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SUPPORTING INFORMATION

Molecular Dynamics Investigations of Regioselectivity of Anionic/Aromatic Substrates by a Family of Enzymes: A Case Study of Diclofenac Binding in CYP2C Isoforms

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Table S1. The binding free energies calculated with different polar solvation models and solute dielectric constants (Energy unit: kcal/mol, with standard deviations)

systems	GB^{HCT} (igb=1)			GB^{OBC1} (igb=2)			GB^{OBC2} (igb=5)		
	$\varepsilon_{in} = 1$	$\varepsilon_{in} = 2$	$\varepsilon_{in} = 4$	$\varepsilon_{in} = 1$	$\varepsilon_{in} = 2$	$\varepsilon_{in} = 4$	$\varepsilon_{in} = 1$	$\varepsilon_{in} = 2$	$\varepsilon_{in} = 4$
CYP2C9_in108_4'oh	-13.3±3.0	-12.5±2.3	-12.2±2.5	-12.2±2.0	-12.2±2.5	-12.4±2.6	-12.3±3.2	-11.4±2.6	-10.5±2.6
CYP2C19_in108_4'oh	-4.6±3.6	-5.1±2.7	-5.5±2.6	-4.7±1.0	-5.1±2.6	-5.8±2.6	-5.0±3.4	-5.7±2.8	-5.8±2.7
CYP2C8_o108_5oh	-8.5±1.8	-8.7±1.9	-9.3±2.0	-7.7±1.7	-8.1±1.8	-8.8±2.0	-8.2±1.9	-8.4±1.8	-8.7±1.9
R^2	0.77	0.72	0.58	0.84	0.80	0.78	0.79	0.74	0.50

Table S2. Decomposition of binding free energy (kcal mol^{-1}) on per-residue basis for CYP2C9_in108_4'oh, CYP2C19_in108_4'oh, and CYP2C8_o108_5oh complexes

(A) CYP2C9_in108_4'oh complex

Residue	ΔE_{vdw}	ΔE_{ele}	ΔE_{GB}	ΔG_{SASA}	ΔG_{bind}
Arg108	0.74	-5.79	1.75	-0.23	-3.53
Phe476	-1.44	-0.88	0.77	-0.25	-1.80
Leu208	-1.00	-0.39	0.20	-0.13	-1.32
Asn204	-0.88	0.33	-0.61	-0.13	-1.29
Leu362	-1.00	-0.45	0.36	-0.13	-1.22
Leu366	-0.83	-1.01	0.91	-0.16	-1.09
Phe114	-0.84	-0.62	0.73	-0.08	-0.81
Thr301	-0.78	-0.86	0.92	-0.06	-0.78
Ala477	-0.55	-0.17	0.06	-0.08	-0.74
Phe100	-0.52	-0.20	0.13	-0.04	-0.63
Ile205	-0.62	0.35	-0.28	-0.05	-0.60
Leu361	-0.63	0.67	-0.60	-0.03	-0.59
Val113	-0.25	-1.15	0.96	-0.03	-0.47
HEM500	-0.70	23.35	-23.09	-0.03	-0.47

(B) CYP2C19_in108_4'oh complex

Residue	ΔE_{vdw}	ΔE_{ele}	ΔG_{GB}	ΔG_{SASA}	ΔG_{bind}
Asn204	-0.10	-4.51	2.89	-0.12	-1.84
Phe114	-1.30	-1.09	1.07	-0.16	-1.48
Gly296	-0.87	-2.43	2.11	-0.08	-1.27
Ala297	-1.54	-0.26	0.75	-0.16	-1.21
Phe476	-1.18	-1.22	1.44	-0.24	-1.20
Arg108	-1.01	-29.15	29.13	-0.16	-1.19
HEM500	-2.17	23.68	-22.53	-0.08	-1.10

Leu366	-0.95	-0.74	0.70	-0.10	-1.09
Glu300	-0.77	16.39	-16.35	-0.08	-0.81
Asp293	-1.06	25.38	-25.01	-0.03	-0.72
Thr301	-0.62	-1.43	1.41	-0.05	-0.69
Val113	-0.72	-0.07	0.20	-0.07	-0.66
Ile362	-0.50	-0.18	0.15	-0.06	-0.59
Phe100	-0.32	-0.12	0.04	-0.03	-0.43

(C) CYP2C8_o108_5oh complex

Residue	ΔE_{vdw}	ΔE_{ele}	ΔG_{GB}	ΔG_{SASA}	ΔG_{bind}
Phe205	-1.99	-0.14	0.18	-0.25	-2.20
Val366	-1.44	-0.37	0.26	-0.30	-1.85
Val296	-1.26	-1.44	1.40	-0.16	-1.46
Thr301	-1.18	0.04	-0.10	-0.12	-1.36
Ala297	-1.18	-0.22	0.30	-0.07	-1.17
Ile113	-1.03	0.01	0.02	-0.17	-1.17
Val362	-0.56	-0.14	-0.07	-0.08	-0.85
Val477	-0.87	0.32	-0.16	-0.12	-0.83
HEM500	-0.98	-1.71	1.98	-0.11	-0.82
Glu300	-0.84	-1.84	2.03	-0.09	-0.74
Leu361	-0.68	0.01	0.17	-0.04	-0.54
Ile476	-0.48	0.13	-0.12	-0.06	-0.53
Leu208	-0.33	-0.04	-0.03	-0.08	-0.48

Figure S1. Binding characteristics of diclofenac for each CYP2C isoform. The crucial residues involved in ligand binding are shown in stick representation. Diclofenac in each conformer are shown in stick representation in blue and yellow, respectively. The black asterisk (*) represents the metabolic site of diclofenac for each isoform.

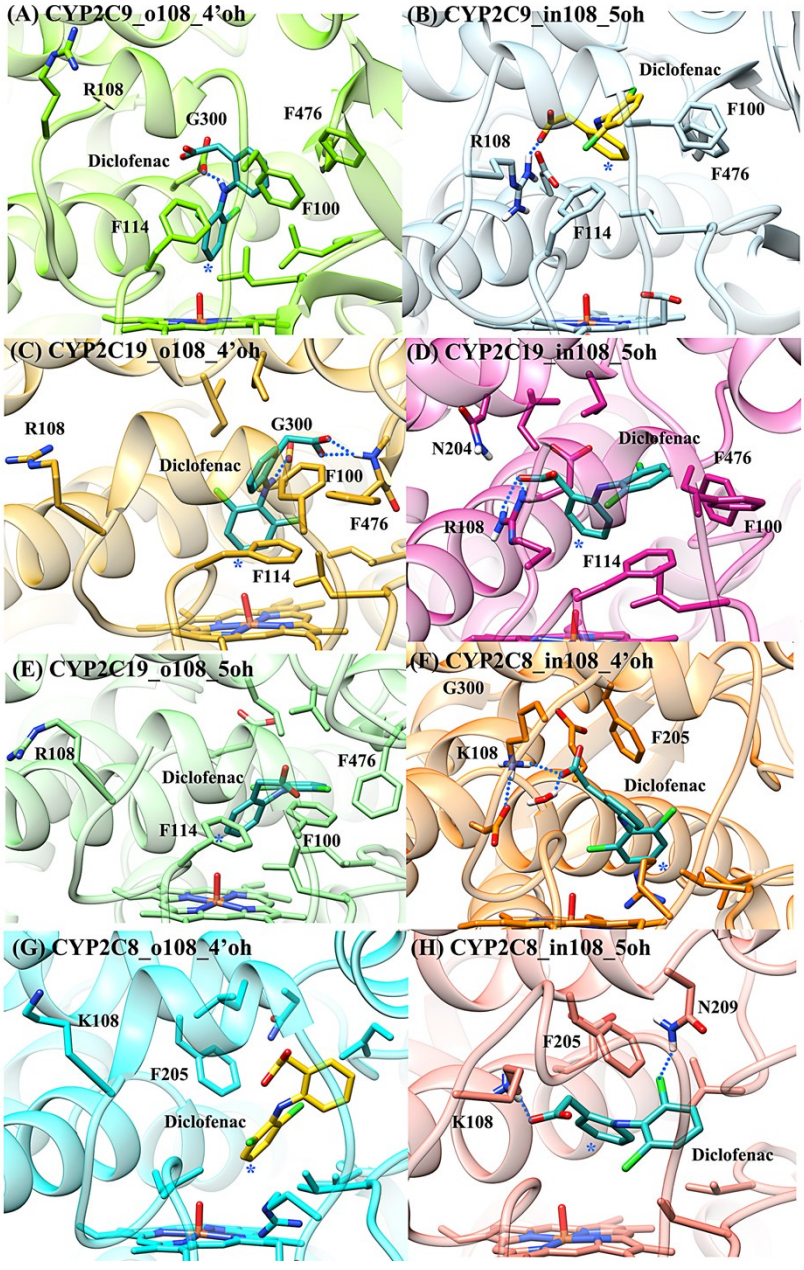

Figure S2. Time-dependent rms deviation values from the starting structures along the simulations for CYP2C enzymes.

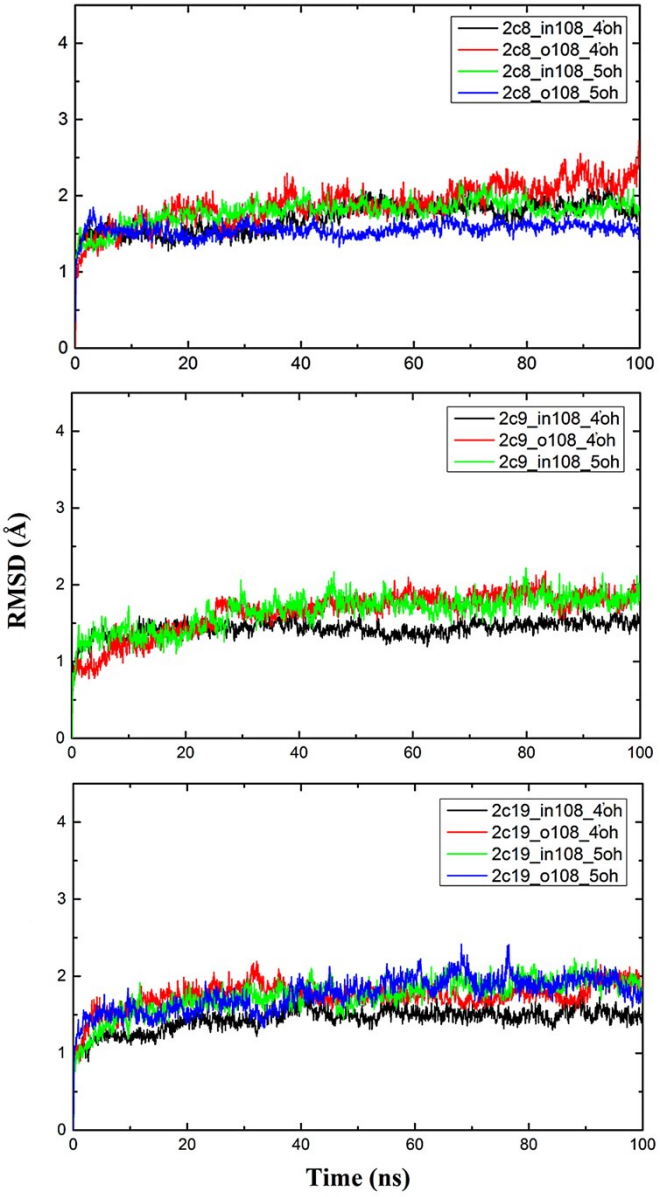

Figure S3. (A) Distinct local structural features of the B-C loop for CYP2C9_in108_4'oh (yellow), CYP2C19_in108_4'oh (salmon pink), and CYP2C8_o108_5oh (blue) complexes. (B) Relocation of the key pivot residues in the B-C loop of CYP2C9_in108_4'oh complex (yellow) compared with those of CYP2C19_108_4'oh complex (salmon pink).

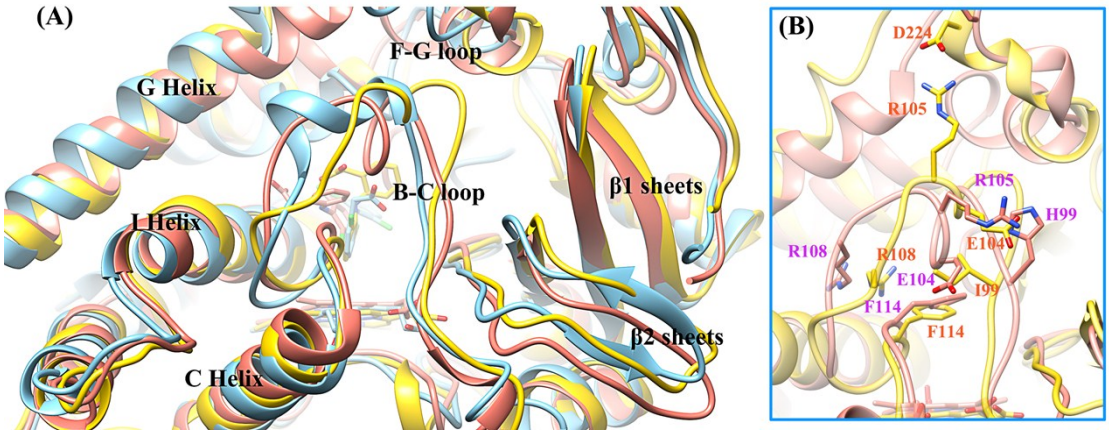

Figure S4. Distances between the centers of the aromatic groups of Phe226 and Trp212 in CYP2C9_in108_4'oh (black), CYP2C19_in108_4'oh (red), and CYP2C8_o108_5oh (green) complexes.

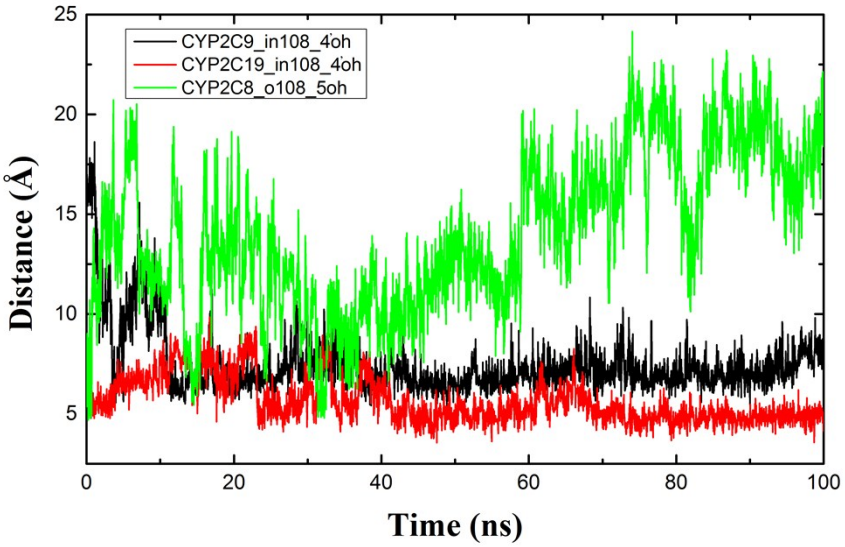

Figure S5. Variations of SASA values of HC1 along MD simulations for CYP2C9_in108_4'oh (black), CYP2C19_in108_4'oh (red), and CYP2C8_o108_5oh (green) complexes.

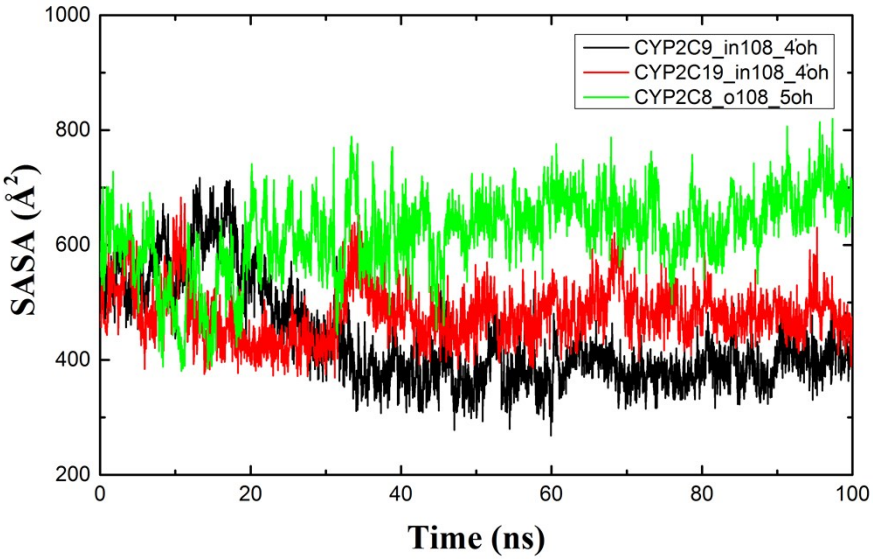

Figure S6. Diagram of the active site volumes for CYP2C isoforms. The volumes were calculated by online tool CASTp (http://sts.bioe.uic.edu/castp/).