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Insight into Estrogen Receptor Beta-Beta and Alpha-Beta Homo and Heterodimerization: A Combined Molecular Dynamics and Sequence Analysis Study

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Abstract

Biological effects of estrogenic ligands are transduced by two estrogen receptors, ER α and ER β ; they transactivate as dimers. Since ER $\beta\beta$ and ER $\alpha\beta$ homo and hetero dimers are known to exhibit anti-proliferative effects, we characterized their dimerization interface in atomic details and explored their ligand induced conformational dynamics. ER $\alpha\beta$ heterodimer is found to be relatively more stable than the ER $\beta\beta$ homodimer and the observed differences are mainly due to loop dynamics. The principal component analysis reveals that, in the essential subspace, the homo and hetero dimer dynamics are distinctively different. The core recognition groove of the dimer interface, formed by helix 9 and helix 10/11, remains unaltered in both homo and hetero dimers. The dimerization surfaces are found to be highly conserved in eukaryotic lineages. Phylogenetic patterns for ER α appear to be very much similar to that of ER β which signifies that the formation of functional heterodimer is evolutionary selected.

Keywords

Estrogen receptors; Homo and hetero dimer; Molecular dynamics; loop dynamics; Phylogeny; Evolution

1. Introduction

Biological functions of estrogenic compounds are generally modulated by two estrogen receptors, namely ER α and ER β . Both the proteins play crucial role in various physiological functions in the reproductive tract, breast, prostate, bone, brain and the cardiovascular system [1-2]. ER β is expressed mainly in the central nervous system, cardiovascular system, immune system, urogenital tract, gastrointestinal tract, kidneys and lungs [3-7]. ER α , on the

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other hand, is mainly expressed in mammary glands [8]. It is to be noted that ER β also shows a considerable expression level in mammary glands [9]. Literature reveals that the biological functions of ER β are quite distinct from the ER α . ER α is known to induce the transcription of pro-proliferative target genes whereas several studies indicate that ER β exerts anti-proliferative and pro-apoptotic [10-12] effects. ER β is known to act as growth modulator in ER α positive breast cancer cells [13]. Despite their marked differences of biological function, both the ER α and ER β share a very similar structural topology and a common mechanism of transactivation. Both ER α and ER β contain five major domains: an N-terminal activation function-1 domain (AF-1), a central DNA-binding domain (DBD), a hinge region, a C-terminal ligand-binding domain (LBD) and a C-terminal trans-activation domain (AF2). The LBD is considered to be the key structural domain that controls ligand-dependent regulation of ER signaling. Ligand binding in the LBD induces conformational changes that facilitate receptor dimerization, either as homodimer (ER α /ER α or ER β /ER β) or heterodimer (ER α /ER β), translocation of dimers to the nucleus, and binding with co-regulatory proteins [14]. This supra-molecular assembly finally interacts to the estrogen response element (ERE) in the promoter region of target genes [14]. Receptor dimerization is one of the crucial steps in ER transactivation and hence the physiological responses of a particular ER depend on the activation of a particular dimer pair by the ligand [13]. Homodimerization of ER α accelerates cellular proliferation [13]. On the other hand, the transcriptional activation of ER β homo-dimer is thought to be protective against hormone-dependent diseases including breast and prostate cancers [10-12]. Additionally, ER β is thought to counteract the stimulatory effects of ER α through the formation of functional hetero-dimer with ER α [15-16]. Experimental evidence suggests that hetero-dimer induced activation of target genes are markedly different from those induced by homo-dimers [17-18]. Thus, the activation and agonist induced dynamics of ER $\alpha\beta$ hetero-dimer and ER $\beta\beta$ homo-dimer need to be explored to design more potent ligand that selectively activate the ER $\alpha\beta$ hetero-dimer and ER $\beta\beta$ homo-dimer.

Structural details of ER α /ER β hetero-dimer are not known due to non availability of crystal structures. Accordingly, the effect of ligand binding in hetero-dimers was never been explored. Recently, we have characterized the essential dimer contacts of ER α homo-dimer in atomic details using *in silico* modeling approach [19]. It is to be noted that the crystal structures of ER β homodimer in the presence of several bound antagonist are available in Protein Data Bank. But the structural dynamics of ER β homodimer in the presence of bound agonist was less explored. In this paper, we characterize the ER $\beta\beta$ homo-dimer and ER $\alpha\beta$ hetero-dimer and their dimerization interface in atomic details using *in silico* modeling techniques. We also explore the ligand induced conformational dynamics of both homo and hetero dimers using all atom molecular dynamics simulation. Our simulation reveals a similar activation mechanism of ER homo- and hetero-dimers in terms of receptor dimerization. We have further explored the sequence similarities and phylogenetic relations between the two ER receptors. The dimerization surface is highly conserved in both the receptors. The observed phylogenetic pattern for ER α parallels with the ER β signifying co-evolution of both the proteins.

2. Materials and Methods

2.1. Modeling the ER homo and hetero dimer

The crystal structure of ER β LBD homo-dimer (PDB ID: 1U3S) where each monomer is bound with a highly potent and selective ER β agonist ligand aryl diphenolic azole (WAY-797) has been considered as ER β LBD dimer agonist conformation in our study. In this structure, Helix 12 is positioned properly to accommodate co-activator proteins. In the crystal structure, a considerable section of residues in the dimerization surface were missing. In chain A, residues 412-419 and in chain B, residues 410-421 were missing. All the missing residues were modelled by using MODELLER 9.9 [20]. No residues were added in the N and C-terminal of the dimer structure. Thus, our final model structure contains 235 residues (263-497) for each chain of ER β dimer.

Crystal structure does not exist for ER α β hetero-dimer. To model one, we primarily superimposed a monomer from ER β LBD homo-dimer (PDB ID: 1U3S) on a monomer from the crystal structure of ER α LBD homo-dimer (PDB ID: 3ERD). This is to emphasize that both 3ERD and 1U3S represent agonist conformations of ER α and ER β , respectively.

2.2. Molecular dynamics simulations

All simulations were performed using GROMACS [21-22] molecular dynamics code with OPLS [23] force field. The parameters for diethylstilbestrol (DES) and aryl diphenolic azole (797) were developed according to the OPLS force-field. Atom definitions, atom types and partial charges were assigned according to the symmetry group analogy in OPLS-AA set [23]. The atomic partial charges are readjusted to maintain the charge neutrality of the whole molecule. The parameters are tested by comparing the GROMACS energy minimized structures with the respective crystal structures. Both the homo and hetero dimers were initially subjected to a short energy minimization *in vacuo* using steepest descent algorithm to remove any bad contacts for added hydrogens. Both the systems were then solvated with SPC explicit water model in a cubic box with periodic boundary conditions. The box dimensions are chosen such that all the protein atoms are at least 1 nm away from the edge of the box. The ionization state of residues was set to be consistent with neutral pH and counter ions were added to neutralize the system. The solvated system was then subjected to a second short energy minimization using steepest descent algorithm to eliminate any bad contacts with added water. After that, a 100 ps position restrained dynamics was carried out where the complex was restrained by restraining forces while the water molecules were allowed to move. It was then followed by 20 ps of NVT simulation at 300 K and 20 ps of NPT simulation to achieve proper equilibration of the simulated system. Final production simulations were performed in the isothermal-isobaric (NPT) ensemble at 300 K, using an external bath with a coupling constant of 0.1 ps. The pressure was kept constant (1 bar) by using pressure coupling with the time-constant set to 1 ps. The LINCS [24] algorithm was used to constrain the bond lengths involving hydrogen atoms, allowing the use of 2.0 fs time step. Electrostatic interactions were calculated using the Reaction-Field method. Van der Waals and Coulomb interactions were truncated at 1.4 nm and the trajectories were stored at every 5 ps.

Structural analysis were carried out by using the in-built tools of GROMACS and the secondary structure assignments were performed with DSSP [25] module integrated with GROMACS. The RMSD matrices were computed on each of the trajectories by the least square fitting of main-chain atoms and the matrices were then processed to extract clusters of similar conformations.

2.3. Principal component analysis (PCA)

Principal Component Analysis (PCA) was performed on each of the dimer trajectories obtained from the MD simulations in order to obtain an insight into the essential dynamics of the simulated system in reduced sub-space. Mass-weighted covariance matrix of atomic positional fluctuations was calculated from each MD trajectory on C α atoms. Then the essential subspace was defined in terms of the calculated eigenvectors.

2.4. Identification of Hotspot residues at the dimer interface

The essential protein-protein interaction interfaces of the homo and hetero dimers were explored by using HotPoint online web-server [26]. The server also predicts hotspot residues that are involved in crucial interactions in the dimer interface.

2.5. Multiple sequence alignments and Phylogenetic analysis

ER α and ER β protein sequences of different organisms were obtained from NCBI (www.ncbi.nlm.nih.gov) protein sequence database. Redundant sequences were removed by using the CD-HIT program [27] using a sequence identity cut-off of 0.95. Multiple sequence alignment (MSA) was carried out using MUSCLE [28] program. The MSA was then used to draw the phylogenetic tree using PhyML [29] web-interface and the generated tree was rendered using TreeDyn [30] program.

3. Result and Discussions

3.1. Analysis of structural stability of homo and hetero dimers during MD simulation

Molecular dynamics simulations have been performed on both ER $\beta\beta$ homo-dimer and ER $\alpha\beta$ hetero-dimer to provide insights into the stability and structural dynamics of the dimers in presence of bound agonist ligand. Fig. 1 depicts the variation of dynamic parameters (RMSD and radius of gyration, R_g) of the simulated system as a function of time.

A root mean square deviation (RMSD) of the protein over the simulation time has been used to analyze the stability of the simulated system. As evident from Fig. 1A, during first 1ns of the simulation, both the systems undergo structural readjustment according to its environments and bound ligands and reach the equilibration. RMSD profiles obtained from MD simulation reveal that ER $\alpha\beta$ hetero-dimer is comparably more stable than the ER $\beta\beta$ homo-dimer. Radius of gyration (R_g) is the mass-weighted root mean square distance of a collection of atoms from their common centre of mass and it defines the overall shape and dimensions of the protein. The plot of variation of radius of gyration of each LBD dimer with simulation time is shown in Fig. 1B. As evident from the figure, ER $\alpha\beta$ hetero-dimer is more stable than the ER $\beta\beta$ homo-dimer which is reminiscent with the observation obtained in RMSD analysis. The R_g of ER $\alpha\beta$ hetero-dimer remains stable at 2.27 ± 0.007 nm

throughout the simulation period signifying a highly stable dimer complex. On the other hand, for ER $\beta\beta$ homo-dimer, during the initial 4ns of simulation, the R_g increases from 2.23 nm to 2.30 nm and in the remaining simulation time it stabilizes at 2.30 ± 0.011 nm. To gain a detailed insight into all the visited conformations during MD simulation for both homo and hetero-dimers, we have computed the RMSD matrices and the results are displayed in Fig. 1C & D. ER $\alpha\beta$ hetero-dimer mainly exists in two very similar conformational clusters. The first cluster represents dimer conformations from first 4ns of simulation. Second one represents conformations of the remaining simulation time. It is to be noted that the conformations between these two clusters are also very similar with an overall RMSD difference of 0.2 nm. In the case of ER $\beta\beta$ homo-dimer, we obtained three conformational clusters from MD simulation. Homo-dimer conformations from first 4ns of simulation form the first cluster. In between 4 to 6 ns of simulation time, the dimer undergoes appreciable conformational changes compared to the initial conformation to generate the second cluster. The average RMSD of the ER $\beta\beta$ homo-dimer between the two conformational clusters is ~ 0.25 - 0.3 nm. In the remaining simulation time, the dimer undergoes further conformational changes to generate the third cluster with an overall RMSD difference of 0.3 - 0.4 nm from the initial structure.

3.2. Residue fluctuation analysis

We then analysed the RMSF (root mean square fluctuations) per residues to identify the regions responsible for the observed structural differences in the ER homo- and hetero-dimer during MD simulation. Also RMSF helps us to understand the structural basis of the observed high fluctuation in RMSD for ER $\beta\beta$ homo dimer. Results are summarized in Fig. 2.

In general, the residue fluctuations for ER $\beta\beta$ dimer are higher than ER $\alpha\beta$ hetero-dimer. It is to be noted that there is a sequence difference between ER α and ER β . Also, the size of their LBD is slightly different: ER α LBD comprises of 245 residues while ER β LBD comprises of 235 residues. Fig 2A reveals that the RMSF profiles of both homo and hetero dimers are qualitatively very similar though the number of amino acids and the sequences are not the same for the two proteins. We then analyzed the critical differences between the two RMSF profiles to infer the observed high RMSD for ER $\beta\beta$ homo-dimer. We found that the high fluctuations of the homo-dimer are mainly due to the loop dynamics; no structural uncoiling has been observed in the MD simulation. Particularly, the loop region between the sheet and helix 6 of chain A in ER $\beta\beta$ homo-dimer (from residue 100 to 117) is found to be highly flexible during the MD simulation but the region is not much flexible for ER $\alpha\beta$ hetero-dimer. Another region of interest is the long loop region between helix 8 and helix 9 (from residue 150 to 166). This region is found to be flexible in both the monomer of the homo and hetero dimer. It is to be noted that more flexibilities have been observed for this region in the homo-dimer in comparison to the hetero-dimer during MD simulation. Also, Helix 12 has been found to be more flexible in ER $\beta\beta$ homo-dimer compared to the hetero-dimer. Recently, we explored the essential dimerization surface between ER $\alpha\alpha$ homo-dimer and found that core recognition groove is formed by helix 9, helix 10 and 11 [31]. Here we observed that the core dimer interaction groove is highly stable for the both homo and hetero dimers during MD simulation.

3.3. Analysis of secondary structure of the dimers during MD simulation

We have further analyzed the secondary structure evolution of the homo and hetero-dimers during the simulation. It is to be noted that the chain B represents ER β in both the homo and hetero dimers and chain A represents ER α in ER $\alpha\beta$ and ER β in ER $\beta\beta$. The secondary structural profiles of chain B is very much similar in ER $\alpha\beta$ and ER $\beta\beta$, as was expected. Alterations in the secondary structures have been observed in chain A. There are more helical content in ER α compared to ER β . Particularly, for homo-dimer, the N-terminal portion of helix 6 loses its helicity in later period of the simulation and appears as helical turn. Another region of interest is the loop connecting helix 8 and helix 9. In the later period of simulation this loop length increases and some of the N-terminal residues of helix 9 lose helicity and appear as turn/bend which is in accordance with the RMSF profile for homo-dimer (Fig. 2). It is to be noted that the general helical topology of both the homo and hetero-dimers remains unaltered during the simulation; only some of the linker helical regions attached with the loop region lose their structure during the later period of simulation for the hetero-dimer.

3.4. Principal component analysis (PCA)

Recently, we have demonstrated that the essential dynamics of ER $\alpha\alpha$ homo-dimer is highly dependent on the nature of the bound ligand in the LBD [31]. The essential motion of the ER $\alpha\alpha$ homo-dimer with bound SERM (4-OHT) in the LBD is distinctively different from the motion of the dimer when a pure antagonist is bound to the LBD [31]. To further characterize the behavior of ER $\beta\beta$ homo and ER $\alpha\beta$ hetero-dimer in the essential subspace, we have performed the principal component analysis (PCA) based on the MD trajectories and the results are displayed in Fig 4. As is evident from the eigenvalue distribution plot for the homo and hetero dimers (Fig. 4A), first few components are more informative with high normalized eigenvalues while the latter components carry very little information about the dimer dynamics (Fig. 4A). First two PCs can explain 67 % of the variance observed in the MD simulation in the case of ER $\beta\beta$ homo-dimer while 53 % variance is explained by the corresponding two PCs for the ER $\alpha\beta$ heterodimer. Fig 4B represents the bi-dimensional projections of the first two PCs. It is evident from the figure that PC1 varies more broadly compared to PC2 in both the dimers. This corroborates well to the eigenvalue distribution plot where PC1 has the highest eigenvalue (Fig. 4A). Interestingly, 2-D projections of PC1 vs PC2 are very different for the homo compared to the hetero-dimers. The 2-D projections have been appeared to be in $\cap$ -shaped distribution pattern for ER $\alpha\beta$ hetero-dimer, while for the ER $\beta\beta$ homo-dimer it appears to be in U-shaped patterns. It is to be noted that the distribution pattern for homo-dimer is also very much similar to the observed 2-D projections for the ER $\alpha\alpha$ homo-dimer when an antagonist ligand is bound to the LBD and the 2-D projections of first two principal components for the hetero-dimer is similar with the distribution pattern of ER $\alpha\alpha$ homo-dimer with bound SERM (4-OHT) to the LBD [31]. Thus, in the essential subspace the ER homo and hetero-dimers are quite different.

3.5. Analysis of essential dimer surface

We then analysed the essential dimer surface for both ER $\beta\beta$ homo dimer and ER $\alpha\beta$ hetero-dimer and results are shown in Fig 5A.

The dimer interface essentially comprises of helix 7, C-terminal region of helix 8, helix 9 and helix 10/11. It is to be noted that in ER, helix 10 and 11 is a contiguous helix. The dimer interface is much larger for the hetero-dimer compared to the ER $\beta\beta$ homo-dimer. Particularly, the N-terminal regions of helix 7 and the C-terminal region of helix 8 lose their contribution from the dimer interface for ER $\beta\beta$ homo-dimer. Analysis of the trajectory reveals that the helicity of these two regions diminish during the later half of the simulation period and the region appears as a highly flexible turn region for ER $\beta\beta$ homo-dimer. It is to be noted that the ER dimerizes in an anti-symmetric orientation where the topology of one monomer is opposite to the other monomer. Electrostatic potential calculation on both ER α and ER β reveals highly similar electrostatic potential profile on the dimer surface for both the receptors. The dimerization surface contains two distinct regions of opposite electrostatic potential on two opposite edges of the surface for both the receptors. The regions of negative potential are mainly due to the negatively charged residues of helix 9 while the positive potential of the surface is mostly contributed by helix 11 and helix 7. Due to the anti-symmetric orientation of the dimer surface in the dimer, the regions of positive potential recognizes the region of negative potential. The very similar surface electrostatics of both the ER receptors signifies a conserved mechanism of receptor dimerization and also rationalizes the formation of functional hetero-dimer.

3.6. “Hotspot” residue analysis

We have further analyzed the crucial “hotspot” residues in the dimerization interface of both ER $\beta\beta$ homodimer and ER $\alpha\beta$ hetero-dimer. Essential “hotspot residues” have been identified from the changes in solvent accessible surface area (SASA) of the monomer and dimerized ER. Upon dimerization, those residues that undergo a reduction in the SASA value $> 15 \text{ \AA}^2$, have been considered to be involved in the dimer interface. Table 1 lists all the crucial residues involved in the dimerization interface of the both homo and hetero-dimers. Residues which undergo changes in SASA by more than $30 \text{ \AA}^2$ is considered to be the “hotspot” residues. Helix 7 does not contain any hotspot residues for hetero-dimer but the ER $\beta\beta$ only contains a single hotspot residue Ala 382. On the other hand, Helix 8 contains three “hotspot” residues for the homo-dimer but there are two “hotspot” residues for the hetero-dimer. Residue Asn 407 in the homo dimer and residue Asn 455 in the hetero dimer play a similar but crucial role in the dimerization process. On the other hand, Met 410 and Pro 412 are crucial for homo dimerization while Tyr 459 is essential for the hetero dimerization. Most of the crucial residues are clubbed into the core recognition groove formed by helix 9 and helix 10/11. In helix 9, conserved Thr 434 for homo-dimer and 483 for hetero-dimer play crucial roles in the dimer interface. In the case of homo dimer, Val 438 of helix 9 is also found to be important. Interestingly, in both homo and hetero-dimer crucial “hotspot” residues are in general occupied same regions in the dimer interface. In the N-terminal end of Helix 10, Ser 452 and Met 453 for homo-dimer and His 501 and Gln 502 for hetero-dimer belongs to the same region of the dimer surface and play crucial role in dimer interface. In helix 10, a highly conserved Ala 456 for homo dimer and Ala 505 for hetero-dimer have been found to be “hotspot” residues for the dimer interface. In helix 11, a conserved Ser 463, His 467 for homo dimer and His 512, His 516 for hetero-dimer belong to the same region of the dimer interface and are highly crucial for dimerization. On the other hand, Met 460 and Leu 509 belong to the same region of the Helix 10/11 and they are highly

crucial for the homo and hetero dimers, respectively. “Hotspot” residues from the other chain (the common ER β in ER $\beta\beta$ and ER $\alpha\beta$) are highly similar in both the homo and hetero dimers. A comparative analysis of the details of the interacting residues has been summarized in table 1.

This conserved interaction profiles of receptor dimerization for both homo and hetero-dimer prompted us to study the conservedness of the dimer surface in sequence level across different organisms.

3.7. Sequence and phylogenetic analysis of ER sequences

To infer specific variations in the ER LBD sequences for different species, multiple sequence alignments and phylogenetic analysis have been carried out. Fig 6 represents a combined phylogenetic analysis of both ER α and ER β . ER sequences and their phylogenetic distribution patterns found in eukaryotic lineages show an interesting feature. ER α and ER β sequences are clubbed differently to form two separate clades. The two proteins ER α and ER β in the same organism are paralogs, due to gene duplication. ER α sequences are orthologs, descendants of a common ancestor, due to speciation, and so are ER β . Both paralogs and orthologs are homologs. It is to be noted that organisms that are closely related during speciation, their ER α sequences are also closely related and clubbed together within a clade while distantly related homologs are generally distributed in different clades. Sequences for both ER α and ER β of *Homo sapiens* are highly similar to *Gorilla gorilla*, *Pan paniscus*, *Pongo pygmaeus*. On the other hand, *Gallus gallus* sequence shows significant similarity with *Coturnix japonica*, *Pseudemys nelson* for both ER α and ER β proteins. Thus, they are clubbed together within a clade. In general, the observed phylogenetic pattern for ER α is very similar with the ER β which signifies a common pattern of evolution. It is to be interpreted by the fact that the formation of functional hetero-dimer can strive the evolutionary process of the both the receptors in a similar way.

ER α and ER β LBD sequences are moderately conserved throughout the eukaryotic lineages and the variations are observed mainly in the loop regions, devoid of any secondary structural elements. This can be rationalised by the fact that the loop regions are highly flexible and, thus, are capable of adapting to variations. Particularly, sequence variations are observed in several loop regions. These regions are the long N-terminal loop region between Helix 1 and Helix 2, the connecting loop between Helix 8 and Helix 9, and the connecting turn between Helix 9 and Helix 10. Interestingly, residues involved in the dimer surface of the LBD are highly conserved. Even, a combined sequence alignments of both the ER α and ER β sequences involved in the dimer interface from different species shows that the dimer interface sequences are highly conserved. “Hotspot residues” identified by in silico analysis are highly conserved and only substitutions to similar amino acids were observed. This signifies that ER LBD dimer interface is less susceptible to mutations and the formation of a functional dimer is an evolutionary selected process. Furthermore, a similar co-evolution pattern observed from phylogenetic analysis and the highly similar dimer contacts imply that the formation of a stable hetero-dimer is also a evolutionary selected process.

4. Conclusions

Transcriptional activity of Estrogen Receptors depends on dimerization [14]. It is well documented that both ER α and ER β form homo and hetero dimers in solution and in vivo [14]. ER α and ER β are co-expressed in the same cells in mouse mammary gland, in rat cardiac myocytes, and fibroblasts suggest that the hetero-dimerization probably occurs in vivo [13, 17]. Even it has been depicted that ER $\alpha\beta$ hetero-dimers bind to ERE sequences with a specificity and affinity similar to those of the respective homo-dimers. We observed that in the presence of agonist ligand, ER forms highly stable hetero-dimer which is more stable than the ER $\beta\beta$ homo-dimers. ER α and ER β have almost identical LBD structural topology and share highly similar electrostatic potential profile for the dimer interface. Combined sequence analysis reveals that the dimer interface of the LBD of both the ER is highly conserved. Interestingly, phylogenetic analysis reveals a co-evolutionary signature between the receptors. This can be rationalized by the fact that the formation of functional hetero-dimers is evolutionary selected process which drives the two proteins to evolve similarly. On therapeutic perspectives, structural and mechanistic insights into the effect of ligand binding on ER $\beta\beta$ homo-dimer and ER $\alpha\beta$ heterodimer are highly essential to design therapeutics. Considering the fact that ER β form heterodimers with ER α and the hetero-dimer complex inhibit cell proliferation, promoting heterodimerization with ER β agonist would be an important strategy to design therapeutics for breast cancer.

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Highlights

- ER forms highly stable hetero-dimer which is more stable than the ER $\beta\beta$ homo-dimers.
- ER α and ER β share very similar electrostatic potential profile of dimer interface.
- In essential subspace, the homo and hetero dimer dynamics are distinctively different.
- The dimer interface of the LBD in both the ERs is highly conserved.
- Phylogenetic analysis reveals a co-evolutionary signature between the two receptors.

Fig. 1. Variations in C_{α} -RMSD (A) and radius of gyration, R_g (B) of ER dimer with simulation time. Black line represents ER $\alpha\beta$ hetero-dimer while the red line represents ER $\beta\beta$ homo-dimer. RMSD matrices of ER $\alpha\beta$ hetero-dimer (C) and ER $\beta\beta$ homo-dimer (D) computed from MD trajectory.

Fig 2.

A. Variations of RMSF profiles of ER homo and hetero-dimer. B. A cartoon representation of ERββ homo-dimer. Regions of high fluctuations are colored in cyan.

Fig 3.
Secondary structure evolution with simulation time. A. ER $\alpha\beta$ hetero-dimer B. ER $\beta\beta$ homo-dimer.

Fig. 4.

A. Normalised Eigen value distribution for the first 20 Eigen vectors obtained from MD trajectory. B: 2-D projection of the first two principal components (1 & 2) for ER $\alpha\beta$ heterodimer (red) while green represents ER $\beta\beta$ dimer.

Fig 5.

The essential dimerization interface of ER homo and hetero dimer interface. Green color represents Chain A and red color represents chain B. Dark green and salmon represents ER $\beta\beta$ homo-dimer interface while light green and salmon represent ER $\alpha\beta$ hetero-dimer. B. & C. represents electrostatic potential of the dimer interface.

Fig 6.
A combined phylogenetic tree for ER alpha and beta across eukaryotic lineages.

ERα	HELIX 7	HELIX 8	HELIX 9	HELIX 10/11
<i>Canis lupus</i>	VLLQLVRRRLQALRL	LKALALANSDSVH	EQLREALHEALLEYEAG	GGGAERRRRAGRLLLTPLLRQTAG
<i>Bufo rangieri</i>	MLVTTATFRFRMMRL	LKSIILLNSGVYTF	HLILDKIIDTLV-HFMG	SLQQQQRRRLAQQLLLSHIRHMSN
<i>Gallus gallus</i>	MLLATAARFRMMNL	LKSIILLNSGVYTF	HRVLDKITDTLI-HLMA	SLQQQHRRRLAQQLLLSHIRHMSN
<i>Felis catus</i>	MLLATSSRFRMMNL	LKSIILLNSGVYTF	HRVLDKITDTLI-HLMA	SLQQQHRRRLAQQLLLSHIRHMSN
<i>Mus musculus</i>	MLLATSSRFRMMNL	LKSIILLNSGVYTF	HRVLDKITDTLI-HLMA	TLQQQHRRRLAQQLLLSHIRHMSN
<i>Homo sapiens</i>	MLLATSSRFRMMNL	LKSIILLNSGVYTF	HRVLDKITDTLI-HLMA	TLQQQHRRRLAQQLLLSHIRHMSN
<i>Papio anubis</i>	MLLATSSRFRMMNL	LKSIILLNSGVYTF	HRVLDKITDTLI-HLMA	TLQQQHRRRLAQQLLLSHIRHMSN
<i>Danio rerio</i>	MLLATVARFRSLKL	LKAIILLNSGAFSF	HRVLDKITDTLI-HLMA	TLQQQHRRRLAQQLLLSHIRHMSN
ERβ				
<i>Protopterus annectens</i>	MMLATTSRIRDCLK	LKAMILLNSSVFPL	QSLLNATTDALVWFIA	PHQQQSTRLAQQLMLLSHIRHASN
<i>Bufo rangieri</i>	MLLATTSRFRELKL	LKVLILLNSNMFL	HQLLNTVIDGLVWVIA	PFRQQSTRLANLLMLLSHVRHASN
<i>Canis lupus</i>	MLLATTSRFRELKL	VKAMVLLNSSMYPL	SHLLNAVTDALVWVIA	PSQQQPVRLANLLMLLSHVRHASN
<i>Homo sapiens</i>	MLLATTSRFRELKL	VKAMILLNSSMYPL	AHLLNAVTDALVWVIA	SSQQQSMRLANLLMLLSHVRHASN
<i>Trachypithecus obscurus</i>	MLLATTSRFRELKL	VKAMILLNSSMYPL	AHLLNAVTDALVWVIA	SSQQQSMRLANLLMLLSHVRHASN
<i>Chlorocebus aethiops</i>	MLLATTSRFRELKL	VKAMILLNSNMYP	AHLLNAVTDALVWVIA	SSQQQSMRLANLLMLLSHVRHASN
<i>Icthyurus punctatus</i>	MLLAGSSRFRELKL	LKALILLNSSMYMT	LRLLDAVTDALVWVIA	STQQQSARLAHLLMLLSHIRHLSN
<i>Danio rerio</i>	MLLAATSRFRELKL	LKAMILLNSNMCLG	LCVLDSDVTDALVWVIA	SFQQRSTRLAHLLMLLSHIRHVS

Fig. 7.

A combined sequence analysis of ER α and ER β essential dimer interface.

Table 1

List of “Hotspot” residues of ER homo and hetero dimer interface with their calculated solvent accessible surface area (SASA). Residues whose SASA change > 30 Å² upon dimerization are marked in bold.

ERββ homo-dimer						ERαβ hetero-dimer					
Residue Number	Residue	Chain	RelCompASA	RelMonomerASA	AASA	Residue Number	Residue	Chain	RelCompASA	RelMonomerASA	ASA
382	A	A	29.47	74.14	44.67	426	D	A	3.41	30.32	26.91
407	N	A	2.13	32.14	30.01	427	M	A	4.33	26.61	22.28
410	M	A	21.27	69.37	48.1	430	A	A	2.08	22.53	20.45
412	P	A	18.7	87.24	68.54	434	R	A	37.2	60.27	23.07
431	N	A	49.52	70.65	21.13	455	N	A	2.43	33.33	30.9
434	T	A	0.07	41.77	41.7	459	Y	A	18.81	71.17	52.36
438	V	A	9.83	52.89	43.06	476	H	A	12.54	34.57	22.03
449	Q	A	38.61	97.03	58.42	483	T	A	0.54	47.43	46.89
452	S	A	6.2	46.92	40.72	487	I	A	28.15	47.06	18.91
453	M	A	15.73	63.18	47.45	501	H	A	8.22	68.67	60.45
456	A	A	0	45.82	45.82	502	Q	A	18.17	63.14	44.97
459	L	A	1.19	43.98	42.79	504	L	A	0	18.62	18.62
460	M	A	6.42	67.15	60.73	505	A	A	0.1	50	49.9
463	S	A	3.94	72.92	68.98	508	L	A	0.1	26.69	26.59
466	R	A	7.75	37.07	29.32	509	L	A	6.22	64.07	57.85
467	H	A	23.47	58.65	35.18	511	L	A	1.56	19.91	18.35
379	M	B	6.22	26.37	20.15	512	S	A	2.15	63.4	61.25
382	A	B	26.4	63.24	36.84	513	H	A	9.52	62.53	53.01
407	N	B	23.5	46.23	22.73	515	R	A	12.85	52.07	39.22
410	M	B	39.34	79.18	39.84	516	H	A	20.58	49.9	29.32
411	Y	B	55.74	71.78	16.04	519	N	A	43.73	65.9	22.17
434	T	B	2.42	52.73	50.31	549	L	A	97.03	119.52	22.49
438	V	B	3.16	62.96	59.8	382	A	B	22.23	47.14	24.91
452	S	B	2.96	67.96	65	386	R	B	28.95	54.23	25.28
453	M	B	13.29	64.64	51.35	407	N	B	2.58	52.74	50.16

ER β homo-dimer						ER α hetero-dimer					
Residue Number	Residue	Chain	RelCompASA	RelMonomerASA	AASA	Residue Number	Residue	Chain	RelCompASA	RelMonomerASA	ASA
453	L	B	0.34	24.42	24.08	410	M	B	11.92	95.95	84.03
456	A	B	0	43.95	43.95	412	P	B	29.95	72.01	42.06
459	L	B	0.22	25.42	25.2	413	L	B	42.35	102.37	60.02
460	M	B	3.11	52.74	49.63	434	T	B	4.73	42.83	38.1
463	S	B	0.45	61.95	61.5	435	D	B	44.43	59.9	15.47
464	H	B	11.05	44.82	33.77	438	V	B	0.1	59.2	59.1
466	R	B	18.35	37.6	19.25	452	S	B	8.33	63.72	55.39
467	H	B	26.1	53.49	27.39	455	L	B	0.73	19.72	18.99
470	N	B	36.38	71.72	35.34	456	A	B	0.6	55.37	54.77
						457	N	B	12.98	34.53	21.55
						459	L	B	0.5	41.03	40.53
						460	M	B	7.82	69.44	61.62
						463	S	B	2.26	59.27	57.01
						464	H	B	8.08	55.04	46.96
						466	R	B	17.67	51.12	33.45
						467	H	B	32.52	53.29	20.77
						470	N	B	35.94	58.1	22.16
						471	K	B	20.65	36.12	15.47