Chem489-503
Metals in Biology and Medicine

Iron-sulfur clusters

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What are iron-sulfur clusters?

- Cofactors found in all life forms
- Contain iron (Fe^{3+} and Fe^{2+}) and sulfide (S^{2-})
- Iron typically exhibits tetrahedral geometry
- Early investigators:
  - Helmet Beinert (Wisconsin-Madison)
  - Richard Holm (Harvard)
  - Eckard Münck (Carnegie Mellon)

[2Fe2S] [3Fe4S] [4Fe4S]

Properties of Fe-S clusters

- Can delocalize electron density over both iron and sulfur
- Makes Fe-S clusters ideal for primary role in biology: mediating electron transfer
- Conserved set of proteins that synthesize Fe-S clusters and insert the intact clusters into Fe-S proteins
- Fe-S protein ligands are typically cysteine thiolates
- Other ligands include Asp, His, Ser, backbone amide, Arg, …
- Clusters are typically stable in two oxidation states
- Brown color
  - S → Fe charge transfer bands (LMCT)
  - More intense for Fe^{3+} oxidation state

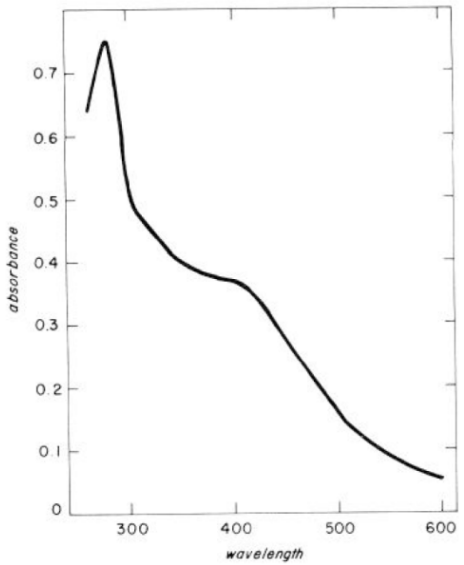


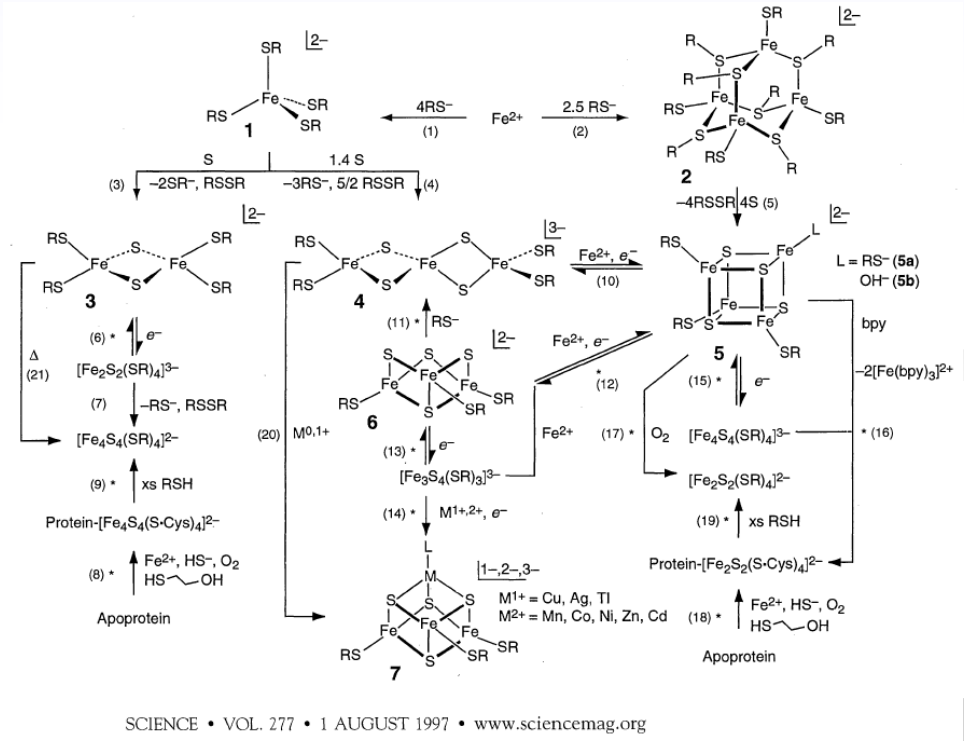
TABLE 1 Functions of some biological [Fe-S] clusters[a]

Function	Examples	Cluster type
Electron transfer	Ferredoxins; redox enzymes	[2Fe-2S]; [3Fe-4S]; [4Fe-4S]
Coupled electron/proton transfer	Rieske protein	[2Fe-2S]
	Nitrogenase	[8Fe-7S]
Substrate binding and activation	(de)Hydratases	[4Fe-4S]
	Radical SAM enzymes	[4Fe-4S]
	Acetyl-CoA synthase	Ni-Ni-[4Fe-4S], [Ni-4Fe-5S]
	Sulfite reductase	[4Fe-4S]-siroheme
Fe or cluster storage	Ferredoxins	[4Fe-4S]
	Polyferredoxins	[4Fe-4S]
Structural	Endonuclease III	[4Fe-4S]
	MutY	[4Fe-4S]
Regulation of gene expression	SoxR	[2Fe-2S]
	FNR	[4Fe-4S]/[2Fe-2S]
	IRP	[4Fe-4S]
	IscR	[2Fe-2S]
Regulation of enzyme activity	Glutamine PRPP amidotransferase	[4Fe-4S]
	Ferrochelatase	[2Fe-2S]
Disulfide reduction	Ferredoxin:thioredoxin reductase	[4Fe-4S]
	Heterodisulfide reductase	[4Fe-4S]
Sulfur donor	Biotin synthase	[2Fe-2S]

Ann Rev Biochem 2005 74:247-281

Formation of Fe-S clusters

- Synthetic Fe-S clusters self-assemble
- Fe-S clusters control protein structure in vicinity of cluster
  - Documented examples of cluster driven protein reorganization
- Unstructured synthetic peptides will correctly assembly clusters
- Apo forms of the $[Fe_2S_2]$ and $[Fe_4S_4]$ proteins can be activated by simple addition of S^{2-} and $Fe^{2+/3+}$
- Why do we need metallochaperone systems for Fe-S cluster assembly?



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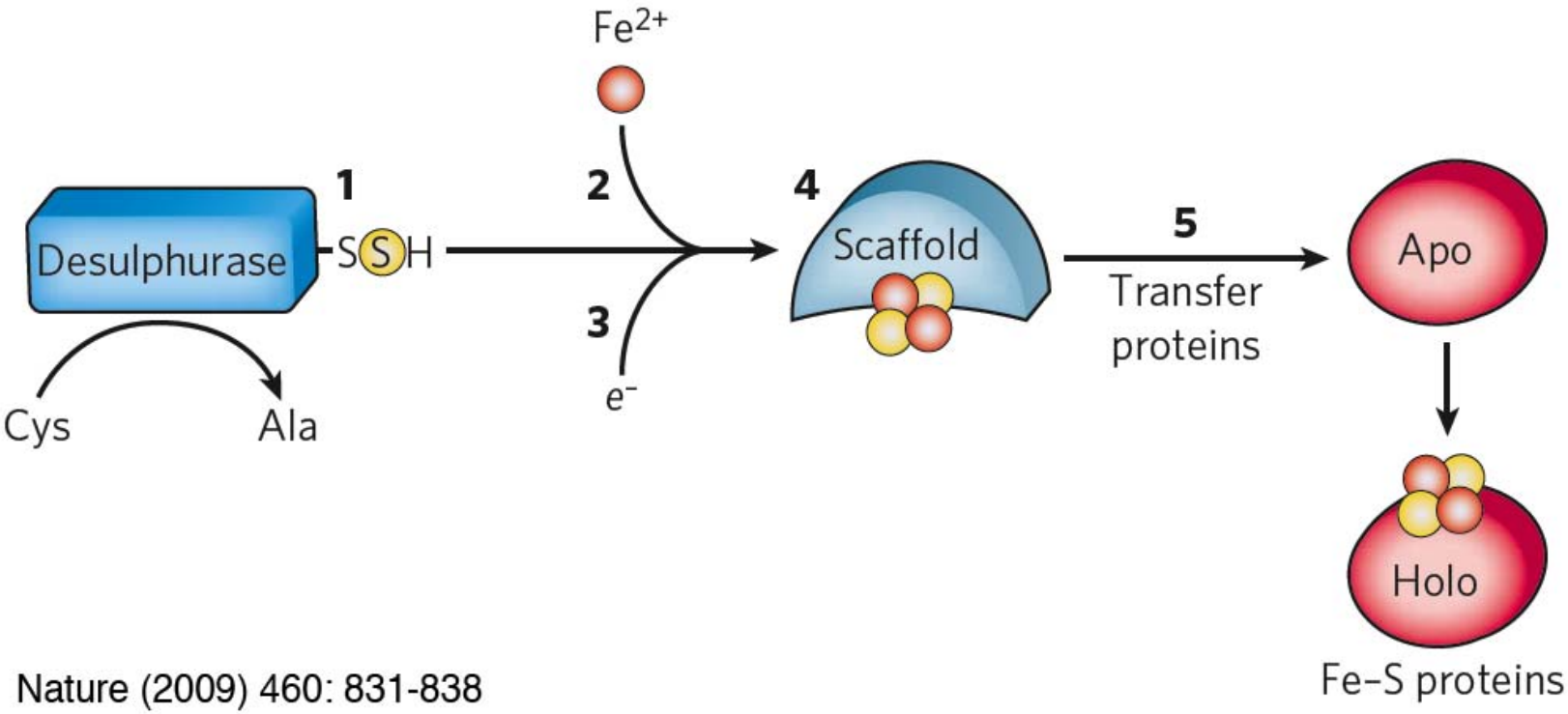
Fe-S cluster biosynthesis

- Free iron and sulfide are toxic to cells
  - Fe^{2+} is prone to Fenton chemistry
  - H_2S is an inhibitor of cytochrome oxidase (similar to CN toxicity)
- Conserved set of Fe-S cluster assembly proteins
  - Build clusters from iron, cysteine, and electrons
  - Recognize, through an unknown mechanism, and insert Fe-S clusters into apo metalloproteins
- Defects in the biogenesis of iron-sulfur clusters
  - Directly associated with mitochondrial dysfunction, cardiovascular and neurodegenerative disease
  - Contribute to genomic instability, the development of cancer, and aging

Challenges for assembly system

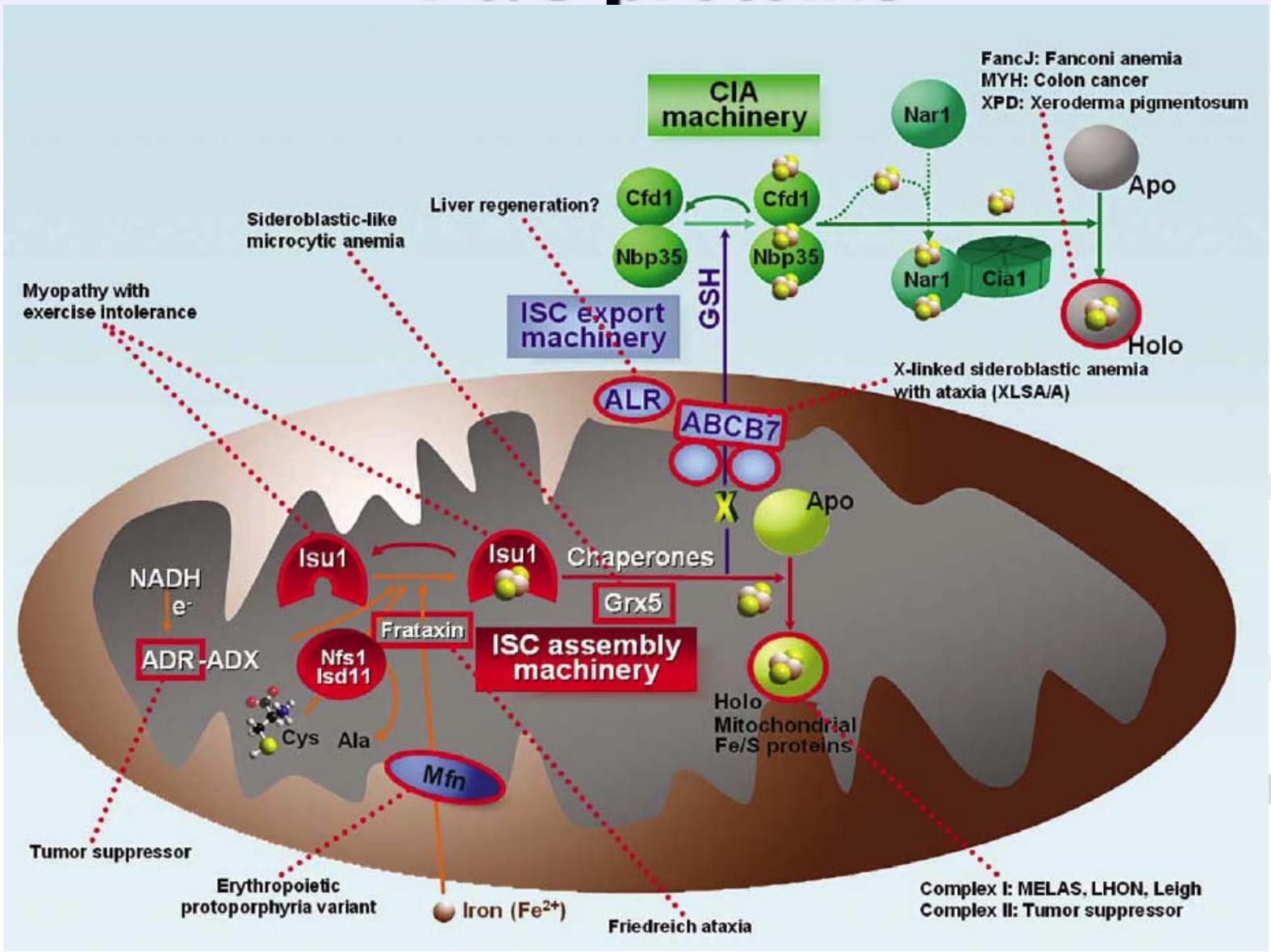
- Must be able to control the reactivity of iron and sulfide to minimize toxicity
- Must be able to recognize different protein scaffolds and environments for cluster insertion
  - Cluster may be on surface or completely buried in protein
- Must be able to insert correct nuclearity cluster into Fe-S protein
  - $[Fe_2S_2]$ and $[Fe_4S_4]$ can both contain 4 cysteine ligands
- Must be able to insert cluster with non-cysteine ligands
  - Rieske centers have 2 His ligands
- Must favor functional state of protein and not undergo local rearrangement
  - Cluster often has incomplete protein ligation to open up coordination site(s) for chemistry
  - Large driving force for cluster assembly
- Must avoid placing cluster in sites designed for other metal cofactors
  - Zn-finger sites: metal is often bound to 4 Cys or 2 Cys 2 His motifs

Components of Fe-S assembly pathways



Nature (2009) 460: 831-838

Diseases resulting from deficient Fe/S proteins

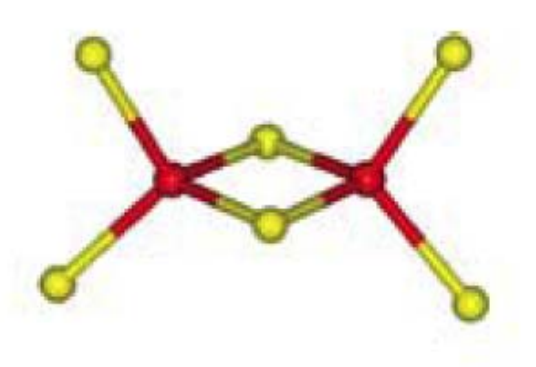


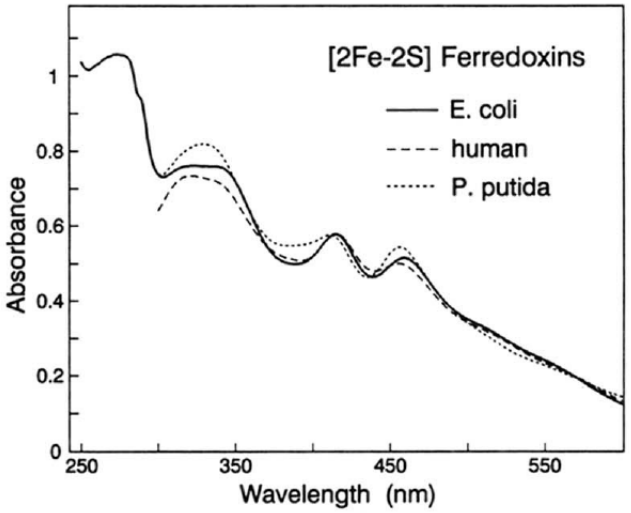
$[Fe_2S_2]$ Ferredoxin (Fd)

- One-electron carrier proteins
- Involved in electron transport chain and oxidoreduction reactions
- First discovered in spinach chloroplast in 1962
- Typically ~12 kDa monomeric protein with 2 Fe and 2 sulfide ions
- Note that there are also proteins with $[Fe_4S_4]$ clusters that are also called ferredoxin

$[Fe_2S_2]$ Ferredoxin (Fd)

- Two sulfide ions bridge the two Iron atoms
- Form a $Fe_2(\mu_2\text{-}S)_2$ core
- Each Fe also has 2 cysteine ligands that connects the cluster to the protein
- Overall geometry about each Fe is tetrahedral
- Fe-S distance is 2.3 Å; Fe-Fe distance is 2.7 Å
- Proteins are brown with S → Fe charge transfer band near 400 nm
  - Broad band in which the intensity of the absorbance band for the oxidized form is 2X that of the reduced form





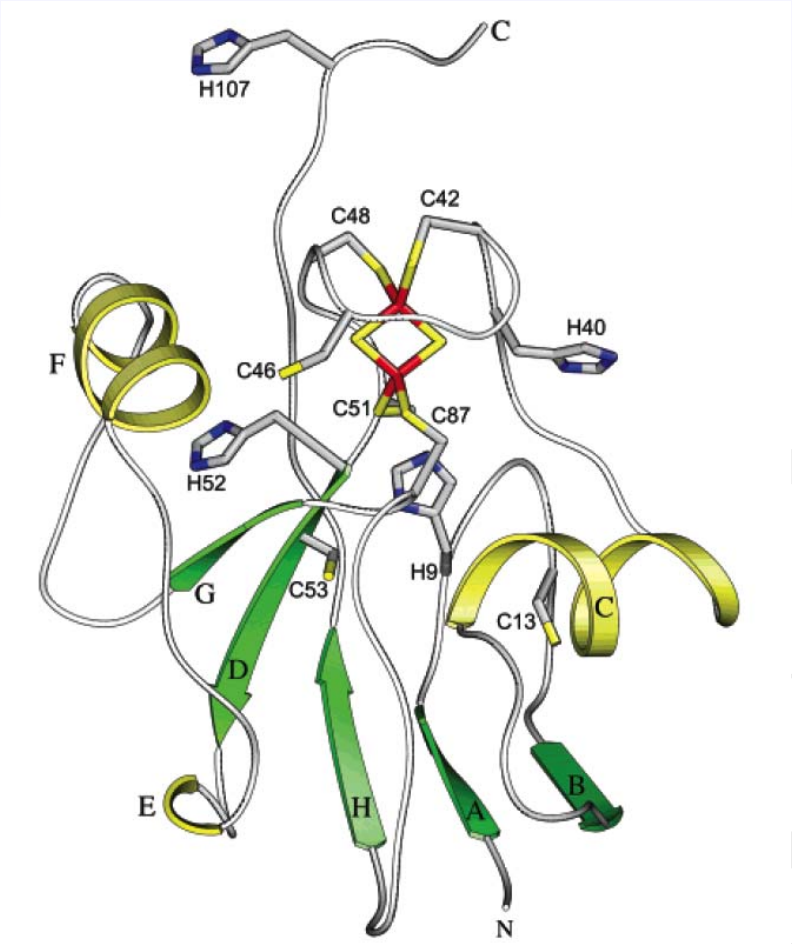
$[Fe_2S_2]$ Ferredoxin (Fd)

- For Fe-S clusters, the redox states of the clusters are indicated by "core" oxidation states
- In the oxidized state
  - Both iron atoms of the cluster are formally Fe^{3+}
  - Both sulfur atoms are formally sulfide (-2 charge)
  - Core oxidation state +2 $[Fe_2S_2]^{2+}$
- The the reduced state
  - One iron atom is Fe^{3+} and the other is Fe^{2+}
  - Both sulfur atoms are formally sulfide (-2 charge)
  - Core oxidation state +1 $[Fe_2S_2]^{1+}$
- The redox potential of the $[Fe_2S_2]^{2+/1+}$ range from -400 to -50 mV

$[Fe_2S_2]$ Ferredoxin (Fd)

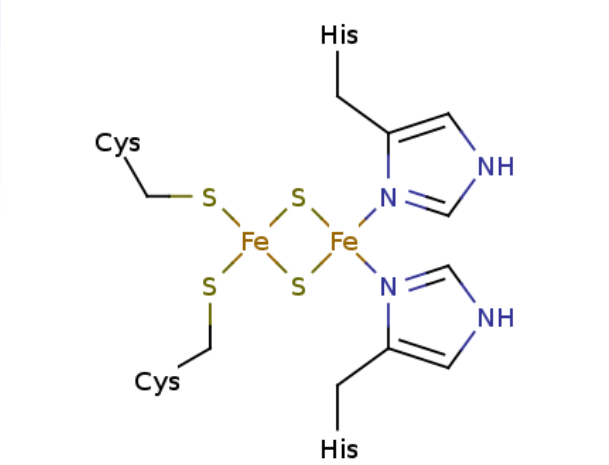
- Fe atoms are antiferromagnetically coupled
- Ferromagnetic and antiferromagnetic coupling
  - The magnetic moments (usually related to the spins of electrons) align with neighboring spins
  - In ferromagnetism the spins are in the same direction
  - In antiferromagnetism the spins are pointing in opposite directions
- $[Fe_2S_2]^{2+}$ state
  - Each Fe^{3+} ions with tetrahedral geometry would be expected to have 5 unpaired electrons and s = 5/2 spin state
  - These irons are antiferromagnetically coupled to give a core spin state S = 0
- $[Fe_2S_2]^{1+}$ state
  - The Fe^{3+} would be s = 5/2 and the Fe^{2+} would be s = 2
  - Antiferromagnetically couple to core S = 1/2 spin state

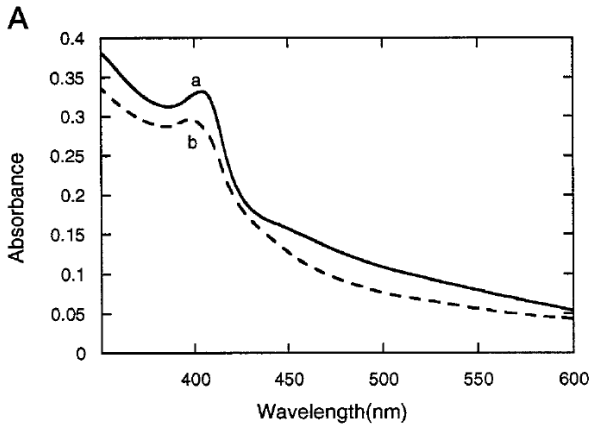
Crystal structure of $[Fe_2S_2]$ ferredoxin



Rieske [Fe_2S_2] center

- Same core structure and oxidation states as [Fe_2S_2] ferredoxin
- Ligated to two cysteines and two histidines
- Redox potential is more positive (+265 to +310 mV) than ferredoxin (-400 to -50 mV)
  - Indicates that the reduces 1+ core oxidation state is more stabilized
  - Can be rationalized as you have replaced two anionic ligands with two neutral ligands
  - A more negatively charge ligand typically stabilizes the higher oxidation state
- Redox potential is pH dependent
  - Data indicates that there are two redox-dependent protonation events
  - Likely that the His ligands undergo protonation upon reduction
- The protein is pink





Rieske [Fe_2S_2] center

- Found in electron transport chain in mitochondria (complex III)
- Accepts electrons from quinone
- Resulting semiquinone serves as a reductant for cytochrome b

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are needed to see this picture.

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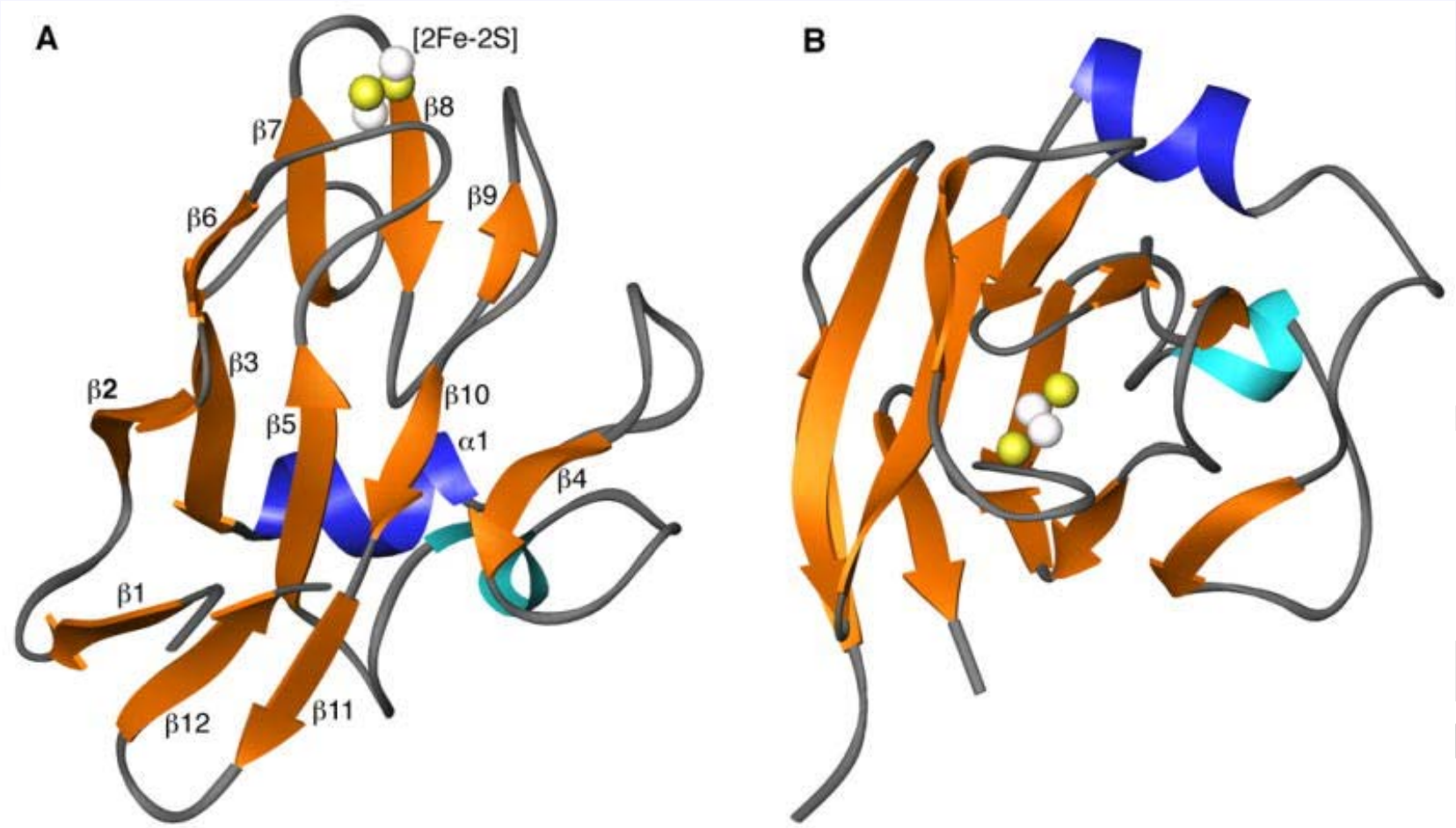
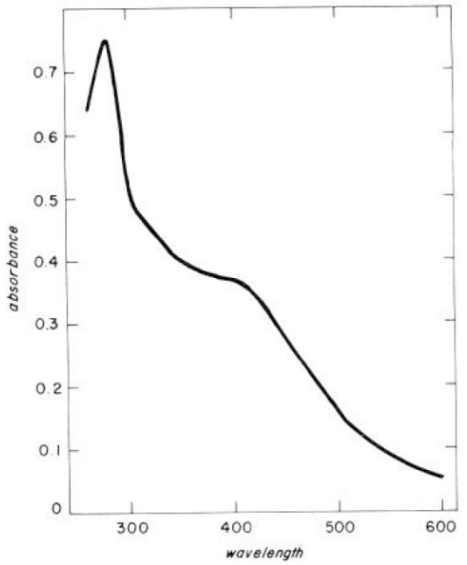
Structure of Rieske domain



$[Fe_4S_4]$ Ferredoxin

- $[Fe_4S_4]$ cluster has cubane structure
  - 4 iron atoms and 4 inorganic sulfides
  - Each iron is tetrahedrally coordinated
  - Typically coordinated by 4 cysteines
  - Other ligands: His, H_2O, substrate, SAM
- Involved in electron transfer
- There are ferredoxin proteins that contain
  - One $[Fe_4S_4]$ cluster
    - Bacillus thermoproteolyticus
  - Two $[Fe_4S_4]$ clusters
    - Clostridium pasteurianum
  - Many $[Fe_4S_4]$ clusters
    - Methanobacterium thermoautotrophicum
    - May be involved in Fe-S cluster storage
- Broad absorbance band near 400 nm
  - Due to S →Fe charge transfer bands



$[Fe_4S_4]$ clusters

- Redox properties: almost always a one electron process
- Can be stabilized in 4 core oxidation states
  - $[Fe_4S_4]^{3+}$ contains 3 Fe^{3+} and 1 Fe^{2+}
    - Is susceptible to cluster degradation to $[Fe_3S_4]$ cluster
  - $[Fe_4S_4]^{2+}$ contains 2 Fe^{3+} and 2 Fe^{2+}
    - Extremely stable (in absence of oxygen)
  - $[Fe_4S_4]^{1+}$ contains 1 Fe^{3+} and 3 Fe^{2+}
  - $[Fe_4S_4]^{0}$ contains 0 Fe^{3+} and 4 Fe^{2+}
- Typically for a given cluster only two adjacent states are stable
- The $[Fe_4S_4]^{2+}$ and $[Fe_4S_4]^{1+}$ states are the most common
- Proteins that contain $[Fe_4S_4]^{2+/1+}$ clusters (E_0 -270 to -750 mV) have traditionally been called ferredoxin
- Proteins that contain $[Fe_4S_4]^{3+/2+}$ clusters (E_0 +50 to +350 mV) known as high potential iron proteins (HIPIP)

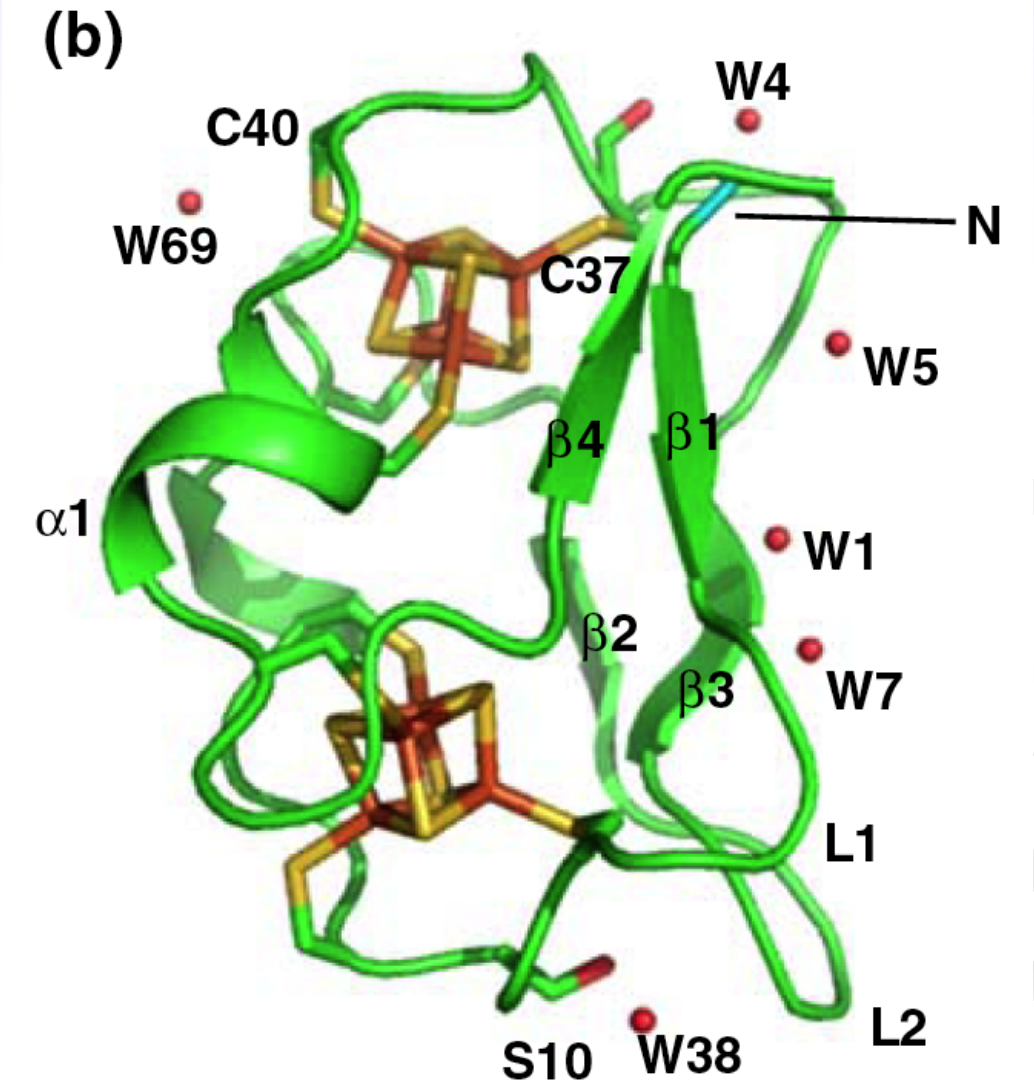
Spin coupling properties

- Ridiculously complicated
- For $[Fe_4S_4]^{1+}$ state (1 Fe^{3+}, 3 Fe^{2+})
  - Pairs of Valence-delocalized $[Fe_2S_2]$ fragments
  - "ferrous pair": two Fe^{2+} ions ferromagnetically couple to generate S = 4 intermediate spin
  - "mixed valence pair": Fe^{3+} and Fe^{2+} ferromagnetically couple to form S = 9/2 intermediate spin
  - The ferrous pair and mixed valence pair then antiferromagnetically couple to generate S = 1/2 system spin state
  - There are also S= 3/2, 5/2, up to 17/2 excited states that are typically not populated at cryogenic temperatures
  - There are examples where the $[Fe_4S_4]^{1+}$ state has a S = 3/2 system spin as the ground state (different EPR properties)

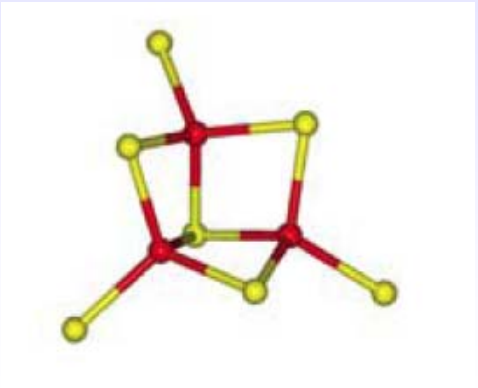
Spin coupling properties

- For $[Fe_4S_4]^{2+}$ state (2 Fe^{3+}, 2 Fe^{2+})
  - Have two "mixed valence pair"
  - Fe^{3+} and Fe^{2+} ferromagnetically couple to form S = 9/2 intermediate spin
  - The mixed valence pairs then antiferromagnetically couple to generate S = 0 system spin state
- For $[Fe_4S_4]^{3+}$ state (3 Fe^{3+}, 1 Fe^{2+}) have a core system spin of 1/2
- $[Fe_4S_4]^{0}$ state (0 Fe^{3+}, 4 Fe^{2+})
  - Found in nitrogenase
  - Appears to have S = 4 system spin as ground state
- Pairs are ferromagnetically coupled and delocalized
- Antiferromagnetically coupling between the pairs is localized
- Most of this is figured out with Mössbauer spectroscopy (Eckerd Munck at Carneige Mellon)

Structure of 8Fe Ferredoxin



Fe_3S_4 Clusters



- Found in bacteria and eukaryotes
- Involved in electron transfer
- Degradation products of $[Fe_4S_4]$ clusters
- 3 Fe ions and 4 inorganic sulfides
  - 1 sulfide bridges all 3 iron atoms
  - 3 sulfides bridge 2 iron atoms
- Iron atoms are typically bound to cysteine ligands
- Easiest to think about a $[Fe_4S_4]$ cluster and remove an iron and cysteine ligand

Fe_3S_4 Clusters

- Redox and electronic properties
- Stable in 2 core oxidation states
- $[Fe_3S_4]^{1+}$
  - Formally 3 Fe^{3+} and 0 Fe^{2+} ions
  - All three irons are antiferromagnetically coupled to yield a S = 1/2 ground state system
- $[Fe_3S_4]^{0}$
  - Formally 2 Fe^{3+} and 1 Fe^{2+} ions
  - Electron delocalizes on two of the irons which ferromagnetically couple to yield a S=9/2 intermediate spin
  - Can think of as $[Fe^{3+}\ Fe^{3+}\ Fe^{3+}]^{1+} + e^- \rightarrow [Fe^{3+}\ \{Fe^{2.5+}\ Fe^{2.5+}\}]^0$
  - The S = 9/2 intermediate spin for the two irons antiferromagnetically couples with the S = 5/2 spin of the other Fe^{3+} to generate a S = 2 ground state system

Fe-S clusters

- Function in electron transport
  - Ferredoxin, Rubredoxin, Rieske
- Function in substrate activation
  - Aconitase
- Function in initiating radical chemistry
  - Radical SAM enzymes such as lysine aminomutase
- Function as substrates
  - Sulfur donor function in Biotin synthase
- Additional functions
  - Gene regulation
  - Part of more complex cofactors such as the catalysts in nitrogenase, [FeFe]-hydrogenase, sulfite reductase, and acetyl-CoA synthase

Aconitase

- Catalyzes the reversible isomerization of citrate and isocitrate
- Cis-aconitate as intermediate

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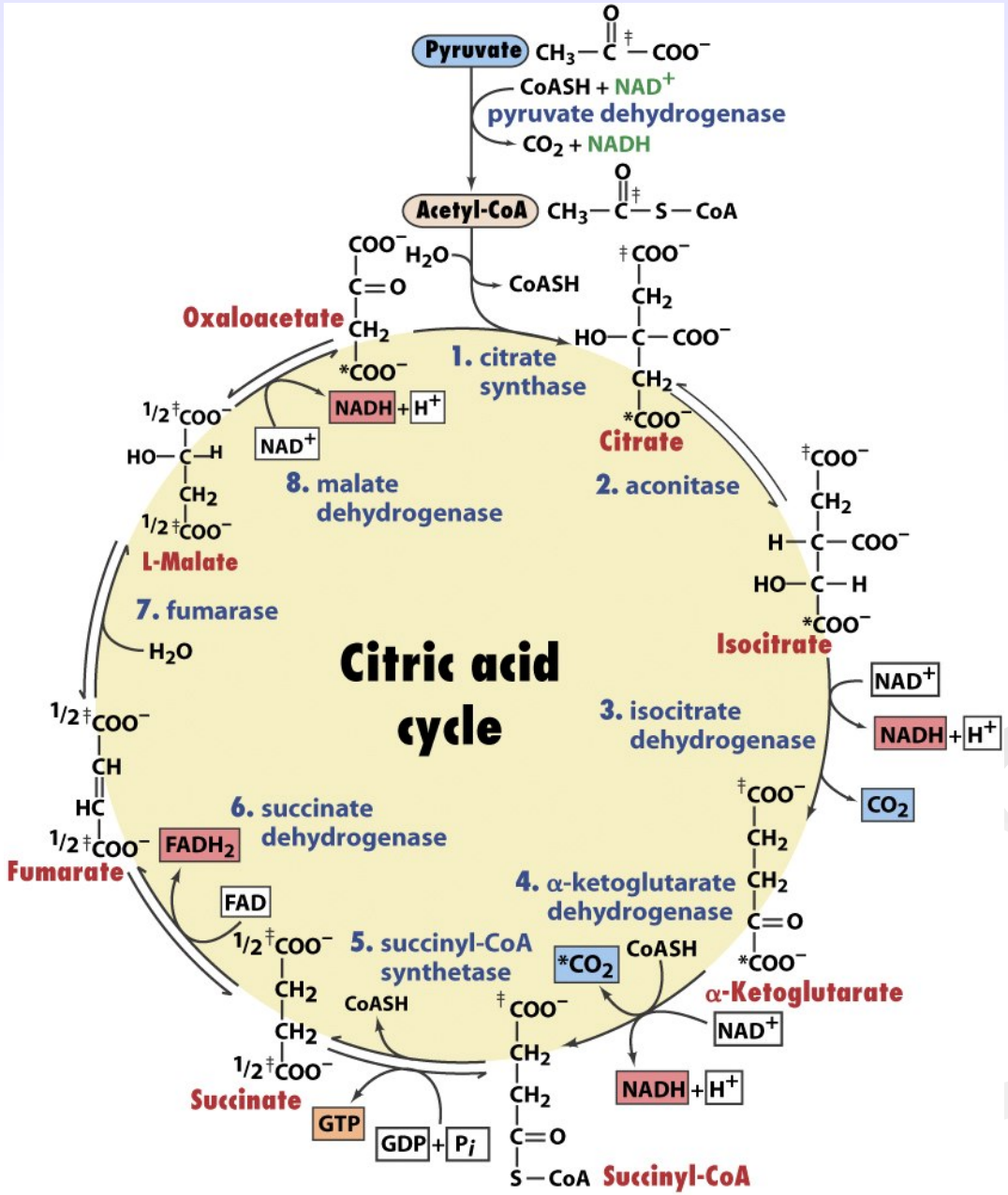

Figure 16-2 Fundamentals of Biochemistry, 2/e
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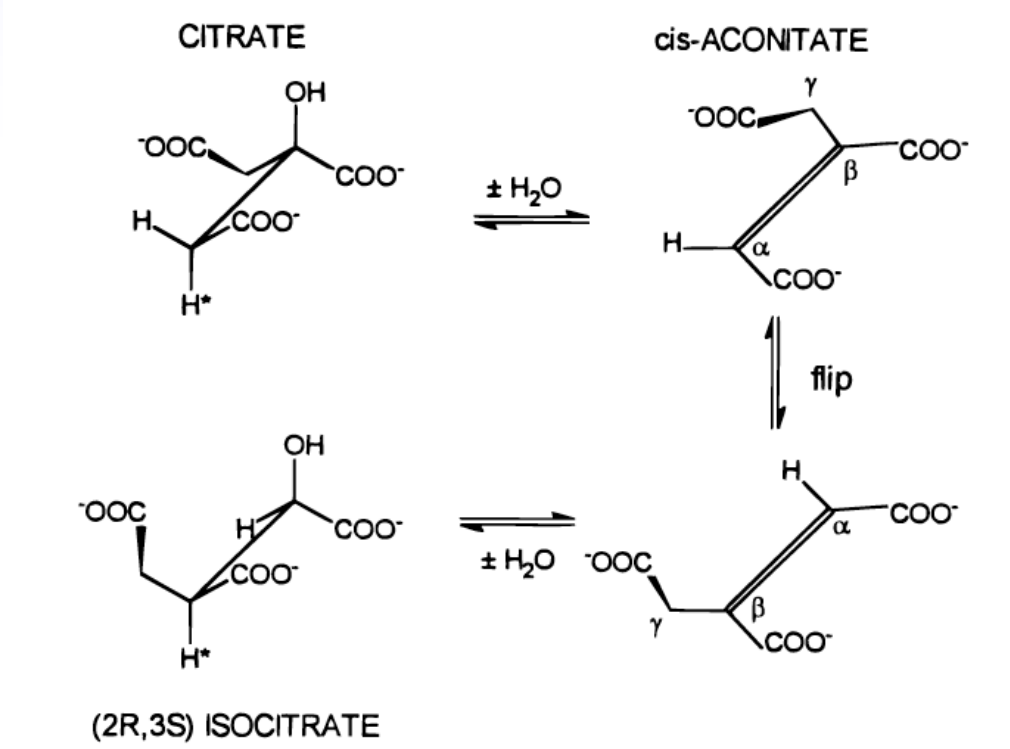
- Citrate is a tertiary alcohol
- It is difficult to oxidize tertiary alcohols because forming the ketone would involve breaking a carbon-carbon bond
- To get around this problem, citrate is isomerized into isocitrate
- Isocitrate is a secondary alcohol which can be easily oxidized to the ketone by NAD^+
- Aconitase catalyzes the dehydration of citrate of form cis-aconitate and then rehydrating the double bond to form isocitrate

Aconitase

- Proton abstracted in dehydration reaction does not readily exchange with solvent
- Stereochemical, radiolabeling, and kinetic experiments suggest that the intermediate “flips” in active site



Aconitase

- Enzyme was apparently unstable
- Found in 1951 that most of the lost activity could be readily recovered by addition of ferrous iron and a reducing agent
- Found in 1972 that the protein contained labile sulfide
- EPR of oxidized form of aconitase (g = 2.018)
- Now known to be of an inactive $[Fe_3S_4]^{1+}$ cluster

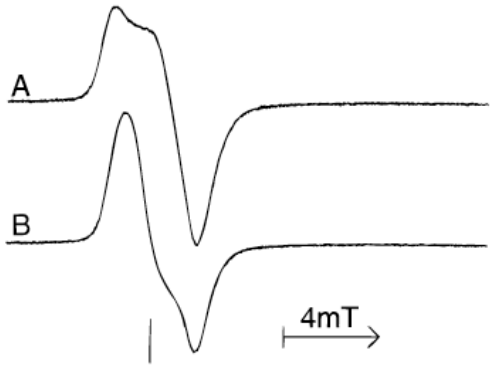
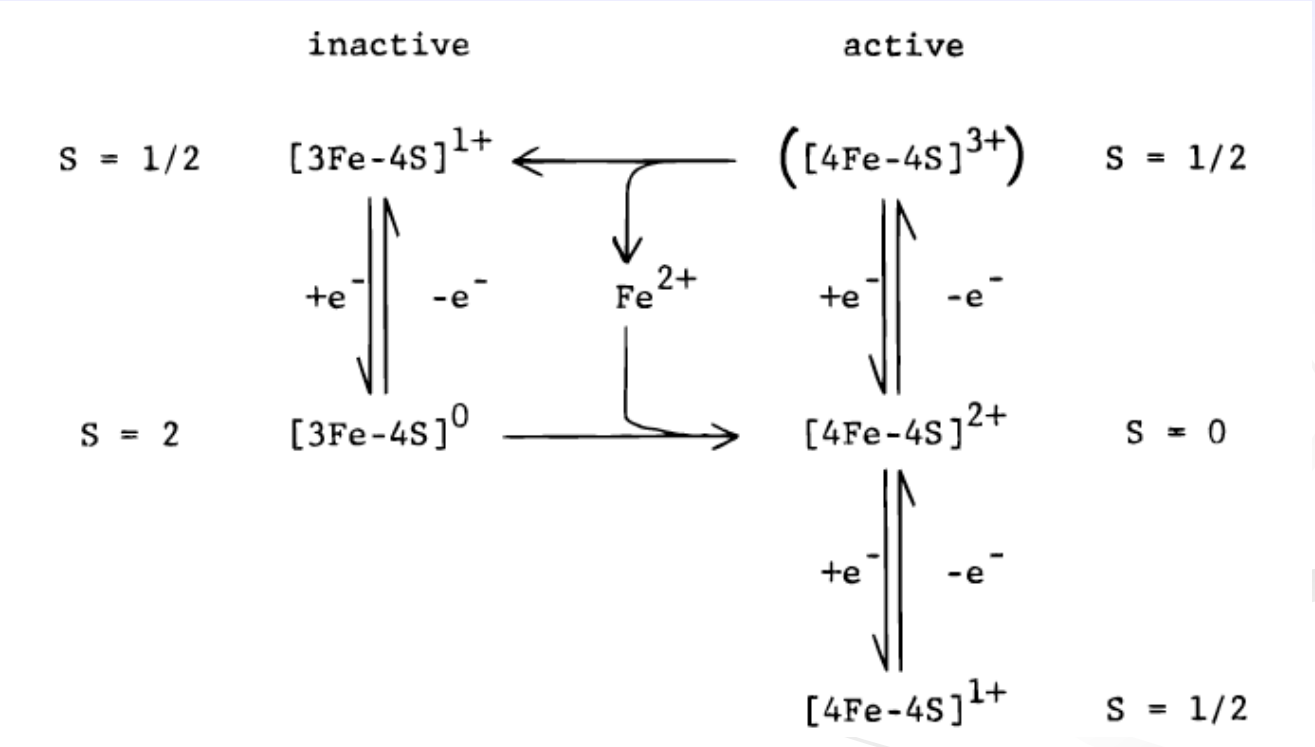
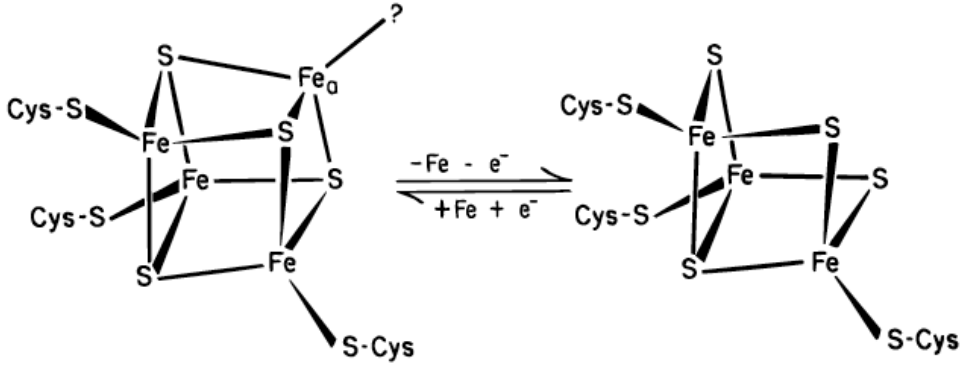


Figure 1. EPR spectra of c-aconitase (32 μM; spectrum A) and m-aconitase (25 μM; spectrum B) in 0.1 M Hepes buffer (pH 7.5). Conditions of spectroscopy: microwave power and frequency, 0.1 mW and 9.177 GHz; modulation amplitude and frequency, 0.5 mT and 100 kHz; time constant, 0.128 s; scanning time, 5 mT/min; temperature, 12 K. The marker is at $g = 2.018$. (Reprinted with permission from ref 25. Copyright 1992 National Academy of Sciences.)

Aconitase


inactive
active
S = 1/2
[3Fe-4S]1+
([4Fe-4S]3+)
S = 1/2
+e-
-e-
Fe2+
+e-
-e-
S = 2
[3Fe-4S]0
[4Fe-4S]2+
S = 0
+e-
-e-
[4Fe-4S]1+
S = 1/2


Cys-S
Cys-S
S
Fe
Fe
Fe
S
S
S
S
Fe
?
S-Cys
-Fe - e-
+Fe + e-
Cys-S
Cys-S
S
Fe
Fe
Fe
S
S
S
S
S-Cys

Substrate binds the Fe-S cluster

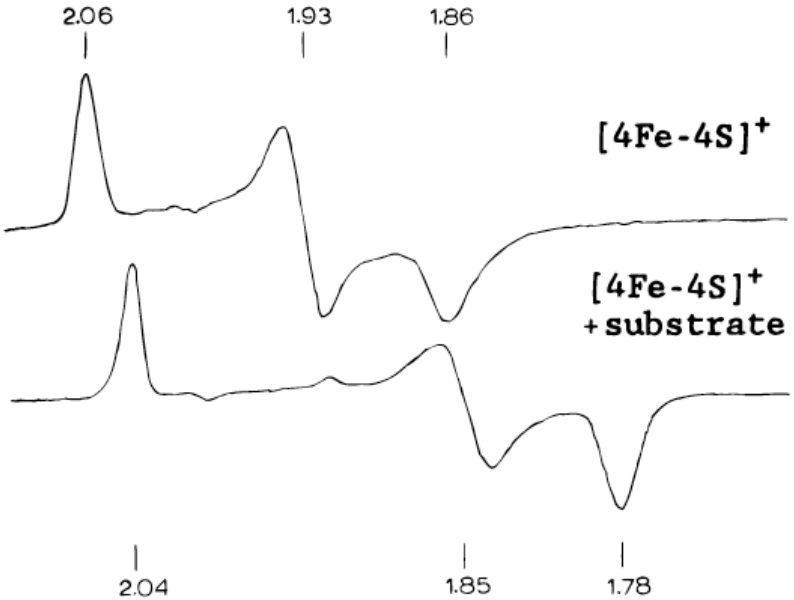
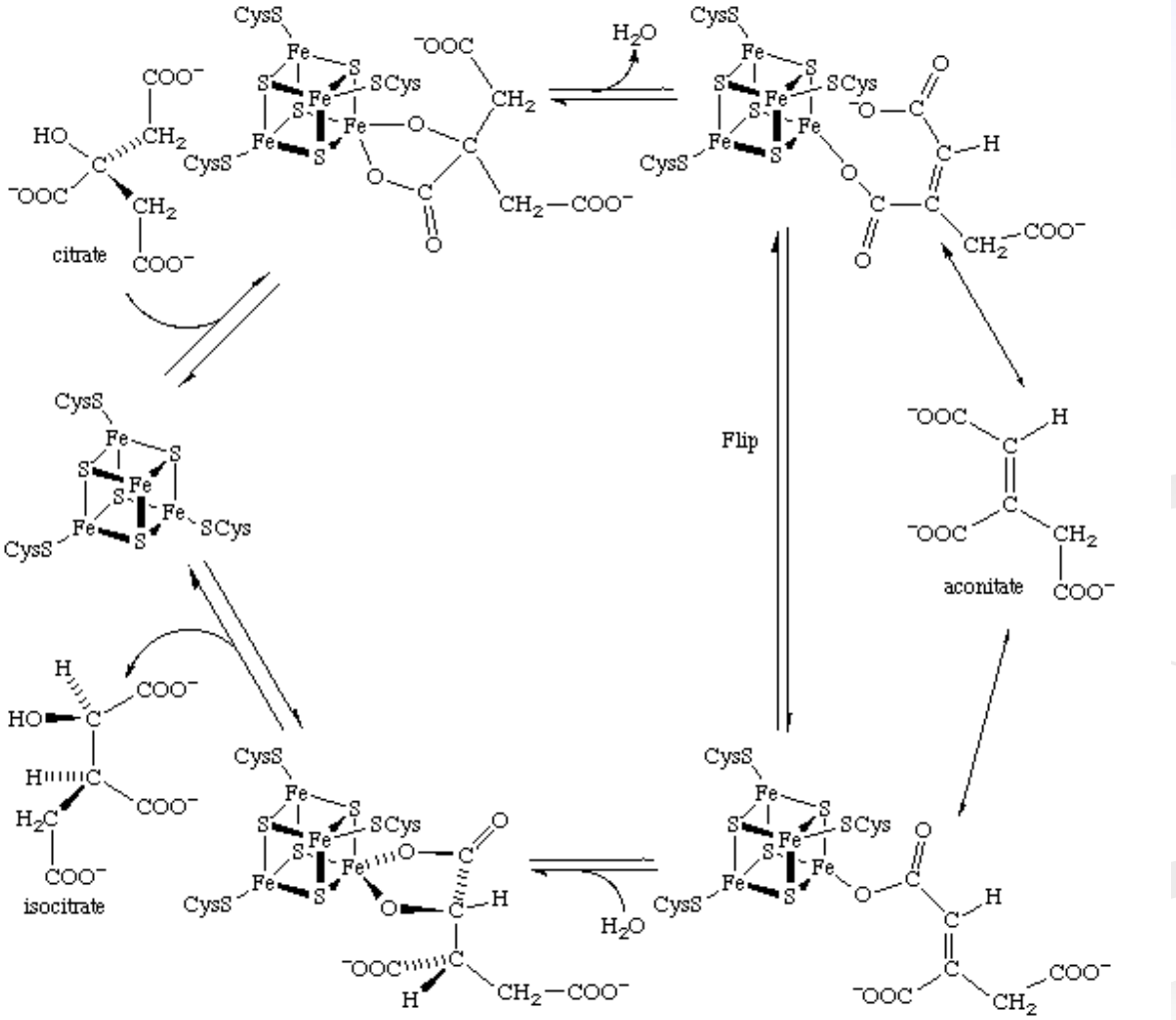
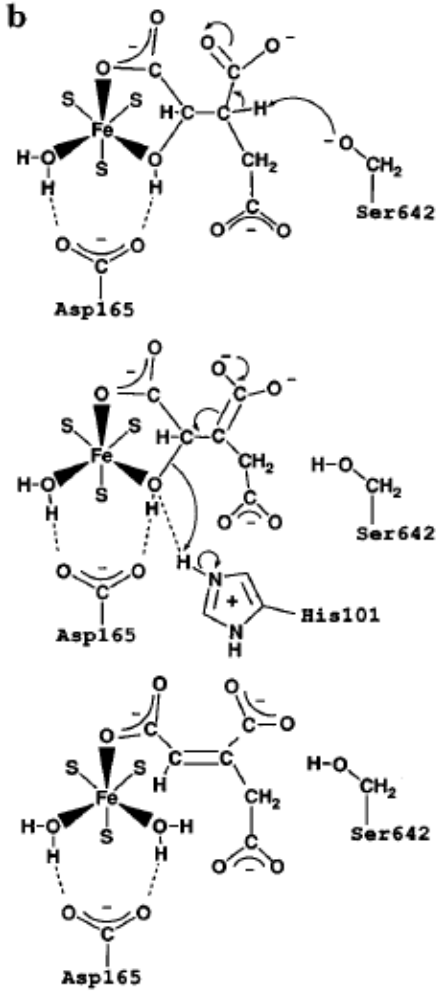


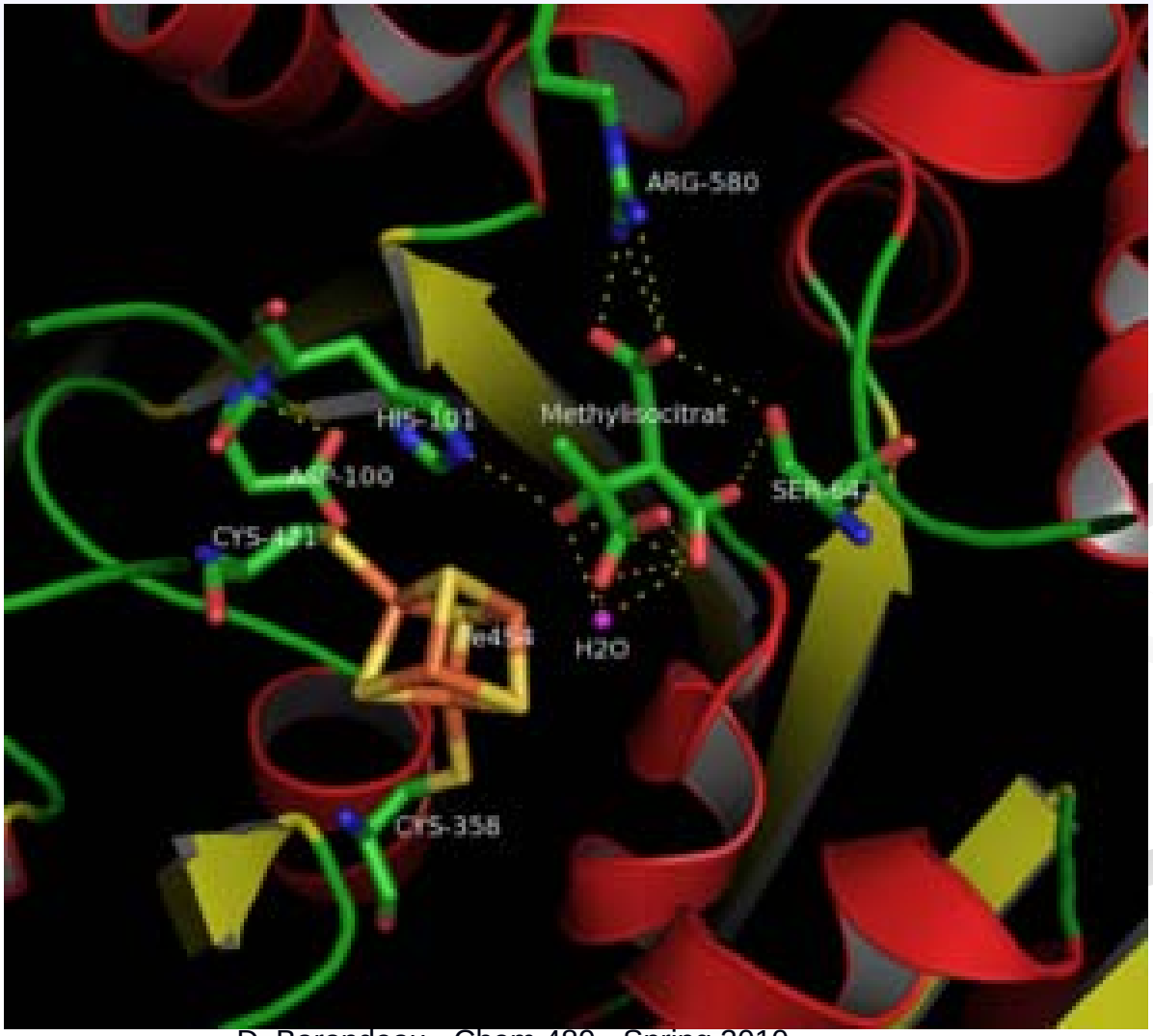
Figure 9. EPR spectra of the $[4Fe\text{-}4S]^+$ cluster of aconitase in the absence (top) and the presence (bottom) of substrate. Prominent features of the spectra are marked on a *g* value scale. Prior to freezing, the samples were photoreduced anaerobically in the presence of deazaflavin and oxalate. The spectra were recorded at a frequency of 9.24 GHz, 1 mW power, and 13 K. (Reprinted with permission from ref 5. Copyright 1989 Fed. Eur. Biochem. Soc.)

Mechanism


citrate
Flip
aconitate
isocitrate
H_2O
CysS
SCys

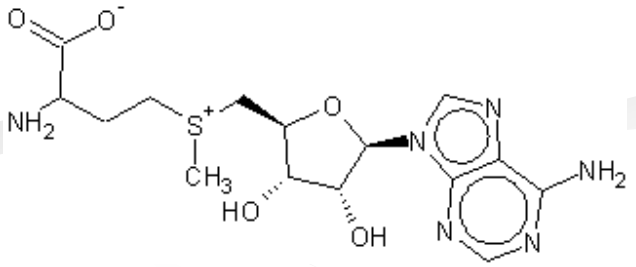

b
Asp165
Ser642
His101

Aconitase (1B0J)



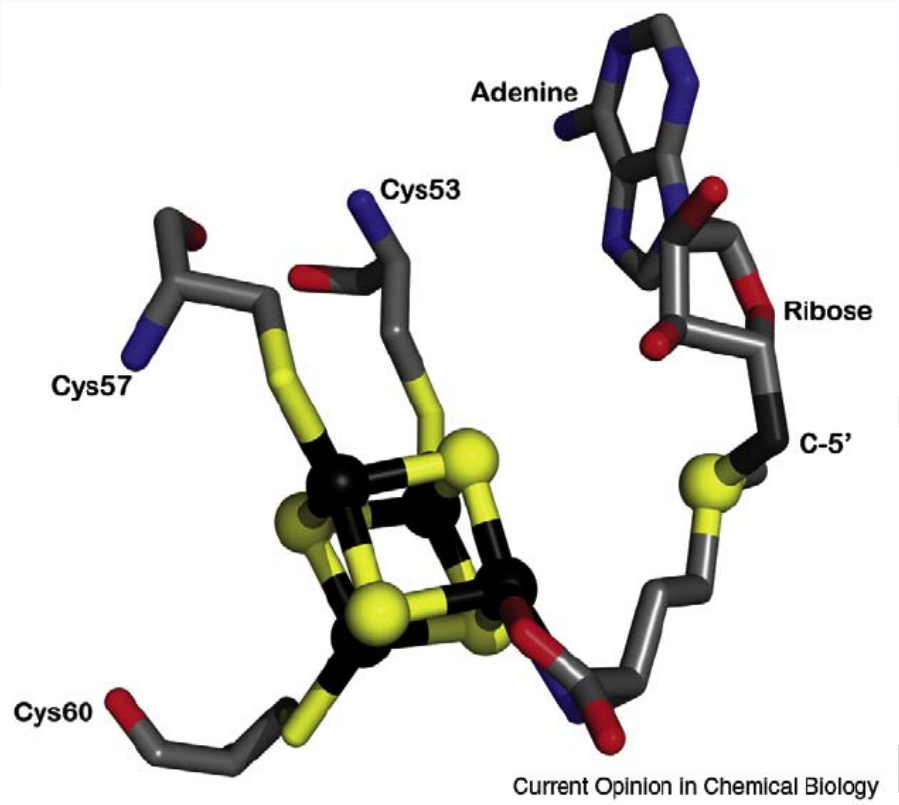
Radical SAM enzymes

- Discovered during early research on lysine metabolism
- Lysine 2,3-aminomutase (LAM)
  - Air sensitive
  - Purified and assayed under blanket of argon at laboratory bench
  - Activity depends on addition of dithionite ($S_2O_4^{2-}$) and s-adenosylmethionine (SAM)
  - SAM functioned as coenzyme and not a substrate (like in methyltransferases)
  - Reaction was typical of B_{12}-dependent rearrangements
  - Functional group undergoes a 1,2 migration concomitant with 1, 2 migration of a hydrogen atom
  - Enzyme did not require coenzyme B_{12}

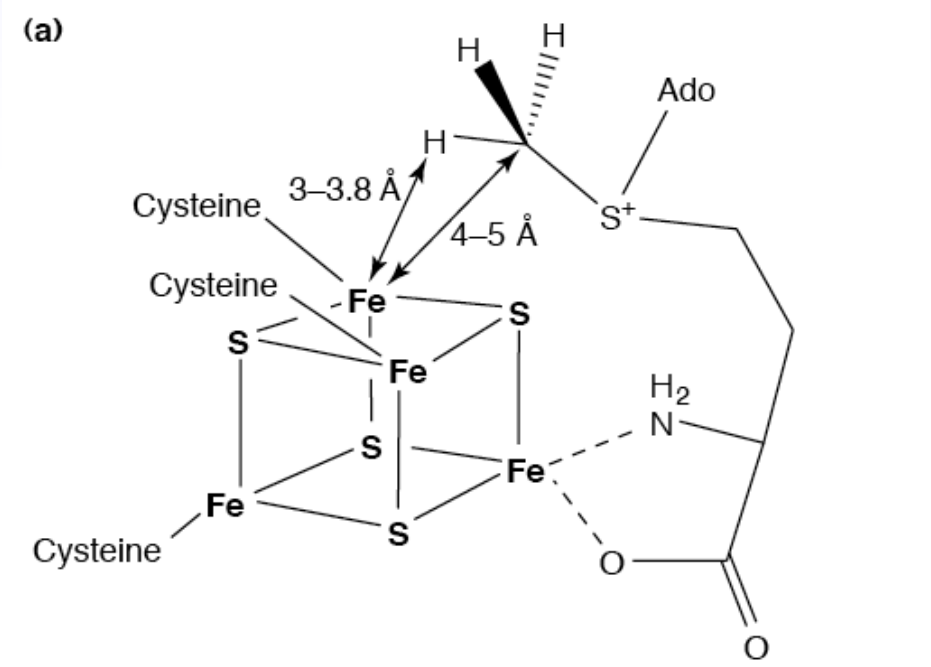


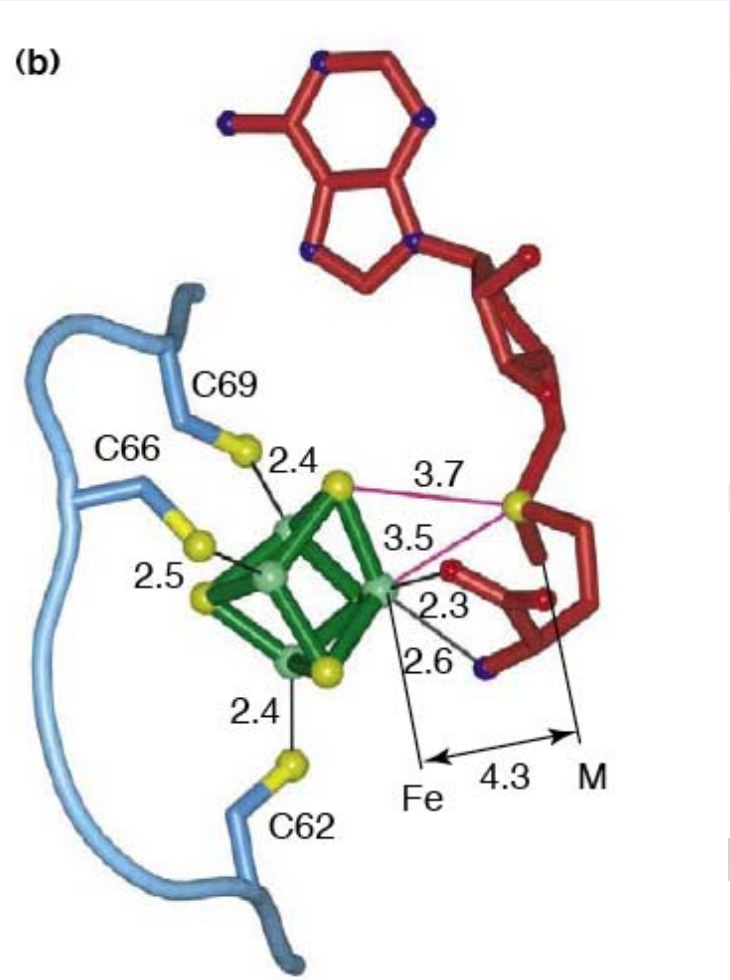
Radical SAM

- Activity enhanced by iron
- Enzyme prepared in anaerobic chamber was 10X more active and contained a visible absorbance band typical of Fe-S clusters
- Addition of acid released hydrogen sulfide
- EPR revealed $[Fe_4S_4]^{1+}$ cluster
- CxxxCxxC motif
- One iron atom is coordinated to SAM by the backbone carboxylate and amino groups



Fe_4S_4-SAM moiety

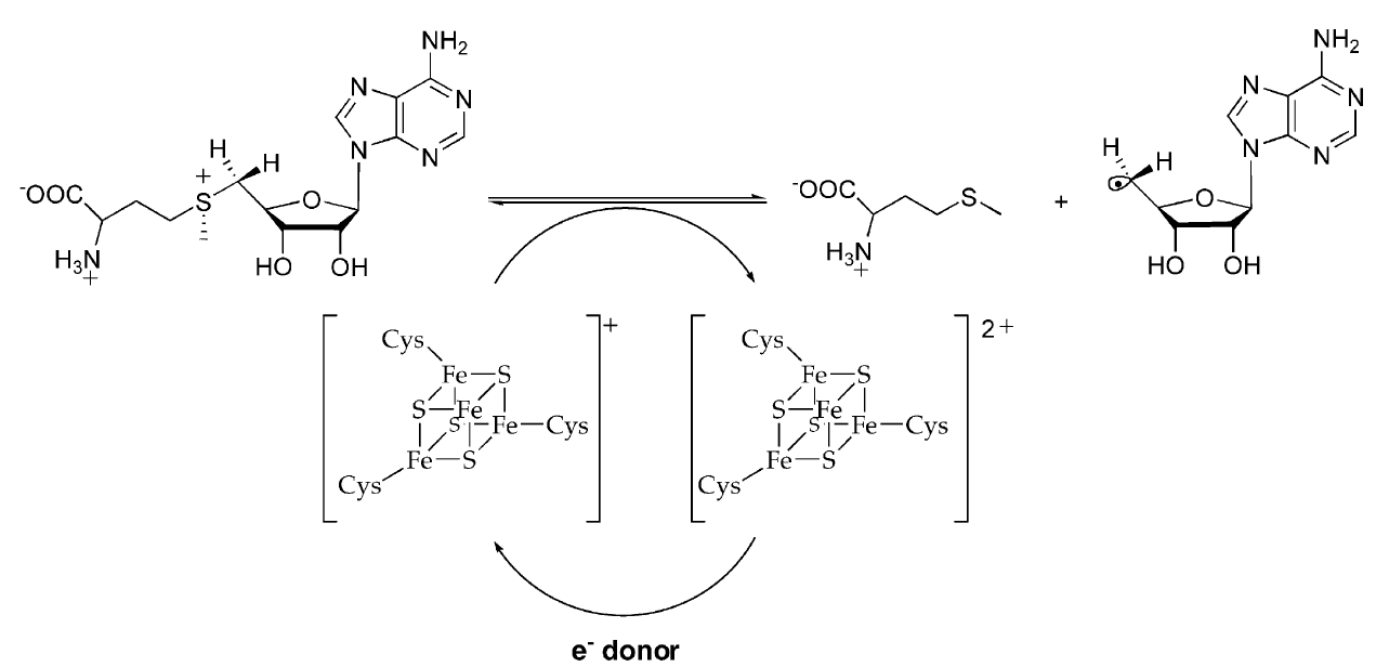




Radical SAM

- Suggested that SAM might be an evolutionary predecessor of adenosylcobalamin
- A predecessor could be expected to exist because the biosynthesis of adenosylcobalamin required thirty steps
  - Work from Ian Scott's lab at TAMU
- Postulated that SAM served as a source of 5'-deoxyadenosyl radical
- Discovered that 5'-deoxyadenosyl moiety in LAM mediates hydrogen atom transfer
- Baker and Frey suggest that SAM is "the poor man's vitamin B_{12}"

Reductive cleavage of SAM



- The homolytic bond dissociation energy (HBDE) for the S-5'-C bond of SAM is ~60 kcal/mol
  - Too elevated to generate the 5'dA• by simple homolysis
  - Addition of low potential electron from cluster induces scission
- In contrast, the Co-C HBDE for coenzyme B_{12} is 31 - 34 kcal/mol

Radical SAM family

- Lability of the cluster
  - Well established that $[Fe_4S_4]$ clusters with three cysteine ligands are less stable than those coordinated by 4 cysteines
  - Radical-SAM $[Fe_4S_4]$ clusters are highly oxygen sensitive
  - Oxidative degradation to $[Fe_3S_4]$ or even $[Fe_2S_2]$ clusters at same site
  - In available structures the $[Fe_4S_4]$ cluster is located near surface
  - Experiments must be conducted under strict anaerobic conditions ($O_2 < 2$ ppm)

TABLE 1 Radical SAM enzymes and associated biological functions

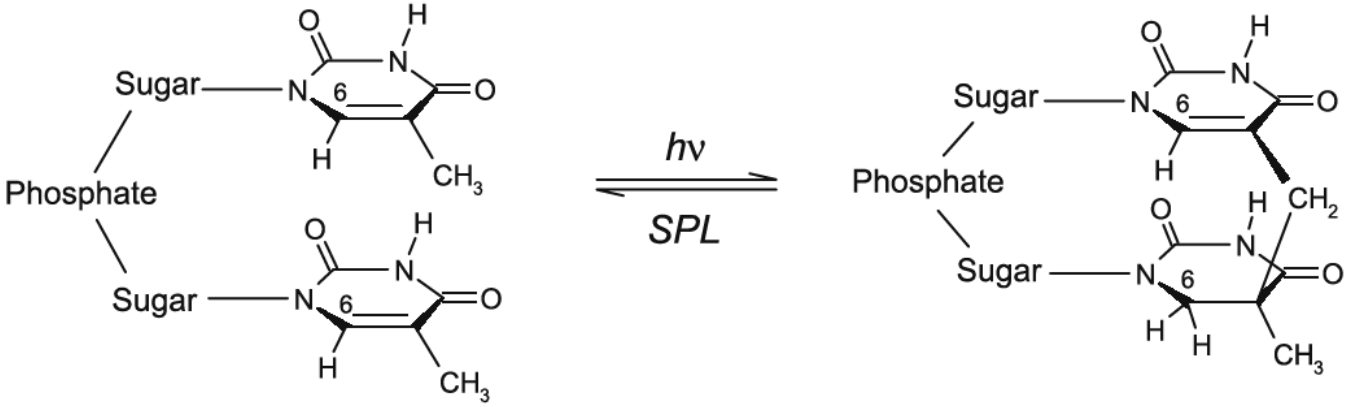
Protein	Function	Reference
LAM	Lysine 2,3aminomutase	Ruzicka *et al.*, 2000
BlsG	Arginine 2,3-aminomutase	Cone *et al.*, 2003
Eam	Glutamate 2,3-aminomutase	Ruzicka & Frey, 2007
SplB	Spore photoproduct lyase	Rebeil *et al.*, 1998
DesII	Desosamine biosynthesis	Trefzer *et al.*,1999
Littorine mutase	Alkaloid biosynthesis	Ollagnier *et al.*, 1998
PFL activase	Glycyl radicalization	Wong *et al.*, 1993
ARR activase	Glycyl radicalization	Eliasson *et al.*, 1990
BssD	Glycyl radicalization	Heider *et al.*, 1999
Glycerol dehydratase activase	Glycyl radicalization	O'Brien *et al.*, 2004
Hydroxyphenylacetate decarboxylase activase	Glycyl radicalization	Yu *et al.*, 2004
BioB	Biotin synthase	Duin *et al.*, 1997
LipA	Lipoyl synthase	Reed & Cronan, 1993
BchE	Bacteriochlorophyll biosynthesis	Suzuki *et al.*, 1997
HemN	Coproporphyrinogen III oxidase	Akhtar, 1994
MoaA	Molybdopterin biosynthesis	Rieder *et al.*, 1998
MiaB	Methylthiolation of tRNA	Esberg *et al.*, 1999
TYW1	Wybusine biosynthesis in $tRNA^{Phe}$	Nona *et al..*, 2006
ThiH	Biogenesis of thiazole in thiamine	Begley *et al.*, 1999
PqqE	Pyrroloquinoline quinone biosynthesis	Goodwin & Anthony, 1998
NifB	Nitrogenase FeMoCo maturation	Allen *et al.*, 1995
AtsB	Formylglycine formation in sulfatases	Fang *et al.*, 2004
ExsD	Succinoglycan production	Becker *et al.*, 1995
SpcY	Spectinomycin biosynthesis	Lyutzkanova *et al.*, 1997
AlbA	Subtilosin biosynthesis	Zhang *et al.*, 1999
SanA	Nicomycin biosynthesis	Möhrle *et al.*, 1995
BcpD	Bialaphos biosynthesis	Thompson & Seto, 1995
MitD/MmcD	Mitomycin C biosynthesis	Mao *et al.*, 1999
OxsB	Oxetanocin biosynthesis	Morita *et al.*, 1999
Fms7	Fortimicin biosynthesis	Kuzuyama *et al.*, 1995
Fom3	Fosfomycin biosynthesis	Kuzuyama *et al.*, 1992
CloN6	Clorobicin biosynthesis	Westrich *et al..*, 2003
Nclk-binding	Cdk5 activator binding	Ching *et al.*, 2000
Best5	Interferon inducible protein	Grewal *et al.*, 200
HydE/HydG	Cofactor maturation/[FeFe] hydrogenase	Posewitz *et al.*, 2004
AviX12	Epimerization in Avilamycin A	Boll *et al.*, 2006
ORF2	Cofactor maturation/amine dehydrogenase	Ono *et al.*, 2006
Elp3	Elongator complex function	Paraskevopoulou *et al.*, 2006
CofG/CofH	Coenzyme F(420) biosynthesis	Graham *et al.*, 2003
PcfB	Maturation of propionicin F	Brede *et al.*, 2004

Radical SAM enzymes

- 3 subclasses
- Class I enzymes
  - analogous to those that use coenzyme B_{12}
  - Cleave SAM reversible
  - At end of cycle SAM is regenerated
- Class II enzymes
  - Cleavage of SAM is irreversible
  - 5'-dA• radical abstracts a hydrogen atom from a glycyl residue, creating a glycyl radical
  - Glycyl radical is stable in the absence of O_2 and is itself regenerated after each turnover
- Class III enzymes
  - SAM is cleaved irreversible for each hydrogen atom abstracted (cosubstrate)

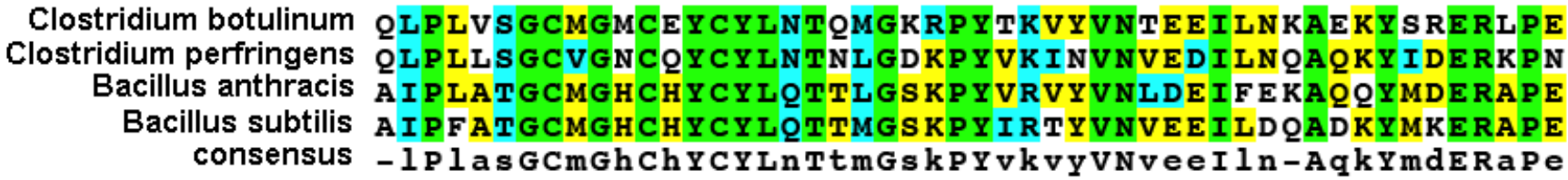
Spore photoproduct lyase

- When nutrients are not abundant some bacteria can go into a metabolically dormant spore state
  - Spores are highly dehydrated
  - Resistant to biocidal agents such as heat, UV radiation, and toxic chemicals
  - Bacteria were successfully cultured from spores isolated from 250 million year old salt crystals
  - *Bacillus subtilis* spores have survived aboard spacecraft when exposed to high vacuum, UV-radiation and cosmic rays of space
  - In spore state these bacteria form a specific type of UV-induced DNA damage called spore photoproduct



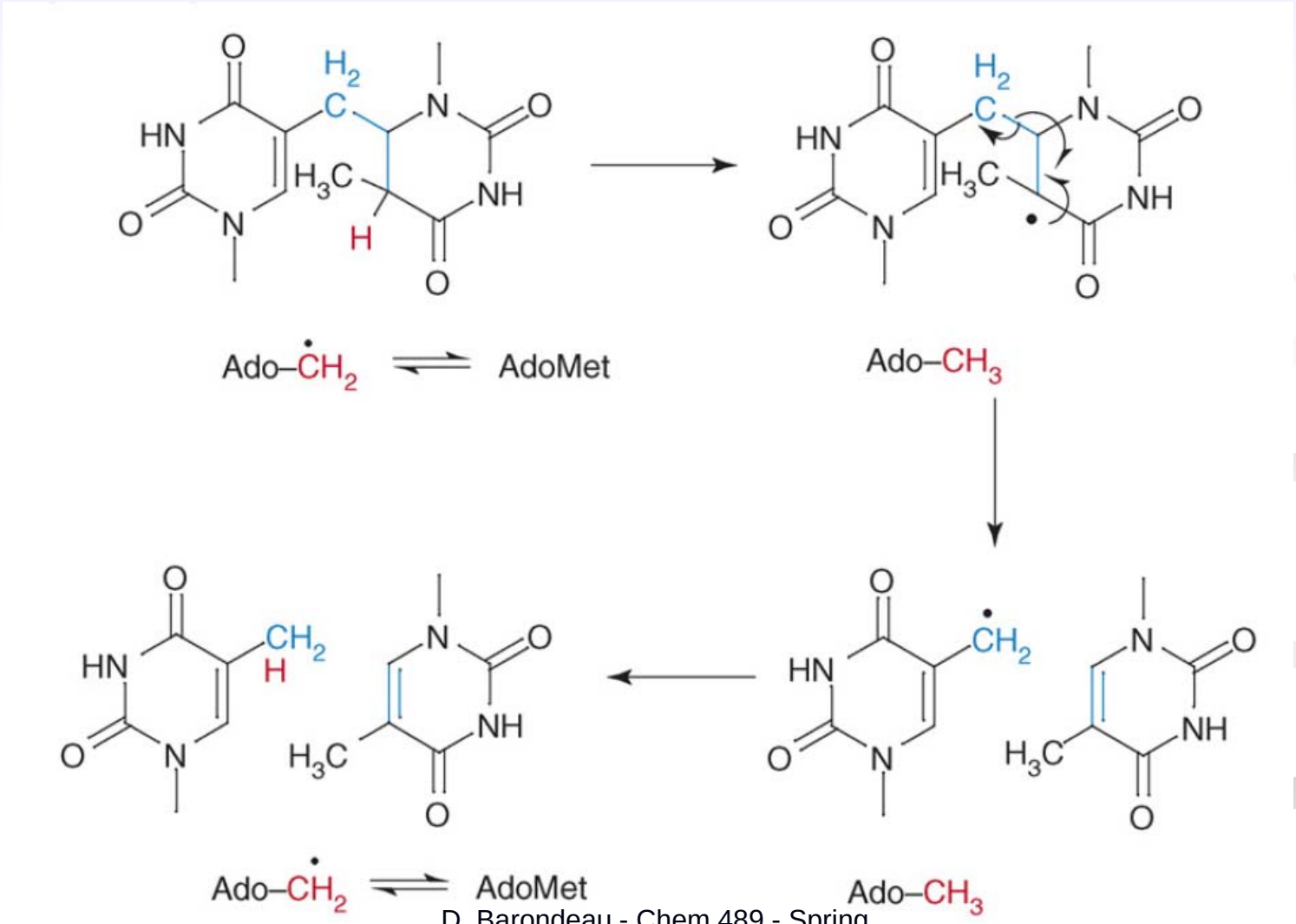
Spore photoproduct lyase

- Spore photoproduct lyase is a DNA repair enzyme found in sporulating bacteria
- Enzyme specifically repairs SP DNA lesions
- SP lyase is packaged in the dormant spore and repairs DNA early in germination
  - Independent of *de novo* protein synthesis
- Deletion of the SP lyase gene results in spores that are significantly more susceptible to UV-induced DNA damage and cell death
- Radical SAM enzyme (CXXXCXXC)



- Has $[Fe_4S_4]^{2+/1+}$ cluster in which one iron is bound to S-adenosylmethionine (SAM)

Spore photoproduct lyase

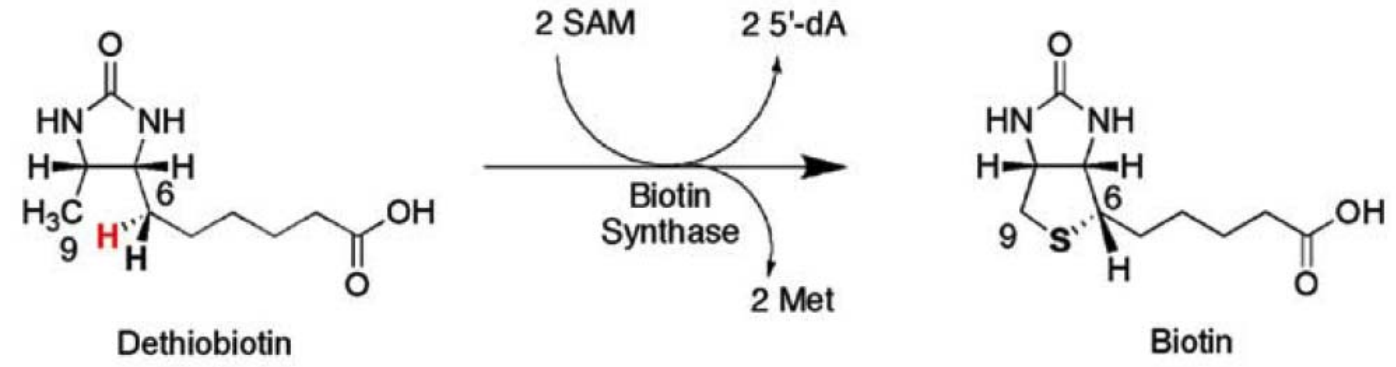


Class III Radical SAM enzymes

- Once defined as those that catalyze the insertion of sulfur atoms into unactivated C-H bonds
- Now apparent that there is far greater diversity of reactions
- Sulfur insertion, decarboxylation, methylthiolation, dehydrogenase, C-C bond formation and various complex transformations involved in the biosynthesis of cofactors and other natural products
- Best studies is probably biotin synthase

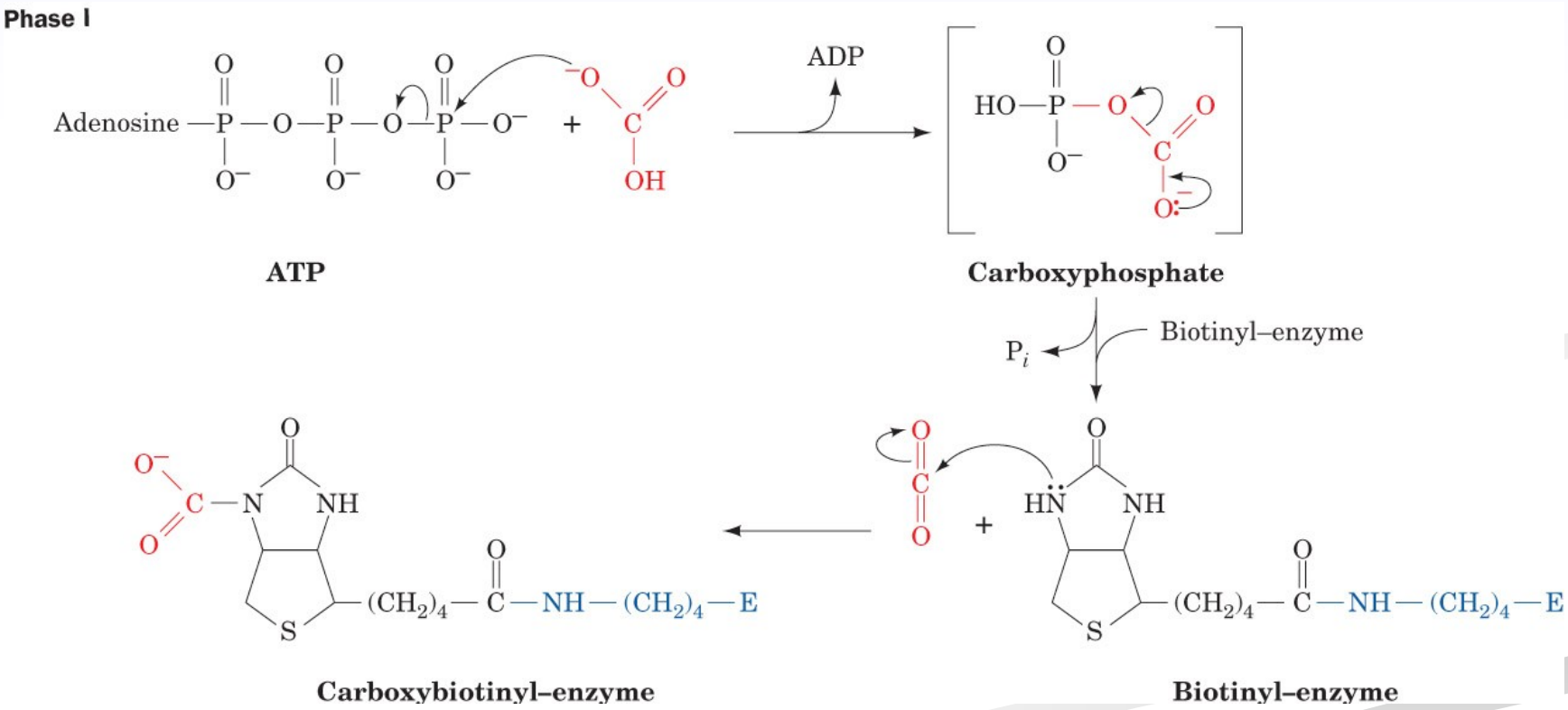
Biotin synthase (BS)

- Converts dethiobiotin (DTB) into biotin through formation of two C-S bonds on 2 non-activated carbons



- Requires SAM, but SAM is not the source of the sulfur
- Most now agree that BS contains 2 clusters a [Fe_4S_4] and [Fe_2S_2]

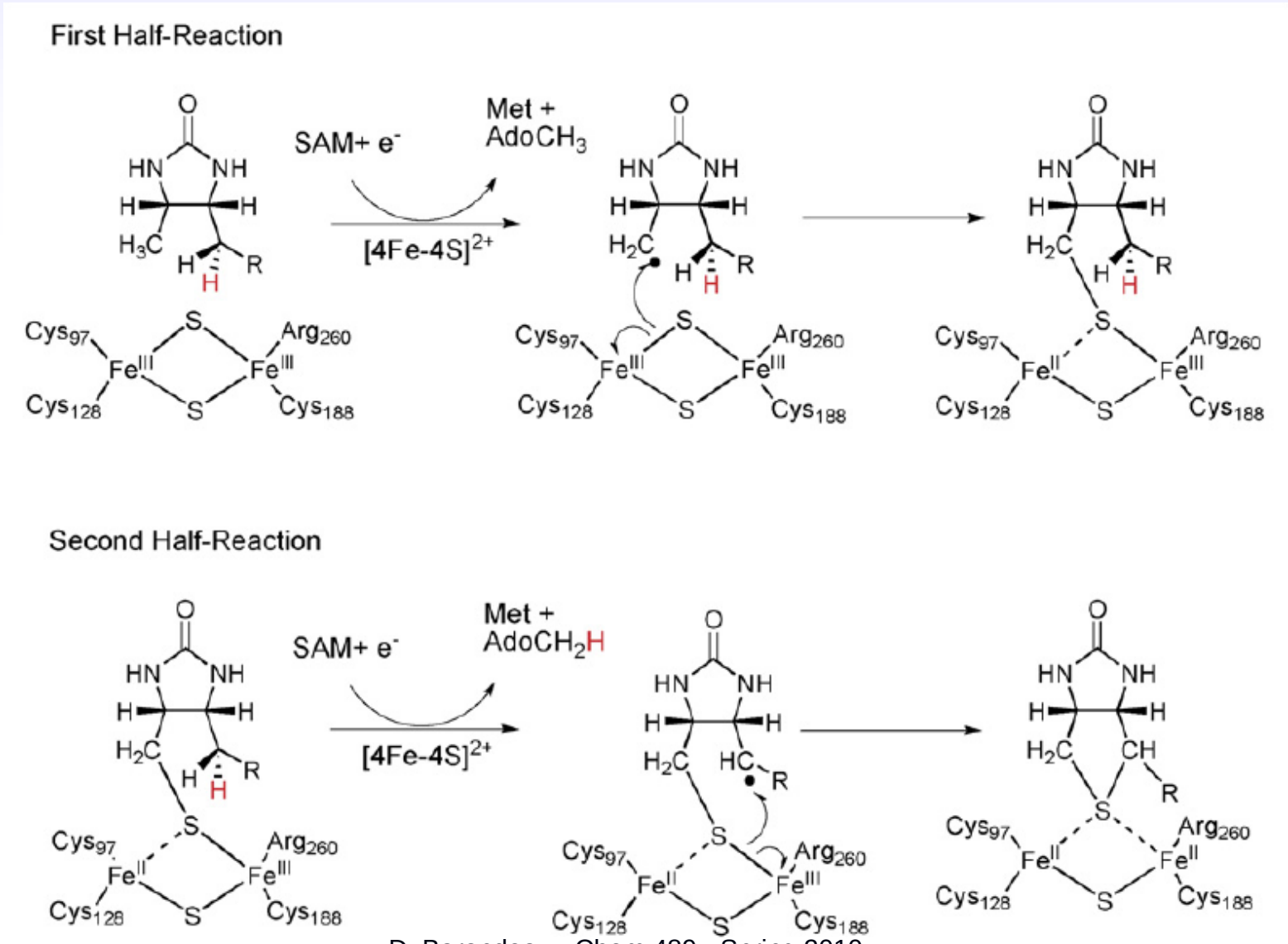
Function of biotin - CO_2 carrier



Biotin Synthase

- Biotin synthase is non-catalytic *in vitro* with less than 1 mole of biotin produced per mole of enzyme
  - No kinetic studies possible
- Biotin synthase itself is the sulfur source
- 2 hypotheses
  - Sulfur could come from [FeS] center
  - Sulfur could come from persulfide of unknown origin
- Reconstitution of $[Fe_2S_2]^{2+}$ using $^{34}S^{2-}$ or $^{35}S^{2-}$ gave protein with labeled cluster that produced labeled biotin in vitro
- During formation of biotin, the amount of $[Fe_2S_2]^{2+}$ decreases, whereas $[Fe_4S_4]^{2+}$ does not
- How the sulfur is transferred is not known (could even form a transient persulfide species)
- In yeast, members of iron-sulfur cluster assembly (Isa1 and Isa2) are required for the activity of biotin synthesis
  - Isa1 and Isa2 suggested to function in regeneration of $[Fe_2S_2]$ cluster

Biotin Synthase mechanism



Biotin synthase structure