COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

DRUG DESIGN

Residues remote from the binding pocket control the antagonist selectivity towards the corticotropin-releasing factor receptor-1

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Table S1. The most conserved residues on the helices of CRF_1R and CRF_2R.

	Conserved residues in CRF_1R	Conserved residues in CRF_2R
TM1	$S130^{1.50}$	$S127^{1.50}$
TM2	$H184^{2.50}$	$H152^{2.50}$
TM3	$E238^{3.50}$	$E205^{3.50}$
TM4	$W265^{4.50}$	$W232^{4.50}$
TM5	$N312^{5.50}$	$N279^{5.50}$
TM6	$K343^{6.50}$	$K310^{6.50}$
TM7	$G385^{7.50}$	$G352^{7.50}$
The superscript of a residue represents the Wootten numbering		

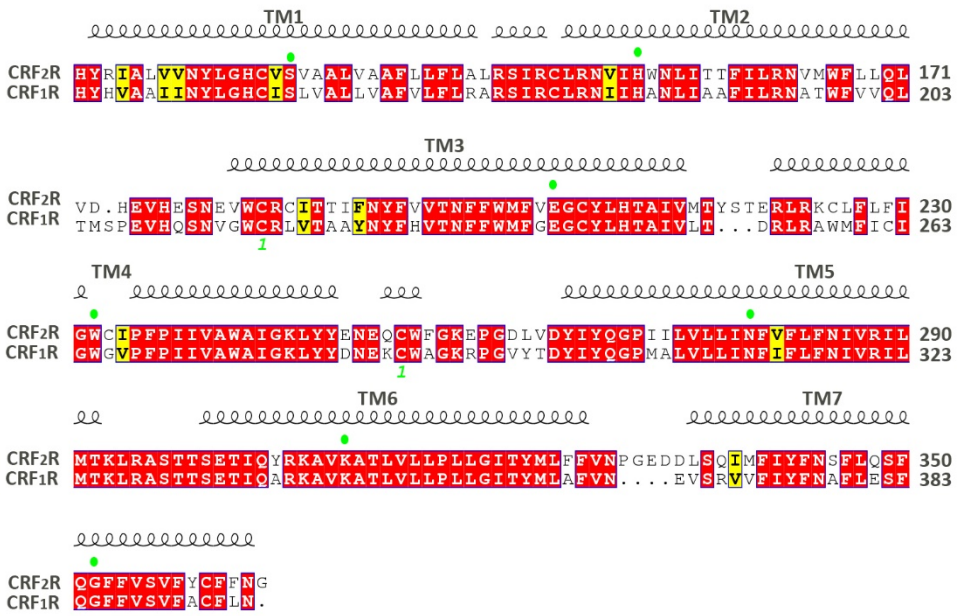
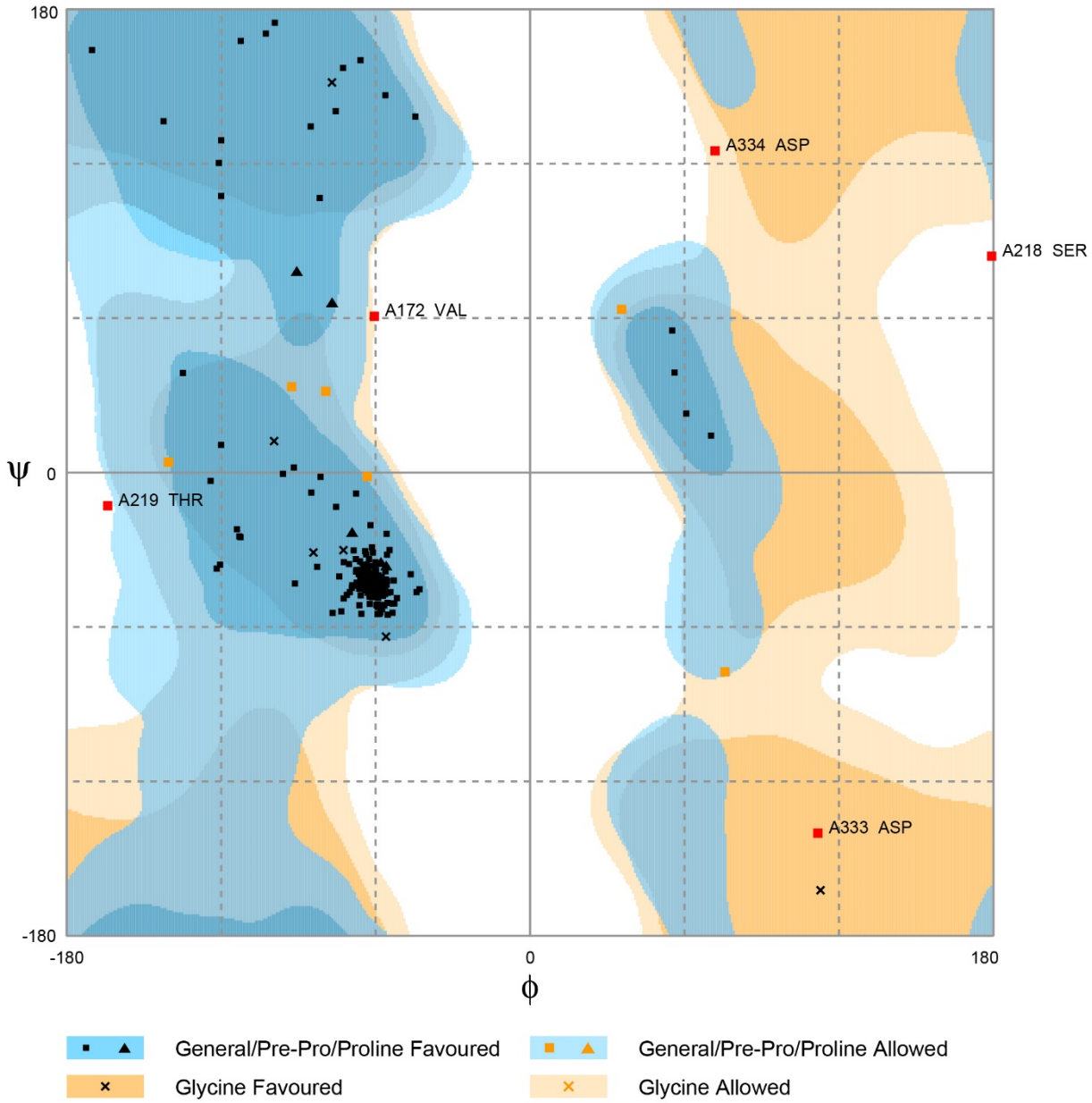


Figure S1. Sequence alignment of CRF_1R with CRF_2R. The identical residues are shown in the red background. The ICL1 of CRF1R is not included. The most conserved residues in each TM are labeled with green dots.



Number of residues in favoured region (~98.0% expected)	: 240 (95.6%)
Number of residues in allowed region (~2.0% expected)	: 6 (2.4%)
Number of residues in outlier region	: 5 (2.0%)

Figure S2. Richardson plot of the modelled structure of CRF_2R.

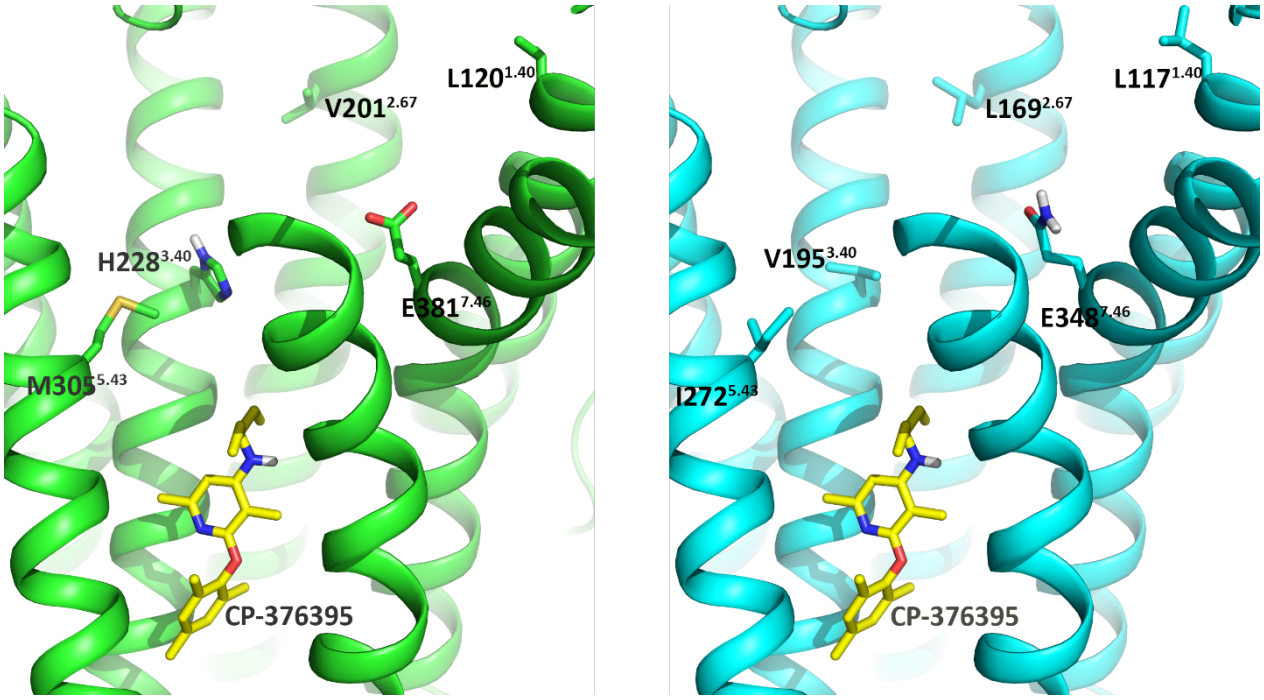


Figure S3. Comparison of the residues in CRF_1R and CRF_2R along the binding pathway of CP-376395. Protein structures are shown in the cartoon mode with the non-conserved residues and the antagonist shown as sticks. The structures of CRF_1R and CRF_2R are colored in green and cyan respectively. The antagonist CP-376395 is colored in yellow.

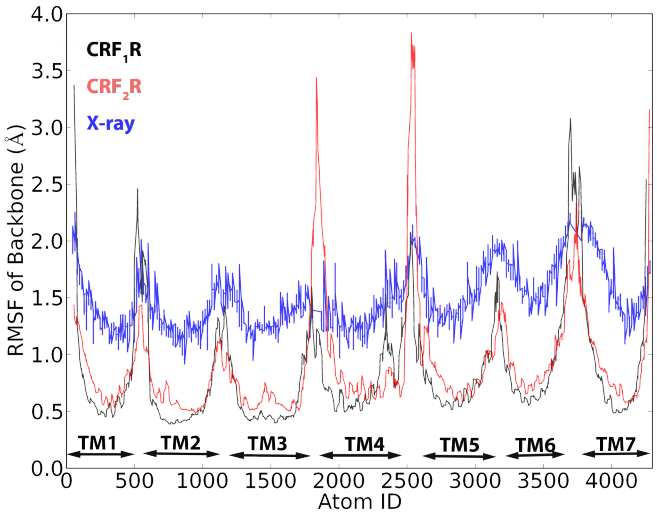


Figure S4. RMSFs of CRF_1R and CRF_2R from the unbiased MD simulations. The RMSF values calculated from the B-factors of the x-ray crystallography structure of CRF_1R are also shown.

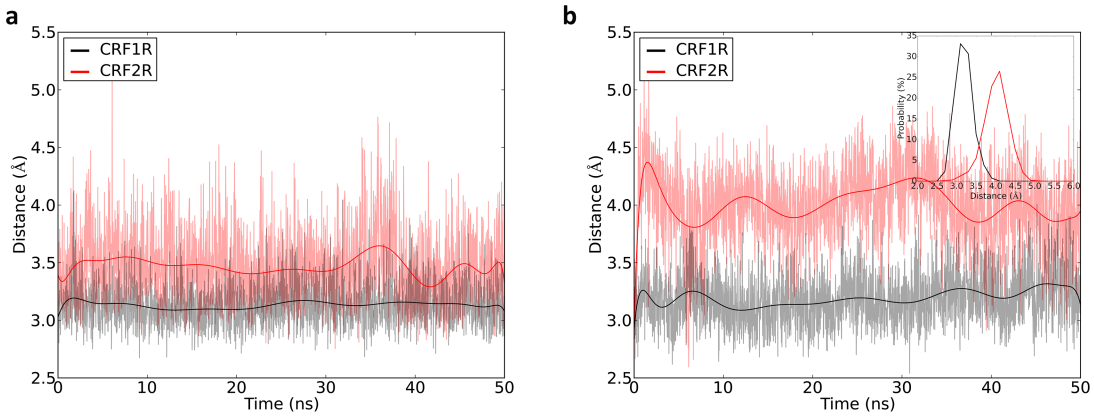


Figure S5. (a) Evolution of the distance between the side chain oxygen atom of Ser$^{1.50}$and the backbone nitrogen atom of Phe$^{7.51}$ in the unbiased MD simulations; (b) Evolution of the distance between the side chain oxygen atom of Ser$^{1.50}$ and the backbone oxygen atom of Ser$^{7.47}$ in the unbiased MD simulations. The probability distributions of the distance are shown in the up right panel.

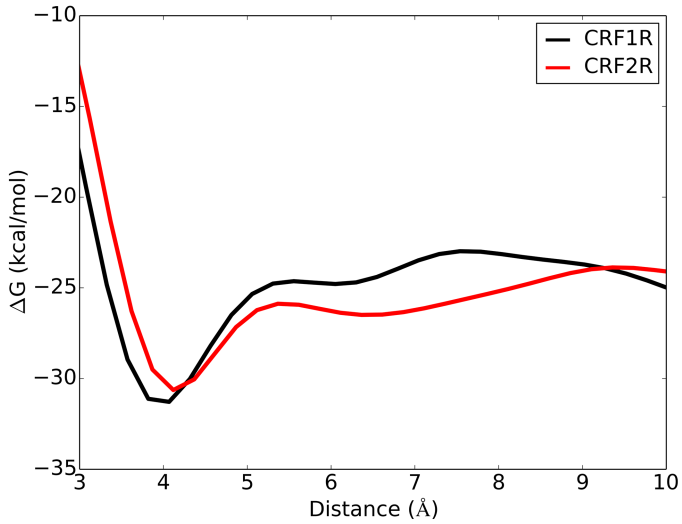


Figure S6. Evolution of the free energy profiles of the two systems with the distance between N^{δ} of $His^{2.50}$ and C^{δ} of $Glu^{3.50}$ as the collective variable. Both receptors prefer to stay in the state with the ionic lock formed.

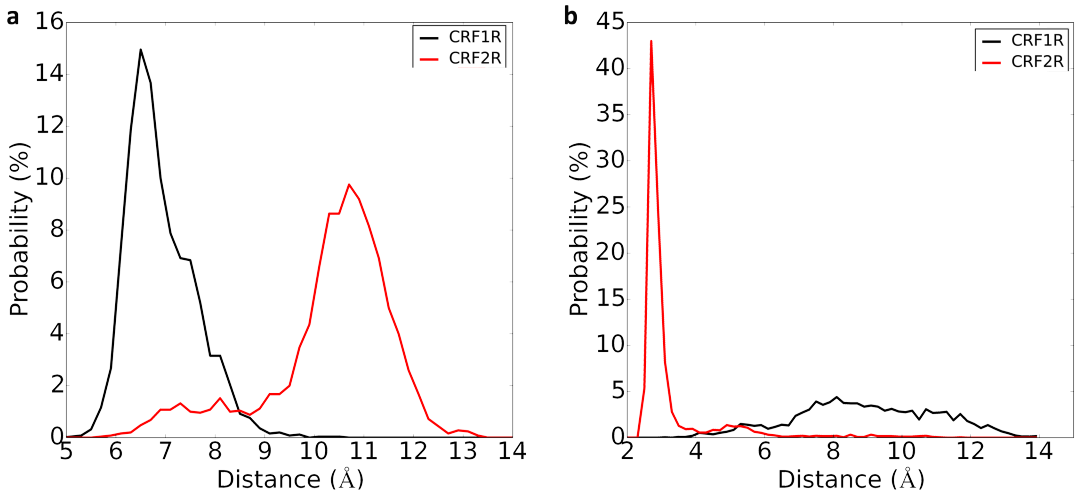


Figure S7. (a)The probability distribution of the distances between the backbone carbon atoms on $Tyr^{6.63}$ and on $His228^{3.40}$ in CRF_1R and that between the backbone carbon atom on $Tyr^{6.63}$ and on $Val228^{3.40}$ in CRF_2R; (b) The probability distributions of the distances between the side chain oxygen atoms on $Tyr^{6.63}$ and on $Gln^{5.50}$ in the two receptors.

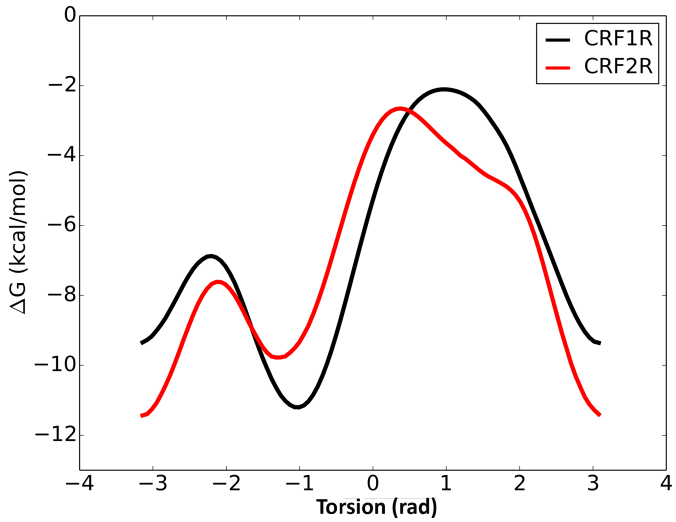


Figure S8. Free energy profiles of the systems with respect to the torsional angle χ_1 of $Tyr^{6.63}$ in CRF_1R and in CRF_2R. The torsional angle χ_1 of $Tyr356^{6.63}$ in CRF_1R prefers to stay around -1 and the torsional angle χ_1 of $Tyr323^{6.63}$ in CRF_2R prefers to stay around $-\pi$ or π.

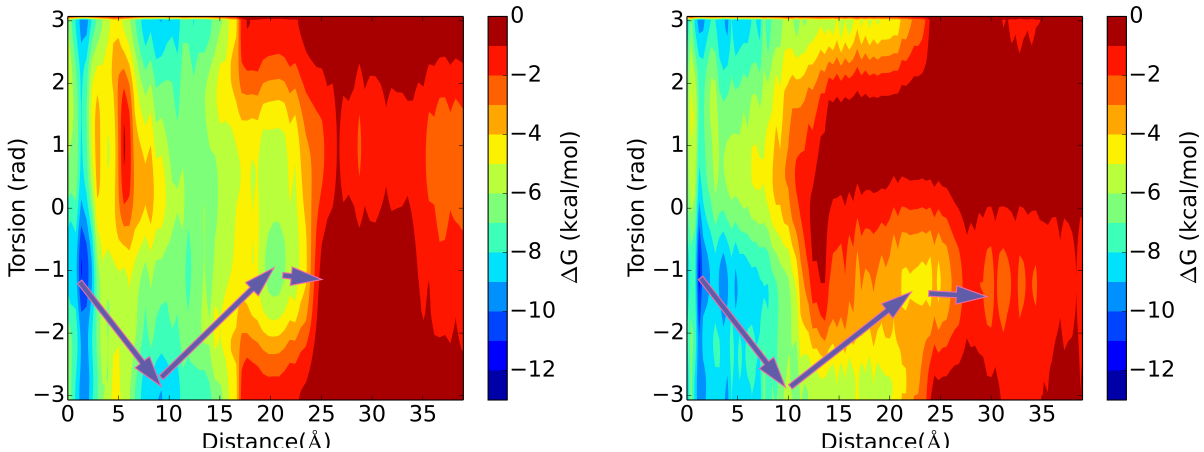


Figure S9. The binding free energy surfaces for the dissociation of CP-376395 from CRF_1R.

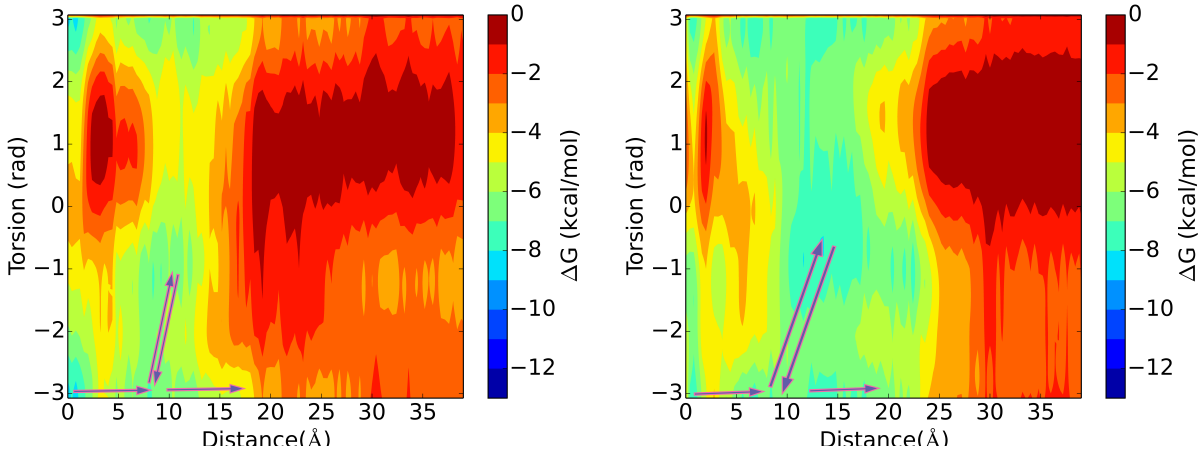


Figure S10. The free energy surfaces for the dissociation of CP-376395 from CRF_2R.