C1net - Conference 4 – Chemical from C1 gas
East Midland Conference, University of Nottingham, 20-23 January 2019

Electron Bifurcation in C1-Fermentations

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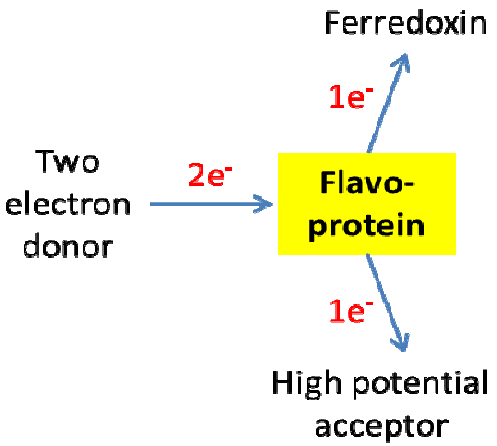
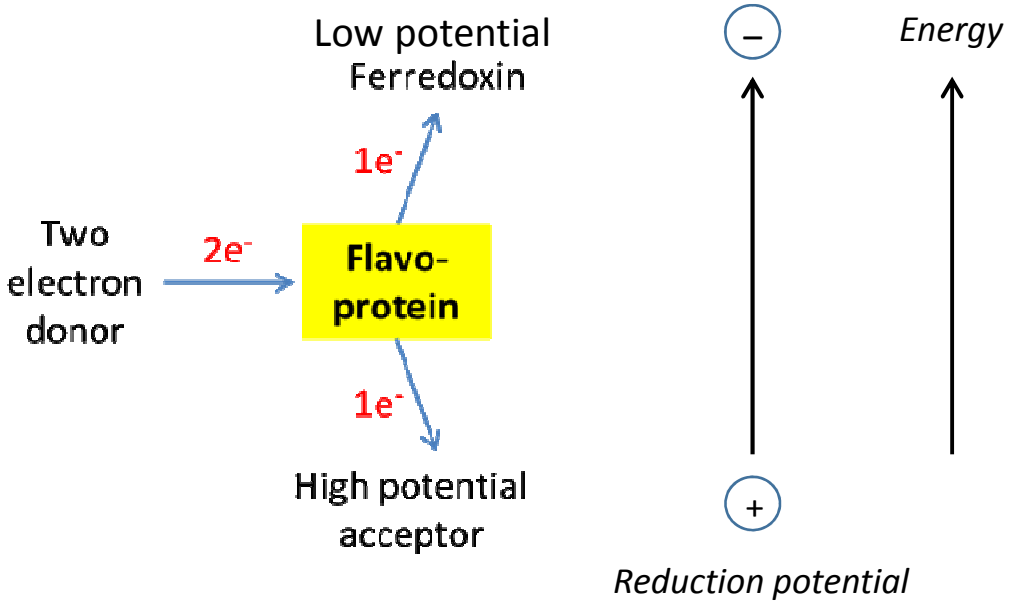
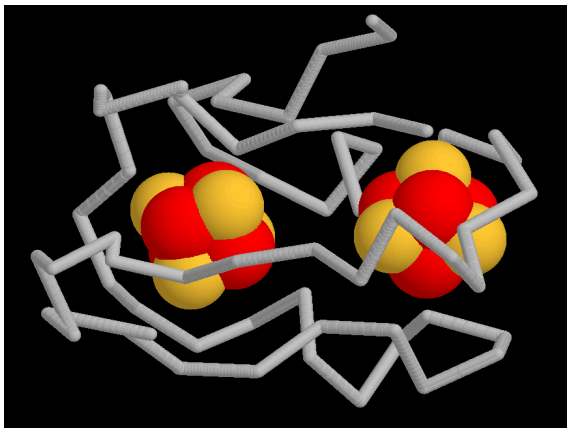


Flavin-Based Electron Bifurcation (FBEB)



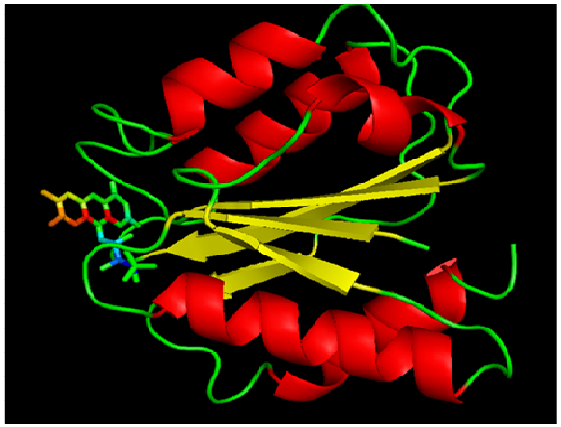
Buckel & Thauer (2018) Chemical Reviews & Frontiers in Microbiology

Ferredoxin or Flavodoxin, the *'energy rich'* electron carriers in anaerobes.



Clostridial ferredoxin with two [4Fe-4S] clusters

$E°'_1$ = –340 mV
$E°'_2$ = –405 mV

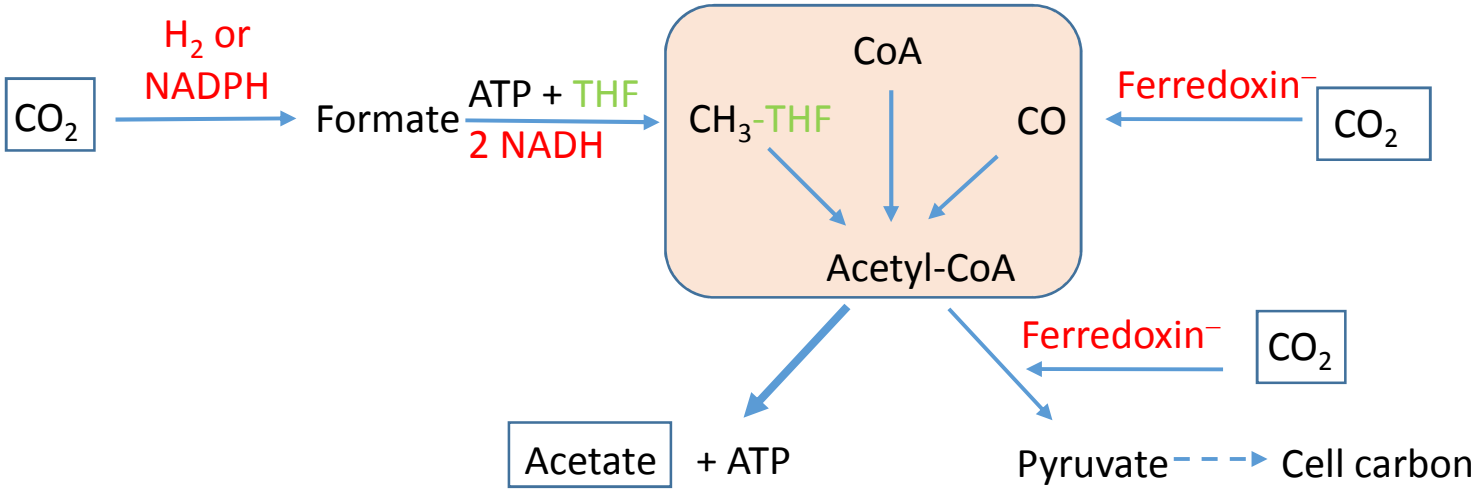


Crystal structure of flavodoxin from *C. beijerinckii*

$E°'_1$ = –60 mV
$E°'_2$ = –420 mV

Ferredoxin (oxidized) + e^- = Ferredoxin$^-$ (reduced)
Flavodoxin (semiquinone) + e^- = Flavodoxin$^-$ (hydroquinone)

Acetogenesis requires reduced ferredoxin



H_2 + 2 Ferredoxin$_{ox}$ = 2 H^+ + 2 Ferredoxin$_{red}^-$; $\Delta G°' \approx 0$.

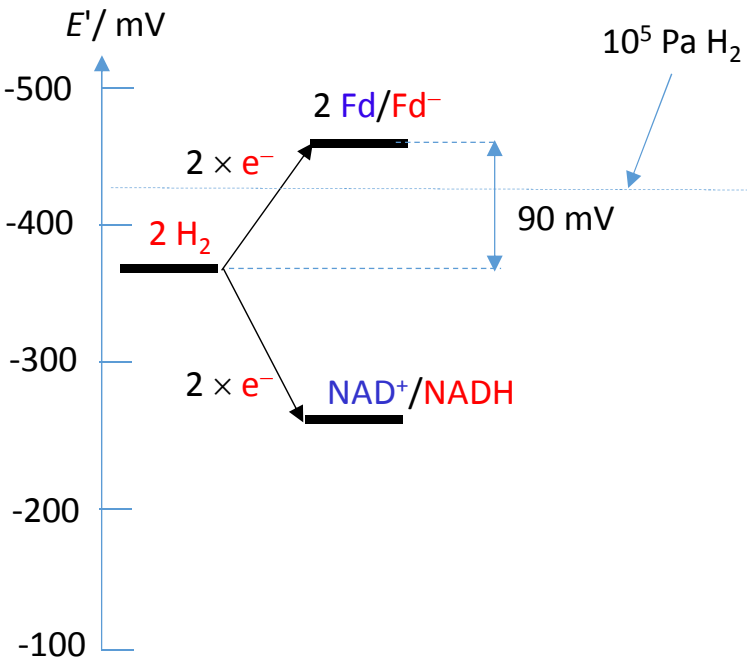
Reduction potential $E°'$ (H_2) = –414 mVolt

$E°'$ (Ferredoxin) = –405 mVolt

Bifurcating Hydrogenases

NAD^+ reductase in acetogens

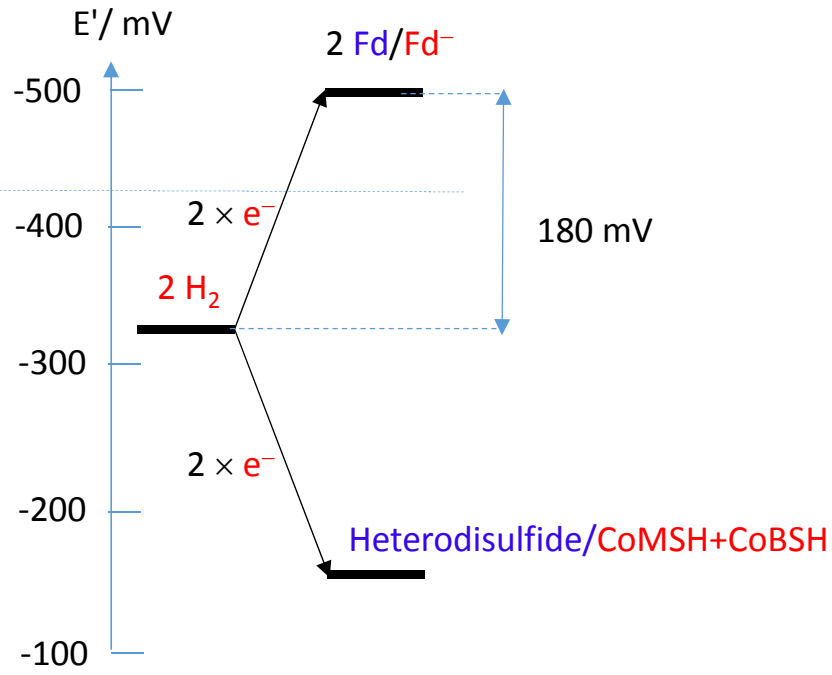
> 250 Pa H_2; E' = –370 mV



Schut and Adams 2009
Schuchmann and Müller 2012
Thauer et al. 2013

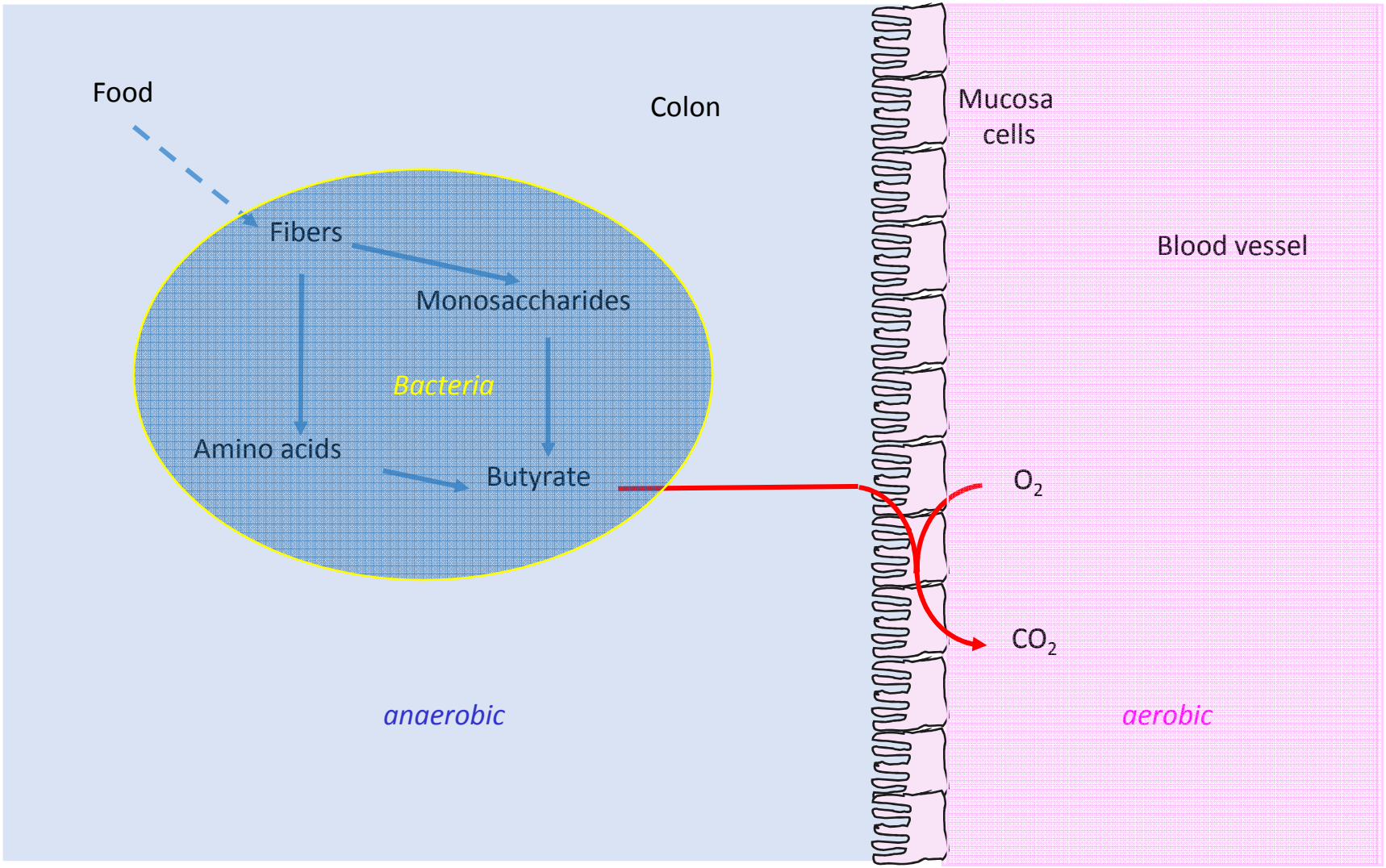
Heterodisulfide reductase in methanogens

> 10 Pa H_2; E' = –320 mV

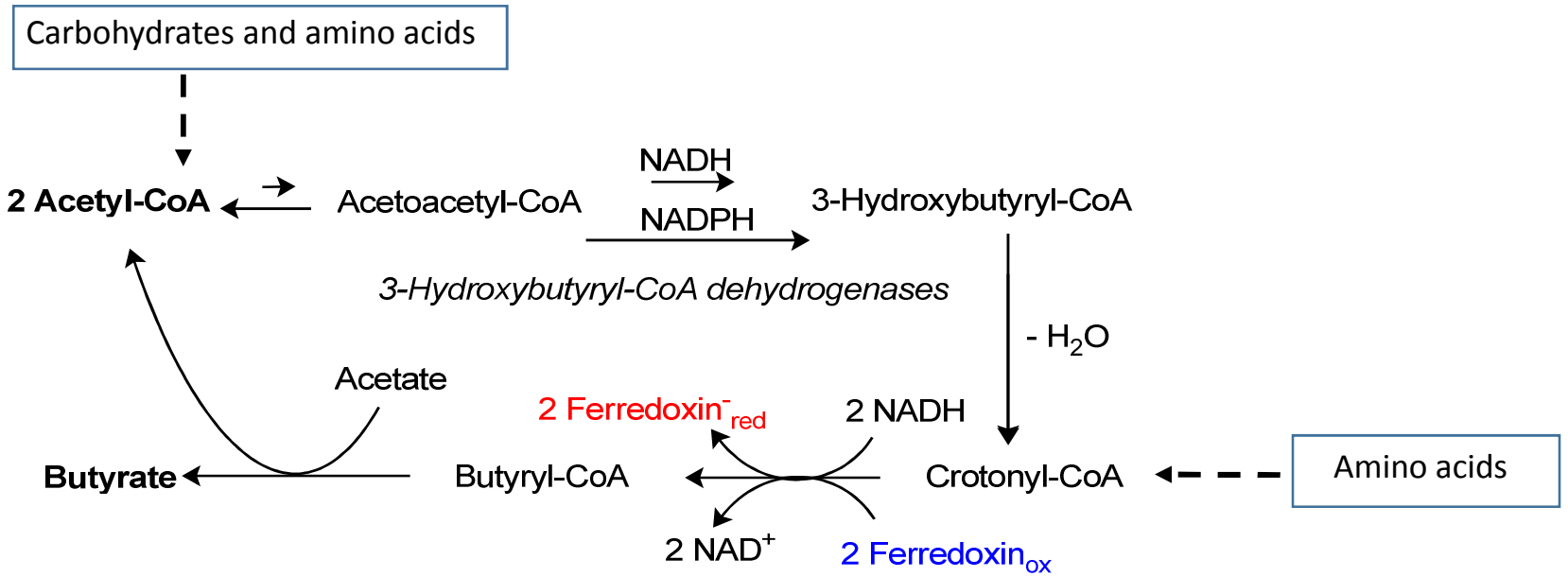


Thauer, Buckel et al. 2008

Butyrate norishes the mucosa cells.



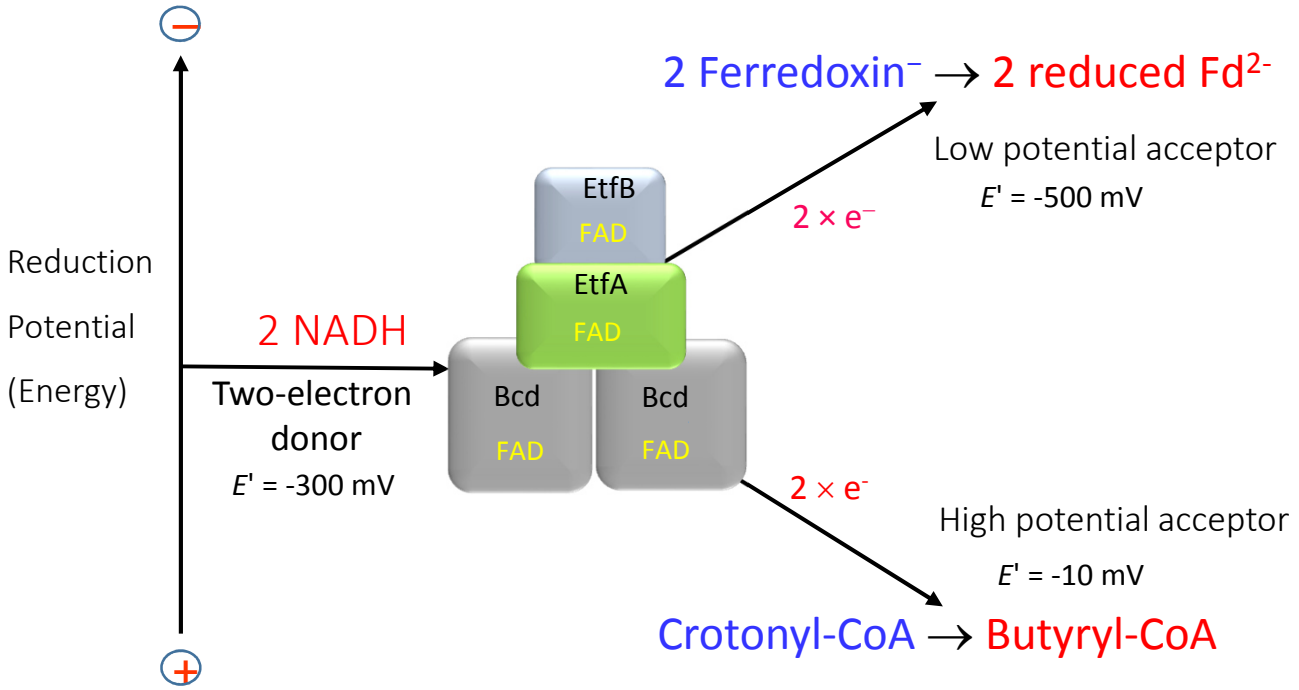
Clostridial butyrate synthesis



Electron bifurcating *EtfAB-Butyryl-CoA dehydrogenase (EtfAB-Bcd)*

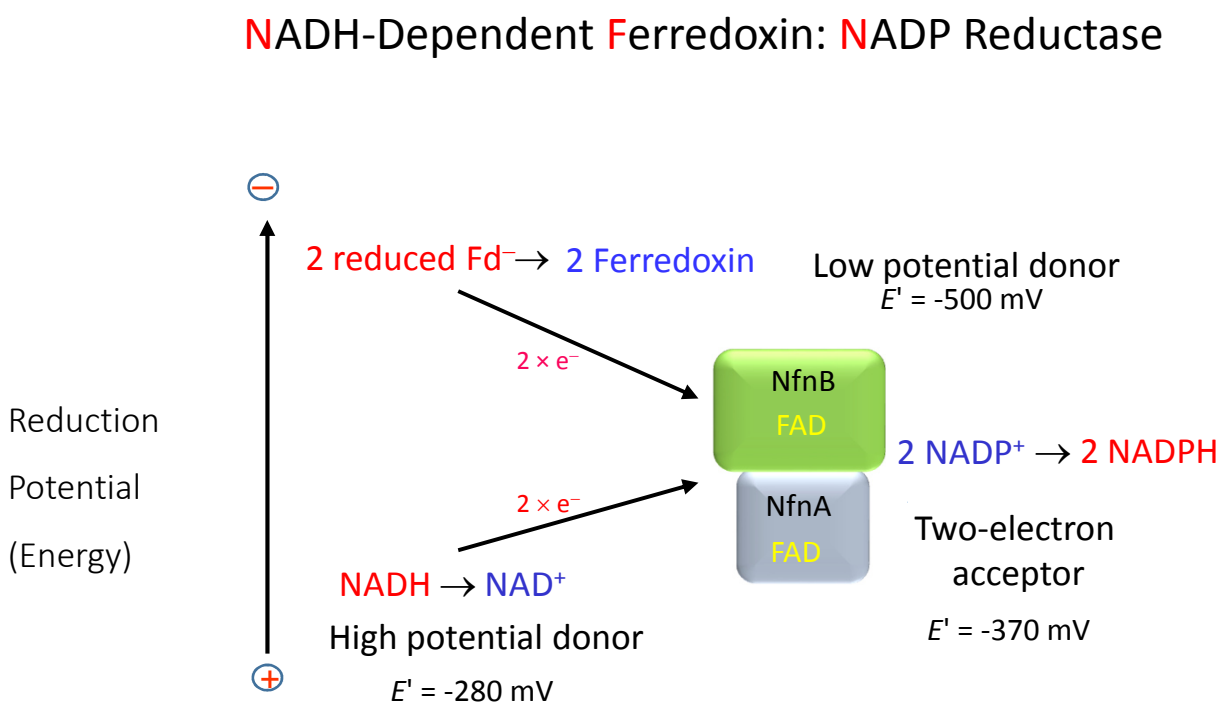
Flavin-based Electron Bifurcation in Clostridia

Electron transferring flavoprotein (EtfAB) and *Butyryl-CoA dehydrogenase* (Bcd)



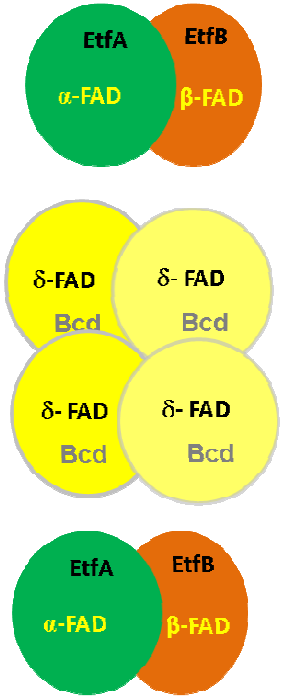
Buckel and Thauer 2008

Flavin-based electron confurcation catalyzed by the NfnAB complex

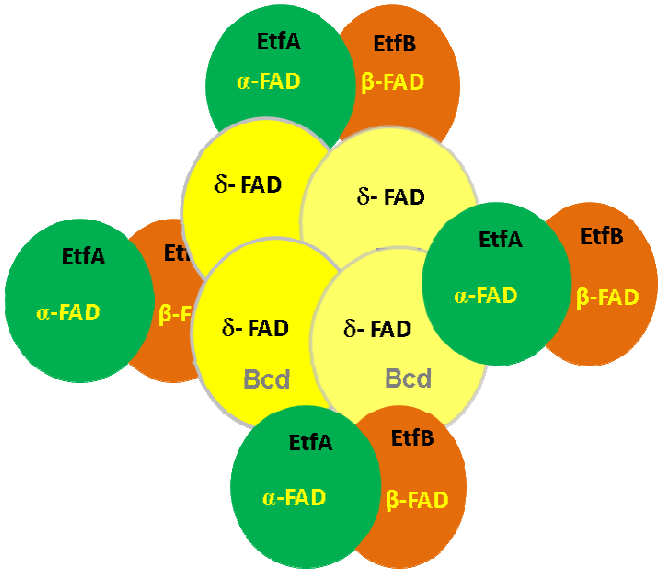


Thauer et al. 2010, 2012; Ermler, Thauer et al. 2015;
BETCy group: Lubner et al. 2017

Electron transferring flavoprotein (Etf) – Butyryl-CoA dehydrogenase (Bcd)

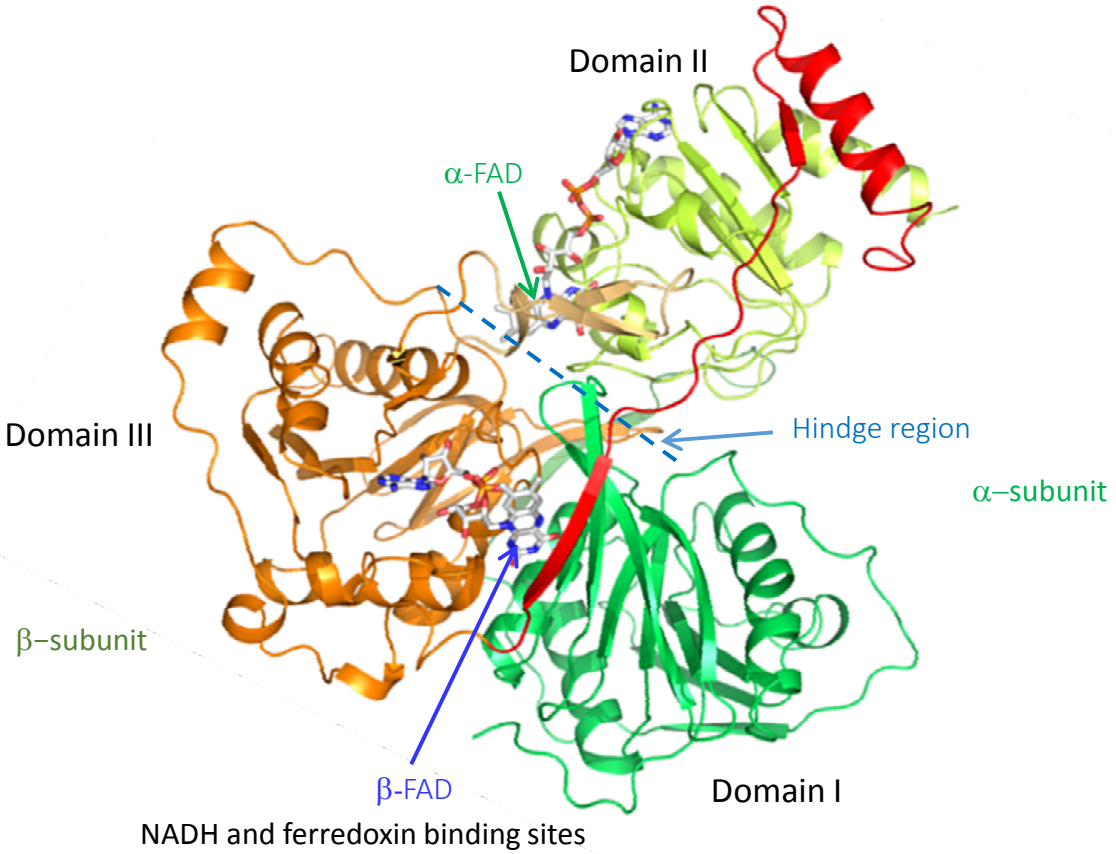


Acidaminococcus fermentans
Megasphaera elsdenii
Gram-negative Firmicutes

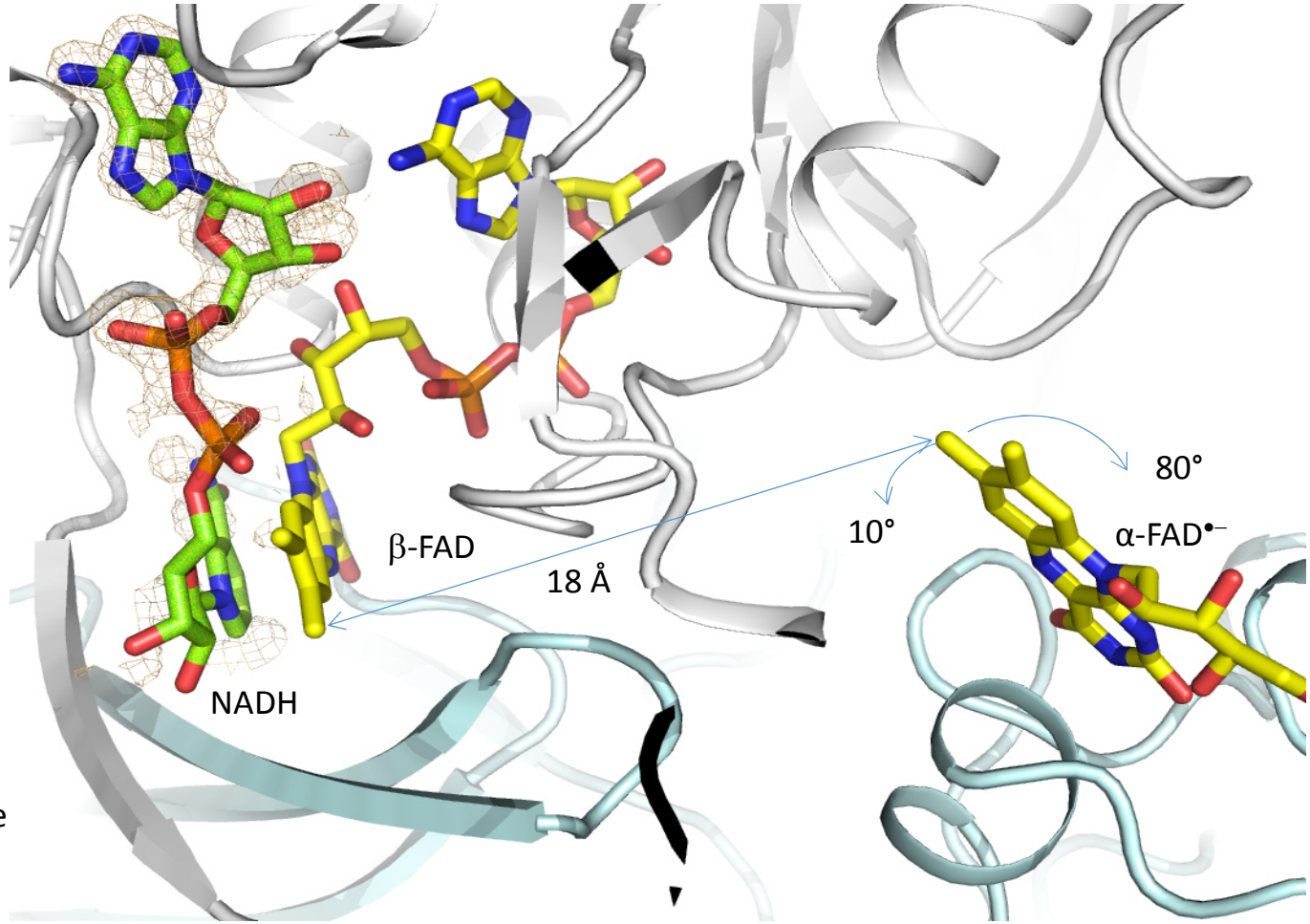


Clostridia
Gram-positive Firmicutes

EtfAB from *Acidaminococcus fermentans*



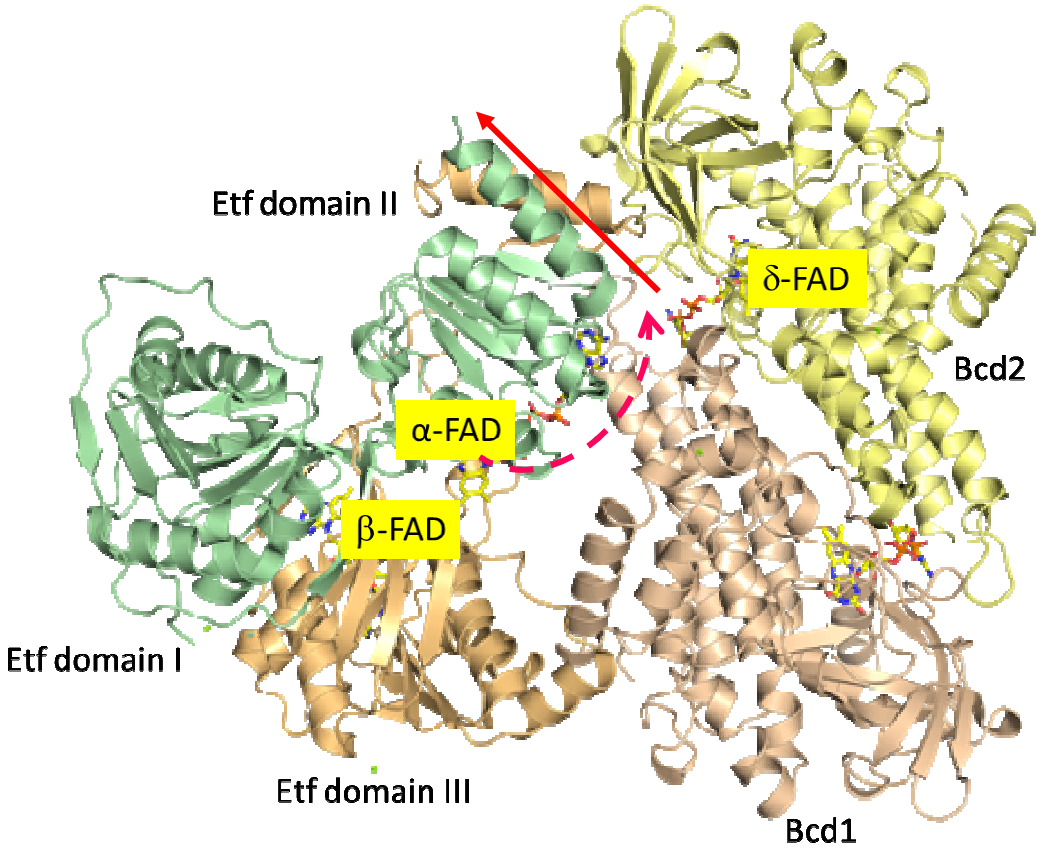
Interaction of NADH with EtfAB from *Acidaminococcus fermentans* the in the bifurcation-like state



Nilanjan Pal Chowdhury & Ulrich Ermler

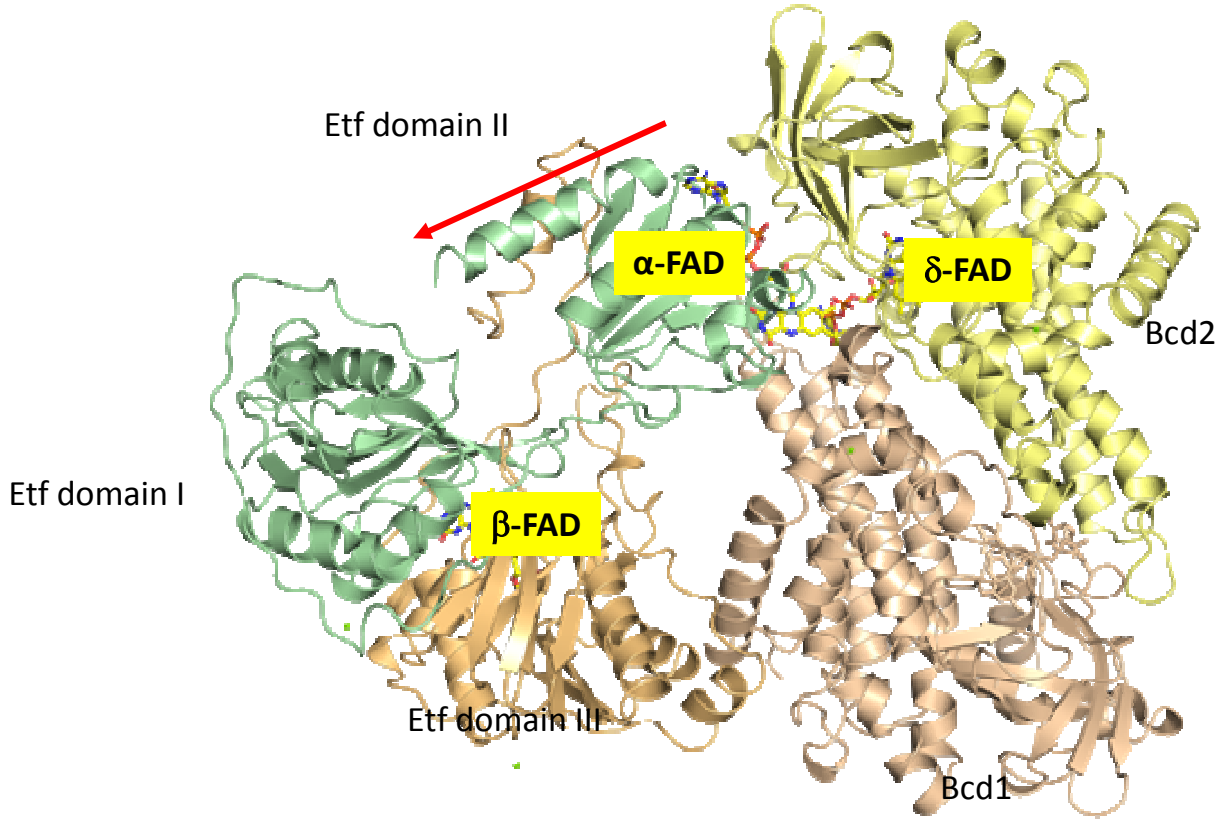
EtfAB-Bcd Complex from *Clostridium difficile*

Bifurcation-like state



EtfAB-Bcd Complex from *Clostridium difficile*

Dehydrogenase-State



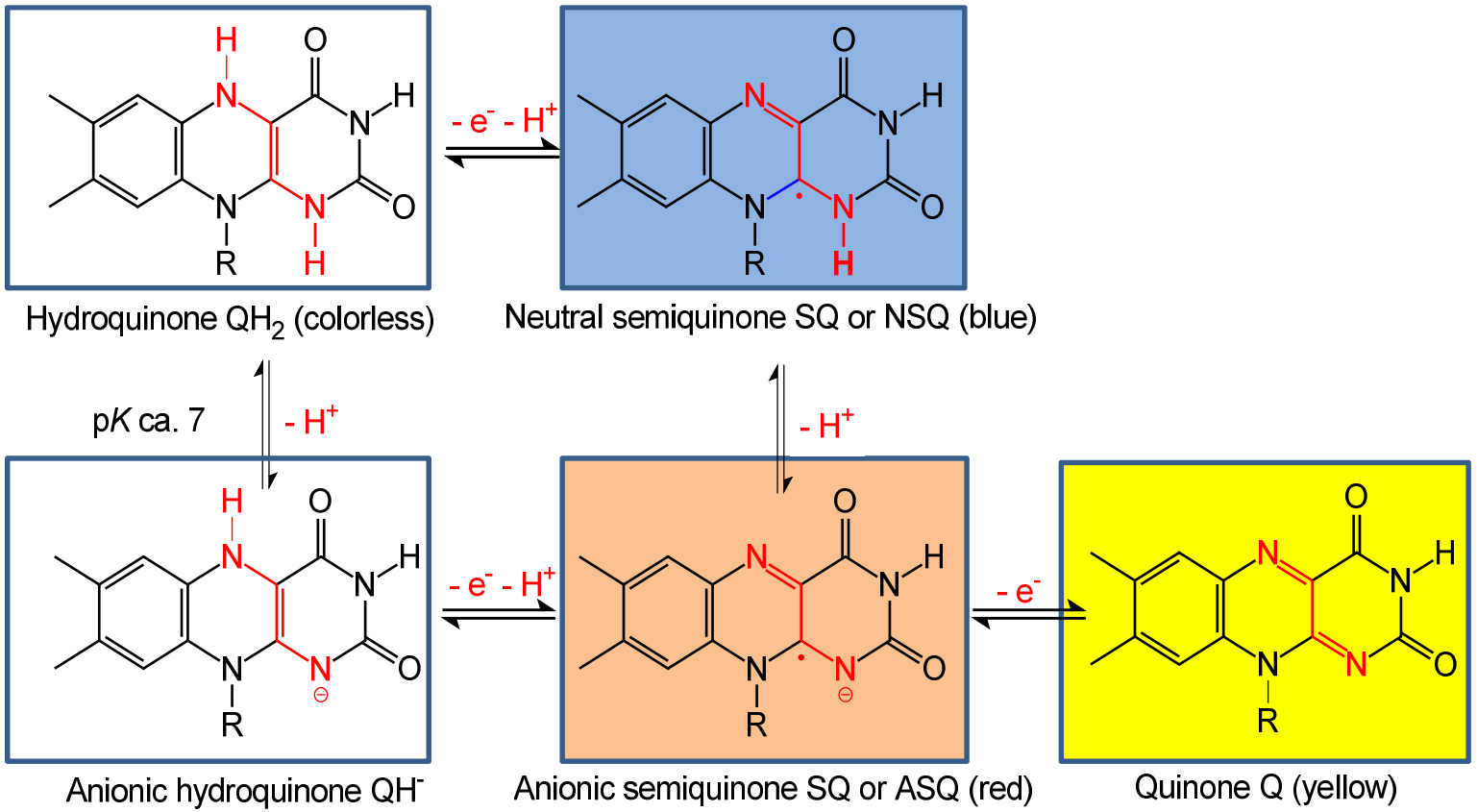
Demmer, J. K., Chowdhury, N. P., Selmer, T., Ermler, U., Buckel, W. *Nature Commun.* 2017, *8*, 1577

Leonor Michaelis
1875 - 1949

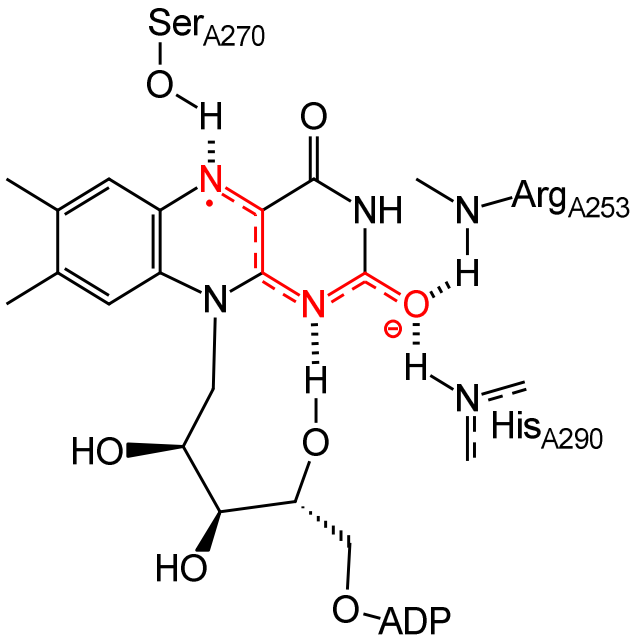
Theory of Oxidation-Reduction:
"The task of the protein be (*a*) to establish the proper geometric configuration between the different prosthetic groups, and (*b*) to aid the formation and stabilization of free radicals."

in The Enzymes, Chemistry and Mechanism of Action (eds Sumner, J. B. & Myrbäck, K.) vol. 2, part 1, pp. 1-54 (Academic Press , New York, **1951**).

Stepwise oxidation of flavin hydroquinone

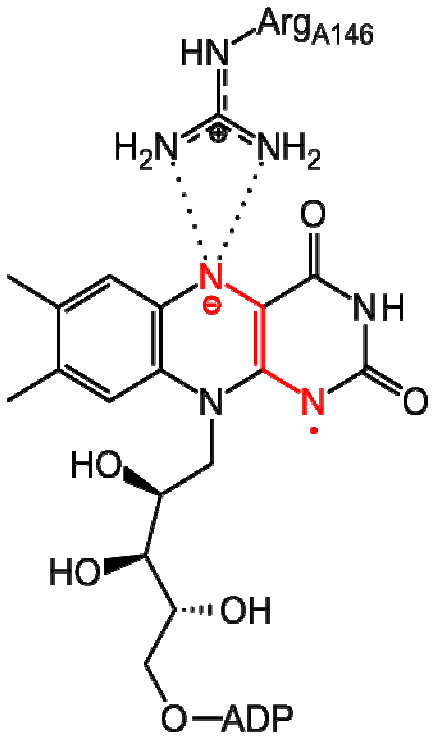


Main interactions of FAD semiquinone anions in EtfAB



Stable α-FAD$^{\bullet-}$

$K_s = 10^{+4}$



Extremely unstable β-FAD$^{\bullet-}$

$K_s \approx 10^{-15}$

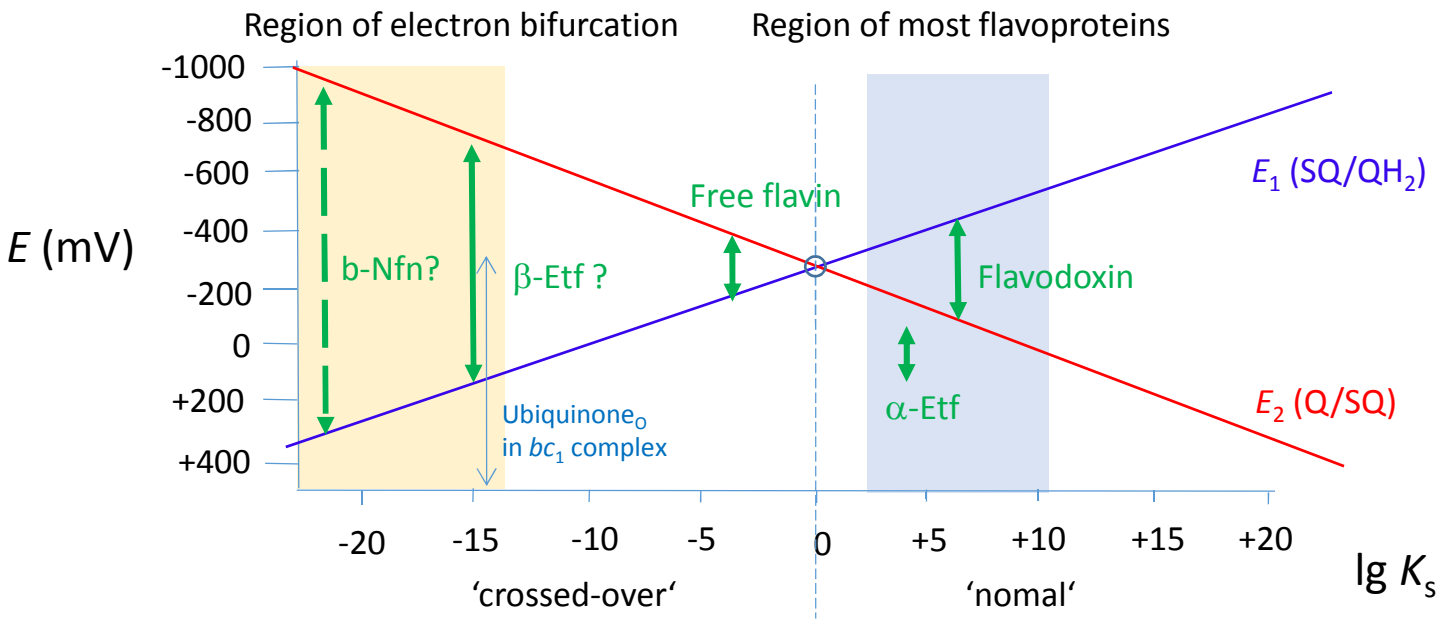
Stability constant $K_s = \dfrac{[SQ]^2}{[Q] \times [HQ]}$

Electron bifurcation requires a very unstable semiquinone

Nernst equation:

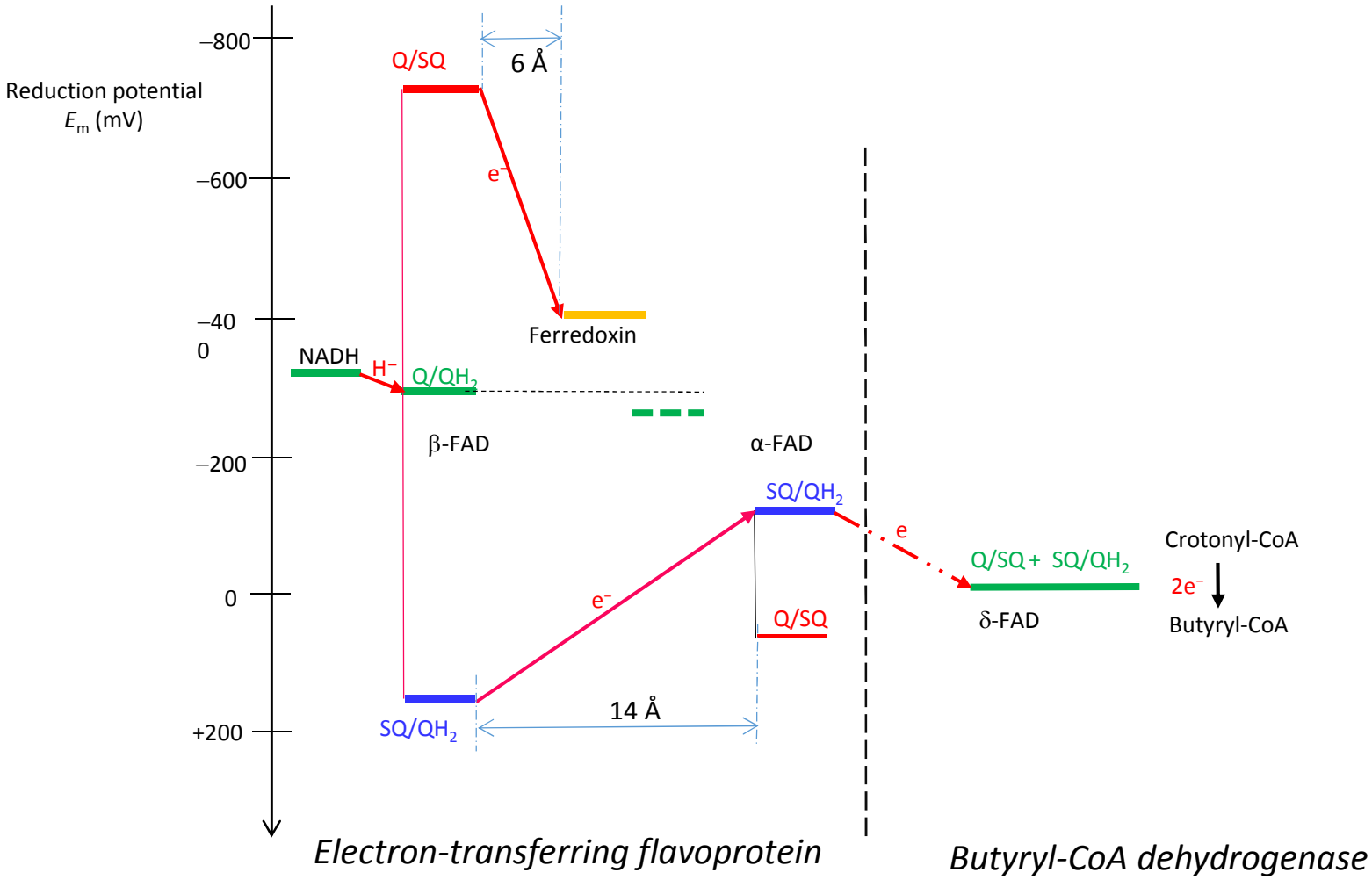
Reduction potential: $E = \pm [2.3\ RT\ (2\ F)^{-1} \times \lg K_s + E_{flavin}] = \pm [0.030 \lg K_s - 0.28]$

$\Delta E = E_1 - E_2 = 2.3\ RT \times F^{-1} \times \lg K_s = 0.059 \lg K_s$

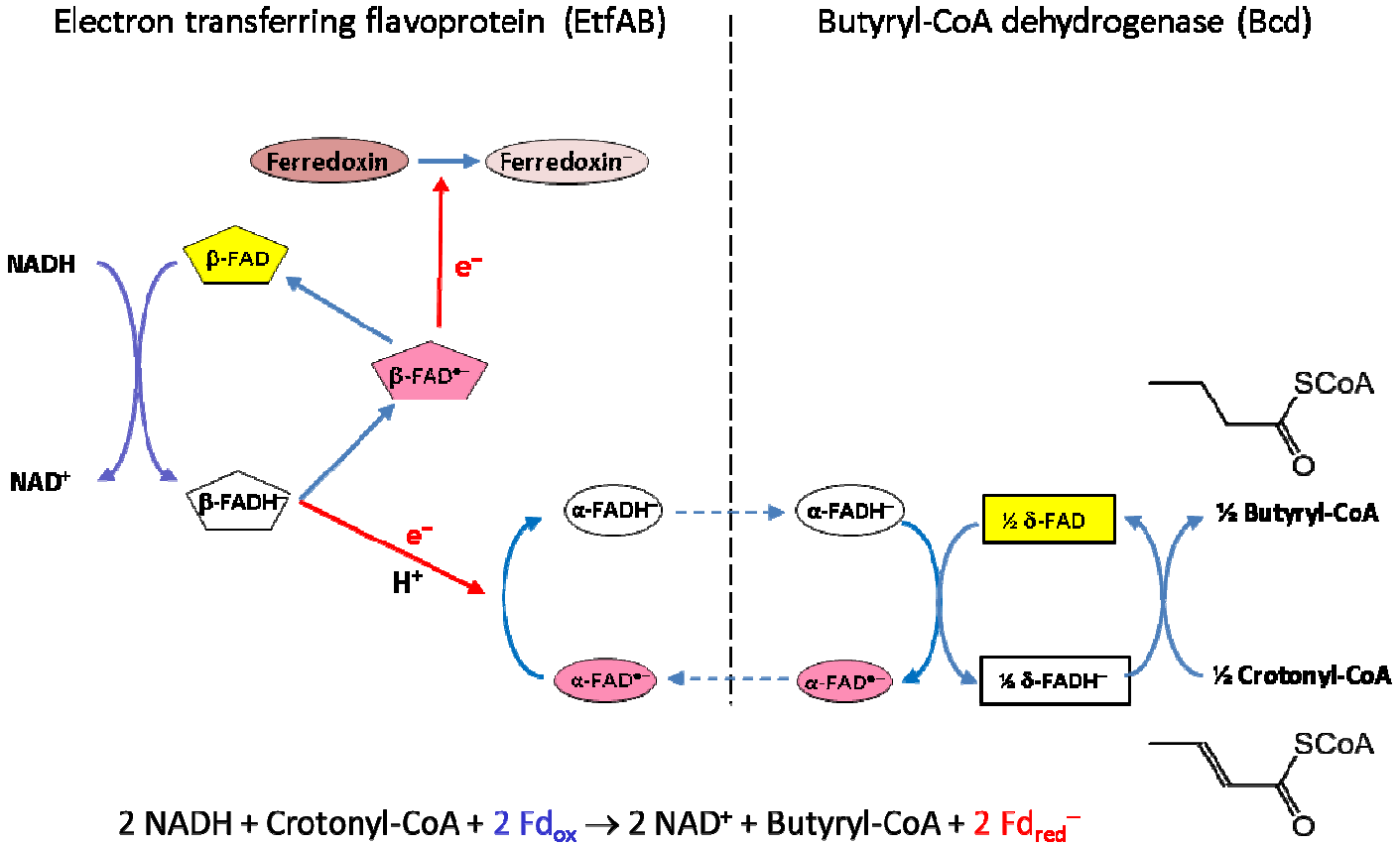


Adapted from Nitschke and Russel 2011

Proposed electron flow in the EtfAB-Bcd complex.



Proposed electron flow in the EtfAB-Bcd complex



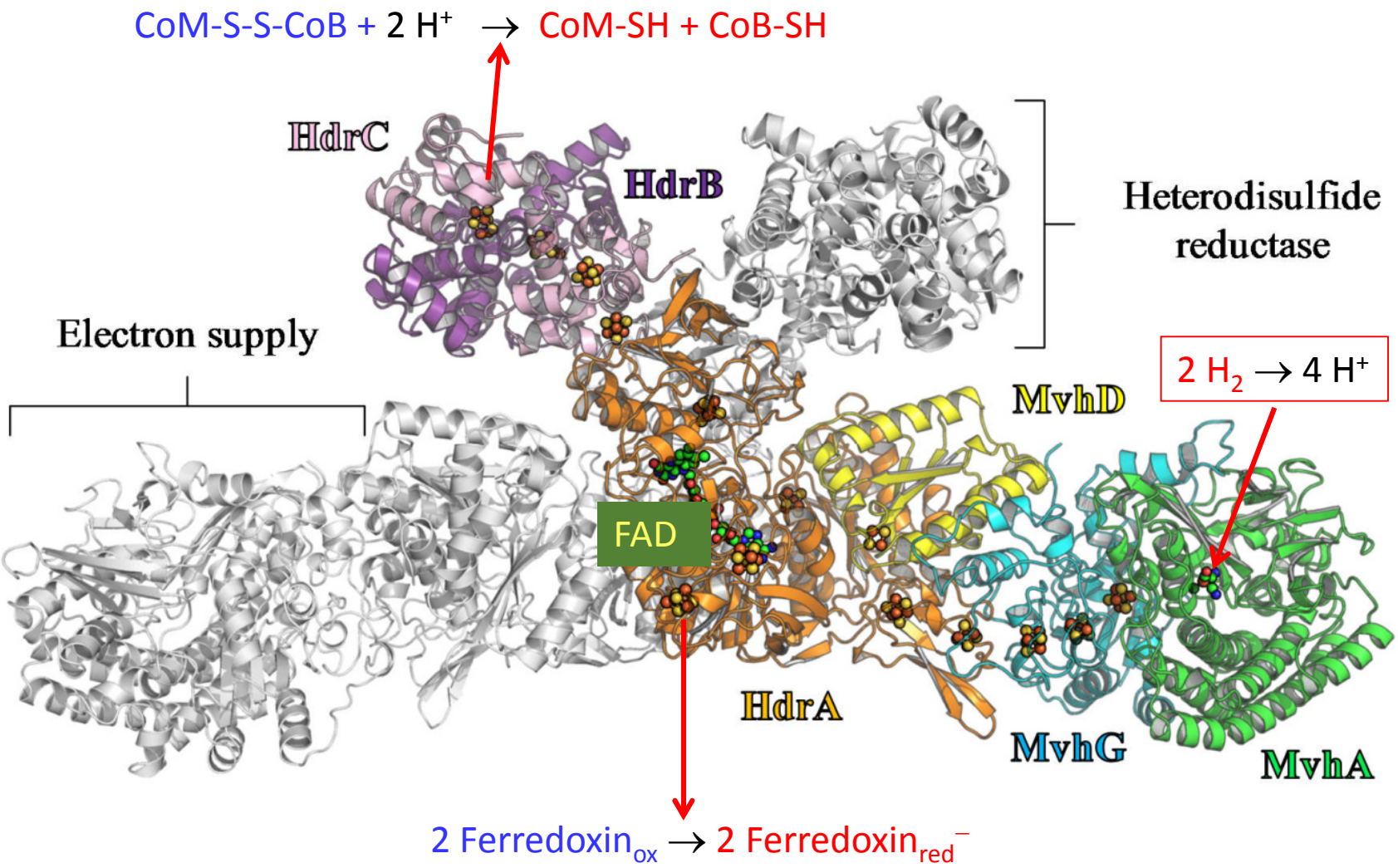
2 NADH + Crotonyl-CoA + 2 Fd_{ox} → 2 NAD^+ + Butyryl-CoA + 2 Fd_{red}^-

The eleven established flavin-based electron-bifurcating systems

System	Donor	High pot. Acceptor	Low pot. Acceptor	Organism	Established by
Butyryl-CoA dehydrogenase/EtfAB	2 NADH	Crotonyl-CoA	Ferredoxin	Clostridia	Buckel & Thauer 2008
Caffeyl-CoA reductase/EtfAB	2 NADH	Caffeyl-CoA	Ferredoxin	*Acetobacterium*	Müller 2010
Lactate dehydrogenase/EtfAB	2 NADH	Pyruvate	Ferredoxin	*Acetobacterium*	Müller 2012
FixABCX	2 NADH	Rhodoquinone Ubiquinone	Ferredoxin	*Rhodospirillum rubrum* *Aztobacter vinelandii*	Nordlund 2004 Ledbetter et al. 2017
Transhydrogenase, Nfn	2 NADPH	NAD^+	Ferredoxin	*Clostridia,* Acetogens *Pyrococcus furiosus*	Thauer 2010 Lubner et al. 2017
HdrABC-[NiFe]-hydrogenase	2 H_2	CoM-SS-CoB	Ferredoxin	Methanogens	Thauer & Buckel 2008
HdrABC-[W/Se]-formate deydrogenase	2 Formate	CoM-SS-CoB	Ferredoxin	Methanogens	Leigh 2010
HdrABC-$F_{420}H_2$	2 $F_{420}H_2$	CoM-SS-CoB	Ferredoxin	*Methanosarcina acetivorans*	Ferry 2017
NADH dehydrogenase: [FeFe]-hydrogenase	2 H_2	NAD^+	Ferredoxin	*Thermotoga, Acetobacterium* *Morella thermoacetica*	Adams ,Müller ,Thauer 2009-10-11
NADPH dehydrogenase: [FeFe]-hydrogenase	2 H_2	$NADP^+$	Ferredoxin	*Clostridium autoethanogenum*	Thauer 2013
NADH-dehydrogenase-[W/Se]-formate-dh	2 Formate	NAD^+	Ferredoxin	*Clostridium acidi-urici*	Thauer 2015

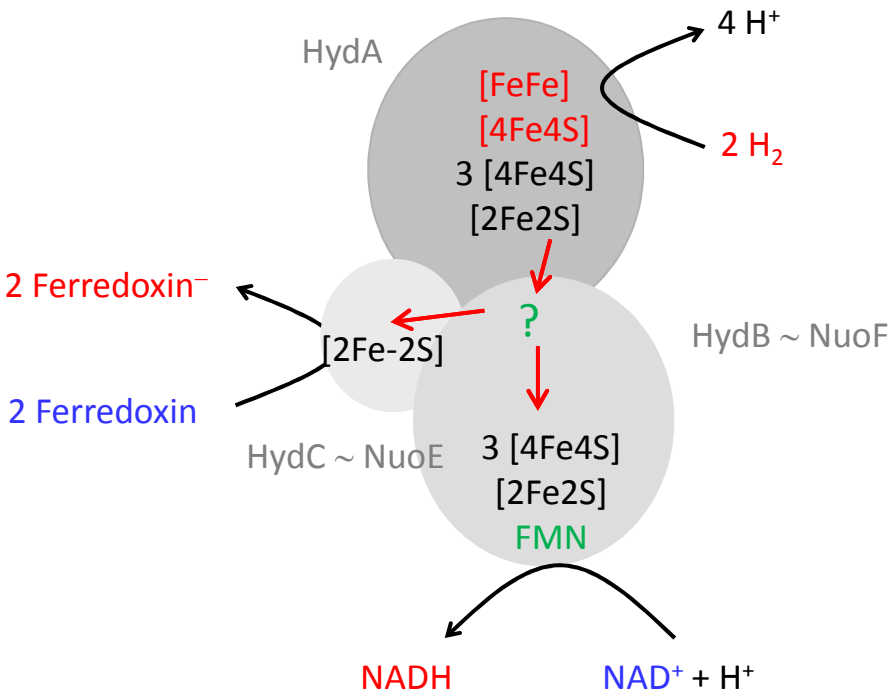
EtfAB **Transhydrogenase** **HdrABC** **NuoF (FMN)**

Crystal structure of the heterodisulfide reductase/hydrogenase complex from *Methanobacterium wolfei*

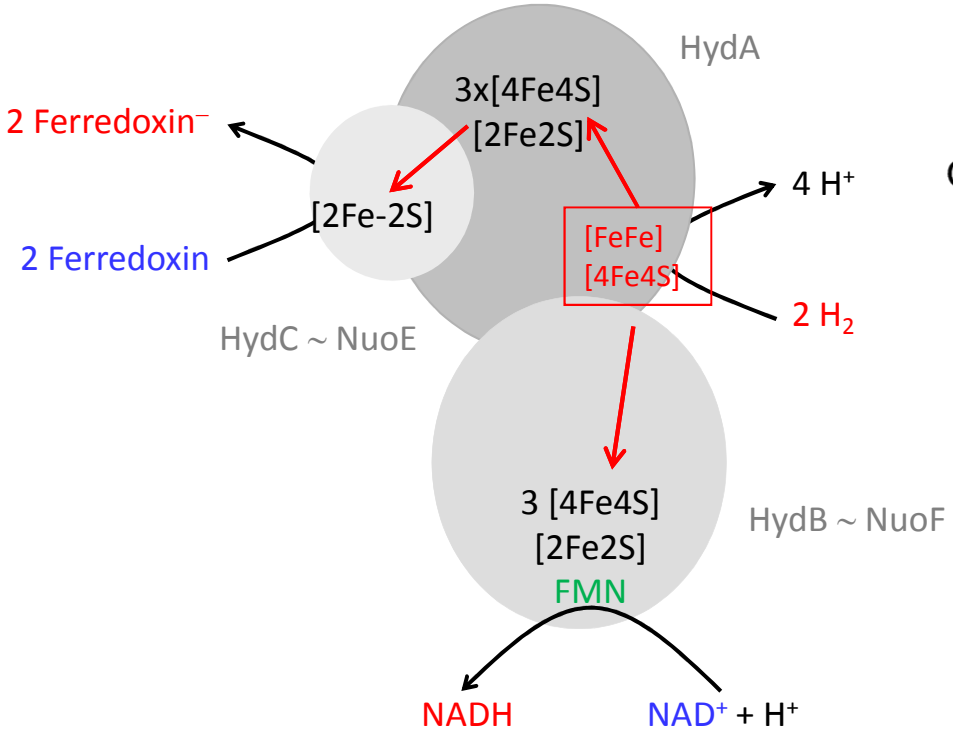


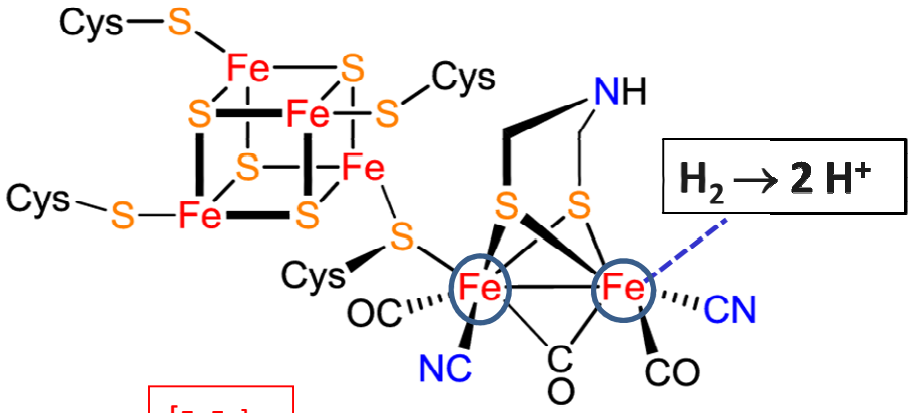
Tristan Wagner, Ulrich Ermer & Seigo Shima, Science 2017

Bifurcating Hydrogenase



Hydrogenase with a Bifurcating H-Cluster ?

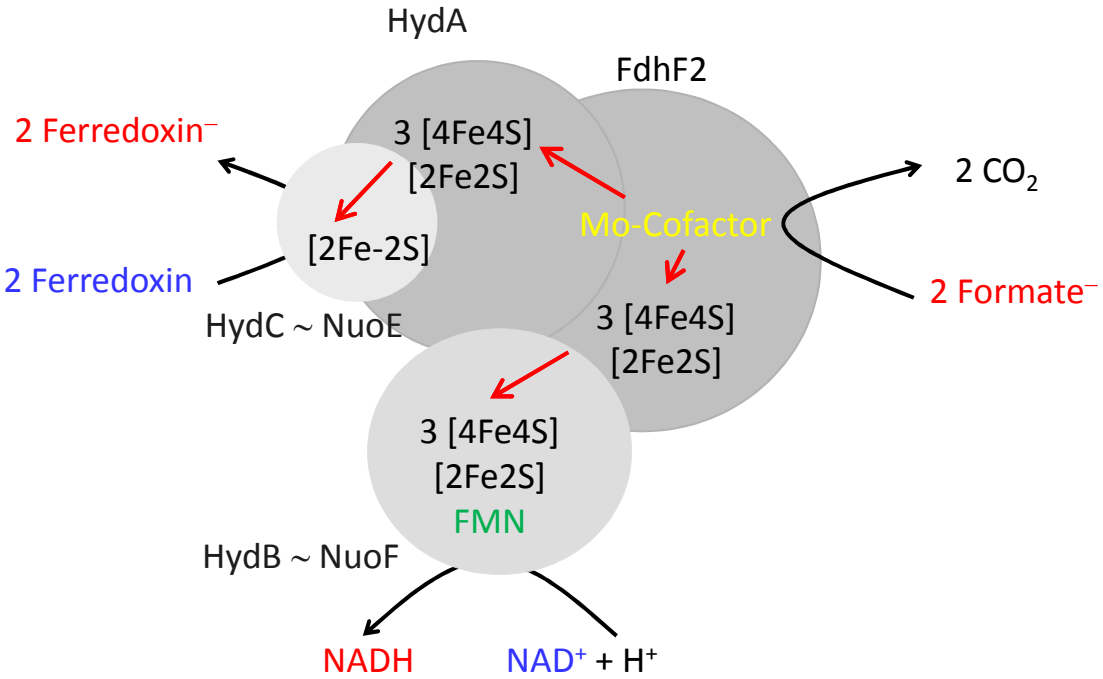




Fe^{II} ----Fe^{II} ~ FAD Hydroquinone
Fe^{III} ----Fe^{II} ~ FAD Semiquinone
Fe^{III} ----Fe^{III} ~ FAD Quinone

Peters, Beratan, Schut & Adams (2018) Chem Com.

Formate Dehydrogenase with a Bifurcating Molybdenum Cofactor ?



Molybdate^{IV} ~ FAD hydroquinone

Molybdate^{V} ~ FAD semiquinone

Molybdate^{VI} ~ FAD quinone

Nitschke, W. and Russell, M. J. 2011
Wang, S., Huang, H., Kahnt, J., and Thauer, R. K. 2013

Acknowledgements

Coworkers

Nilanjan Pal Chowdhury

Amr Mohammed Mowafy

Collaborations

Ulrich Ermler and Julius Demmer, MPI für Biophysik, Frankfurt

Thorsten Selmer, FH Aachen

Rudolf K. Thauer, MPI Marburg

Wolfgang Nitschke, CNRS Marseilles