Supporting Information

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



Fig. S2. Superposition of the Co(II)Cbl _{S-S}MeH^{CT} shown in green with (A) MeCo(III)Cbl _{S-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-S}MeH^{CT}, colored as in Fig. 2. The superpositions are based on selected residues from the β-strands of the AdoMet domain.

	1078	1093 1094	4 1097					1139
Ecoli/1-1227	AQH <mark>DDY</mark> NK I	MVKALADRL	AEAFAEYL	HERVRK	VY <mark>WGY</mark> APN <mark>E</mark>	N L S N E E L I R E N Y	QGIRP	A P G Y P
Salmonellatyphimurium/1-1256	AQH <mark>DDY</mark> NK I	MVKA I A <mark>DR</mark> L	AEAFAEYL	HERVRK	CVY <mark>WGY</mark> APNE	S L S N D E L I R E N Y	QGIRP	APGYP
Photobacteriumprofundum/1-1223	AKG <mark>DDY</mark> NA I	MVQA I ADRL	AEAFAECM	HETVRK	CD I <mark>W</mark> G <mark>Y</mark> A P E E	S L ANE D L I RE K Y	QGIRP	A P <mark>G Y P</mark>
Vibriocholerae/1-1226	AQG <mark>DDY</mark> NA I	MI QA VA <mark>DR</mark> L	AEAFAEYL	HEKVRK	E I <mark>W</mark> G <mark>Y</mark> A S D E	NL SNDEL IRER <mark>Y</mark>	QGIRP	A P <mark>G Y P</mark>
Methylococcus/1-1237	RVH <mark>DDY</mark> SGI	ML KA L A <mark>D R</mark> L	AEAFAERM	H Q R V <mark>R</mark> R	E F WG Y A P E E	S L DNE A L I A E A Y	R G I R P	A P <mark>G Y P</mark>
Nitrosococcusoceani/1-1232	RQY <mark>DDY</mark> NS I	L L KA I A D R L	AEAFAECM	HERVRK	E F WH Y A P D E	A L T N E E L I S E N Y	R G V <mark>R</mark> P	A P <mark>G Y P</mark>
Azotobactervinelandii/1-1278	DQG <mark>DDY</mark> SSI	MVKALADRL	AEACAEWL	HERVRK	E Y <mark>W</mark> G <mark>Y</mark> A P N <mark>E</mark>	RLSNEELIKEQY	KGIRP	A P <mark>G Y P</mark>
Pseudomonasputida/1-1235	DKG <mark>DDY</mark> SSI	MVKALADRL	AEACAEWL	H E Q V <mark>R</mark> K	E H <mark>W</mark> G <mark>Y</mark> A R D E	HL DNE AL I KE Q <mark>Y</mark>	SGIRP	A P <mark>G Y P</mark>
Mycobacterium/1-1264	AAN <mark>DDY</mark> SA I	LLESLADRL	AEAFAERM	H Q R V <mark>R</mark> K	E F <mark>W</mark> G <mark>Y</mark> Q P D <mark>E</mark>	Q L D N E A L I G E K Y	SGIRP	APGYP
MycobacteriumAvium/1-1257	AAL DDY SA I	LLESIADRL	AEAFAERM	H Q R V <mark>R</mark> K	E F <mark>W</mark> G <mark>Y</mark> Q P D <mark>F</mark>	QL DNDAL IDEK <mark>Y</mark>	R G I R P	A P <mark>G Y P</mark>
NitrosospiraMultiformis/1-1267	E A H <mark>D D Y</mark> S A I	ILKALADRL	AEAFAEHM	HWR I <mark>R</mark> R	E F <mark>W</mark> G F V K D F	N L S N E Q L V A E E Y	QGIRP	A P <mark>G Y P</mark>
Nitrosomonaseuropaea/1-1237	A A N <mark>D D Y</mark> S A I	ILKALADRL	AEAFAEHM	H A R V <mark>R</mark> R	E F <mark>W</mark> G <mark>Y</mark> V K D <mark>F</mark>	S L DNE Q L I DE Q Y	LGIRP	A P <mark>G Y P</mark>
NitrobacterhamburgensisX14/1-120	51 NAN <mark>DDY</mark> SSI	LVKALADRL	AEAFAERM	H Q R V <mark>R</mark> K	E F <mark>W</mark> G <mark>Y</mark> A R D <mark>F</mark>	A L T N D Q L I KE D Y	VGIRP	A P <mark>G Y P</mark>
Chlorobiumferrooxidans/1-1228	L E Q <mark>D D Y</mark> H K I	MT QAL ADR L	AEAFAEML	HEKVRR	E L WG Y A P D E	A F K P E E L S G E K Y	QGIRP	A P G Y P
Prosthecochlorisvibrioformis/1-122	24 KEH <mark>DDY</mark> HR I	MALALADRL	AEAFAEML	HEKVRR	E L <mark>W</mark> G <mark>Y</mark> A P G <mark>E</mark>	I L GT GE <mark>L</mark> L S E K <mark>Y</mark>	QGIRP	A P <mark>G Y P</mark>
Chlorobiumphaeobacteroides/1-122	27 A E H <mark>D D Y</mark> H R I	MVQALADRL	AEAFAEML	H Q R V <mark>R</mark> K	E L <mark>W</mark> G <mark>Y</mark> A I D <mark>E</mark>	N L T K K Q L L N E K Y	RGIRP	APGYP
Blastopirellulamarina/1-1239	ADFDDYNSI	MT KALADRL	AEAFAEWL	HARARL	D - WGFGADE	N L S K E E L I A E K Y	R G I R P	A A <mark>G Y P</mark>
Clostridiumbeijerincki/1-1213	ASG <mark>DDY</mark> GAT	MVILLADRL	AEAFAEYV	HEKVRK	E Y <mark>W</mark> A <mark>Y</mark> S P D E	N L F I E E I F K G K Y	R G I R P	A I GY P
Homosapiens/1-1265	DDG <mark>DDY</mark> SSI	MVKAL GDRL	AEAFAEEL	HERVR	E L WAYCGSE	Q L D V A D L R R L R Y	KGIRP	APGYP
Pantroglodytes/1-1265	DDG <mark>DDY</mark> SSI	MVKAL GDRL	AEAFAEEL	HERVR	E L WAYCGSE	Q L D V A D L R R L R Y	K <mark>GIR</mark> P	A P <mark>G Y P</mark>
Macaca/1-1204	DDG <mark>DDY</mark> SSI	MVKAL GDRL	AEAFAEEL	HERVRR	E L WAYCGSE	Q L D V A D L R R L R Y	EGIRP	A P G Y P
Ratus/1-1253	DDG <mark>DDY</mark> SSI	MVKAL GDRL	AEAFAEEL	HERVR	E L WAYCGSE	Q L G V T D L R K L R Y	EGIRP	A P G Y P
Musmusculus/1-1257	DDG <mark>DDY</mark> SSI	MVKAL <mark>GDR</mark> L	AEAFAEEL	HERVR	E L WA Y S R S E	Q L G V P D L R R L R Y	EGIRP	A P G Y P
Bostaurus/1-1265	E E C <mark>D D Y</mark> S S I	MVKAL <mark>GDR</mark> L	AEAFAEEL	HERAR	E L <mark>W</mark> G <mark>Y</mark> C S G E	Q L A V A D L R R L R Y	EGIRP	A P <mark>G Y P</mark>
Daniorerio/1-1263	KQG <mark>DDY</mark> RS I	MVKALADRL	AEAFAEEL	H V R V <mark>R</mark> R	DLWGYSSEE	D L P A S D L H K L R Y	EGIRP	A A <mark>G Y P</mark>
Caenorhabditiselegans/1-1249	KNH <mark>DDY</mark> AS I	MVKALADRL	A E A Y A E Y L	HKE VRT	TLWGYSTNE	D L T E S D L L S I K Y	QGIRP	ACGYP
Leishmania_infantum/1-1252	KDN <mark>D</mark> S <mark>Y</mark> RS I	MIKALADR F	AEAFTEM	HRIIRT	DLWGYAEK	T A E T V D L I RMQ Y	QGIRP	A P <mark>G Y</mark> P
Dictyosteliumdiscoideum/1-1260	KEN <mark>DDY</mark> SSI	MAKALADR L	AEALAEAV	HEDVRR	E HWAYEKD	A L S N E D L F K I K Y	KGIRP	A P <mark>G Y</mark> P
Ostreococcuslucimarinus/1-1252	AAN <mark>D</mark> DYSYI	MAEALADRL	AEAFAELL	HERVRK	DDWGYAKDE	S F N C E D L L K V K Y	QGIRP	A P G Y P

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.

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Fig. 54. Overlay of the Co(II)Cbl _{S-S}MeH^{CT} structure, in green, with the AquoCo(III)Cbl _{S-S}MeH^{CT} structure, in light gray, and with the MeCo(III)Cbl _{S-S}MeH^{CT} structure (PDB ID 3BUL) [Datta 5, 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 _{S-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.

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Table S1. Data collection and refinement statistics^a

Protein	AquoCob(III) _{s-s} MetH ^{CT}	Cob(II) _{s-s} MetH ^{CT} + AdoHcy
Diffraction data		
Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2
Unit cell parameters	a = 107.5	<i>a</i> = 107.0
	<i>b</i> = 107.5	<i>b</i> = 107.0
	c = 143.8	c = 141.2
	$\alpha = \beta = \gamma = 90$	$lpha=eta=\gamma=$ 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>Ι/σ</i> ^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 (Ų)	111	74
Average cobalamin B-factor (Ų)	113	78
Average AdoHcy B-factor (Ų)	-	98

^aStatistics for the highest resolution shell are enclosed in parentheses.

 ${}^{b}R_{sym} = \Sigma | I - \& # 12296; I \& # 12297; | \Sigma I,$ where I = observed intensity, and & # 12296; I & # 12297; = 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.

^eRMSD, root mean square deviation.

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