Crystal Packing of the c_6 -type cytochrome OmcF from *Geobacter sulfurreducens* is mediated by an N-terminal Strep-tag II

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Synopsis The structure of the Strep-tag II-tagged monoheme *c*-type cytochrome OmcF from *Geobacter sulfurreducens* was determined by Fe SAD on a rotating anode X-ray source. Packing analysis reveals that the N-terminal Strep-tag II is the exclusive mediator of crystal contacts along the *c*-axis, providing crystal-contacts to three monomers simultaneously.

Abstract The putative outer membrane *c*-type cytochrome OmcF of *Geobacter sulfurreducens* contains a single heme group and shows homologies to soluble cytochromes c_6 , a class of electron transfer proteins typically found in cyanobacterial photosynthetic electron transfer chains. OmcF was overexpressed heterologously in *Escherichia coli* as an N-terminal Strep-tag II fusion protein and isolated using streptactin affinity chromatography followed by size exclusion chromatography. The structure was solved by Fe SAD using data collected to a resolution of 1.86 Å on a rotating Cu-anode X-ray generator. In the crystal, packing interactions in one dimension were exclusively mediated through the Strep-tag II sequence. The tag and linker regions were in contact with three further monomers of OmcF, leading to a well-defined electron density map for this engineered and secondary-structure-free region of the molecule.

Keywords: *c*-type cytochrome; Fe SAD phasing; dissimilatory metal reduction; *Geobacter sulfurreducens*

1. Introduction

Molecular dioxygen is the most efficient terminal electron acceptor available for a respiratory energy metabolism that couples electron transport along a redox potential gradient to the translocation of protons across a biological membrane. In anoxic environments, the best electron acceptor is nitrate, NO_3^- , followed by metal ions such as Fe^{3+} or Mn^{4+} (Lovley, 1991, 1993, 1997). While gaseous dioxygen and soluble nitrate can be easily taken up by organ-

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isms, the oxides of iron and manganese are present in the form of insoluble minerals and hence are hard to access (Lovley *et al.*, 2004). Nevertheless, in spite of the obvious difficulties involved in respiring a solid substrate, bacteria have evolved three distinct strategies to achieve the reduction of metal oxides (Newman, 2001, DiChristina, 2005, DiChristina *et al.*, 2005). The most straightforward approach is to charge electrons onto small, redox-active compounds that are subsequently expelled from the cell. Quinones and humic acids are used for this purpose, and some of these carriers have been found to perform a double function as redox-active antibiotics (Newman & Kolter, 2000, Hernandez & Newman, 2001). The second possible strategy reverses the principle and makes use of siderophores. These again are small carrier molecules, but now acting as strong chelators for metal ions and being used to leech metals out of minerals that are subsequently reimported into the cell and reduced by periplasmic enzymes (Varma & Chincholkar, 2007, Chiu *et al.*, 2001). Both of these methods are by design limited in their control of electron and substrate flow and are therefore less efficient than the third mechanism, for which the term ‘dissimilatory metal reduction’ (DMR) was coined (Lovley, 1993). Hereby, electron transfer systems cross both membranes of the Gram-negative, metal-reducing bacteria and shuttle electrons to the outside of the cell where they can be used to reduce the minerals *in situ*. This requires distinct transfer chains, terminating in metal reductases that are located on the outside of the bacterial cell.

The considerable interest in bacterial metal reduction originates not only from the geologically relevant role of DMR organisms in shaping the global environment during the entire history of life on Earth, but in particular also from the wide range of possible, biotechnological applications. Due to the low substrate specificity of metal-reducing bacteria, other – and potentially hazardous – metals can be reduced, including chromium, cadmium and uranium, opening a range of possible applications in bioremediatory processes, with first, encouraging results being at hand (Lovley, 1995, 2001, Lovley & Coates, 1997). Furthermore, DMR bacteria have been shown to adhere to graphite electrodes and transfer electrons, opening various technological possibilities from biosensors up to ‘soil batteries’ that power electronic hardware in the field (Bond *et al.*, 2002, Bond & Lovley, 2003, 2005). In anoxic environments, DMR bacteria were found to dominate bacterial populations under conditions favouring respiration of mineral oxides (Coates *et al.*, 1996, Lovley *et al.*, 2004). The genera *Geobacter* and *Shewanella* were the first to be identified as true metal reducers, and once their genome sequences had become available, a striking abundance of genes encoding *c*-type cytochromes became obvious (Heidelberg *et al.*, 2002, Methé *et al.*, 2003). The genome of the δ -proteobacterium *Geobacter sulfurreducens* PCA, the paradigmatic model organism for DMR, has been annotated with no less than 111 genes for this class of proteins, in which one or multiple heme groups are covalently linked to a protein chain *via* binding motives of the general pattern C-X_{*n*}-C-H ($2 \leq n \leq 5$) (Méthé *et al.*, 2003). Several of these proteins have been stud-

ied as model systems for understanding the packing and interaction of heme groups in larger and more complex cytochromes (Heitmann & Einsle, 2005).

A total of 31 of the *c*-type cytochromes of *G. sulfurreducens* were predicted to be localized in the outer membrane (Méthé *et al.*, 2003). Of these, the 104 amino acid OmcF is among the smallest, and it has been shown that its deletion has a significant effect on the ability of the bacteria to grow by reduction of Fe(III) citrate and also on the expression of several other cytochromes, including the putative metal reductases OmcB, OmcC and OmcS (Kim *et al.*, 2005b). Based on its sequence, OmcF (GSU2432; naming scheme of the genome according to <http://www.tigr.org>) was grouped with the family of cytochromes c_6 , whereby its closest relatives were found in other *Geobacteraceae*. Outside this genus, OmcF shows similarities to cyanobacterial cytochromes c_6 (28.9 % sequence identity with *Synechocystis* sp. PCC6803 cytochrome c_6), but also to plant-type cytochromes c_{6A} (21 % sequence identity with the protein from *Arabidopsis thaliana*). In cyanobacteria, cytochromes c_6 are found as electron carriers to cytochrome *c* oxidase and in both cyanobacteria and algae they transfer electrons from the cytochrome *b₆f* complex to photosystem I. In a subsequent publication, the localization of OmcF at the outer membrane was revised and an inner membrane location was proposed instead (Ding *et al.*, 2006). Using a search algorithm for lipoproteins, a putative lipidation site was indeed predicted at amino acid Cys 19 of the OmcF sequence (Juncker *et al.*, 2003b).

Here we report the crystal structure of OmcF refined to a resolution of 1.86 Å, recombinantly expressed in *Escherichia coli* as a soluble, Strep-tag II fusion protein. The structure was solved by single-wavelength anomalous dispersion of iron (Fe SAD), using data collected to high redundancy on a copper rotating anode generator. OmcF shows the typical characteristics of a c_6 -type cytochrome, but surprisingly its crystallization was found to be strictly dependent on the affinity tag, which participates actively in crystal contact formation.

2. Materials and Methods

2.1. Expression and purification of recombinant OmcF

The gene encoding *omcF* was amplified by polymerase chain reaction from genomic DNA of *G. sulfurreducens* PCA, using the forward primer 5'-AAAGAATTCCGGAGGCGGC GAGCTGTTCG-3' that introduced an EcoRI restriction site (underlined) and removed the first 75 nucleotides that comprise both the leader sequence predicted by SignalP (Bendtsen *et*

al., 2004) and the lipid attachment site as predicted by LIPOP (Juncker *et al.*, 2003a). The reverse primer, 5'-AAACTCGAGTCATGGGAAGCTC GCCAC-3', introduced an XhoI restriction site (underlined) after the stop codon. The fragment was then cloned into a modified pET22b(+) expression vector (Merck-Novagen, Darmstadt, Germany) that provided an *ompA* leader sequence and N-terminal Strep-tag II. Protein was expressed in *E. coli* BL21(DE3) containing the accessory plasmid pEC86 that encodes the eight *ccm*-genes required for the maturation of *c*-type cytochromes (Thöny-Meyer *et al.*, 1995). Freshly transformed bacteria were used to inoculate 5 ml overnight cultures incubated at 37° C. Expression cultures were grown without induction, aerobically in 2L Erlenmeyer flasks filled with 1L LB medium in an incubator at 25° C and 220 rpm for 20 hours. Cell pellets were resuspended in 2 ml of binding buffer (20 mM Tris/HCl buffer at pH 8.0) per gram cells (wet weight) and disrupted by five passages through a microfluidizer (Microfluidics, Newton, MA) at a pressure of 0.55 MPa. Debris and membranes were removed by centrifugation at $100,000 \times g$ for 1 hour. The supernatant was applied to a StrepTactin column (IBA, Göttingen, Germany) (Schmidt & Skerra, 2007) with a bed volume of 16 ml using the Äkta prime system (GE Healthcare, Freiburg, Germany). The column was developed with a step of 2.5 mM desthiobiotin in binding buffer and the eluted peak fraction was further concentrated using Vivaspinn centrifugal concentration devices (Vivascience/Sartorius, Göttingen, Germany) with a cutoff of 5 kDa. The concentrated sample was subsequently applied to size exclusion chromatography (Superdex S75 16/60, GE Healthcare, Freiburg, Germany) with 150 mM NaCl in 20 mM Tris/HCl buffer at pH 8.0. The concentration of the purified protein was determined by the bicinchoninic acid method (Smith *et al.*, 1985) and the samples were analyzed by SDS-PAGE (Laemmli, 1970) and heme staining (Goodhew *et al.*, 1986).

2.2. Spectroscopy and redox titrations

Electron excitation spectra were recorded on an Ultrospec 2100 pro spectrophotometer (GE Healthcare, Freiburg, Germany) for different protein concentrations and for samples fully reduced by addition of 5 mM Na-dithionite or fully oxidized with 10 mM $K_3[Fe(CN)_6]$. Molar extinction coefficients were calculated based on the absorption values of different samples at isosbestic points. Potentiometric redox titrations were carried out according to a modified protocol of Dutton (Dutton, 1978). 2 ml of 24 μ M OmcF in 150 mM NaCl and 20 mM Tris/HCl buffer at pH 8.0 and a mixture of 5 redox mediators (methyl viologen, juglone, 1,4-naphthoquinone, phenazine methosulfate and 1,2-naphthoquinone), each at a concentration of 0.4 μ M, were used in an anoxic cuvette. Exclusion of dioxygen was assured by retaining a positive pressure of 0.1 bar of N_2 in the cuvette throughout the experiment. Reduction was achieved by stepwise addition of 25 mM $Na_2S_2O_4$ solution (pH 8.0), while 10 mM

$\text{K}_3[\text{Fe}(\text{CN})_6]$ were used for reoxidation. Spectra were recorded using an OceanOptics S2000 fibre optic CCD spectrometer. The fraction of reduced protein was calculated from the relative change of absorption at 552 nm (α -band). The custom-made Ag/AgCl redox electrode (Sensortechnik Meinsberg, Germany) was calibrated with quinhydrone. All obtained redox potentials were corrected for the standard hydrogen electrode.

2.3. Crystallization and structure determination of OmcF

Purified protein was used for crystallization screens at a concentration of $10 \text{ mg}\cdot\text{ml}^{-1}$ in sitting-drop vapor diffusion experiments. Drops of $1 \text{ }\mu\text{l}$ protein and $1 \text{ }\mu\text{l}$ reservoir solution were mixed and equilibrated against $300 \text{ }\mu\text{l}$ of reservoir solution. Within three days of incubation at 293 K , clusters of crystal needles and small plates appeared in conditions containing $1.4 - 1.8 \text{ M } (\text{NH}_4)_2\text{SO}_4$, $2 \text{ } \%$ (w/v) PEG 400 and 100 mM of HEPES/NaOH buffer at pH 7.0 to 7.5. Serial dilutions of crushed crystals from these conditions were used for microseeding into crystallization conditions with lowered ammonium sulfate concentrations. Drops of $1 \text{ }\mu\text{l}$ protein and $1 \text{ }\mu\text{l}$ reservoir solution were incubated against $300 \text{ }\mu\text{l}$ of the reservoir for 2 hours and then $0.2 \text{ }\mu\text{l}$ of diluted (1:100) crystal slush were added. Crystals obtained by microseeding still grew in clusters, but few individual, three-dimensional crystals could be isolated for X-ray diffraction experiments on a MicroMax 007 rotating copper anode X-ray source (Rigaku, Tokyo, Japan) equipped with a mar345dtb image plate detector (mar research, Hamburg, Germany). Crystals were cryo-protected by transfer into 2.4 M lithium sulfate, $2 \text{ } \%$ PEG 400 and 100 mM HEPES/NaOH at pH 7.0, and cooled to 100 K in a nitrogen gas stream for data collection. Diffraction data were integrated and scaled using the programs DENZO and SCALEPACK (Otwinowski & Minor, 1997). SHELXD was used to localize the heme iron atom (Schneider & Sheldrick, 2002), and initial phasing was carried out with SHELXE (Sheldrick, 2002). A single heme group was manually fitted into the electron density map, allowing the identification of additional, weak peaks in the anomalous difference electron density map as sulfur atoms of cysteine and methionine. The positional information of these iron and sulfur atoms was used for phase calculations with SHARP (La Fortelle *et al.*, 1997), and SOLOMON (Abrahams & Leslie, 1996) was used for subsequent density modification. The structural model was built in COOT (Emsley & Cowtan, 2004) and refined using REFMAC5 (Murshudov *et al.*, 1997). Electrostatic potential maps were calculated using DELPHI (Honig & Nicholls, 1995) and figures were created in PyMOL (DeLano, 2002).

3. Results and Discussion

3.1. Structure Solution by Fe SAD

Data from a single, diffracting crystal of OmcF were collected on a mar345dtb image plate detector using Cu-K α radiation. The protein crystallized in the orthorhombic space group P2₁2₁2 with one monomer per asymmetric unit (Table 1). As amino acid sequence comparisons with other cytochromes *c*₆ indicated strong homologies, structure solution by molecular replacement was attempted as soon as data completeness reached 70 %. A moderately clear solution (CC = 0.24; *R* = 0.55) was obtained using MOLREP (Collaborative Computational Project No. 4, 1994), with cytochrome *c*_{6A} from *Arabidopsis thaliana* as a search model (PDB code 2CE0). However, the resulting crystal packing showed significant gaps in one dimension and the solution was consequently discarded and data collection was continued in order to obtain high redundancy data for in-house phase determination by Fe SAD (Dauter *et al.*, 2002). At a multiplicity of 21 (Table 1), the heme iron atom could be straightforwardly localized using SHELXD, and a molecular model was built into an electron density map obtained after phasing with SHARP and density modification with SOLOMON (see Materials and Methods). The example of OmcF thus emphasizes the feasibility of using intrinsic iron atoms for SAD phasing on in house X-ray equipment. At $\lambda = 1.54 \text{ \AA}$, the anomalous signal for iron is significantly stronger than the one for sulfur (3.3 vs. 0.6 electrons), such that Fe SAD phasing using diffraction data from a home source should be possible in most cases.

3.2. Biochemical and Structural Properties of OmcF

Oxidized and reduced electron excitation spectra of OmcF show strong similarities with spectra of previously characterized *c*₆ cytochromes (Fig. 1). The fully reduced spectrum exhibits three characteristic absorption maxima at 552 nm (α -), 523 nm (β -) and 417 nm (γ - or Soret-band). The molar extinction coefficient for the fully reduced α -band was determined to 23,800 M⁻¹·cm⁻¹, in good agreement with previously reported values for cytochromes *c*₆ (Pettigrew & Moore, 1987, Moore & Pettigrew, 1990). The γ -band undergoes a hypsochromic shift to 410 nm in the oxidized spectrum. The absence of a shoulder around 610 nm points towards a *low spin* configuration, as expected for a six-coordinate Fe(III) in heme. The midpoint redox potential of OmcF was determined to 121.2 $\pm$ 0.8 mV by potentiometric redox titration (Fig. 2). This value is approximately 200 mV lower than those previously reported for cytochromes *c*₆ (Pettigrew & Moore, 1987, Moore & Pettigrew, 1990, Reuter & Wiegand, 2001), but well in accordance with the published midpoint potential of cytochromes *c*_{6A} from *A. thaliana* (Molina-Heredia *et al.*, 2003). At such low potential, the plant protein is unable to shuttle electrons between the cytochrome *b*₆*f* complex and photosystem I, and consequently a regulatory role in photosynthesis or a function as a redox sensor was proposed (Weigel *et al.*,

2003). A sensory role was also proposed for OmcF (Kim *et al.*, 2005a), although dissimilatory iron reduction does not require as positive potentials as photosynthesis. Most recently, two novel c_6 -type cytochromes from *Synechococcus* sp. PCC7002 were characterized and identified as members of two new groups of c_6 -type cytochromes (Bialek *et al.*, 2008). One of these, PetJ2, exhibits a midpoint potential similar to cytochromes c_{6A} , although its sequence identity to OmcF is low (22.4 %).

In the electron density map obtained for OmcF, all residues of the protein were clearly defined (Fig. 3, 4). The globular part of the structural model has a diameter of approximately 30 Å and comprises 79 amino acids, starting at residue 26 of the original OmcF sequence. In accordance with the classical fold of cytochromes c_6 it consists of 4 α -helical elements connected by 3 loop regions, one of which contains the CAGCH heme binding motif starting at Cys 35, immediately following the first α -helix. The distal axial ligand of the heme iron, Met 79, is located within the last loop region. This fold is highly similar to known structures of cytochromes c_6 from unicellular algae and cyanobacteria (Kerfeld *et al.*, 1995, Beißinger *et al.*, 1998) (Fig. 4). In addition to the canonical cytochrome fold, both the linker regions and affinity tag (19 amino acids) that were part of the expressed construct of OmcF were clearly defined in electron density (Fig. 3; see below).

Close to the heme group a tyrosine residue, Tyr 71, is oriented nearly parallel to the porphyrin plane, with its hydroxyl-group coordinated to a water molecule bound between the heme propionates. Previously characterized cytochromes c_6 have a glutamine residue at the corresponding position, while in cytochromes c_{6A} , a small, hydrophobic residue (Val, Ile, Leu) was found (Chida *et al.*, 2006). Mutating this position in cytochrome c_6 and c_{6A} modulates the midpoint redox potential of the heme group (Worrall *et al.*, 2007). Tyr 71 and its π -stacking with the porphyrin ring is the most significant difference between OmcF and cytochromes c_6 , indicating that this feature may be a prime contribution to the significantly lower redox potential of OmcF. The family of cytochromes c_6 shows high structural conservation, to the effect that all structures presented to date align with root-mean-squared deviations of their backbone positions of below 2 Å (Table 2). In this list, OmcF is the only protein from a non-photosynthetic organism, and yet the protein shows higher homologies to cytochromes c_6 than to other bacterial monoheme cytochromes of known structure.

3.3. Crystallization of OmcF strictly depended on the affinity tag

The structural model of *G. sulfurreducens* OmcF quickly revealed that the solutions obtained by molecular replacement had actually been correct and that in the crystal packing layers of protein were indeed separated by marked gaps (Fig. 5). These gaps were bridged exclusively by the N-terminal extension of the protein consisting of the Strep-tag II affinity tag

used for protein purification and a 9 amino acid linker sequence that was introduced during the cloning procedure. Crystallization of OmcF therefore necessarily depended on the presence of this linker and tag region. In crystal structures of engineered proteins, affinity tags rarely have defined electron density, indicating structural flexibility. While presumably flexible in solution as well, the Strep-tag II of OmcF was able to form favorable crystal contacts with a total of three monomers within the crystal packing, making it a crucial element in crystal formation in this case (Fig. 6). In this process, the strongly positive electrostatic surface potential of OmcF (not shown) may combine favorably with electron-rich amino acid side chains in the affinity tag to allow for stable packing interactions.

In detail, the number of direct hydrogen-bonding interactions between the Strep-tag II and its three interaction partners is limited to two each with the first and second molecules, and three with the third (Table 3, Fig. 7). The first residues of the linker, Ala -5 to Ser -1, span the gap observed in the crystal packing (Fig. 5), reaching the next layer of protein where all interactions of the N-terminal segment are taking place. Intramolecular hydrogen bonds induce two structured conformations within this segment. One of these is a 3_{10} -helical turn from Gly -9 to Thr -6, and out of this structure the two hydrogen bonds to Gln 41 of the adjacent monomer are formed (Table 3, Fig. 7). A second structural unit comprises the region from Gln -13 to Lys -10 within the Strep-tag II. Here, the side chains of Gln -13, Glu -11 and Lys -10 form multiple hydrogen bonding interactions to backbone amide groups, positioning the side chain of Glu -11 such that a tight salt bridge is formed with Arg 66 from the second monomer in the crystal packing (Table 3, Fig. 7). The interaction with the third monomer crucially depends on a coordinated water molecule, the exact one described above to reside between the two propionate side chains of the heme group and Tyr 71 (Fig. 7). In addition, the aromatic side chains of Phe -12 and Trp -17, but also Pro -3 and Pro -14 contribute to extensive van-der-Waals interactions that add favorably to the stability of the observed packing arrangement. Structured affinity tags are rarely observed in crystal structures, and if so, do not commonly participate in building up the crystal lattice, as they are not likely to form rigid structures. An exception was found in the structure of the yeast cytochrome bc_1 complex, crystallized with a C-terminally Strep-tagged antibody Fv fragment (Hunte *et al.*, 2000). In this structure, crystal packing of the large membrane complex was mediated in one dimension by the bound Fv fragment, but indeed half of a Strep-tag I sequence at the C-terminus of this fragment was in hydrogen-bonding interaction with a neighboring bc_1 complex, contributing – although not exclusively – to the interaction between the molecules in the crystal.

Figure 1 Electron excitation spectra of recombinant *G. sulfurreducens* OmcF. Ferricyanide-oxidized (dots) and dithionite-reduced (line) spectra showed typical features of a *c*-type cytochrome, with a bathochromic shift of the Soret band upon reduction and an absorption maximum of the α -band in the reduced state at 552 nm. A shoulder above 610 nm in the oxidized spectrum – indicative for *high spin* Fe(III) – was not observed.

Figure 2 Potentiometric redox titration of *G. sulfurreducens* OmcF monitored at 552 nm. A non-linear fit of the titration data with the Nernstian equation yields a midpoint potential of 121.2 ± 0.8 mV with respect to the standard hydrogen electrode.

Figure 3 Crystal structure of *G. sulfurreducens* OmcF. The cartoon representation shows the elongated, yet fully defined N-terminal region consisting of two amino acids originating from the cloning process (AS), the 8 amino acid Strep-tag II (WSHPQFEK) and a 9 amino acid linker region (GAE-TAVPNS). The cytochrome itself folds into the canonical, globular fold of cytochromes c_6 .

Figure 4 Stereo representation of the cytochrome c_6 domain of *G. sulfurreducens* OmcF. The single heme group of OmcF is covalently linked to the peptide chain via a bonding motif with the sequence

CAGCH (residues 35-39). His 39 functions as a proximal axial ligand to the heme iron atom, while the distal axial ligand is Met 79. Every tenth residue in the sequence is marked.

Figure 5 Packing interactions in crystals of *G. sulfurreducens* OmcF. The space group is $P2_12_12$, with the *c*-axis pointing upward. The Strep-tag II affinity tag is omitted, emphasizing that it is required to provide packing along *c*. (a) shows a view along *b*, while (b) is rotated 45°, providing a view along the diagonal of the *ab* plane. One unit cell is shown in both figures.

Figure 6 The Strep-tag II affinity tag mediates essential crystal contacts in *G. sulfurreducens* OmcF. The elongated, N-terminal extension consisting of the affinity tag and a 9 amino acid linker region bridges a gap in the crystal packing along the *c*-axis (Fig. 5) to reach the next layer of proteins, where it makes specific contact with three OmcF monomers (green, blue and yellow). The residues of the Strep-tag II sequence (WSHPQFEK) are shown with a red surface.

Figure 7 Stereo representation showing the interactions of the linker (blue) and Strep-tag II region (red) with three neighboring monomers, as detailed in Table 3. Hydrogen bonding interactions within the linker/tag are shown in blue, interactions with other monomers in red. Five of the seven observed hydrogen bonding interactions are formed by the Strep-tag II, the other two by the linker region.

Table 1 Data collection and refinement statistics.

Data collection statistics	
X-ray wavelength [Å]	1.5418
resolution limits [Å]	30 – 1.86 (1.96 – 1.86)
space group	P 2 ₁ 2 ₁ 2
unit cell dimensions	<i>a</i> = 53.48 Å, <i>b</i> = 40.13 Å, <i>c</i> = 42.09 Å
no. of observations	321,255
no. of unique reflections	14,750
Multiplicity	21.78
completeness [%]	99.7 (98.5)
<i>R</i> _{sym}	0.105 (0.4952)
<i>R</i> _{p.i.m.}	0.022 (0.1202)
<i>I</i> / <i>σ</i> (<i>I</i>)	23.50 (6.06)
Refinement statistics	
<i>R</i> _{cryst}	0.172
<i>R</i> _{free}	0.216
r.m.s.d. in bond lengths [Å]	0.018
r.m.s.d. in bond angles [°]	1.79
Cruikshank's DPI [Å]	0.15

average B factors [$\text{\AA}^2$]	22.1
no. of protein atoms	777
no. of water molecules	124
Ramachandran statistics (%); residues in	
most favoured regions	88
additionally allowed regions	12
generously allowed regions	0
disallowed regions	0

^a Values given in parentheses represent the highest resolution shell. R_{sym} is the residual for symmetry-equivalent reflections according to $R_{\text{sym}} = (\sum |I_{\text{hkl}} - \langle I_{\text{hkl}} \rangle| / \sum I_{\text{hkl}})$. R_{pim} is calculated according to (Weiss & Hilgenfeld, 1997). The diffracton precision index (DPI) is calculated according to (Cruickshank, 1999). For the calculation of R_{free} , 5 % of reflections were chosen at random to constitute a test set.

Table 2 Comparison of structures of the cytochrome c_6 family.

PDB ID	Organism	Type of organism	Resolution [$\text{\AA}$]	r. m. s. d. [$\text{\AA}$]	Sequence identity [%]	Reference
1GDV	<i>Porphyra yezoensis</i>	red alga	1.56	0.88	25.2	(Yamada <i>et al.</i> , 2000)
2V08	<i>Phormidium laminosum</i>	cyanobacterium	2.00	1.10	21.7	(Worrall <i>et al.</i> , 2007)
1F1F	<i>Arthrospira maxima</i>	cyanobacterium	2.70	0.73	21.6	(Sawaya <i>et al.</i> , 2001)
1CYI	<i>Chlamydomonas reinhardtii</i>	green alga	1.90	0.91	21.5	(Kerfeld <i>et al.</i> , 1995)
1C6S	<i>Synechococcus elongatus</i>	cyanobacterium	NMR	1.77	21.4	(Beißinger <i>et al.</i> , 1998)
1CTJ	<i>Monoraphidium braunii</i>	green alga	1.10	1.11	20.9	(Frazão <i>et al.</i> , 1995)
1LS9	<i>Cladophora glomerata</i>	green alga	1.30	1.16	20.0	(Dikiy <i>et al.</i> , 2002)
1C6R	<i>Scenedesmus obliquus</i>	green alga	1.90	1.07	18.3	(Schnackenberg <i>et al.</i> , 1999)
2CE0	<i>Arabidopsis thaliana</i>	higher plant	1.24	1.84	16.6	(Marcaida <i>et al.</i> , 2006)

Table 3 Listing of direct hydrogen bonds formed by the Strep-tag II / linker region ^a

Strep(II)-tag / linker pos.	Symmetry mate no.	Symmetry mate pos.	Distances
His -15, N _{ε2}	A	Tyr 71, O _η	2.4 $\text{\AA}$, H ₂ O, 2.5 $\text{\AA}$
His -15, N _{ε2}	A	heme propionates	2.4 $\text{\AA}$, H ₂ O, 2.5 $\text{\AA}$ / 2.7 $\text{\AA}$
Glu -11, O _{ε1}	B	Arg 66, N _{η1}	2.7 $\text{\AA}$
Glu -11, O _{ε2}	B	Arg 66, N _{η2}	2.8 $\text{\AA}$
Lys -10, N	C	Ala 101, O	2.9 $\text{\AA}$
Glu -7, O	C	Gln 41, N _{ε2}	2.9 $\text{\AA}$
Ala -5, O	C	Gln 41, N	2.8 $\text{\AA}$

^a Numbering of the tag and linker region are -15 to -1, with the Strep-tag II sequence encompassing residues -17 to -10. The original protein sequence of OmcF starts with residue Gly26, in accordance with the original gene sequence.

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