Supporting Information

Lipid molecules can induce an opening of membrane-facing tunnels in cytochrome P450 1A2

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Supplementary tables and figures

Table S1. Simulation protocol of the CG model of membrane-bound P450 1A2.

Step	Length [ns]	Minimization [number of steps]	Steps size [fs]	Fixed atoms
1		1000		
2	2		20	protein and lipids
3	10		20	protein
4	20		20	residues 39 to 513
5	20		20	residues 39 to 513
6*	2000 - 4000	1000	20	

* this step corresponds to the production phase

Table S2. Simulation protocol of all-atom model of the P450 1A2 catalytic domain.

Step	Length [ns]	Minimization [number of steps]	Steps size [fs]	Fixed atoms	Constrained atoms	Size of constraints [kcal/mol/Å^2]
1		1000	0.1			
2	0.0005		0.1	protein		
3	0.05		1	protein		
4	0.05		1		protein	1
5	0.05		1		backbone	1
6	0.2		1			
7	0.1		2			
8*	230		2			

* this step corresponds to the production phase

Table S3. Simulation protocol of the all-atom model of the membrane-bound P450 1A2.

Step	Length [ns]	Minimization [number of steps]	Steps size [fs]	Fixed atoms	Constrained atoms	Size of constraints [kcal/mol/Å^2]
1		10000				
2	0.05		1	protein and lipids		
3	0.05		1		protein and lipids	1
4	0.05		1		protein	1
5	0.05		1		backbone	1
6	0.05		1		Cα atoms	1
7	0.05		1			
8*	230 or 420		2			

* this step corresponds to the production phase

Table S4. Probability of tunnel opening in P450 1A2 calculated separately for individual trajectories. All tunnels were detected using methane-sized probe (1.9 Å). [a]

Tunnel	Membrane-free P450 1A2 [%]					Membrane-bound P450 1A2 [%]				
Replica number	1s	2s	3s	4s	5s	1m	2m	3m	4m	5m
Simulation time [ns]	230	230	230	230	230	420	230	230	230	230
Membrane-facing										
2b			7			25				
2d						26				
4					3	38		2		
5						3				
Solvent-facing										
2c	49	46	2	7	31	15	10	5	< 1	34
2e								4		
s	< 1	7		1	12			2		3
w			6		1					

[a] average error of the opening probability assignment was estimated to be 1 percentage point

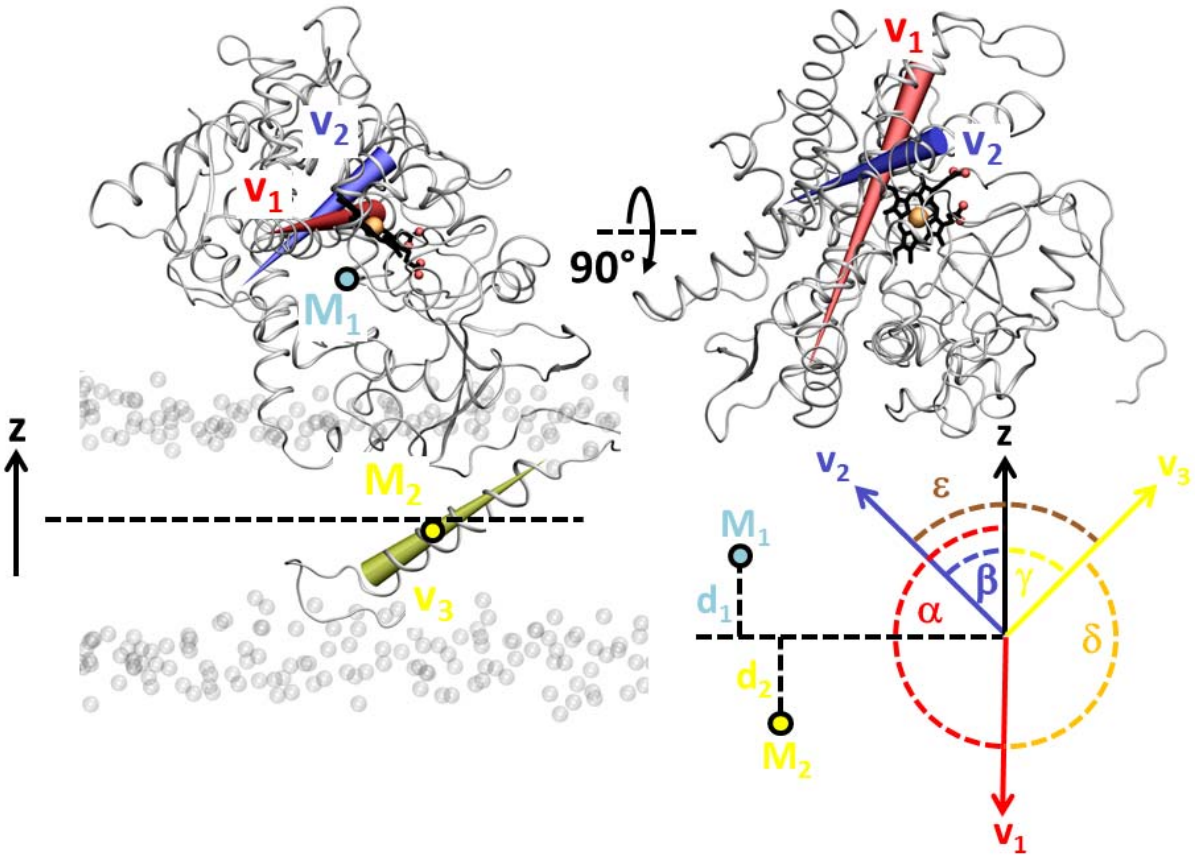

Figure S1. Definition of vectors (v_1-v_3) and centers of mass (M_1-M_3) describing the orientation of the P450 1A2–cyt b_5 complex in the phospholipid bilayer. Vector v_1 (blue cone) connects one helical turn in helix C and one in helix F, i.e. the center of the C_α atoms of residues 127-131 and 197-201 of P450 1A2, respectively. Vector v_2 (red cone) connects the centers of the first and last helical turns in helix I defined by centers of the C_α atoms of residues 305-309 and 332-336 of P450 1A2, respectively. Vectors v_3 (yellow cone) is placed along P450 1A2 TM helix (residues 7-33). Center of mass of the P450 1A2 catalytic domain (C_α atoms of residues 34-513) and TM helix (C_α atoms of residues 7-33) are named M_1 (light blue point) and M_2 (yellow point), respectively. P450 1A2 (light grey) is shown as a cartoon. Bead representing a phosphate group in DLPC molecules is shown as light grey ball. Heme cofactor of P450 1A2 is shown as black sticks. Oxygen atoms of heme propionates are shown as red balls and iron atom as orange balls. Dashed black line shows center of the phospholipid bilayer.

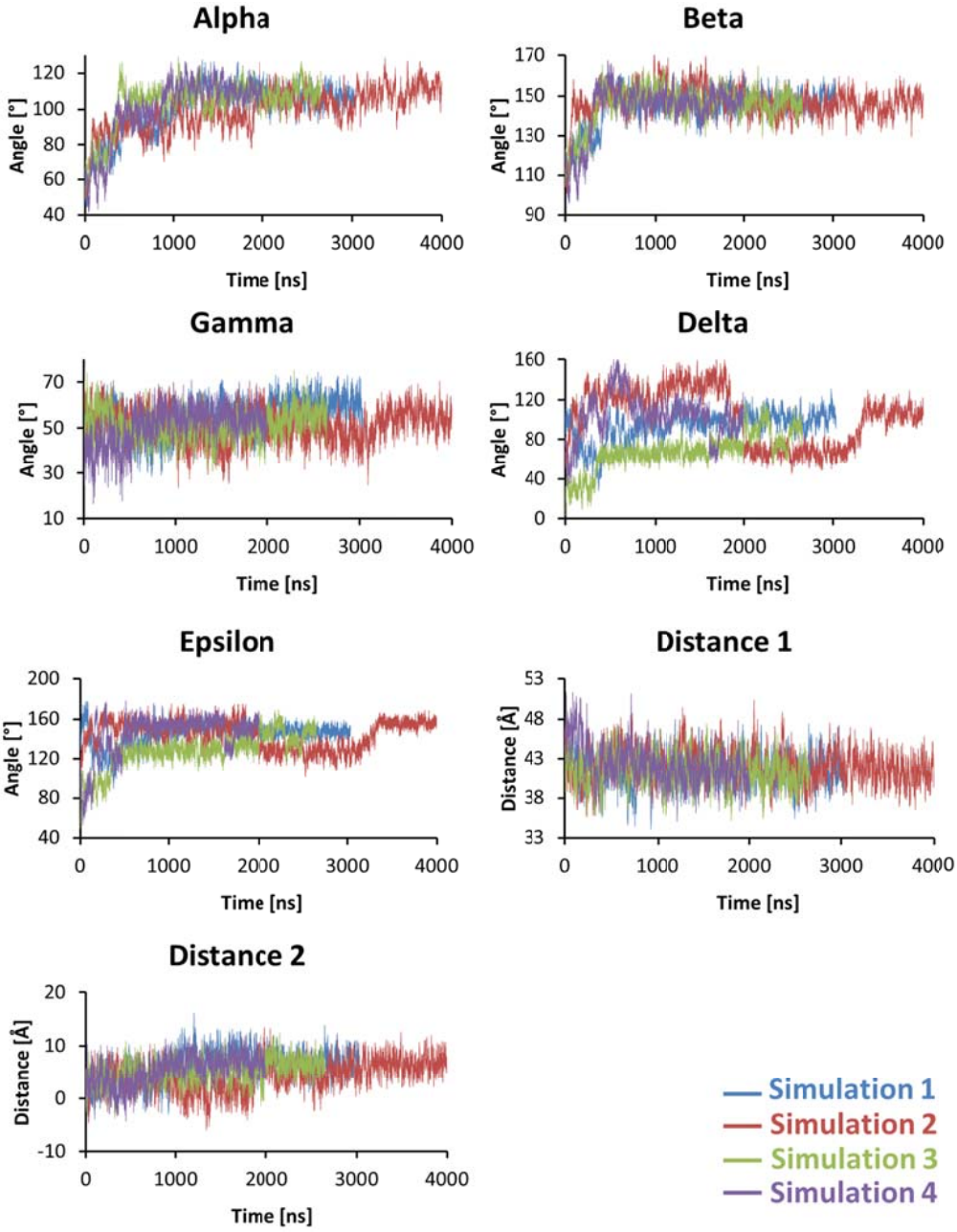

Figure S2. The convergence of full-length membrane-bound P450 1A2 during CG MD simulation monitored as a time evolution of seven geometric parameters. These parameters defined in Figure S1 relate rigid-body positions of the catalytic and TM domains of P450 1A2 with the phospholipid membrane.

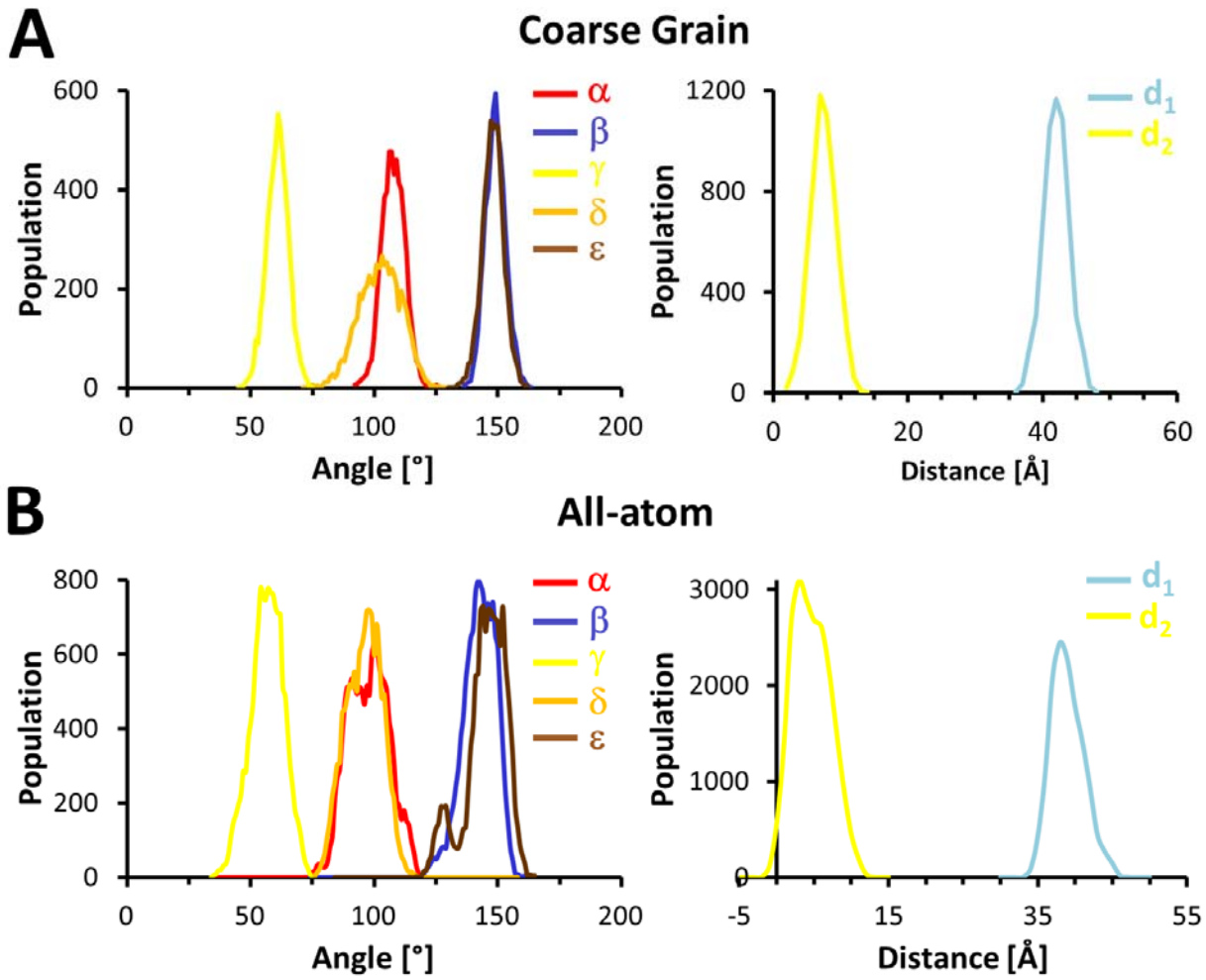

Figure S3. Distribution of geometric parameters describing the orientation of the full-length membrane-bound P450 1A2 with respect to the membrane in the CG (A) and all-atom (B) model. The peak values of the seven parameters of the selected structure are as follows; $\alpha = 107°$, $\beta = 149°$, $\gamma = 65°$, $\delta = 103°$, $\varepsilon = 147°$, $d_1 = 42$ Å, $d_2 = 7$ Å.

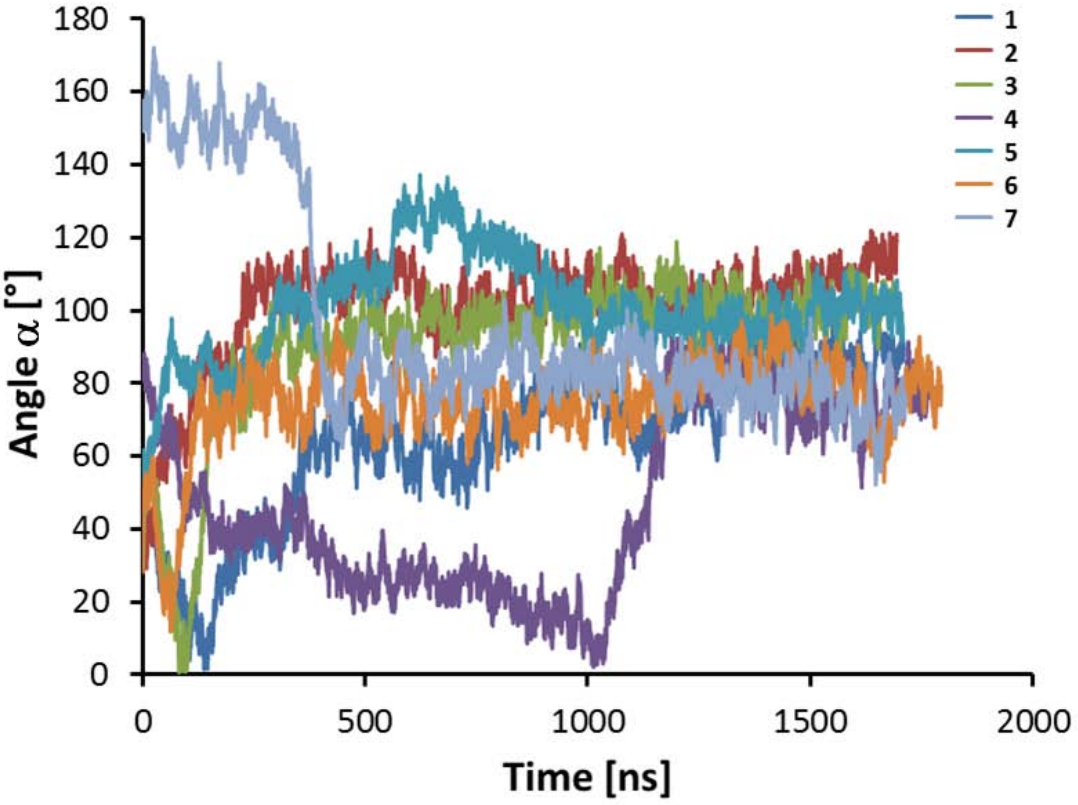

Figure S4. Convergence of the orientation of the catalytic P450 1A2 domain with respect to the membrane during seven self-assembly CG MD simulations monitored as a time evolution of the angle α.

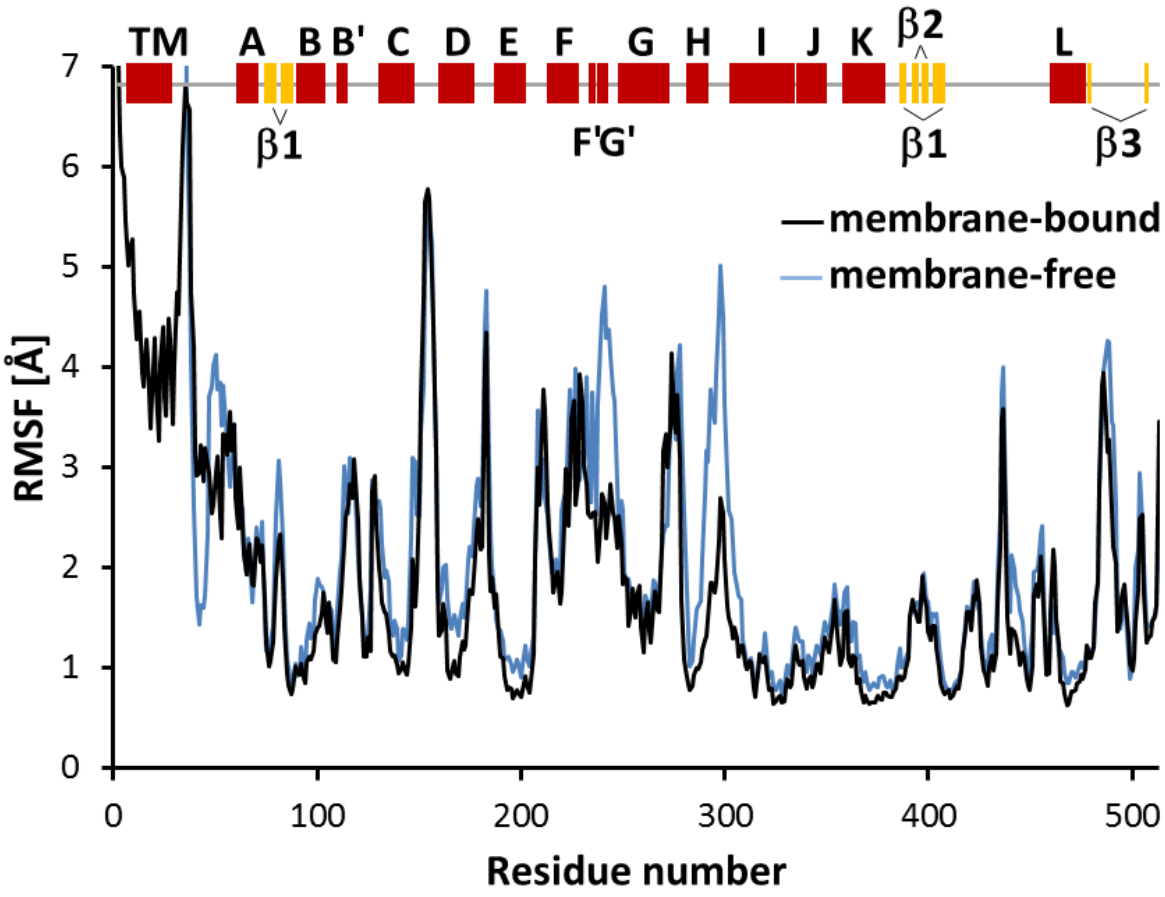

Figure S5. Flexibility of the membrane-bound and -free P450 1A2. The root-mean square fluctuation (RMSF) of backbone Cα atoms measured during the all-atom simulations. The initial structural alignment that was used for generating the average structure was done for backbone atoms of residues 34 to 513. Location of secondary structures is shown in the upper part (helices – red, beta strands – yellow, and coils – grey lines)

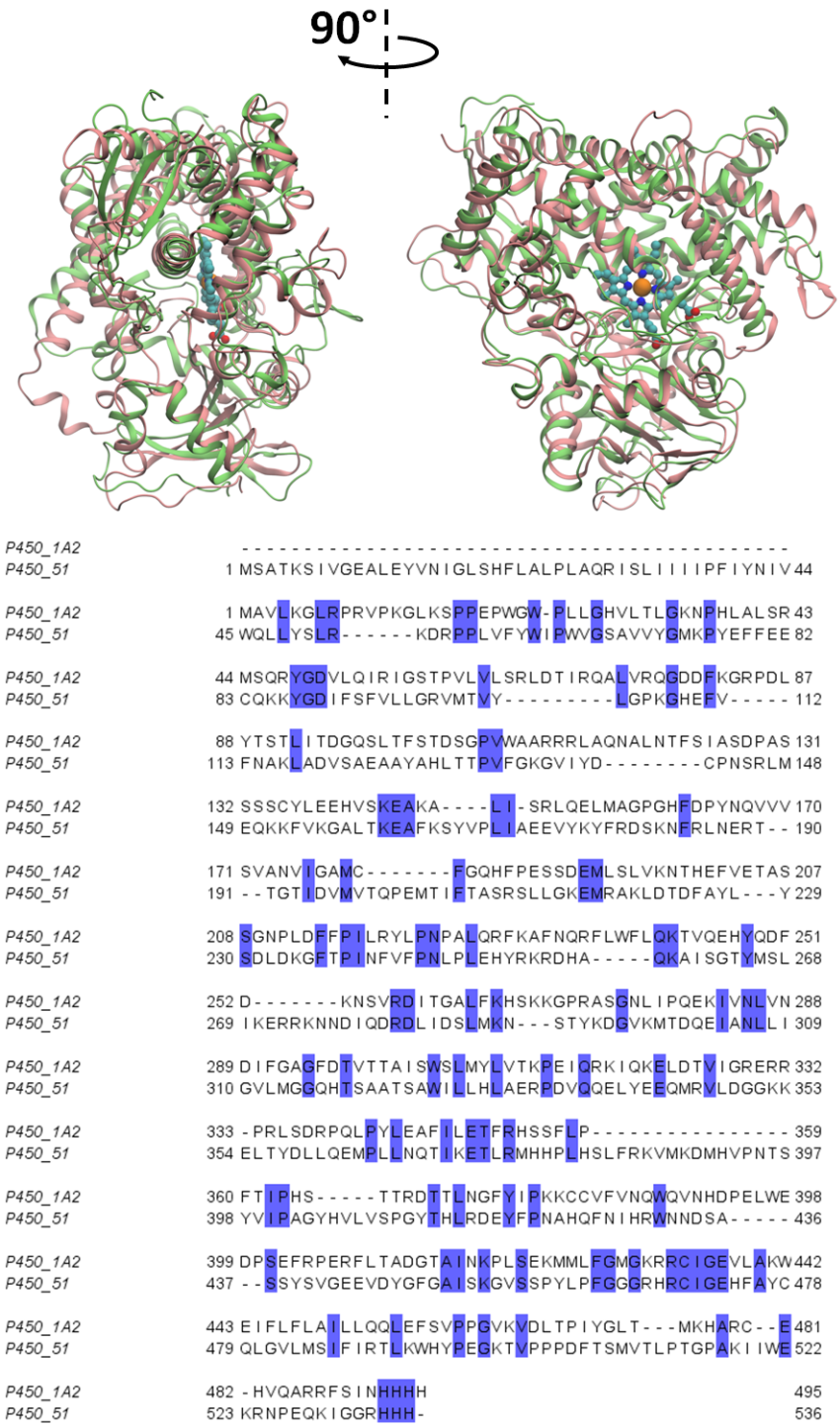

Figure S6. Structural and sequence alignment of the catalytic domain of P450 1A2 (pink) and lanosterol 14α-demethylase – cytochrome P450 51A1 (lime). Heme cofactor of P450 1A2 is shown in ball-sticks representation and colored according to atom types. Identical residues in the sequence alignment are colored in blue.

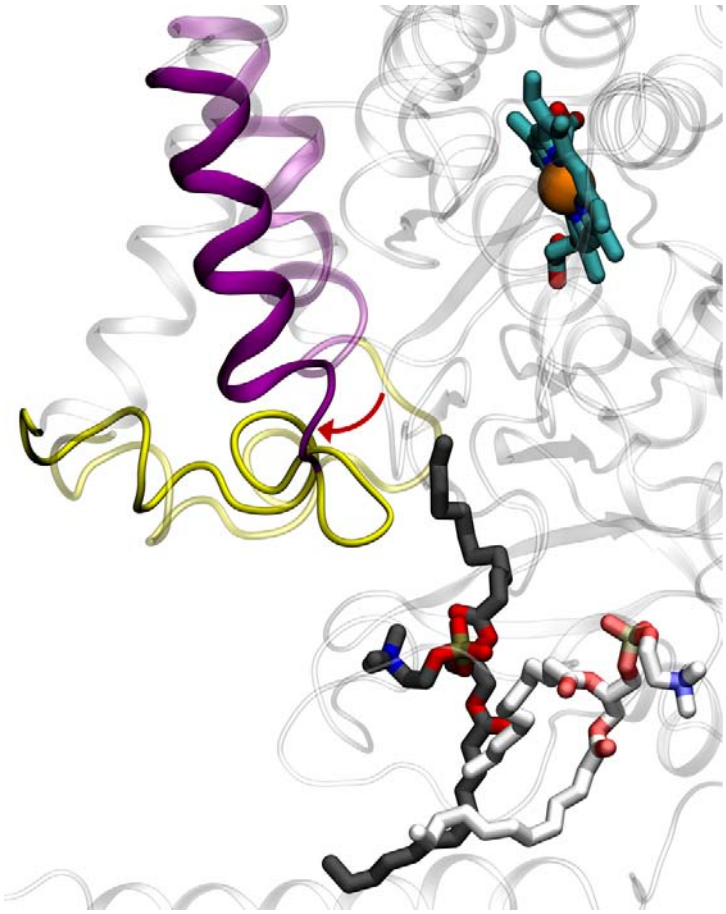

Figure S7. Structural rearrangement of F helix and FG loop upon DLPC molecule intruding into the catalytic domain of the membrane-bound P450 1A2. The dislocated F helix (purple) and FG loop (yellow) are shown in opaque cartoon representation, while their initial positions are shown in a transparent representation. The red arrow shows direction of their movement. The intruding DLPC molecule, in the initial structure (white carbon atoms) and being mostly buried (grey carbon atoms), is shown in sticks representation and colored according to atom types. Heme cofactor is shown in sticks representation and colored according to atom types (cyan carbon atoms).

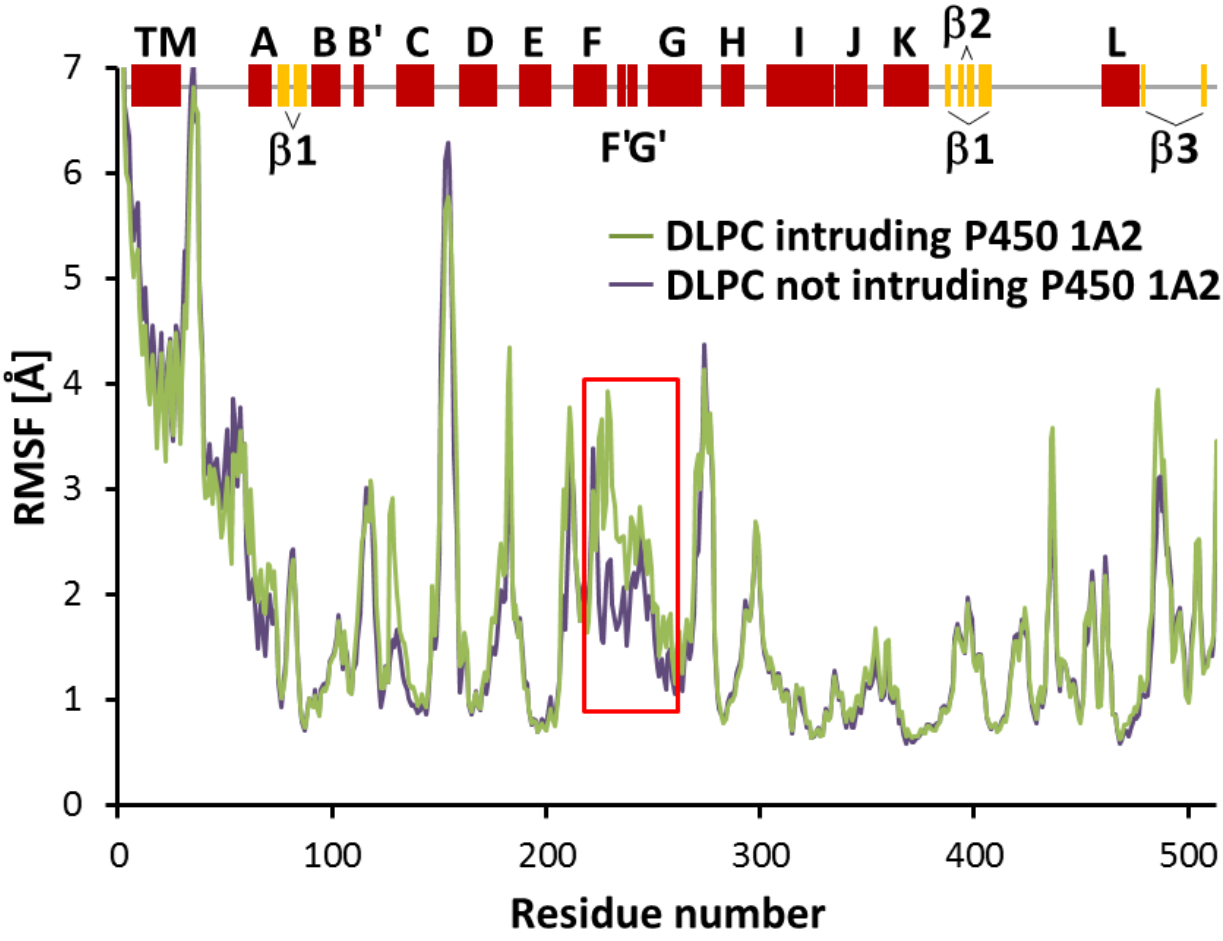

Figure S8. Change in flexibility of the membrane-bound P450 1A2 upon DLPC molecule intrusion. The RMSF (Root Mean Square Fluctuation) of backbone Cα atoms measured during the all-atom simulations number 2m till 5m (DLPC not intruding) and simulation number 1m (DLPC intruding tunnel 2d). Initial structural alignment used for a generation of an average structure was done for backbone atoms of residues 34 to 513. Location of secondary structures is shown in the upper part (helices – red, beta strands – yellow, and coils – grey lines).

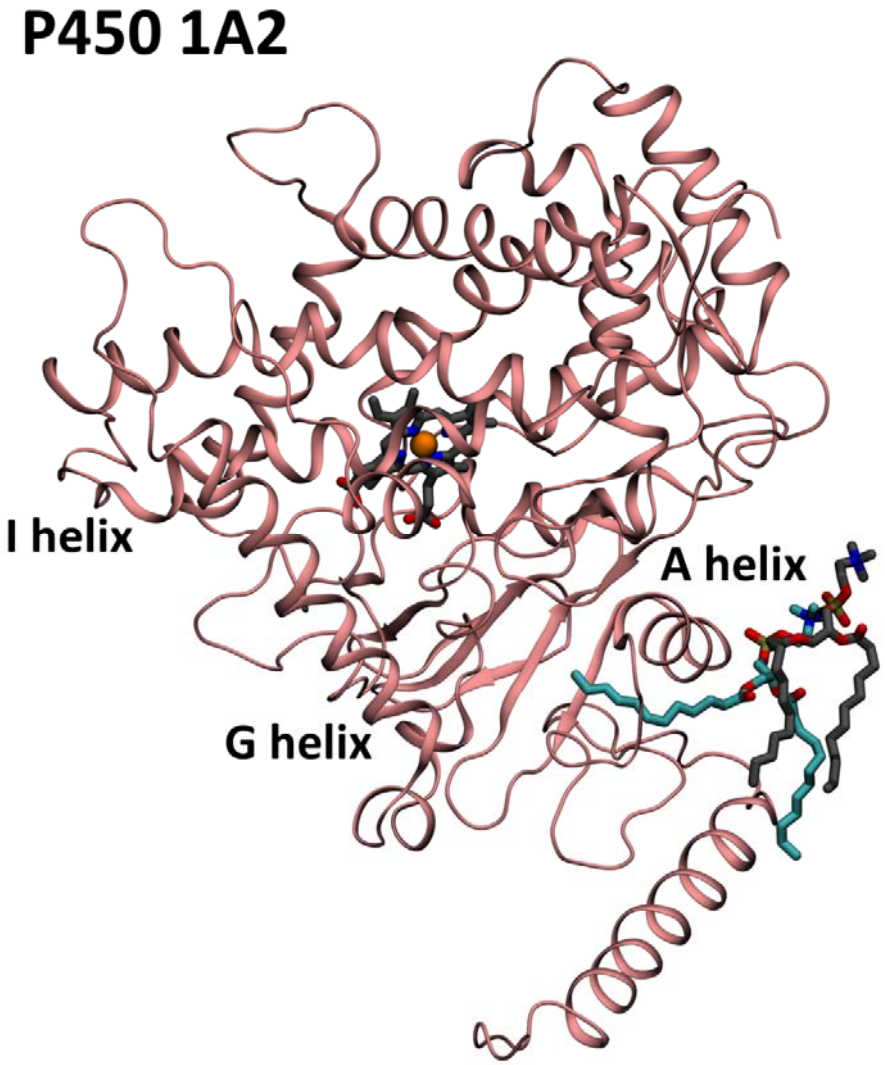

Figure S9. Spontaneous intrusion of a DLPC into the tunnel 2d reproduced at elevated temperature. The structure represents a snapshot (at 45 ns) from the all-atom MD simulation conducted at 333 K. Initial and resulting position of DLPC molecule is shown in gray and cyan color, respectively. Other DLPC molecules, solvent and ions are not shown.

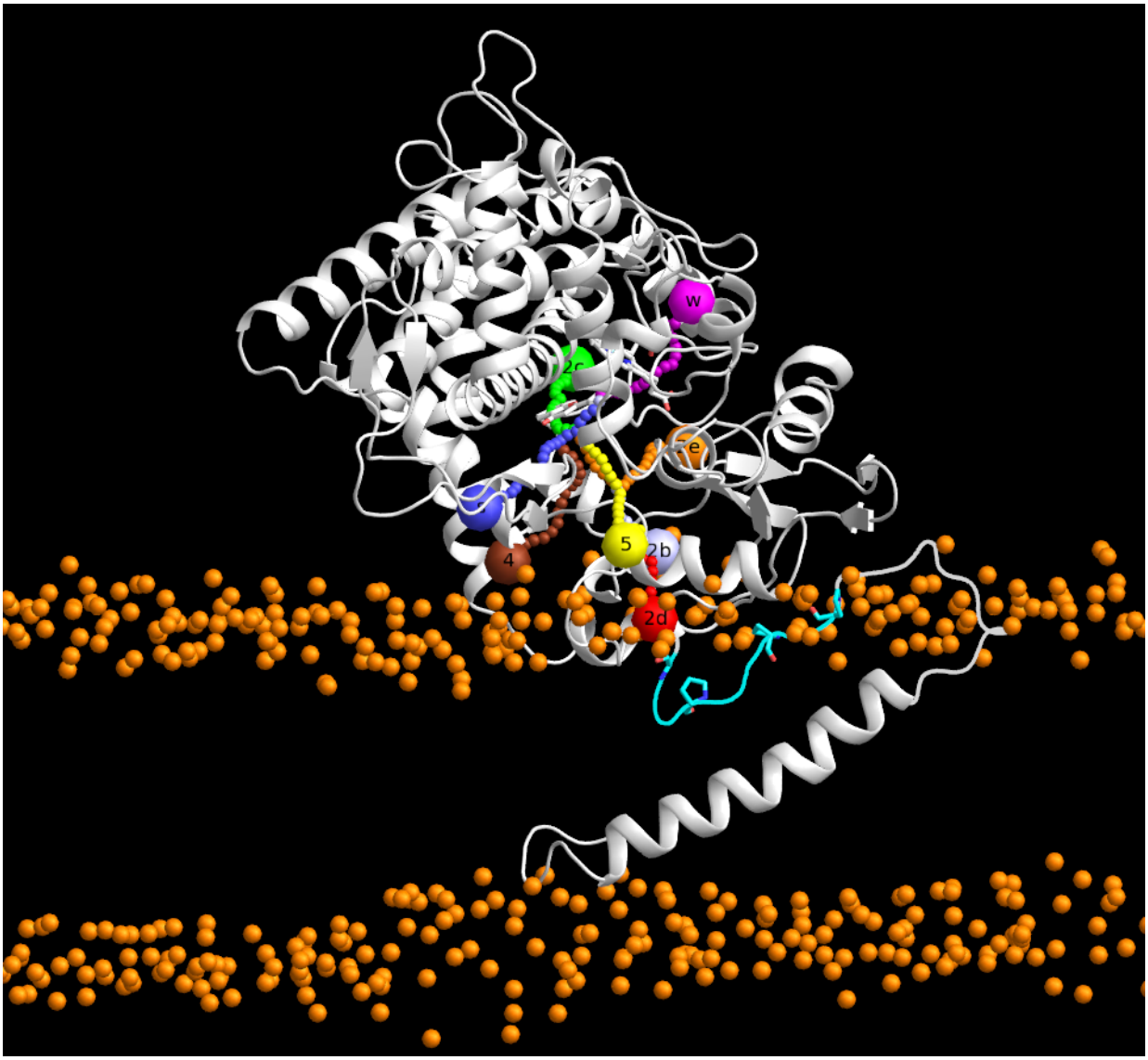

Figure S10. Interactive 3D model of the membrane-bound P450 1A2. Entrances and paths of individual tunnels are indicated by balls and curved sticks, respectively. P450 1A2 is shown in cartoon representation. The heme cofactor is shown in stick. Phosphate atoms of lipid molecules are shown as orange balls. DLPC molecules, K^+ and Cl^- ions and water molecules are not shown. Tunnel paths were extracted from trajectories of all-atom MD simulations; but the representative structure was generated from the MD snapshot closest to the trajectory average. Because, most tunnels were open only for short time period, only some of them are fully open in this snapshot, resulting in visual overlap of the tunnel path with the protein backbone.

Viewing tips: *Use Adobe Acrobat Reader v9 or higher to enable interactive mode. Click on the model, accept the security warning and click the model again; use left button to rotate and wheal button to zoom.*