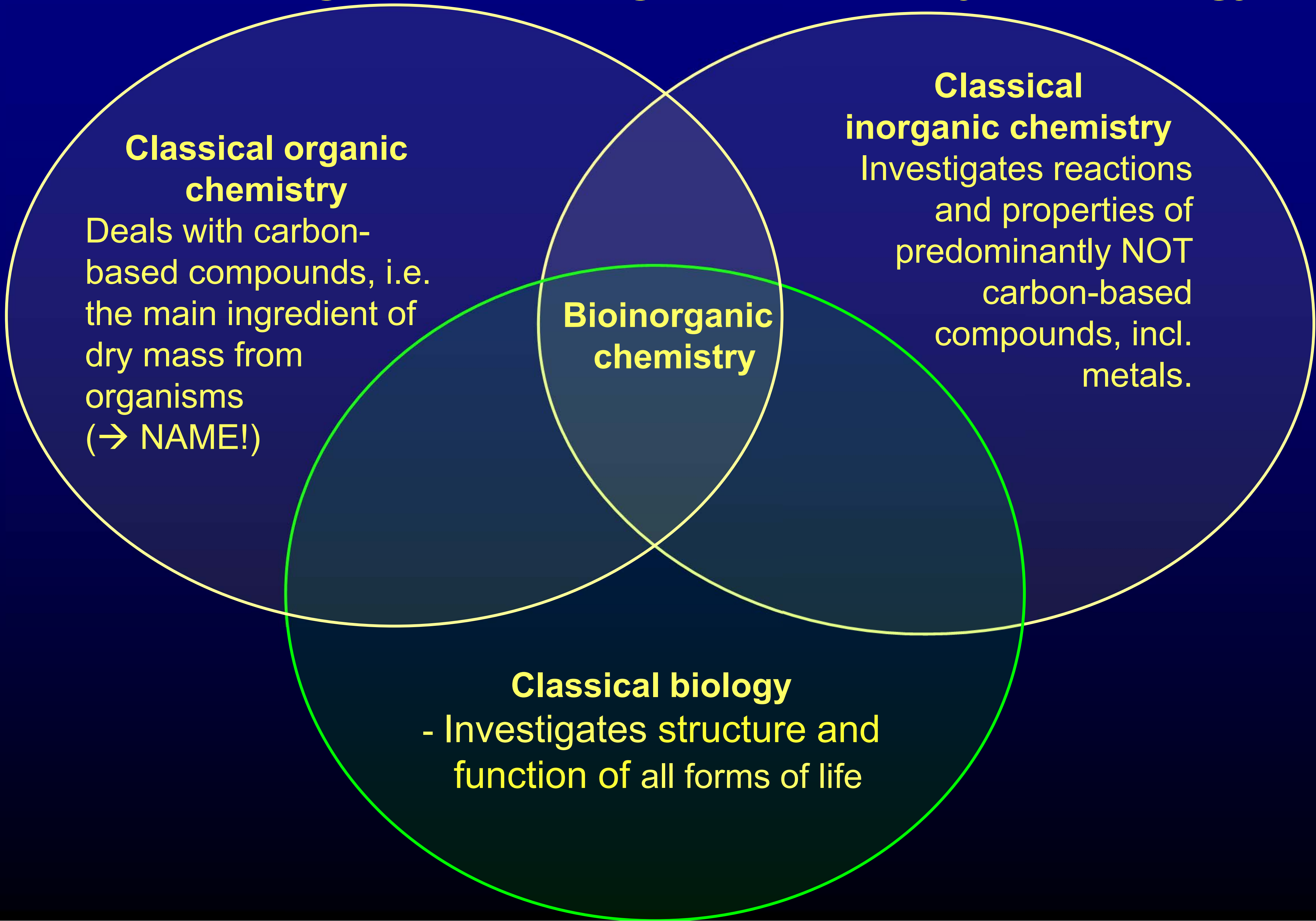
Basics of metalloprotein biochemistry

Bioinorganic Chemistry versus
Classical organic and inorganic Chemistry and Biology



Themes of bioinorganic chemistry research

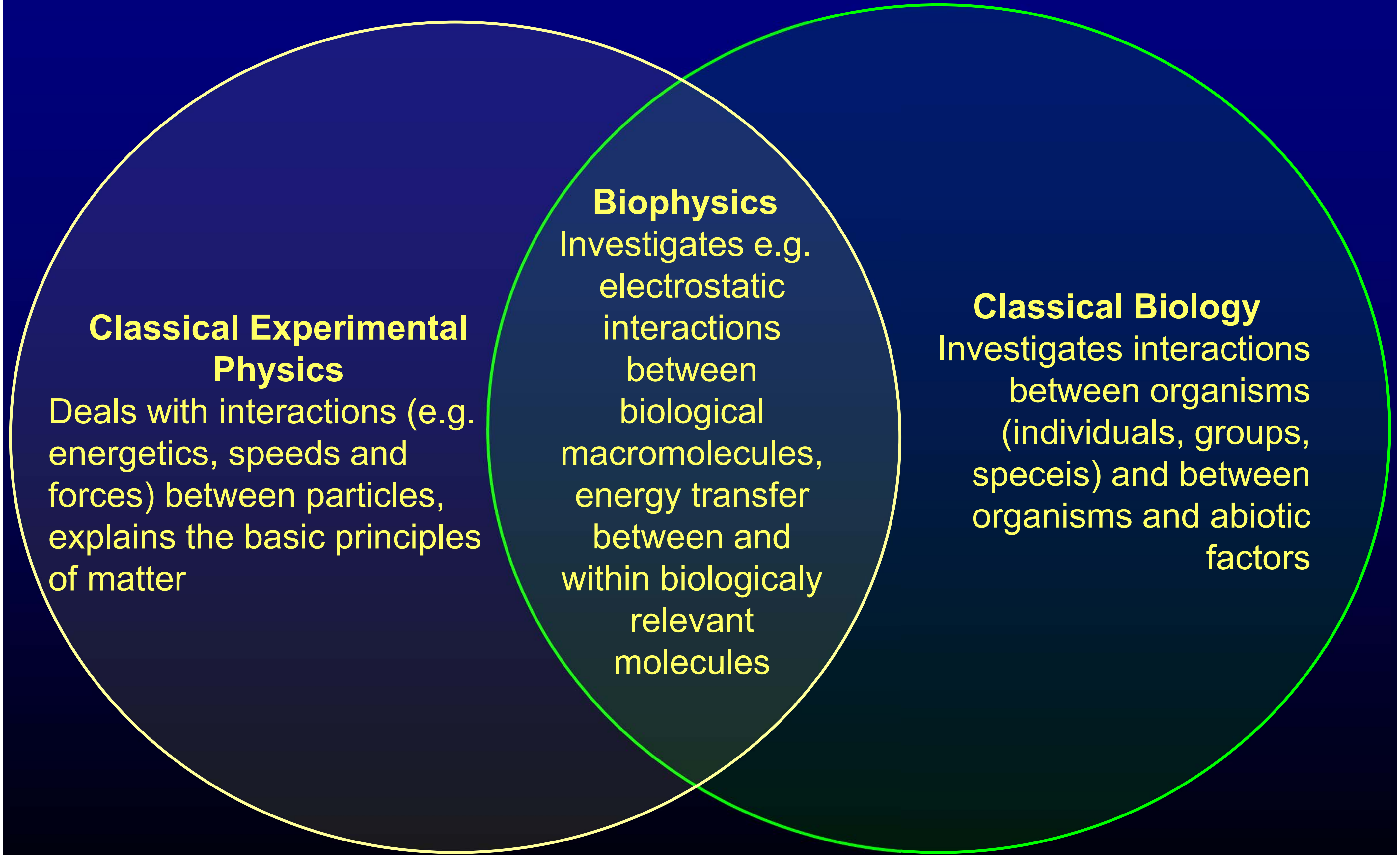
Metal coordination in biological ligands

→ Metal(loid) transport
→ Metal(loid) storage
→ Metal-based catalysis in biology, usually via metal-based active sites in enzymes
→ Metals as structural elements in proteins
→ Metal(loid) deficiency and toxicity
→ Metal(loid) detoxification

Methods used for investigating these questions include for example (in solutions, in models systems, but also in living cells)

- UV/VIS absorption and fluorescence spectroscopy (→ electronic transitions to/from excited states)
- X-ray absorption and emission spectroscopy (→ ionisation energies = X-ray absorption edges and emission bands, their element-specific characterisitics and their modification by redox state and neighbouring atoms)
- EPR spectroscopy (→ analysis of the ligand environment of paramagnetic metal ions)
- NMR spectroscopy (→ analysis of the environment of NMR-active nuclei)

Biophysics versus
Classical Experimental Physics and Classical Biology

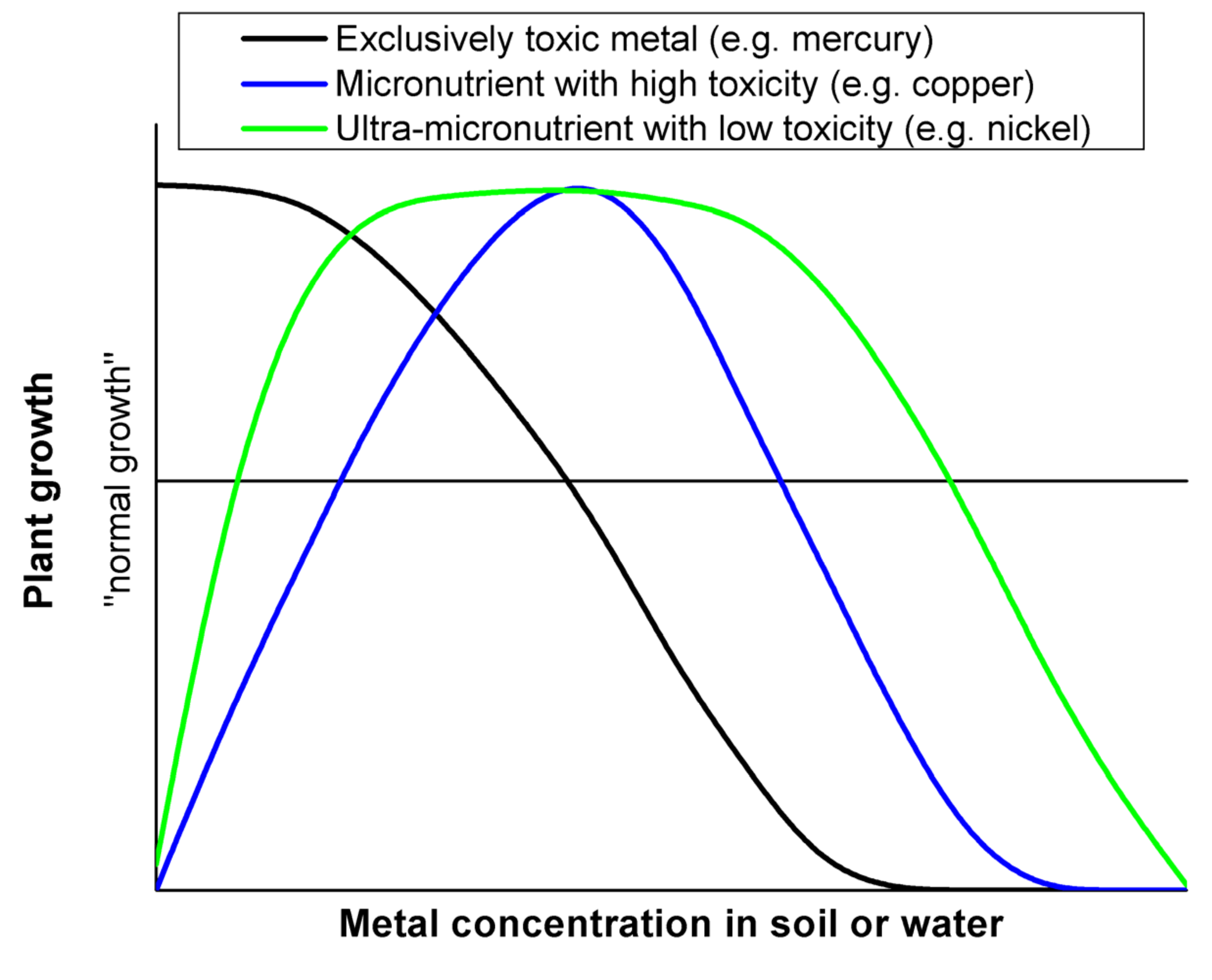


Themes of biophysical research

Energetics and kinetics of biological processes
→ transport (e.g. of metals)
→ catalysis in biology, usually via metal-based active sites in enzymes
→ reversible coupling of biologically relevant molecules without bond formation/breakage
→ protein folding

Methods used for investigating these questions include for example (in solutions, in models systems, but also in living cells)
- UV/VIS absorption and fluorescence spectroscopy (→ electronic transitions to/from excited states → e.g. analysis of chromophore coupling)
- X-ray absorption spectroscopy (→ ionisation energies = X-ray absorption edges and emission bands, their element-specific characteristics and their modification by redox state and neighbouring atoms)
- EPR spectroscopy (→ e.g. spin labelling for analysis of protein folding)
- NMR spectroscopy (→ e.g. analysis of kinetics of protein (re-/un-)folding)

Dose-response principle for transition metals

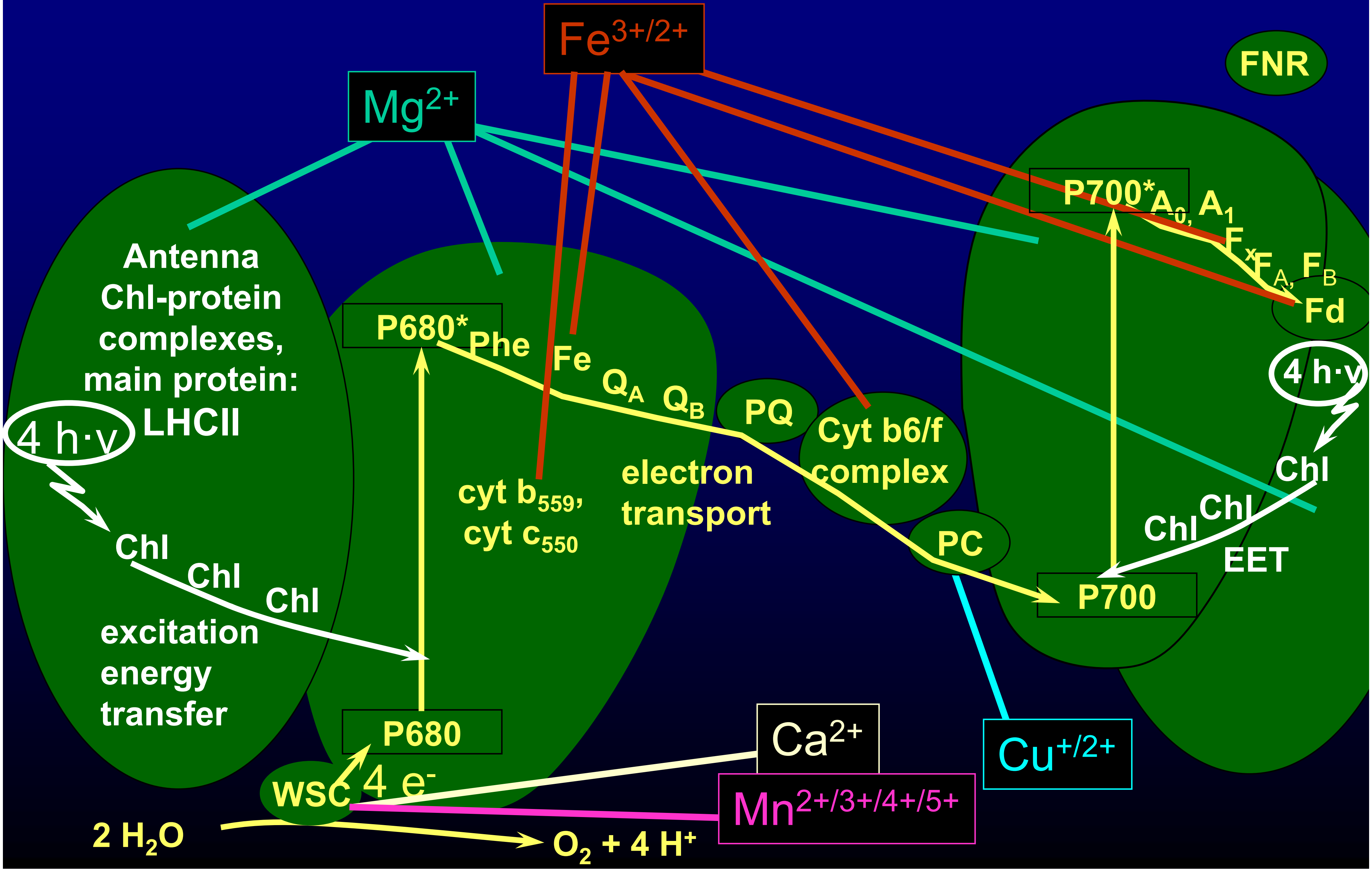


Küpper H, Kroneck PMH, 2005, Metal ions Life Sci 2, 31-62

Why Investigate Metals in Biology ?

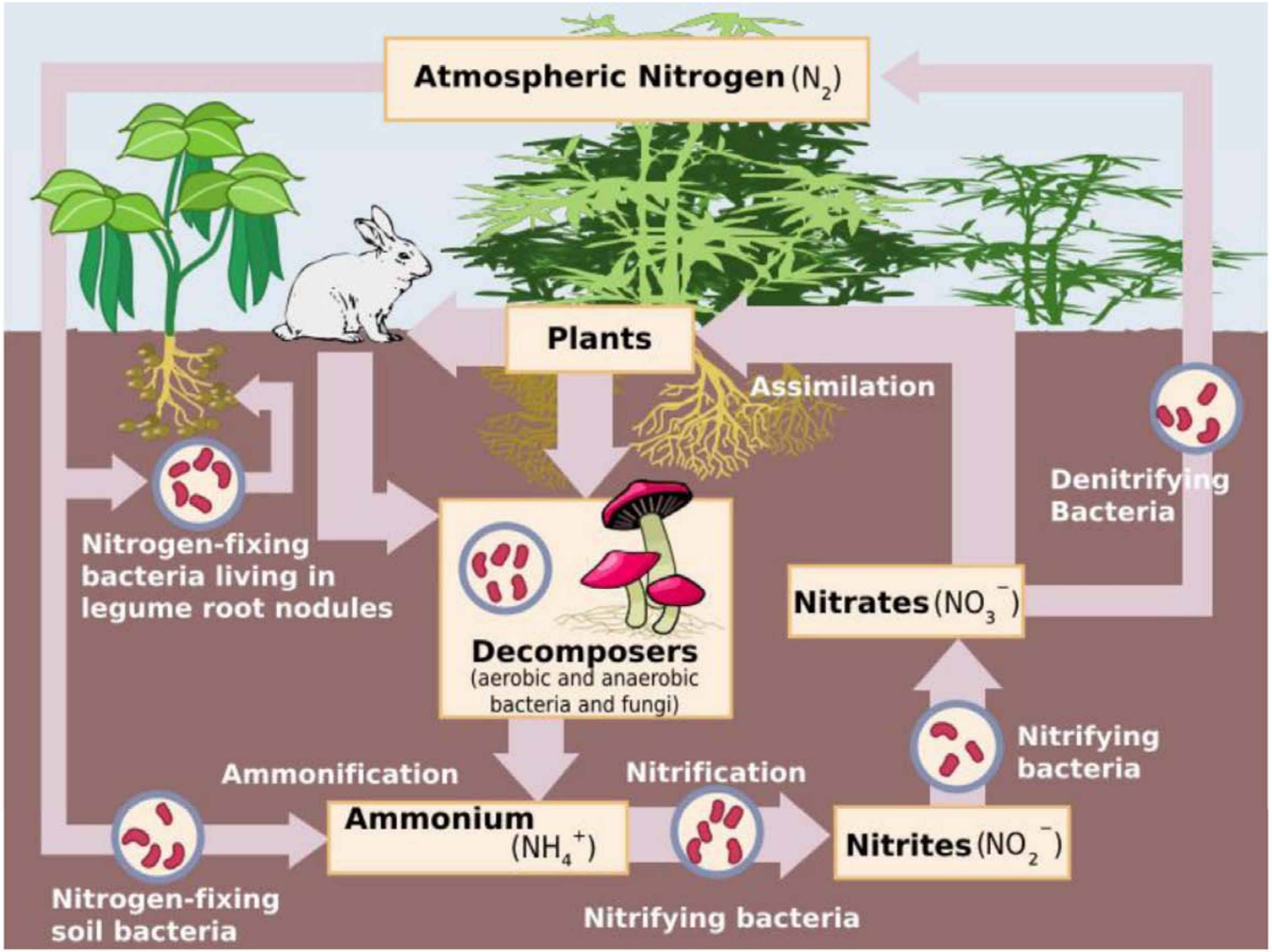
- **There is hardly any important process in nature which does not depend on a metal ion; ~ 1/3 of the proteins of the human genome depend on metal ions**
- **Novel Materials, Structures and Reactions**
- **Trigger - Signaling - Sensing - Regulation**
- **Acid-Base Catalysis**
- **Redox – Proton & Electron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

Case 1: Metal sites in photosynthetic proteins



Case 2: Nitrogen Fixation - Nitrogenase

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



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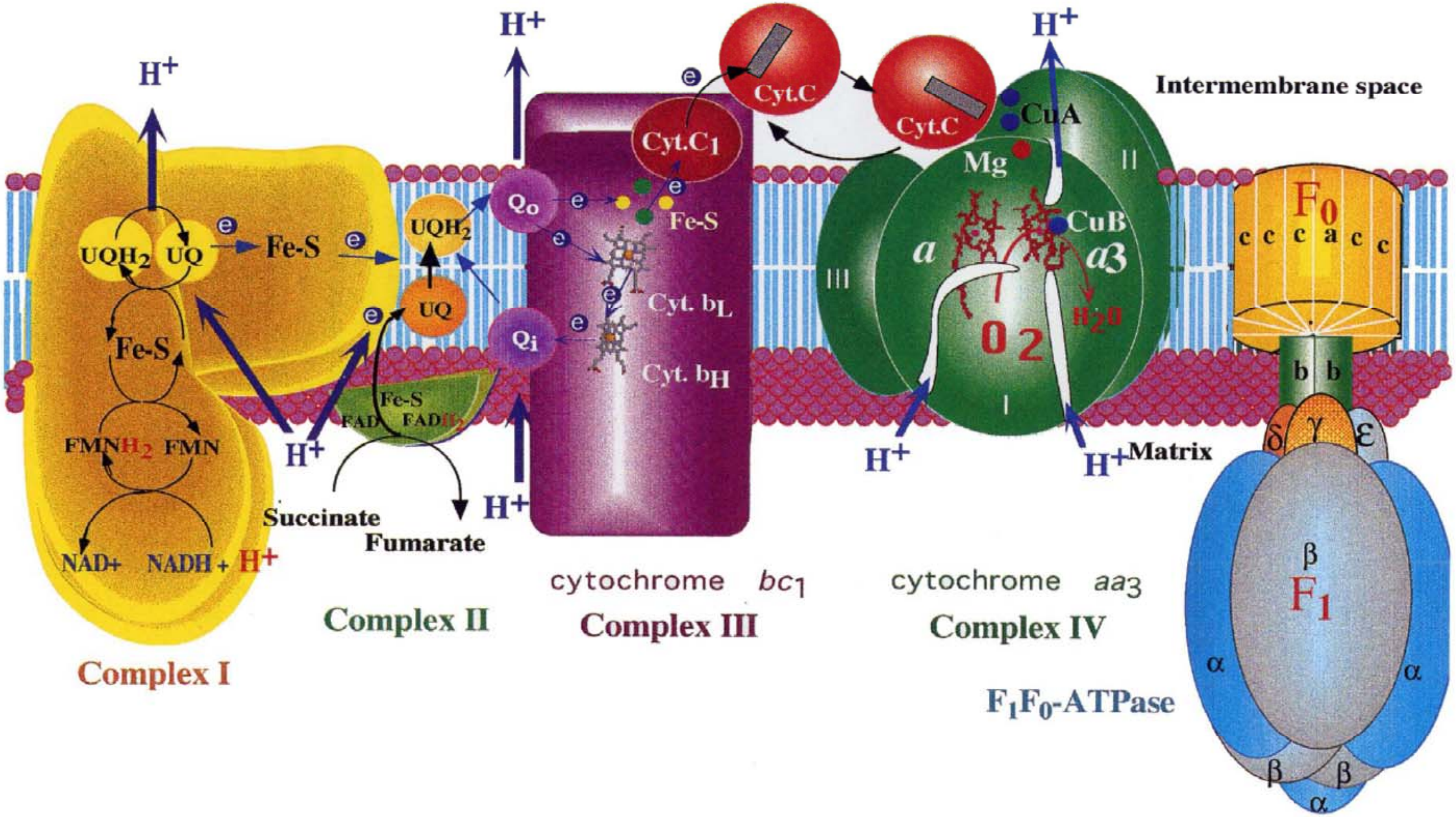
Case 3: Respiration – Reduction of O_2 to H_2O

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

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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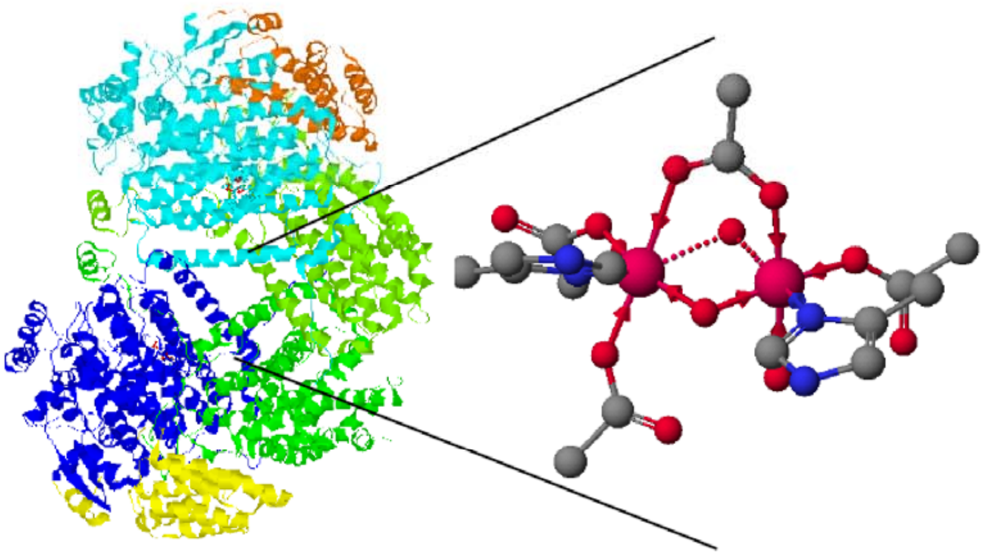
Basic Features of a Metal Protein Complex

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

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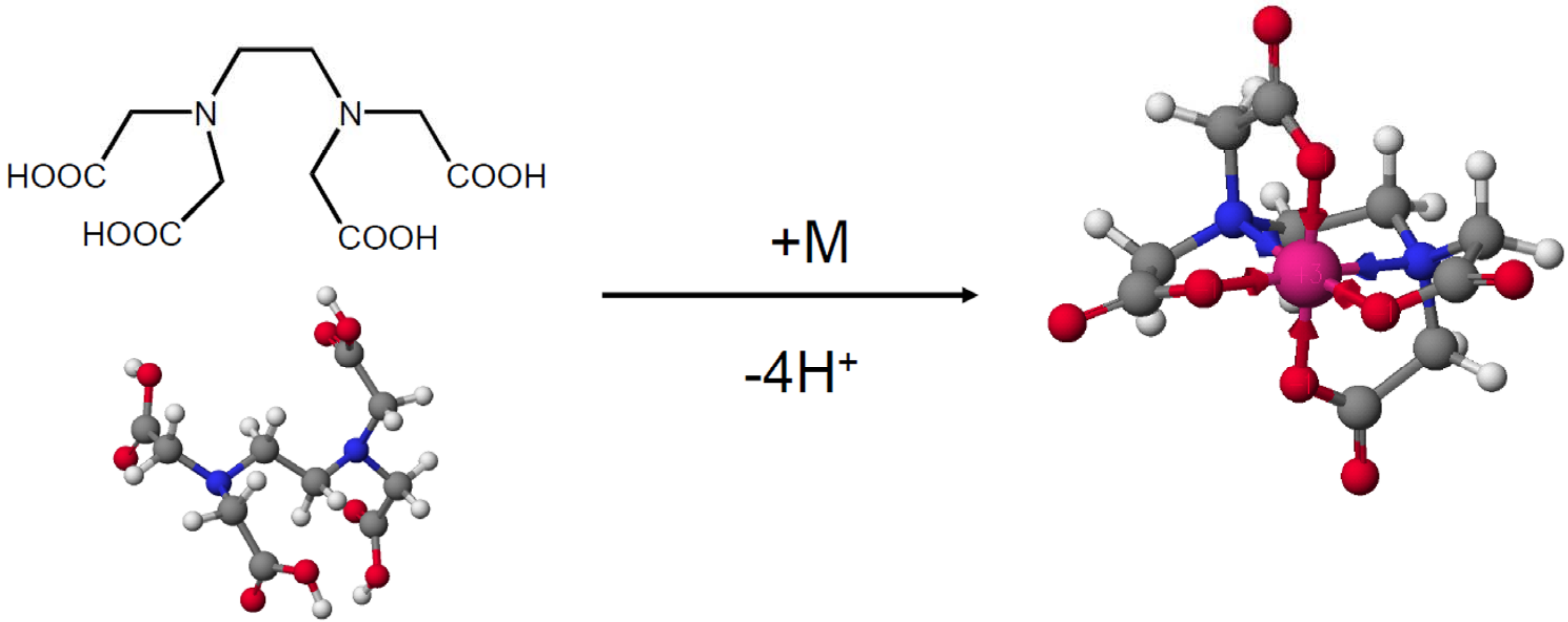
Strong chelating ligand: EDTA



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

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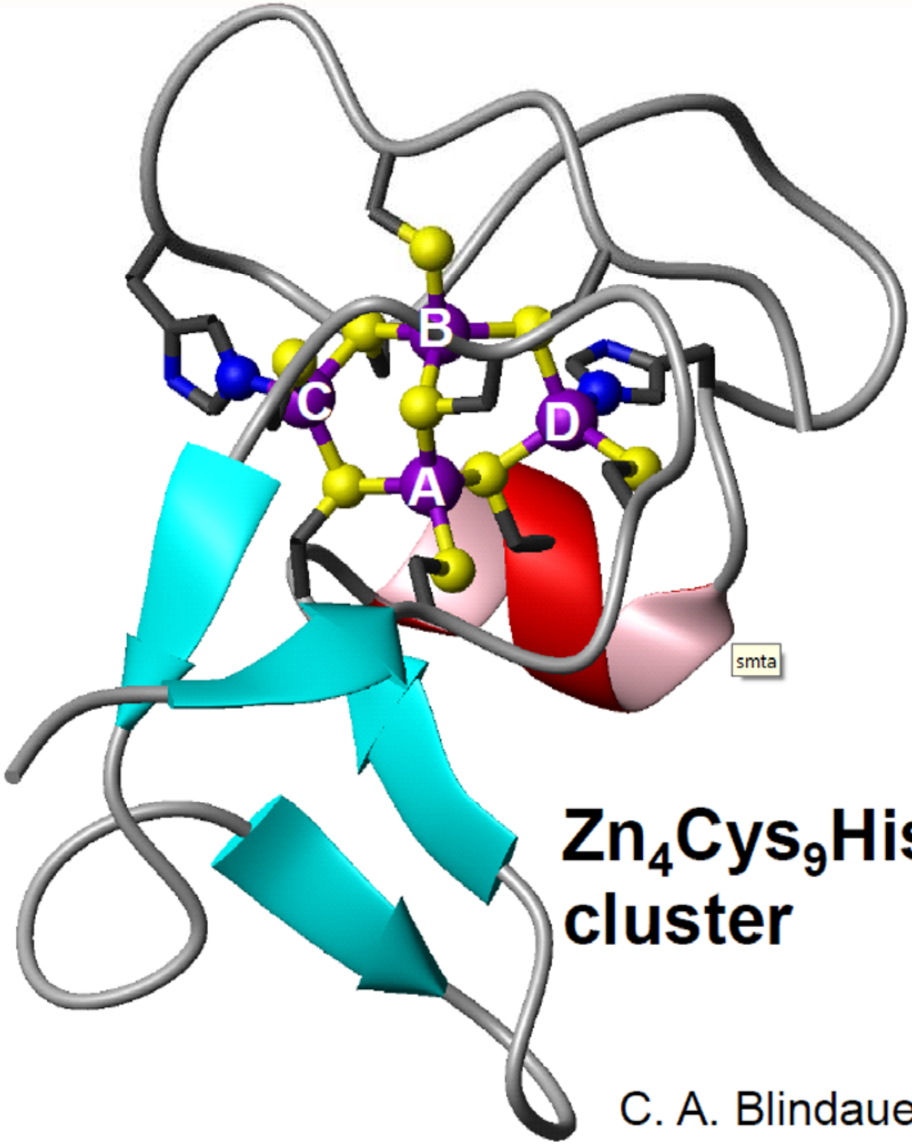
Protein Chelate: Bacterial Metallothionein (MT)

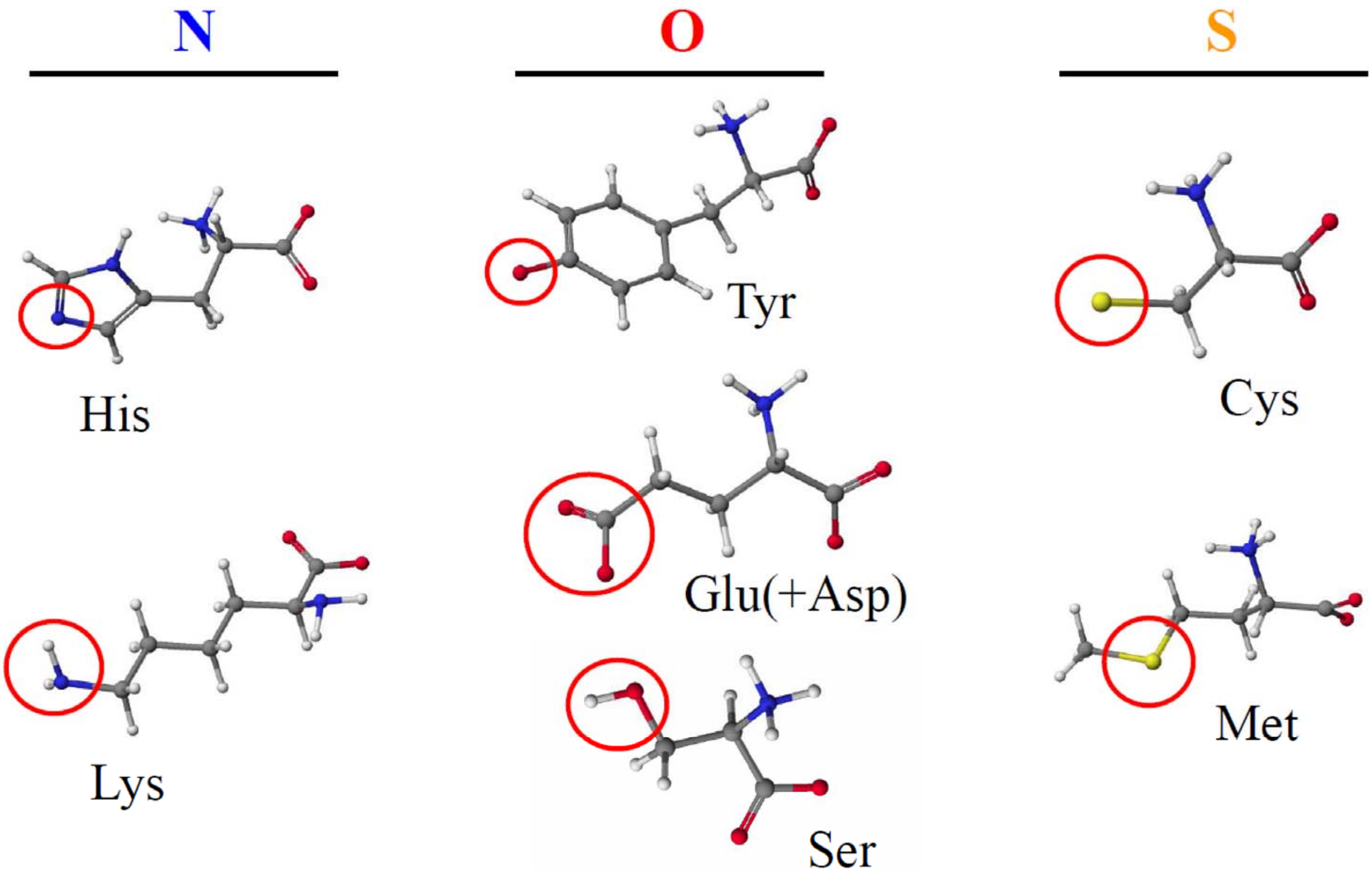


$Zn_4Cys_9His_2$ cluster

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)

Protein Ligands – Amino Acid Residues



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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-COO^-, CO_3^{2-} NH_3, NO_3^-, R-NH_2, R-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-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^-

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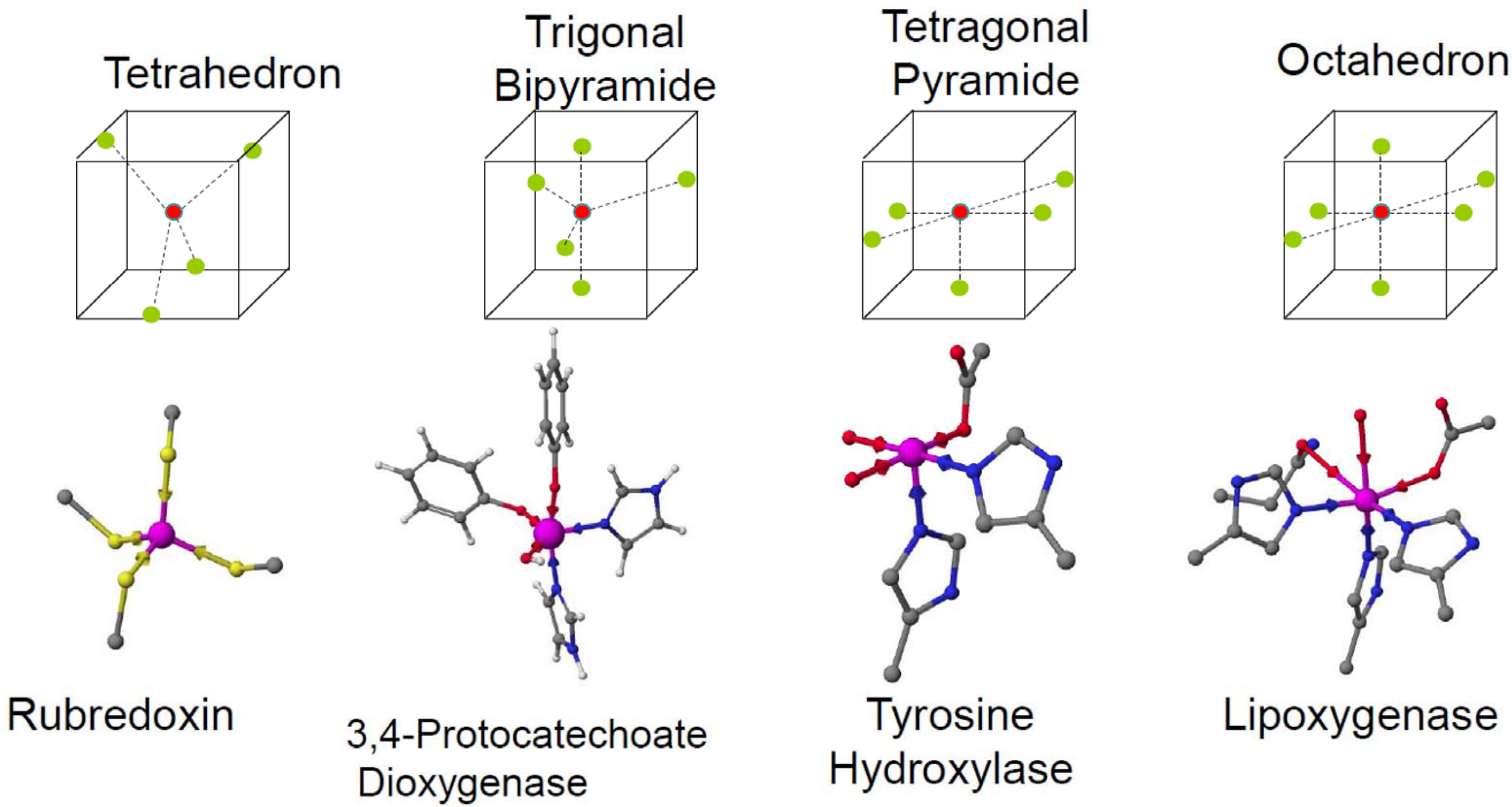
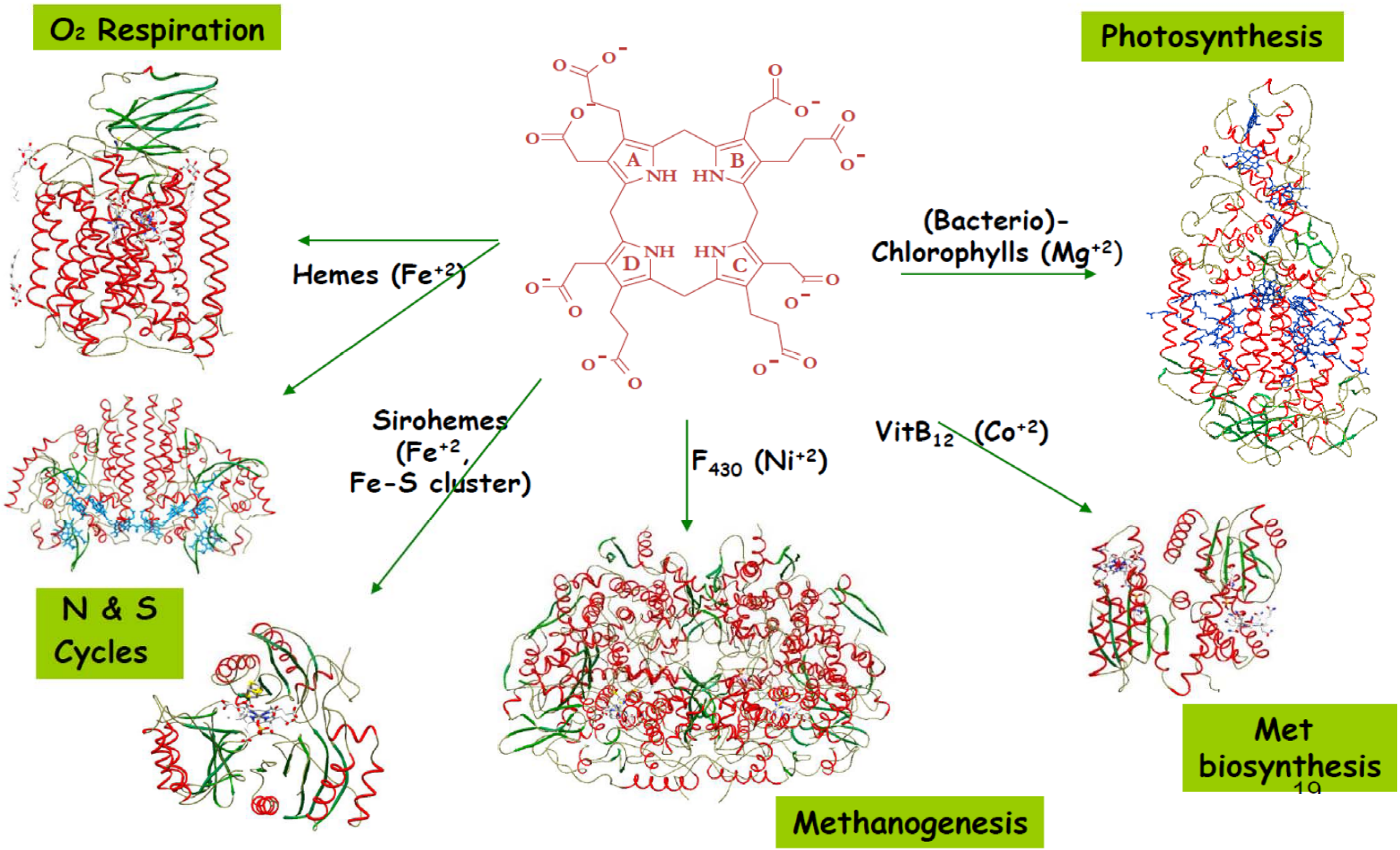
Geometry is important: Iron Proteins



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

Tetrapyrrole - Versatile Ligand in Biology



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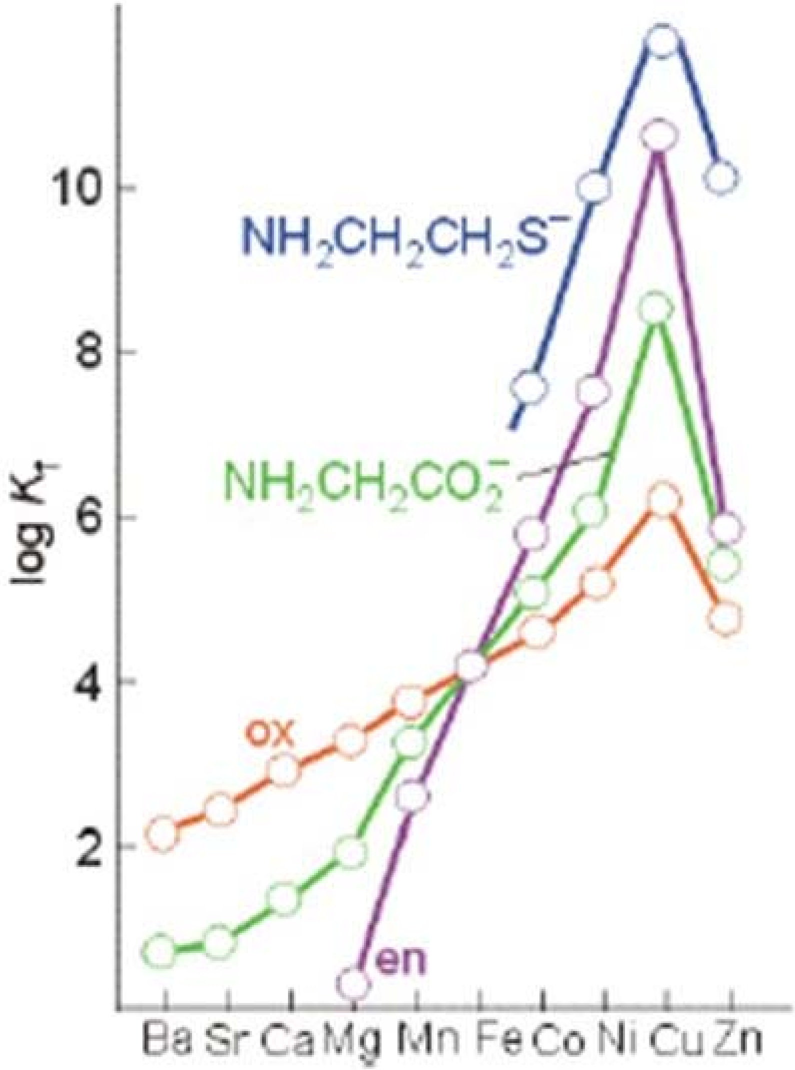
Stability of Metal Ion Complexes: The Irving-Williams Series



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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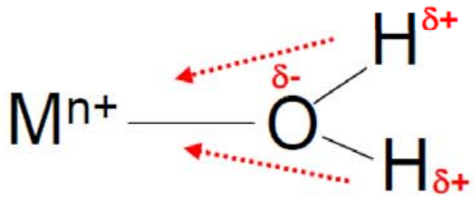
Modulation of pK_a

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

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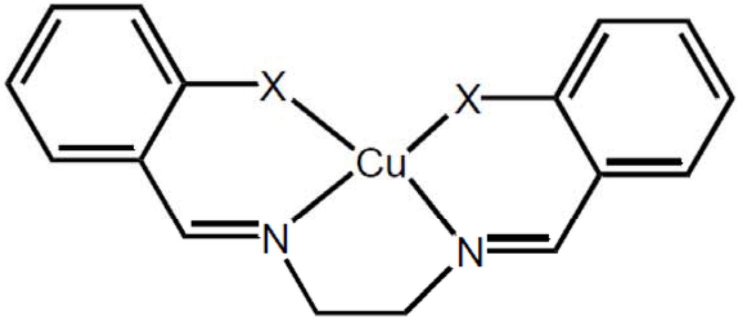
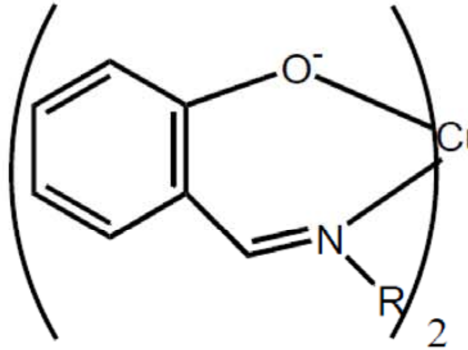
Modulation/tuning of Redox Potentials $E_{1/2}$





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

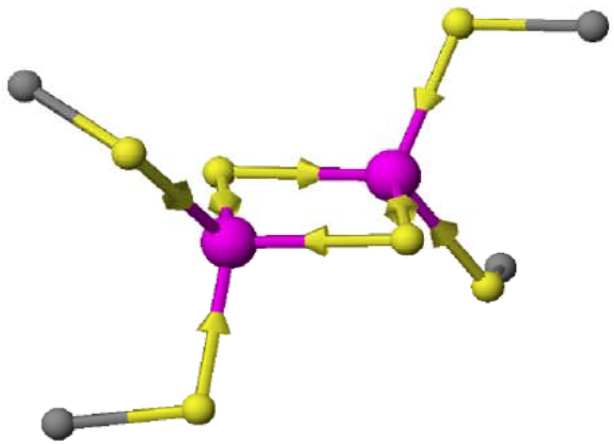
R=*i*-Pr : $E_{1/2}$ = -0.74 V

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

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

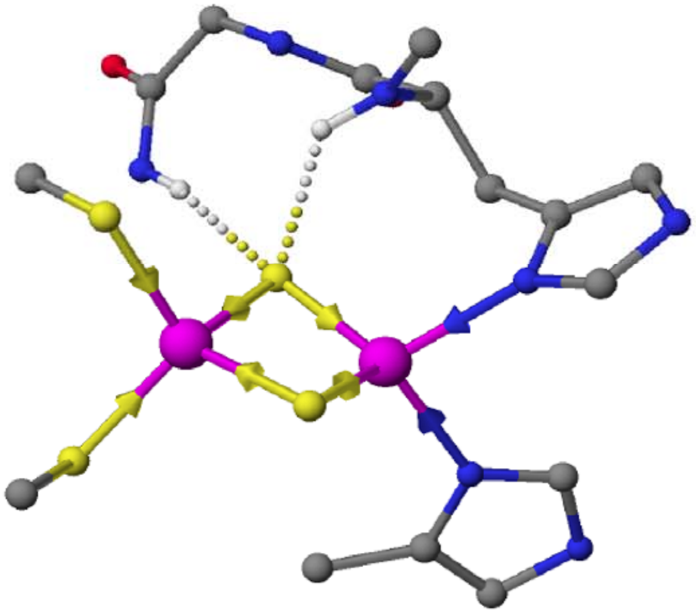
Modulation of Redox potentials (H bridges)

2Fe-2S Ferredoxin



$E^{0'}$ ~ -400 mV

2Fe-2S Rieske



$E^{0'}$ ~ +280 mV

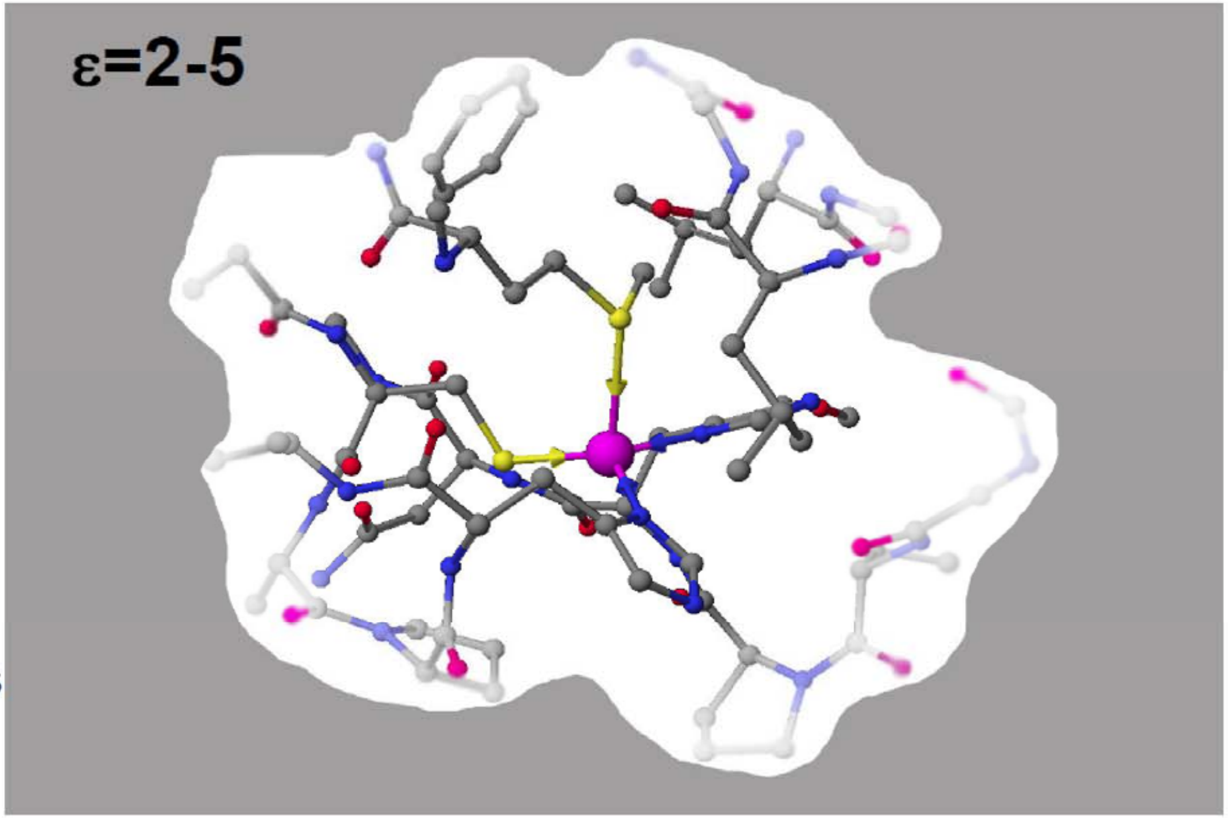
(+150 mV without H bridges)

(a) Stephens, P.J.; Jollie, D.R.; Warshel, A. (**1996**) *Chem. Rev.*, 96, 2491

(b) Link, T.A. (**1999**) *Adv. Inorg. Chem.*, 47, 83

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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Proteins Tune the Properties of Metal Ions

Coordination number

- The lower the higher the Lewis acidity

Coordination geometry

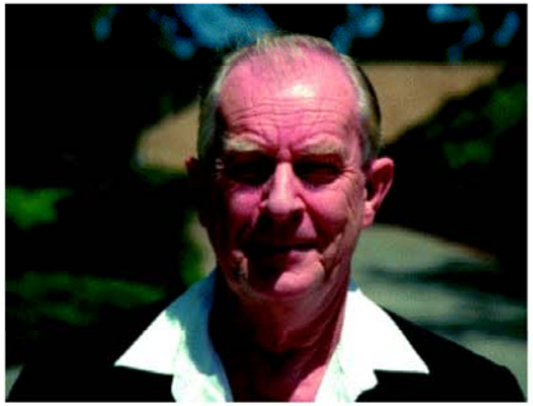
- Proteins can dictate distortion
- Distortion can change reactivity of metal ion

Weak interactions - Second Shell Effects

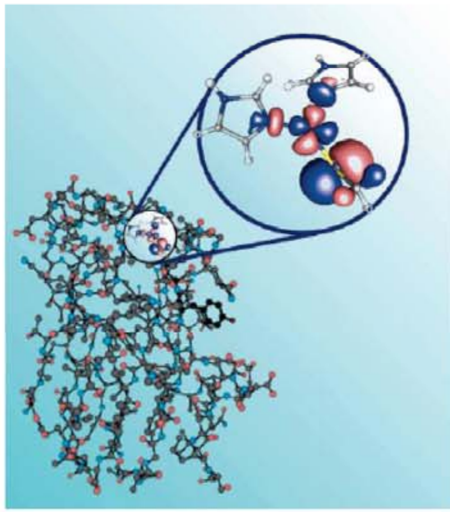
- Hydrogen bonds to bound ligands
- Hydrophobic residues: dielectric constant can change stability of metal-ligand bonds

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



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