has shown that the N-terminal halves of helix H10 of the heterodimeric partners are in close contact with RXR and share a high structural similarity. In contrast, the C-terminal halves of helix H10, with structural variance between FXR and other nuclear receptors, are not involved in the dimer interface. Furthermore, sequence alignment, as well as ConSurf Server analysis, indicates that the helix H10 is one of the most conserved region in the LBDs among FXRs from different species and also different nuclear receptors, highlighting the important roles of RXR heterodimerization in the nuclear receptor signaling. Taken together, the structure of the FXR/RXR LBDs heterodimer provide a framework to develop the pharmaceutical agents in treating the FXR/RXR–related metabolic diseases.

**Experimental procedures**

**Protein preparation**

The human RXRα LBD (residues 225–462) was expressed from the T7 promoter of pACYCDUET-1 vector (Novagen), and the human FXR LBD (residues 243–472) was expressed as N-terminal 6×His fusion protein from the expression vector pET24a (Novagen). BL21 (DE3) cells transformed with these two expression plasmids were grown in LB broth at 25 °C to an A600 of ~1.0 and induced with 0.1 mM isopropyl 1-thio-β-d-galactopyranoside at 16 °C. The cells were harvested and sonicated in 200 ml off extraction buffer (20 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, and 25 mM imidazole) per 6 liter of cells. The lysate was centrifuged at 20,000 rpm for 30 min, and the supernatant was loaded on a 5-ml NiSO4-loaded HiTrap HP column (GE Healthcare). The column was washed with extraction buffer, and the protein was eluted with a gradient of 25–500 mM imidazole. The FXR/RXR LBD was further purified with a Q-Sepharose column (Amersham Biosciences), followed by gel filtration with a HiLoad 26/60 Superdex 200- pg column (Amersham Biosciences). To prepare the protein–ligand complex, we added a 5-fold molar excess of WAY-362450 (Targetmol, China) and 9-cis-RA (Sigma) to the purified protein, followed by filter concentration to 10 mg ml⁻¹. The FXR/RXR LBD was complexed with 2-fold molar of a SRC2 peptide (KHKLHRLIQDSS) before filter concentration.

**Coactivator-binding assays**

The binding of the various coregulator peptide motifs to FXR LBD in response to ligands was determined by AlphaScreen™ assays using a hexahistidine detection kit from PerkinElmer as described before (7). The experiments were conducted with ~20–40 nM receptor LBD and 20 nM biotinylated cofactor pep-
tides in the presence of 5 μg ml⁻¹ donor and acceptor beads in a buffer containing 50 mM MOPS, 50 mM NaF, 0.05 mM CHAPS, and 0.1 mg ml⁻¹ BSA, all adjusted to a pH of 7.4. EC₅₀/Iₐ₅₀ values for the effects of ligand binding to FXR or FXR/RXR were constructed from a nonlinear least square fit of the data based on an average of three repeated experiments. The relative affinity of a SRC2-2 peptide (KEKKHIL-HRLLQDSS) to FXR or FXR/RXR was determined by competition of a biotin-labeled second LXXLL motif of SRC1 (Biotin-SPSSHSLTERHKILHRLLQEGSP), with increasing concentrations of unlabeled SRC2-2 peptide in the presence of FXR ligand WAY-362450 and RXR.

**Transient transfection assay**

293T cells were maintained in DMEM containing 10% fetal bovine serum and were transiently transfected using Lipofectamine 2000 (Invitrogen). All mutant FXR plasmids were created using the QuikChange site-directed mutagenesis kit (Stratagene). Before 24 h of transfection, 24-well plates were plated (5 × 10⁴ cells/well). For nuclear receptor luciferase reporter assay, the cells were cotransfected with plasmids encoding full-length human FXR or FXR/RXR was determined by competition of a biotin-labeled second LXXLL motif of SRC1 (Biotin-SPSSHSLTERHKILHRLLQDSS) to FXR or FXR/RXR was determined by competition of a biotin-labeled second LXXLL motif of SRC1 (Biotin-SPSSHSLTERHKILHRLLQEGSP), with increasing concentrations of unlabeled SRC2-2 peptide in the presence of FXR ligand WAY-362450 and RXR.

**Crystallography and structure determination**

The crystals of FXR/RXR heterodimer complex were grown at room temperature in hanging drops containing 1.0 μl of the ligand–protein solutions and 1.0 μl of well buffer containing 0.2 M sodium thiocyanate and 25% (w/v) PEG 8000. The crystals were directly flash frozen in liquid nitrogen for data collection. The observed reflections were reduced, merged, and scaled with DENZO and SCALEPACK in the HKL2000 package (33). The structures were determined by molecular replacement in the CCP4 suite (34). Manual model building was carried out with Coot (35), followed by Refmac5 (36) refinement in the CCP4 suite.

**Author contributions**—W. Z., Y. Lu, S. T., F. M., Y. W., S. X., and Y. Li data curation; W. Z. and Y. Li formal analysis; W. Z. supervision; W. Z. and Y. Li validation; W. Z. and Y. Li investigation; W. Z. and Y. Li methodology; W. Z. and Y. Li writing—original draft; W. Z., Y. Lu, S. T., F. M., Y. W., S. X., and Y. Li writing—review and editing; Y. Li conceptualization; Y. Li funding acquisition; Y. Li project administration.

**Acknowledgments**—We thank the staff at BL19L1 of the Shanghai Synchrotron Radiation Source for assistance in data collection.

**References**

1. Makishima, M., Okamoto, A. Y., Repa, J. I., Tu, H., Learned, R. M., Luk, A., Hull, M. V., Lustig, K. D., Mangelsdorff, D. J., and Shan, B. (1999) Identification of a nuclear receptor for bile acids. Science 284, 1362–1365 CrossRef Medline

2. Parks, D. J., Blanchard, S. G., Bledsoe, R. K., Chandra, G., Consler, T. G., Kliever, S. A., Stimmel, J. B., Willson, T. M., Zavacki, A. M., Moore, D. D., and Lehmann, J. M. (1999) Bile acids: natural ligands for an orphan. Science 284, 1365–1368 CrossRef Medline

3. Wang, H., Chen, J., Hollister, K., Sowers, L. C., and Forman, B. M. (1999) Endogenous bile acid ligands for the nuclear receptor FXR/BAR. Mol. Cell 3, 543–553 CrossRef Medline

4. Zhang, Y., Lee, F. Y., Barrera, G., Lee, H., Vales, C., Gonzalez, F. J., Willson, T. M., and Edwards, P. A. (2006) Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. Proc. Natl. Acad. Sci. U.S.A. 103, 1006–1011 CrossRef Medline

5. Cariou, B., van Harmelen, K., Duan-Sandoval, D., van Dijk, T. H., Grefhorst, A., Abdelkarim, M., Caron, S., Torpier, G., Fruchart, J. C., Gonzalez, F. I., Kuipers, F., and Staels, B. (2006) The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. J. Biol. Chem. 281, 11039–11049 CrossRef Medline

6. Watanabe, M., Houten, S. M., Wang, L., Moschetta, A., Mangelsdorff, D. J., Heyman, R. A., Moore, D. D., and Auwerx, J. (2004) Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J. Clin. Invest. 113, 1408–1418 CrossRef Medline

7. Jin, L., Feng, X., Rong, H., Pan, Z., Jinba, Y., Qiu, L., Zheng, W., Lin, S., Wang, R., Wang, Z., Wang, S., Liu, H., Li, S., Xie, W., and Li, Y. (2013) The antiparasitic drug ivermectin is a novel FXR ligand that regulates metabolism. Nat. Commun. 4, 1937 CrossRef Medline

8. Lefebvre, P., Cariou, B., Lien, F., Kuipers, F., and Staels, B. (2009) Role of bile acids and bile acid receptors in metabolic regulation. Physiol. Rev. 89, 147–191 CrossRef Medline

9. Wang, Q., Blackford, J. A., Jr., Song, L. N., Huang, Y., Cho, S., and Simons, S. S. Jr. (2004) Equilibrium interactions of corepressors and coactivators with agonist and antagonist complexes of glucocorticoid receptors. Mol. Endocrinol. 18, 1376–1395 CrossRef Medline

10. Mangelsdorff, D. J., and Evans, R. M. (1995) The RXR heterodimers and orphan receptors. Cell 83, 841–850 CrossRef Medline

11. Shulman, A. I., and Mangelsdorff, D. J. (2005) Retinoid X receptor heterodimers in the metabolic syndrome. N. Engl. J. Med. 353, 604–615 CrossRef Medline

12. Yamada, S., and Kakuta, H. (2014) Retinoid X receptor ligands: a patent review (2007–2013). Expert. Opin. Ther. Pat. 24, 443–452 CrossRef Medline

13. Gamp, R. T., Jr., Montana, V. G., Lambeth, M. H., Miller, A. B., Bledsoe, R. K., Milburn, M. V., Kliever, S. A., Willson, T. M., and Xu, H. E. (2000) Asymmetry in the PPAR–RXR crystal structure reveals the molecular basis of heterodimerization among the nuclear receptors. Mol. Cell 5, 545–555 CrossRef Medline

14. Bourguet, W., Vivat, V., Wurtz, J. M., Chambon, P., Grommeyer, H., and Moras, D. (2000) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. Mol. Cell 5, 289–298 CrossRef Medline

15. Svensson, S., Ostberg, T., Jacobsson, M., Norström, C., Stefansson, K., Hallén, D., Johansson, I. C., Zachrisson, K., Ogg, D., and Jendehede, L. (2003) Crystal structure of the heterodimeric complex of LXRα and RXRβ ligand-binding domains in a fully agonistic conformation. EMBO J. 22, 4625–4633 CrossRef Medline

16. Wallace, B. D., Betts, L., Talmage, G., Pollet, R. M., Holman, N. S., and Redinbo, M. R. (2013) Structural and functional analysis of the human nuclear xenobiotic receptor PXR in complex with RXRα. J. Mol. Biol. 425, 2561–2577 CrossRef Medline

17. Suino, K., Feng, L., Reynolds, R., Li, Y., Cha, J. Y., Repa, J. I., Kliever, S. A., and Xu, H. E. (2004) The nuclear xenobiotic receptor CAR: structural determinants of constitutive activation and heterodimerization. Mol. Cell 16, 893–905 CrossRef Medline

18. Xu, R. X., Lambert, M. H., Wisely, B. B., Warren, E. N., Weinert, E. E., Waitt, G. M., Williams, J. D., Collins, L. M., Moore, L. B., Willson, T. M., and Moore, J. T. (2004) A structural basis for constitutive activity in the human CAR/RXR heterodimer. Mol. Cell 16, 919–928 CrossRef Medline

19. Putcha, B. D., Wright, E., Brunzelle, J. S., and Fernandez, E. J. (2012) Nuclear receptor CAR/RXR are shown to be antiparasitic drug ivermectin is a novel FXR ligand that regulates metabolism. Nat. Commun. 4, 1937 CrossRef Medline

20. Kojetin, D. J., Matta-Camacho, E., Hughes, T. S., Srinivasan, S., Nwachuku, J. C., Cavett, V., Nowak, J., Chalmers, M. J., Marciano, D. P.
Kamenecka, T. M., Shulman, A. I., Rance, M., Griffin, P. R., Bruning, J. B., and Nettles, K. W. (2015) Structural mechanism for signal transduction in RXR nuclear receptor heterodimers. *Nat. Commun.* **6**, 8013 CrossRef Medline

21. Flatt, B., Martin, R., Wang, T. L., Mahaney, P., Murphy, B., Gu, X. H., Foster, P., Li, J., Pircher, P., Petrowski, M., Schulman, I., Westin, S., Wrobel, J., Yan, G., Bischoff, E., Daige, C., and Mohan, R. (2009) Discovery of XL335 (WAY-362450), a highly potent, selective, and orally active agonist of the farnesoid X receptor (FXR). *J. Med. Chem.* **52**, 904–907 CrossRef Medline

22. Chao, E. Y., Caravella, J. A., Watson, M. A., Campobasso, N., Ghisletti, S., Billin, A. N., Galardi, C., Wang, P., Laﬂitte, B. A., Iannone, M. A., Goodwin, B. J., Nichols, J. A., Parks, D. J., Stewart, E., Wiethe, R. W., et al. (2008) Structure-guided design of N-phenyl tertiary amines as transrepression-selective liver X receptor modulators with anti-inﬂammatory activity. *J. Med. Chem.* **51**, 5758–5765 CrossRef Medline

23. Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., and Ben-Tal, N. (2016) ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Res.* **44**, W344–W350 CrossRef Medline

24. Gronemeyer, H., Gustafsson, J. A., and Laudet, V. (2004) Principles for modulation of the nuclear receptor superfamily. *Nat. Rev. Drug Discovery* **3**, 950–964 CrossRef Medline

25. Rocchi, S., Picard, F., Vamecq, J., Gelman, L., Potier, N., Zeyer, D., Dubouchaud, S. G., Desreumaux, P., Moras, D., Renaud, J. P., Chard, S. G., Desreumaux, P., Moras, D., Renaud, J. P., Blanchard, S. G., Desreumaux, P., Moras, D., Renaud, J. P., et al. (2001) A unique PPAR γ ligand with potent insulin-sensitizing yet weak adipogenic activity. *Mol. Cell* **8**, 737–747 CrossRef Medline

26. Pavlin, M. R., Brunzelle, J. S., and Fernandez, E. I. (2014) Agonist ligands mediate the transcriptional response of nuclear receptor heterodimers through distinct stoichiometric assemblies with coactivators. *J. Biol. Chem.* **289**, 24771–24778 CrossRef Medline

27. Mudaliar, S., Henry, R. R., Sanyal, A. J., Morrow, L., Marschall, H. U., Kopnes, M., Adorini, L., Sciaccia, C. I., Clopton, P., Castelloe, D., Dillon, P., Prazinski, M., and Shapiro, D. (2013) Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and non-alcoholic fatty liver disease. *Gastroenterology* **145**, 574–582.e1 CrossRef Medline

28. Neuschwander-Tetri, B. A., Loomba, R., Sanyal, A. J., Lavine, J. E., Van Natta, M. L., Abdelmalek, M. F., Chalasani, N., Dasarathy, S., Diehl, A. M., Hameed, B., Kowdley, K. V., McCullough, A., Terrault, N., Clark, J. M., Tonascia, J., et al. (2015) Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* **385**, 956–965 CrossRef Medline

29. Fernandez, E. J. (2018) Allosteric pathways in nuclear receptors: potential targets for drug design. *Pharmacol. Ther.* **183**, 152–159 CrossRef Medline

30. Grober, J., Zaghini, I., Fujii, H., Jones, S. A., Kliwer, S. A., Willson, T. M., Ono, T., and Besnard, P. (1999) Identification of a bile acid-responsive element in the human ileal bile acid-binding protein gene. *J. Biol. Chem.* **274**, 29749–29754 CrossRef Medline

31. Ohno, T., Shirakami, Y., Shimizu, M., Kubota, M., Sakai, H., Yasuda, Y., Kochi, T., Tsurumi, H., and Moriwaki, H. (2012) Synergistic growth inhibition of human hepatocellular carcinoma cells by acyclic retinoid and GW4064, a farnesoid X receptor ligand. *Cancer Lett.* **323**, 215–222 CrossRef Medline

32. Rizzo, G., Renga, B., Antonelli, E., Passeri, D., Pellicciari, R., and Fiorucci, S. (2005) The methyl transferase PRMT1 functions as co-activator of farnesoid X receptor (FXR)/9-cis retinoid X receptor and regulates transcription of FXR responsive genes. *Mol. Pharmacol.* **68**, 551–558 CrossRef Medline

33. Otwinowski, Z. a., M., W. (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **276**, 307–326 CrossRef

34. Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterson, E. A., Powell, H. R., et al. (2007) Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* **63**, 220–230 CrossRef Medline

35. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. *Acta Crystallogr. D Biol. Crystallogr.* **60**, 2126–2132 CrossRef Medline

36. Murshudov, G. N., Skubakov, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F., and Vagin, A. A. (2011) REFMAC5 for the refinement of macromolecular crystal structures. *Acta Crystallogr. D Biol. Crystallogr.* **67**, 355–367 CrossRef Medline