Supplementary Information

Dynamic changes in binding interaction networks of sex steroids establish their nonclassical effects

Mónika Bálint^{1,2,†}, Norbert Jeszenői^{3,†}, István Horváth⁴, István M. Ábrahám^{3,*}, Csaba Hetényi^{1,*}

*Corresponding authors. <u>istvan.abraham@aok.pte.hu</u> <u>csabahete@yahoo.com</u>

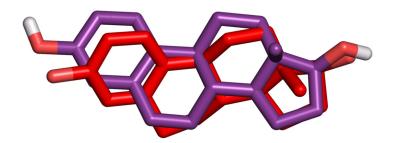
[†]Equal contribution.

¹Department of Pharmacology and Pharmacotherapy, University of Pécs, Szigeti út 12, 7624 Pécs, Hungary.

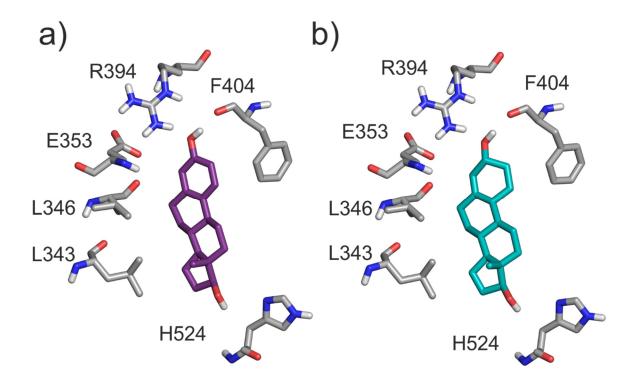
²Department of Biochemistry, Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary.

³MTA NAP-B Molecular Neuroendocrinology Group, Institute of Physiology, Szentágothai Research Center, Center for Neuroscience, University of Pécs, Szigeti út 12, 7624 Pécs, Hungary. ⁴Chemistry Doctoral School, University of Szeged, Dugonics tér 13, 6720 Szeged, Hungary.

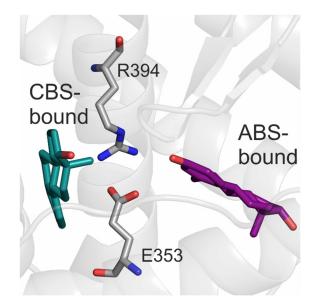
Supplementary Figures



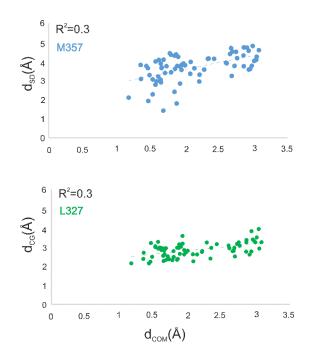
Supplementary Figure S1 Conformation of E2 obtained with blind docking (magenta) compared to the reference structure (red), from X-ray structure (1gwr).



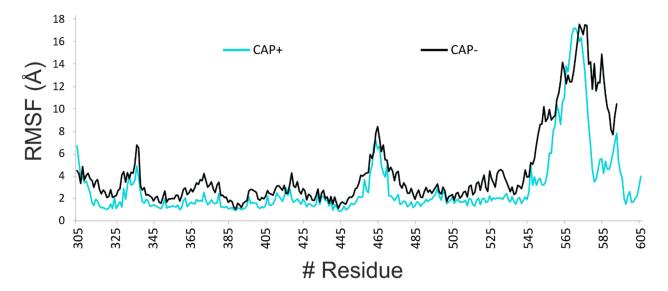
Supplementary Figure S2 Binding conformations of E2 (a, magenta) and EN (b, teal) in CBS are presented. The main interacting amino acids are in sticks with grey carbon atoms. In contrast with ABS (Fig. 4, main text), the two molecules (E2 and EN) bind to CBS in a similar orientation.



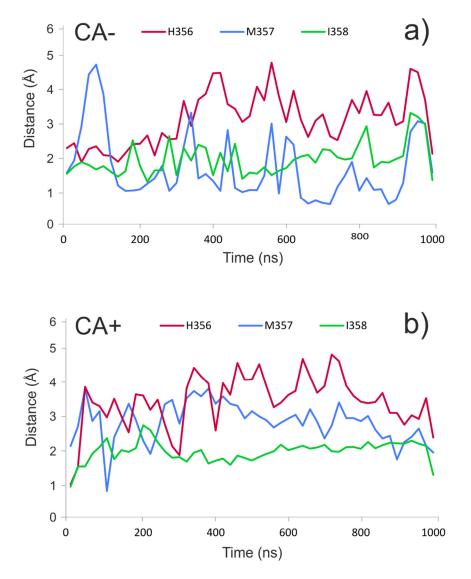
Supplementary Figure S3 Docked conformations of E2 are presented in magenta (ABS) and teal (CBS). The two binding sites are separated by R394 and E353.



Supplementary Figure S4 Correlations observed in simulations with EN, case CA-. Similarly to the main text, points were taken until the ligand reached the d_{LIM} dissociation criteria, which was 70 ns in this case. Correlations are plotted for the movements of L327 and H357. Although the correlation is not as strong as observed in CA+ scenario described in the main text, the role of both M357 and L327 appears to be important in ligand dissociation, even without the presence of CA.



Supplementary Figure S5 RMSF of 3q95 during 1µs ligand-free CA- and CA + simulations. RMSF was calculated and presented in the same way as in Fig. 3 of the main text. Similarly to Fig. 3, high RMSF values were observed in L1, L2 and in the F domain. The above-presented similarities in the RMSF between 3q95 and 2b23 demonstrates that the two crystallographic structures have a similar dynamic behaviour in additon to their excellent structural alignement of 0.5 Å (Methods).



Supplementary Figure S6 Distances of actual positions of M357 (SD), H356 (CE1), and I358 (CG) sidechain atoms calculated from their initial positions during ligand-free simulations CA-(a) and CA+ (b) (PDB structure 3q95). The calculations were performed in the same way as presented in Methods of the main text. In the CA- simulations (a), it can be observed that H356 is the most mobile residue among the three analyzed, I358 and M357 have high mobility explained by the absence of the restraining factor CA (see main text, Section 1). In CA+ (b) simulations, the mobility of I358 decreases due to the prescence of CA, similarly to Fig. 3 and its distance from the initial position remains constant. On the other hand, an increase in the distances is observed for H356 and M357, the two amino acids that switch inside the ABS, in the presence of CA. The above observations support the proposed mechanism and the observations presented in the main text (Fig. 3).

Supplementary Tables

#Rank	$\Delta G_{scoring}(k)$	ccal/mol)*
#Kalik	E2	EN
1	-8.16	-8.75
2	-7.56	-8.1
3	-6.69	-6.56
4	-6.54	-6.56
5	-6.18	-6.07
6	-6.15	-5.66
7	-5.96	-
8	-5.67	-
9	-5.64	-
10	-5.01	-
11	-4.88	-

Supplementary Table S1 Ranks obtained from blind docking of E2 and EN to hERα

*The ranks are ordered by their AutoDock 4.2 scoring values.

11	v			1	5			0
CA		+	-		+	-	-	-
ABS		+	+	-	-	÷		-
CBS		+	+	-	-			+
seed1	2.7	10.0	10.0	10.0	5.6	1.9	10.0	10.0
seed2	0.8	6.6	2.6	10.0	1.7	6.2	10.0	1.8
seed3	3.6	10.0	7.6	10.0	6.7	3.2	10.0	10.0
seed4	1.4	10.0	7.0	10.0	6.4	6.0	5.5	10.0
seed5	2.0	10.0	6.5	10.0	10.0	2.3	10.0	10.0
all seeds	2.1	9.3	6.7	10.0	6.1	3.9	9.1	8.4

Supplementary Table S2 Residence frequency values for EN calculated using RMSD

*Bold numbers represent the simulations with RF values closest to the average RF.

Supplementary	Table S3 Residence fr	equency values	for EN c	calculated using d _{COM}
---------------	-----------------------	----------------	----------	-----------------------------------

CAP	-	F	-		+	-	+	-
ABS	-	F	+	_	+	-		-
CBS	-	F	+	_	-			+
seed1	2.7	10.0	10.0	10.0	6.6	2.3	10.0	10.0
seed2	0.8	6.6	3.8	10.0	6.3	6.3	10.0	1.8
seed3	5.9	10.0	7.6	10.0	10.0	9.6	10.0	10.0
seed4	1.5	10.0	7.0	10.0	7.0	9.1	5.4	10.0
seed5	2.0	10.0	8.3	10.0	10.0	5.1	10.0	10.0
all seeds	2.6	9.3	7.2	10.0	8.0	6.5	9.1	8.4

*Bold numbers represent the simulations with RF values closest to the average RF. These simulations were selected as reference, and used to present results in Table 2 and 3 of the main text.

	v			1	2			0
CAP:	-	ł	-		+	-	+	-
ABS	-	ł	+	-	+	-		-
CBS	-	ł	+	-	-			+
seed1	0.7	10.0	10.0	10.0	0.9	0.1	10.0	10.0
seed2	0.7	10.0	10.0	10.0	2.2	4.1	10.0	10.0
seed3	0.2	10.0	0.3	10.0	0.2	0.2	10.0	10.0
seed4	1.1	10.0	2.3	10.0	0.1	1.4	10.0	10.0
seed5	1.3	10.0	0.7	10.0	0.1	0.1	10.0	10.0
all seeds	0.8	10.0	4.6	10.0	0.7	1.2	10.0	10.0

Supplementary Table S4 Residence frequency values for E2 calculated using RMSD

*Bold numbers represent the simulations with RF values closest to the average RF.

Supplementary	Table S5 Ro	esidence frequend	cy values for l	E2 calculated	using d _{COM}
---------------	-------------	-------------------	-----------------	---------------	------------------------

CAP:	-	ŀ	-		+	-	+	-
ABS	-	ŀ	+	-	+	-		-
CBS	-	ŀ	+	÷	-			-
seed1	1.03	10.0	10.0	10.0	8.7	0.1	10.0	10.0
seed2	0.74	10.0	10.0	10.0	5.3	9.5	10.0	10.0
seed3	3.56	10.0	2.2	10.0	0.3	0.2	10.0	10.0
seed4	1.74	10.0	2.7	10.0	0.1	1.7	10.0	10.0
seed5	1.72	10.0	0.8	10.0	0.1	0.1	10.0	10.0
all seeds	1.76	10.0	5.1	10.0	2.9	2.3	10.0	10.0
secus								

*Bold numbers represent the simulations with RF values closest to the average RF. These simulations were selected as reference, and used to present results in Table 2 and 3 of the main text.

Supplementary Table S6 Simulation times corresponding to $d_{COM} \ge 5$ Å and $d_{COM} \ge 10$ Å values

		Time (ns)					
d _{COM}		EN E2					
		CA+	CA-	CA+	CA-		
5	t_2	27.2	70.3	4.1	6.2		
10	t ₃	40.0	89.9	96.7	-		

Supplementary Table S7 Velocity (Å/ns) values of EN and E2, during CA-/ABS+/CBS+ and CA+/ABS+/CBS+ simulations

-									
	Velocity (Å/ns)								
	EN E2								
v	Time interval	CA+	CA-	CA+	CA-				
v_1	t ₂ -t ₁	0.18	0.07	1.22	0.81				
V 2	t3-t2	0.39	0.26	0.10	-				
V 3	t3-t1	0.25	0.11	0.05	-				

PDB code	Method	Resolution(Å)	Residues
1A52	X-ray	2.8	297-554
1AKF	model	-	309-547
1ERE	X-ray	3.1	301-553
1ERR	X-ray	2.6	301-553
1G50	X-ray	2.9	304-550
1GWQ	X-ray	2.45	301-548
1GWR	X-ray	2.4	305-549
1HCP	NMR	-	180-254
1HCQ	X-ray	2.4	180-262
1L2I	X-ray	1.95	297-554
1PCG	X-ray	2.7	304-547
1QKT	X-ray	2.2	304-551
1QKU	X-ray	3.2	301-550
1R5K	X-ray	2.7	297-554
1 S J0	X-ray	1.9	307-554
1UOM	X-ray	2.28	301-553
1X7E	X-ray	2.8	305-549
1X7R	X-ray	2	305-549
1XP1	X-ray	1.8	307-554
1XP6	X-ray	1.7	307-554
1XP9	X-ray	1.8	307-554
1XPC	X-ray	1.6	307-554
1XQC	X-ray	2.05	301-553
1YIM	X-ray	1.9	307-554
1YIN	X-ray	2.2	307-554
1ZKY	X-ray	2.25	298-554
2AYR	X-ray	1.9	304-551
2B1V	X-ray	1.8	298-554
2B1Z	X-ray	1.78	298-554
2B23	X-ray	2.1	298-554
2BJ4	X-ray	2	305-533
2FAI	X-ray	2.1	298-554
2G44	X-ray	2.65	298-554
2G5O	X-ray	2.3	298-554
2I0J	X-ray	2.9	304-547
2IOG	X-ray	1.6	309-554
2IOK	X-ray	2.4	301-554
2JF9	X-ray	2.1	304-533
2JFA	X-ray	2.55	304-533

Supplementary Table S8 List of hERα structures in the PDB

2LLO NMR - 287-305 2OCF X-ray 2.95 298-595 2OUZ X-ray 2 301-553 2P15 X-ray 1.94 298-554 2POG X-ray 1.84 304-551 2Q6J X-ray 2.7 298-554 2Q70 X-ray 1.95 304-551 2QA6 X-ray 2.6 298-554 2QA6 X-ray 1.85 298-554 2QA8 X-ray 1.89 298-554 2QGT X-ray 2.4 304-551 2QGT X-ray 2.39 298-554 2QGW X-ray 2.39 298-554 2QGW X-ray 2.7 298-554 2QR9 X-ray 2.3 298-554 2QR9 X-ray 2.3 298-554 2QXM X-ray 2.3 298-554 2QZO X-ray 1.7 298-554 2QZO X-ray	PDB code	Method	Resolution(Å)	Residues
2OUZ X-ray 1.94 298-554 2POG X-ray 1.84 304-551 2Q6J X-ray 1.84 304-551 2Q6J X-ray 1.95 304-551 2QA6 X-ray 2.6 298-554 2QA8 X-ray 1.85 298-554 2QA8 X-ray 1.85 298-554 2QAB X-ray 1.89 298-554 2QGT X-ray 2.4 304-551 2QGT X-ray 2.39 298-554 2QGW X-ray 2.39 298-554 2QGW X-ray 2.3 298-554 2QR9 X-ray 2.3 298-554 2QXS X-ray 1.72 298-554 2QZO X-ray 1.72 298-554 2QZO X-ray 1.72 298-551 2R6Y X-ray 2 304-551 2YAT X-ray 1.69 298-307 3CBO X-ray	2LLO	NMR	-	287-305
2P15X-ray1.94298-5542POGX-ray1.84304-5512Q6JX-ray2.7298-5542Q70X-ray1.95304-5512QA6X-ray2.6298-5542QA8X-ray1.85298-5542QA8X-ray1.89298-5542QA7X-ray2.4304-5512QG7X-ray2.15298-5542QGWX-ray2.39298-5542QGWX-ray2.7298-5542QGWX-ray2.7298-5542QR9X-ray2.3298-5542QXMX-ray2.3298-5542QXXX-ray1.77298-5542QXXX-ray1.77298-5542QXXX-ray1.72298-5542QXXX-ray1.72298-5542QXXX-ray1.72298-5542QXXX-ray1.72298-5542QXXX-ray1.72298-5542QXXX-ray1.69298-3073CBVX-ray1.69298-3073CBNX-ray1.65298-3073CBPX-ray1.42298-3073CBPX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9297-5543GSAX-ray2.3299-5533Q95X-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1	20CF	X-ray	2.95	298-595
2POGX-ray1.84304-5512Q6JX-ray2.7298-5542Q70X-ray1.95304-5512QA6X-ray2.6298-5542QA8X-ray1.85298-5542QA8X-ray1.89298-5542QE4X-ray2.4304-5512QGTX-ray2.39298-5542QGWX-ray2.39298-5542QGWX-ray2.39298-5542QR9X-ray2298-5542QXMX-ray2.3298-5542QXXX-ray1.75298-5542QXSX-ray1.77298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QKSX-ray1.72298-5542QKSX-ray1.72298-5542QKOX-ray1.72298-5542QEYX-ray2304-5512R6YX-ray2.6301-5512YATX-ray1.69298-3073CBMX-ray1.69298-3073CBPX-ray1.65298-3073CBPX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9297-5543L03X-ray2.3299-5533QS8X-ray2.3299-5533Q95X-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1	20UZ	X-ray	2	301-553
2Q6J X-ray 2.7 298-554 2Q70 X-ray 1.95 304-551 2QA6 X-ray 2.6 298-554 2QA8 X-ray 1.85 298-554 2QA8 X-ray 1.89 298-554 2QA4 X-ray 2.4 304-551 2QGT X-ray 2.15 298-554 2QGW X-ray 2.39 298-554 2QGW X-ray 2.39 298-554 2QGW X-ray 2 298-554 2QR9 X-ray 2 298-554 2QXS X-ray 1.85 298-554 2QXS X-ray 1.72 298-554 2QZO X-ray 1.72 298-554 2QZO X-ray 1.72 298-554 2QZO X-ray 1.72 298-554 2QZO X-ray 1.72 298-551 2R6Y X-ray 2.6 301-551 2YJA X-ray	2P15	X-ray	1.94	298-554
2Q70X-ray1.95304-5512QA6X-ray1.85298-5542QA8X-ray1.85298-5542QABX-ray1.89298-5542QGTX-ray2.4304-5512QGTX-ray2.39298-5542QGWX-ray2.39298-5542QGWX-ray2.7298-5542QF4X-ray2.7298-5542QGWX-ray2298-5542QR9X-ray2298-5542QXMX-ray2.3298-5542QXMX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QKWX-ray2304-5512R6YX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.65298-3073CBOX-ray1.65298-3073CBOX-ray1.65298-3073CBDX-ray2.4299-5513ERDX-ray2.3298-5503HM1X-ray2.3298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533Q95X-ray2.3299-5533Q95X-ray2.12.92-5523UUCX-ray2.1302-5523UUCX-ray2.1302-552	2POG	X-ray	1.84	304-551
2QA6 X-ray 2.6 298-554 2QA8 X-ray 1.85 298-554 2QAB X-ray 1.89 298-554 2QE4 X-ray 2.4 304-551 2QGT X-ray 2.39 298-554 2QGW X-ray 2.39 298-554 2QGW X-ray 2.7 298-554 2QR9 X-ray 2 298-554 2QXM X-ray 2 298-554 2QXM X-ray 1.85 298-554 2QXM X-ray 1.85 298-554 2QXM X-ray 1.7 298-554 2QXS X-ray 1.72 298-554 2QZO X-ray 1.72 298-554 2QZO X-ray 1.72 298-551 2R6W X-ray 2.6 301-551 2YJA X-ray 1.82 299-551 3CBM X-ray 1.65 298-307 3CBD X-ray 1.65 298-307 3CBD X-ray 1.65 298-550<	2Q6J	X-ray	2.7	298-554
QA8X-ray1.85298-5542QABX-ray1.89298-5542QE4X-ray2.4304-5512QGTX-ray2.15298-5542QGWX-ray2.39298-5542QGWX-ray2.7298-5542QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXMX-ray1.7298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.65298-5012R6YX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.9297-5543ERDX-ray2.4299-5513ERDX-ray1.9297-5543HLVX-ray3298-5503L03X-ray2.3298-5503OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.1302-5523UUCX-ray2.1<	2Q70	X-ray	1.95	304-551
2QABX-ray1.89298-5542QE4X-ray2.15298-5542QGTX-ray2.39298-5542QGWX-ray2.39298-5542QGWX-ray2.7298-5542QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXMX-ray1.7298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QEYX-ray1.72298-5542QEOX-ray1.65298-5512R6YX-ray2.6301-5512YATX-ray1.69298-3073CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.3297-5543HLVX-ray3298-5503L03X-ray1.9297-5533OSAX-ray2.3299-5533Q95X-ray2.03299-5533Q95X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.1302-5523UUCX-ray2.1 <td>2QA6</td> <td>X-ray</td> <td>2.6</td> <td>298-554</td>	2QA6	X-ray	2.6	298-554
QE4X-ray2.4304-5512QGTX-ray2.15298-5542QGWX-ray2.39298-5542QFX-ray2.7298-5542QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXMX-ray1.72298-5542QXSX-ray1.77298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QKYX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.69298-3073CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9297-5543GSAX-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.3299-5533Q97X-ray2.12.13UU7X-ray2.1302-5523UUCX-ray2.1302-552	2QA8	X-ray	1.85	298-554
2QGTX-ray2.15298-5542QGWX-ray2.39298-5542QH6X-ray2.7298-5542QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXSX-ray1.7298-5542QZOX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542R6WX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.69298-3073CBOX-ray1.65298-3073CBOX-ray1.65298-3073CBOX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9297-5543OS8X-ray2.3299-5533OS9X-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.1298-5543UU7X-ray2.1302-5523UUCX-ray2.1302-552	2QAB	X-ray	1.89	298-554
2QGWX-ray2.39298-5542QH6X-ray2.7298-5542QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542R6WX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.69298-3073CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073CBPX-ray1.42298-5513ERDX-ray2.03297-5543HLVX-ray3298-5503L03X-ray1.9297-5543GS8X-ray2.03299-5533OSAX-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.1298-5543UU7X-ray2.1302-5523UUCX-ray2.1302-552	2QE4	X-ray	2.4	304-551
2QH6X-ray2.7298-5542QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542QKWX-ray2304-5512R6WX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.69298-3073CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543HLVX-ray3298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533OS9X-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.1302-5523UUCX-ray2.1302-552	2QGT	X-ray	2.15	298-554
2QR9X-ray2298-5542QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542QZOX-ray1.72298-5542R6WX-ray2304-5512YATX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.69298-3073CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543HLVX-ray3298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533OS9X-ray2.3299-5533Q95X-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1302-5523UUCX-ray2.1302-552	2QGW	X-ray	2.39	298-554
2QSEX-ray1.85298-5542QXMX-ray2.3298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542R6WX-ray2304-5512R6YX-ray2304-5512YATX-ray2304-5512YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1298-5543UUCX-ray2.1302-5523UUCX-ray2.1302-552	2QH6	X-ray	2.7	298-554
2QXMX-ray2.3298-5542QXSX-ray1.7298-5542QZOX-ray1.72298-5542R6WX-ray2304-5512R6YX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.65298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1298-5543UUCX-ray2.1302-5523UUCX-ray2.1302-552	2QR9	X-ray	2	298-554
2QXSX-ray1.7298-5542QZOX-ray1.72298-5542R6WX-ray2304-5512R6YX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBOX-ray1.42298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray1.9297-5543HLVX-ray1.9297-5543HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533Q95X-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.1302-5523UUCX-ray2.1302-552	2QSE	X-ray	1.85	298-554
2QZOX-ray1.72298-5542R6WX-ray2304-5512R6YX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1302-5523UUAX-ray2.1302-5523UUCX-ray2.1302-552	2QXM	X-ray	2.3	298-554
2R6WX-ray2304-5512R6YX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBOX-ray1.42298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533OS9X-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1302-5523UUAX-ray2.1302-5523UUCX-ray2.1302-552	2QXS	X-ray	1.7	298-554
2R6YX-ray2304-5512YATX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533Q95X-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.1302-5523UUCX-ray2.1302-552	2QZO	X-ray	1.72	298-554
2YATX-ray2.6301-5512YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.1298-5543UU7X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.1302-5523UUCX-ray2.1302-552	2R6W	X-ray	2	304-551
2YJAX-ray1.82299-5513CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533Q95X-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.1302-5523UUCX-ray2.1302-552	2R6Y	X-ray	2	304-551
3CBMX-ray1.69298-3073CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OSAX-ray2.3299-5533Q95X-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	2YAT	X-ray	2.6	301-551
3CBOX-ray1.65298-3073CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543UU7X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	2YJA	X-ray	1.82	299-551
3CBPX-ray1.42298-3073DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543UU7X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3CBM	X-ray	1.69	298-307
3DT3X-ray2.4299-5513ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543UU7X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3CBO	X-ray	1.65	298-307
3ERDX-ray2.03297-5543ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.3299-5533Q97X-ray2.1298-5543UU7X-ray2.1298-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3CBP	X-ray	1.42	298-307
3ERTX-ray1.9297-5543HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3DT3	X-ray	2.4	299-551
3HLVX-ray3298-5503HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3ERD	X-ray	2.03	297-554
3HM1X-ray2.33298-5503L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3ERT	X-ray	1.9	297-554
3L03X-ray1.9298-5503OS8X-ray2.03299-5533OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3HLV	X-ray	3	298-550
30S8X-ray2.03299-55330S9X-ray2.3299-55330SAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3HM1	X-ray	2.33	298-550
3OS9X-ray2.3299-5533OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3L03	X-ray	1.9	298-550
3OSAX-ray2.3299-5533Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3OS8	X-ray	2.03	299-553
3Q95X-ray2.05298-5543Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3OS9	X-ray	2.3	299-553
3Q97X-ray2.1298-5543UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	30SA	X-ray	2.3	299-553
3UU7X-ray2.2302-5523UUAX-ray2.05302-5523UUCX-ray2.1302-552	3Q95	X-ray	2.05	298-554
3UUAX-ray2.05302-5523UUCX-ray2.1302-552	3Q97	X-ray	2.1	298-554
3UUC X-ray 2.1 302-552	3UU7	X-ray	2.2	302-552
•	3UUA	X-ray	2.05	302-552
3UUD X-ray 1.6 302-552	3UUC	X-ray	2.1	302-552
	3UUD	X-ray	1.6	302-552

PDB code	Method	Resolution(Å)	Residues
4AA6	X-ray	2.6	182-252
4DMA	X-ray	2.3	303-549
4IU7	X-ray	2.29	303-549
4IUI	X-ray	2.3	303-549
4IV2	X-ray	2.14	303-549
4IV4	X-ray	2.3	303-549
4IVW	X-ray	2.06	303-549
4IVY	X-ray	1.95	303-549

Supplementary Video legends

Supplementary Video 1

The three-dimensional structure of the $hER\alpha$, with important structural elements highlighted in red. ABS and CBS binding sites are also presented in the video.

Supplementary Video 2

An example 100-ns-long molecular dynamics (MD) simulation of EN dissociation from the ABS. The simulation started from a CA+/CBS+/ABS+ starting complex (Fig. 1). Disruption of H-bonds with K449, and L327 can be observed at 25 and 30 ns, respectively.

Supplementary Video 3

The three-dimensional structure of the flickering gate is composed by T1, L1, and H3 structural elements. Its closed (blue) and opened (red) states show considerable differences at T1 and L1 conformations. EN is presented with red (ABS-bound) and blue (unbound) sticks.