Structure of the open conformation of a functional chimeric NADPH cytochrome P450 reductase

Louise Aigrain¹, Denis Pompon¹, Solange Moréra^{2*} & Gilles Truan^{1*†}

¹Centre de Génétique Moléculaire, FRE3144, and ²Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), CNRS, Gif-sur-Yvette, France

Two catalytic domains, bearing FMN and FAD cofactors, joined by a connecting domain, compose the core of the NADPH cytochrome P450 reductase (CPR). The FMN domain of CPR mediates electron shuttling from the FAD domain to cytochromes P450. Together, both enzymes form the main mixed-function oxidase system that participates in the metabolism of endo- and xenobiotic compounds in mammals. Available CPR structures show a closed conformation, with the two cofactors in tight proximity, which is consistent with FAD-to-FMN, but not FMN-to-P450, electron transfer. Here, we report the 2.5 Å resolution crystal structure of a functionally competent yeast–human chimeric CPR in an open conformation, compatible with FMN-to-P450 electron transfer. Comparison with closed structures shows a major conformational change separating the FMN and FAD cofactors from 86 Å.

Keywords: NADPH cytochrome P450 reductase; electron transfer; redox partner complex

EMBO reports (2009) 10, 742–747. doi:10.1038/embo.2009.82

INTRODUCTION

Cytochromes P450 (P450) are monooxygenases that metabolize various molecules, including drugs and pollutants (Guengerich, 2008). The generic reaction catalysed by P450 is the oxidation of a wide range of organic compounds, using molecular oxygen and two electrons that are supplied by various redox partners (Hannemann *et al*, 2007). For eukaryotic microsomal P450, the membrane-bound form of NADPH cytochrome P450 reductase (CPR) is the primary source of electrons (Iyanagi, 2005). CPR is a multidomain enzyme, composed of a FMN-containing flavodoxin-like domain associated with a FAD-containing ferredoxin reductase-like domain (Smith *et al*, 1994) probably originating from individual horizontal gene transfer or exon fusion. A folded connecting domain joins the two catalytic parts. By using its two

flavin cofactors and a peculiar internal electron transfer cycle, CPR is able to split the dielectronic flux of NADPH by sequential electron transfer to external acceptors (Brenner *et al*, 2008).

Three-dimensional structures of oxidized forms of soluble rat and yeast CPR have been published (Wang *et al*, 1997; Lamb *et al*, 2006). The two flavins in their catalytic domains are in close proximity to one another, in a suitable position for interflavin electron transfer. However, docking attempts to accommodate a potential electron acceptor such as P450 while maintaining a compatible distance for flavin-to-haeme electron transfer have been, so far, unsuccessful. The generally accepted idea to account for this discrepancy is a concerted movement of the domains leading to an opening of the structure and, consequently, the exposure of the FMN domain to the solvent and ultimately to the P450. The role of the linker domain could therefore include the control of these movements (Gutierrez *et al*, 2003) and their synchronization with the catalytic cycle of the CPR, and, more precisely, with the redox states of the flavins. Interestingly, although their structures are highly similar, the redox properties of the flavins and the details of the catalytic cycles vary between yeast and human CPR (Louerat-Oriou *et al*, 1998). By designing a chimeric enzyme composed of the yeast FMN domain and the human connecting/FAD domain, we planned to analyse the effects of domain substitutions on the catalytic cycles and redox potentials of the CPR. Furthermore, this domain substitution might also perturb the dynamics and frequency of domain movements of natural CPR.

RESULTS

We designed a chimeric CPR (YH for yeast–human) composed of the residues Ser44–Leu211 of the yeast-CPR FMN domain and the residues Gly232–Ser677 of the human-CPR FAD domain (see supplementary information online). The protein was expressed, purified and crystallized as described in the Methods section. This chimeric CPR lacks the amino-terminal membrane-spanning domain required to facilitate crystallization as described previously (Wang *et al*, 1997; Lamb *et al*, 2006).

Crystal structure of the open conformation of YH CPR

The structure of YH was solved to 2.5 Å using molecular replacement and two search models: the yeast FMN domain

¹Centre de Génétique Moléculaire, FRE3144, and ²Laboratoire d'Enzymologie et Biochimie Structurales (LEBS), CNRS, 1 Avenue de la Terrasse, Gif-sur-Yvette Cedex 91198, France

*These authors contributed equally to this work

†Corresponding author. Tel: +33 1 69823665; Fax: +33 1 69823682;

E-mail: gilles.truan@cgm.cnrs-gif.fr

Table 1 | Crystallographic data and refinement parameters

Space group	C2
Unit-cell parameters (Å, °)	$a = 146.8, b = 60.7, c = 78.8, \beta = 103.4$
Resolution (Å)	20–2.5 (2.64–2.5)
Number of observed reflections	85,573 (13,223)
Number of unique reflections	23,129 (3,583)
R_{merge} (%) [*]	10.4 (50.9)
Completeness (%)	97.4 (94.9)
I/σ	12.7 (4.2)
R_{cryst} (%) [†]	22.2
R_{free} (%) [‡]	29
r.m.s. bond deviation (Å)	0.008
r.m.s. angle deviation (°)	1.09
Average B (Å ²)	
Protein	34.6
Flavin mononucleotide	22.1
FAD	19.1
Solvent	28.2

Values for the highest resolution shell are in parentheses. ^{*} $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the i th observed amplitude of reflection hkl and $\langle I(hkl) \rangle$ is the mean amplitude for all observations i of reflection hkl ; [†] $R_{\text{cryst}} = \sum \|F_{\text{obs}}\| - \|F_{\text{calc}}\| / \sum \|F_{\text{obs}}\|$. [‡]5% of the data were set aside for free R-factor calculation.

(residues 47–211 from Protein Data Bank (PDB) code 2BF4) and the rat FAD domain (residues 243–678 from PDB code 1AMO). A copy of each domain was positioned in the asymmetric unit with good packing, indicating that the asymmetric unit contained a monomer of YH. The rat FAD sequence was changed into human sequence and renumbered from residues 223 to 657, but not 658 as the rat sequence has an additional residue (see supplementary information online). The hinge region of 11 residues from 212 to 222 in the YH structure belongs to the human domain and corresponds to the human hinge region from residues 232 to 242. This linker region, which is not defined in the rat structure (also residues 232–242 in 1AMO) and that links the connecting domain to the FMN domain, was easily built in the electron density map. Crystallographic data and refinement parameters are available in Table 1. Crystal contacts and packing confirm that the YH is monomeric, as observed in solution by size exclusion chromatography.

The YH structure contains one FMN and one FAD molecule, as reported for the rat CPR structure (Wang *et al*, 1997). No secondary FMN binding site was visible, which is in contrast to the yeast CPR structure that has been reported previously (Lamb *et al*, 2006). NADP⁺ is not visible in the structure, which is consistent with its absence during protein purification and crystallization. Both rat and yeast CPR structures were solved in a closed conformation in which the FMN domain is embedded in the FAD domain crevice, resulting in a distance of 4 Å between FMN and FAD ligands (Fig 1A). Conversely, the structure of the YH CPR presents the two catalytic domains in an open conformation, leading to the separation of 86 Å between FMN and

Fig 1 | Ribbon representations of the rat, yeast and yeast-human structures. (A) Rat (Protein Data Bank (PDB) code 1AMO, light blue) and yeast (PDB code 2BF4, brown) structures were aligned onto their FAD domains. (B) The yeast-human (YH) structure is represented in the same orientation (FAD domains aligned) showing the main conformational change (see the arrow representing the measured distance between flavin mononucleotide (FMN) and FAD cofactors). FMN, FAD and NADP⁺ are shown in pink.

FAD cofactors (Fig 1B). No crystal symmetric FAD and FMN cofactors are in the vicinity of those from the asymmetric unit. Structural comparison of the YH connecting/FAD domain with the rat counterpart reveals that both folds superimpose well, with a root mean square (r.m.s.) deviation of 0.9 Å for 428 C-α atoms (Fig 2A). Likewise, the FMN domains of the YH and the yeast CPR are very similar, with an r.m.s. deviation of 0.46 Å for 165 C-α atoms (Fig 2B). Furthermore, the FMN and FAD cofactors of the YH are positioned identically and in the same conformation as their equivalent in the catalytic domains of the rat and yeast CPR. Therefore, we conclude that there are almost no structural changes in the individual catalytic domains of the YH, but instead a large rearrangement of the two catalytic domains. The flexible residues 218–220 from the hinge region seem to be responsible for the domain movement (Fig 1B). The absence of the NADP⁺ ligand has no visible effect on the FAD domain fold and Try 656 is still able to stack against the FAD isoalloxazine ring, as observed in the rat and yeast CPR.

Fig 2 | Comparison of the FAD and FMN domains between yeast–human CPR, rat and yeast equivalent domains. (A) Alignment of the FAD domain of the rat CPR (Protein Data Bank (PDB) code 1AMO; residues 243–678, blue) on the FAD domain of the chimeric yeast–human (YH) NADPH cytochrome P450 reductase (CPR; residues 220–657, red). (B) Alignment of the FMN domain of the yeast CPR (PDB code 2BF4; residues 47–211, blue) with the FMN domain of the chimeric YH CPR (residues 47–211, red).

YH coupling with cytochrome *c* and hCYP 3A4

To ascertain that the open structure of YH represents a functional enzyme, YH activity was measured using artificial (cytochrome *c*) and natural (human cytochrome P450 (hCYP) 3A4) electron acceptors. Tests were designed to verify not only that YH transferred single electrons, but also that it could drive successive functional catalytic cycles with multiple, sequential and synchronized electron transfers. Table 2 shows the production of 6 β -hydroxy-testosterone by a reconstituted system composed of hCYP 3A4 and human cytochrome b5 mixed with yeast, human or YH CPR. For the natural forms (yeast and human) the soluble and membrane-bound forms were compared. Yeast and human membrane-bound forms of CPR are active and transfer electrons to hCYP 3A4, although the yeast enzyme is less efficient. As shown previously with fusion enzymes (Dodhia *et al*, 2006; Inui *et al*, 2007), with *in vivo* complementation (Kunic *et al*, 2001) or in a reconstituted system (Lamb *et al*, 2001), soluble CPRs are able to transfer electrons to microsomal enzymes. Interestingly, the removal of the membrane segment decreases the activity for the human CPR, whereas the activity of the yeast soluble or membrane-bound CPR is approximately the same under the

Table 2 | Comparison of the testosterone 6 β -hydroxylation activity of human P450 3A4 (hCYP 3A4) reconstituted with human cytochrome b5 and various CPR

	Hm CPR	Hs CPR	Ym CPR	Ys CPR	HY CPR
Normalized activity*	100	52.8	25.9	21.9	7.9

CPR, cytochrome P450 reductase; Hm, human membrane-bound cytochrome P450 reductase; Hs, human soluble cytochrome P450 reductase; YH CPR, soluble chimeric yeast–human cytochrome P450 reductase; Ym, yeast membrane-bound cytochrome P450 reductase; Ys, yeast soluble cytochrome P450 reductase.

*Tests were carried out as described in the Methods section. 6 β -Hydroxytestosterone produced in the various tests was quantified after high performance liquid chromatography separation of the reaction products. For each CPR, the hCYP 3A4 specific activity was calculated. The ratio between each specific activity and that calculated with Hm CPR was computed and is reported in the table. The 100% (Hm CPR) corresponds to a turnover of 25 μ mol per μ mol of hCYP 3A4 per min.

experimental conditions tested. Soluble and membrane-bound yeast CPRs are less able to support hCYP 3A4 activity than the human forms, which is in agreement with results reported previously (Louerat-Oriou *et al*, 1998). More important is the ability of YH CPR to efficiently transfer electrons to hCYP 3A4. Furthermore, control reactions without NADPH or YH CPR did not show any detectable 6 β -hydroxylation activity, indicating an obligate electron transfer reaction through YH CPR and P450. This result shows that the YH chimeric protein can efficiently drive successive electronic cycles and transfers to P450. The decrease in efficiency of the YH CPR is moderate, ranging from three- to ninefold compared with the soluble yeast and human forms, respectively.

The possibility of YH CPR dimer formation, potentially resulting in intermolecular electron transfer between FAD and FMN domains belonging to separated CPR monomers, can be ruled out. Cytochrome *c* reductase activity, which involves obligatory electron transfer from the FMN domain to cytochrome *c*, was found to be linearly dependent on YH CPR concentration (Fig 3). In the nM range for YH concentrations, if self-exchange occurred between the two YH monomers at $1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ rate of self-exchange (Bertini *et al*, 1993), the initial velocity of electron transfer to cytochrome *c* would be $1 \times 10^{-3} \text{ s}^{-1}$, which is incompatible with the measured velocity of 1.93 s^{-1} . Steady-state parameters were also measured ($K_m = 0.34 \mu\text{M}$, $V_{\max} = 2.00 \text{ nmol s}^{-1}$). Therefore, we are confident that the YH CPR transfers electrons from FAD to the FMN of the same protein, similar to parental CPR. Taken together these results show that the YH CPR, constructed from individual domains originating from evolutionarily distant CPR, can still be functionally competent, while showing clearly different (open versus closed) conformation in the crystals.

DISCUSSION

To understand the role and properties of individual CPR domains in the catalytic cycle and, in particular, in the stabilization of the previously crystallized closed conformations, we designed, produced and crystallized a chimeric CPR composed of the yeast FMN domain and the human connecting/FAD domain (YH CPR). YH crystallized in the open conformation, which is compatible with the putative conformation of the CPR inside a binary complex with redox partners. The structural modifications observed in the open conformation lead to the positioning of the FMN cofactor in contact with the solvent, in a spatial position compatible with

Fig 3 | Linearity of cytochrome *c* reductase activity towards yeast-human concentration. Various tests were carried out at different yeast-human (YH) concentrations (from 0.5 to 50 nM) and at various cytochrome *c* concentrations. Cytochrome *c* was always added in excess (100 times) compared with YH concentration. Initial velocities were determined and reported as a function of YH concentration. Data points are fitted by a linear regression ($R^2 = 0.99$), giving a slope of 1.93 s^{-1} . The inset contains a plot of the cytochrome *c* reduction velocity as a function of its concentration. Points were fitted by a hyperbola equation ($R^2 = 0.99$).

electron transfer with approaching redox partners. A model of a binary complex between a P450 and the FMN domain of the CPR (lacking the connecting/FAD domain) has been published previously (Sevrioukova *et al*, 1999). In this structure (PDB code 2BVY), the FMN domain binds to the crevice located on the proximal face of the P450, minimizing the distance between the two cofactors. Structures in the closed conformation cannot be aligned on the FMN domain of this FMN-P450 complex without a clash between the structures. However, as shown in Fig 4, the new open conformation of the CPR is compatible—that is, there is no clash between the structures—with a bimolecular complex with a P450. We can hypothesize that the opening movement of the CPR probably corresponds to a motion of the FAD domain because the FMN domain is located immediately after the membrane-embedded N-terminus of the CPR. This idea is enforced further by the fact that P450 is also membrane bound; hence, the supposed contact region between the FMN domain and P450 should be located near to the membrane surface.

Careful examination of the atomic details of the connecting domain of YH did not reveal any clear structural modifications that could explain the movement between the two domains. Thus, the hinge region linking the FMN and connecting/FAD domains seems to be responsible for the spatial reorganization of the two domains.

As biochemical properties of the human and yeast CPR clearly differ, especially in terms of the redox potentials of FMN cofactors and coupling efficiency with human cytochrome P450, it is therefore possible that the chimeric protein is more stable in the opened conformation. Electron transfer activity of YH chimeric CPR toward cytochrome *c* and hCYP 3A4 shows that our enzyme can perform intra- (FAD to FMN, closed conformation) and inter- (FMN to haem, open conformation) electron transfers and should therefore be able to close and open itself during its catalytic cycle.

The slightly lower activity of YH CPR might result from some shift in the equilibrium between the open and closed forms, which could slow down electron transfer from FAD to FMN. An alternative hypothesis is that some coupling between the redox-state cycling of the FAD and the open versus closed conformational stabilization was lost in the chimeric structure due to some perturbation of the interactions between the two catalytic domains. This hypothesis is supported by examples of other multidomain flavin-containing proteins, such as yeast cytochrome *b*₂, which show strong conformational change on FMN reduction (Hazzard *et al*, 1994) or the nitric oxide synthase in which conformational changes have also been shown (Welland *et al*, 2008). The control of the conformational changes in multidomain electron transfer proteins by their cofactors redox states constitutes an exciting topic that can be more thoroughly investigated now that the CPR structure is available in both open and closed conformations.

METHODS

Cloning, expression and purification. Chimeric genes were constructed as follows. A first PCR reaction, designed to amplify separately the yeast FMN (residues 44–212) and human connecting/FAD domains (residues 232–677), was carried out. Primers at the 3' end of the yeast FMN domain and at the 5' end of the human connecting/FAD domain contained a 20-base extension similar to the 5' end of the human connecting/FAD domain and 3' end of the yeast FMN domain, respectively. Therefore, both amplified matrices had 40 bases in common at the junction point. Primary PCR products were then used as matrices for a second amplification (see supplementary information online; Fig 1). Secondary PCR products were digested and cloned into pET15b in frame with the 6-histidine-tag. DNA sequencing confirmed the absence of any undesired mutations. The constructed plasmid was transformed to BL21 (DE3) competent *Escherichia coli* cells to allow the expression of the soluble YH CPR.

Protein expression and purification. Protein expression was carried out at 29 °C in Terrific Broth medium complemented by 2.5 mg l^{-1} riboflavin and $100 \text{ } \mu\text{g ml}^{-1}$ ampicillin over 36 h. Cultures were spun down for 10 min at 7,500 r.p.m. and suspended in 20 mM Na/K phosphate buffer pH 7.4 (buffer A). Cell lysis was achieved in buffer A containing an antiprotease cocktail with a constant cell-disruption system (Constant Cell Ltd, Aberdeenshire, UK). Cell debris was removed by centrifugation at 8,500 r.p.m. for 1 h at 4 °C. His-tagged proteins were bound on a TALON Polyhistidine-Tag Purification Resin (Clontech, Mountain View, CA, USA) equilibrated with buffer A, containing 0.25 M NaCl and 0.25 M KCl, and eluted with a 0.33 M imidazole step gradient. Fractions containing YH CPR were desalted using a HiPrep desalting column (Amersham Pharmacia Biotech, Pittsburgh, PA, USA). After concentration using a Vivaspin-15 centrifugal concentrator (Sartorius, Goettingen, Germany), the protein solution was purified through gel filtration chromatography using a Sephacryl S-400 resin (Amersham Pharmacia Biotech) equilibrated with buffer A, containing $1 \text{ } \mu\text{M}$ FMN and FAD, 1 mM DTT and 1 mM EDTA. Purity of the sample was determined by SDS-PAGE and examination of the 280/250 nm ratio measured by optical spectroscopy (typically 5). Soluble YH CPR was then concentrated to 10 mg ml^{-1} and stored at 4 °C until crystallization assays were carried out.

Fig 4 | Potential view of yeast-human NADPH cytochrome P450 reductase in a binary complex with a P450. (A) P450 BM (*Bacillus megaterium*) 3 haem domain (Protein Data Bank (PDB) code 2BVY) is shown in pink; (B) aligned FMN domains of yeast-human NADPH cytochrome P450 reductase (YH CPR; PDB code 3FJO) is shown in green and of BM3 CPR (PDB code 2BVY) in pink; (C) FMN domain of yeast CPR (PDB code 2BF4) shown in yellow; and (D) FAD domain of YH CPR (PDB code 3FJO). The FMN domain of YH chimeric CPR structure (green) was aligned with the FMN domain (pink) of the FMN-P450 complex (PDB code 2BVY). The FAD domain of the yeast CPR structure was then aligned onto the FAD domain of YH, but was omitted in the figure for clarity. The FMN domain of yeast CPR (C) shown in yellow represents the closed conformation, whereas the FMN domain of YH CPR (B) represents the open conformation.

Cytochrome *c* reductase activity. Cytochrome *c* reductase activity was measured at 37 °C and followed at 550 nm ($\Delta\epsilon = 21,000 \text{ M}^{-1} \text{ cm}^{-1}$) with YH concentrations ranging from 0.5 to 50 nM. YH was diluted directly in a spectrophotometer cuvette containing 20 mM NaPO_4 buffer, pH 7.4, supplemented with 0.5 units of catalase and 0.5 units of super oxide dismutase. Cytochrome *c* was added at concentrations ranging from 0.15–40 μM . The reaction was started by the addition of 200 μM NADPH and was monitored for 2 min in a Carry 300 spectrophotometer (Varian, Palo Alto, CA, USA).

Enzymatic reconstitution of cytochrome P450 activity. The P450 reductase activity tests were carried out in reconstituted systems made of sonicated lipid micelles of dilauroylphosphatidylcholine (1 mg ml⁻¹) in 10 mM MOPS buffer pH 7.4. Each sample contained 100 nM human P450 3A4 (Invitrogen, Carlsbad, CA, USA), 30 mM MgCl_2 and 200 μM testosterone. The molar ratio between CPR and P450 varied from 0 to 60 and the cytochrome *b*₅ concentration was 200 nM. The reaction was started by the addition of 2 mM NADPH and, after 30 min of incubation at 37 °C, the reaction was stopped by the addition of 1 volume of acetonitrile. Testosterone and 6 β -hydroxytestosterone were quantified by reverse-phase high performance liquid chromatography on an XTerra MS C18 column (Waters, Milford, MA, USA) using pure testosterone and 6 β -hydroxytestosterone as standards.

Crystallization and data collection. Commercial crystallization kits (Qiagen, Hilden, Germany) were screened in sitting-drop vapour diffusion experiments using a nanodrop robot (Cartesian Proteomic Solutions, Saint-Marcel, France) at 20 °C. Small crystals appeared within five days under only one condition (0.1 M Tris-HCl pH 8.5, 28% (wt/vol) polyethylene glycol (PEG) 6000 and 0.1 M LiCl). The crystals were improved using an additive screen (Hampton Research, Aliso Viego, CA, USA) and were then

optimized manually in the presence of 8% hexafluoroethanol after equilibration against a reservoir solution consisting of 0.01 M Tris-HCl pH 8.5, 20% (wt/vol) PEG 6000 and 250 mM LiCl.

Crystals were flash-frozen in a cryoprotecting solution containing 0.01 M Tris-HCl pH 8.5, 250 mM LiCl, 20% PEG 6000 and 20% PEG 400. Data collection experiments were carried out at 100 K on the PROXIMA I beamline (Synchrotron SOLEIL, Saint-Aubin, France). 180 ° of data were collected in 1 ° frames, with 1 s per frame exposure. Diffraction intensities were evaluated using the program XDS (Kabsch, 1993) and processed further using the CCP4 program suite (Collaborative Computational Project, Number 4, 1994). Data collection and processing statistics are given in Table 1.

Structural determination and refinement procedure. Molecular replacement using PHASER allowed a copy of the yeast FMN domain (residues 47–211 from PDB code 2BF4) and of the rat FAD domain (residues 243–678 from PDB code 1AMO) to be positioned in the asymmetric unit. The rat FAD sequence was changed into human sequence and renumbered from residues 223 to 657. The human hinge region of 11 residues (212–222), which links the FAD domain to the FMN domain and is not defined in the rat CPR structure (residues 232–242 from PDB code 1AMO), was easily built in the electron density map. Model refinement was carried out using Refmac and the electron density maps were evaluated using COOT (Emsley & Cowtan, 2004). In the final model, the first three residues (44–46) at the N-terminus, and five residues (481–485) and three residues (578–580) from surface loops are disordered; data parameters are given in Table 1. The atomic coordinates and structure factors (PDB code 3FJO) have been deposited in the Protein Data Bank (<http://www.rcsb.org>).

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We are grateful to A. Thompson for help in data collection on PROXIMA I. L.A. is a PhD student financed by the French Ministry of Research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Bertini I, Gaudemer A, Luchinat C, Piccioli M (1993) Electron self-exchange in high-potential iron-sulfur proteins. Characterization of protein I from *Ectothiorhodospira vacuolata*. *Biochemistry* **32**: 12887–12893
- Brenner S, Hay S, Munro AW, Scrutton NS (2008) Inter-flavin electron transfer in cytochrome P450 reductase—effects of solvent and pH identify hidden complexity in mechanism. *FEBS J* **275**: 4540–4557
- Collaborative Computational Project, Number 4 (1994) The CCP4 suite: programs for protein crystallography. *Acta Crystallogr* **50**: 760–763
- Dodhia VR, Fantuzzi A, Gilardi G (2006) Engineering human cytochrome P450 enzymes into catalytically self-sufficient chimeras using molecular Lego. *J Biol Inorg Chem* **11**: 903–916
- Emsley P, Cowtan K (2004) Coot: model-building tools for molecular graphics. *Acta Crystallogr* **60**: 2126–2132
- Guengerich FP (2008) Cytochrome p450 and chemical toxicology. *Chem Res Toxicol* **21**: 70–83
- Gutierrez A, Munro AW, Grunau A, Wolf CR, Scrutton NS, Roberts GC (2003) Interflavin electron transfer in human cytochrome P450 reductase is enhanced by coenzyme binding. Relaxation kinetic studies with coenzyme analogues. *Eur J Biochem* **270**: 2612–2621
- Hannemann F, Bichet A, Ewen KM, Bernhardt R (2007) Cytochrome P450 systems—biological variations of electron transport chains. *Biochim Biophys Acta* **1770**: 330–344
- Hazzard JT, McDonough CA, Tollin G (1994) Intramolecular electron transfer in yeast flavocytochrome b2 upon one-electron photooxidation of the fully reduced enzyme: evidence for redox state control of heme-flavin communication. *Biochemistry* **33**: 13445–13454
- Inui H, Maeda A, Ohkawa H (2007) Molecular characterization of specifically active recombinant fused enzymes consisting of CYP3A4, NADPH-cytochrome P450 oxidoreductase, and cytochrome b5. *Biochemistry* **46**: 10213–10221
- Iyanagi T (2005) Structure and function of NADPH-cytochrome P450 reductase and nitric oxide synthase reductase domain. *Biochem Biophys Res Commun* **338**: 520–528
- Kabsch W (1993) Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J Appl Crystallogr* **26**: 795–800
- Kunic B, Truan G, Breskvar K, Pompon D (2001) Functional cloning, based on azole resistance in *Saccharomyces cerevisiae*, and characterization of *Rhizopus nigricans* redox carriers that are differentially involved in the P450-dependent response to progesterone stress. *Mol Genet Genomics* **265**: 930–940
- Lamb DC, Kim Y, Yermilitskaya LV, Yermilitsky VN, Lepesheva GI, Kelly SL, Waterman MR, Podust LM (2006) A second FMN binding site in yeast NADPH-cytochrome P450 reductase suggests a mechanism of electron transfer by diflavin reductases. *Structure* **14**: 51–61
- Lamb DC, Warrilow AG, Venkateswarlu K, Kelly DE, Kelly SL (2001) Activities and kinetic mechanisms of native and soluble NADPH-cytochrome P450 reductase. *Biochem Biophys Res Commun* **286**: 48–54
- Louerat-Oriou B, Perret A, Pompon D (1998) Differential redox and electron-transfer properties of purified yeast, plant and human NADPH-cytochrome P-450 reductases highly modulate cytochrome P-450 activities. *Eur J Biochem* **258**: 1040–1049
- Sevrioukova IF, Li H, Zhang H, Peterson JA, Poulos TL (1999) Structure of a cytochrome P450-redox partner electron-transfer complex. *Proc Natl Acad Sci USA* **96**: 1863–1868
- Smith GC, Tew DG, Wolf CR (1994) Dissection of NADPH-cytochrome P450 oxidoreductase into distinct functional domains. *Proc Natl Acad Sci USA* **91**: 8710–8714
- Wang M, Roberts DL, Paschke R, Shea TM, Masters BS, Kim JJ (1997) Three-dimensional structure of NADPH-cytochrome P450 reductase: prototype for FMN- and FAD-containing enzymes. *Proc Natl Acad Sci USA* **94**: 8411–8416
- Welland A, Garnaud PE, Kitamura M, Miles CS, Daff S (2008) Importance of the domain-domain interface to the catalytic action of the NO synthase reductase domain. *Biochemistry* **47**: 9771–9780