

## Supplementary Data

Table 2

Effects of reduction and ligand- or detergent-binding on the UV/Visible spectrum and spin state of the haem iron for detergent-free CYP74C3 and a comparison with other CYP74 enzymes, Coral AOS, CYP2B4 and CYP1A2

Cytochrome	Ligand or Detergent	Ligand (mM)	Soret (nm)	Visible (nm)	Spin state	Reference
CYP74C3	Native	-	391	508, 545	High	This work
	Reduced	-	408	547	High	This work
	Emulphogene (5.0 mM)	-	393, 420(s)	568, 545(s)	Low	This work
	Imidazole	480	366, 426	544, 547	Low	This work
	KCN	500	365, 434	539, 566	Low	This work
	Thiazole	500	422	537, 564	Low	This work
	Reduced+CO	~1	452	560	Low	This work
	O <sub>2</sub>	~1	422	535, 564	Low	This work
	DMPHP	250	375, 453	563	Low	This work
	Pyridine	375	422	480, 557	Low	This work
	BI	250	423	541, 576	Low	This work
CYP74B1 (Pepper HPL)	KCNS	1000	374, 414	530	Mixture	This work
	Azide	1000	407	553	Mixture	This work
	Native	-	n.d.	n.d.	n.d.	[24]
	Triton (3.2 mM)	-	393	512, 540	High	[24]
	Reduced+Triton	-	410	550	High	[24]
	Imidazole+Triton	540	360, 424	544, 576	Low	[24]
CYP74B3 (Tomato HPL)	KCNS+Triton	200	364, 433	555	Low	[24]
	Reduced+Triton+NO	~1	431	541, 571	Low	[24]
	Native	-	n.d.	n.d.	n.d.	[21]
	Triton (4.8 mM)	-	390	500(s)	Low	[21]
CYP74B4 (Alfalfa HPL)	Reduced+Triton	-	405	550(s)	Low	[21]
	Reduced+Triton+CO	~1	447	No feature	Low	[21]
	Native	-	418	No feature	Low	[13,49]
CYP74C2 (Melon HPL)	Reduced	-	420	No feature	Low	[13,49]
	Triton (3.2 mM)	-	390	No feature	High/low*	[49]
	Reduced+Triton	-	416	No feature	Low	[49]
	Pyridine	-	390	No feature	Low	[49]
	Pyridine+Triton	-	418	No feature	Low	[49]
	Native	-	n.d.	n.d.	n.d.	[28]
Flax AOS <sup>†</sup>	Emulphogene (1.6 mM)	-	418	537, 570	Low	[28]
	Reduced+Emulphogene+CO	~1	447	No feature	Low	[28]
	Native	-	n.d.	n.d.	n.d.	[48]
AOS domain of coral AOS-LOX fusion (Coral AOS)	Emulphogene (1.6-4.0 mM)	-	391	512, 540	High	[48]
	Reduced+Emulphogene	-	407	No feature	High	[48]
	Reduced+Emulphogene+CO	~1	450	No feature	Low	[48]
	Native	-	406	500, 534	High	[47]
	Reduced+CO	~1	427	544, 569	Low	[47]
CYP2B4	Azide	200	408	500	Mixture	[47]
	KCNS	200	370, 420	550, 578	Low	[47]
	Native	-	418	535, 568	Low	[50,53]
	Reduced	-	413	544	Low	[50]
	<i>n</i> -octyl glucoside (10 mM)	-	425	No feature	Low	[54]
	Imidazole	50	359, 426	No feature	Low	[50]
	BI	0.05	359, 423	540, 574	Low	[50]
	DMPHP	0.5	377, 452	560	Low	[50]
	KCN	50	365, 436	559	Low	[50]
	Reduced+CO	~1	368, 451	554	Low	[50]
CYP1A2	Native	-	394	No feature	High	[50,53]
	Reduced	-	411	542	Low	[50]
	Renex 690 (1.6-8.1 mM)	-	418	No feature	Low	[53]
	Imidazole	200	356, 424	No feature	Low	[50]
	BI	1.3	361, 423	539, 575	Low	[50]
	DMPHP	0.33	370, 450	~550	Low	[50]
	KCN	90	368, 436	553	Low	[50]
	Reduced+CO	~1	366, 447	549	Low	[50]

CYP74C3 concentration was 3.25  $\mu$ M, except for reactions of CYP74C3 with Emulphogene, which were at 10.2  $\mu$ M and all reactions with CYP74C3 were carried out in 100 mM sodium phosphate buffer, pH 6.5 at 25°C; (s), shoulder; \* Discrepancy between EPR and UV/Visible spectroscopy data; <sup>†</sup> CYP74A1 was not characterised as a recombinant enzyme; n.d., not determined.

**Fig. 7. Sedimentation velocity analysis of CYP74C3 in detergent-buffer**

Effect of detergent-buffer on sedimentation coefficient distribution of purified CYP74C3 and fit using MULTIG (x). The fitted function (-----) is plotted onto the raw data (—).

