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, 2nd 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 web sites

http://http://www.ebi.ac.uk/pdbe/

comprehensive database of all published protein structures

http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi

comprehensive databases (genomes, genes, proteins, inherited diseases...etc) and various search tools

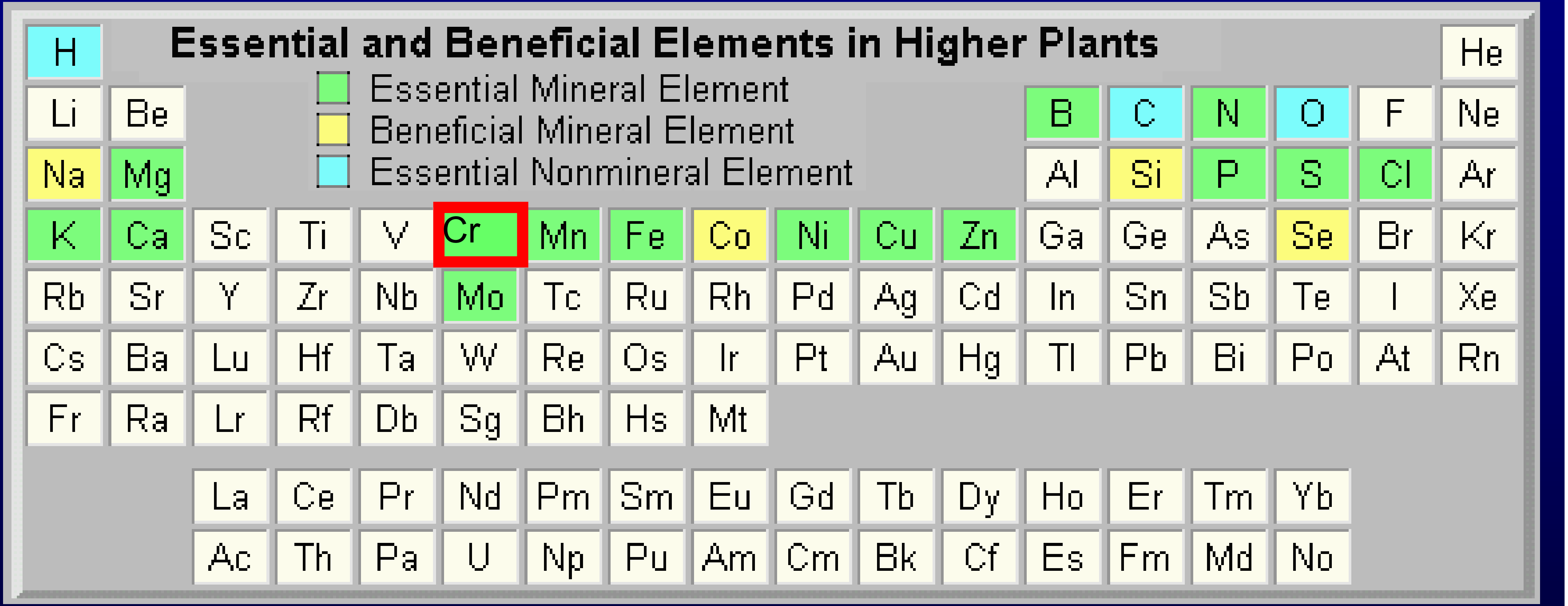
http://www.brenda-enzymes.org/

comprehensive enzyme database including information on metal requirements

http://www.webelements.com

periodic table of the elements including useful information on each element

Elements that are known to be essential for plants



Essential and Beneficial Elements in Higher Plants

🟩 Essential Mineral Element
🟨 Beneficial Mineral Element
🟦 Essential Nonmineral Element

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		

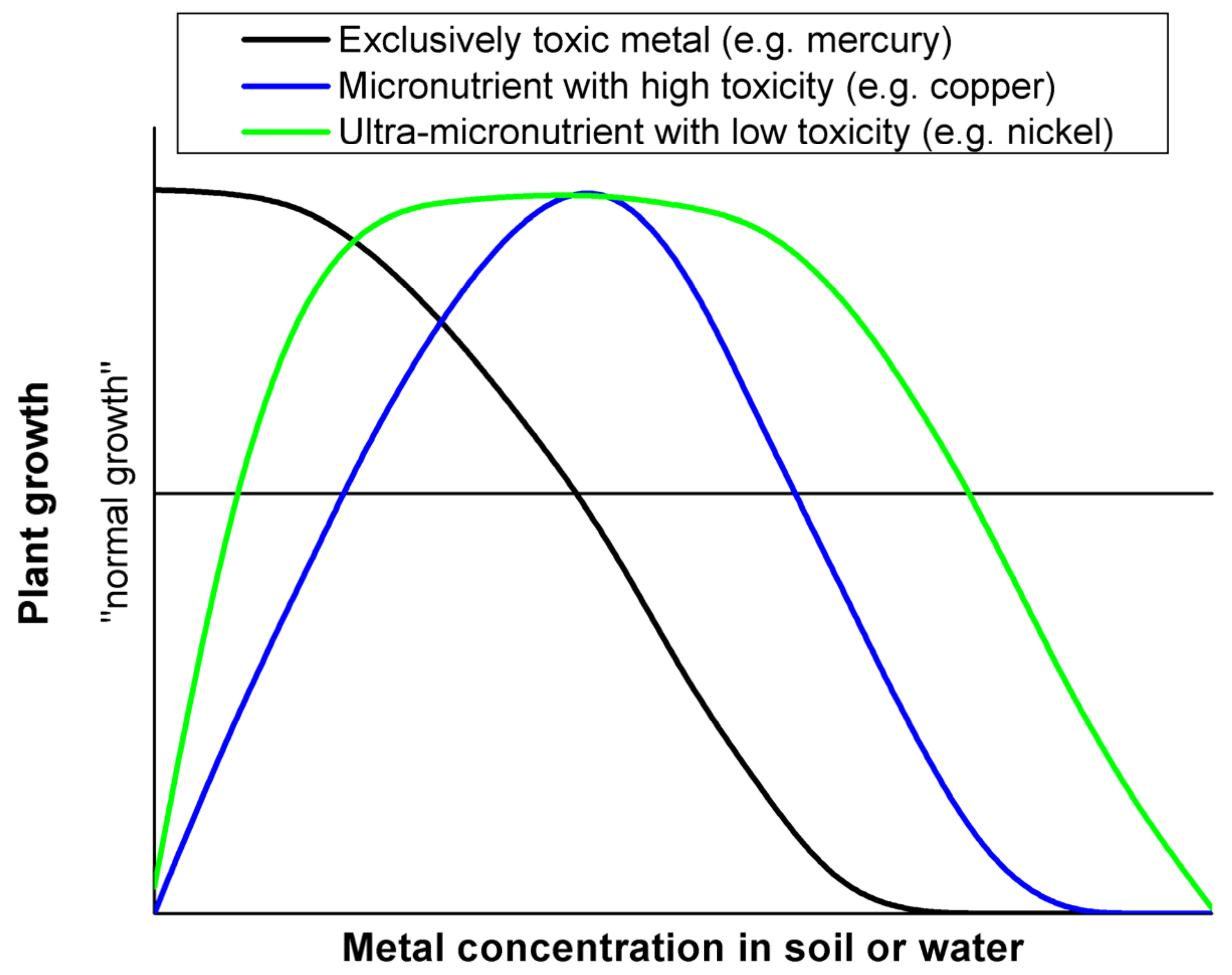
→ In plants, chromium has so far always been regarded as only toxic, not beneficial or even essential. This should be reconsidered. In animals (incl. humans), an essential role of chromium is debated since the 1960s (still no consensus has been reached). This suggested role is activation of insulin,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

periodic table from http://soils.wisc.edu/facstaff/barak/soilscience326/periodic.gif

Why Investigate Metals in Biology ?

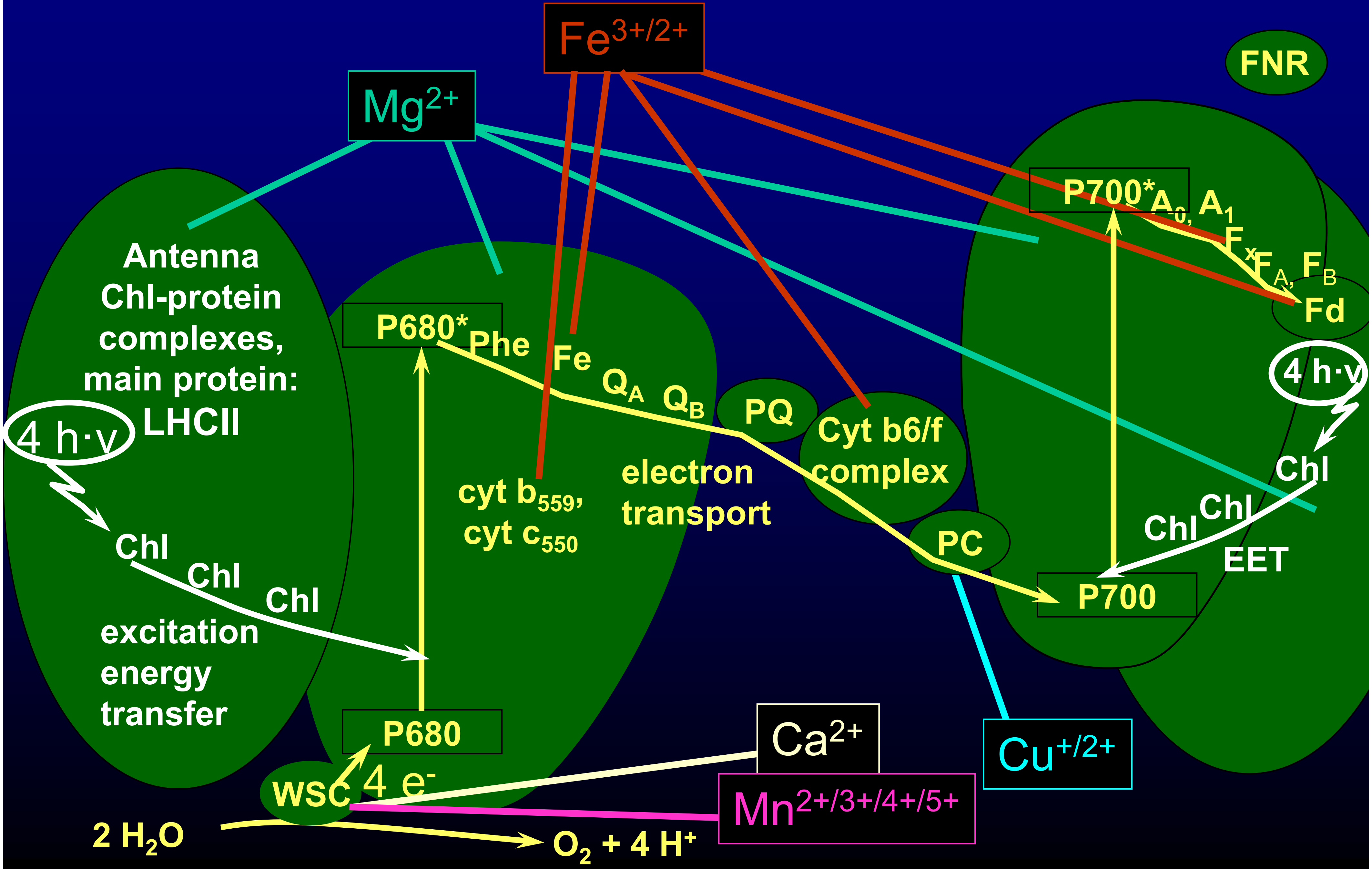
- There is hardly any important process in nature which does not depend on a metal ion; ~ 1/3 of the proteins of the human genome depend on metal ions
- Novel Materials, Structures and Reactions
- Trigger - Signaling - Sensing - Regulation
- Acid-Base Catalysis
- Redox – Proton & Electron Transfer (coupled,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

Dose-response principle for transition metals



Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

Case 1: Metal sites in photosynthetic proteins



$Fe^{3+/2+}$

Mg^{2+}

FNR

Antenna
Chl-protein
complexes,
main protein:
LHCII

4 h·v

Chl

Chl

Chl

excitation
energy
transfer

P680*

Phe

Fe

Q_A

Q_B

PQ

Cyt b6/f
complex

$cyt\ b_{559}$,
$cyt\ c_{550}$

electron
transport

P680

wsc

4 e^-

$2\ H_2O$

$O_2 + 4\ H^+$

Ca^{2+}

$Mn^{2+/3+/4+/5+}$

PC

$Cu^{+/2+}$

P700*

A_0, A_1

F_x

F_A, F_B

Fd

4 h·v

Chl

Chl

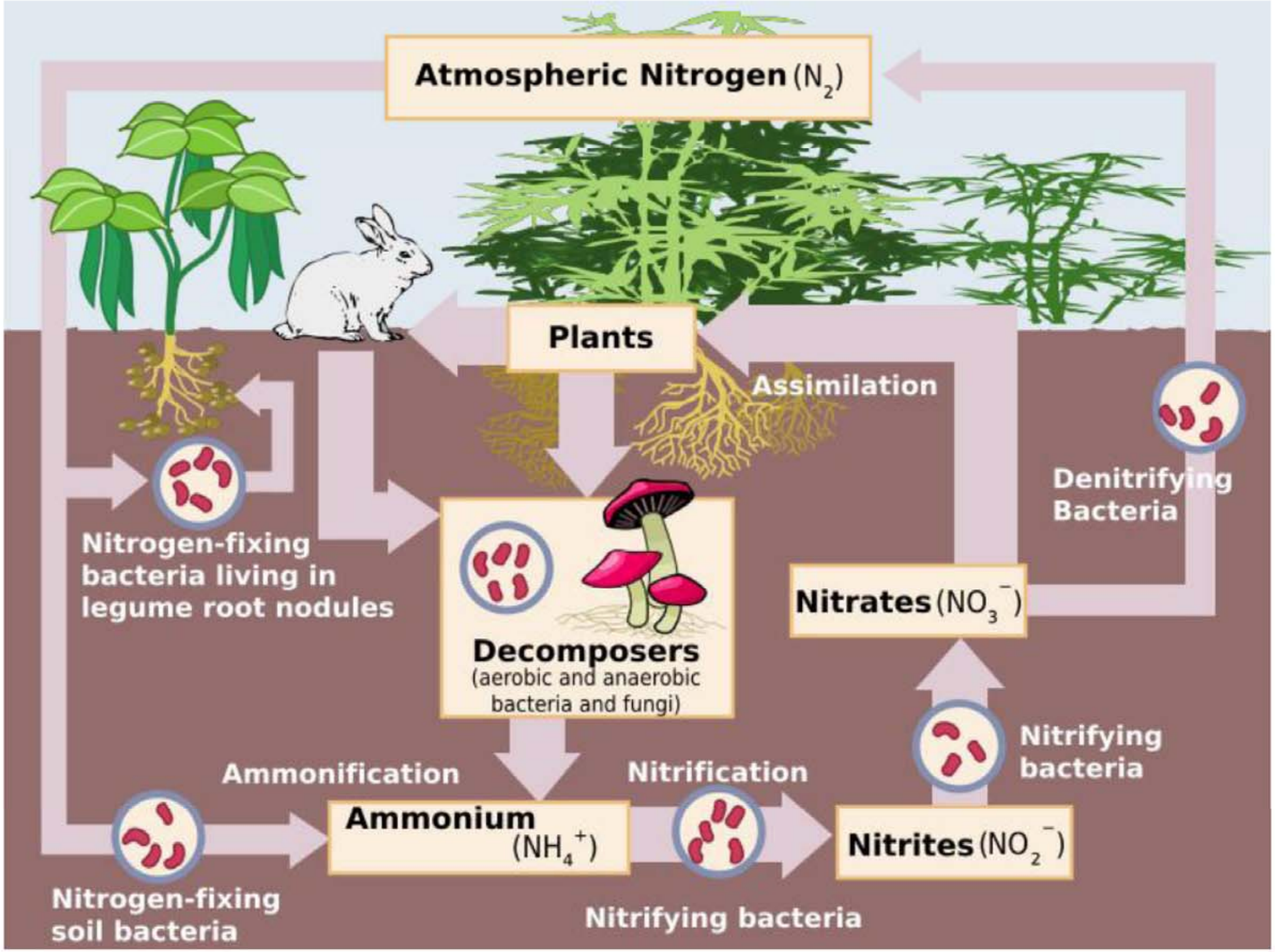
Chl

EET

P700

Case 2: Nitrogen Fixation - Nitrogenase

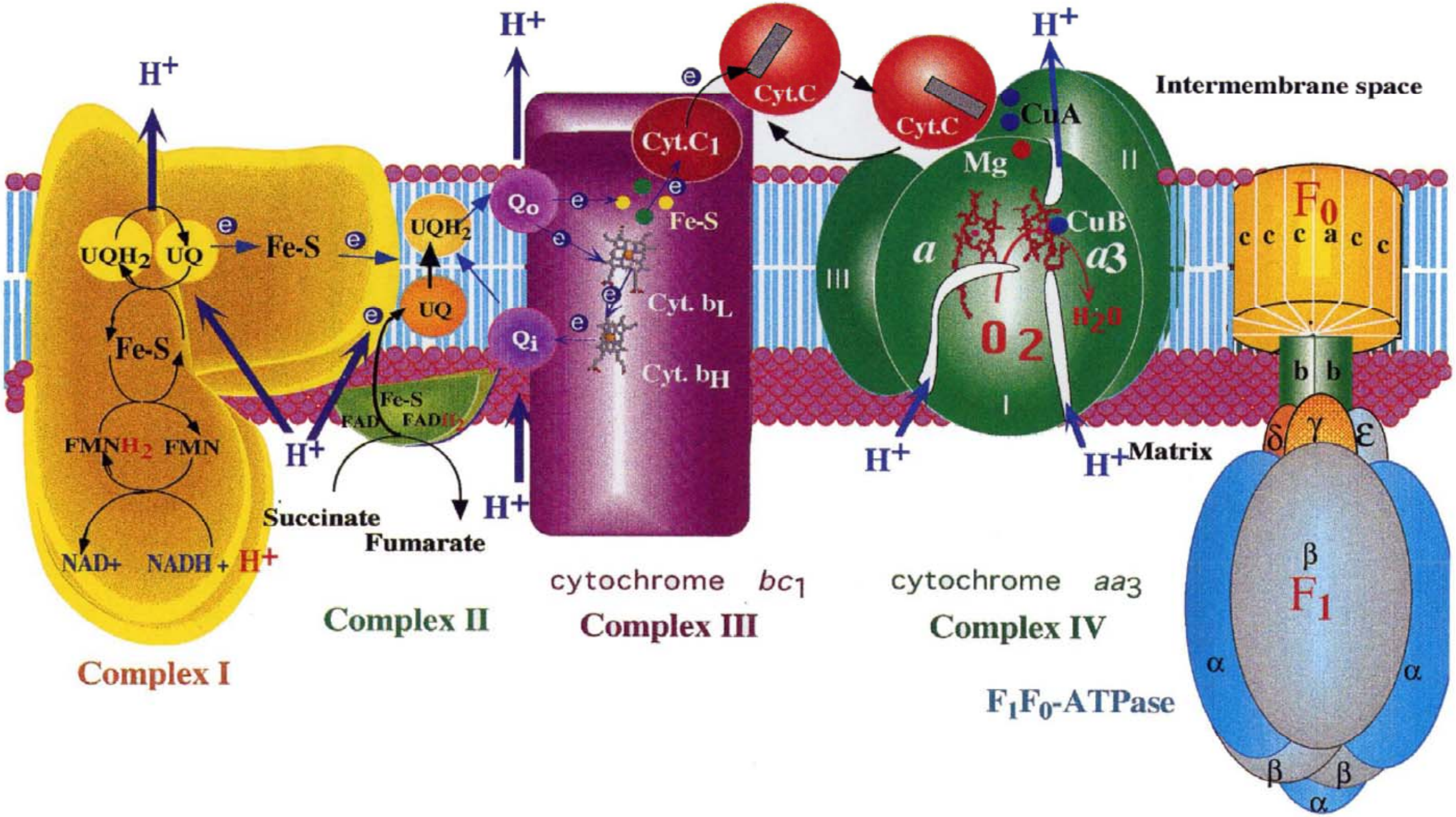
http://en.wikipedia.org/wiki/Nitrogen_cycle



Lecture slide from Peter Kroneck

Case 3: Respiration – Reduction of O_2 to H_2O

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



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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
- Sterochemically Flexible
  - Large Variety of Structures.
  - Little Reorganization
  - Facile Ligand Addition/Dissociation
- Facilitate Reactions of Bound Ligands

Lecture slide from Peter Kroneck

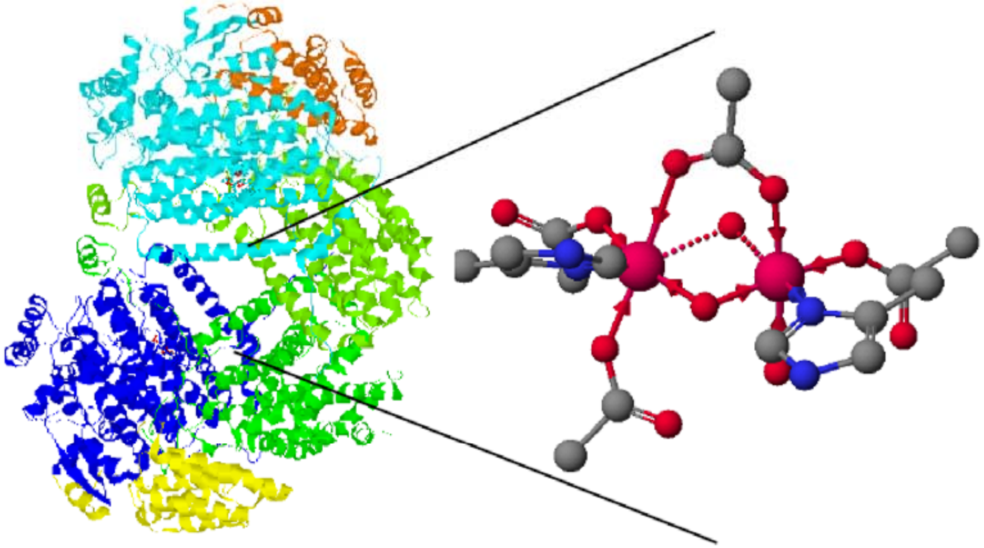
Basic Features of a Metal Protein Complex

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

Prot-L| --------M

δ- δ+

strong attraction



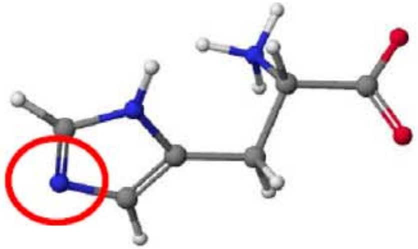
$CH_4 + O_2 + 2e^- + 2H^+ \rightarrow H_3COH + H_2O$

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

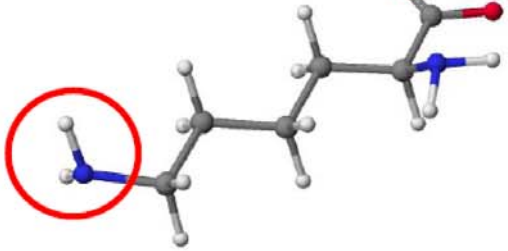
Lecture slide from Peter Kroneck

Protein Ligands – Amino Acid Residues

N

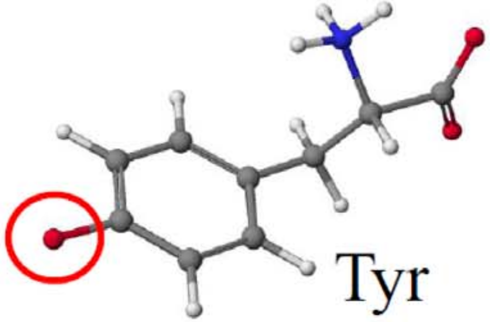


His

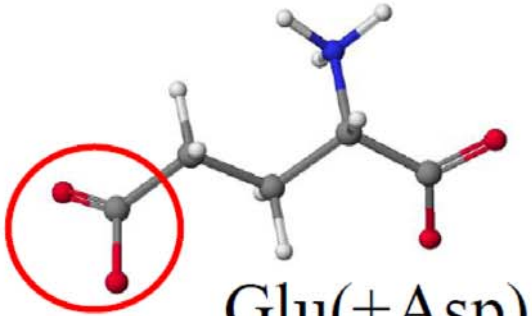


Lys

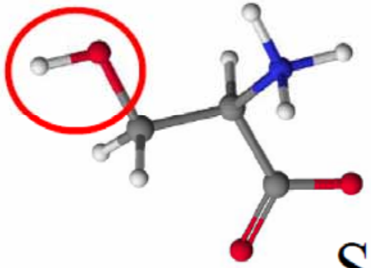
O



Tyr

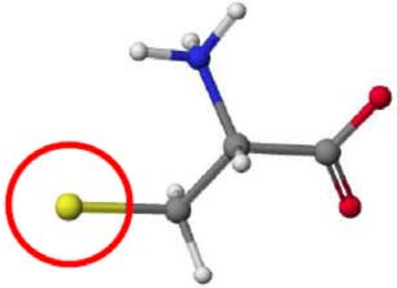


Glu(+Asp)

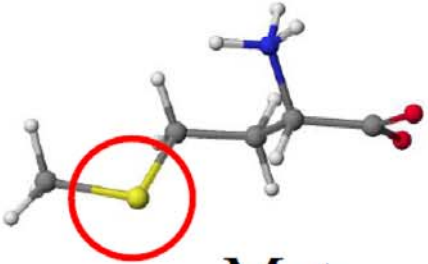


Ser

S



Cys



Met

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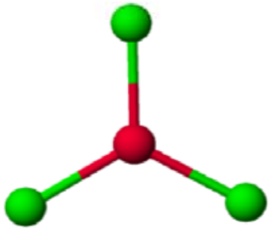
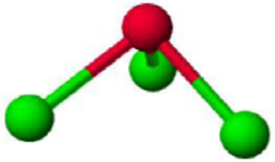
Geometry – Coordination Number

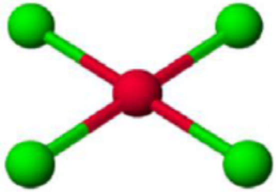
3

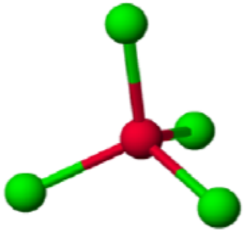

Trigonal


Trigonal pyramidal

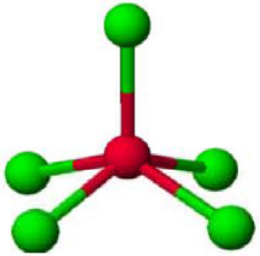

T-shape

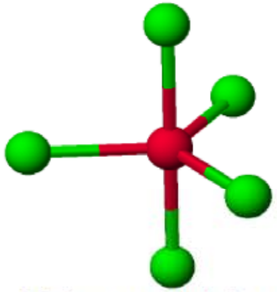
4


Square planar

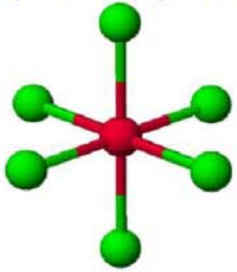

Tetrahedral

5


Square pyramidal


Trigonal bipyramidal

6


Octahedral

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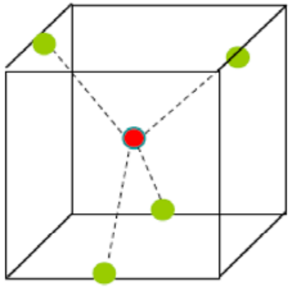
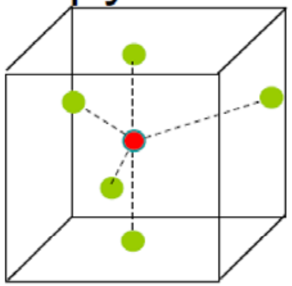
Geometry is important: Iron Proteins

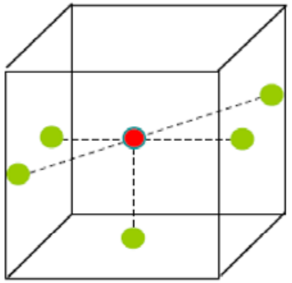
Tetrahedron



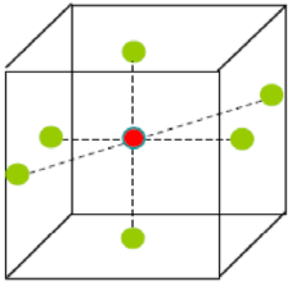
Trigonal Bipyramide

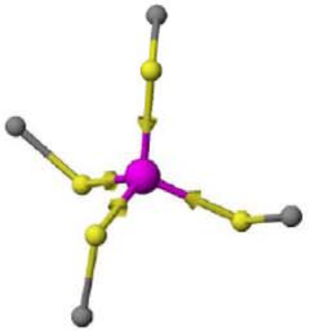


Tetragonal Pyramide

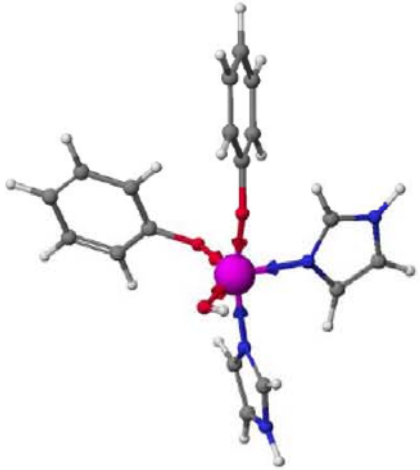


Octahedron



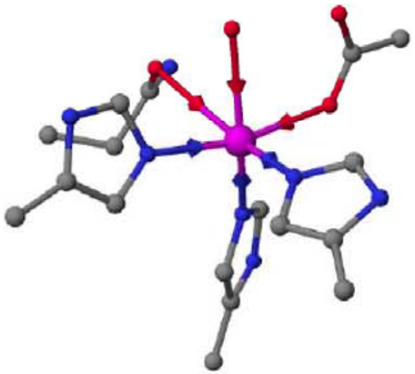


Rubredoxin



3,4-Protocatechoate Dioxygenase

Tyrosine Hydroxylase

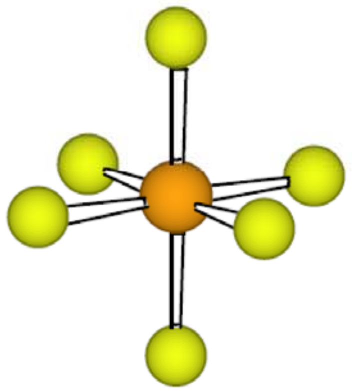


Lipoxygenase

EI Solomon, *et al.* (**2000**), Chem. Rev., *100*, 235–350

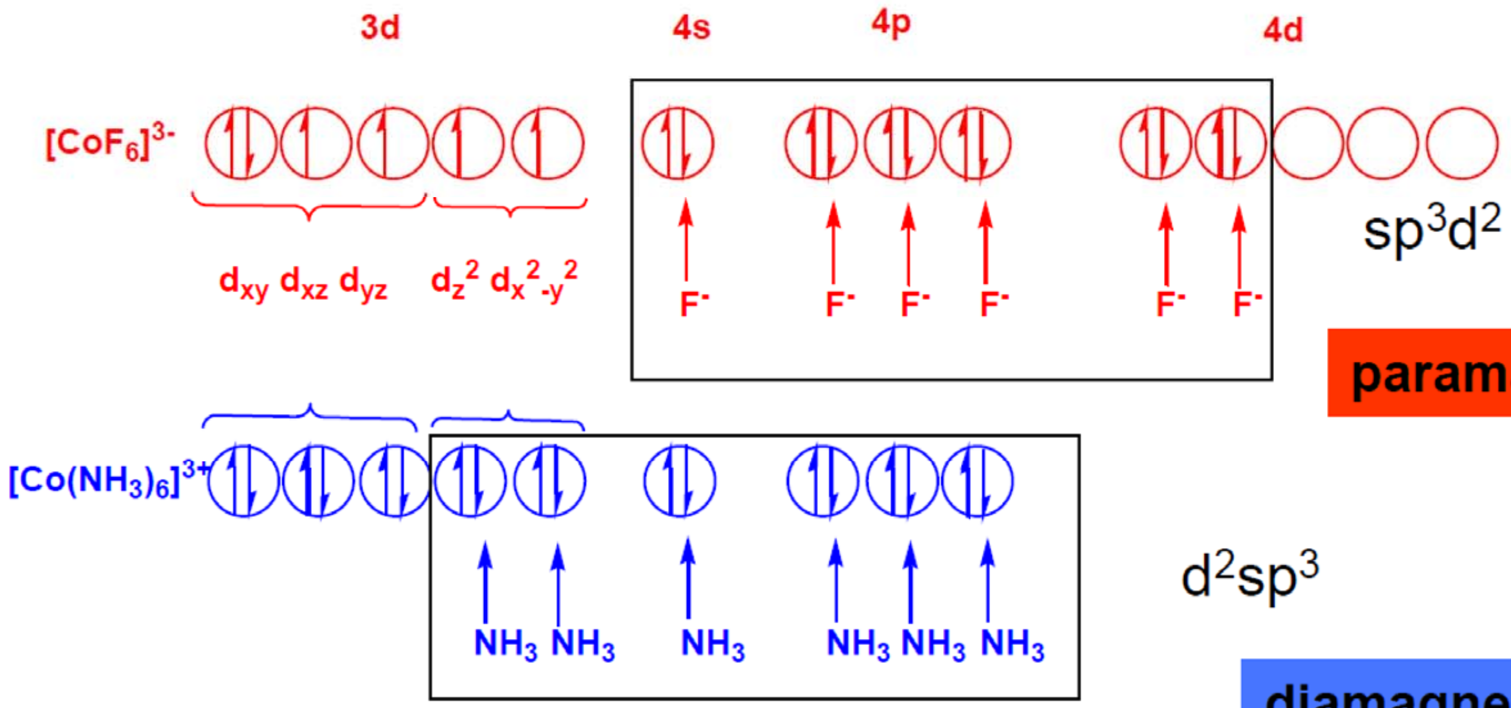
Remember: Valence Bond Theory

L. Pauling



$[Co(NH_3)_6]^{3+}$ and $[CoF_6]^{3-}$

d^6



sp^3d^2

paramagnetic, S=2

d^2sp^3

diamagnetic, S=0

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

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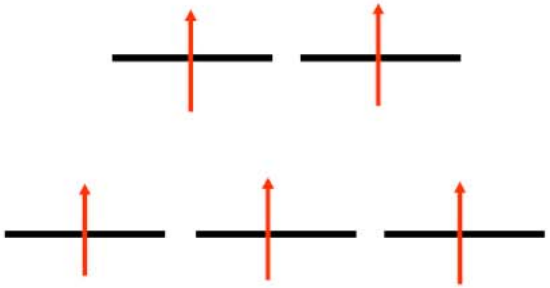
Color and Magnetism

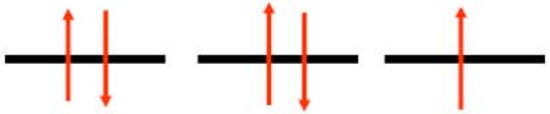
Variable Spin States of Metal Centers

For a d^5 configuration, Fe(III)



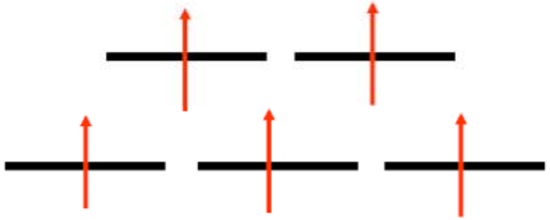
High-Spin, S= 5/2

OR



Low-Spin, S= 1/2

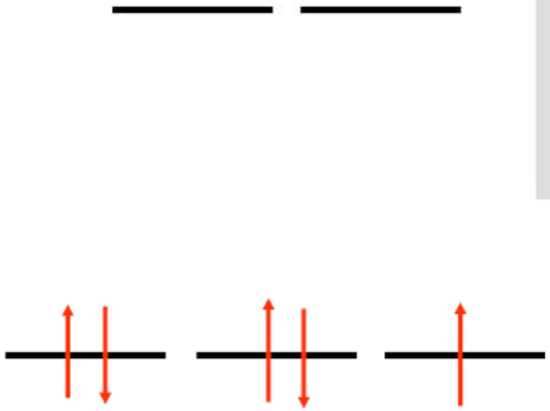
Depending on the METAL ION ENVIRONMENT, balance of Crystal Field Splitting, 10Dq and Spin-Pairing Energy, P



10Dq < P

Weak Field

High Spin



10Dq > P

Strong Field

Low-Spin

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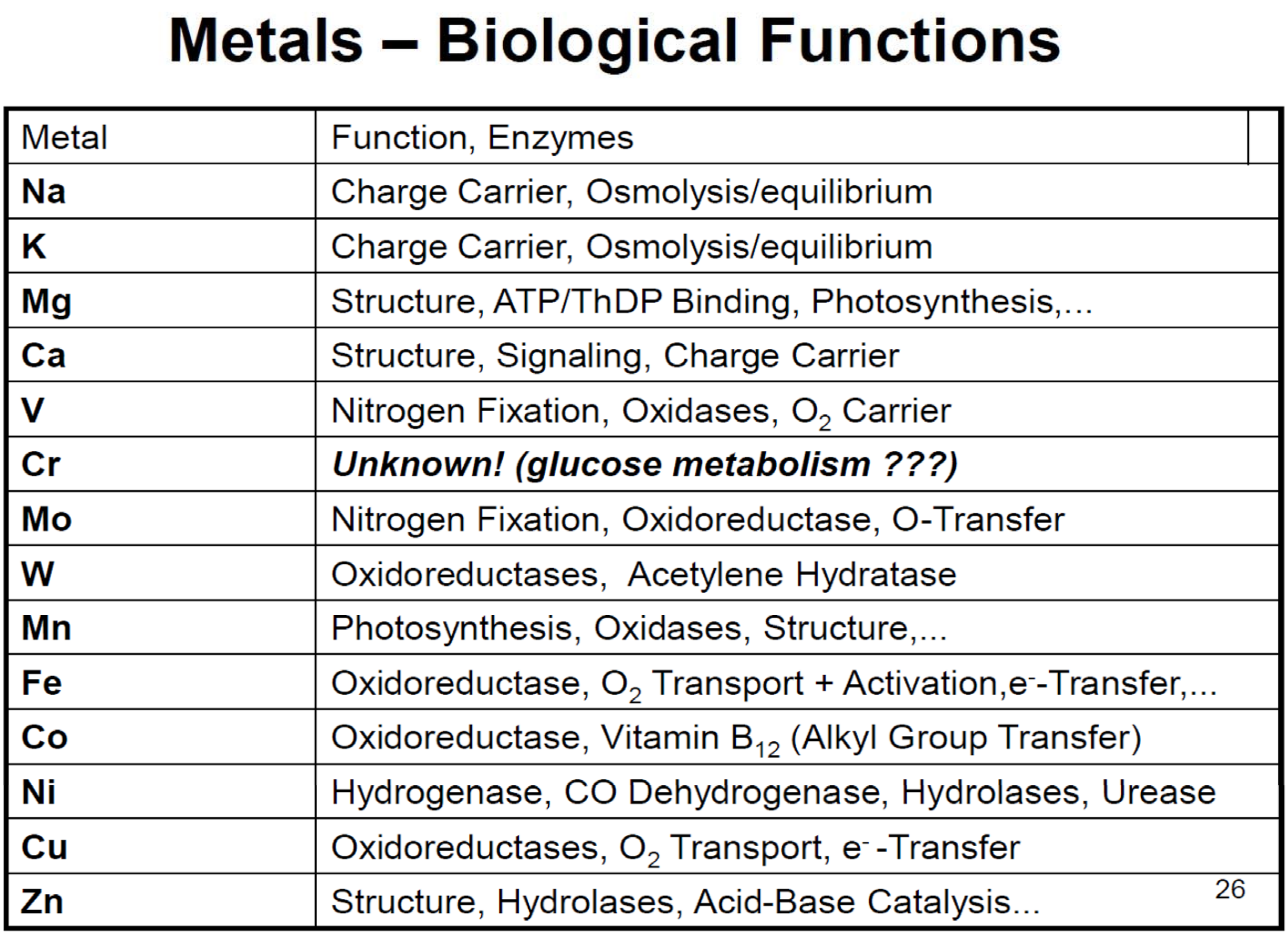

Metals – Biological Functions

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_2 Carrier
Cr	***Unknown! (glucose metabolism ???)***
Mo	Nitrogen Fixation, Oxidoreductase, O-Transfer
W	Oxidoreductases, Acetylene Hydratase
Mn	Photosynthesis, Oxidases, Structure,...
Fe	Oxidoreductase, O_2 Transport + Activation,e^--Transfer,...
Co	Oxidoreductase, Vitamin B_{12} (Alkyl Group Transfer)
Ni	Hydrogenase, CO Dehydrogenase, Hydrolases, Urease
Cu	Oxidoreductases, O_2 Transport, e^- -Transfer
Zn	Structure, Hydrolases, Acid-Base Catalysis...

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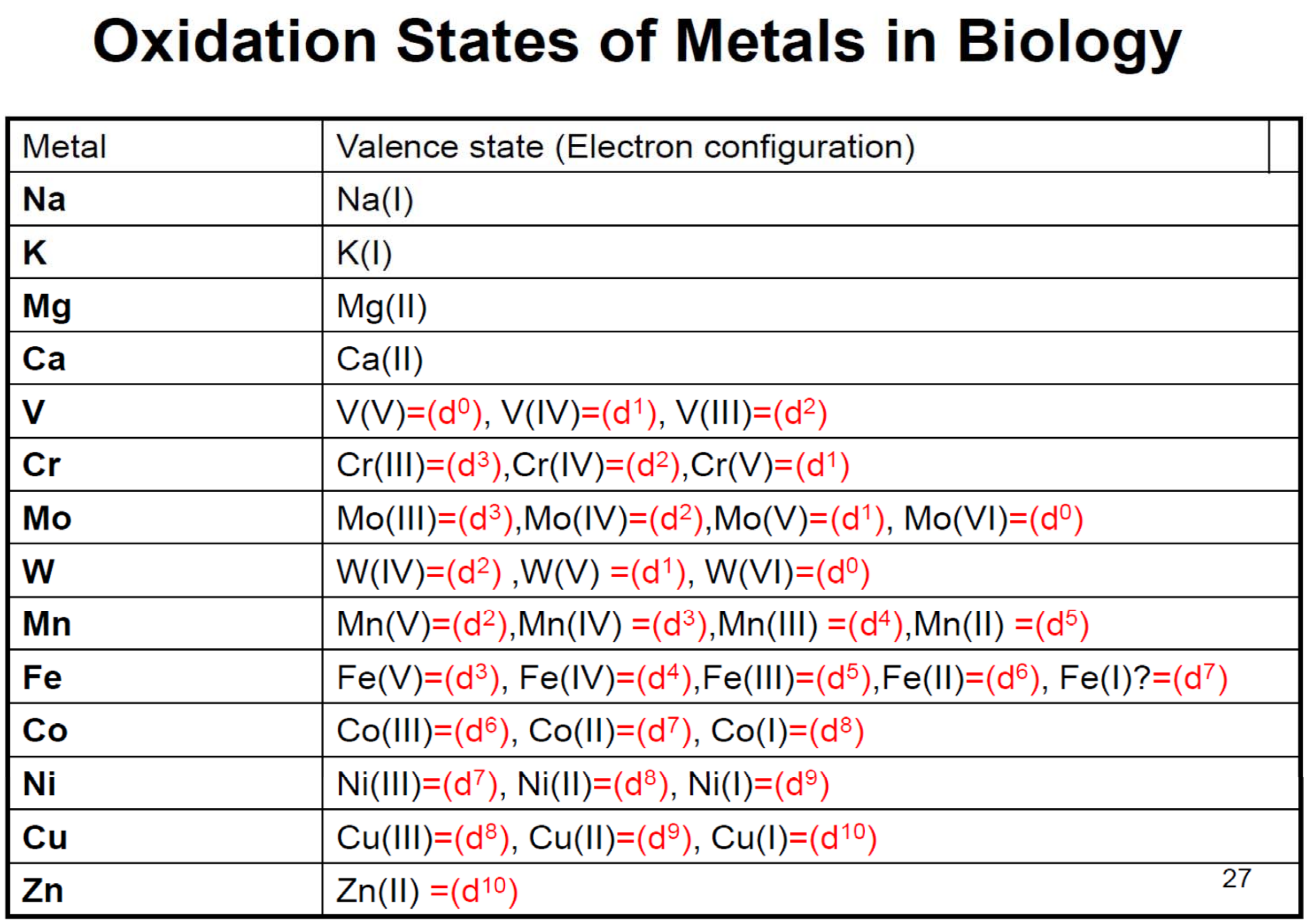

Oxidation States of Metals in Biology

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

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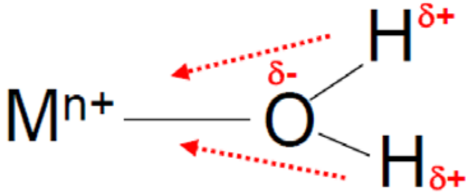
Exogenous ligands

	Ligand	pK_a
Acid/base	$H_2O/OH^-, O^{2-}$	14,~34
	HCO_3^-/CO_3^{2-}	10.3
	HPO_4^{2-}/PO_4^{3-}	12.7
	H_3CCOO^-/H_3CCOOH	4.7
	HO_2^-/H_2O_2	11.6
	NH_3/NH_4^+	9.3
	N_3^-/N_3H	4.8
	$F^-, Cl^- Br^-, I^-/XH$	3.5, -7, -9, -11
Neutral	O_2, CO, NO, RNC	



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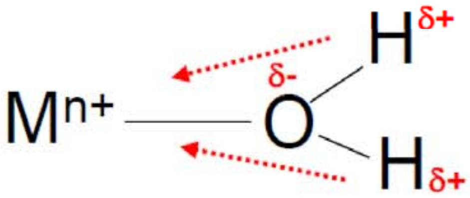
Modulation of pK_a

$$H_2O + M^{n+} \underset{+H^+}{\overset{-H^+}{\rightleftarrows}} HO^- \text{-}M^{n+}$$

Metal	pK_a
none	14.0
Ca^{2+}	13.4
Mn^{2+}	11.1
Cu^{2+}	10.7
Zn^{2+}	10.0

4 orders of magnitude !



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Kinetic Control

$$[M^{n+}(H_2O)_m] \underset{+H_2O}{\overset{-H_2O}{\rightleftharpoons}} [M^{n+}(H_2O)_{m-1}]$$

Metal	k (s^{-1})
K^+	$1x10^9$
Ca^{2+}	$3x10^8$
Mn^{2+}	$2x10^7$
Fe^{2+}	$4x10^6$
Co^{2+}	$3x10^6$
Ni^{2+}	$4x10^4$
Fe^{3+}	$2x10^2$
Co^{3+}	$<10^{-6}$

15 orders of magnitude!

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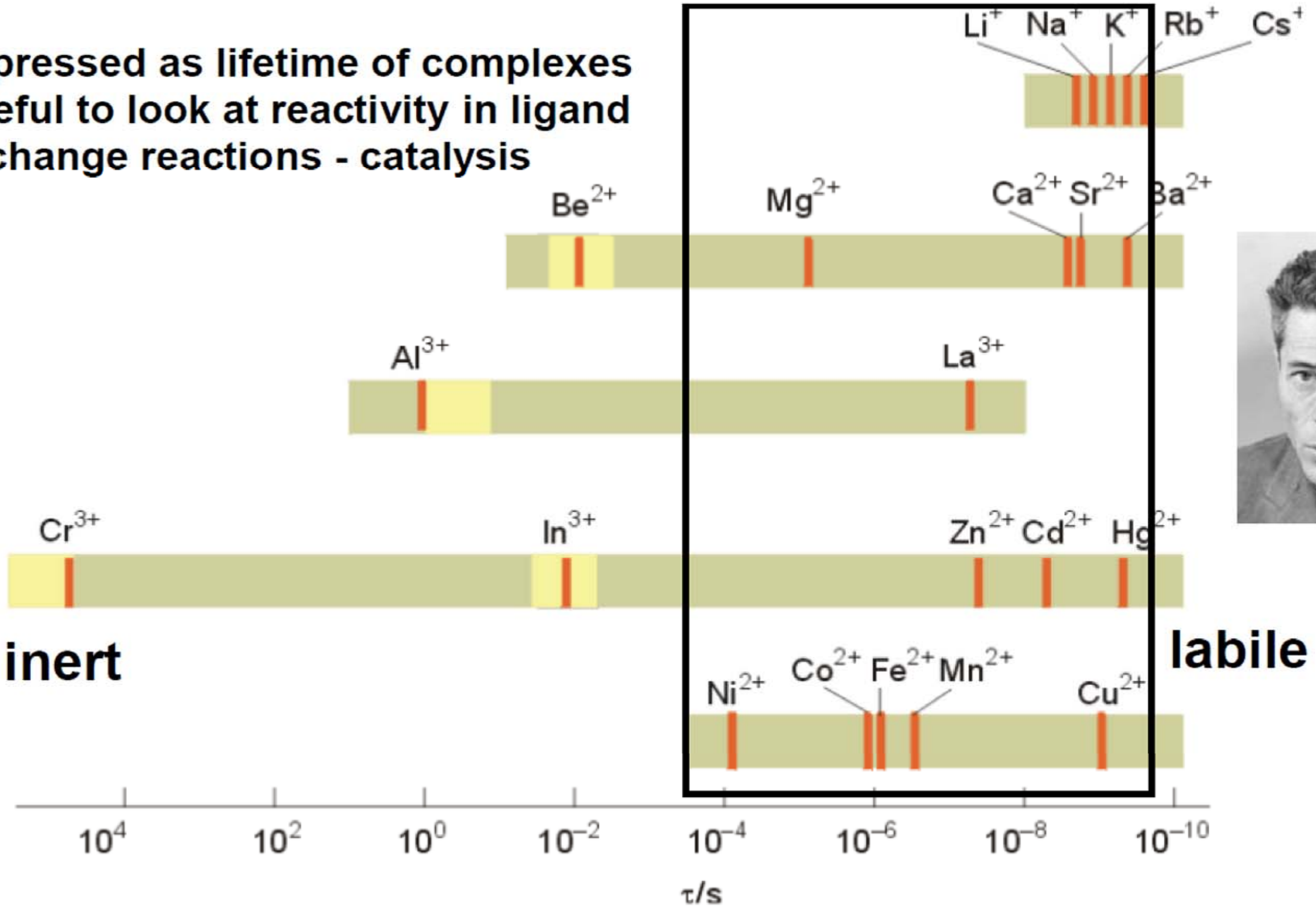
Water exchange rates

M. Eigen, Nobel Prize Lecture 1967

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



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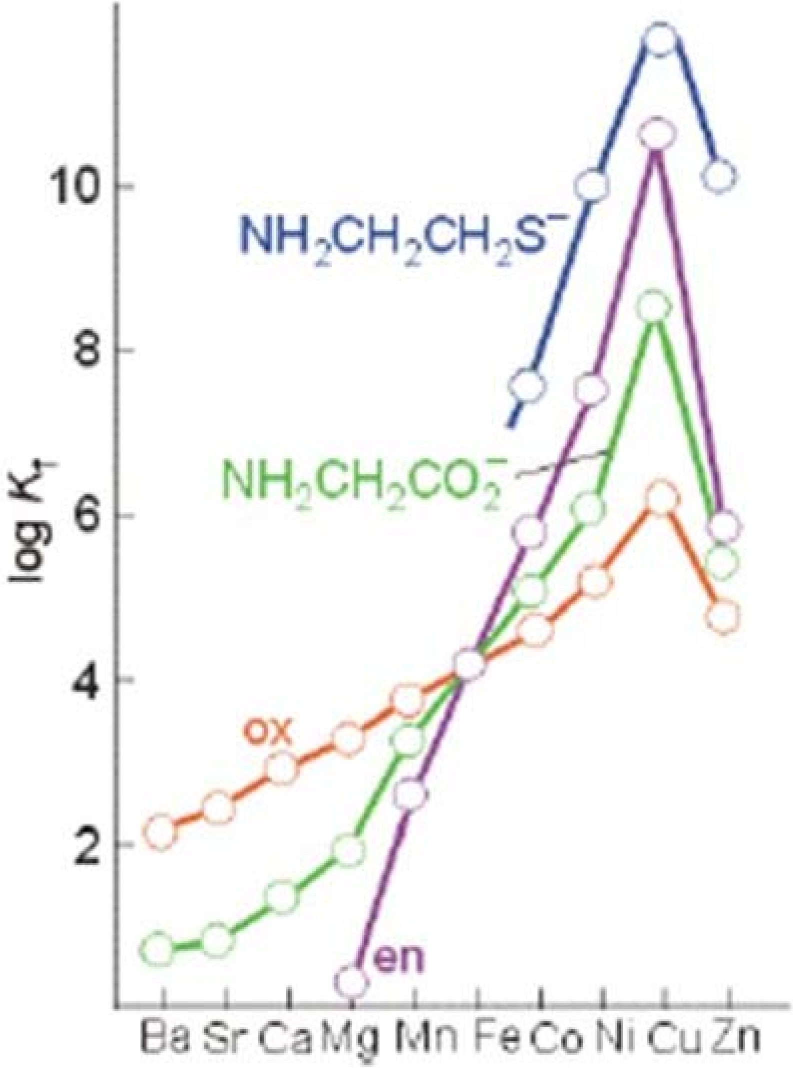
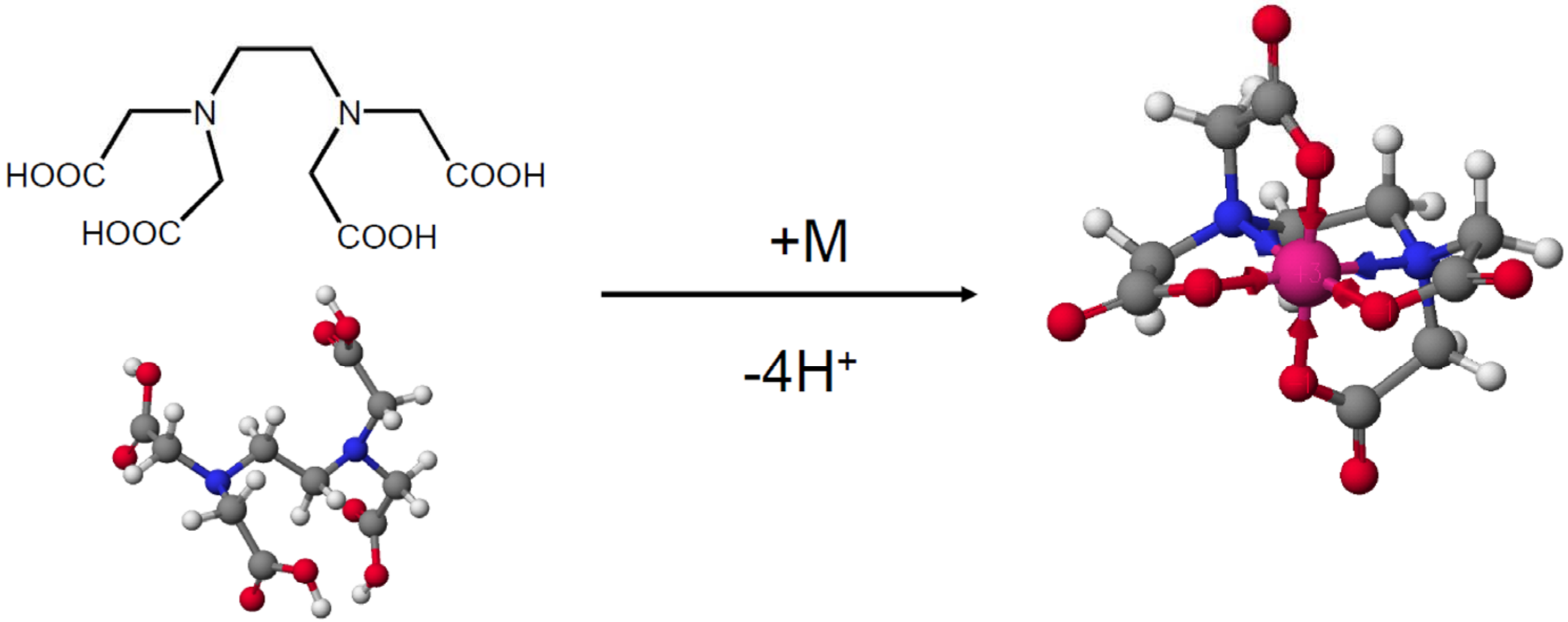
Stability of Metal Ion Complexes: The Irving-Williams Series



Strong chelating ligand: EDTA



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

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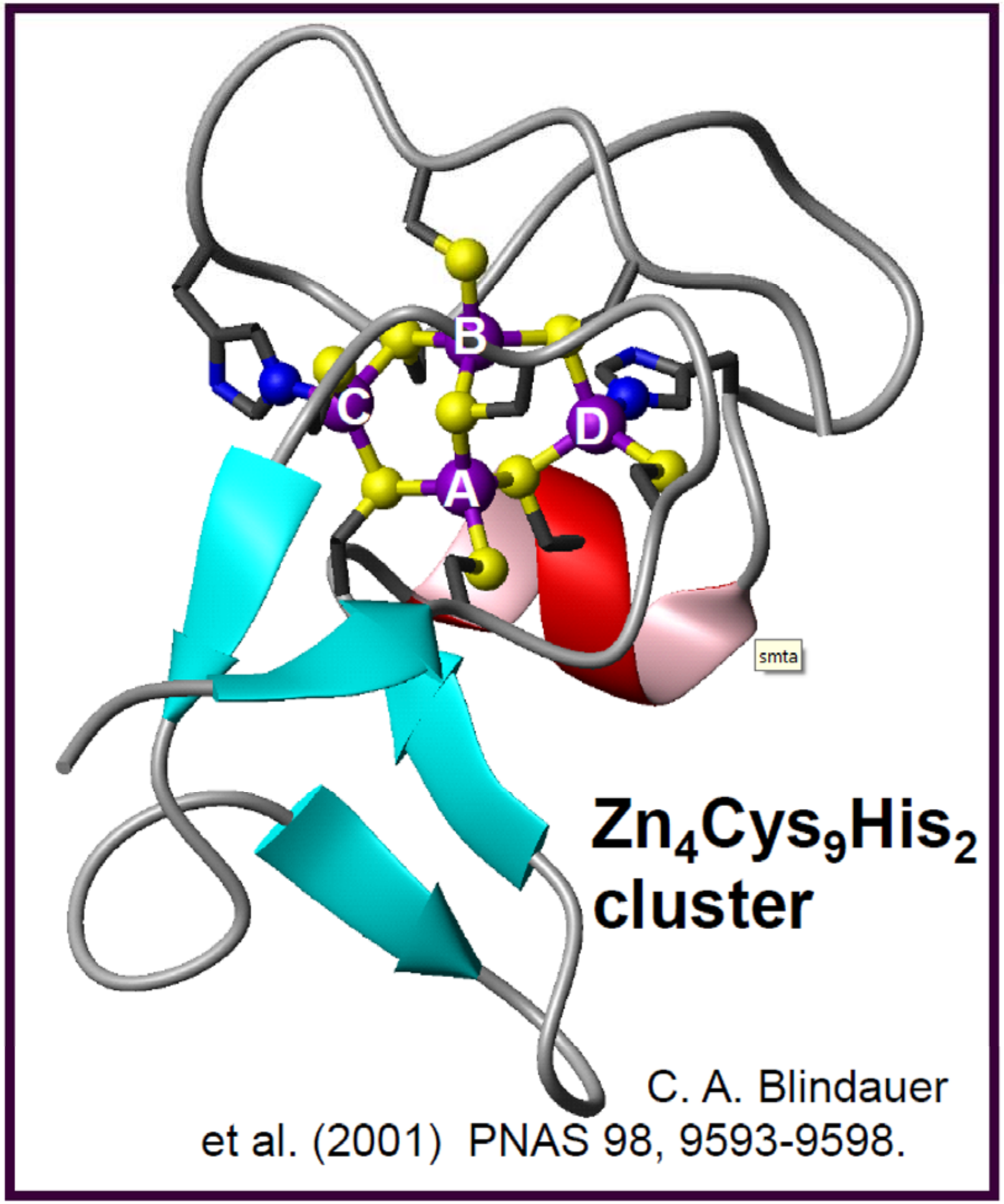
Protein Chelate: Bacterial Metallothionein (MT)



$Zn_4Cys_9His_2$ cluster

C. A. Blindauer et al. (2001) PNAS 98, 9593-9598.

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

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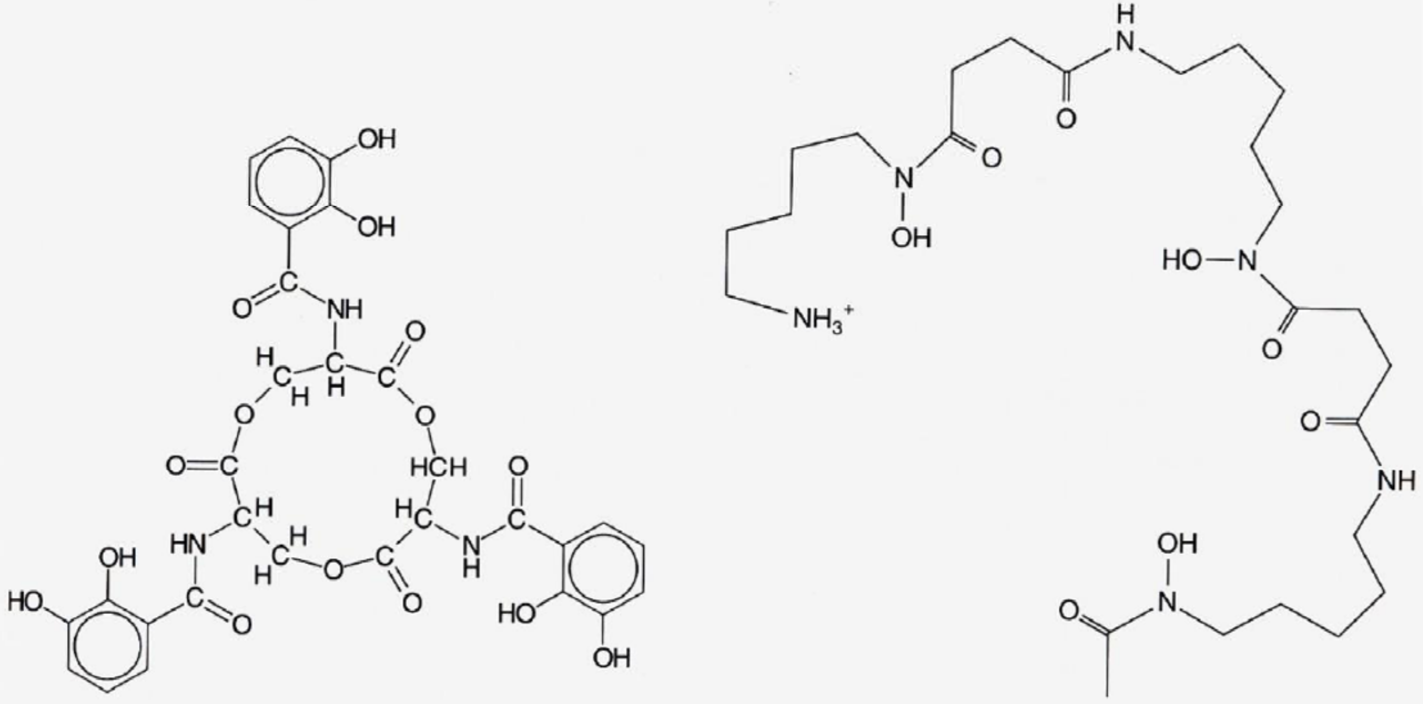
Biological Chelate: Siderophores



(a) Enterobactin **(b)** Desferrioxamin

Extremely stable complex of Enterobactin/Fe^{3+} K~ 10^{49}

Release of Fe through a) degradation of ligand, or b) protonation and reduction to Fe^{2+} which binds much weaker to the siderophore.

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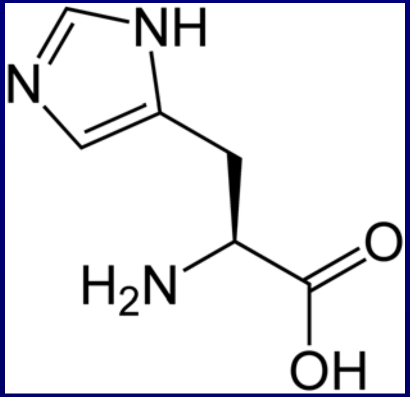
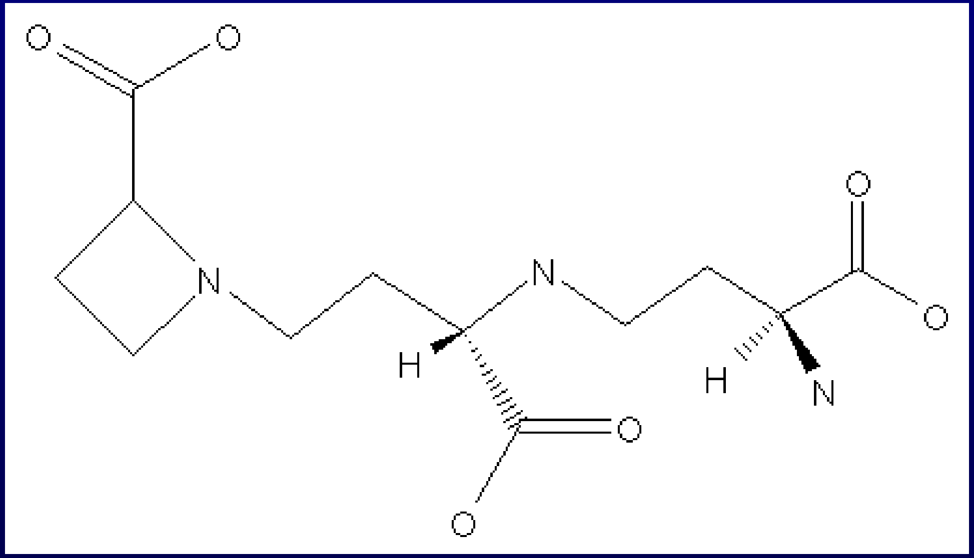
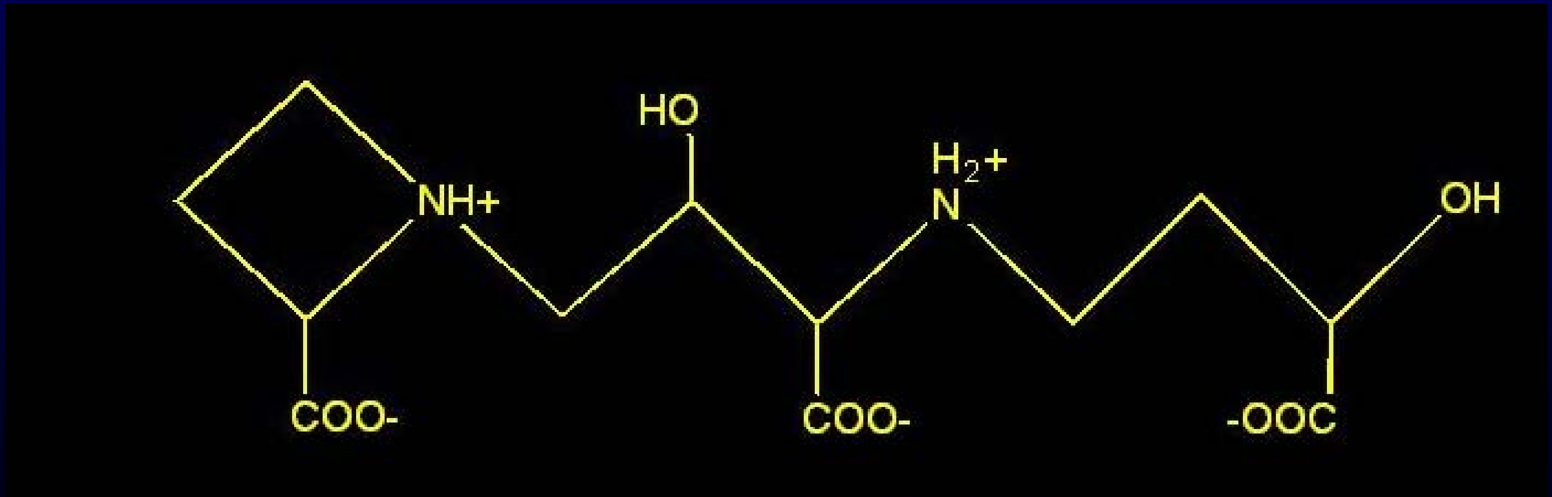
Long-distance transport ligands in plants



Histidine

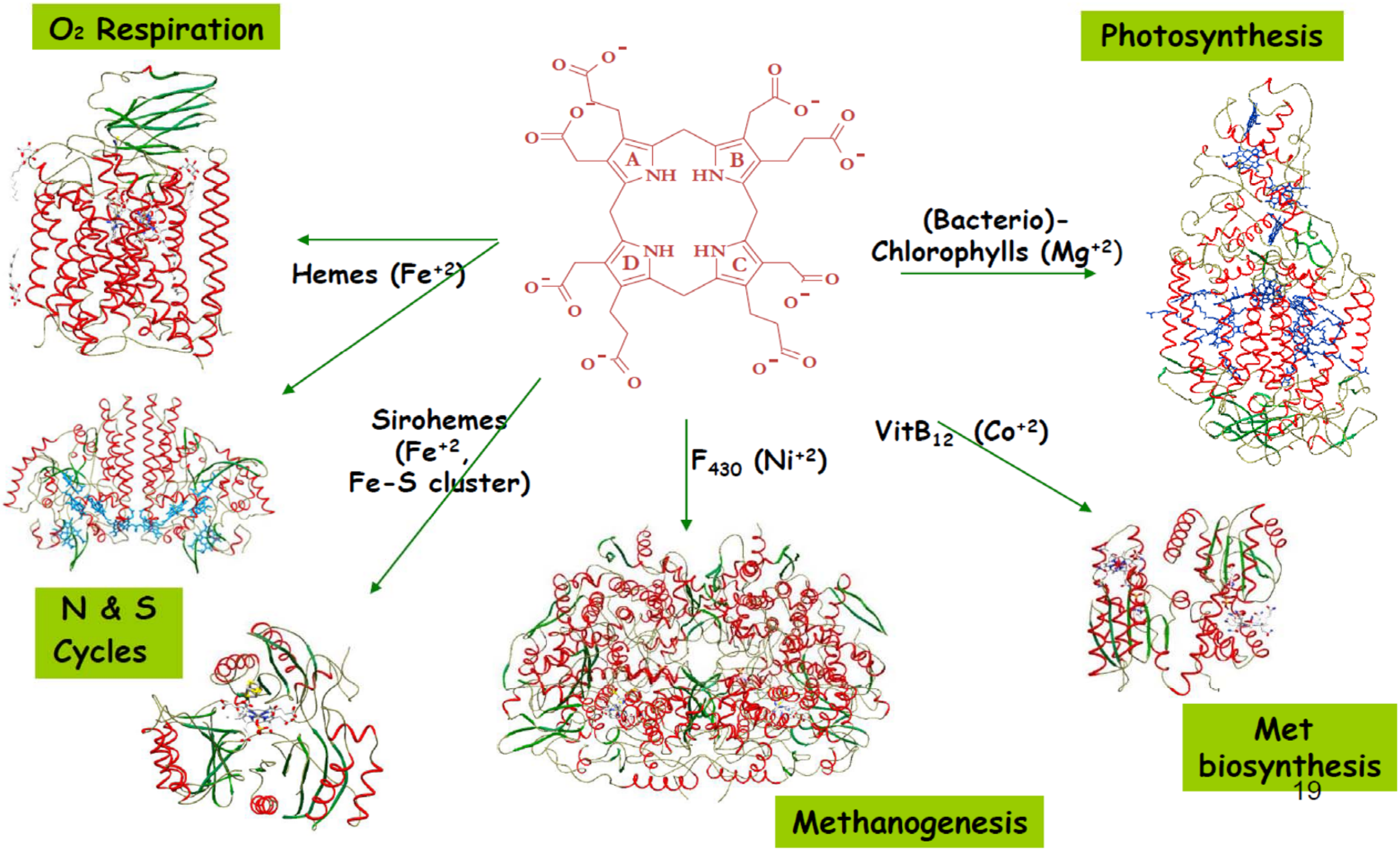


Nicotianamine



Mugineic acid

Tetrapyrrole - Versatile Ligand in Biology



O₂ Respiration

Hemes (Fe^{+2})

N & S Cycles

Sirohemes
$(Fe^{+2}$,
Fe-S cluster)

F_{430} (Ni^{+2})

Methanogenesis

(Bacterio)-
Chlorophylls (Mg^{+2})

Photosynthesis

$VitB_{12}$ (Co^{+2})

Met biosynthesis

Lecture slide from Peter Kroneck

Hard and Soft Acid-Base (HSAB) Principle

„Hard“ Ligands prefer „hard“ Metal ions

→ Ionic Bonds

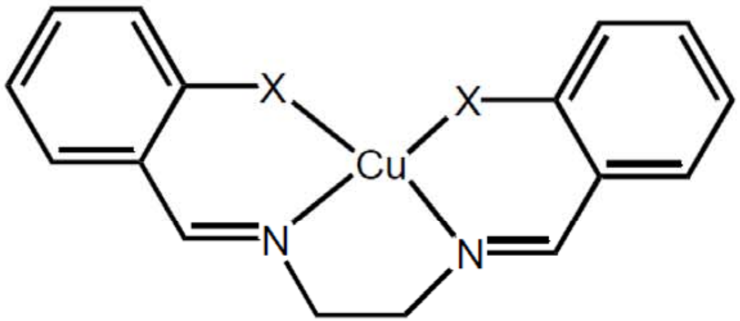
„Soft“ Ligands prefer „soft“ Metal ions

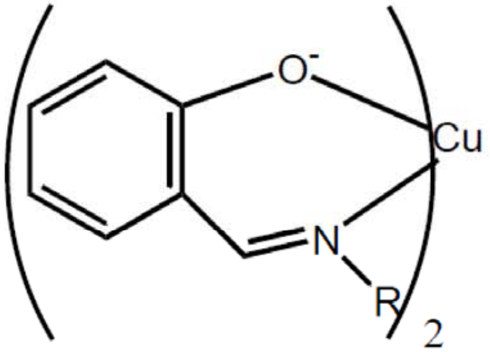
→ Covalent Bonds

Metal	Ligand
Hard	**Hard**
H^+, Na^+, K^+, Mg^{2+}, Ca^{2+} Mn^{2+}, Cr^{3+}, Co^{3+}, Fe^{3+}	H_2O, OH^-, $R\text{-}COO^-$, CO_3^{2-} NH_3, NO_3^-, $R\text{-}NH_2$, $R\text{-}O^-$, ROR
Borderline	**Borderline**
Fe^{2+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+} Co^{2+}, Cu^{2+}	NO_2^-, N_2, SO_3^{2-}, N_3^-, $Ph\text{-}NH_2$ Imidazole
Soft	**Soft**
Cu^+, Pt^{2+}, Au^+, Hg^{2+}, Cd^{2+}	R_2S, RS^-, R_3P, CN^-, SCN^-, O^{2-} S^{2-}, R^-, H^-

Lecture slide from Peter Kroneck

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





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

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

- **Soft Ligand (RS^-) stabilizes Cu(I) state**
- **Positive Potential**

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

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

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

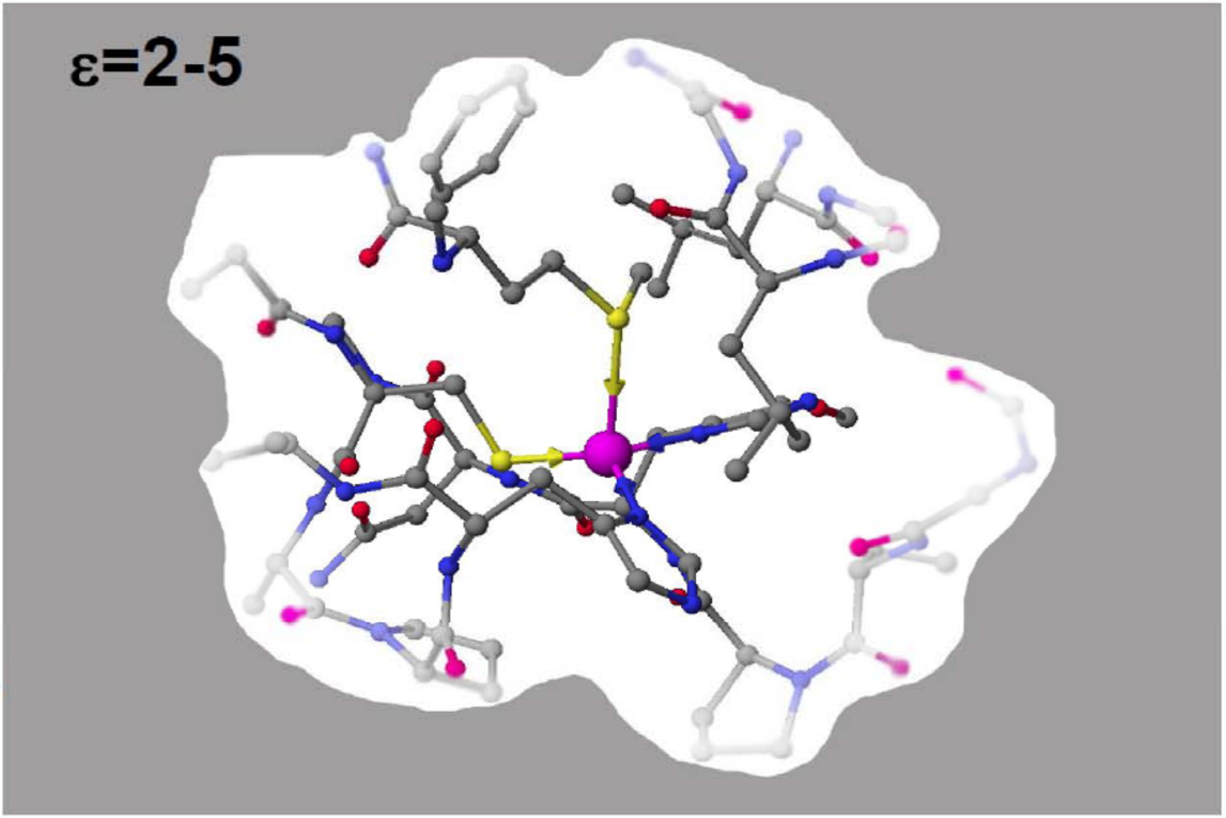
R=*t*-Bu : $E_{1/2}$ = -0.66 V

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

Lecture slide from Peter Kroneck

Influence of Protein Environment

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



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

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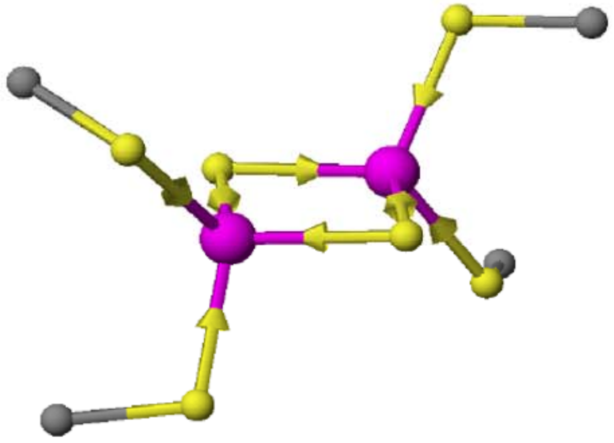
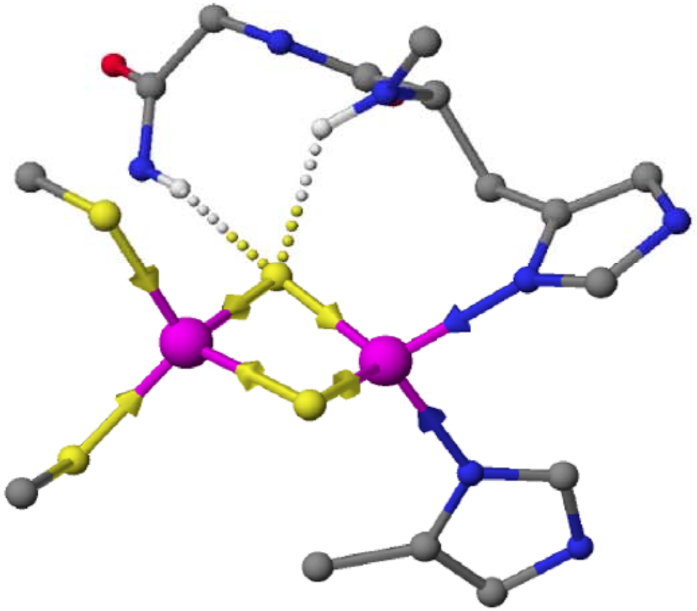
Modulation of Redox potentials (H bridges)

2Fe-2S Ferredoxin



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

2Fe-2S Rieske



$E^{0`} \sim +280$ 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

Lecture slide from Peter Kroneck

Proteins Tune the Properties of Metal Ions

Coordination number

- The lower the higher the Lewis acidity

Coordination geometry

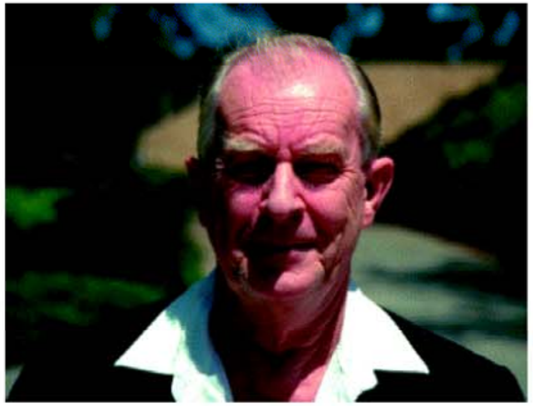
- Proteins can dictate distortion
- Distortion can change reactivity of metal ion

Weak interactions - Second Shell Effects

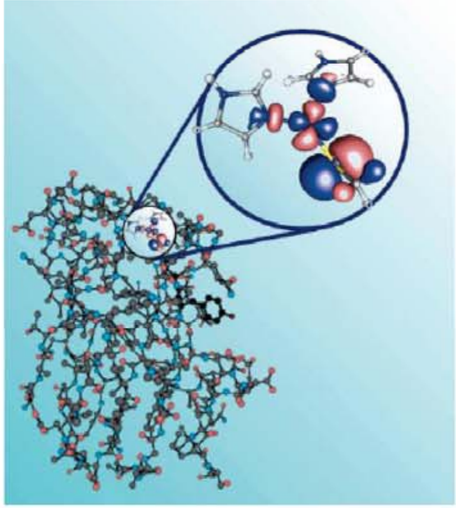
- Hydrogen bonds to bound ligands
- Hydrophobic residues: dielectric constant can change stability of metal-ligand bonds

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



Lecture slide from Peter Kroneck

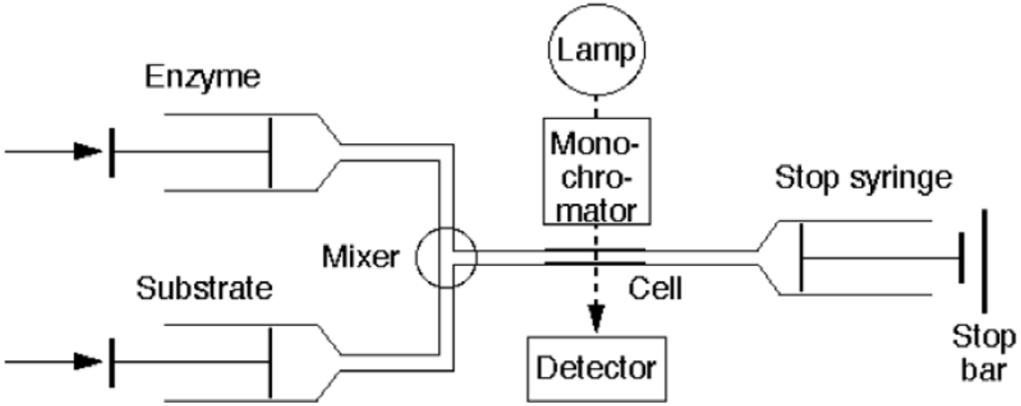
How to study *Fast Reactions* (ms-µs)

- **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)

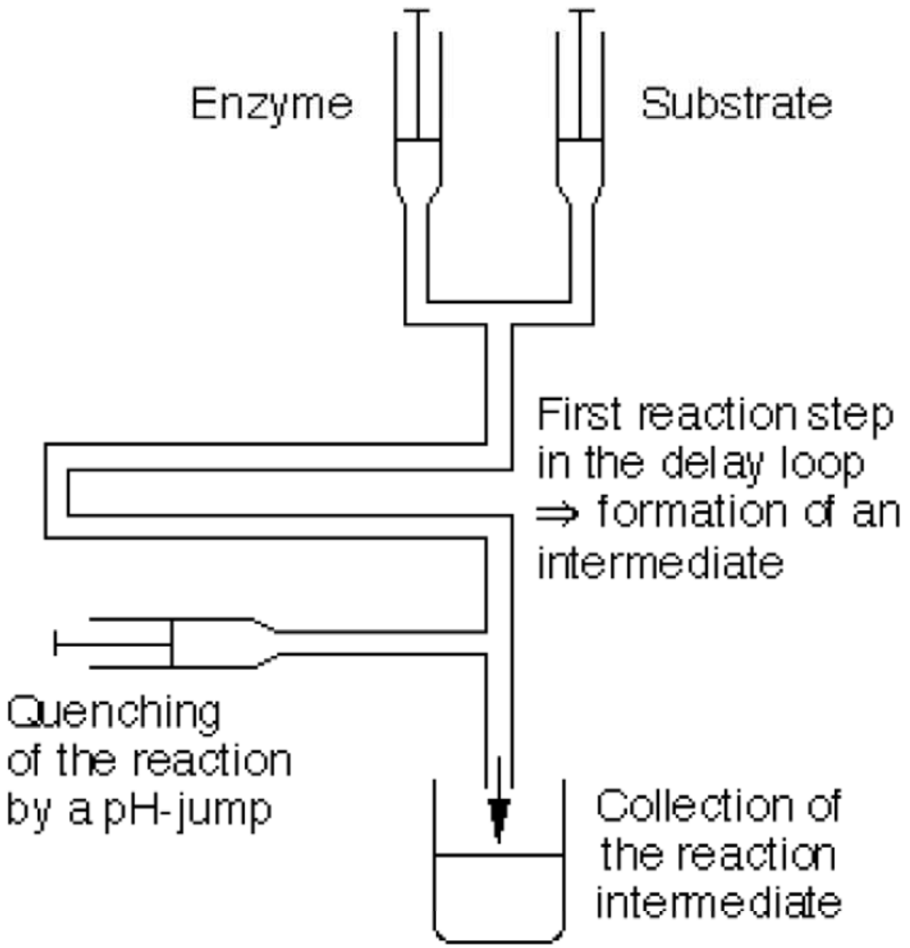
D. Ballou, Ann Arbor

- 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

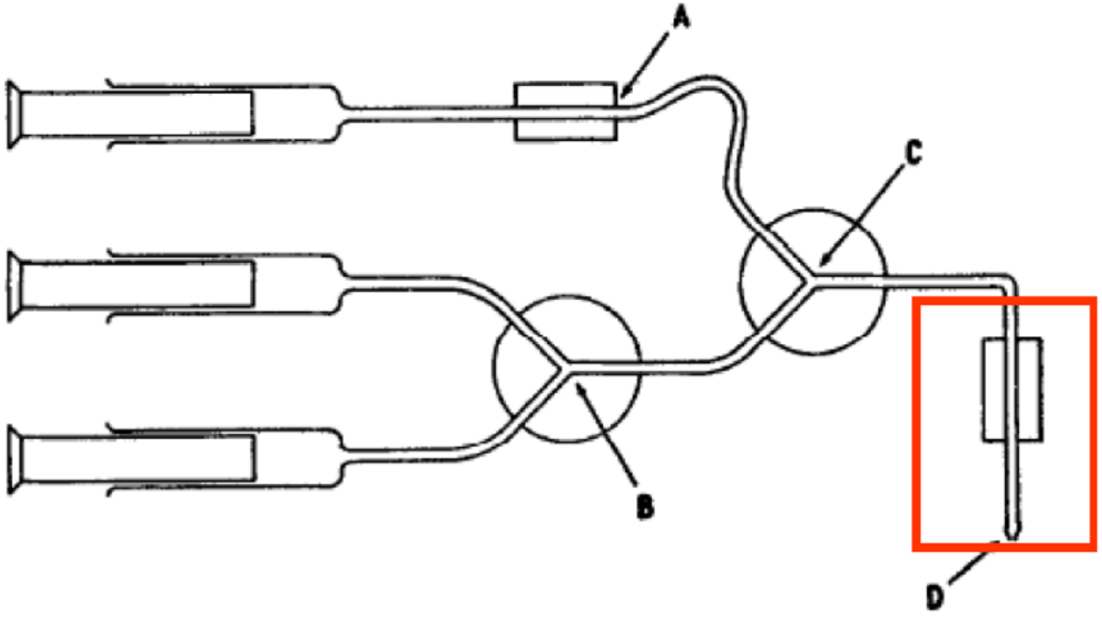


The figure shows a typical set-up for the detection and analysis of reaction intermediates. Kinetic information on a time scale of about 1 ms and longer is obtainable with conventional quench-flow devices. Higher time resolution can be achieved with microvolume (microcapillary) mixers.

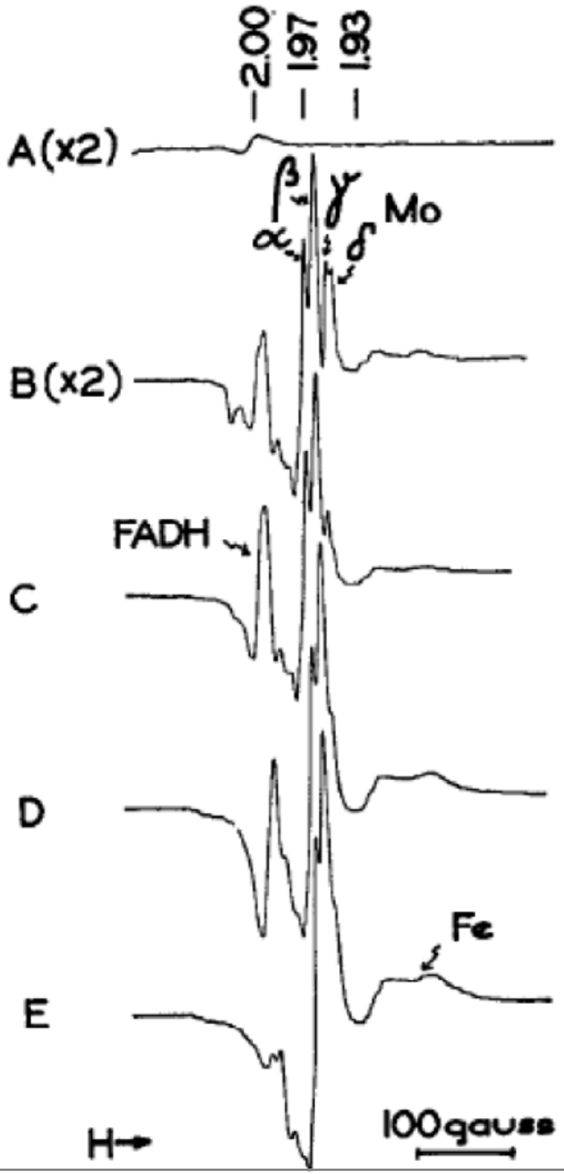
Lecture slide from Peter Kroneck

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

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



Isopentane Bath at minus 140-150 C°



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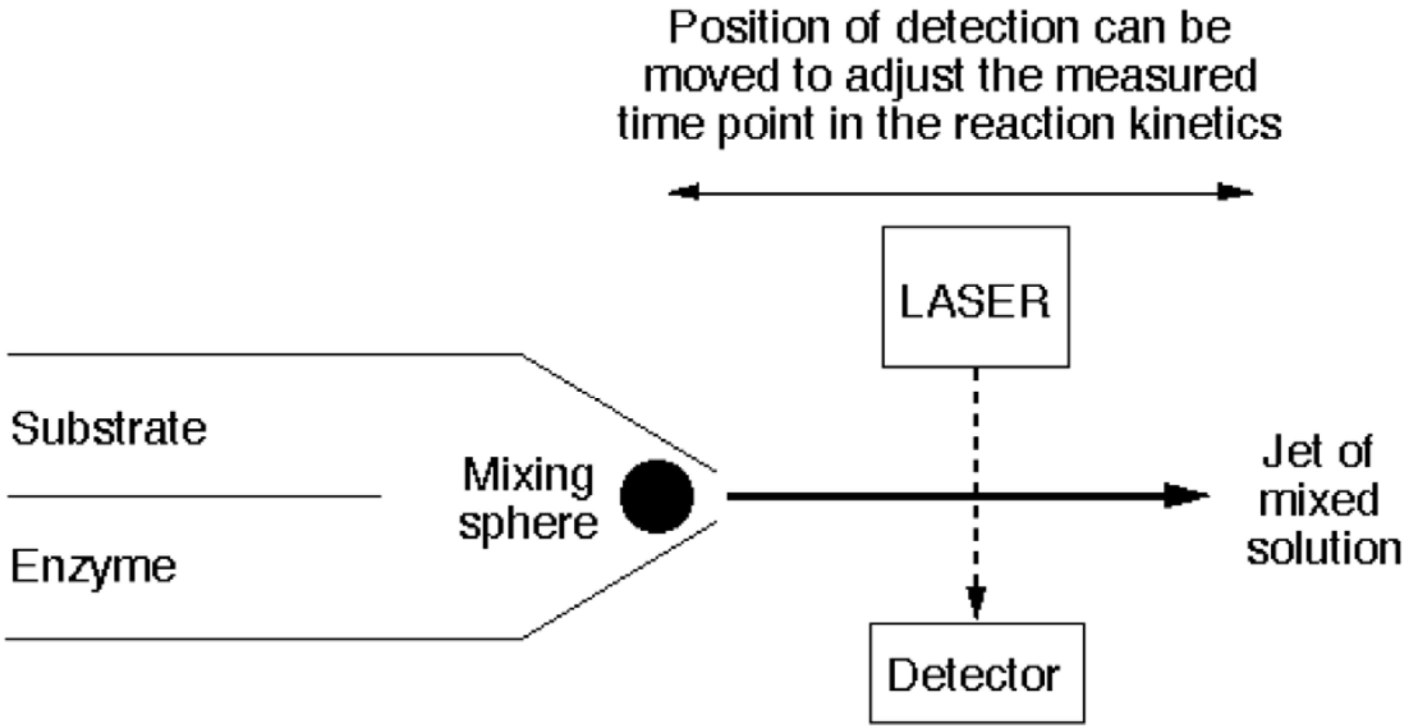
From Milliseconds to Microseconds (1)

- **Unfortunately, the dead time of common stopped-flow devices is usually around <u>2-5 milliseconds</u>. 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 <u>continuous-flow mixing</u>: 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-1, a dead length of 100 mm corresponds to a dead time of 1-10 µs. 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 µs.

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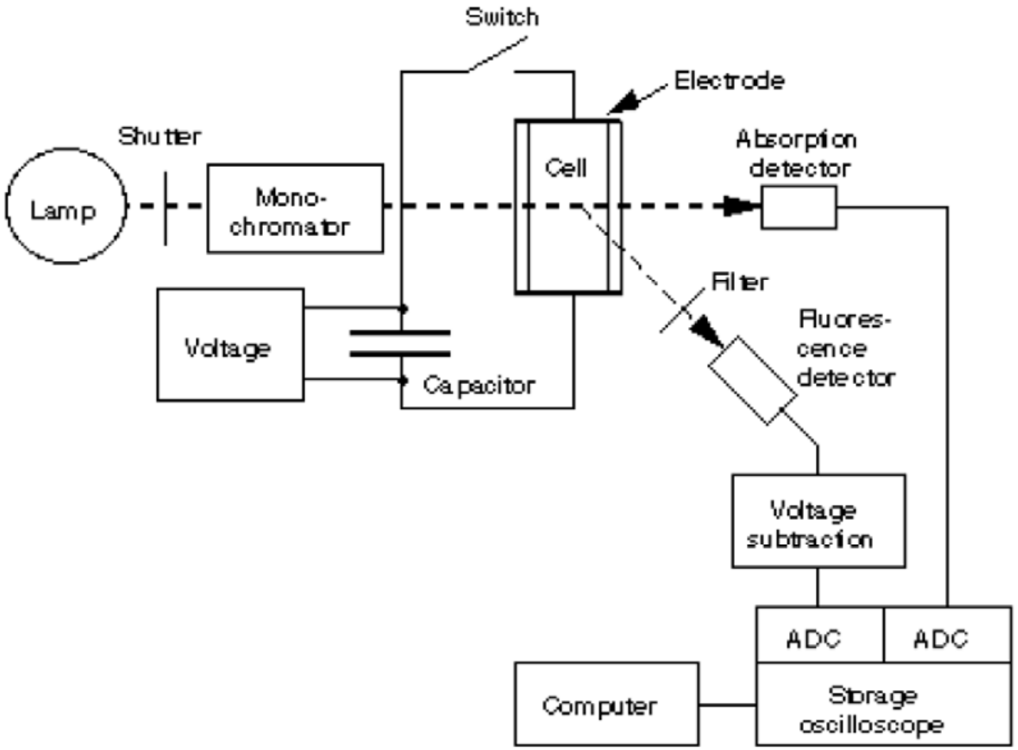
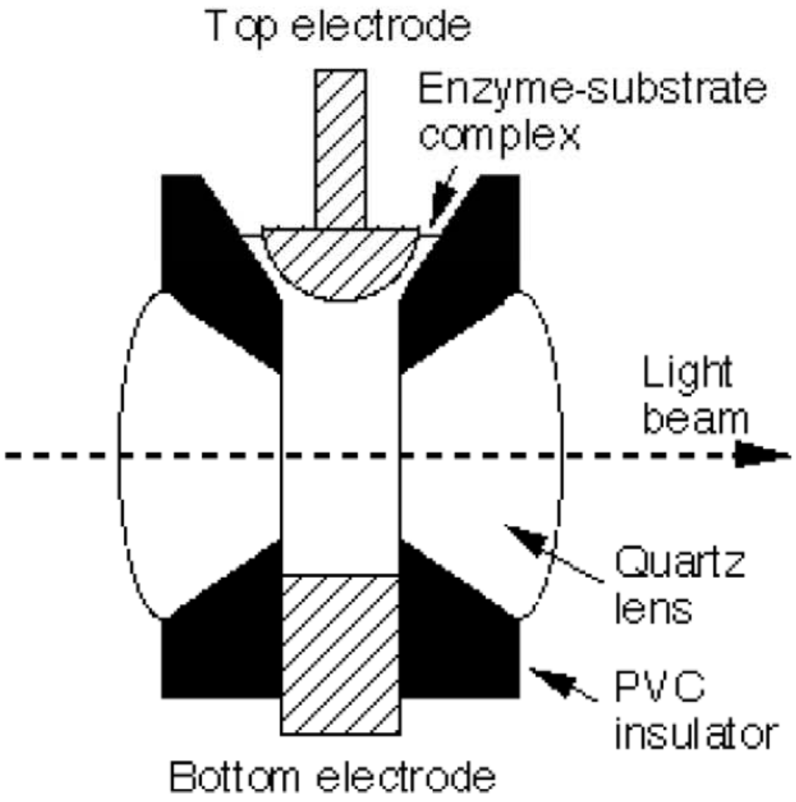
Electrical-discharge-induced T-jump method pioneered by Manfred Eigen (Göttingen)



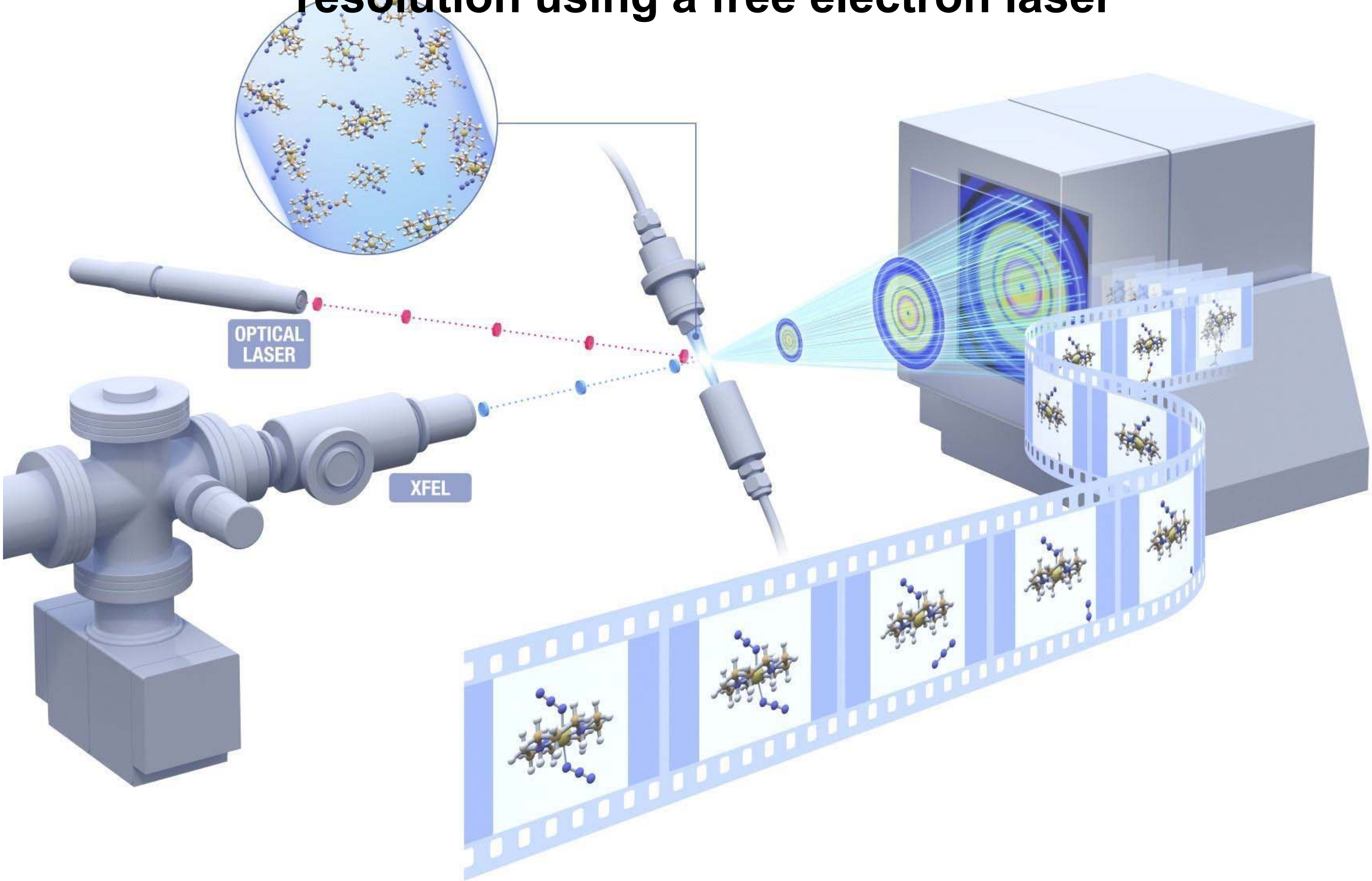
Solutions with electrolytes may have sufficient electrical conductivity to be heated by a rapid electrical discharge. Joule heating with rise times of 1 ms or faster are achieved in 100 mM KCl. A capacitor is charged by a power supply up to a specific voltage and then rapidly discharged through the sample 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



Information from DESY (Germany)

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