Supplementary Information for

An enzyme-trap approach allows isolation of intermediates in cobalamin biosynthesis.

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Supplementary Methods.

NMR.

For the data collection on precorrin-7, precorrin-8 and HBA resonance assignments were completed using $^{13}$C-$^1$H HSQC and HMBC as well as $^1$H-$^1$H DQF-COSY, ROESY, NOESY and TOCSY NMR experiments. $^{13}$C-$^1$H HSQC datasets were recorded using $^1$H and $^{13}$C spectral widths of 6000.6 Hz and 18000.1 Hz with 1536 and 256 complex pairs, respectively. $^{13}$C-$^1$H HMBC datasets were recorded using $^1$H and $^{13}$C spectral widths of 6000.6 Hz and 36215.5 Hz with 1536 and 200 complex pairs, respectively. All $^1$H-$^1$H correlation datasets were recorded with both direct and indirect dimension spectral widths of 6000.6 Hz and 4096 and 256 complex pairs, respectively.

Precorrin-8 and HBA assignments

Evidence for tautomerism was visible in both precorrin-8 and HBA NMR data but this was less problematic and widespread as that observed for precorrin-7. Assignments for precorrin-8 and HBA were completed via the confirmation of contacts between neighbouring groups. Previously published assignments were used to support findings, but differences were seen, in particular for methylene groups (Supplementary Table 1).

NMR analysis of precorrin-8 and HBA fully supports a methylation at position C5 with HMBC contacts evident between the two methyl groups at positions C5 and C7. The C5 methylation position is also in agreement with assignments published previously$^1$. All methyl and methine positions could be assigned with additional assistance of DQF-COSY, TOCSY and HMBC datasets. The methine at position C3 was not visible in the HSQC but was present in the DQF-COSY and contacts were also confirmed through
HMBC data supporting line broadening being evident in the HSQC due to conformational exchange. All methylenes could be assigned but not unambiguously.

**NMR analysis of CobE in the presence and absence of intermediates.**

For the analysis of intermediates binding to CobE, all NMR data were obtained at 298 K using a 14.1 T (600 MHz $^1$H) Bruker Avance III NMR spectrometer equipped with a QCI-F cryoprobe. Standard $^{15}$N,$^1$H HSQC experiments were acquired of CobE in the presence and absence of intermediates. NMR assignments for tetrapyrroles were obtained using $^1$H homonuclear DQF-COSY, NOESY (500 ms) and TOCSY (80 ms) and $^{13}$C,$^1$H heteronuclear HSQC, HMBC and HSQC-TOCSY (80 ms) experiments. Mixing/constant time periods are shown in parentheses. NMR data processing was obtained using TopSpin 3.0 and NMR data analysis using the CCPN analysis suite, version 2.0.

$^{15}$N,$^1$H HSQC spectra of CobE before and after addition of HBA, precorrin-7 and precorrin-6B (Supplementary Fig. 4a-d) support binding of the intermediates by this protein and significant structural change in CobE upon interaction as signified by the changes in chemical shifts. In addition, across Supplementary Fig. 4a-c these chemical shift changes in the HSQC of CobE shown similarities and differences upon binding each intermediate as shown in Supplementary Fig. 4d. Although these data support a common binding site in CobE, it also suggests the protein adapts to each intermediate it binds as the chemical shift changes are subtly different across all three intermediates. This will also reflect the relative affinities of each intermediate for CobE.

**NMR Titration of CobE with HBA**
Constant CobE concentration of 0.2 mM and HBA concentrations of 0.0, 0.00625 mM, 0.0125 mM, 0.025 mM, 0.05 mM, 0.1 mm, 0.15 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, 0.8 mM and 1.0 mM. Two binding events were identified due to an insufficient simultaneous fit of all data to the below equation. Therefore, each event was individually fitted to the known equation\(^2\) using Kaleidagraph software version 4.2:

\[
\Delta = \Delta_o \left( \frac{K_d + [L] + [P]}{2[P]} \right) - \sqrt{\left(\frac{(K_d + [L] + [P])^2 - 4[P][L]}{2[P]}\right)}
\]

where \(\Delta\) is the observed change in chemical shift, \(\Delta_o\) is the maximum shift and \([L]\) and \([P]\) are ligand and protein concentrations. Levenberg–Marquardt fitting was used to obtain both \(\Delta_o\) and \(K_d\) and optimal data fits are shown in Figure S4 where NMR titration data identifies a primary tight binding event and secondary loose binding event. The tight binding event correlates with fluorescence quenching data for CobE with HBA (Supplementary Fig. 5) despite data being influenced by the secondary binding event.

**Crystallography, CobE.**

The gene encoding the *Pseudomonas aeruginosa* CobE was cloned and overexpressed, and the protein purified and crystallized as described earlier\(^3\). To express Se-Met labelled protein, the *cobE::pET14b* vector was transformed to the methionine auxotroph *E. coli* B834 (DE3) host strain.

The same purification protocol was used for both native and Se-Met-modified proteins. The sonicated cells were centrifuged at 14000 \(\times\) g to remove cell debris and the CobE
containing supernatant (further clarified using 0.45 µl filters) was applied to a nickel resin HiTrap Chelating HP 5 ml column (Pharmacia) for purification. The column was washed with buffer containing 20 mM TRIS-HCl, pH 8.5, containing 0.2 M NaCl and 1 mM β-mercaptoethanol. The protein was eluted from the column with a 10-500 mM imidazole gradient. The N-terminal His₆-tag was cleaved by digestion with thrombin overnight at room temperature and removed using a HiTrap Chelating HP 5 ml column. The fractions containing CobE were loaded onto a Superdex 75 gel filtration column which had been previously equilibrated with 20 mM TRIS-HCl buffer pH 8.5, containing 0.2 M NaCl and 10 mM ditho-1,4-threitol (DTT). The protein eluted as a single peak and the relevant fractions were pooled and concentrated to 12 mg ml⁻¹.

Sitting-drop vapour diffusion was used to screen the crystallization conditions with pure CobE concentrated to 12 mg ml⁻¹. Initial screening was conducted using Crystal Screen 1, Crystal Screen 2, PEG/Ion Screen (Hampton Research) and Clear Strategy Screen. Those conditions that resulted in the most promising types of precipitate were subsequently optimized by application of hanging-drop vapour diffusion, with a range of pH and types and concentration of precipitant. Crystals suitable for X-ray diffraction were obtained from 0.1 M MES buffer pH 6.9, 2 M ammonium sulfate and 5% dioxane in the form of clusters of rods of average dimensions 0.4 x 0.2 x 0.1 mm³ which appeared in the drops after 5-10 days. The Se-Met-containing protein crystallized under the same conditions as the native.

Crystals were mounted in nylon CryoLoop (Hampton Research), immersed in mother liquor supplemented with 27% (v/v) glycerol as cryoprotectant and vitrified. Complete data sets were collected at ESRF ID14-1 at 100 K with an ADSC Quantum 4 detector.
The crystals diffracted X-rays to a resolution of 1.9 Å and belong to the primitive orthorhombic space group $P2_12_12_1$, with unit cell parameters $a = 31.86$, $b = 41.07$, $c = 87.41$ Å. There is one molecule per asymmetric unit giving a Matthews coefficient of 1.9 Å$^3$/Da, corresponding to a solvent content of ~ 35%.

The crystals of the Se-Met-labelled protein are isomorphous to the native with essentially identical cell parameters and diffract to a resolution of 1.7 Å. A single wavelength dataset was collected at 100 K on beam line ID14-1 at the ESRF with an ADSC Quantum 4 detector.

After the peak corresponding to a single selenium site was found in the anomalous difference Patterson synthesis based on the single wavelength dataset, three-wavelength MAD data to a resolution of 2.5 Å were collected on beam line BM14 at the ESRF at 100 K using a MarMosaic CCD detector. Based on the absorption spectrum for the Se K edge, two energies were selected: the peak (0.97925 Å) and the inflection point (0.97944 Å). A third energy was selected at a wavelength point remote from the absorption edge, 0.95372 Å. All intensity data were indexed, integrated and scaled using the HKL programs DENZO and SCALEPACK.
Supplementary Results

Supplementary Figure 1. SDS-PAGE of purified proteins trapping intermediates.

D, SDS7 Dalton marker (Sigma), molecular weight are indicated on the left side of the gel in kDa. J, CobJ* (28.5 kDa) trapping Factor IV; M, CobM* (29.8 kDa); F, CobF* (29.6 kDa); K, CobK* trapping precorrin-6B (28.3 kDa); L, CobL* (43.9 kDa); H, CobH* trapping HBA (24.1 kDa); E, CobE* (15.8 kDa). E1, CobE* trapping precorrin-7; E2, CobE* trapping precorrin-8 and E3, CobE* trapping HBA.
Supplementary Figure 2. Expression and characterization of CobH-HBA complex.

A plasmid encoding CobA-I-G-J-M-F-K-L-H* allows the production of HBA within bacterial cells giving them an orange color. Purification of the His-tagged CobH on a nickel column gives rise to an orange colored solution that can be used for protein crystallization studies. Crystallographic studies resulted in the structure determination of the HBA-bound CobH.
Supplementary Figure 3. Role of CobE in the stabilization of precorrin-8 and the structure determination of the protein.

Figure 3a. Spectrum of free precorrin-8 recorded over a period of 15 hours, after t = 0, 1, 3, 5, 7, 9 and 15 hours. The main absorption peak at 418 nm decreases with time. Spectra were recorded every two hours but essentially all of the precorrin-8 had bleached by the end of the period.
**Supplementary Figure 3b.** Stabilization of precorrin-8 by CobE. When bound to CobE precorrin-8 absorbs around 389 nm but the compound is largely stabilized such that only about 20% is lost over the 15 hour period in comparison to (a) above.
**Supplementary Figure 3c.** Transformation of precorrin-6B into HBA by incubation with CobL and CobH. Precorrin-6B (black spectrum) is transformed initially into precorrin-8 and then into HBA (orange spectrum). Spectra were recorded after 0, 10, 20 minutes and 1, 6 and 14 hours.
Supplementary Figure 3d. Enhancement of HBA production from precorrin-6B in presence of CobE. The same experiment as in (c) above except it was conducted in the presence of CobE. The yield of HBA is about twice that observed with the incubation in the absence of CobE.
Supplementary Figure 4. NMR spectra of CobE in the presence and absence of bound intermediates.

Figure 4a. $^{15}$N,$^1$H HSQC of 0.4 mM CobE at 25°C/pH 7.5 in the absence (blue) and presence (red) of HBA (1:1 stoichiometric ratio)
Supplementary Figure 4b. $^{15}\text{N,}^{1}\text{H} \text{HSQC}$ of 0.4 mM CobE at 25°C/pH 7.5 in the absence (blue) and presence (red) of precorrin-6B.
Supplementary Figure 4c. $^{15}$N,$^1$H HSQC of 0.4 mM CobE at 25°C/pH 7.5 in the absence (blue) and presence (red) of precorrin-7.
Supplementary Figure 4d. $^{15}$N,$^1$H HSQC of 0.4 mM CobE at 25°C/pH 7.5 in the presence of three different intermediates, HBA (blue), precorrin-6B (red) and precorrin-7 (green).
Supplementary Figure 5. Fluorescence quenching of HBA with CobE.

Kₐ = 0.077 ± 0.015 μM. The titration of CobE into HBA was performed at a constant concentration of 0.2 μM in 20 mM Hepes pH7.5 and 100 mM NaCl. The quenching of HBA fluorescence was monitored using a FluoStar optima (BMG Labtech) plate reader (excitation at 520 nm, emission at 600-620 nm).
Supplementary Figure 6. NMR spectra of CobE at different concentrations of HBA. 

$^{15}$N HSQC spectra of CobE + HBA at 0.0, 0.00625 mM, 0.0125 mM, 0.025 mM, 0.05 mM (a) and 0.1 mM, 0.15 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, 0.8 mM and 1.0 mM (b).

(a) PRIMARY BINDING EVENT, $K_d = 120$ n
(b) SECONDARY BINDING EVENT, $K_d = 309 \mu M$
Supplementary Figure 7. Activity of CobL variants.

CobK:precorrin-6B (PC6B) was incubated with a number of CobL variants in the presence of S-adenosylmethionine. The spectra colored in black, with a maximum at 348nm, are typical of precorrin-6B bound to CobK. The blue spectra are precorrin-7 (max at 373nm) while the red spectra are precorrin-8 (max at 381). All species were analyzed by mass spectroscopy to confirm their identity.
Supplementary Figure 8. Sequence alignment of CobL and orthologues from the anaerobic pathway.

Amino-acid sequence alignment of the *R. capsulatus* CobL with the anaerobic pathway orthologues CbiE and CbiT from *Salmonella enterica*. The sequence of the dissected *cobL* gene products, CobL\(^N\) and CobL\(^C\), are also shown for completeness. CobL\(^N\) is the N-terminal part of CobL corresponding to CbiE, whereas CobL\(^C\) is the C-terminal part of CobL corresponding to CbiT. The two independent point mutations, G257R and E276A, inactivate the C-terminal region of the protein by disrupting the S-adenosylmethionine binding site and therefore are active CbiE only-like proteins. The secondary structure elements are represented above the multiple alignment.
Supplementary Figure 9. Modeling of full-length CobL based on CobL<sup>C</sup> and CbiE. The structure of CobL modeled using the solved structure of the C-terminal domain, which forms a 222 tetramer, plus two dimers of the closest canonical methyltransferase, *Archaeglobus fulgidus* CbiE. Each of the four protein chains in the tetramer are in a different color with SAH represented as stick model.
Supplementary Figure 10. Major and minor forms of precorrin-7

NMR analysis reveals that precorrin-7 exists in a major and minor tautomeric form.
Supplementary Figure 11. HSQC, HMBC and TOCSY NMR spectra of precorrin-7.
$^{13}$C-$^1$H HMBC Precorrin-7

$^{13}$C chemical shift / ppm

$^1$H chemical shift / ppm
$^1$H-$^1$H TOCSY Precorrin-7
Supplementary Figure 12. UV-vis spectra of HBA and C5 desmethyl-HBA.

C5-Desmethylhydrogenobyrinic acid

Hydrogenobyrinic acid

DesMethyl-HBA 867.4 (M+H)

HBA 881.4 (M+H)
Supplementary Tables

**Supplementary Table 1a. NMR assignment tables for HBA**

### CH₃ assignments

| ¹³C signal | δ¹³C (ppm) | δ¹'H (ppm) |
|------------|------------|------------|
| C1         | 21.89      | 1.205      |
| C2         | 16.23      | 1.357      |
| C5         | 13.75      | 2.110      |
| C7         | 18.89      | 1.492      |
| C12'       | 18.72      | 1.288      |
| C12''      | 30.89      | 1.093      |
| C15        | 13.76      | 2.161      |
| C17        | 19.30      | 1.335      |

### CH assignments

| ¹³C signal | δ¹³C (ppm) | δ¹'H (ppm) |
|------------|------------|------------|
| C3         | 55.73      | 3.250      |
| C8         | -          | -          |
| C10        | 89.46      | 5.592      |
| C13        | 52.91      | 3.159      |
| C18        | 40.28      | 2.855      |
| C19        | 68.60      | 4.309      |

### CH₂ assignments

Brackets represent more than one possible assignment due to overlap. The most likely assignment proceeds in the brackets.

| ¹³C signal | δ¹³C (ppm) | δ¹'H (ppm) |
|------------|------------|------------|
| C2'        | 43.42      | 2.378      |
| C3'        | 24.49      | 2.066      |
| C3''       | 34.55      | 2.566      |
| C7'        | 44.64      | 2.570      |
| C8'        | 34.50      | (2.471, 2.568) |
| C8''       | 34.50      | (2.568, 2.471) |
| C13'       | 24.01      | (1.846, 2.066) |
| C13''      | 24.01      | (2.066, 1.846) |
| C17'       | 33.92      | 2.627      |
| C17''      | 31.18      | 2.313      |
| C18'       | 33.95      | 2.624      |
Supplementary Table 1b. NMR assignment tables for Precorrin-8

**CH₃ assignments**

| ¹³C signal | δ ¹³C (ppm) | δ ¹H (ppm) |
|------------|-------------|-------------|
| C1         | 19.39       | 1.469       |
| C2         | 16.74       | 1.110       |
| C5         | 18.41       | 2.036       |
| C7         | 18.78       | 1.330       |
| C11        | 26.09       | 1.551       |
| C12        | 11.60       | 2.100       |
| C15        | 18.72       | 1.615       |
| C17        | 19.03       | 1.164       |

**CH assignments**

| ¹³C signal | δ ¹³C (ppm) | δ ¹H (ppm) |
|------------|-------------|-------------|
| C3         | 57.85       | 3.210       |
| C15        | 37.11       | 4.087       |
| C18        | 39.20       | 2.506       |
| C19        | 81.56       | 4.298       |

**CH₂ assignments**

Brackets represent more than one possible assignment due to overlap. The most likely assignment proceeds in the brackets.

| ¹³C signal | δ ¹³C (ppm) | δ ¹H (ppm) |
|------------|-------------|-------------|
| C2’        | 57.41       | 3.586       |
| C3’        | 39.29       | (2.842, 3.427) |
| C3’’       | 39.28       | (3.427, 2.842) |
| C7’        | 39.81       | 3.051       |
| C8’        | (25.48, 29.26) | (2.153, 2.377) |
| C8’’       | (29.26, 25.48) | (2.377, 2.153) |
| C10        | 34.26       | 2.488       |
| C13’       | 21.73       | 3.673       |
| C13’’      | 21.70       | 2.876       |
| C17’       | 34.90       | (1.637, 1.912) |
| C17’’      | 34.90       | (1.912, 1.637) |
| C18’       | 37.89       | 2.621       |
Supplementary Table 1c. NMR assignment tables for Precorrin-7.

All assignments are for the major form of precorrin-7 (a) unless otherwise stated (b)

**CH$_3$ assignments**

| $^{13}$C signal | $\delta^{13}$C (ppm) | $\delta^1$H (ppm) |
|-----------------|----------------------|-------------------|
| C1              | 20.16                | 1.509             |
| C2              | 27.41                | 1.350             |
| C7              | 20.44                | 1.298             |
| C11             | 21.49                | 1.418             |
| C12             | 25.08                | 1.242             |
| C15             | 19.48                | 1.162             |
| C17             | 20.29                | 1.163             |

**CH assignments**

| $^{13}$C signal | $\delta^{13}$C (ppm) | $\delta^1$H (ppm) |
|-----------------|----------------------|-------------------|
| C3              | 57.91                | 3.894             |
| C5              | 60.63                | 3.251             |
| C8 (b form)     | 53.74                | 3.354             |
| C15             | 60.16                | 3.633             |
| C18             | 54.45                | 3.034             |
| C19             | 49.76                | 3.171             |

**CH$_2$ assignments**

Brackets represent more than one possible assignment due to overlap. The most likely assignment proceeds in the brackets.

| $^{13}$C signal | $\delta^{13}$C (ppm) | $\delta^1$H (ppm) |
|-----------------|----------------------|-------------------|
| C2'             | 42.87                | 2.980             |
| C3'             | -                    | -                 |
| C3''            | -                    | -                 |
| C7'             | (33.57, -)           | (1.772, 1.918, - )|
| C8'             | (33.57, -)           | (1.772, 1.918, - )|
| C8''            | (33.57, -)           | (1.772, 1.918, - )|
| C10             | (21.62, 37.80)       | 2.637             |
| C13'            | 44.64                | 2.922             |
| C13''           | 44.56                | 2.426             |
| C17'            | (34.10, 13.43)       | (3.103, 2.018)    |
| C17''           | (13.43, 34.10)       | (2.018, 3.103)    |
| C18'            | 21.47                | 1.728             |
**Supplementary Table 2.** List of strains and plasmids

| Description                          | Reference            |
|--------------------------------------|----------------------|
| JM109                                | Promega              |
| BL21 star (DE3) pLysS                | Invitrogen           |
| B834(DE3)                            | Novagen              |
| pET3a                                | Novagen              |
| pET14b                               | Novagen              |
| pET3a/cobA-I-G-J-M-F-K               | This study           |
| pET3a/cobA-I-G-J-M-F-K-L             | This study           |
| pET3a/cobA-I-G-J*                    | This study           |
| pET3a/cobA-I-G-J-M*                  | This study           |
| pET3a/cobA-I-G-J-M-F*                | This study           |
| pET3a/cobA-I-G-J-M-F-K*              | This study           |
| pET3a/cobA-I-G-J-M-F-K-L*            | This study           |
| pET3a/cobA-I-G-J-M-F-K-L^c*          | This study           |
| pET3a/cobA-I-G-J-M-F-K-L-E*          | This study           |
| pET3a/cobA-I-G-J-M-F-K-L^E*          | This study           |
| pET3a/cobA-I-G-J-M-F-K-L-H*          | This study           |
| pET3a/cobA-I-G-J-M-F-K-L^H*          | This study           |
| pET3a/cobA-I-G-J-M-F-K-L^H-E*        | This study           |
| pET3a/cobA                          | This study           |
| pET3a/cobI                          | This study           |
| pET14b/cobE from B. melitensis       | This study           |
| pET14b/cobE from P. aeruginosa       | Vévodová *et al*     |
| pET3a/cobG from P. denitrificans or B. melitensis | Schroeder *et al* |
| pET14b and pET3a/cobF               | This study           |
| pET14b and pET3a/cobM               | This study           |
| pET14b and pET3a/cobK               | This study           |
| pET14b and pET3a/cobL               | This study           |
| pET14b and pET3a/cobH               | This study           |
| pET14b/cobL_{G257R}                 | This study           |
| pET14b/cobL_{E276A}                 | This study           |
| pET14b/cobL_{N} (cbiE like)          | This study           |
| pET14b and pET3a/cobL^C (cbiT like)  | This study           |

All the genes are from *R. capsulatus* except cobG, which is either from either *P. denitrificans* or *B. melitensis*, and cobE, which is either from *B. melitensis* or *Pseudomonas aeruginosa*. An asterix indicates that the gene is fused to a Hexa-Histidine-tag coding sequence. Sequencing analysis was performed by GATC Biotech in Germany.
### Supplementary Table 3a Data collection, phasing and refinement statistics for CobE

| Data collection | Native | Crystal 1 | Se-Met |
|-----------------|--------|-----------|--------|
| **Space group** | P2₁2₁2₁ | P2₁2₁2₁ |        |
| **Cell dimensions** |        |           |        |
| \(a, b, c (\text{Å})\) | 31.9, 41.1, 87.4, 90.0, 90.0, 90.0 | 31.7, 41.4, 88.0, 90.0, 90.0, 90.0 |        |
| \(a, b, g (^{\circ})\) | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 |        |
| **Wavelength** | 0.9340 | 0.97925 | 0.97944 |
| **Resolution (Å)** | 50-1.9 | 35-2.5 | 35-2.5 |
| **\(R_{\text{sym}}\) or \(R_{\text{merge}}\)** | 0.075 | 0.050 | 0.043 |
| **\(I / \bar{I}\)** | 26.8 | 30.9 | 34.5 |
| **Completeness (%)** | 98.3 | 99.7 | 99.8 |
| **Redundancy** | 7.0 | 13.3 | 3.7 |
| **Refinement** | | |        |
| **Resolution (Å)** | 45.0-1.70 |        |        |
| **No. reflections** | \(R_{\text{work}} / R_{\text{free}}\) | 0.19/0.27 |        |
| **No. atoms** | | |        |
| Protein | 1194 |        |        |
| Ligand/ion | 2 sulfate, 3 glycerol |        |        |
| Water | 97 |        |        |
| **B-factors** | | |        |
| Protein | 31.9 |        |        |
| **R.m.s. deviations** | | |        |
| Bond lengths (Å) | 0.015 |        |        |
| Bond angles (°) | 1.759 |        |        |

One native and one Se-Met labeled crystal were used. *Highest-resolution shell is shown in parentheses.*
**Supplementary Table 3b**  Data collection and refinement statistics for CobL$^C$

| **Data collection** | **CobL$^C$** |
|---------------------|--------------|
| Space group         | C2           |
| Cell dimensions     |              |
| $a$, $b$, $c$ (Å)   | 114.35, 44.80, 84.11 |
| $a$, $b$, $g$ (°)   | 90.0, 119.75, 90.0 |
| Resolution (Å)      | 72.93-2.70   |
| $R_{sym}$ or $R_{merge}$ | 6.0(24.1) |
| $I / sI$            | 14.6(5.3) |
| Completeness (%)    | 99.9(100.0) |
| Redundancy          | 3.6(3.7) |

| **Refinement**      |              |
| Resolution (Å)      | 2.7          |
| No. reflections     | 10402        |
| $R_{work}$ / $R_{free}$ | 18.5/26.5  |
| No. atoms           |              |
| Protein             | 2790         |
| Ligand/ion          | 2 SAH, 2 glycerol, 1 tetraethylene glycol |
| Water               | 31           |
| $B$-factors         |              |
| Protein             | 38.6         |
| R.m.s. deviations   |              |
| Bond lengths (Å)    | 0.0125       |
| Bond angles (°)     | 1.606        |

One crystal was used. *Highest-resolution shell is shown in parentheses.
Supplementary Table 3c. Data collection and refinement statistics for CobH complexes

|                        | CobH/HBA          | CobH/5-desmethyl HBA |
|------------------------|-------------------|----------------------|
| **Data collection**    |                   |                      |
| Space group            | C2                | C2                   |
| **Cell dimensions**    |                   |                      |
| a, b, c (Å)            | 70.3, 66.0, 48.5  | 70.5, 66.7, 48.1     |
| a, b, g (°)            | 90.0, 90.0, 90.0  | 90.0, 99.3, 90.0     |
| Resolution (Å)         | 47.89-1.68        | 47.79-1.45 (1.49-1.77) |
|                        | (1.77-1.68) *     | 1.45                 |
| **Rsym or Rmerge**     | 5.9(17.4)         | 2.9(45.8)            |
| **I / sI**             | 16.9(6.6)         | 20.0(2.2)            |
| **Completeness (%)**   | 97.4(95.1)        | 97.8(98.3)           |
| **Redundancy**         | 4.4(4.1)          | 3.4(2.4)             |
| **Refinement**         |                   |                      |
| **Resolution (Å)**     | 1.68              | 1.45                 |
| **No. reflections**    | 24344             | 37503                |
| **Rwork / Rfree**      | 16.6/20.5         | 16.1/17.8            |
| **No. atoms**          |                   |                      |
| Protein                | 1541              | 1592                 |
| Ligand/ion             | HBA, 2 glycerol   | Des-HBA, 1 sulfate   |
| Water                  | 223               | 279                  |
| **B-factors**          |                   |                      |
| Protein                | 17.4              | 21.1                 |
| **R.m.s. deviations**  |                   |                      |
| Bond lengths (Å)       | 0.011             | 0.010                |
| Bond angles (°)        | 1.82              | 1.08                 |

* One crystal was used for each structure. *Highest-resolution shell is shown in parentheses.

References

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