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Supplemental Material

Differential *in Vitro* Biological Action, Coregulator Interactions, and Molecular Dynamic Analysis of Bisphenol A (BPA), BPAF, and BPC Ligand–ERα Complexes

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Figure S1. Working model of the MARCoNI assay. Cell lysates ware isolated from the EGFP-tagged full-length human ERα-transfected U2OS cells. For MARCoNI, 25 μL assay mixtures that contain cell lysates, 25 nM of Alexa488-conjugated GFP-antibody (Invitrogen), and 0.2 mM ligand (E2, BPA, BPAF or BPS, pre-diluted in DMSO, final concentration 2%) were prepared on ice. The ligand-modulated coregulator interactions with the EGFP-tagged ERα was assessed using a PamChip® plate which contained the 154 coregulator-derived binding peptides. The positive interaction means that ligand increases peptide binding and negative interaction means that the ligand decreases peptide binding to ERα.

Figure S2. Ligand interactions from the reported X-ray crystal structures. The ligand interactions with ER that were found in the X-ray crystal structures (A) E2, (B) BPA, and (C and D) BPAF in two conformations.

Figure S3. Root mean square deviations (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 RMSD calculations and all backbone heavy atoms of each residue were used to calculate the average RMSD at each time point.

Figure S4. Ligand free conformations of ERα (starting with aqueous agonist form). Starting conformation is in white and the final conformation is in red. The three helices that are involved in creating the coregulator surface are marked.

Figure S5. Ligand free conformations of ERα (starting with antagonist form). Starting conformation is in white and the final conformation is in red.

Figure S6. Root mean square deviations (RMSD) of various ligand bound-ER structures. 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. For BPA, BPAF, and BPS, the black curve corresponds to the starting ligand in conformation "1" and the red curve to the ligand in conformation "2".

Figure S7. E2-bound conformation of ERα (in which h12 is in agonist conformation). The final conformation of ERα with E2 from the MD simulation.

Figure S8. BPA-bound conformations of ERα. The final conformation of ERα with BPA started in conformation 1 is shown in the blue ribbon; The final conformation of ERα with BPA started in conformation 2 is shown in the red ribbon. BPA is shown in space filling in the ligand binding site of ERα.

Figure S9. BPAF-bound conformations of ERα. The final conformation of ERα with BPAF started in conformation 1 is show in the blue ribbon; The final conformation of ERα with BPAF started in conformation 2 is shown in the red ribbon. BPAF is shown in space filling in the ligand binding site of ERα.

Figure S10. BPS-bound conformations of ERα. The final conformation of ERα with BPS started in conformation 1 is shown in the blue ribbon; The final conformation of ERα with BPS started in conformation 2 is shown in the red ribbon. BPS is shown in space filling in the ligand binding site of ERα.

Figure S11. Root mean square deviations (RMSD) of the ligands in their aqueous environments. Starting structures were used as the reference structures for these calculations and all heavy atoms of the ligand were used to calculate the average RMSD at each time point.

Additional File - Excel Document.

Table S1. qPCR primers used in this study

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

Table S2. 74 peptides that positively interact[a] with the E2/ERα complex derived from 32 coregulator

Peptide	Relative binding value[b]	Coregulator
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 CBP_57_80	1.3 24.4	CBP: CREB-binding protein
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 MED1_632_655	11.2 21.1	TRIP-2: thyroid receptor-interacting protein 2
MLL2_4175_4197 MLL2_4702_4724	2.7 3.0	MLL2/KMT2B: myeloid/lymphoid or mixed-lineage leukemia protein 2
NCOA1_1421_1441 NCOA1_620_643 NCOA1_677_700 NCOA1_737_759	5.6 45.1 5.5 47.5	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA2_628_651 NCOA2_677_700 NCOA2_733_755	12.8 9.7 8.6	SRC-2/NCoA-2: nuclear receptor co-activator 2
NCOA3_104_123_N-KKK NCOA3_609_631 NCOA3_609_631_C627S NCOA3_673_695 NCOA3_725_747	2.5 21.1 13.1 5.8 6.3	SRC-3/NCoA-3: nuclear receptor co-activator 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 NELFB_80_102	2.2 4.9	NELF-B: negative elongation factor B
NR0B1_1_23 NR0B1_136_159 NR0B1_68_90_C69S	7.3 3.9 6.8	DAX-1: nuclear receptor subfamily group B member 1
NR0B2_106_128 NR0B2_201_223_C207S NR0B2_9_31_C9S/C11S	12.2 11.1 3.4	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NRBF2_128_150	10.4	NRBF-2: nuclear receptor subfamily 0 group B member 2

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

[a]Positive interactions defined as: binding value>50, relative binding >1, p-value <0.05

[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO

For complete results, see Excel Table S2

Table S3. 8 peptides that positively interact[a] with the BPA/ERα complex derived from 7 coregulators

Peptide	Relative binding value[b]	Coregulator
NCOA1_620_643 NCOA1_737_759	2.0 2.5	SRC-1/RIP160: nuclear receptor co-activator 1
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

[a]Positive interactions defined as: binding value>50, relative binding >1, p-value <0.05
[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO
For complete results, see Excel Table S2

Table S4. 9 peptides that positively interact[a] with the BPAF/ERα complex derived from 9 coregulators

Peptide	Relative binding value[b]	Coregulator
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

[a]Positive interactions defined as: binding value>50, relative binding >1, p-value <0.05
[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO
For complete results, see Excel Table S2

Table S5. 25 peptides that positively interact[a] with the BPS/ERα complex derived from 14 coregulators

Peptide	Relative binding value[b]	Coregulator
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 NCOA1_620_643 NCOA1_677_700	1.3 3.6 1.6	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA2_628_651 NCOA2_677_700 NCOA2_733_755	1.9 1.7 1.4	SRC-2/NCoA-2: nuclear receptor co-activator 2
NCOA3_609_631 NCOA3_609_631_C627S NCOA3_725_747	2.4 1.7 1.2	SRC-3/NCoA-3: nuclear receptor co-activator 3
NR0B1_1_23 NR0B1_136_159 NR0B1_68_90_C69S	1.6 1.2 1.6	DAX-1: nuclear receptor subfamily group B member 1
NR0B2_106_128	2.1	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NRIP1_1055_1077 NRIP1_120_142 NRIP1_253_275_C263S	1.9 1.6 1.9	RIP140: receptor-interacting protein 1
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 PRGC1_134_154	1.9 1.5	PGC1α: PPAR-g co-activator 1-alpha
PRGC2_146_166	1.5	PGC1β: PPAR-g co-activator 1-beta
TIP60_476_498	1.4	Tip60: 60 kDa Tat interactive protein

[a]Positive interactions defined as: binding value>50, relative binding >1, p-value <0.05

[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO

For complete results, see Excel Table S2

Table S6. 35 peptides that negatively interact[a] with the E2/ERα complex derived from 22 coregulators

Peptide	Relative binding value[b]	Coregulator
ANDR_10_32	0.6	AR: Androgen receptor
CENPR_159_177	0.4	CENP-R: nuclear receptor-interaction factor 3
CHD9_1023_1045 CHD9_855_877	0.4 0.4	CHD9: PPAR-alpha-interacting complex protein 320 kDa
CNOT1_140_162 CNOT1_1626_1648 CNOT1_1929_1951_C1932S CNOT1_557_579	0.2 0.3 0.3 0.3	NOT1H: negative regulator of transcription subunit 1 homolog
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 MAPE_300_322 MAPE_454_476_C472S MAPE_91_113	0.7 0.3 0.2 0.2	OIP4: OPA-interacting protein 4
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 NCOR1_2039_2061_C2056S NCOR1_2251_2273	0.2 0.2 0.3	N-CoR1: nuclear receptor corespressor 1
NCOR2_2123_2145 NCOR2_2330_2352 NCOR2_649_671_C649S	0.5 0.3 0.6	N-CoR2/SMRT: nuclear receptor corespressor 2
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 PELP1_258_280	0.3 0.4	PELP1: proline-, glutamic acid-, and leucine-rich protein 1
PR285_2216_2238_C2219S	0.6	PDIP1: PPAR-gamma DBD-interacting protein 1
TGFI1_325_347_C334S/C346S TGFI1_443_461_C452S/C455S	0.5 0.4	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	0.5	TREF1: transcriptional-regulating factor 1

[a]Negative interactions defined as: binding value>50, relative binding <1, p-value <0.05

[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO

For complete results, see Excel Table S2

Table S7. 44 peptides that negatively interact[a] with the BPA/ERα complex derived from 28 coregulators

Peptide	Relative binding value[b]	Coregulator
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 CNOT1_1929_1951_C1932S CNOT1_557_579	0.2 0.3 0.4	NOT1H: negative regulator of transcription subunit 1 homolog
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 MAPE_300_322 MAPE_454_476_C472S	0.6 0.8 0.3	OIP4: OPA-interacting protein 4
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 NCOR1_2039_2061 NCOR1_2039_2061_C2056S NCOR1_2251_2273	0.6 0.5 0.5 0.3	N-CoR1: nuclear receptor corespressor 1
NCOR2_2123_2145 NCOR2_2330_2352	0.5 0.2	N-CoR2/SMRT: nuclear receptor corespressor 2
NR0B2_9_31_C9S/C11S	0.6	SHP: small heterodimer partner/nuclear receptor subfamily member 2
NRIP1_700_722 NRIP1_701_723 NRIP1_924_946	0.8 0.8 0.7	RIP140: receptor-interacting protein 1
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 PELP1_168_190 PELP1_258_280 PELP1_496_518_C496S PELP1_56_78_C71S	0.3 0.6 0.4 0.5 0.6	PELP1: proline-, glutamic acid-, and leucine-rich protein 1
PPRC1_1159_1181	0.6	PRC: PGC-related coactivator
PR285_1062_1084 PR285_2216_2238_C2219S	0.3 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
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 TRRAP_971_993	0.6 0.7	STAF40: transformation/transcription domain-associated protein

[a]Negative interactions defined as: binding value>50, relative binding <1, p-value <0.05

[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO

For complete results, see Excel Table S2

Table S8. 75 peptides that negatively interact[a] with the BPAF/ERα complex derived from 38 coregulators

Peptide	Relative binding value[b]	Coregulator
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 CNOT1_1626_1648 CNOT1_1929_1951_C1932S	0.2 0.3 0.3	NOT1H: negative regulator of transcription subunit 1 homolog
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 MAPE_300_322 MAPE_454_476_C472S MAPE_91_113	0.3 0.4 0.2 0.4	OIP4: OPA-interacting protein 4
MED1_591_614 MED1_632_655	0.5 0.4	TRIP-2: thyroid receptor-interacting protein 2
MLL2_4175_4197	0.4	MLL2/KMT2B: myeloid/lymphoid or mixed-lineage leukemia protein 2
NCOA1_1421_1441 NCOA1_620_643 NCOA1_677_700	0.5 0.4 0.4	SRC-1/RIP160: nuclear receptor co-activator 1
NCOA2_628_651 NCOA2_677_700	0.5 0.4	SRC-2/NCoA-2: nuclear receptor co-activator 2
NCOA3_609_631_C627S NCOA3_673_695	0.3 0.4	SRC-3/NCoA-3: nuclear receptor co-activator 3
NCOR1_2039_2061 NCOR1_2039_2061_C2056S NCOR1_2376_2398 NCOR1_662_684_C662S	0.3 0.4 0.5 0.4	N-CoR1: nuclear receptor corespressor 1
NCOR2_2330_2352 NCOR2_649_671_C649S	0.3 0.5	N-CoR2/SMRT: nuclear receptor corespressor 2
NELFB_328_350 NELFB_428_450	0.4 0.4	NELF-B: negative elongation factor B
NR0B1_1_23 NR0B1_136_159 NR0B1_68_90_C69S	0.5 0.5 0.4	DAX-1: nuclear receptor subfamily member 1
NR0B2_106_128 NR0B2_201_223_C207S NR0B2_9_31_C9S/C11S	0.4 0.4 0.2	SHP: small heterodimer partner/nuclear receptor subfamily member 2

NRIP1_1055_1077 NRIP1_120_142 NRIP1_121_143_P124R NRIP1_253_275_C263S NRIP1_368_390 NRIP1_488_510 NRIP1_700_722 NRIP1_701_723 NRIP1_805_831 NRIP1_924_946 NRIP1_924_946_C945S	0.5 0.3 0.4 0.5 0.4 0.3 0.4 0.4 0.4 0.4 0.4	RIP140: receptor-interacting protein 1
NSD1_894_916	0.5	NSDP1: NR-binding SET domain-cotaining protein
PAK6_248_270	0.5	PAK 6: serine/threonine –protein kinase
PELP1_168_190 PELP1_258_280 PELP1_496_518_C496S PELP1_56_78_C71S PELP1_571_593_C575S/C581S	0.4 0.5 0.4 0.5 0.3	PELP1: proline-, glutamic acid-, and leucine-rich protein 1
PR285_2216_2238_C2219S	0.2	PDIP1: PPAR-gamma DBD-interacting protein 1
PRGC1_130_155 PRGC1_134_154 PRGC2_146_166	0.5 0.5 0.4	PGC1α: PPAR-g co-activator 1-alpha
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 TGFI1_443_461_C452S/C455S	0.5 0.6	TGFI1: transforming growth factor beta-1-induced transcript 1
TIF1A_373_395_C394S TIF1A_747_769	0.2 0.4	TIF1-a: transcription intermediary factor 1-alpha
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 TRRAP_971_993	0.6 0.5	STAF40: transformation/transcription domain-associated protein
TRXR1_132_154	0.2	TR: thioredoxinreduactase TR1
ZNHI3_89_111	0.4	TRIP-3: thyroid receptor interacting protein 3

[a]Negative interactions defined as: binding value>50, relative binding <1, p-value <0.05

[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO

For complete results, see Excel Table S2

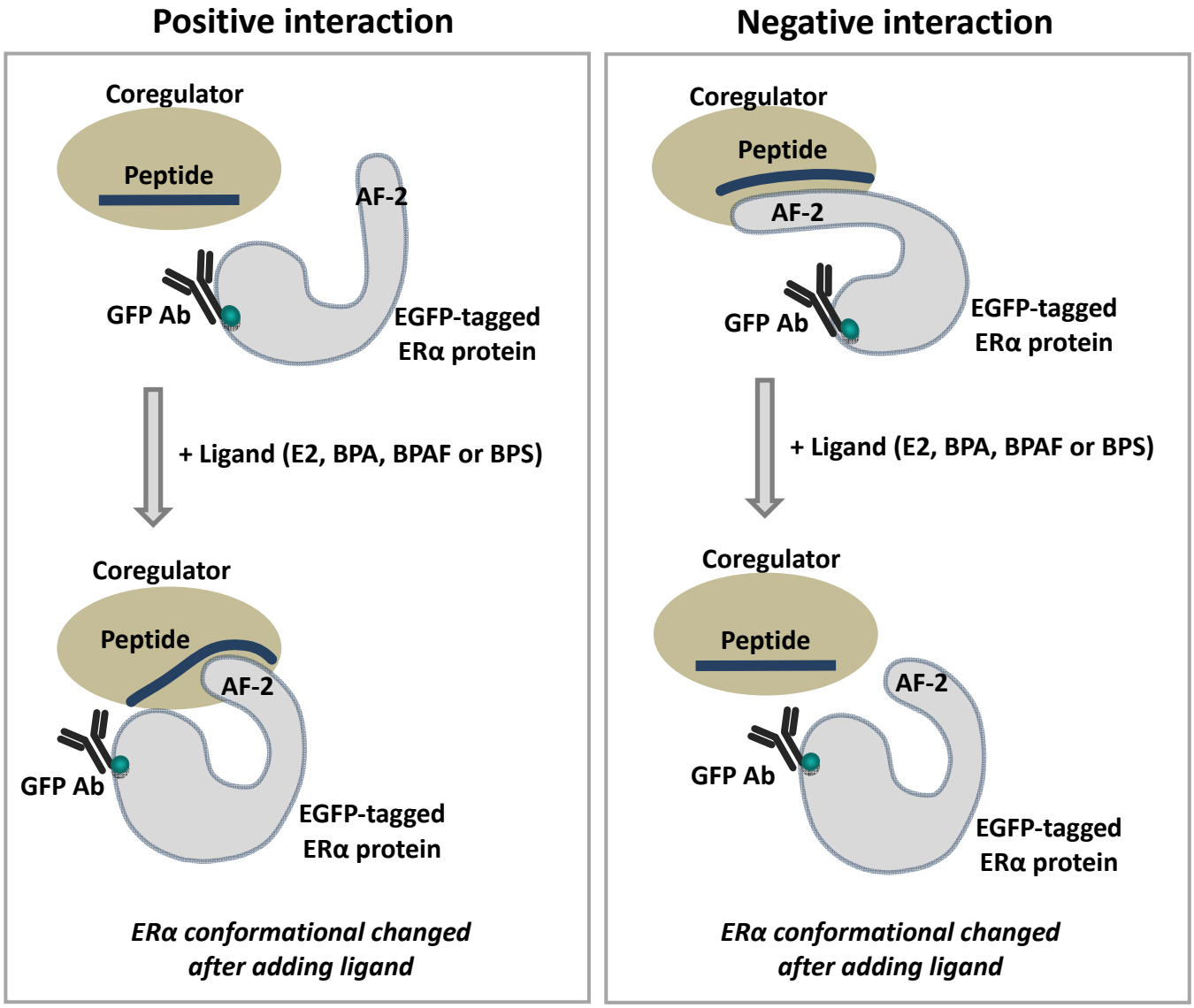
Table S9. 31 peptides that negatively interact[a] with the BPS/ERα complex derived from 23 coregulators

Peptide	Relative binding value[b]	Coregulator
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 CNOT1_1929_1951_C1932S	0.4 0.3	NOT1H: negative regulator of transcription subunit 1 homolog
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 MAPE_300_322 MAPE_454_476_C472S MAPE_91_113	0.7 0.7 0.4 0.3	OIP4: OPA-interacting protein 4
MGMT_86_108	0.7	MGMT: methylated-DNA-protein-cysteine methyltransferase
NCOR1_2039_2061 NCOR1_2251_2273 NCOR1_662_684_C662S	0.6 0.3 0.6	N-CoR1: nuclear receptor corespressor 1
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 PELP1_258_280	0.4 0.4	PELP1: proline-, glutamic acid-, and leucine-rich protein 1
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 TGFI1_443_461_C452S/C455S	0.7 0.6	TGFI1: transforming growth factor beta-1-induced transcript 1
TIF1A_373_395_C394S	0.5	TIF1-α: transcription intermediary factor 1-alpha

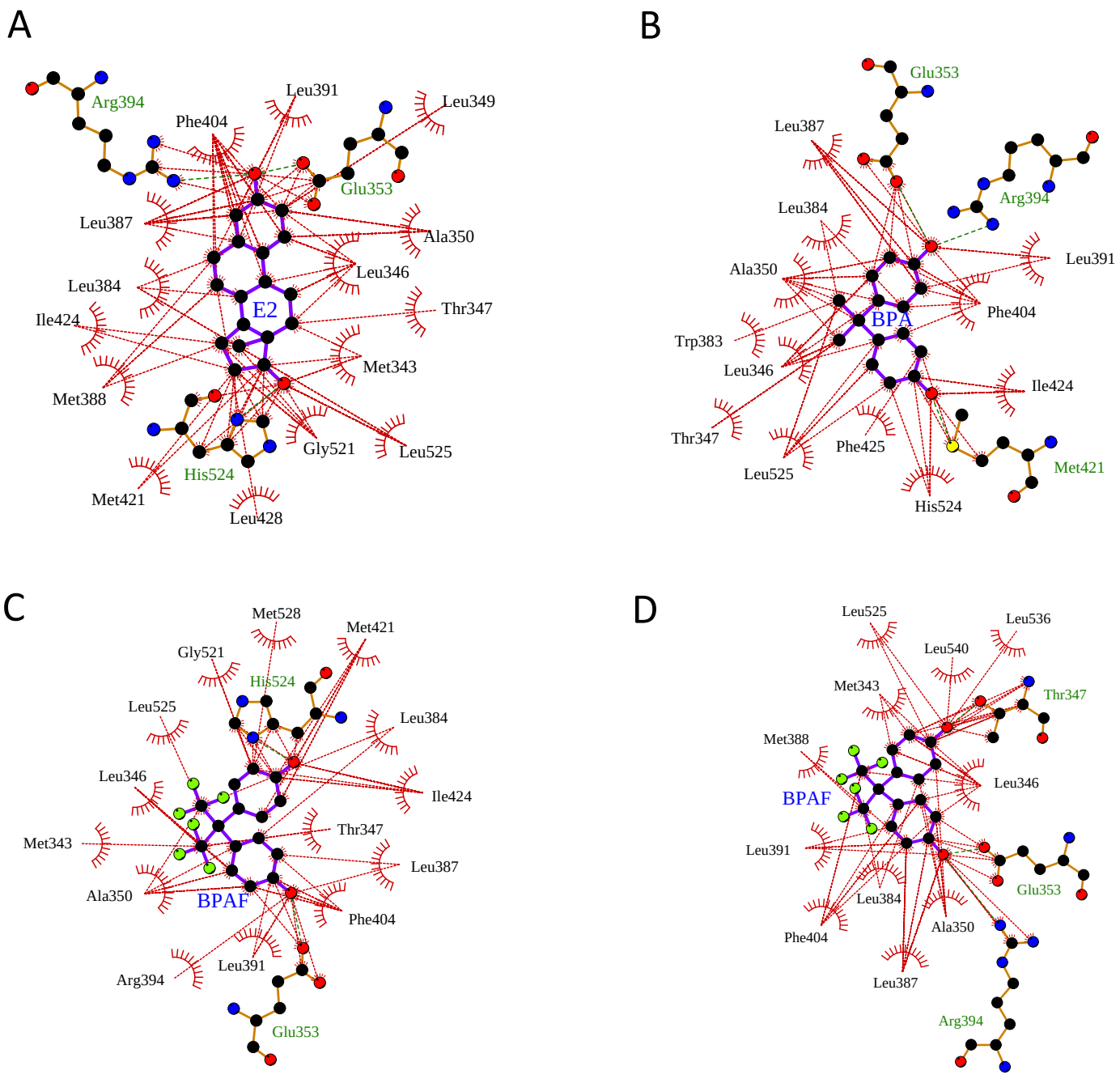
[a]Negative interactions defined as: binding value>50, relative binding <1, p-value <0.05

[b]Relative binding value shows each peptide binding relative to the control, 2% DMSO

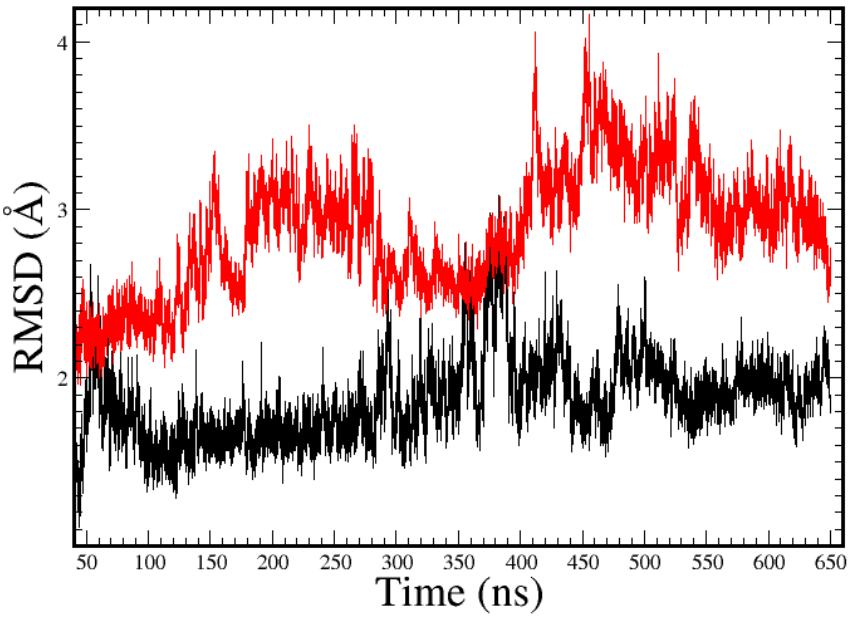
For complete results, see Excel Table S2



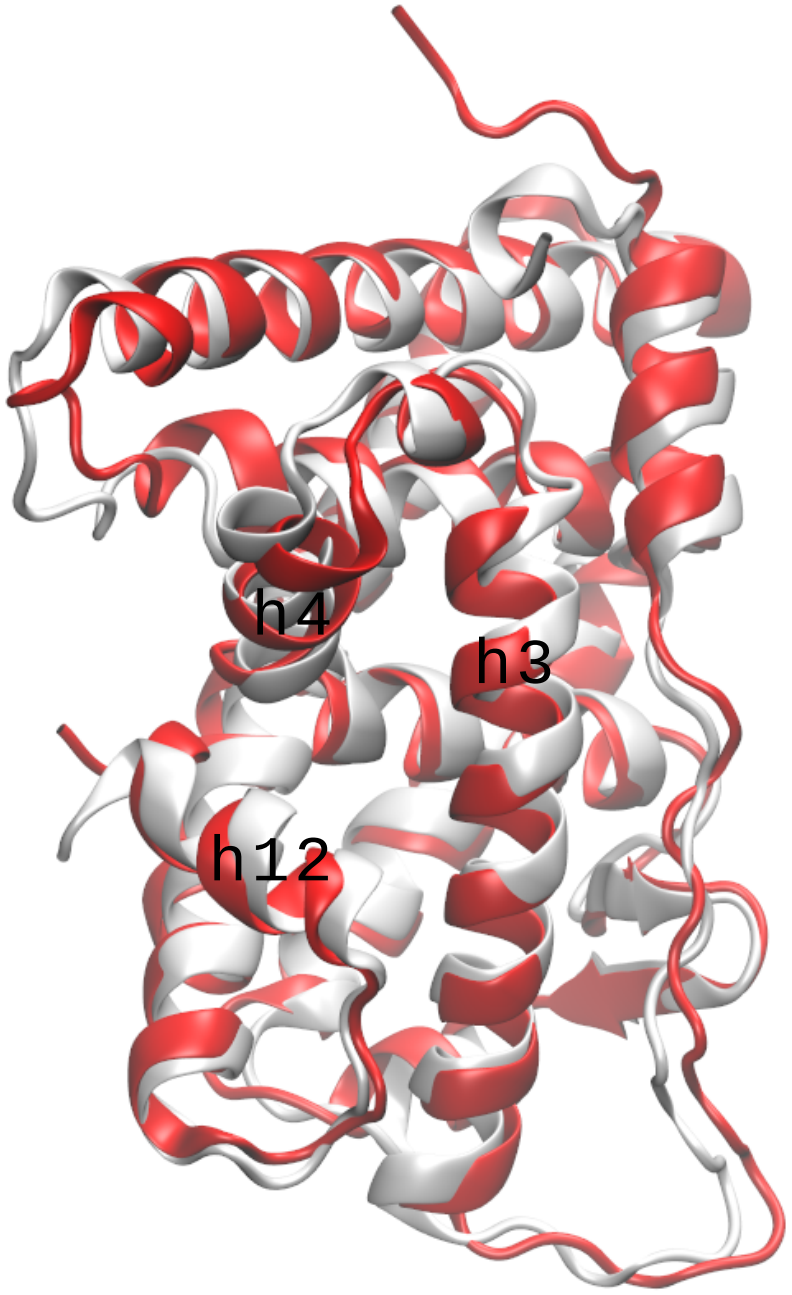
Supplemental Figure S1. Working model of the MARCoNI assay. Cell lysates ware isolated from the EGFP-tagged full-length human ERα-transfected U2OS cells. For MARCoNI, 25 µL assay mixtures that contain cell lysates, 25 nM of Alexa488-conjugated GFP-antibody (Invitrogen), and 0.2 mM ligand (E2, BPA, BPAF or BPS, pre-diluted in DMSO, final concentration 2%) were prepared on ice. The ligand-modulated coregulator interactions with the EGFP-tagged ERα was assessed using a PamChip® plate which contained the 154 coregulator-derived binding peptides. The positive interaction means that ligand increases peptide binding and negative interaction means that the ligand decreases peptide binding to ERα.



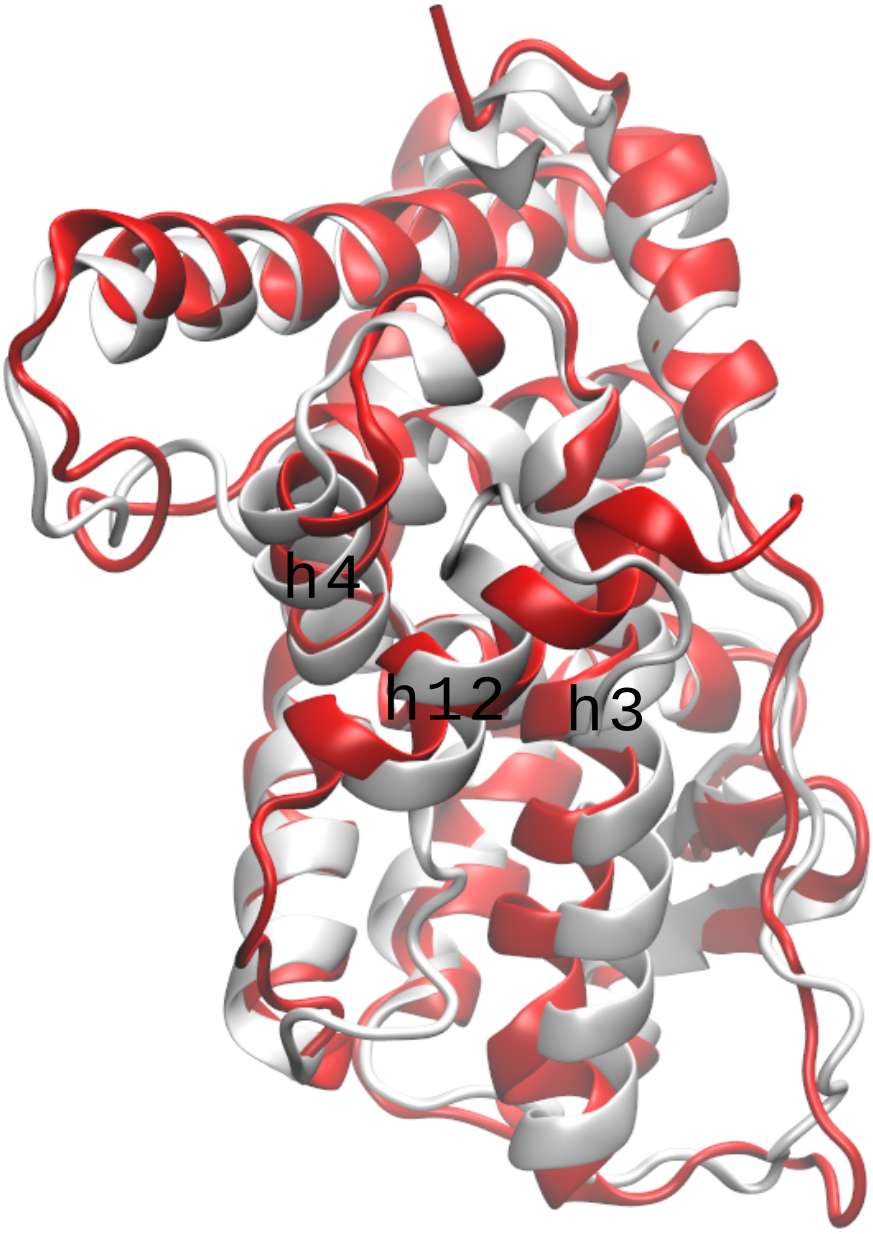
Supplemental Figure S2. Ligand interactions from the reported X-ray crystal structures. The ligand interactions with ER that were found in the X-ray crystal structures (A) E2, (B) BPA, and (C and D) BPAF in two conformations.



Supplemental Figure S3. Root mean square deviations (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 RMSD calculations and all backbone heavy atoms of each residue were used to calculate the average RMSD at each time point.

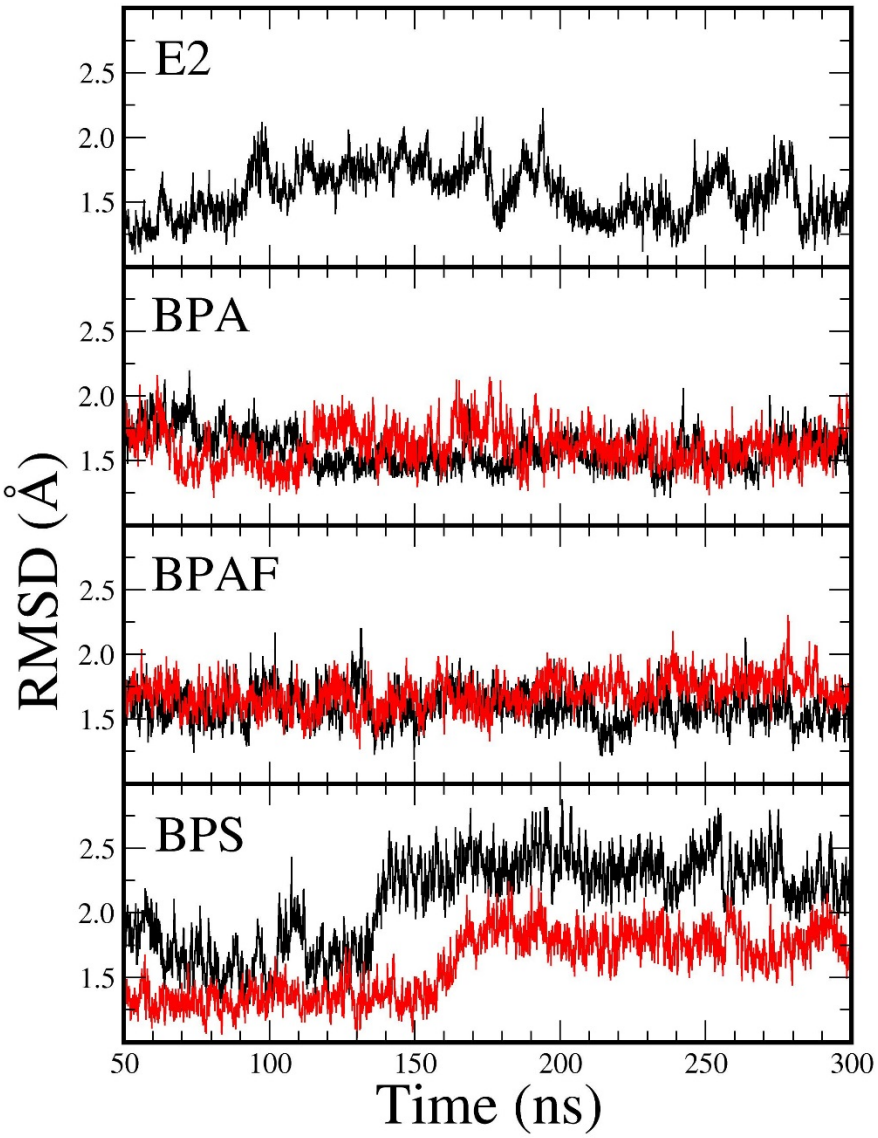


Supplemental Figure S4. Ligand free conformations of ERα (starting with aqueous agonist form). Starting conformation is in white and the final conformation is in red. The three helices that are involved in creating the coregulator surface are marked.

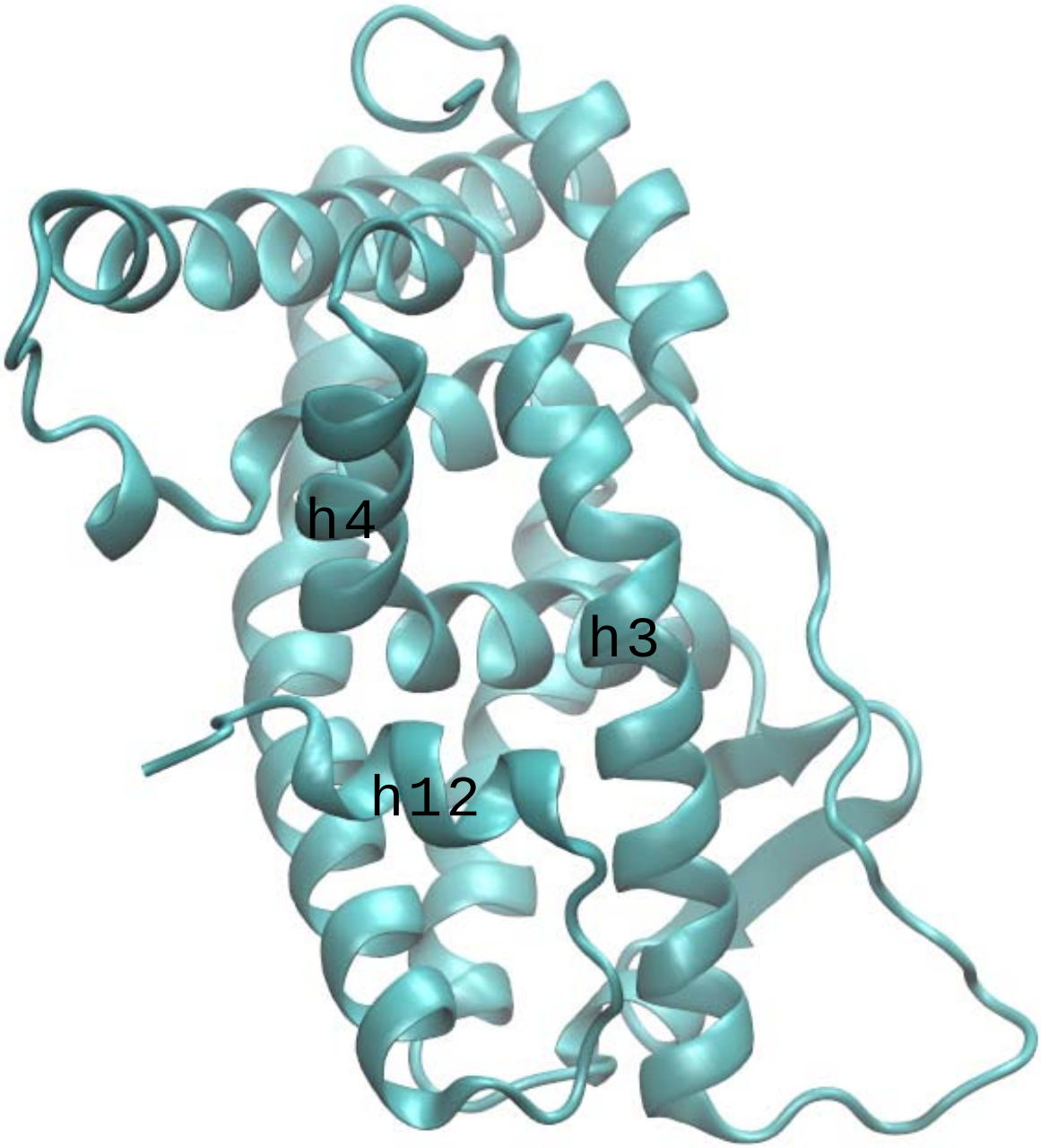


Supplemental Figure S5. Ligand free conformations of ERα (starting with antagonist form).

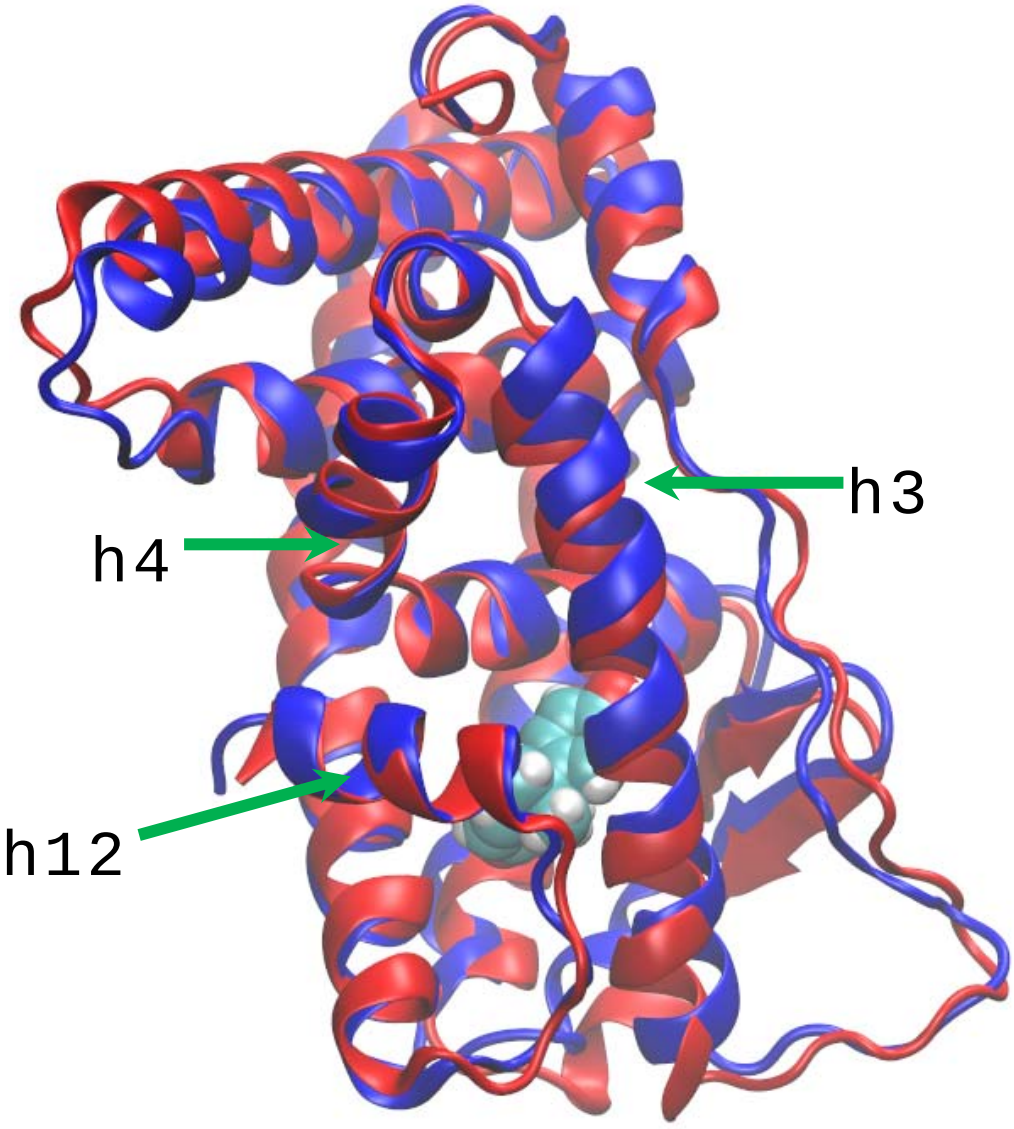
Starting conformation is in white and the final conformation is in red.



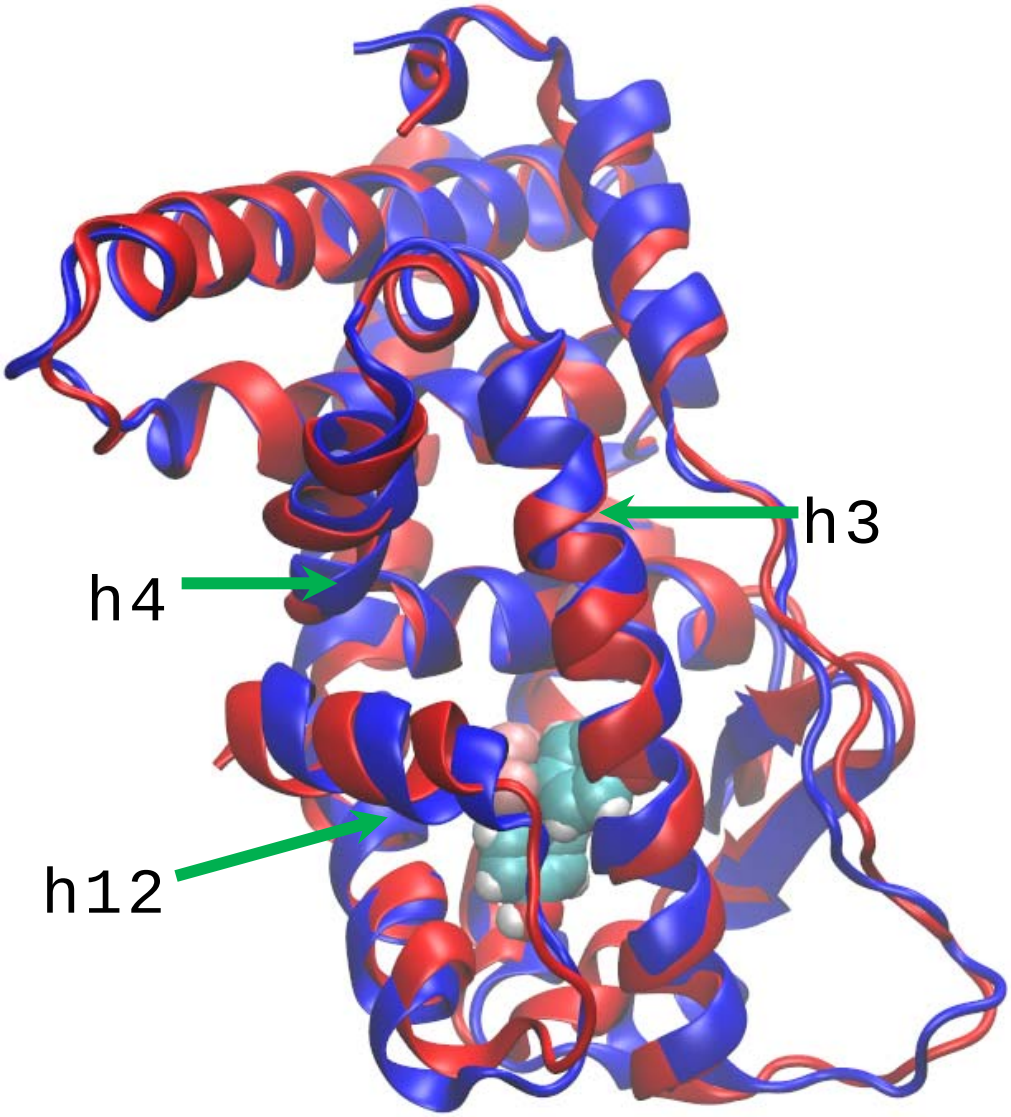
Supplemental Figure S6. Root mean square deviations (RMSD) of various ligand bound-ER structures. The X-ray crystal structures were used as the reference structures for these calculations and all backbone heavy atoms of each residue were used to calculate the average RMSD at each time point. For BPA, BPAF, and BPS, the black curve corresponds to the starting ligand in conformation "1" and the red curve to the ligand in conformation "2".



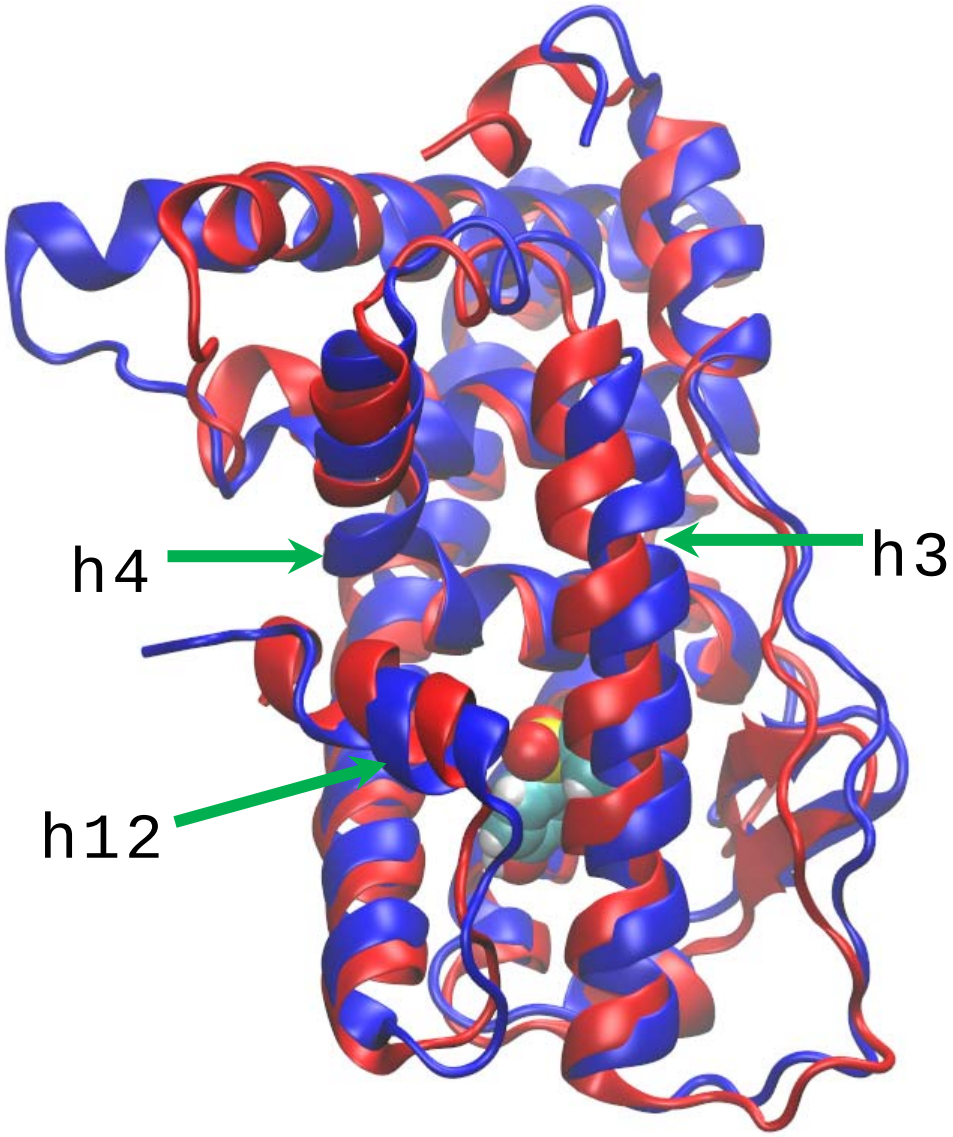
Supplemental Figure S7. E2-bound conformation of ERα (in which h12 is in agonist conformation). The final conformation of ERα with E2 from the MD simulation.



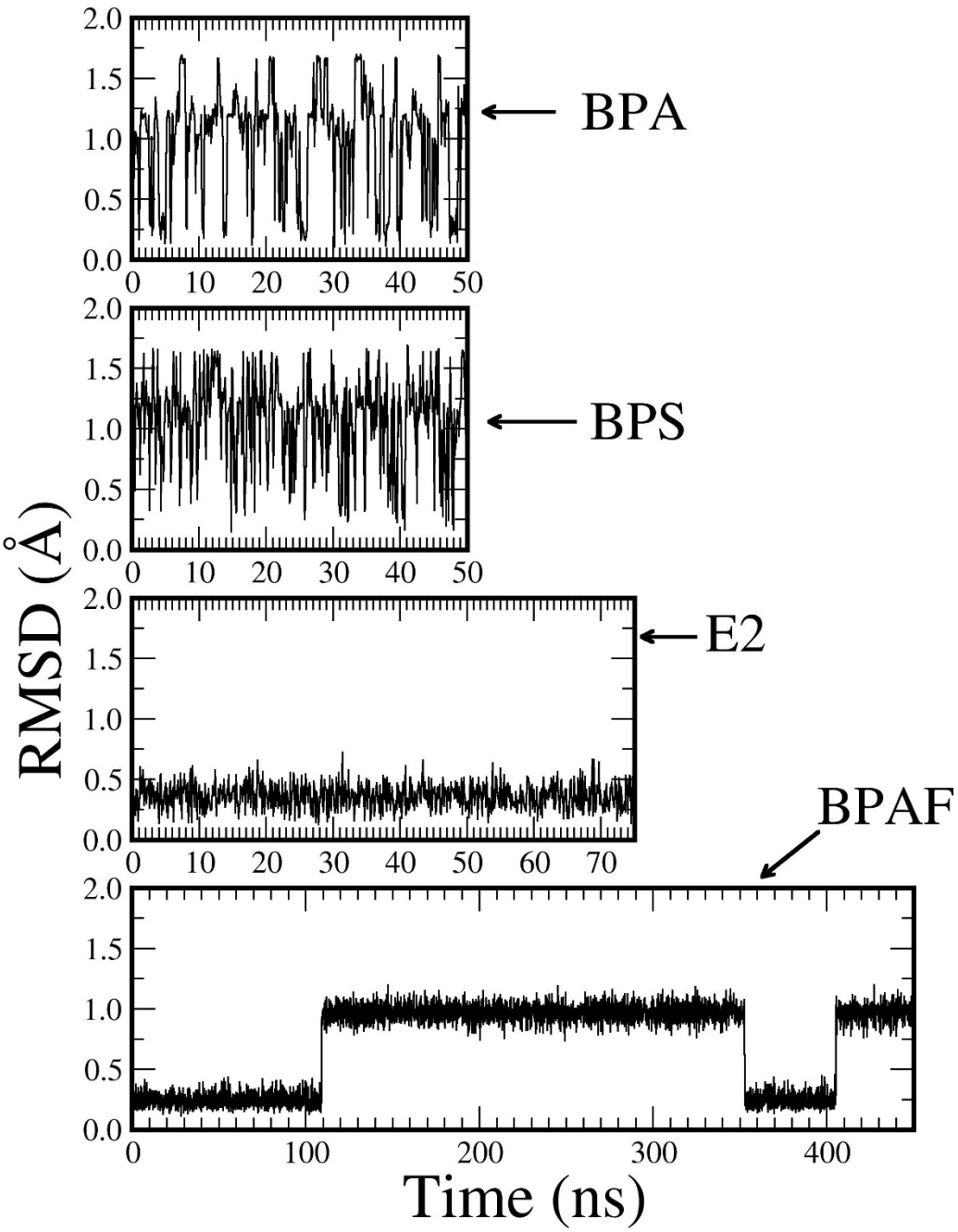
Supplemental Figure S8. BPA-bound conformations of ERα. The final conformation of ERα with BPA started in conformation 1 is shown in the blue ribbon; The final conformation of ERα with BPA started in conformation 2 is shown in the red ribbon. BPA is shown in space filling in the ligand binding site of ERα.



Supplemental Figure S9. BPAF-bound conformations of ERα. The final conformation of ERα with BPAF started in conformation 1 is show in the blue ribbon; The final conformation of ERα with BPAF started in conformation 2 is shown in the red ribbon. BPAF is shown in space filling in the ligand binding site of ERα.



Supplemental Figure S10. BPS-bound conformations of ERα. The final conformation of ERα with BPS started in conformation 1 is shown in the blue ribbon; The final conformation of ERα with BPS started in conformation 2 is shown in the red ribbon. BPS is shown in space filling in the ligand binding site of ERα.



Supplemental Figure S11. Root mean square deviations (RMSD) of the ligands in their aqueous environments. Starting structures were used as the reference structures for these calculations and all heavy atoms of the ligand were used to calculate the average RMSD at each time point.