## **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 <sub>S-S</sub>MeH<sup>CT</sup> structure.



**Fig. S2.** Superposition of the Co(II)Cbl <sub>S-S</sub>MeH<sup>CT</sup> shown in green with (A) MeCo(III)Cbl <sub>S-S</sub>MeH<sup>CT</sup> (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 <sub>S-S</sub>MeH<sup>CT</sup>, 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	<b>AEAFAEML</b>	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	HERVRR	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 <sub>S-S</sub>MeH<sup>CT</sup> structure, in green, with the AquoCo(III)Cbl <sub>S-S</sub>MeH<sup>CT</sup> structure, in light gray, and with the MeCo(III)Cbl <sub>S-S</sub>MeH<sup>CT</sup> 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 <sub>S-S</sub>MeH<sup>CT</sup>/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  $1\sigma$ ) and red (at  $3\sigma$ ) 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<sup>a</sup>

Protein	AquoCob(III) <sub>s-s</sub> MetH <sup>CT</sup>	Cob(II) <sub>s-s</sub> MetH <sup>CT</sup> + AdoHcy
Diffraction data		
Space group	P4 <sub>3</sub> 2 <sub>1</sub> 2	P4 <sub>3</sub> 2 <sub>1</sub> 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> <sup>a</sup>	19.44 (3.17)	27.20 (5.82)
R <sub>sym</sub> (%) <sup>a,<sup>b</sup></sup>	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 <sub>cryst</sub> <sup>c</sup>	28.2	28.2
R <sub>free</sub> <sup>d</sup>	32.1	30.0
No. protein atoms	4,572	4,546
No. water molecules	22	26
RMSD bond lengths (Å) <sup>e</sup>	0.009	0.009
RMSD bond angles (deg.) <sup>e</sup>	1.10	1.40
Average protein B-factor (Ų)	111	74
Average cobalamin B-factor (Ų)	113	78
Average AdoHcy B-factor (Ų)	-	98

<sup>a</sup>Statistics 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.

<sup>e</sup>RMSD, root mean square deviation.

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