Iron Chelation in Biology

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Iron Chelation in Biology

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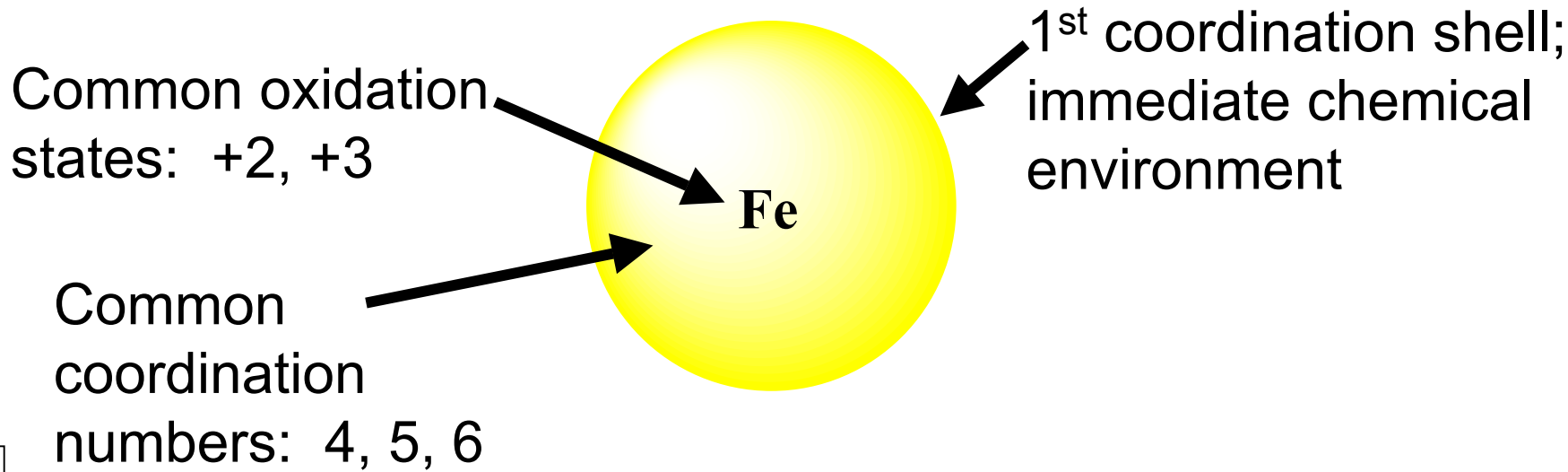
Introduction: Biological Iron Coordination Chemistry

- Iron is the second most abundant metal on the earth's surface, falling closely behind aluminum and in near equivalent concentration to calcium and sodium. It is an essential element for virtually every living cell.

- The biochemistry of iron is controlled to a large extent by its coordination chemistry; *i.e.* the immediate chemical environment in the first coordination shell. This first coordination shell controls iron's biological activity in small molecule storage (e.g.O_2), electron transport, and catalysis.



References [1,2]

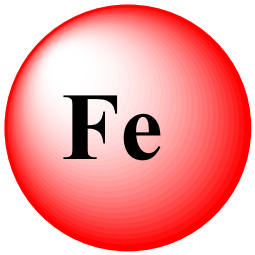
Introduction: Biological Iron Coordination Chemistry

Examples of the extensive use of iron in biological systems, all of which are controlled or mediated by chelation, are as follows:

- redox chemistry involved in simple electron-transfer reactions;
- redox chemistry involved in reactions with O_2, ranging from O_2 transport and storage to O_2 reduction by cytochrome oxidase, and O atom insertion catalyzed by cytochrome P_{450}; and
- substrate activation by the electrophilic behavior of iron; for example, hydrolase enzymes such as purple acid phosphatase.

References [1,2]

Introduction:
Biological Iron Coordination Chemistry

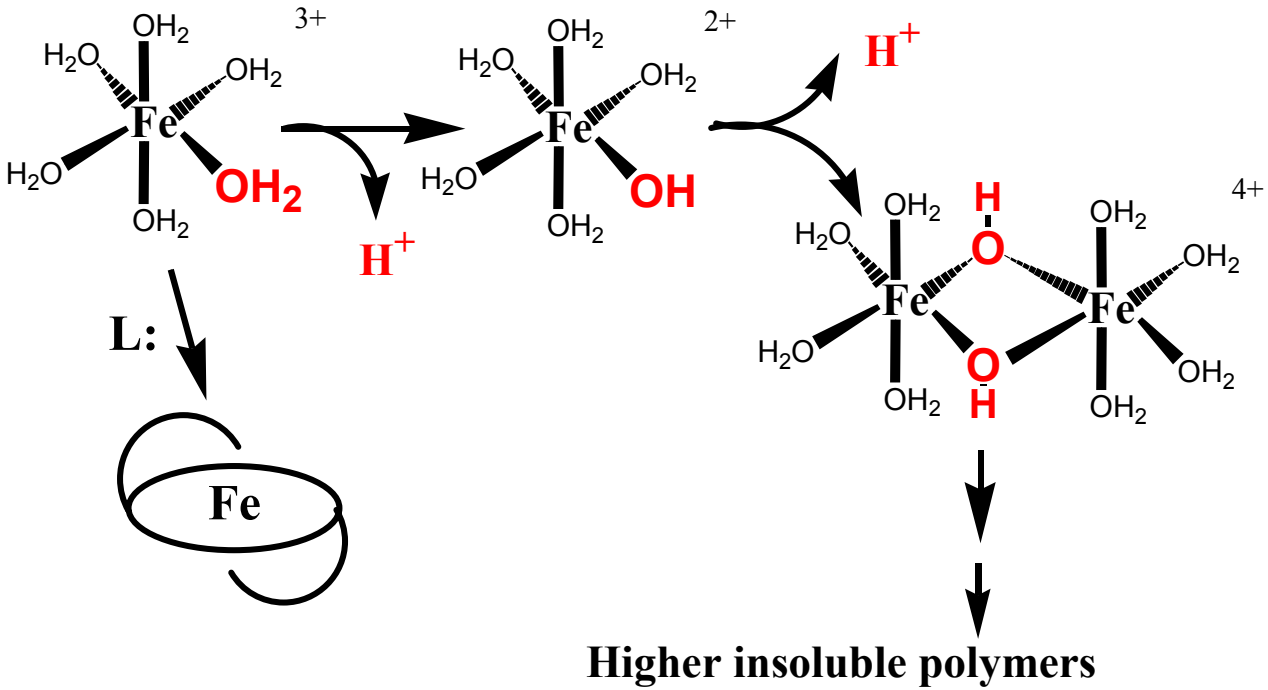


The first coordination shell

- Prevents hydrolysis/precipitation
- Influences molecular recognition
- Controls redox potential
- Controls mobility

Iron Chelation and Solubility

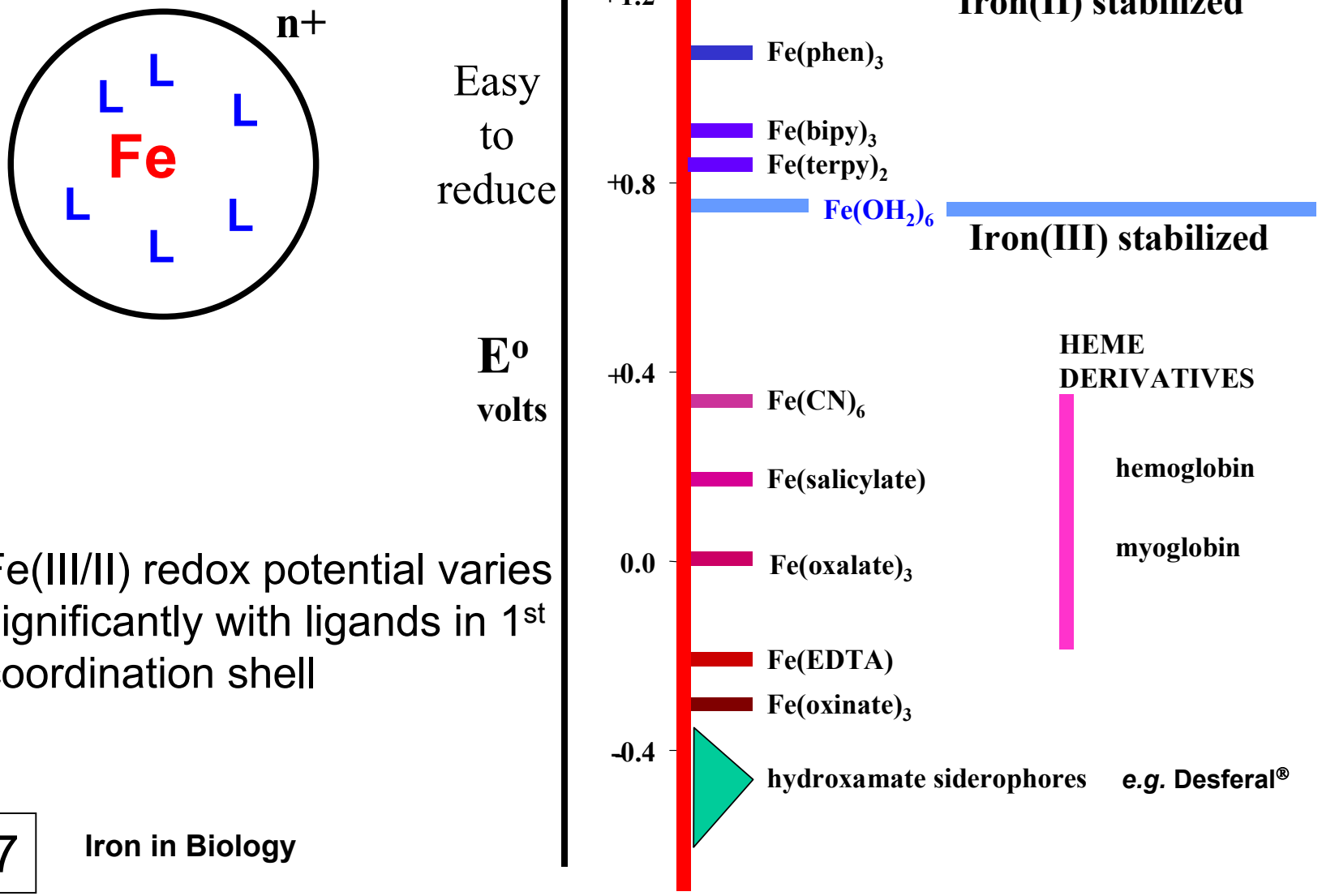
- Fe insoluble due to hydrolysis



$[Fe_{aq}^{3+}]_{tot} = 10^{-10}$ M @ pH 7

- Strong chelators prevent hydrolysis and precipitation

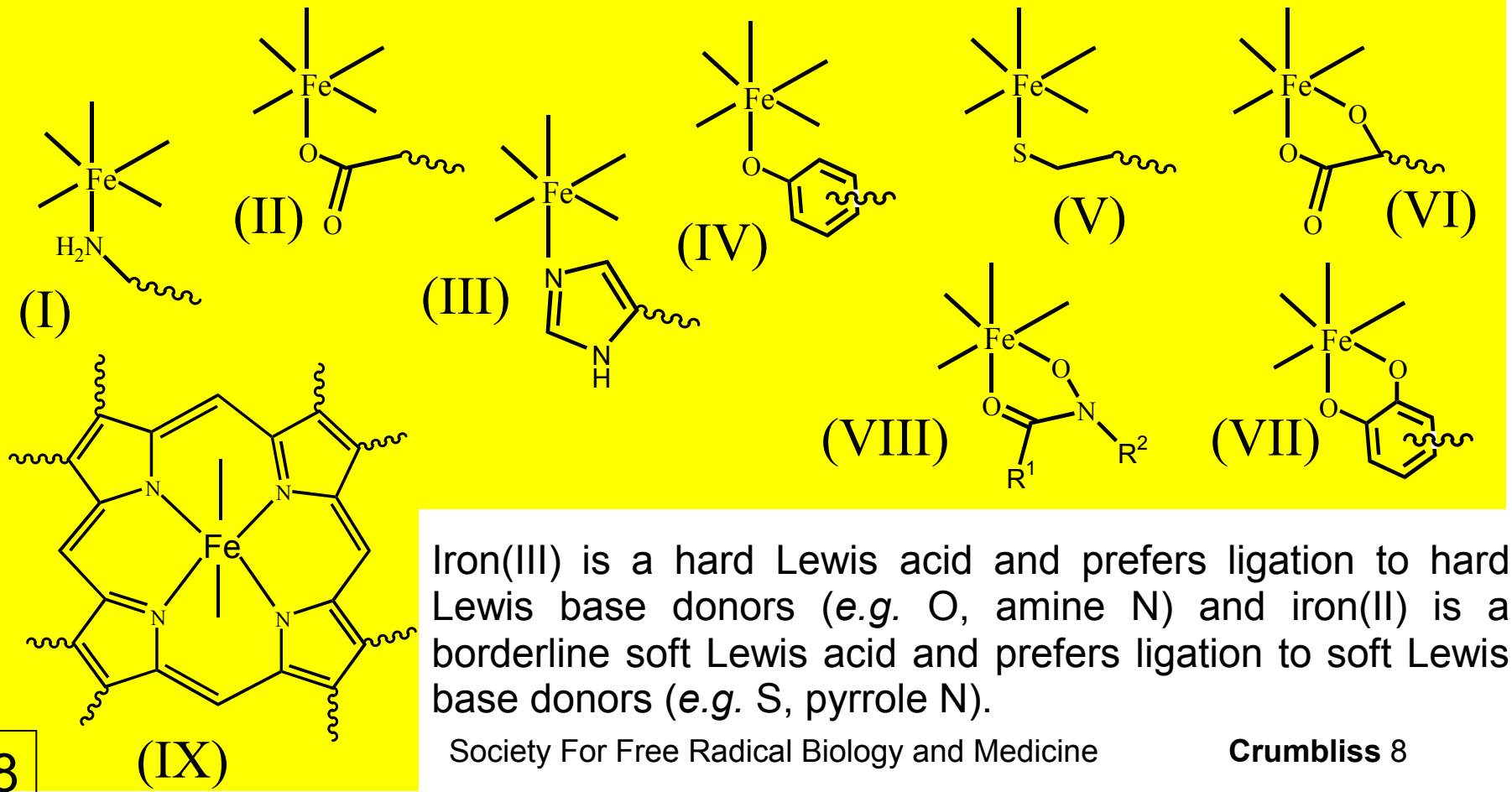
Iron Chelation and Redox Potential



Fe(III/II) redox potential varies significantly with ligands in 1st coordination shell

Common Iron Ligands in Biology

Common iron ligand donor groups in biology include amino acid side chains, such as amine (I), carboxylate (II), imidazole (III), phenol (IV), and thiol (V). Other ligating groups include α-hydroxy carboxylate (VI), catecholate (VII), hydroxamate (VIII) and porphyrin (IX).



Iron(III) is a hard Lewis acid and prefers ligation to hard Lewis base donors (*e.g.* O, amine N) and iron(II) is a borderline soft Lewis acid and prefers ligation to soft Lewis base donors (*e.g.* S, pyrrole N).

Iron Chelate Stability Definitions

Compilations of metal-ligand complex stabilities, such as that edited by Martell and Smith, use pH independent equilibrium constants, β_{FeLH}, as defined below for the reaction between Fe(III) and a hexadentate triprotic ligand, LH_3, in aqueous solution.

$$Fe(OH_2)_6^{3+} + L^{3-} \rightleftharpoons FeL \qquad \beta_{110} = \frac{[FeL]}{[Fe(OH_2)_6^{3+}][L^{3-}]}$$

However, in an *in vivo* or *in vitro* situation protons compete for the Fe(III) binding sites and the degree of complexation of the metal will be influenced by the ligand pK_a values and the pH of the medium.

$$Fe(OH_2)_6^{3+} + H_3L \rightleftharpoons FeL + 3\,H^+ \qquad K = \frac{[FeL]\,[H^+]}{[Fe(OH_2)_6^{3+}][H_3L]}$$

Since stability constants β and K are determined as concentration quotients, their units differ on changing the denticity of the ligand. Consequently, β_{110} for a hexandentate ligand and β_{130} for a bidentate ligand cannot be directly compared. A pFe scale circumvents this problem and the problem of H^+ competition due to different ligand pK_a values. The pFe value for a particular ligand is the negative log of the free Fe(III) concentration at a fixed set of conditions: [total ligand] = 10 μM, [total Fe(III)] = 1 μM, and pH = 7.4. A high pFe value denotes a stable chelate complex.

Panel 17 illustrates the influence of pH on Fe(III)-siderophore complex stability, using pFe values to express the stability of the complex.

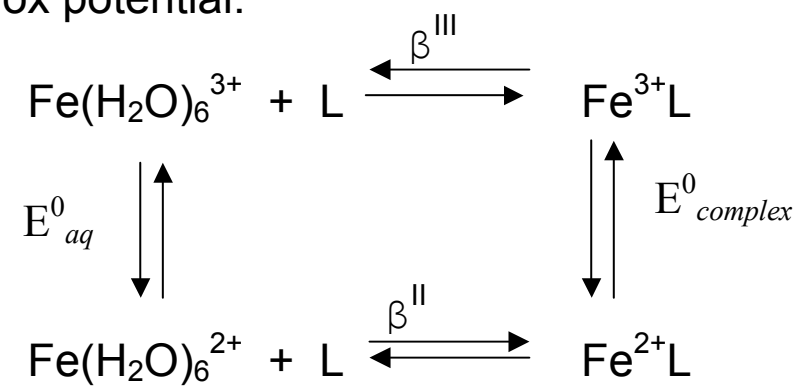
References [3,4,5,6]

Iron Chelation and Redox Control

Why is it important?

A mechanism for preventing iron from participating in a catalytic cycle to produce toxic hydroxyl radicals and/or reactive oxygen species (ROS) (*e.g. via* the Fenton reaction or Haber Weiss cycle) is to control its redox potential by selective chelation. Through chelation, the redox potential for iron may be removed from the region where it can undergo redox cycling and produce hydroxyl radicals and ROS. This is illustrated in **Panel 12**.

From the following thermochemical cycle, Equation (1) can be derived which relates the redox potential of an Fe complex to the chelator's ability to discriminate between Fe(III) and Fe(II), as expressed by β^{III} and β^{II}. This relationship illustrates that the selectivity of a chelator for Fe(III) over Fe(II) increases with decreasing redox potential.



$$E^0_{aq} - E^0_{complex} = 59 \log(\beta^{III}/\beta^{II}) \quad [1]$$

Reference [6]

Iron Chelation and Redox Control

Why is it important?

From Equation (1) it is evident that the redox potential and stability of an iron complex are inter-related.

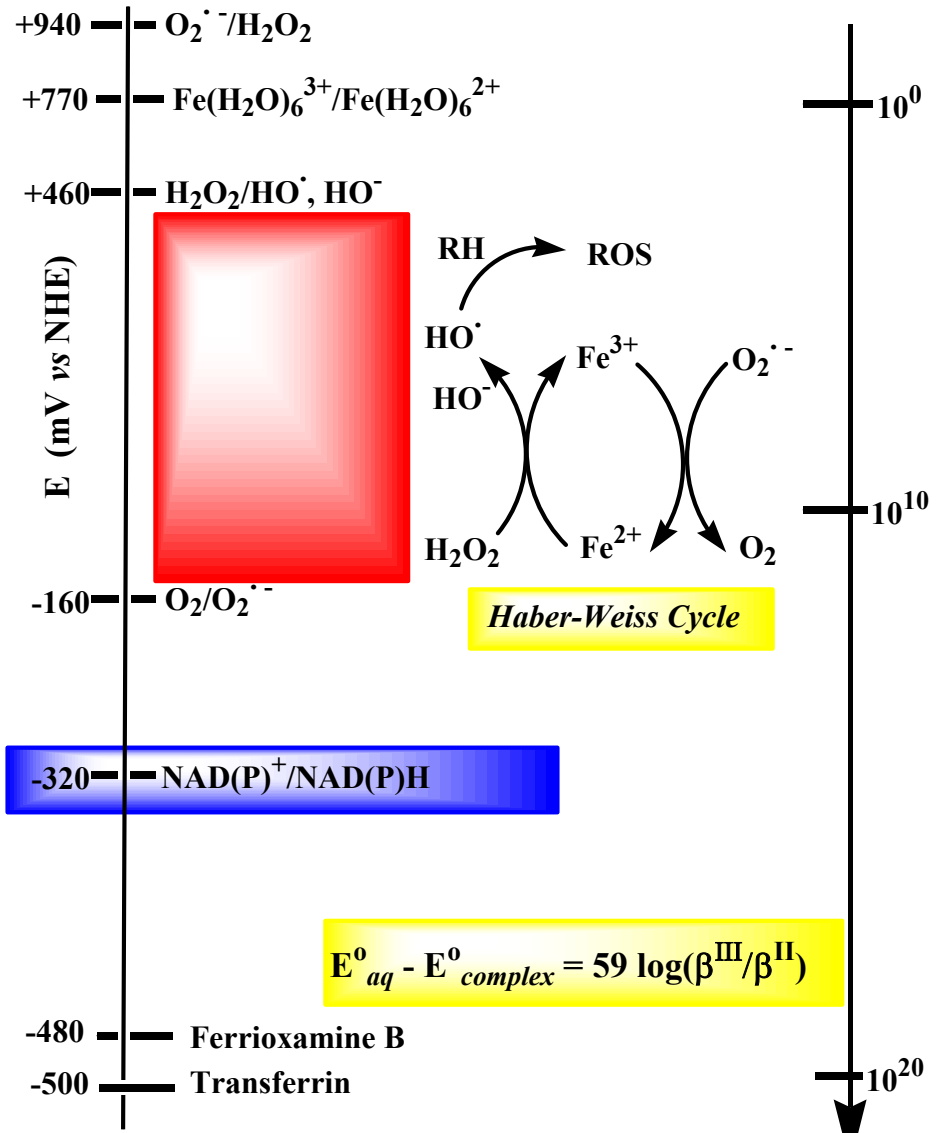
These inter-relationships are important in characterizing the biological chemistry of iron because controlling the oxidation state of iron is a method of controlling both the thermodynamic and kinetic stability of a coordination compound. This is illustrated in **Panel 13**. As a result, the redox potential of a complex may be viewed as a measure of the sensitivity of a ***molecular level switch*** for changing the chemical environment of the iron (1^{st} coordination shell). Data in **Panel 13** show that for high spin complexes, changing the oxidation state of iron from +2 to +3 changes both the kinetic lability and thermodynamic stability of an iron chelate complex.

Reference [6]

Iron Chelation and Redox Control

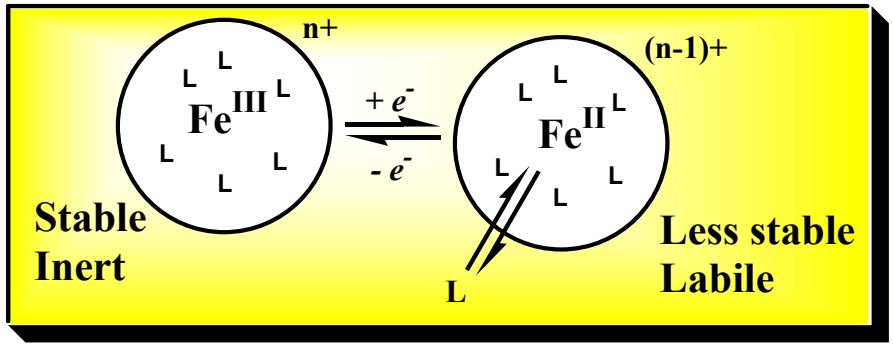
Why control E^0?

- *Prevent redox cycling & ROS production*
- *Fe(III) selectivity*
- *Control stability*
- *Control ligand exchange kinetics*
- *Control "switch" sensitivity*



Reference [6]

Oxidation State Influence on Chelate Stability



Thermodynamics

Illustration of the loss of several orders of magnitude of stability on reduction of high spin Fe(III) complex to Fe(II).

Fe(III)transferrin	log K @ pH 7.4 = 20
Fe(II)transferrin	log K @ pH 7.4 = 3
Fe(III)ferrioxamine B	log β_{110} = 30.6
Fe(II)ferrioxamine B	log β_{110} = 10.3

Kinetics

Illustration of an increase in 1st coordination shell lability on reduction of Fe(III) to Fe(II).

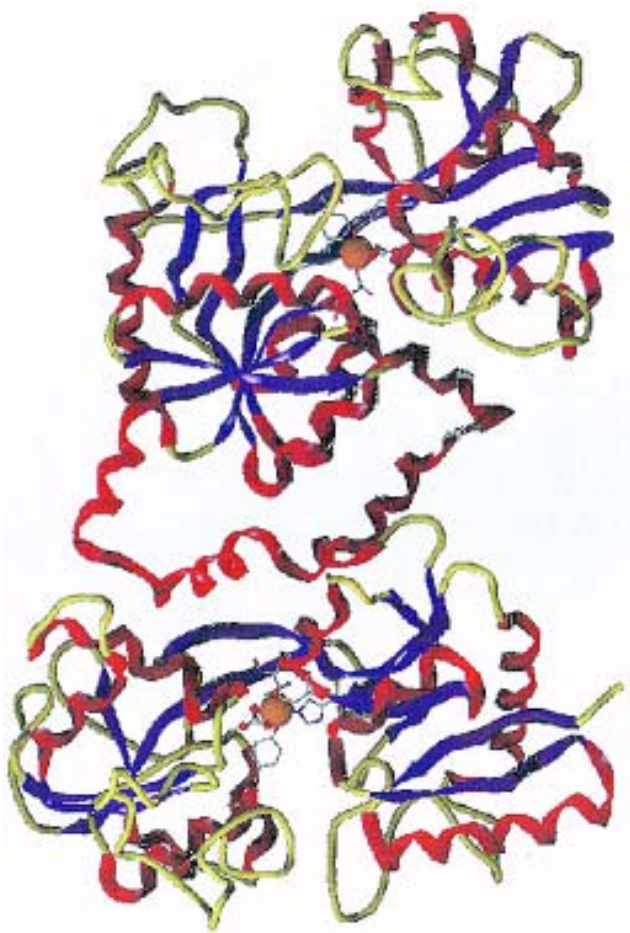
$Fe(OH_2)_6^{3+} + {}^*OH_2 \xrightleftharpoons{t_{1/2} = 4\ ms} Fe(OH_2)_5({}^*OH_2)^{3+} + OH_2$

$+e^-$ / $-e^-$

$Fe(OH_2)_6^{2+} + {}^*OH_2 \xrightleftharpoons{t_{1/2} = 0.2\ \mu s} Fe(OH_2)_5({}^*OH_2)^{2+} + OH_2$

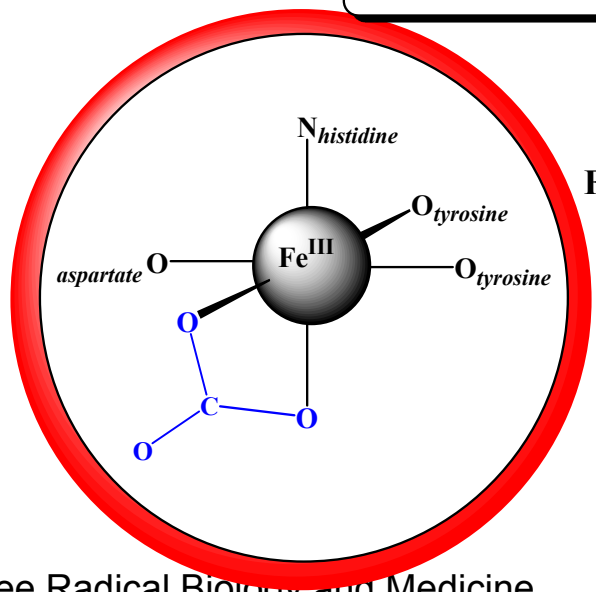
References [7,8,9,10,11]

Iron Chelation and Transport



In humans, the host protein transferrin (Tf) is produced in excess of circulating free iron and sequesters extracellular iron at extremely high affinity (K_d ~10^{-20} M). This chelation of iron prevents it from precipitation and also has a bacteriostatic effect by keeping iron as an essential nutrient from being available to bacterial pathogens.

Human Transferrin Fe(III) Binding Site

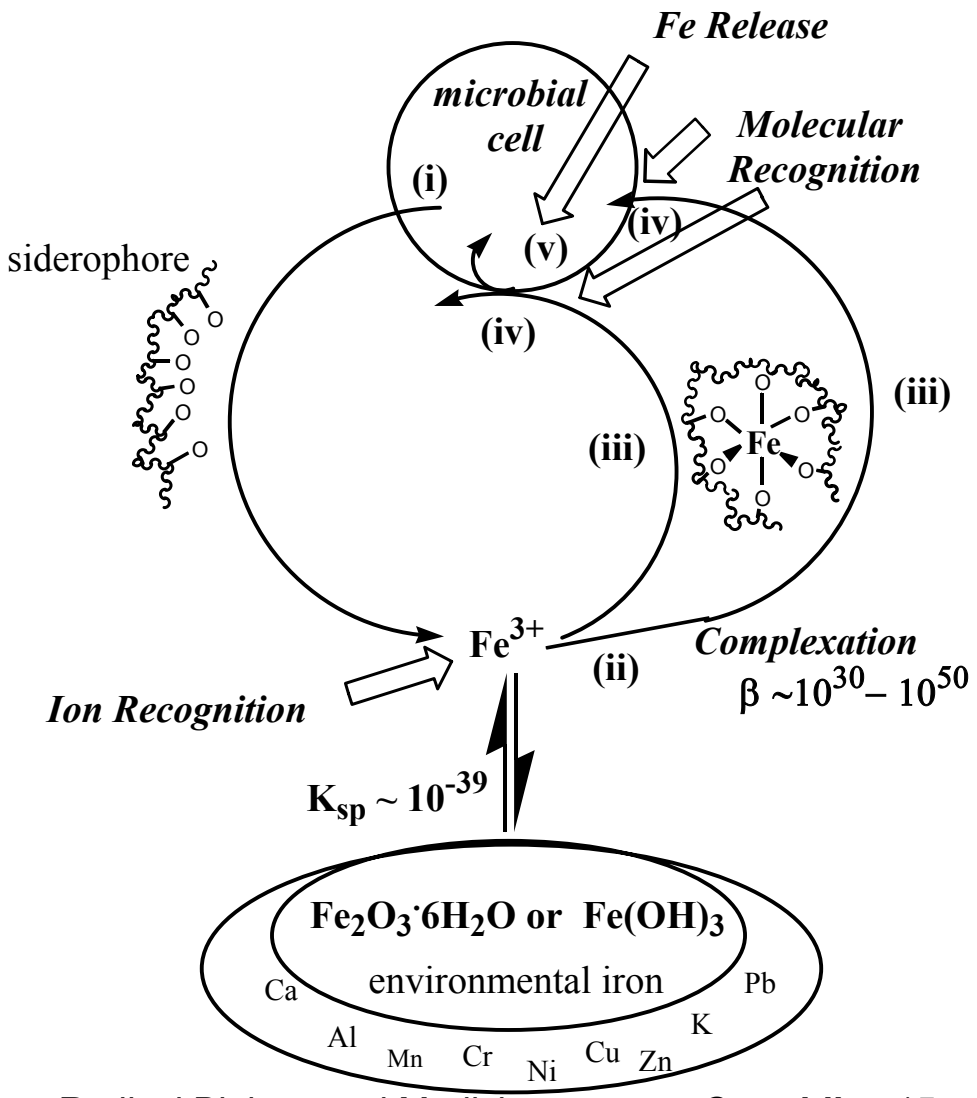


$$Fe^{3+} + \text{apo-Tf} + CO_3^{2-} \xrightarrow{K_b \sim 10^{20}\ M^{-1}} Fe^{III}Tf(CO_3^{2-})$$

Reference [7]

Iron Chelation and Transport

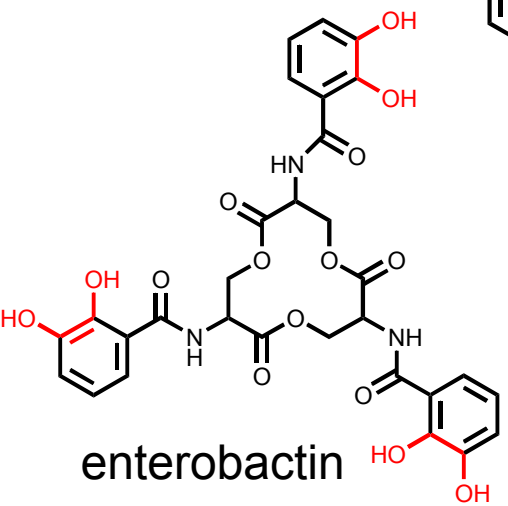
Microbes solubilize environmental iron by a chelation process, whereby the microbe secretes chelators called siderophores which have a high and specific affinity for Fe(III). Siderophore mediated iron acquisition by microbes is illustrated here where the cell synthesizes and releases a polydentate siderophore (i) which solubilizes insoluble iron deposits by chelation (ii). The Fe(III) chelate diffuses back to the cell (iii) where it is recognized by a cell receptor (iv) and the iron is released into the metabolic processes within the cell (v).



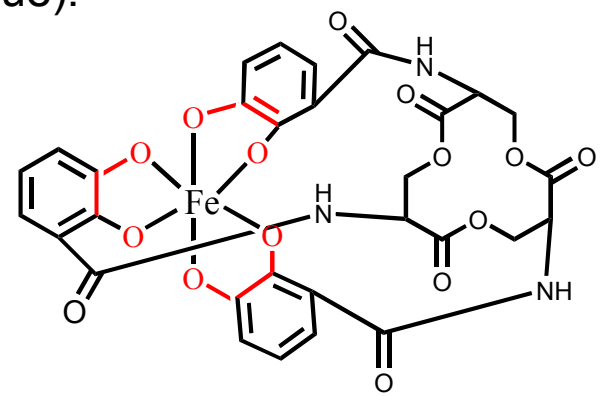
References [4,5,6]

Iron Chelation and Transport

Siderophores, microbially synthesized Fe(III) specific chelators, are low molecular weight molecules that usually incorporate bidentate catechol, hydroxamic acid, and/or α-hydroxy carboxylic acid donor groups. These chelators exhibit high Fe(III) complex stabilities (high β and pFe) to enhance delivery of iron to the cell, and large negative redox potentials (**Panels 7, 12** and **18**) for Fe(III) complexing specificity and to prevent redox cycling leading to the production of toxic hydroxyl radicals and ROS (**Panels 10**, **11** and **12**). Shown below are the structures of two hexadentate siderophores; enterobactin, a tris catecholate (in red), and ferrioxamine B, a tris hydroxamate (in blue).

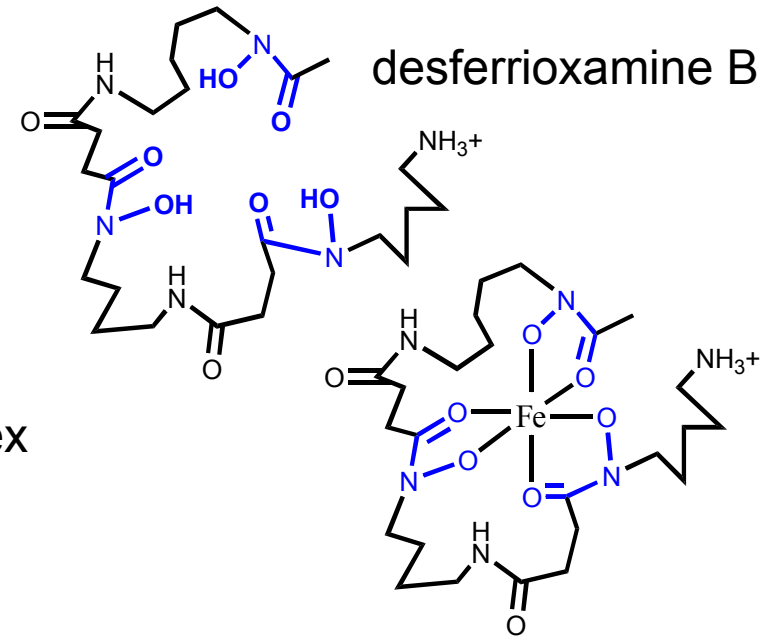


enterobactin



Fe(III)-enterobactin complex

$\beta_{110} = 10^{49}$; pFe = 35.5



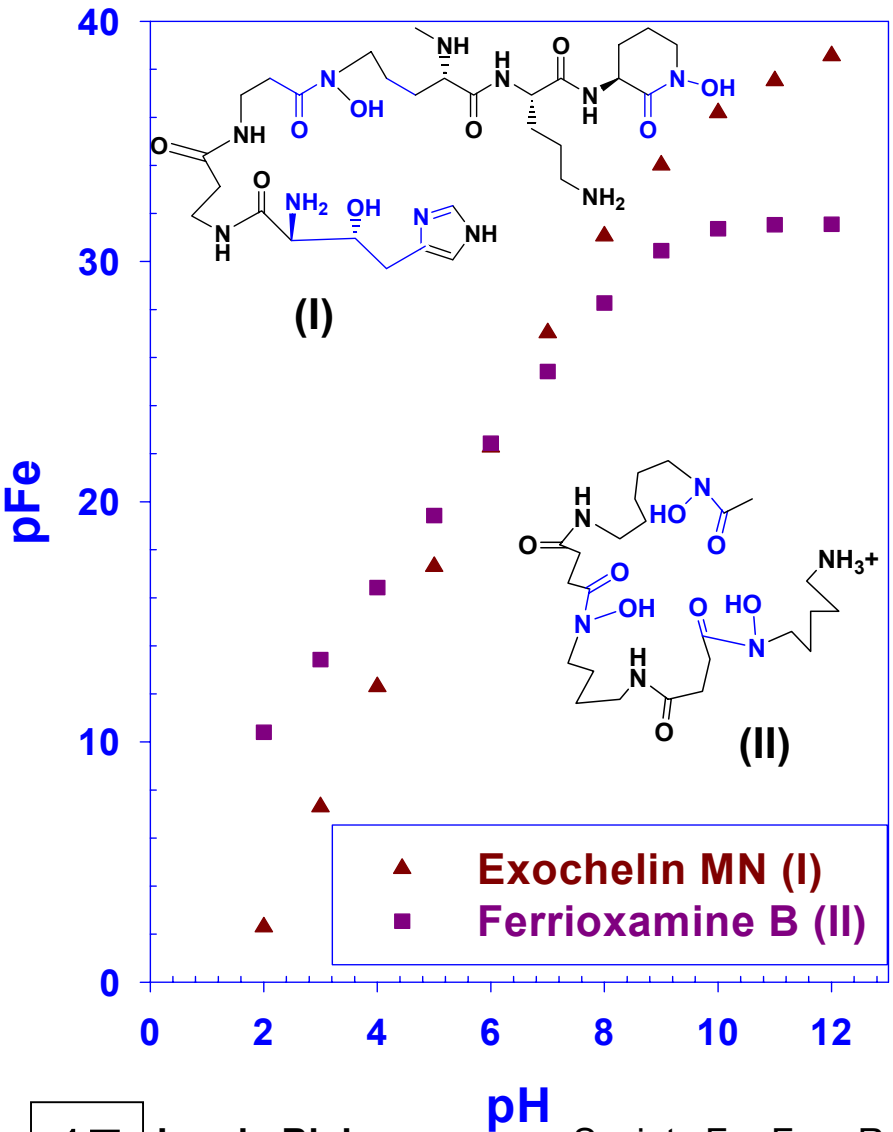
desferrioxamine B

ferrioxamine B complex

$\beta_{110} = 10^{30.2}$; pFe = 26.6

References [4,5,6]

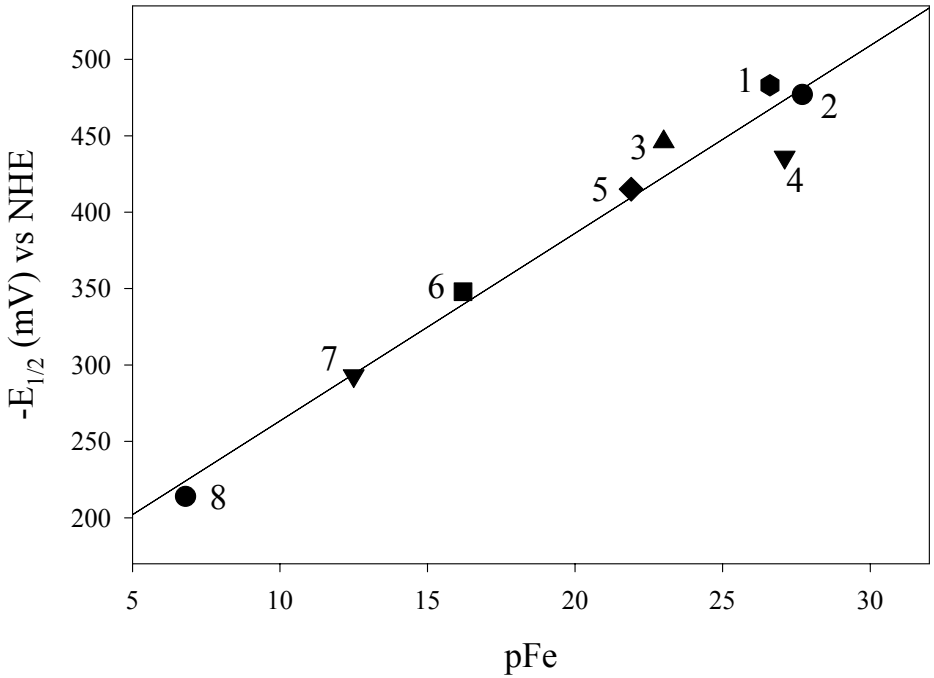
Influence of pH on Fe(III)-Chelate Stability



Plot of Fe(III) complex stability, expressed as pFe (**Panel 9**), as a function of pH for two siderophores, exochelin MN (I) and ferrioxamine B (II). Although they have approximately the same stability at pH 6.0, above this pH exochelin MN has a higher affinity for Fe(III) and below this value ferrioxamine B exhibits a higher affinity. This is due to different levels of competition from H^+ for the Fe(III) binding sites, due to different pK_a values for the donor groups (shown in blue) in these two siderophore chelators.

Reference [9,12]

Influence of Fe(III)-Chelate Stability on E^0



1. Fe(desferrioxamine B)$^+$
2. Fe(Desferrioxamine E)
3. Fe_2(alcaligin)$_3$
4. Fe(saccharide-trihydroxamate)
5. Fe_2(rhodotorulic acid)$_3$
6. Fe(N-methylacetohydroxamate)$_3$
7. Fe(acetohydroxamate)$_3$
8. Fe(L-lysinehydroxamate)$_3$

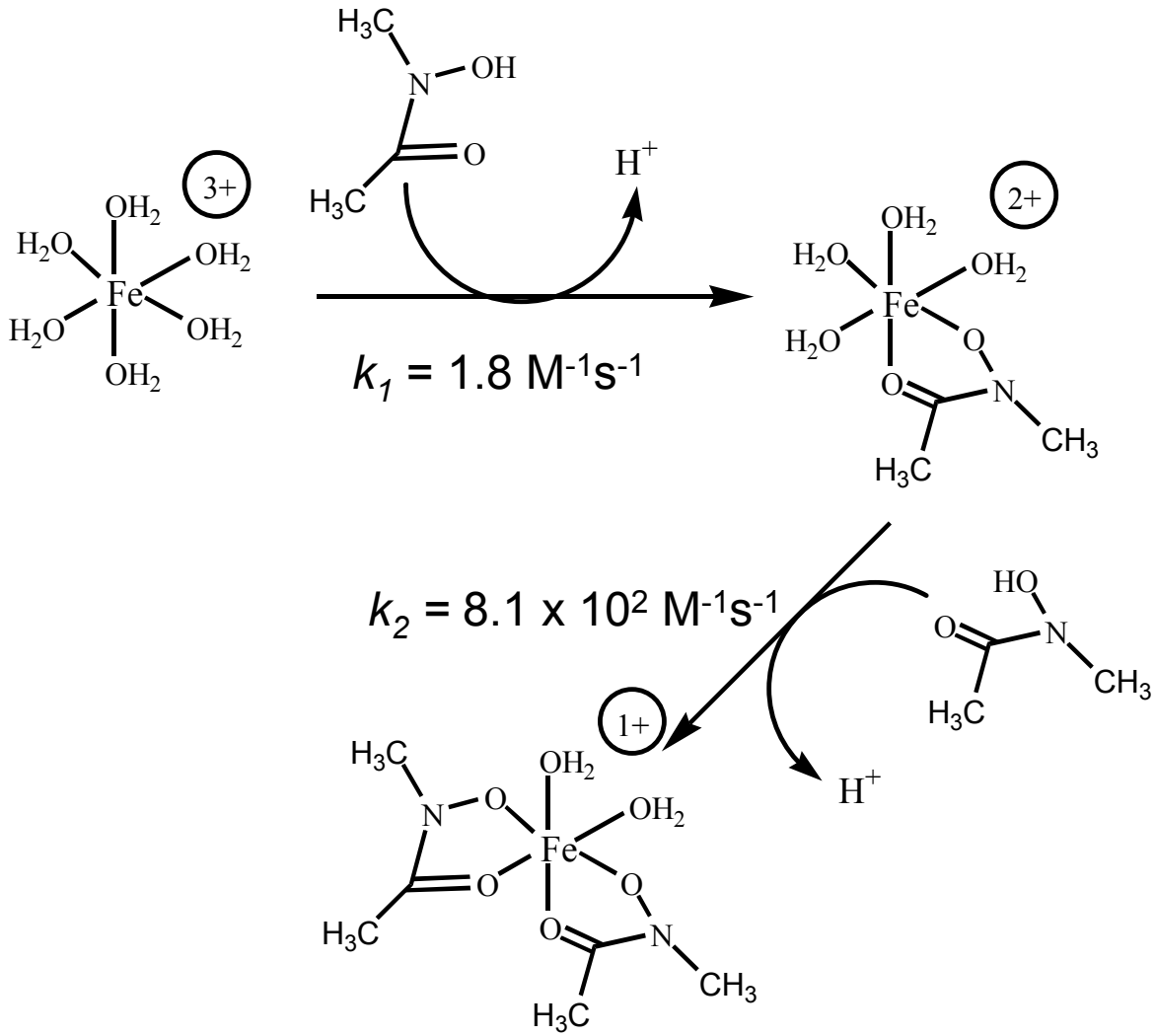
Plot of the reversible Fe(III/II) redox potential ($-E_{1/2}$) as a function of the stability of the complex, as expressed by pFe values (**Panel 9**). Data are for hexadentate (1,2,4), tetradentate (3,5) and bidentate (6,7,8) hydroxamic acid siderophores and siderophore mimics. Note that:

- as the stability of the Fe(III)-complex increases, the complex becomes more difficult to reduce; and
- the stability of the complex decreases with decreasing denticity.

References [6,10,13,14]

Influence of Fe(III)-Chelation on Kinetics

Iron chelate formation places a strong electron donor in the first coordination shell, which labilizes the remaining aquated coordination sites. This is illustrated here for the reaction of hexa(aquo)iron(III) with N-methylacetohydroxamic acid, a siderophore mimic. Incorporation of the bidentate hydroxamate group in the first coordination shell labilizes the remaining aquo ligands by a factor of ~500 (k_2/k_1).



Reference [5,15]

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Acknowledgements

I thank my co-workers, some of whose names appear in the References, for their hard work, questions, ideas, and intellectual stimulation. Our work in this area is supported by the National Science Foundation, the National Instutites of Health, and the American Chemical Society Petroleum Research Fund.