Appendix B

Dansyl probe syntheses and characterization and D-8-Ad:P450cam structure determination

Acknowlegements. The structure of the D-8-Ad:P450cam conjugate was determined by Anna-Maria A. Hays.

Syntheses.

Adamantane-1-carboxylic acid [4-(5-dimethylamino-naphthalene-1-sulfonylamino)-butyl]-amide (**1**): (D-4-Ad) 0.100 g (0.312 mmole) **3**, 74.5 mg (0.37 mmole) 1-adamantyl carbonyl chloride, and 0.11 mL (0.62 mmole) N,N-diisopropylethylamine were dissolved in 5 mL dry DMF under Ar and stirred overnight at ambient temperature. The reaction mixture was diluted with 25 mL CH_2Cl_2, washed twice with water, and the organic phase concentrated under reduced pressure. The crude product was purified via flash chromatography using 9:1 MeOH:CH_2Cl_2 as eluent to give the product as a pale yellow-green solid. Yield 35.6 mg (24 %) ^{1}H NMR ($CDCl_3$) 8.53 (1H, d, J=8.4 Hz) 8.31 (1H, d, J=8.4 Hz) 8.22 (1H, dd, J=0.9, 7.2 Hz) 7.55 (1H, dd, J= 7.5, 8.4 Hz) 7.51 (1H, dd, J= 7.2, 8.4 Hz) 7.18 (1H, d, J=7.5 Hz) 5.63 (1H, m) 5.30 (1H, t, J=6.0 Hz) 3.11 (2H, m) 2.89 (2H, m) 2.88 (6H, s) 2.00 (3H, m) 1.77 (6H, m) 1.68 (6H, m) 1.42 (4H, m) ^{13}C NMR ($CDCl_3$) 178.43, 152.16, 134.95, 130.58, 130.06, 129.81, 128.57, 123.44, 119.07, 115.41, 45.68, 43.10, 40.77, 39.45, 38.73, 36.72, 28.33, 26.99, 26.90. ESI-MS (m/z) 484.3 ($M+H^+$).

Adamantane-1-carboxylic acid [4-(5-dimethylamino-naphthalene-1-sulfonylamino)-octyl]-amide (**2**): Was prepared from **4** and 1-adamantyl carbonyl chloride in a manner identical to **1**. Yield 45%. ^{1}H NMR ($CDCl_3$) 8.53 (1H, d, J=8.4) 8.29 (1H, d, J=8.7) 8.24 (1H, dd, J=7.5, 1.2 Hz) 7.56 (1H, dd, J=7.5, 8.7 Hz) 7.52 (1H, dd, J=7.2, 8.4 Hz) 7.18

(1H, d, J=7.2 Hz) 5.58 (1H, m) 4.77 (1H, t, J=5.7 Hz) 3.17 (2H, m) 2.88 (6H, s) 2.87 (2H, m) 2.02 (3H, m) 1.90 (3H, m) 1.82 (3H, m) 1.70 (6H, m) 1.38 (4H, m) 1.14 (8H, m)) ^{13}C NMR ($CDCl_3$) 178.17, 152.20, 134.98, 130.57, 130.07, 129.86, 123.45, 118.98, 115.40, 45.67, 43.48, 40.77, 39.50, 38.83, 36.75, 36.65, 29.73, 29.17, 28.99, 28.36, 28.06, 26.86, 26.50. ESI-MS (m/z) 540.3 ($M+H^+$).

5-Dimethylamino-Naphthalene-1-sulfonic acid (4-amino-butyl)-amide (**3**):

Following the preparation by Ikunaga *et al*.,[2] 200 mg (0.75 mmole) dansyl chloride and 1.49 mL 1,4-diaminobutane (14.8 mmole) were dissolved in 5 mL CH_2Cl_2 and stirred for 2 hours under argon. The reaction mixture was loaded directly onto a flash silica column, and eluted using 4:1:1 CH_2Cl_2:MeOH:Et_3N to give the product as a pale yellow-green oil. Yield 0.104 g (44 %) ^1H NMR ($CDCl_3$) 8.49 (1H, d, J=8.4 Hz) 8.36 (1 H, d, J=8.7 Hz) 8.20 (1H, d, J=7.5 Hz) 7.49 (1H, dd, J= 7.5, 8.7 Hz) 7.48 (1H, dd, J = 7.2, 8.4 Hz) 7.13 (1H, d, J=7.2 Hz) 5.3 (3H, overlapping m) 2.85 (6H, s) 2.84 (2H, m) 2.73 (2H, t, J=6.3 Hz) 1.52 (4H, m) ^{13}C NMR ($CDCl_3$) 152.00, 135.28, 130.25, 130.02, 129.81, 129.49, 128.32, 123.39, 119.28, 115.31, 45.61, 43.01, 40.61, 28.36, 27.22. ESI-MS (m/z) 322.2 ($M+H^+$).

5-Dimethylamino-naphthalene-1-sulfonic acid (4-amino-octyl)-amide (**4**):[3] Was prepared from 1,8-diaminooctane and dansyl chloride in an identical fashion to **3**. Yield 66%. ^1H NMR ($CDCl_3$) 8.49 (2H, d, J=8.4 Hz) 8.32 (2H, d, J=8.4 Hz) 8.20 (2H, dd,

J=0.9, 7.2) 7.52 (2H, dd, J=8.4, 7.5 Hz) 7.48 (2H, dd, J=7.2, 8.4 Hz) 7.14 (2H, d, J=7.5 Hz) 5.5 (3H, overlapping m) 2.85 (6H, s) 2.82 (2H, m) 2.75 (2H, t, J=7.2 Hz) 1.49 (2H, m) 1.33 (2H, m) 1.11 (8H, m) ^{13}C NMR ($CDCl_3$) 152.09, 135.25, 130.42, 130.05, 129.87, 129.60, 128.51, 123.43, 119.18, 115.36, 45.65, 43.39, 40.99, 30.44, 29.64, 28.95, 28.82, 26.51, 26.35. ESI-MS (m/z) 378.3 ($M+H^+$).

P450cam:D-8-Ad Crystallization and Data Collection. The C334A P450cam:D-8-Ad complex was formed at a molar ratio of 1:1 (400 μM) at room temperature and crystallized by hanging drop vapor diffusion at 4° C. Crystals were obtained under 0.1 M citrate (pH 5.5), 200 mM KCl, 13% (wt/vol) polyethylene glycol (PEG; molecular weight = 8,000). For diffraction experiments, crystals were soaked in a solution containing 0.75 M citrate (pH 5.5), 150 mM KCl, 10% (wt/vol) PEG 8000, and 25% (wt/vol) PEG 400 for 1 minute and flash frozen in liquid nitrogen. Data were collected on an Raxis IV detector equipped with Osmic confocal mirrors and Xstream cryo-device (100K) using CuK_α radiation (λ = 1.5418 Å) from a Ru200 X-ray generator operated at 50 kV, 100 mA. Data were processed using DENZO and SCALEPACK.[4] The space group was $P2_12_12_1$ with cell dimensions: a = 64.95, b = 75.31, c = 93.17 $Å^3$ (Matthews coefficient (V_M) = 2.50; solvent content = 49.9%).

Structure Determination. The structure was solved by molecular replacement using the program AMoRE[5] with camphor-bound P450cam (PDB code 2cpp) as the initial model. After initial rigid body refinement in CNS,[6] further refinement was carried out by iterative cycles of simulated annealing and B factor refinement using CNS and manual fitting using XFIT.[7] The heme and D-8-Ad were located in $|F_o|-|F_c|$ electron density omit maps and further refined by simulated annealing and manual fitting. The difference

electron density map ($|F_{obs}|-|F_{calc}|$) of the D-8-Ad is well defined and continuous, and the average B-factor for D-8-Ad is moderately low (38 $Å^2$) confirming the high occupancy of the ligand. The final model, which includes residues (11 – 414) of P450cam, D-8-Ad, heme, and 301 waters, gave R_{factor}/R_{free} values of 20.2 and 24.7.

Table B.1. Diffraction and Refinement Statistics for P450cam complexed with D-8-Ad

Diffraction Data:	
PDB code	
Resolution (Å)	20 - 2.2
Unit Cell (Å)	a=64.95, b=75.31, c=93.17
Space Group	$P2_12_12_1$
Reflections (Total/Unique)	115720 / 21045
Multiplicity	5.2
Completeness (%)	93.3 (63.8) *
R_{sym}	0.102 (0.266)*
$I/\sigma(I)$	13.9 (2.5)*
Refinement Statistics:	
R_{factor}§	20.2 (28.5) *
R_{free}¶	24.7 (33.0) *
Average B (from Wilson plot, $Å^2$)	26.2
No. of protein atoms and Ave B, ($Å^2$)	3200, 25.4
No. of waters and Ave B, ($Å^2$)	301, 34.0
No. of heme atoms and Ave B, ($Å^2$)	43, 16.5
No. of D-8-Ad atoms and Ave B, ($Å^2$)	38, 38.9
Rms bonds, angles†	0.006 Å , 1.3°

* Outer shell statistics (2.30 – 2.20 Å)

§ $R = ?||F_{obs}| - |F_{calc}||/?|F_{obs}|$ for all reflections (no σ cutoff).

¶ Free R calculated using 4.8% as test set.

† rms deviations from ideal bond and angle restraints.

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