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The Promiscuity of Allosteric Regulation of Nuclear Receptors by Retinoid X Receptor

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Abstract

The promiscuous protein retinoid X receptor (RXR) displays essential allosteric regulation of several members in the nuclear hormone receptor superfamily via heterodimerization and (anti)cooperative binding of cognate ligands. Here, the structural basis of the positive allostery of RXR and constitutive androstane receptor (CAR) is revealed. In contrast, a similar computational approach had previously revealed the mechanism for negative allostery in the complex of RXR and thyroid receptor (TR). By comparing the positive and negative allostery of RXR complexed with CAR and TR respectively, we reported the promiscuous allosteric control involving RXR. We characterize the allosteric mechanism by expressing the correlated dynamics of selected residue–residue contacts which was extracted from atomistic molecular dynamics simulation and statistical analysis. While the same set of residues in the binding pocket of RXR may initiate the residue–residue interaction network, RXR uses largely different sets of contacts (only about one-third identical) and allosteric modes to regulate TR and CAR. The promiscuity of RXR control may originate from multiple factors, including (1) the frustrated fit of cognate ligand *9c* to the RXR binding pocket and (2) the different ligand-binding features of TR (loose) versus CAR (tight) to their corresponding cognate ligands.

Graphical abstract

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INTRODUCTION

Allosteric regulation is an essential biomolecular process occurring in biophysical activities and biochemical reaction networks.^{1–3} At the molecular level, allostery corresponds to a phenomenon of structural changes induced by the binding of effector molecule(s). Such changes of conformations include both the ground state shift and change of fluctuation around the ground state. Both cooperative ligand binding and allosteric enzymatics⁴ can be critical for various biological functions such as transport⁵ and signaling.^{6–8} Indeed, a special group of sensing proteins possess multiple, easily accessible conformations. Often, external signals and environmental changes can trigger these proteins to change their structure and/or flexibility.⁹ These “dynamic” proteins (molecular switches) are essential for the initial biological responses to many stimulations. Allostery (“communication” between different parts of a protein or protein complex) provides a trigger mechanism. This control of binding or catalytic properties at a remote site, due to the binding status of the effector site, can be essential for the regulation of biochemical reactions. Although the foundation of allostery has been established for nearly a century, many challenges still exist. New discoveries are being made even for well-studied systems, such as the oxygen binding to hemoglobin (Hb).⁵ For example, an extremely novel low-pH triggered transition of homotropic allostery (from cooperative binding to anticooperative binding of oxygen) was discovered to account for the root effect of fish Hb.¹⁰ Indeed, many advanced scenarios of allostery, such as multistate allostery,^{11–13} negative allostery,^{8,10,14} and promiscuity^{15,16} of allostery, have not yet been explored in detail. Fully understanding the mechanism of allosteric control in these more complicated, but practical situations can lead to rational drug design which can alter biochemical pathways and aid in the regulation of homeostasis.

The nuclear hormone receptor (NR) superfamily is a group of allosteric proteins that sense the presence of important small molecules such as lipophilic hormones and trigger the downstream event of gene transactivation accordingly.¹⁷ An extensive collection on NR research can be found at www.nursa.org. These proteins play important biological roles in development, differentiation, and metamorphosis.^{17–19} One novel aspect of allostery in the NR family is the promiscuous protein retinoid X receptor (RXR). As a common subunit of NR heterodimeric complexes, RXR displays essential allosteric regulation of many other members in the superfamily. Thus, it is one of the most important nuclear receptors. For example, RXR acts as an obligate heterodimeric partner with thyroid hormone receptor (TR)²⁰ and with constitutive androstane receptor (CAR).²¹ As shown in Figure 1, the ligand binding domain (LBD) of RXR forms heterodimeric complexes with the corresponding LBD of those NR proteins and influences the ligand-binding properties (and thus the activities) of these proteins through heterotropic allostery.²² Despite the fact that all LBDs of

the NR proteins share a structural fold of 12 helices and other common secondary structural features,²³ the binding of RXR and its cognate ligand *9c* has drastically different effects depending on RXR's binding partners. For example, the heterodimer RXR:TR can be activated upon the binding of cognate ligand *t3* to TR, however the transactivation levels decreases in the presence of *9c*-bound RXR. The molecular origin of this phenomenon can be traced to the negative cooperativity between these two binding sites, which was demonstrated using experimental structural biology and biophysical methods.²⁰ Intriguingly, RXR has different heterotropic allosteric effects for other NR proteins. While negatively regulating the binding event of TR with *t3*, RXR positively regulates CAR and its corresponding cognate ligand *tcp*.²⁴ Besides CAR and TR, RXR also affects more than a dozen other important nuclear receptors that recognize important ligands: PXR (pregnane X), PPARs (fatty acids), VDR (vitamin D), RAR (retinoic acid), etc.²¹ Thus, understanding the promiscuity of RXR (how one protein can be multifunctional and regulate multiple binding partners) is essential for NR biology.

Despite the importance of allostery in molecular biology, the direct and efficient methods of uncovering the mechanism are few. Often conventional (experimental and computational) techniques fall short in efficiently and cost-effectively identifying the regulatory mechanism of protein complexes, especially in more complicated scenarios of allostery. Here, we examine the allosteric control using a multiscale computational method: an atomistic molecular dynamics simulation and coarse-grained statistical analysis.²⁵ The all-atom modeling is important to faithfully track the small ligand-protein, intraprotein, interprotein, and protein-DNA interactions.^{26–30} Whereas the traditional bioinformatics approaches^{31,32} and direct coarse-grained simulations,^{33–35} though having various advantages, often miss the resolution to account for the specific chemistry of small molecules and fine details of protein structures that are essential for many allosteric mechanisms. The subsequent step of statistical analysis will perform data reduction and extract the correlation between different residue-residue (or ligand) contact events and express the allostery as collective modes of the forming and breaking of dynamic contacts.

Our method, termed CAMERRA (computation of allosteric mechanism by evaluating residue-residue associations), has been demonstrated to reveal the level of remote interaction between the modulating and functional sites along with potential communication channels or networks between them in two cases. Unlike a conventional analysis which calculates the covariance matrix of C_{α} atoms (a two-point correlation analysis),^{28,29} CAMERRA performs a higher order (4-point) correlation analysis, which is a new and clearer way to locate the correlated movements among four residues and the allosteric communications.³⁶ Particularly, we have applied CAMERRA to uncover the negative allosteric mechanism of the RXR:TR complex in a previous study³⁶ and the positive allostery of ricin subunits in another study.^{25,37} Here, we report the positive allostery of the RXR:CAR complex using the same protocol. Below, we will make a direct comparison between RXR:CAR and RXR:TR with the focus on the allosteric mechanism of the promiscuous RXR subunit.

SYSTEMS AND METHODS

Systems

To reveal the negative allosteric mechanism of RXR, we have previously studied the complex of the ligand binding domains, RXR(*9c*):TR(*t3*) [PDB: 3UVV], where ligand 3,3', 5-triiodo-L-thyronine (*t3*) is bound to TR, and 9-cis-retinoic acid (*9c*, a vitamin A metabolite) is bound to RXR.²⁰ In contrast, we have applied a similar method to study the current case of positive allostery using the complex RXR(*9c*):CAR(*tcp*) [PDB: 1XLS].²¹ The protein RXR and its cognate ligand *9c* is identical in each study. An identical human form of RXR (hRXR) is adopted in both complexes, while chicken TR (cTR) and mouse CAR (mCAR) are chosen as the other subunit of the dimer complex due to the availability of structural information and biophysical data. The agonist ligand of mCAR, 1,4-bis[2-(3,5-dichloro- pyridyloxy)]benzene (*tcp*, also termed *tcpobop*), an insecticide contaminant, is selected. Note that throughout the rest of the paper, we use the convention that protein names are capitalized and ligands are italicized. While there are clearly many other NR complexes involving human RXR that can be potentially studied in the near future, the comparison of the two systems (with known structure and allosteric properties) that we examined here can provide basic clues on the multifunctionality of RXR control.

Besides the modeling of the wild-type dimeric complex RXR:CAR, glycine mutants were used to enhance the sampling of conformational space and to scan the interaction between the binding pocket of RXR and *9c*. Because of the lack of an apo structure, RXR(0):CAR(*tcp*), one would need to perform a long time simulation to obtain such structures from a holo structure, with the ligand removed as the starting point. Also, the comparison of the apo form of RXR and other NR proteins suggests that the transition from apo to holo form involves large scale motion. A large part of it, the open/close of a “gate” of the ligand-binding pocket may not have a direct connection to allosteric motion. We thus focus on subtle perturbation and point mutations of the holo form to obtain a set of conformations that are very close to the holo form but are approaching the apo-form. Glycine mutants can remove the direct protein–ligand interactions and thus perturb the protein–ligand interaction at the residue–ligand level. These apo-approaching mutants used here are identical to those of RXR:TR complexes that we have studied previously.³⁶ As listed in Table S1 of the supporting information in ref 36, these 18 residues (V265, C269, A271, A272, Q275, L309, I310, F313, R316, A327, I345, F346, V349, C432, L433, H435, L436, F439) that constitute the binding pocket were selected by examining the crystal structure and representative snapshots of the wild-type RXR:TR. Through the simulations of these glycine mutants which have a weakened protein–ligand interaction, we can obtain a large ensemble of structures that are closer to the apo form ensemble. Note that the residue–ligand interactions may not be completely removed as glycine can potentially provide a backbone hydrogen bond with the carboxyl end of *9c*. Together, these side-chain deletion mutants allow us a more efficient detection of the allosteric mechanism expressed as residue–residue interaction networks.

Simulation Setup

For the RXR:CAR complex we used Chain A (RXR: human) and Chain E (CAR: mouse) of PDB 1XLS²¹ via the xleap module of AMBER 10.³⁸ The force fields for protein and water are AMBER ff99SB and TIP3P, respectively. There are four separate entities in each system: protein RXR (chain A, internal index 1–232, corresponding to standard index 227–458), protein CAR (chain E, internal index 233–474, corresponding to standard residue index 117–358), ligand *9c* (internal index 475) and ligand *tcp* (internal index 476). For a comparison, in the protein complex RXR(*9c*):TR-(*tcp*) examined in the previous study, we have the identical index of RXR while protein TR has internal index 233–491 (standard index 147–405).

The size of each system is roughly that of the wild type (92165 atoms, including 15 Na⁺ counterions and 28147 water molecules. The same number of water and counterions (except mutant R316G has one more Na⁺) was used for each mutant. The same solvated and energy-minimized wild-type structure was used as the starting point for every mutant studied here. From this structure, 18 single-point glycine mutant complexes were constructed with the xleap module. For each of the 19 systems (including wild-type), minimization, heating, and equilibration (for approximately 1 ns) were conducted and followed by a 25 ns NPT production simulation at constant room temperature and pressure (300 K and 1 atm) using NAMD2.7.³⁹ The temperature was regulated by the Langevin thermostat while the pressure was also held constant and regulated through Berendsen barostat. Long-range interactions were treated using the particle mesh Ewald method, and all bonds involving hydrogen atoms were constrained using the SHAKE algorithm. Snapshots are taken every 1 ps during production runs. The simulation protocol of RXR:CAR complexes and parameters are identical to those of RXR:TR. Although individual simulations of 25 ns are short, we hope that collectively 19 perturbative systems can provide an ensemble of conformations for capturing the allostery via CAMERRA. The level of convergence was studied in the previous report.³⁶

Analysis

Since we have reported in detail how we have applied the CAMERRA (computation of allosteric mechanism by evaluating residue–residue associations) method to study RXR:TR complex,³⁶ we will only mention the essential concepts and terminology that are needed to describe our results. Our central hypothesis is that allostery can be captured by the propagation of collective motions of a subset of dynamic residue–residue contacts (forming and detaching), as illustrated in Figure 2. Thus, we perform a higher order (4-point) correlation analysis to examine the correlated motion between four objects *i*, *j*, *k*, and *l*. For example, we want to inspect if residues *i* and *j* form contact when residues *k* and *l* form contact. In contrast, a traditional two-point correlation analysis often recognizes large-scale (soft-mode) domain motions of proteins and may not focus on the allosteric communication.

After gathering simulation snapshots, the first step of the CAMERRA analysis was selecting dynamic contacts. Since we are performing a 4-point correlation analysis, the computation complexity is $O(N^4)$ from principle, which is much higher than the traditional $O(N^2)$ of a typical C_α covariance calculation. Here *N* is system size (the number of residues). We need

to reduce the number of contacts to make the calculation manageable. We first read in each simulation snapshot and reduce the Cartesian information to contact information, that is, a size of $N \times N$ contact matrix u . Here $u_{ij}(t)$ can be 1 or 0 depending on whether two residues i and j formed a contact or not at time t . Contacts are deemed formed when any atoms of these two residues come closer than the distance cutoff 4.2 Å. We can then perform statistics of all the snapshots and isolate contacts that are, on average forming between 20% to 80% during the simulation. We term these group of (total m) contacts “dynamic contacts.” Thus, we filter out (from all possible contacts) those contacts that are rarely formed and those contacts that are always formed during the simulation. Thus, the computational complexity is reduced to $O(m^2)$.

RESULTS AND DISCUSSION

Since the results of RXR:TR systems have been reported previously,³⁶ we will focus on analyzing the data of RXR:CAR here. We will still mention the RXR:TR results whenever it is necessary in order to make a comparison between the two complexes.

The Selection of Dynamic Residue–Residue Contacts

As mentioned, the first step of tracking the (anti)cooperativity between two binding sites via CAMERRA is selecting “dynamic” contacts at the resolution of residue. To start, we rendered each simulation snapshot, which contains the Cartesian coordinates of all the atoms of the systems, to the binary information on residue–residue or residue–ligand contacts (either formed or not). For a system of N residues (including amino acid residues and ligands), there are a total $N \times (N - 1)/2$ pairs of potential contacts. For the RXR-(9c):CAR(*tcp*) system $N = 232 + 242 + 2 = 476$, while for the RXR(9c):TR(*t3*) system $N = 232 + 259 + 2 = 493$. By averaging over snapshots, we then calculated how often each of these contacts were formed, and selected the contacts that formed between 20% and 80% during the simulation. As a result, we found a total $m = 489$ and 494 dynamic contacts satisfying the cutoff criterion for RXR:TR and RXR:CAR, respectively. The nearly identical number of dynamic contacts being selected reflects the fact that while the RXR:CAR system has a smaller total residue number N , it has a more compact structure and thus has a higher number of contacts formed per residue.

Figure 3 displays the selected dynamic contacts on a contact map. One important question that we would like to address is whether or not RXR uses the same regulation mechanism to interact with CAR and TR. We can partially address this question by a direct comparison of the dynamic contacts selected for RXR:TR and RXR:CAR. We assume that the allosteric structural changes (including both the first order ground-state shift and the second order fluctuation effects) can be described by the collective motions of these dynamic contacts’ breaking and forming events. If the selected contacts of RXR:CAR and those of RXR:TR are different, we thus conclude that RXR uses different communication channel(s) for regulating CAR and TR. We found that there are 215 dynamic intraprotein contacts of RXR for the RXR:TR system while there are 219 for the RXR:CAR system. Only about a third of them are shared by the two sets of contacts. Several regions show drastically different patterns of contacts being selected. For example, the RXR:TR system has a cluster of

intraprotein contacts between H3 and H11, whereas the RXR:CAR regulation uses a cluster of contacts in H2 that interacted with H3.

Besides the intraprotein contacts of RXR, we also can make a comparison of the dynamic contacts between ligand *9c* and RXR. In the case of RXR:TR, four dynamic contacts (W305, I324, L433, F439) were selected. Here, we found two contacts (W305 and I324) that were obtained in the RXR:CAR complex. Although for the RXR:CAR complex, L433 and F439 did not meet the strict criterion for selecting dynamic contacts, both contacts are close to the cutoff values. The contact percentage of L433-*9c* was shown to only be slightly lower than the 20% while the F439-*9c* was slightly higher than 80%. Thus, it is interesting to point out that the RXR:TR and RXR:CAR complexes share some of the same sets of residues, and the dynamic contacts between protein–ligand is conserved.

For a comparison of the interprotein contacts between RXR:CAR versus RXR:TR, we located 46 interfacial dynamic contacts between the RXR:CAR system, whereas RXR:TR only has 33 dynamic contacts. For the RXR:CAR complex, these 46 interfacial contacts are formed by 28 distinct RXR residues with residues of CAR, whereas the RXR:TR interface only has 21 RXR residues that contribute to the 33 interfacial contacts of the RXR:TR system. Note that all 21 residues but one belong to the 28 interfacial residues of RXR selected from the RXR:CAR system. It seems that allosteric network of residue–residue interaction is stronger in the RXR:CAR system than in the RXR:TR system.

PCA Analysis of Contacts Reveals the Promiscuity of RXR Regulation

Parallel to the RXR:TR study, we performed CAMERRA analysis on the combined snapshots generated from 19 systems (wild-type + 18 mutants). We first calculated the covariance matrix C (size $m \times m$, $m = 494$) of dynamic contacts. Here, $C_{\alpha\beta}$ represents the correlation between contact events α and β ($= 1, 2, \dots, m$), that is, $C_{\alpha\beta} = \langle (u_{\alpha} - \langle u_{\alpha} \rangle) \times (u_{\beta} - \langle u_{\beta} \rangle) \rangle$. Contact status $u = 1$ when a particular contact is formed and 0 otherwise. A principal component analysis (PCA)⁴⁰ can diagonalize this symmetric matrix of size $m \times m$ and locate the most important collective motions of contact breaking and forming by identifying the largest modes of contact fluctuation.^{35,36} Each mode of motion is expressed by each eigenvector (principal component, PC) of matrix C , which expresses a mode of motion. The corresponding eigenvalue indicates the amplitude of the motion and thus the significance of that mode. Eigenvectors of contact PCA are analogous to the vibrational modes of Cartesian PCA.

A three-dimensional representation of the dominating allosteric motion of the RXR:CAR system (the normalized eigenvector PC1) is shown in Figure 4a. Here the dynamic contacts beyond the cutoff amplitude are shown as cylinders, which are colored by the values of the corresponding PC1 components. Here red indicates contact breaking while blue contact forming. It is very interesting to point out that the locations of “sensitive regions”, the high amplitude regions with strong red and blue cylinders, of PC1 for the RXR:CAR complex is drastically different from the PC1 result of RXR:TR reported previously. In the current RXR:CAR case, we can see that the most sensitive region is at the interface between RXR and CAR, especially between H10 and H9'. In comparison, the most sensitive region of

RXR:TR is the ligand binding pocket of TR where the second region is the binding pocket of RXR. There is relatively little interfacial communication in the RXR:TR case.

In Figure 4b, we focus on displaying the dynamics of protein–ligand interaction, i.e., the contact forming and breaking between binding pockets and their corresponding ligands. Since multiple protein residues are interacting with the same ligand, we use partial summation of the PC1 components to express the level of contact interaction change between the protein and ligand. We define the protein–ligand contact dynamics in mode PC1

as $a_k = \sum_i \sum_{j=1}^{i-1} PC1_{ij} \delta_{j,k} = \sum_i PC1_{ik}$. Here k is the internal index of the corresponding ligand and δ is the Kronecker delta function. We can see that ligand binding is positively correlated in the RXR:CAR system; that is, when the contacts between *9c* and RXR are formed, so are the contacts between *tcp* and CAR. This is in contrast to RXR:TR, which exhibits negative cooperative binding. It is important to emphasize the nature of values expressed by the ordinates displayed here. Since the eigenvectors are all normalized, a larger matrix (large m) will lead to relatively smaller values of PC1 components. The values themselves are sensitive to m , and they have little direct meaning and should be mainly used for comparing the relative values. To connect the normalized values to the range of fluctuation of these contacts, one can look at eigenvalues and PC projections, such as Figure 4 of ref 36. We estimate the amplitude of (anti)cooperative ligand-binding motion expressed in Figure 4b for RXR:TR and RXR:CAR is a fluctuation of a few contact units. Also, the sign of the PC is not important, a complete switch of red and blue (contact breaking and forming) still represents the same “vibration” mode.

Glycine Scanning Reveals Frustrated Fit Binding of RXR

Besides collectively contributing to the ensemble of conformations for PCA, these 18 mutants can be studied and compared to the wild-type to obtain the effect of perturbation. As we mentioned, these glycine mutants systematically weakened the protein–ligand *9c* interaction so we could study the apo form effect from the viewpoint of the holo form. Here, we focus on reporting the effect of mutation from two related properties (J_{50} and n_{CN}) that are derived directly from residue–residue contacts. Since both properties use contact data as input information, they fit into our scheme of analysis. The current results expressed using contacts are corroborated by data obtained from other methods such as computational B-factor analysis³⁶ and by experimental data as well.²⁰ As described previously, J_{50} indicates how many of the contacts are formed more than 50% during the simulation. Whereas, the number of contacting neighbors (n_{CN}) describes, on average, how many contacting neighbor residues each residue has, including sequential neighbors; that is,

$$n_{CN} = (2/N) \times \sum_{i=1}^N \sum_{j=1}^{i-1} \langle u_{ij}(t) \rangle$$

As shown in Figure 5, parameters J_{50} and n_{CN} show an overall positive correlation as they both are indicative of the level of contacts formed and larger values indicate more contacts are formed. Since the number of contacting neighbors is a per residue property, large ordinates indicate a more packed structure. In panel a, we can see that RXR:CAR is more packed than RXR:TR, whereas a larger J_{50} for RXR:TR indicates TR has a larger size than CAR and thus more contacts overall. Conventionally, a side-chain deletion mutant will

remove contact(s) and thus decrease the level of contacts. Thus, one typically expects all the mutants will be at the lower-left side of the wide-type. However, as we reported previously, the RXR:TR system showed that wide-type is less compact than all the mutants, which is consistent with B-factor analysis and experimental evidence showing that the apo form is more ordered than the holo form. We have argued that this natural “misfit” of the cognate ligand *9c* to the binding pocket is crucial for the negative allosteric control of TR-*t3* binding. The propagation of the frustration induced by *9c* to the TR side loosens up the structural integrity of the *t3* binding pocket. It is interesting to report here that in the case of RXR:CAR, the wild-type falls in the middle of Figure 5b. Since wild-type is not at the upper-right corner, it confirmed the effect of frustrated binding of the ligand *9c*. On the other hand, unlike the negative allostery, the wild-type did not show at the lower-left corner. We believe this phenomenon comes from two competing factors that affect the relative compactness of mutants. Although the presence of *9c* weakens some contacts for the wild-type system due to frustration, the positive allostery (cooperative binding) leads to more contact formation at other parts of the complex. Thus, the partial cancellation may provide the wild-type in having a median compactness relative to the mutants for the RXR:CAR system. The previous crystallography study reported that the CAR protein has an unusually small ligand binding pocket relative to other NR proteins.²¹ Thus, we postulate that the frustrated fit of *9c* to RXR can loosen up the overly tight binding pocket of CAR and induce positive allostery. Whereas, for TR, it loosens the binding pocket of TR and induces negative allostery, which is corroborated by experimental evidence.²⁰ By comparing the relative position of individual mutants in Figure 5 panel b versus panel c, we can see the order of compactness is partially preserved. For example, the positions of H435G and C269G are consistently high in these panels. Both residues (H435 and C269) are bulky and close to the aromatic end of *9c*, thus the glycine replacement alleviates the frustration.

CONCLUDING REMARKS

Multifunctional allosteric signaling proteins such as RXR play essential regulatory roles in biology. To decode how the LBD domain of RXR is able to have positive allosteric control over a group of proteins while having negative allosteric control over another group, we studied cooperative binding of complex RXR(*9c*):CAR(*tcp*) (wild-type and a group of glycine scanning mutants). We also compared our results with the previously obtained data on the anticooperative binding of complex RXR(*9c*):TR(*t3*). We applied the method CAMERRA that isolates important allosteric modes by performing PCA on selected dynamic contacts. The two-stage scheme of CAMERRA [(1) selecting dynamic contacts and (2) rendering important contact collective modes] was able to uncover the allosteric mechanism and distinguish the positive versus negative allostery. We found that the “frustrated fit” of *9c* to the binding pocket RXR is a common feature observed in both systems. However, the dynamic intraprotein contacts (of RXR) selected are largely different (about two-thirds of the contacts). Further, the allosteric modes and sensitive areas expressed by PC 1s for these two systems are drastically different. Ultimately, the difference between the response of TR and the response of CAR may originate from the relative tightness of their binding pocket.

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Figure 1. Binding of RXR and its cognate ligand *9c* has different effects on TR versus CAR. (a) The negative heterotropic allostery of RXR(*9c*):TR(*t3*) complex (upper) and the positive heterotropic allostery of RXR(*9c*):CAR(*tcp*) complex (lower). The structure of the corresponding complexes are shown in panels b and c.

Figure 2.

Method of CAMERRA can locate allosteric motions by tracking the correlated dynamics of residue–residue contacts. As shown in panel a, there are contacts breaking (red) and forming (green) between two residues (dark dots) and between the residue and ligand (light dots) during a simulation. Correlated contact formations between the protein and ligand indicates a cooperative binding (b). The correlation of a contact formation at one binding pocket and the contact breaking at another indicates an anticooperative binding (c).

Figure 3.

Comparison of dynamic contacts. (a) The dynamic contacts obtained between residues (internal index) for the RXR:CAR (black circle) and RXR:TR (red cross) systems. (b) The zoomed-in view of the intraprotein contacts of RXR. Here, the mapping to the nomenclature of the standard secondary structure²³ is also shown on the x -axis.

Figure 4.

(a) The dominating mode (PC1) of covariance matrix of the dynamic contacts is displayed in a three-dimensional representation (two different viewpoints). The components of PC1 are displayed as color-coded cylinders between residues in the complex structure. Note that only those with >0.03 or <-0.03 are shown explicitly here and we have omitted to display a few contacts with near zero values (white cylinders). (b) The comparison of the dynamics of protein–ligand contacts in eigenvector PC1. The ordinate is the normalized contact dynamics (sum of selected PC1 elements involving protein–ligand contacts). The same sign (both

positive) of contact dynamics indicates the cooperative binding nature of the ligand binding. As a comparison, the corresponding results³⁶ of RXR(*9c*):TR(*t3*) shows anticooperative binding and negative allostery.

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Figure 5.

Effect of mutation on contact formation: J_{50} versus n_{CN} . The overall comparison is shown in panel a, while the zoomed-in views on the RXR:CAR and the RXR:TR systems are shown in panels b and c, respectively.