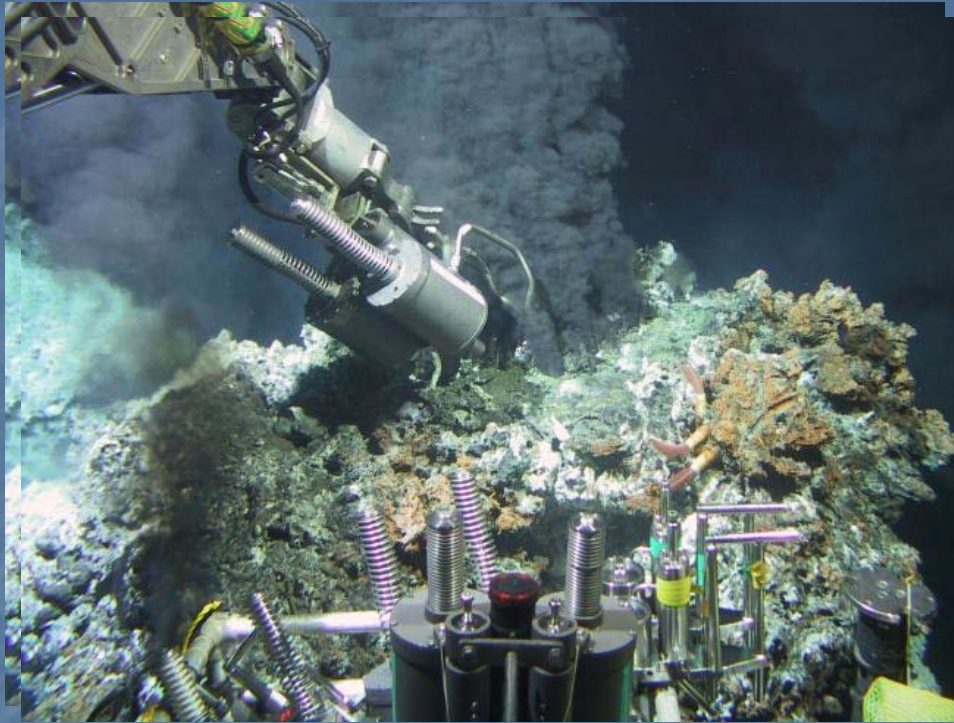


Life in Hot Carbon Monoxide: Comparative genomics and physiology of CO utilizing thermophiles



Frank Robb

University of Maryland School of Medicine, and
Institute of Marine and Environmental Technology

St Petersburg State University,
September 12, 2014

The 3rd Kamchatka Workshop
Sponsored by:
NSF, ISME, Finnzymes, NEB, CRDF



Kamchatka 2005: Biodiversity, Molecular Biology and Biogeochemistry of Thermophiles
August 20-26, 2005, Petropavlovsk-Kamchatksy, Russia

OUTLINE

1. Hydrogen and Biohydrogen
2. Carboydotrophs –Physiology and Genomics
3. Regulation of Overflow Metabolism
4. Chaperonin "Class III"

Hydrogen: Green and clean

- Hydrogen is the cleanest fuel.
- The use of hydrogen as common fuel holds promise for the future, but also problems.
- One major hurdle to overcome in using hydrogen is developing economic and clean ways of producing enough hydrogen.
- Biohydrogen production - using biological processes as means of hydrogen production.

Industrial Hydrogen Production

■ *Steam-Reforming Reactions*

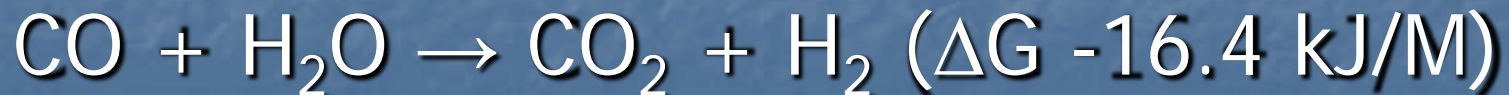
□ Methane:



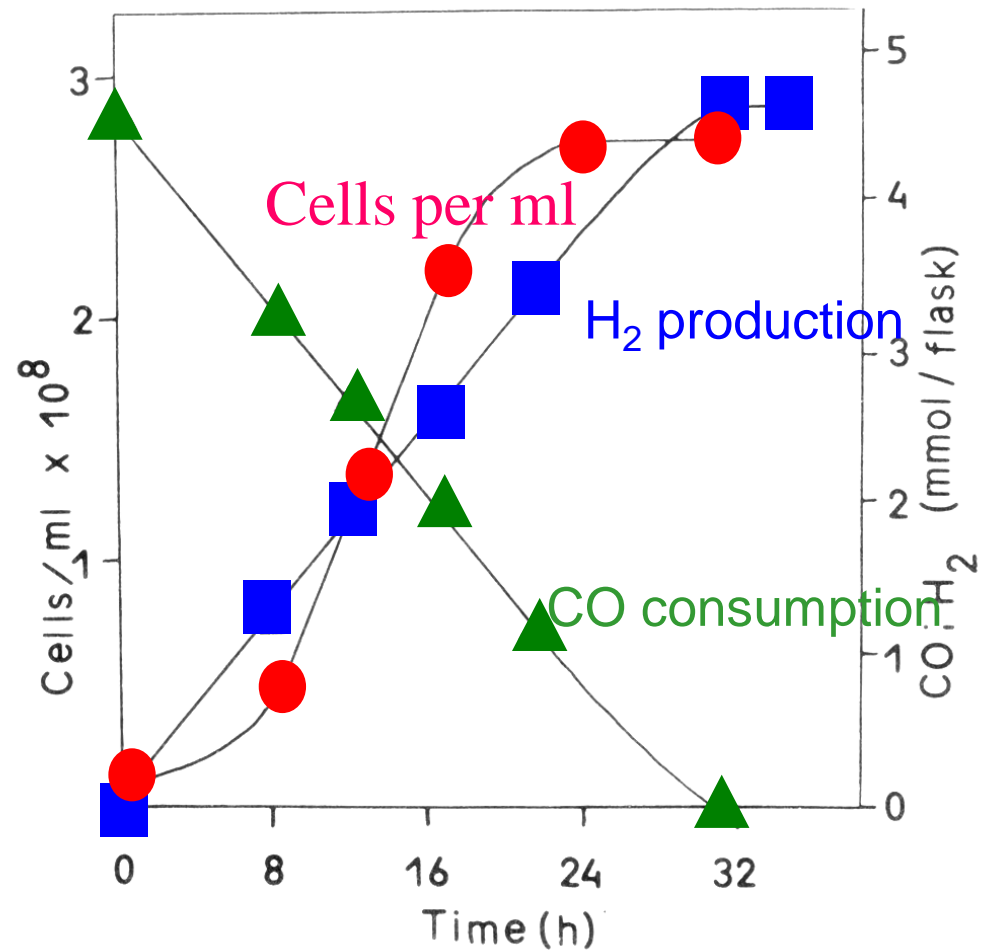
□ Propane:



■ *Water-Gas Shift Reaction*



- Obligate chemolithotroph
- Produces H_2 and CO_2
- Acetate is not produced
- Very high CO concentrations are not inhibitory
- Low GC (41.5%) Gram positive, sporulating bacterium



Carboxydotherrnus hydrogenoformans:
Growth with CO as sole carbon and
energy source.



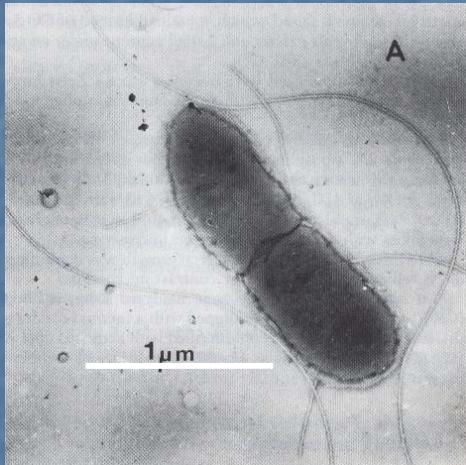
Thermophilic cyanobacterial mat

Hydrogenogenic CO-trophs

*Carboxydotherrmus
hydrogenoformans*

(Svetlichnyi et al., 1991)

Kuril Islands, Kamchatka
T: 40-80 ° C [70-72°C]
pH: 6.6-8.0 [7.0]



*Thermosinus
carboxydivorans*
strain Nor1^T

(Sokolova et al., 2004)

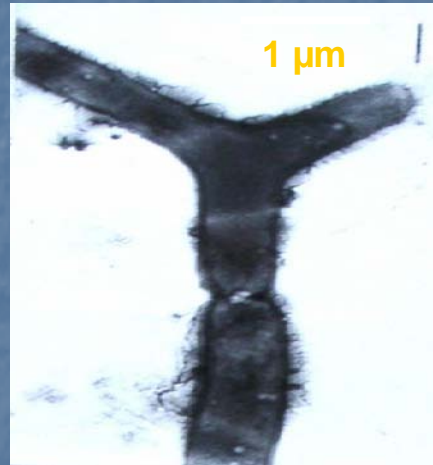
Norris Basin, YNP
40-68 ° C [60°C]
6.5-7.6 [6.8-7.0]



*Caldanaerobacter
subterraneus*
subs. *pacificus*

(Sokolova et al., 2001;
Fardeau et al., 2004)

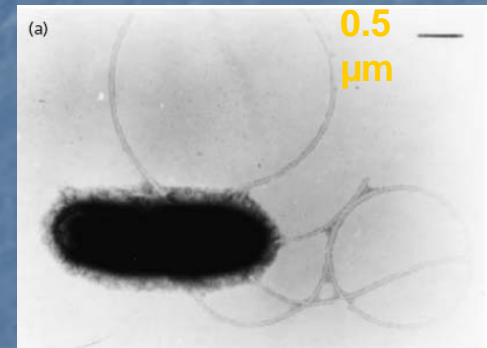
Okinawa Trough
50-80°C [70°C]
5.8-7.6 [6.8-7.1]



*Carboxydocella
thermautotrophica* 41^T

(Sokolova et al., 2002)

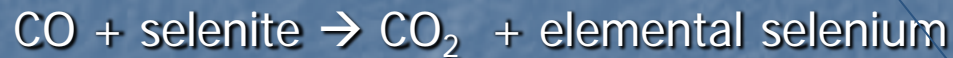
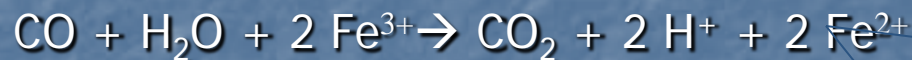
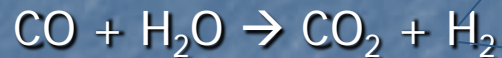
Geyser Valley,
Kamchatka
40-68 ° C [58 ° C]
6.5-7.6 [7.0]



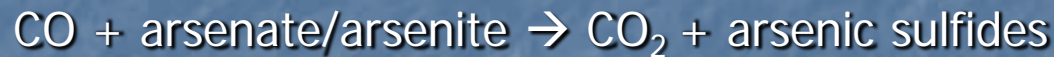
Carboxydotr

Carboxydothemus hydrogenoformans
Thermanaerobacterium sp. ST01
Thermosinus carboxydivorans Nor1
Caldanaerobacter subterraneus subs. Pacificum
Carboxydocella thermautotrophica
Carboxydocella sporoproducens
Rubrivivax gelatinosus
Rhodospirillum rubrum
Dictyoglomus carboxydivorans
Thermofilum sp.
Carboxydocella sp. 930
Carboxydocella sp. 961
Carboxydocella sp. 1244
Carboxydocella sp. 1503
Carboxydothemus siderophilus st. 1315

Anaerobic:



Thermosinus carboxydivorans Nor1
Carboxydocella ferrireducens st. 019
Carboxydothemus siderophilus st. 1315
Carboxydothemus ferrireducens



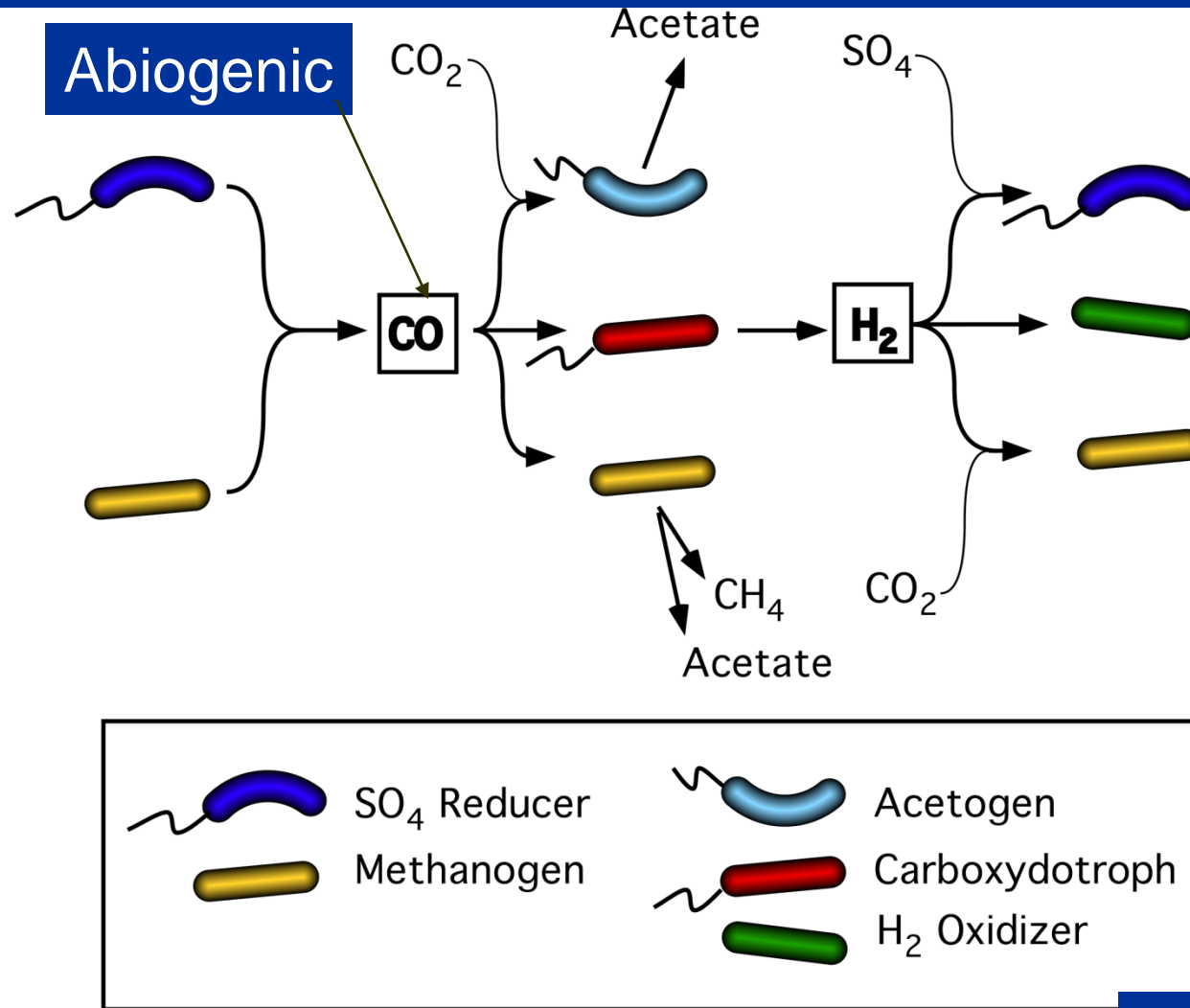
Moorella thermoacetica

Biosynthesis:



Methanosarcina acetivorans

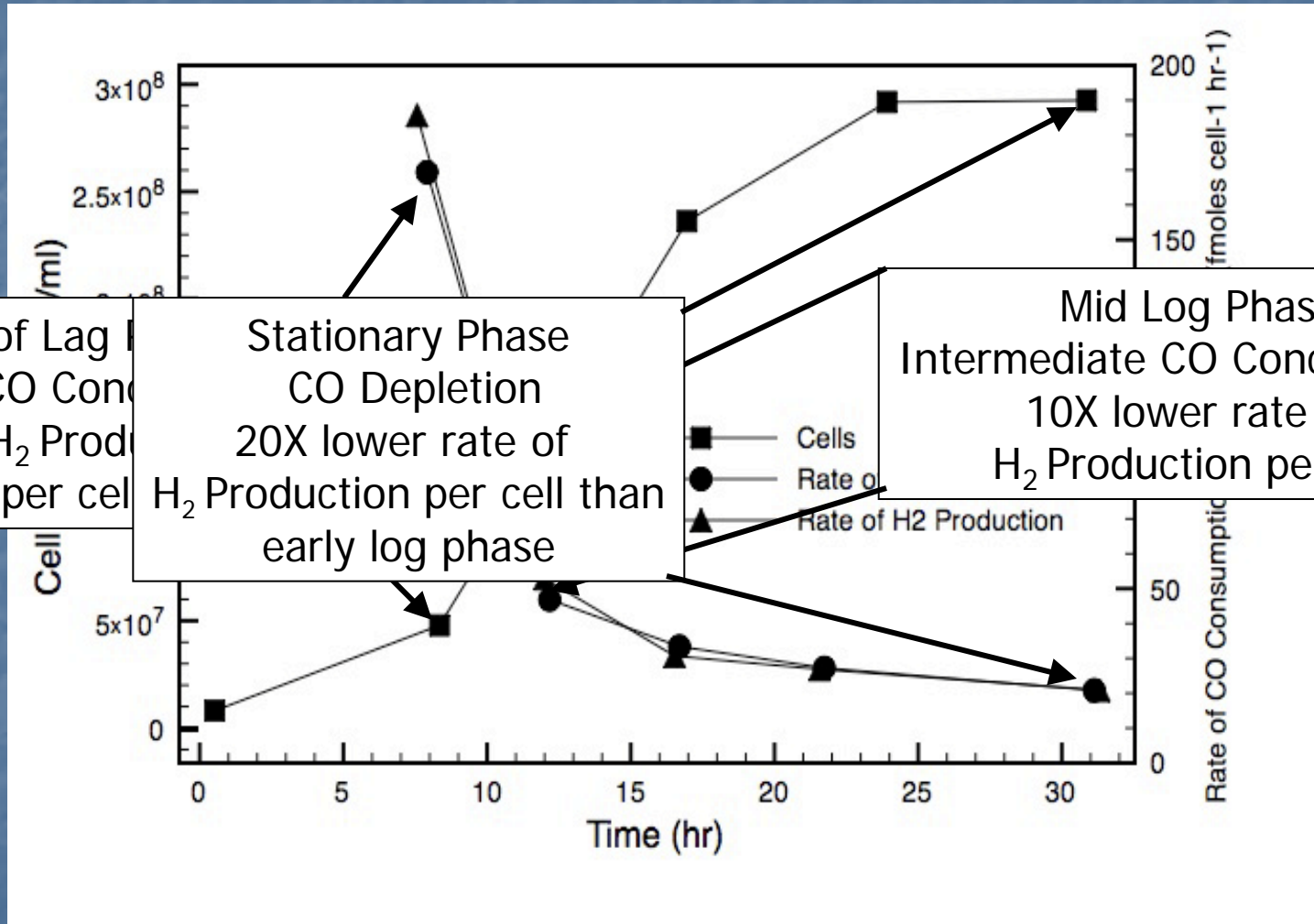
Role of Anaerobic Carbon Monoxide-Utilizing Microbes in Thermophilic Ecosystems



"Take and Give" Mutualism

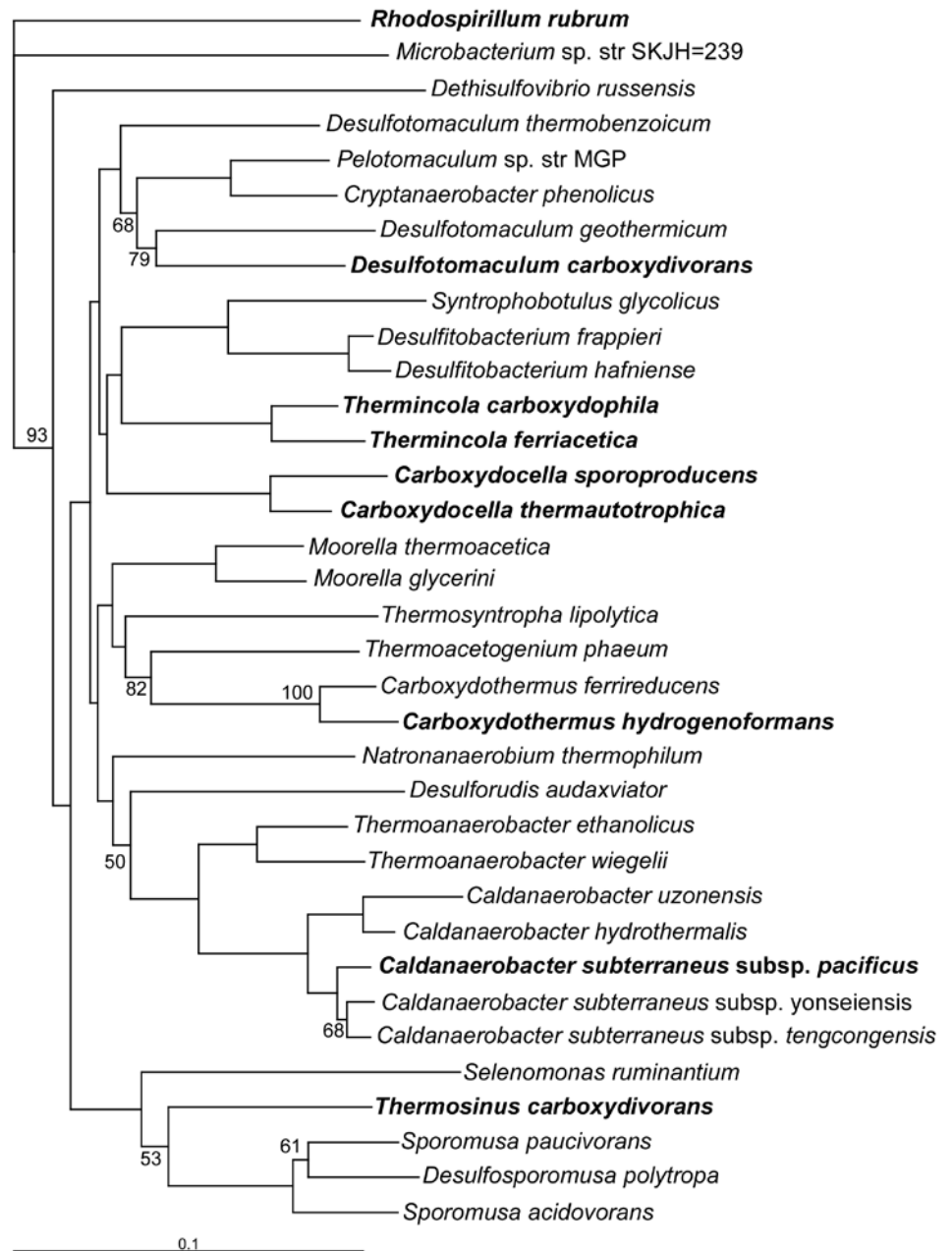
- Sulfate reducers can produce up to 6000 ppm CO during normal growth (10-100X higher than abiogenic CO levels).
- When CO is in excess, rapid depletion of CO levels will spare the community from CO-toxicity.
- This fresh supply of H₂ can then be fed back into sulfate reduction pathways which in turn can resupply the hydrogenogens with CO.
- When biogenic CO is not a dominant CO source, *C. hydrogenoformans* can survive on geothermal CO via CO-sensing.

Rates of H₂ Production

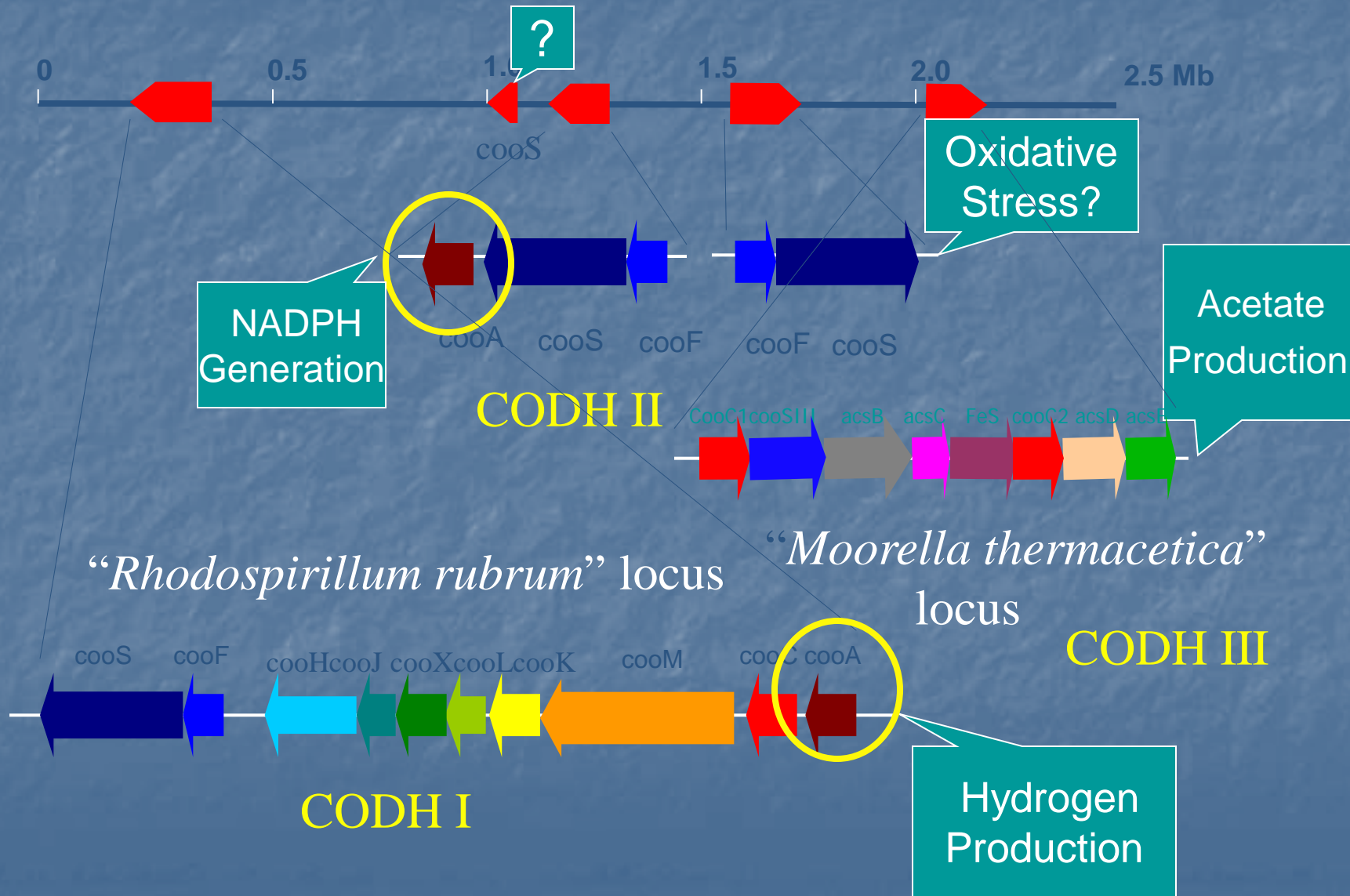


Overflow Metabolism?

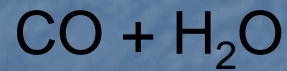
Diversity of CO-Utilizing Thermophiles



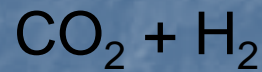
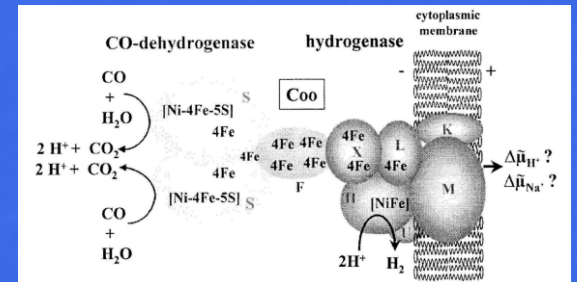
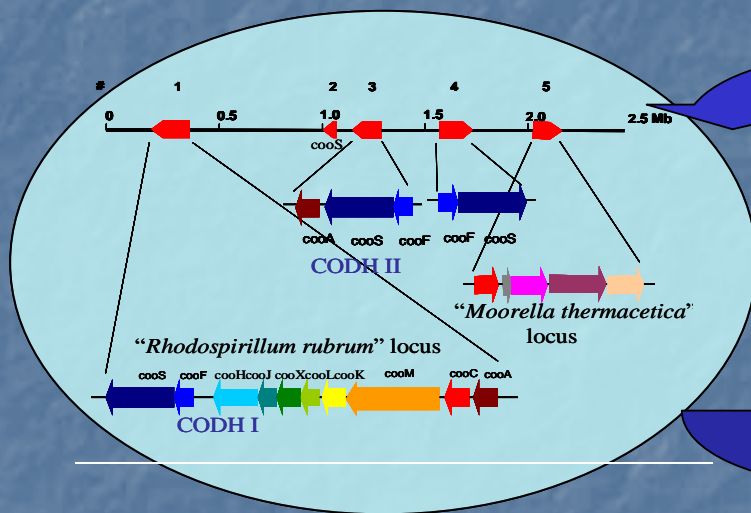
Relative location and gene composition of the CODH clusters in the *Carboxydothemus hydrogenoformans* genome.



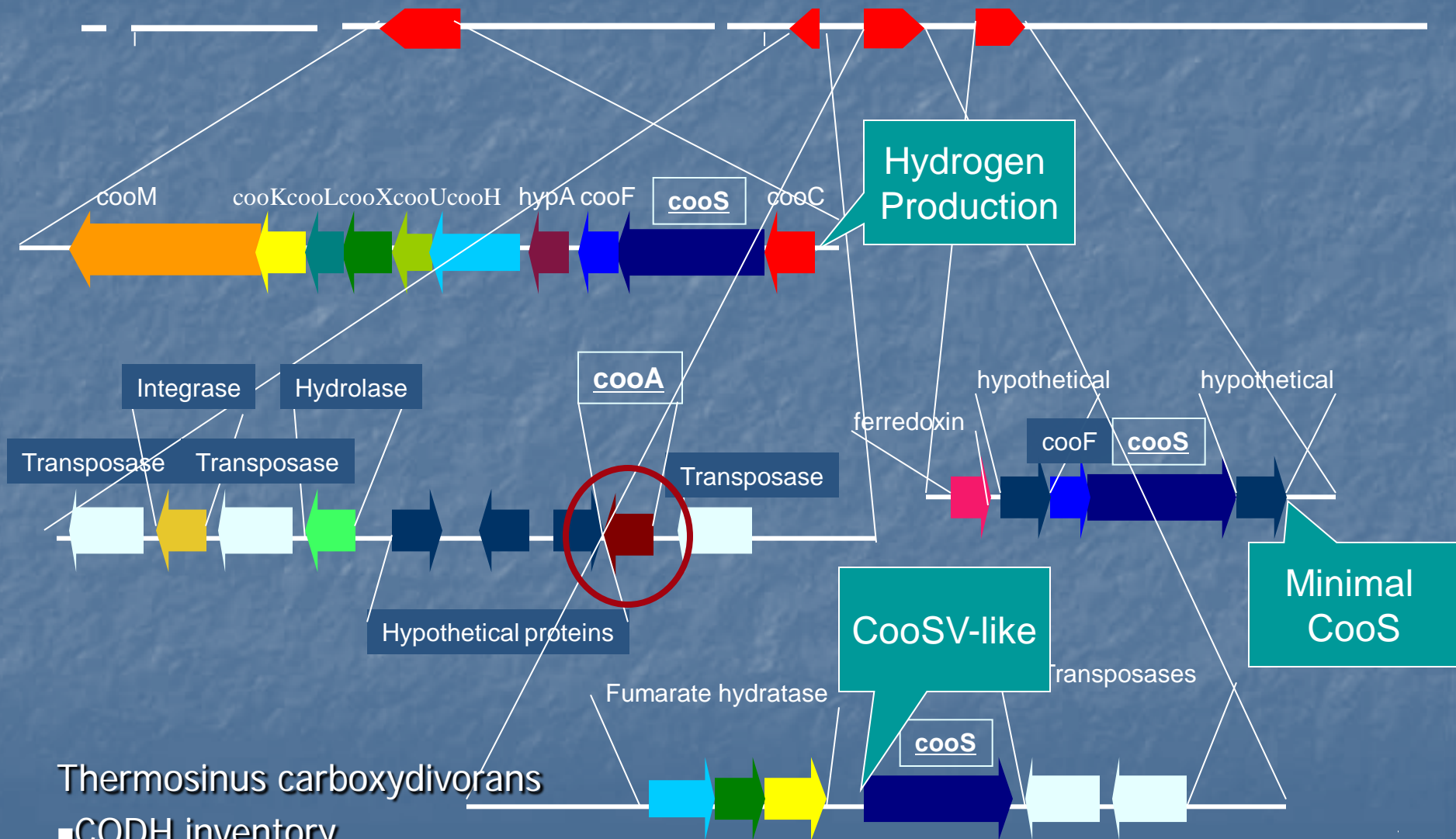
Genomics/ Transcriptomics



Proteomics



locus_tag	Name	Mowse Score	Peptide matches	% Coverage
CHY_0807	chaperonin, 60 kDa	367	4	11
CHY_0085	CO dehydrogenase subunit, cooS II	288	8	23
CHY_0666	alanine dehydrogenase	282	5	16
CHY_2312	translation elongation factor Tu	258	6	25
CHY_0789	aldehyde ferredoxin oxidoreductase	232	6	13
CHY_1373	fumarate reductase, Cluster B	197	5	12
CHY_0269	dehydrogenase, FMN-dependent family	176	4	11
CHY_0284	enolase	169	4	14
CHY_0695	glutamate formiminotransferase	162	6	8
CHY_0128	fructose-1,6-bisphosphate aldolase, class II	161	2	11
CHY_1824	CO dehydrogenase subunit, cooS I	149	3	9
CHY_1998	acetyl-CoA carboxylase, biotin carboxylase	145	2	6
CHY_1353	electron transfer flavoprotein, alpha subunit	142	3	9
CHY_1839	glycerol kinase	134	4	12
CHY_1374	fumarate hydratase,Cluster B	111	2	17
CHY_1226	CO dehydrogenase subunit acsD	109	1	5
CHY_1222	CO dehydrogenase subunit acsB	106	2	2
CHY_1223	CO dehydrogenase subunit acsC	72	2	3



Thermosinus carboxydivorans

■ CODH inventory

■ *cooA*

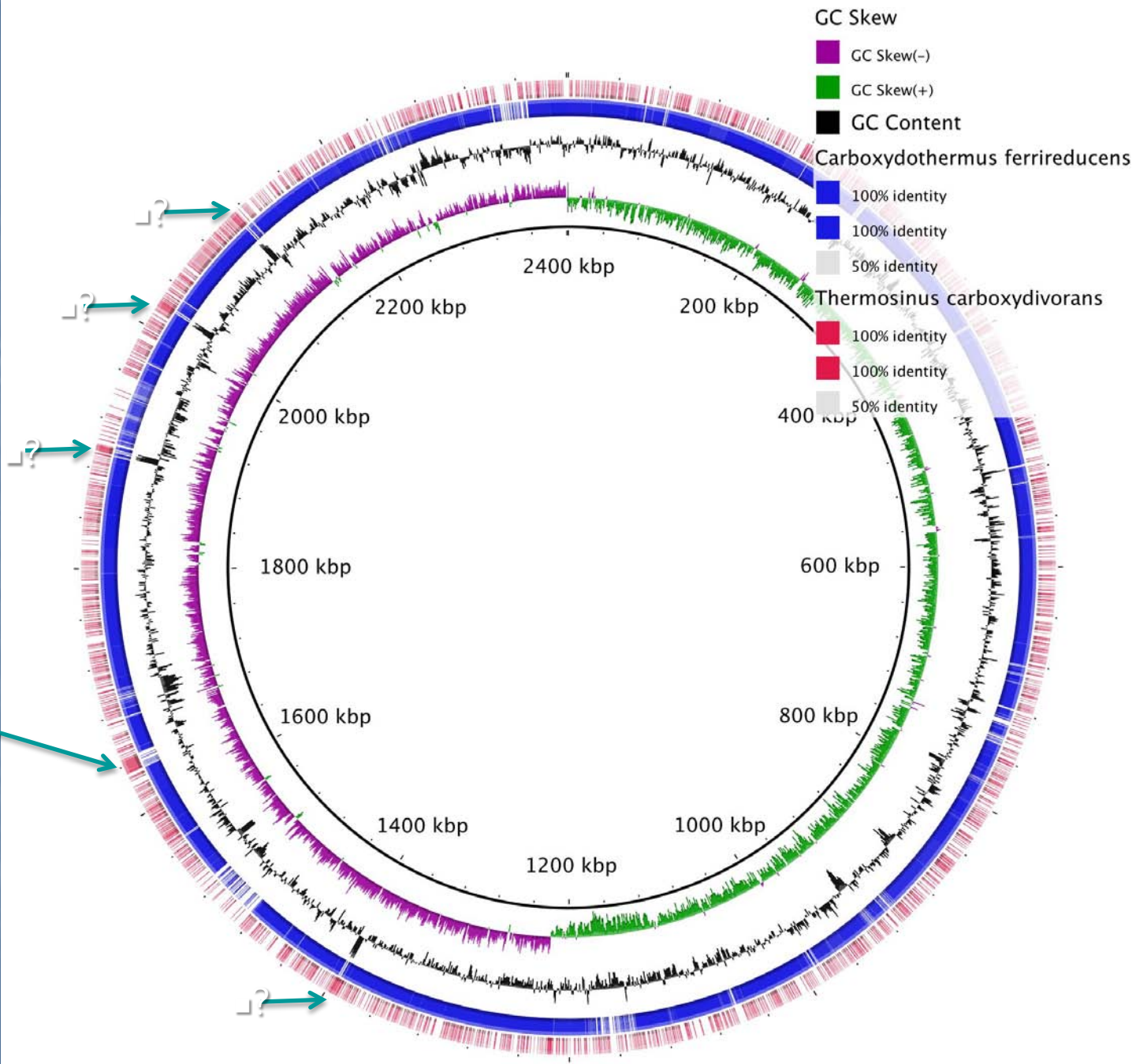
Unique Aspects of *Thermosinus* CODHs

- Three CODHs
 - One is linked to a hydrogenase (Similar to *C. hydrogenoformans* CODH1) (Contig 51).
 - One is similar to the CODHV from *C. hydrogenoformans* and is not linked to any other CO-related genes(Contig 46).
 - One is considerably smaller (482 a.a. compared to the average *cooS* (622-712 a.a.)). This contains all of the conserved domains necessary for a *cooS* (Contig 54).
- No CODH/ACS

CODH1 Gene cluster

- High similarity between *T. carboxydivorans* CODH1 and *C. hydrogenoformans* CODH1 (88% identity on the nucleotide level).
- Percent GC
 - *T. carboxydivorans* - 51.6 %
 - *C. hydrogenoformans* - 42.0 %
 - *T. carboxydivorans* CODH1 operon - 43.4 %

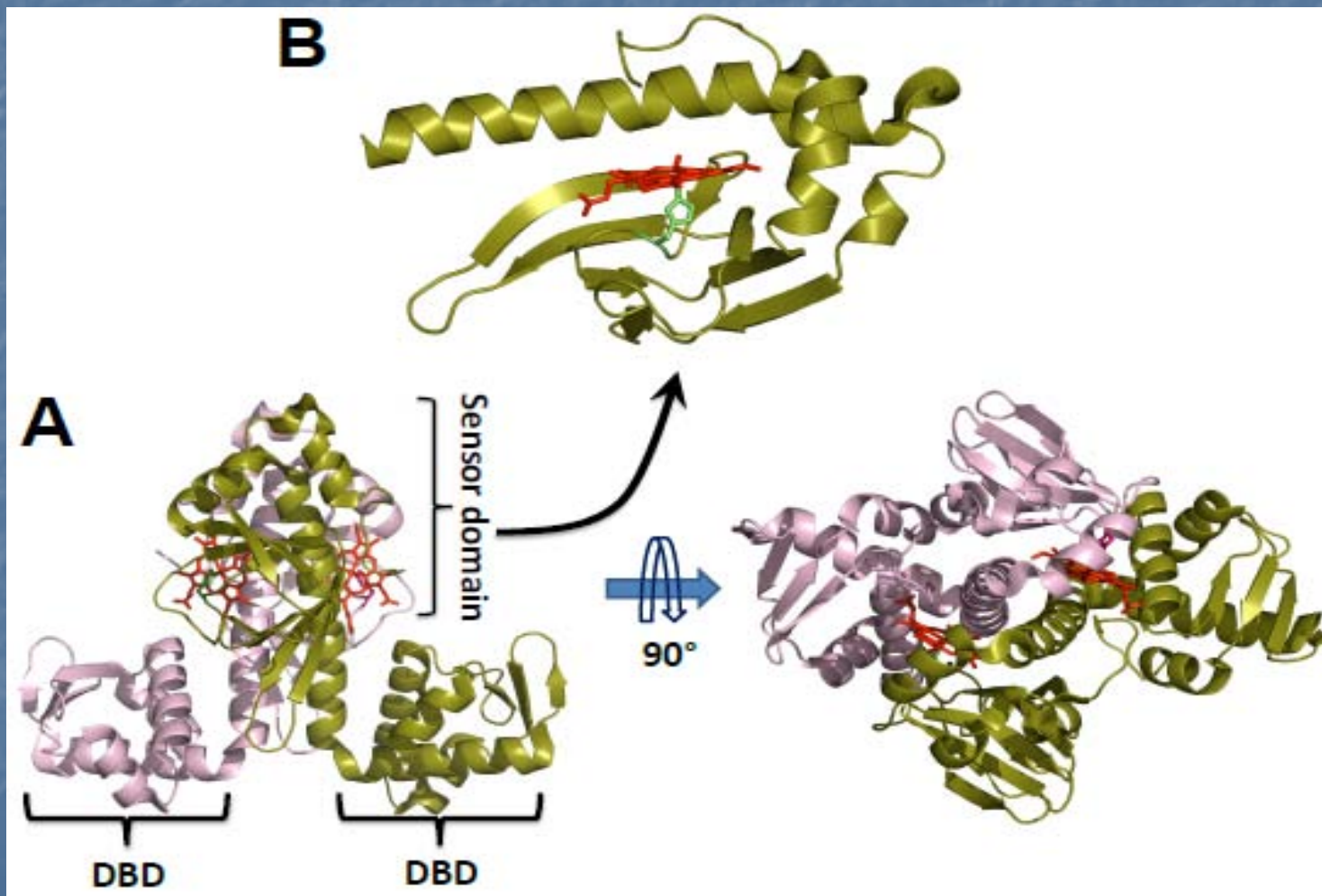
■CODH1



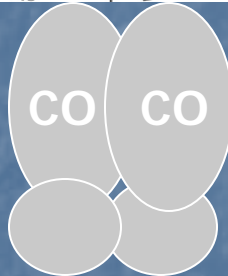
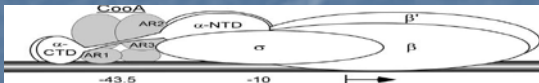
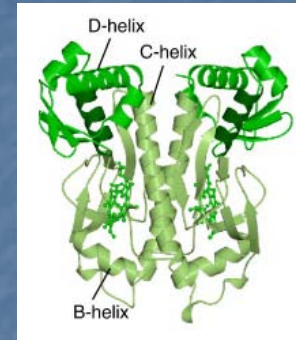
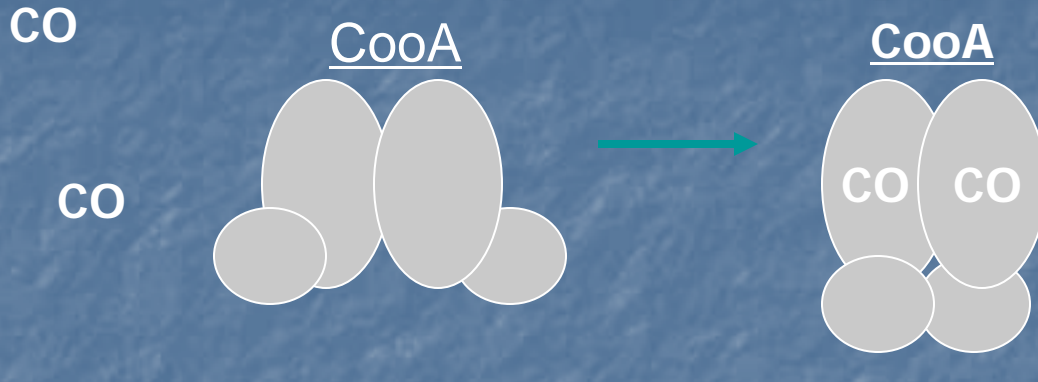
Organisms with CooA Homologs

Organism	Number of CooA Homologs	Original Description	CODH/ACS
<i>Carboxydotherrnus hydrogenoformans</i>	2 (CooA-1 and CooA-2)	Thermophilic hydrogenogen	Yes
<i>Carboxydotherrnus ferrireducens</i>	1 (CooA-2)	Thermophilic Iron reducer	Yes
<i>Thermosinus carboxydivorans</i>	1 (CooA-1)	Thermophilic hydrogenogen	No
<i>Rhodospirillum rubrum</i>	1 (CooA-1)	Phototrophic purple non-sulfur bacteria	No
<i>Azotobacter vinelandii</i>	1	Aerobic nitrogen fixer	No
<i>Desulfovibrio vulgaris</i>	1	Sulfate reducer	No
<i>Desulfovibrio desulfuricans</i>	1	Sulfate reducer	No
<i>Desulfitobacterium hafniense</i>	2 (One CooA-2 homolog)	Reductive dechlorinator	Yes

CooA



Action of CooA



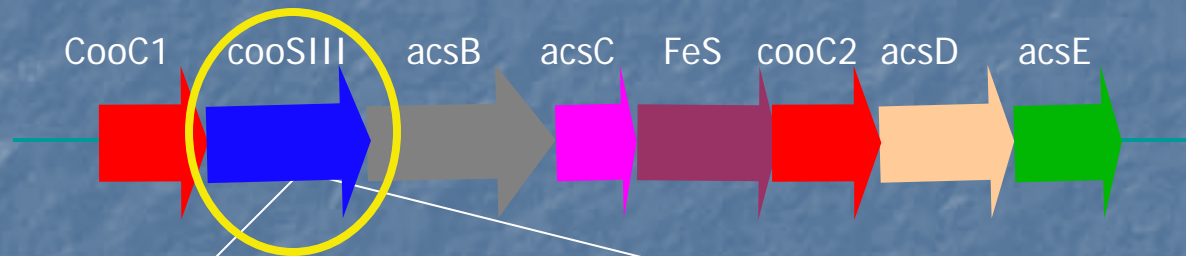
cooF promoter – **TGTCA NNNNNN CGACA**
cooM promoter – **TGTCG NNNNNN TGACG**

Dysfunctional Genomics:

Frameshifts in the *C. hydrogenoformans* genome

- 20 frameshift mutations were found in the genome.
- The sequenced strain was grown in medium supplemented with pyruvate to encourage growth.
- One of the frameshifts was in the CODH/ACS gene cluster.

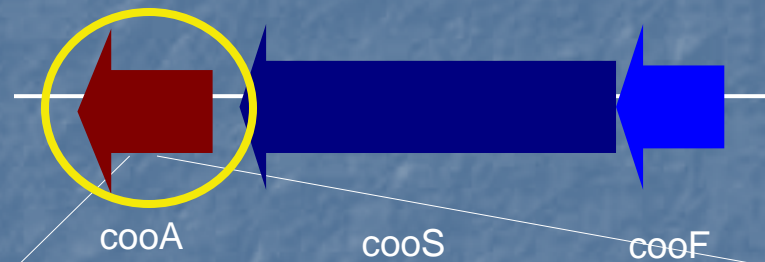
CODH/ACS Catalytic Subunit Frameshift



Sequenced strain
Genome sequence
DSMZ archived strain

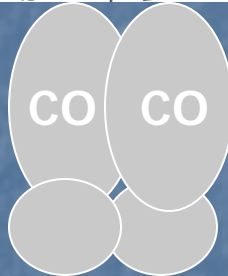
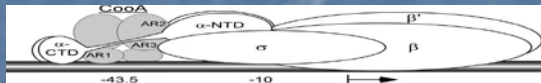
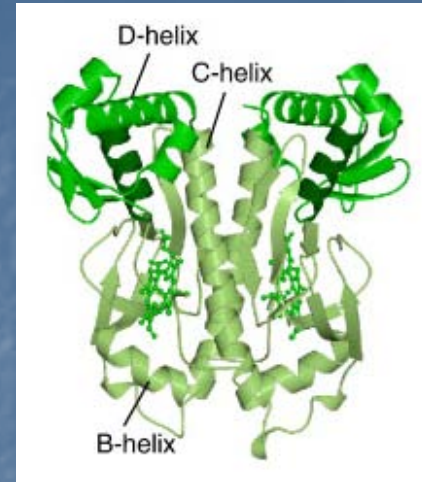
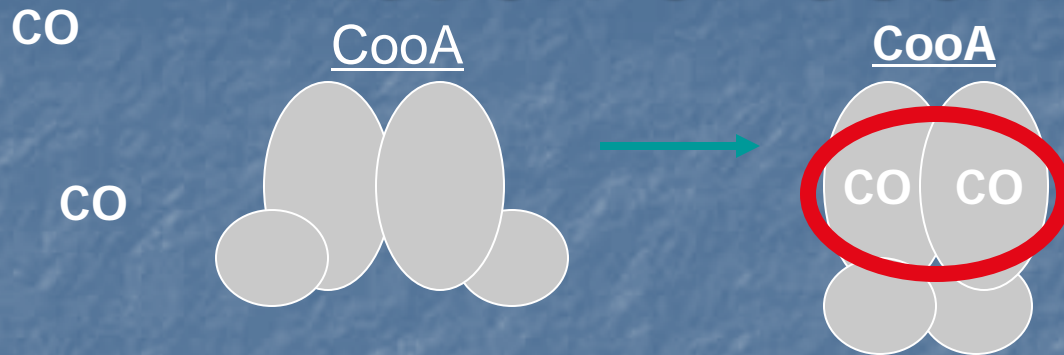
```
*****
2AF  GGCTGG-ATACTCAAGGAAAAGACGATATGAC
CH   GGCTGG-ATACTCAAGGAAAAGACGATATGAC
DF   GGCTGGATACTCAAGGAAAAGACGATATGAC
ruler  ....460.....470.....480.
```

CooA-2 Frameshift



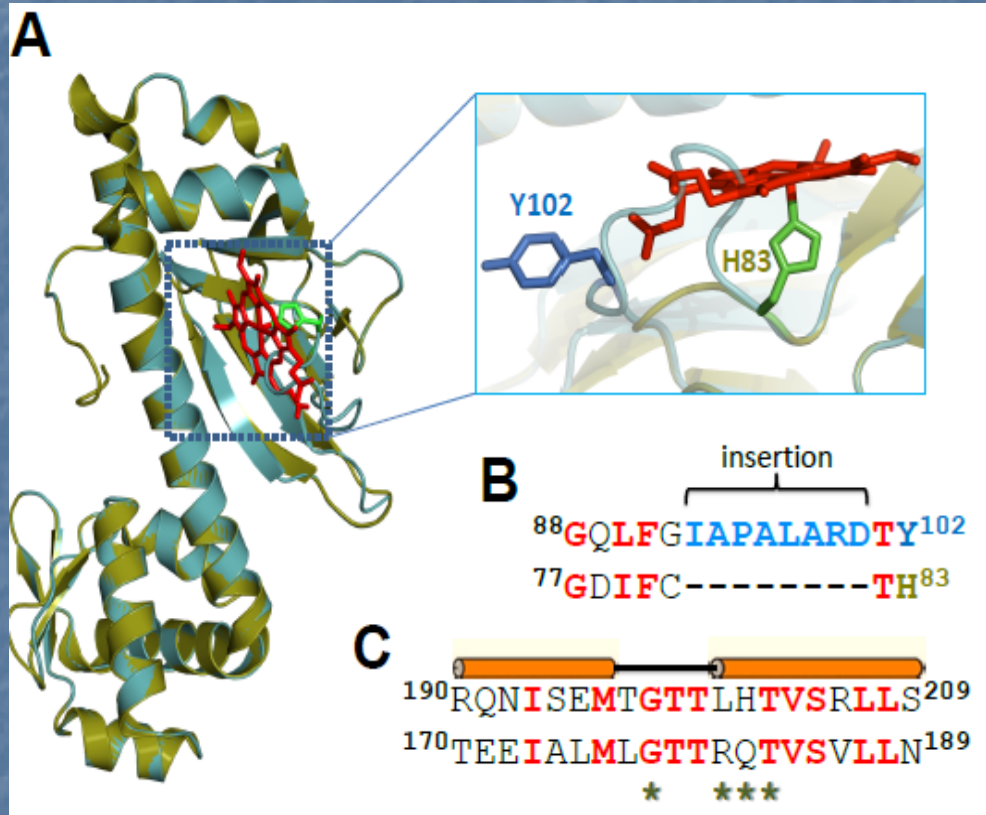
1	CooA-2.Z-29	AAGGAGTTTATCCCCAGTTTAAAGAGATTTACCTAAAGAAAAAAGAAATAGCCTTTTCCCCGGGAATCT
2	CooA-2.DSMZ	AAGGAGTTTATCCCCAGTTTAAAGAGATTTACCTAAAGAAAAA-GAAATAGCCTTTTCCCCGGGAATCT
	ruler70.....80.....90.....100.....110.....120.....130....

Action of CooA



cooF promoter – **TGTCA NNNNNN CGACA**
cooM promoter – **TGTCG NNNNNN TGACG**

CooA and NnrR



■ (A) Superposition of Ch-CooA (olive) and NnrR (teal). The boxed region corresponding to the heme binding pocket is expanded on the right. The proximal heme ligand of Ch-CooA is His-83 (green) and that of NnrR is Tyr-102 (blue)

■ (B) Amino acid sequence in the vicinity of heme ligands of Ch-CooA (top) and NnrR (bottom). The unique insertion of NnrR is highlighted in blue. Conserved residues are in red.

■ (C) Helix-loop-helix (orange cylinder, helix) region essential for DNA binding.

"Red" *E. coli*

Expression of CooA1

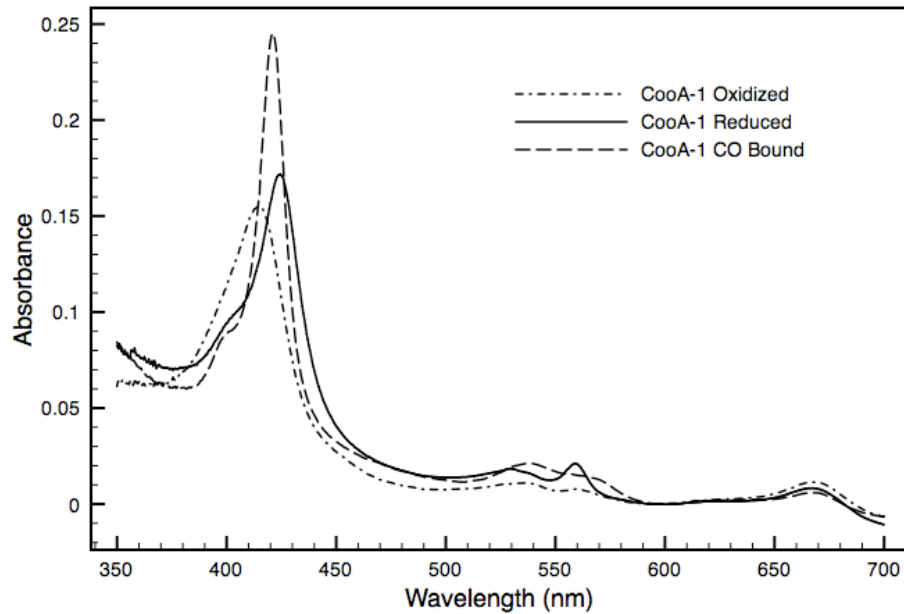
E. Coli VJS6737 grown anaerobically at 30°C on MOPS minimal medium supplemented with Amp. (100µg/ml), 2% CO, and varying levels of IPTG.



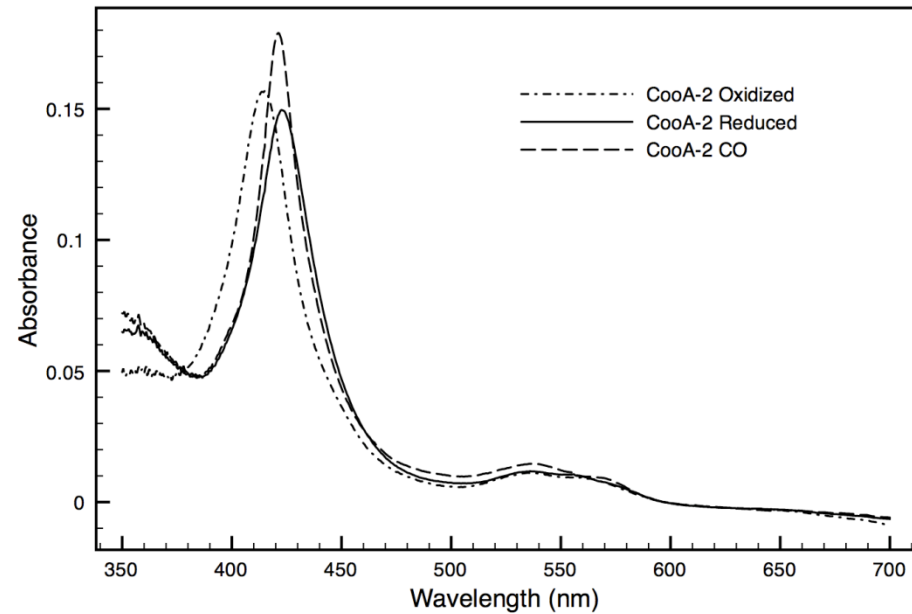
A - No IPTG
B - 25µM IPTG
C - 50µM IPTG
D - 100µM IPTG
E - 250µM IPTG
F - 500µM IPTG

CO binding Spectrum of CooA-1 and CooA-2

CooA-1



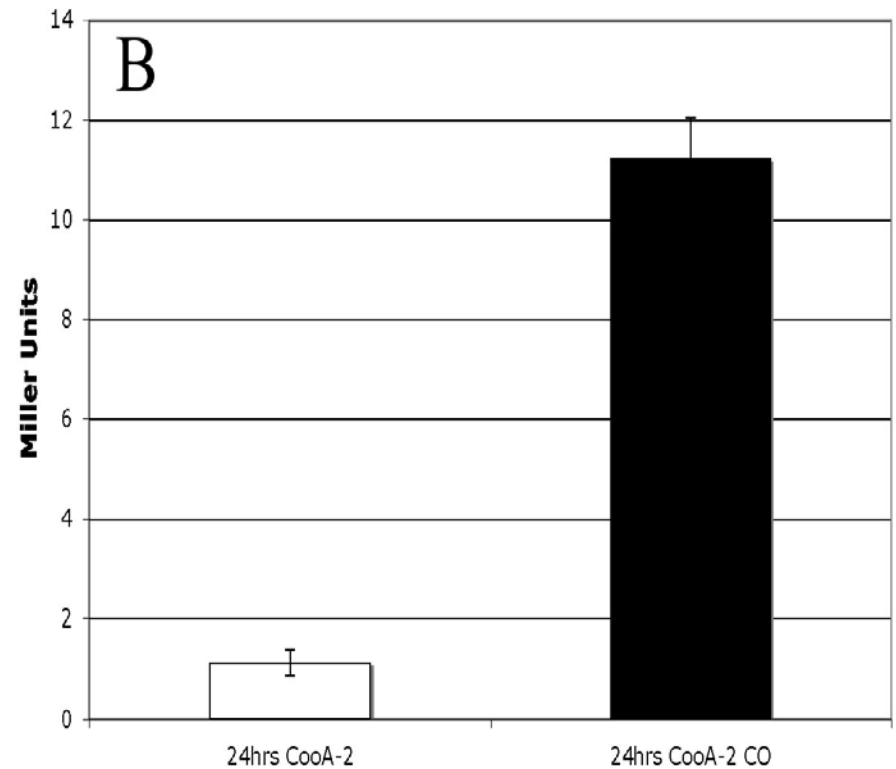
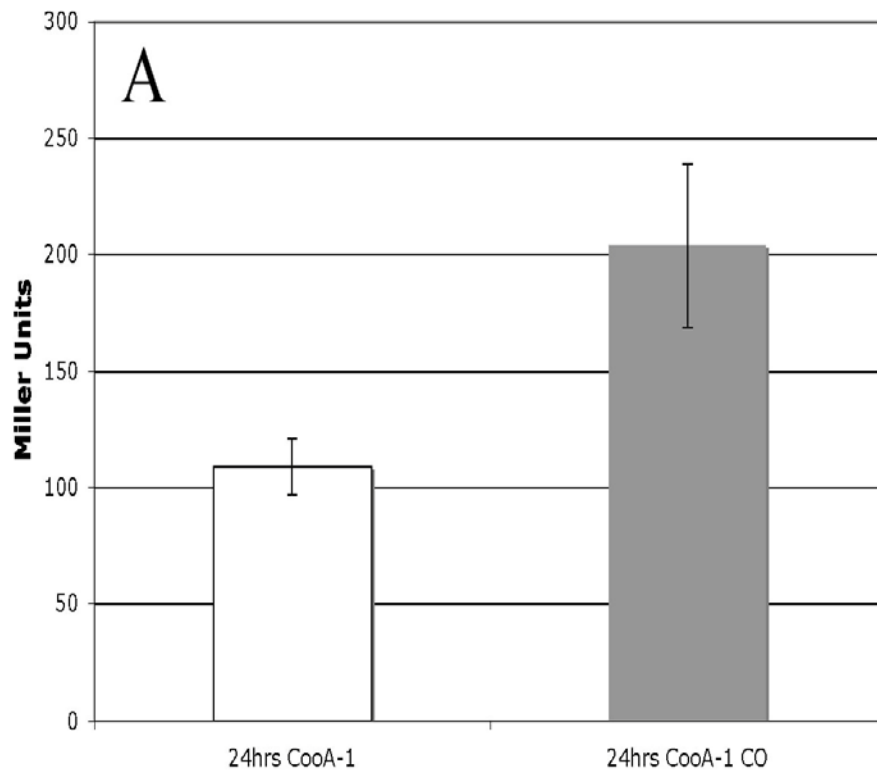
CooA-2



R. rubrum *cooF* promoter *lacZ* fusions

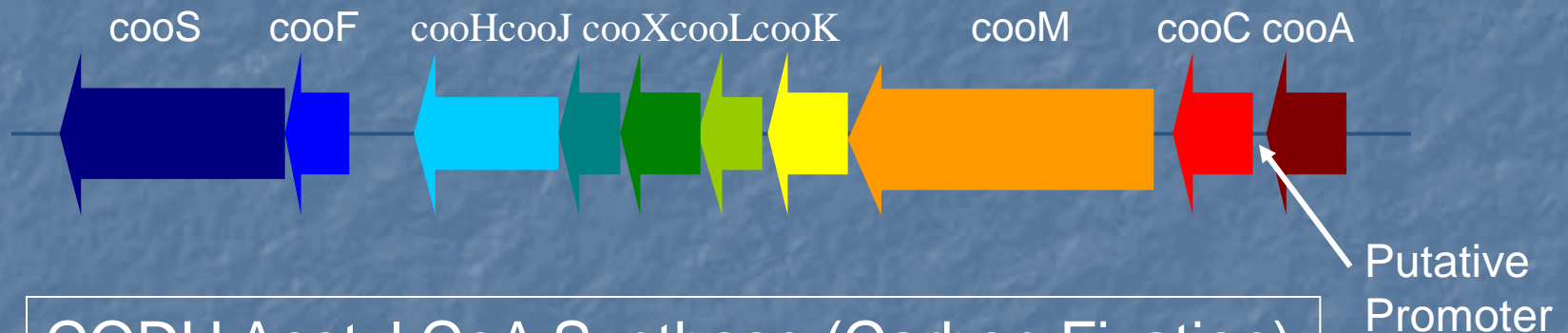
CooA-1

CooA-2

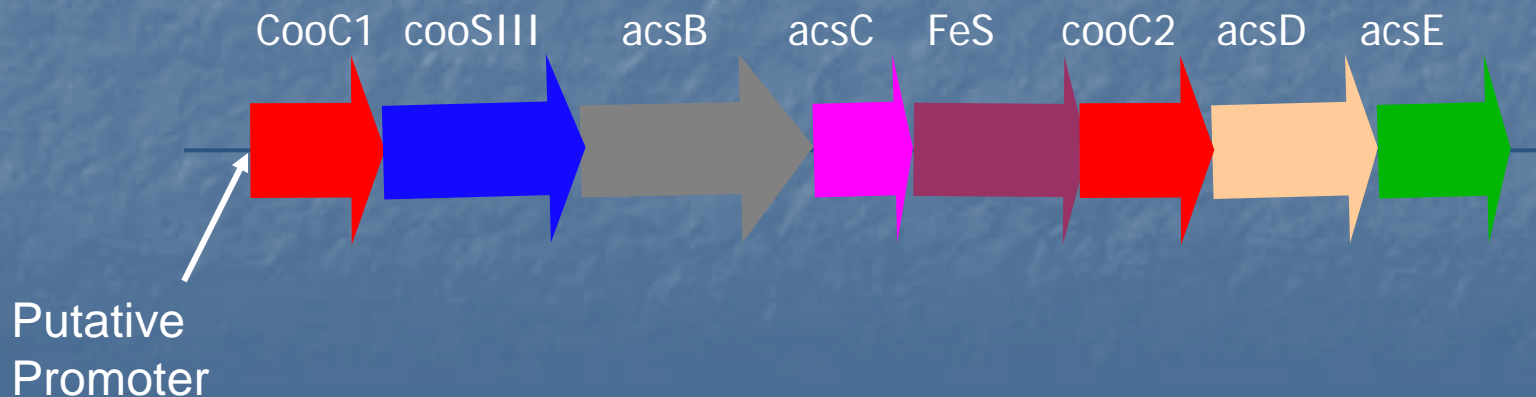


Promoters used in DNA Binding Experiments

Hydrogenase-linked CODH (Energy conservation)



CODH Acetyl CoA Synthase (Carbon Fixation)

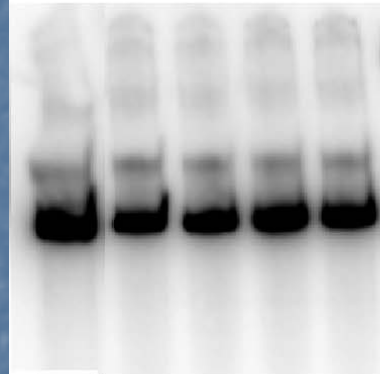


Mobility Shifts

A

CooA-1 ACS 25°C

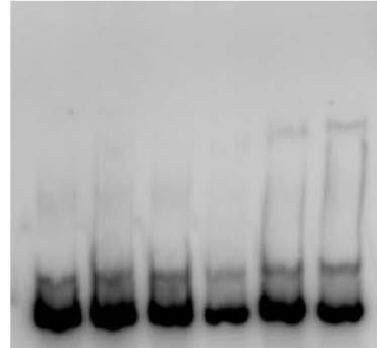
- 



B

CooA-1 *cooC* 25°C

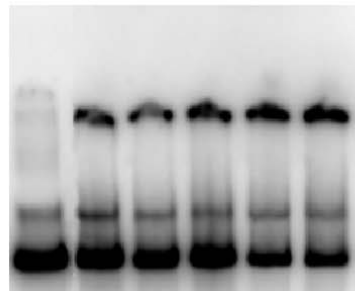
- 



C

CooA-2 ACS 25°C

- 



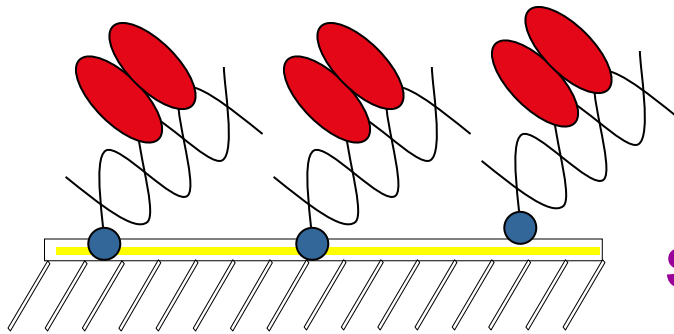
D

CooA-2 *cooC* 25°C

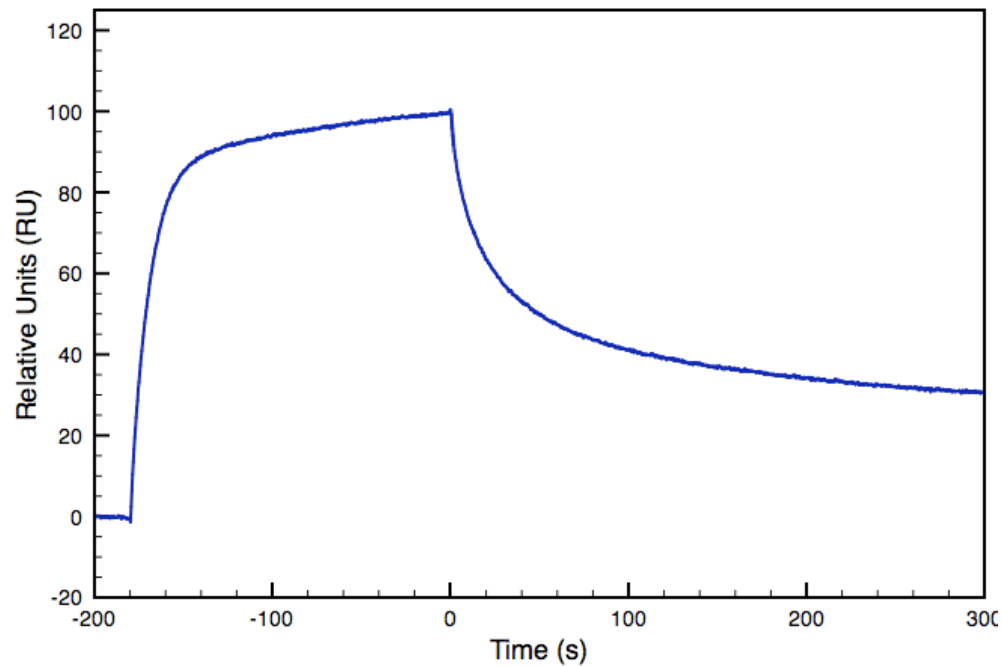
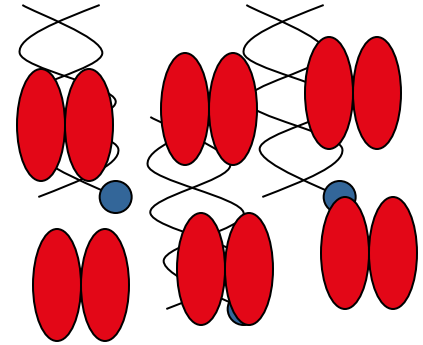
- 



CooA Biacore Experiment

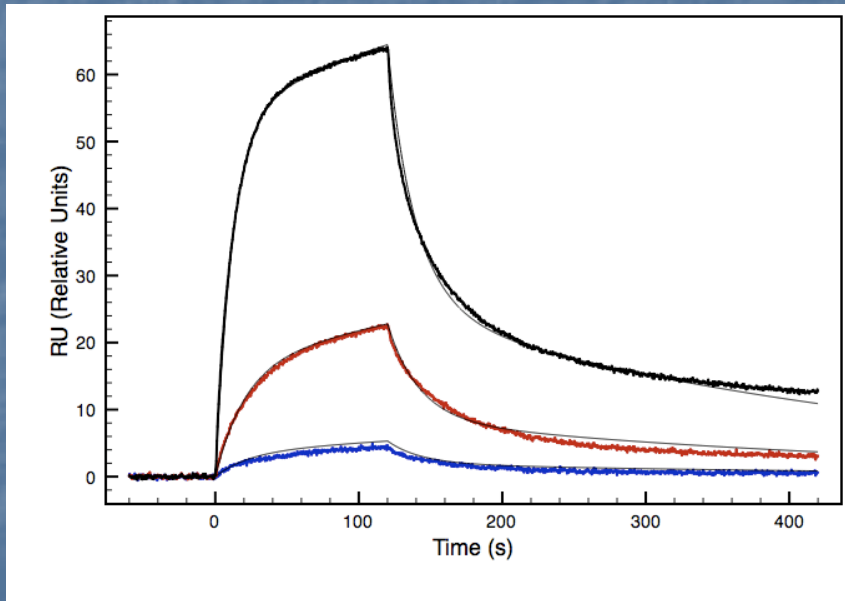


Streptavidin Chip

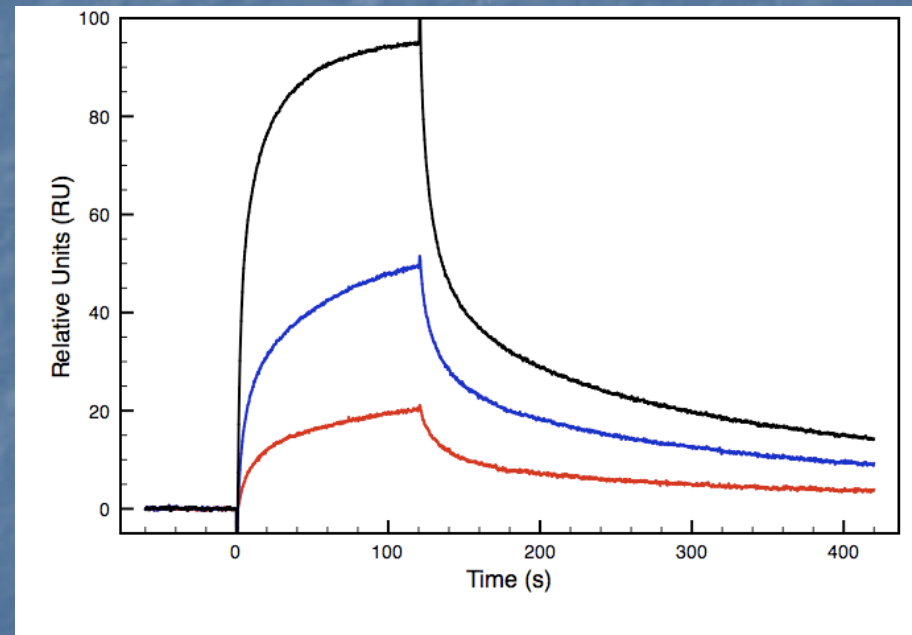


Binding to the Hydrogenase Promoter

CooA-1 *cooC*
Hydrogenase promoter

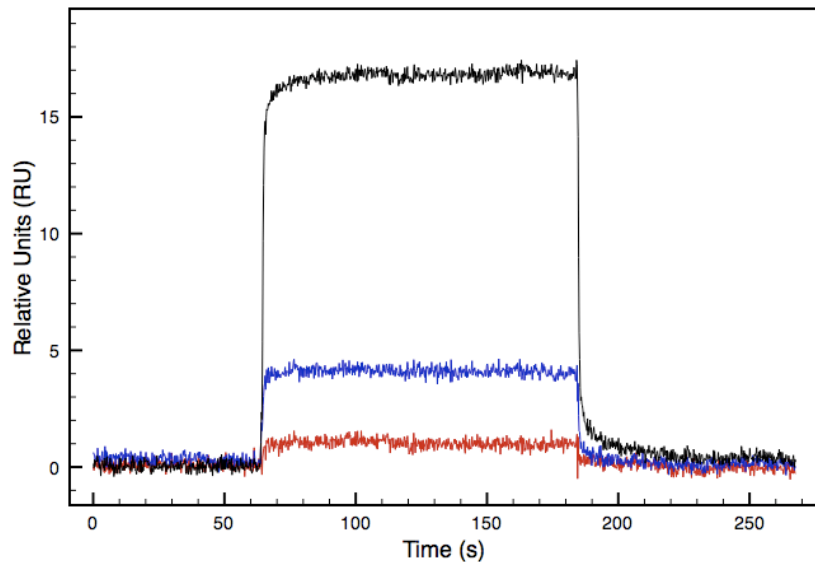


CooA-2 *cooC*
Hydrogenase promoter

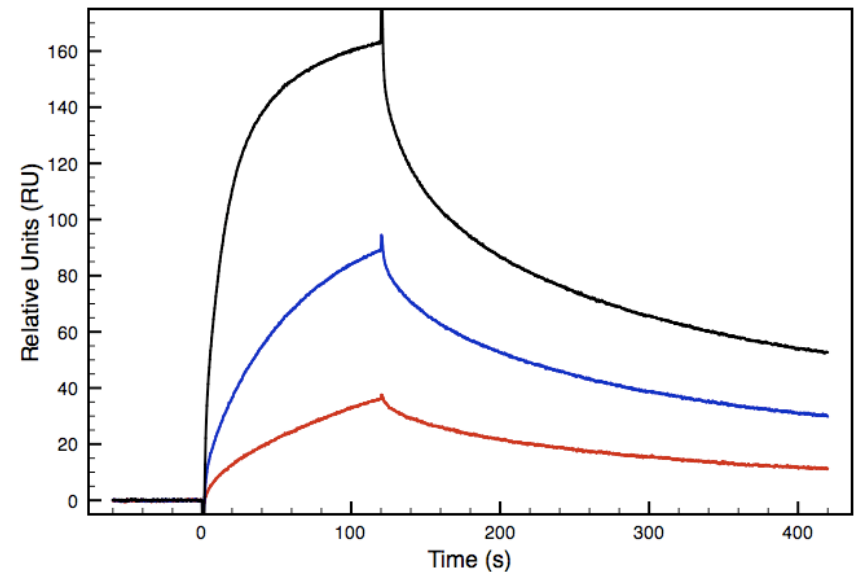


Binding to the CODH/ACS promoter

CooA-1 *cooC*
CODH/ACS promoter



CooA-2 *cooC*
CODH/ACS promoter

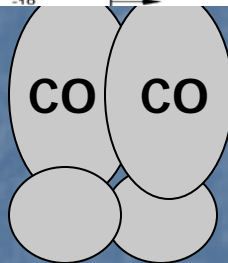
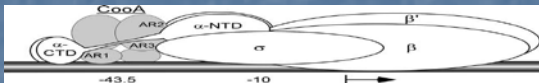
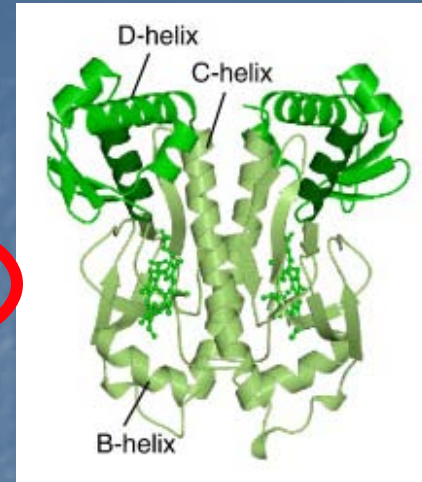
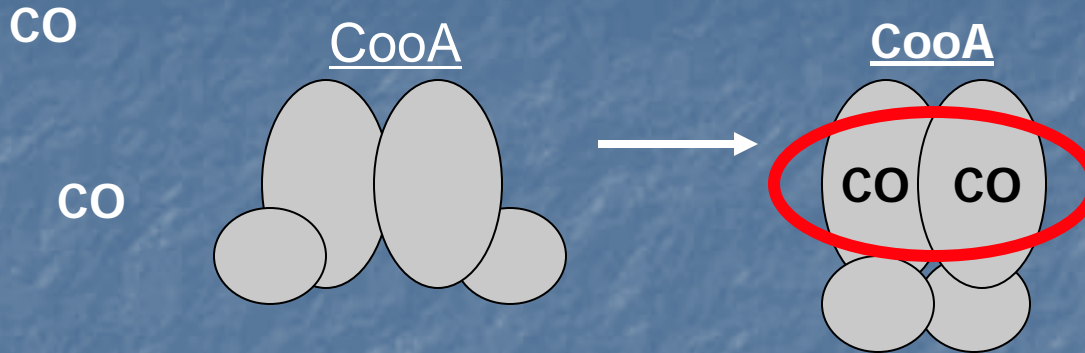


Binding Parameters for CooA Binding to the Hydrogenase and ACS promoters

	CooA-1 (Hydrogenase)	CooA-2 (Hydrogenase)	CooA-2 (CODH/ACS)
k_a^1 (1/Ms)	3.168×10^5	2.823×10^6	7.460×10^5
k_d^1 (1/s)	0.06273	0.2061	0.04123
k_a^2 (1/s)	0.004339	0.005667	0.008221
k_d^2 (1/s)	0.003120	0.004122	0.003576
K_D (nM)	82.8	30.7	16.8
R_{max} (RU)	110.4	112.3	178.7
χ^2 (RU ²)	0.536	3.4	5.07

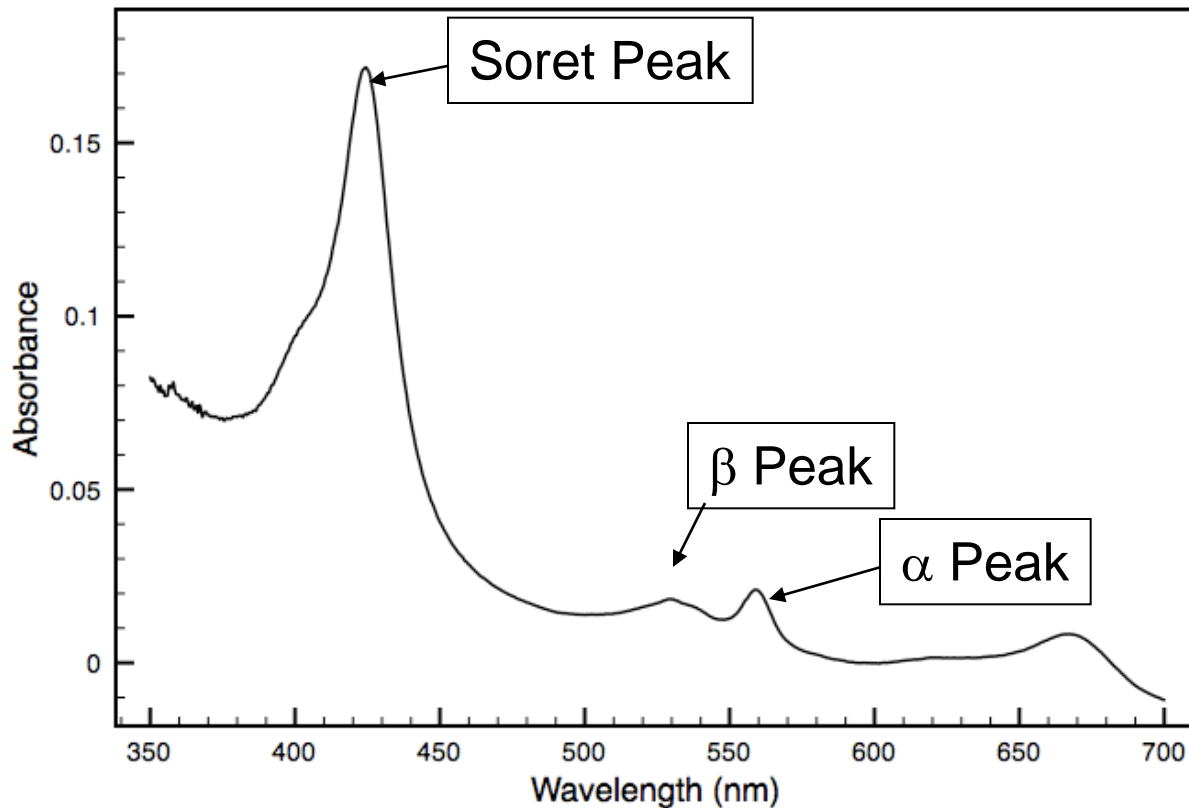
R. rubrum CooA binding to the *cooF* promoter $K_D = 12.7$ nM

Action of CooA



cooF promoter – **TGTCA NNNNNN CGACA**
cooM promoter – **TGTCG NNNNNN TGACG**

The Anatomy of a CooA Spectrum



$$Y = \frac{[\text{CO}]^n}{K_H + [\text{CO}]^n}$$

Y - Fractional Saturation

K_H - CO binding
disassociation constant

n - Cooperativity

$n = 1$ (no cooperativity)

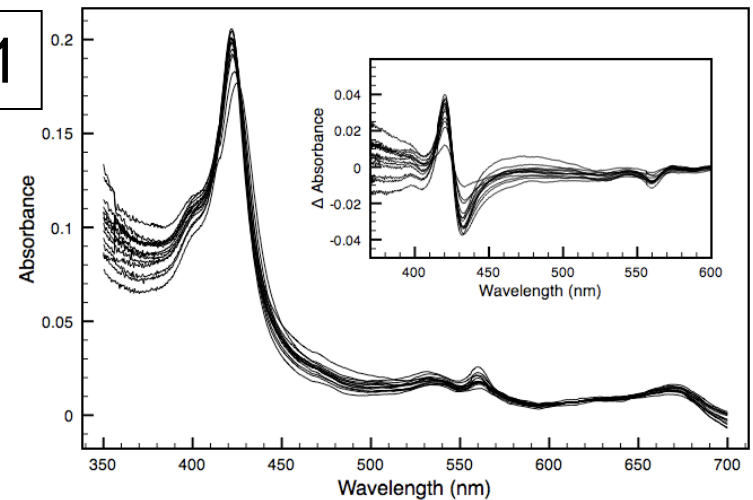
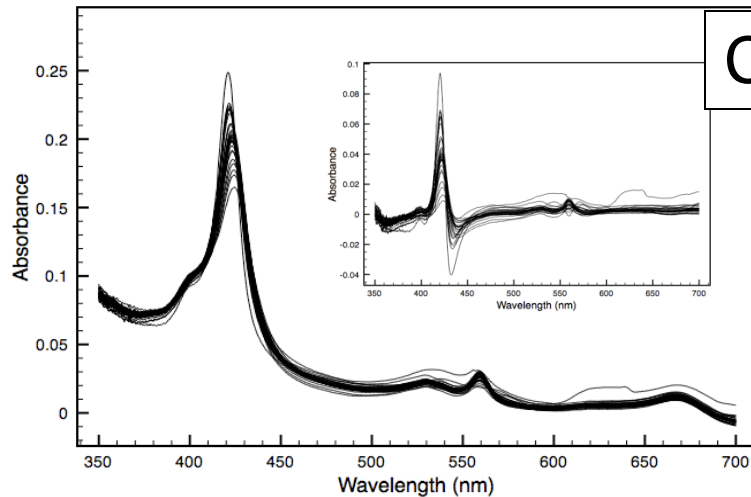
$n = 2$ (fully cooperative)

CO Binding to CooA

25°C

70°C

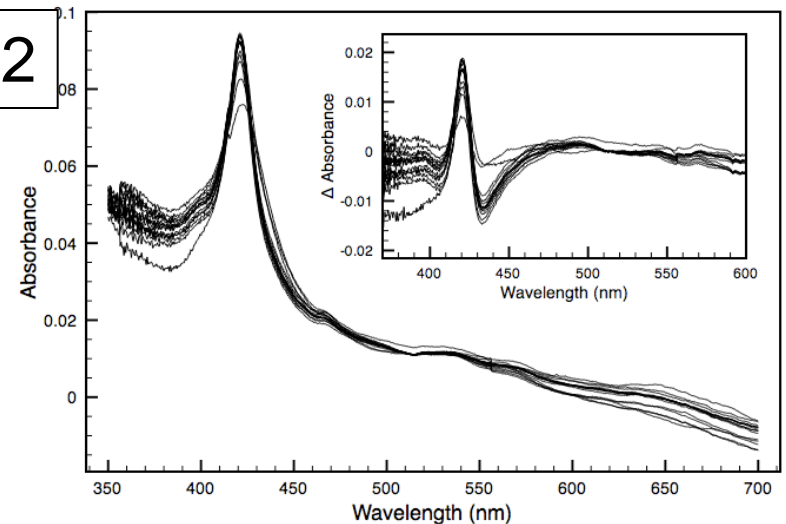
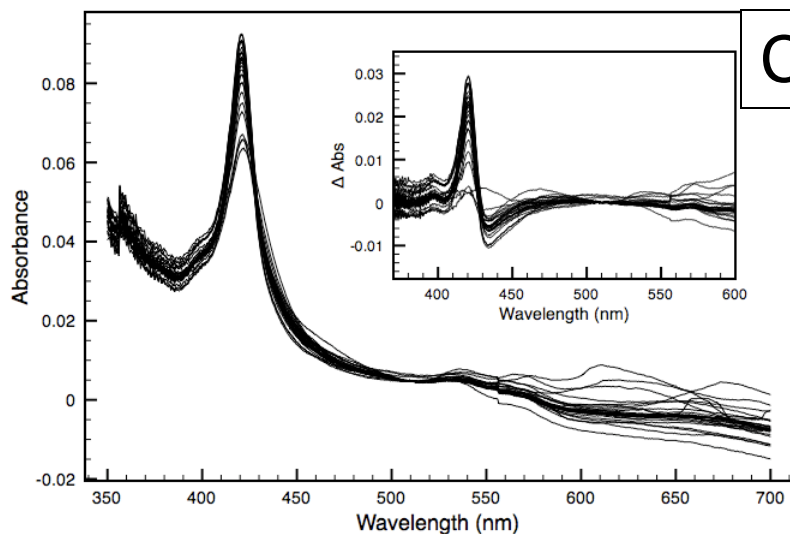
CooA-1



25°C

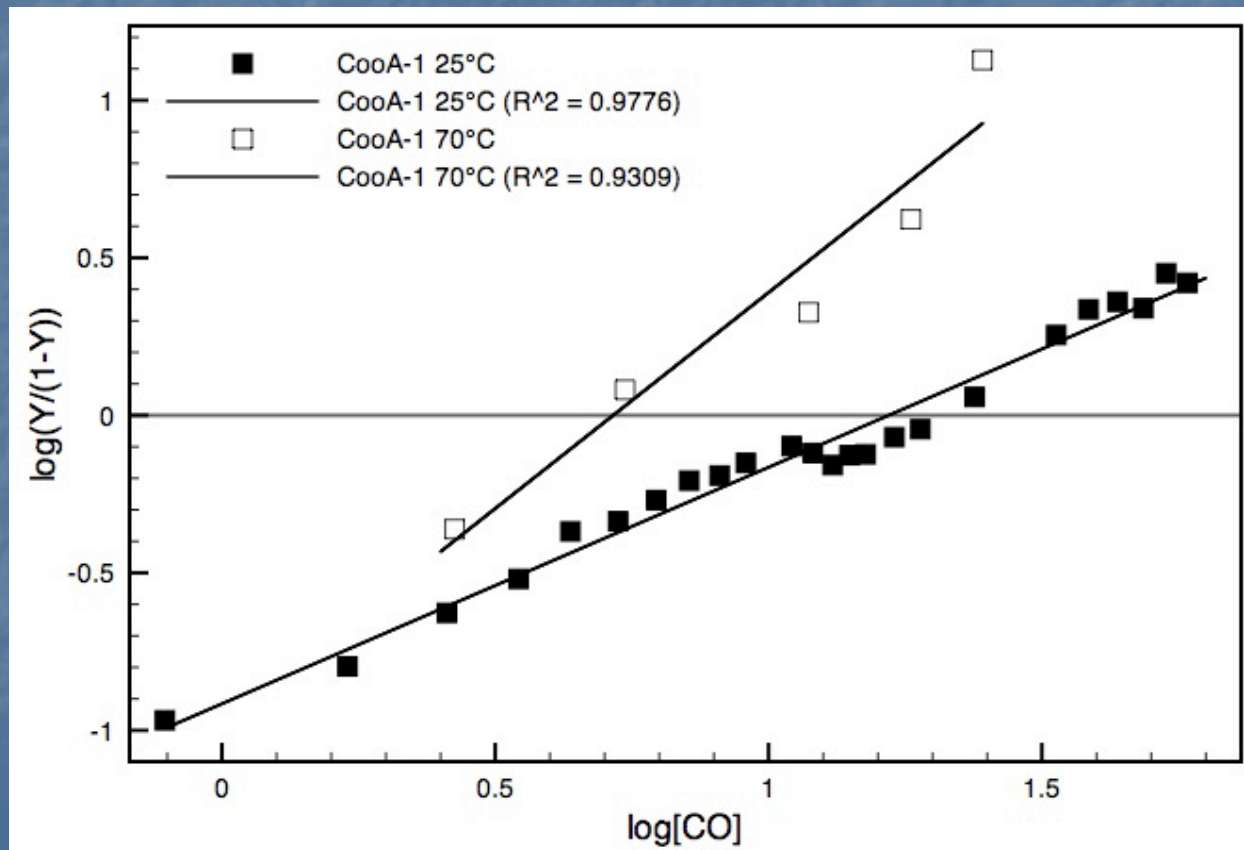
55°C

CooA-2



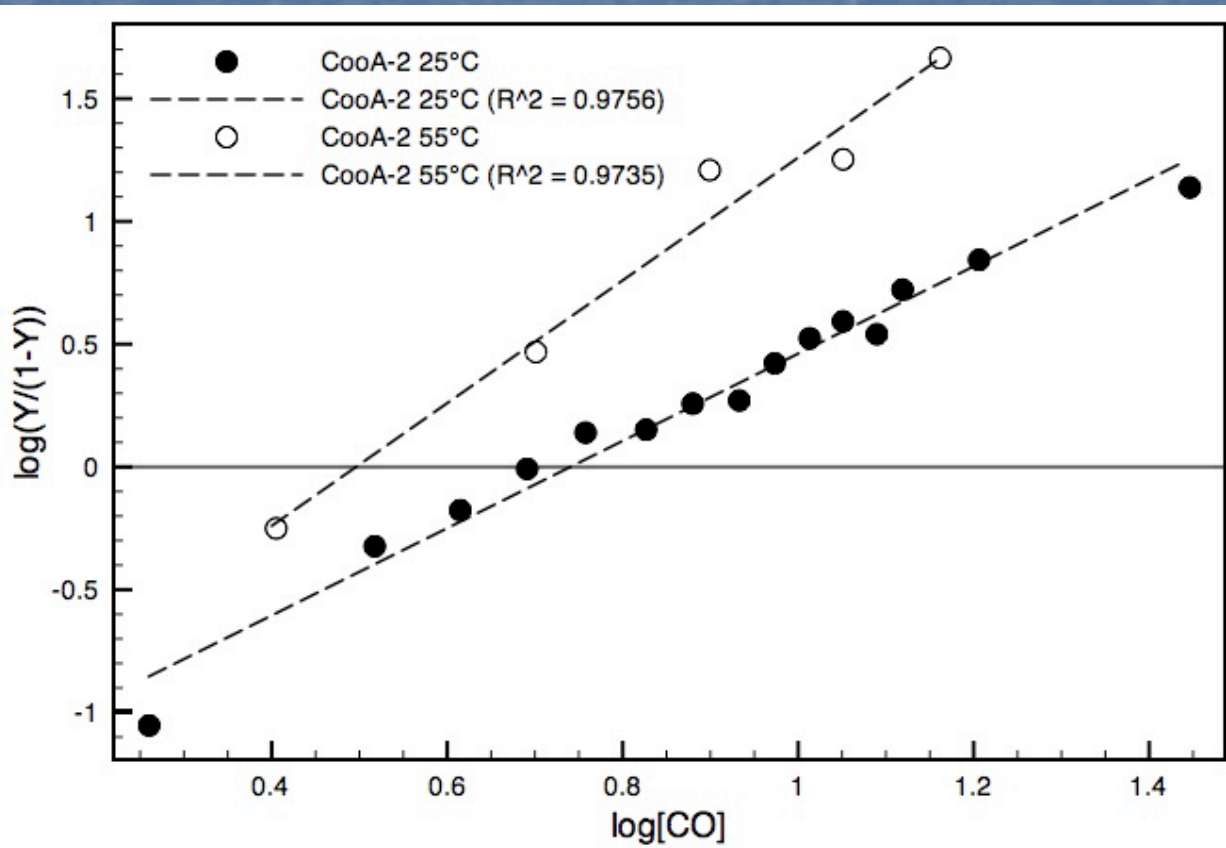
Determination of CO Binding Affinity (K_H) for CooA-1

$$K_{H25^\circ\text{C}} = 16.5 \mu\text{M} \quad K_{H70^\circ\text{C}} = 5.2 \mu\text{M}$$



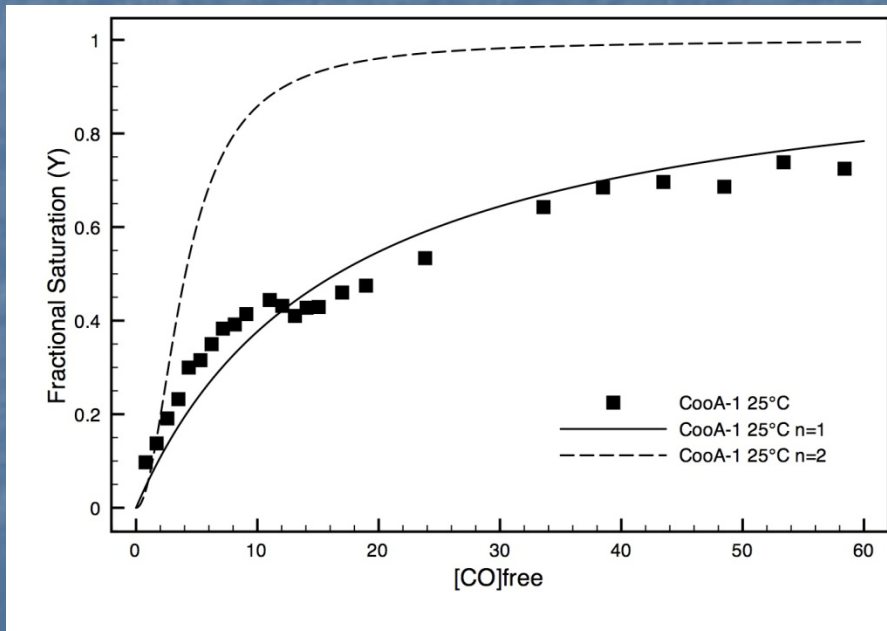
Determination of CO binding affinity (K_H) for CooA-2

$$K_{H25^\circ\text{C}} = 5.5 \mu\text{M} \quad K_{H55^\circ\text{C}} = 3.1 \mu\text{M}$$



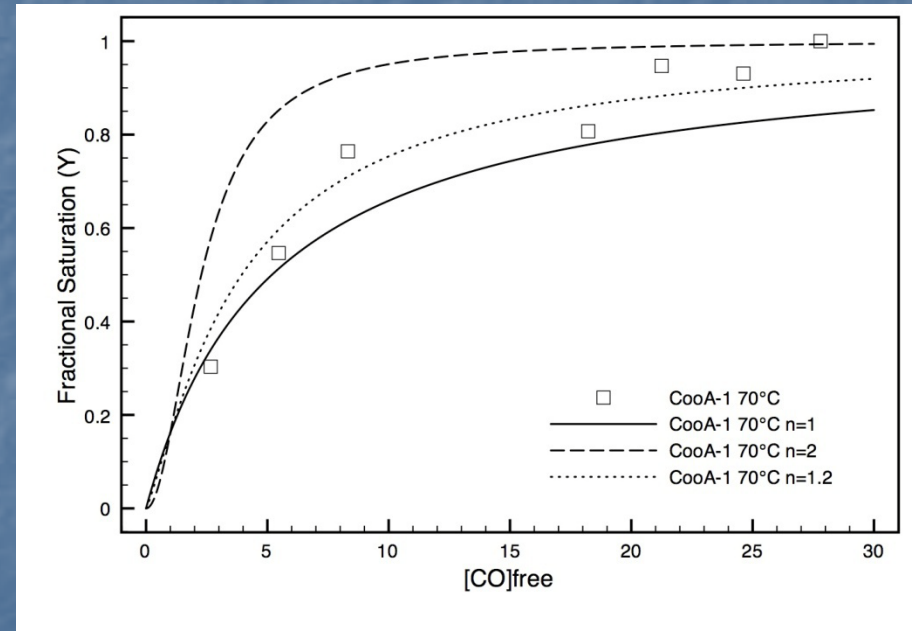
Determination of Cooperativity (n) for CO Binding to CooA-1

CooA-1 25°C



$n = 1$

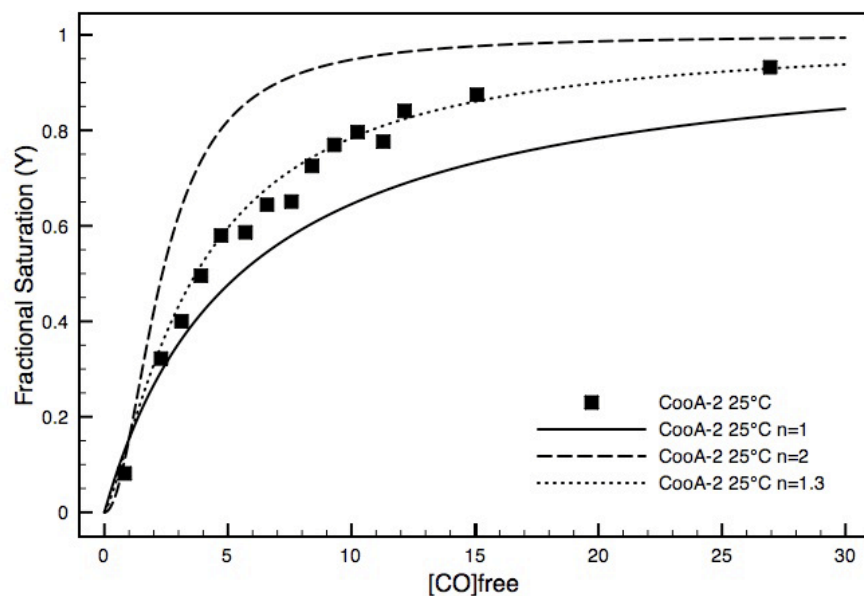
CooA-1 70°C



$n = 1.2$

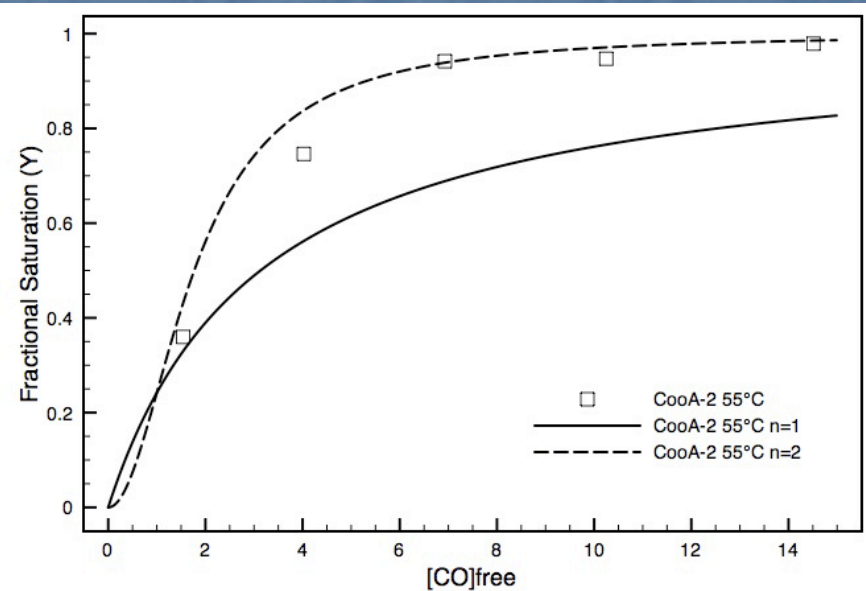
Determination of Cooperativity (n) for CO Binding to CooA-2

CooA-2 25°C



$$n = 1.3$$

CooA-2 55°C



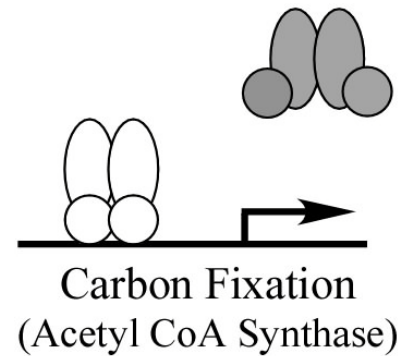
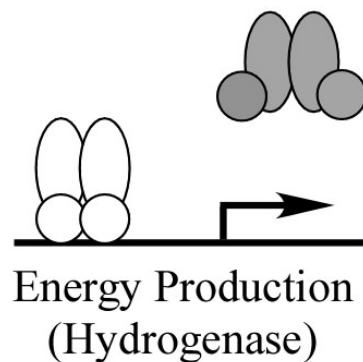
$$n = 2$$

CO Binding Constants

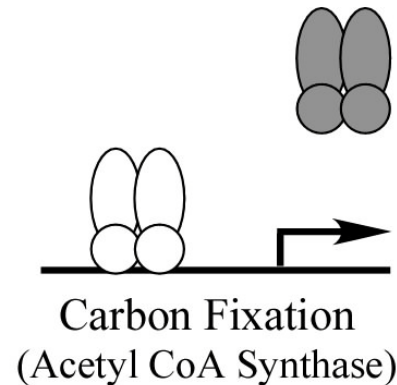
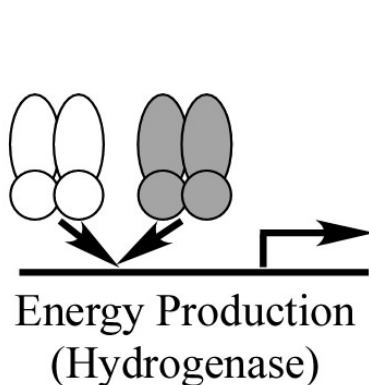
Constant	25°C	Elevated Temperature
K_H CooA-1	16.5 μM	5.2 μM (70°C)
K_H CooA-2	5.5 μM	3.1 μM (55°C)
K_H <i>R. rubrum</i> CooA	2.2 μM	-
n CooA-1	1	1.2
n CooA-2	1.3	2
n <i>R. rubrum</i> CooA	1.4	-

Model for Interplay Between CooA Homologs for Regulation of Multiple CODH Homologs

Limiting CO Concentrations (Geothermal CO)



High CO Concentrations (Biogenic CO)

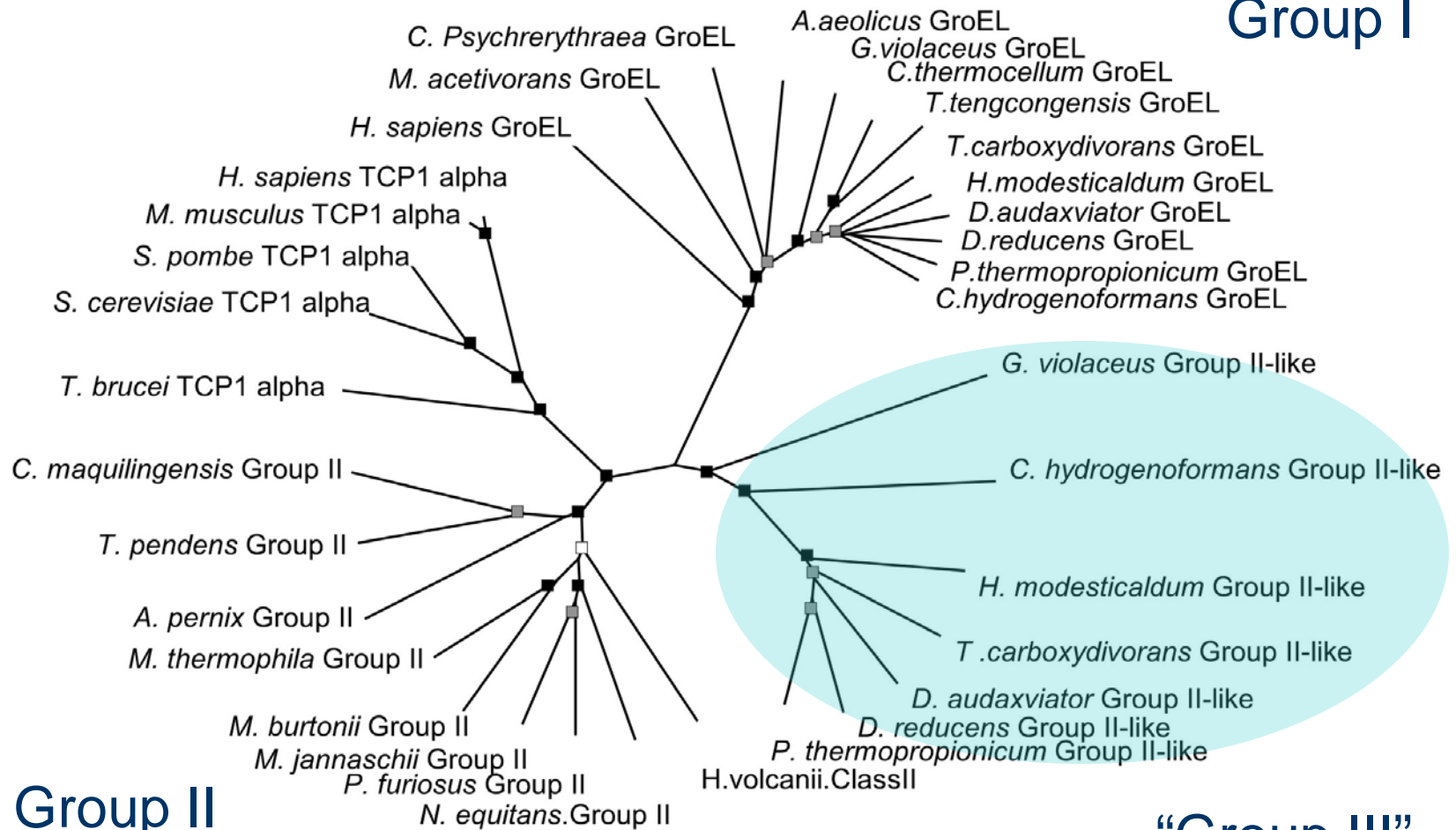


Summary

- CooA-1 is specialized for activating maximal expression of the CODH-linked hydrogenase at high CO concentrations.
- CooA-2 binds CO with high affinity under low CO concentrations and is able to activate both energy production via the hydrogenase as well as carbon fixation via the CODH/ACS.
- Multiple CooAs allow *C. hydrogenoformans* to shunt CO towards energy conservation or carbon acquisition pathways depending on availability of CO, thus enabling efficient growth on CO over a wide range of concentrations.

Chaperonin Tree

Group I



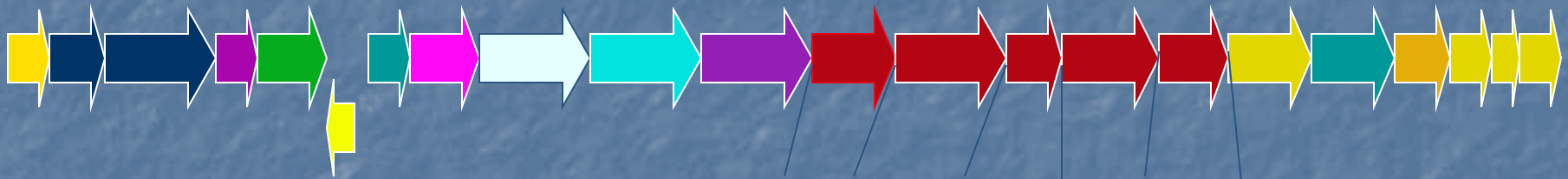
Group II

“Group III”

- Bootstrap >98%
- Bootstrap 75-98
- Bootstrap 50-75

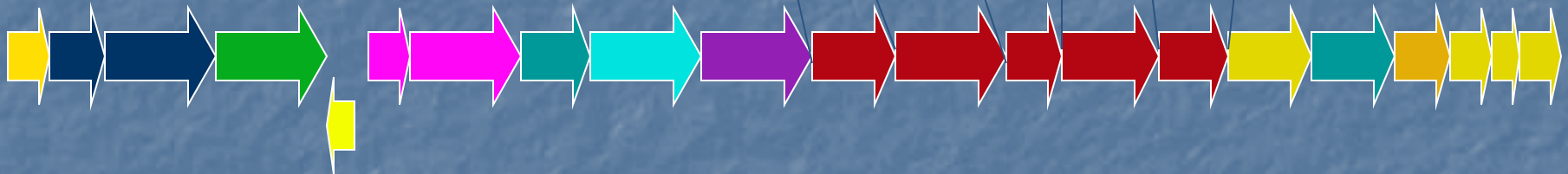
Novel HSP60

C. hydrogenoformans



HtrC HSP60 GrpE DnaK DnaJ

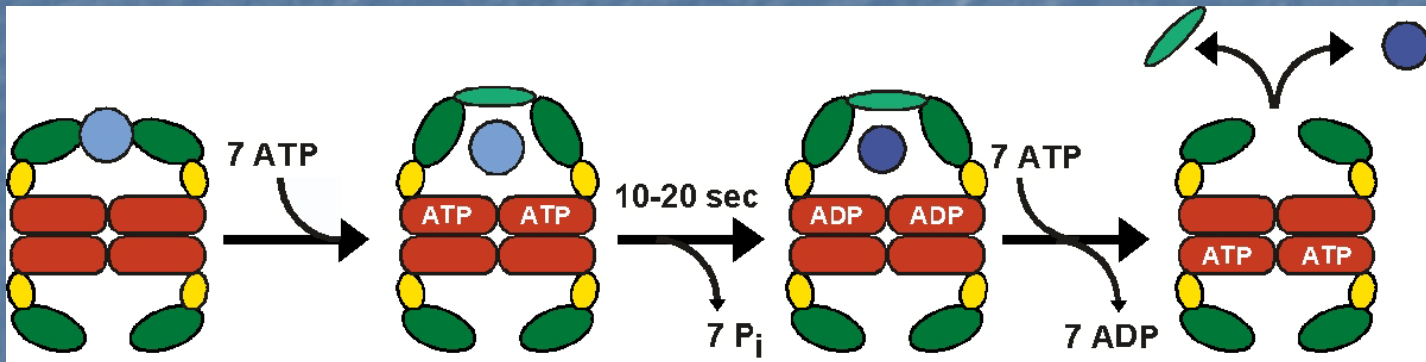
T. carboxydivorans



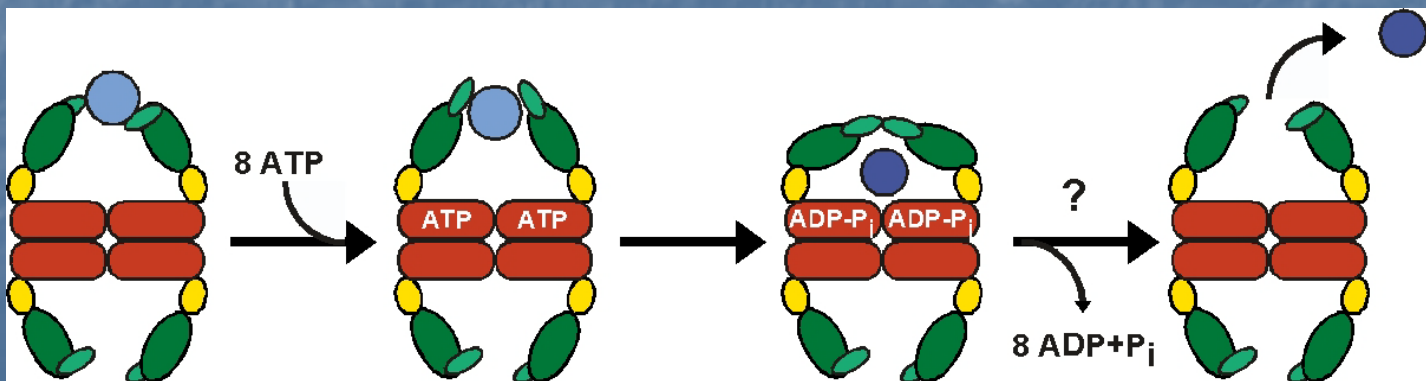
Chaperonin background

Chaperonin (HSP60) - An ATP dependent Foldase

Group I - Bacteria, Archaea, In mitochondria of Eukaryotes



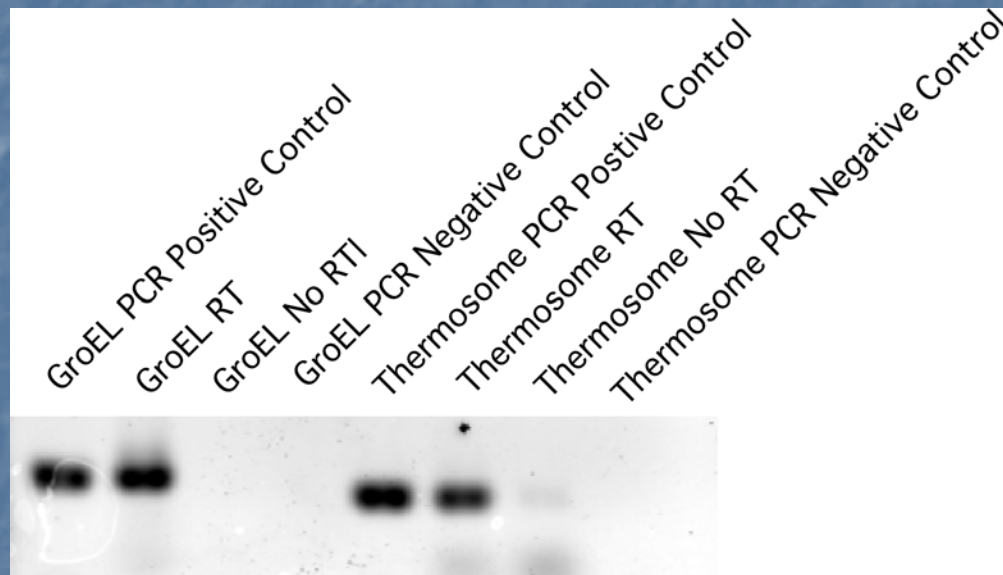
Group II - Archaea, Eukaryotes



Characteristics of HSP60

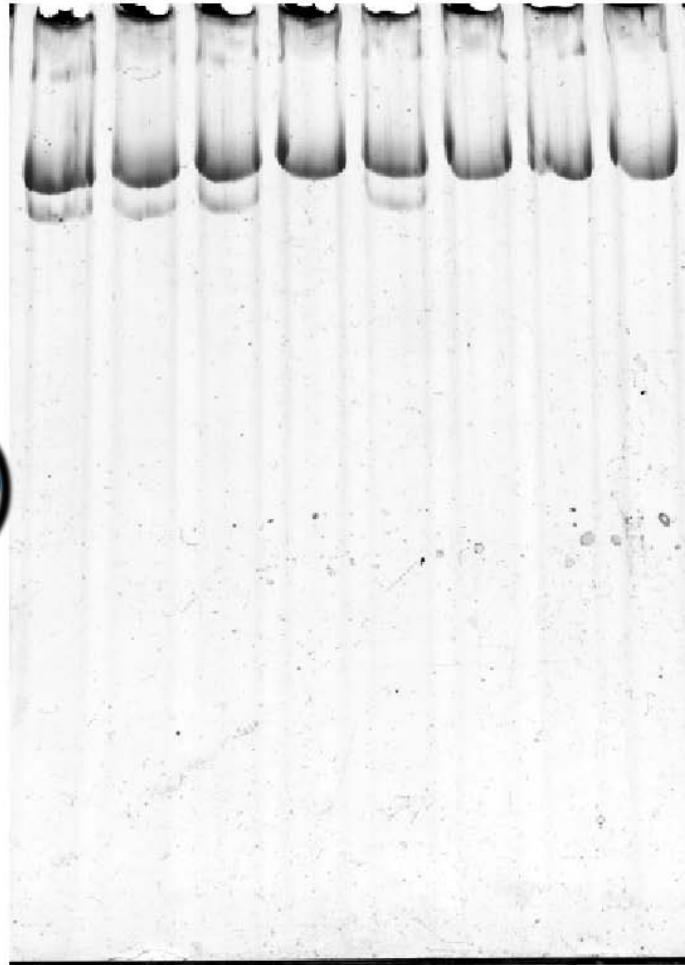
- ATP dependent Foldase
- Oligomers form a double barrel structure.
- Group I
 - Dependent upon co-chaperone GroES for folding of non-native proteins.
 - Oligomeric structure is composed of two seven member rings.
- Group II
 - Contains a helical protrusion that acts as built-in lid allowing these HSP60s to act in a GroES-independent manner.
 - Oligomeric structure is composed of two eight or nine member rings (depending on species).

Expression in *C. hydrogenoformans*



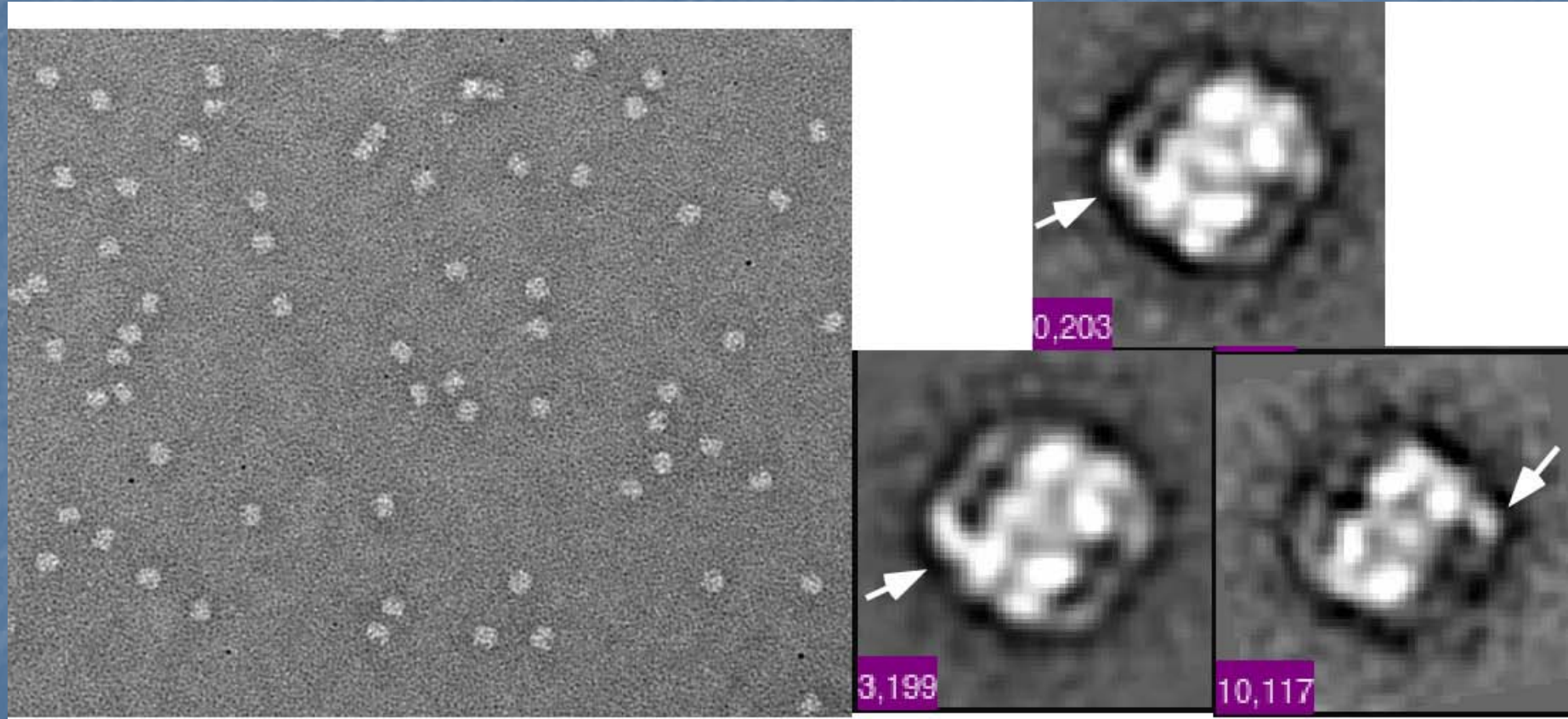
Lid Domain (Native PAGE)

Open —▶
Closed —▶▶



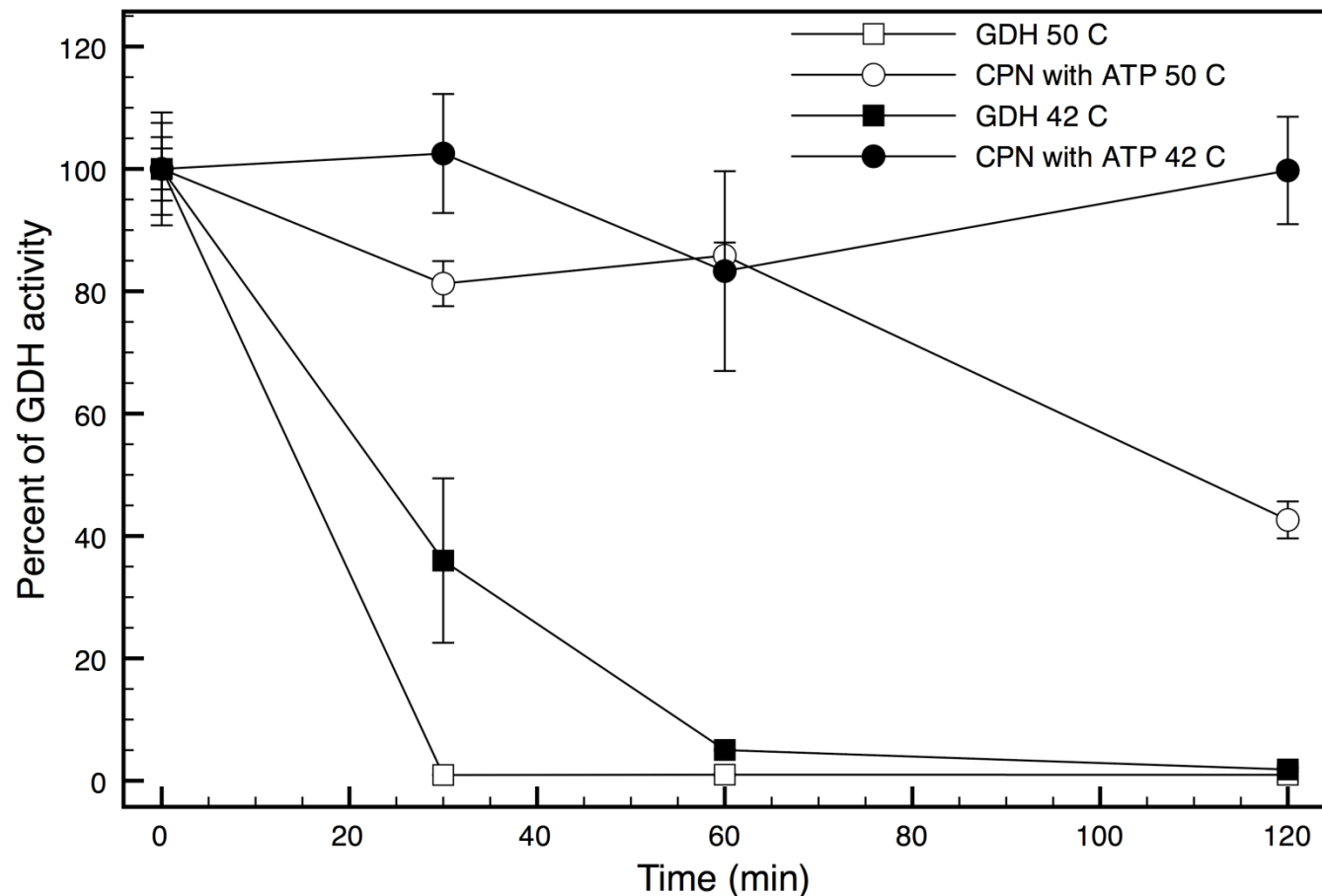
KCl	-	+	-	-	+	-	+	+
MgCl ₂	-	-	+	-	+	+	-	+
ATP	-	-	-	+	-	+	+	+

Lid Domain: Electron Microscopy Single Particle Analysis

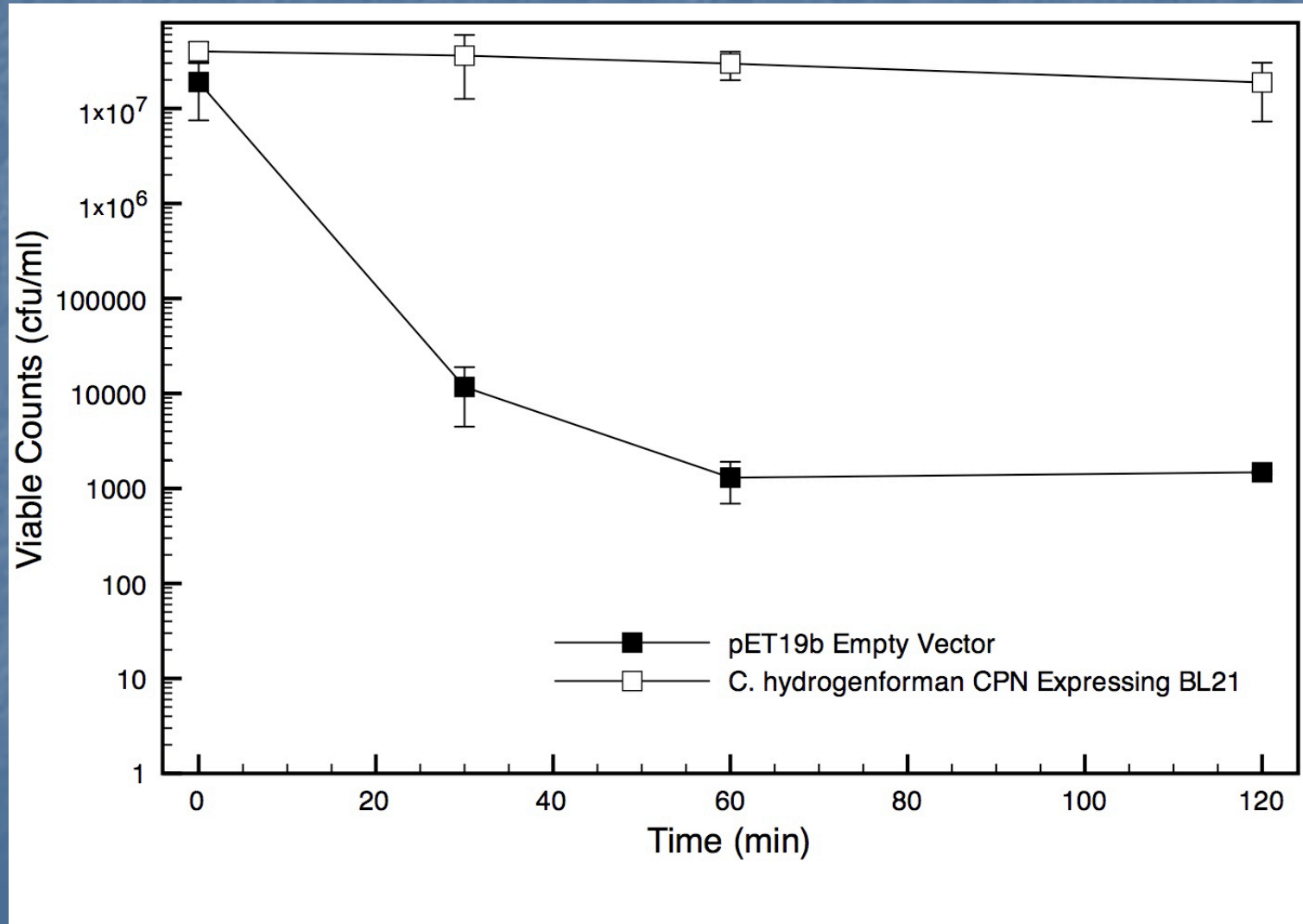


C. hydrogenoformans Group III chaperonin

Glutamate Dehydrogenase Protection Assays.



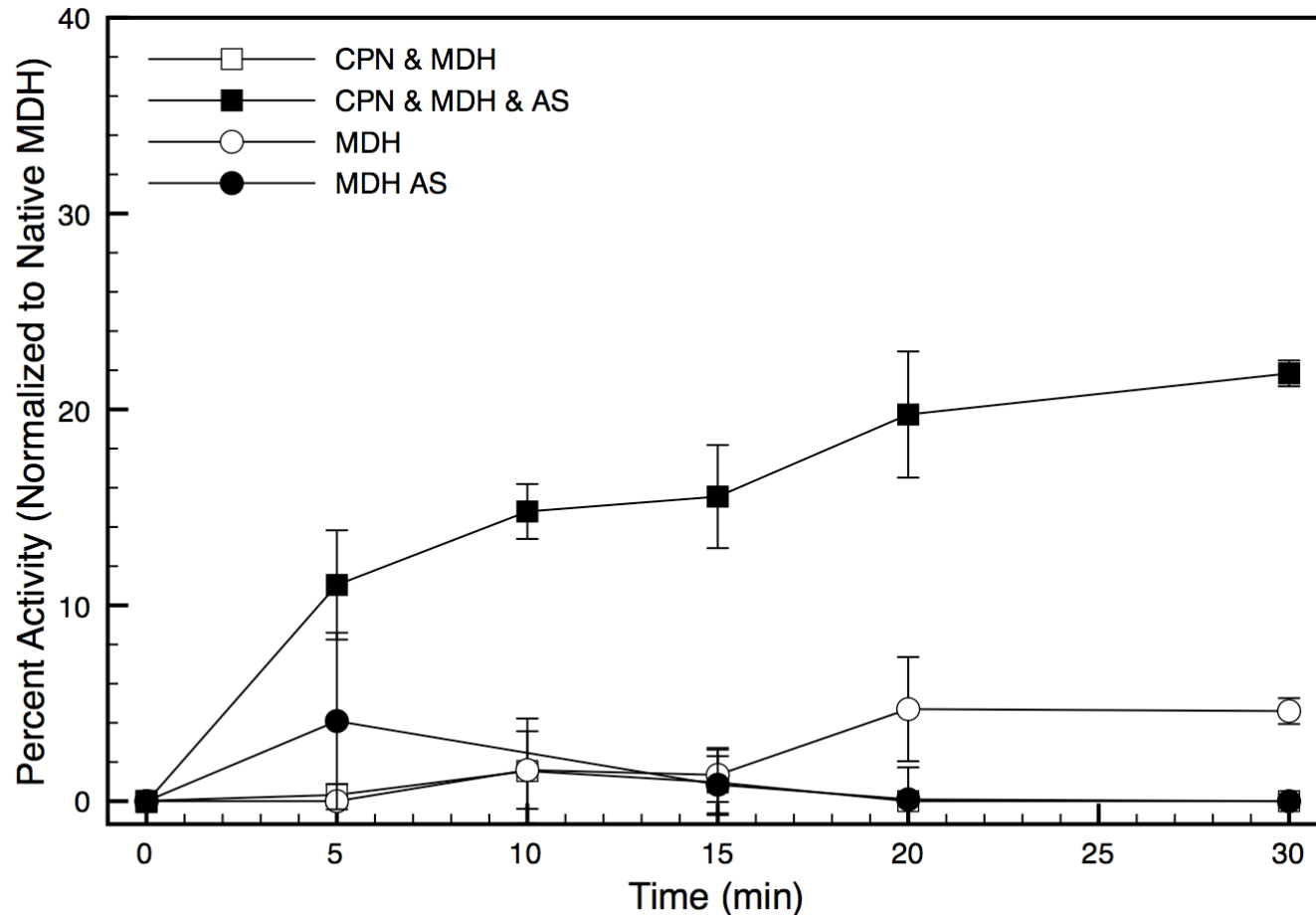
Enhanced Survival of *E. coli* at 50°C.



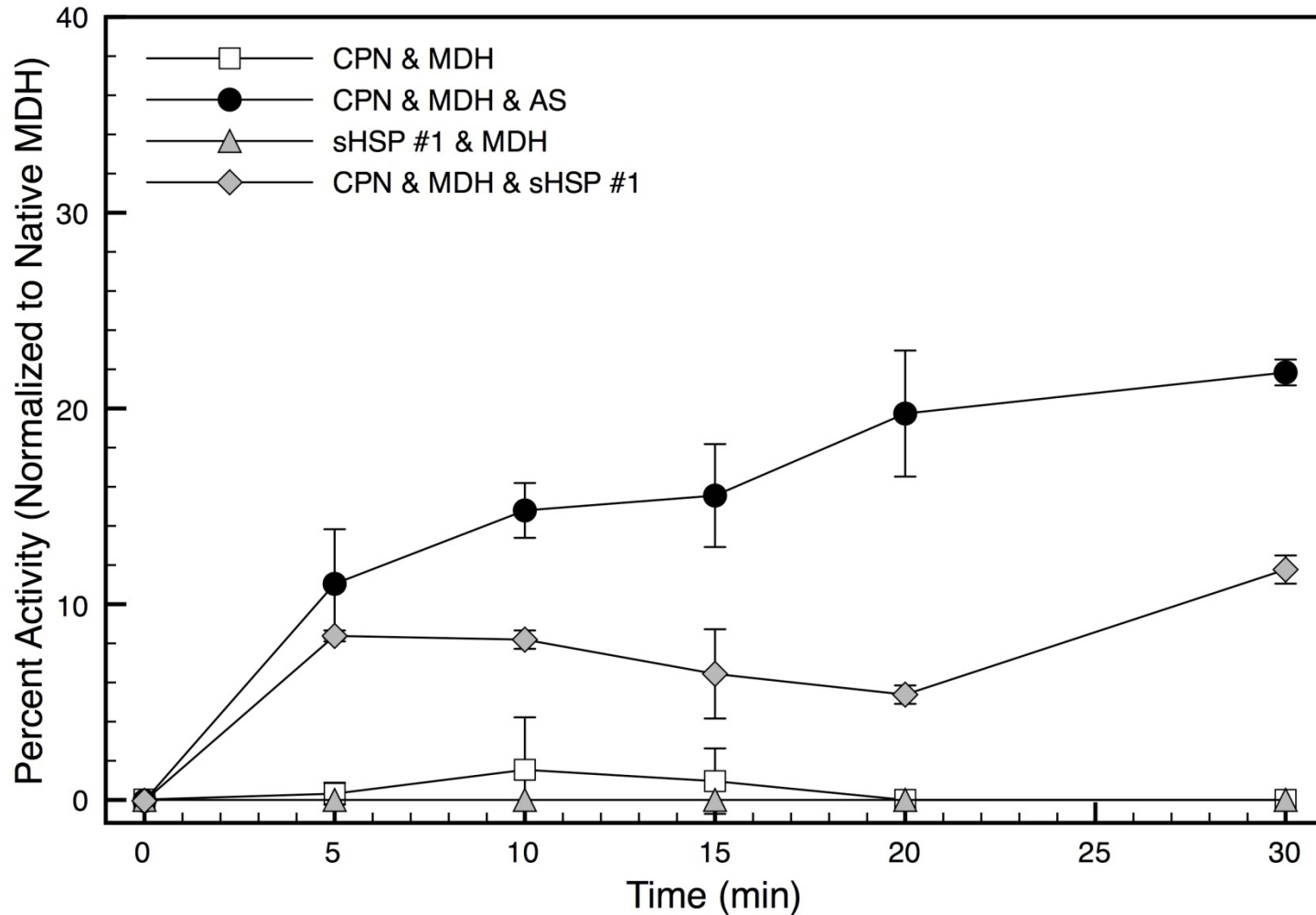
Malate Dehydrogenase Refolding Assays

- MDH from porcine heart was denatured in 4M Guanidine HCl at 37°C for 1 hr.
- Denatured MDH was diluted 1:100 into reaction mixtures containing *C. hydrogenoformans* Group III HSP60.
- Folding reactions were incubated at 42°C for 30 minutes, sampled at 5 min intervals
- MDH activity was measured at 25°C

Refolding (Malate Dehydrogenase)



Refolding (Cooperation with sHSP)



Conclusions

- Diverse thermophilic firmicutes oxidize CO and produce H₂ or reduce metals.
- Multiple CO-responsive transcriptional activators allow for efficient use of CO via multiple pathways over a wide range of CO concentrations.
- A novel "Class III" HSP60 was annotated in the genomes of a select group of Firmicutes Carboxydotrophs dominate: Represents at least a very deep branch in Class II CPN

<http://www.imet.usmd.edu/people/robb.html>

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