Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information

Structural insights into tumor-specific chaperoning activity of Gamma Synuclein in protecting Estrogen receptor alpha 36 and promoting Tamoxifen resistance in breast cancer

Manivel Panneerselvam, Kannan Muthu and Krishna Ramadas*

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Supplementary Figures and Figure Legends:

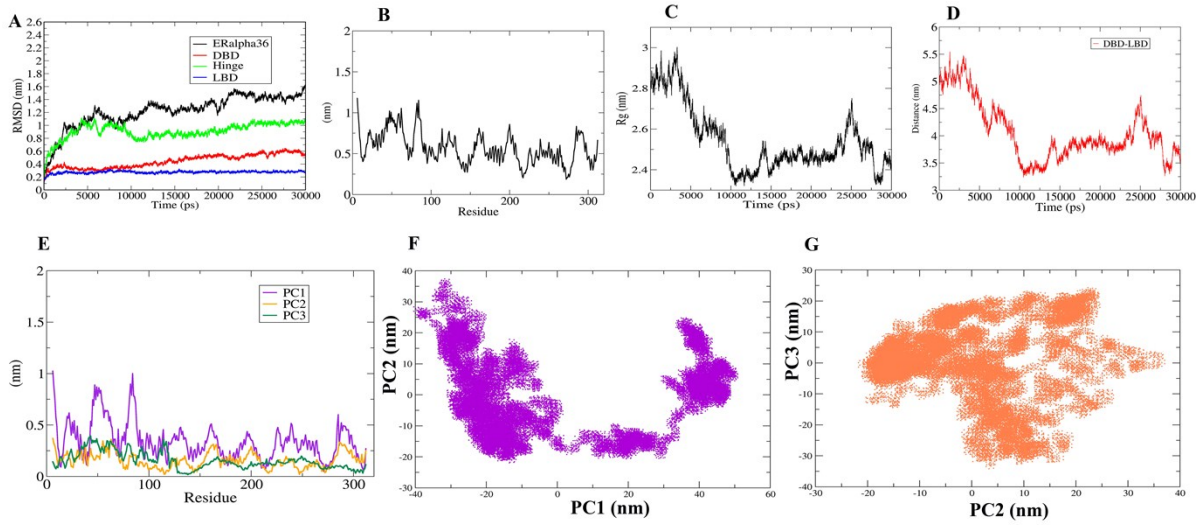

Figure S1. RMSD, RMSF, Radius of Gyration and Projection of PCAs of ERα36 in chaperone-free state.

(A) Backbone RMSD and (B) RMSF shown as a function of time depicts fluctuations in hinge region (76-132). (C) Depicts the Radius of gyration plot highlights changes seen in compactness of structure indicating the presence of varied conformations. (D) Shows decrease in the distance between the DBD and LBD. (E) Shows the fluctuations seen in the residues calculated along first three principal components. (F) and (G) shows the projection of PCAs, highlights the presence of structural variations seen in phase space of these vectors.

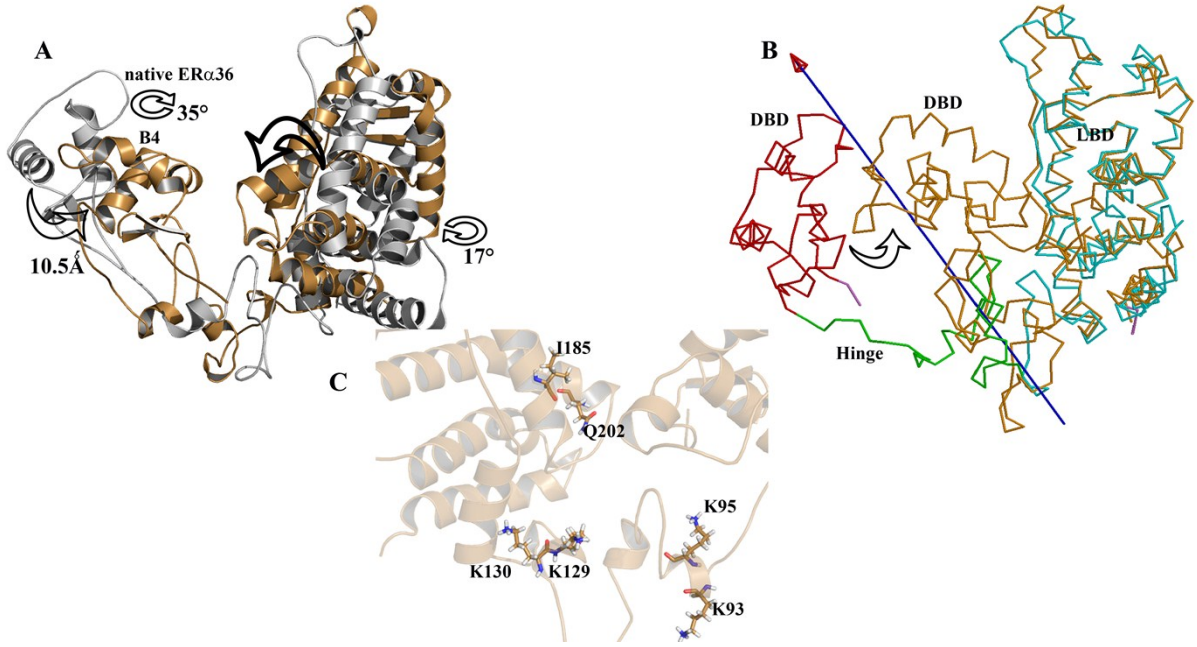

Figure S2: Overview of domain motions observed in ERα36 and their implications.

(A) Displays the rotation and displacement of DBD, and rotation in LBD of ERα36 and (B) displays the movement of DBD towards LBD. (C) Displays the exposure of ubiquitin binding residues in the structure of chaperone-free ERα36.

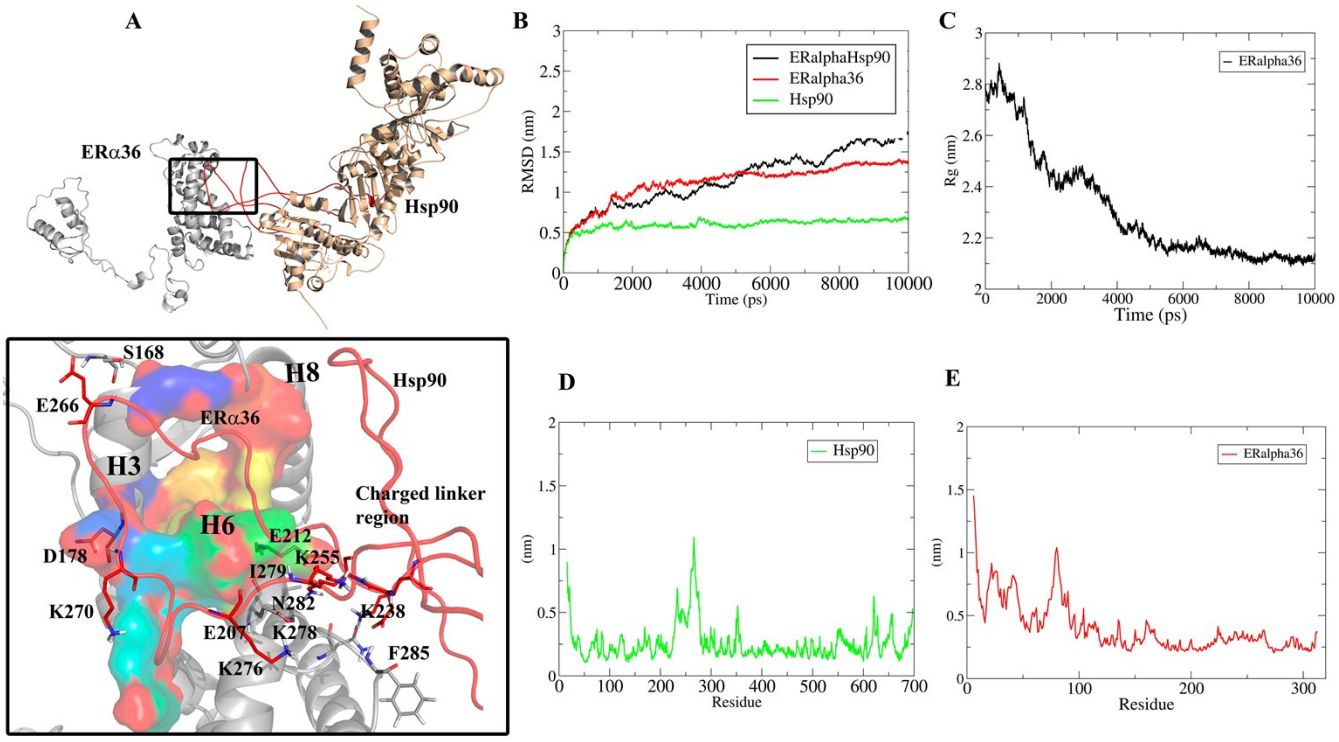

Figure S3: Conformation of ERα36-Hsp90 complex.

(A) The predicted molecular assembly of ERα-36-Hsp90 complex, where charged linker region of Hsp90 establishes interaction with LBD of ERα-36. (B) Displays the interaction between the residues of Hsp90 and ERα36. (C) Backbone RMSD plot displays the variations seen across the time scale of 10ns in the ERα36-Hsp90 complex and in the monomers of the complex. (D) Highlights the changes seen in the radius of gyration plotted across the time. (E) And (F) displays the fluctuations of residues in both ERα-36 and Hsp90 respectively.

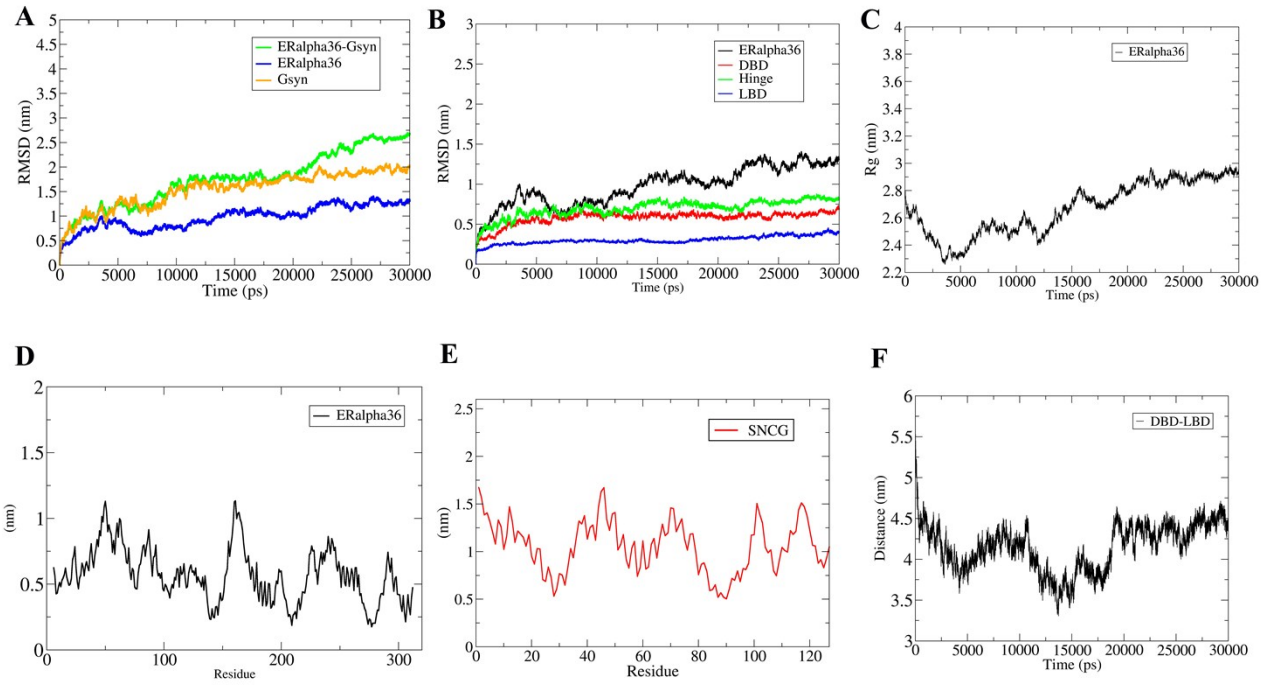

Figure S4: MD analysis of stability and conformational changes in ERα36-SNCG complex.

(A) Backbone RMSD plot displays the variations seen in ERα36-SNCG complex caused by SNCG (denoted as Gsyn in the figure) binding. (B) Variations seen in the structure of ERα36 indicate that hinge region exhibits much fluctuation than DBD and LBD. (C) Displays the changes seen in the Radius of gyration that plotted across time. (D) and (E) display the RMSF for residues of ERα36 and SNCG. (F) depicts the decrease in the distance between DBD and LBD of ERα36 in presence of SNCG.

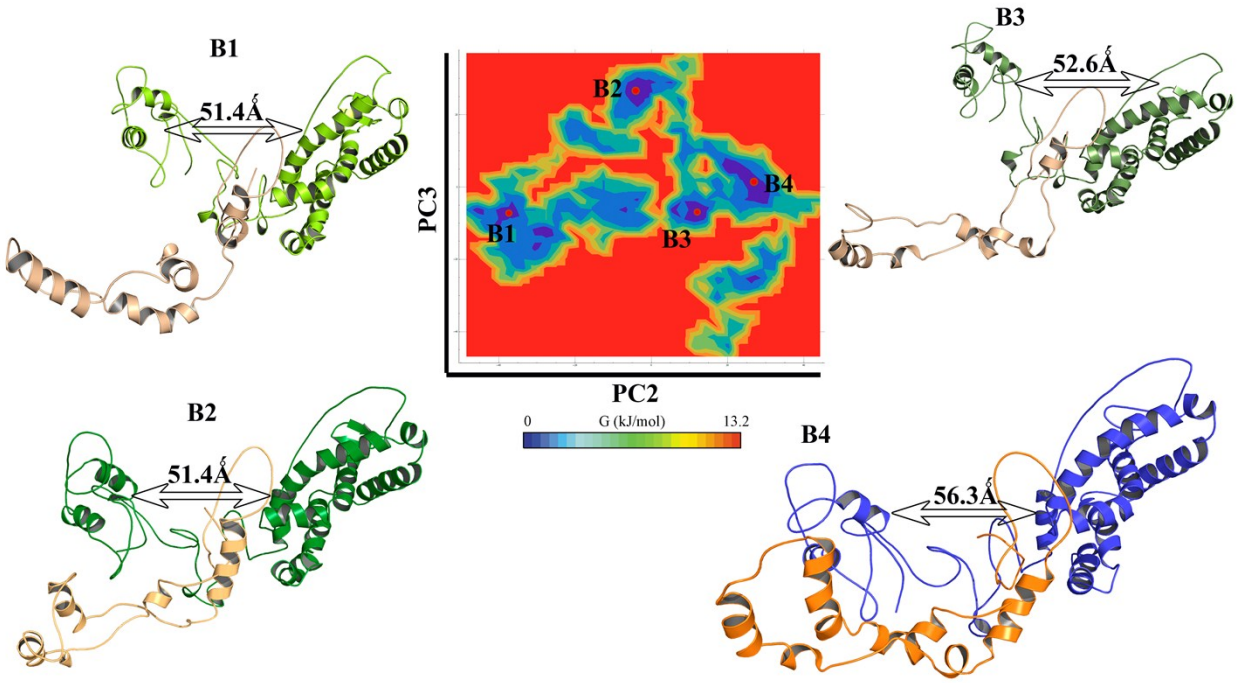

Figure S5: Two-dimensional FEL plot and the conformation of ERα36 imposed by SNCG.

Two dimensional FEL generated by PC2 and PC3 displays the presence of four energy basins and structures retrieved from them indicates that in presence of SNCG, the distance between DBD and LBD has not decreased, and the co-activator groove has not been masked by DBD.

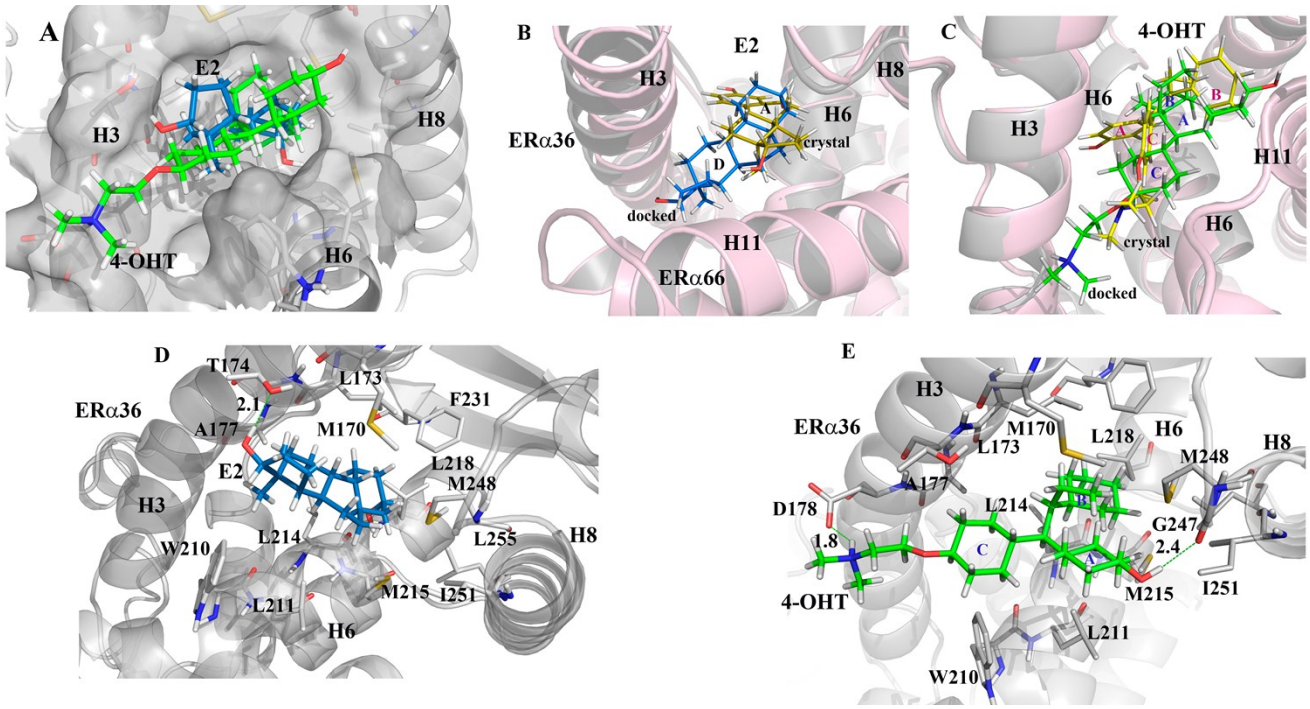

Figure S6: Binding conformation of E2 and 4-OHT with ERα36.

(A) The interacting mode of EST (blue), 4-OHT (green) with ERα-36. (B) The comparison of crystal E2 binding mode between ERα-66 (light pink) and ERα36 (grey). (C) Comparison of crystal and docked binding mode of 4-OHT between ERα66 and ERα36. (D) Interactions observed between E2 and ERα36. (E) Interactions observed between 4-OHT and ERα36. Hydrogen bonds are represented in green color.

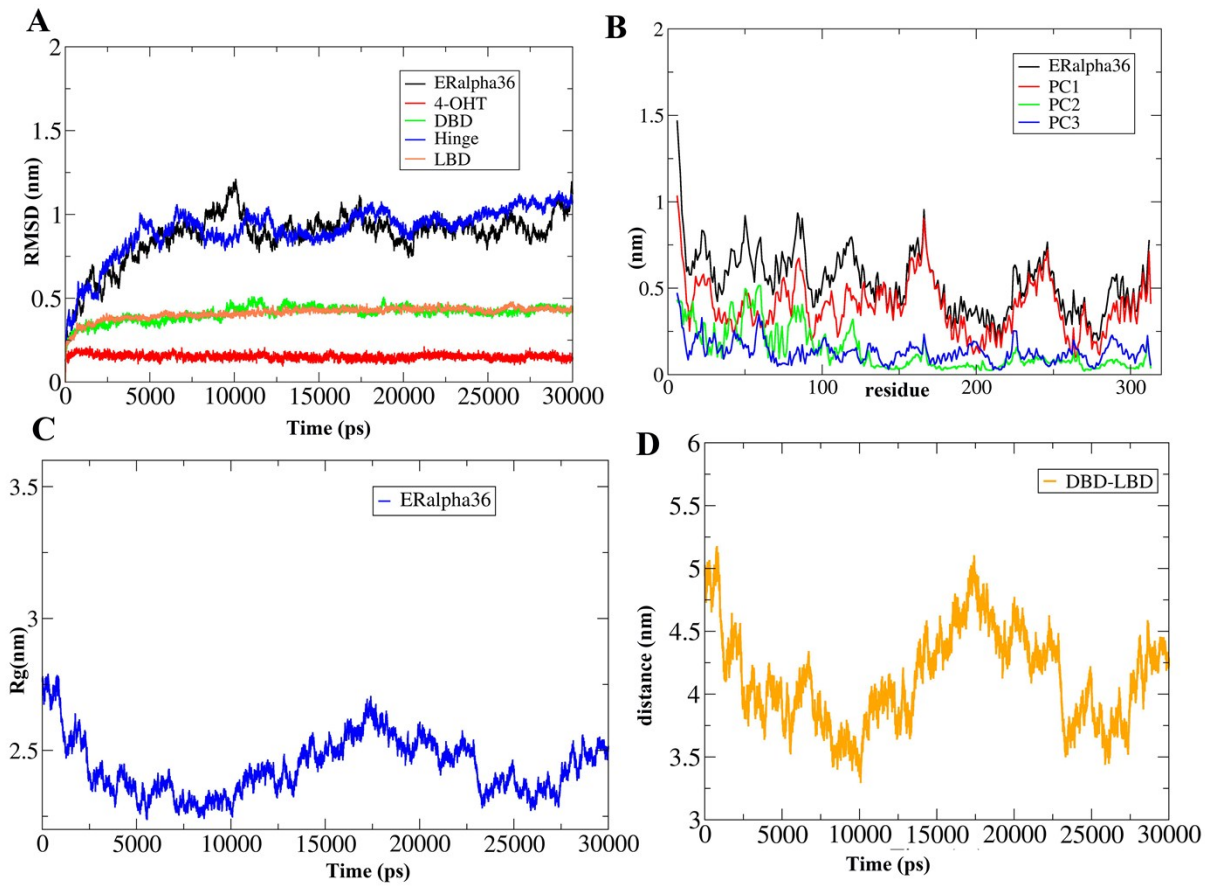

Figure S7: Supplementary Figure 5: Stability and fluctuations of ERα36-4-OHT complex.

(A) Backbone RMSD analysis of deviations seen in ERα36. (B) Fluctuations of ERα36 residues. (C) Radius of gyrations of ERα36. (D) distance between DBD and LBD of ERα-36 seen in 4-OHT bound complex.

Supplementary Tables:

Table S1: Percentage of motions covered by each principal components

Principal Components	ERα-36 native simulation		ERα-36-SNCG complex simulation			ERα-36-Tamoxifen simulation		
	Eigen values	Percentage of Motion (%)	Eigen values	Percentage of Motion (%)		Eigen values	Percentage of Motion (%)	
PC1	755.801	57.36	1881.02	62.08		581.917	63.95	
PC2	155.32	69.15	549.586	80.22		114.412	76.52	
PC3	151.904	80.68	255.227	88.64		73.8176	84.63	
PC4	78.7215	86.66	132.355	93.01		70.3356	92.36	
PC5	36.0152	89.39	53.3263	94.77		26.4106	95.26	
PC6	19.9266	90.90	45.3005	96.26		12.7835	96.54	
PC7	14.1735	91.98	39.0357	97.55		11.6517	97.36	
PC8	10.1596	92.75	30.1864	98.55		7.43254	98.18	
PC9	7.1341	93.29	25.6867	99.40		6.11318	98.85	
PC10	6.67725	93.80	18.1257	100		5.07426	99.41	

Table S2: RMSIP value calculated between eigenvectors from MD of ERα36.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
PC1	0.0348	0.0348	0.104	0.174	0.122	0.0174	0.0174	0.087	0.0174	0.104
PC2	0.139	0.104	0.087	0.0348	0.104	0.0348	0.0522	0.087	0.191	0
PC3	0.157	0.226	0.191	0.087	0.226	0.157	0.0696	0.0522	0.157	0.122
PC4	0.209	0.0522	0.122	0.122	0.139	0.418	0.0522	0.313	0.244	0.191
PC5	0.0348	0.139	0.191	0.087	0.279	0.0696	0.0696	0.261	0.383	0.261
PC6	0.122	0.383	0.313	0.209	0.104	0.122	0.0696	0.0522	0.0174	0.0522
PC7	0.609	0.4	0.087	0.191	0.104	0.104	0.0348	0.226	0.087	0.0348
PC8	0.522	0.54	0.0522	0.296	0.122	0.313	0.104	0.0696	0.0522	0.0348
PC9	0.0348	0.209	0.0696	0.627	0.244	0.0348	0.244	0.174	0.0696	0.0174
PC10	0.0348	0.261	0.696	0.0348	0.191	0.0696	0.261	0.157	0	0.0696
RMSIP	**0.663059**									

Table S3: Interface and binding statistics for ERα36 – SNCG and ERα36 – Hsp90 Complexes

	Interface residues[a]		Interface area[b] (Å²)	Δ^iG [c] kcal/mol	Δ^iG [d] P-Value kcal/mol	Internal energy complex[e] kcal/mol	Binding energy[f] kcal/mol	Buried surface area[g] (Å²)
ERα36 SNCG complex	ERα-36	SNCG	1016.7	-7.9	0.871	-15897.1	-43325.4	1911.96
	112	96						
ERα36 Hsp90 complex	ERα-36	Hsp90	1270.5	-11.1	0.698	-40424	-79953.8	2639.31
	40	33						

Values a-d is obtained from the results of PDBePISA server analysis and values e-g are obtained from the HADDOCK analysis

c. Denotes the difference in the total solvation energies (solvation free energy gain upon complexation) between monomer and complexed structures and the negative value is an indicator of hydrophobic nature of interface.

d. Refers to the probability value of the observed solvation free energy gain and P-value >0.5 denotes that the interface is hydrophobic in nature.

e. Refers to the total Internal energy of the complex.

f. Refers to the binding energy of the complex.

g. Refers to the buried surface area, which is obtained by calculating the difference between the sum of the solvent accessible surface area for monomers and complex.

Table S4: Intermolecular contact observed in ERα36 – SNCG and ERα36 – Hsp90 Complexes

Complex	Hydrogen bonds			Salt bridges		
ERα36 – SNCG Complex	**ERα36**	**Dist (Å)**	**SNCG**	**ERα36**	**Dist (Å)**	**SNCG**
	K189[HZ3]	1.61	E98[OE2]	K189[NZ]	3.35	E98[OE1]
	Q202[HE22]	2.00	E98[OE2]	K189[NZ]	2.63	E98[OE2]
	R114[HH12]	2.45	E116[OE2]	R114[NH1]	3.21	E116[OE2]
	R114[HH22]	1.71	E116[OE2]	R114[NH2]	2.69	E116[OE2]
	R114[HH11]	1.67	E117[OE2]	R114[NH1]	2.62	E117[OE2]
	S132[N]	2.88	E120[OE1]	R114[NE]	3.90	E117[OE2]
	L133[N]	2.96	E120[OE1]	K 189[NZ]	2.64	D 127[OD1]
	A134[N]	2.64	E120[OE2]	K 130[NZ]	2.58	D127[OD2]
	R295[HH22]	2.41	E120[O]	E 207[OE1]	2.89	R 96[NH2]
	R295[HH12]	1.75	E120[O]			
	K189[HZ1]	1.60	D127[OD1]			
	K130[HZ2]	1.66	D127[OD2]			
	E207[OE1]	2.48	R96[HH22]			
	D196[OD1]	2.58	A122[N]			
	D196[OD1]	2.77	Q123[N]			
	T198[OG1]	1.89	Q123[HE21]			
	V195[O]	2.99	D127[N]			
ERα36 – Hsp90 Complex	**ERα36**	**Dist (Å)**	**Hsp90**	**ERα36**	**Dist (Å)**	**Hsp90**
	S168[N]	2.84	E 266[OE1]	H289[NE2]	3.85	E244[OE1]
	F285[N]	3.17	D 254[OD1]	H289[NE2]	3.31	E244[OE2]
	N282[OD1]	1.70	K 238[HZ1]	D212[OE2]	2.56	K255[NZ]
	N282[O]	2.14	K 255[HZ1]	E207[OE2]	2.63	K276[NZ]
	I279[O]	1.89	K 255[HZ3]	E207[OE1]	2.71	K278[NZ]
	E212[OE2]	1.58	K 255[HZ2]			
	D178[OD1]	2.81	K 270[N]			
	D207[OE2]	1.62	K 276[HZ2]			
	D 207[OE1]	1.73	K 278[HZ2]			