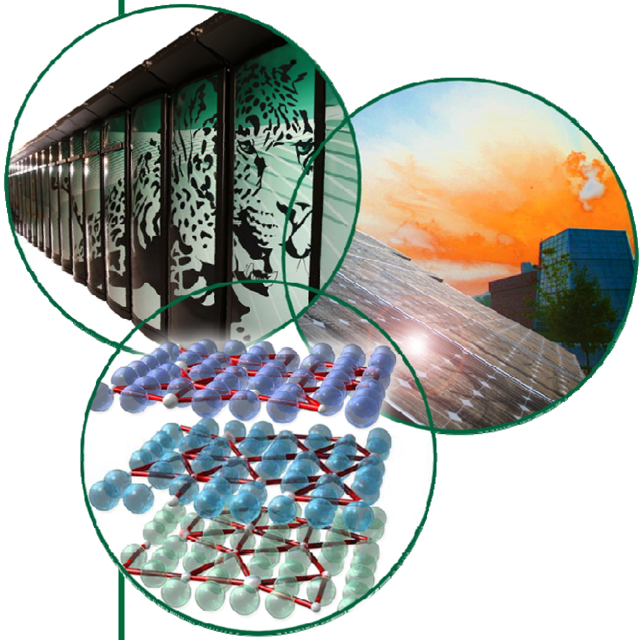
The Genetic Basis for Bacterial Mercury Methylation

The Team

Jerry Parks
Alex Johs
Mircea Podar
Romain Bridou - MU
Richard Hurt
Steve Smith - MU
Stephen Tomanicek
Yun Qian
Steve Brown
Craig Brandt
Cindy Gilmour - SERC
Tony Palumbo
Jeremy Smith
Judy Wall - MU
Dwayne Elias
Liyuan Liang



Subsurface Biogeochemical Research

Oak Ridge National Laboratory

SBR Science Focus Area (SFA) Program

Biogeochemical and Molecular Mechanisms Controlling Contaminant Transformation in the Environment

U.S. DEPARTMENT OF ENERGY

OAK RIDGE NATIONAL LABORATORY
MANAGED BY UT-BATTELLE FOR THE DEPARTMENT OF ENERGY

Outine of the Methylmercury Problem and Progress

- History 1908-2013
- Biogeochemistry - Geogenic and Anthropogenic Activities
- Chemical Reasoning - Jerry Parks, Alex Johs & Mircea Podar
- Structural Bioinformatics – Alex Johs & Jerry Parks
- Comparative Genomics – Mircea Podar, Jerry Parks & Alex Johs
- Microbiology – Romain Bridou, Richard Hurt, Dwayne Elias, Steve Smith and Judy Wall

The Mercury Problem

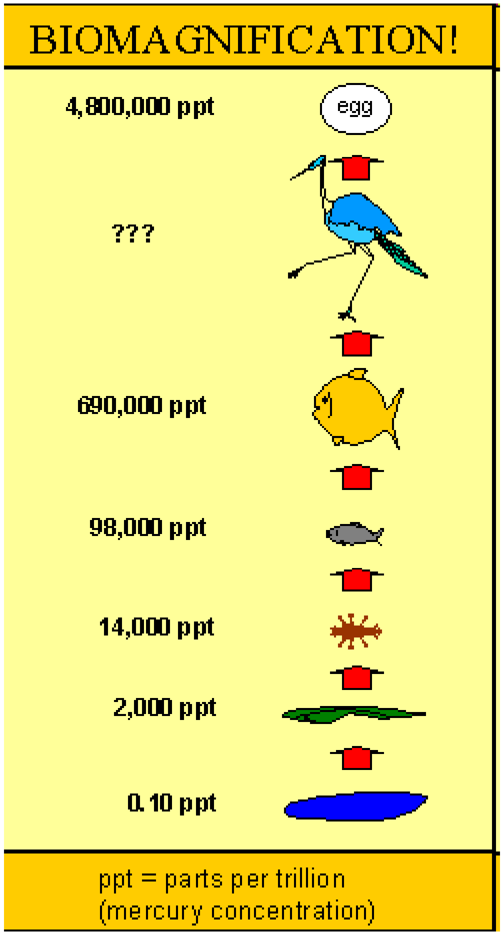
Hg toxic heavy metal

Hg^{2+} converted in sediments and soils to methylmercury, MHg^{+}, a neurotoxin

MHg^{+} bioaccumulates in the food chain

EPA “safe level” = 5800 ppt

Methylmercury is rapidly taken up but only slowly eliminated from the body by fish and other aquatic organisms



Methylmercury in the organs and tissues causes birth defects & disorders of the brain, reproductive system, immune system, kidney, and liver at extremely low levels in food.

Minamata Bay Disease

- **In 1908, a chemical company started making acetylene, acetaldehyde,vinyl chloride, etc., in Minamata, Japan**

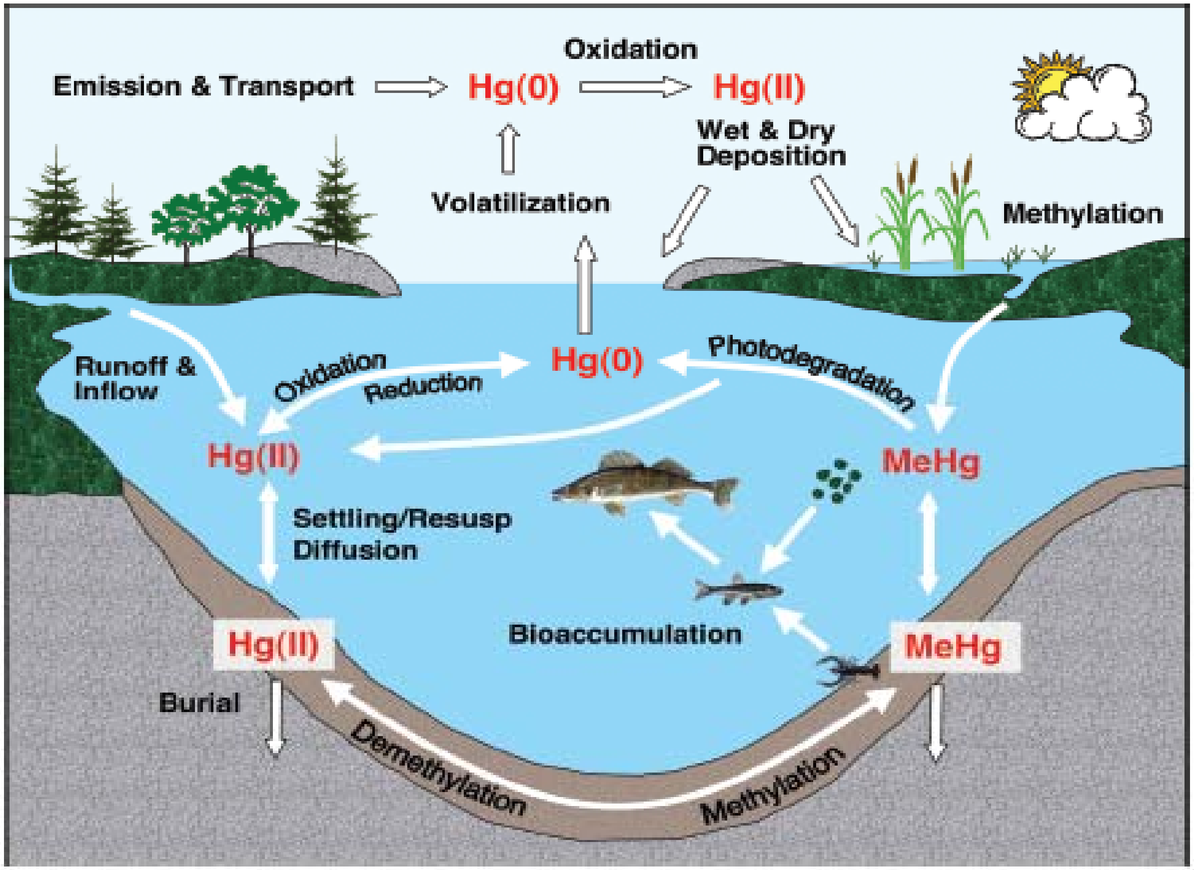
- **In 1956, an “epidemic” was recognized and researchers at Kumamoto University began to investigate causes.**

- **By 1959 mercury poisoning was recognized.**

Photographic essay (1971-73)
by W. Eugene Smith in *LIFE*

In 1972, Iraq had a major mercury poisoning with estimates of nearly 10,000 with 100,000 people having permanent brain damage.

Biogeochemistry

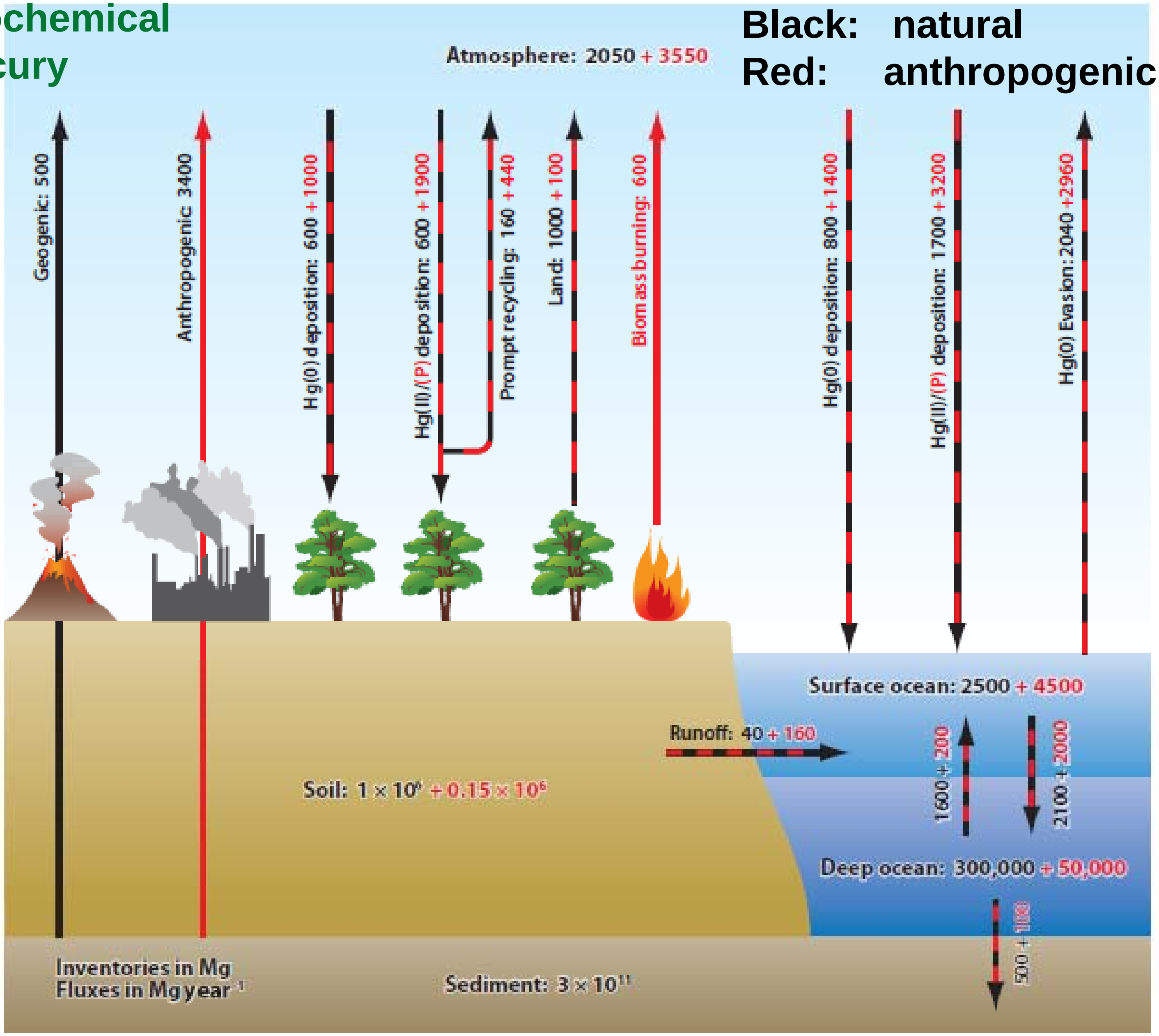


Engstrom, D.R. (2007) PNAS 104(42):16,394-5
Commentary

Global biogeochemical cycle for mercury

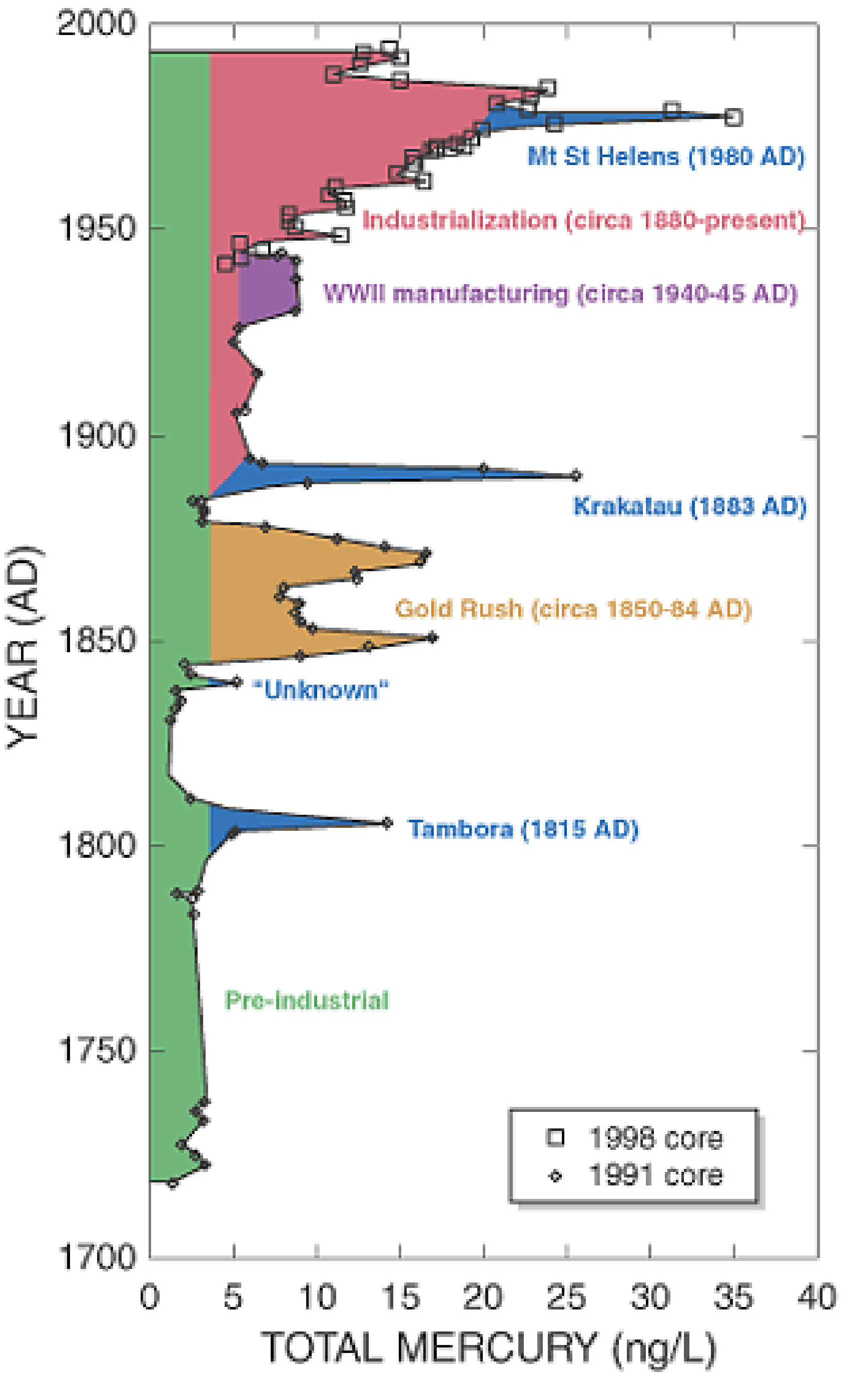
Black: natural
Red: anthropogenic

Nos. = megagrams/year



Selin, N.E. (2009) Annu Rev. Environ. Resour. 34:43-63

Atmospheric mercury deposition



David Krabbenhoft and Paul Schuster, 2002. **USGS Toxic Substances Hydrology Program**

How do bacteria methylate mercury?

A question posed for 45 years!

Chemical reasoning

$CH_3^+ + Hg^0 \rightarrow CH_3Hg^+$

$CH_3^\cdot + Hg^{\cdot +} \rightarrow CH_3Hg^+$

$CH_3{:}^- + Hg^{2+} \rightarrow CH_3Hg^+$

Cofactors that transfer methyl groups

SAM/AdoMet	S-adenosylmethionine	CH_3^+
MeTHF	N^5-methyltetrahydrofolate	CH_3^+
MeCbl	methylcobalamin	CH_3^+, $CH_3^{\cdot}$, $CH_3{:}^-$

(Wood, JM. Science, 183:1049. 1974)
(Ridley, Dizikes and Wood, 1977)

Methylcobalamin methylates Hg non-enzymatically

MeCbl + Hg(II) → MeHg

Wood, Kennedy and Rosen, 1968, *Nature* 220:173-4

Jensen and Jernelov, 1969, *Nature* 223:753-4

Bartha and coworkers (circa 1993-1994)

***Desulfovibrio desulfuricans* LS**

Key results:

- **The source of the methyl group in $^{14}CH_3Hg^+$ was $^{14}CH_3$-THF**

- **Reductive acetyl-CoA (Wood-Ljungdahl) pathway apparently involved**

- **40 kDa corrinoid protein identified but not characterized**

(Choi and Bartha, 1993; Choi and Bartha, 1994a,b)

Unexplained inconsistencies that delayed progress

- No clear evolutionary relationship of strains with the capacity to methylate Hg

- No apparent correlation between bacteria expressing a reductive acetylCoA pathway and methylation of Hg

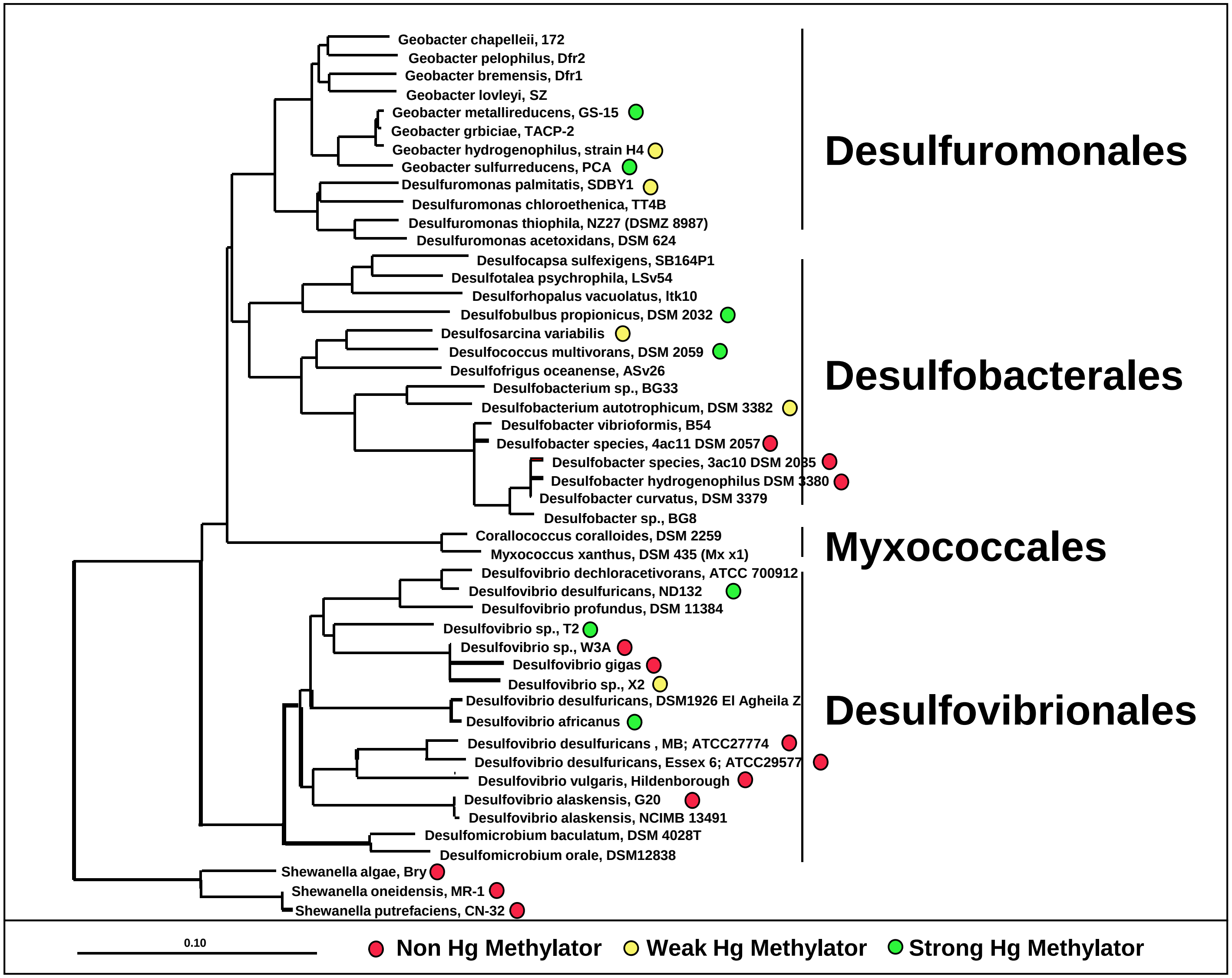
Technical difficulties that delayed progress

- Genomes of known methylators only recently available

- Limited genetic analysis available in methylating bacteria

- Methylmercury assays not trivial

Phylogenetic relationships among mercury methylating deltaproteobacteria



Assimilating Dr. Bartha's data – What chemistry would result in mercury methylation?

$$CH_3^- + Hg^{2+} \rightarrow CH_3Hg^+$$

Methylate cofactor:

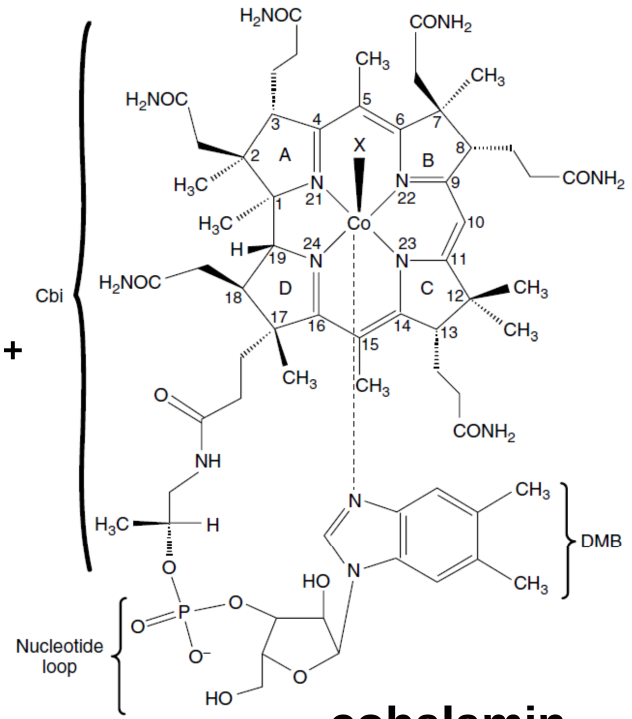
$CH_3^+ + Co(I)\text{-protein} \rightarrow CH_3\text{-}Co(III)\text{-protein}$

Transfer methyl group to Hg^{2+}:

$CH_3\text{-}Co(III)\text{-protein} + Hg^{2+} \rightarrow Co(III)\text{-protein} + CH_3Hg^+$

Regenerate Co(I):

$Co(III)\text{-protein} + 2\,e^- \rightarrow Co(I)\text{-protein}$

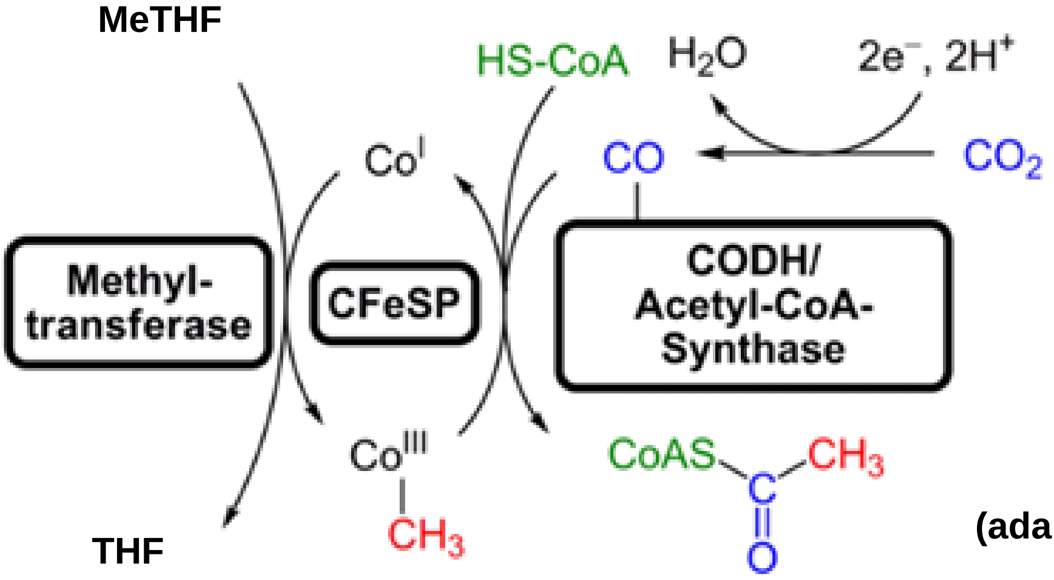


cobalamin

Need to find one or more proteins that can:

1. Stabilize cofactor for methyl transfer
2. Provide low-potential electrons to (re)generate Co(I)

Methyl transfers in the reductive Acetyl-CoA pathway

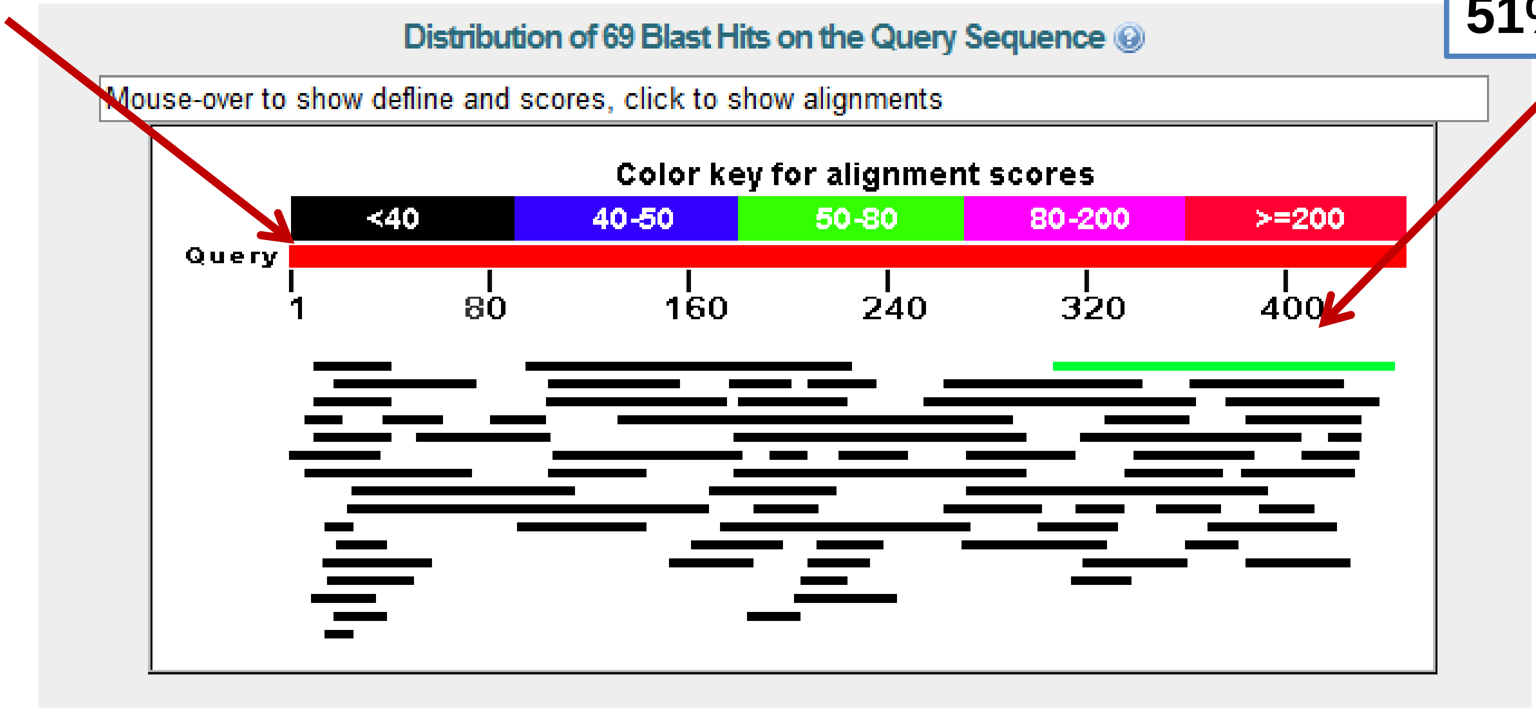


(adapted from Ragsdale, 2008)

Two CH_3^+ transfers

A gene encoding a CFeSP-like protein is present in the genome of *Desulfovibrio desulfuricans* ND132

Chain A
CfsA



Distribution of 69 Blast Hits on the Query Sequence

Mouse-over to show defline and scores, click to show alignments

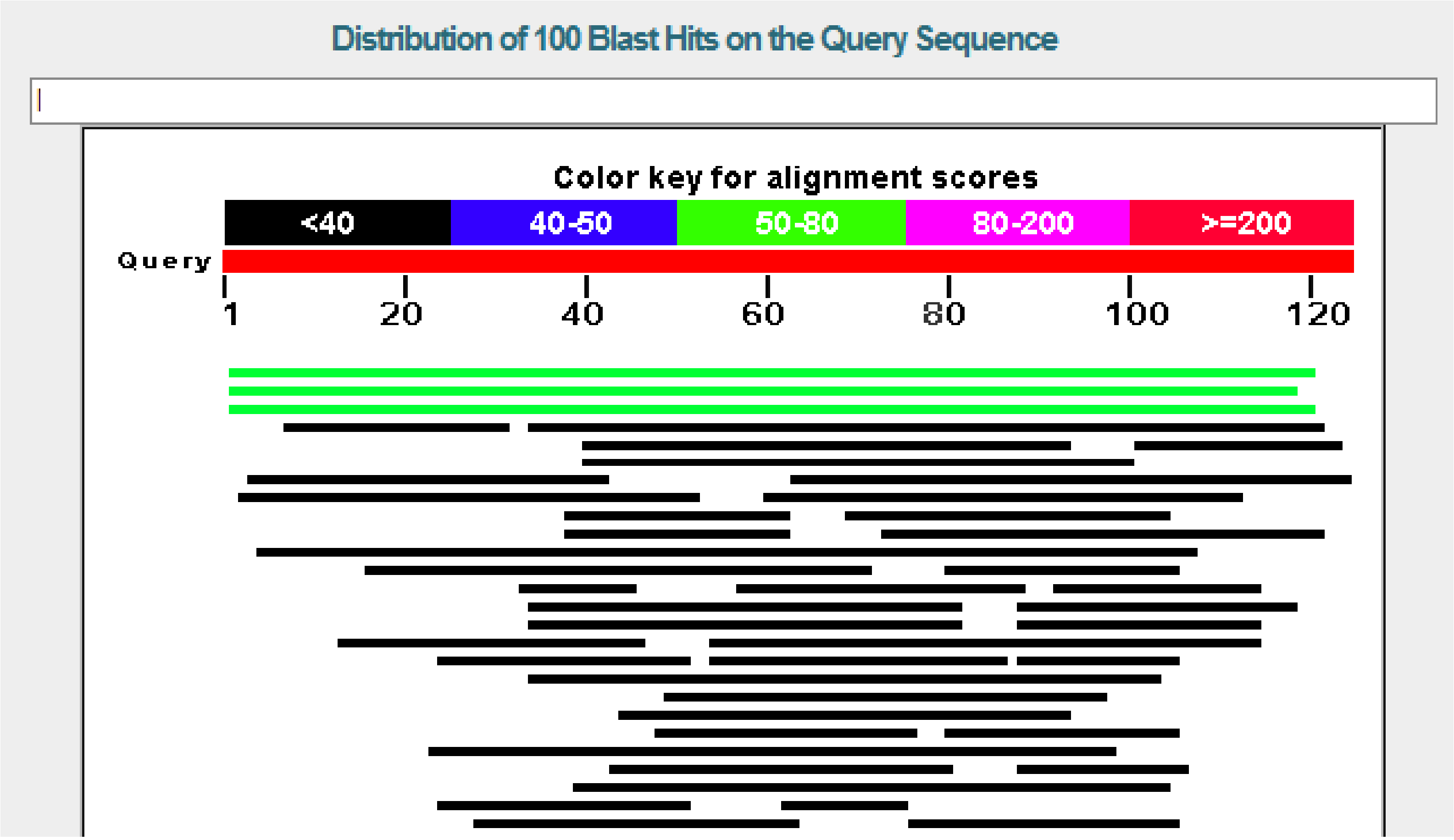
28% id
51% sim

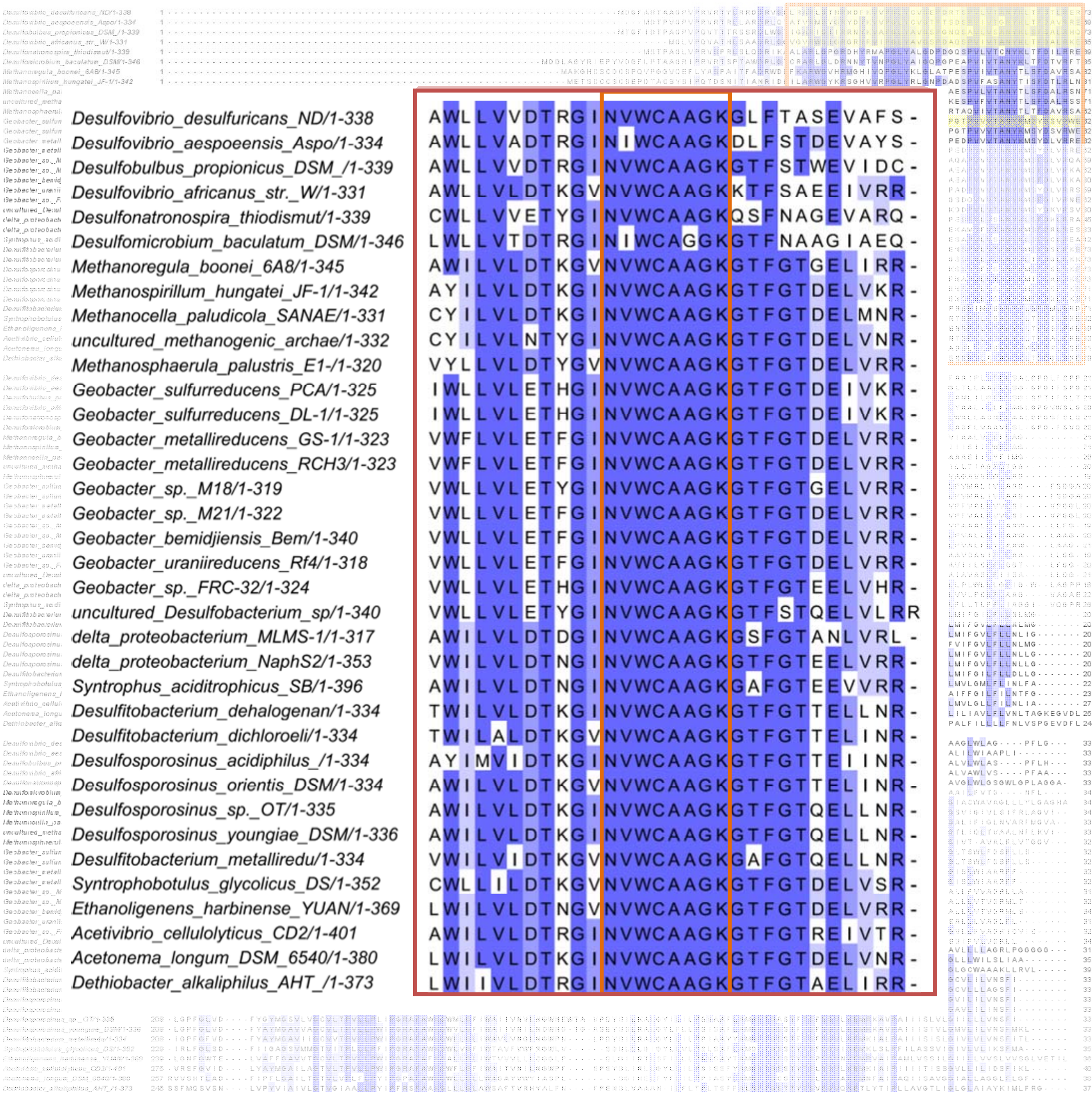
This gene is unique to confirmed methylators among the *Desulfovibrio*

Desulfovibrio aespoeensis Aspo-2

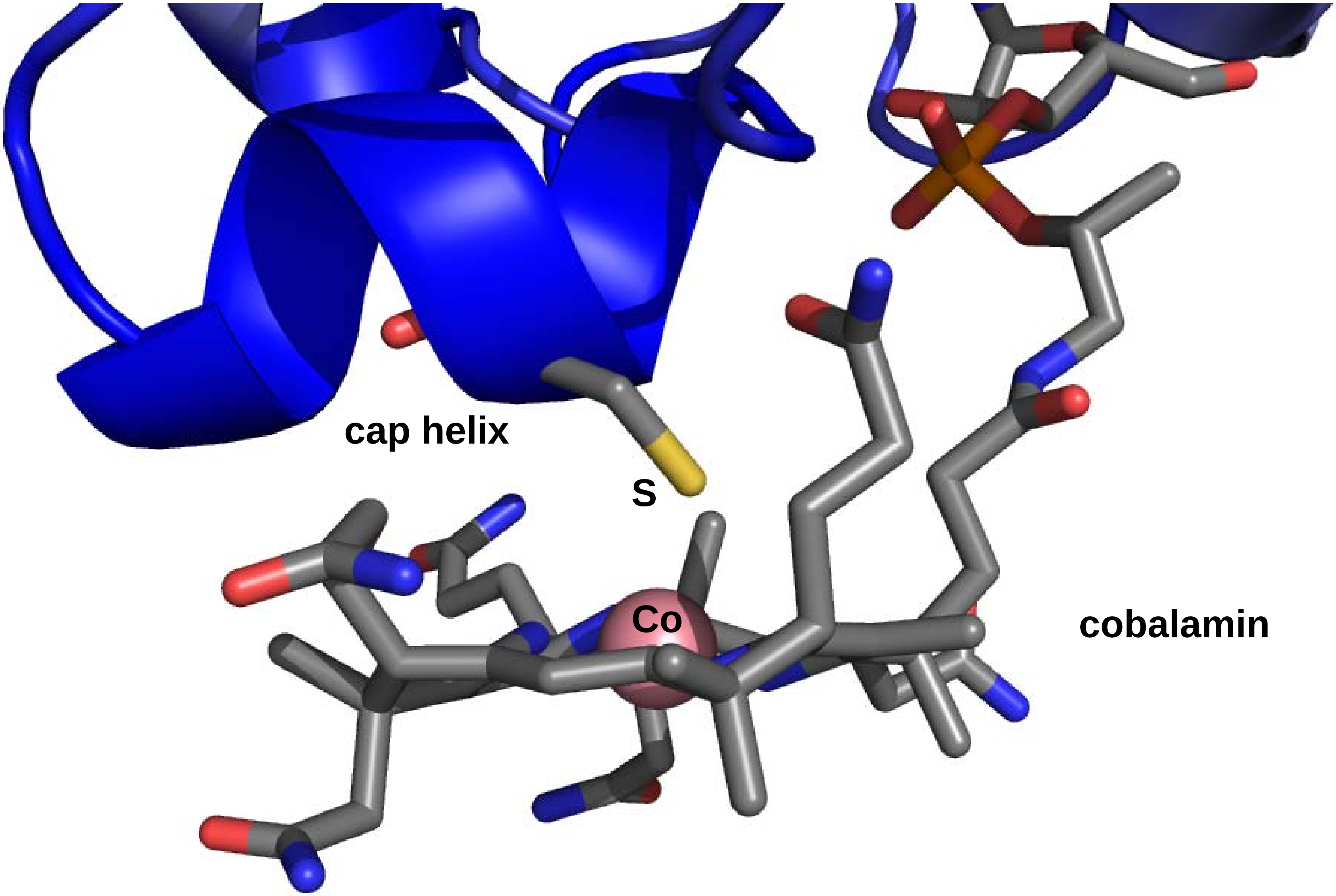
Desulfovibrio africanus str. Walvis Bay

Desulfovibrio desulfuricans ND132



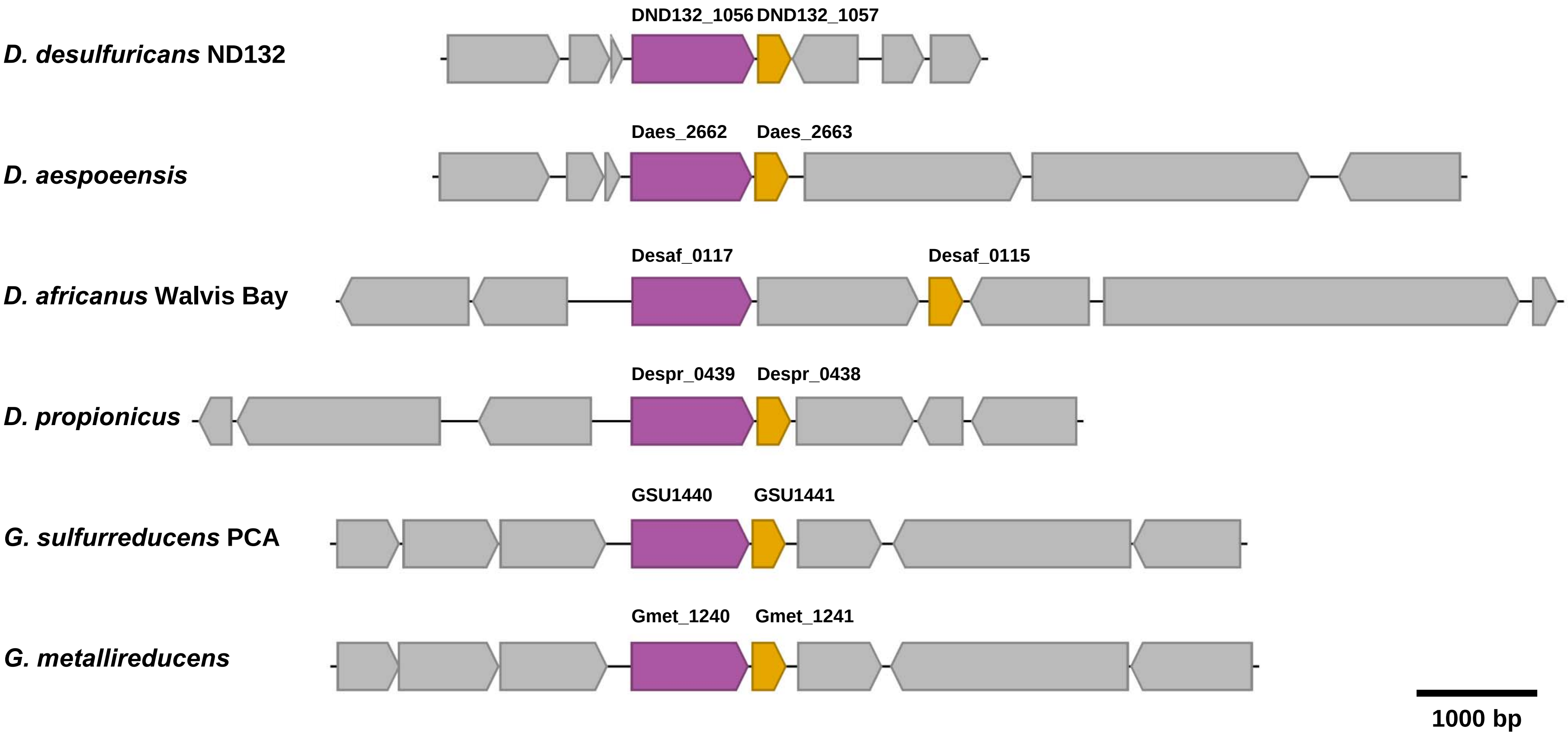

Desulfovibrio_desulfuricans_ND/1-338 AWLLVVDTRGINVWCAAGKGLFTASEVAFS-
Desulfovibrio_aespoeensis_Aspo/1-334 AWLLVADTRGINIWCAAGKDLFSTDEVAYS-
Desulfobulbus_propionicus_DSM_/1-339 AWLLVVDTRGINVWCAAGKGTFSTWEVIDC-
Desulfovibrio_africanus_str._W/1-331 AWLLVLDTKGVNVWCAAGKKTFSAEEIVRR-
Desulfonatronospira_thiodismut/1-339 CWLLVVETYGINVWCAAGKQSFNAGEVARQ-
Desulfomicrobium_baculatum_DSM/1-346 LWLLVTDTRGINIWCAGGKGTFNAAGIAEQ-
Methanoregula_boonei_6A8/1-345 AWILVLDTKGVNVWCAAGKGTFGTGELIRR-
Methanospirillum_hungatei_JF-1/1-342 AYILVLDTKGINVWCAAGKGTFGTDELVKR-
Methanocella_paludicola_SANAE/1-331 CYILVLDTKGINVWCAAGKGTFGTDELMNR-
uncultured_methanogenic_archae/1-332 CYILVLNTYGINVWCAAGKGTFGTDELVNR-
Methanosphaerula_palustris_E1-/1-320 VYLLVLDTYGVNVWCAAGKGTFGTDELVRR-
Geobacter_sulfurreducens_PCA/1-325 IWLLVLETHGINVWCAAGKGTFGTDEIVKR-
Geobacter_sulfurreducens_DL-1/1-325 IWLLVLETHGINVWCAAGKGTFGTDEIVKR-
Geobacter_metallireducens_GS-1/1-323 VWFLVLETFGINVWCAAGKGTFGTDELVRR-
Geobacter_metallireducens_RCH3/1-323 VWFLVLETFGINVWCAAGKGTFGTDELVRR-
Geobacter_sp._M18/1-319 VWLLVLETYGINVWCAAGKGTFGTGELVRR-
Geobacter_sp._M21/1-322 VWLLVLETFGINVWCAAGKGTFGTDELVRR-
Geobacter_bemidjiensis_Bem/1-340 VWLLVLETFGINVWCAAGKGTFGTDELVRR-
Geobacter_uraniireducens_Rf4/1-318 VWLLVLETFGINVWCAAGKGTFGTDELVRR-
Geobacter_sp._FRC-32/1-324 VWLLVLETHGINVWCAAGKGTFGTEELVHR-
uncultured_Desulfobacterium_sp/1-340 VWLLVLETYGINVWCAAGKGTFSTQELVLRR
delta_proteobacterium_MLMS-1/1-317 AWILVLDTDGINVWCAAGKGSFGTANLVRL-
delta_proteobacterium_NaphS2/1-353 VWILVLDTNGINVWCAAGKGTFGTEELVRR-
Syntrophus_aciditrophicus_SB/1-396 AWILVLDTNGINVWCAAGKGAFGTEEVVRR-
Desulfitobacterium_dehalogenan/1-334 TWILVLDTKGINVWCAAGKGTFGTTELLNR-
Desulfitobacterium_dichloroeli/1-334 TWILALDTKGVNVWCAAGKGTFGTTELINR-
Desulfosporosinus_acidiphilus_/1-334 AYIMVIDTKGINVWCAAGKGTFGTTEIINR-
Desulfosporosinus_orientis_DSM/1-334 AWILVLDTKGVNVWCAAGKGTFGTTELINR-
Desulfosporosinus_sp._OT/1-335 AWILVLDTKGINVWCAAGKGTFGTQELLNR-
Desulfosporosinus_youngiae_DSM/1-336 AWILVLDTKGINVWCAAGKGTFGTQELLNR-
Desulfitobacterium_metallireduc/1-334 VWILVIDTKGVNVWCAAGKGAFGTQELLNR-
Syntrophobotulus_glycolicus_DS/1-352 CWLLILDTKGVNVWCAAGKGTFGTDELVSR-
Ethanoligenens_harbinense_YUAN/1-369 LWILVLDTNGVNVWCAAGKGTFGTDELVRR-
Acetivibrio_cellulolyticus_CD2/1-401 AWILVLDTRGINVWCAAGKGTFGTREIVTR-
Acetonema_longum_DSM_6540/1-380 LWILVLDTKGINVWCAAGKGTFGTDELVNR-
Dethiobacter_alkaliphilus_AHT_/1-373 LWIIVLDTRGINVWCAAGKGTFGTAELIRR-

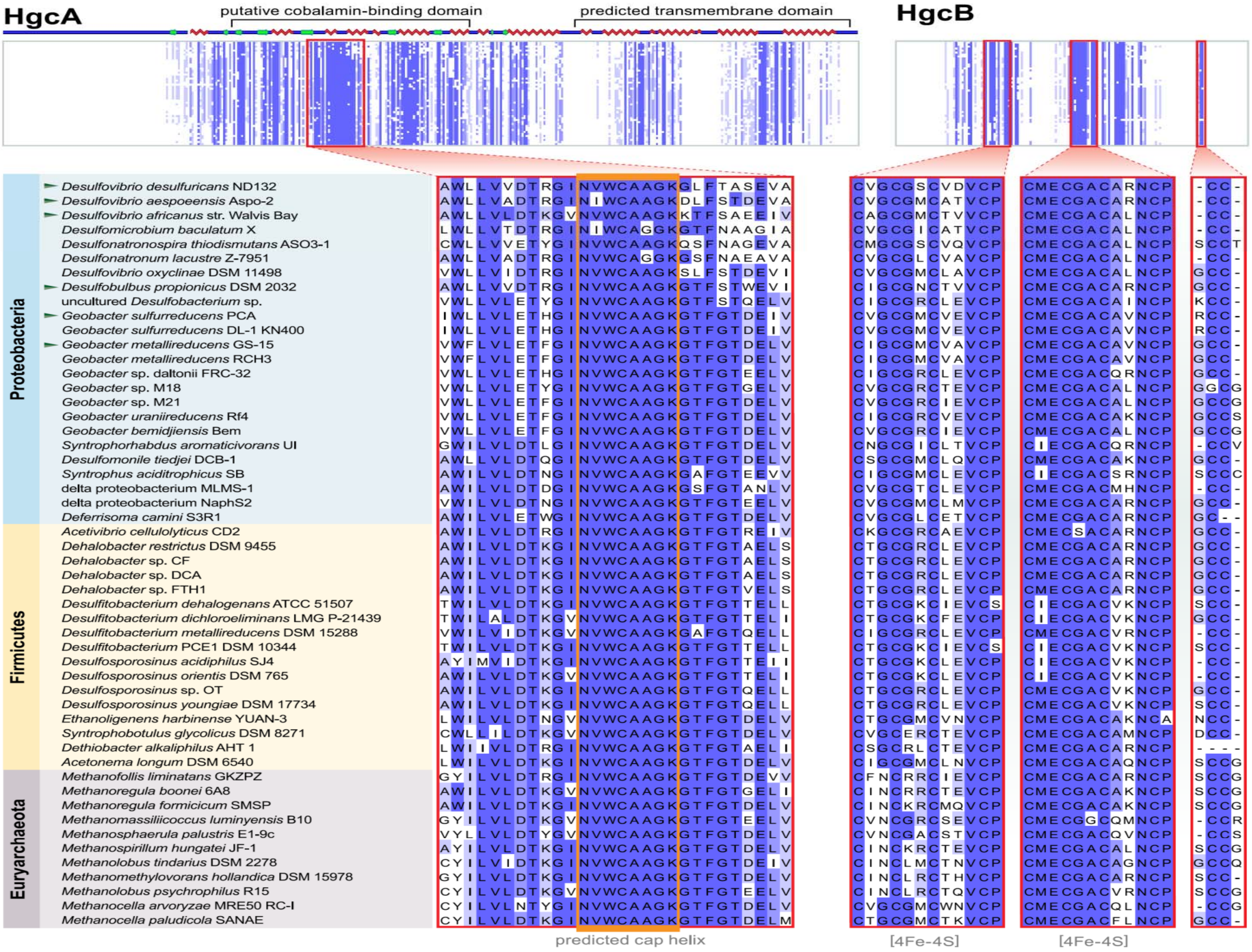
Homology model of HgcA



Comparative genomics

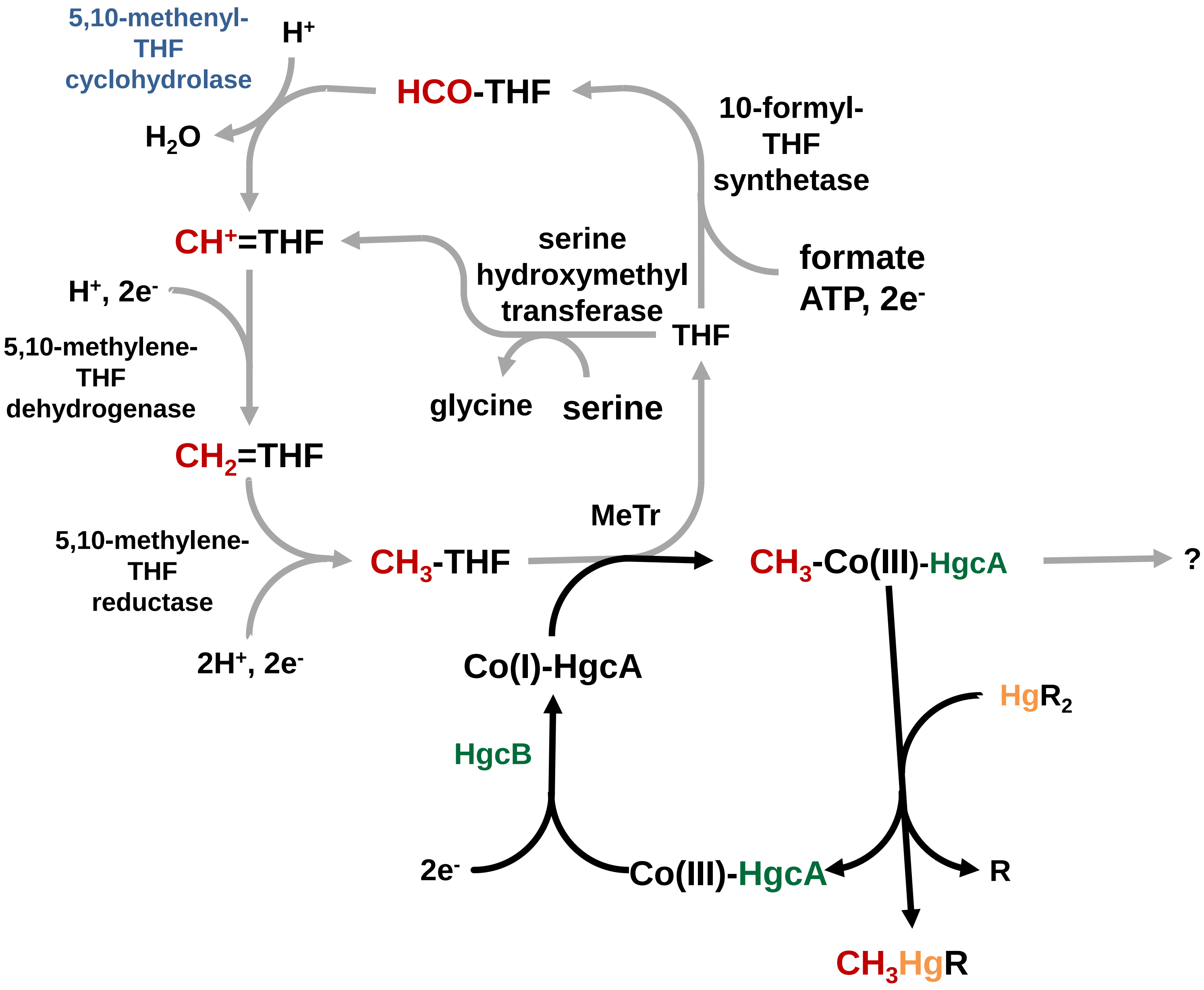
Whole genomes of six confirmed methylators vs several non-methylators





Proteobacteria	► *Desulfovibrio desulfuricans* ND132	AWLLVVDTRGINVWCAAGKGLFTASEVA	CVGCGSCVDVCP	CMECGACARNCP	-CC-
	► *Desulfovibrio aespoeensis* Aspo-2	AWLLVADTRGINIWCAAGKDLFSTDEVA	CVGCGMCATVCP	CMECGACARNCP	-CC-
	► *Desulfovibrio africanus* str. Walvis Bay	AWLLVLDTKGVNVWCAAGKKTFSAEEIV	CAGCGMCTVVCP	CMECGACALNCP	-CC-
	Desulfomicrobium baculatum X	LWLLVTDTRGINIWCAGGKGTFNAAGIA	CVGCGICATVCP	CMECGACALNCP	-CC-
	Desulfonatronospira thiodismutans ASO3-1	CWLLVVETYGINVWCAAGKQSFNAGEVA	CMGCGSCVQVCP	CMECGACALNCP	SCCT
	Desulfonatronum lacustre Z-7951	AWLLVADTRGINVWCAGGKGSFNAEAVA	CVGCGLCVAVCP	CMECGACALNCP	-CC-
	Desulfovibrio oxyclinae DSM 11498	VWLLVIDTRGINVWCAAGKSLFSTDEVI	CVGCGMCLAVCP	CMECGACALNCP	GCC-
	► *Desulfobulbus propionicus* DSM 2032	AWLLVVDTRGINVWCAAGKGTFSTWEVI	CIGCGNCTVVCP	CMECGACARNCP	GCC-
	uncultured *Desulfobacterium* sp.	VWLLVLETYGINVWCAAGKGTFSTQELV	CIGCGRCLEVCP	CMECGACAINCP	KCC-
	► *Geobacter sulfurreducens* PCA	IWLLVLETHGINVWCAAGKGTFGTDEIV	CVGCGMCVEVCP	CMECGACAVNCP	RCC-
	Geobacter sulfurreducens DL-1 KN400	IWLLVLETHGINVWCAAGKGTFGTDEIV	CVGCGMCVEVCP	CMECGACAVNCP	RCC-
	► *Geobacter metallireducens* GS-15	VWFLVLETFGINVWCAAGKGTFGTDELV	CIGCGMCVAVCP	CMECGACAVNCP	GCC-
	Geobacter metallireducens RCH3	VWFLVLETFGINVWCAAGKGTFGTDELV	CIGCGMCVAVCP	CMECGACAVNCP	GCC-
	Geobacter sp. daltonii FRC-32	VWLLVLETHGINVWCAAGKGTFGTEELV	CIGCGRCLEVCP	CMECGACQRNCP	GCC-
	Geobacter sp. M18	VWLLVLETYGINVWCAAGKGTFGTGELV	CVGCGRCTEVCP	CMECGACALNCP	GGCG
	Geobacter sp. M21	VWLLVLETFGINVWCAAGKGTFGTDELV	CVGCGRCIEVCP	CMECGACALNCP	GCCG
	Geobacter uraniireducens Rf4	VWLLVLETFGINVWCAAGKGTFGTDELV	CIGCGRCVEVCP	CMECGACAKNCP	GCCS
	Geobacter bemidjiensis Bem	VWLLVLETFGINVWCAAGKGTFGTDELV	CVGCGRCIEVCP	CMECGACALNCP	GCCG
	Syntrophorhabdus aromaticivorans UI	GWILVLDTLGINVWCAAGKGTFGTDELV	CNGCGICLTVCP	CIECGACQRNCP	-CCV
	Desulfomonile tiedjei DCB-1	AWLLVLDTQGINVWCAAGKGTFGTDELV	CSGCGMCLQVCP	CMECGACAKNCP	GCC-
	Syntrophus aciditrophicus SB	AWILVLDTNGINVWCAAGKGAFGTEEVV	CIGCGMCLEVCP	CIECGACSRNCP	SCCC
	delta proteobacterium MLMS-1	AWILVLDTDGINVWCAAGKGSFGTANLV	CVGCGTCLEVCP	CMECGACMHNCP	-CC-
	delta proteobacterium NaphS2	VWILVLDTNGINVWCAAGKGTFGTEELV	CVGCGMCLMVCP	CMECGACARNCP	GCC-
	Deferrisoma camini S3R1	AWILVLETWGINVWCAAGKGTFGTDELV	CVGCGLCETVCP	CMECGACARNCP	GC--
Firmicutes	*Acetivibrio cellulolyticus* CD2	AWILVLDTRGINVWCAAGKGTFGTREIV	CKGCGRCAEVCP	CMECSACARNCP	GCC-
	Dehalobacter restrictus DSM 9455	AWILVLDTKGINVWCAAGKGTFGTAELS	CTGCGRCLEVCP	CMECGACARNCP	GCC-
	Dehalobacter sp. CF	AWILVLDTKGINVWCAAGKGTFGTAELS	CTGCGRCLEVCP	CMECGACARNCP	GCC-
	Dehalobacter sp. DCA	AWILVLDTKGINVWCAAGKGTFGTAELS	CTGCGRCLEVCP	CMECGACARNCP	GCC-
	Dehalobacter sp. FTH1	AWILVLDTKGINVWCAAGKGTFGTVELS	CTGCGRCLEVCP	CMECGACARNCP	GCC-
	Desulfitobacterium dehalogenans ATCC 51507	TWILVLDTKGINVWCAAGKGTFGTTELL	CTGCGKCIEVCS	CIECGACVKNCP	SCC-
	Desulfitobacterium dichloroeliminans LMG P-21439	TWILALDTKGVNVWCAAGKGTFGTTELI	CTGCGKCFEVCP	CIECGACVKNCP	GCC-
	Desulfitobacterium metallireducens DSM 15288	VWILVIDTKGVNVWCAAGKGAFGTQELL	CIGCGRCLEVCP	CMECGACVRNCP	-CC-
	Desulfitobacterium PCE1 DSM 10344	TWILVLDTKGINVWCAAGKGTFGTTELL	CTGCGKCIEVCS	CIECGACVKNCP	SCC-
	Desulfosporosinus acidiphilus SJ4	AYIMVIDTKGINVWCAAGKGTFGTTEII	CTGCGKCLEVCP	CIECGACVKNCP	-CC-
	Desulfosporosinus orientis DSM 765	AWILVLDTKGVNVWCAAGKGTFGTTELI	CTGCGKCLEVCP	CIECGACVKNCP	-CC-
	Desulfosporosinus sp. OT	AWILVLDTKGINVWCAAGKGTFGTQELL	CTGCGRCLEVCP	CMECGACVKNCP	GCC-
	Desulfosporosinus youngiae DSM 17734	AWILVLDTKGINVWCAAGKGTFGTQELL	CTGCGRCLEVCP	CMECGACVKNCP	SCC-
	Ethanoligenens harbinense YUAN-3	LWILVLDTNGVNVWCAAGKGTFGTDELV	CTGCGMCVNVCP	CMECGACAKNCA	NCC-
	Syntrophobotulus glycolicus DSM 8271	CWLLILDTKGVNVWCAAGKGTFGTDELV	CVGCERCTEVCP	CMECGACAMNCP	DCC-
	Dethiobacter alkaliphilus AHT 1	LWIIVLDTRGINVWCAAGKGTFGTAELI	CSGCRLCTEVCP	CMECGACARNCP	----
	Acetonema longum DSM 6540	LWILVLDTKGINVWCAAGKGTFGTDELV	CIGCGMCLNVCP	CMECGACAQNCP	SCCG
Euryarchaeota	*Methanofollis liminatans* GKZPZ	GYILVLDTRGINVWCAAGKGTFGTDEVV	CFNCRRCIEVCP	CMECGACARNCP	SCCG
	Methanoregula boonei 6A8	AWILVLDTKGVNVWCAAGKGTFGTGELI	CINCRRCTEVCP	CMECGACAKNCP	SCCG
	Methanoregula formicicum SMSP	AWILVLDTKGINVWCAAGKGTFGTDELV	CINCKRCMQVCP	CMECGACAKNCP	SCCG
	Methanomassiliicoccus luminyensis B10	GYILVLDTKGVNVWCAAGKGTFGTEELV	CVNCGRCSEVCP	CMECGGCQMNCP	-CCR
	Methanosphaerula palustris E1-9c	VYLLVLDTYGVNVWCAAGKGTFGTDELV	CVNCGACSTVCP	CMECGACQVNCP	-CCS
	Methanospirillum hungatei JF-1	AYILVLDTKGINVWCAAGKGTFGTDELV	CINCKRCTEVCP	CMECGACALNCP	SCCG
	Methanolobus tindarius DSM 2278	CYILVIDTKGINVWCAAGKGTFGTDEIV	CINCLMCTNVCP	CMECGACAGNCP	GCCQ
	Methanomethylovorans hollandica DSM 15978	GYILVLDTKGINVWCAAGKGTFGTDELV	CINCLRCTHVCP	CMECGACARNCP	SCC-
	Methanolobus psychrophilus R15	CYILVLDTKGVNVWCAAGKGTFGTEELV	CINCLRCTQVCP	CMECGACVRNCP	SCCG
	Methanocella arvoryzae MRE50 RC-I	CYILVLNTYGINVWCAAGKGTFGTDELV	CVGCGMCWNVCP	CMECGACQLNCP	-CCG
	Methanocella paludicola SANAE	CYILVLDTKGINVWCAAGKGTFGTDELM	CTGCGMCTKVCP	CMECGACFLNCP	GCC-

Proposed mercury methylation pathway



5,10-methenyl-THF cyclohydrolase

H^+

H_2O

HCO-THF

10-formyl-THF synthetase

formate

ATP, $2e^-$

CH^+=THF

serine hydroxymethyl transferase

THF

glycine

serine

H^+, $2e^-$

5,10-methylene-THF dehydrogenase

CH_2=THF

5,10-methylene-THF reductase

$2H^+$, $2e^-$

CH_3-THF

MeTr

CH_3-Co(III)-HgcA

?

Co(I)-HgcA

HgcB

$2e^-$

Co(III)-HgcA

HgR_2

R

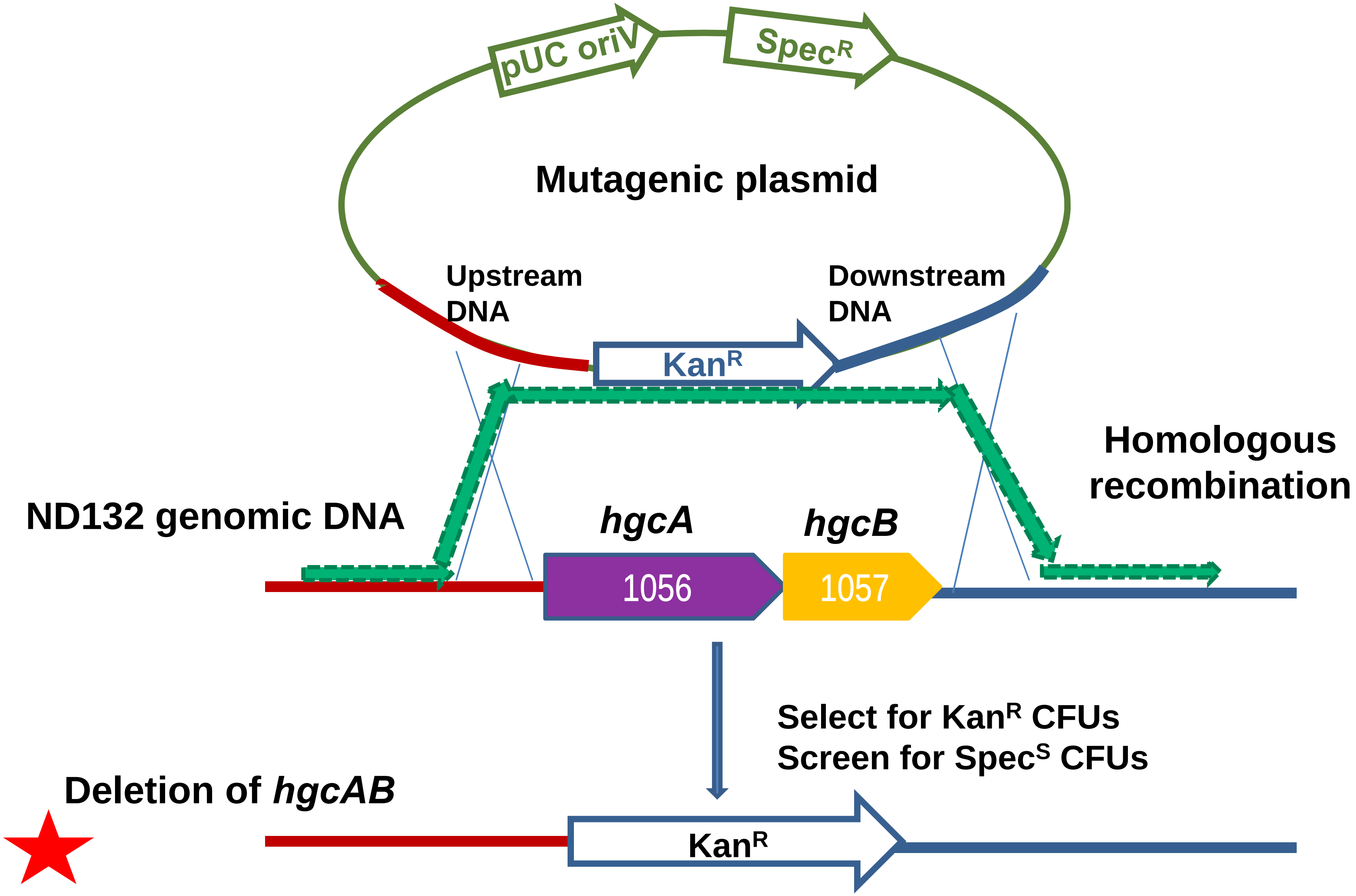
CH_3HgR

Confirmation that these two genes, *hgcAB,* were necessary for methylation of mercury!

Genetic development of
***D. desulfuricans* ND132**

***Geobacter sulfurreducens* PCA**
genetics available

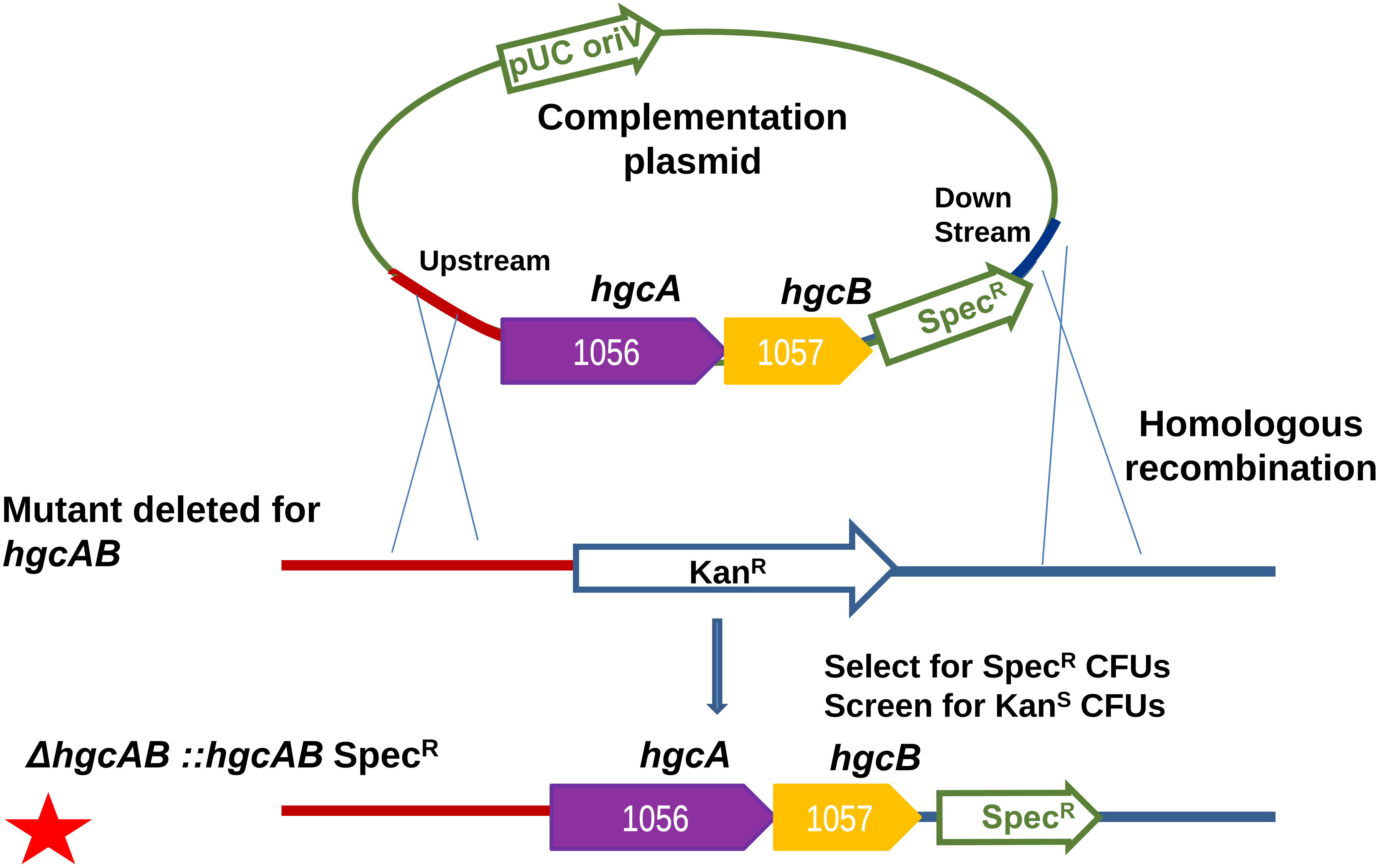
Marker exchange deletions in ND132



Select for Kan^R CFUs
Screen for Spec^S CFUs

Deletion of *hgcAB*

Complementation of deletions



Complementation plasmid

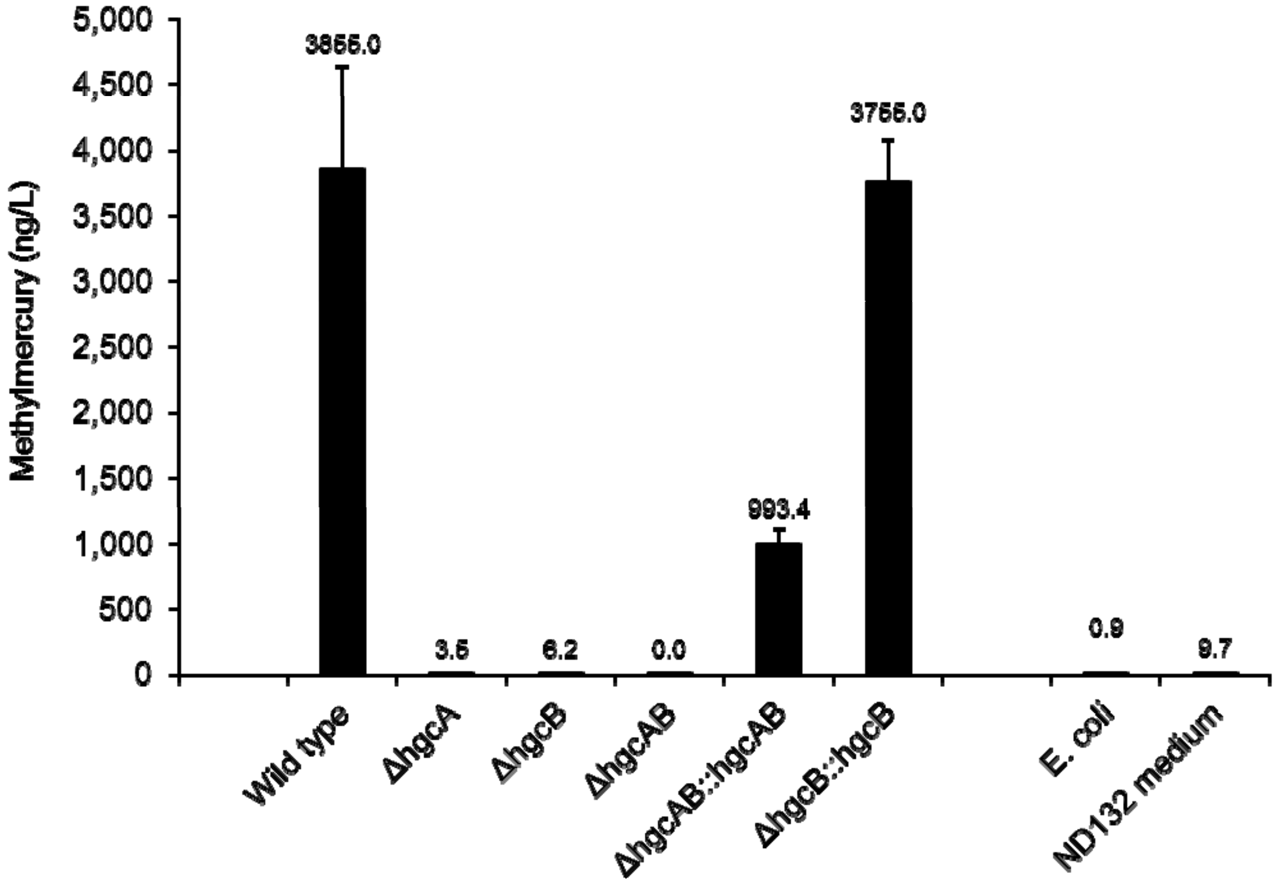
Homologous recombination

Mutant deleted for *hgcAB*

Select for Spec^{R} CFUs
Screen for Kan^{S} CFUs

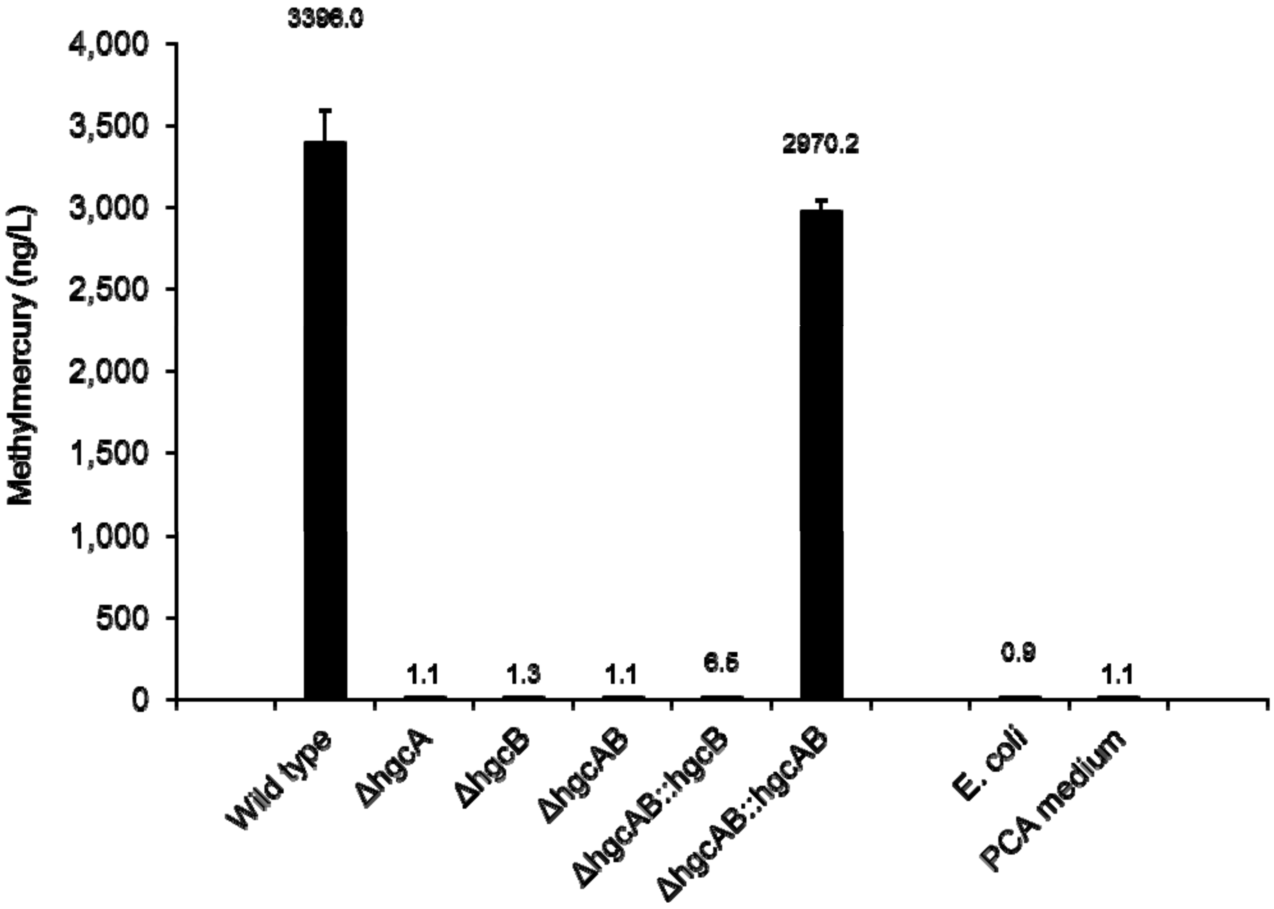
***ΔhgcAB ::hgcAB* Spec^{R}**

Production of methylmercury by *D. desulfuricans* ND132 mutants



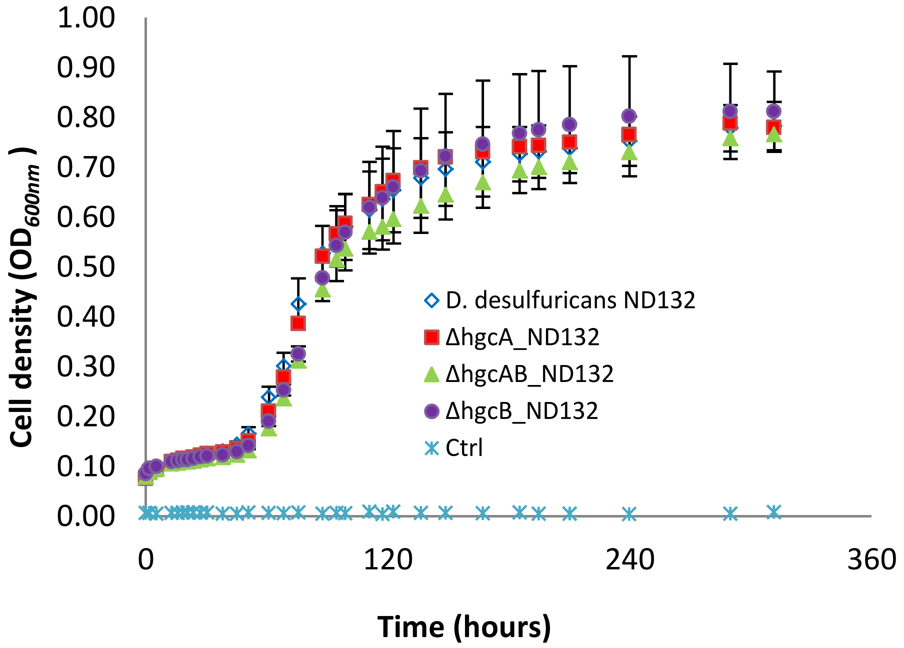
Conclude: *hgcA* and *hgcB* are both necessary for methylation of Hg

Production of methylmercury by *G. sulfurreducens* PCA mutants



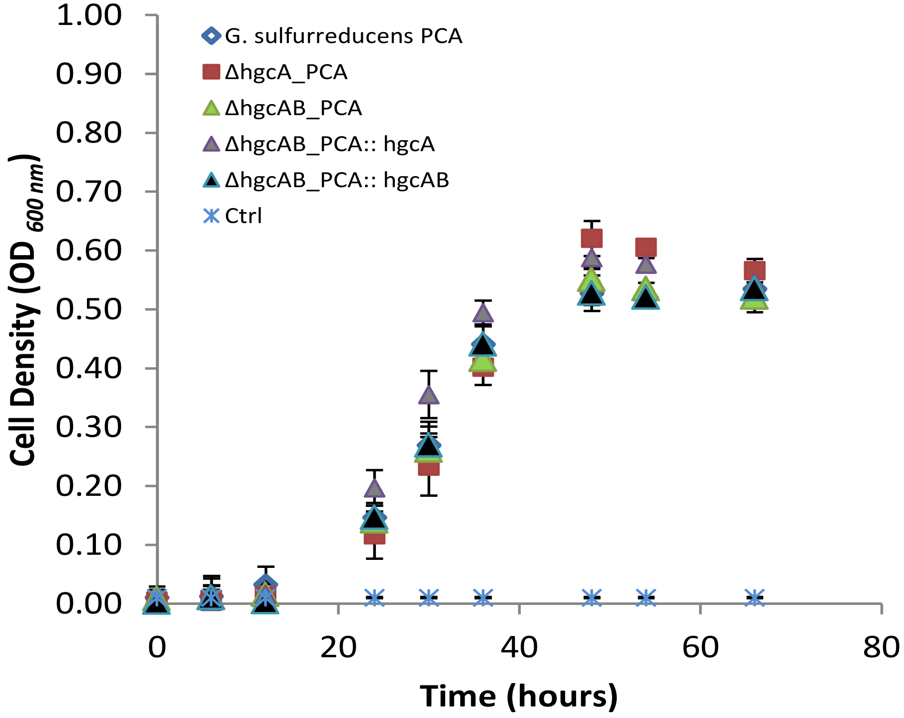
Conclude: *hgcA* and *hgcB* are both necessary for methylation of Hg

Growth of deletion mutants



***D. desulfuricans* ND132**

Growth with pyruvate/fumarate



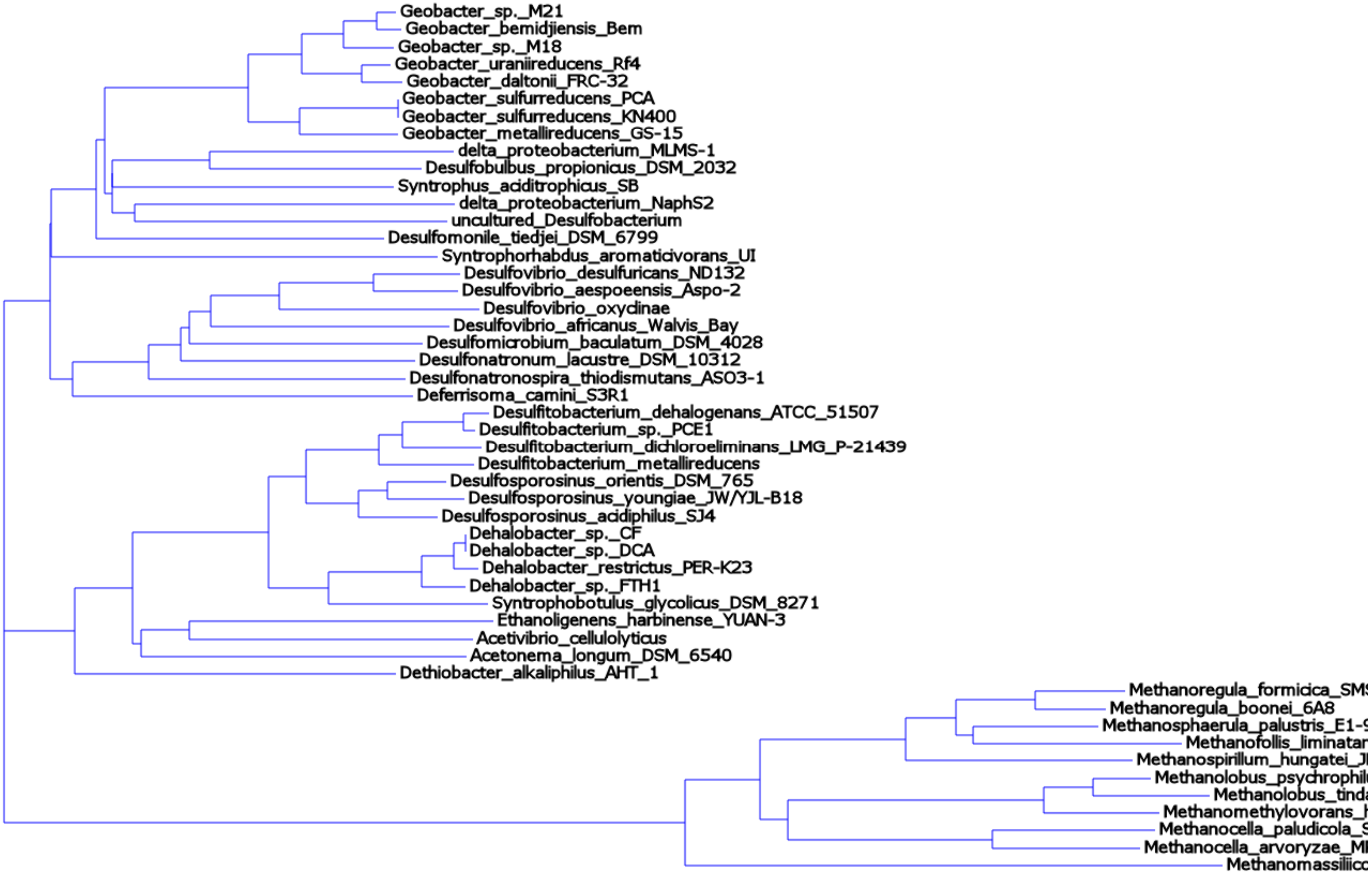
***G. sulfurreducens* PCA**

Growth with acetate/fumarate

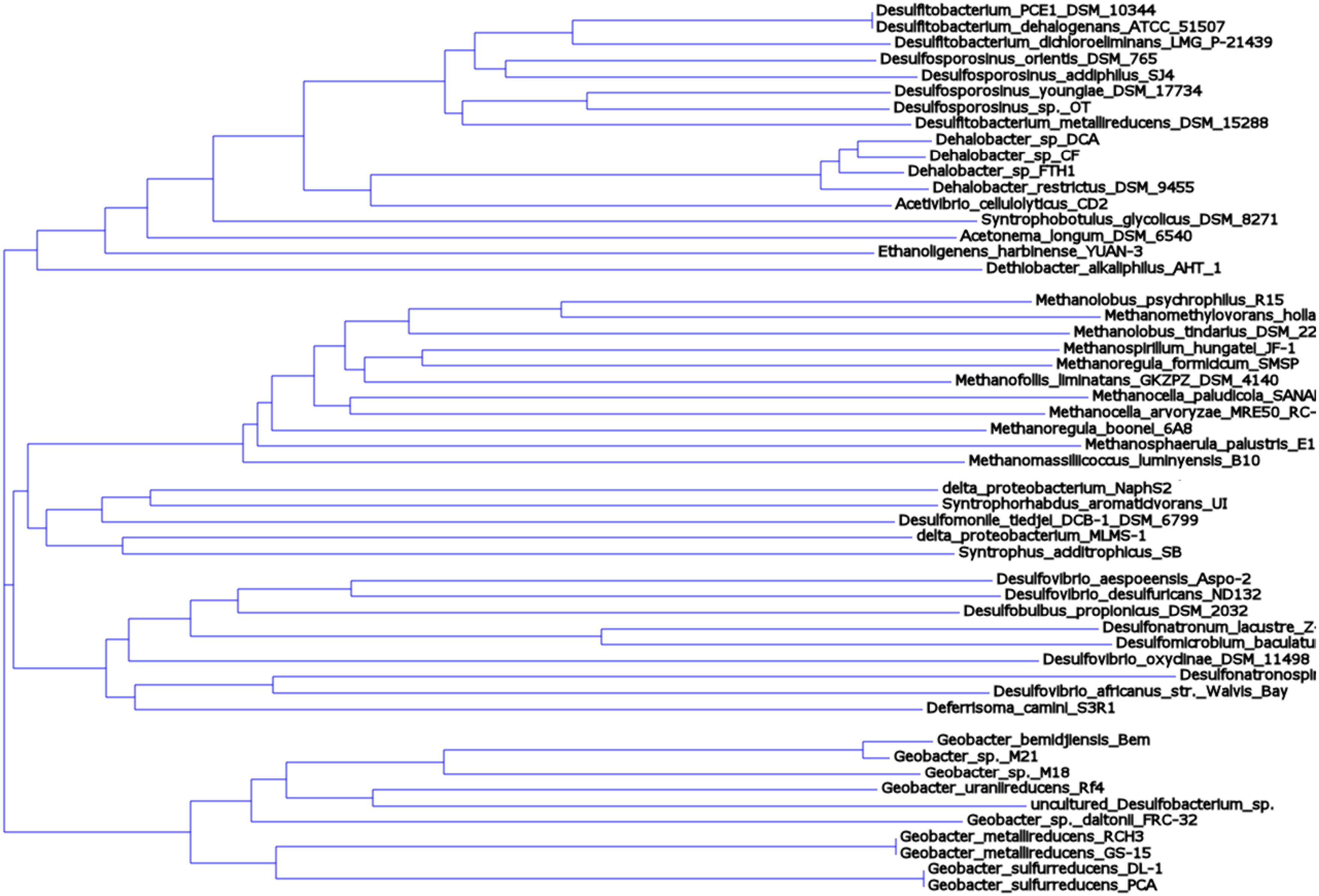
So far….perfect correlation between presence of *hgcAB* and ability to methylate mercury

Testing microbes with complete sequences

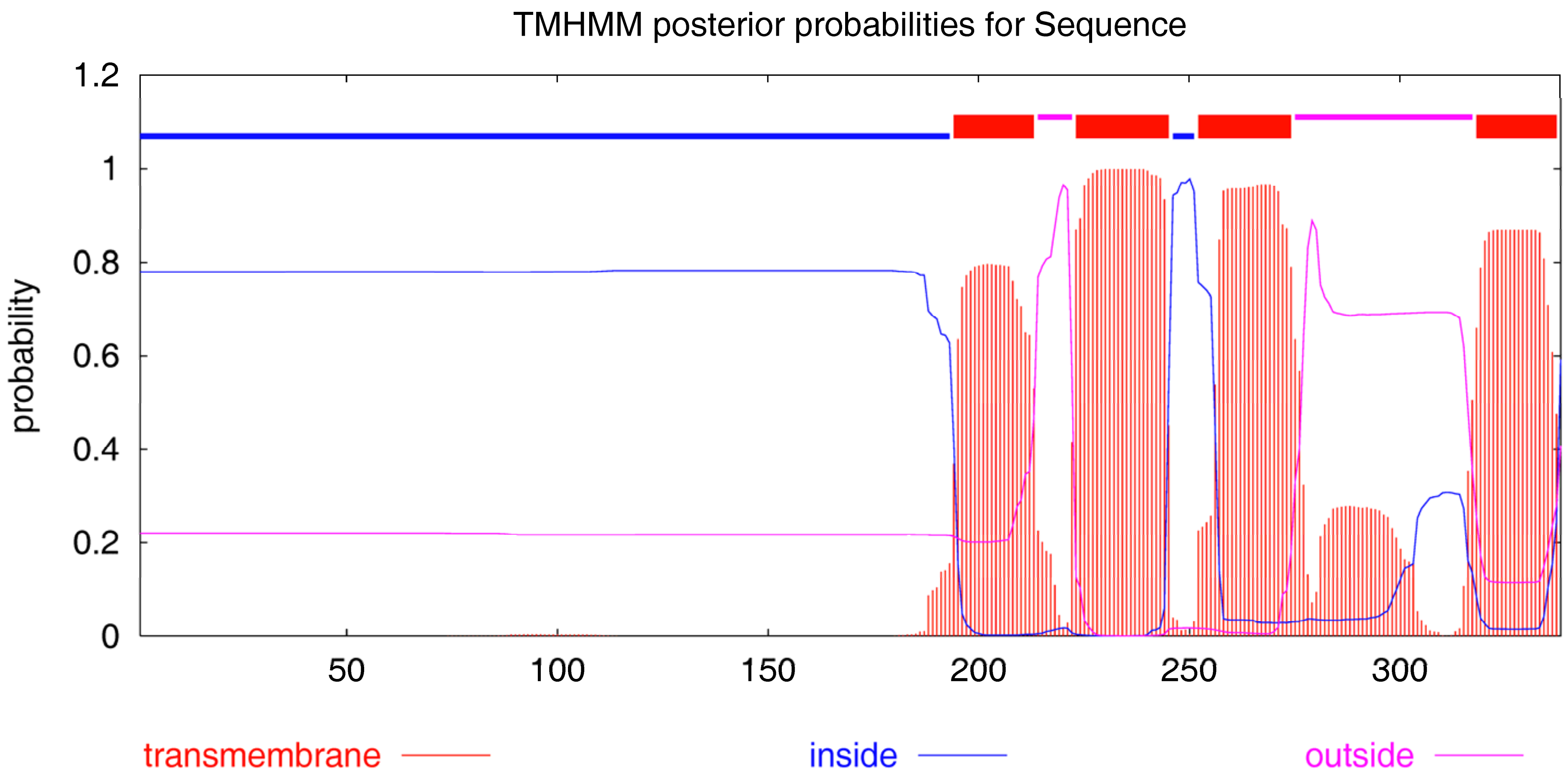
Phylogenetic tree: 16S rRNA of mercury methylating microbes



Phylogenetic tree: HgcA sequences



What is the role of the C-terminal domain in HgcA?

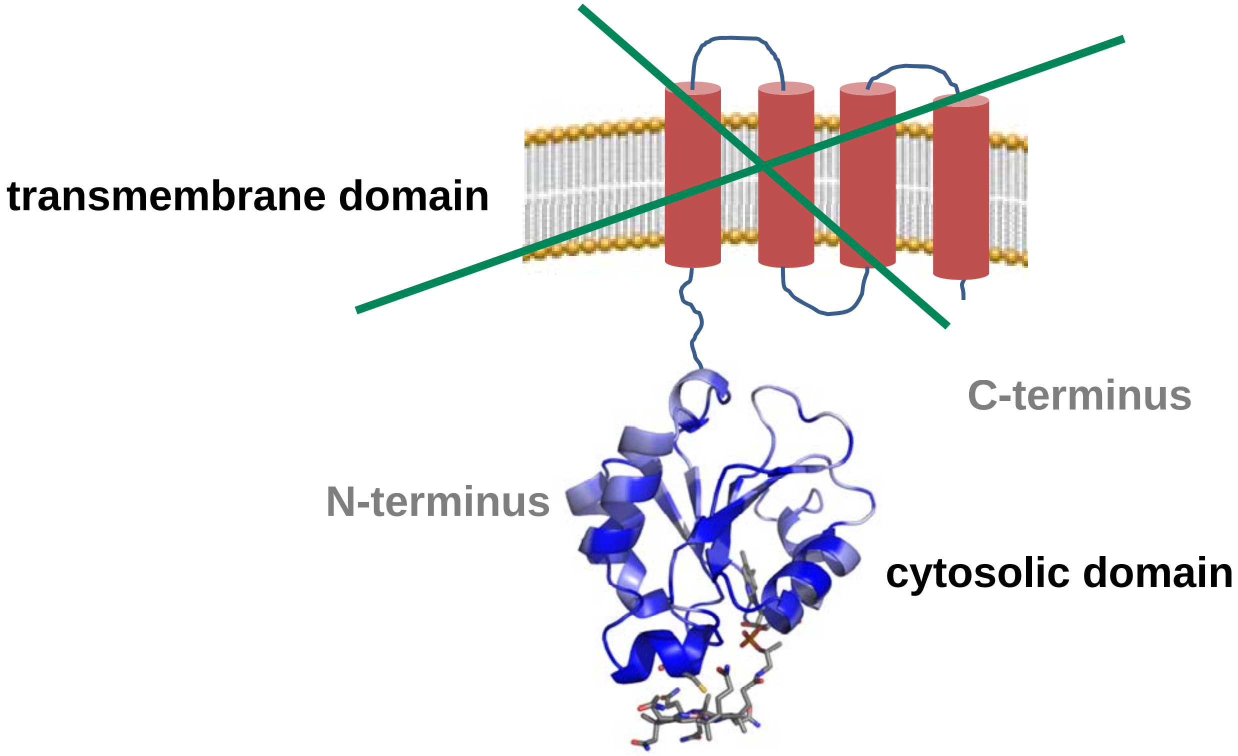


Membrane protein with 4 transmembrane helices

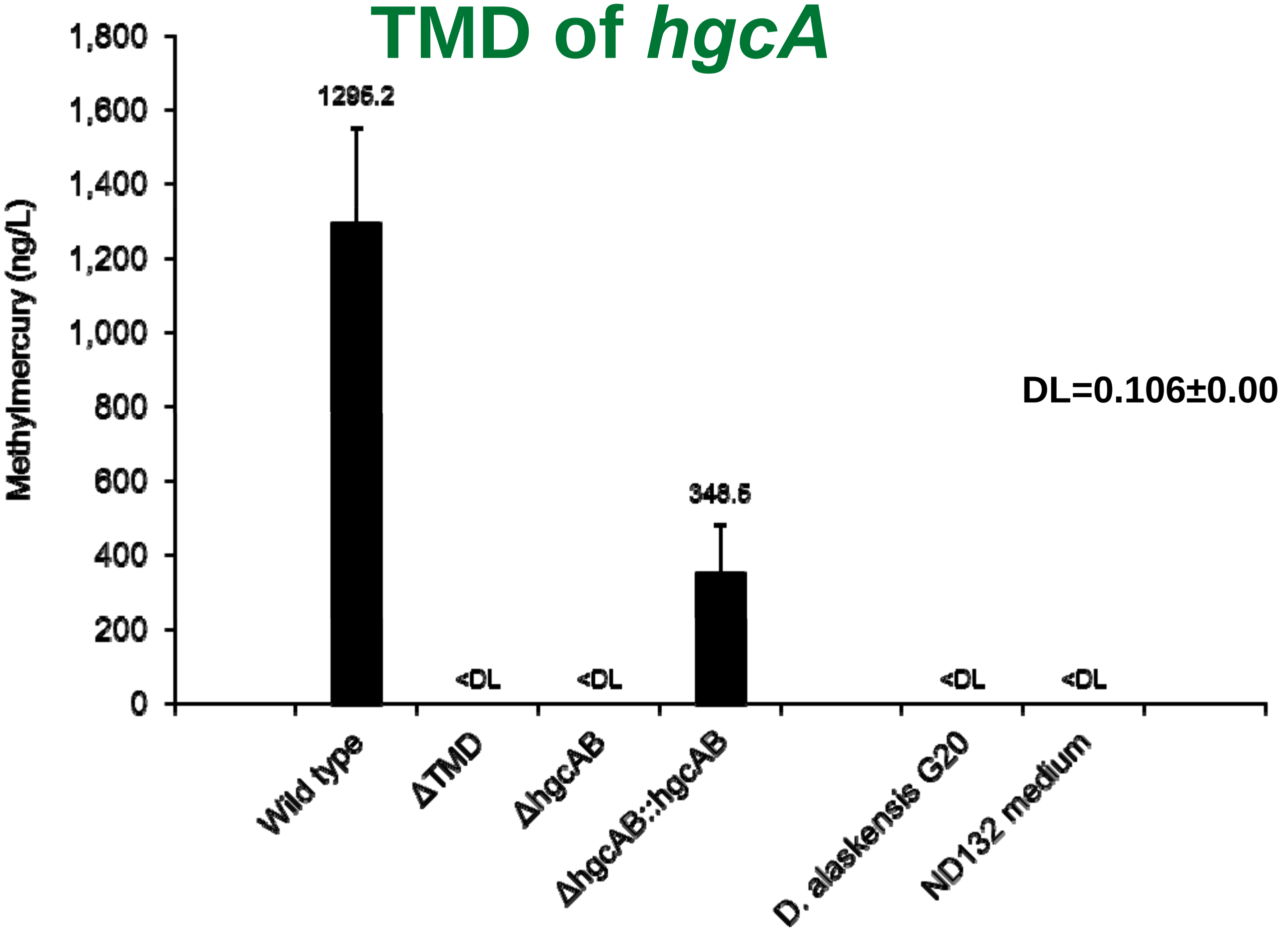
Sequence analysis of HgcA

>gi|323458404|gb|EGB14269.1| CO dehydrogenase/acetyl-CoA synthase delta subunit, TIM barrel [Desulfovibrio desulfuricans ND132]

**MDGFARTAAGPVPRVRTYLRRDDRVGDLRARLGTNRHDFKVVPGLYCVGEPDRTSPVLVTANYKLTFDTL
RERLTSIDAWLLVVDTRGINVWCAAGKGLFTASEVAFSVNAVRLHQVVEHRELILPQLAATGVAAREVER
ICGFKVLWGPIRARDLPAFLRNGNKADEAMRGVTFTLRERAALIPVELYQLRKPLFAAIPLLFLLSALGP
DLFSPPALWQRGISAVTATLVGALAGSVLVPLFLNRLPWRQFWPKGALVGGAAGTLAALYLPVHGWADPL
ALTLWATAVASWQAMNFTGSTPYTSPSGVEKEMRRGMPLQALAALAAAGLWLAGPFLG**



Production of methylmercury by *D. desulfuricans* ND132 mutant lacking TMD of *hgcA*



DL=0.106±0.005 ng/L

Conclude: Transmembrane domain required for methylation by ND132 *hgcA*

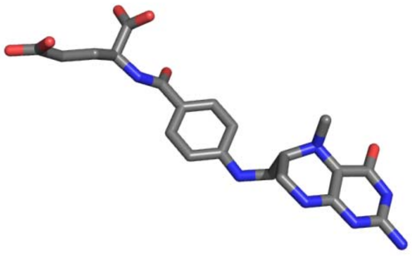
Assay: 2 h incubation and measured by EPT-GC-AFS

What are the implications?

- This discovery will likely change how mercury research is performed globally.
- The proposed mechanism is *new chemistry*, which, if confirmed, opens up new areas of research.
- We now have a potential biomarker for methylmercury generation.
- Correlating gene, protein and organism abundances with methylmercury formation rates and yields will lead to improved and more sensitive biogeochemical models!

Many questions remain regarding the molecular mechanism of mercury methylation

Methyl donor?
MeTr?
CH_3-THF

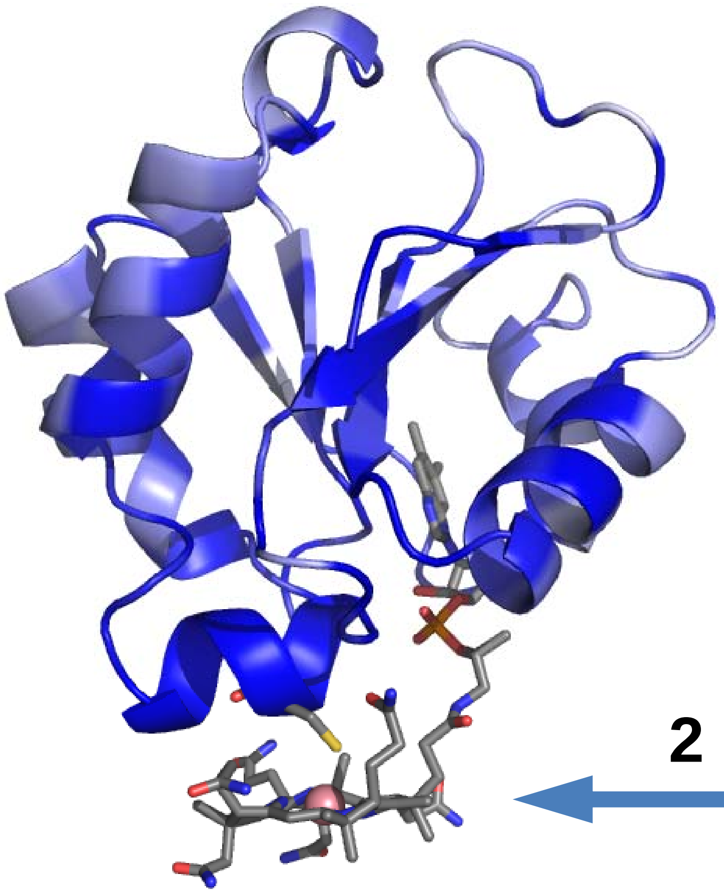


Reaction mechanism?

Function ?

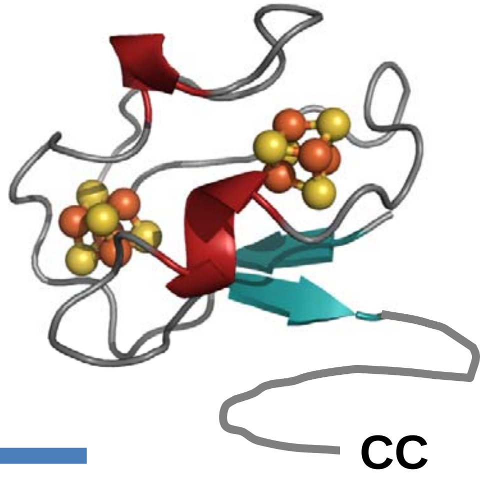
Structure?

Model of HgcA



Co(III) → Co(I)

Model of HgcB



2 e^- ←