Supporting Information (SI)

Insights into the structure and molecular mechanisms of the ligand-estrogen receptor α complex with bisphenol A and two analogues

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Materials and Methods

Chemicals. 17β-Estradiol (E2) was purchased from Sigma-Aldrich and ICI 182,780 (ICI) was purchased from Tocris Bioscience. Bisphenol A (BPA), bisphenol AF (BPAF), and bisphenol S (BPS) used in this study were provided by the Midwest Research Institute via a contract with the National Toxicology Program (NTP). All 10 mM stock solutions were made in DMSO and kept in -20 °C.

Plasmids. The expression vectors pcDNA3 was purchased from Invitrogen. An internal control plasmid for transfection efficiency, pRL-TK renilla luciferase (pRL-TK Luc), was purchased from Promega. The luciferase reporter plasmid, 3xERE Luc (synthetic vitellogenin ERE-TATA fused to a luciferase reporter gene) and full-length human ERα expression plasmid, pcDNA/hERα, have been described previously (39). The following plasmids were gifts from Dr. D. McDonnell (Duke University): pcDNA/SRC-1, -2, -3, and pcDNA/PGC1α, β.

Cell lines and tissue culture. Ishikawa/vec and Ishikawa/ERα cells were maintained in phenol red-free DMEM:F12 medium (Invitrogen) supplemented with 10% FBS and 1 mg/mL Geneticin (G418; Invitrogen). HepG2 cells were maintained in phenol red-free Minimum Essential Medium (MEM; Invitrogen) supplemented with 10% Fetal Bovine Serum (FBS; Gemini Bio- Products) and 4 mM L-glutamine (Invitrogen). MCF-7 and BG-1 FR cells were maintained in phenol-red free DMEM:F12 medium (Invitrogen) supplemented with 10% FBS and 4 mM L-glutamine. Recombinant U2OS/ERα cells were maintained in phenol red–free Dulbecco's modified essential medium (DMEM; Invitrogen) supplemented with 10% FBS and 4 mM L-glutamine. For cell-starving conditions, 5 or 10% Charcoal/Dextran stripped FBS (sFBS; Gemini Bio-Products) was substituted for FBS in the medium.

Transient transfection and luciferase reporter analysis.

For ER-negative HepG2 cells, a total of 0.5 μg of DNA, including 0.2 μg of ERα or ERβ, 0.2 μg of 3xERE Luc and 0.1 μg of pRL-TK Luc plasmids were transfected into the cells. For Ishikawa/vec, Ishikawa/ERα stable cells, and ER-positive cells (MCF-7 and BG-1 FR), a total of 0.3 μg of DNA, including 0.2 μg of 3xERE Luc and 0.1 μg of pRL-TK Luc plasmids were transfected. After 8 hours, cells were changed to fresh 10% sFBS medium overnight and then treated with vehicle control (DMSO, final concentration <0.01%), E2, or BPAs (1, 10, 100 and 1000 nM).

For coregulator experiments in HepG2 cells, a total of 0.8 μg of DNA, including 0.1 μg of ERα, 0.4 μg of either SRC-1, SRC-2, SRC-3, PGC1α, or PGC1β, 0.2 μg of 3xERE Luc, and 0.1 μg of pRL-TK plasmids were transfected into the cells. After 8 hours, cells were changed to fresh 10% sFBS medium overnight and then treated with vehicle control (DMSO, final concentration <0.01%), E2 (10 nM), or BPAs (100 and 1000 nM) for 18 hours.

Luciferase assays were performed using the Dual Luciferase Reporter Activity System (Promega). Transfection efficiency was normalized against the renilla luciferase. Fold changes were calculated relative to vehicle controls. All experiments were repeated at least three times. Data shown are the average of triplicate determinations in a representative experiment. Values were calculated relative to vehicle control and presented as ± standard error of the mean (SEM).

RNA extraction and qPCR analysis. Total RNA was extracted using RNeasy Mini Kit (Qiagen). First-strand cDNA synthesis was performed using Superscript reverse transcriptase according to the manufacturer's protocol (Invitrogen). The mRNA levels were measured using SYBR green assays (Applied Biosystems). The sequences of primers used in qPCR and ChIP qPCR are shown in the table below. Cycle threshold (Ct) values were obtained using the ABI PRISM 7900 Sequence Detection System and analysis software (Applied Biosystems). Each sample was normalized against β-actin expression. Experiments were repeated three times and results are presented as mean ± SEM. Changes of endogenous gene expression by E2 were calculated as fold change relative to the vehicle control group in each cell line.

qPCR primers

Gene symbol and GenBank accession number	Sequence (5' – 3')
Human *WISP2/CNN5* (NM_003881)	F: TGAGCGGCACACCGAAGAC R: ACAGCCATCCAGCACCAG
Human *PGR* (NM_000926.4)	F: GACGTGGAGGGCGCATAT R: GCAGTCCGCTGTCCTTTTCT
Human *GREB1* (NM_014668)	F: CAAAGAATAACCTGTTGGCCC R: GACATGCCTGCGCTCTCATAC
Human *β-actin* (NM_001101)	F: GACAGGATGCAGAAGGAGATCAC R: GCTGATCCACATCTGCTGGAA
Human *FOLR2* (NM_000803)	F: CCTCTGGAGTCACTCATACAAGG R: CCTGGGCTGAATCAAACCAC
Human *FN3K* (NM_022158)	F: TTCATCCCGCAGGTGAATGAG R: TGCCTCTCGGTCAGCATAGT
Human *KRT20* (NM_019010)	F: GGACGACACCCAGCGTTTAT R: CGCTCCCATAGTTCACCGTG

Table S1*A*. 74 peptides that positively interact with the E2/ERα complex derived from 32 coregulators

74 peptides	Relative binding value	32 coregulator descriptions
BL1S1_1_11	5.8	BLOC-1: biogenesis of lysosome-related organelles complex-1 subunit 1
BRD8_254_276	34.3	TrCP120: Thyroid hormone receptor coactivating protein 120kDa
CBP_2055_2077	1.3	CBP: CREB-binding protein
CBP_57_80	24.4	
CENPR_1_18	1.5	CENP-R: Centromere protein R
EP300_69_91	16.5	P300: Histone acetyltransferase p300/E1A-associated protein p300
GNAQ_21_43	5.4	Gαq : Guanine nucleotide-binding protein alpha-q
HAIR_745_767_C755S/C759S	18.3	H: Protein hairless
IKBB_277_299	3.1	NFkB:NF-kappa-B inhibitor beta
ILK_131_153	4.5	ILK-1/p59ILK: Integrin-liked protein kinase
JHD2C_2054_2076	19.0	TRIP-8: thyroid receptor-interacting protein 8
LCOR_40_62	34.5	LCoR: ligand-dependent corepressor
MED1_591_614	11.2	TRIP-2: thyroid receptor-interacting protein 2
MED1_632_655	21.1	
MLL2_4175_4197	2.7	MLL2/KMT2B: Myeloid/lymphoid or mixed-lineage leukemia protein 2
MLL2_4702_4724	3.0	
NCOA1_1421_1441	5.6	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA1_620_643	45.1	
NCOA1_677_700	5.5	
NCOA1_737_759	47.5	
NCOA2_628_651	12.8	SRC-2/NCoA-2: nuclear receptor co-activator 2
NCOA2_677_700	9.7	
NCOA2_733_755	8.6	
NCOA3_104_123_N-KKK	2.5	SRC-3/NCoA-3: nuclear receptor co-activator 3
NCOA3_609_631	21.1	
NCOA3_609_631_C627S	13.1	
NCOA3_673_695	5.8	
NCOA3_725_747	6.3	
NCOA6_875_897	7.6	NCoA-6: nuclear receptor co-activator 6
NCOR1_2376_2398	2.8	N-CoR1: Nuclear receptor corepressor 1
NELFB_428_450	2.2	NELF-B: Negative elongation factor B
NELFB_80_102	4.9	
NR0B1_1_23	7.3	DAX-1: nuclear receptor subfamily group B member 1
NR0B1_136_159	3.9	
NR0B1_68_90_C69S	6.8	
NR0B2_106_128	12.2	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NR0B2_201_223_C207S	11.1	
NR0B2_9_31_C9S/C11S	3.4	
NRBF2_128_150	10.4	NRBF-2: Nuclear receptor subfamily 0 group B member 2

Table S1*A*. *Continued*

74 peptides	Relative binding value	32 coregulator descriptions
NRIP1_1055_1077	6.7	RIP140: receptor-interacting protein 1
NRIP1_120_142	15.3	
NRIP1_121_143_P124R	7.3	
NRIP1_173_195	6.6	
NRIP1_173_195_C177S	4.0	
NRIP1_253_275_C263S	21.3	
NRIP1_368_390	5.1	
NRIP1_488_510	8.1	
NRIP1_700_722	4.4	
NRIP1_701_723	4.4	
NRIP1_805_831	4.3	
NRIP1_924_946	3.2	
NRIP1_924_946_C945S	4.5	
NSD1_894_916	7.4	NSDP1: NR-binding SET domain-cotaining protein
PELP1_168_190	1.2	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PELP1_20_42	2.0	
PELP1_446_468	9.5	
PNRC1_306_327	1.7	PNRC1/B4-2: Proline-rich nuclear receptor coactivator 1
PPRC1_151_173	6.3	PRC: PGC-1 related coactivator
PR285_1105_1127	6.1	PDIP1: PPAR-gamma DBD-interacting protein 1
PRGC1_130_155	5.8	PGC1α: PPAR-g co-activator 1-alpha
PRGC1_134_154	5.2	
PRGC2_146_166	7.1	PGC1β: PPAR-g co-activator 1-alpha
PRGC2_338_358	6.0	
PROX1_57_79	12.7	PROX1: homeobox prospero-like protein
RBL2_875_897_C879S/C894S	1.4	
TIF1A_747_769	4.6	
TIP60_476_498	12.1	Tip60: 60 kDa Tat interactive protein
TREF1_168_190	43.7	TReP132: Transcriptional –regulating factor 1
TRXR1_132_154	13.1	TR: Thioredoxinreduactase TR1
UBE3A_649_671	2.8	E6-AP: E6AP ubiquitin protein ligase
WIPI1_119_141	19.2	WIPI-1: WD repeat phosphoinositide-interacting protein 1
WIPI1_313_335_C318S	6.1	
ZNHI3_89_111	5.5	TRIP-3: Thyroid receptor interacting protein 3
ZNT9_449_471	2.1	ZnT-9/HUEL: Zinc transporter 9/Humanembrynic lung protein

Table S1*B*. 8 peptides that positively interact with the BPA/ERα complex derived from 7 coregulators

8 peptides	Relative binding value	7 coregulator descriptions
NCOA1_620_643	2.0	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA1_737_759	2.5	
NCOA2_628_651	1.3	SRC-2/RIP160: nuclear receptor co-activator 1
NCOA6_875_897	1.5	NCoA-6/RAP250: nuclear receptor co-activator 6
NR0B2_106_128	1.1	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NRIP1_1055_1077	1.1	RIP140: receptor-interacting protein 1
PELP1_446_468	1.7	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PRGC1_134_154	1.1	PGC1α: PPAR-g co-activator 1-alpha

Table S1*C*. 9 peptides that positively interact with the BPAF/ERα complex derived from 9 coregulators

9 peptides	Relative binding value	9 coregulator descriptions
KIF11_832_854_C854S	1.9	KIF11: kinesin-like protein KIF11
MAPE_356_378	1.9	MAPE: melanoma antigen preferentially express protein
NCOA3_104_123_N-KKK	1.5	SRC-3/NCoA-2: nuclear receptor co-activator 3
PELP1_446_468	1.7	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PPRC1_1159_1181	1.5	PRC: PGC-related coactivator
PRDM2_948_970	1.6	PRDP2/MTB-ZF: PR domain-containing protein 2
PRGR_102_124	3.4	PR: progesterone receptor
TF65_437_459	2.2	TF65: transcription factor p65
ZNT9_449_471	1.5	ZnT-9/HUEL: zinc transporter 9

Table S1*D*. 25 peptides that positively interact with the BPS/ERα complex derived from 14 coregulators

25 peptides	Relative binding value	14 coregulator descriptions
JHD2C_2054_2076	1.5	TRIP-8: thyroid receptor-interacting protein 8
LCOR_40_62	3.1	LCoR: ligand-dependent corepressor
MED1_591_614	1.9	TRIP-2: thyroid receptor-interacting protein 2
NCOA1_1421_1441	1.3	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA1_620_643	3.6	
NCOA1_677_700	1.6	
NCOA2_628_651	1.9	SRC-2/NCoA-2: nuclear receptor co-activator 2
NCOA2_677_700	1.7	
NCOA2_733_755	1.4	
NCOA3_609_631	2.4	SRC-3/NCoA-3: nuclear receptor co-activator 3
NCOA3_609_631_C627S	1.7	
NCOA3_725_747	1.2	
NR0B1_1_23	1.6	DAX-1: nuclear receptor subfamily group B member 1
NR0B1_136_159	1.2	
NR0B1_68_90_C69S	1.6	
NR0B2_106_128	2.1	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NRIP1_1055_1077	1.9	RIP140: receptor-interacting protein 1
NRIP1_120_142	1.6	
NRIP1_253_275_C263S	1.9	
NSD1_894_916	1.2	NSDP1: NR-binding SET domain-cotaining protein
PELP1_446_468	1.6	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PRGC1_130_155	1.9	PGC1α: PPAR-g co-activator 1-alpha
PRGC1_134_154	1.5	
PRGC2_146_166	1.5	PGC1β: PPAR-g co-activator 1-beta
TIP60_476_498	1.4	Tip60: 60 kDa Tat interactive protein

Table S2*A*. 35 peptides that negatively interact with the E2/ERα complex derived from 22 coregulators

35 peptides	Relative binding value	22 coregulator descriptions
ANDR_10_32	0.6	AR: Androgen receptor
CENPR_159_177	0.4	CENP-R: Nuclear receptor-interaction factor 3
CHD9_1023_1045	0.4	CHD9: PPAR-alpha-interacting complex protein 320 kDa
CHD9_855_877	0.4	
CNOT1_140_162	0.2	NOT1H: Negative regulator of transcription subunit 1 homolog
CNOT1_1626_1648	0.3	
CNOT1_1929_1951_C1932S	0.3	
CNOT1_557_579	0.3	
DHX30_49_70	0.4	DHX30: DEAH box protein 30
GELS_376_398	0.7	ADF: Actin-depolymerizing factor
HAIR_553_575_C567S	0.4	H: Hairless
MAPE_249_271	0.7	OIP4: OPA-interacting protein 4
MAPE_300_322	0.3	
MAPE_454_476_C472S	0.2	
MAPE_91_113	0.2	
MEN1_255_277	0.6	MEN1: Menin
MGMT_86_108	0.4	MGMT: Methylated-DNA-protein-cysteine methyltransferase
MTA1S_388_410_C393S/C396S	0.7	MTA1: Metastasis-associated protein
NCOR1_2039_2061	0.2	N-CoR1: Nuclear receptor corespressor 1
NCOR1_2039_2061_C2056S	0.2	
NCOR1_2251_2273	0.3	
NCOR2_2123_2145	0.5	N-CoR2/SMRT: Nuclear receptor corespressor 2
NCOR2_2330_2352	0.3	
NCOR2_649_671_C649S	0.6	
NELFB_328_350	0.5	NELF-B: Negative elongation factor B
NR0B2_237_257	0.3	SHP: small heterodimer partner/nuclear receptor subfamily member 2
PAK6_248_270	0.7	PAK-6: Serine/threonine-protein kinase p21-activated kinase 6
PCAF_178_200	0.2	PCAF: P300/CBP-associated factor
PELP1_142_164	0.3	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PELP1_258_280	0.4	
PR285_2216_2238_C2219S	0.6	PDIP1: PPAR-gamma DBD-interacting protein 1
TGFI1_325_347_C334S/C346S	0.5	TGFI1: Transforming growth factor beta-1-induced transcript 1
TGFI1_443_461_C452S/C455S	0.4	
TIF1A_373_395_C394S	0.5	TIF1-a: Transcription intermediary factor 1-alpha
TREF1_850_872	0.5	TREF1: Transcriptional-regulating factor 1

Table S2*B*. 44 peptides that negatively interact with the BPA/ERα complex derived from 28 coregulators

44 peptides	Relative binding value	28 coregulator descriptions
ANDR_10_32	0.8	AR: Androgen receptor
BL1S1_1_11	0.8	BLOC-1: biogenesis of lysosome-related organelles complex-1 subunit 1
CBP_345_368_C367S	0.6	CBP: CREB-binding protein
CENPR_159_177	0.3	CENP-R: Centromere protein R
CNOT1_1626_1648	0.2	NOT1H: Negative regulator of transcription subunit 1 homolog
CNOT1_1929_1951_C1932S	0.3	
CNOT1_557_579	0.4	
DHX30_49_70	0.5	DHX30: DEAH box protein 30
GELS_376_398	0.5	ADF: Actin-depolymerizing factor
GNAQ_21_43	0.7	Gαq : Guanine nucleotide-binding protein alpha-q
HAIR_553_575_C567S	0.4	H: Hairless
IKBB_277_299	0.7	NFkB:NF-kappa-B inhibitor beta
MAPE_249_271	0.6	OIP4: OPA-interacting protein 4
MAPE_300_322	0.8	
MAPE_454_476_C472S	0.3	
MGMT_86_108	0.5	MGMT: Methylated-DNA-protein-cysteine methyltransferase
MTA1S_388_410_C393S/C396S	0.3	MTA1: Metastasis-associated protein
NCOR1_1925_1946	0.6	N-CoR1: Nuclear receptor corespressor 1
NCOR1_2039_2061	0.5	
NCOR1_2039_2061_C2056S	0.5	
NCOR1_2251_2273	0.3	
NCOR2_2123_2145	0.5	N-CoR2/SMRT: Nuclear receptor corespressor 2
NCOR2_2330_2352	0.2	
NR0B2_9_31_C9S/C11S	0.6	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NRIP1_700_722	0.8	RIP140: receptor-interacting protein 1
NRIP1_701_723	0.8	
NRIP1_924_946	0.7	
NSD1_894_916	0.6	NSDP1: NR-binding SET domain-cotaining protein
PCAF_178_200	0.3	PCAF: P300/CBP-associated factor
PELP1_142_164	0.3	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PELP1_168_190	0.6	
PELP1_258_280	0.4	
PELP1_496_518_C496S	0.5	
PELP1_56_78_C71S	0.6	
PPRC1_1159_1181	0.6	PRC: PGC-related coactivator
PR285_1062_1084	0.3	PDIP1: PPAR-gamma DBD-interacting protein 1
PR285_2216_2238_C2219S	0.4	
PRDM2_948_970	0.6	PRDP2/MTB-ZF: PR domain-containing protein 2
RAD9A_348_370	0.4	hRAD9: DNA repairexonuclease rad9 homolog A
TGFI1_325_347_C334S/C346S	0.7	TGFI1: Transforming growth factor beta-1-induced transcript 1
TIF1A_373_395_C394S	0.5	TIF1-a: Transcription intermediary factor 1-alpha
TREF1_850_872	0.7	TREF1: Transcriptional-regulating factor 1
TRRAP_770_792	0.6	
TRRAP_971_993	0.7	STAF40: Transformation/transcription domain-associated protein

Table S2*C*. 75 peptides that negatively interact with the BPAF/ERα complex derived from 38 coregulators

75 peptides	Relative binding value	38 coregulator descriptions
ANDR_10_32	0.4	AR: Androgen receptor
BL1S1_1_11	0.8	BLOC-1: biogenesis of lysosome-related organelles complex-1 subunit 1
CBP_345_367_C367S	0.7	CBP: CREB-binding protein
CENPR_159_177	0.4	CENP-R: Centromere protein R
CHD9_1023_1045	0.5	CHD9: PPAR-alpha-interacting complex protein 320 kDa
CNOT1_140_162	0.2	NOT1H: Negative regulator of transcription subunit 1 homolog
CNOT1_1626_1648	0.3	
CNOT1_1929_1951_C1932S	0.3	
DHX30_49_70	0.4	DHX30: DEAH box protein 30
GNAQ_21_43	0.3	Gαq : Guanine nucleotide-binding protein alpha-q
HAIR_553_575_C567S	0.2	H: Hairless
IKBB_277_299	0.3	NFkB: NF-kappa-B inhibitor beta
MAPE_249_271	0.3	OIP4: OPA-interacting protein 4
MAPE_300_322	0.4	
MAPE_454_476_C472S	0.2	
MAPE_91_113	0.4	
MED1_591_614	0.5	TRIP-2: thyroid receptor-interacting protein 2
MED1_632_655	0.4	
MLL2_4175_4197	0.4	MLL2/KMT2B: Myeloid/lymphoid or mixed-lineage leukemia protein 2
NCOA1_1421_1441	0.5	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA1_620_643	0.4	
NCOA1_677_700	0.4	
NCOA2_628_651	0.5	SRC-2/NCoA-2: nuclear receptor co-activator 2
NCOA2_677_700	0.4	
NCOA3_609_631_C627S	0.3	SRC-3/NCoA-3: nuclear receptor co-activator 3
NCOA3_673_695	0.4	
NCOR1_2039_2061	0.3	N-CoR1: Nuclear receptor corespressor 1
NCOR1_2039_2061_C2056S	0.4	
NCOR1_2376_2398	0.5	
NCOR1_662_684_C662S	0.4	
NCOR2_2330_2352	0.3	N-CoR2/SMRT: Nuclear receptor corespressor 2
NCOR2_649_671_C649S	0.5	
NELFB_328_350	0.4	NELF-B: Negative elongation factor B
NELFB_428_450	0.4	
NR0B1_1_23	0.5	DAX-1: nuclear receptor subfamily member 1
NR0B1_136_159	0.5	
NR0B1_68_90_C69S	0.4	
NR0B2_106_128	0.4	
NR0B2_201_223_C207S	0.4	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NR0B2_9_31_C9S/C11S	0.2	
NRIP1_1055_1077	0.5	RIP140: receptor-interacting protein 1
NRIP1_120_142	0.3	
NRIP1_121_143_P124R	0.4	
NRIP1_253_275_C263S	0.5	
NRIP1_368_390	0.4	
NRIP1_488_510	0.3	
NRIP1_700_722	0.4	
NRIP1_701_723	0.4	
NRIP1_805_831	0.4	
NRIP1_924_946	0.4	
NRIP1_924_946_C945S	0.4	
NSD1_894_916	0.5	NSDP1: NR-binding SET domain-cotaining protein

Table S2*C*. *Continued*

75 peptides	Relative binding value	38 coregulator descriptions
PAK6_248_270	0.5	PAK 6: Serine/threonine –protein kinase PAK 6
PELP1_168_190	0.4	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PELP1_258_280	0.5	
PELP1_496_518_C496S	0.4	
PELP1_56_78_C71S	0.5	
PELP1_571_593_C575S/C581S	0.3	
PR285_2216_2238_C2219S	0.2	PDIP1: PPAR-gamma DBD-interacting protein 1
PRGC1_130_155	0.5	PGC1α: PPAR-g co-activator 1-alpha
PRGC1_134_154	0.5	
PRGC2_146_166	0.4	
PROX1_57_79	0.3	PROX1: homeobox prospero-like protein
RAD9A_348_370	0.4	hRAD9: DNA repairexonuclease rad9 homolog A
TGFI1_325_347_C334S/C346S	0.5	TGFI1: Transforming growth factor beta-1-induced transcript 1
TGFI1_443_461_C452S/C455S	0.6	
TIF1A_373_395_C394S	0.2	TIF1-a: Transcription intermediary factor 1-alpha
TIF1A_747_769	0.4	
TIP60_476_498	0.6	Tip60: 60 kDa Tat interactive protein
TREF1_850_872	0.7	TREF1: Transcriptional-regulating factor 1
TRIP4_149_171_C171S	0.4	ASC-1: Activating signal cointegrator 1
TRRAP_770_792	0.6	STAF40: Transformation/transcription domain-associated
TRRAP_971_993	0.5	protein
TRXR1_132_154	0.2	TR: Thioredoxinreduactase TR1
ZNHI3_89_111	0.4	TRIP-3: Thyroid receptor interacting protein 3

Table S2*D*. 31 peptides that negatively interact with the BPS/ERα complex derived from 23 coregulators

31 peptides	Relative binding value	23 coregulator descriptions
BL1S1_1_11	0.8	BLOC-1: biogenesis of lysosome-related organelles complex-1 subunit 1
CBP_345_367_C367S	0.6	CBP: CREB-binding protein
CHD9_1023_1045	0.6	CHD9: PPAR-alpha-interacting complex protein 320 kDa
CNOT1_1626_1648	0.4	NOT1H: Negative regulator of transcription subunit 1 homolog
CNOT1_1929_1951_C1932S	0.3	
DHX30_49_70	0.5	DHX30: DEAH box protein 30
GELS_376_398	0.5	ADF: Actin-depolymerizing factor
GNAQ_21_43	0.7	Gαq : Guanine nucleotide-binding protein alpha-q
HAIR_553_575_C567S	0.4	H: Hairless
MAPE_249_271	0.7	OIP4: OPA-interacting protein 4
MAPE_300_322	0.7	
MAPE_454_476_C472S	0.4	
MAPE_91_113	0.3	
MGMT_86_108	0.7	MGMT: Methylated-DNA-protein-cysteine methyltransferase
NCOR1_2039_2061	0.6	N-CoR1: Nuclear receptor corespressor 1
NCOR1_2251_2273	0.3	
NCOR1_662_684_C662S	0.6	
NCOR2_2330_2352	0.3	N-CoR2/SMRT: Nuclear receptor corespressor 2
NELFB_328_350	0.5	NELF-B: Negative elongation factor B
NRIP1_700_722	0.8	RIP140: receptor-interacting protein 1
PAK6_248_270	0.8	PAK 6: Serine/threonine –protein kinase PAK 6
PCAF_178_200	0.2	PCAF: P300/CBP-associated factor
PELP1_142_164	0.4	PELP1: Proline-, glutamic acid-, and leucine-rich protein 1
PELP1_258_280	0.4	
PPRC1_1159_1181	0.6	PRC: PGC-related coactivator
PR285_1062_1084	0.4	PDIP1: PPAR-gamma DBD-interacting protein 1
PRDM2_948_970	0.6	PRDP2/MTB-ZF: PR domain-containing protein 2
RAD9A_348_370	0.4	hRAD9: DNA repairexonuclease rad9 homolog A
TGFI1_325_347_C334S/C346S	0.7	TGFI1: Transforming growth factor beta-1-induced transcript 1
TGFI1_443_461_C452S/C455S	0.6	
TIF1A_373_395_C394S	0.5	TIF1-α: Transcription intermediary factor 1-alpha

Table S3. Coregulator PELP1 in the ligand-ERα complex by MARCoNI assay

			Relative binding values			
Peptides	Peptide sequences	Motifs	E2	BPA	BPAF	BPS
PELP1_20_42	GTGGLSAVSSGPRLRLLLLESVS	LxxLL33	2.0	-	-	-
PELP1_56_78_C71S	VHPPNRSAPHLPGLMSLLRLHGS	LxxLL69	-	0.6	0.5	-
PELP1_142_164	QDPPATMELAVAVLRDLLRYAAQ	LxxLL155	0.3	0.3	-	0.4
PELP1_168_190	LFRDISMNHLPGLLTSLLGLRPE	LxxLL181	1.2	0.6	0.4	-
PELP1_258_280	ESWEQELHSLLASLHTLLGALYE	LxxLL271	0.4	0.4	0.5	0.4
PELP1_446_468	AGMLQGGASGEALLTHLLSDISP	LxxLL459	9.5	1.7	1.7	1.6
PELP1_496_518_C496S	SPFFLQSLHGDGPLRLLLLPSIH	LxxLL509	-	0.5	0.4	-
PELP1_571_593_C575S/C581S	TSSRSRRELYSLLLALLLAPSPR	LxxLL584	-	-	0.3	-

Red (relative binding value >1) shows positive interaction; Blue (relative binding value <1) shows negative interaction

Figure S1

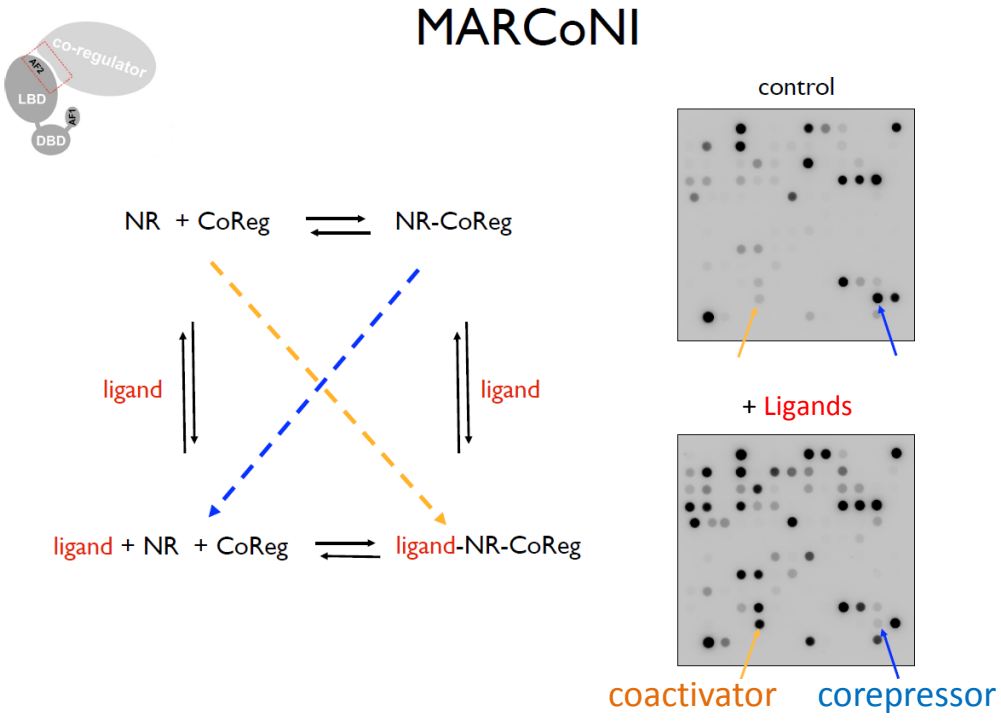

Figure S1. Working model of the MARCoNI assay for an agonist.

Figure S2

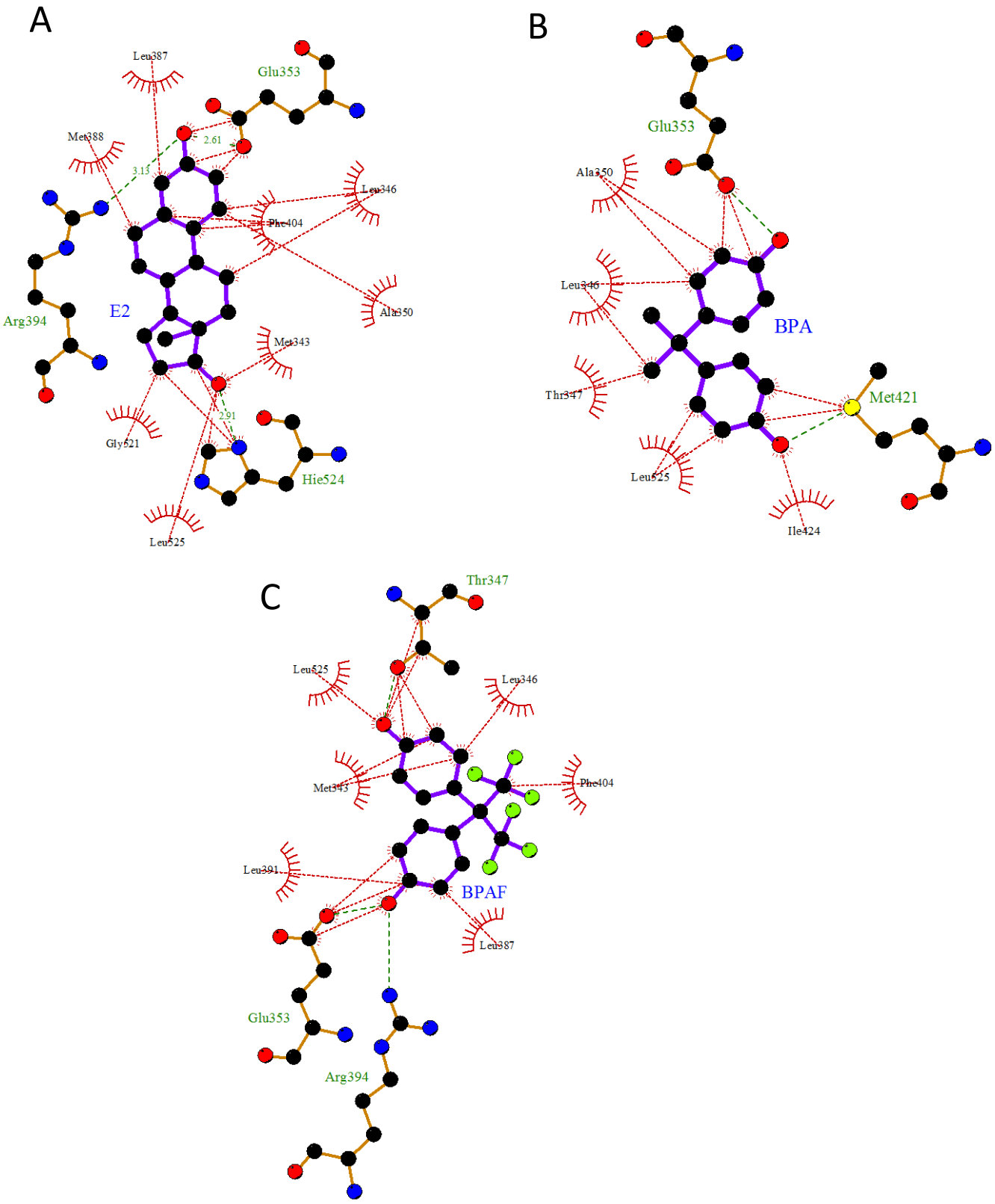
A
Leu387
Glu353
Met388
2.61
3.15
Leu346
Phe404
Ala350
Arg394
E2
Met343
2.91
Gly521
Hie524
Leu525
B
Glu353
Ala350
Leu346
BPA
Thr347
Met421
Leu525
Ile424
C
Thr347
Leu525
Leu346
Met343
Phe404
Leu391
BPAF
Leu387
Glu353
Arg394

Figure S3

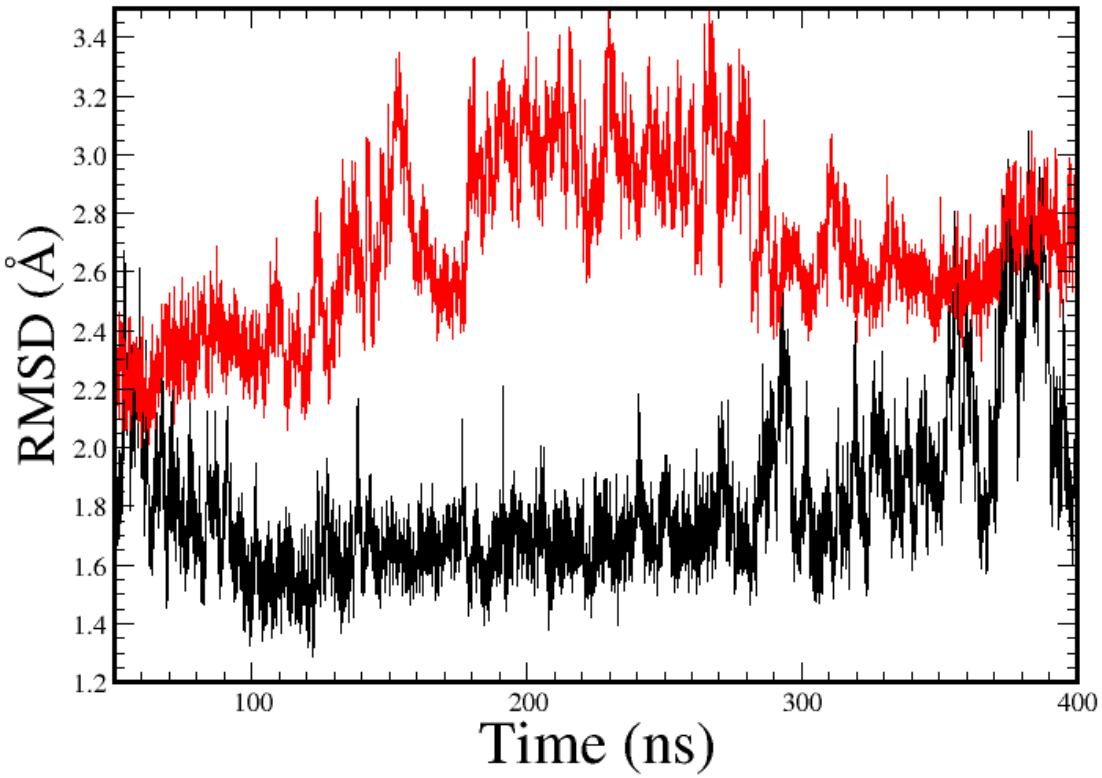

Figure S3. Root mean square deviation (RMSD) of ligand-free ER structures started at agonist (black) and antagonist (red) forms The X-ray crystal structures were used as the reference structures for these calculations and all backbone heavy atoms of each residue were used to calculate the average RMSD at each time point.

Figure S4

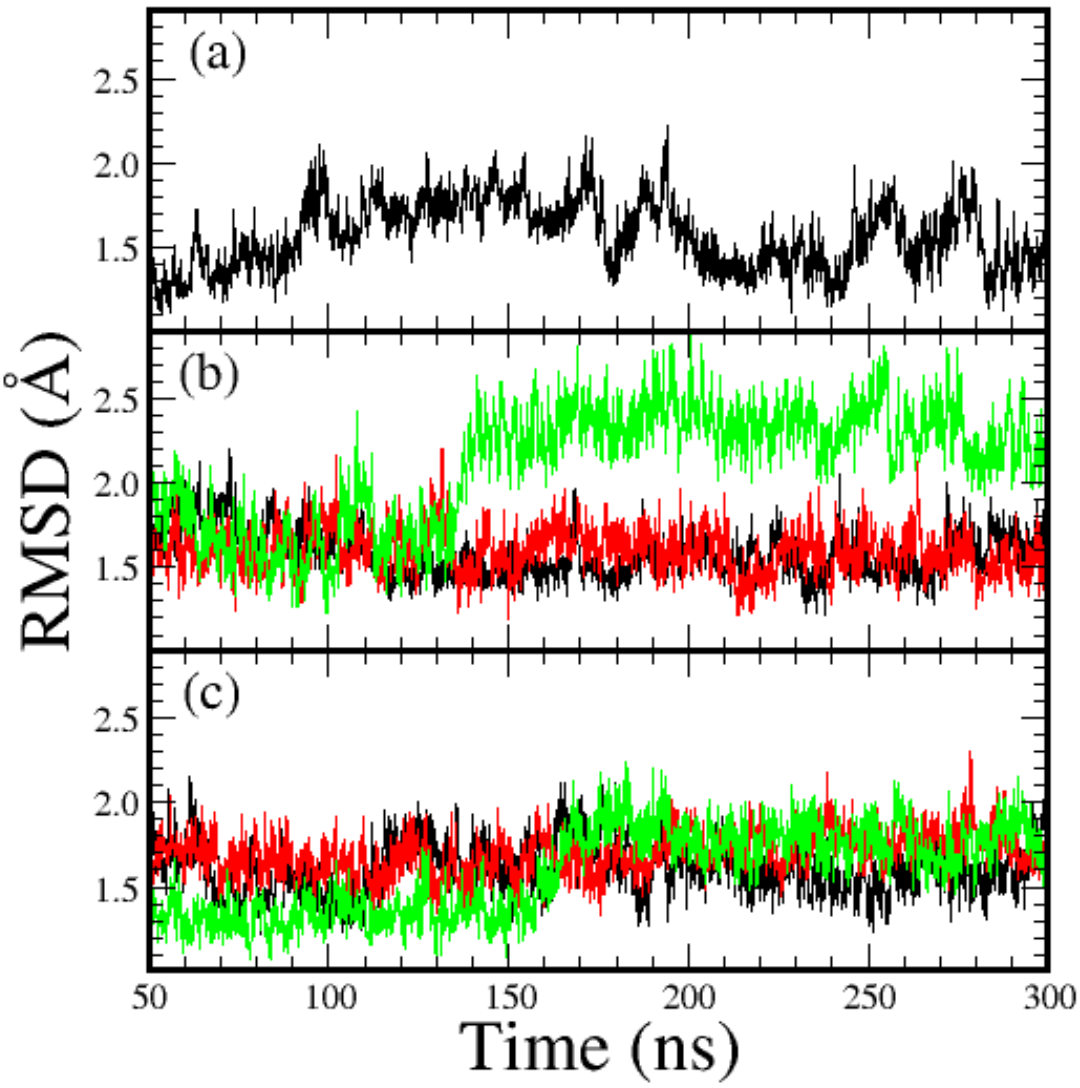

Figure S4. Root mean square deviation (RMSD) of various ligand bound ER structures in which ligand is at the regular ligand binding site. The X-ray crystal structures were used as the reference structures for these calculations and all backbone heavy atoms of each residue were used to calculate the average RMSD at each time point.

Figure S5

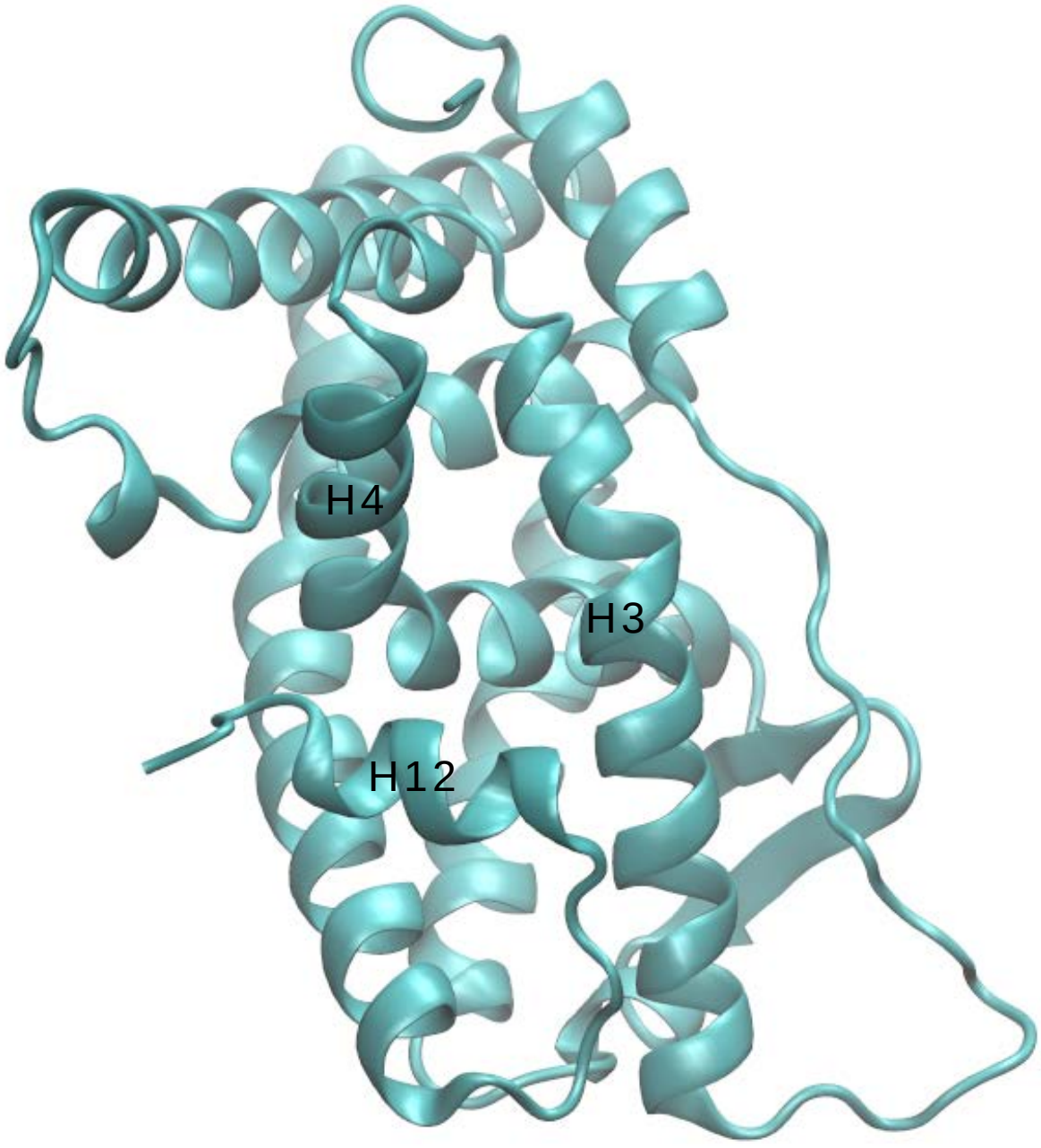

Figure S5. E2-bound conformation of ERα (in agonist form).

Figure S6

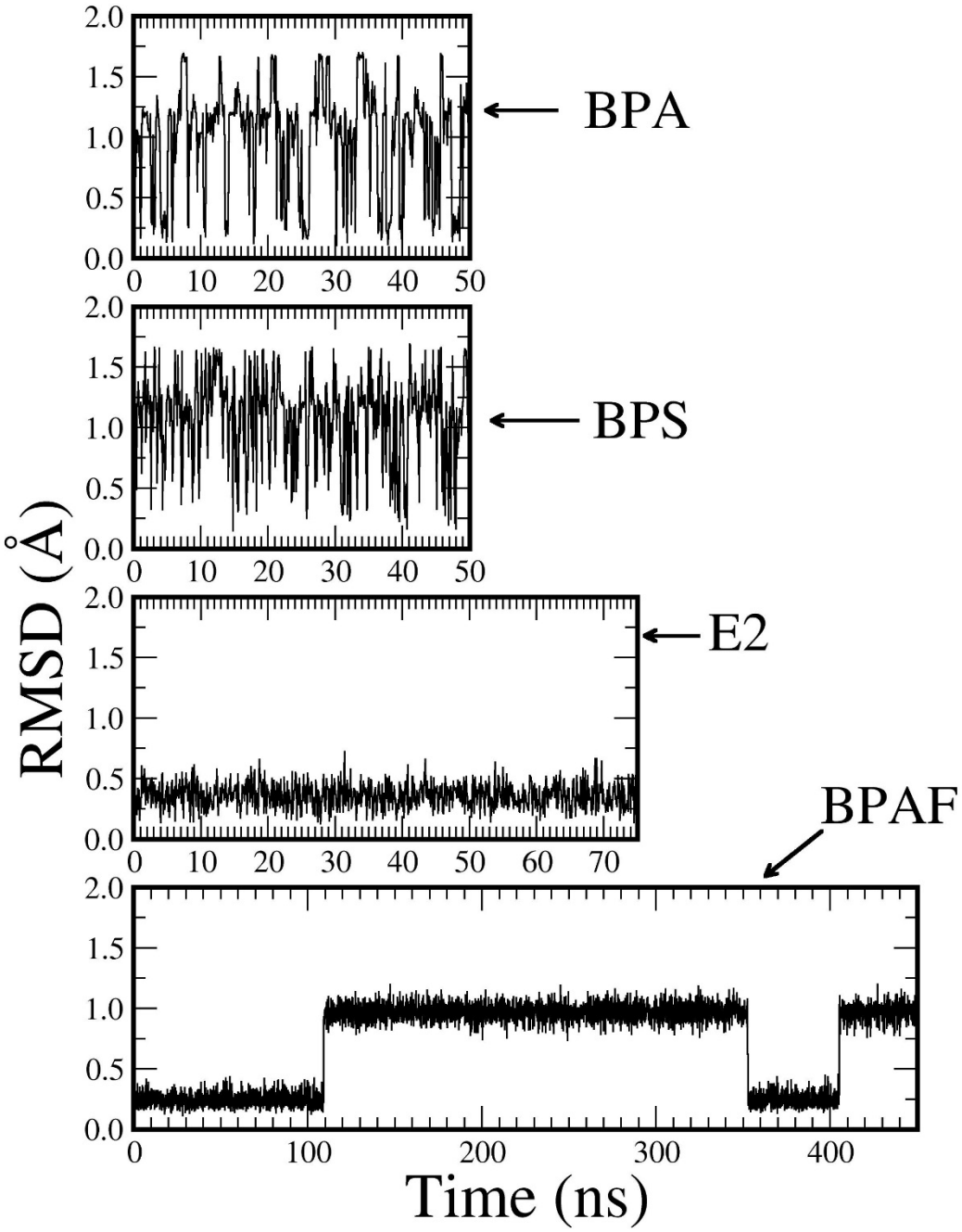

Figure S6. Root mean square deviation (RMSD) of the ligands in their aqueous environments. Starting structures were used as the reference structures for these calculations and all heavy atoms were used to calculate the average RMSD at each time point.

Figure S7

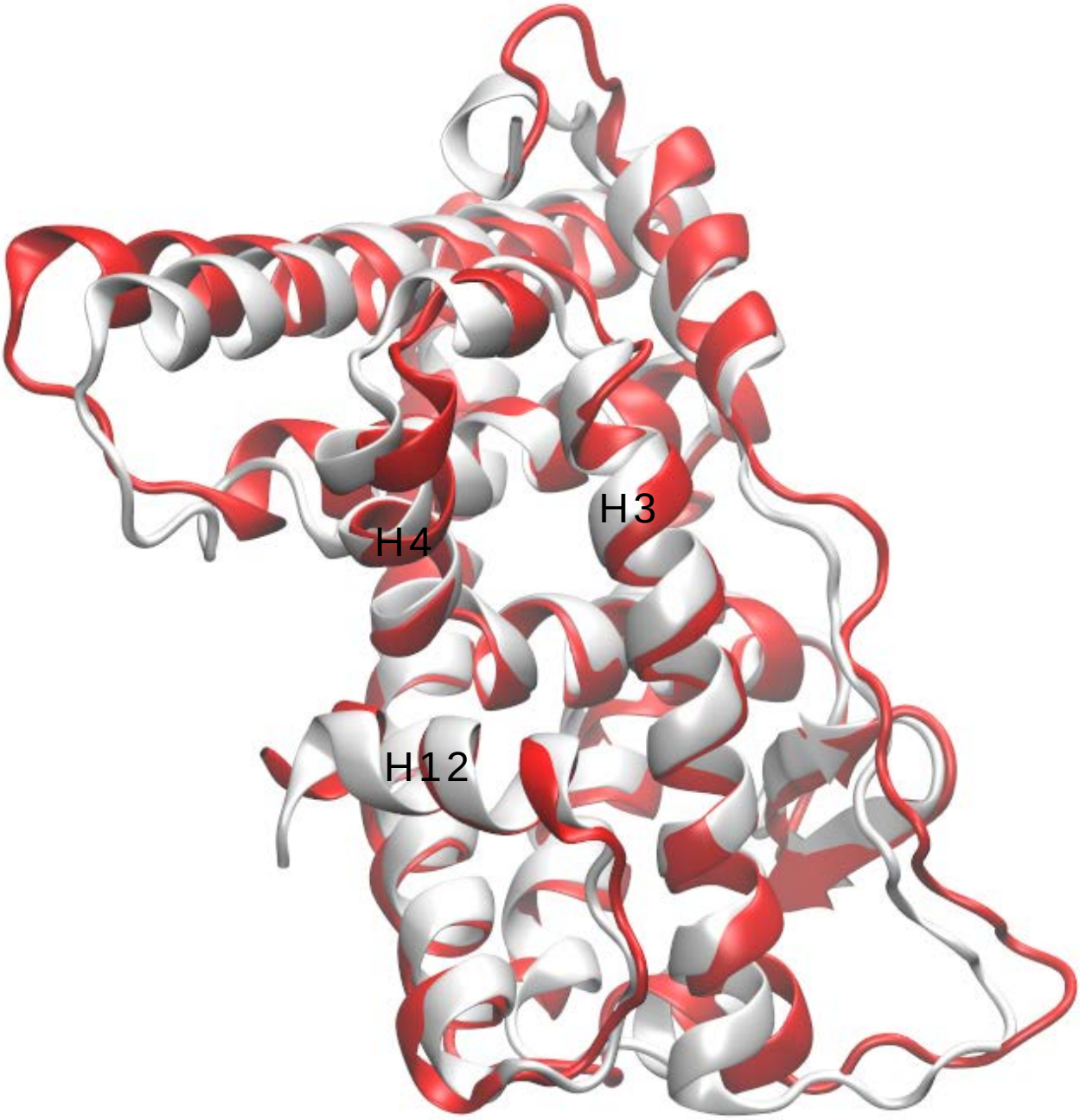

Figure S7. Ligand free conformation of ERα (starting with aqueous agonist form). Starting conformation is in white and the final conformation is in red.

Figure S8

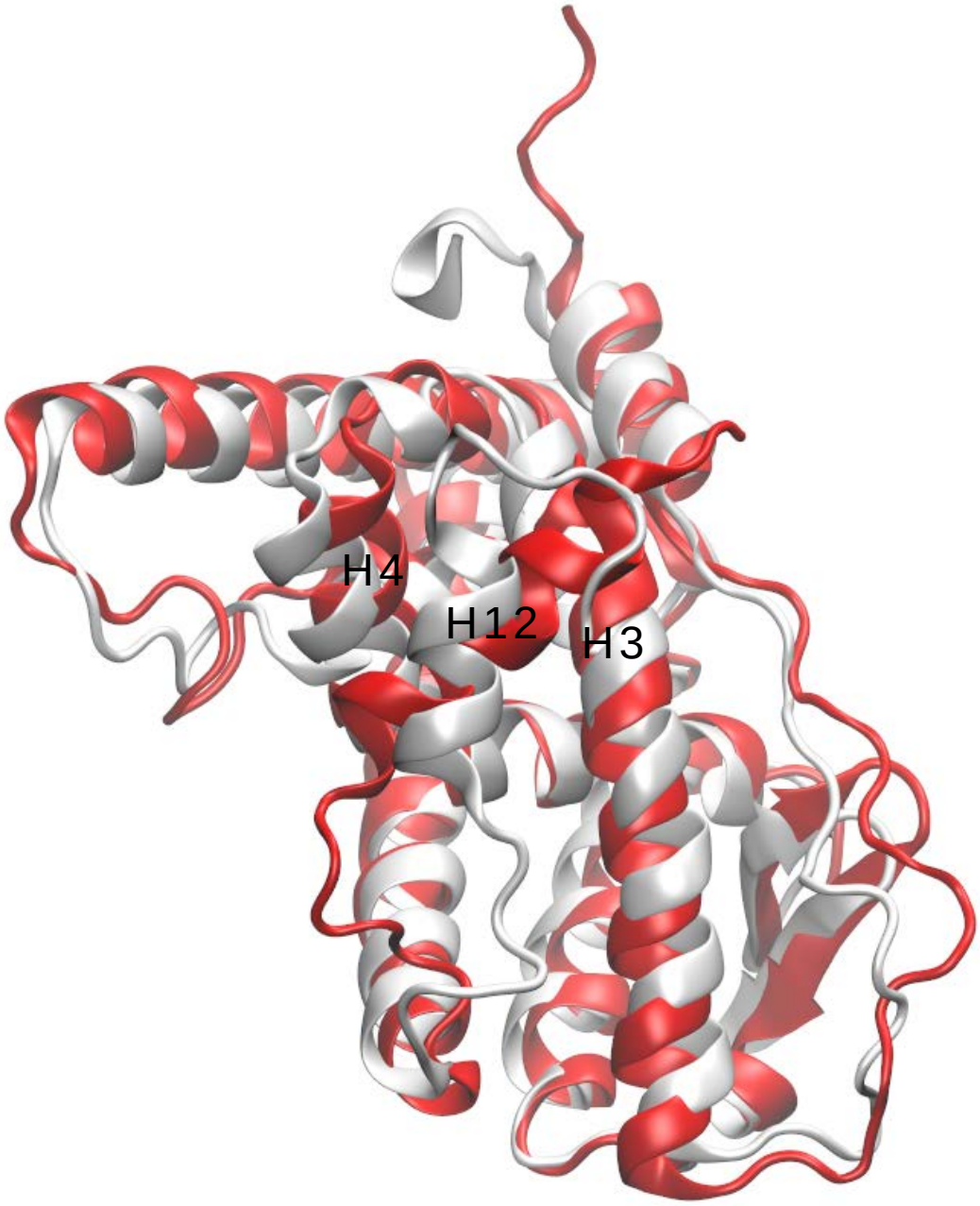

Figure S8. Ligand free conformation of ERα (starting with antagonist form). Starting conformation is in white and the final conformation is in red.

Figure S9

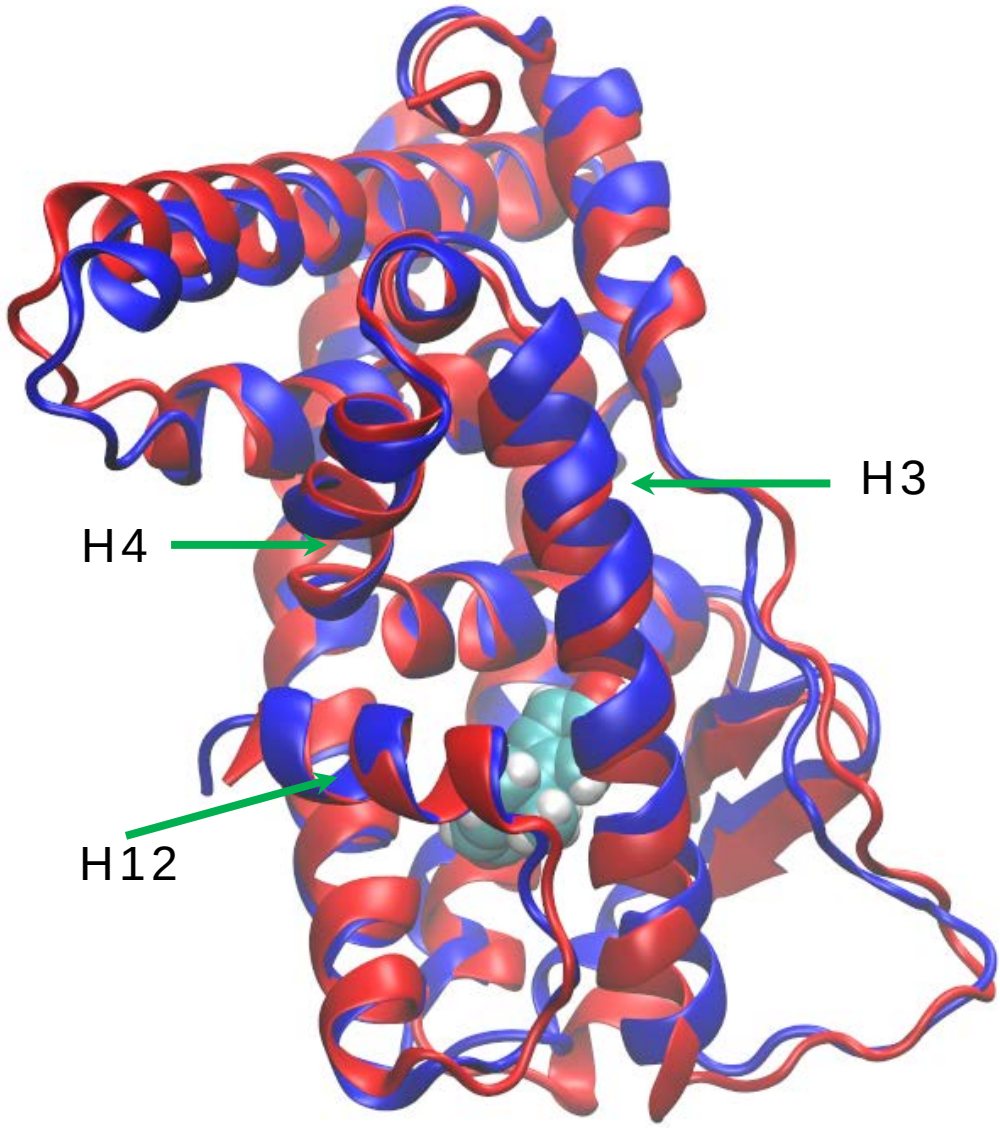

Figure S9 BPA-bound conformations of ERα (Blue-BPA in agonist conformation; Red-BPA in antagonist conformation). BPA is shown in space filling in the ligand binding site of ERα.

Figure S10

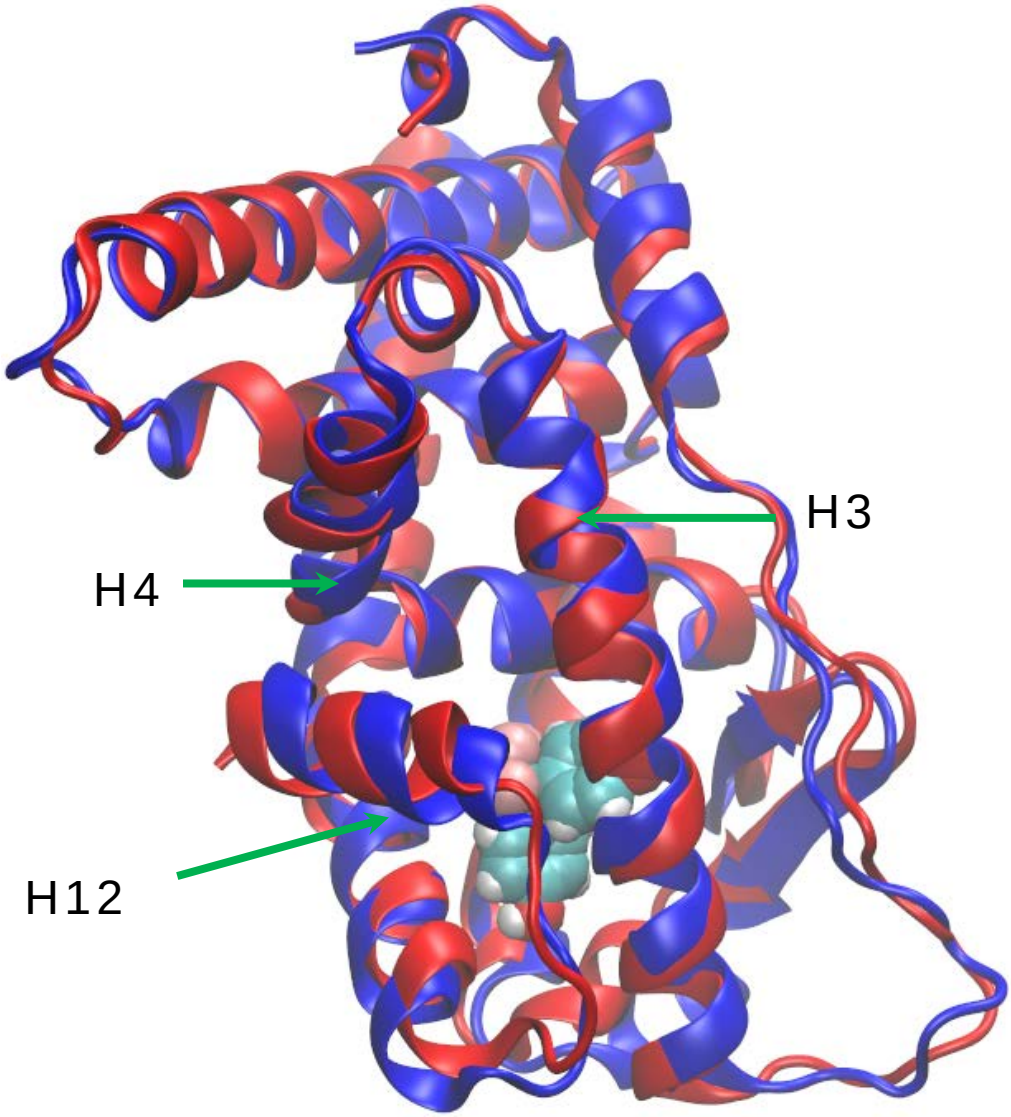

Figure S10 BPAF-bound conformations of ERα (Blue-BPAF in agonist conformation; Red-BPAF in antagonist conformation). BPA is shown in space filling in the ligand binding site of ERα.

Figure S11

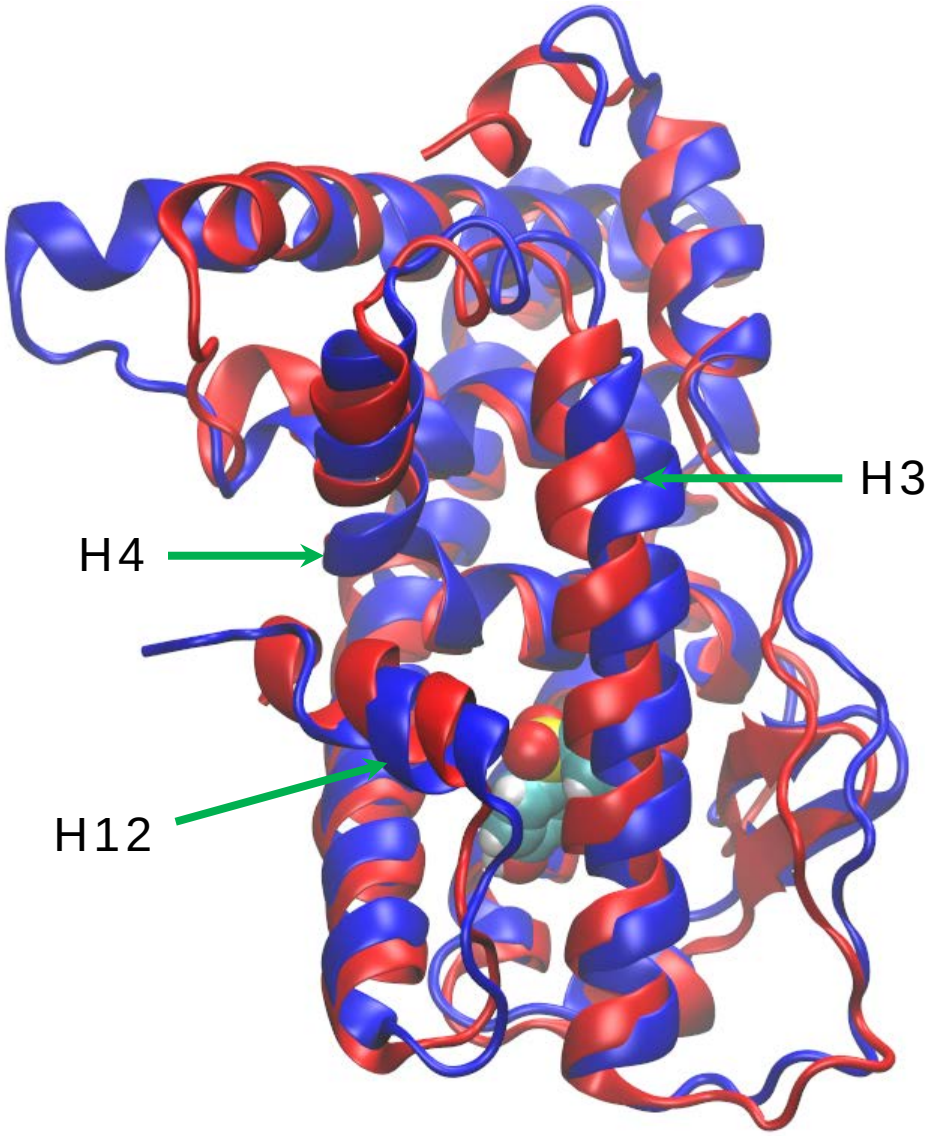

Figure S11 BPS-bound conformations of ERα (Blue-BPS in agonist conformation; Red-BPS in antagonist conformation). BPA is shown in space filling in the ligand binding site of ERα.

Figure S12

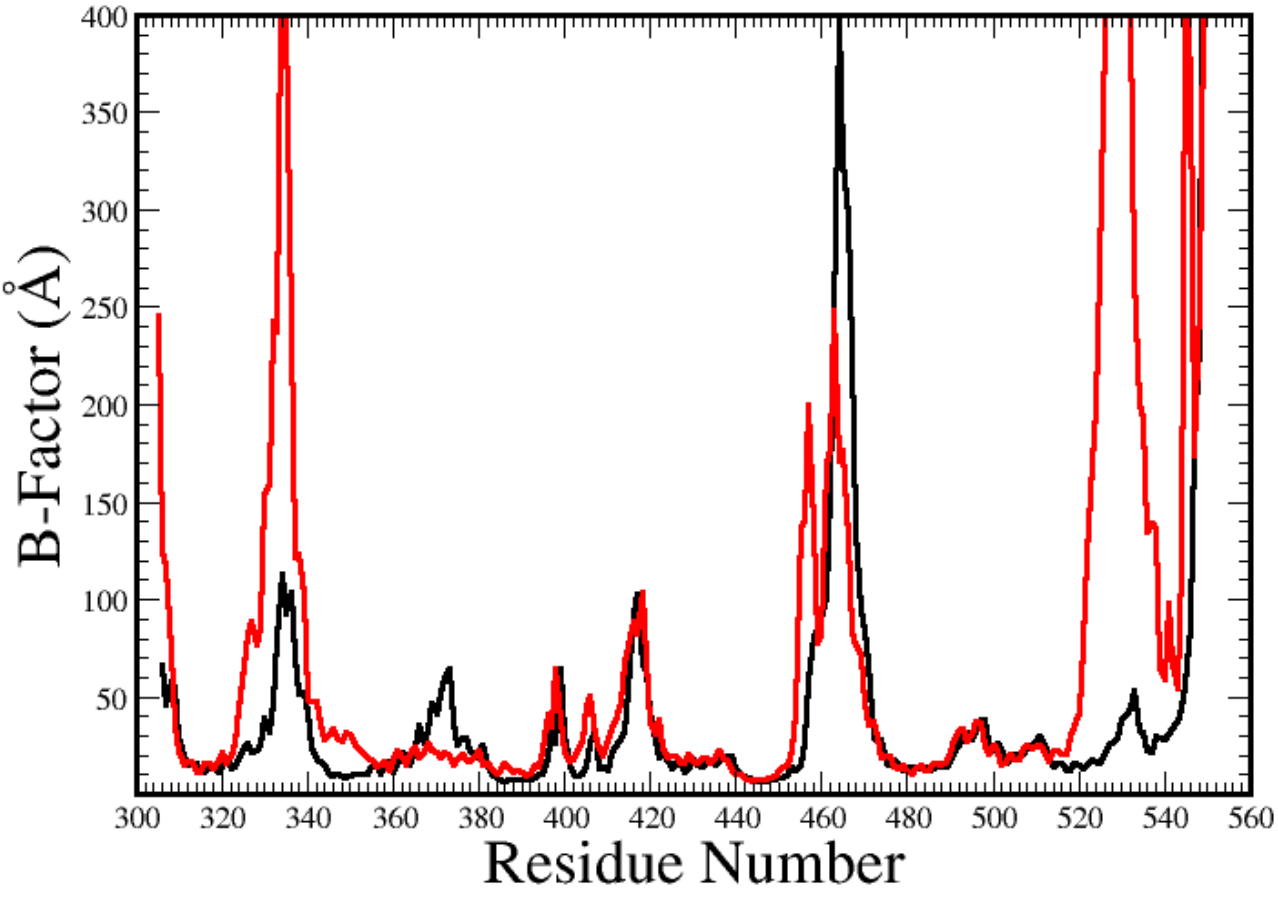

Figure S12 B-factors of backbone atoms of the ligand-free conformations of ERα (Black-in agonist conformation; Red-in antagonist conformation)

Figure S13

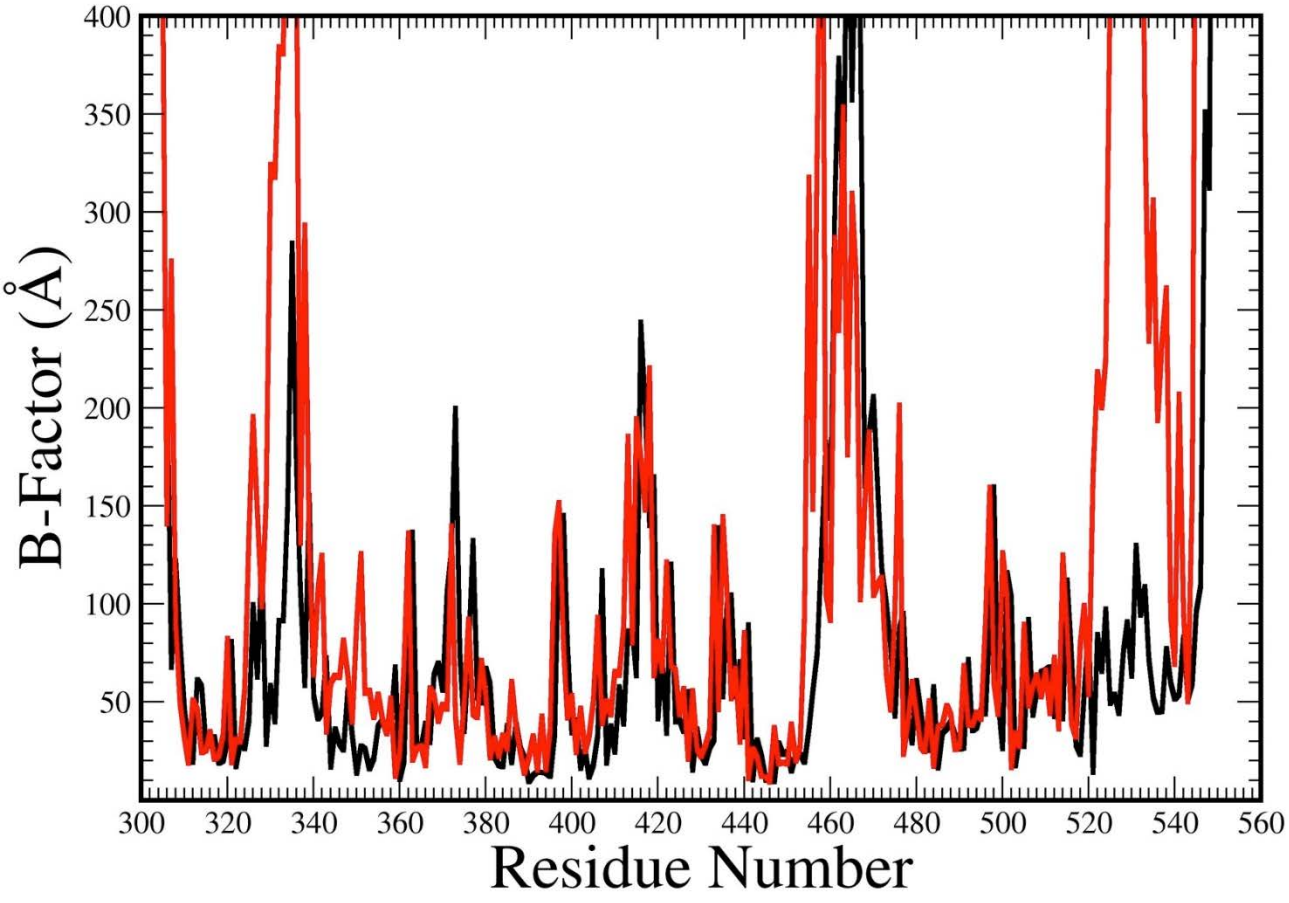

Figure S13 B-factors of side chain atoms of the ligand-free conformations of ERα (Black-in agonist conformation; Red-in antagonist conformation)

Figure S14

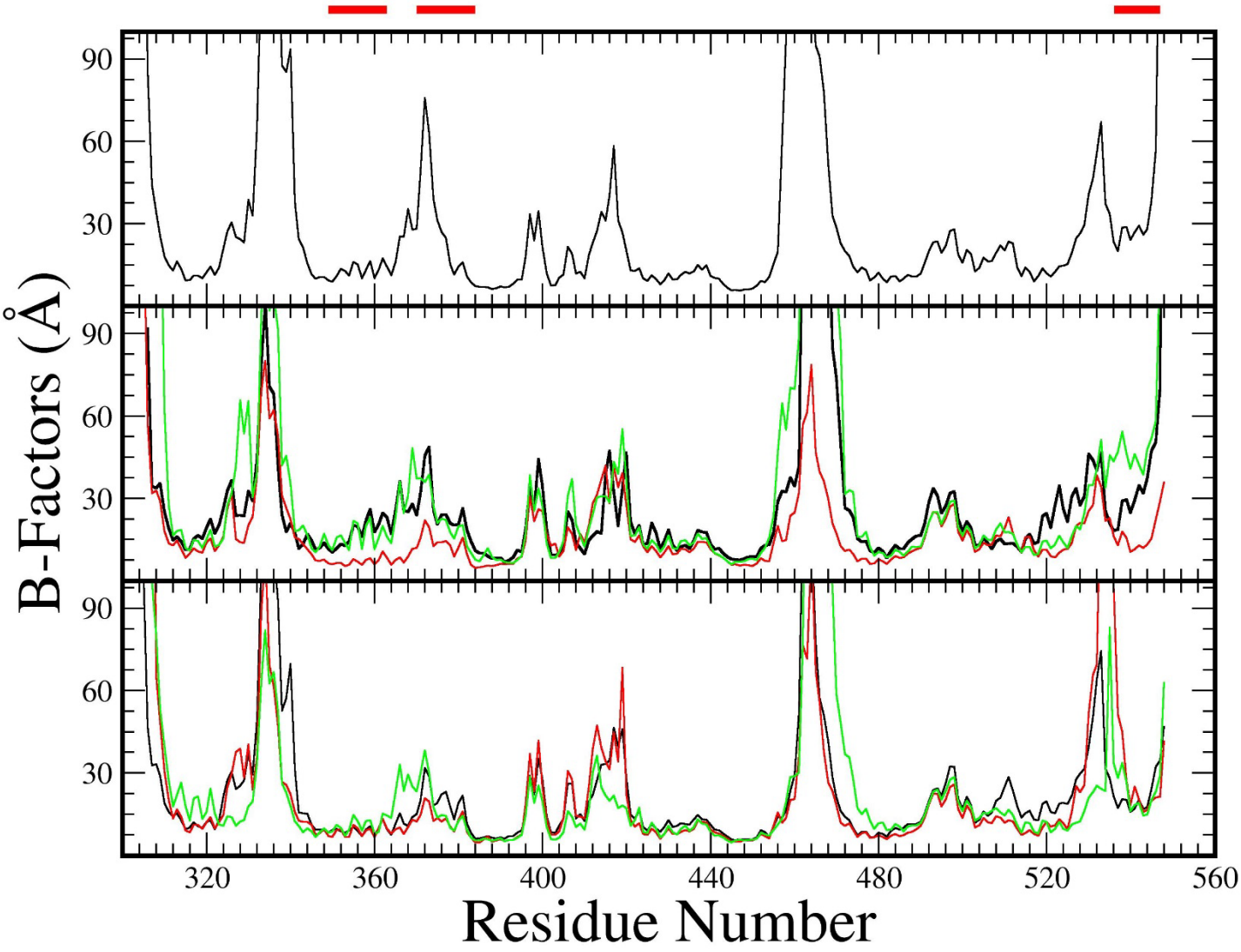

Figure S14 B-factors of backbone atoms of the ligand-bound conformations of ERα (Black-in agonist conformation; Red-in antagonist conformation)

Figure S15

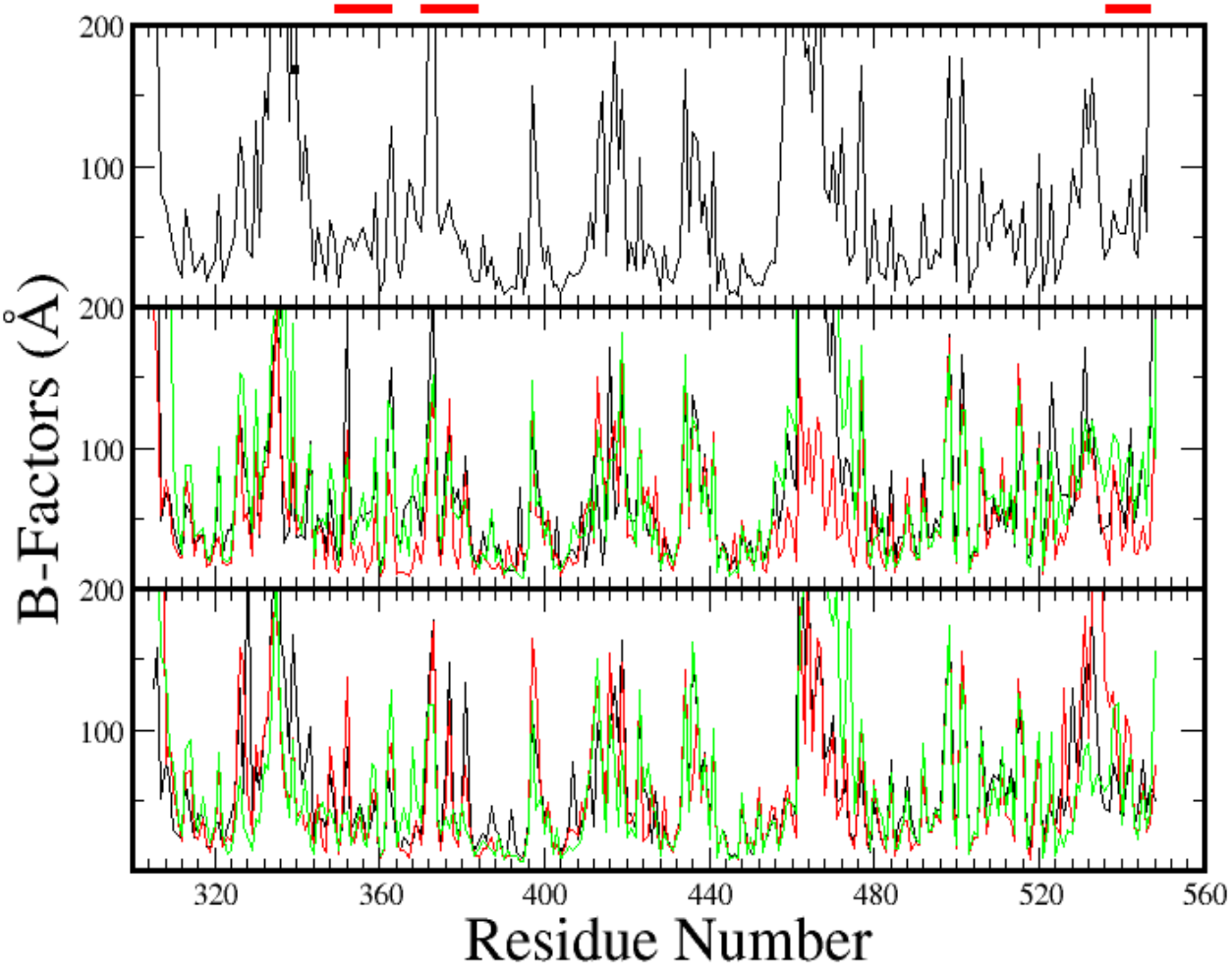

Figure S15 B-factors of side chain atoms of the ligand-bound conformations of ERα.