ROR γ structural plasticity and druggability

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Abstract

Retinoic acid receptor-related orphan receptor γ (ROR γ) is a transcription factor regulating the expression of the pro-inflammatory cytokine IL-17 in human T helper 17 (Th17) cells. Activating ROR γ can induce multiple IL-17-mediated autoimmune diseases but may also be useful for anticancer therapy. Its deep immunological functions make ROR γ an attractive drug target. Over 70 crystal structures have been published describing atomic interactions between ROR γ and agonists and inverse agonists. In this review, we focus on the role of dynamic properties and plasticity of the ROR γ orthosteric and allosteric binding sites by examining structural information from crystal structures and simulated models. We discuss the possible influences of allosteric ligands on the orthosteric binding site. We find that high structural plasticity favors the druggability of ROR γ , especially for allosteric ligands.

Keywords: ROR γ ; plasticity; druggability; orthosteric binding pocket; allosteric binding pocket.

1. Introduction

Retinoid-related orphan receptor γ (ROR γ) is a member of the nuclear receptor (NR) superfamily in the human genome, with two isoforms, the ubiquitous ROR γ and the thymus-specific ROR γ t. The first isoform is believed to act as a negative regulator of adipocyte differentiation to impact adipogenesis and insulin sensitivity [1]. The second isoform plays vital roles in promoting T cell differentiation into the T helper 17 (Th17) subtype and regulating IL-17A gene transcription in Th17 cells [2]. Overexpression of ROR γ and ROR γ t can lead to obesity-associated insulin resistance and multiple autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, Crohn's disease, and psoriasis. On the other hand, high ROR γ t activity can sometimes be beneficial and has been shown to enhance antitumor immunity by increasing IL-17, GM-CSF secretion, and decreasing PD-1 expression [3,4]. The pathological functions and the therapeutic potential of ROR γ make it an intriguing drug target.

The ROR γ isoforms only differ in the first 21 residues, which are truncated in ROR γ t. To simplify the discussion in this article, we use the term "ROR γ " to represent both isoforms and describe the ROR γ isoforms with residue numbers. Similar to other NRs, ROR γ is composed of an N-terminal domain (NTD, 1 - 30 a.a.), a DNA-binding domain (DBD, 31 - 95 a.a.), a hinge domain (HD, 96 - 265 a.a.), a ligand-binding domain (LBD, 266 - 505 a.a.) and a C-terminal domain (CTD, 506 - 518 a.a.). The knowledge obtained from other NRs facilitates scientists to

quickly recognize the orthosteric binding site located in the LBD. Under the engagement of pharmaceutical companies, including Merck, Takeda, and Biogen, in developing novel ROR γ -targeting drugs, hundreds of small-molecule modulators binding in the orthosteric site have been developed and structurally investigated. However, numerous highly active compounds were discontinued or suspended for further development in the stages of preclinical studies or clinical trials, owing to off-target effect, toxicity, and poor therapeutic efficacy [5] [6]. It is disappointing that no compound has yet passed Phase II clinical trials. More recently, two allosteric binding sites have been discovered in the LBD [7] and HD [8], and relative inverse agonists were developed. In contrast to the orthosteric site conserved in the NRs, these allosteric sites exist in unique binding pockets different from other NRs, resulting in better binding selectivity. Also, the non-overlapping locations of the sites avoid competition between allosteric inverse agonists and endogenous agonists, leading to lower dose usage in treatments and potentially developing fewer side effects [9]. Therefore, exploring allosteric ROR γ modulators is a hot research topic now.

The flexibility of a protein is necessary for accomplishing its function [10–12]. NRs are multidomain proteins, and activation of the transcriptional function is achieved through a signal initiated by an agonist ligand binding in the orthosteric site and then transferred from LBD to DBD under the mediation of the HD and coactivator proteins. The whole process involves multiple steps of intra- and inter-domain actions accompanied by conformational changes. Studying these changes will not only shed light on the mechanisms of protein activity but also aid in identifying new allosteric binding sites for drug discovery.

No full-length three-dimensional experimental structure of ROR γ is currently available. The LBD is the only domain that has been successfully co-crystallized with ligands. Over 100 crystal structures of ROR γ LBD-ligand complexes are published in the Protein Data Bank, including 69 orthosteric inverse agonists, 18 orthosteric agonists, and 9 allosteric inverse agonists. Many of the released modulators have been classified by chemotype and described in a recent review [13]. Here, complementarily, we focus on ROR γ itself and use the crystal structures as snapshots to glimpse its structural plasticity. We discuss the relationship between druggability and plasticity of ROR γ by covering its orthosteric and allosteric binding pockets. We also summarize computational and modeling results that provide more insight into structural dynamics and its role in druggability.

2. The plasticity of the ROR γ orthosteric binding site affects druggability

The orthosteric binding site of ROR γ is in the core of the globular LBD, and the pocket is mainly embraced by α -helixes H3, H5, H7, and H11 with the critical H12 in the vicinity of one pocket exit (Fig. 1). Since the putative endogenous agonists, cholesterol analogs (hydroxycholesterols [14], 4 α -carboxy, 4 β -methyl-zymosterol [15]), were found binding in this site, thousands of small molecules have been screened as potential ligands, and the structure-activity relationships (SARs) have been thoroughly studied. It is believed that a ROR γ agonist binding in the orthosteric site facilitates contact of the coactivator with the LBD surface, initiating the transcriptional function. In contrast, a ROR γ inverse agonist in the same site achieves inhibition

by preventing the coactivator binding to the LBD. Depending on the ligand type, H12 changes its conformation to act as an on-and-off switch. Meanwhile, the shape of the pocket is plastic and can demonstrate induced fit binding.

2.1 The dynamics of H12

For a long period, H12 was dominantly considered as a static helix distal from the orthosteric pocket in apo NR LBDs, basing on artificial crystal-packing interactions in co-crystals of the RAR γ LBD-ligand complexes [16]. This changed in 2003 when Kallenberger *et al.* proposed a more dynamic model based on tracking the H12 movement in the PPAR γ LBD by fluorescence anisotropy techniques [17]. They found that H12 is highly dynamic and disordered in the apo LBD, but agonist binding induces H12 to become a helix and lie on the pocket exit with a well-defined pose, facilitating recruitment of the coactivator to the LBD surface and consequently activating transcription.

This dynamic model also applies to the H12 behavior in the ROR γ LBD. NMR results showed that the H12 chemical shifts in the unbound LBD were missing or shifted in backbone ^{15}N -TROSY and methyl- ^{13}C -HSQC spectra, demonstrating disorder [18]. This was reflected in the difficulty of crystallizing the apo LBD in the absence of the coactivator [18,19]. HDX-MS results also demonstrated reduced dynamic properties of H12 when the ROR γ LBD transitioned from the apo state to the agonistic state, supporting a disorder-to-order transition [20]. However, the H12 dynamic properties in the apo and inverse-agonist states differed, suggesting that H12 may not have a discrete conformation when an inverse agonist binds in the orthosteric site. In some co-crystals of ROR γ LBD with inverse agonists, H12 could not be modeled due to missing electron density [21]; in many cases, H12 had to be truncated to cocrystallize the ROR γ LBD with inverse agonists [22–24].

The term “agonist lock” is introduced to describe the atomic mechanism of LBD agonism [22]. In the agonist-bound LBD, a hydrogen bond formed between His⁴⁷⁹ and Tyr⁵⁰² locks H12 in the correct position under the assistance of Phe⁵⁰⁶ (Fig. 2), facilitating coactivator recruitment. It is now commonly accepted that ROR γ orthosteric binders mainly regulate the transcriptional function by manipulating the stability of the lock. The results of molecular dynamics (MD) simulations further confirm it by showing lower fluctuations in interaction energy for the lock in the agonist-bound LBD and higher fluctuations for the lock in the unbound or inverse agonist-bound LBD [25]. Furthermore, they provide evidence for the existence of the lock in the unbound state, even if energetically unfavorable, implying that the H12 conformation in the unbound LBD crystal could be one of many conformations switching in solution.

2.2 The plasticity of the orthosteric binding pocket on accepting modulators

The pocket in apo ROR γ features a druggable volume of 940-Å³ with 61% hydrophobicity [18]. The agonists co-crystallized with the LBD only partially occupy it (Fig. 3). In contrast, the size and molecular weight features of ROR γ inverse agonists deviate more than the agonists. To

decipher the SAR rules, we take a closer look at the conformational changes of the orthosteric site induced by modulators.

Among 18 co-crystals of LBD-agonist complexes, all the LBDs share similar conformations with backbone C α RMSDs lower than 1.2 Å. The binding modes of the agonists are similar, even for the 12 which have diverse non-sterol scaffolds. The modulators interact non-covalently with H3, H5, H6, and H7 to stabilize the whole pocket and hydrophobically contact His⁴⁷⁹ while remaining distant from Tyr⁵⁰² (Fig. 4). Both the pocket and the agonist lock are also observed in the ligand-less state bound to a coactivator peptide. It suggests a rigid, uniform model of ROR γ LBD required to achieve activation, which must be considered in the structure-based drug discovery of ROR γ agonists. To date, the known agonists were found accidentally or were synthesized based on the scaffolds of inverse agonists. Virtual screening may be helpful for the discovery of diverse agonist chemotypes. We will discuss the applications of virtual screening in a later section.

We also compared the conformations of orthosteric pockets of LBD-inverse agonist complexes from crystal structures. Based on the pairwise backbone C α RMSDs, we assigned them into three pocket conformation modes (Fig. 5). Most of them are in Mode I with RMSDs < 2 Å, implying a stable pocket structure. The Mode I pocket shows minor global difference to the apo pocket, but we can still observe subtle conformational changes induced by modulators. The inverse agonists in this group pushed or pulled H3, H4, H5, and/or H7 to slightly deviate from the original positions (Fig. 6A). Although H11 retains the backbone pose, the side chain of His⁴⁷⁹ may flip (Fig. 6D), which breaks the agonist lock [26]. Even if the lock is retained, the shifts of H3, H4, and the side chains of Lys336 and Glu504 disfavor the non-covalent contacts to the coactivator [27,28]. In the Mode II models (RMSDs: 2 - 3 Å), the movement of the related helices becomes more evident (Fig. 6B), and the side chain of His⁴⁷⁹ points to the direction opposite to the agonist lock (Fig. 6E), leading to a broken lock [19,29]. For the Mode III pocket, in addition to the displaced helices alike to Mode II, part of H11 turns into a loop, where His⁴⁹⁷ is situated (Fig. 6C, F). This dramatic change impairs the lock [30,31].

These modes construct a noticeably plastic structure of the orthosteric pocket for accepting inverse agonists. The flexibility is contributed from the limited shifts of H3, H4, H5, and H7, and the more dramatic changes of H11, H11', H12. Because the helices contact each other, a moderate movement of one helix may cause a large scale shift. Most inverse agonists not only form hydrogen-bonds to or hydrophobically contact His⁴⁷⁹ to affect the agonist lock, but also indirectly affect the more displaceable helices through the more rigid helices, resulting in further pushing Tyr⁵⁰² away from His⁴⁷⁹ and complete dysfunction of the lock. Therefore, the pocket plasticity offers several discrete ways for inverse agonists to block protein activation and can accommodate a broad range of inverse agonist chemotypes. This suggests that discovering inverse agonists should be easier than agonists, which has turned out to be historically accurate.

3. The plasticity of ROR γ allosteric binding sites increases druggability

Drug companies have made extensive efforts to develop drugs targeting the ROR γ orthosteric binding site for treating autoimmune diseases and cancers, but numerous challenges have arisen in cell-based assays, animal experiments, and clinical trials. A serious problem is off-target effects. Despite that the agonist lock is unique to the ROR subfamily, the orthosteric binding site is structurally conserved in most NRs. Some modulators under development have been found to tightly bind other NRs. While chemists are trying to optimize the structures to improve selectivity, other researchers are transferring attention to investigate the druggability of allosteric binding sites.

3.1 The AF-2 allosteric binding site

Helix H12 in the ROR γ LBD is also named activation function-2 (AF-2) for its role in interacting with cofactors and regulating activation. In 2015, Scheepstra *et al.* identified a novel allosteric binding site on AF-2 when investigating the SAR of a series of ROR γ inverse agonists (**1**, **2**, **3**, **4**) (Fig. 7) [7]. This site is a shallow pocket nested by H3, H4, H5, H11, lidded by H11' and H12, and mainly filled with hydrophobic residues (Fig. 8). Ligands fit in the pocket through π - π stacking, hydrophobic effects, and several hydrogen bonds. They force H12 to relocate, which dramatically extends the distance between His⁴⁷⁹ and Tyr⁵⁰², for example, from 2.8 Å to 16.8 Å, and interrupts the agonist lock (Fig. 9). Recently, more active analogs and modulators with different scaffolds targeting the site have been developed. Interestingly, all allosteric inverse agonists directly hydrogen-bond to Ala⁴⁹⁷ and/or Phe⁴⁹⁸ on H11' with or without one-to-two more bonds to the residues on H3 and H4. Ala⁴⁹⁷ is not conserved in the ROR family, making it a potential interacting target for improving drug selectivity. There may be other ligand binding modes available in this pocket still waiting to be discovered.

We compared the binding modes of nine ligands. Eight ligands were co-crystallized with the LBD [7,32–35]. A ninth was modeled from MD simulations [36]. The eight co-crystal structures overlap with the backbone RMSDs < 1.5 Å, supporting the same conformation of the AF-2 allosteric pocket that occurs in all the structures. By comparing the volumes of the allosteric inverse agonists with the orthosteric ones, the pocket appears to be only tolerant to a narrow range of molecules. However, we believe the pocket can adjust to accept a broader range of ligands for two reasons. First, the pocket is surprisingly pliable. It is smaller in the unbound state until a ligand induces expansion. The structural difference between the unbound and the bound LBDs is 2.7 – 3.0 Å in backbone RMSDs, demonstrating a notable conformational change before and after ligand binding. Specifically, induced by **1** (Fig. 8), for instance, helices H3, H4, H5, and H11 shift towards the pocket center, H11' loses the helical shape, and H12 is pushed to the environment by **1**. Second, the AF-2 pocket is connected to the LBD orthosteric pocket through a short tunnel, that can accommodate parts of some ligands. The AF-2 pocket is also connected to the solvent environment by gaps between the helices, which allows ligands to protrude from the pocket. Thus, the pocket is extendable depending on the ligand chemistry.

For allosteric inverse agonists that bind in the AF-2 pocket, the mechanism for inhibiting transcriptional activation by ROR γ remains to break the agonist lock by disrupting the interaction

between His⁴⁷⁹ and Tyr⁵⁰². Like the orthosteric pocket, the flexibility of the AF-2 pocket mainly comes from the dynamic properties of H11, H11', and H12. However, some characteristics make the AF-2 pocket superior for drug targeting. In contrast to the buried orthosteric pocket, the AF-2 pocket is located on the surface of the LBD, which makes it directly accessible to ligands. The dynamic H11' and H12 helices act as a mobile forearm and hand to hold the ligand. What is more, H11' is more accessible for a ligand sitting in this pocket than the orthosteric one. H11' is another unique feature only existing in the ROR family and is not conserved between ROR α , ROR β , and ROR γ [18,36]. Targeting the residues in H11' provides opportunities for increased drug selectivity.

3.2 An allosteric binding site in the ROR γ HD

Previous research on NRs has revealed the vital role of the HD in the regulation of DBD-DNA interactions via posttranslational modifications [37–39]. It is not surprising that mutating Ser¹¹³ and Leu¹¹⁴ in the HD abolished ubiquitination at Lys⁹⁰, which is required for TH17 differentiation [40]. Based on this discovery, Lao *et al.* performed high-throughput screening (HTS) targeting the LBD and HD [8]. The SAR of the most active molecules (**10**) from the screen were explored through docking against a homology model of the full-length ROR γ structure created using I-TASSER software [41]. The docking results predicted binding in an allosteric pocket in the HD between the DBD and LBD, where **10** hydrogen-bonds to Gln²²³ and hydrophobically contacts Leu²⁴⁴. Unfortunately, no crystallography was performed to validate the predicted binding mode.

To the best of our best knowledge, this is the first report of an allosteric binding site in an NR HD. The paper only provides limited structural data on describing the allosteric pocket and the atomic mechanism of the inhibition. Thus, to understand the interactions of **10** with Ser¹¹³ and Leu¹¹⁴, we tried to reproduce the reported complex through docking **10** into a full-length ROR γ model also produced from I-TASSER (Fig. 10A). One of the top-ranked binding poses occupies a position close to Lao's but interacts with the surrounding residues differently (Fig. 10B-E). The carbonyl oxygen of **10**, instead of the carboxyl oxygen as shown in the paper, hydrogen-bonds closely to Gln²²³. Leu²⁴⁴ is too far to interact with **10**. In our protein model, Ser¹¹³ and Leu¹¹⁴ are located at the edge of the pocket. We propose that the ligand occupies the adjacent space and prevent the ubiquitination-related enzymes from accessing these two residues. Meanwhile, we found that the structure of the modeled LBD deviated significantly from the crystal structures, which would adversely affect the performance of docking. As a control experiment, we also docked **10** into an inverse-agonist bound LBD crystal structure [42] and found it was predicted to bind in the orthosteric site (Fig. 10F). We conclude that docking alone cannot decide the correct binding site for **10**. Further experimental research is needed to confirm whether it binds in the originally proposed allosteric site.

Despite the unresolved question of the proper binding site for **10**, the study by Lao *et al.* is important for raising the possibility of new allosteric binding pockets in ROR γ and other NRs. As a linker between the DBD and LBD, the HD is a flexible loop that may easily reshape itself to accommodate the chemistry of a ligand. Aside from the simulated model used above, I-TASSER

also produced two other top-ranked models with HDs adopting different conformations (Fig. 10A). **10** can be docked at different sites in the DBD-LBD gap (Fig. 10C, D). In the absence of a crystal structure, these simulated protein models represent potential conformations of the whole ROR γ , which may or may not correspond to reality. The docking results may not provide answers to the binding mode of **10** but do demonstrate a high degree of plasticity of a potential allosteric pocket in the HD. Additionally, the HD is the least conserved domain in NRs (even in the ROR subfamily). Hence, drugs targeting an allosteric site in the ROR γ HD may provide better selectivity.

3.3 Other potential allosteric binding sites

It is generally true that every member of the NR superfamily is activated by an agonistic signal transferred from the LBD to the DBD, mediated by a coactivator protein and the HD. Interrupting any step in this process can block activation.

In the agonist-bound conformation of ROR γ , the coactivator peptide binds to a shallow, hydrophobic pocket formed by H3, H4, and H12 of the LBD (Fig. 11). Its small surface area makes it a challenging site for drug design. Also, the pocket is sequentially and structurally conserved in the ROR family, suggesting low selectivity as a drug target site. However, it still can be useful in drug targeting by joining it to the neighboring AF-2 allosteric site (Fig. 12). This extended pocket introduces more contact area to ligands, providing opportunities to improve binding affinity.

In each NR, the DBD contains two zinc finger motifs that recognize and bind to a specific DNA sequence, called the hormone response element (HRE). Recently, Ban *et al.* reported developing an allosteric inverse agonist targeting the DBD of the androgen receptor by taking advantage of a pocket visible in the DBD-HRE complex crystal structure [43]. This raises the fascinating possibility of identifying allosteric DBD binding pockets in other NRs. Several questions need to be answered before attempting this for ROR γ . It is known that mutations at Ser³⁸, Lys⁵², and Lys⁹⁰ decrease the binding of the ROR γ DBD to the HRE [40,44,45]. The residues are situated in the zinc finger motif, SUMOylation site, and ubiquitination site of the DBD, respectively, which are potential candidate sites for drug targeting. Unfortunately, no co-crystal structure of the ROR γ DBD-HRE complex is available to explore the druggability of these sites. A more pressing issue is that the high conservation of the DBD (especially for these three residues) in the ROR subfamily reduces the opportunity of designing selective drugs.

4. The helices acting as the arm and hand of the LBD

Two ligand-binding sites have been identified in the ROR γ LBD, including the orthosteric site and the AF-2 allosteric site. Interestingly, the plasticity of both pockets is determined by the dynamic properties of helices H11, H11' and H12 as induced by modulators. When an agonist binds in the orthosteric site, the helices fix it in the pocket through forming a rigid triangular net constructed of hydrogen bonds with or without water mediation, hydrophobic packing, and π stacking effects. When an inverse agonist binds in either site, the triangular net is broken, and the helices display diverse degrees of freedom. Anchored by H10 in one end and restricted by the other helices, H11 can only move in a limited range of angles, serving as an arm in the LBD. In

contrast, H11' and H12 are as mobile as a free hand connecting to a moving arm. Because it is the C-terminal end of the protein, H12 is presumably more active than H11'. Alternatively, H12 may be bound to the LBD surface by interacting with either ligand and/or residues. Researchers must also be cautious about interpreting the dynamic properties of H12 due to artefactual crystal packing interactions in crystal structures. Nevertheless, H11' is found disordered in all co-crystals bound with allosteric inverse agonists and most of the co-crystals with orthosteric inverse agonists, whether H12 is in the helical conformation or not. H11' heavily relies on hydrogen bonding from H11 and H12 to retain it in the helical conformation, so disruption from modulators can release it from these restraints. Monitoring the conformational change of H11' may help to predict modulator activity.

5. The role of protein plasticity in the computer-aided discovery of ROR γ -targeting drugs

In contrast to the classical HTS method of screening chemicals for ROR γ -targeting drugs, virtual screening (VS) accomplishes the searching process *in silico* by docking a library of drug-like small compounds into a structural model, scoring each compound by mathematic algorithms. It immensely saves time and cost in the initial screening stages of narrowing down the chemical pool. In 2014, the Xu group conducted VS on a 220,000-compound database against the orthosteric pocket in an inverse-agonistic LBD crystal model using the Schrödinger Glide software, resulting in 115 hits [46]. In 2016, they did another VS to a five-times larger database against the same model by using Schrödinger Glide and Phase simultaneously, resulting in 28 hits [47]. Most of the hits showed moderate or strong activity in biological and biochemical assays, but the models of the atomic structures for the best hits were only modeled. The pharmacophore of an allosteric inverse agonist (**8**) bound in the AF-2 site was also discovered by VS against the crystal model induced by **1**, and its SAR was thoroughly studied by X-ray crystallography [35]. These illustrate the feasibility of applying VS in the discovery of ROR γ LBD-targeting modulators. It is important to note that a feature of VS is that the programs usually dock the compounds against a rigid model to speed up the screening process. It seems indifferent to the search of new agonists since the crystal models before and after ligand binding are almost identical. However, some potential inverse agonists could be omitted by the program if the docked model is remarkably different from the real shape of the bound pocket. Therefore, the plasticity of the pocket needs to be considered in VS to avoid missing some important hits. The state-of-the-art docking methods incorporate multiple model templates and machine learning scoring functions to markedly improve the effectiveness of predicting ligand activity and even the quantitative predictions of binding affinity [48].

Another *in silico* method, MD, has aided the study of the H11, H11', H12 flexibility affected by different types of ligands and the atomic mechanisms on activating or inactivating ROR γ and other NRs [25,49,50]. It is also an alternative method to study the SAR for a protein-ligand complex that lacks a co-crystal structure. For example, an MD simulation on the complex of **10** and the full-length ROR γ homology model (Fig. 10B) will provide a view on the possible atomic interactions, the induced conformational change of the protein, and the stability of the

complex, because the ligand may drop from the pocket during the simulation if the binding is not strong enough.

Conclusion

The ROR γ gene was first discovered in 1994 [51], and its role in immune homeostasis was unclear until 2006 [2]. Although enormous progress has been made to understand it structurally and biologically since that time, much mystery is still waiting to be uncovered. In this review, we highlighted the role of the protein structural plasticity in ligand binding and activity and drug design. ROR γ is highly plastic and can form multiple modulator binding pockets. Consequently, it adopts diverse conformations and fulfills different duties. This characteristic makes ROR γ a superb drug target for treating autoimmune diseases and cancers. X-ray crystallography is still the gold standard technique in studying atomic protein-ligand interactions, but its strict requirement on protein stability makes crystallization of the full-length ROR γ unreachable for now. Fortunately, other methods such as homology modeling and cryo-electron microscopy are developing to compensate for the deficiency. Other *in silico* methods, such as MD and VS, also play increasingly important roles in investigating the dynamics and drug screening of ROR γ . Under a combination of experimental and computational methods, we expect that the understanding of ROR γ will be substantially improved in the near future, and more ROR γ -targeting drugs will be developed with better clinical performance.

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Fig. 1 The three-layer folding structure of apo ROR γ LBD (PDB 5X8U, green) showing helix H12 near a coactivator peptide (orange).

Fig. 2 The agonist lock of ROR γ LBD.

Fig. 3 Basic physical parameters of RORgamma modulators.

Fig.4 No significant conformational change is observed between apo LBD (PDB 5X8U, gray) and the agonist-bound conformation of LBD (PDB 4S14, cyan).

Fig. 5 RMSD comparison diagram of helixes H1 - H11 (265-490 a.a.) in the inverse-agonist bound LBDs. Light green color represents RMSD < 2 Å, yellow for 2 - 3 Å, orange for 3 - 4 Å, and red for RMSD ≥ 5 Å.

Fig. 7 Allosteric inverse agonists binding in the AF-2 site.

Fig.8 . The location of the AF-2 binding pocket.

Fig.9 Tyr502 is pushed to a position too far away from His479 to form the agonist lock.

Figure 10. An inverse agonist (**10**) potentially binds in an allosteric pocket of the ROR γ HD. (A) The modulator **10** was docked into the pocket in three different I-TASSER-created full-length ROR γ homology models. (B - E) The top-ranked binding poses of **10** in different models are shown with non-covalent interactions to the surrounding residues. Residues Ser¹¹³ and Leu¹¹⁴ are presented in orange, while Gln²²³ and Leu²⁴⁴ are in light blue. (F) **10** is docked into the orthosteric pocket (3B0W).

Fig.11 The coactivator-binding pocket. The agonist-bound LBD structure (3KYT) is presented with the hydrophobic surface shown in red for high hydrophobicity and blue for high hydrophilicity.

Fig.12 The extended AF-2 binding pocket. The LBD structure (4YPQ) is presented with the hydrophobic surface shown in red for high hydrophobicity and blue for high hydrophilicity.