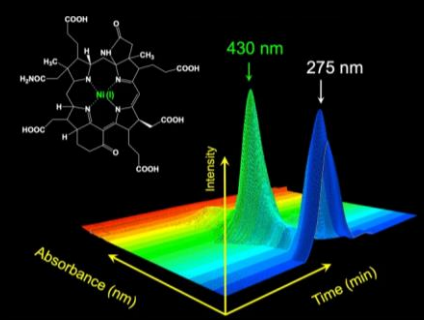
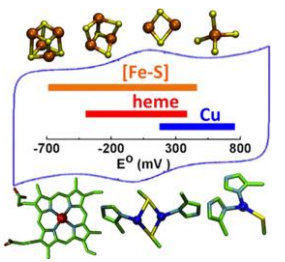


Workshop on Biomolecular Spectroscopy

EC Duin
2022



Part A

Metallocofactors and Metalloproteins

Content

Introduction

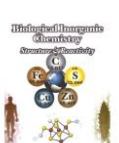
One-third of all known proteins require metal ions as cofactors for biological function.

Holm, R.H., Kennepohl, P., Solomon, E.I.
(1996) Chem. Rev. 96, 2239-2314.

Field: ***Bioinorganic Chemistry***

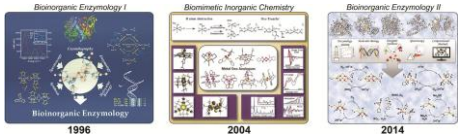
Journal: Journal of Biological Inorganic Chemistry

Text Book: Biological Inorganic Chemistry (Bertini, Gray, Stiefel, Valentine)

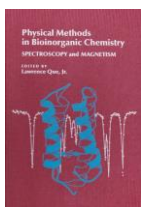


Resources

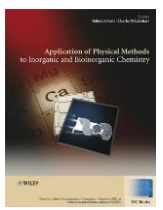
- Chemical reviews:
  - 1996, Vol 96, issue 7
  - 2004, Vol 104, issue 2
  - 2014, Vol 114, issue 7



- Methods in Enzymology



- Advances in Inorganic Chemistry



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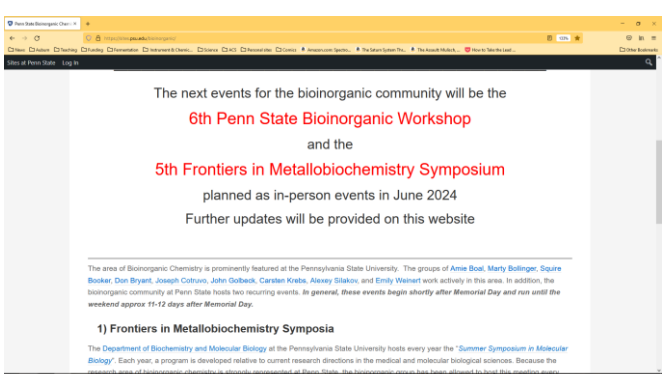
Symposia



The next events for the bioinorganic community will be the

6th Penn State Bioinorganic Workshop

and the

5th Frontiers in Metallobiochemistry Symposium

planned as in-person events in June 2024

Further updates will be provided on this website

The area of Bioinorganic Chemistry is prominently featured at the Pennsylvania State University. The groups of Amie Boal, Marty Bollinger, Squire Booker, Don Bryant, Joseph Cotruvo, John Golbeck, Carsten Krebs, Alexey Silakov, and Emily Weinert work actively in this area. In addition, the bioinorganic community at Penn State hosts two recurring events. ***In general, these events begin shortly after Memorial Day and run until the weekend approx 11-12 days after Memorial Day.***

1) Frontiers in Metallobiochemistry Symposia

The Department of Biochemistry and Molecular Biology at the Pennsylvania State University hosts every year the "*Summer Symposium in Molecular Biology*". Each year, a program is developed relative to current research directions in the medical and molecular biological sciences. Because the

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Function of Metalloproteins/enzymes

- ***electron transfer*** – uptake, release, and storage of electrons;
- ***catalytic*** – substrate binding, activation, and turnover;
- ***sensing*** – ligand binding and regulation;
- ***structural*** – configuration of protein tertiary and/or quaternary structure;
- ***storage*** – uptake, binding, and release of metals in soluble form;
- ***dioxygen binding*** – metal-O_2 coordination and release;

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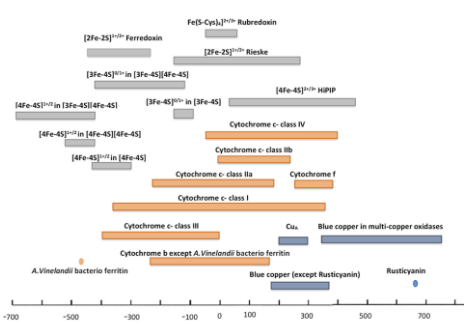
Function in Electron Transport



Liu et al (2014) Chem. Rev., 114, 4366−4469

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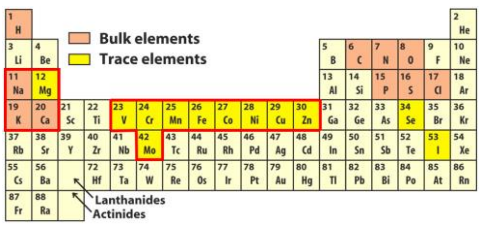
Metal Ions in Proteins

- Bulk elements: ***Na***, ***K*** and ***Ca*** ions.
- Trace elements: ***Mg***, ***V***, ***Cr***, ***Mn***, ***Fe***, ***Co***, ***Ni***, ***Cu***, ***Zn***, and ***Mo*** ions.
- Due to toxicity, the concentration of free metals in the cytosol itself is very low. This is mainly due for the transition metals, not for Na^+, K^+, and Ca^{2+}.



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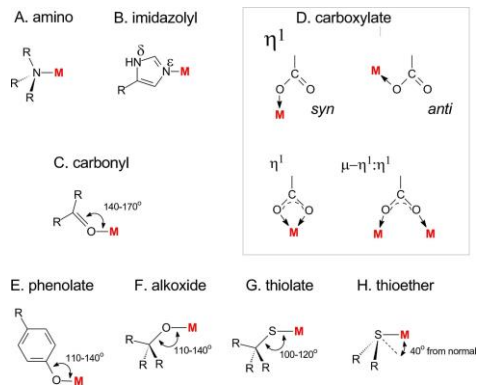
Coordination



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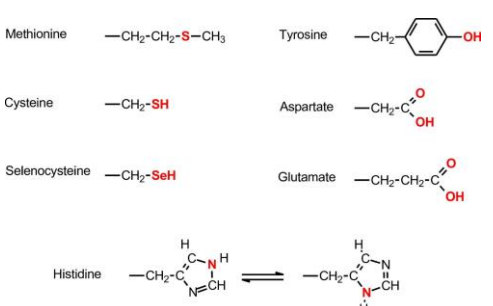
Amino Acids Involved in Coordination



Other groups: carbonyl, carboxylate and amino groups in backbone

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Mechanisms of Enzyme Action

Enzymes use two (or three) main ways to catalyze reactions:

1) ***Transition-state stabilization through binding energy or through an entatic state of the enzyme***

2) ***Rearrangements of covalent bonds during an enzyme-catalyzed reaction***

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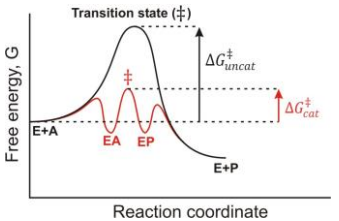
Transition-State stabilization

- What really sets enzymes apart from most other catalysts is the formation of a specific ***enzyme-substrate complex***.
- *However, in order to catalyze reactions, an enzyme must be complementary to the* ***reaction transition state (‡)****.*



- Some weak interactions are formed in the EA complex, but the full complement of such interactions between substrate and enzyme is formed only when the substrate reaches the transition state.
- This is also the basis for the ***reaction specificity***, the ability to discriminate between a substrate and a competing molecule.

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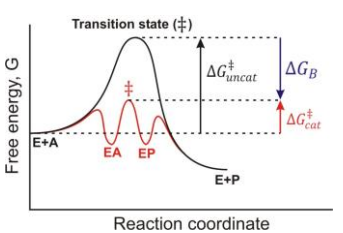
Transition-State stabilization

Binding energy, ΔG_B

- Much of the energy required to lower activation energies is derived from weak, noncovalent interactions between the transition state and enzyme: hydrogen bonds and hydrophobic and ionic interactions.
- Formation of each weak interaction in the EA complex is accompanied by release of a small amount of free energy that provides a degree of stability to the interaction: ***binding energy.***



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Metal Ion Catalysis

- Can act as electrophilic catalysts stabilizing the increased electron density or negative charge that can develop during reactions
- Provides a powerful nucleophile at neutral pH. Coordination to a metal ion can increase the acidity of a nucleophile with an ionizable proton:

$$M^{2+} + NucH \rightleftharpoons M^{2+}(NucH) \rightleftharpoons M^{2+}(Nuc^{-}) + H^{+}$$

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Metal Ion Catalysis

Properties of the metal–ion sites in proteins involved in Electron Transfer:

metal oxidation state	bond stability	typical number and type of side chain ligands	typical coordination geometry
Zn^{2+}	high	3: His, Cys, (Glu)	severely distorted tetrahedron
Cu^{1+}	high	3,4: His, Cys, Met	severely distorted tetrahedron
Cu^{2+}	high	3,4: His, (Cys)	distorted square planar arrangement
Fe^{2+}, Ni^{2+}, Co^{2+}, Mg^{2+}	low	4-6: His, Glu, Asp	distorted octahedron
Fe^{3+}	high	4-6: Glu, Asp, Tyr, Cys	distorted octahedron

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Metal Ion Catalysis

- The metal centers are often ***coordinatively unsaturated***; one residue is missing for forming a regular coordination number of 4 (tetrahedron, square) or 6 (octahedron).
- For catalytic activity the open site is essential for coordination of the substrate. In the 'resting state' this site might be occupied by an easily removable ligand like H_2O.
- ***Deviation of the coordination geometry from the ideal symmetries***.
- A certain degree can be expected due to the presence of different amino acid ligands and the generally unsymmetrical environment provided by the protein.
- Pronounced distortion, however, has been proposed to be important for catalysis

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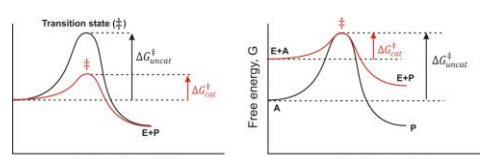
Metal Ion Catalysis: Entatic State



- The active center of the enzyme already largely features the (complementary) geometry necessary to reach the critical high-energy transition state of the substrate.
- In the ***'entatic'*** (strained) state of the enzyme, much of the energy needed to reach that transition state is already stored and distributed over many chemical bonds.

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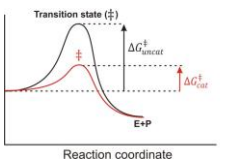
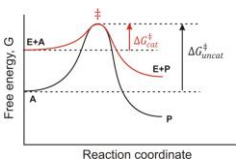
Metal Ion Catalysis: Entatic State





- Small remaining geometrical changes between the initial and transition state of the enzyme/substrate complex then result in only a small activation energy.
- For this reason, the active state of a metalloenzyme should not contain a regular (=low-energy, relaxed) coordination environment of the metal that is involved in catalysis; on the contrary, the main goal should be a destabilization of the initial state.

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Proteins Involved in Electron Transfer

- Full coordination sphere
- Geometrical distortion represents a compromise between the prefered geometries for the oxidized and reduced metals center
- Example: Cu

Cu^{1+}	Cu^{2+}
d^{10}	d^{9}
tetrahedral or trigonal geometry	square planar or square pyramidal
soft sulfur ligands	nitrogen ligands

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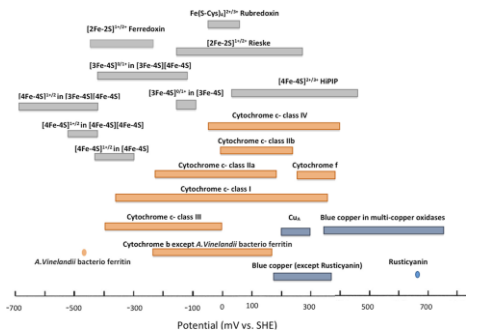
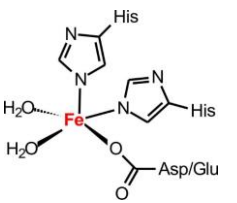
Examples

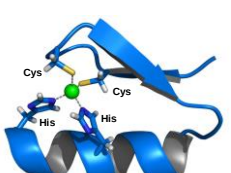


Non-heme Fe/2-His-1-Carboxylate Facial Triad Motif

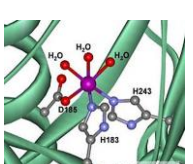
- Several proteins contain a versatile structural motif that consists of two His and one Asp/Glu residues, which occupy an octahedral face of the active site metal center.
- Mononuclear non-heme iron enzymes represent a large subset of metalloenzymes that exhibit this motif.



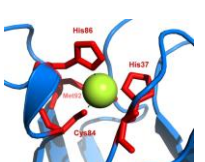
Mono Nuclear



Zinc finger motif found in several proteins

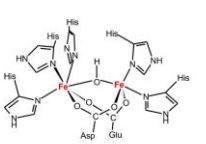


Fe in deacetoxycepalosporin C synthase



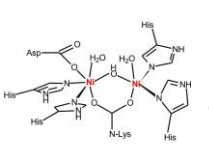
Cu in plastocyanin

Dinuclear



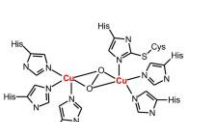
Dinuclear Fe site in hemerythrin

O_2 transportation in marine invertebrates



Dinuclear Ni site in urease

Converts urea into ammonia and bicarbonate



Dinuclear Cu site in Hemocyanin

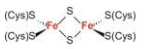
O_2 transportation in some crustaceans

NiFe site in hydrogenase urease

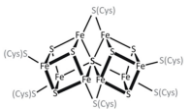
Converts H_2 into H^+

Iron-sulfur Clusters

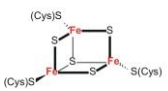
Non-heme Fe



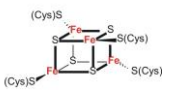
[2Fe-2S]



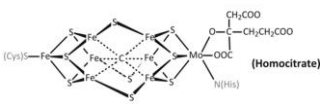
P-Cluster
[8Fe-7S]



[3Fe-4S]



[4Fe-4S]



FeMoco
[Mo-8Fe-9S-C-homocitrate]

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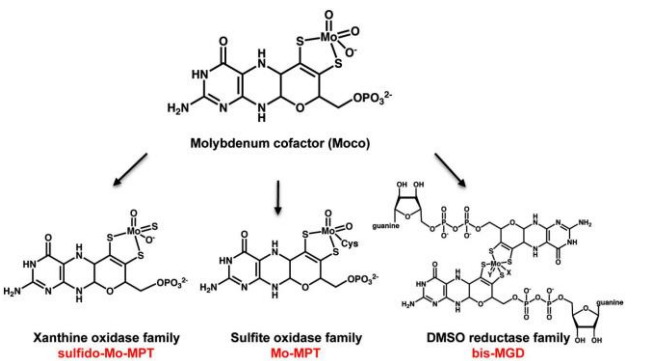
Pterins



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Weirdos

Clusters in CO Dehydrogenase

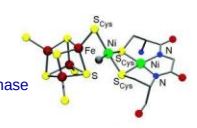


Figure 1. A-Cluster of CODH

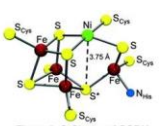
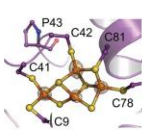
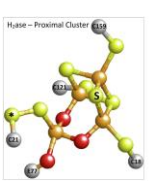


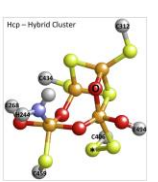
Figure 2. C-Cluster of CODH



Non-cuboidal [4Fe-4S] cluster
in heterodisulfide reductase



Proximal cluster
(to the NiFe site)
in some hydrogenases)



Hybrid cluster in hybrid
cluster protein

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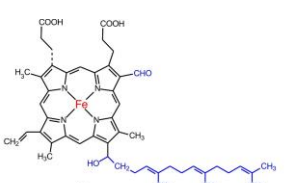
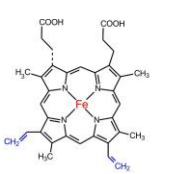
Tetrapyrroles: Hemes

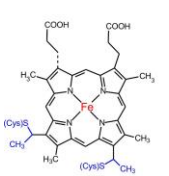
pyrrole



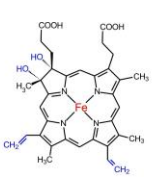
Heme *a*



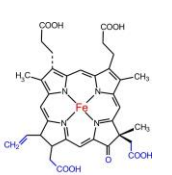
Heme *b*



Heme *c*



Heme *d*



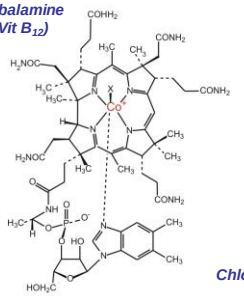
Heme d_1

28

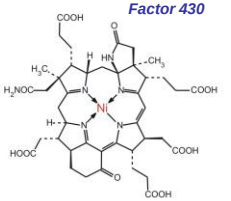
Tetrapyrroles

pyrrole

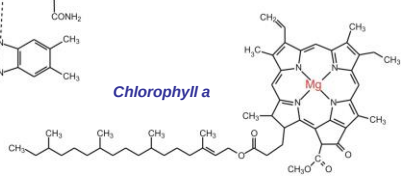
Cobalamine (Vit B_{12})



Factor 430



Chlorophyll a



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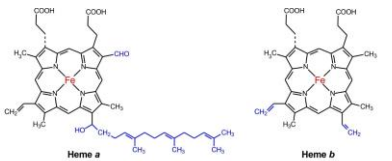
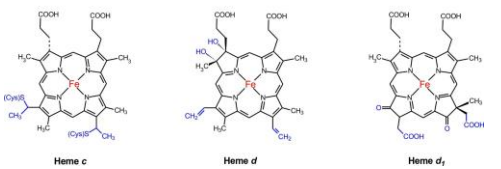
Cytochromes





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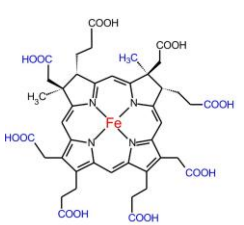
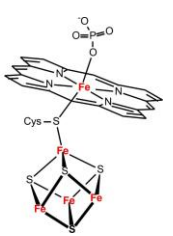
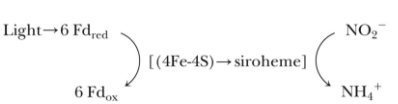
Siroheme





Found in nitrite reductase: Nitrite is reduced (with 6 electrons) to ammonium while bound to siroheme.



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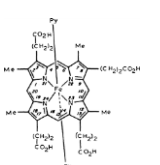
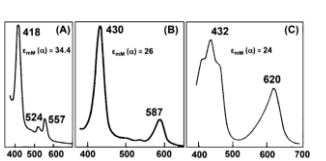
Classification

- ***Cytochromes*** are classified on the basis of the electronic absorption maxima of the ***heme macrocycle***, such as *a*, *b*, *c*, *d*, *f*, and *o* types of heme.
- More specifically, these letter names represent characteristic absorbance maxima in the UV−vis electronic absorption spectrum when the heme iron is coordinated with pyridine in its reduced (ferrous) state, designated as the "pyridine hemochrome" spectrum.
- (A) heme *b* or *c*, (B) heme *a*, (C) heme d_1





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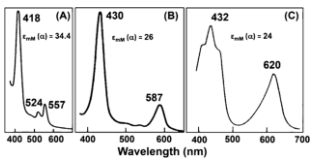
Classification



pyridine hemochromogen				
heme	position of α peak (nm)	ε_{mM} (at α peak)	α peak (nm) of reduced protein	example
protoheme IX (*b*)	557	34.4	557–563	cyt b_6f complex
heme *c*	550	29.1	549–561	cyt *c*
heme *a*	587	26	587–611	cyt aa_3 oxidase
heme *d*	613		630–635	cyt *bd* oxidase
heme d_1	620	24	625	cyt cd_1 nitrite reductase
heme *o*	553		560	cyt bo_3 oxidase

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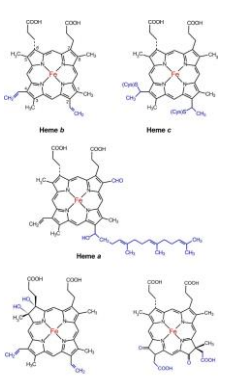
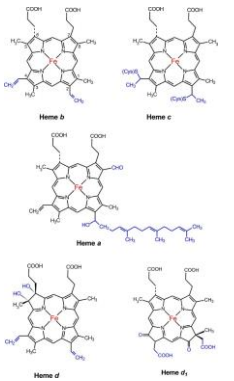
Types

- The *b*-type cytochromes have four methyl substitutions at positions 1, 3, 5, and 8, two vinyl groups in positions 2 and 4, and two propionate groups at positions 6 and 7, resulting in a 22-π-electron porphyrin.
- Hemes *a* and *c* are biosynthesized as derivatives of heme *b*.

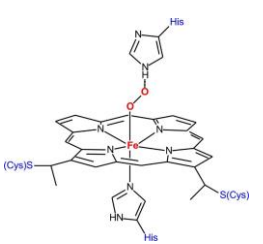


Types

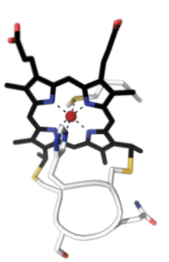
- Covalent cross-linking of the vinyl groups at β-pyrrole positions 2 and 4 of heme *b* with Cys residues from the protein yields heme *c*, where the vinyl groups of heme *b* are replaced by thioether bonds.
- The covalent cross-linking of the two Cys residues from the protein to the porphyrin ring occurs at the highly conserved -Cys-x-x-Cys-His- sequences (x=any amino acid).
- This cross-linking covalently attaches heme *c* to the protein. The histidine residue in the conserved sequence serves as an axial ligand to the heme iron.



Consensus Sequences for Hemes



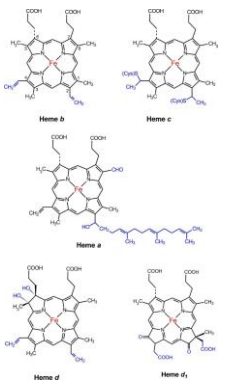
- -**C**-xx-**C**-**H**-



Cytochrome c family

Types

- In heme *a*, the vinyl group at position 2 of the porphyrin ring of heme *b* is replaced by a hydroxyethylfarnesyl side chain while the methyl group at position 8 is oxidized to a formyl group
- These substituents make heme *a* more hydrophobic as well as more electron withdrawing than heme *b* due to the presence of farnesyl and formyl groups, respectively.
- Heme *o* differs from heme *a* by having a methyl group at ring position 8 instead of the formyl group.



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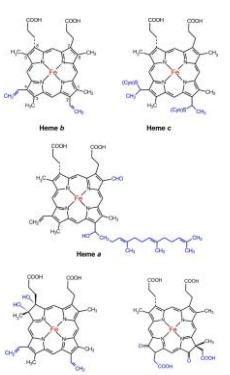
Types

- In heme *d*, two cis-hydroxyl groups are inserted at positions 5 and 6 on the β-pyrrole, which renders heme *d* as a 20-π-electron ***chlorin***.
- Heme d1 contains two ketone groups in place of the vinyl groups at positions 2 and 4, while two acetate groups are added to positions 1 and 3 of the tetrapyrrole macrocycle, resulting in 18-π-electron ***isobacteriochlorins***.



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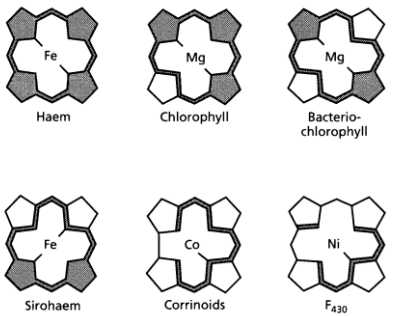
Conjugated System of Tetrapyrroles



Thauer (1998) Microbiology 144, 2377-2406.

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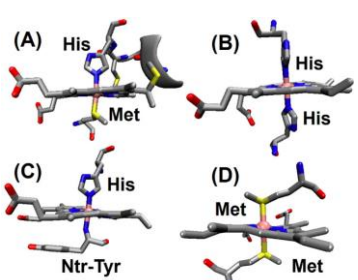
Types

- Heme *f* is similar to heme *c*, with the difference in the ligands that coordinate to the heme iron at the axial position (called axial ligands) make hemes *c* and *f* spectroscopically distinct.

A. Class I cytochromes *c*
B. Cytochromes *b* and multiheme cytochromes *c*
C. Cytochrome *f*
D. Cytochrome in bacterioferritin



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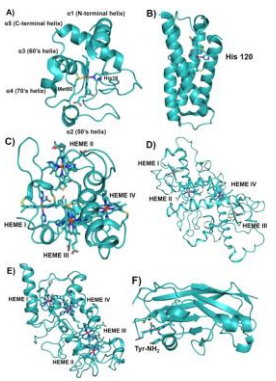
C-type Cytochromes

Class I

- The class I (A) include small (8–120 kDa) soluble proteins containing a single 6-coordinate low-spin (6cLS) heme moiety and display a range of reduction potentials from −390 to +450 mV versus standard hydrogen electrode (SHE)
- Conserved -Cys-x-x-Cys-His- sequence
- Some examples where Met is replace with Asn, His, or absent.
- Can be part of bigger complex



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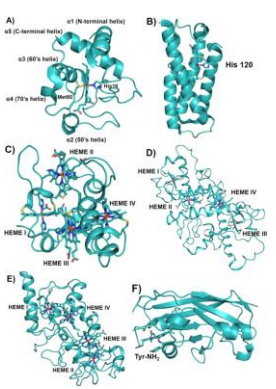
C-type Cytochromes

Class II

- C-terminal -Cys-x-x-Cys-His- sequence
- Four α-helices and a left-handed twisted overall structure
- The second axial ligand to the heme iron is variable
- Reduction potentials ranging from −5 to +230 mV



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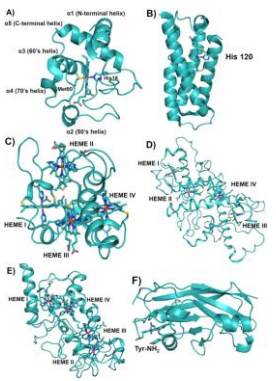
C-type Cytochromes

Class III

- Includes proteins containing multiple hemes with bis-His ligation
- Reduction potentials: −20 to −380 mV.
- Shown structure of cyt c_3 from *Desulfovibrio*, which acts as a natural electron acceptor and donor in hydrogenases and ferredoxins.
- Contains 4 hemes which are located in close proximity to each other



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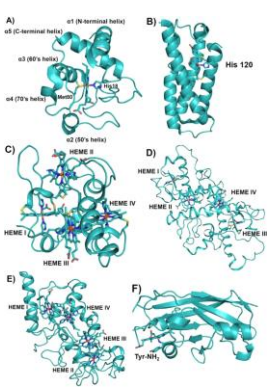
C-type Cytochromes

Class IV

- Large molar mass (~35–40 kDa) cytochromes that contain other prosthetic groups in addition to *c*-type hemes such as flavocytochromes *c* and cytochromes *cd*.
- The cyt *c* in the reaction center (RC) from *Rhodpseudomonas viridis* consists of four c-type heme moieties covalently bound to subunit C of the RC.
- Three of the hemes have His/Met axial ligation, while the fourth heme is bis-His-ligated.



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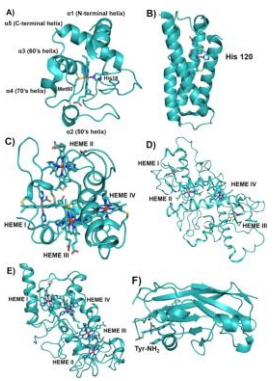
C-type Cytochromes

C_{554}

- Class of its own
- One of the hemes is HS, and the other three are 6cLS with reduction potentials of +47, +47, −147, and −276 mV, respectively.

Cyt f

- -Cys-x-x-Cys-His-
- Unique β-sheet fold
- An unusual second axial ligation to the heme iron, an N-terminal $-NH_2$ group of a Tyr residue



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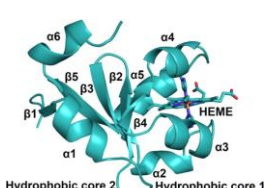
B-type Cytochromes

Cyt b_5

- ET hemoproteins
- bis-His-ligated *b*-type heme
- reduction potentials that span a range of ~400 mV.
- The structures have two hydrophobic cores on each side of a β-sheet that belong to the α + β class.
- The larger hydrophobic core contains the heme binding crevice, while the smaller hydrophobic core is proposed to have only a structural role.



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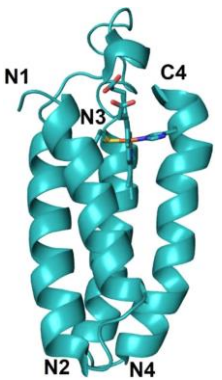
B-type Cytochromes

Cyt b_{562}

- Unknown function (*E. coli*)
- 6cLS heme with His and Met axial ligands
- Structurally homologous to cyt *c′* (class II) that contains a covalently bound 5cHS *c*-type heme
- In the oxidized unfolded state, the heme of cyt b_{562} is converted to 5cHS with His as the only axial ligand.



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Control of Redox Chemistry

Heme type

- In some cases exchanging *b* and *c* in active site can change potential but not generally the case.
- Heme *a* has higher potential (~160 mV) due to electron withdrawing acyl groups.

Axial Ligands

- Met ligation can raise potential by 100-150 mV (replacing His)

Solvent exposure

- Lower dielectric constant of proteins relative to aqueous solution
- Water exposure destabilizes the charged ferric site over the neutral ferrous state of the heme.

Control of Redox Chemistry

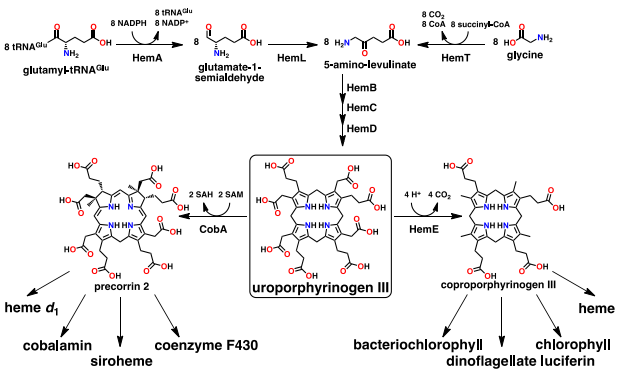
Second Coordination Sphere

- Hydrogen bonding to axial ligands
- Aromatic interaction with axial ligands

Charges and Electrostatics

- In general, negative local charges stabilize the ferric state and lower the reduction potential, and the magnitude of this effect can be comparable to that of ligand substitution or ligand secondary coordination sphere effects.
- Change in pH can change these local charges
- Nearby hemes can interact and shift potential by 50-60 mV

Tetrapyrrole Synthesis

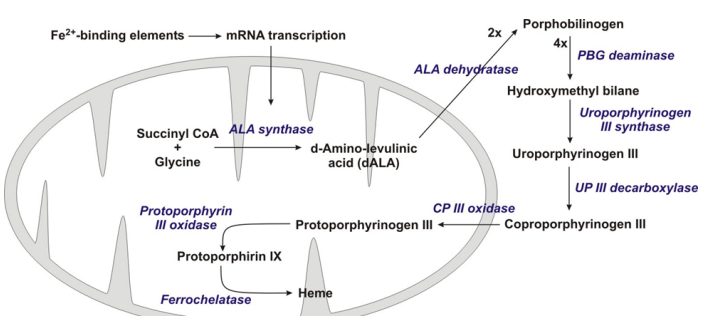


Control of Redox Chemistry

Heme Distortion/Ruffling

- Heme distortion or ruffling plays an important role in the electronic structure of the porphyrins, due to decreased delocalization of the π electrons.
- One example: protein-induced heme distortion can account for up to a 170 mV increase in potential in the heme nitric oxide/oxygen binding protein. (Olea (2010) J. Am. Chem. Soc. 2010, 132, 12794)

Porphyrin Synthesis



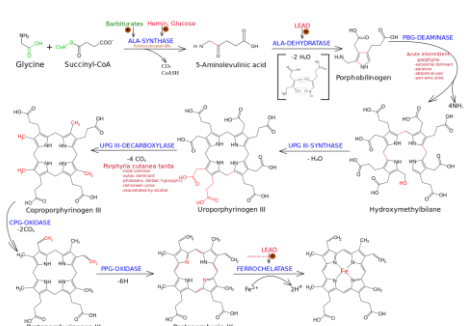
Porphyrin Synthesis

- In humans, this pathway serves almost exclusively to form heme. In other species, it also produces similar substances such as cobalamin (vitamin B_{12}).

- The pathway is initiated by the synthesis of D-Aminolevulinic acid from glycine and succinyl-CoA from the citric acid cycle.
- The reaction is catalyzed by ***ALA synthase***.
- This is the ***rate-limiting step*** in heme synthesis and is regulated by intracellular iron levels and heme concentration.
- Excess produced heme is oxidized to hematin (Fe^{3+}-OH), which subsequently can be converted into hemin (Fe^{3+}-Cl). Hemin and heme inhibit ALA synthase allosterically. Hemin also inhibits production of ALA synthase.

Porphyrin Synthesis

- The phorphyrias are a group of disorders caused by abnormalities in heme biosynthesis
- Excessive accumulation and excretion of porphyrins or their precursors
- Excreted by different routes dependent on their water solubility



Porphyrin Synthesis

- A low-iron level, e.g., in iron deficiency, leads to decreased porphyrin synthesis, which prevents accumulation of the toxic intermediates.

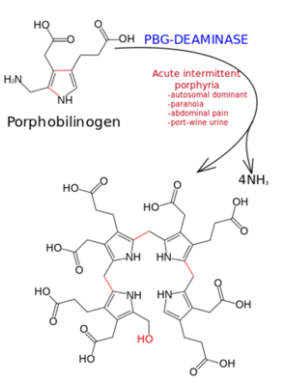
ALA dehydratase

- Condenses two molecules of ALA to yield porphobilinogen (PBG)
- ALA dehydratase is a zinc containing enzyme and is severely inhibited by lead ions.

Porphyrin Synthesis

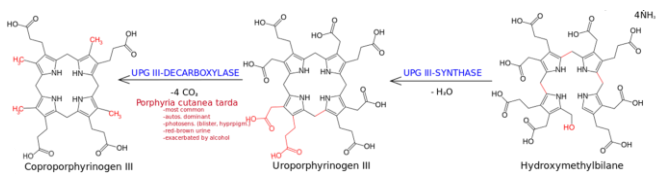
Porhobilinogen deaminase

- Catalyzes condensation of four porphobilinogen molecules in a symmetrical head-to-tail arrangement to form a straight-chain tetrapyrrole, hydroxymethylbilane.

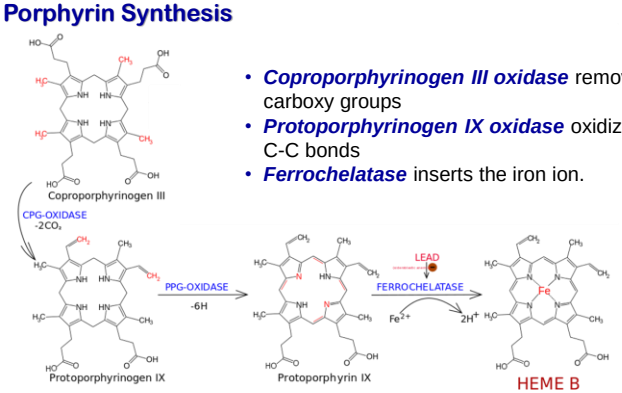


Porphyrin Synthesis

- ***Uroporphyrinogen III synthase*** closes the tetrapyrrole ring.
- ***Uroporphyrinogen III decarboxylase*** replaces acetyl groups with methyl groups



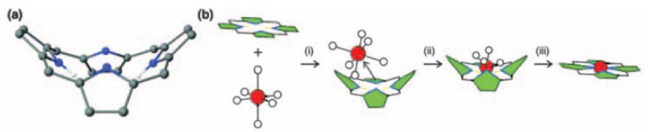
Porphyrin Synthesis



- ***Coproporphyrinogen III oxidase*** removes two carboxy groups
- ***Protoporphyrinogen IX oxidase*** oxidizes four C-C bonds
- ***Ferrochelatase*** inserts the iron ion.

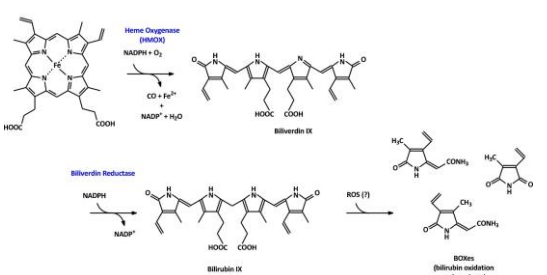
Ferrochelatase

- The final step is the insertion of the iron ion by ***ferrochelatase***



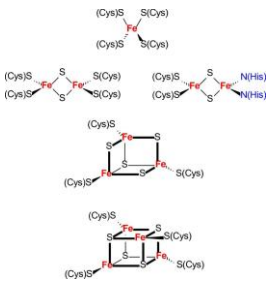
- Out-of-plane saddle structure in which two pyrrole rings with unprotonated nitrogens (blue spheres) point upwards, while the other two, protonated (blue and white spheres) point downwards. (b) Steps in the mechanism for incorporation of the metal ion (red) into the porphyrin (pyrrole rings in green)

Catabolism



- When heme proteins are degraded in mammals, the polypeptides are hydrolyzed to amino acids while the heme groups are freed of their iron and are converted to bilirubin
- After transport to the liver, bilirubin is coupled to glucuronic acid and the conjugated bilirubin is excreted into bile as the principle bile pigment.

Iron-sulfur Clusters



- ***Rubredoxins (Rd):*** $[1Fe–4S]^{3+,2+}$
- ***Ferredoxins (Fd):***
  - low-potential $[2Fe–2S]^{2+,+}$, $[3Fe–4S]^{+,0}$, $[4Fe–4S]^{2+,+}$, [3Fe–4S][4Fe–4S] and [4Fe–4S][4Fe–4S])
- ***Rieske proteins:*** high-potential $[2Fe–2S]^{2+,+}$
- ***High-potential iron–sulfur proteins (HiPIPs):*** $[4Fe–4S]^{3+,2+}$

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Fe-S Clusters

- Fe–S proteins are among the oldest metalloproteins on earth. The early atmosphere, under which both sulfur and iron were abundant, enabled the spontaneous assembly of these two elements into clusters, mainly containing four iron and four sulfur atoms.
- Fe–S proteins can be reconstituted by addition of $FeCl_3$ and Na_2S in a reductive environment.
- The presence of iron and sulfur in the solution is sufficient for formation of a [4Fe–4S] cluster.
- Despite the straightforward in vitro assembly, the assembly of the Fe–S clusters in vivo is a more precise and complex process.

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Biosynthesis

- Set of ***isc genes*** (***i***ron ***s***ulfur ***c***luster formation)
- Other systems are available: SUF, NIF, and CIA.
- Similar sets of genes are present in microorganisms from all three kingdoms, and in higher organisms examined, e.g. yeast, human, and Arabidopsis.
- In higher organisms cluster formation takes place in the mitochondrion.

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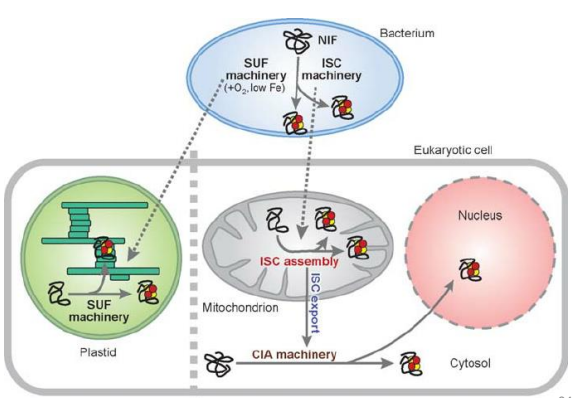
Biosynthesis



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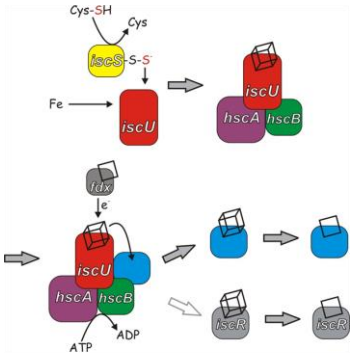
Isc System

- IscU: Contains three highly conserved Cys residues that form a scaffold for cluster assembly.
- IscS: Sulfur source. Uses cysteine as a substrate to form an enzyme-bound persulfide.
- HscA and HscB: Molecular chaperones. Extensive sequence similarity to chaperones DnaK and DnaJ.
- Fdx: [2Fe-2S] containing ferredoxin. Redox active, needed for cluster transfer.



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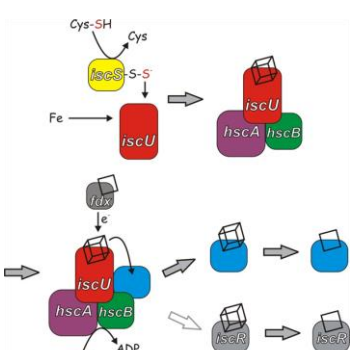
Isc System

- IscR: Negative regulatory protein. When a [2Fe-2S] cluster is present on IscR the isc genes are not expressed. Loss of the [2Fe-2S] cluster results in a dramatic increase in expression of the isc genes.

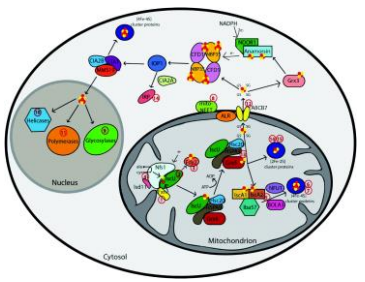
- In many organisms, iron delivery is facilitated by Frataxin (*contradictory data in the literature*)



66

Isc System

- ***Common theme: metal containing cofactor is pre-made on a scaffolding protein.***



- Studies in yeast, zebrafish and plants have shown that up to 20 different proteins are involved in eukaryotic Fe–S cluster biogenesis.
- For some of these the function is unknown. There also seems to be a lot of duplication and identical steps are catalyzed by different enzymes in different organisms.
- Most proteins are essential and deletion is lethal

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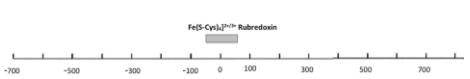
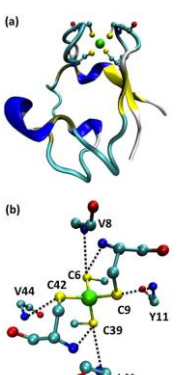
Rubredoxin

- Small protein (45-54 amino acids)
- Mono-iron center
- Distorted tetrahedral
- Two Cys-x-x-Cys-Gly segments

- Fe(II)/Fe(III)
- Reduction potentials: -100 to +50 mV vs SHE
- No structural changes upon change in redox state





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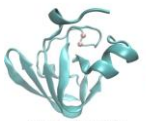
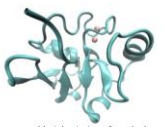
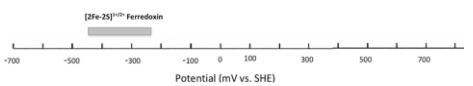
2Fe Ferredoxins


Plant-type ferredoxin
PDB: 3AV8


Vertebrate-type ferredoxin
PDB: 1CJE

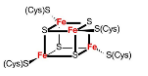

Thioredoxin-like ferredoxin
PDB: 1F37

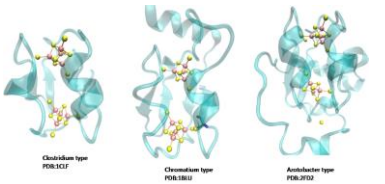
- The reduction potentials are between −460 and −300 mV.
- For thioredoxin-like class average is around −300 mV



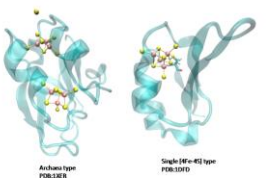
69

3Fe, 4Fe, 7Fe and 8Fe Ferredoxins





- The cluster takes the form of a distorted cube
- The cysteine ligands are arranged in a C-x_2-C-x_2-C motif, the so-called classic [4Fe–4S] motif.



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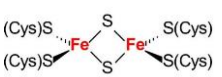
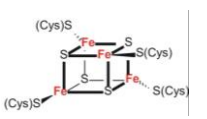
Consensus Sequences for [2Fe-2S] Clusters



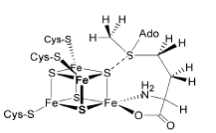
- -**C**-xxxx-**C**-xx-**C**-x_{29}-**C**- plant-type
- -**C**-xxxxx-**C**-xx-**C**-$x_{36/37}$-**C**- hydroxylase/vertebrate-type
- -**C**-$x_{10\text{-}12}$-**C**-$x_{29\text{-}34}$-**C**-xxx-**C**- Thioredoxin-type

70

Consensus Sequences for 4Fe Clusters

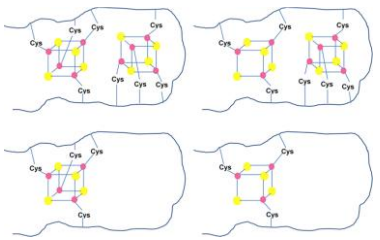


- -**C**-xx-**C**-xx-**C**-xxx-**C**-P- 4Fe-Ferredoxins
- -**C**-xxx-**C**-xx-**C**- Radical-SAM family (Coproporphyrinogen III oxidase, biotin synthase)



72

Consensus Sequences for 4Fe Clusters



- Two repeats: -**C**-xx-**C**-xx-**C**-xxx-**C**-P-x_n-**C**-xx-**C**-xx-**C**-xxx-**C**-P-
- An Asp replaces the second Cys in the consensus sequence for the 3Fe clusters

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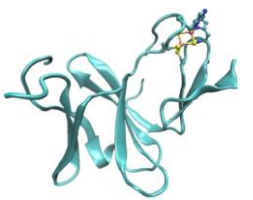
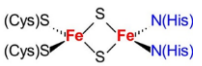
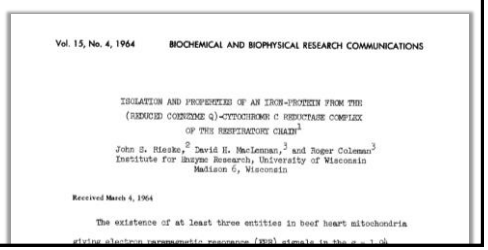
Rieske [2Fe-2S] Clusters



Always part of larger enzyme.





Vol. 15, No. 4, 1964 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

ISOLATION AND PROPERTIES OF AN IRON-PROTEIN FROM THE (REDUCED COENZYME Q)-CYTOCHROME C REDUCTASE COMPLEX OF THE RESPIRATORY CHAIN[1]

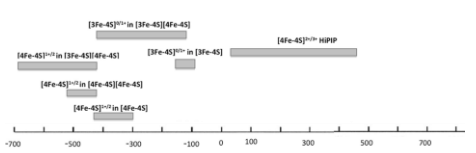
John S. Rieske,[2] David H. MacLennan,[3] and Roger Coleman[3]
Institute for Enzyme Research, University of Wisconsin
Madison 6, Wisconsin

Received March 4, 1964

The existence of at least three entities in beef heart mitochondria

3Fe, 4Fe, 7Fe and 8Fe Ferredoxins

- The reduction potential of [4Fe–4S] clusters usually ranges from −250 to −650 mV, with an average of −400.
- The common reduction potential for [3Fe–4S] clusters ranges from −50 to −450 mV, with an average of −100 to −150.
- The reduction potential of the [3Fe–4S] cluster can be is pH-dependent.
- The pH dependence can be related to proton transfer via the conserved Asp next to the cluster or protonation of the cluster itself.



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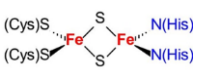
Rieske [2Fe-2S] Clusters



- Rieske proteins can be found in *bc* complexes such as the bc_1 complex of mitochondria and bacteria, the b_6f complex of chloroplast, and corresponding subunits in menaquinone oxidizing bacteria.
- Three residues other than Fe–S ligands are also conserved in this class of Rieske proteins, two of which are cysteine residues that form a disulfide bond important in the stability of the protein, and the other is a Gly:

  -**Cys**-x-**His**-x-Gly-Cys-$x_{12–44}$-**Cys**-x-Cys-**His**-

- Rieske-type proteins that are part of water-soluble dioxygenases:

  -**Cys**-x-**His**-$x_{16–17}$-**Cys**-x-x-**His**-

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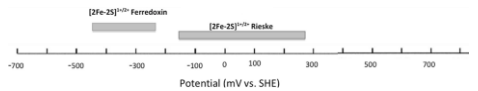
Rieske [2Fe-2S] Clusters

- Reduction potentials of Rieske centers vary in a wide range of −100 to +490 mV



- The difference between the overall charge of the cluster (0/–1 in the case of Rieske proteins vs −2/–3 in the case of ferredoxins) and electronegativity of the ligands (histidine vs cysteine) is the main reason for the higher reduction potential of Rieske proteins. Different H-bonds to bridging or terminal sulfurs and solvent exposure of the clusters are the main determinants of different reduction potentials within the Rieske family.
- pH-dependent reduction potential, attributed to deprotonation of a group in contact with the Rieske complex

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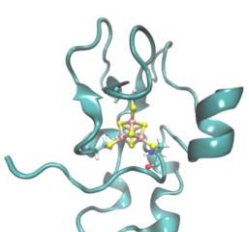
HiPIP

- Small proteins (6–11 kDa)
- Characteristic HiPIP fold
- -Cys-x_2-Cys-$x_{8–16}$-Cys-$x_{10–13}$-Gly-Trp/Tyr-Cys
- Several loops around the protein make a hydrophobic pocket for the protein to accommodate the cluster
- HiPIPs have conserved $NH_{amide}\cdots S$ H-bonds to the coordinating sulfurs.
- These H-bonds stabilize the reduced form of the protein by decreasing the electron density on sulfurs, thereby increasing the reduction potential.



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Redox Potentials

Reduction potential affected by:

- Geometry
  - Changes when more Fe ions are present
- Coordination:
  - Ferredoxin vs. Rieske
  - Replacing Cys with Met in rubredoxins variants changes potential by 100-200 mV

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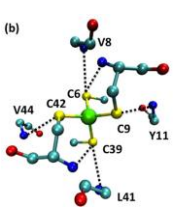
Redox Potentials

Reduction potential affected by:

- The overall protein fold
  - Solvent accessibility
  - H-bonding network: replacing Val44 with Ala in rubredoxin causes change of 50 mV
  - The presence of a fifth Cys residue close to a 4Fe cluster can lead to formation of a SH···S H-bond and tune the activity by lowering the reduction potential.
  - Overall charge of the protein. Placing charged residues in the second coordination sphere changes the potential



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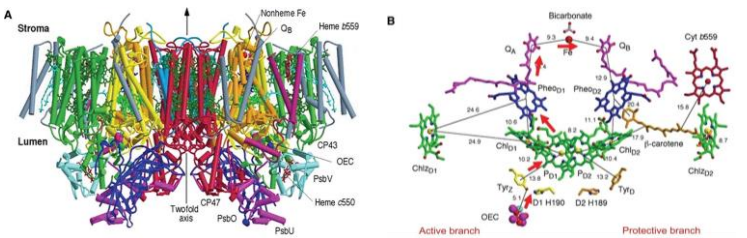
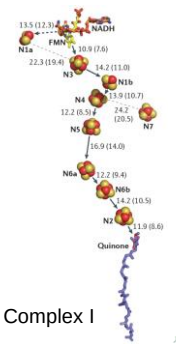
Giant Metalloprotein Complexes

Photosystem II

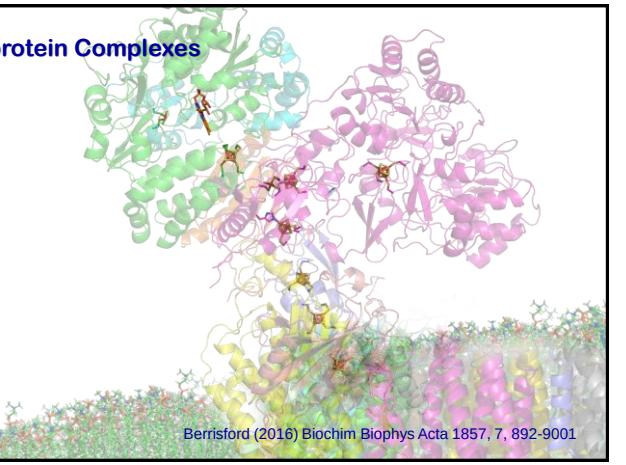


Barber (2012) Cold Spring Harb Symp Quant Biol 77: 295-307

Giant Metalloprotein Complexes



Complex I

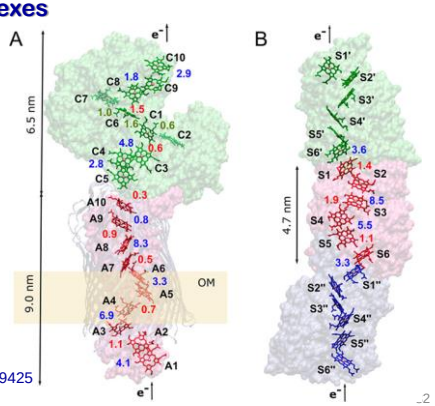


Berrisford (2016) Biochim Biophys Acta 1857, 7, 892-9001

Giant Metalloprotein Complexes

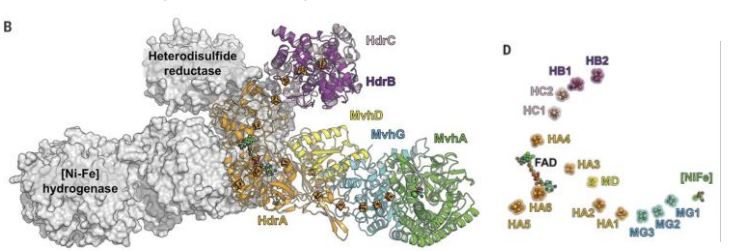
Multi-heme proteins

A. MtrCAB complex from *Shewanella baltica* OS185
B. OmcS polymer containing three repeat units from *Geobacter sulfurreducens*



Jiang (2021) J. Phys. Chem. Lett. 11, 9421-9425

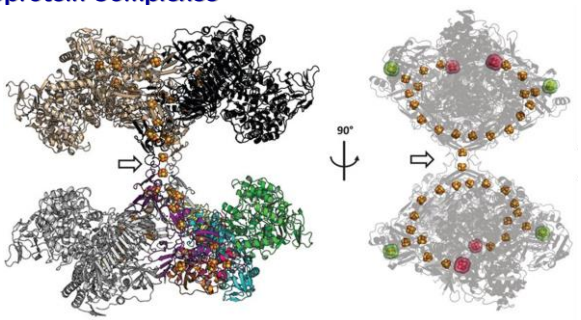
Giant Metalloprotein Complexes



- Hydrogenase:heterodisulfide reductase complex from *Methanothermobacter marburgensis*

Wagner (2017) Science, 357, 699-703

Giant Metalloprotein Complexes



- Formyl-methanofuran dehydrogenase from *Methanothermobacter wolfeii*

Wagner (2016) Science, 354, 114-117

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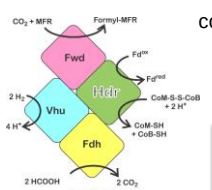
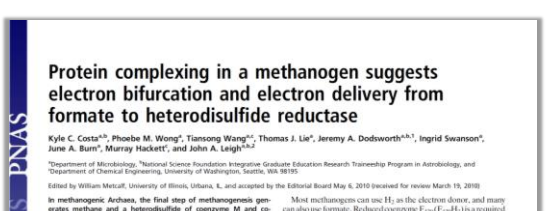
Mega Metalloprotein Complexes



Methanococcus maripaludis contains a complex that contains: heterodisulfide reductase, formylmethanofuran dehydrogenase, F_{420}-nonreducing hydrogenase, and formate dehydrogenase.



PNAS

Protein complexing in a methanogen suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase

Kyle C. Costa[a,b], Phoebe M. Wong[a], Tiansong Wang[a,c], Thomas J. Lie[a], Jeremy A. Dodsworth[a,b,1], Ingrid Swanson[a], June A. Burn[a], Murray Hackett[c], and John A. Leigh[a,b,2]

[a]Department of Microbiology, [b]National Science Foundation Integrative Graduate Education Research Traineeship Program in Astrobiology, and [c]Department of Chemical Engineering, University of Washington, Seattle, WA 98195

Edited by William Metcalf, University of Illinois, Urbana, IL, and accepted by the Editorial Board May 6, 2010 (received for review March 19, 2010)

In methanogenic Archaea, the final step of methanogenesis generates methane and a heterodisulfide of coenzyme M and coenzyme B (CoM-S-S-CoB). Reduction of this heterodisulfide by

Most methanogens can use H_2 as the electron donor, and many can also use formate. Reduced coenzyme F_{420} ($F_{420}H_2$) is a required intermediate and can be generated from H_2 by F_{420}-reducing hy-