

Supporting Information

Determining Molecular Structures of Cytochrome P450 and Its Complex Associated with Model Cell Membranes

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S1. Fitting Parameters of SFG and ATR-FTIR Measurements.

Table S1. Fitting parameters of SFG ssp and ppp spectra from trunc-CYP2B4 associated with a POPC lipid bilayer. Overall fitting R^2 : 0.97 for ssp and 0.99 for ppp.

	<i>Peak Center (cm⁻¹)</i>	<i>Amplitude (a.u.)</i>	<i>Peak Width (cm⁻¹)</i>	<i>Peak Assignment</i>
<i>ssp</i>	1650.9	51.1	10.9	Trunc-CYP2B4 α -helix
<i>ppp</i>	1649.4	116.2	14.0	Trunc-CYP2B4 α -helix

Table S2. Fitting parameters of SFG ssp and ppp spectra from trunc-CYP2B4 associated with a DLPC lipid bilayer. Overall fitting R^2 : 0.84 for ssp and 0.78 for ppp.

	<i>Peak Center (cm⁻¹)</i>	<i>Amplitude (a.u.)</i>	<i>Peak Width (cm⁻¹)</i>	<i>Peak Assignment</i>
<i>ssp</i>	1651.1	19.8	9.2	Trunc-CYP2B4 α -helix
<i>ppp</i>	1635.0	10.0	12.0	Trunc-CYP2B4 β -sheet
	1650.0	28.0	8.0	Trunc-CYP2B4 α -helix

Table S3. Fitting parameters of SFG ssp and ppp spectra from trunc-CYP2B4/trunc-FBD complex associated with a POPC lipid bilayer. Overall fitting R^2 : 0.95 for ssp and 0.96 for ppp.

	<i>Peak Center (cm⁻¹)</i>	<i>Amplitude (a.u.)</i>	<i>Peak Width (cm⁻¹)</i>	<i>Peak Assignment</i>
<i>ssp</i>	1605.0	0.5	6.0	FBD α -helix
	1621.0	5.4	8.3	FBD α -helix, CYP2B4 anti-parallel β -sheet
<i>ppp</i>	1645.6	19.0	12.0	CYP2B4 α -helix
	1590.0	4.5	9.0	FBD parallel β -sheet
	1609.0	12.4	12.0	FBD parallel α -helix
	1629.5	39.0	16.8	FBD α -helix, CYP2B4 anti-parallel β -sheet
	1644.2	24.9	13.7	CYP2B4 α -helix

Table S4. Fitting parameters of ATR-FTIR s and p spectra from trunc-CYP2B4 associated with a POPC lipid bilayer. Overall fitting R^2 : 0.99 for s and 0.99 for p.

	<i>Peak Center (cm⁻¹)</i>	<i>Amplitude (a.u.)</i>	<i>Peak Width (cm⁻¹)</i>	<i>Peak Assignment</i>
<i>s</i>	1645.9	0.030	13.1	CYP2B4 random coil
	1653.7	0.0019	3.0	CYP2B4 α -helix
<i>p</i>	1648.4	0.051	13.2	CYP2B4 random coil
	1655.5	0.0057	3.1	CYP2B4 α -helix

Table S5. Fitting parameters of ATR-FTIR s and p spectra from trunc-CYP2B4 associated with a DLPC lipid bilayer. Overall fitting R²: 0.99 for s and 0.99 for p.

	<i>Peak Center (cm⁻¹)</i>	<i>Amplitude (a.u.)</i>	<i>Peak Width (cm⁻¹)</i>	<i>Peak Assignment</i>
<i>s</i>	1645.3	0.061	13.3	CYP2B4 random coil
	1654.3	0.0049	2.7	CYP2B4 α -helix
<i>p</i>	1647.4	0.101	13.5	CYP2B4 random coil
	1656.2	0.011	3.4	CYP2B4 α -helix

Table S6. Fitting parameters of ATR-FTIR s and p spectra from trunc-CYP2B4/trunc-FBD complex associated with a POPC lipid bilayer. Overall fitting R²: 0.99 for s and 0.99 for p.

	<i>Peak Center (cm⁻¹)</i>	<i>Amplitude (a.u.)</i>	<i>Peak Width (cm⁻¹)</i>	<i>Peak Assignment</i>
<i>s</i>	1609.6	0.00016	2.8	FBD α -helix
	1645.8	0.030	13.1	CYP2B4 random coil
	1655.6	0.0023	2.0	CYP2B4 α -helix
<i>p</i>	1608.0	0.0022	3.3	FBD
	1647.8	0.048	13.3	CYP2B4 random coil
	1655.9	0.0066	3.2	CYP2B4 α -helix

S2. Parameters and Results for the HADDOCK Simulation.

Crystal structures of the trunc-CYP2B4 (PDB ID: 3MVR) and the trunc-FBD (PDB ID: 1AMO, residue 64–235) were used as input structures for the HADDOCK simulation. Table S7 lists the residues involved in intramolecular interactions (binding interface) between the two proteins in the trunc-CYP2B4/trunc-FBD complex, obtained from previous publication.¹

Table S7. Ambiguous interaction restraints used in HADDOCK simulation.

	FBD	CYP2B4
AMBIGUOUS INTERACTION RESTRAINTS	88, 116, 143, 146, 175, 177, 179	122, 126, 133, 135, 137, 422, 433

Table S8. Energy profile of lowest energy cluster obtained from HADDOCK simulation for the modeling of CYP2B4-FBD complex.

HADDOCK score	-93.5 +/- 2.7
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Cluster size	18
RMSD from the overall lowest-energy structure (Å)	0.5 +/- 0.3
Van der Waals energy (kcal/Mol)	-33.3 +/- 2.8
Electrostatic energy (kcal/Mol)	-352.3 +/- 13.5
Desolvation energy (kcal/Mol)	6.7 +/- 1.6
Restraints violation energy (kcal/Mol)	34.9 +/- 22.08
Buried Surface Area (Å ²)	1327.4 +/- 49.9

Table S9. Minimum distance between ligand heme and FMN of 18 structures output from HADDOCK.

Energy Order	Protein Number	Distance (nm)	Energy Order	Protein Number	Distance (nm)
1	39	1.36	10	158	1.35
2	8	1.38	11	113	1.35
3	54	1.36	12	111	1.38
4	50	1.37	13	55	1.30
5	94	1.33	14	122	1.35
6	63	1.37	15	47	1.42
7	101	1.37	16	144	1.37
8	66	1.37	17	28	1.46
9	11	1.37	18	83	1.36
Avg Distance (nm)	1.34 ± 0.03				

S3. Hamiltonian Matching Results.

Table S10. Matched orientations of trunc-CYP2B4/trunc-FBD complex associated with a POPC lipid membrane.

Energy Order	Protein Number	Highest Matching Score	Matched Hamiltonian Orientation	$\alpha/^\circ$	$\beta/^\circ$	$\theta_{heme}/^\circ$	$\theta_p/^\circ$
1	11	0.45	(80°, 210°) (100°, 30°)	74.84	118.01	61.01	104.15
2	50	0.44	(80°, 30°) (100°, 210°)	79.05	120.22	60.17	103.48
3	28	0.44	(80°, 150°) (100°, 330°)	78.66	120.85	61.28	104.89
4	8	0.44	(80°, 20°) (100°, 200°)	73.63	111.68	51.83	102.29
5	55	0.44	(80°, 200°) (100°, 20°)	77.02	114.25	51.49	103.54
6	144	0.44	(80°, 210°) (100°, 30°)	75.35	119.89	64.15	109.85
7	101	0.43	(80°, 30°) (100°, 210°)	79.17	120.20	60.06	104.67
8	54	0.43	(80°, 330°) (100°, 150°)	76.45	118.80	60.53	102.97
9	158	0.43	(80°, 330°) (100°, 150°)	84.41	123.24	58.27	104.03
10	122	0.43	(80°, 330°) (100°, 150°)	77.77	118.85	58.52	100.52
11	63	0.42	(80°, 150°) (100°, 330°)	80.21	120.70	59.70	102.42
12	83	0.42	(80°, 340°) (100°, 160°)	75.63	111.53	48.53	101.19
13	39	0.42	(80°, 200°) (100°, 20°)	76.54	114.03	51.97	103.51
14	66	0.42	(80°, 160°) (100°, 340°)	78.80	113.73	48.59	102.87
15	111	0.42	(80°, 20°) (100°, 200°)	75.01	110.94	48.37	103.78

16	47	0.42	(80°, 20°) (100°, 200°)	82.36	116.60	47.92	106.17
17	113	0.41	(80°, 20°) (100°, 200°)	75.77	112.14	49.75	101.82
18	94	0.41	(80°, 160°) (100°, 340°)	79.46	114.70	50.65	103.12
Avg				77.79	116.69	55.15	103.63
STD				2.75	3.76	5.51	2.04

α , β , θ_{heme} and θ_p were only calculated for the first matched orientation (top one in each cell) of each structure.

Table S11. Matched orientations of trunc-CYP2B4 associated with a POPC lipid membrane.

Protein Number	Highest Matching Score	Matched Hamiltonian Orientation	$\alpha/^\circ$	$\beta/^\circ$	$\theta_{heme}/^\circ$
101	1.62	(30°, 330°), (150°, 150°)	58.64	98.43	42.82
39	1.61	(30°, 330°), (150°, 150°)	60.11	100.66	44.81
113	1.6	(30°, 330°), (150°, 150°)	57.92	96.16	39.17
28	1.6	(30°, 180°), (150°, 0°)	53.59	93.46	39.18
66	1.58	(30°, 170°), (150°, 350°)	53.05	92.78	38.28
8	1.57	(30°, 340°), (150°, 160°)	49.88	89.85	36.14
158	1.57	(30°, 170°), (150°, 350°)	54.19	93.81	39
50	1.56	(30°, 330°), (150°, 150°)	58.45	98.46	42.96
144	1.56	(40°, 0°), (140°, 180°)	57.5	97.98	42.64
94	1.55	(20°, 200°), (160°, 20°)	55.54	95.45	40.96
111	1.54	(30°, 330°), (150°, 150°)	59.32	98.85	42.32
83	1.54	(30°, 170°), (150°, 350°)	60.52	98.76	41.96
63	1.52	(30°, 170°), (150°, 350°)	50.3	90.61	37.73
54	1.51	(30°, 190°), (150°, 10°)	56.41	95.03	38.89
122	1.47	(30°, 180°), (150°, 0°)	52.69	91.52	36.39
11	1.45	(50°, 0°), (130°, 180°)	58.55	97.69	41.48
55	1.44	(40°, 340°), (140°, 160°)	60.63	100.28	43.32
47	1.4	(70°, 0°), (110°, 180°)	60.14	101.58	45.87
Avg			56.52	96.19	40.77
STD			3.39	3.45	2.72

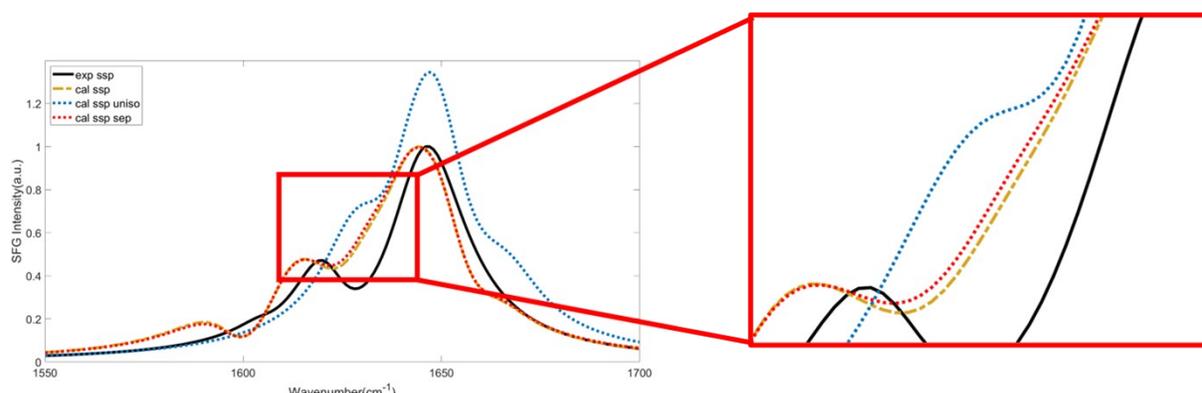
α , β , θ_{heme} and θ_p were only calculated for the first matched orientation (top one in each cell) of each structure.

Table S12. Matched orientations of trunc-CYP2B4 associated with a DLPC lipid membrane.

Protein Number	Highest Matching Score	Matched Hamiltonian Orientation	$\alpha/^\circ$	$\beta/^\circ$	$\theta_{heme}/^\circ$
122	1.33	(40°, 170°), (140°, 350°)	44.4	80.09	25.63

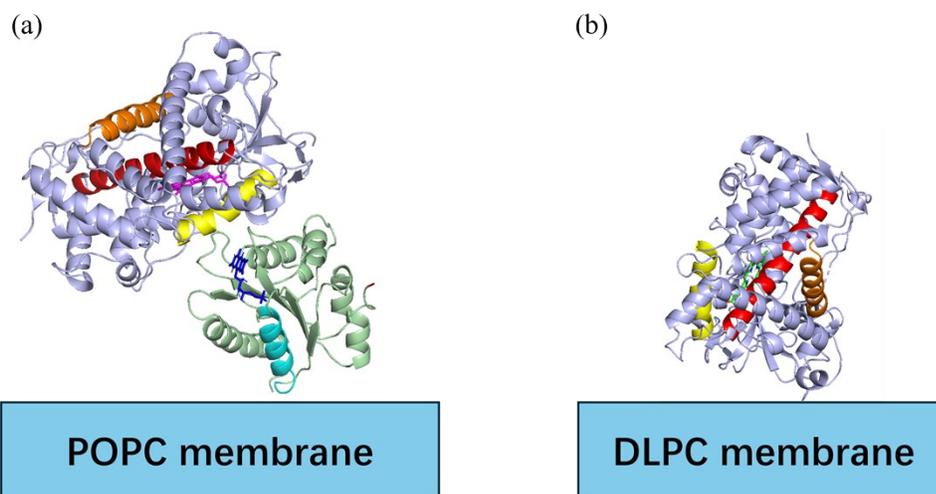
101	1.32	(50°, 330°), (130°, 150°)	43.62	78.5	24.43
83	1.32	(50°, 160°), (130°, 340°)	36.3	68.04	15.34
47	1.31	(90°, 330°), (90°, 150°)	38.03	66.69	12.12
144	1.31	(60°, 350°), (120°, 170°)	43.45	77.1	21.93
28	1.31	(50°, 170°), (130°, 350°)	43.26	73.78	18.43
54	1.3	(50°, 170°), (130°, 350°)	41.62	71.83	16.55
50	1.3	(60°, 330°), (120°, 150°)	37.01	68.56	15.12
39	1.28	(60°, 330°), (120°, 150°)	37.78	70.73	17.2
63	1.28	(40°, 170°), (140°, 350°)	46.4	82.01	27.78
66	1.28	(50°, 160°), (130°, 340°)	38.33	71.94	18.51
111	1.28	(50°, 330°), (130°, 150°)	44.05	78.88	24.05
94	1.27	(40°, 180°), (140°, 0°)	45.01	74.96	18.95
158	1.27	(50°, 160°), (130°, 340°)	39.26	72.94	19.4
8	1.26	(60°, 330°), (120°, 150°)	36.57	69.14	15.94
11	1.26	(70°, 340°), (110°, 160°)	43.5	77.72	23.28
113	1.26	(50°, 330°), (130°, 150°)	35.2	70.16	17.71
55	1.25	(60°, 340°), (120°, 160°)	47.47	80.68	24.27
Avg			41.48	74.10	19.81
STD			3.75	4.67	4.19

α , β , θ_{heme} and θ_p were only calculated for the first matched orientation (top one in each cell) of each structure.



S4. Spectra Comparison

Figure S1 Enlargement of part of the spectra shown in Figure 4b in the main text to show that calculate SFG spectra with and without considering the coupling between trunc-FBD and trunc-CYP2B4 are slightly different.



S5. Structure Visualization.

Figure S2. Structure of (a) PC11 associated with a POPC lipid membrane at orientation of (100°, 30°), and (b) trunc-CYP2B4 from PC28 associated with a DLPC lipid membrane at orientation of (60°, 170°). Trunc-FBD and trunc-CYP2B4 are in pale green and light blue, respectively. FMN is in blue and heme is in magenta. α_1 helix of trunc-FBD is in cyan. C-terminus of trunc-FBD is in blue dot. I helix, C, and F helix of trunc-CYP2B4 are in red, yellow and orange, respectively.

S6. Reference.

- (1) Prade, E.; Mahajan, M.; Im, S.; Zhang, M.; Gentry, K. A.; Anantharamaiah, G. M.; Waskell, L.; Ramamoorthy, A. A Minimal Functional Complex of Cytochrome P450 and FBD of Cytochrome P450 Reductase in Nanodiscs. *Angew. Chem. Int. Ed.* **2018**, *57* (28), 8458–8462.