Supporting Information

Romão et al. 10.1073/pnas.1014298108

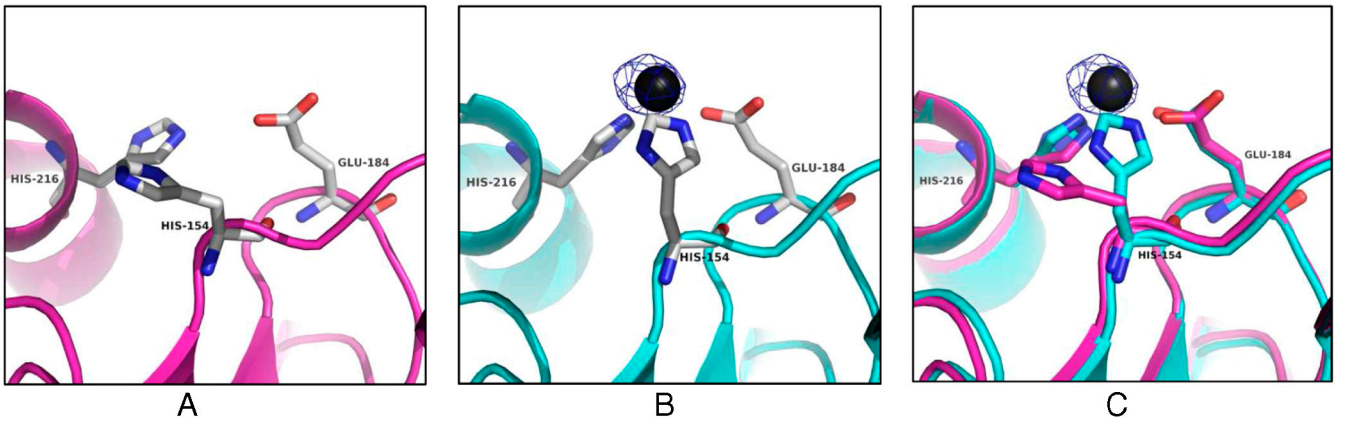

Fig. S1. The cobalt site in the *Desulfovibrio vulgaris* CbiK^{P} crystal structure is highlighted. (*A*) Native protein, (*B*) protein cocrystallized with cobalt (the cobalt ion is shown as a dark gray sphere), and (*C*) the superposition showing the shift of His154.

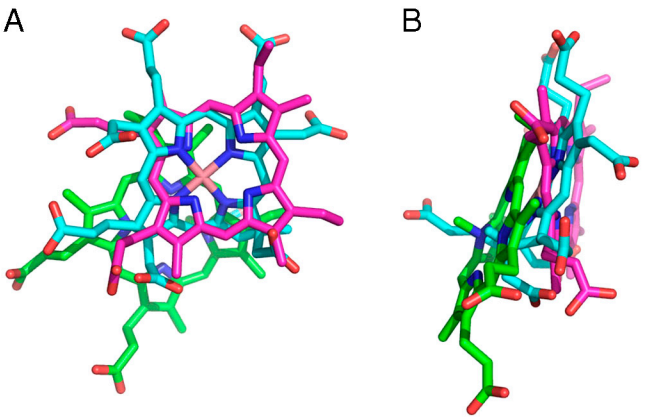

Fig. S2. The relative position and orientation of the porphyrins bound to cobalto-chelatase CbiK, human ferrochelatase and bacterial chelatase. This figure was produced by superimposing the chelatase structures CbiK, Glu343Lys mutant of the human ferrochelatase (PDB code 2HRE), and bacterial chelatase (PDB code 1C1H) using DaliLite and then drawing the porphyrins, metallated sirohydrochlorin bound to CbiK (cyan), protoporphyrin IX bound to human ferrochelatase (magenta), and N-methyl mesoporphyrin bound to bacterial ferrochelatase (green). *B* is rotated by 90 degrees around the y-axis compared to *A*. When looking down on the face of the tetrapyrrole ring (*A*), metallated SHC (cyan) is positioned close to protoporphryin IX (magenta) bound to the Glu343Lys mutant of human ferrochelatase but the C and D rings are toward the center of the molecule as opposed to rings B and C; i.e., it is rotated approximately 90°. The N-methyl mesoporphyrin does not bind so deeply and is rotated around by another approximately 90°.

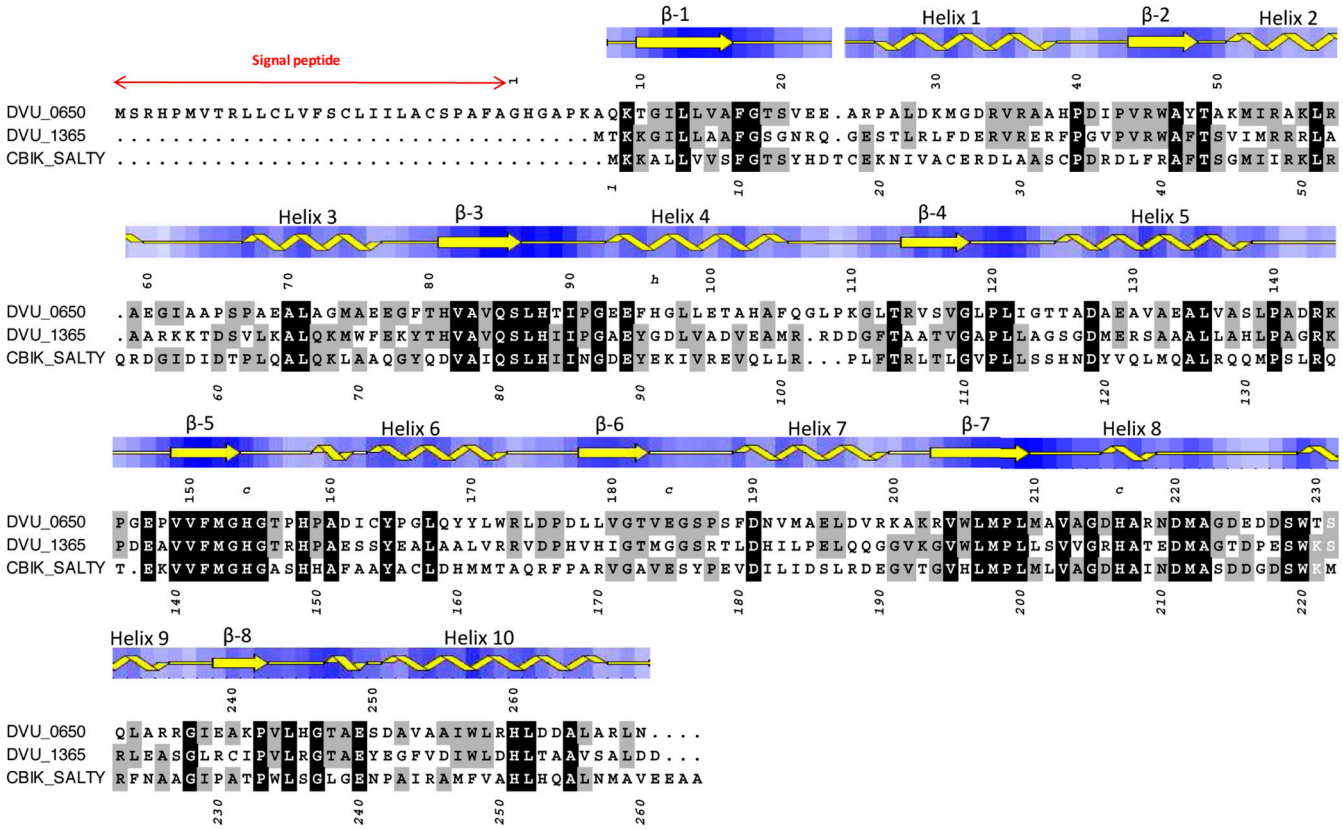

Fig. S3. Amino acid sequence alignment of Dv–CbiK^{P} (DVU0650) with Dv–CbiK^{C} (DVU1365) and Se–CbiK (CbiK SALTY). The numbering presented in the top and in the bottom of the alignment refers to residues of *D. vulgaris* CbiK^{P} and *Salmonella enterica* CbiK amino acid sequences, respectively. The secondary structure of *D. vulgaris* CbiK^{P} is displayed in yellow. The symbols *C* and (*) denote the *D. vulgaris* CbiK^{P} amino acid residues involved in heme and cobalt binding, respectively. The strictly conserved residues are within black boxes and the conserved residues are within dark gray boxes.

Table S1. Crystallographic data statistics

	Af–CbiX	Af–CbiX–SHC	Se–CbiK–SHC	Dv–CbiK	Dv–CbiK–Co			
Beamline	DLS	DLS	DLS	ESRF-ID14EH1	ESRF-BM14			
Wavelength (Å)	0.9835	1.0079	0.9780	0.9340	1.42363	1.60458	1.60556	1.61019
Space group	$P4_22_12$	$P2_12_12_1$	$P6_322$	I422	I422			
Cell parameters	51.2, 101.9	47.0, 99.0, 116.7	110.6, 89.5	121.1, 120.2	121.4, 121.0	121.4, 121.1	121.4, 121.0	121.4, 121.0
Molecules/au	1	4	1	1	1			
Resolution	29.5–1.6	50.3–2.0	47.9–2.0	42.8–1.9	49.45–2.4	49.45–2.4	49.45–2.4	49.45–2.4
*R_{merge}	0.054 (0.126)	0.147 (0.616)	0.116 (0.702)	0.085 (0.872)	0.067 (0.411)	0.092 (0.583)	0.082 (0.502)	0.093 (0.620)
†R_{pim}	0.023 (0.052)	0.061 (0.249)	0.061 (0.407)	0.016 (0.194)	0.036 (0.227)	0.031 (0.193)	0.035 (0.225)	0.050 (0.348)
Multiplicity	6.4 (6.7)	7.0 (6.8)	8.0 (6.5)	28.0 (20.3)	7.7 (7.6)	14.9 (14.6)	14.9 (14.6)	7.6 (7.4)
$I/s(I)$	23.6 (13.3)	8.2 (2.9)	10.4 (2.6)	7.6 (0.9)	10.9 (1.9)	10.9 (1.9)	10.9 (1.9)	10.9 (1.9)
Wilson B (Å^2)	13.6	25.3	23.9	25.5	36.2	38.2	37.0	35.5
Completeness (%)	99.4 (100.0)	100.0 (100.0)	99.4 (97.4)	99.9 (99.2)	97.5 (96.6)	99.8 (98.5)	99.8 (98.6)	98.0 (98.8)
Observed reflections	118464 (17616)	253190 (37169)	176900 (20356)	988948 (102646)	134448 (18669)	268739 (37274)	268613 (37210)	133606 (18422)
Unique reflections	18511 (2649)	37028 (5432)	22224 (3111)	35365 (5059)	17470 (2469)	18035 (2546)	18046 (2556)	17558 (2491)

Values in parentheses are for the highest resolution shell.

*$R_{merge} = (\Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|)/(\Sigma_{hkl}\Sigma_i I(hkl))$, where $I_i(hkl)$ is the intensity measured for a each unique Bragg reflection with indices (hkl), $\langle I(hkl)\rangle$ is the average intensity for multiple measurements of this reflection.

†$R_{pim} = \Sigma_{hkl}[n/(n-1)]^{1/2}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i I_i(hkl)$ (1), where n is the multiplicity, other variables as defined for R_{merge}.

1 Weiss, MS (2001) Global indicators of X-ray data quality. *J Appl Crystallogr* 34:130–135.

Table S2. Refinement statistics

	Af–CbiX	Af–CbiX–SHC	Se–CbiK–SHC	Dv–CbiK	Dv–CbiK–Co
Resolution (Å)	1.6	2.0	1.9	1.9	2.4
R-factor (%)	18.7	21.9	19.8	17.4	17.0
R-free (%)	21.5	28.2	24.2	19.9	22.0
Rmsd bonds (Å)	0.029	0.021	0.024	0.020	0.020
Rmsd angles (°)	2.402	2.384	2.179	1.767	1.989