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

QMMM study of hydride mechanism in flavocytochrome b_2

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Supporting Information

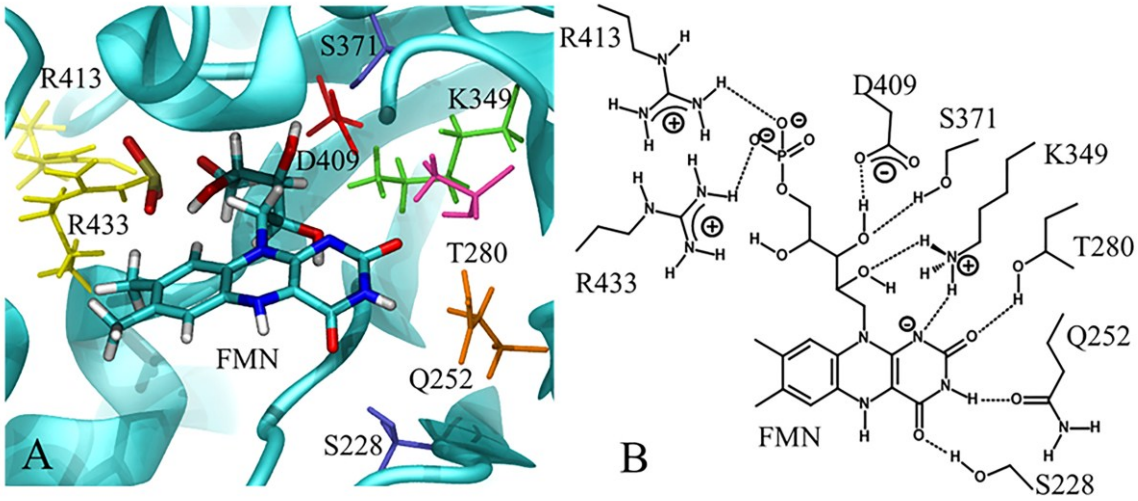

Figure S1: 3D (left) and schematic (right) representation of FMN in the active site pocket and closed residues. 3D visualization comes from snapshot from reactant state MD treated by VMD.

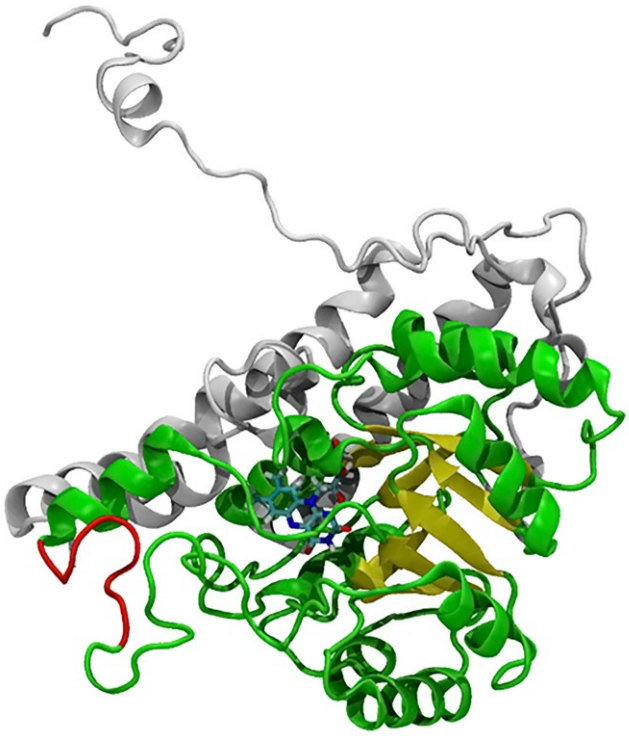

Figure S2: Fcb2 flavodomain structure from our simulations. The triosephosphate isomerase (TIM) barrel is in yellow (for β strands) and green (α helices and loops). The reconstructed residues of loop 4, absent from the crystallographic structure, are in red.

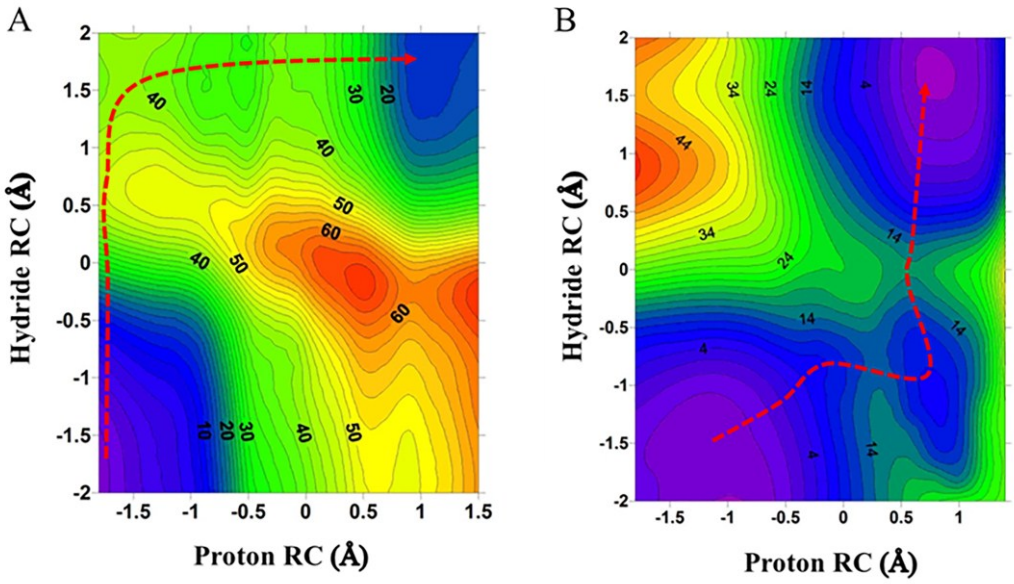

Figure S3: AM1/MM (A) and M06-2X:AM1/MM (B) PES of a hydride transfer mechanism in Fcb2 for a distal position of R289. Isoenergetic lines are drawn for each 2 kcal/mol, from purple minima to red maxima.

	WT R289 distal						WT R289 Proximal						D282N						Y254L					
	R		TS		P		R		TS		P		R		TS		P		R		TS		P	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
H3-O3	0.97	0.03	1.56	0.04	2.41	0.4	0.97	0.03	1.08	0.03	4.52	1.19	0.97	0.03	1.01	0.03	2.43	0.48	0.96	0.03	1.04	0.03	3.3	0.23
H3-NεH373	3.23	0.6	1.12	0.03	1.01	0.03	5.45	1.78	1.6	0.04	1.01	0.03	3.77	0.73	1.79	0.04	1.02	0.03	4.35	1.52	1.76	0.04	1	0.03
αH-αC	1.13	0.03	1.44	0.04	3.89	0.63	1.13	0.03	1.61	0.04	3.84	0.69	1.13	0.03	1.45	0.04	4.16	0.63	1.13	0.03	1.62	0.04	4.12	0.57
H2-N5FMN	3.37	0.48	1.26	0.04	1.01	0.03	4.23	1.1	1.13	0.03	1.01	0.03	3.81	0.52	1.24	0.04	1.01	0.03	4.52	1.12	1.14	0.03	1.01	0.03
Ca-O3	1.42	0.02	1.27	0.02	1.24	0.02	1.43	0.02	1.32	0.02	1.24	0.02	1.42	0.03	1.37	0.02	1.24	0.02	1.42	0.02	1.32	0.02	1.24	0.02
N5-C4a	1.31	0.02	1.4	0.02	1.42	0.03	1.31	0.02	1.4	0.03	1.42	0.02	1.31	0.02	1.35	0.03	1.42	0.02	1.31	0.02	1.4	0.02	1.41	0.02
C4a-C10	1.5	0.03	1.44	0.03	1.42	0.02	1.5	0.03	1.45	0.03	1.42	0.02	1.5	0.03	1.48	0.03	1.42	0.02	1.5	0.03	1.45	0.03	1.41	0.02
C10-N1	1.34	0.02	1.36	0.02	1.38	0.02	1.34	0.02	1.36	0.02	1.38	0.02	1.34	0.02	1.35	0.02	1.38	0.02	1.34	0.02	1.36	0.02	1.38	0.02
O1-NεR376	2.9	0.14	2.94	0.14	2.94	0.16	3.11	0.47	3.07	0.22	3.85	0.6	2.89	0.13	2.91	0.12	2.95	0.15	2.87	0.12	2.93	0.13	2.81	0.1
O1-NH1R376	4.92	0.16	5.06	0.16	4.97	0.2	5.13	0.48	5.07	0.21	5.83	0.57	4.76	0.14	4.86	0.14	4.95	0.19	4.88	0.14	5.08	0.15	4.86	0.14
O1-NH2R376	3.48	0.31	3.7	0.22	3.49	0.29	3.87	0.47	3.67	0.25	4.8	0.55	3.15	0.23	3.28	0.2	3.45	0.27	3.39	0.22	3.76	0.23	3.43	0.24
O2-NεR376	3.75	0.37	3.57	0.21	3.85	0.29	3.47	0.3	3.79	0.22	3.22	0.35	4.06	0.26	3.96	0.22	3.9	0.29	3.82	0.12	3.59	0.23	3.9	0.24
O2-NH1R376	5.01	0.22	4.93	0.14	5.07	0.23	4.84	0.17	5.07	0.15	4.75	0.19	5.2	0.21	5.08	0.16	5.1	0.22	5.04	0.16	5	0.18	5.12	0.2
O2-NH2R376	2.89	0.16	2.86	0.12	2.93	0.21	2.85	0.11	2.96	0.14	2.96	0.17	3.01	0.19	2.91	0.13	2.94	0.2	2.9	0.14	2.94	0.16	3.01	0.19
O1-NεR289	4.82	0.35	5.33	0.23	4.45	0.31	4.96	0.16	5.02	0.15	5.11	0.24	3.92	0.33	4.59	0.22	4.65	0.25	5.76	0.33	5.64	0.3	5.38	0.48
O1-NH1R289	6.29	0.52	7.04	0.27	5.79	0.31	3.58	0.25	3.74	0.2	3.9	0.29	5.21	0.33	5.67	0.23	5.88	0.25	5.76	0.33	7.36	0.29	7.14	0.48
O1-NH2R289	4.77	0.59	6.06	0.39	4.41	0.32	2.88	0.13	2.91	0.13	2.95	0.21	3.78	0.37	4.18	0.26	4.52	0.31	4.39	0.32	6.31	0.49	6.24	0.55
O2-NεR289	4.92	0.31	4.98	0.22	4.14	0.26	5.11	0.22	5.05	0.15	4.85	0.19	4.63	0.43	5.01	0.3	4.42	0.39	4.49	0.5	5.14	0.27	5.37	0.41
O2-NH1R289	5.66	0.46	6.21	0.26	4.76	0.26	2.99	0.18	2.98	0.14	2.93	0.17	5.03	0.31	5.2	0.26	4.89	0.25	5.06	0.39	6.39	0.3	6.73	0.44
O2-NH2R289	3.95	0.52	4.86	0.34	3.04	0.26	3.72	0.31	3.63	0.2	3.28	0.29	3.41	0.42	3.74	0.32	3.34	0.39	3.49	0.46	5.04	0.44	5.4	0.48
O2-OY143	2.95	0.23	3.11	0.41	4.42	0.76	3.12	0.57	2.97	0.21	4.33	0.97	3.29	0.58	2.89	0.23	3.93	1.17	3.34	0.59	3.48	0.51	3.7	0.59
O1-WAT491	2.91	0.17	3.15	0.3	3	0.21				-			2.85	0.17	2.93	0.16	3.07	0.28	2.9	0.19	3.73	0.79	3.33	0.64
O3-Y254	3.04	0.3	2.82	0.12	3.08	0.31	4.32	1.01	3	0.16	3.58	0.87	3.84	0.51	2.9	0.16	3.15	0.4	7.74	0.49	6.65	0.35	6.73	0.47
O3-Q377	4.2	0.96	4.39	0.61	5.31	0.75	4.37	1.26	4.1	0.43	3.58	0.87	3.93	0.71	6.23	0.48	5.86	0.76	4.86	0.87	4.18	0.32	3.28	0.27
NεH373-D289	3.93	0.64	4.29	0.7	4.87	0.44	3.75	0.25	3.59	0.45	3.78	0.25	4.94	1.08	5.07	0.5	5.04	0.47	3.72	0.37	3.3	0.21	3.91	0.39
NδH373-D289	3.44	0.58	3.18	0.36	2.93	0.28	3.33	0.21	3.15	0.39	3.24	0.2	3.58	0.46	3.41	0.29	3.68	0.52	3.71	0.32	3.86	0.3	3.3	0.23

Table S1: Average intra and intermolecular distances in the active site and associated standard deviation from MDs simulations at reactants (R), transition state (TS) or products (P) for WT, D282N and Y254L enzymes. For WT enzyme with R289 in a distal position, TS refers to the transition state of the hydride transfer step of the step-wise mechanism. Distances are provided in ångströms.

	WT R289 distal			WT R289 Proximal			D282N			Y254L		
	R	**TS**	**P**	**R**	**TS**	**P**	**R**	**TS**	**P**	**R**	**TS**	**P**
R376	-116.3	-103.8	-102.6	-106.7	-97.8	-95.7	-110.1	-108.2	-95.7	-113.219	-98.5	-107.7
R289	-48.0	-33.7	-54.3	-90.2	-75.7	-86.8	-58.1	-48.9	-43.4	-55.5	-27.7	-30.3
Y254	-5.2	-8.6	-6.3	-3.3	-5.6	-6.9	-3.2	-6.1	-5.4	-0.2	0.1	0.1
Y143	-6.8	-3.00	6.3	-6.6	-5.5	4.1	-6.6	-9.8	-1.3	-5.4	2.5	4.7
D282	19.3	9.2	6.8	22.4	23.3	-5.3	0.3	-1.5	-1.6	24.1	28.8	1.7
Q377	-14.2	-3.8	-6.5	-20.0	-10.1	-14.1	-11.3	-9.9	3.7	-16.3	-8.9	-8.0
K349	-108.4	-109.7	-113.2	-104.3	-114.0	-114.1	-111.7	-115.9	-122.4	-100.6	-115.6	-112.5
S228	-5.0	-8.8	-6.7	-6.3	-5.5	-9.6	-4.3	-4.6	-7.2	-3.1	-8.2	-5.9
Q252	-4.1	-2.7	-4.1	-3.1	-2.7	-0.7	-3.6	-3.0	-2.0	-3.1	-2.8	1.6
T280	-11.3	-10.9	-10.2	-10.0	-10.5	-9.108	-9.9	-10.3	-11.7	-8.5	-10.8	-9.0

Table S2: Average electrostatic interactions (in kcal/mol) between residues in or close to the active site pocket and QM part (L-lactate, FMN and H373 side chain) from MDs simulations at reactants (R), transition state (TS) or products (P) for WT, D282N and Y254L Fcb2. For WT enzyme with R289 in a distal position, TS refers to the transition state of the hydride transfer step of the step-wise mechanism.

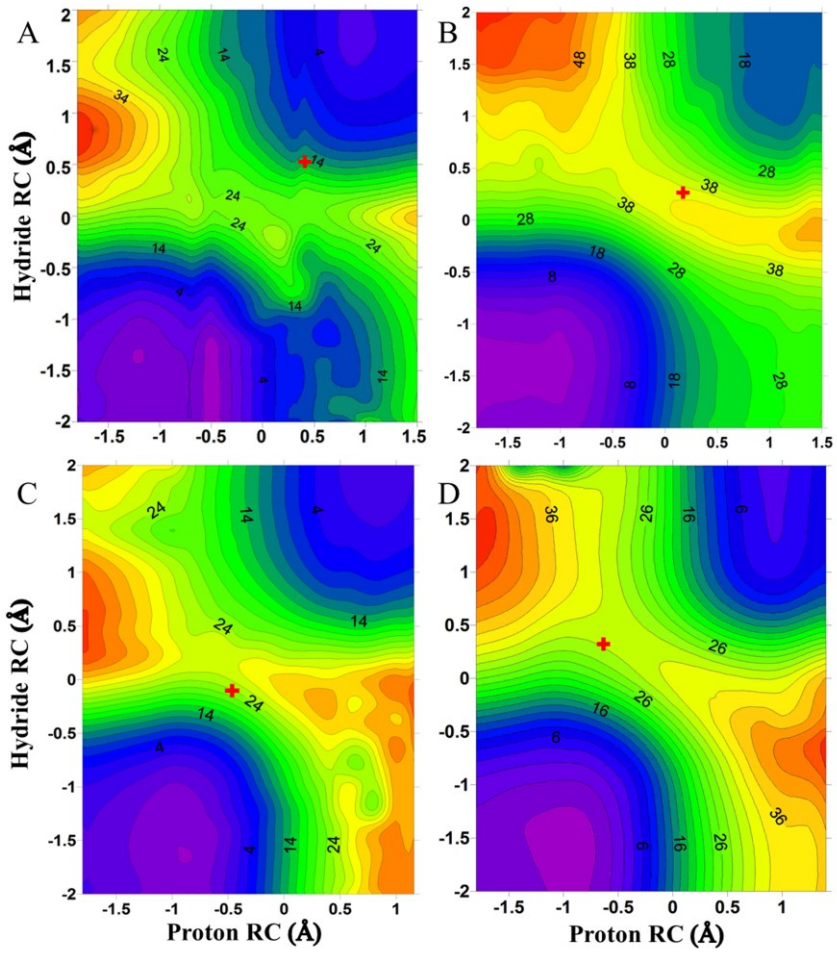

Figure S4: M06-2X:AM1/MM free energy surfaces of the hydride transfer mechanism in Fcb2 for a distal position of R289 (A), a proximal position of R289 (B), D282N variant (C) and Y254L variant (D). Localization of TSs at M06-2X/MM level is indicated with red crosses.