Supplemental Material

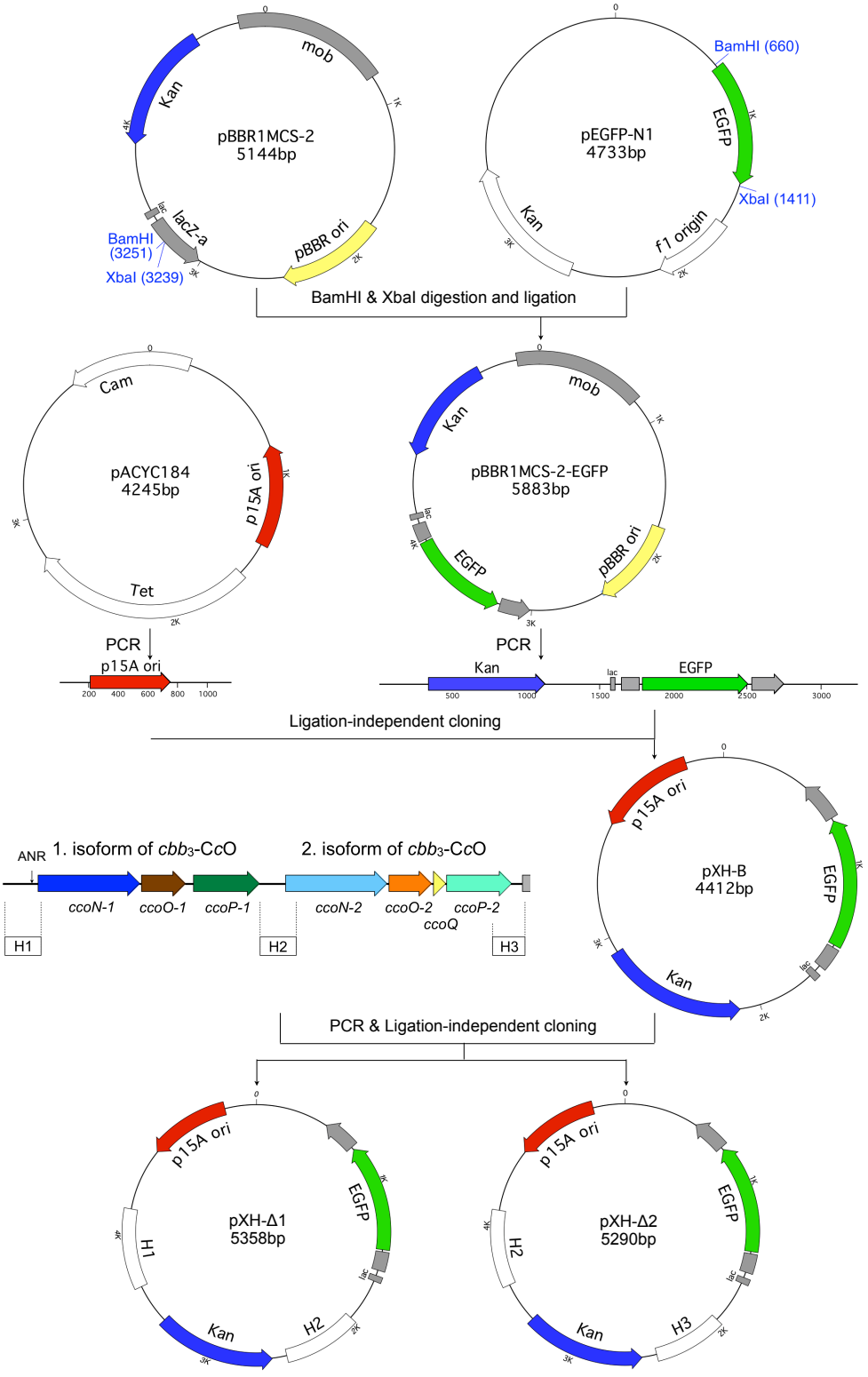

FIG S1: Schematic representation of the construction of the suicide plasmids. For details, see text.

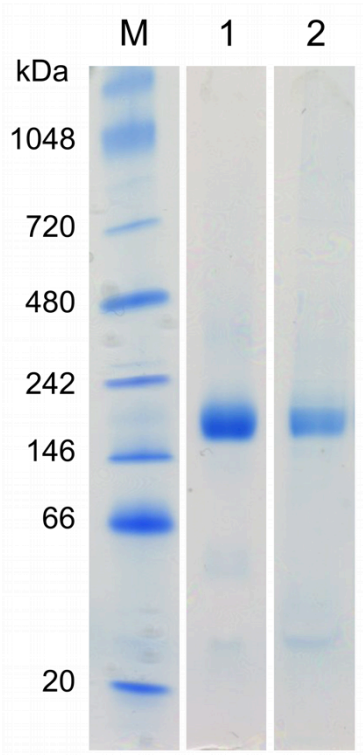

FIG S2: BN-PAGE gel of purified recombinant *cbb*$_3$-isoforms. Cbb$_3$-1 (lane 1) and Cbb$_3$-2 (lane 2) were solubilized and purified with DDM as described in Materials and Methods, and analyzed on a blue native PAGE gel (4-16% Bis-Tris gel). The molecular weights (kDa) of the marker proteins (lane M) are indicated on the left.

A N Subunit

1st isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-1)

```
1   MNTATS-TAY SYKVVRQFAI MTVVWGIVGM GLGVFIAAQL AWPFLNFDLP WTSFGRLRPL HTNAVIFAFG GCALFATSYY
81  SVQRTCQTTL FAPKLAAFTF WGWQLVILLA AISLPLGFTS SKEYAELEWP IDILITIVWV AYAVVFFGTL AKRKVKHIYV
161 GNWFFGAFIL TVAILHVVNN LEIPVTAMKS YSLYAGATDA MVQWWYGHNA VGFFLTAGFL GIMYYFVPKQ AERPVYSYRL
241 SIVHFWALIT VYIWAGPHHL HYTALPDWAQ SLGMVMSLIL LAPSWGGMIN GMMTLSGAWH KLRSDPILRF LVVSLAFYGM
321 STFEGPMMAI KTVNALSHYT DWTIGHVHAG ALGWVAMVSI GALYHLVPKV FGREQMHSIG LINTHFWLAT IGTVLYIASM
401 WVNGIAQGLM WRAINDDGTL TYSFVESLEA SHPGFVVRMI GGAIFFAGML VMAYNTWRTV QAAKPAEYDA AAQIASAWSH
481 PQFEK
```

2nd isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-2)

```
1   MSTAISETAY NYKVVRQFAI MTVVWGIIGM GLGVFIAAQL VWPSLNLDLP WTSFGRLRPL HTNAVIFAFG GCALFATSYY
81  VVQRTCQARL FSDGLAAFTF WGWQAVIVLA VITLPMGYTS SKEYAELEWP IDILITLVWV SYIAVFFGTI MKRKAKHIYV
161 GNWFFGAFIL VTAMLHIVNN LEIPVSLFKS YSIYAGATDA MVQWWYGHNA VGFFLTTGFL GMMYYFVPKQ AERPVYSYRL
241 SIVHFWALIT LYIWAGPHHL HYTALPDWAQ SLGMVMSIIL LAPSWGGMIN GMMTLSGAWH KLRTDPILRF LVVSLAFYGM
321 STFEGPMMAI KTVNALSHYT DWTIGHVHAG ALGWVAMITI GSMYHLIPKV FGREQMHSVG LINAHFWLAT IGTVLYIASM
401 WVNGITQGLM WRAINEDGTL TYSFVEALEA SHPGFIVRAV GGAFFLAGML LMAYNTWRTV RAAKSAQYDT AAQIASAWSH
481 PQFEK
```

B O Subunit

1st isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-1)

```
1   MKSHEKLEKN VGLLTLFMIL AVSIGGLTQI VPLFFQDSVN EPVEGMKPYT ALQLEGRDLY IREGCVGCHS QMIRPFRAET
81  ERYGHYSVAG ESVYDHPFLW GSKRTGPDLA RVGGRYSDDW HRAHLYNPRN VVPESKMPSY PWLVENTLDG KDTAKKMSAL
161 RMLGVPYTEE DIAGARDSVN GKTEMDAMVA YLQVLGTALT NKR
```

2nd isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-2)

```
1   MKNHEILEKN IGLLTLFMIL AVSIGGLTQI VPLFFQDAVN EPVEGMKPYT ALQLEGRDLY IREGCVGCHS QMIRPFRAET
81  ERYGHYSVAG ESVYDHPFLW GSKRTGPDLA RVGGRYSDDW HRAHLYNPRN VVPESKMPSY PWLVENTLDG KDTAKKMSAL
161 RMLGVPYTEE DIAGARDAVR GKTEMDAMVA YLQVLGTALT NKR
```

C P Subunit

1st isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-1)

```
1   MSTFWSGYIA LLTLGTIVAL FWLIFATRKG ESAGTTDQTM GHAFDGIEEY DNPLPRWWFL LFIGTLVFGI LYLVLYPGLG
81  NWKGVLPGYE GGWTQEKQWE REVAQADEKY GPIFAKYAAM SVEEVAQDPQ AVKMGARLFA NYCSICHGSD AKGSLGFPNL
161 ADQDWRWGGD AASIKTSILN GRIAAMPAWG QAIGEEGVKN VAAFVRKDLA GLPLPEGTDA DLSAGKNVYA QTCAVCHGQG
241 GEGMAALGAP KLNSAAGWIY GSSLGQLQQT IRHGRNGQMP AQQQYLGDDK VHLLAAYVYS LSQKPEQLAN Q
```

2nd isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-2)

```
1   MTSFWSWYVT LLSLGTIAAL VWLLLATRKG QRPDSTEETV GHSYDGIEEY DNPLPRWWFM LFVGTVIFAL GYLVLYPGLG
81  NWKGILPGYE GGWTQVKEWQ REMDKANEQY GPLYAKYAAM PVEEVAKDPQ ALKMGGRLFA SNCSVCHGSD AKGAYGFPNL
161 TDDDWLWGGE PETIKTTILH GRQAVMPGWK DVIGEEGIRN VAGYVR-SLS GRDTPEGISV DIEQGQKIFA ANCVVCHGPE
241 AKGVTAMGAP NLTDNV-WLY GSSFAQIQQT LRYGRNGRMP AQEAILGNDK VHLLAAYVYS LSQQPEQ
```

D Q Subunit

2nd isoform of *cbb*$_3$-*Cc*O (Cbb$_3$-2)

```
1   MMEIGTLRGL GTILVVVAFI GVVLWAYSSK RKQSFDEAAN LPFADDETDA KKREEEASRS KK
```

FIG S3: Peptide mass fingerprinting of the two *cbb*$_3$-isoforms (Cbb$_3$-1 and Cbb$_3$-2). Amino acid sequences of the four subunits CcoN, CcoO, CcoP and CcoQ, are shown in A-D, respectively. The first amino acid on each line is numbered at the left. The unique residues associated with both *cbb*$_3$-isoforms are shown as enlarged characters. The sequence coverage obtained using simultaneous nLC-ESI- and nLC-MALDI-MS/MS is indicated in red (ESI only), blue (MALDI only) and purple (detected in both ESI and MALDI-MS). The eight amino acids of the Strep-tag II are indicated by black underlining. The CcoQ subunit was detected only in Cbb$_3$-2.

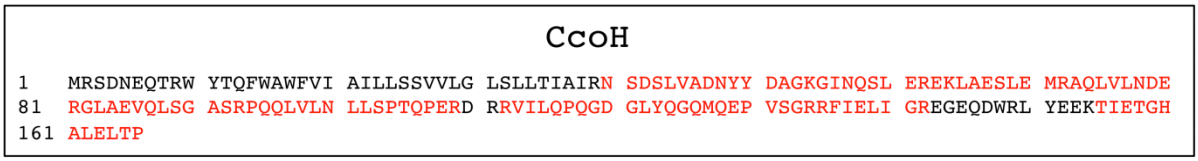

FIG S4: Sequence coverage obtained for the assembly protein CcoH in both isolated *cbb*$_3$-isoforms. The first amino acid on each line is numbered on the left. Peptides detected by ESI-MS peptide mass fingerprinting are shown in red.

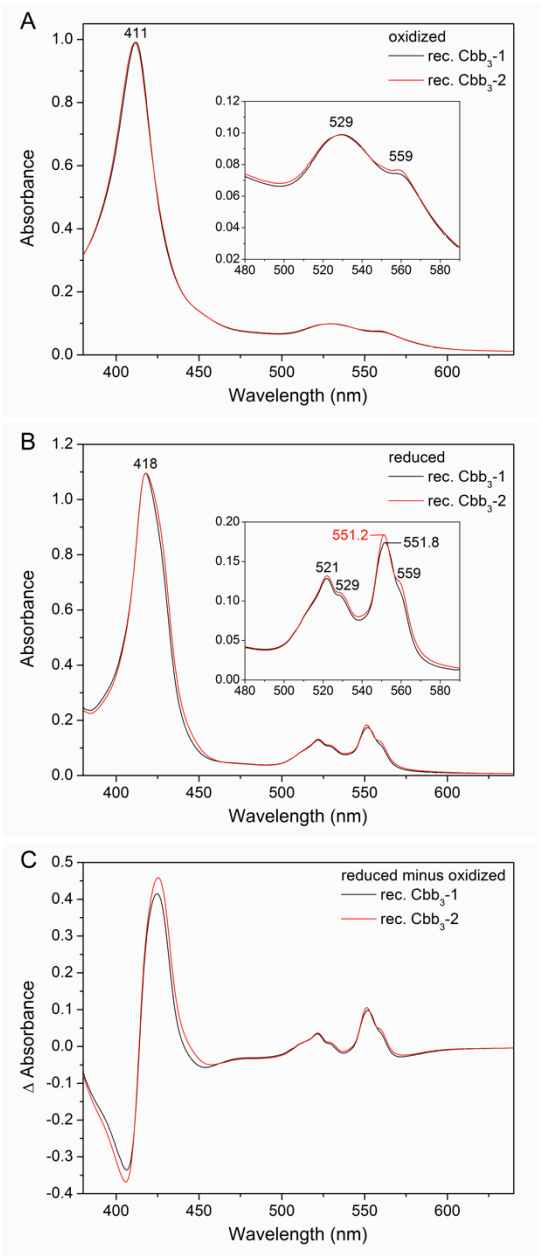

FIG S5: UV-Vis spectra of both recombinant *cbb*$_3$-isoforms. The absorption spectra of 2 µM recombinant Cbb$_3$-1 (black line) and Cbb$_3$-2 (red line) were recorded from 380 to 640 nm at 25°C. The insert shows an enlarged view of the α and β bands from 480 to 590 nm. (A) The *cbb*$_3$-C*c*Os are fully oxidized by adding potassium hexacyanoferrate (III). (B) The *cbb*$_3$-C*c*Os are fully reduced by adding sodium dithionite. (C) The reduced minus oxidized difference spectra of *cbb*$_3$-C*c*Os.

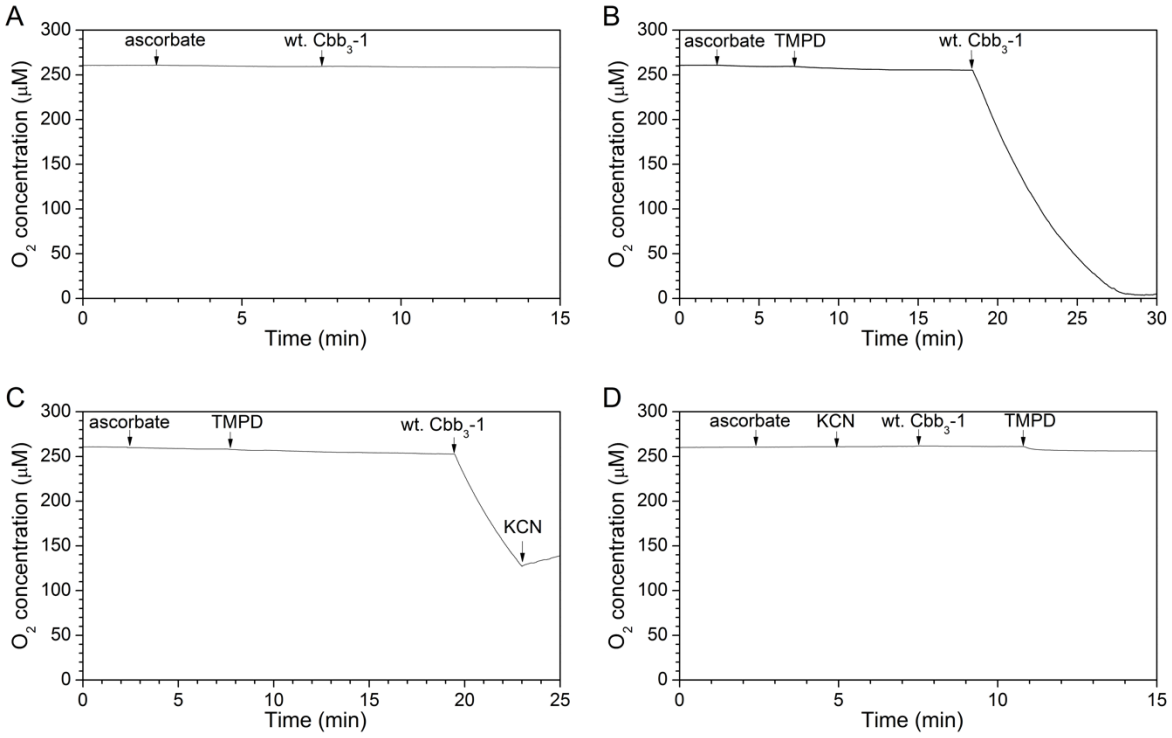

FIG S6: Measurements of oxygen reductase activity of *cbb*$_3$-C*c*O. The addition of 3 mM ascorbate, 1 mM TMPD, 8.3 nM *cbb*$_3$-C*c*O and 1 mM KCN is indicated by arrows. (A) No oxygen consumption was observed in the absence of TMPD. (B) The reaction was initiated by adding *cbb*$_3$-C*c*O to a reaction mixture containing ascorbate and TMPD. (C) Inhibition of the *cbb*$_3$-C*c*O by KCN during the turnover. After addition of KCN, the concentration of oxygen was slowly increased because of the back diffusion of oxygen in the reaction mixture. (D) No oxygen consumption was present when KCN was added before the reaction initiation.

TABLE S1: Oligonucleotides used in this work

	Oligonucleotide	Sequence (5'-3')[a]	Description of amplicon
Primers for generation of pXH-B	1_Fw pBBR	CCGCCCTATACCTTGTCTGC	Kan^r and EGFP from pBBR1MCS-2-EGFP
	1_Rev pBBR	GGAAGTCCAGCGCCAGAAAC	
	1_Fw 184 H2	TGGCGCTGGACTTCCGTGATGCTGCCAACTTACTG	p15A origin from pACYC184
	1_Rev 184 H1	CAAGGTATAGGGCGGGACGATGAGCGCATTGTTAG	
Primers for generation of pXH-Δ1 and pXH-Δ2	3181 CCW	TGCCACCTGGGATGAATGTC	linearized pXH-B, 5' insertion site
	3228 CW	TGTTTCTGGCGCTGGACTTC	
	1969 CCW	TCTCATGCTGGAGTTCTTCG	linearized pXH-B, 3' insertion site
	2016 CW	CGAAGCCCAACCTTTCATAG	
	3228 CW/cbb3I-F	CCAGCGCCAGAAACACTTGCAGATGGGCCACTCG	H1-flanking arm, 5' homologous regions
	3181 CCW/Cbb3I-R	TCATCCCAGGTGGCATGTATGGGCTTCCATCCAC	
	3228 CW/cbb3II-F	CCAGCGCCAGAAACACTCGCCGCCTATGTTTACAG	H2-flanking arm, 5' homologous regions
	3181 CCW/cbb3II-R	TCATCCCAGGTGGCACAGGCCCATCCCAATGATTC	
	2016 CW/cbb3II-F	AAAGGTTGGGCTTCGCTCGCCGCCTATGTTTACAG	H2-flanking arm, 3' homologous regions
	1969 CCW/cbb3II-R	AACTCCAGCATGAGACAGGCCCATCCCAATGATTC	
	2016 CW/cbb3III-F	AAAGGTTGGGCTTCGTGATGCCTGGCTGGAAAGAC	H3-flanking arm, 3' homologous regions
	1969 CCW/cbb3III-R	AACTCCAGCATGAGAGATACGTGCCAACCAGGATC	
Primers for generation of pXH22	7_Fw (M6263)	CTTGCAGATGGGCCACTCGAGGCTTGTC	Amplification of *ccoNOP*-1 from genomic DNA
	6_Rev (M6263)	AAACGGCGGCAAGTATGGAGAAAGCAG	
	1.C Strep Fw	CGCGCAGATCGCCAGCGCTTGGAGCCACCCGCAGTTCGAAAAATGAGGAGCCTAGG	Strep-tag II at C-terminus of *ccoN*-1
	1.C Strep Rev	CCTAGGCTCCTCATTTTTCGAACTGCGGGTGGCTCCAAGCGCTGGCGATCTGCGCG	
	ccoN-1-N BamHI	AGTGGATCCCAAGGCGCTCAGCCATTTCG	Clone of *ccoNOP*-1 + Strep-tag II into pBBR1MCS
	ccoP-1-C HindIII	CGTAAGCTTTCGCAGAGTAGCGGAAGTTG	
Primers for generation of pXH26	5_Fw (PCR101)	CGGCCTGGGGCCAAGCCATCGGCGAAG	Amplification of *ccoNOQP*-2 from genomic DNA
	4_Rev (PCR101)	AGTCATCGAGTGCGTACCACGGCGGAAG	
	2.C Strep Fw	CCGCGCAGATCGCTAGCGCTTGGAGCCACCCGCAGTTCGAAAAATGAGGAACGGATAG	Strep-tag II at C-terminus of *ccoN*-2
	2.C Strep Rev	CTATCCGTTCCTCATTTTTCGAACTGCGGGTGGCTCCAAGCGCTAGCGATCTGCGCGG	
	IF ccoN-2-N	TAGAACTAGTGGATCTCCGCTACTCTGCGACTATC	Clone of *ccoNOQP*-2 + Strep-tag II into pBBR1MCS
	IF ccoP-2-C	CGGTATCGATAAGCTGTCCAGCTGCGAATGGTACG	
Primers for generation of pXH39, promoter exchange	pXH22-Pro-N	GTATGGGCTTCCATCCAC	Promoter region from *ccoNOP*-1 (pXH22)
	pXH22-Pro-C	ATCGATACCGTCGACCTC	
	IF-Pro-N	GATGGAAGCCCATACATGAGCACAGCAATCAG	Structural genes from *ccoNOQP*-2 (pXH26)
	IF-Pro-C	GTCGACGGTATCGATGTCCAGCTGCGAATGGTACG	
General sequencing primers for *ccoNOP*-1	lacZa_C	TACGACTCACTATAGGGCGAATTGGAGC	
	1.Seq	CCTCGGGATCATGTACTACTTC	
	2.Seq	TCGGTGGTGCGATCTTCTTC	
	3.Seq	AGCCGTCAGGGACTTCAATCG	
	4.Fw	GCCATCGGCGAAGAAGGCGTGAAGAAC	
General sequencing primers for *ccoNOQP*-2	lacZa_C1	GCGGGCCTCTTCGCTATTAC	
	5.Seq	TTCTTCGGCACCATCATGAAGC	
	10.Fw	CAACGAAGACGGCACCCTGACCTACTCC	
	7.Seq	GGTAACGCATGATGGAAATCGG	
	lacZa_N1	AGGTTTCCCGACTGGAAAGC	

[a] Homologous regions used for the ligation independent cloning are shown in red. The nucleotide sequence encoding the Strep-tag II (with a two amino acid linker) is shown in blue. Restriction enzymes sites are underlined.

TABLE S2: Purification of Strep-tagged recombinant Cbb_3-1

Purification step	**Volume (ml)**	**Total protein concentration (mg/ml)[a]**	**Total protein (mg)**	**Total activity (U)[b]**	**Specific activity (U/mg)**	**Yield (%)**	**Purification fold**
Crude cell extract	250.0	13.66	3415.0	4052	1.2	-	-
Crude membrane	107.5	10.09	1084.7	3484	3.2	100	1.0
Solubilization	380.0	2.33	885.4	2913	3.3	82	1.0
Strep-Tactin	50.0	0.17	8.5	412	48.5	0.78	15.2
Q Sepharose	23.0	0.33	7.6	433	57.1	0.70	17.8
Superdex 200	9.6	0.78	7.5	544	72.6	0.69	22.7

[a] Protein concentration determined by BCA assay using BSA as protein standard.

[b] Enzyme activity was measured using an oxygen electrode. One U is defined as the amount of Cbb_3-1 required to reduce 1 μmol of O_2 in 1 min.