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Supplementary Information for

How Mutations Affect Structural Characteristics and Substrate Binding of CYP21A2? An Investigation by Molecular Dynamics Simulations

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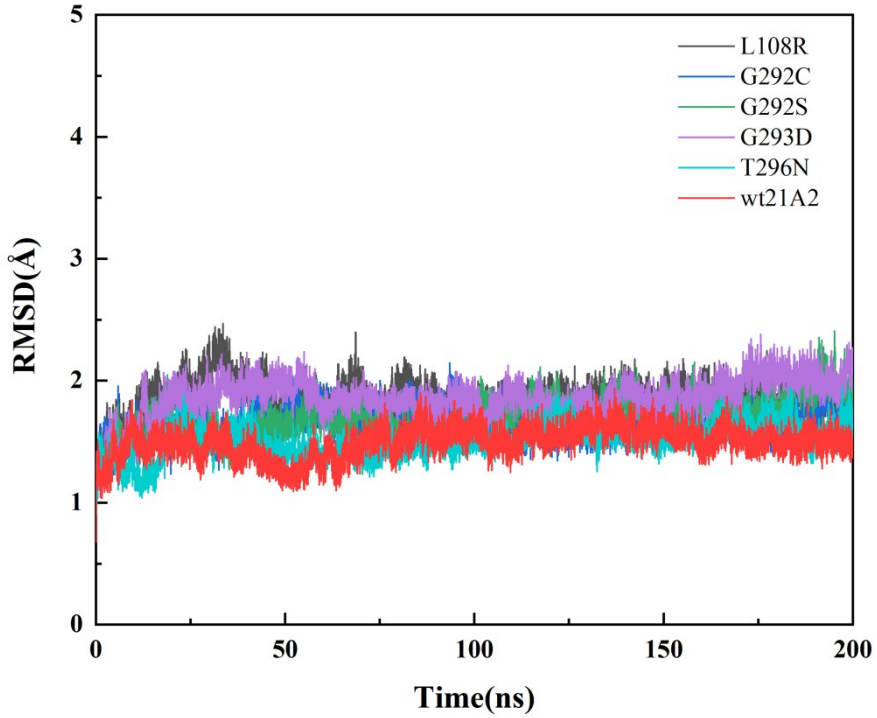

Figure S1. RMSD variations from the starting structures along the simulations for wt21A2, L108R, G292C, G292S, G293D, and T296N.

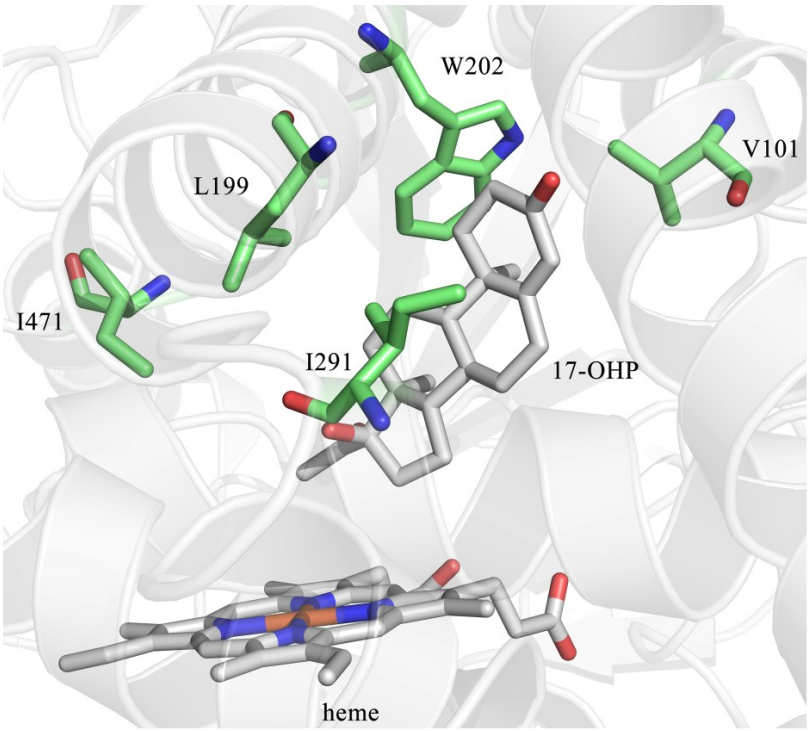

Figure S2. Structural of the substrate binding site in CYP21A2. Hydrophobic residues provide a hydrophobic environment for the substrate binding region. Hydrophobic residues are colored in green.

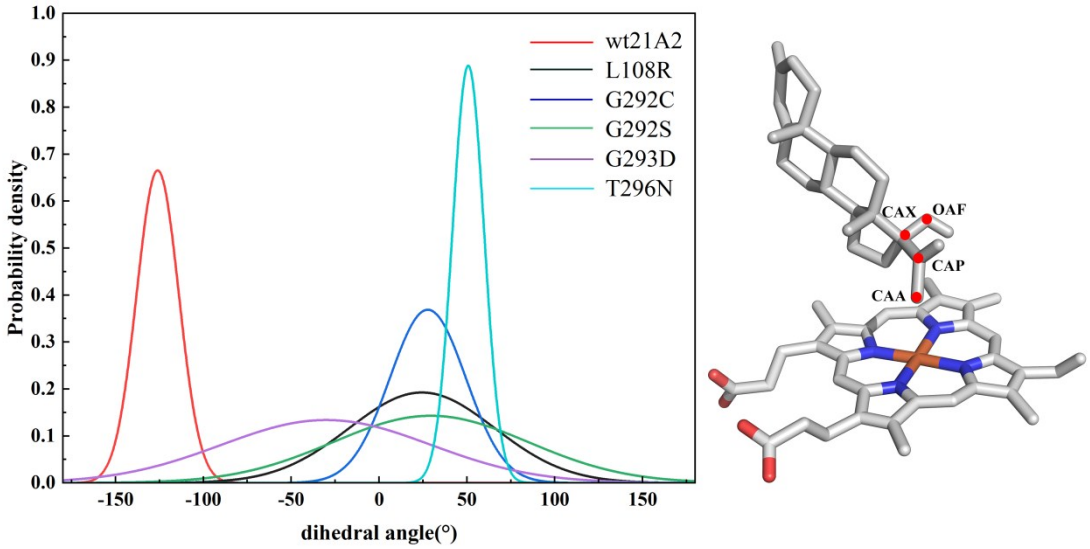

Figure S3. Probability distribution of the substrate dihedral angle for wt21A2, L108R, G292C, G292S, G293D, and T296N. The four atoms that determine the substrate orientation are shown in red dots on the right side.

Table S1. Energy contributions (kcal/mol) of key residues to the binding energy

Residue	wt21A2	L108R	G292C	G292S	G293D	T296N
Val101	-1.34	-0.78	-1.32	-1.12	-1.04	-1.42
Ser109	-1.07	-1.03	-0.96	-1.15	-1.00	-1.21
Leu199	-1.22	-1.37	-1.03	-1.13	-1.37	-1.12
Trp202	-2.81	-3.11	-3.10	-3.59	-3.17	-3.3
Arg234	-0.92	-0.15	-0.78	-0.22	0.18	-0.30
Ile291	-2.00	-1.83	-2.04	-1.87	-0.87	-2.24
Thr296	-4.16	-0.82	-1.88	-0.54	-0.61	-3.43
Ile471	-1.02	-0.46	-1.06	-0.79	-0.52	-0.76
HEM	-6.35	-3.85	-1.40	-2.69	-4.11	-3.30

Table S2. (A) Decomposition of binding energy (kcal/mol) of key residue for wt21A2

Residue	Van der Waal	Electrostatic	Polar solvation	Non-polar solvation	Total
Val101	-1.12	0.12	-0.26	-0.08	-1.34
Ser109	-0.91	0.17	-0.24	-0.09	-1.07
Leu199	-1.27	-0.06	0.22	-0.11	-1.22
Trp202	-2.48	-0.48	0.31	-0.16	-2.81
Arg234	-0.41	-6.84	6.38	-0.05	-0.92
Ile291	-2.42	0.22	0.41	-0.21	-2.00
Thr296	-0.48	-2.85	-0.71	-0.12	-4.16
Ile471	-0.77	-0.29	0.18	-0.13	-1.02
HEM	-4.31	-4.94	3.08	-0.18	-6.35

Table S2. (B) Decomposition of binding energy (kcal/mol) of key residue for L108R

Residue	Van der Waal	Electrostatic	Polar solvation	Non-polar solvation	Total
Val101	-0.87	0.29	-0.10	-0.10	-0.78
Ser109	-0.92	0.16	-0.17	-0.11	-1.03
Leu199	-1.49	-0.09	0.34	-0.14	-1.37
Trp202	-2.85	-0.30	0.27	-0.23	-3.11
Arg234	-0.22	-6.65	6.79	-0.08	-0.15
Ile291	-2.51	-2.12	3.06	-0.26	-1.83
Thr296	-0.70	-0.16	0.13	-0.09	-0.82
Ile471	-0.50	-0.04	0.13	-0.05	-0.46
HEM	-3.40	-0.61	0.32	-0.15	-3.85

Table S2. (C) Decomposition of binding energy (kcal/mol) of key residue for G292C

Residue	Van der Waal	Electrostatic	Polar solvation	Non-polar solvation	Total
Val101	-1.14	0.15	-0.24	-0.09	-1.32
Ser109	-0.72	0.02	-0.18	-0.08	-0.96
Leu199	-1.23	-0.06	0.34	-0.09	-1.03
Trp202	-2.73	-0.45	0.27	-0.20	-3.10
Arg234	-0.22	-6.30	5.78	-0.05	-0.78
Ile291	-2.22	0.24	0.18	-0.24	-2.04
Thr296	-0.63	-0.98	-0.20	-0.08	-1.88
Ile471	-1.11	0.02	0.17	-0.14	-1.06
HEM	-1.76	-1.02	1.45	-0.07	-1.40

Table S2. (D)Decomposition of binding energy (kcal/mol) of key residue for G292S

Residue	Van der Waal	Electrostatic	Polar solvation	Non-polar solvation	Total
Val101	-1.15	0.23	-0.06	-0.13	-1.12
Ser109	-1.00	0.07	-0.13	-0.09	-1.15
Leu199	-1.32	-0.09	0.37	-0.09	-1.13
Trp202	-3.03	-0.40	0.06	-0.21	-3.59
Arg234	-0.47	-6.40	6.70	-0.04	-0.22
Ile291	-2.16	0.09	0.40	-0.19	-1.87
Thr296	-0.47	0.15	-0.18	-0.05	-0.54
Ile471	-0.79	0.05	0.06	-0.11	-0.79
HEM	-2.53	-0.32	0.23	-0.07	-2.69

Table S2. (E) Decomposition of binding energy (kcal/mol) of key residue for G293D

Residue	Van der Waal	Electrostatic	Polar solvation	Non-polar solvation	Total
Val101	-1.04	0.21	-0.09	-0.12	-1.04
Ser109	-0.97	0.22	-0.16	-0.09	-1.00
Leu199	-1.53	-0.01	0.28	-0.11	-1.37

Trp202	-2.97	-0.37	0.39	-0.23	-3.17
Arg234	-0.50	-6.50	7.24	-0.05	0.18
Ile291	-2.64	-0.66	2.63	-0.20	-0.87
Thr296	-0.57	0.19	-0.16	-0.08	-0.61
Ile471	-0.46	0.00	0.03	-0.10	-0.52
HEM	-3.40	-2.22	1.68	-0.16	-4.11

Table S2. (F) Decomposition of binding energy (kcal/mol) of key residue for T296N

Residue	Van der Waal	Electrostatic	Polar solvation	Non-polar solvation	Total
Val101	-1.26	0.17	-0.21	-0.12	-1.42
Ser109	-1.00	0.07	-0.18	-0.09	-1.21
Leu199	-1.23	-0.11	0.30	-0.08	-1.12
Trp202	-2.95	-0.41	0.26	-0.20	-3.30
Arg234	-0.41	-6.91	7.05	-0.03	-0.30
Ile291	-2.59	-0.08	0.64	-0.22	-2.24
Thr296	-0.52	-6.36	3.58	-0.13	-3.43
Ile471	-0.85	0.04	-0.02	-0.08	-0.30
HEM	-2.66	0.27	-0.81	-0.10	-3.30

Table S3. Hydrogen Bonds Occupancies of Crucial Residues for wt21A2, L108R, G292C, G292S, G293D, and T296N

	Occupied (%)					
H-bond	Wt21A2	L108R	G292C	G292S	G293D	T296N
D288@O-L/R108@H	NA	75.10	NA	NA	NA	NA
D288@O-G/C/S292@H	77.95	NA	91.65	86.4	NA	77.35
L289@O-G/D293@H	99.15	NA	99.25	98.40	98.40	98.15
G/C/S292@O- T/N296@H	98.80	41.33	89.20	88.65	41.55	97.40
G/D293@O-A298@H	NA	73.37	NA	NA	98.75	NA
G/D293@O-A297@H	NA	31.19	NA	15.00	NA	NA
G/D293@O-T/N296@H	NA	8.64	NA	NA	46.30	NA
T/N296@O-T297@H	NA	20.10	21.15	15.15	77.20	NA
T/N296@O-17-OHP@H	98.70	NA	18.30	NA	NA	99.40
17-OHP@O-R234@H	97.15	99.52	86.75	97.50	99.50	99.40