Supporting Information

Koutmos et al. 10.1073/pnas.0906132106

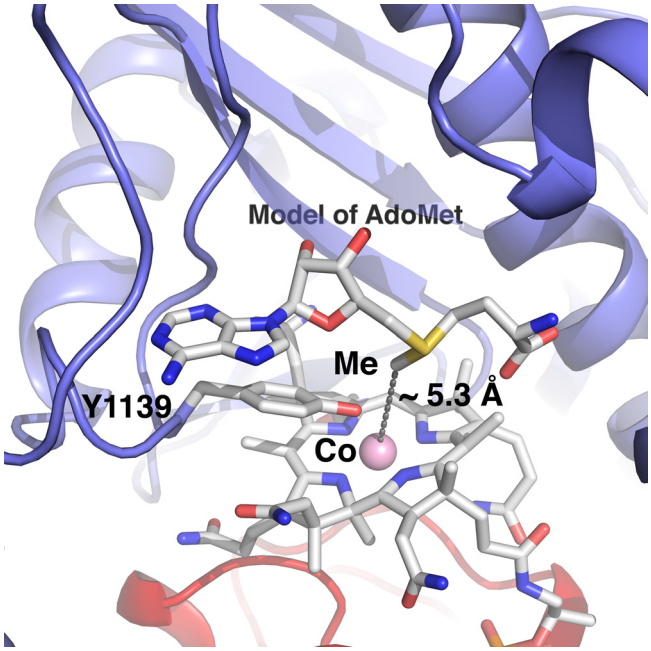

Fig. S1. AdoMet was modeled in the AdoMet/AdoHcy pocket on the basis of the position of the AdoHcy in the Co(II)Cbl $_{5\text{-}S}$MeH^{CT} structure.

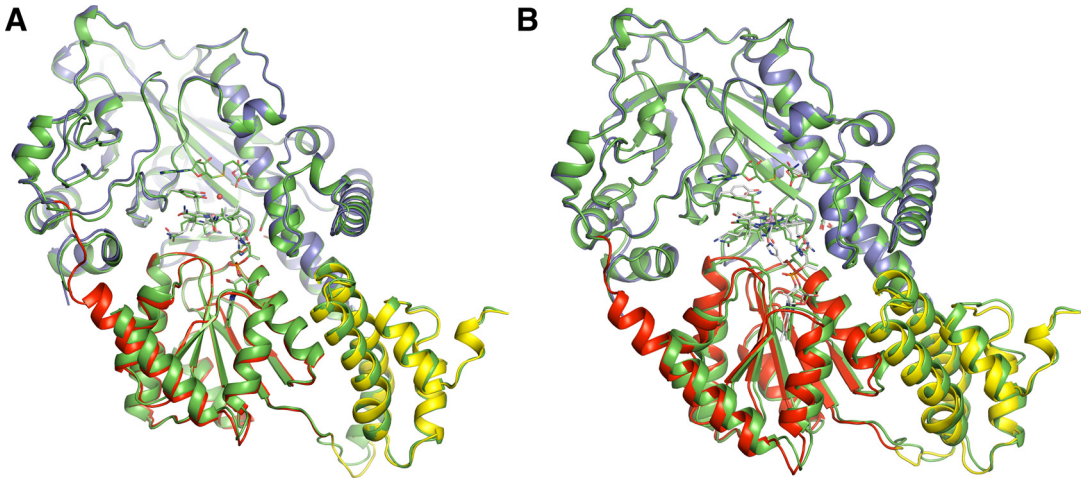

Fig. S2. Superposition of the Co(II)Cbl $_{S\text{-}S}$MeH^{CT} shown in green with (*A*) MeCo(III)Cbl $_{S\text{-}S}$MeH^{CT} (PDB ID 3BUL) [Datta S, Koutmos M, Pattridge KA, Ludwig ML, Matthews RG (2008) A disulfide-stabilized conformer of methionine synthase reveals an unexpected role for the histidine ligand of the cobalamin cofactor. *Proc Natl Acad Sci USA* 105:4115–4120] colored as in Fig. 2, and with (*B*) AquoCo(III)Cbl $_{S\text{-}S}$MeH^{CT}, colored as in Fig. 2. The superpositions are based on selected residues from the β-strands of the AdoMet domain.

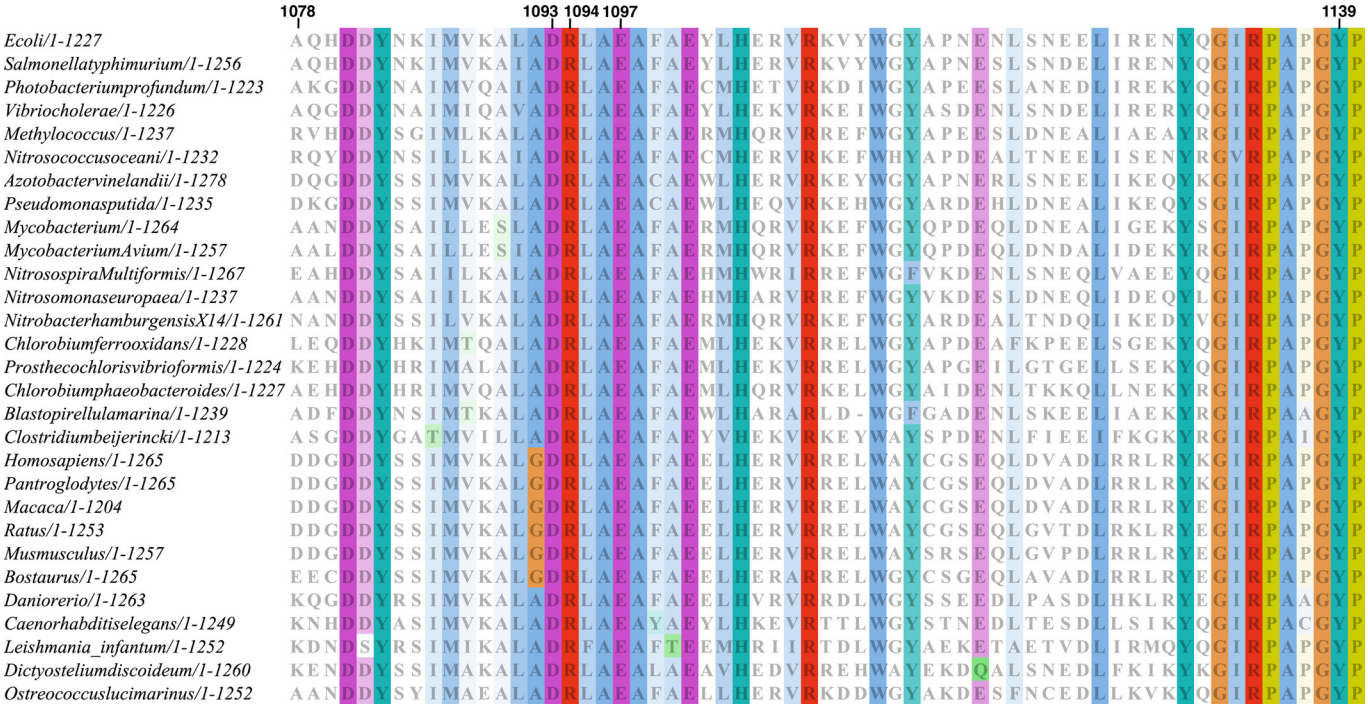

Fig. S3. Sequence alignment of MetH enzymes from various sources. Invariant and conserved residues are highlighted according to the clustalx coloring scheme (1). Invariant residues D1093, E1097, and Y1139 in *E. coli* are indicated in the figure. These alignments are selected from a multiple alignment performed in CLUSTALW (2) using MetH sequences obtained from the NCBI database.

1. Chenna R, et al. (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* 31:3497–3500.

2. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.

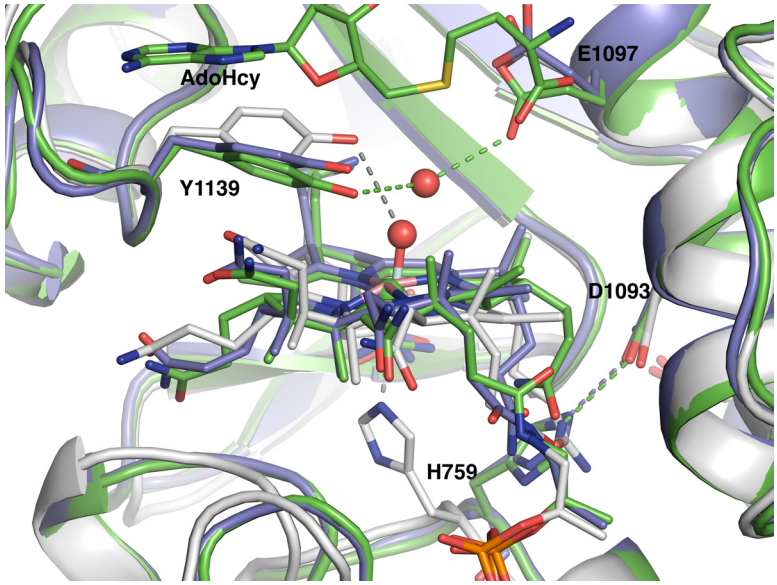

Fig. S4. Overlay of the Co(II)Cbl $_{5\text{-}S}$MeH^{CT} structure, in green, with the AquoCo(III)Cbl $_{5\text{-}S}$MeH^{CT} structure, in light gray, and with the MeCo(III)Cbl $_{5\text{-}S}$MeH^{CT} structure (PDB ID 3BUL) [Datta S, Koutmos M, Pattridge KA, Ludwig ML, Matthews RG (2008) A disulfide-stabilized conformer of methionine synthase reveals an unexpected role for the histidine ligand of the cobalamin cofactor. *Proc Natl Acad Sci U S A* 105:4115–4120], in blue. Residues from the β-strands of the AdoMet domain were used for the superposition. Of note are (*i*) the 2 different positions of H759, a ''His-on'' position in $_{5\text{-}S}$MeH^{CT}/AquoCo(III)Cbl and a ''His-off'' position in the other 2 structures in which H759 hydrogen bonds to D1093; (*ii*) the relative movement and different positions of Y1139 in all 3 structures; and (*iii*) the movement of the cobalamin domain but not the cobalamin cofactor.

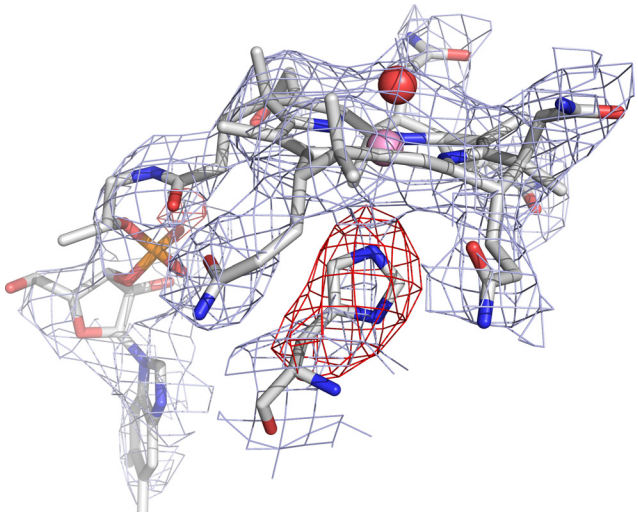

Fig. S5. Electron density and ball and stick model of the AquoCo(III)Cbl cofactor. The blue (at 1σ) and red (at 3σ) contours represent electron density from a weighted $2F_{obs}$-F_{calc} and F_{obs}-F_{calc} omit map, respectively. His-759 was omitted from the model before the calculation of the composite omit map.

Table S1. Data collection and refinement statistics[a]

Protein	AquoCob(III) $_{S\text{-}S}$MetH^{CT}	Cob(II) $_{S\text{-}S}$MetH^{CT} + AdoHcy
Diffraction data		
Space group	$P4_32_12$	$P4_32_12$
Unit cell parameters	$a = 107.5$	$a = 107.0$
	$b = 107.5$	$b = 107.0$
	$c = 143.8$	$c = 141.2$
	$\alpha = \beta = \gamma = 90$	$\alpha = \beta = \gamma = 90$
Data range (Å)	50–3.25	50–2.70
Measured reflections	131,794	519,512
Unique reflections	13,886	23,160
Average redundancy	9.5	22.4
Completeness (%) [a]	99.9 (99.9)	100.0 (99.9)
I/σ [a]	19.44 (3.17)	27.20 (5.82)
R_{sym} (%)[a,b]	8.8 (77.8)	8.5 (62.2)
Refinement		
Number of reflections	13,846	23,159
Working set	13,153	22,029
Test set	693	1,130
R_{cryst}[c]	28.2	28.2
R_{free}[d]	32.1	30.0
No. protein atoms	4,572	4,546
No. water molecules	22	26
RMSD bond lengths (Å)[e]	0.009	0.009
RMSD bond angles (deg.)[e]	1.10	1.40
Average protein B-factor (Å^2)	111	74
Average cobalamin B-factor (Å^2)	113	78
Average AdoHcy B-factor (Å^2)	-	98

[a]Statistics for the highest resolution shell are enclosed in parentheses.
[b]$R_{sym} = \Sigma|I - \langle I \rangle|/\Sigma I$, where I = observed intensity, and $\langle I \rangle$ = average intensity obtained from multiple measurements.
[c]$R_{cryst} = \Sigma||F_{obs}|-|F_{calc}||/\Sigma|F_{obs}|$, where F_{calc} and F_{obs} are the calculated and observed structure factor amplitudes, respectively.
[d]R_{free}, R-factor based on 5% of the data excluded from refinement.
[e]RMSD, root mean square deviation.