Supplementary Information for:

An alternative conformation of ERβ bound to estradiol reveals H12 in a stable antagonist position

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Content:

Tables

Table S1. X-ray data collection and refinement statistics

Resolution range	41.91 - 2.5 (2.59 - 2.5)
Space group	P 3_2 2 1
Unit cell	83.811 83.811 168.531 90 90 120
Total reflections	157,355 (15,493)
Unique reflections	24,355 (2,389)
Multiplicity	6.5 (6.5)
Completeness (%)	99.76 (100.00)
Mean I/sigma(I)	11.88 (1.62)
Wilson B-factor	56.41
R-merge	0.1027 (1.285)
R-meas	0.1117 (1.394)
R-pim	0.04344 (0.5345)
CC1/2	0.997 (0.575)
CC*	0.999 (0.854)
Reflections used in refinement	24,352 (2,391)
Reflections used for R-free calcualtion	1,198 (106)
R-work	0.2052 (0.3158)
R-free	0.2428 (0.3492)
CC(work)	0.961 (0.700)
CC(free)	0.954 (0.599)
Number of non-hydrogen atoms	3664
macromolecules	3561
ligands	40
solvent	63

Protein residues	462
RMS(bonds)	0.002
RMS(angles)	0.61
Ramachandran favored (%)	97.12
Ramachandran allowed (%)	2.88
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.51
Clashscore	4.96
Average B-factor	64.50
macromolecules	64.80
ligands	50.46
solvent	56.32

[a] Values in parentheses are for the highest resolution shell.

[b] $R_{merge} = \Sigma \mid I_j - \langle I \rangle \mid / \Sigma \langle Ij \rangle$, where I_j is the observed intensity of an individual reflection and <I> is the average intensity of that reflection.

[c] Root-mean-square-deviation.

Figures

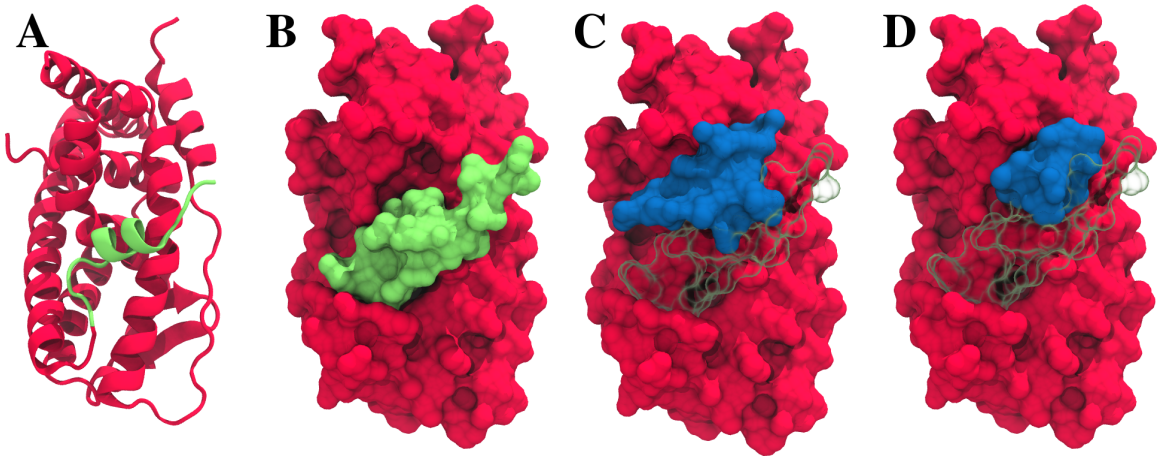

Figure S1. Alternative H12 conformation partially overlaps with the coactivator groove. Alternative ERβ LBD-E2 conformation shown as cartoon (**A**) and in surface representation (**B**) with H12 highlighted in green and the rest of LBD structure in red. Alternative ERβ LBD-E2 structure superimposed with the canonical ERβ LBD in complex with E2 (PDB id: 3OLS) and KB095284 (PDB id: 4ZI1) ligands bound, respectively, to coactivator SRC1 Box2 (**C**) and CIA12 coactivator peptide (**D**) displayed as blue surfaces.

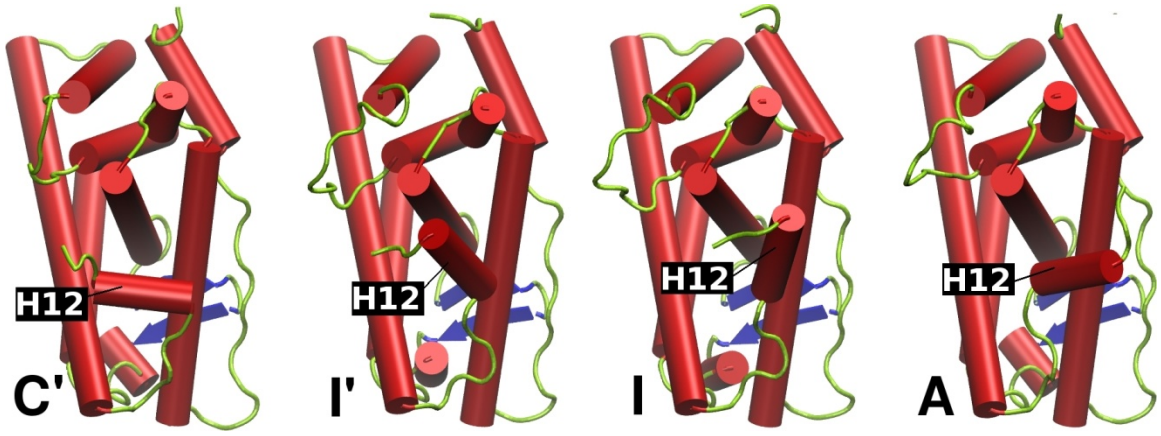

Figure S2. Representative structures of the global and local minimum of free-energy landscape maps.

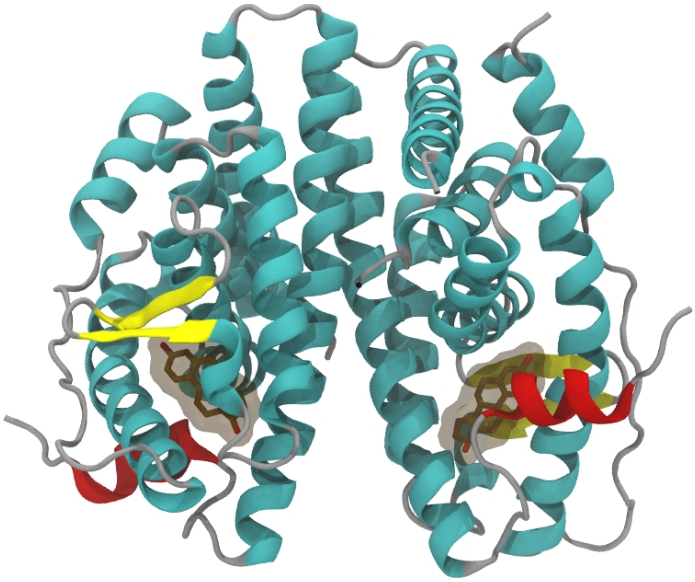

Figure S3. Physiologically relevant homo-dimeric ERβ LBD-E2 structure in alternative conformation.

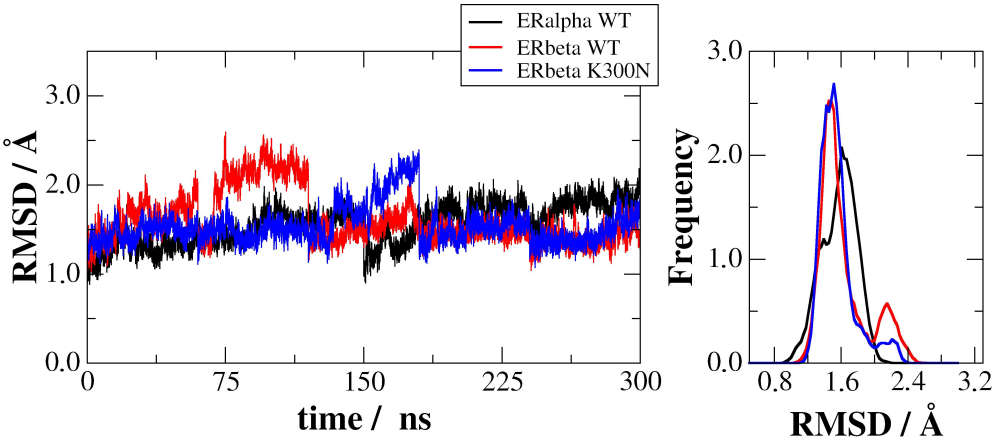

Figure S4. The overall stabilities of ER-LBD structures during the conventional MD simulations were verified via the calculations of backbone RMSD with respect to the canonical crystal structures (PDB id: 1QKU for wild-type ERα LBD-E2 and PDB id: 3OLS for wild-type and K300N mutant of ERβ LBD-E2). The results are displayed in

plots of RMSD as a function of time (with three 100-ns concatenate trajectories for each system) and RMSD distributions.

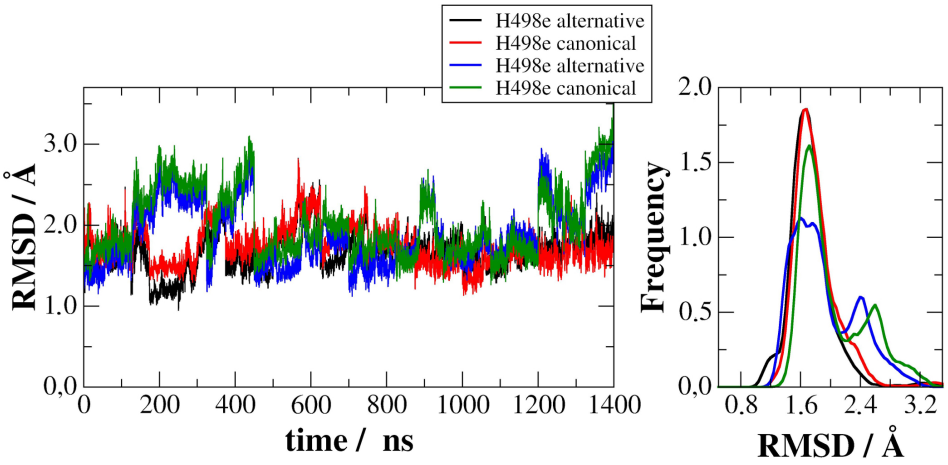

Figure S5. The overall stabilities of LBD structures during ABF MD simulations (in H498e and H498+ protonation states) were verified via the calculations of backbone RMSD with respect to the canonical (PDB id: 3OLS) and the alternative ERβ LBD-E2. Since H12 conformations were biased ensemble sampled during these simulations, they were not included in this analysis. The results are displayed in plots of RMSD as function of time and RMSD distributions.