Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Table S1. Accelerated (aMD) and classical (cMD) simulations performed on an ERα monomer and a dimer starting from an unfolded conformation of H12.[1]Conclusions about the H12 conformational change.[2] Simulations that were extended for achieving a better convergence and were used for a convergence control of the aMD runs.

Simulation number	Configuration	Starting position and time of the performed aMD and cMD simulations	Conclusions[1]
		aMD and cMD of Monomer	
1	Monomer 1 (aMDm1)	70 ns	No H12 transition
2	Monomer 2 (aMDm2)	100 ns	No H12 transition
3	Monomer 3 (aMDm3)	100 ns	No H12 transition
4	Monomer 4 (aMDm4)	100 ns	No H12 transition
5	Monomer 5 (aMDm5)	70 ns	No H12 transition
6	Monomer 6 (aMDm6)	70 ns	No H12 transition
7	Monomer 7 (aMDm7)	100 ns	No H12 transition
8	Monomer 8 (aMDm8)	100 ns	No H12 transition
9	Monomer 9 classical MD, (cMDm9)	100 ns	No H12 transition
		aMD of a Dimer	
10	Dimer 1 (aMD1)	140 ns [2] extended to 250 ns	Transition, path 2, agonist
11	Dimer 2 (aMD2)	100 ns [2] extended to 250 ns	Transition, path 1, antagonist
12	Dimer 3 (aMD3)	70 ns [2] extended to 250 ns	No H12 transition
13	Dimer 4 (aMD4)	150ns [2] extended to 250 ns	Transition, path 1, agonist
14	Dimer 5 (aMD5)	70 ns	Transition, path 1, agonist
15	Dimer 6 (aMD6)	70 ns	Transition, path 2, agonist
16	Dimer 7 (aMD7)	70 ns	Transition, path 1, agonist
17	Dimer 8 (aMD8)	70 ns	No H12 transition
18	Dimer 9 (aMD9)	100 ns	No H12 transition

Table S2. Performed accelerated (aMD) and classical (cMD) simulations with different initial structures, H12 positions and protocols. The simulations in a monomer followed previously employed protocols (see Methods for more details).[1] Conclusions about the H12 conformational change.[2] We used unfolded helix 12 definition as seen in a pdb id 1a52 structure, instead of an apo as was initially suggested.

Simulation number	Configuration	Starting position and time of the performed aMD and cMD simulations	Conclusions[1]
		Start from an antagonist structure	
1	ER, with E_2 ligand (aMDm9)	70 ns	No significant change
2	ER, with E_2 ligand (aMDm10)	60 ns	No significant change
3	ER with E_2 ligand, water buffer of 20 Å (aMDm11)	70 ns	No significant change
		Start from an agonist structure	
4	ER without a ligand (aMDd12)	100 ns	No significant change
5	ER without a ligand (aMDd13)	100 ns	No significant change
		Start from pdb id 1a52[2]	
6	ER without a ligand (aMDd14)	70 ns	Change, but H12 not agonist
7	ER with estradiol (E_2) (aMDd15)	80 ns	Change, but H12 not agonist
8	ER with E_2 and 12A cut-off (aMDd16)	50 ns	Change, but H12 not agonist
9	ER with E_2 and 7 additional H12 residues included (aMDd17)	60 ns	Change, but H12 not agonist
10	ER with E_2 executed at 450K (aMDd18)	50 ns	Defolding of ER
11	ER with E_2 executed at 400K (aMDd19)	50 ns	No defolding, but H12 is the same
12	ER with E_2; SA of H12 at 800K (aMDd20)	50 ns	No H12 in an agonist form
13	ER with E_2 and a co-activator (aMDd21)	100 ns	No H12 in an agonist form
14	ER with E_2; classical MD (cMDd22)	100 ns	The same as aMD
15	ER without ligand; classical MD (cMDd23)	100 ns	The same as aMD
16	ER with E_2; classical MD; confirmation (cMDd24)	50 ns	The same as aMD

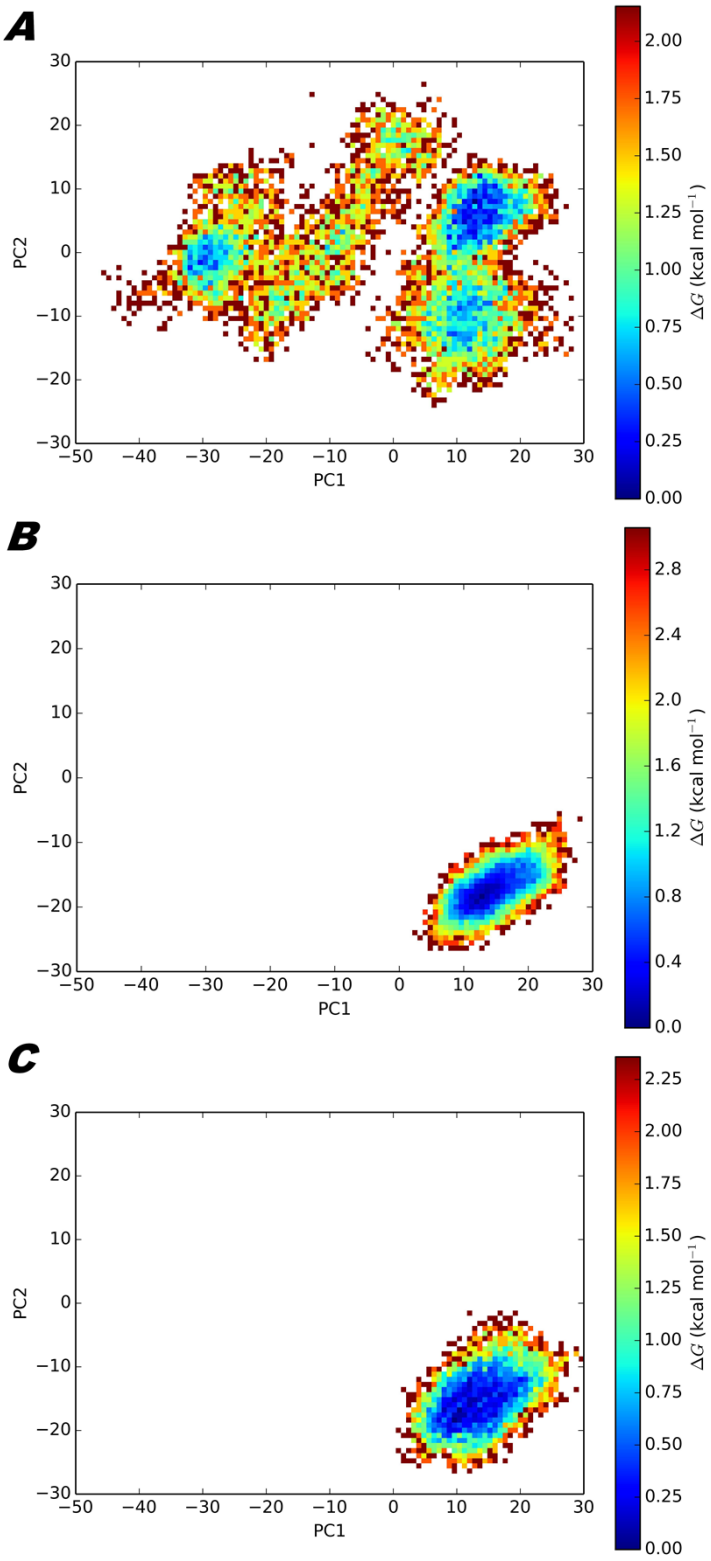

Figure S1. PCA analysis based on the trajectories of *(A)* aMDm3, *(B)* cMDm9 and *(C)* aMDm1 runs, respectively. Only residues 312 ÷ 530 were included. Note the difference in the sampling between aMDm1 and aMDm3. Both cMDm9 and aMDm1 explored the space under H11, where fewer structural transformations was observed, but the aMDm3 describes several flexible states above H11, which explains the difference between aMDm3 and aMDm1 runs and demonstrates how H12 conformations affect the remaining part of the LBD (see also Figure S2).

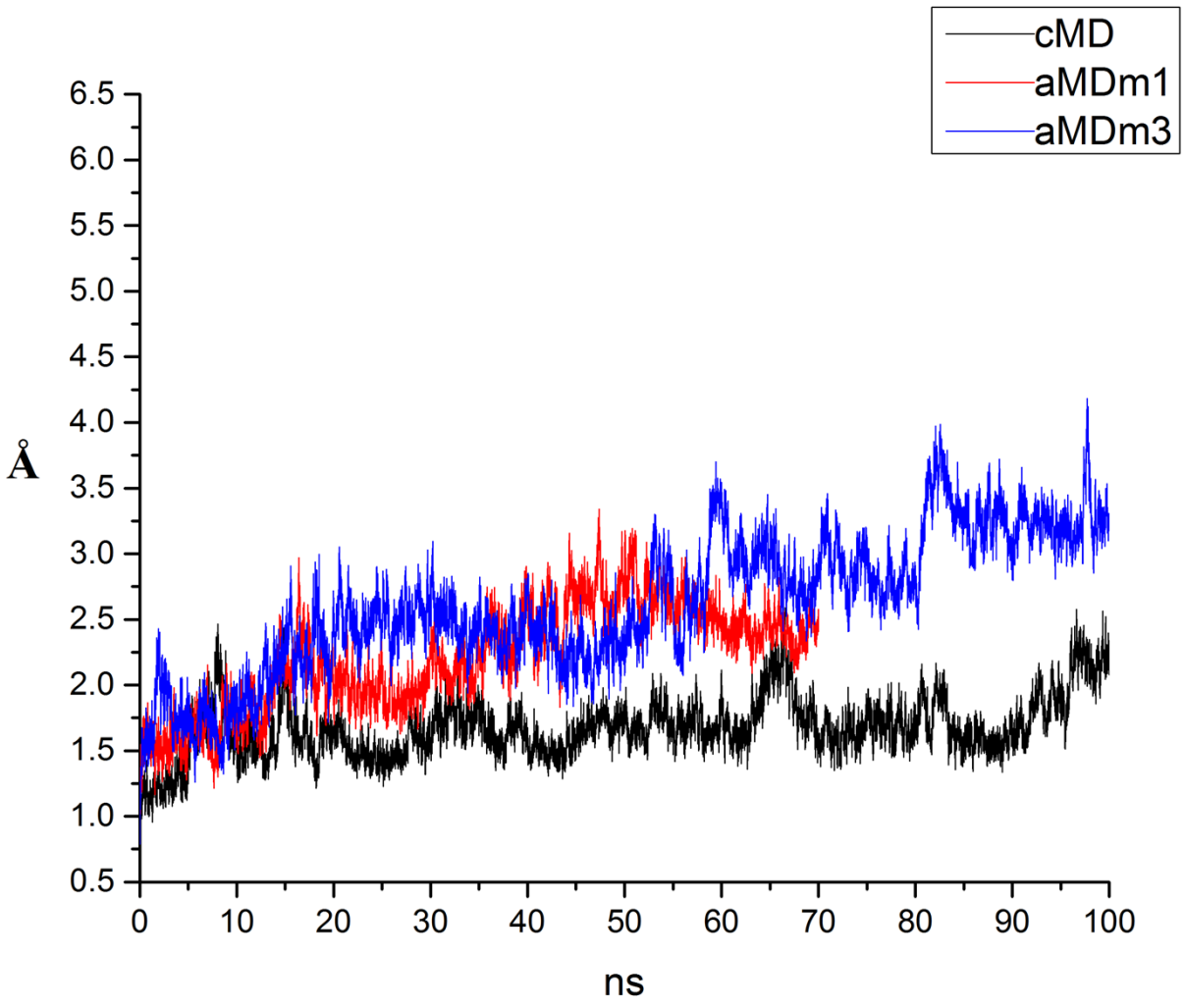

Figure S2. RMSD analysis based on the trajectories of *(A)* aMDm3, *(B)* cMD (cMDm9) and *(C)* aMDm1 runs, respectively. Only residues 312 ÷ 530 were included, which demonstrates how the changes in the H12 motion affect the remaining LBD substructures. Note that aMDm3 showed higher deviations due to the H12 interactions with H11 and H7/H8.

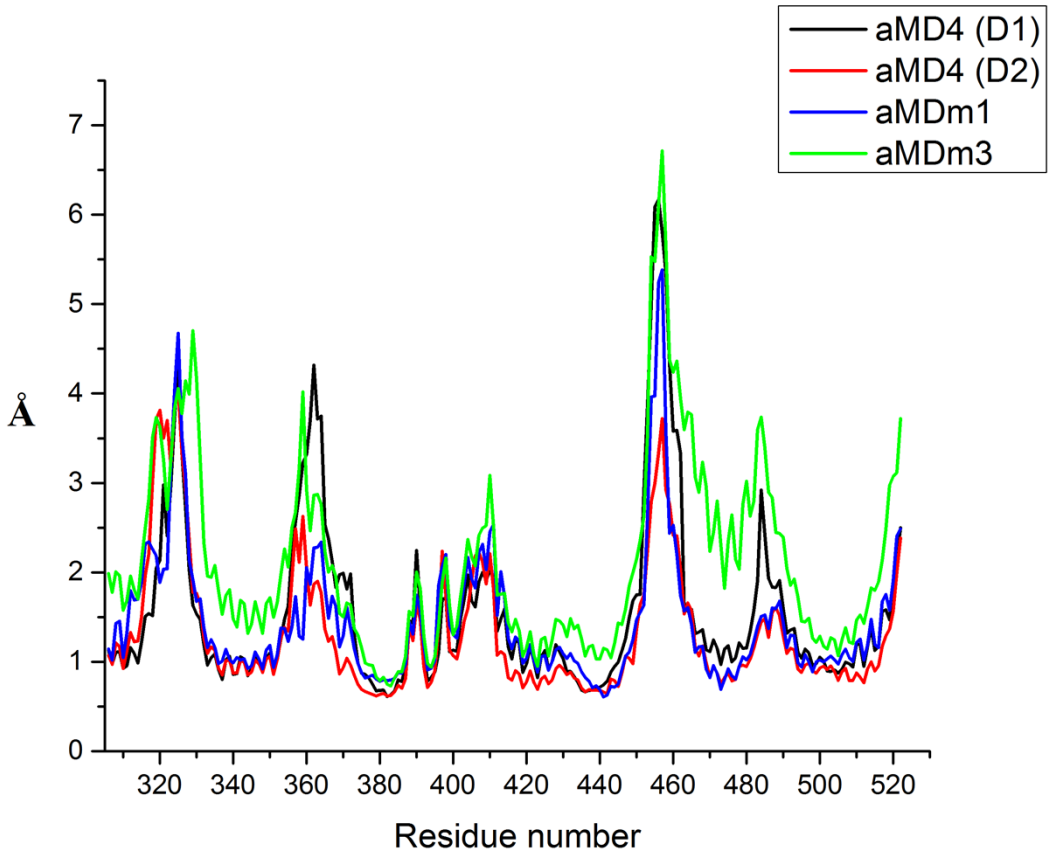

Figure S3. Root mean square fluctuation (RMSF) of residues in the ERα, indicated by monomer simulations aMDm1 (blue) and aMDm3 (green), and by dimer runs aMD4 (D1) (black) and aMD4 (D2) (red). Note that these fluctuations represent only the structural changes detected by the aMD approach, and not the real dynamics. These changes show the degree of transformation of the individual substructural elements.

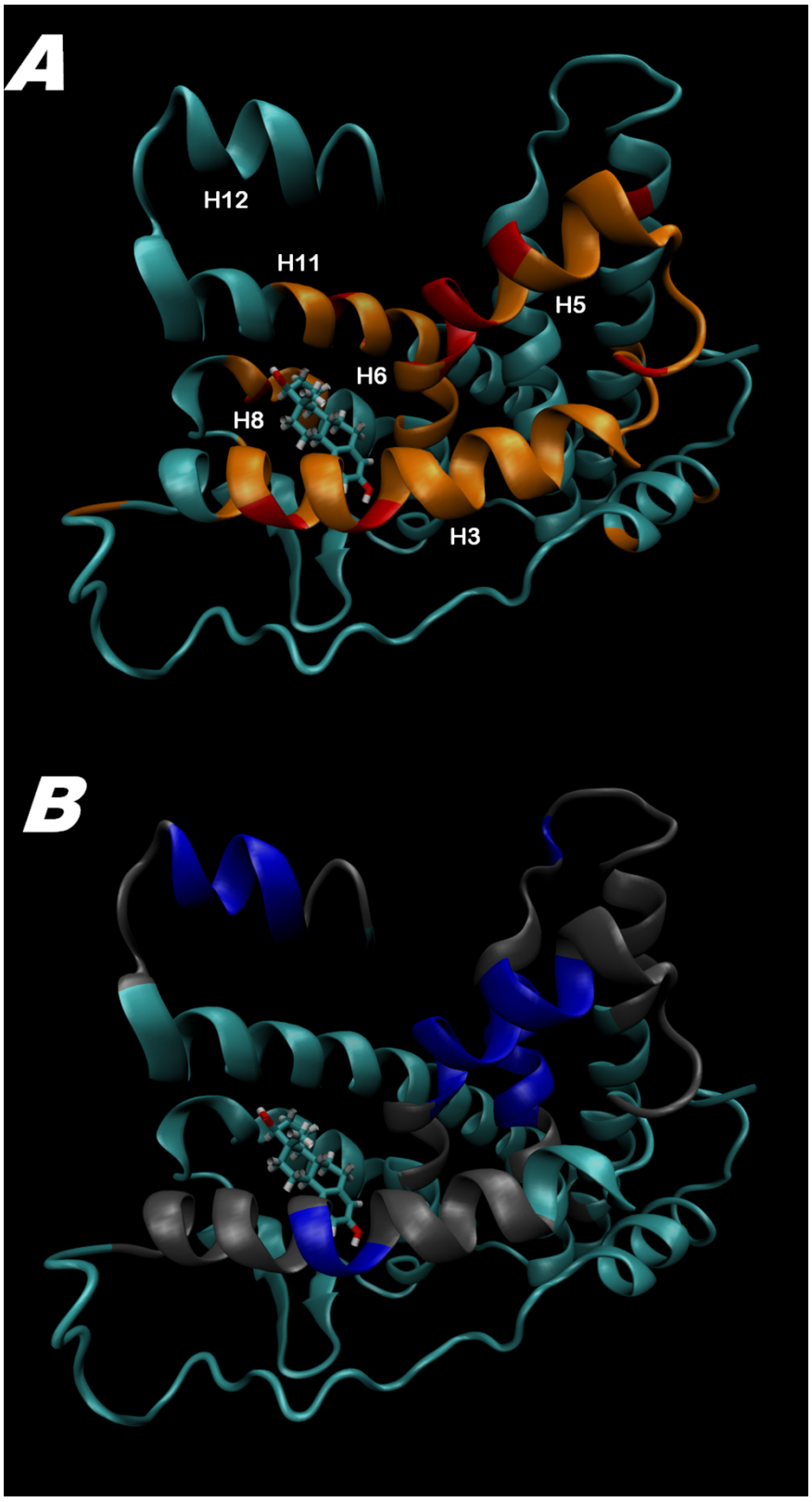

Figure S4. Identified correlations projected on an ERα monomer structure obtained by an aMDm3 simulation ***(A).*** Identified positive correlations. The residues with a correlative coefficient (r) in the range of 0.5÷0.7 are colored in orange, whereas those with a r≥0.7 are in red. ***(B)*** Identified negative correlations. The residues with a correlative coefficient in the range of -0.5÷-0.7 are colored in gray, whereas those with a r=≤-0.7 are in blue.

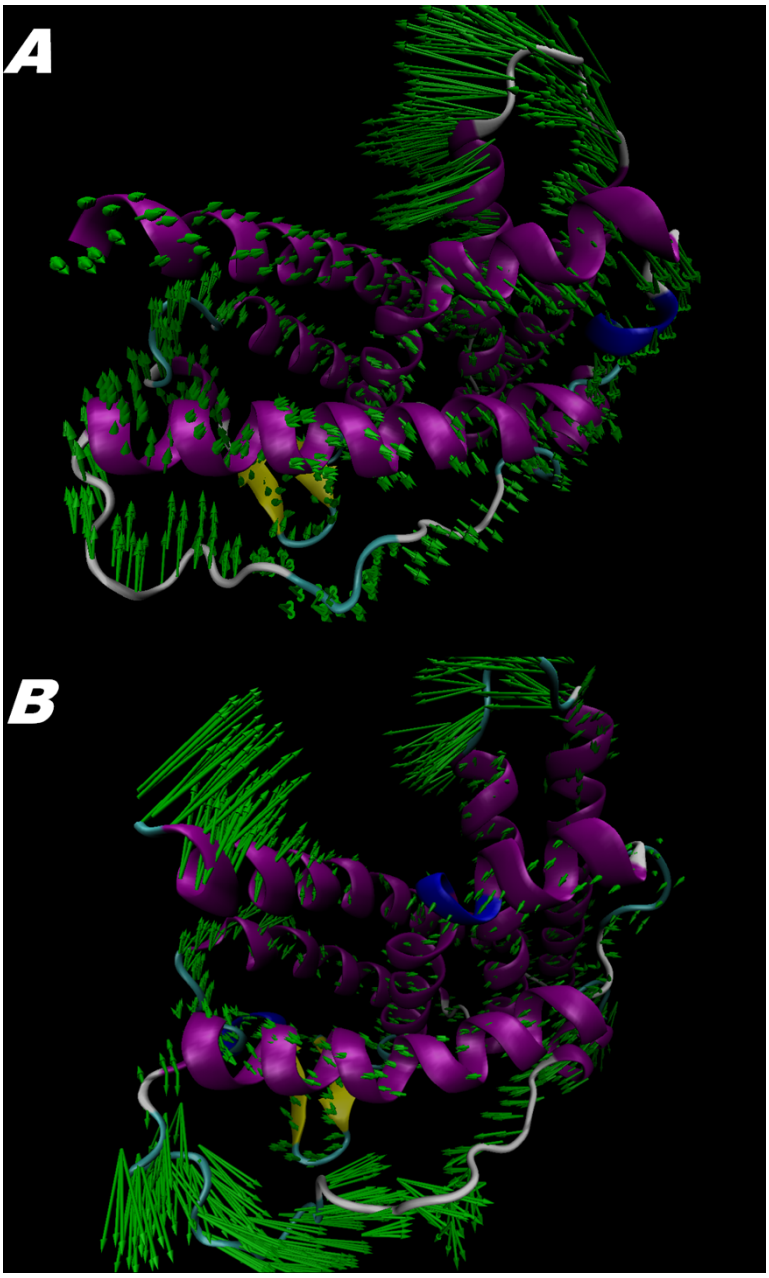

Figure S5. Observed protein motions represented by the projections of the 1st principle component (PC1) onto the protein structures obtained by ***(A)*** aMDm1 and ***(B)*** aMDm3 runs, respectively. H12 was omitted due to the large structural and therefore PC1 deviations observed, which restrict the visualization of the remaining structural elements, which are the subject of this discussion.

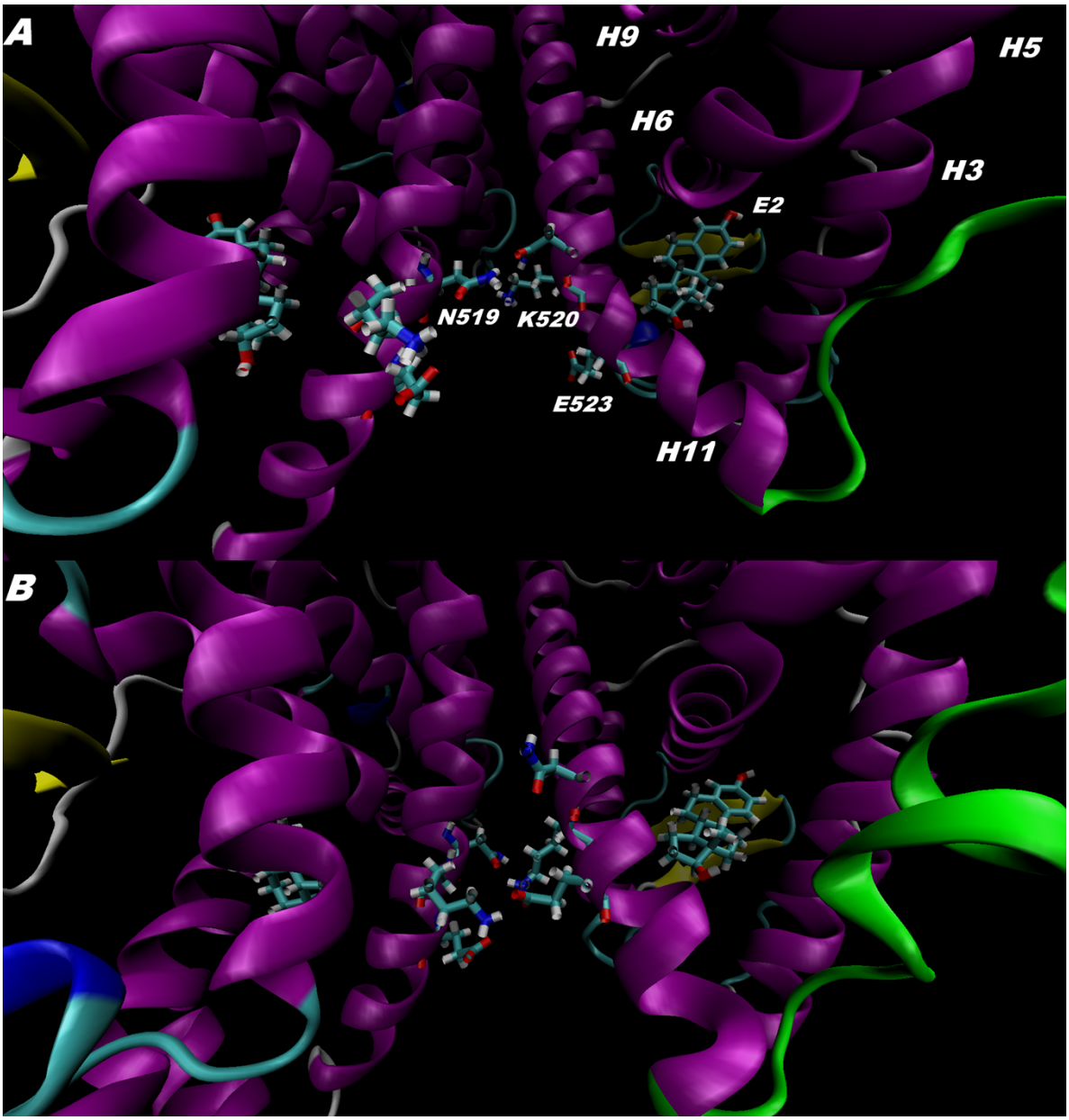

Figure S6. Some of **the** identified residues in the central part of H11, which contribute and correspond to the H12 transitions from ***(A)*** an under H11 position to an antagonist state and ***(B)*** a near antagonist-like state.

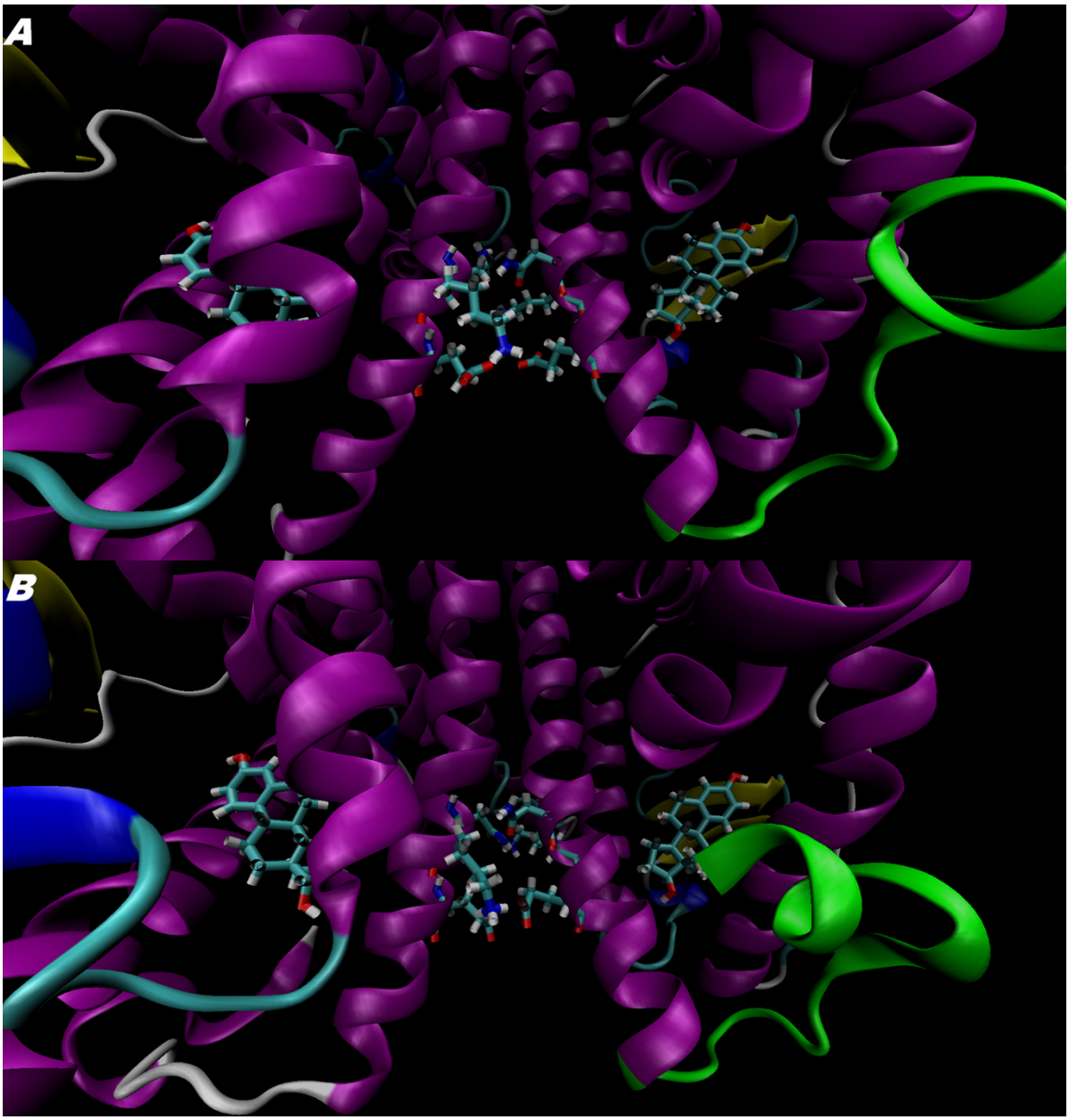

Figure S7. Some of **the** identified residues in the central part of H11, which contribute and correspond to the H12 transitions from ***(A)*** an antagonist state to an agonist and ***(B)*** a near agonist-like state. Residues numbering is shown on Figure S4.

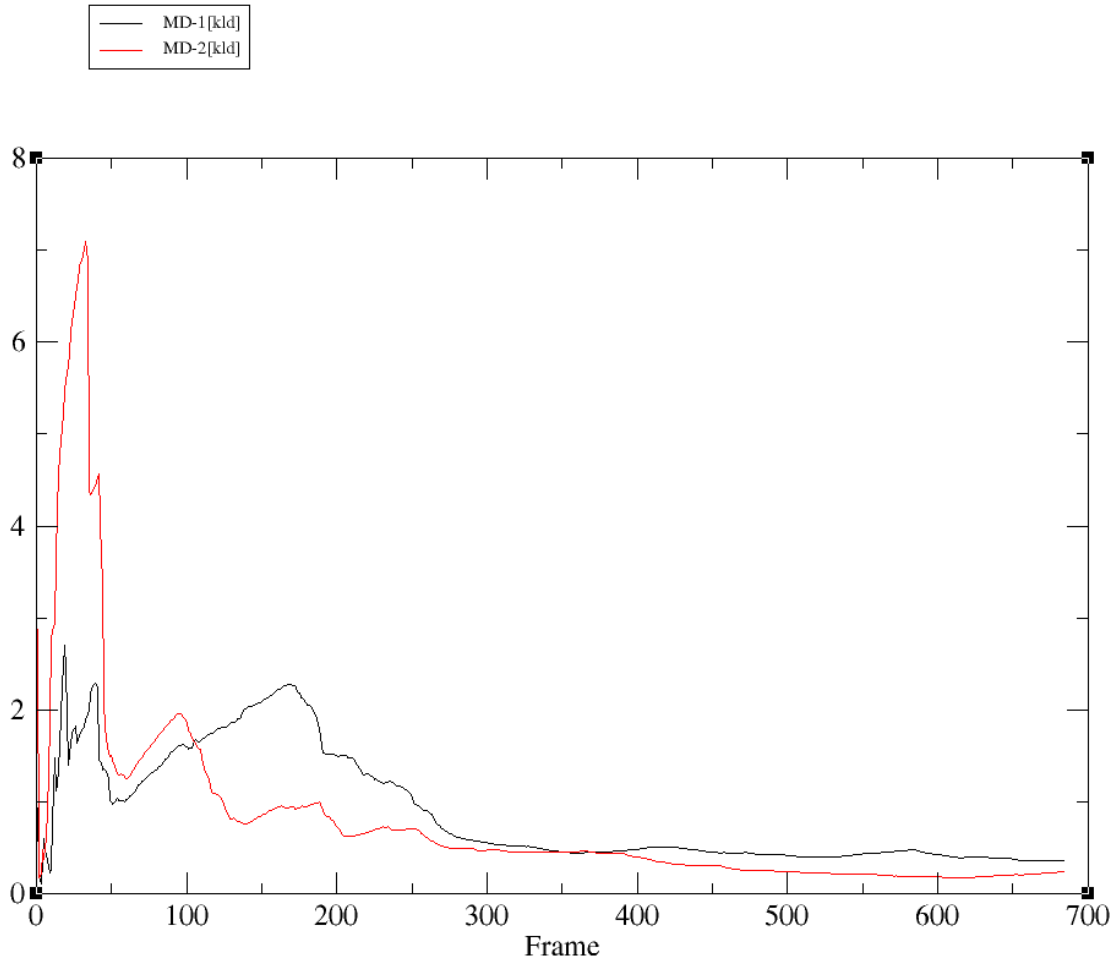

Figure S8. The Kullback−Leibler divergence (KLD) of the first two principal component projection histograms from the independent simulations aMD4 and aMD5 versus time. PC1 is in black, whereas PC2 is in red. Each frame represents 100 ps, i.e. 700 frames are equal to 70 ns.

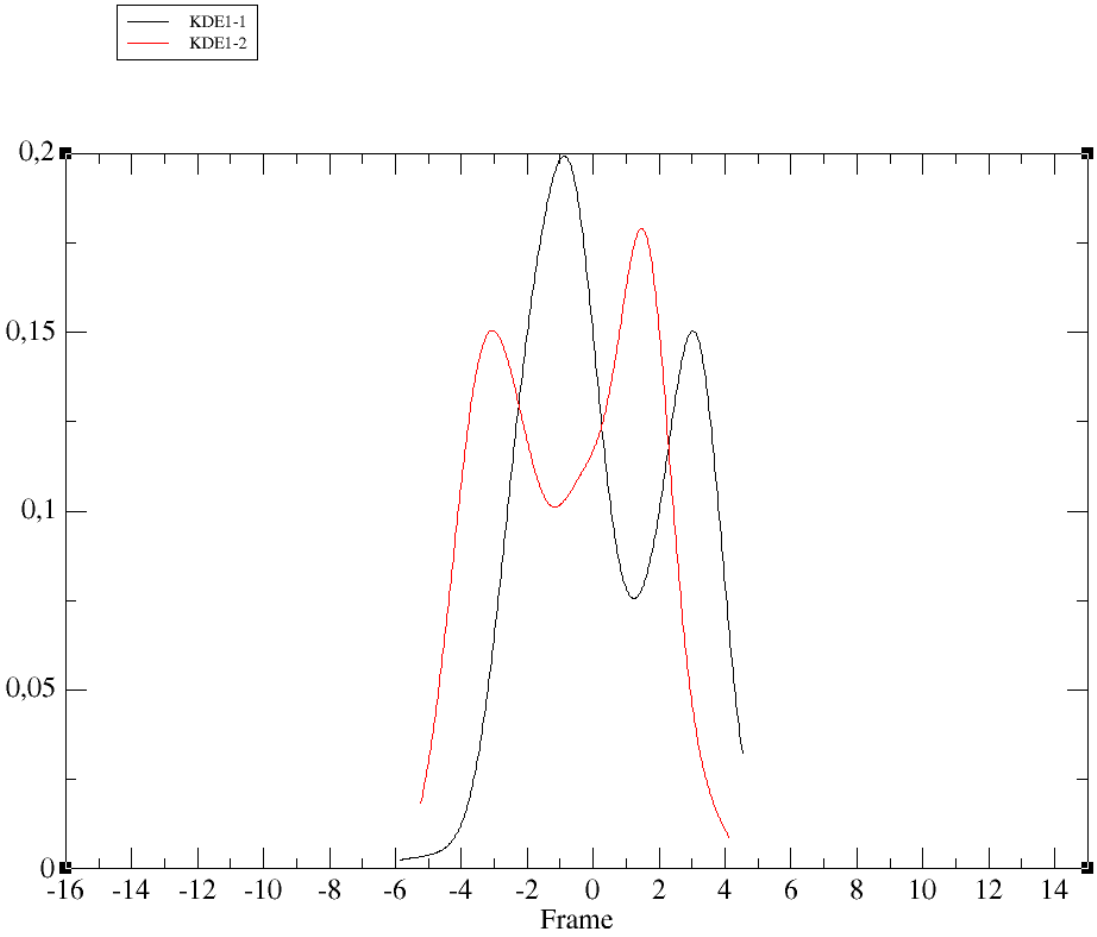

Figure S9. The Kullback−Leibler divergence (KLD) PC histogram from PC analysis in the Cartesian space, calculated from the combined aMD4 and aMD5 simulation trajectories.