Supplemental Information

STRUCTURAL DIFFERENCES BETWEEN SOLUBLE AND MEMBRANE BOUND CYTOCHROMES P450

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Table S1. Structural parameters plotted at Figure 3A (angle between C_β-C_γ bond of the heme A-propionate and the mean heme plane) and Figure 3B (the height of the center of the beta-domain over the mean heme plane).

Cytochrome P450	HBR, number[a]	Angle, deg	Height, Å
	Membrane	**bound**	**enzymes**
1A2	H388	-59.90	2.06
1B1	H401	-56.90	2.78
2A6	R372	-68.8(6.6)	2.4(0.21)
2A13	H372	-65.2(1.4)	2.48(0.31)
2B4	H369	-56.1(22.8)	2.99(0.69)
2B6	H369	-67.40	3.74
2C5	H365	-51.2(15.8)	2.75(0.57)
2C8	H368	-64.9(0.7)	2.49(0.02)
2C9	H368	-37.2(15.4)	2.33(0.20)
2D6	H376	-59.0(1.7)	2.28(0.15)
2E1	H370	-65.5(3.1)	2.62(0.44)
2R1	H381	-47.2(3.6)	3.96(0.23)
3A4	R375	-69.4(3.6)	3.64(0.27)
7A1	R364	-62.9(3.3)	1.49(0.07)
11A1	R396	-73.3(9.1)	3.77(0.04)
19A1	R375	-74.80	4.12
24A1	R396	-70.9(4.1)	2.35(0.49)
46A1	R372	31.8(58.3)	3.91(0.36)
51G*(Leishm.)*	R360	64.4(2.5)	5.36(0.51)
51G(*Tr.Cruzi*)	R361	59.8(6.6)	5.42(0.56)
51G(*Tr.Bruz.)*	R361	62.0(9.5)	6.00(0.62)
51A1(Human)	R382	46.0(51.4)	6.01(0.81)
	Soluble	**enzymes**	
51A(*M.tuberc.)*	R326	64.6(5.2)	7.14(1.34)
101A1	R299	64.8(3.1)	7.78(0.82)
101C1	R285	63.7(2.9)	8.27(0.38)
101D1	R307	63.5(3.0)	6.83(0.12)
101D2	R306	60.6(6.4)	5.87(0.14)
102A1	L333	66.5(4.3)	4.26(0.41)
105A1	R297	57.0(2.6)	6.04(0.41)
105D6	R295	53.3	5.27
105K1	R298	63.2(1.0)	4.45(0.05)
105P1	R290	66.1	4.35
107A1	R293	63.3(1.6)	7.76(0.34)
107L1	R296	57.9(6.6)	8.7(0.46)
107H1	R286	65.9(2.0)	6.09(0.09)

108A1	R319	-76.8	3.18
113A1	R293	61.0	6.70
119A1	R259	59.7(0.7)	5.29(0.01)
119A2	R259	35.3	6.2(0.52)
120A1	R323	59.7(3.6)	5.53(0.62)
121	R286	55.9(0.7)	5.88(0.09)
124	R320	-72.3	6.26
125A1	R318	-43.3	5.20
130	R295	65.5(1.4)	6.22(0.05)
134	R285	-70.0(6.9)	4.65(0.23)
142	R282	-59.3	6.54
146	R287	61.1(1.1)	5.24
151A1	R296	-70.2(1.6)	2.87(0.6)
154A1	R295	-68.6	5.39
154C1	R295	44.3(1.3)	7.11(0.27)
158A1	R298	-1.7(60.6)	5.3(0.93)
158A2	R295	-57.2(2.5)	3.97(0.24)
165B3	R289	60.2(1.6)	6.30(0.02)
165C4	R298	61.3(3.0)	7.31(0.18)
165D3	R279	56.9	5.93
167A1	R307	64.3	8.25
170A1	R343	-20.6(4.2)	5.30(0.4)
175A	R273	53.3(2.7)	4.61(0.4)
176A	R289	39.1(14.2)	8.21(0.35)
199A	R303	54.4	7.26
231A	R247	59.5(3.7)	6.80(0.88)
245A	R306	53.9	7.22
248	R285	62.9	8.0
PimD	R288	89.0(9.0)	6.29(1.11)
VitDh	R289	64.1(1.4)	8.13(0.49)
	Exceptions	**(not shown**	**in Figure 3)**
8A1(Human)	R359		3.5(1.0)
8A1(Zebra fish)	R339		5.4(1.6)
55A3	R292		6.63(1.01)
74A1(*Arabidops.Th.)*	G393		1.81(0.26)
74A1(*Part. Argent.)*	G349		2.19(0.01)
152A1	A295		2.3

[a] HBR, heme binding residue in the sequence of corresponding cytochrome P450. Angles and heights are shown as averaged over multiple structures when available with the standard deviations shown in parentheses.

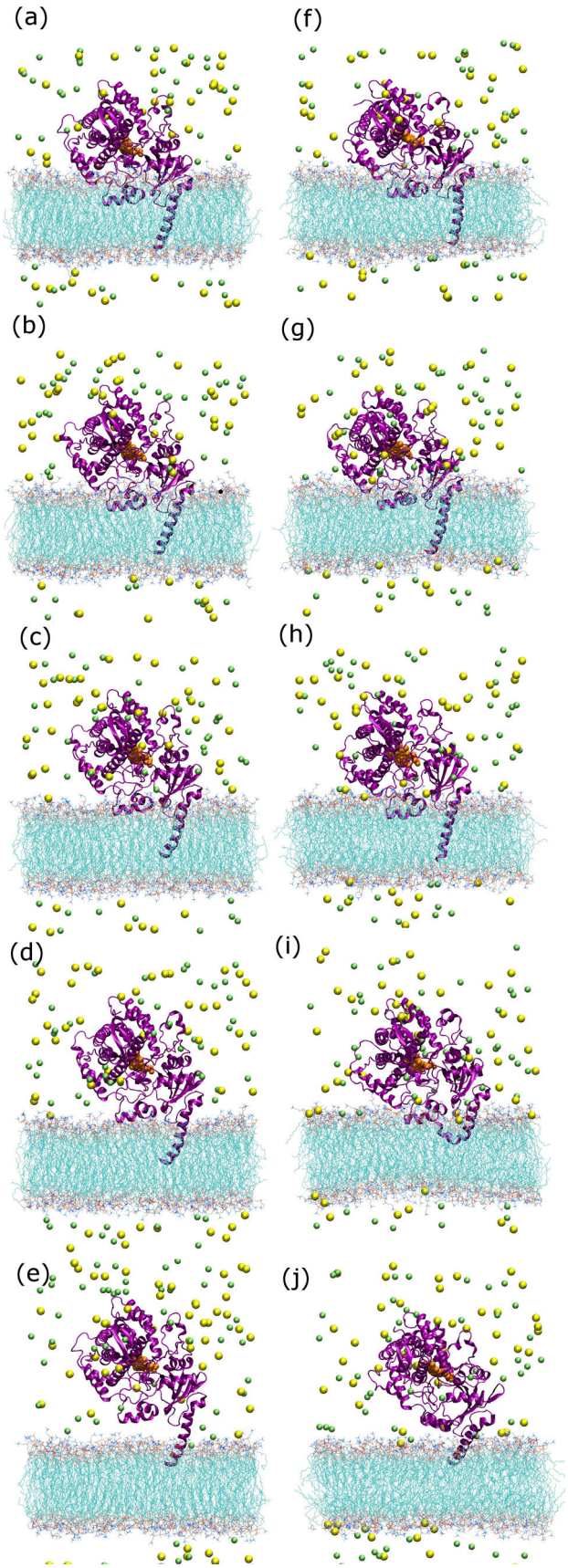
(a)
(f)
(b)
(g)
(c)
(h)
(d)
(i)
(e)
(j)

Figure S1: Five 20-ns molecular dynamics simulations of CYP3A4 inserted at various depths of a POPC lipid bilayer were done to determine an appropriate initial starting structure for further studies. The starting systems of these simulations are shown in (a)-(e) with (b) being the so-called "ideal" depth of insertion and that which most closely matched the Orientation of Proteins in Membranes database. In (a) the protein is inserted 5A deeper into the membrane and (b)-(e) have the protein inserted 5 Å, 10 Å, and 20 Å shallower, respectively. The resulting structures following 20-ns of simulation are shown in (f)-(j). Both the 5 Å deeper (f) and 5 Å shallower (h) simulations moved closer to the "ideal" insertion depth (g). The 10 Å (d and i) and 20 Å (e and j) appeared to be moving out of the membrane.

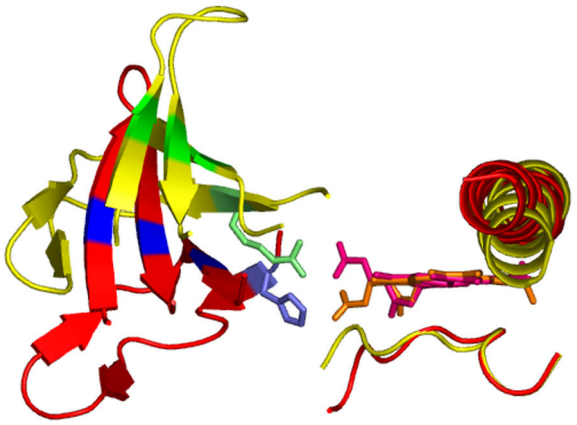

Figure S2. Fragments of the CYP101 structure (yellow) and that of CYP2E1 (red) are superimposed within the heme plane coordinate frame. The different orientations of the A-propionate side chains in the membrane bound mammalian CYP2E1 and bacterial soluble CYP101 are determined by the height over the heme plane of corresponding heme binding residues Arg299 CYP101, green) and His370 (CYP2E1, blue). This difference is due to the significantly lower position of the entire beta-domain in CYP2E1. Four Cα atoms on the twist axis of the beta-domain which have been used for calculation of the height of the beta-sheet above the heme plane are colored green in CYP101 and blue in CYP2E1 (more details in the text).

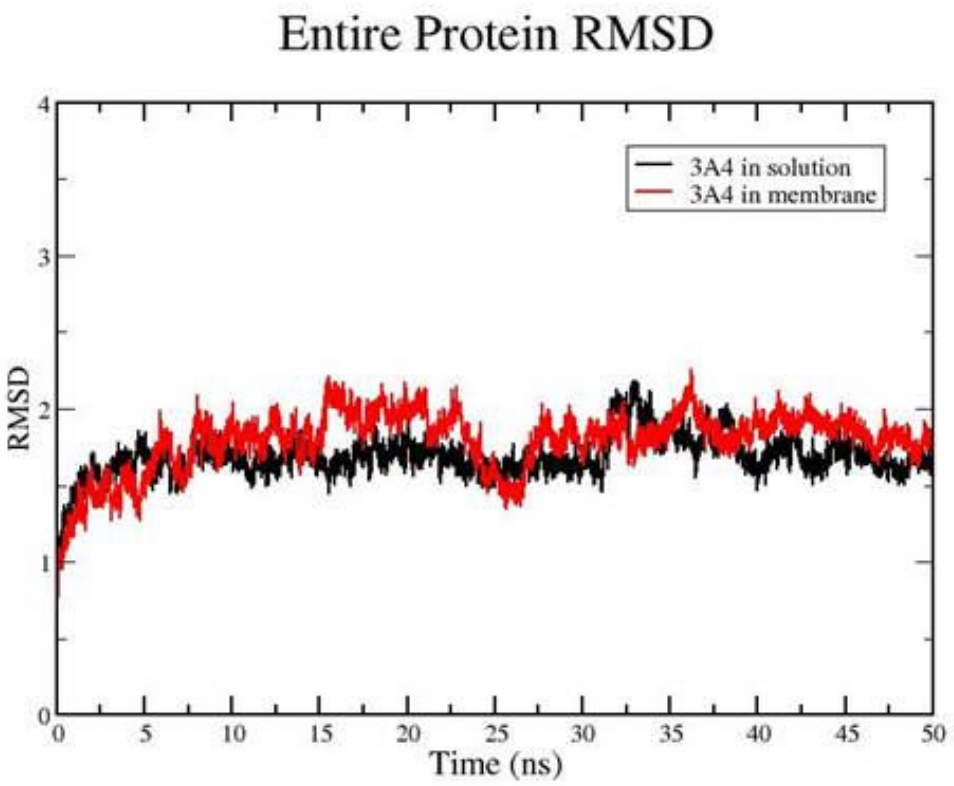

Figure S3. RMSD of CYP3A4 protein (Cα atoms only) over the course of the 50 ns molecular dynamics simulation. CYP3A4 in aqueous solution is shown in black and CYP3A4 embedded in a membrane is shown in red. Both simulations reach an equilibrium structure within 10 ns as shown by the initial rise in RMSD followed by plateau.

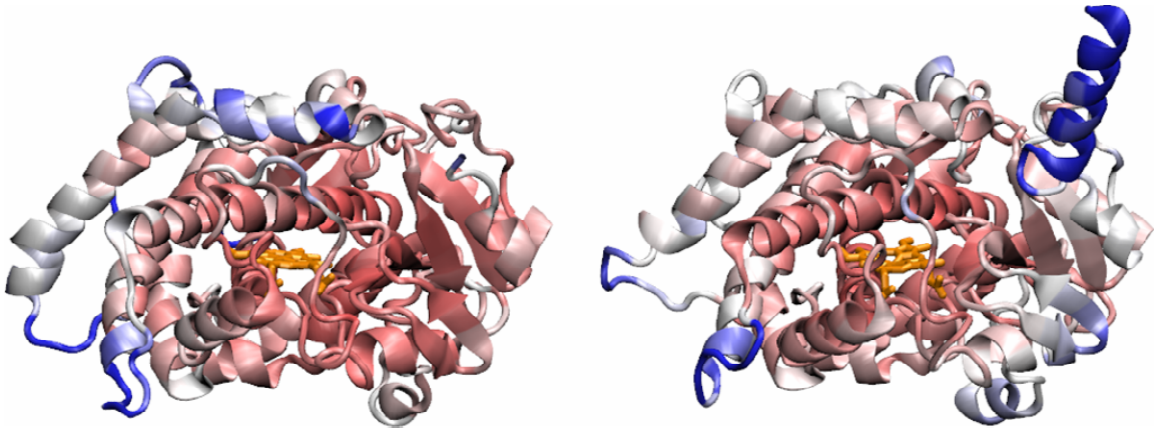

Figure S4

Residue RMSD (Cα atoms only) averaged over the course of the entire 50 ns molecular dynamics simulation for (left) 3A4 in solution and (right) 3A4 in membrane. The proteins residues are colored on a gradient based on their RMSD from red (RMSD < 1) to blue (RMSD > 4). The per residue RMSD shows that the core of the protein is very stable irrespective of whether the simulation is done in solution or in a membrane. However there are structural differences that arise with more global movements which can only be seen when looking at the relative movements of the residues over time (see Figure S5).

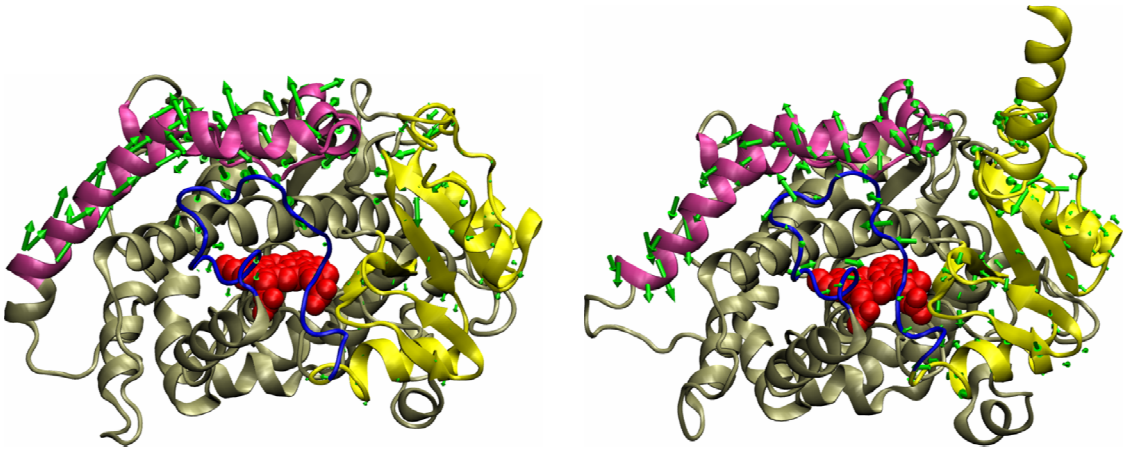

Figure S5

Principal component analysis (green arrows) calculated using in-house Matlab scripts shows the movement of protein residues (Cα atoms only) over the course of the 50 ns 3A4 in solution (left) and 3A4 in membrane (right) simulations. One can see a distinct difference in the movement of the F-G helices (pink) which results from a direct interaction with the lipid bilayer in the case of the 3A4 in membrane simulations. The movement of the F-G helices in the 3A4 in membrane simulation results in the correlated movement of the B-C loop which closes the 2e channel.