

# Basics of coordination chemistry in biological systems

Hendrik Küpper, Advanced Course on Bioinorganic Chemistry & Biophysics of Plants, summer semester 2025  
based on a lecture of Peter Kroneck, Universität Konstanz

# ***"Bioinorganic Chemistry & Biophysics of Plants"***

## **Introductory Books & References**

**Frausto da Silva, Williams, 2001**

**The biological chemistry of the elements, Oxford University Press**

**H. B. Gray, E. I. Stiefel, J. Selverstone Valentine, I. Bertini, 2007**

**Biological Inorganic Chemistry: Structure and Reactivity, University Science Books**

**R. R. Crichton, 2012**

**Biological Inorganic Chemistry, 2<sup>nd</sup> edition, Elsevier**

**Messerschmidt, Huber, Poulos, Wieghardt (eds), 2001; 2004; 2009 on-line**

**Handbook of Metalloproteins, John Wiley & Sons, LTD**

**Chemical Reviews, 1996; 2004**

**Special Issues on Bioinorganic Enzymology, 96, 2237; 104, 347**

# Useful websites for bioinorganic chemistry

→ <https://www.wwpdb.org/> Since 1971, the Protein Data Bank archive (PDB) has served as the single repository of information about the 3D structures of proteins, nucleic acids, and complex assemblies.

→ <https://www.brenda-enzymes.info/introduction.php>, <https://www.brenda-enzymes.org/>  
BRENDA is the main collection of enzyme functional data available to the scientific community. It is available free of charge via the internet

→ <https://www.softpedia.com/get/Others/Home-Education/Freshney-Periodic-Table.shtml> The best FREEWARE periodic table I know of, it contains a lot of information about each element incl. many physicochemical properties, isotopes, uses, history,...

→ <https://alphafoldserver.com/> AlphaFold Server allows you to model a structure consisting of many biological molecules via AI. This includes binding sites for common biologically important metals, but it does NOT reveal binding constants etc!

→ <https://combio.life.nctu.edu.tw/MIB2/> Metal Ion-Binding site prediction and modeling server. Very simple, easy to use web tool for getting a first glance a likely metal-binding residues incl. predicted binding constants. There are newer and more powerful tools, but they are much harder to use

# Elements that are known to be essential for plants

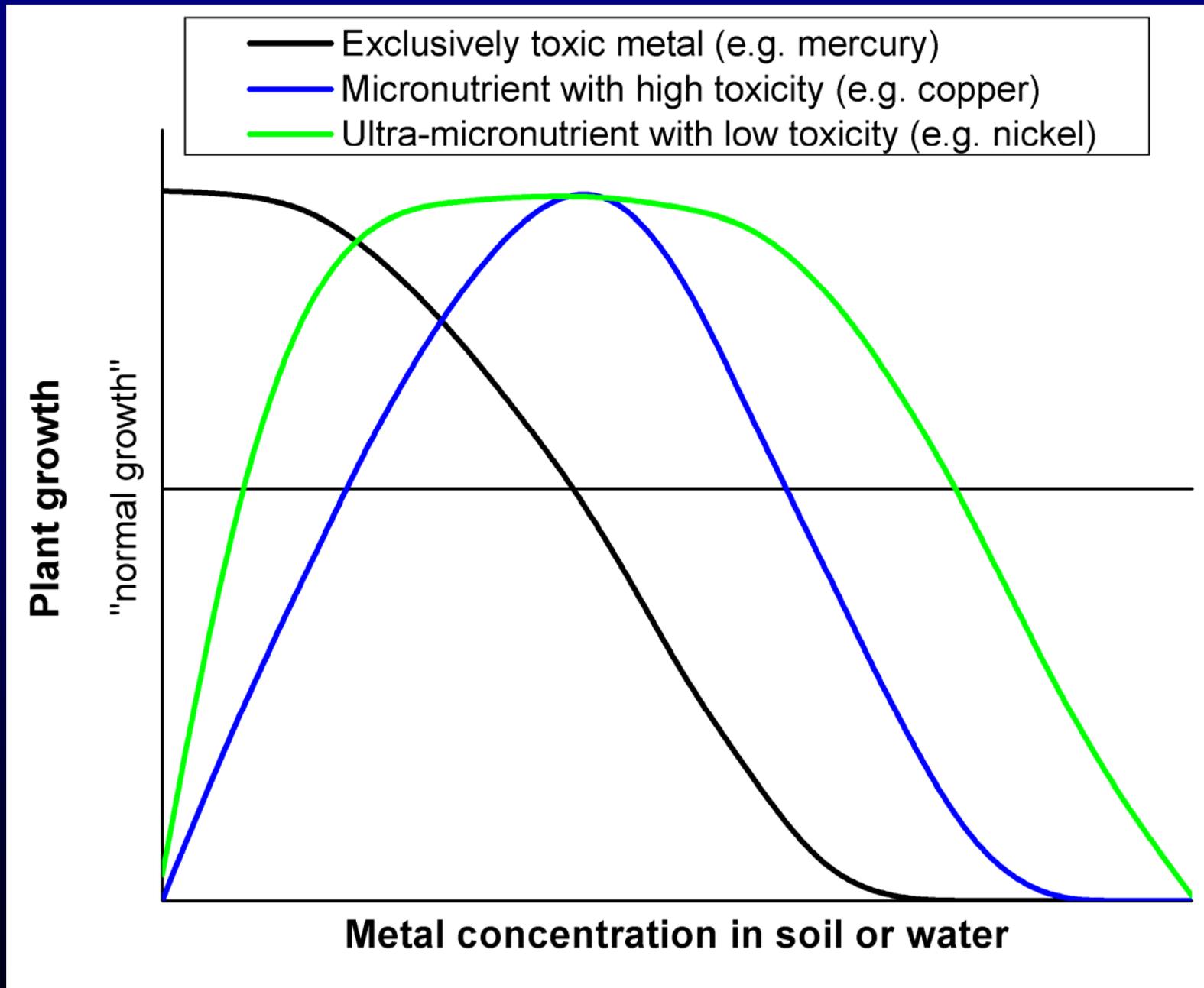
Essential and Beneficial Elements in Higher Plants																	
H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Lr	Rf	Db	Sg	Bh	Hs	Mt									
		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb		
		Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No		

→ In plants, chromium has so far always been regarded as only toxic, not beneficial or even essential. This should be reconsidered. In animals (incl. humans), an essential role of chromium is debated since the 1960s (still no consensus has been reached). This suggested role is activation of insulin, which does not exist in plants.

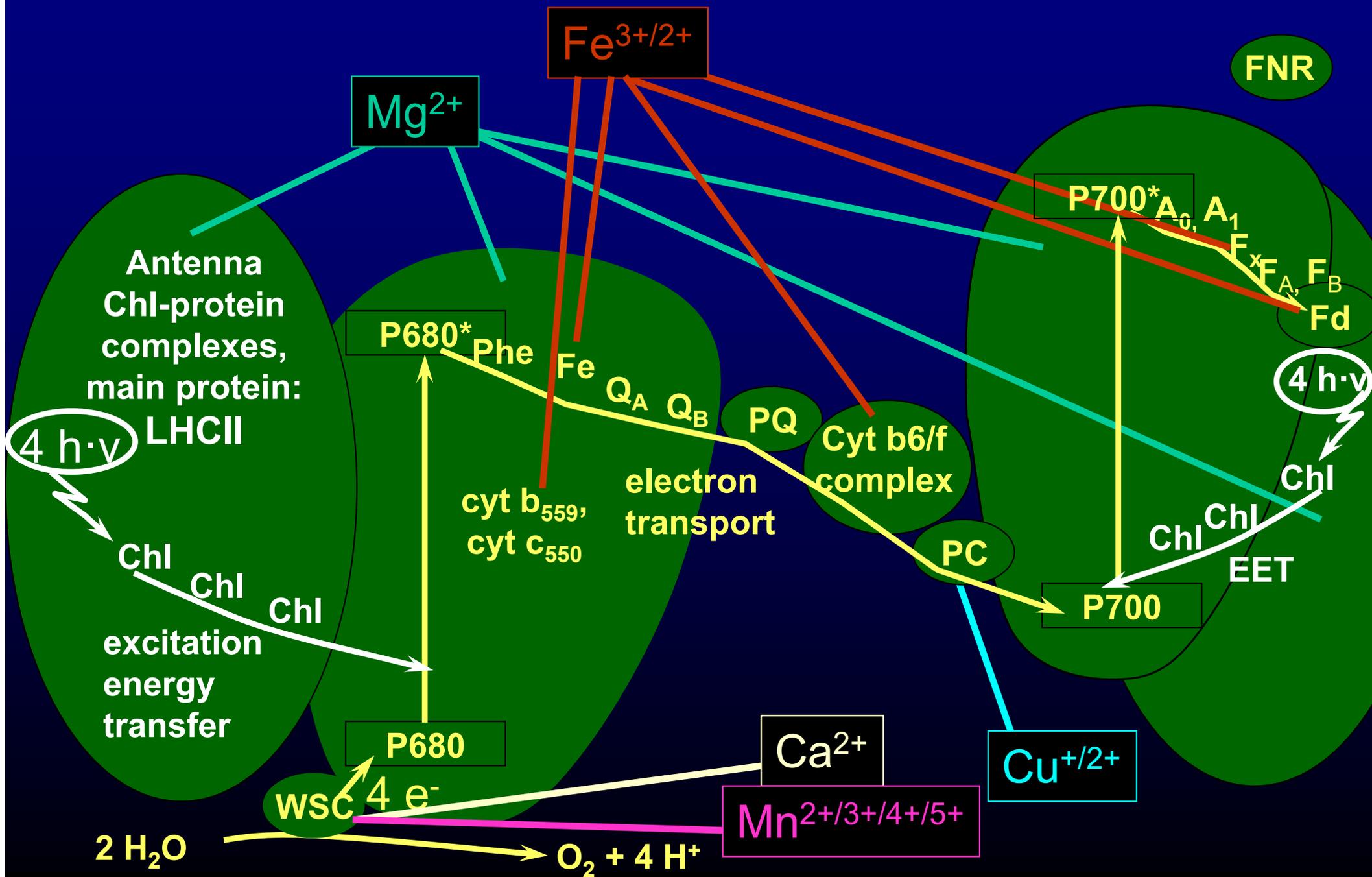
# Why Investigate Metals in Biology ?

- **There is hardly any important process in nature which does not depend on a metal ion; ~ 1/3 of the proteins of the human genome depend on metal ions**
- **Novel Materials, Structures and Reactions**
- **Trigger - Signaling - Sensing - Regulation**
- **Acid-Base Catalysis**
- **Redox – Proton & Electron Transfer  
(coupled, conservation of energy)**

# Dose-response principle for transition metals

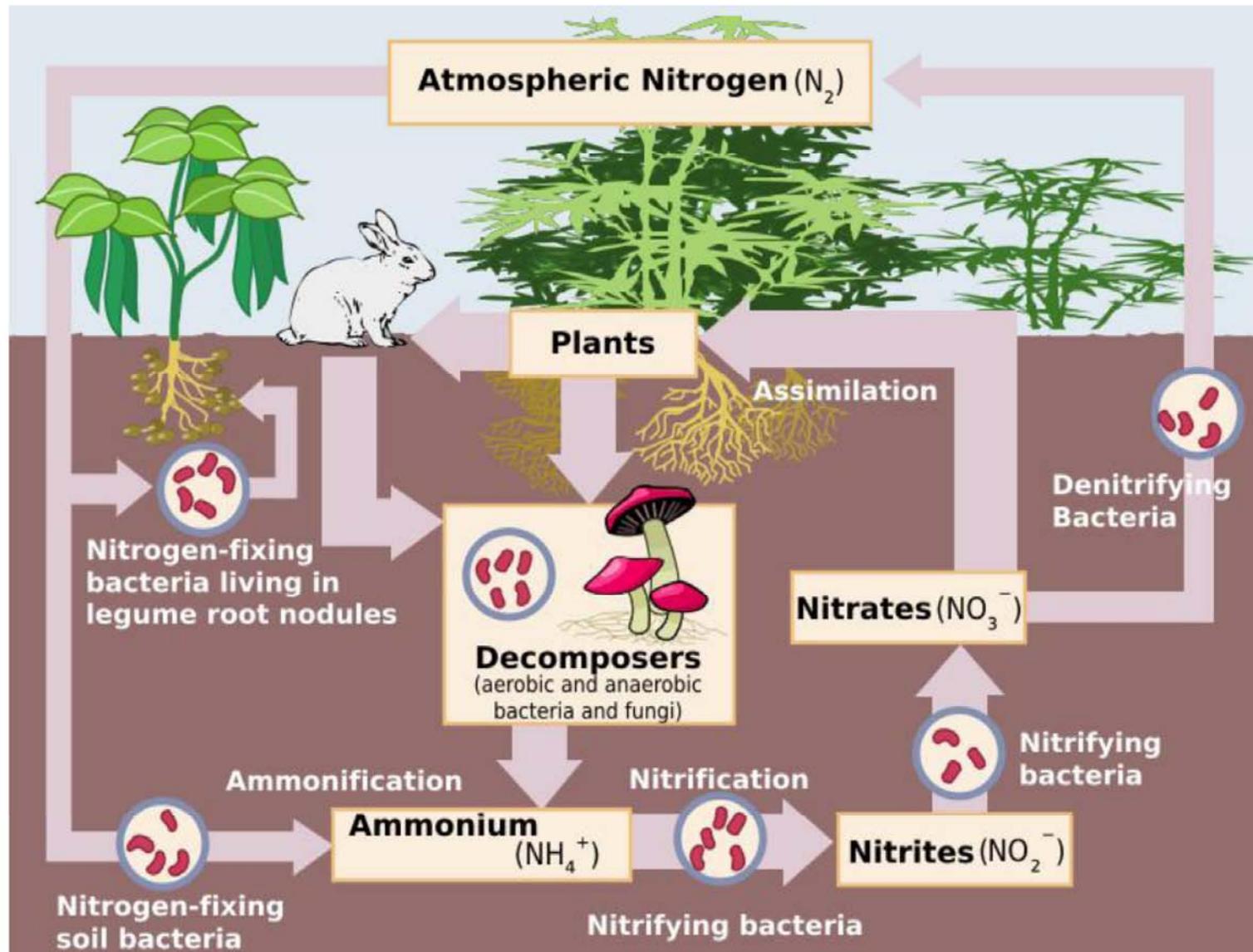


# Case 1: Metal sites in photosynthetic proteins



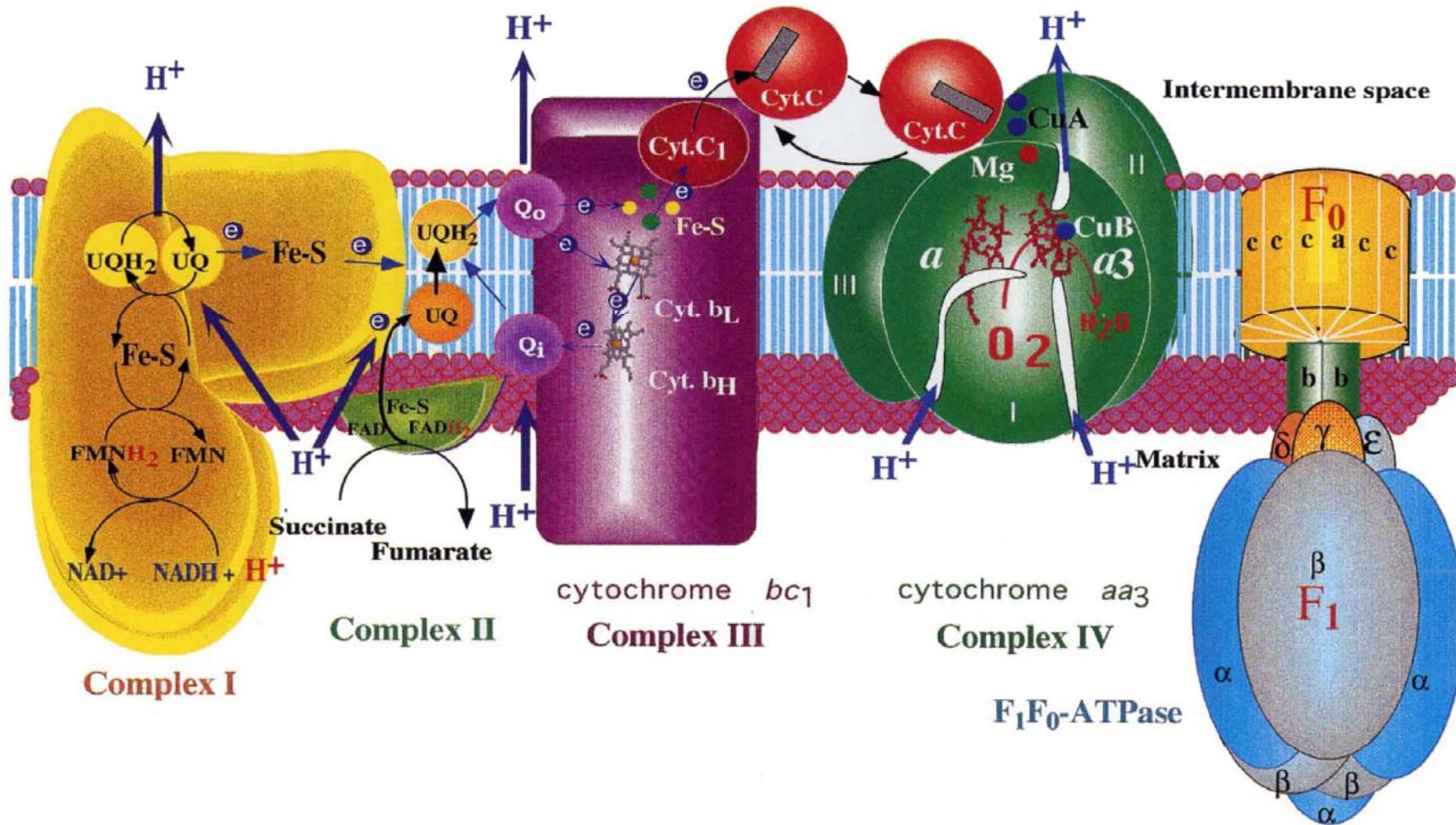
# Case 2: Nitrogen Fixation - Nitrogenase

[http://en.wikipedia.org/wiki/Nitrogen\\_cycle](http://en.wikipedia.org/wiki/Nitrogen_cycle)



# Case 3: Respiration – Reduction of $O_2$ to $H_2O$

## Synthesis of ATP – proton-coupled electron transfer (PCET)

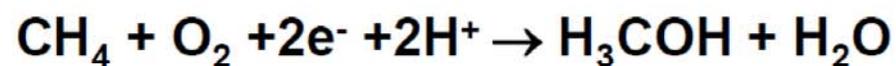
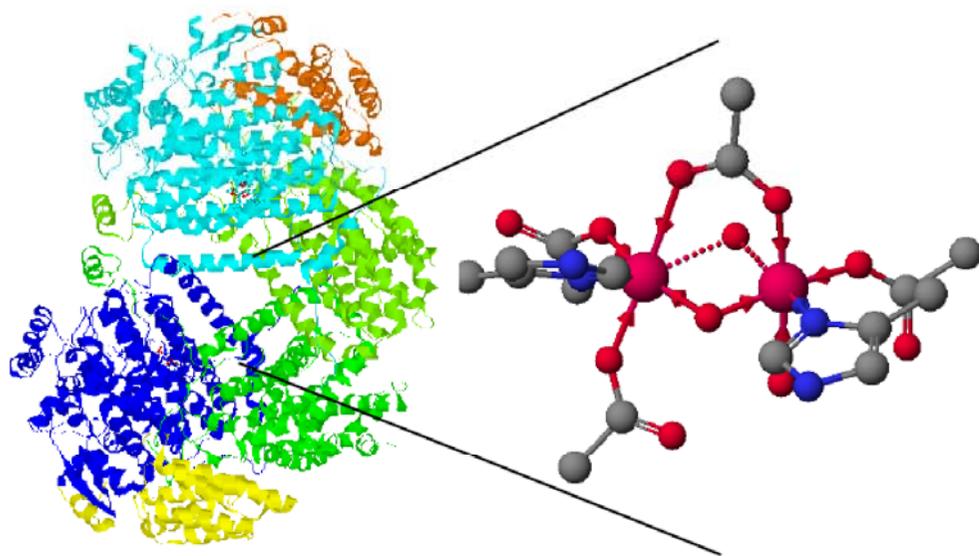
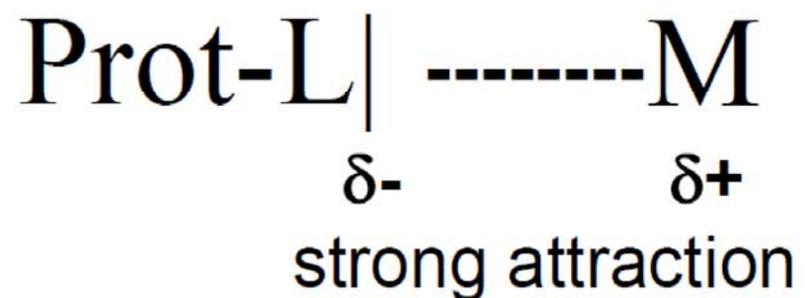


# Why (Transition)Metal Ions ?

- **Positively Charged**
    - Lewis Acids
    - Stabilization of Anions
  - **Loosely Bound Electrons**
    - Redox Active
    - Multiple Redox States
    - Easily tunable Redox Potential
  - **Coupled Redox/Acid Base Chemistry**
- **Open Shell Systems**
    - No Problems with Spin Restriction
  - **Stereochemically Flexible**
    - Large Variety of Structures.
    - Little Reorganization
    - Facile Ligand Addition/Dissociation
  - **Facilitate Reactions of Bound Ligands**

# Basic Features of a Metal Protein Complex

Chem. Rev. 1996, 96, 2239-2314 (1996) RH Holm, P Kennepohl, E I Solomon, Structural and Functional Aspects of Metal Sites in Biology

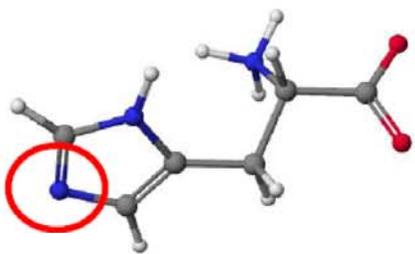


**Chemistry at the Catalytic Center  
(Active site) of the Iron Enzyme  
Methane Monooxygenase**

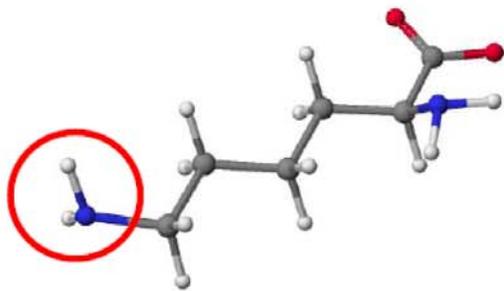
# Protein Ligands – Amino Acid Residues

**N**

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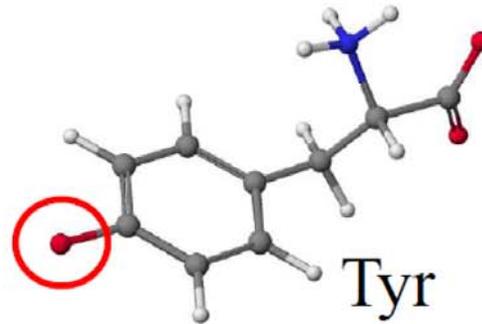
His



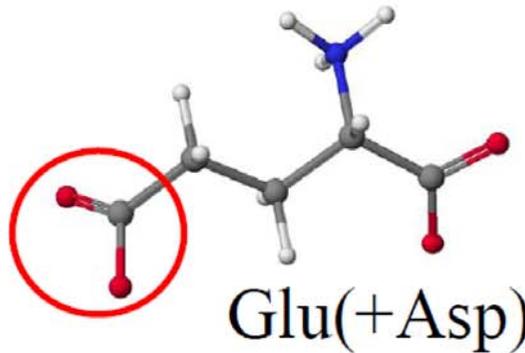
Lys

**O**

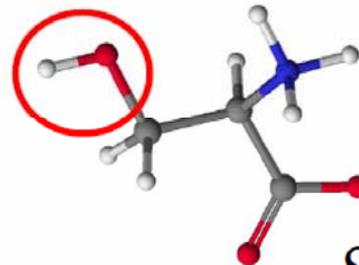
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Tyr



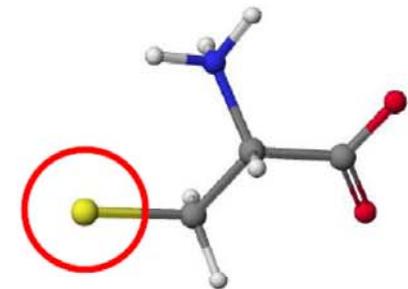
Glu(+Asp)



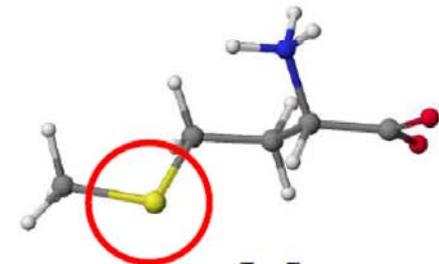
Ser

**S**

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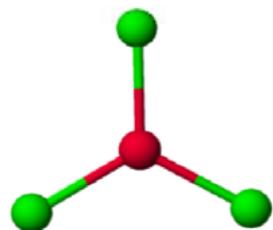
Cys



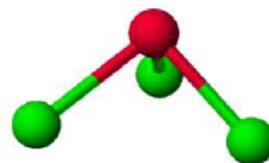
Met

# Geometry – Coordination Number

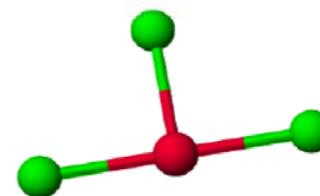
3



Trigonal

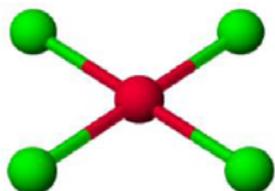


Trigonal pyramidal

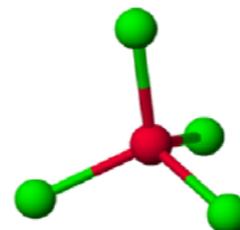


T-shape

4

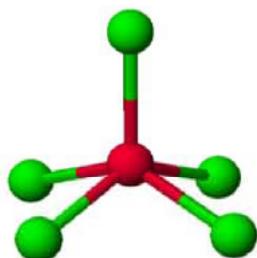


Square planar

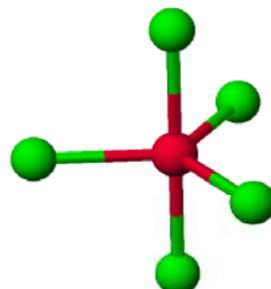


Tetrahedral

5

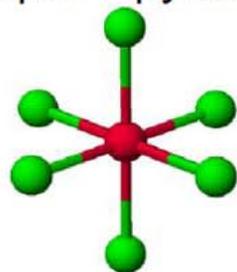


Square pyramidal



Trigonal bipyramidal

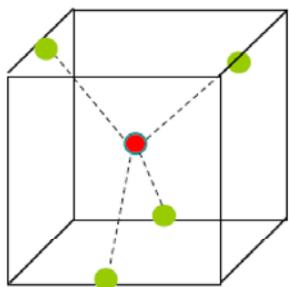
6



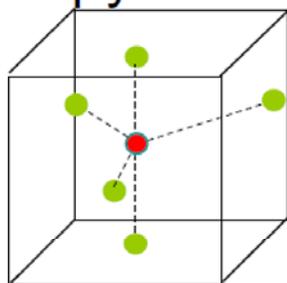
Octahedral

# Geometry is important: Iron Proteins

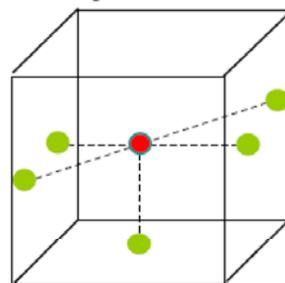
Tetrahedron



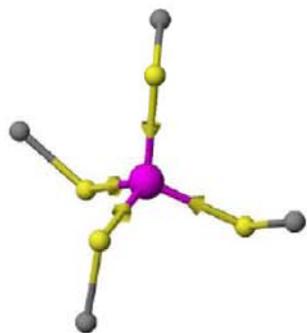
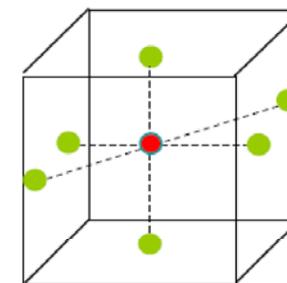
Trigonal Bipyramide



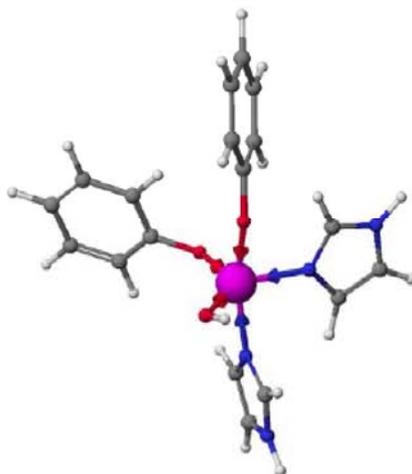
Tetragonal Pyramide



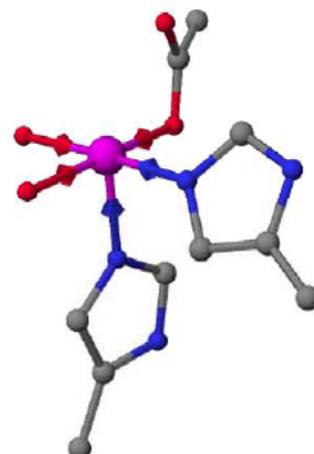
Octahedron



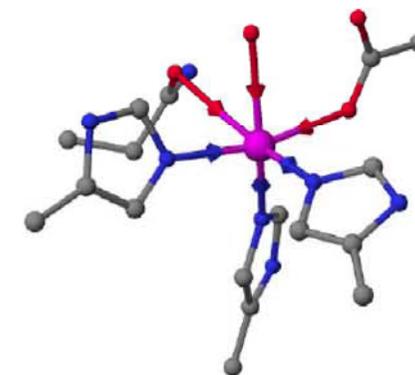
Rubredoxin



3,4-Protocatechoate  
Dioxygenase



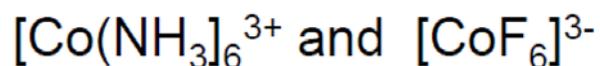
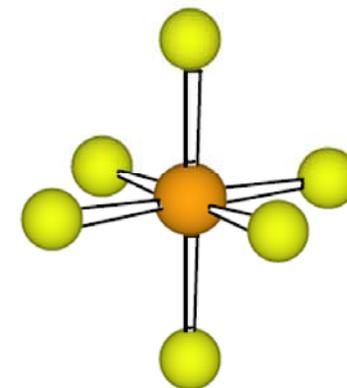
Tyrosine  
Hydroxylase



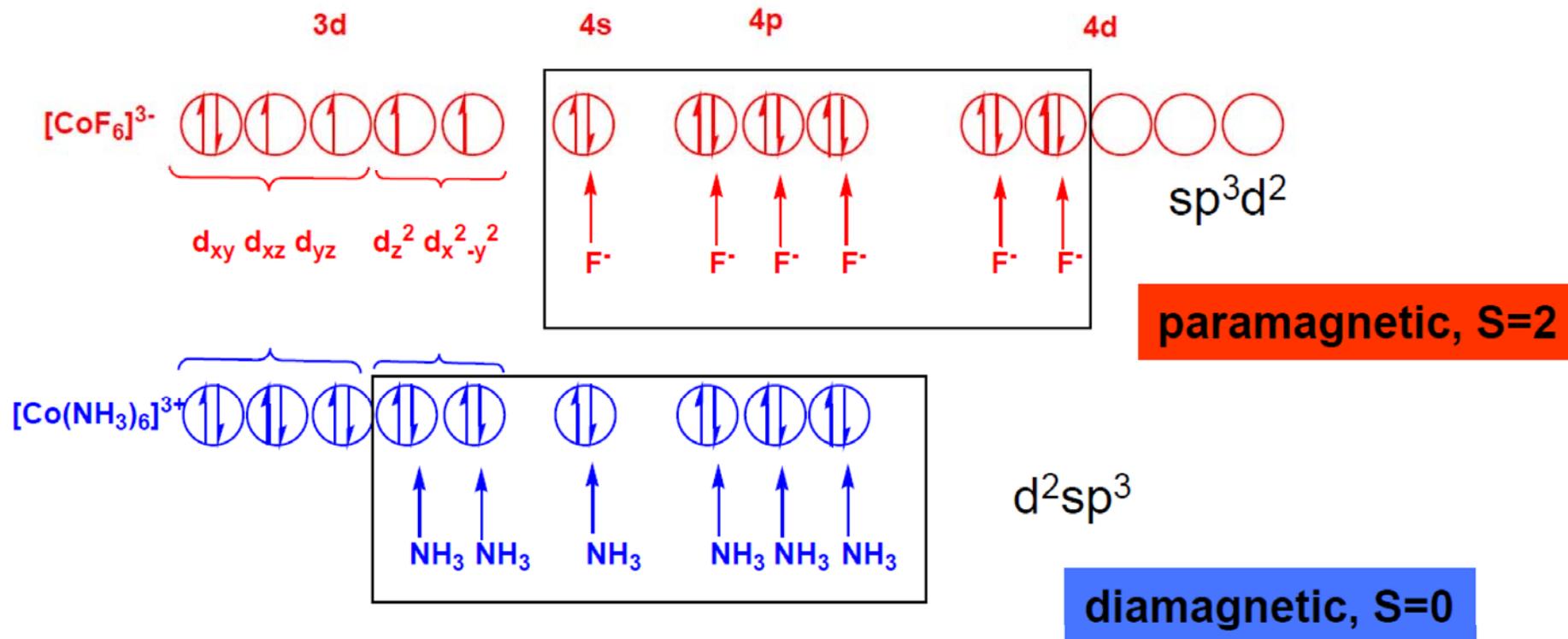
Lipoxygenase

# Remember: Valence Bond Theory

*L. Pauling*



$d^6$

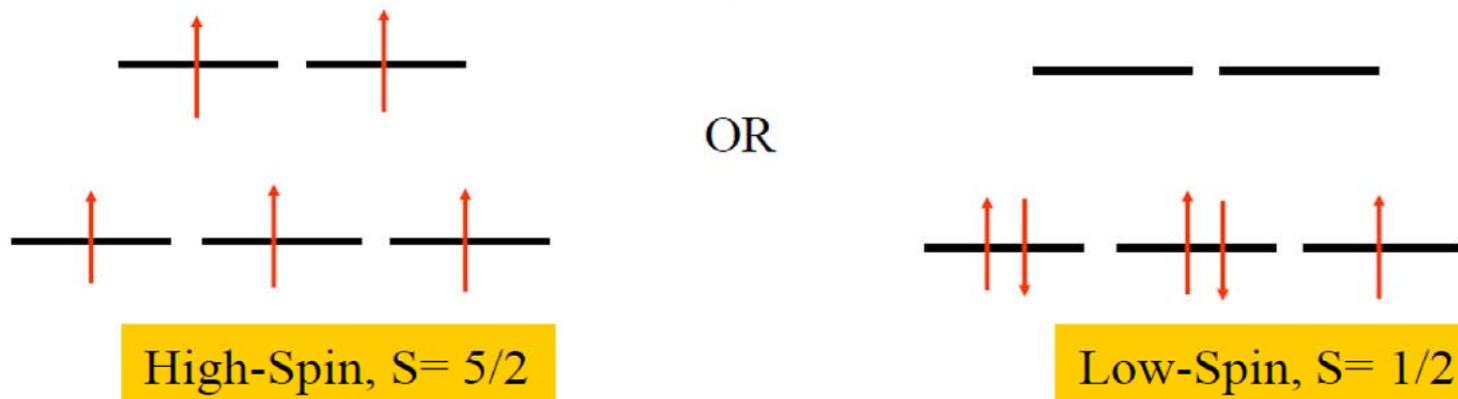


$sp^3d^2$  and  $d^2sp^3$  hybridization

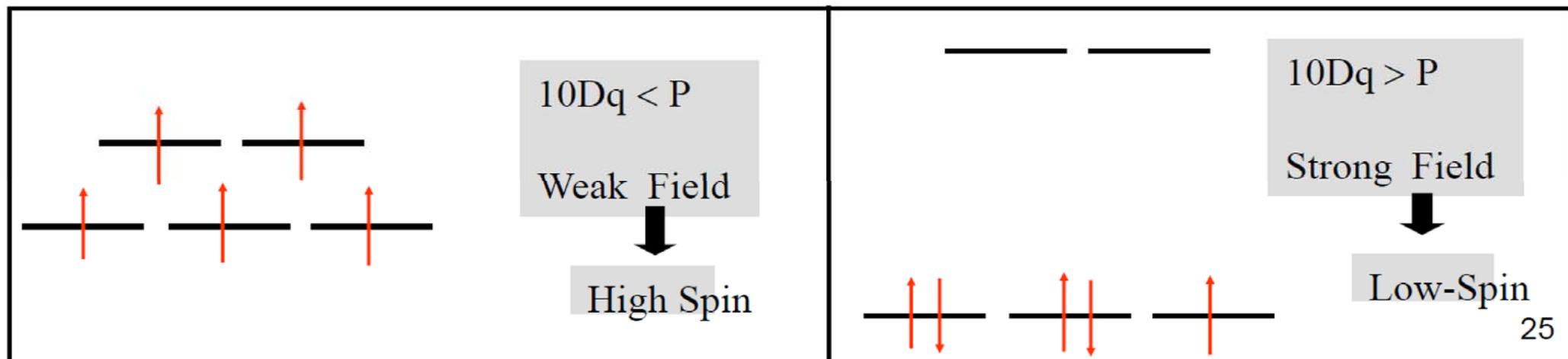
# Color and Magnetism

## Variable Spin States of Metal Centers

### For a $d^5$ configuration, Fe(III)



Depending on the METAL ION ENVIRONMENT, balance of Crystal Field Splitting,  $10Dq$  and Spin-Pairing Energy,  $P$



# Metals – Biological Functions

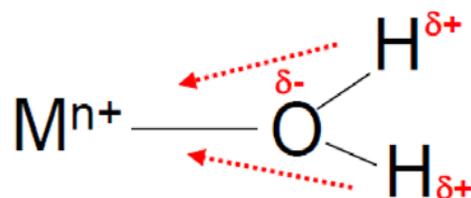
Metal	Function, Enzymes
Na	Charge Carrier, Osmolysis/equilibrium
K	Charge Carrier, Osmolysis/equilibrium
Mg	Structure, ATP/ThDP Binding, Photosynthesis,...
Ca	Structure, Signaling, Charge Carrier
V	Nitrogen Fixation, Oxidases, O <sub>2</sub> Carrier
Cr	<b><i>Unknown! (glucose metabolism ???)</i></b>
Mo	Nitrogen Fixation, Oxidoreductase, O-Transfer
W	Oxidoreductases, Acetylene Hydratase
Mn	Photosynthesis, Oxidases, Structure,...
Fe	Oxidoreductase, O <sub>2</sub> Transport + Activation, e <sup>-</sup> -Transfer,...
Co	Oxidoreductase, Vitamin B <sub>12</sub> (Alkyl Group Transfer)
Ni	Hydrogenase, CO Dehydrogenase, Hydrolases, Urease
Cu	Oxidoreductases, O <sub>2</sub> Transport, e <sup>-</sup> -Transfer
Zn	Structure, Hydrolases, Acid-Base Catalysis...

# Oxidation States of Metals in Biology

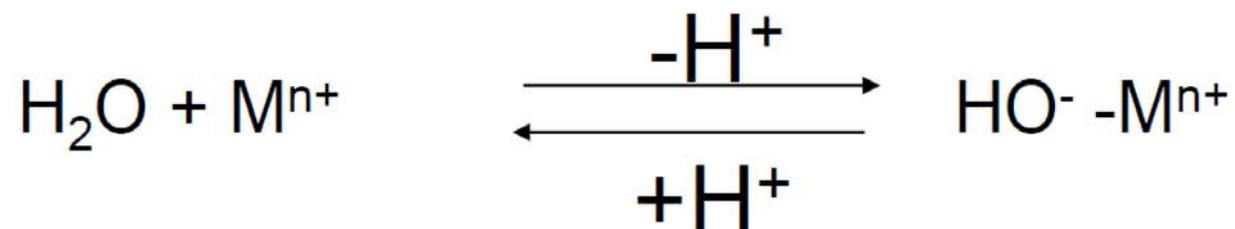
Metal	Valence state (Electron configuration)
<b>Na</b>	Na(I)
<b>K</b>	K(I)
<b>Mg</b>	Mg(II)
<b>Ca</b>	Ca(II)
<b>V</b>	V(V)=(d <sup>0</sup> ), V(IV)=(d <sup>1</sup> ), V(III)=(d <sup>2</sup> )
<b>Cr</b>	Cr(III)=(d <sup>3</sup> ), Cr(IV)=(d <sup>2</sup> ), Cr(V)=(d <sup>1</sup> )
<b>Mo</b>	Mo(III)=(d <sup>3</sup> ), Mo(IV)=(d <sup>2</sup> ), Mo(V)=(d <sup>1</sup> ), Mo(VI)=(d <sup>0</sup> )
<b>W</b>	W(IV)=(d <sup>2</sup> ), W(V)=(d <sup>1</sup> ), W(VI)=(d <sup>0</sup> )
<b>Mn</b>	Mn(V)=(d <sup>2</sup> ), Mn(IV)=(d <sup>3</sup> ), Mn(III)=(d <sup>4</sup> ), Mn(II)=(d <sup>5</sup> )
<b>Fe</b>	Fe(V)=(d <sup>3</sup> ), Fe(IV)=(d <sup>4</sup> ), Fe(III)=(d <sup>5</sup> ), Fe(II)=(d <sup>6</sup> ), Fe(I)?=(d <sup>7</sup> )
<b>Co</b>	Co(III)=(d <sup>6</sup> ), Co(II)=(d <sup>7</sup> ), Co(I)=(d <sup>8</sup> )
<b>Ni</b>	Ni(III)=(d <sup>7</sup> ), Ni(II)=(d <sup>8</sup> ), Ni(I)=(d <sup>9</sup> )
<b>Cu</b>	Cu(III)=(d <sup>8</sup> ), Cu(II)=(d <sup>9</sup> ), Cu(I)=(d <sup>10</sup> )
<b>Zn</b>	Zn(II)=(d <sup>10</sup> )

# Exogenous ligands

	Ligand	pK <sub>a</sub>
Acid/base	H <sub>2</sub> O/OH <sup>-</sup> , O <sup>2-</sup>	14, ~34
	HCO <sub>3</sub> <sup>-</sup> /CO <sub>3</sub> <sup>2-</sup>	10.3
	HPO <sub>4</sub> <sup>2-</sup> /PO <sub>4</sub> <sup>3-</sup>	12.7
	H <sub>3</sub> CCOO <sup>-</sup> /H <sub>3</sub> CCOOH	4.7
	HO <sub>2</sub> <sup>-</sup> /H <sub>2</sub> O <sub>2</sub>	11.6
	NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	9.3
	N <sub>3</sub> <sup>-</sup> /N <sub>3</sub> H	4.8
	F <sup>-</sup> , Cl <sup>-</sup> , Br <sup>-</sup> , I <sup>-</sup> /XH	3.5, -7, -9, -11
Neutral	O <sub>2</sub> , CO, NO, RNC	

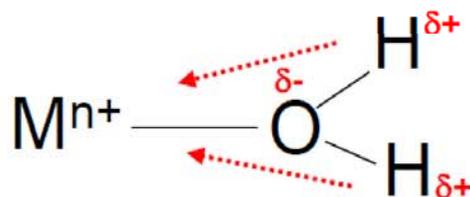


# Modulation of pK<sub>a</sub>

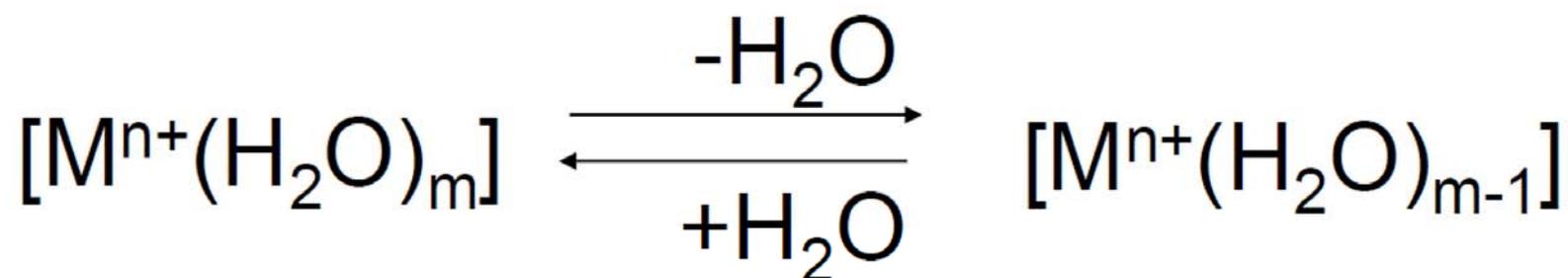


Metal	pK <sub>a</sub>
none	14.0
Ca <sup>2+</sup>	13.4
Mn <sup>2+</sup>	11.1
Cu <sup>2+</sup>	10.7
Zn <sup>2+</sup>	10.0

} 4 orders of magnitude !



# Kinetic Control



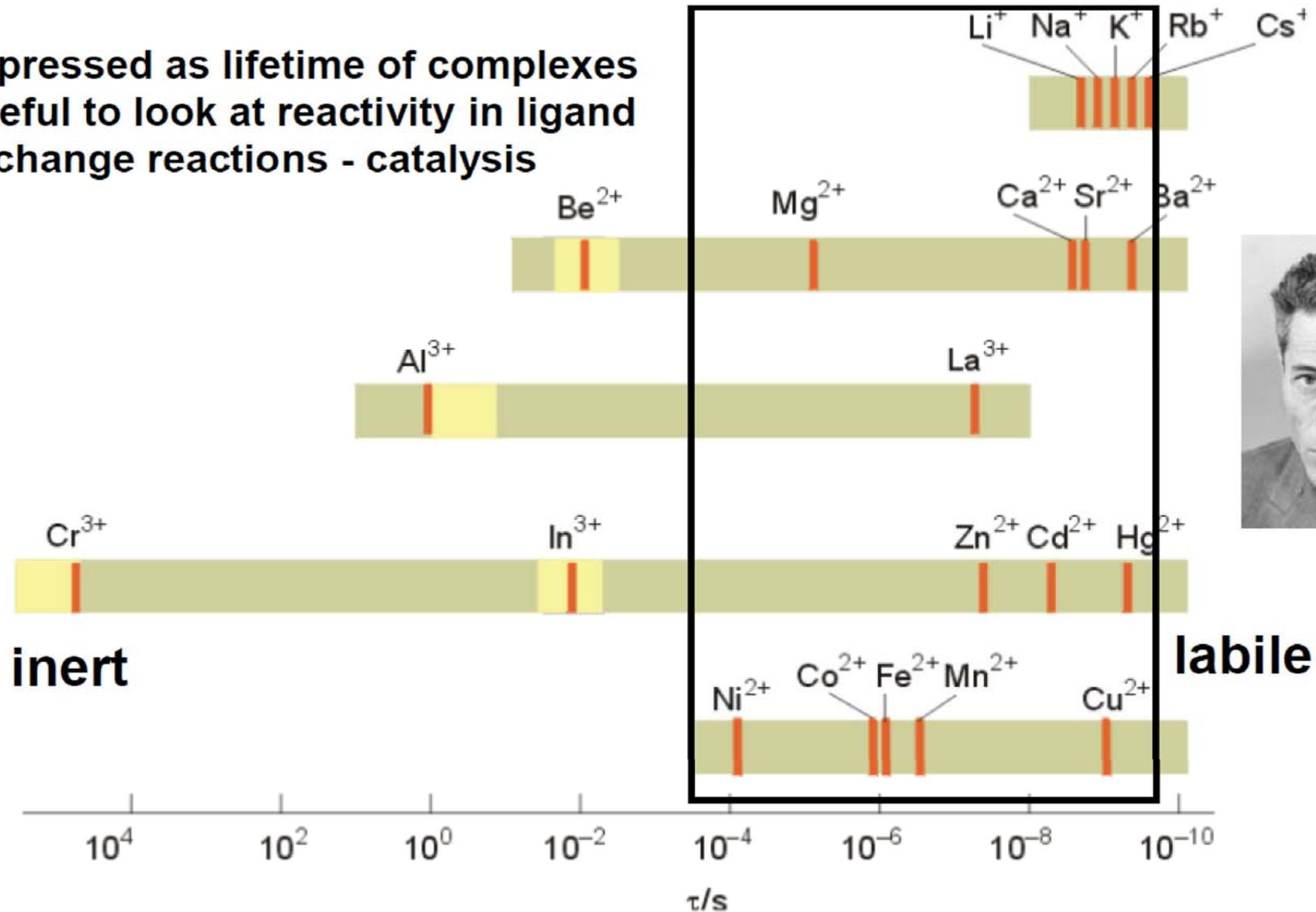
Metal	k (s <sup>-1</sup> )
K <sup>+</sup>	1x10 <sup>9</sup>
Ca <sup>2+</sup>	3x10 <sup>8</sup>
Mn <sup>2+</sup>	2x10 <sup>7</sup>
Fe <sup>2+</sup>	4x10 <sup>6</sup>
Co <sup>2+</sup>	3x10 <sup>6</sup>
Ni <sup>2+</sup>	4x10 <sup>4</sup>
Fe <sup>3+</sup>	2x10 <sup>2</sup>
Co <sup>3+</sup>	<10 <sup>-6</sup>

15 orders of magnitude!

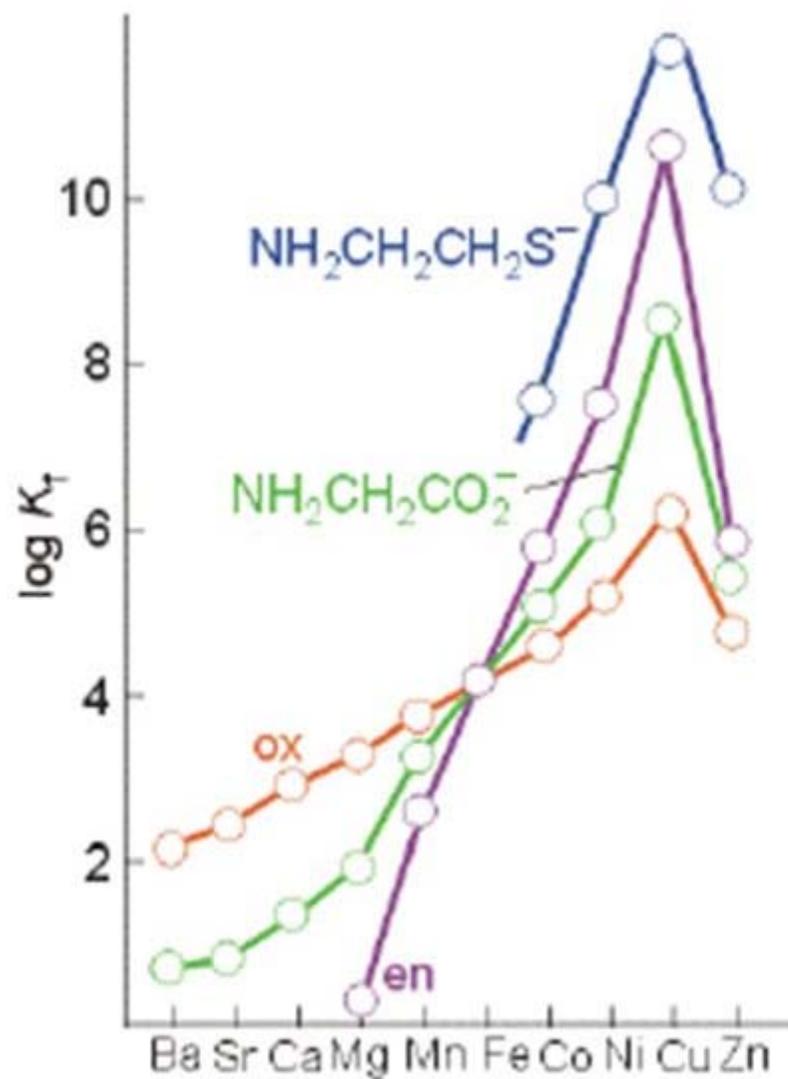
# Water exchange rates

M. Eigen, Nobel Prize Lecture 1967

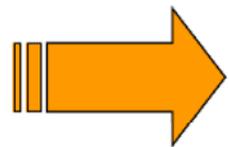
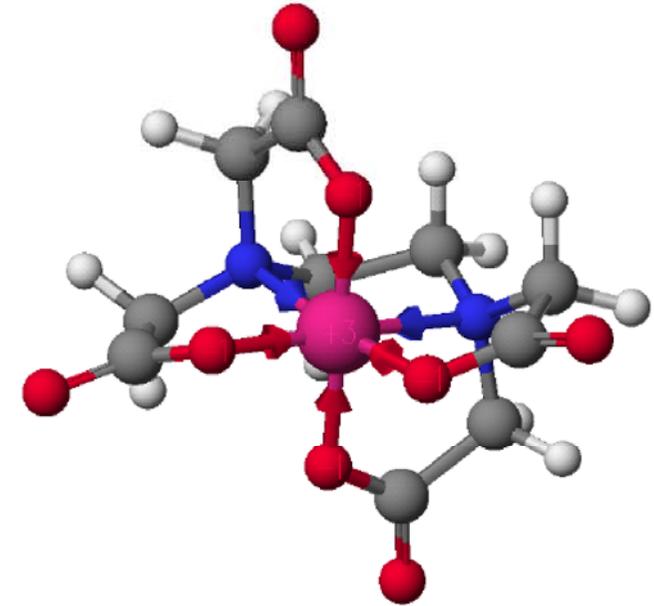
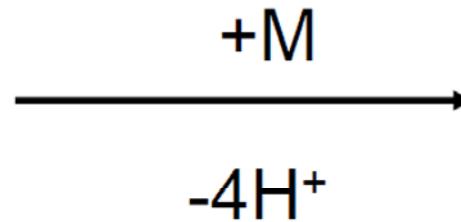
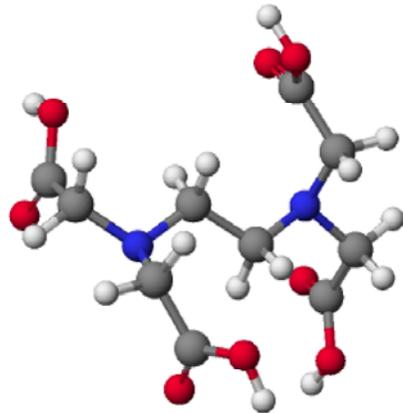
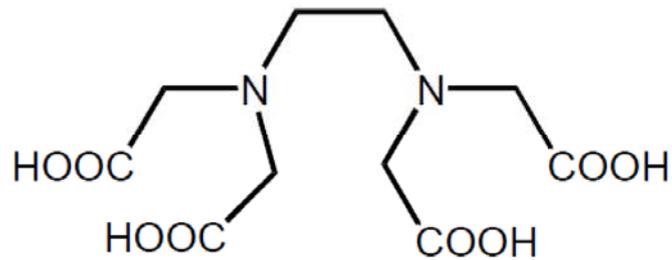
Expressed as lifetime of complexes  
Useful to look at reactivity in ligand  
exchange reactions - catalysis



# Stability of Metal Ion Complexes: The Irving-Williams Series

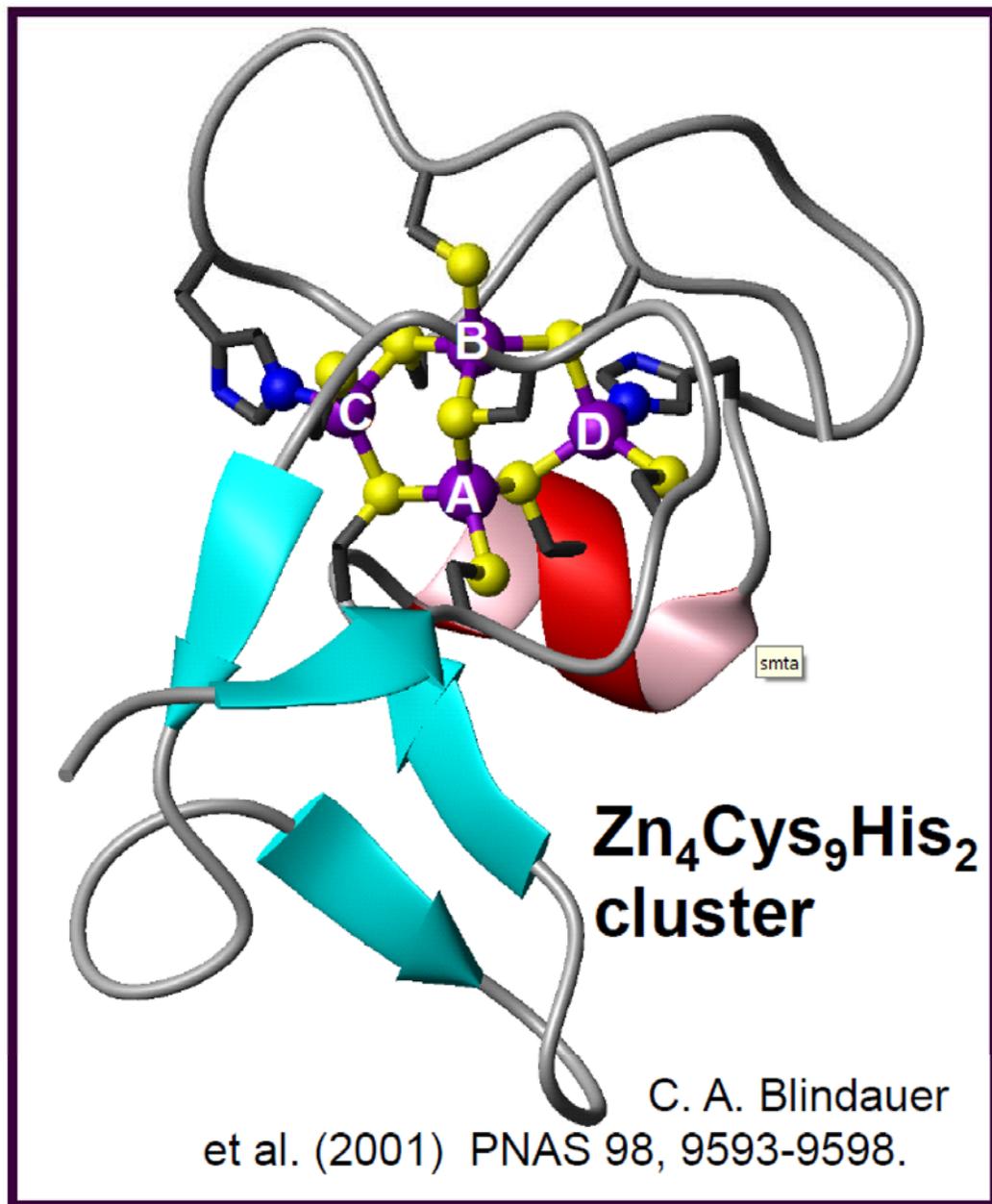


# Strong chelating ligand: EDTA



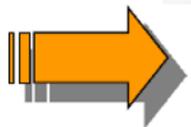
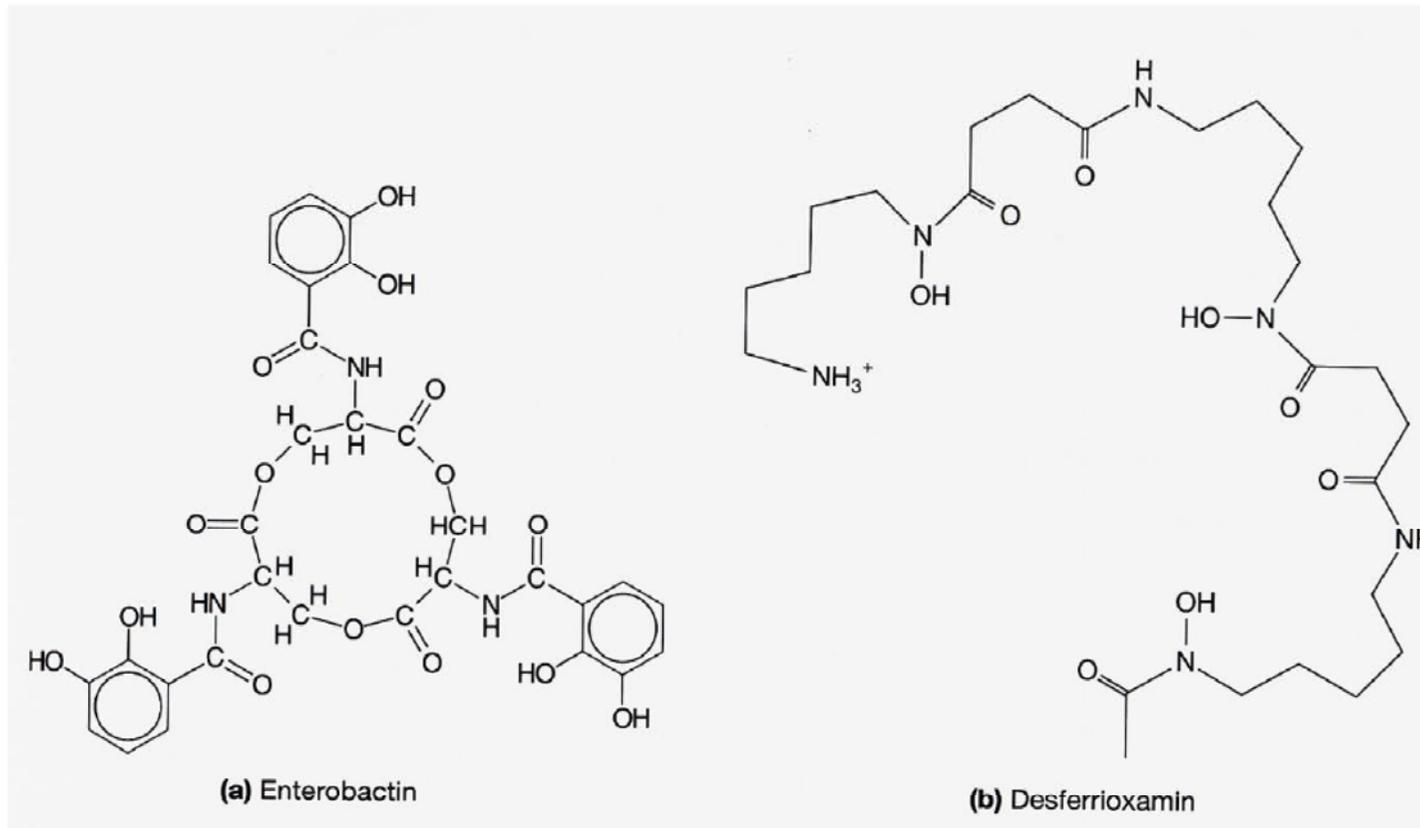
**Hexadentate Ligand => strong complexing agent; can be applied to remove metal ions from biological samples (proteins, nucleic acids).**

# Protein Chelate: Bacterial Metallothionein (MT)



- 55 amino acids
- One domain
- Not only Cys, but also 2 His
- Cluster similar to mammalian MT: Essentially a distorted piece of mineral (ZnS)

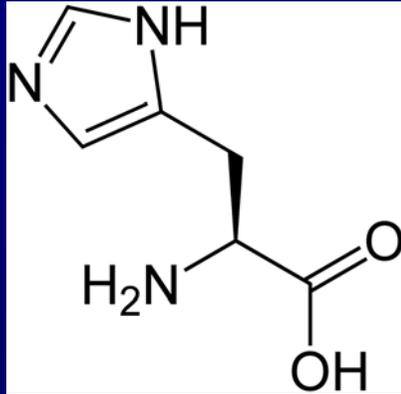
# Biological Chelate: Siderophores



**Extremely stable complex of Enterobactin/Fe<sup>3+</sup>  $K \sim 10^{49}$**

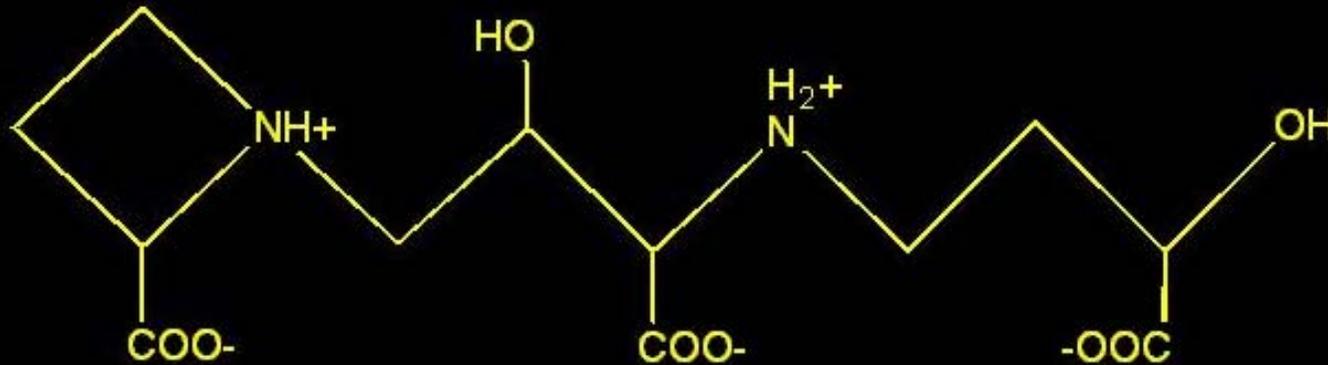
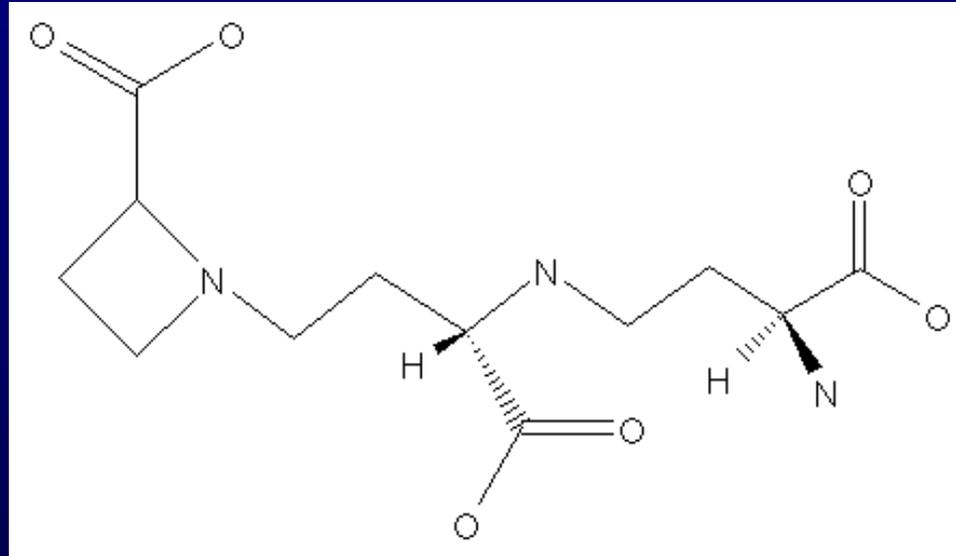
**Release of Fe through a) degradation of ligand, or b) protonation and reduction to Fe<sup>2+</sup> which binds much weaker to the siderophore.**

# Long-distance transport ligands in plants



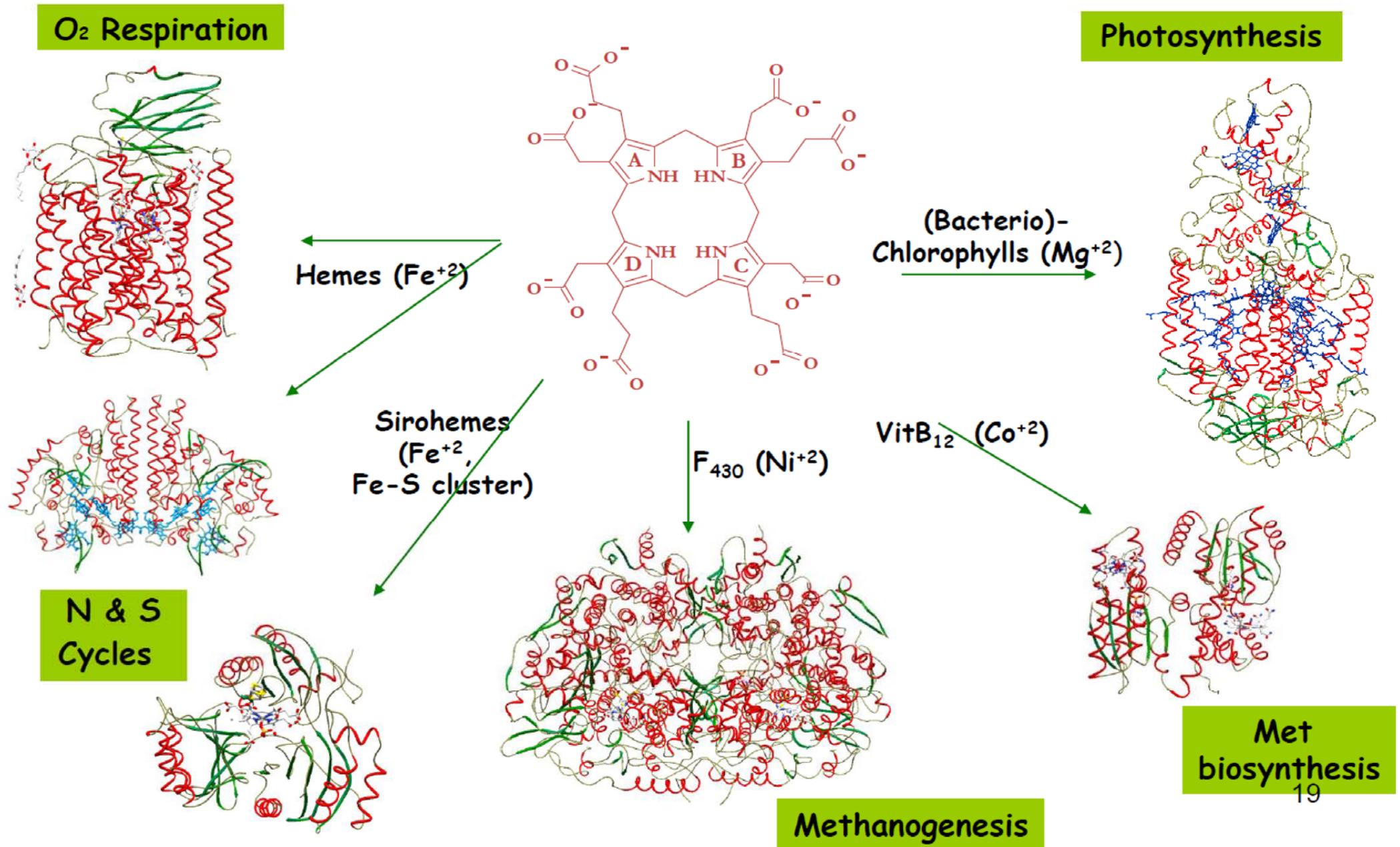
Histidine

Nicotianamine



Mugineic acid

# Tetrapyrrole - Versatile Ligand in Biology



# Hard and Soft Acid-Base (HSAB) Principle

„Hard“ Ligands prefer „hard“ Metal ions

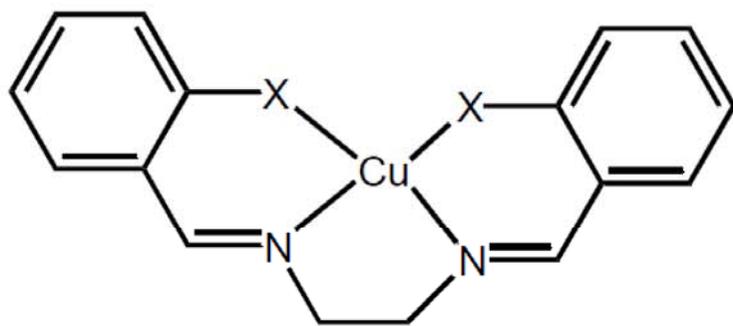
—————> Ionic Bonds

„Soft“ Ligands prefer „soft“ Metal ions

—————> Covalent Bonds

Metal	Ligand
<b><u>Hard</u></b> H <sup>+</sup> , Na <sup>+</sup> , K <sup>+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> Mn <sup>2+</sup> , Cr <sup>3+</sup> , Co <sup>3+</sup> , Fe <sup>3+</sup>	<b><u>Hard</u></b> H <sub>2</sub> O, OH <sup>-</sup> , R-COO <sup>-</sup> , CO <sub>3</sub> <sup>2-</sup> NH <sub>3</sub> , NO <sub>3</sub> <sup>-</sup> , R-NH <sub>2</sub> , R-O <sup>-</sup> , ROR
<b><u>Borderline</u></b> Fe <sup>2+</sup> , Ni <sup>2+</sup> , Zn <sup>2+</sup> , Mg <sup>2+</sup> , Ca <sup>2+</sup> Co <sup>2+</sup> , Cu <sup>2+</sup>	<b><u>Borderline</u></b> NO <sub>2</sub> <sup>-</sup> , N <sub>2</sub> , SO <sub>3</sub> <sup>2-</sup> , N <sub>3</sub> <sup>-</sup> , Ph-NH <sub>2</sub> Imidazole
<b><u>Soft</u></b> Cu <sup>+</sup> , Pt <sup>2+</sup> , Au <sup>+</sup> , Hg <sup>2+</sup> , Cd <sup>2+</sup>	<b><u>Soft</u></b> R <sub>2</sub> S, RS <sup>-</sup> , R <sub>3</sub> P, CN <sup>-</sup> , SCN <sup>-</sup> , O <sup>2-</sup> S <sup>2-</sup> , R <sup>-</sup> , H <sup>-</sup>

# Modulation/tuning of Redox Potentials $E_{1/2}$

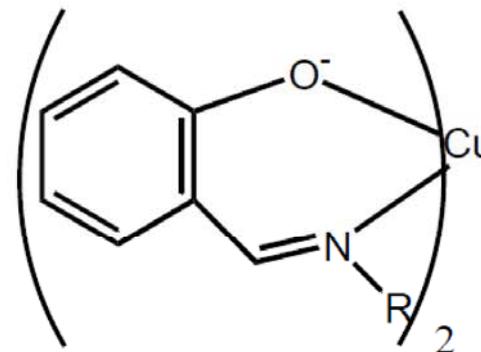


$$X=O^-: E_{1/2} = -1.21 \text{ V}$$

$$X=S^-: E_{1/2} = -0.83 \text{ V}$$

➡ **Soft Ligand ( $RS^-$ )  
stabilizes Cu(I) state**

➡ **Positive Potential**



$$R=CH_3 : E_{1/2} = -0.90 \text{ V}$$

$$R=C_2H_5 : E_{1/2} = -0.86 \text{ V}$$

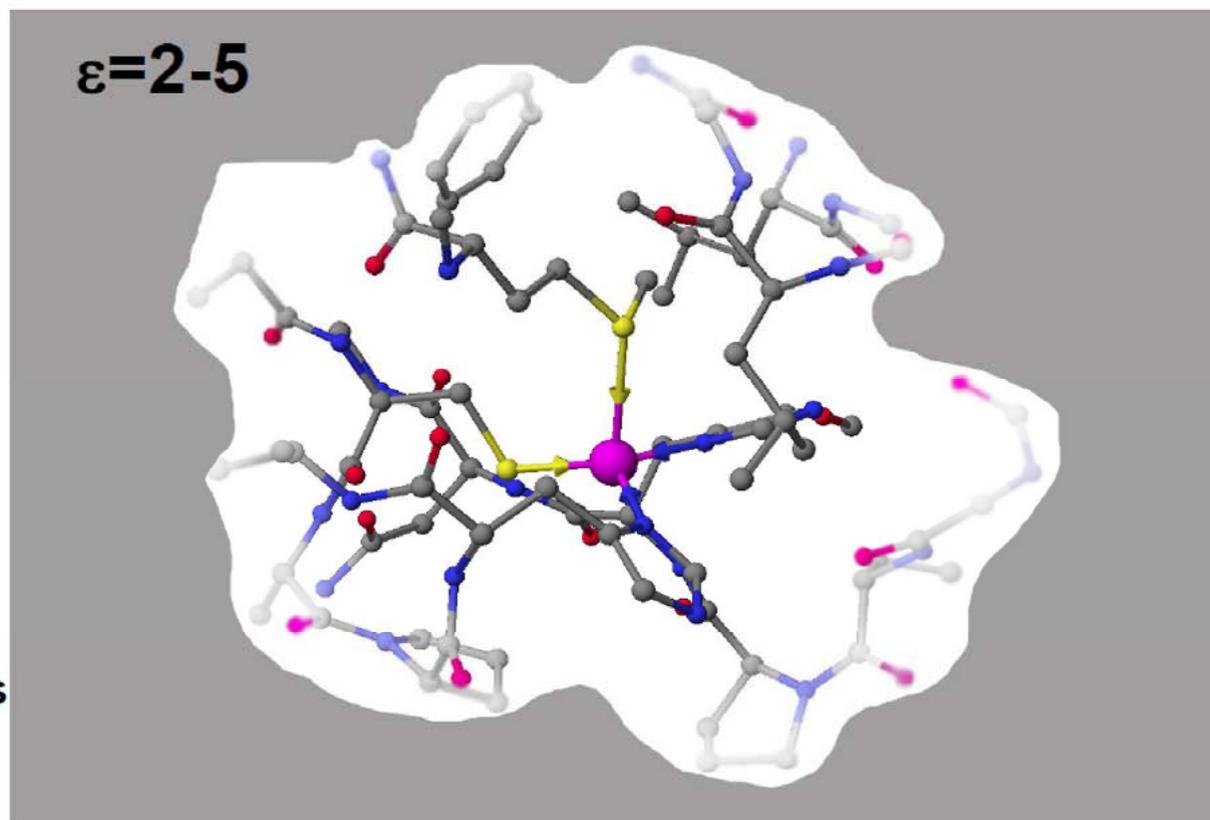
$$R=i\text{-Pr} : E_{1/2} = -0.74 \text{ V}$$

$$R=t\text{-Bu} : E_{1/2} = -0.66 \text{ V}$$

➡ **Steric hindrance forces  
tetrahedral geometry,  
stabilizes Cu(I)**

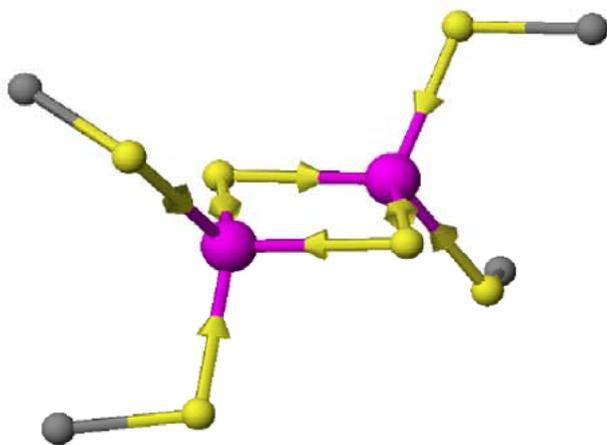
# Influence of Protein Environment

- Stabilization of unfavorable metal-ligand combinations
- Low polarity
  - Hydrophobic chemistry
- Preformed sites
  - „Entatic State“
- Substrate specific channels and bindings sites
- Fine-tuned acid/base chemistry
- Local production of intermediates – transition states



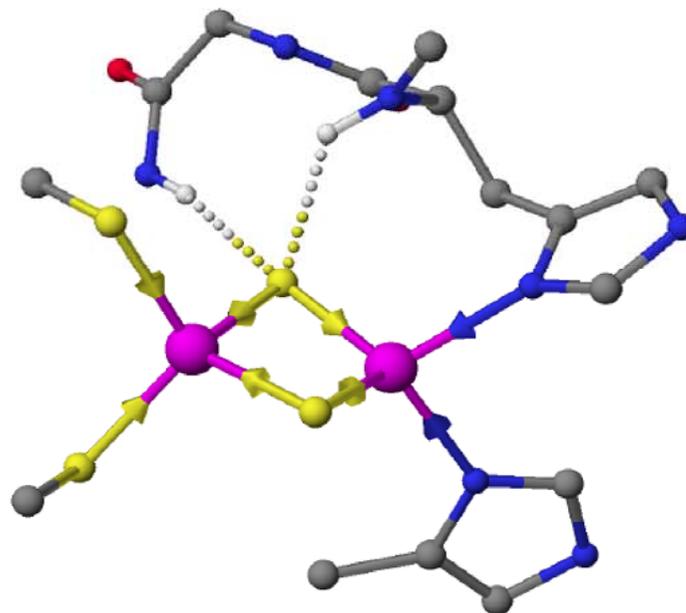
# Modulation of Redox potentials (H bridges)

## 2Fe-2S Ferredoxin



$E^0 \sim -400 \text{ mV}$

## 2Fe-2S Rieske



$E^0 \sim +280 \text{ mV}$

(+150 mV without H bridges)

- (a) Stephens, P.J.; Jollie, D.R.; Warshel, A. (1996) *Chem. Rev.*, 96, 2491  
(b) Link, T.A. (1999) *Adv. Inorg. Chem.*, 47, 83

# Proteins Tune the Properties of Metal Ions

## Coordination number

- The lower the higher the Lewis acidity

## Coordination geometry

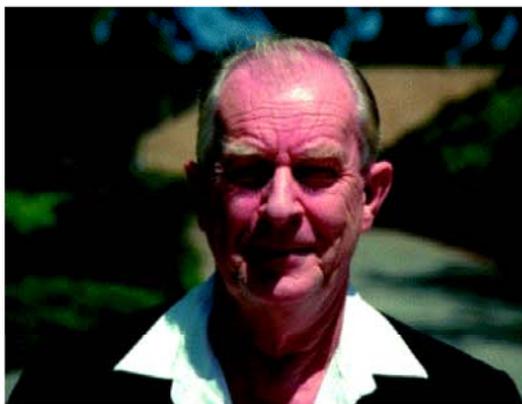
- Proteins can dictate distortion
- Distortion can change reactivity of metal ion

## Weak interactions - Second Shell Effects

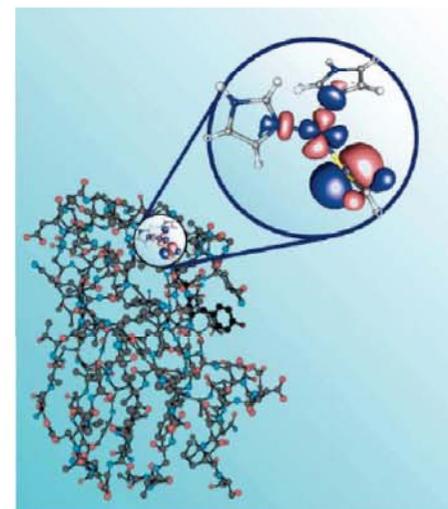
- Hydrogen bonds to bound ligands
- Hydrophobic residues: dielectric constant can change stability of metal-ligand bonds

# Conclusion

**The structural and functional properties of metal ions in biological systems can be understood by combining the principles of coordination chemistry with the knowledge of the unique environment created by biomolecules**



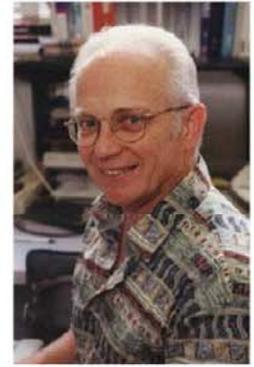
**Bo G. Malmström, Göteborg, 1927-2000**



## **How to study *Fast Reactions* (ms- $\mu$ s)**

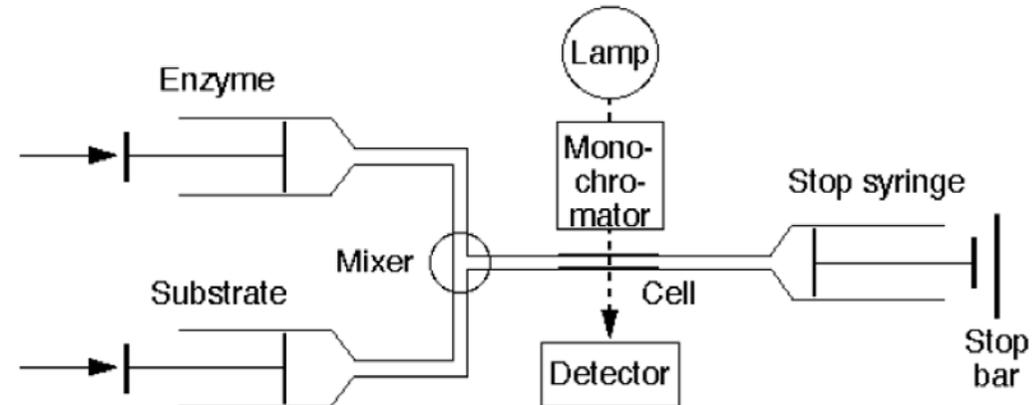
- Most kinetic techniques for the examination of rapid enzyme kinetics exploit stopped-flow, continuous-flow mixing or relaxation experiments.**
- Novel biophysical methods and devices of fast reaction kinetics have been developed in the recent years, including microcapillary mixing heads for the investigation of particularly precious enzymes, quench-flow double-jump for trapping of intermediates, and highly stable optics for temperature-jumping of fast enzyme reactions.**

# Stopped-Flow (Spectrophotometry)



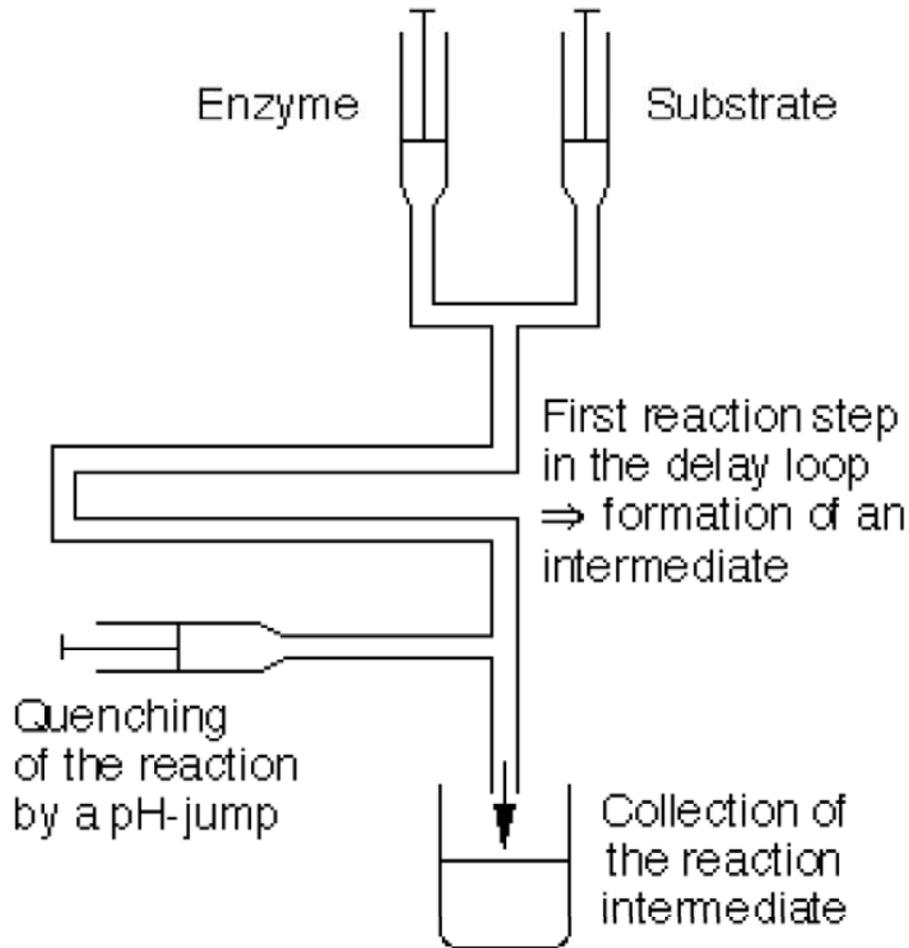
D. Ballou, Ann Arbor

- One of the oldest ways of studying kinetics of enzymes is to mix solutions of the protein and of the substrate. The reaction is followed optically, e.g. UV/vis, IR, light scattering, fluorescence, or CD.
- Two syringes containing a solution of enzyme and substrate, respectively, are simultaneously pushed. Turbulence in the T-mixer, where both liquids join together, causes a rapid mixing. After a certain amount of liquid has passed through the mixer, the position of the stop syringe triggers a stop signal and a signal for the detector to start recording the reaction kinetics in the sample cell.



# Rapid-Quench

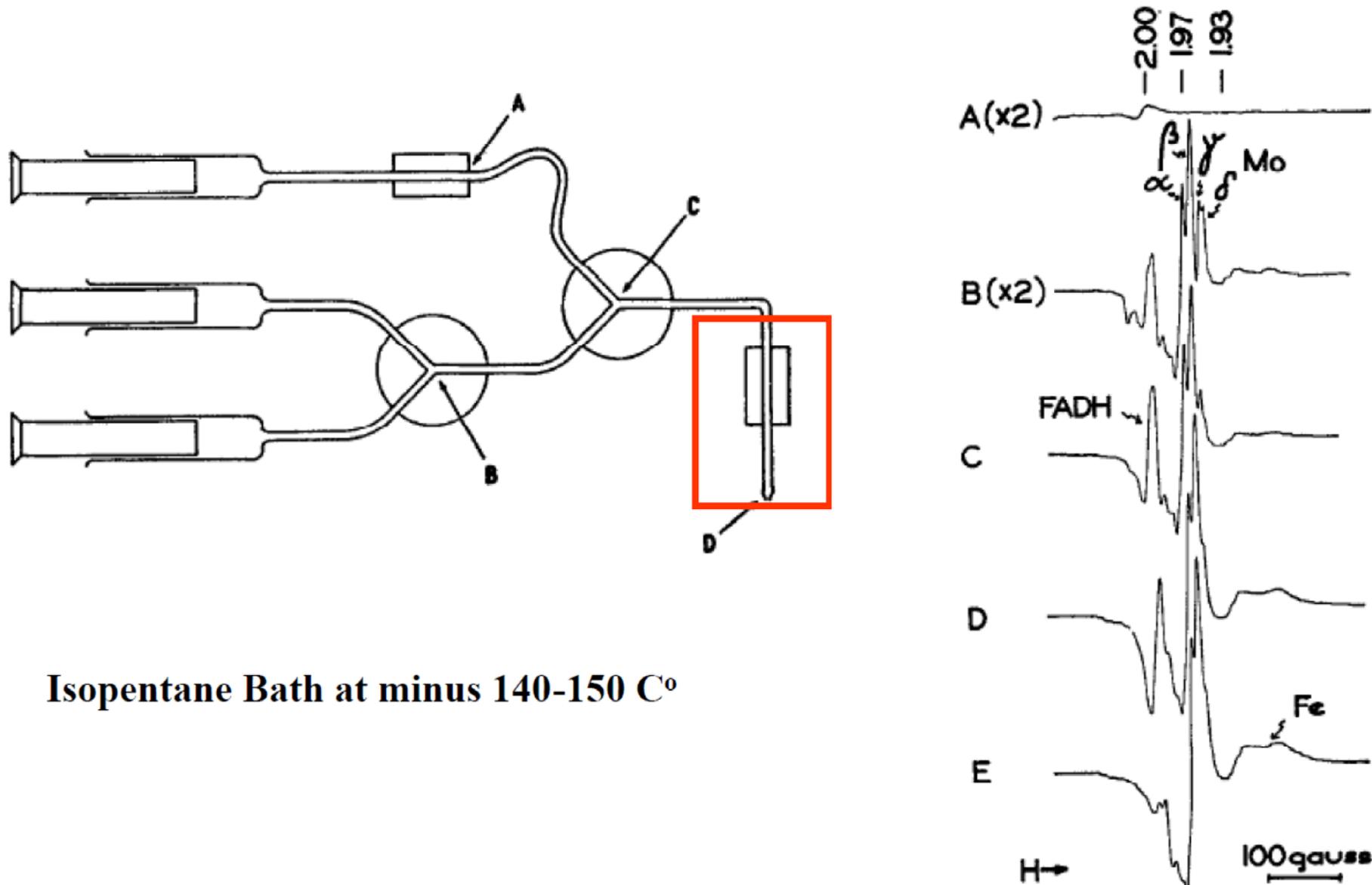
Change in pH, temperature



**The figure shows a typical set-up for the detection and analysis of reaction intermediates. Kinetic information on a time scale of about 1 ms and longer is obtainable with conventional quench-flow devices. Higher time resolution can be achieved with microvolume (microcapillary) mixers.**

# Rapid-Freeze EPR (Triple Mix) – 26-1400 ms

Palmer et al., J. Biol. Chem., 239, 2657, 1964

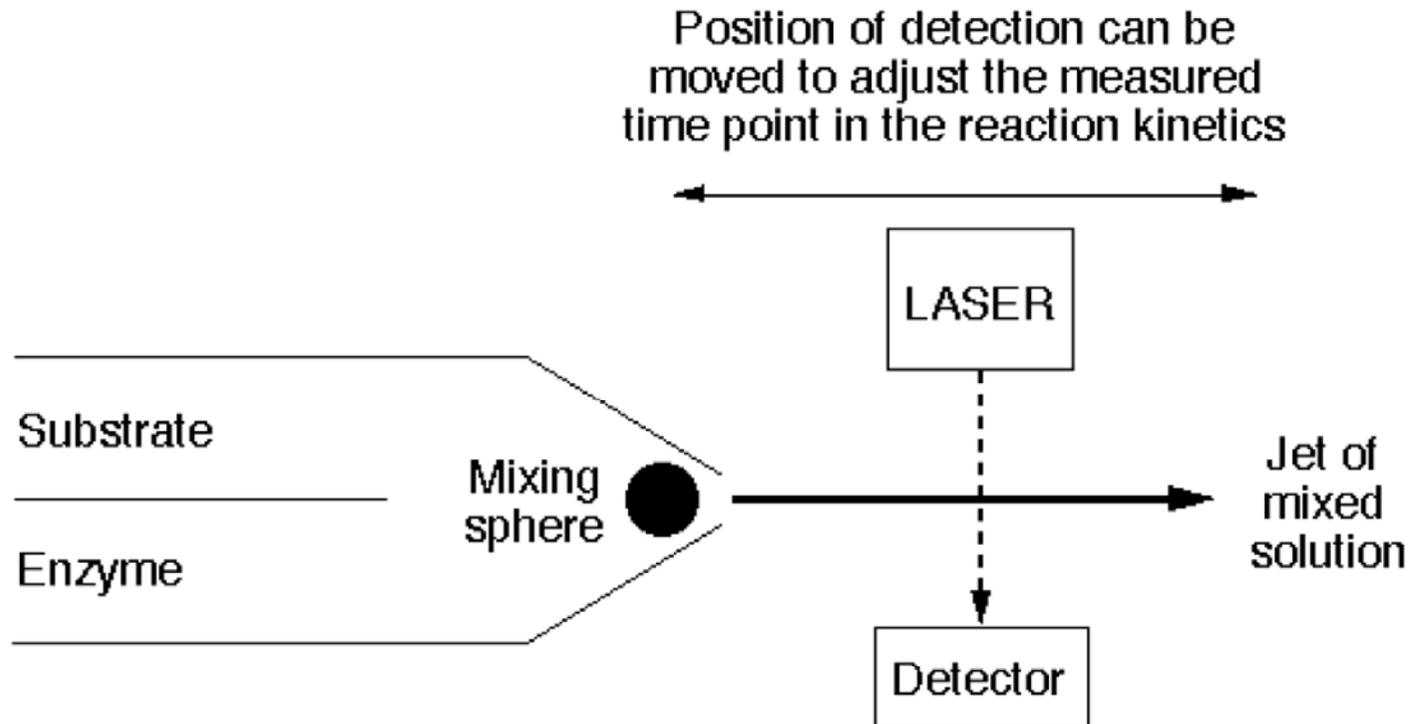


Isopentane Bath at minus 140-150 C°

# From Milliseconds to Microseconds (1)

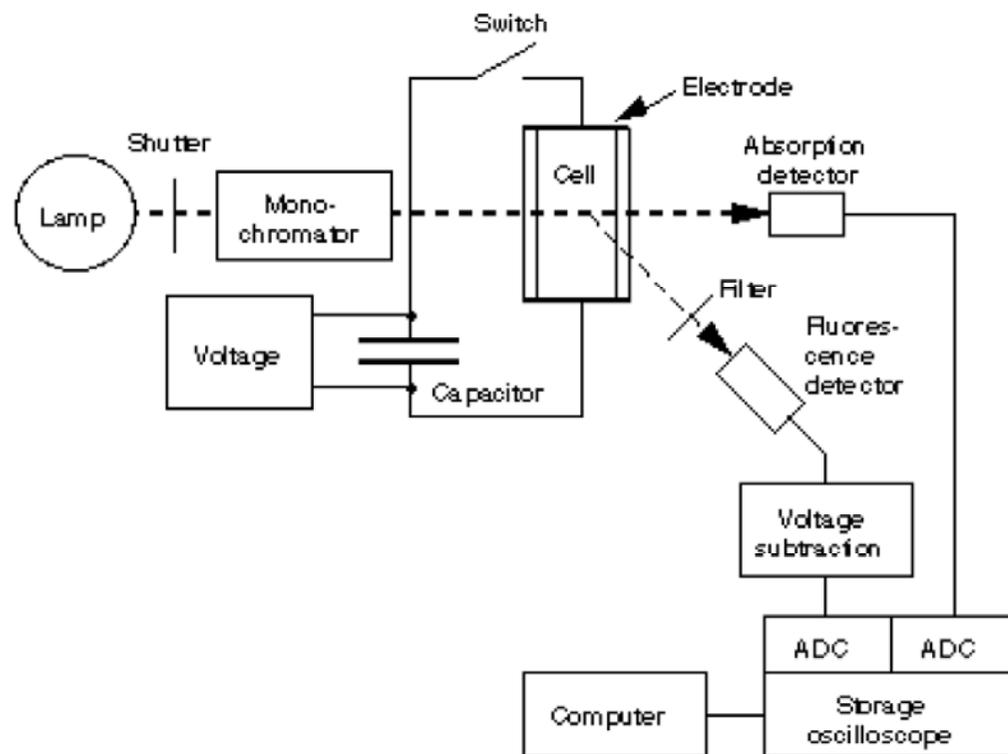
- Unfortunately, the dead time of common stopped-flow devices is usually around 2-5 milliseconds. Faster mixing requires stronger turbulence in the mixing chamber. Decreasing the size of the tubes and increasing the speed of flow would require impracticably high pressures.
- Ultrafast continuous-flow mixing: Solutions of protein and substrate are gently joined together and passed through a tube with a decreasing cross-section. At the end of the tube, the laminar flow is changed into a highly turbulent flow by passing the liquid over a sphere of only a few 10 mm diameter. The mixed solution forms a continuous free jet. Each position in the jet corresponds to a certain time point in the reaction kinetics. Kinetic traces are recorded by moving the LASER/detector system along the jet. Because in free air the jet is stable over a few cm, reactions may be followed from microseconds to milliseconds.

# From Milliseconds to Microseconds (2)



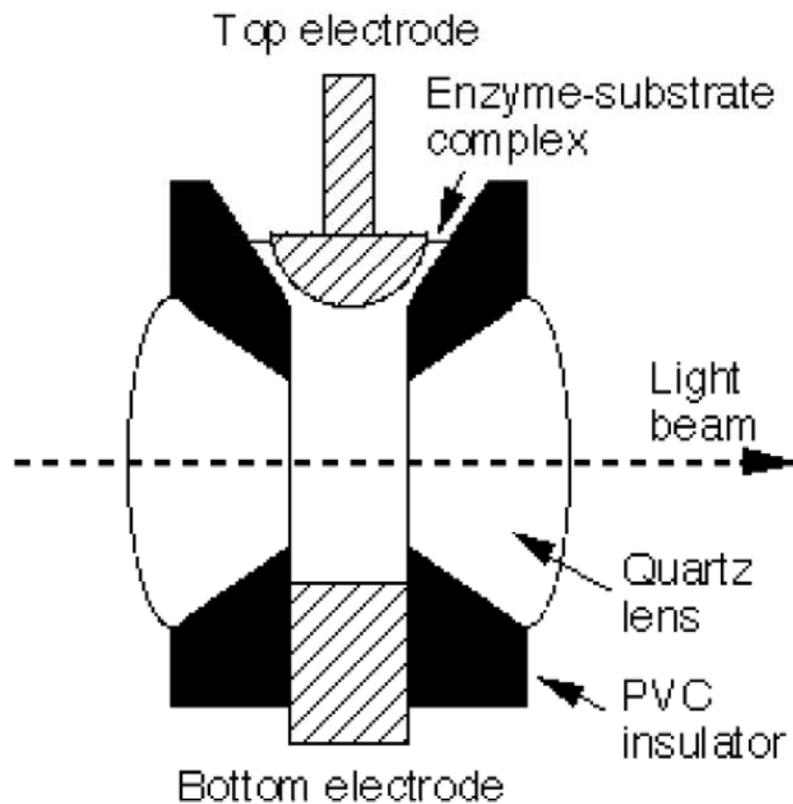
**The size of the mixing sphere may be as small as a few micrometers. At a flow speed of 10-100 m s<sup>-1</sup>, a dead length of 100 mm corresponds to a dead time of 1-10  $\mu$ s. Using continuous-flow rather than stopped-flow avoids pressure waves at high flow speeds. These modifications led to a 100-fold reduction of the mixing time down to about 10  $\mu$ s.**

# Electrical-discharge-induced T-jump method pioneered by **Manfred Eigen** (Göttingen)



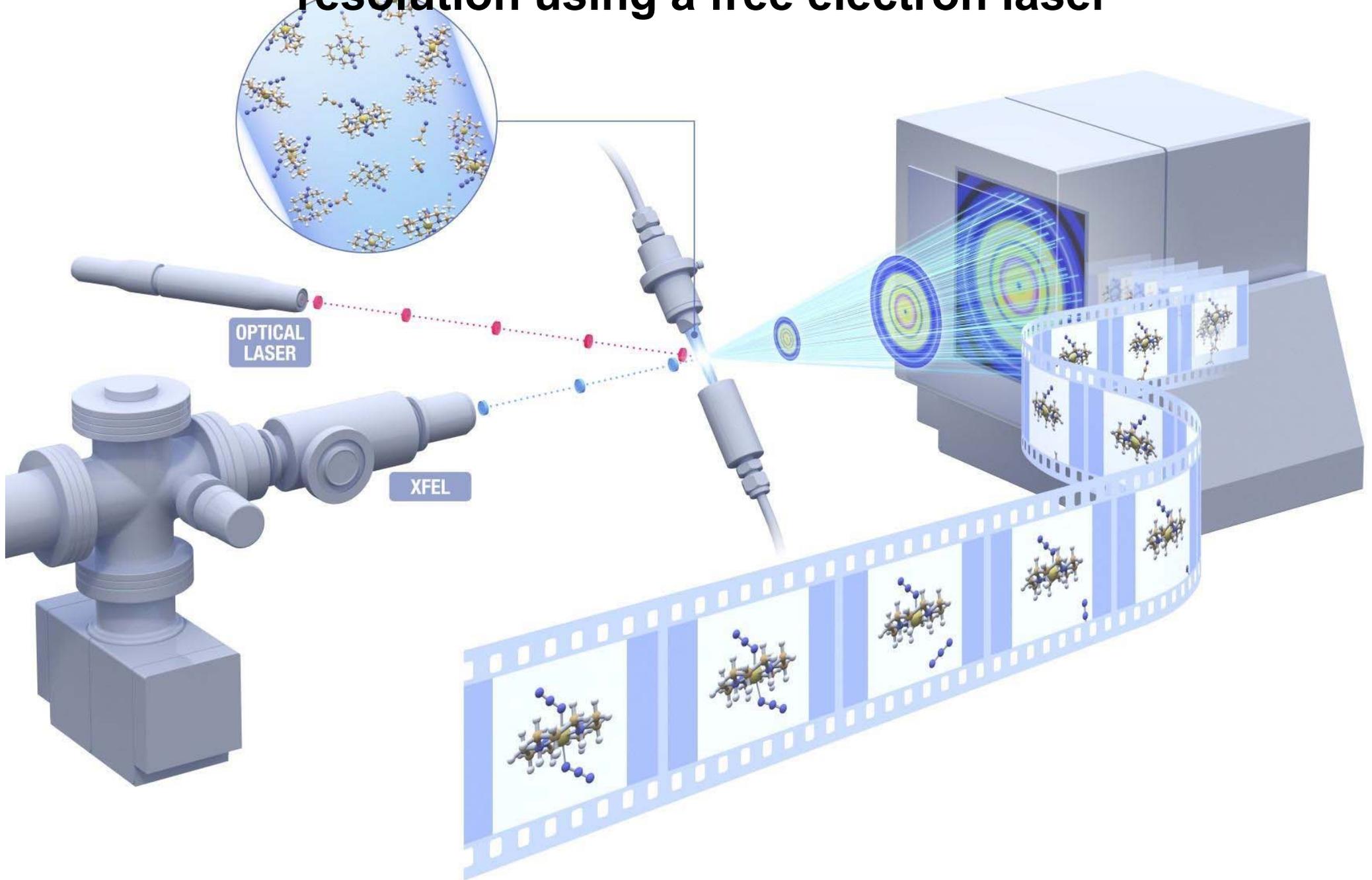
Solutions with electrolytes may have sufficient electrical conductivity to be heated by a rapid electrical discharge. Joule heating with rise times of 1 ms or faster are achieved in 100 mM KCl. A capacitor is charged by a power supply up to a specific voltage and then rapidly discharged through the sample cell. The electrical discharge causes heating by 1-20°C with rise times of 500 ns - 10 ms. The kinetics is followed by absorption or fluorescence detection.

# T-Jump Cell



**Design of the sample cell in a modern T-jump apparatus pioneered by Manfred Eigen and DIA-LOG. In order to avoid pressure due to thermal expansion upon T-jump, the top of the cell is not sealed. Fluorescence detection is perpendicular to the excitation beam.**

# The future: Filming chemical reactions with femtosecond resolution using a free electron laser



**All slides of my lectures can be downloaded  
from my workgroup homepage**

Biology Centre CAS → Institute of Plant Molecular Biology → Departments  
→ Department of Plant Biophysics and Biochemistry,  
*or directly*

**[http://webserver.umbr.cas.cz/~kupper/AG\\_Kuepper\\_Homepage.html](http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html)**