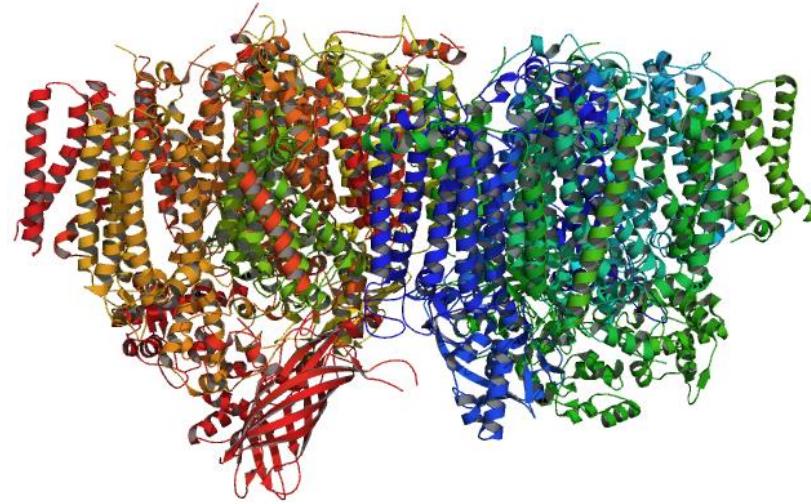
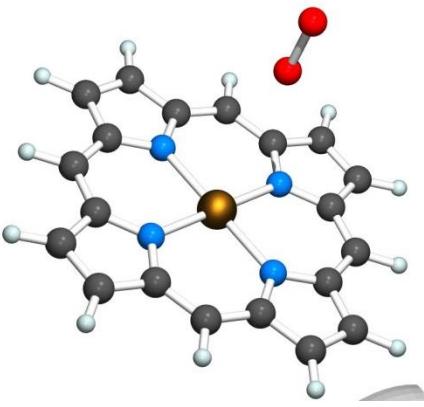
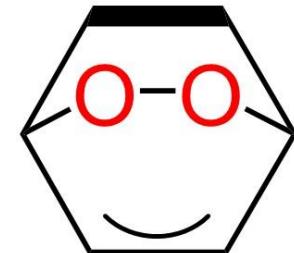


# Bioinorganic Chemistry (BIC)

## II. Metal Ions & Proteins: Binding, Folding, Transport & Storage



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Department of Chemistry



# **Review on Part I**

**Bioinorganic Chemistry:** usually **metal**-containing molecules & their reactions in **biological systems**. **Many 1<sup>st</sup>-row transition metals** were found to involve in bioinorganic systems.

**General Roles of the Metal(s):** **structures, functions, chemical reactions & medicine.**

**Thermodynamics & Kinetics of Metal-Ligand bond:** depends on the identity of metals & ligands (e.g. HSAB, chelate effect, ligand effect); **Various metal-ligand binding** for various biological **functions**.

**Combinations of Physical Methods:** Observations & characterization of bioinorganic systems.

# **Overview of Common Sources of Biological Ligands ( $M-L_{bio}$ )**

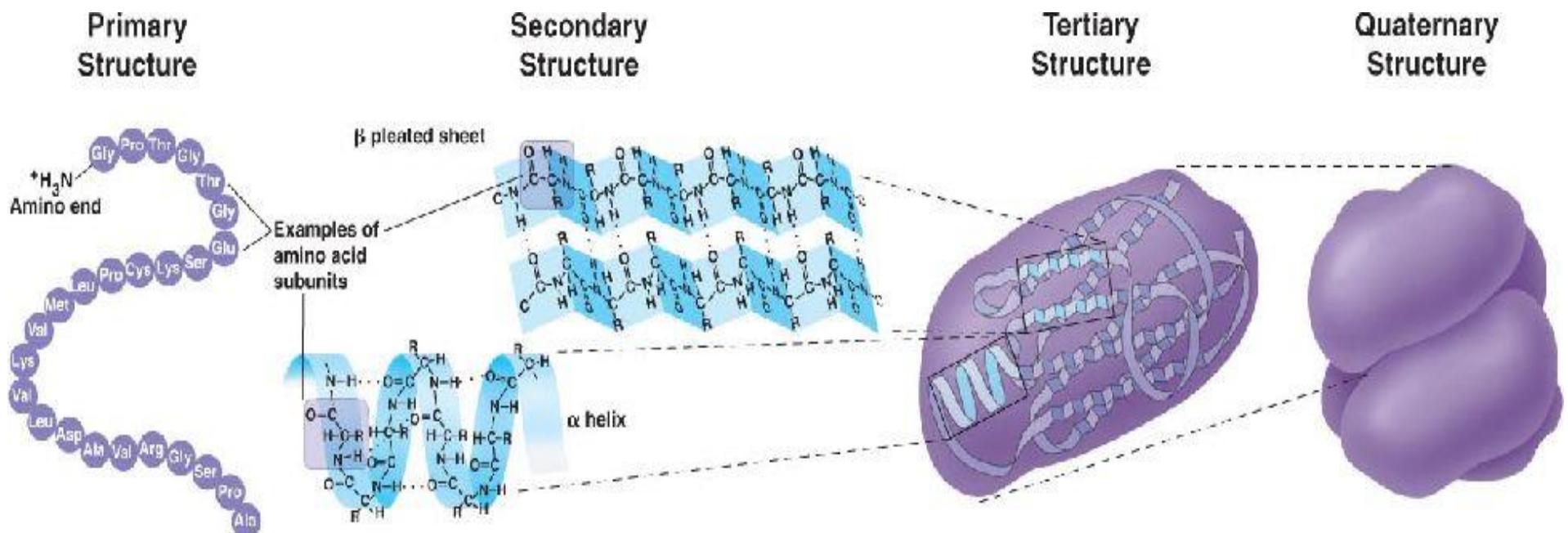
# Protein Structure

**Primary structure:** sequence of amino acids (AAs)

**Secondary structure:** through-space arrangement of nearby amino acids residues

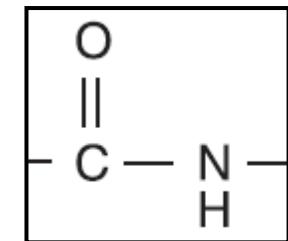
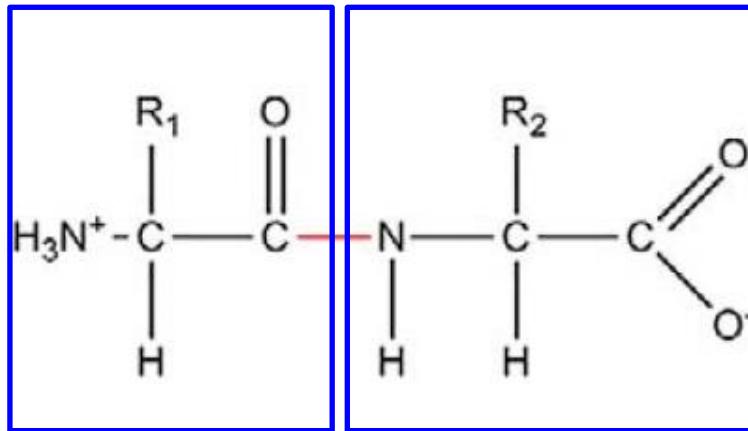
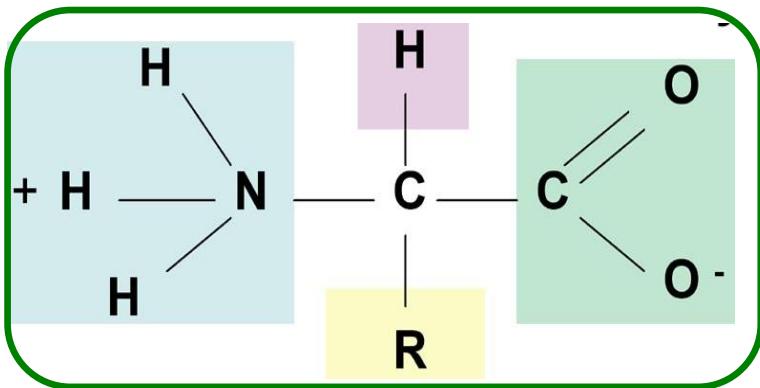
**Tertiary structure:** through-space arrangement of pre-organized secondary structures

**Quaternary structure:** through-space organization of tertiary structures (subunits)

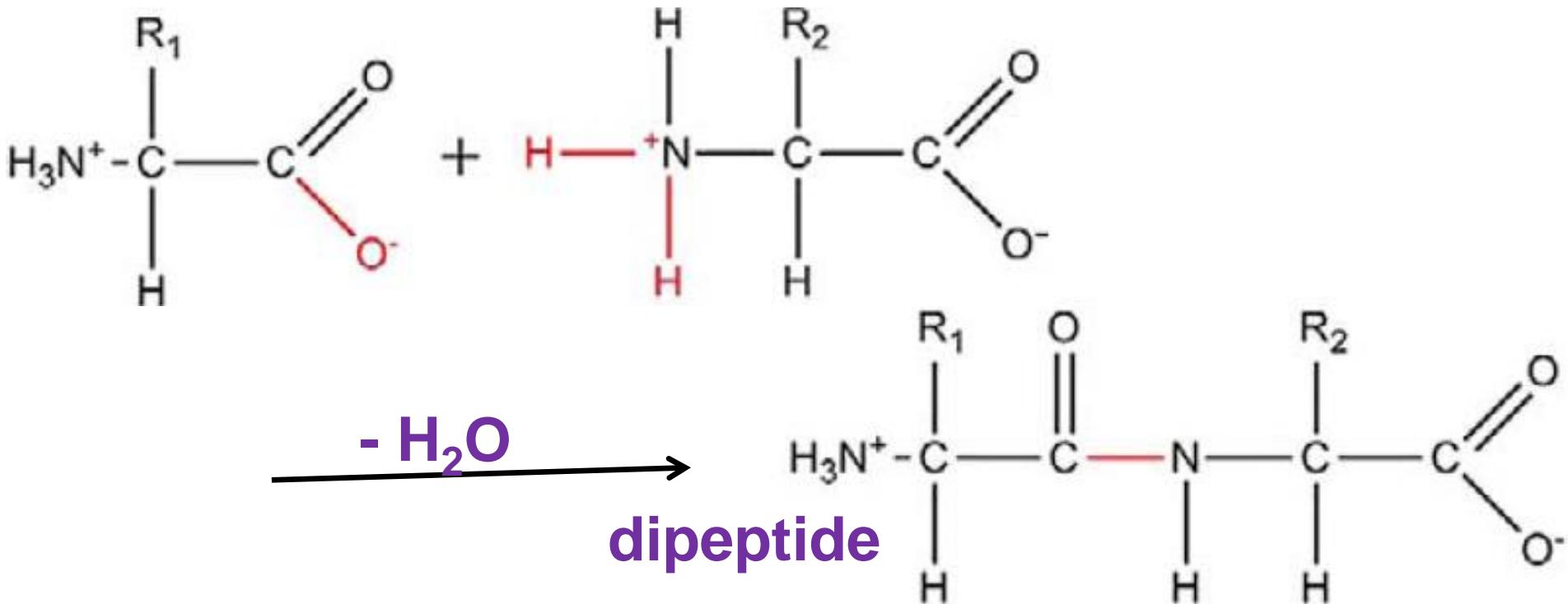


# Amino Acids: Building Units of Proteins

Generic form of AAs:

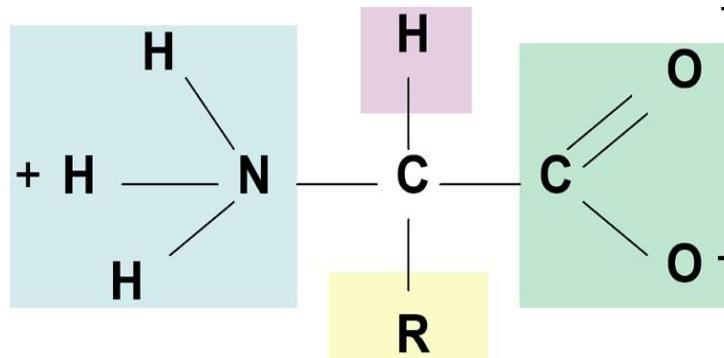


Peptide  
bond

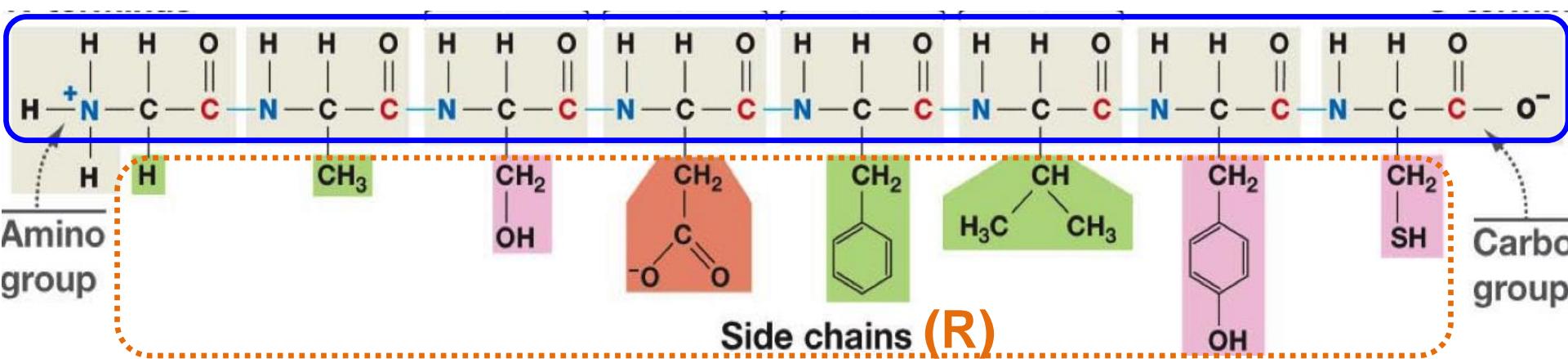


# Amino Acids: Building Units of Proteins

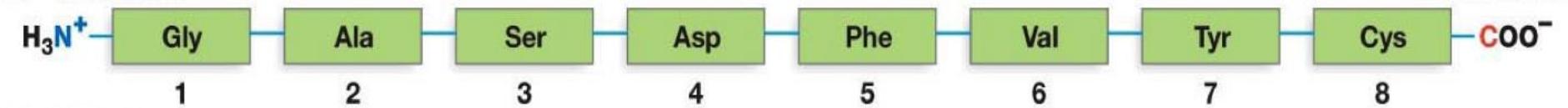
Generic form:



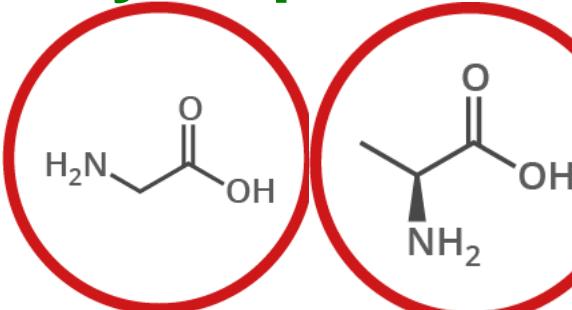
Backbone



N-terminus



# Hydrophobic R



**GLYCINE G**  
**(Gly, G)**



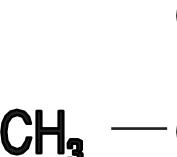
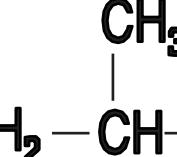
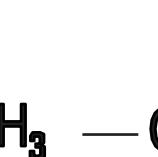
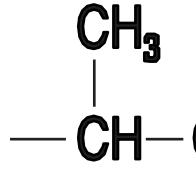
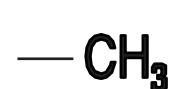
## VALINE V (Val, V)



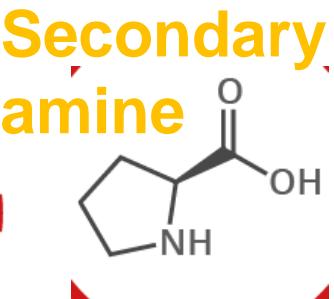
## **ISOLEUCINE (Ile, I)**



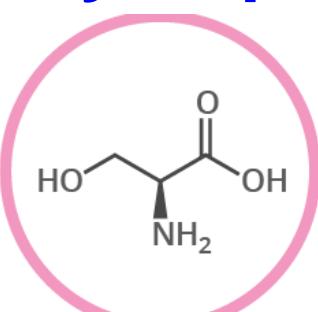
R: — H



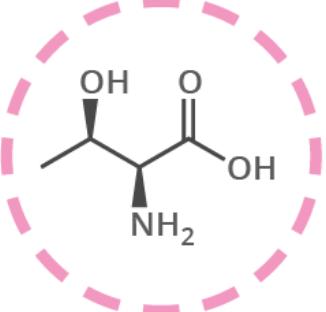
# Aliphatic



# Hydrophilic R

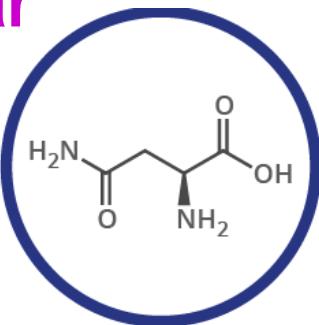


## SERINE S (Ser, S)

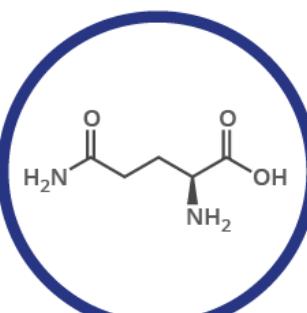


# THREONINE (Thr, T)

# Polar

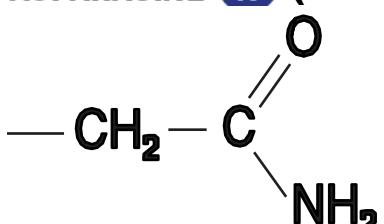
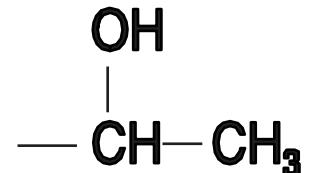


**ASPARAGINE N (Asn, N)**

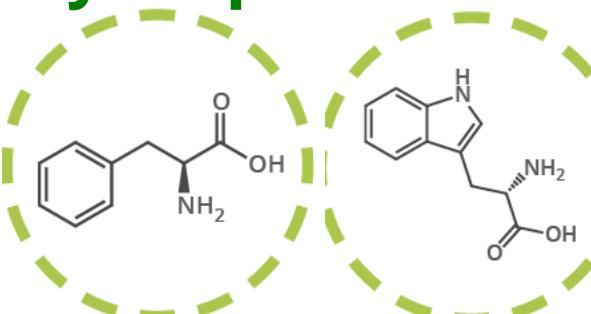


## GLUTAMINE Q (Gln, Q)

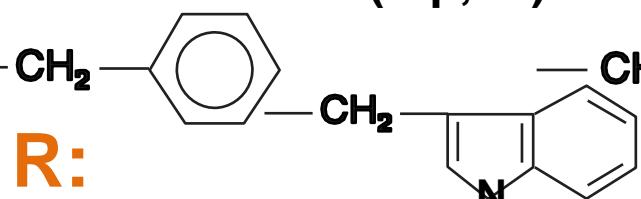
R+



# Hydrophobic      Aromatic      S-containing      Se-containing

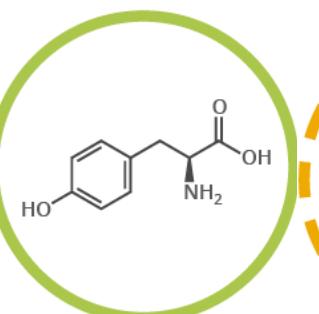


PHENYLALANINE F TRYPTOPHAN W  
(Phe, F) (Trp, W)



R:

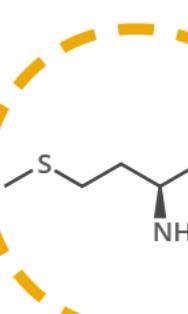
# Aromatic



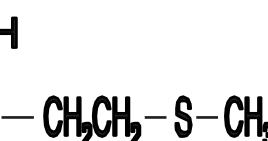
TYROSINE Y  
(Tyr, Y)



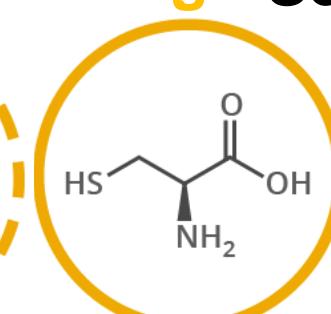
# S-containing



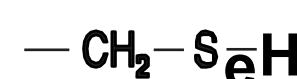
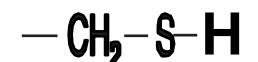
METHIONINE M  
(Met, M)



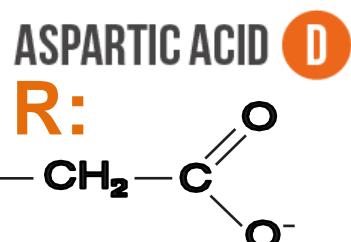
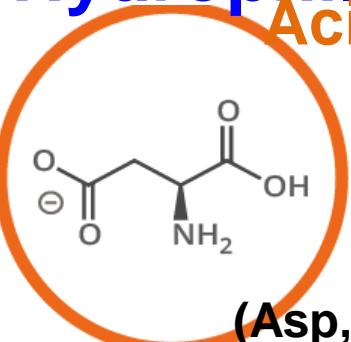
# Se-containing



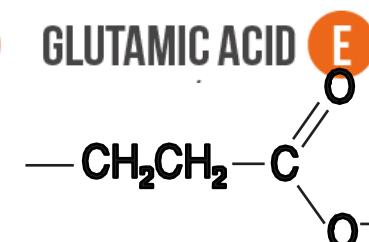
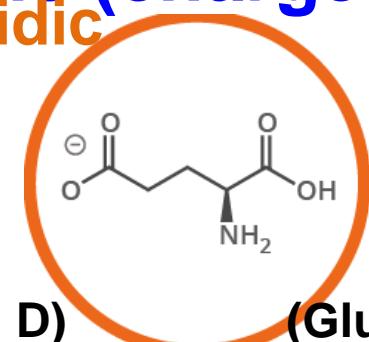
CYSTEINE C Selenocysteine  
(Cys, C) (Sec, U)



# Hydrophilic (charged) Acidic

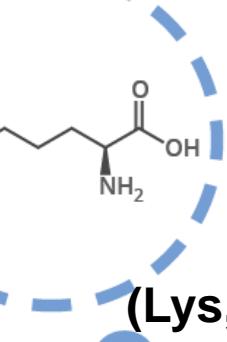


ASPARTIC ACID D

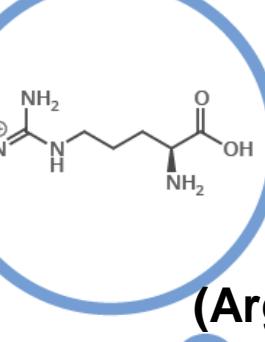


GLUTAMIC ACID E

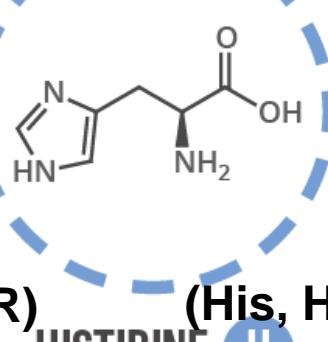
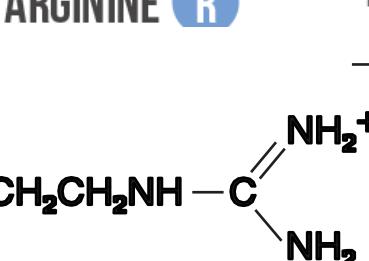
# Basic



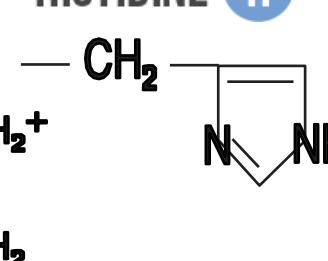
LYSINE K



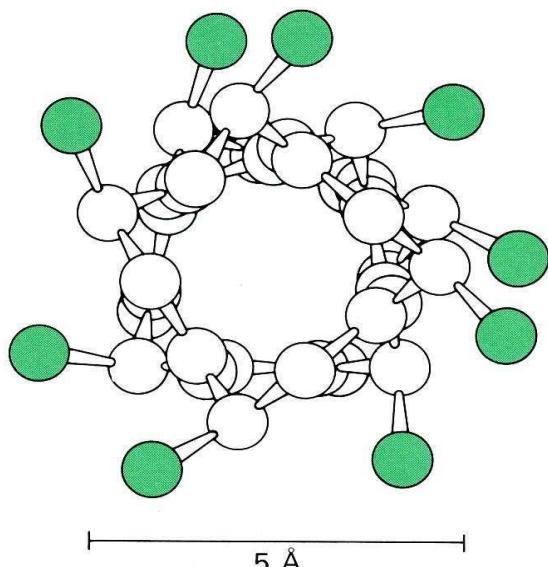
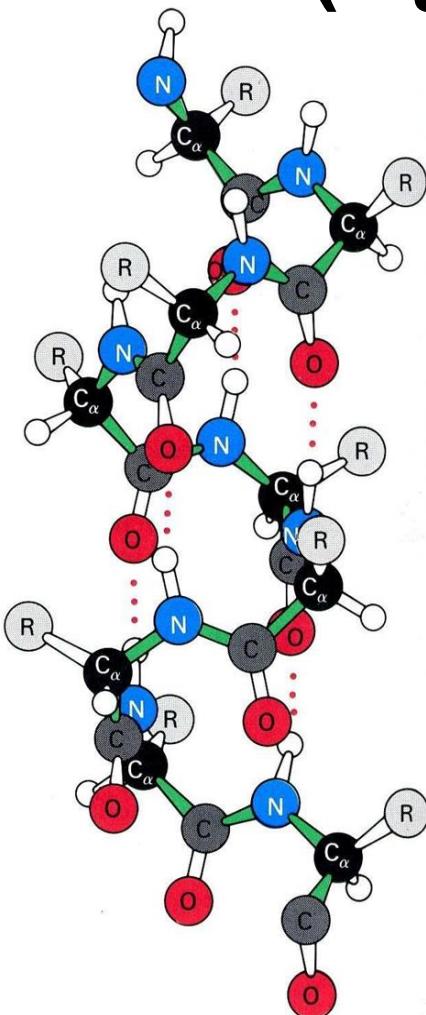
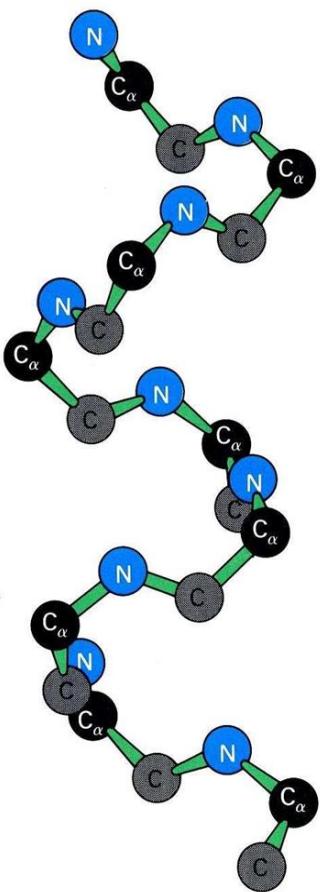
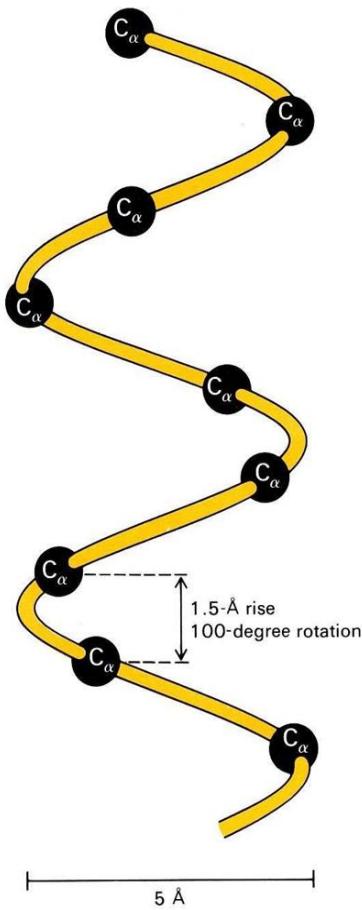
ARGININE R



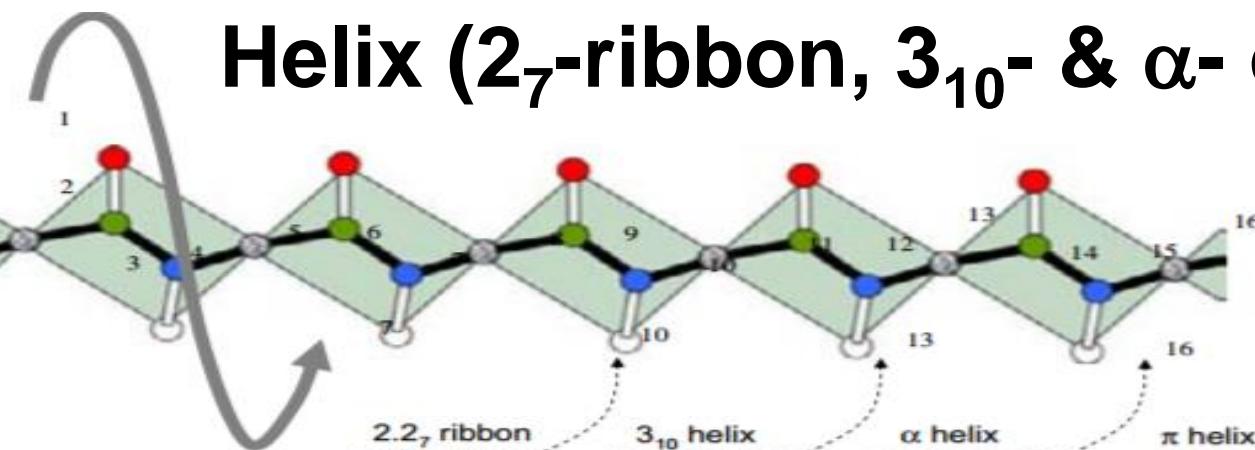
HISTIDINE H



# Secondary Structure: Helix (e.g. $\alpha$ - & $3_{10}$ -)



# Helix ( $2_7$ -ribbon, $3_{10}$ - & $\alpha$ - or $\pi$ -helix)

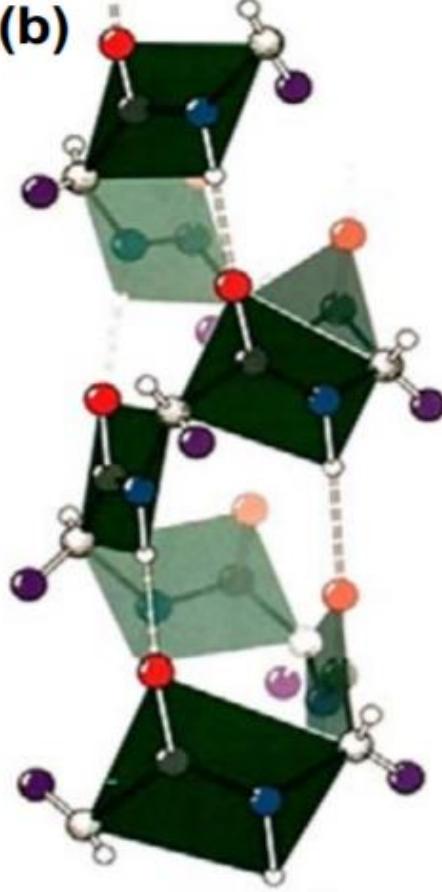


(a)



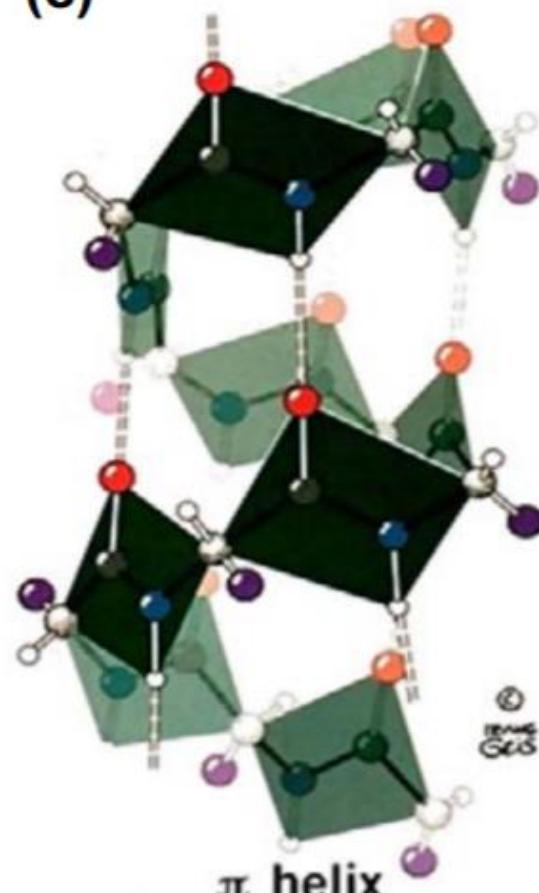
$3_{10}$  helix

(b)

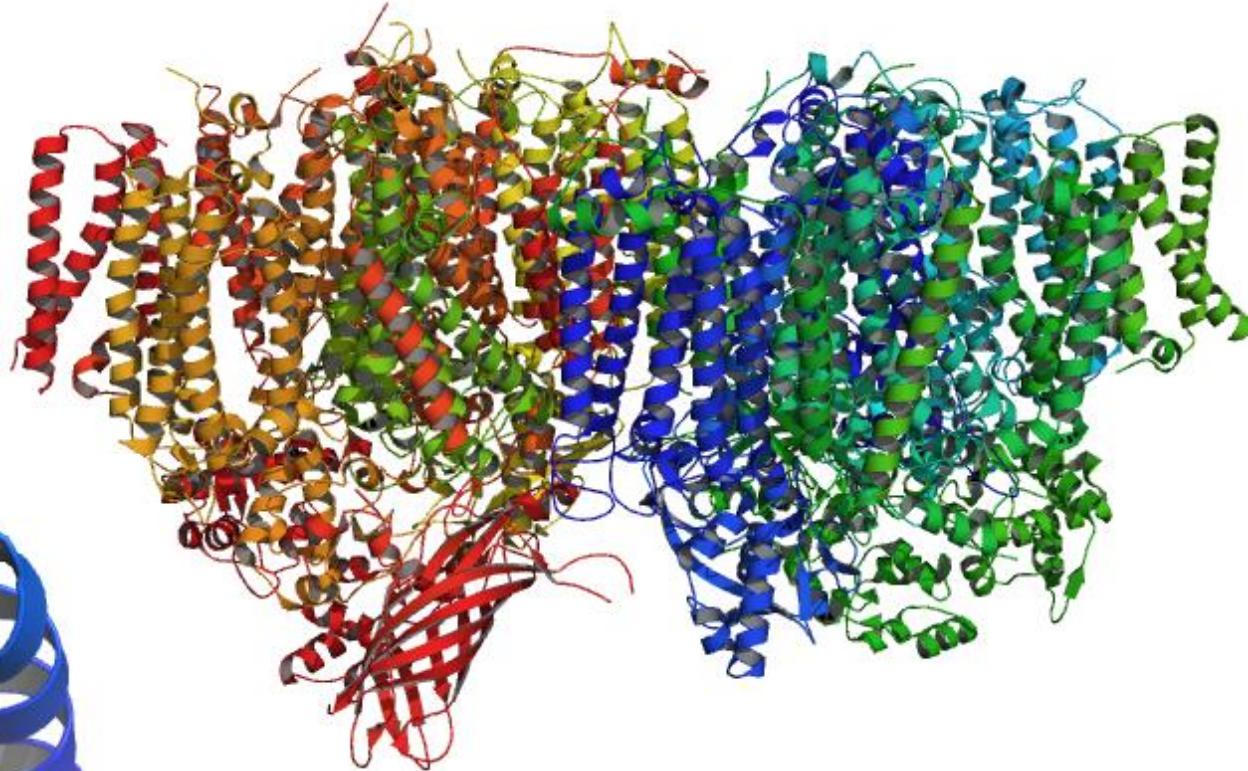
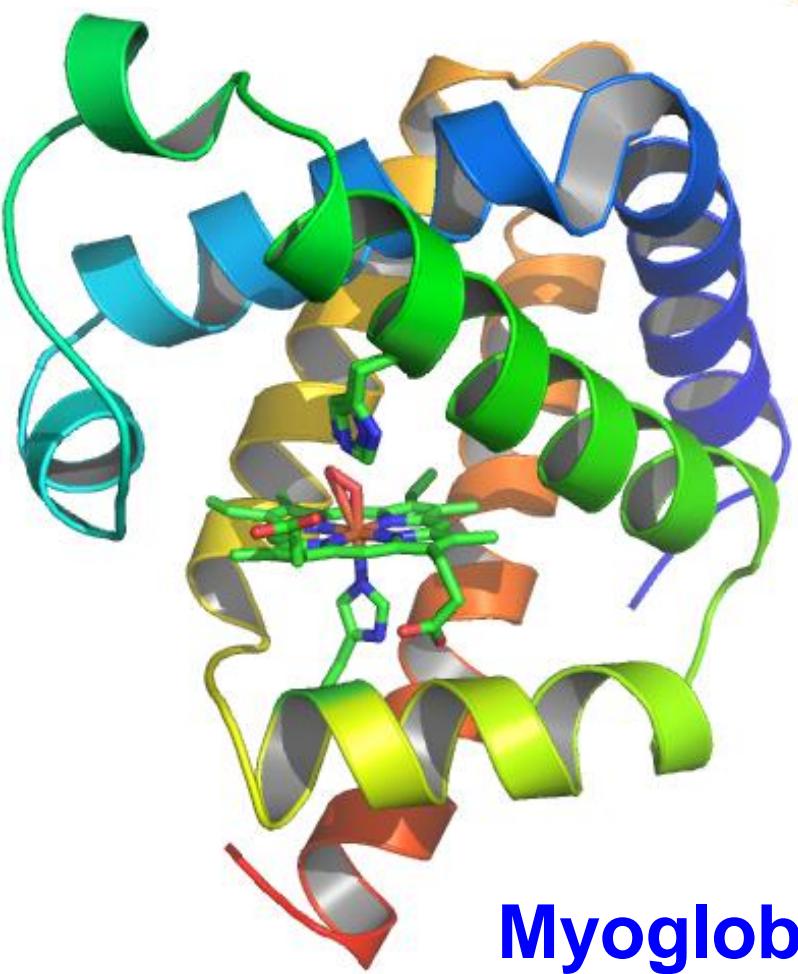


$\alpha$  helix

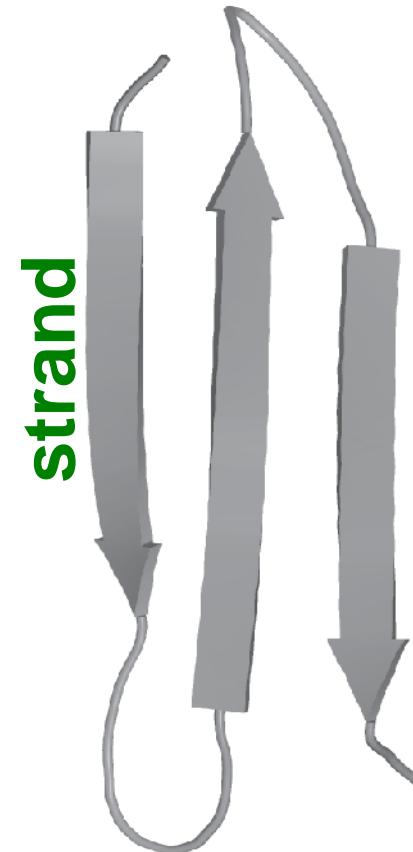
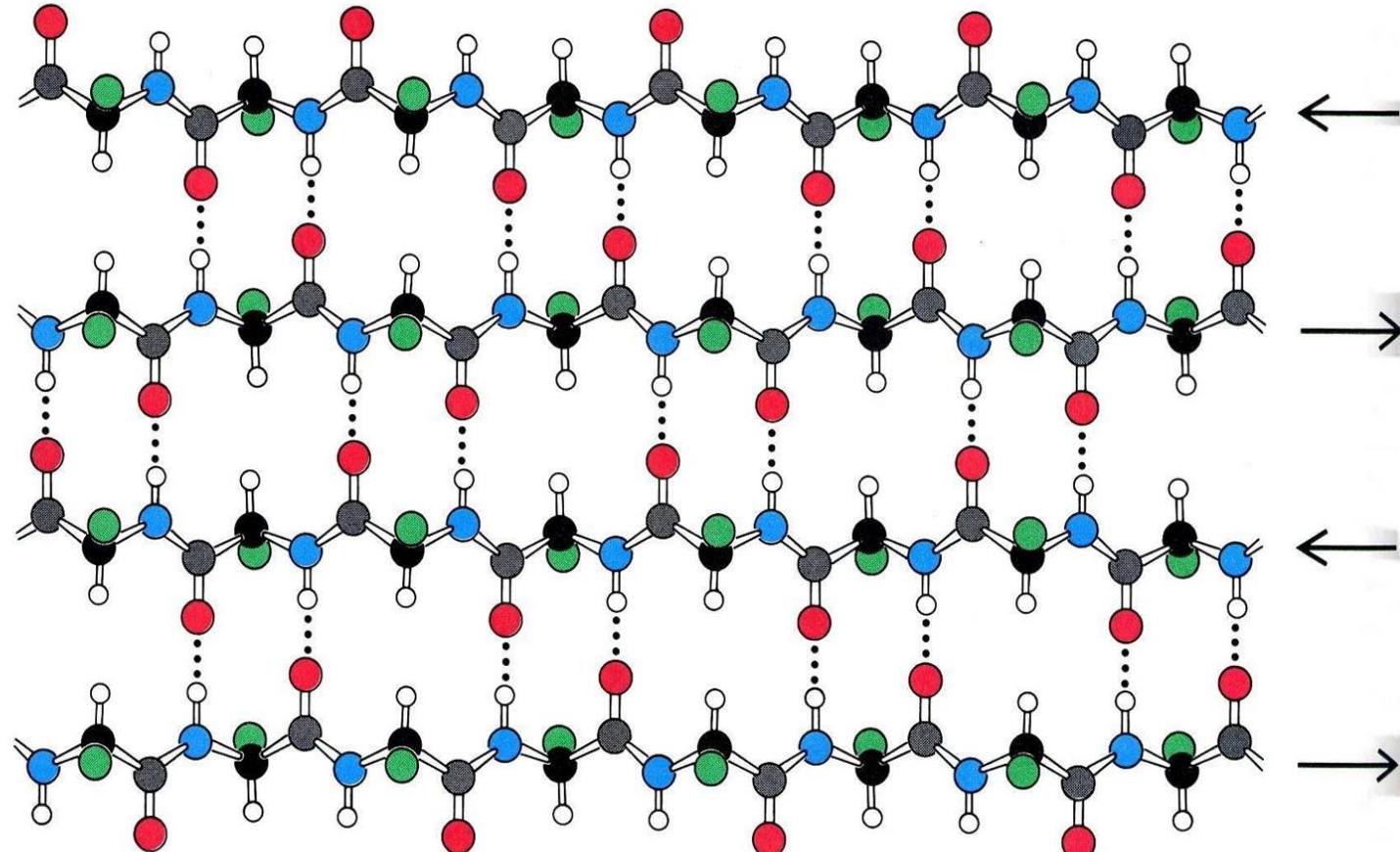
(c)



$\pi$  helix

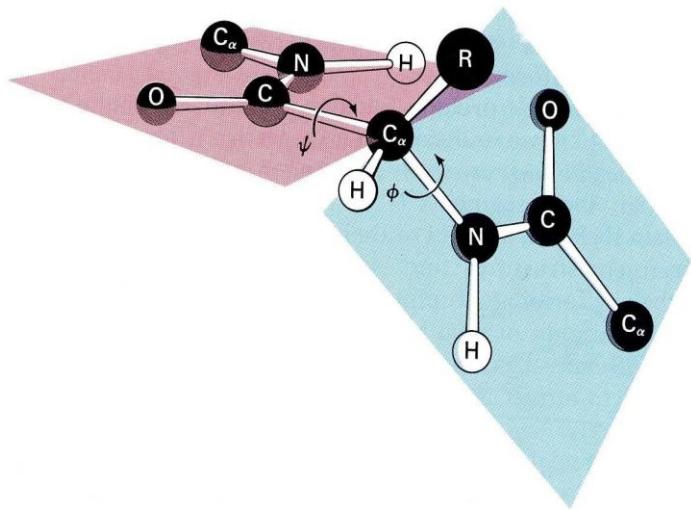
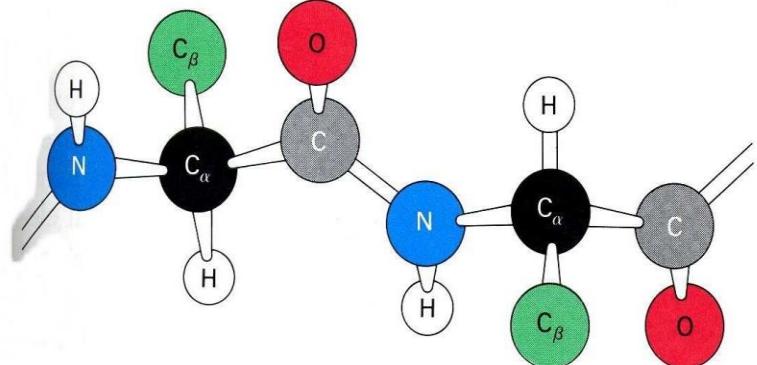


# Secondary Structure: $\beta$ -Sheet

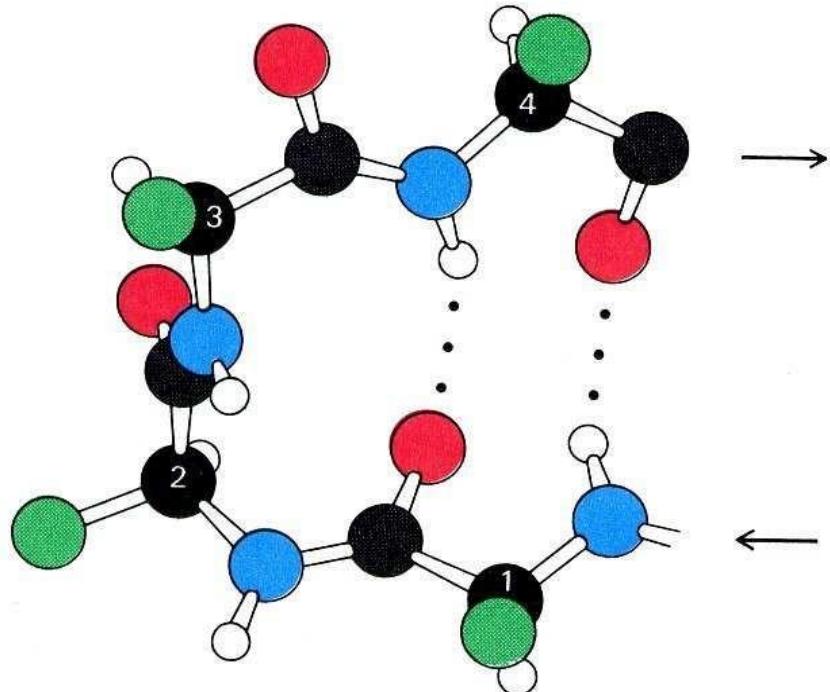


Antiparallel  $\beta$  pleated sheet. Adjacent strands run in opposite directions. Hydrogen bonds between NH and CO groups of adjacent strands stabilize the structure. The side chains (shown in green) are above and below the plane of the sheet.

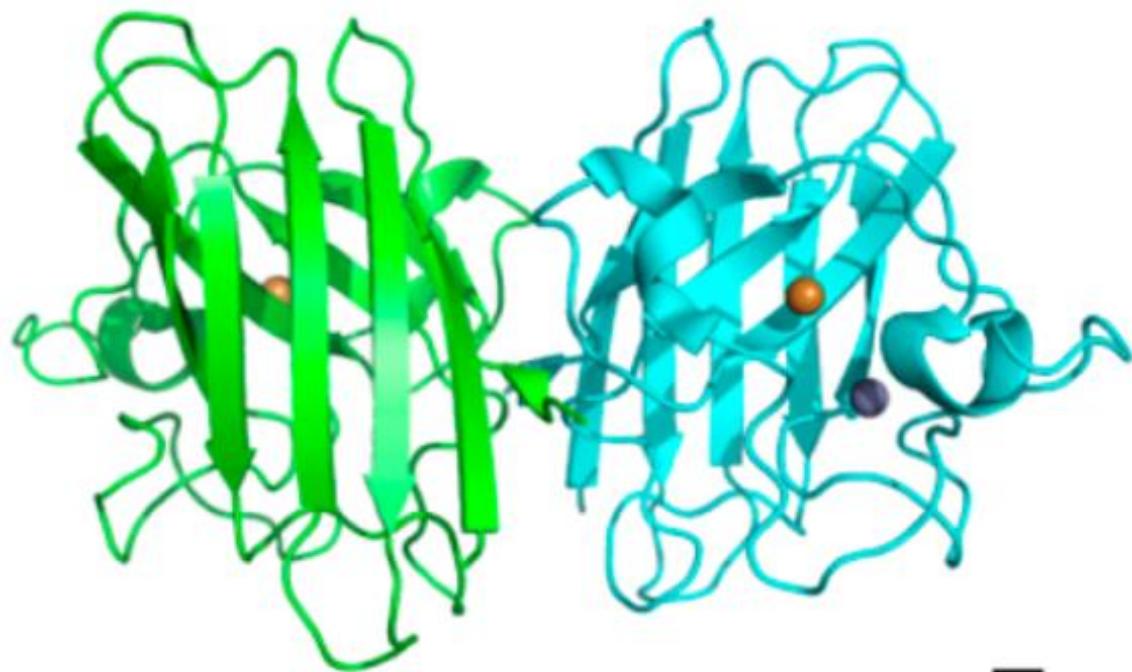
# $\beta$ -Sheet & $\beta$ -Turn (hairpin loop)



Conformation of a dipeptide unit in a  $\beta$  pleated sheet. The polypeptide chain is almost fully stretched out.

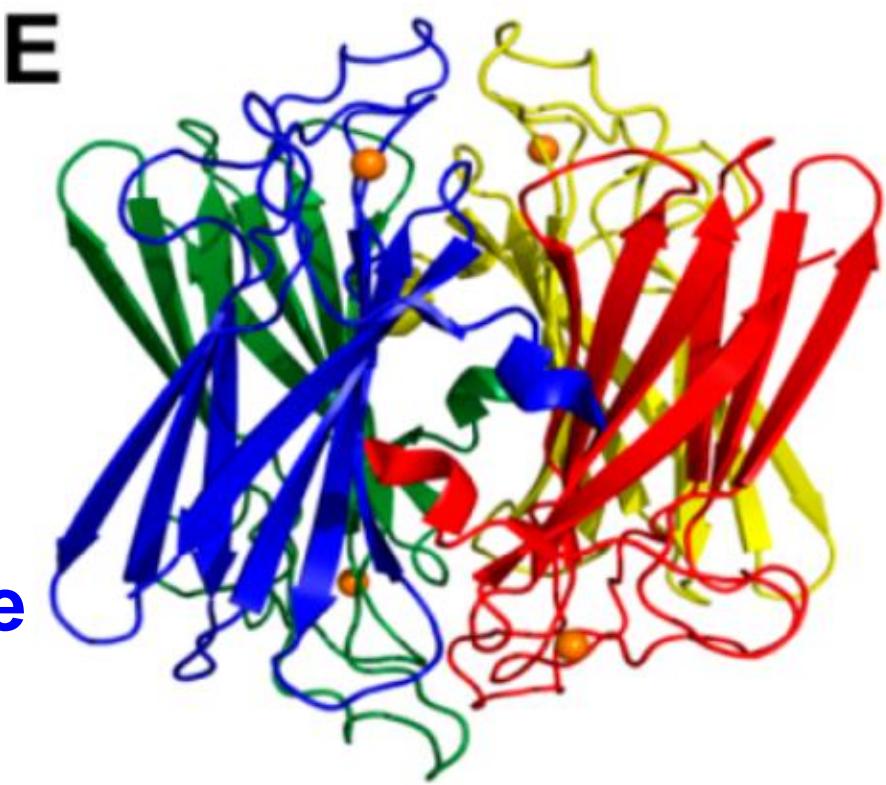


Structure of a  $\beta$ -turn. The CO group of residue 1 of the tetrapeptide shown here is hydrogen bonded to the NH group of residue 4, which results in a hairpin turn.

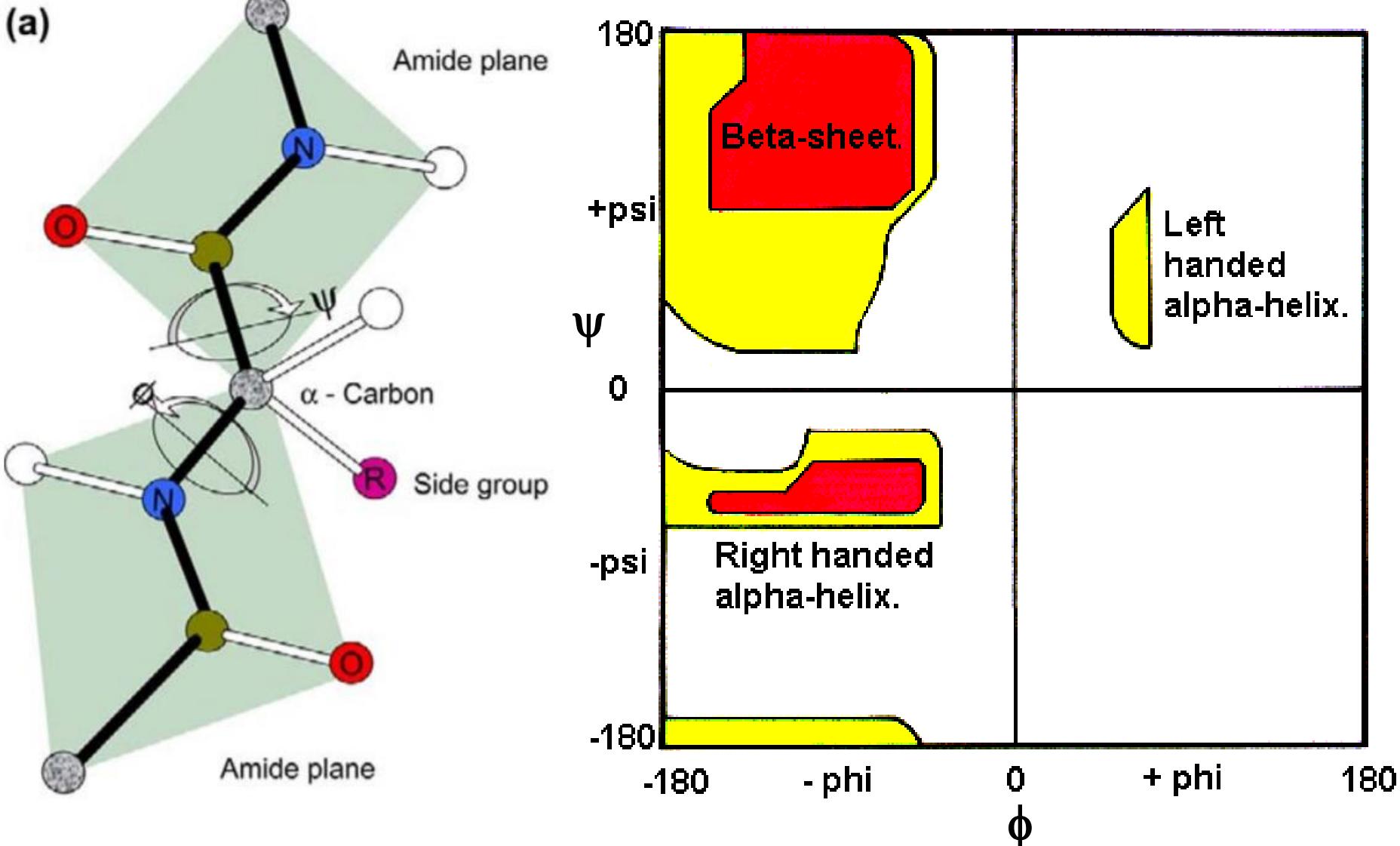


CuZn superoxide  
dismutase (SOD)

*P. furiosus* 1Fe-  
Superoxide Reductase  
(SOR)



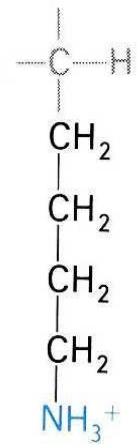
## The Ramachandran Plot.



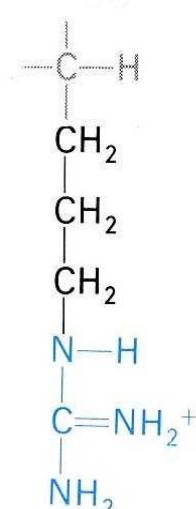
**Two dihedral angles** of the backbone determine the secondary structure

# Tertiary Structure

## Positively-charged R

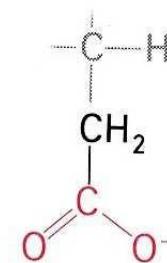


Lysine  
(Lys, K)

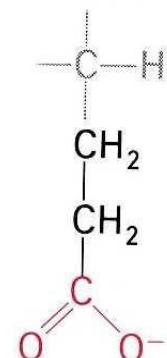


Arginine  
(Arg, R)

## Negatively-charged R



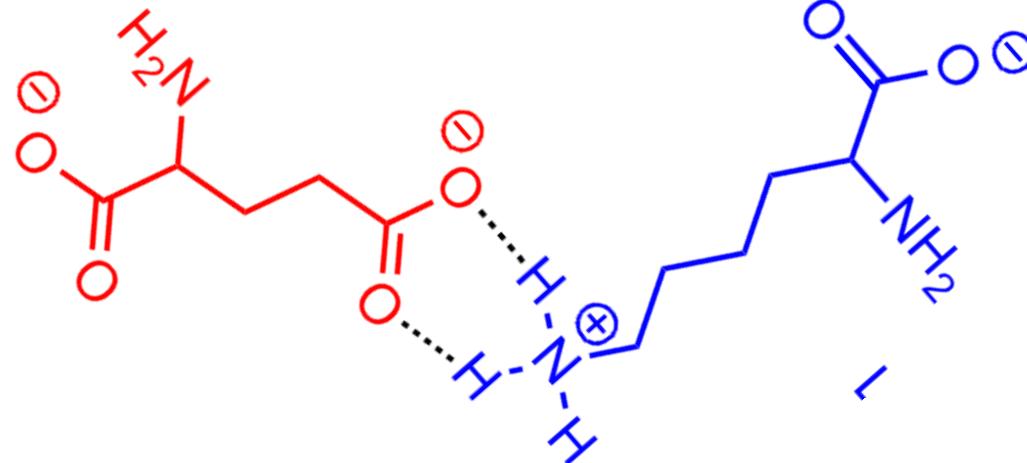
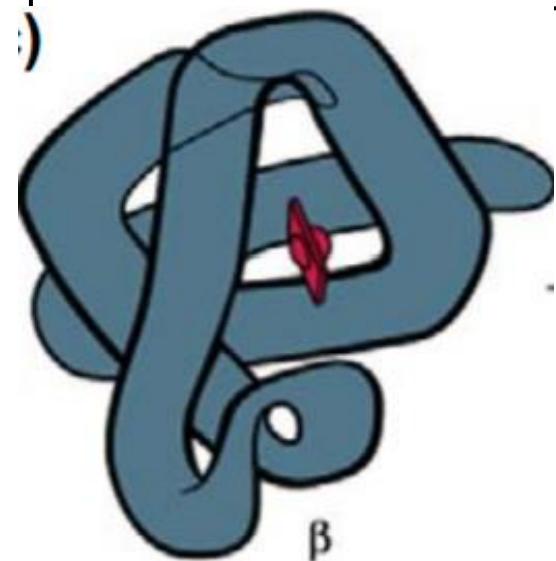
Aspartate  
(Asp, D)



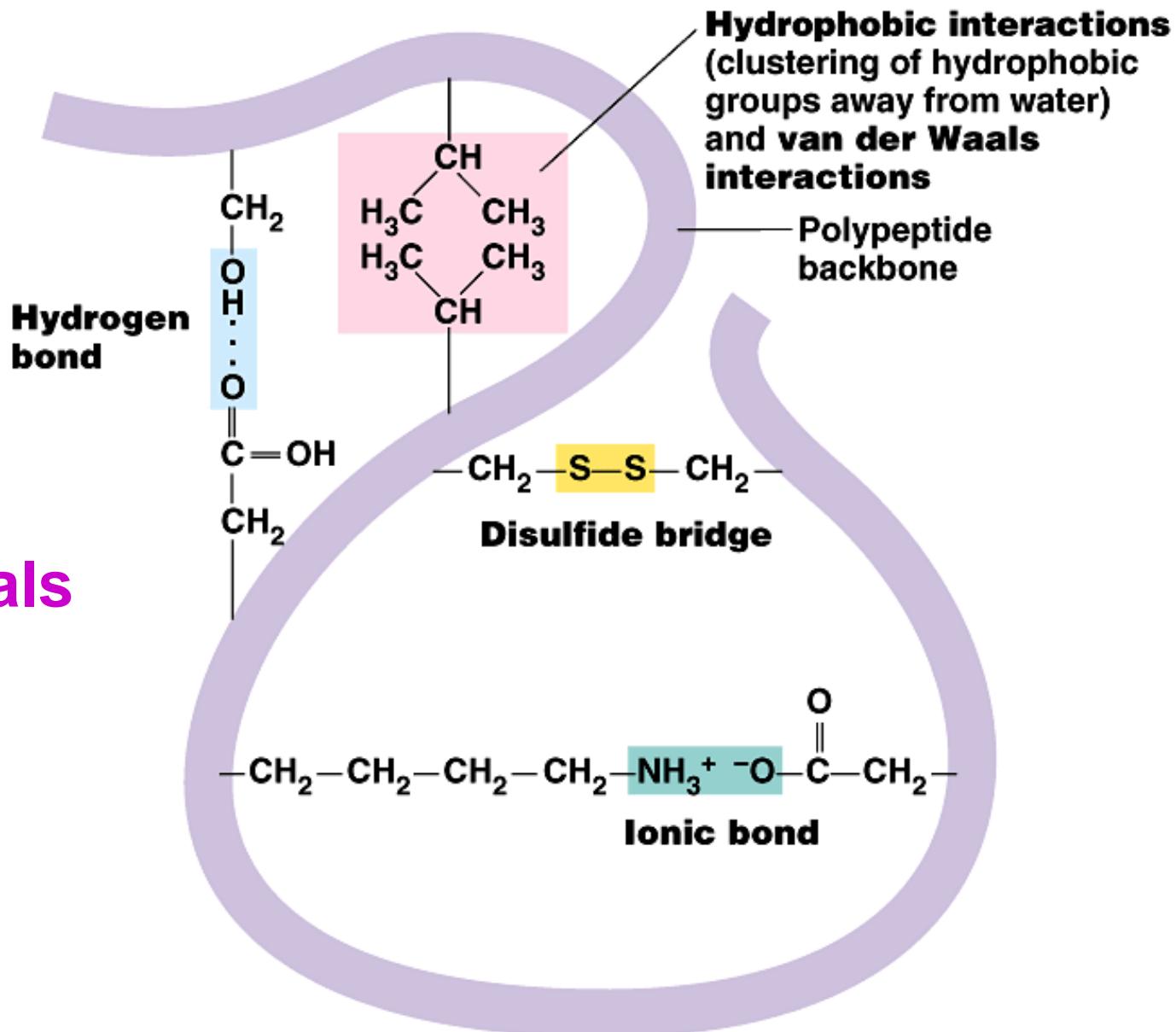
Glutamate  
(Glu, E)

### 1. Ionic bonds: Salt bridges

Cationic  
His too



## 2. Hydrogen bonds

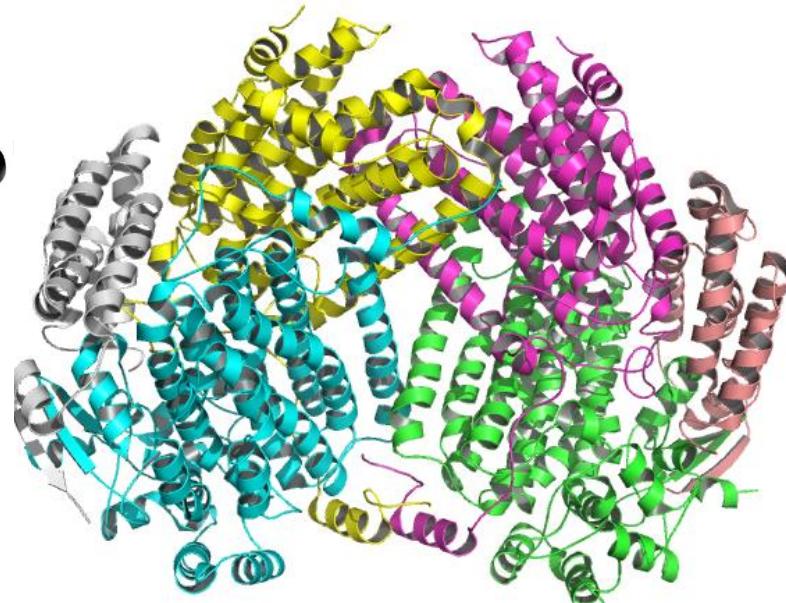
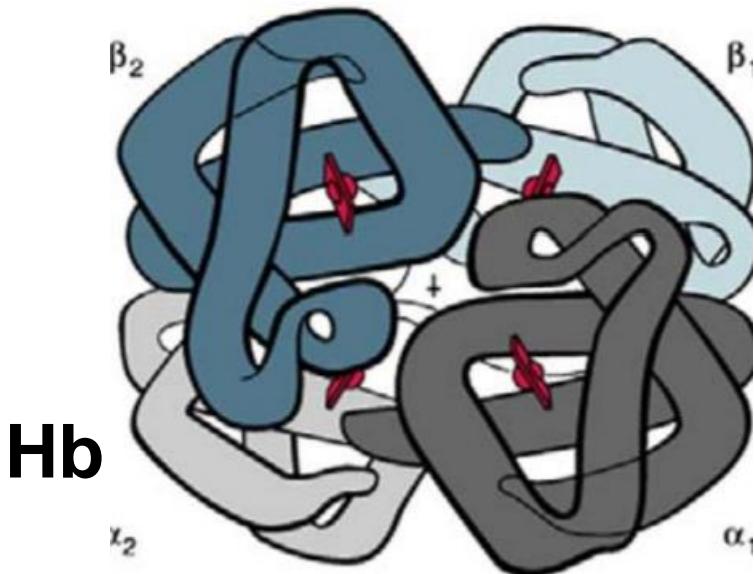
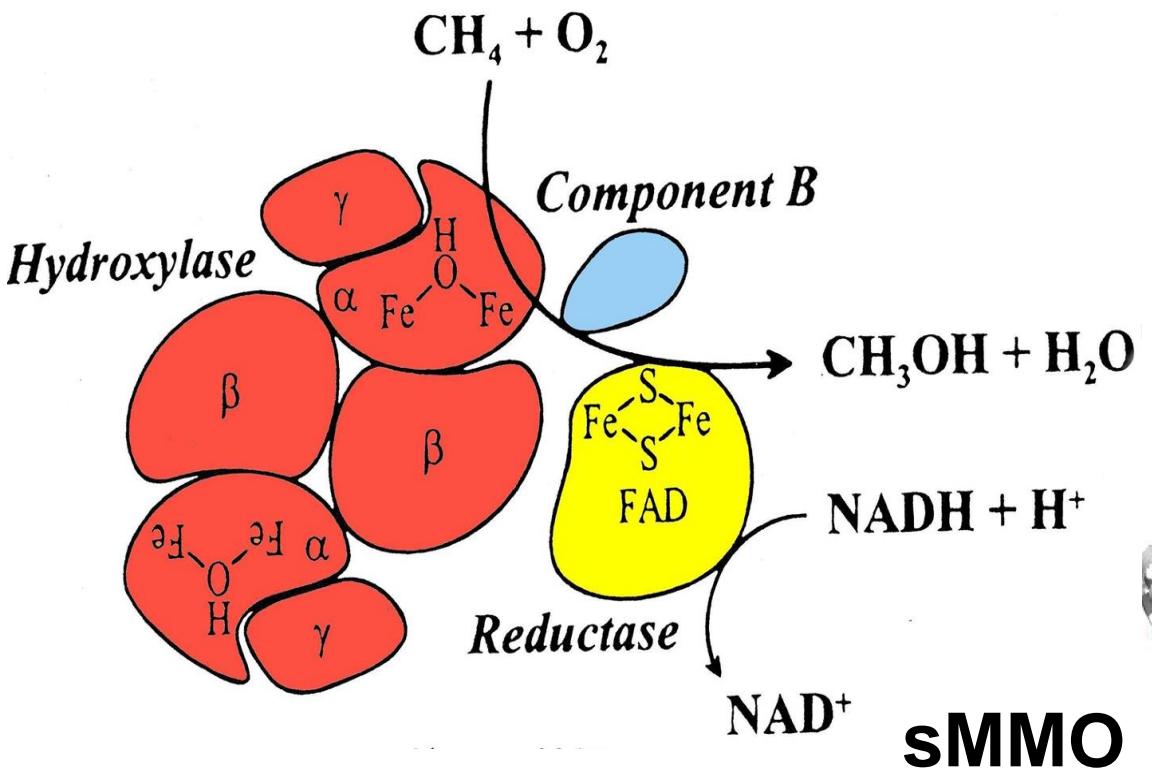


## 3. Van der Waals (hydrophobic) interactions

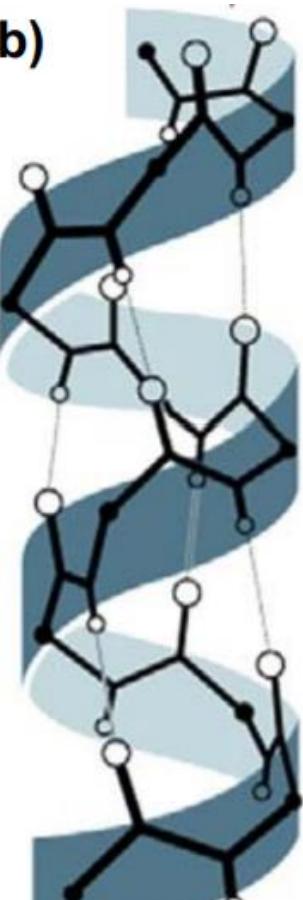
## 4. Others: Disulfide bond (R-S-H + H-S-R → R-S-S-R)

# Quaternary Structure

Assembly of subunits (tertiary structure): Hemoglobin (Hb, Fe) & soluble Methane Mono-Oxygenase (sMMO, Fe)

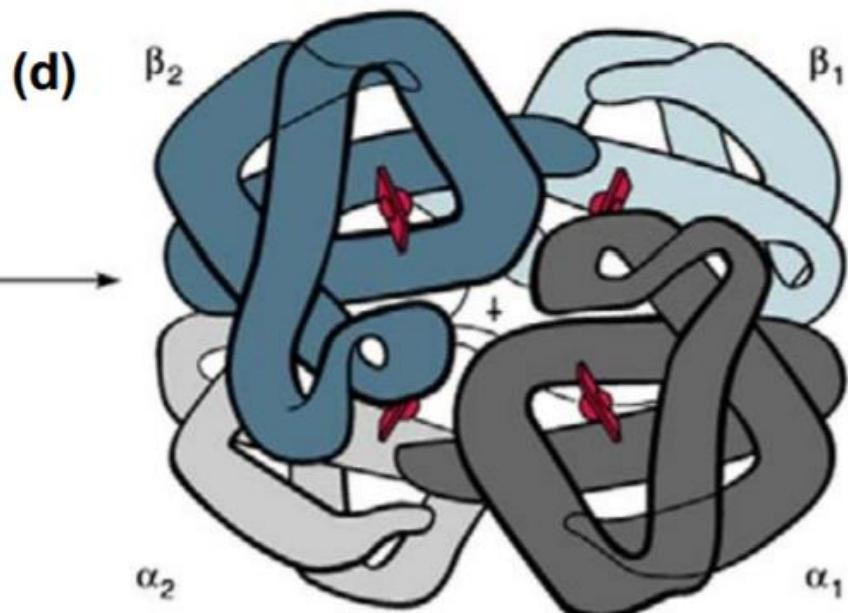


## Secondary structure



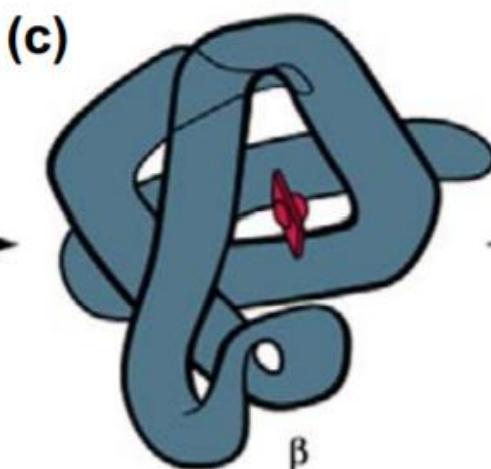
Secondary  
structure  
(helix)

## Quaternary structure



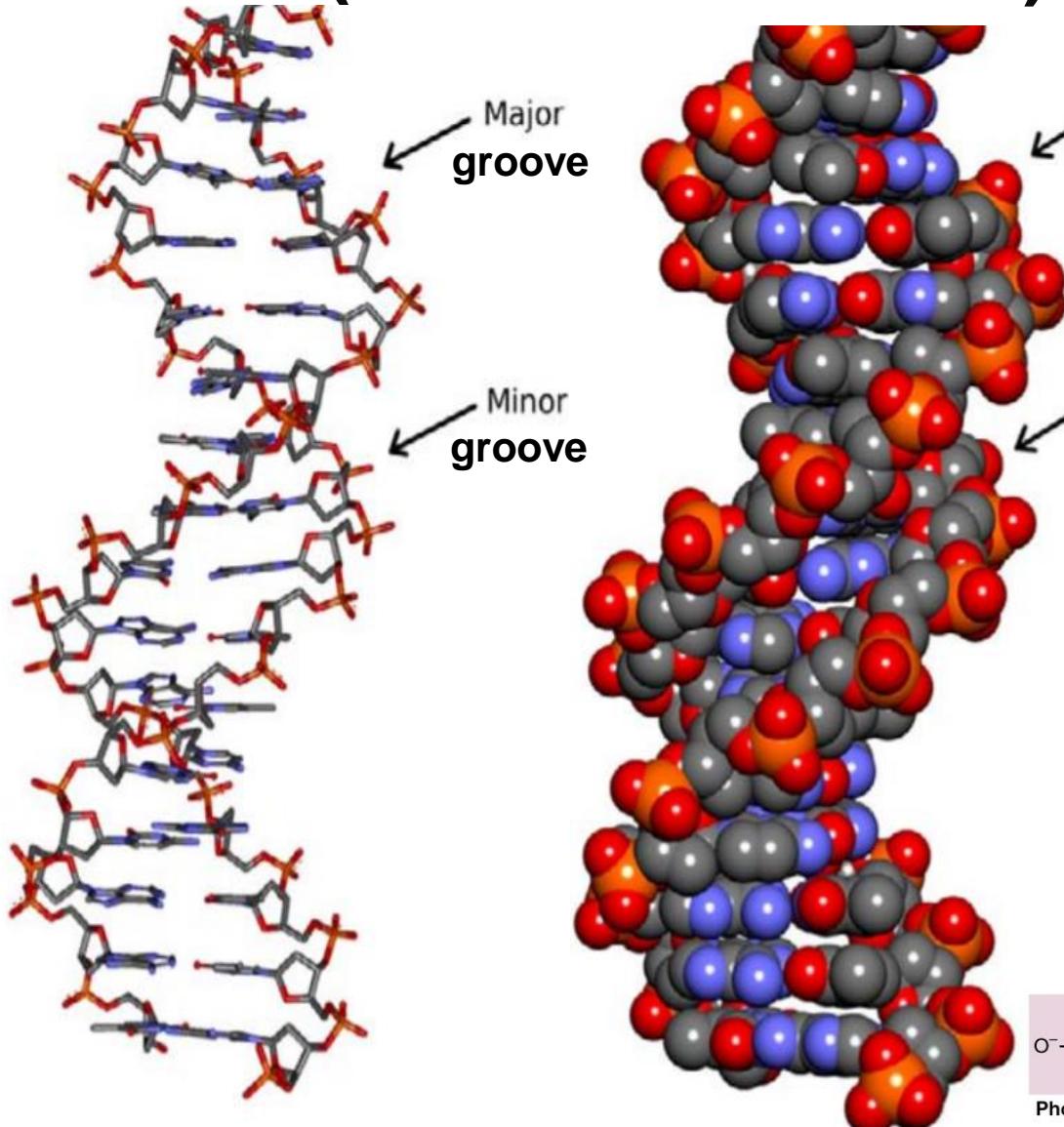
Quaternary structure:  
the four separate chains  
of hemoglobin assembled  
into an oligomeric protein

## Tertiary structure

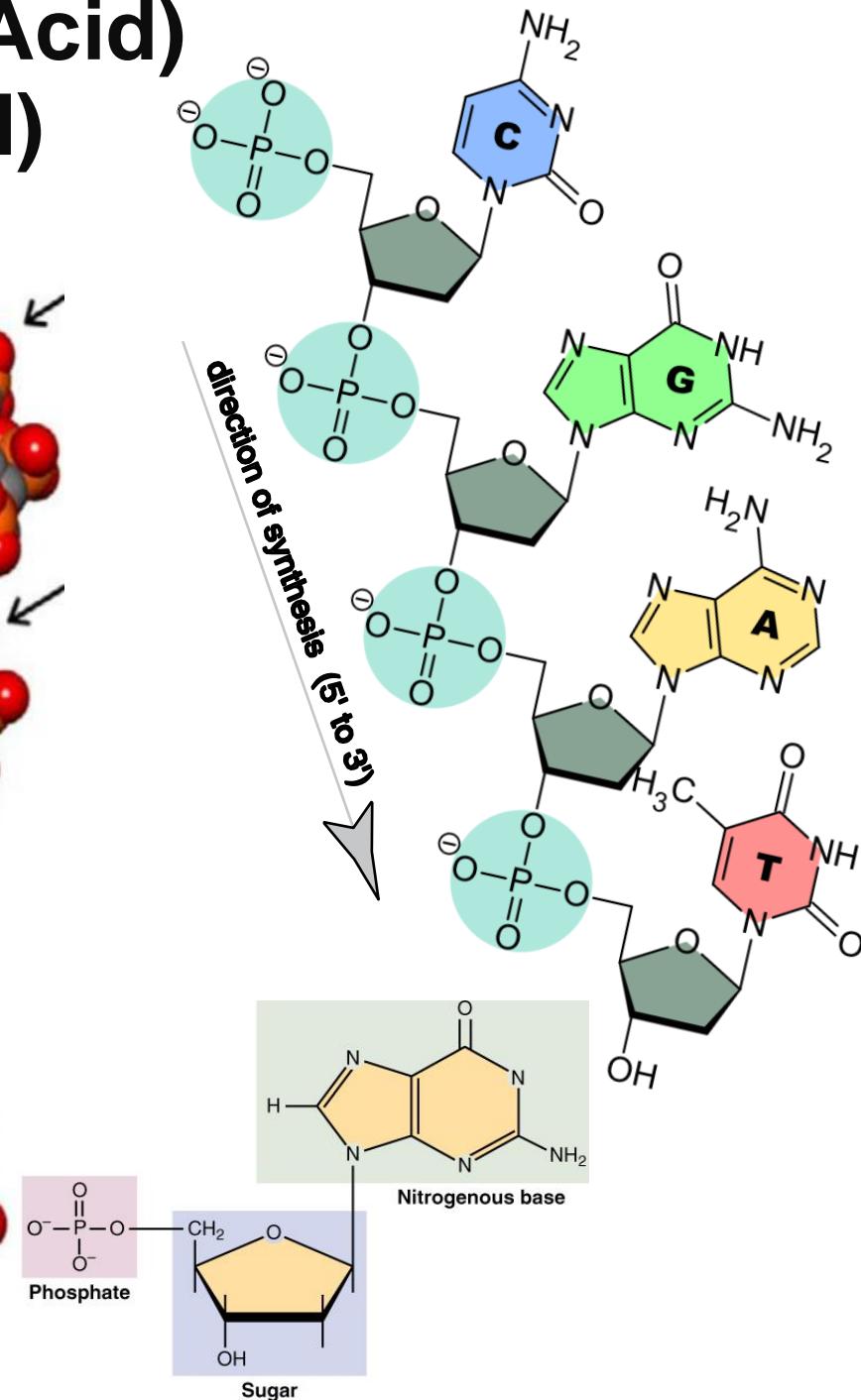


Tertiary structure:  
one complete protein chain  
( $\beta$  chain of hemoglobin)

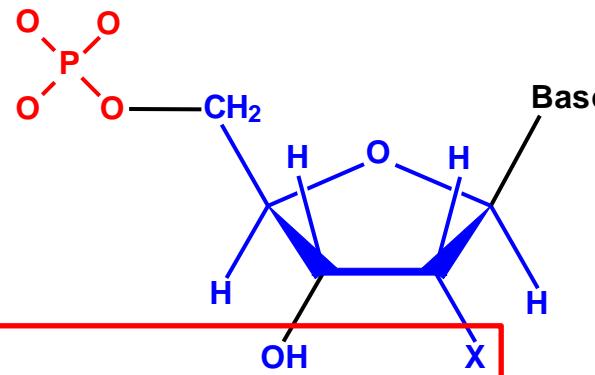
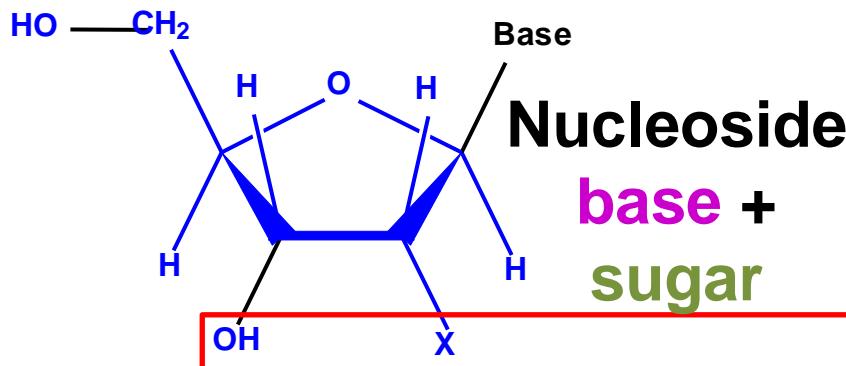
# DNA (Deoxyribonucleic Acid) & RNA (Ribonucleic Acid)



Double-helix DNA



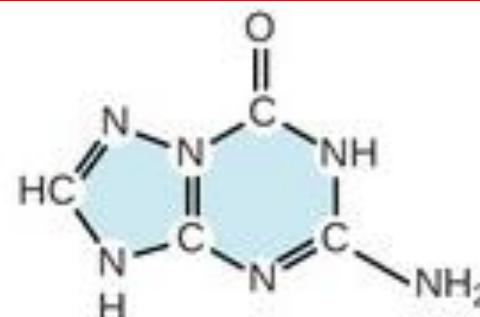
# Building Units of DNA & RNA



**Base  
Purines:**



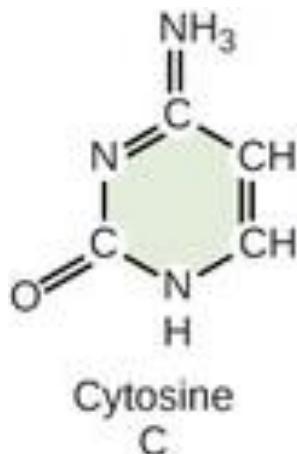
Adenine  
A



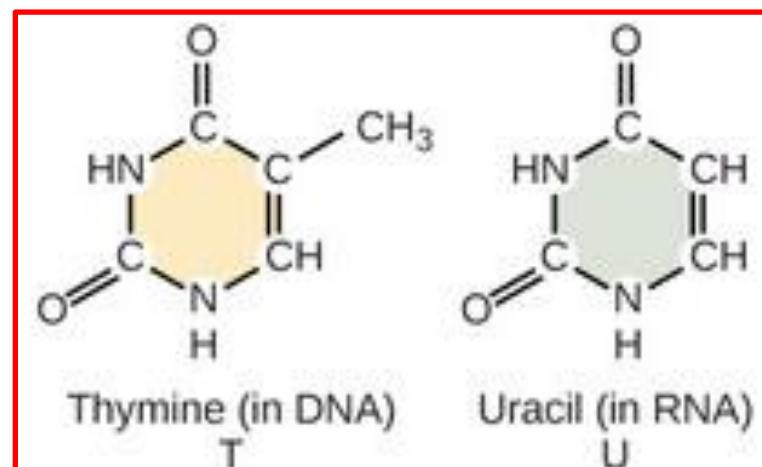
Guanine  
G

X = OH: Ribose  
X = H: Deoxyribose

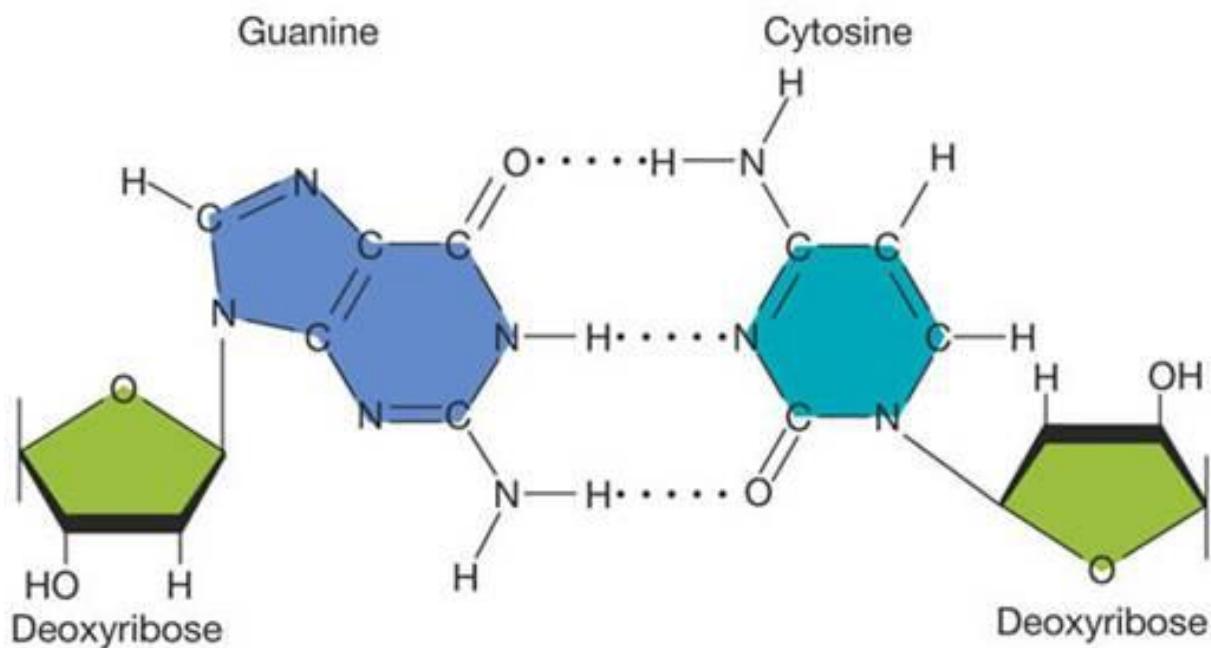
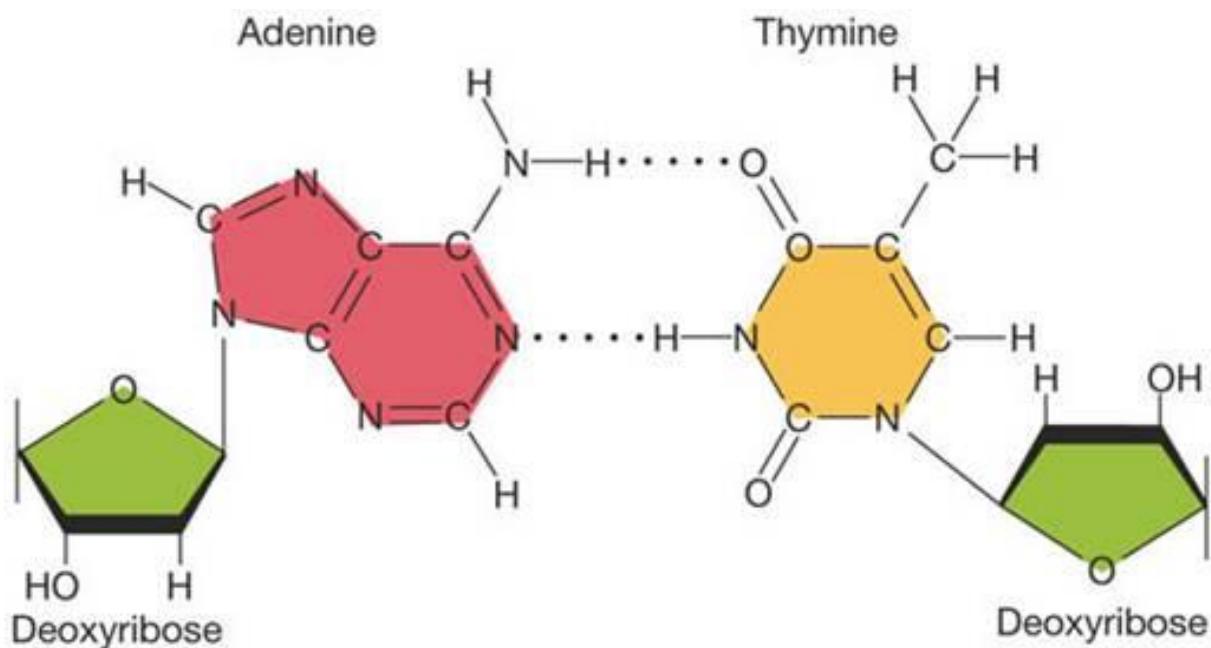
**Pyrimidines:**

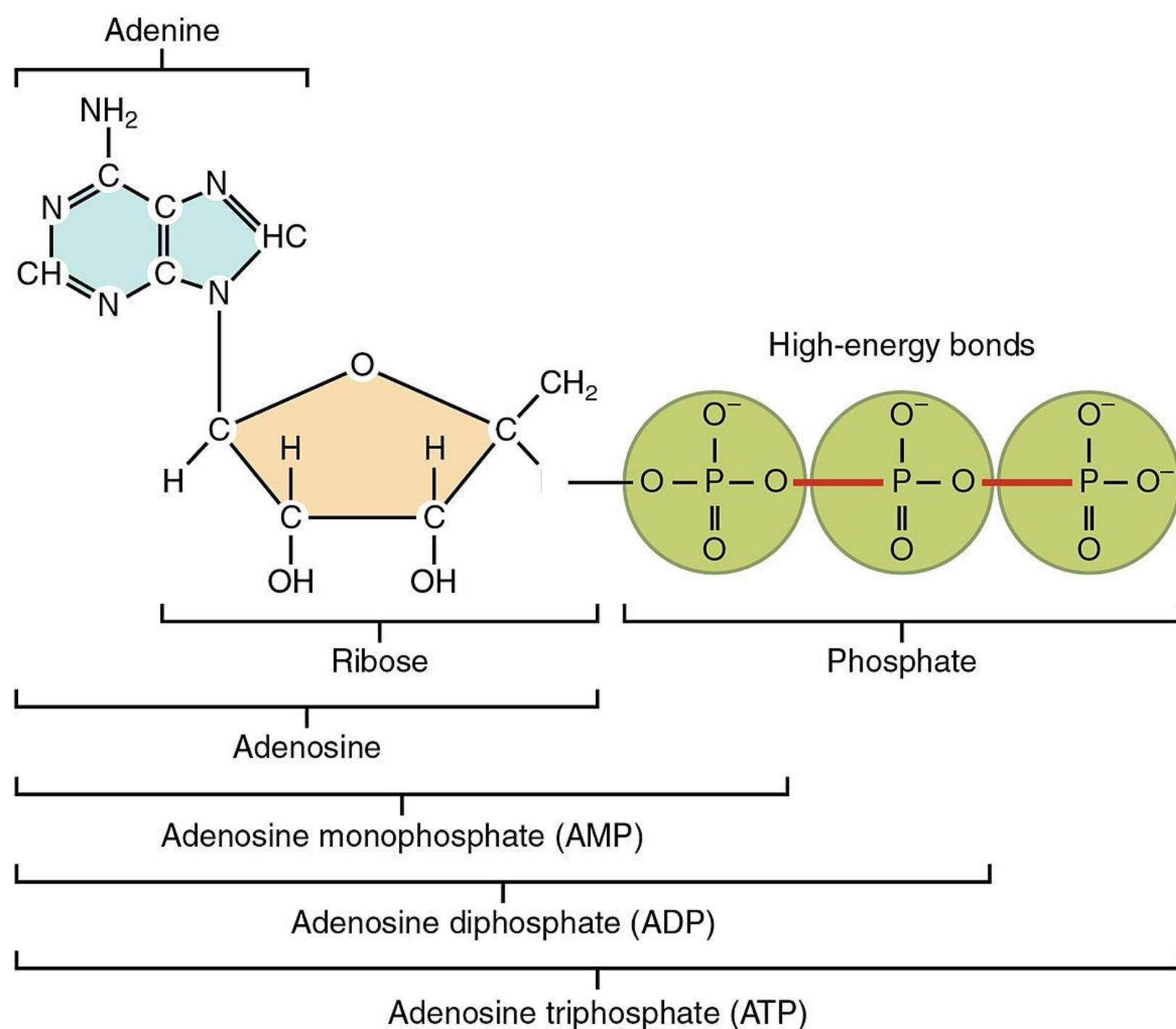


Cytosine  
C



# Base-Pairings (A-T & C-G)

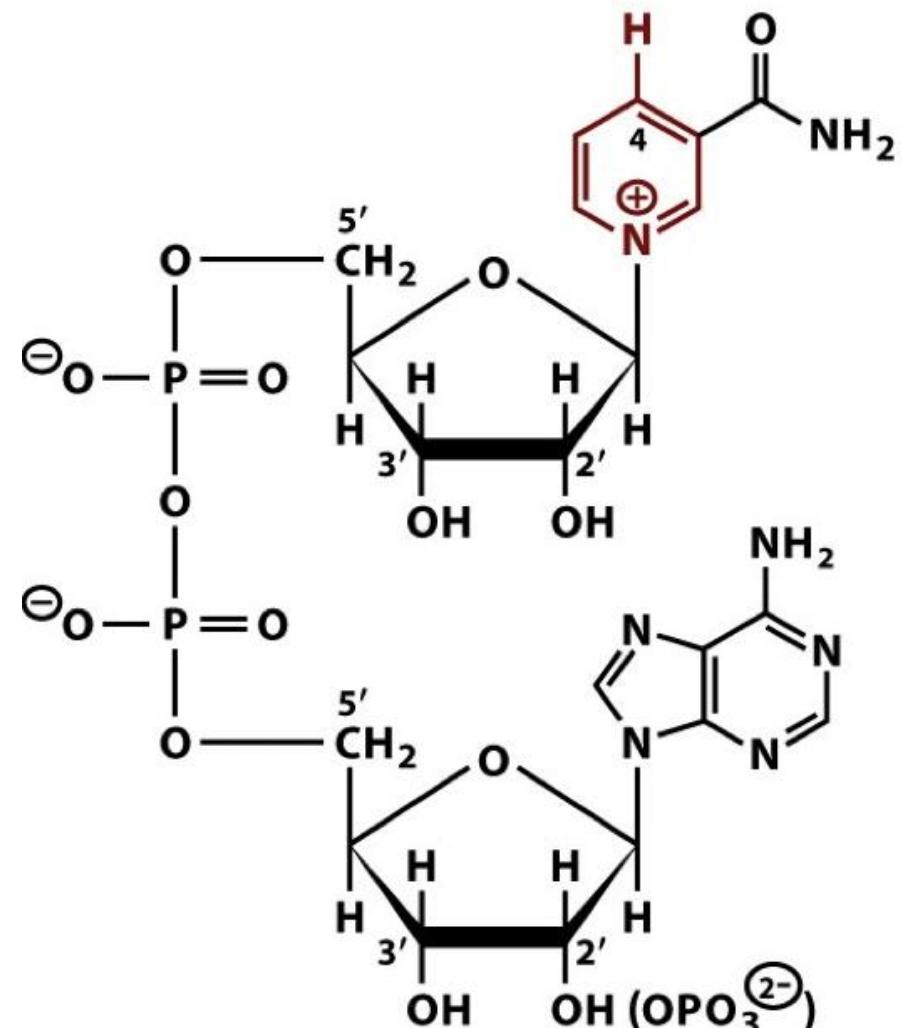




# NAD<sup>+</sup>/NADH or NADP<sup>+</sup>/ NADPH

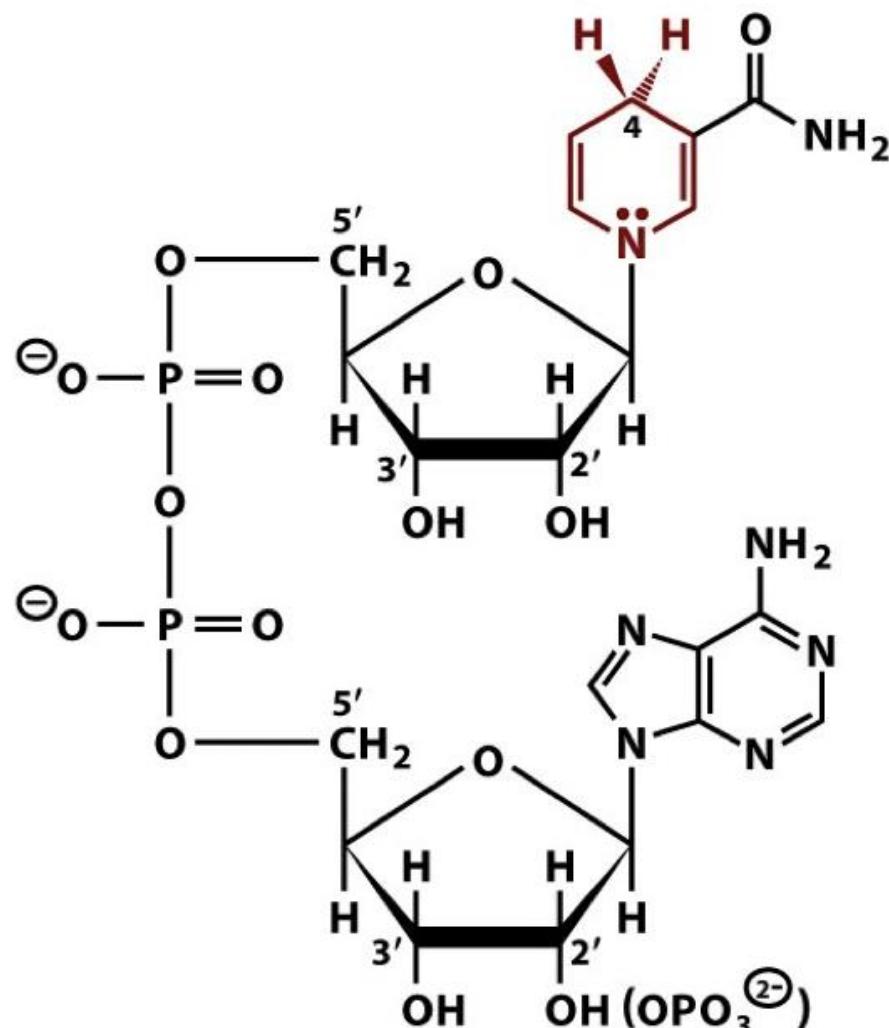
(Nicotinamide Adenine Dinucleotide, hydride/e<sup>-</sup> acceptor)

*Oxidized form*



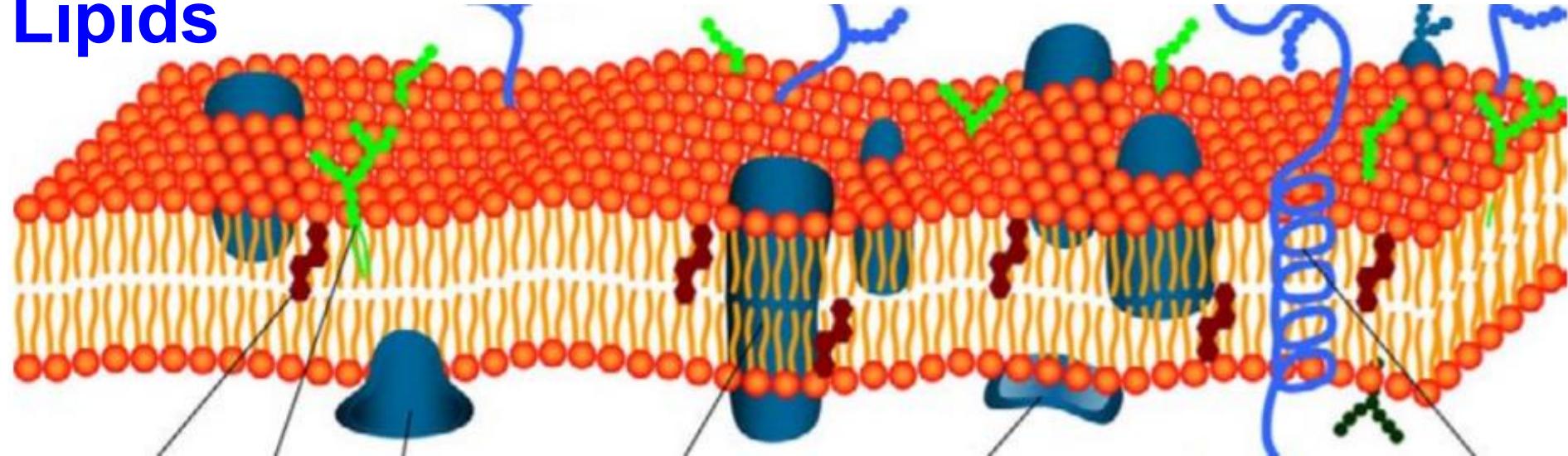
NAD<sup>+</sup> (NADP<sup>+</sup>)

*Reduced form*

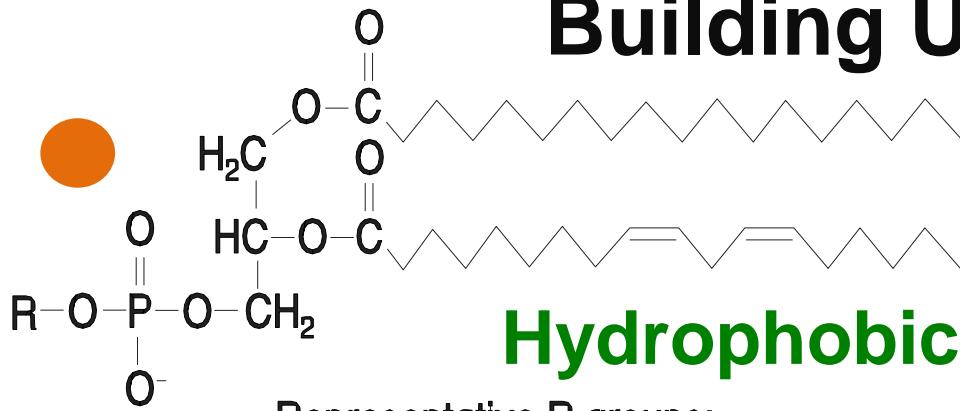


NADH (NADPH)

# Lipids



# Building Units



## Representative R groups:

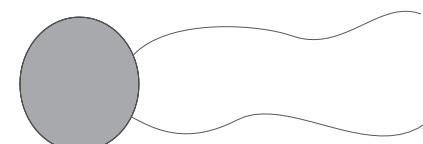
# Hydrophilic (charged)

ethanolamine	$\text{--CH}_2\text{CH}_2\text{NH}_3^+$
choline	$\text{--CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3^+$
serine	$\text{--CH}_2\text{CHNH}_3^+$
	$\searrow$ $\text{COO}^-$
glycero	$\text{--CH}_2\text{CHOHCH}_2\text{OH}$

(saturated fatty acid,  
e.g., stearic acid, 18:0)

(polyunsaturated fatty acid,  
e.g., linoleic acid, 18:2)

## Cartoon representation



# SATURATED FAT

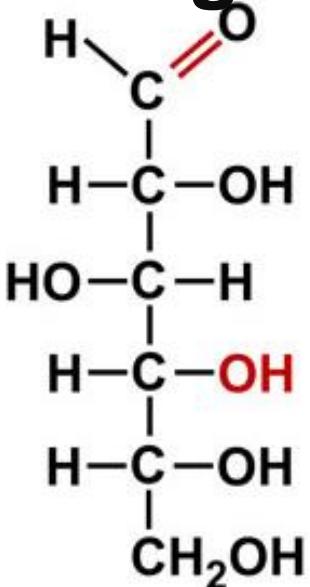
VS

# UNSATURATED FAT

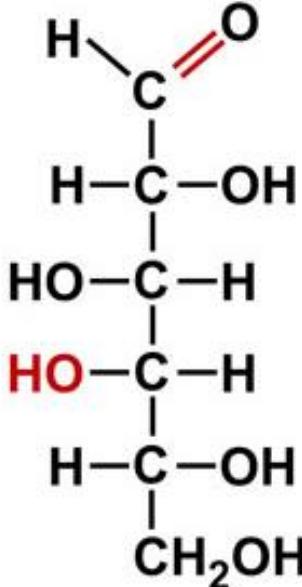


# Building Units of Carbohydrates

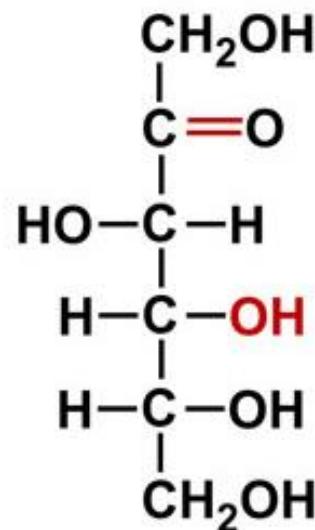
Open  
Form



葡萄糖 Glucose



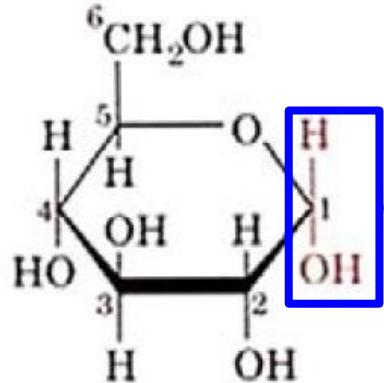
Galactose 半乳糖



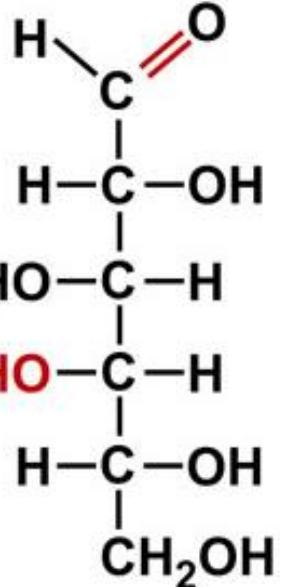
Fructose 果糖

Ring  
Forms

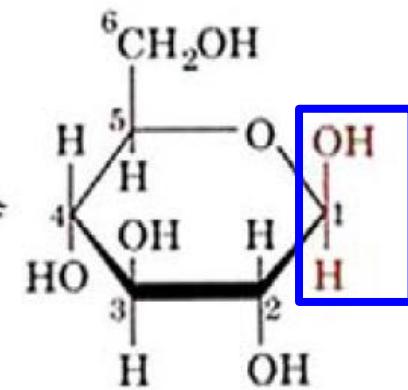
c)



$\alpha$ -D-Glucopyranose

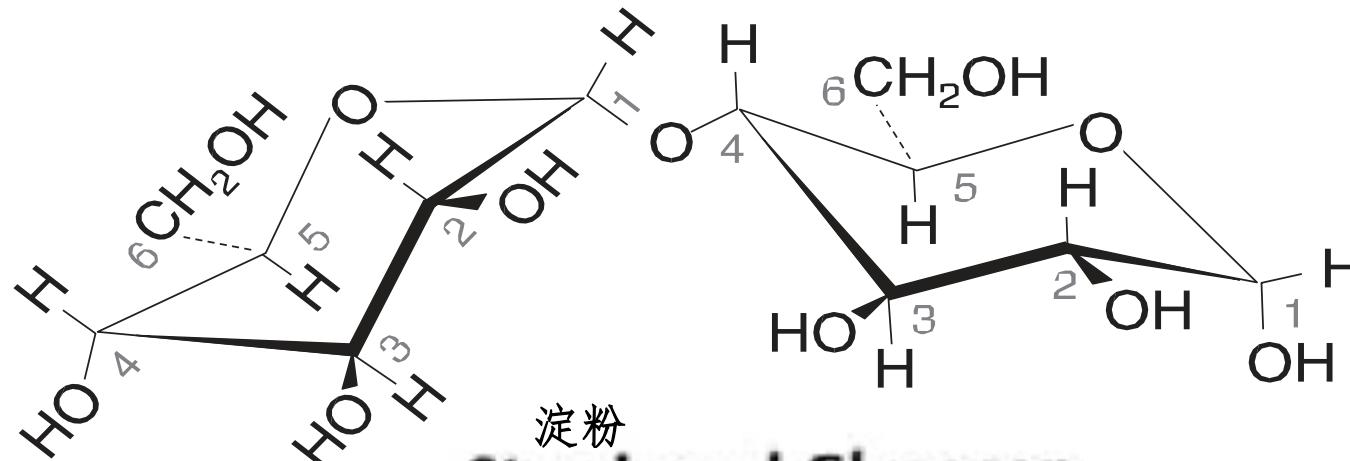


D-Glucose

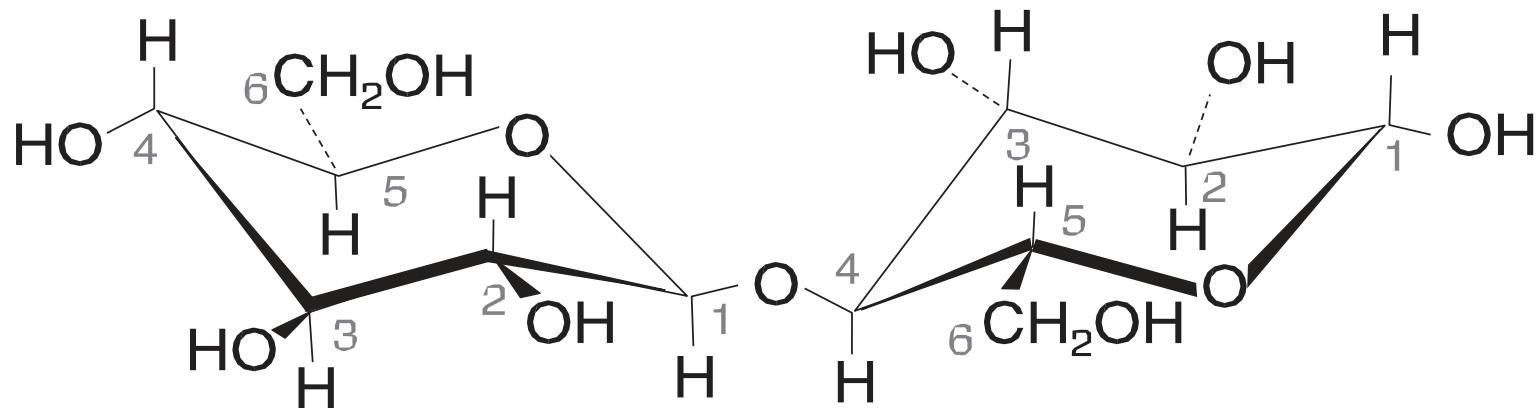


$\beta$ -D-Glucopyranose

# Linkage (C1-->C4) of two monosaccharides



淀粉  
Starch and Glycogen  
( $\alpha$ -1,4 linkages)



Cellulose 纤维素  
( $\beta$ -1,4 linkages)

# **Coordination of Proteins to Metals**

# Coordination of Proteins to Metals

Direct coordination of amino acids (usually its side-chain) or coordination through cofactors.

## Side-chain & their intrinsic $pK_a$

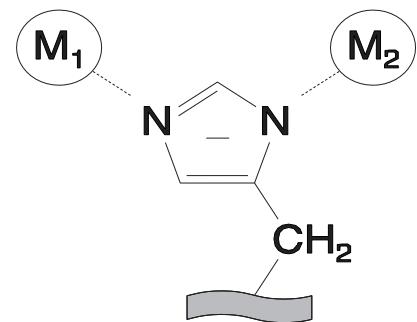
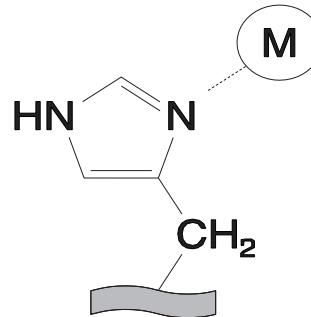
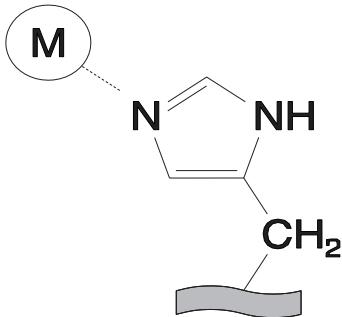
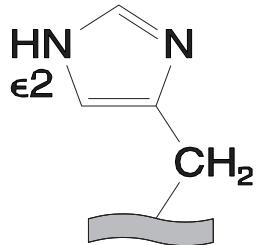
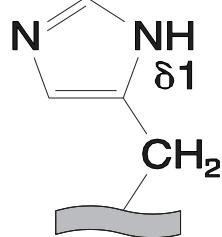
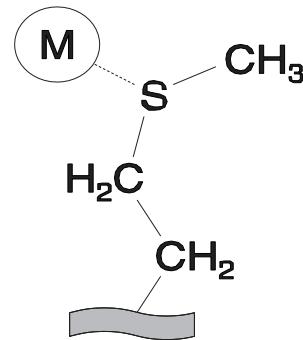
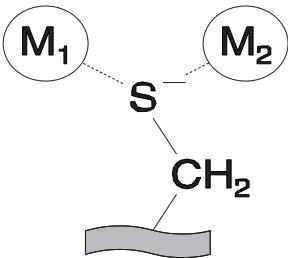
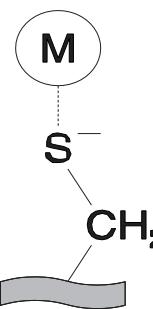
Free Amino Acid	$pK_a$
Cys	8.3
Met	Met <sup>a</sup>
His	His <sup>+</sup> /His
	His/His <sup>-</sup>
Asp	Glu
	Asp
Glu	3.9
Tyr	10.1
Ser	13.0
Thr	13.0
Asn	Asn <sup>a</sup>
	Gln <sup>a</sup>
Arg	Lys <sup>+</sup> /Lys
	Arg <sup>+</sup> /Arg

Chemical structures of amino acid side chains:

- Cys: SC(C)C
- Met: CC(C)SS
- His: C1=CN=NH=C1
- Asp: CC(=O)CO
- Glu: CCC(=O)CO
- Tyr: Oc1ccc(CC)c(O)c1
- Ser: CC(O)C
- Thr: CC(O)C(C)C
- Asn: CC(=O)NCC
- Gln: CCC(=O)NCC

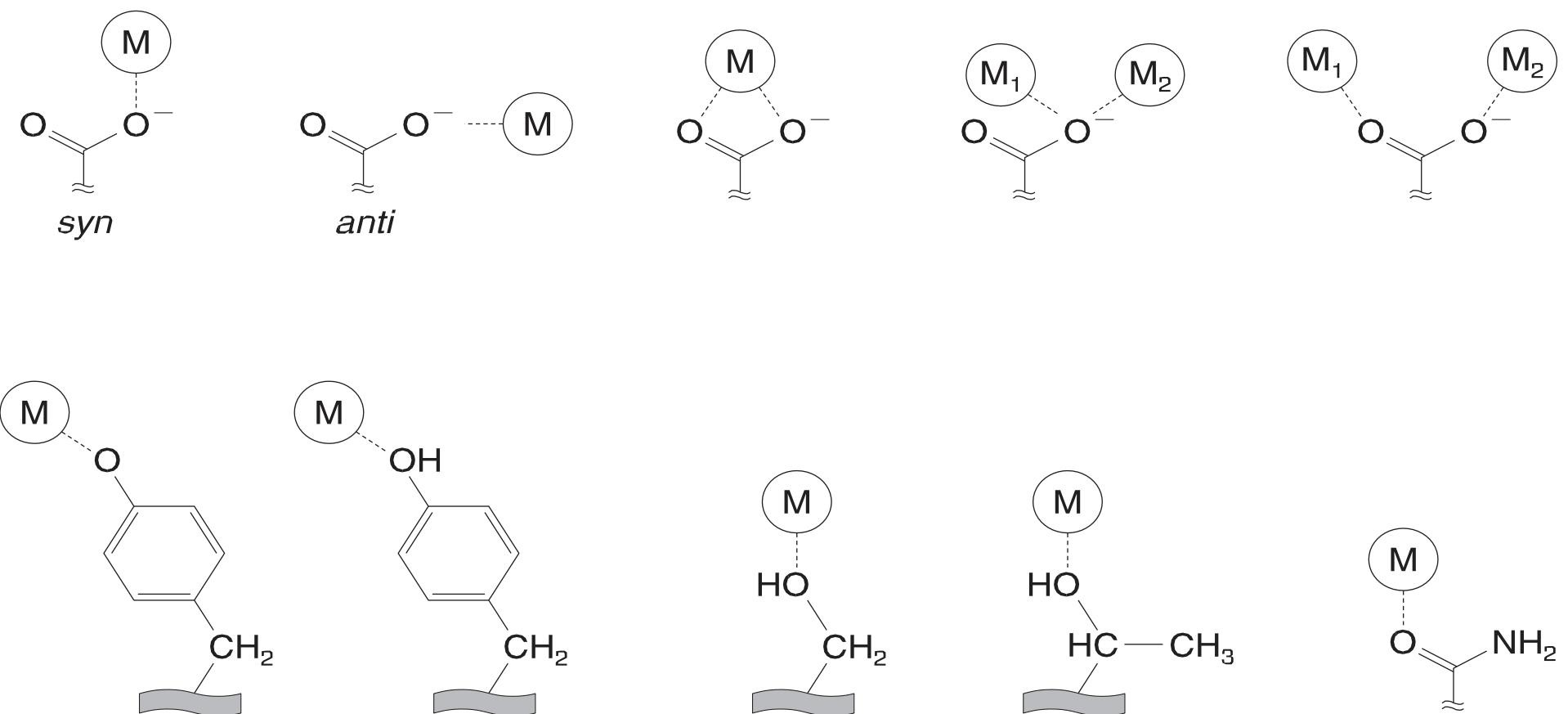
**Real  $pK_a$  depends on their own surrounding or bonds**

# Coordination modes

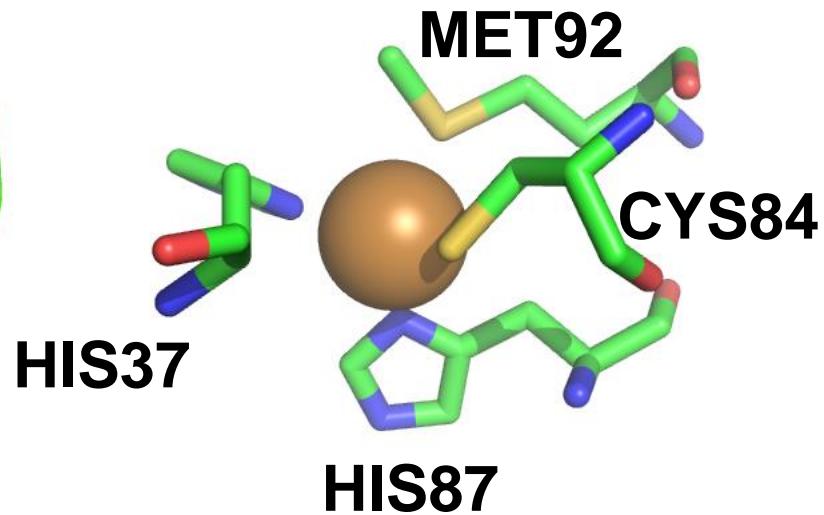
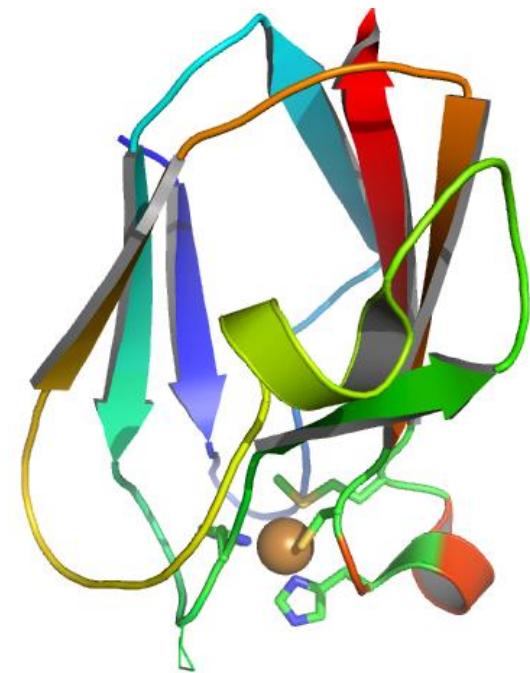


**Less  
common**

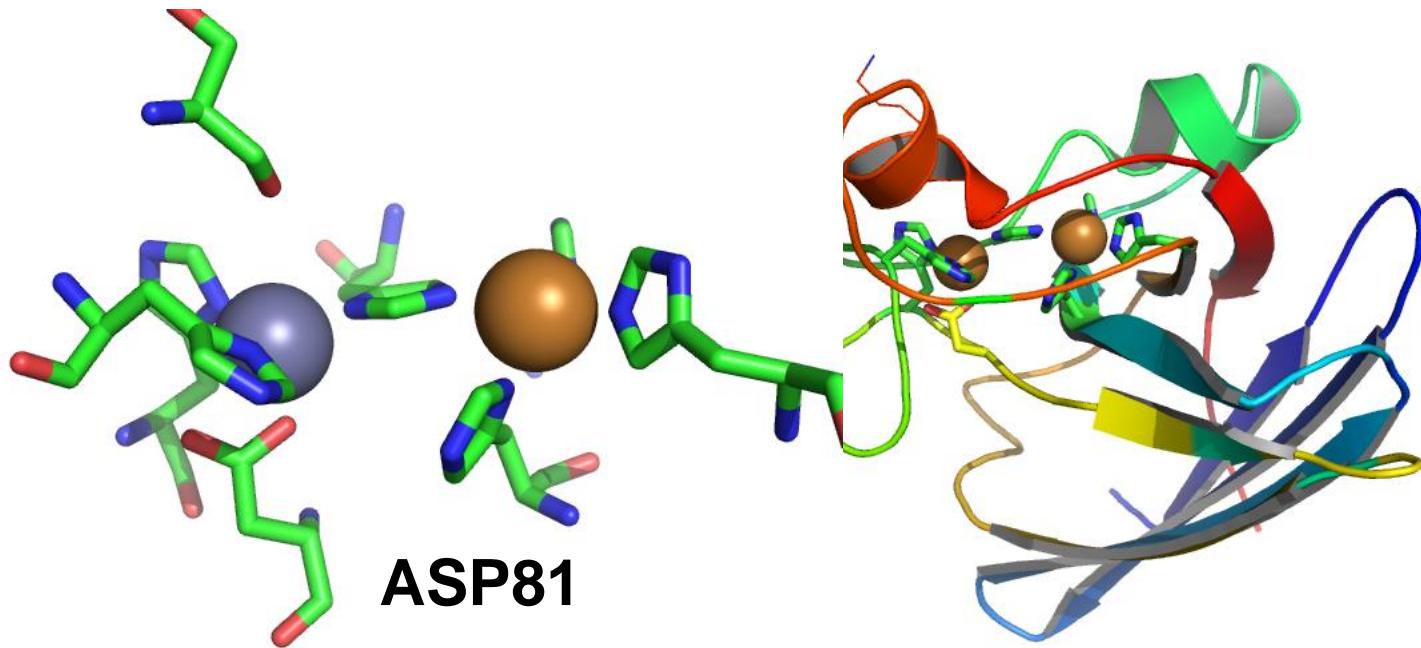
# Coordination modes



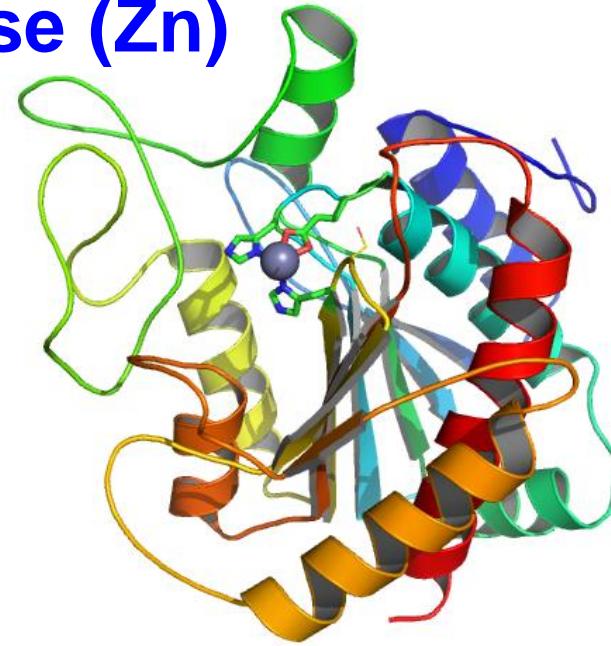
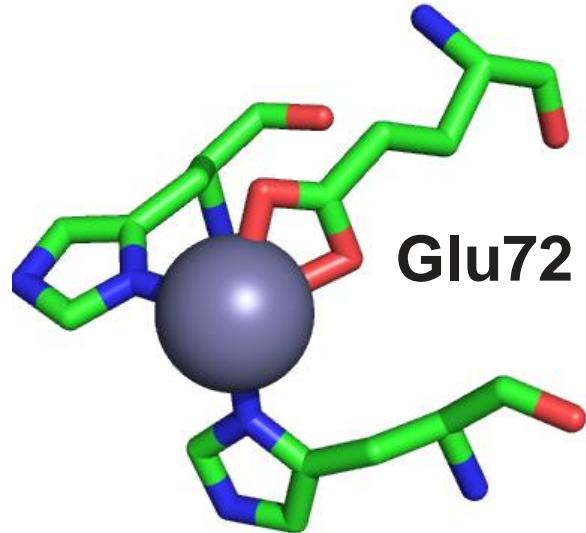
**Plastocyanin**  
**(Cu, ET agent**  
in photo-  
synthesis)



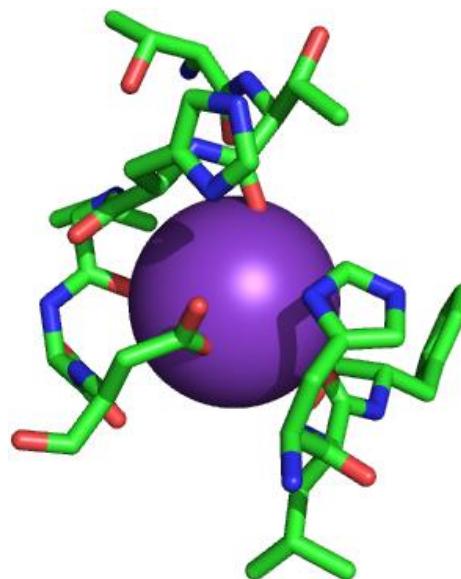
**Cu, Zn**  
**Superoxide**  
**Dismutase**  
**(SOD)**



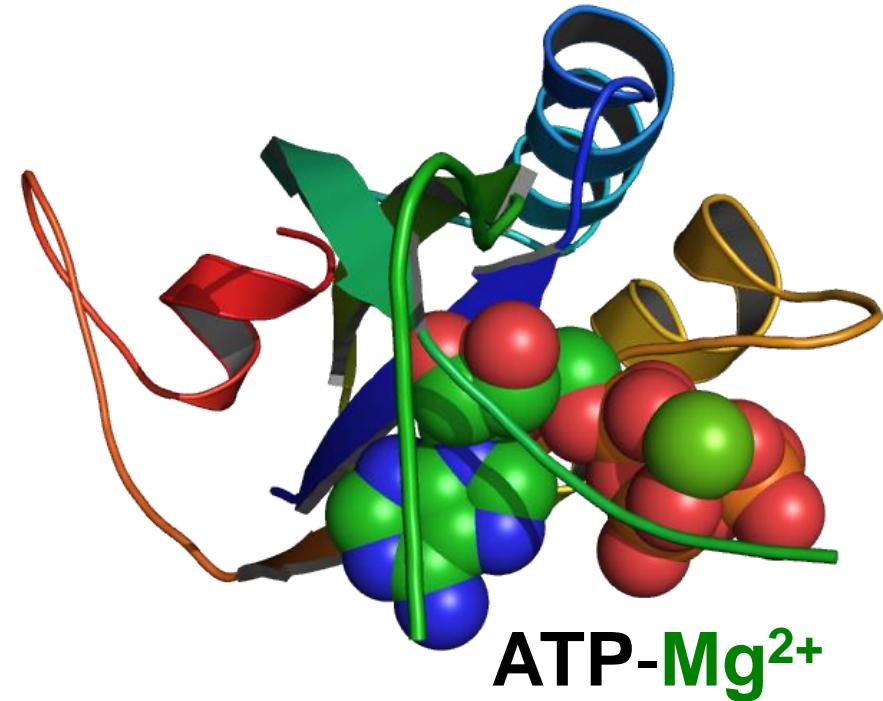
# Carboxypeptidase (Zn)



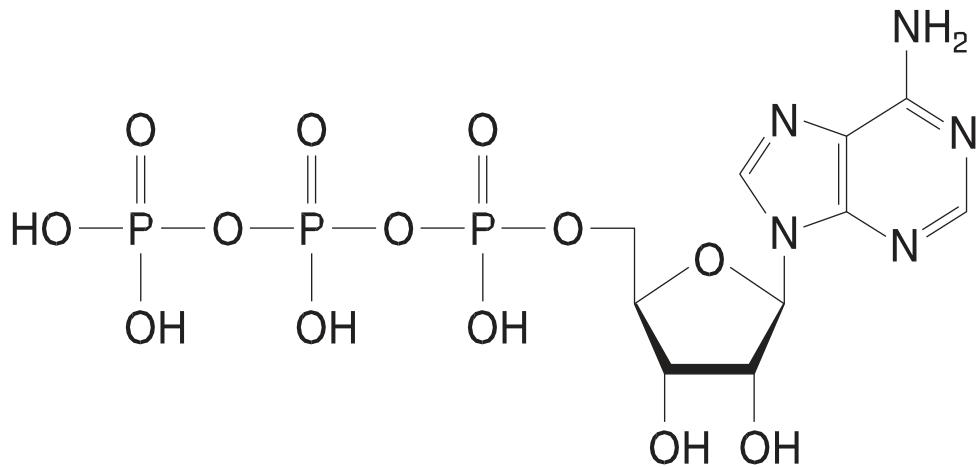
# K in dialkylglycine decarboxylase



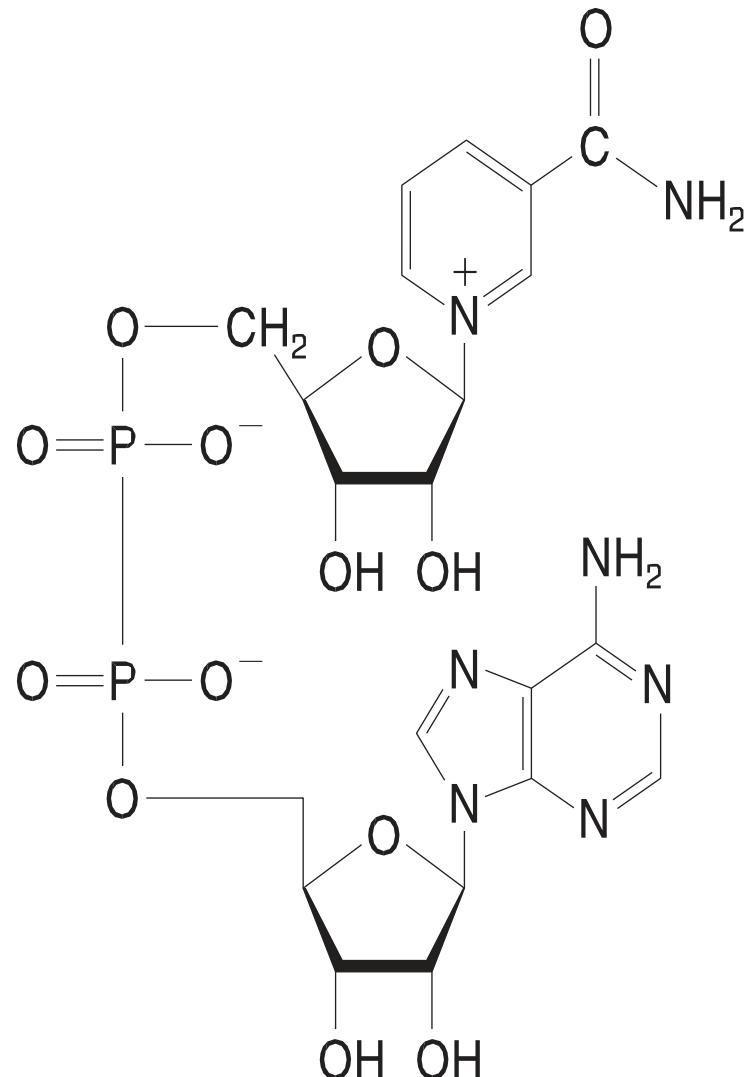
# Coordination via cofactors



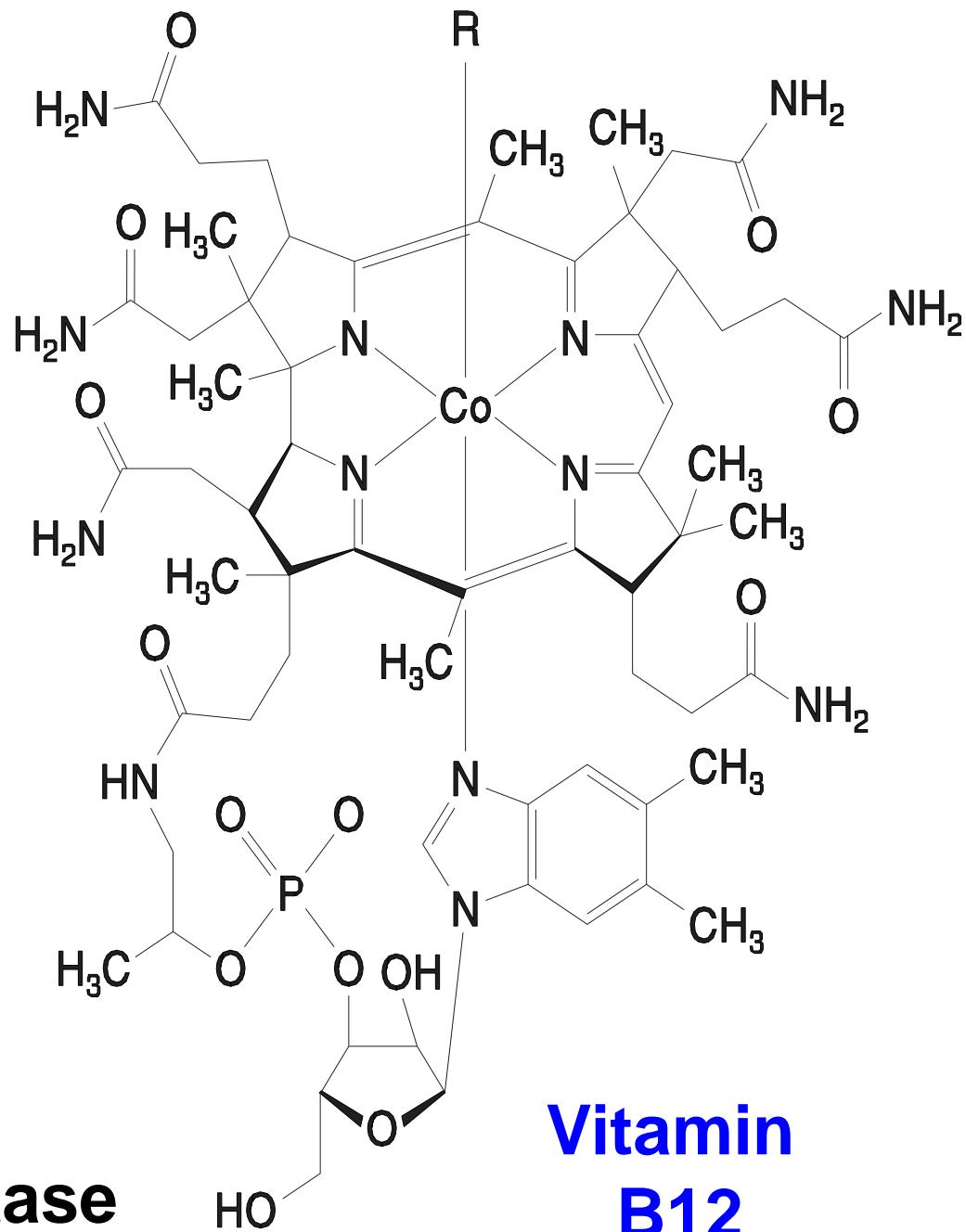
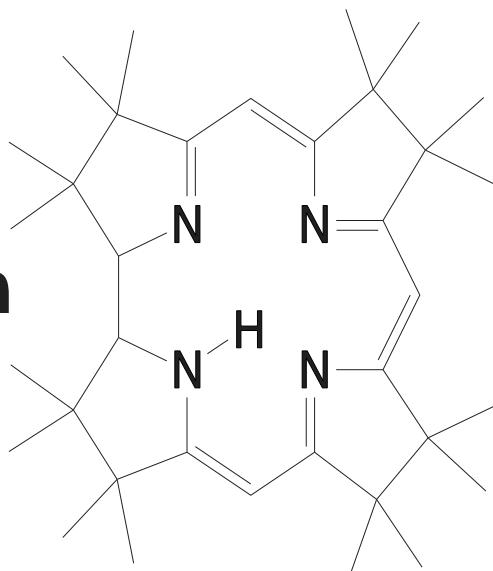
Adenosine triphosphate (ATP)



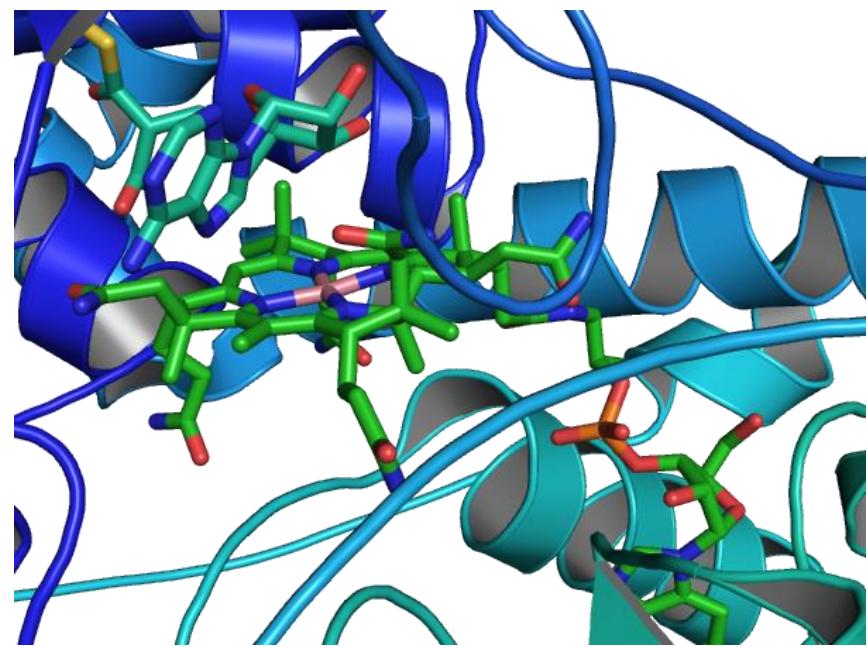
Nicotinamide adenine dinucleotide (NAD<sup>+</sup>)



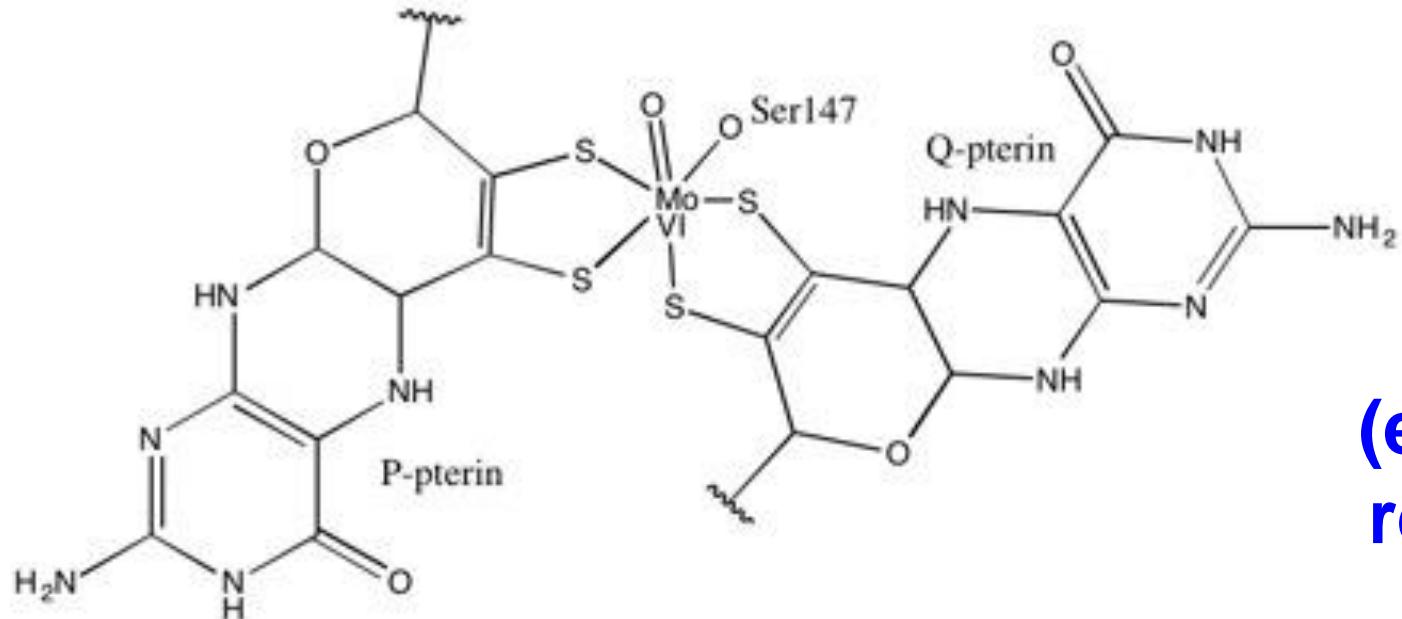
**corrin  
ring**



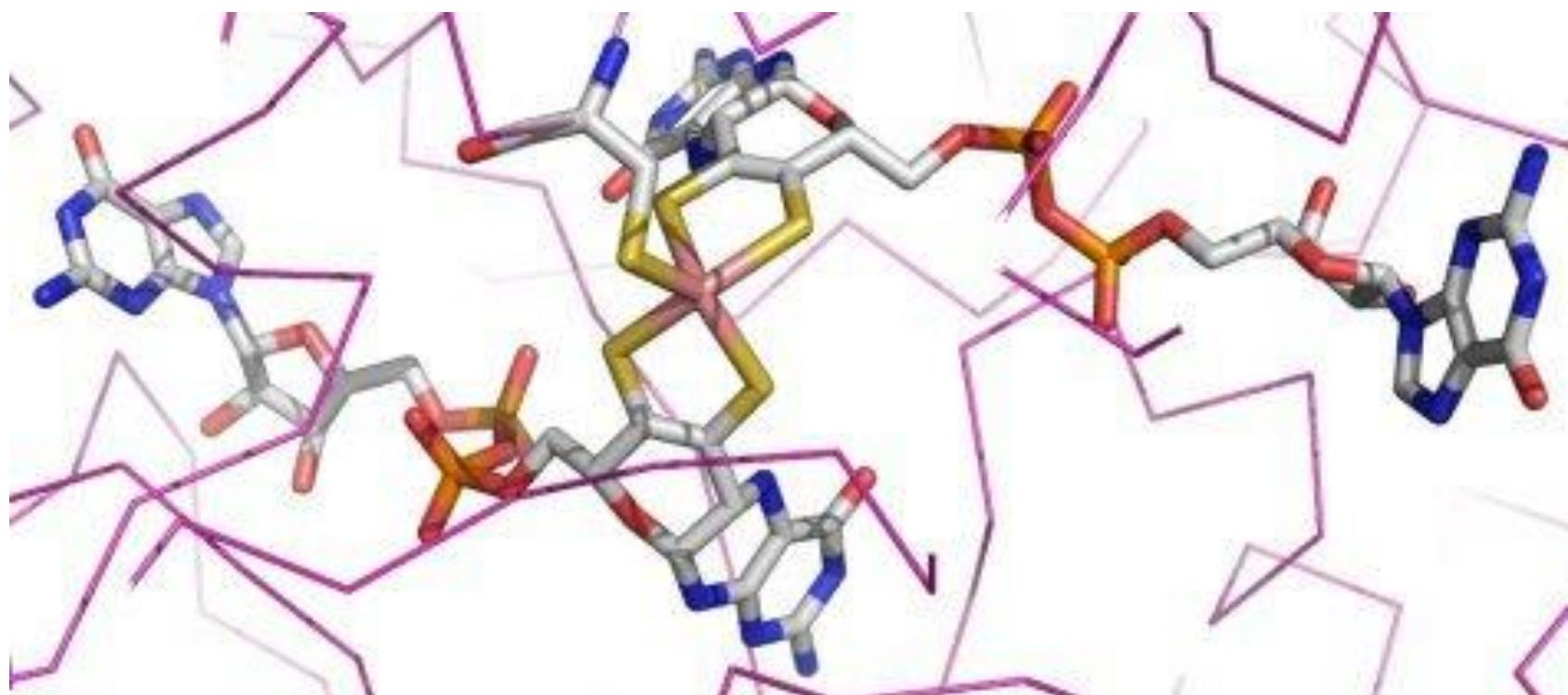
**Vitamin  
B12**



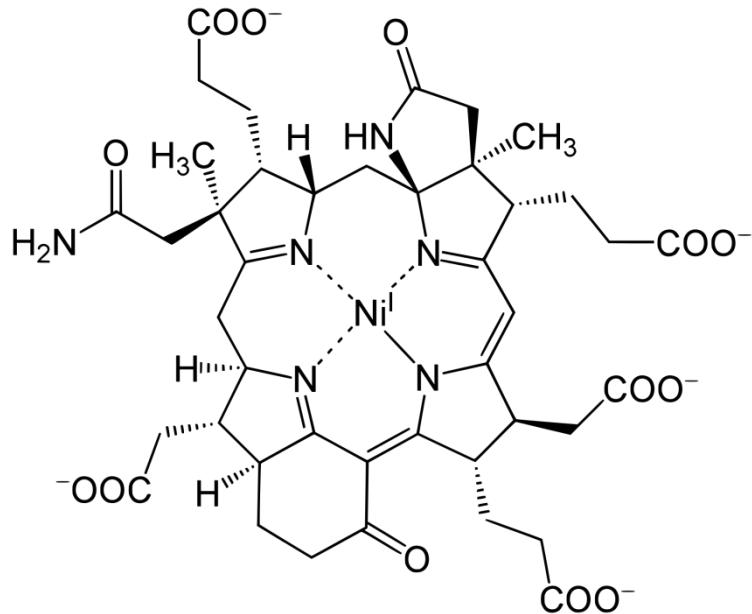
**Methylmalonyl-CoA mutase  
(MMCM)**



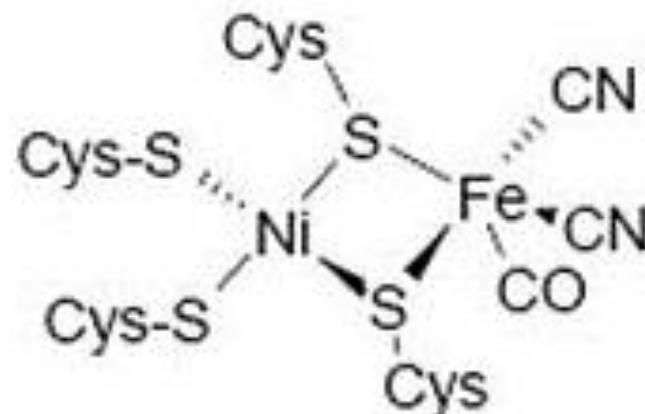
Mo or W  
(e.g. DMSO  
reductase)



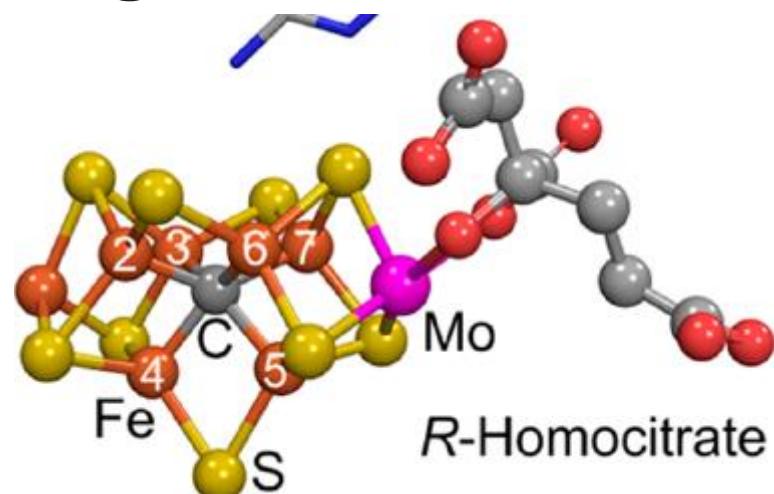
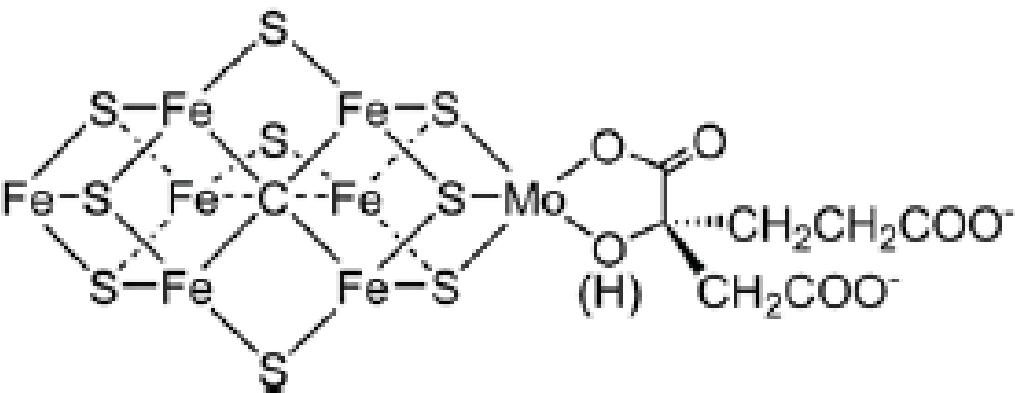
# F430 nickel cofactor



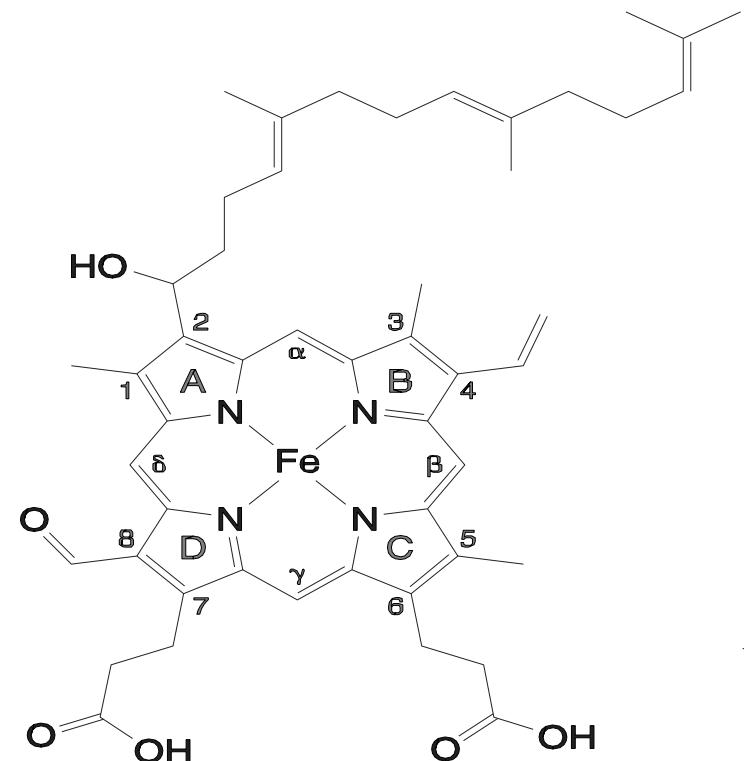
# [NiFe] hydrogenases



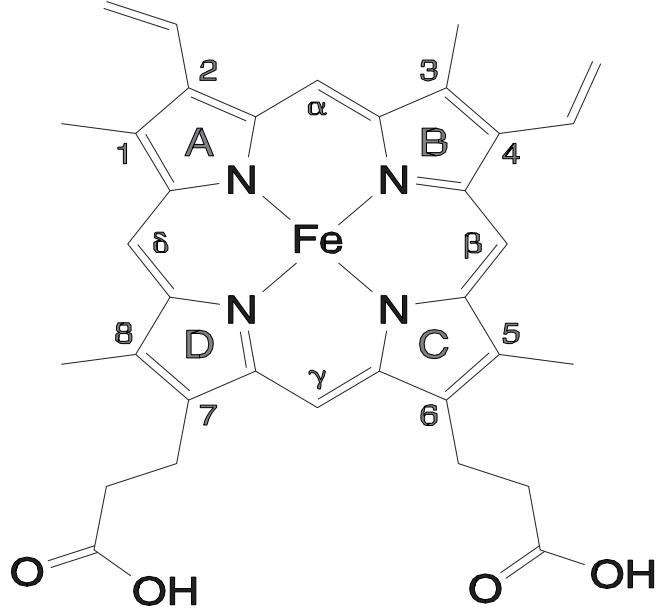
# Fe-Mo cofactor of Nitrogenase



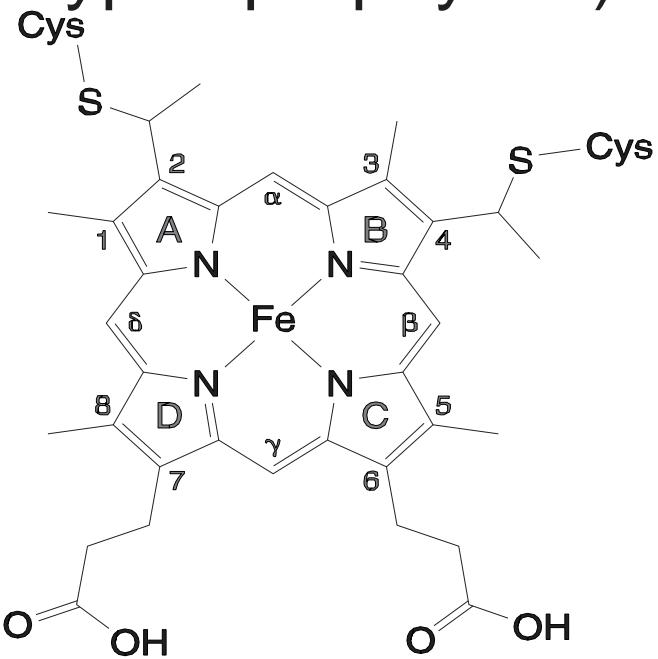
# Hemes: Porphyrins (only show few types porphyrins)



**Heme a**

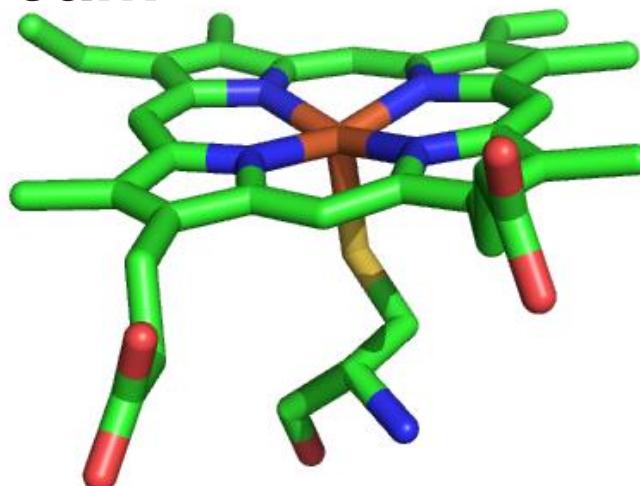
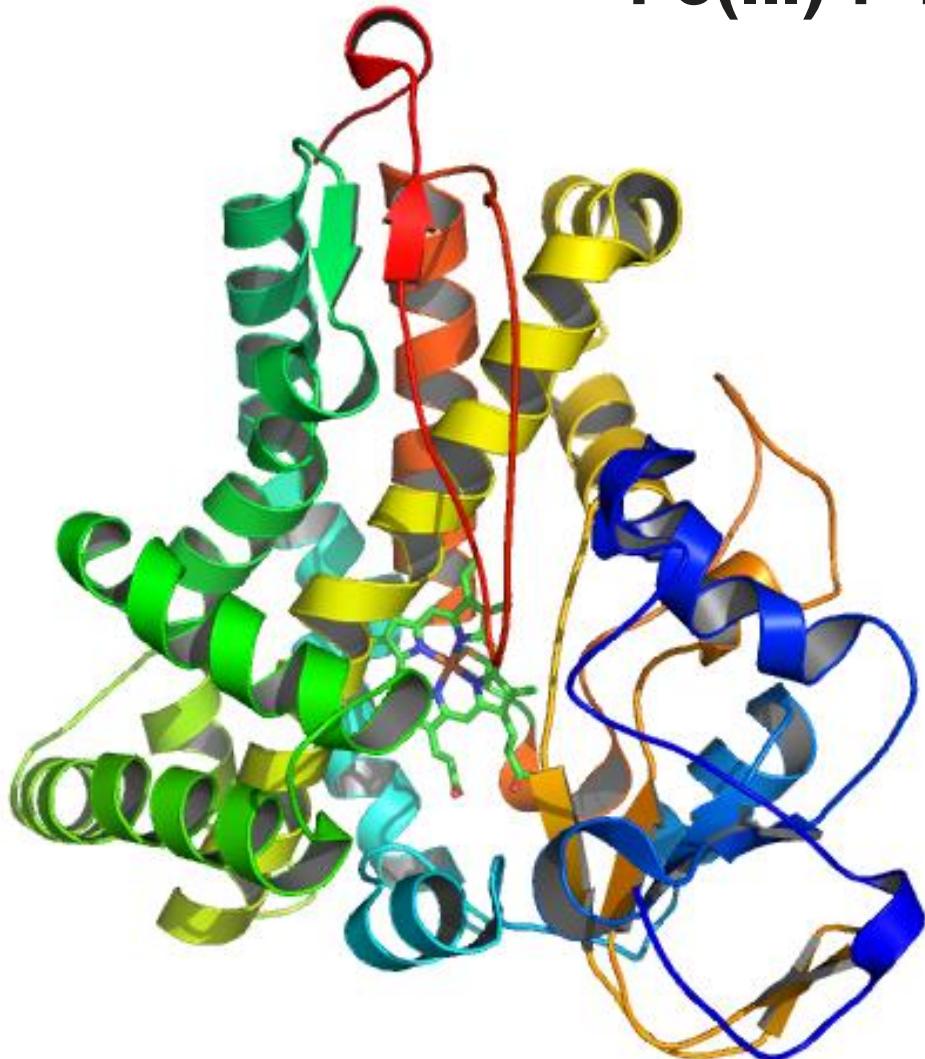


**Heme b**

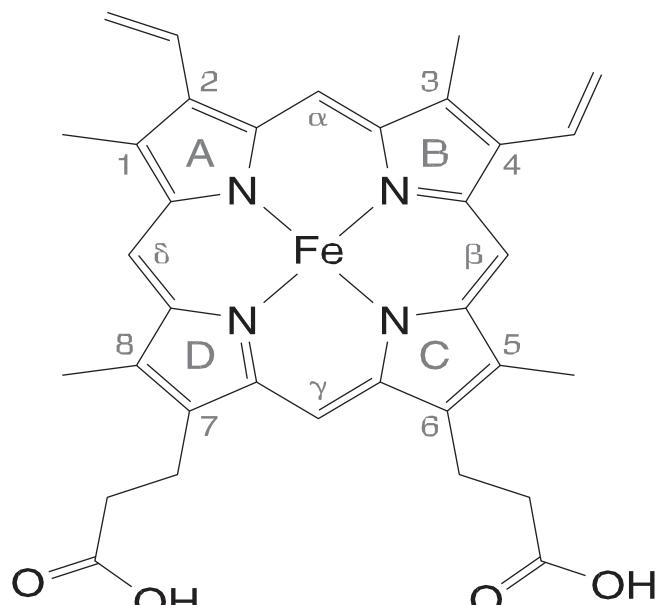


**Heme c**

# Fe(III) P450cam



Cys357

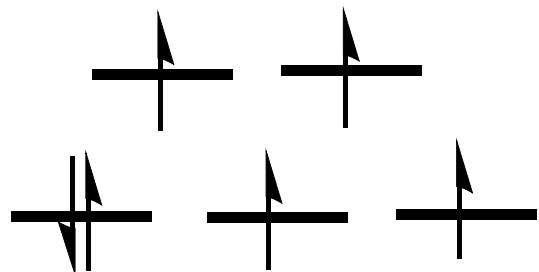


Heme *b*

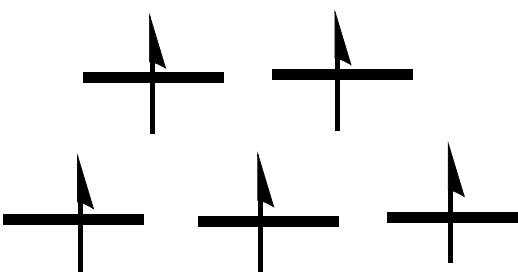
Protein Class/Family	Function	Heme Type	Axial Ligation	Formal Iron Oxidation Spin States
Globins (Section XI.4)	O <sub>2</sub> transport and storage	<i>b</i>	His Nε	Fe(II) ( <i>S</i> = 2)
Nitrophorin (Section XIV.3)	Nitric oxide transport	<i>b</i>	His Nε	
Catalases (Section XI.3)	H <sub>2</sub> O <sub>2</sub> + H <sub>2</sub> O <sub>2</sub> → 2 H <sub>2</sub> O + O <sub>2</sub>	<i>b</i>	Tyr Oη	Fe(III) ( <i>S</i> = 5/2)
Peroxidases (Section XI.3)	A–H <sub>2</sub> + H <sub>2</sub> O <sub>2</sub> → A + 2 H <sub>2</sub> O	<i>b</i> <sup>b</sup>	His Nε (+H <sub>2</sub> O)	Fe(III) ( <i>S</i> = 5/2)
Chloroperoxidases (Section XI.3)	AH + X <sup>–</sup> + H <sup>+</sup> + H <sub>2</sub> O <sub>2</sub> → AX + 2 H <sub>2</sub> O	<i>b</i>	Cys Sγ	Fe(III) ( <i>S</i> = 5/2)
Nitric oxide synthases (Section XIV.3)	L-Arg + 2 O <sub>2</sub> + $\frac{3}{2}$ NADPH → citrulline + nitric oxide + $\frac{3}{2}$ NADP <sup>+</sup>	<i>b</i>	Cys Sγ	Fe(III) ( <i>S</i> = 5/2)
P450 proteins (Sections VI.3, IX.3, XI.5)	RH + O <sub>2</sub> + 2 H <sup>+</sup> + 2 e <sup>–</sup> → ROH + H <sub>2</sub> O	<i>b</i>	Cys Sγ (+H <sub>2</sub> O or OH <sup>–</sup> )	Fe(III) ( <i>S</i> = 5/2)

**S:** spin quantum number

## Fe 3d orbitals



High-spin Fe(II)



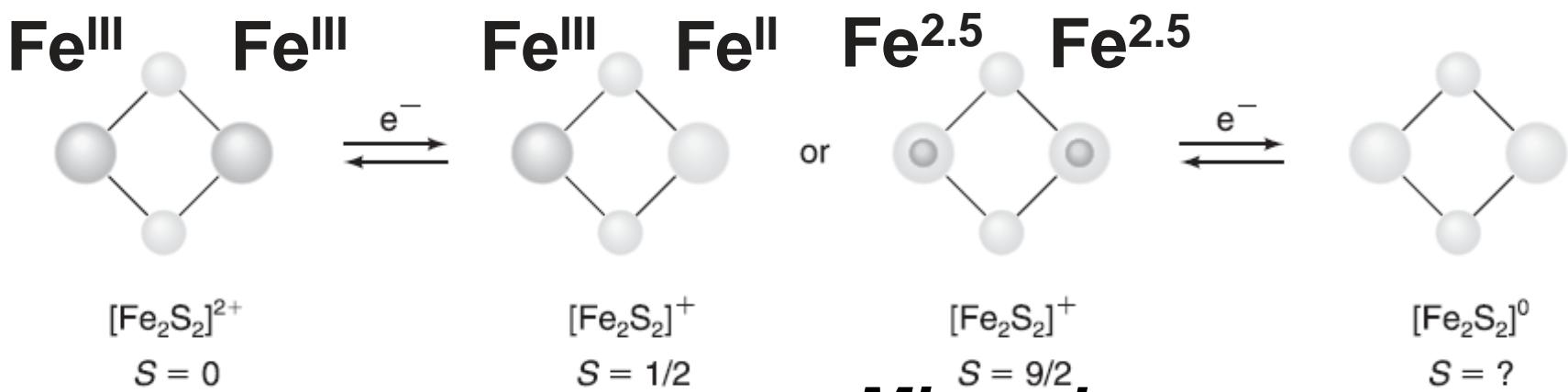
High-spin Fe(III)

Cytochrome <i>c</i> oxidase (heme <i>a</i> <sub>3</sub> ) (Section XI.6)	$O_2 + 4 e^- + 4 H^+ \rightarrow 2 H_2O$	<i>a</i> <sub>3</sub>	His Nε	Fe(III) ( <i>S</i> = 5/2) Spin coupled to Cu <sub>B</sub>
Cytochrome <i>bo</i> quinol oxidase (heme <i>o</i> )	$O_2 + 4 e^- + 4 H^+ \rightarrow 2 H_2O$	<i>o</i>	His Nε	Fe(III) ( <i>S</i> = 5/2) ?
Cyt <i>c</i> 554 (heme 2) (Sections IV, XII.3)	oxidation of ammonia to nitrite	<i>c</i>	His Nε	Fe(III) ( <i>S</i> = 5/2)
Nitrite reductases (Section XII.4)	$NO_2^- + 8 H^+ + 6 e^- \rightarrow NH_4^+ + 2 H_2O$	Siroheme	Cys Sγ	Fe(II) ( <i>S</i> = 1 or <i>S</i> = 2) Spin coupled to [Fe <sub>4</sub> S <sub>4</sub> ] <sup>+</sup>
Hydroxylamine oxidoreductase (heme P460) (Section XII.3)	$NH_2OH + H_2O \rightarrow NO_2^- + 4 e^- + 5 H^+$	P460	His Nε	Fe(III) ( <i>S</i> = 5/2 or <i>S</i> = 3/2) Spin coupled to heme 6
Cytochrome <i>cd</i> <sub>1</sub> nitrite reductase (Section XII.4)	$NO_2^- + 2 H^+ + e^- \rightarrow NO + 2 H_2O$ and $O_2 + 4 H^+ + 4 e^- \rightarrow 2 H_2O$	<i>d</i> <sub>1</sub>	His Nε or His Nε + Tyr Oη	Fe(III) ( <i>S</i> = 1/2)
Sulfite reductases (Section XII.5)	$HSO_3^- + 6 H^+ + 6 e^- \rightarrow HS^- + 3 H_2O$ $3 HS^- + 3 H^+ + 2 e^- \rightarrow S_3O_6^{2-} + 3 H_2O$ $2 S_3O_6^{2-} + 4 H^+ + 4 e^- \rightarrow S_2O_3^{2-} + 3 H_2O$	Siroheme	Cys Sγ	Fe(II) ( <i>S</i> = 1 or <i>S</i> = 2) Spin coupled to [Fe <sub>4</sub> S <sub>4</sub> ] <sup>+</sup>

# Iron-sulfur proteins (learn more in part IV)

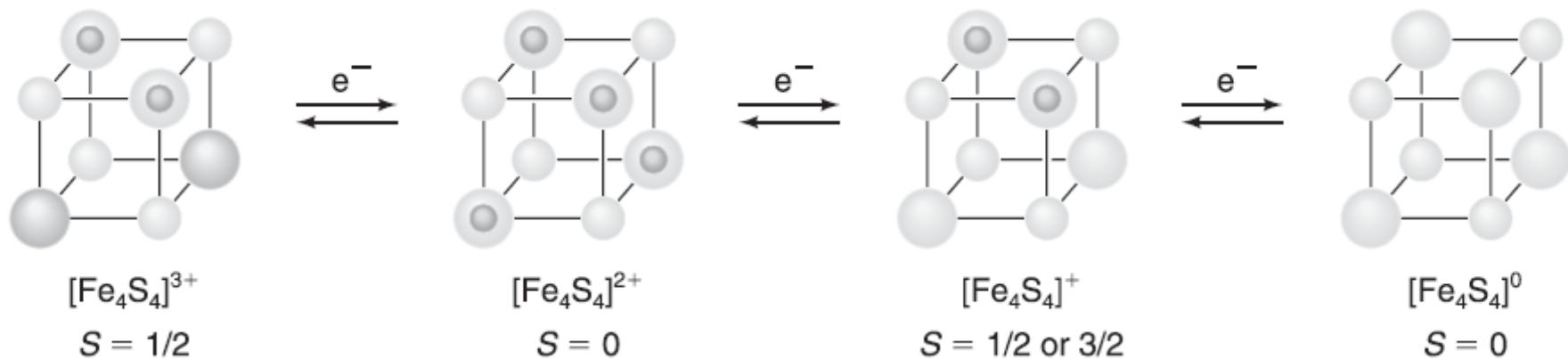
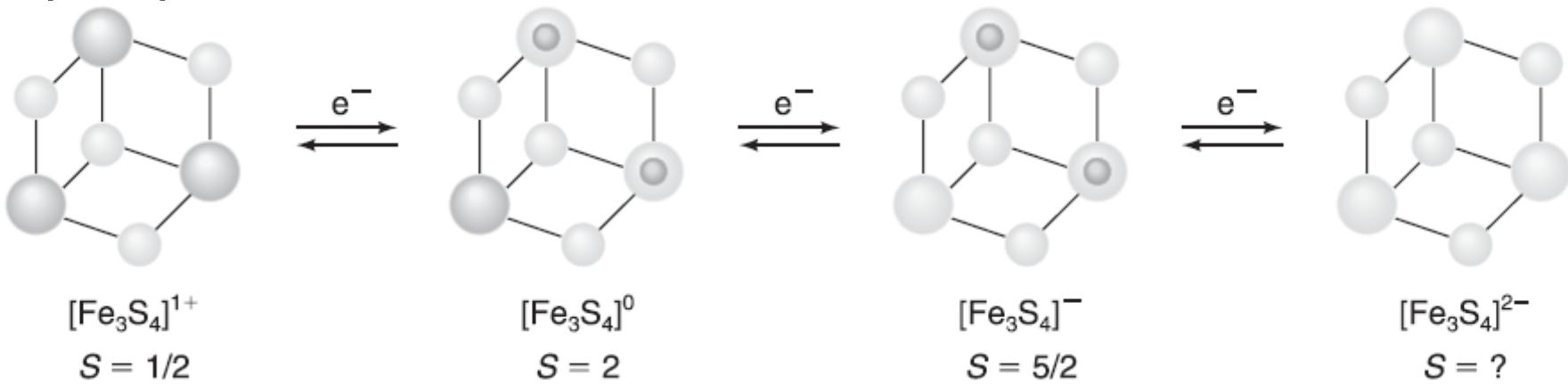
Typical chemical and spectroscopic characteristics of iron-sulfur proteins

Protein	Cluster	Oxidation state	Formal valence	EPR g values (temp)
Rubredoxin	1Fe-0S	Oxidized	Fe <sup>3+</sup>	4.3, 9 (< 20 K)
		Reduced	Fe <sup>2+</sup>	None
2-Iron ferredoxin	2Fe-2S	Oxidized	2Fe <sup>3+</sup>	None
		Reduced	1Fe <sup>3+</sup> , 1Fe <sup>2+</sup>	1.89, 1.95, 2.05 (< 100 K)
3-Iron ferredoxin	3Fe-4S	Oxidized	3Fe <sup>3+</sup>	1.97, 2.00, 2.02 (< 20 K)
		Reduced	2Fe <sup>3+</sup> , 1Fe <sup>2+</sup>	None
4-Iron ferredoxin	4Fe-4S	Oxidized	3Fe <sup>3+</sup> , 1Fe <sup>2+</sup>	2.04, 2.04, 2.12 (< 100 K)
		Intermed.	2Fe <sup>3+</sup> , 2Fe <sup>2+</sup>	None
		Reduced	1Fe <sup>3+</sup> , 3Fe <sup>2+</sup>	1.88, 1.92, 2.06 (< 20 K)



**S:** spin quantum number

## Mix-valence



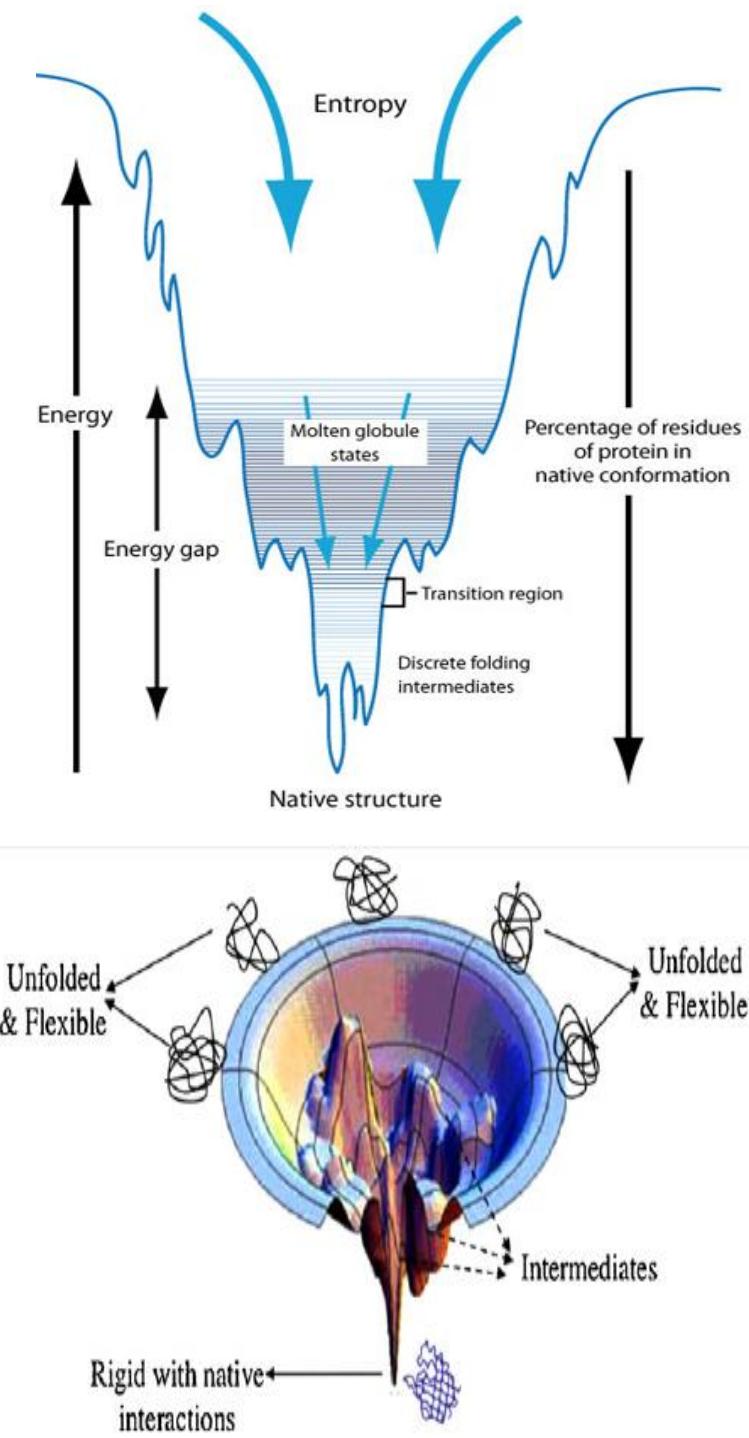
Some reactions that are catalyzed by Fe/S center-containing redox enzymes

enzyme	catalyzed reaction
hydrogenases	$2 \text{ H}^+ + 2 \text{ e}^- \rightleftharpoons \text{H}_2$
nitrogenases	$\text{N}_2 + 10 \text{ H}^+ + 8 \text{ e}^- \rightleftharpoons 2 \text{ NH}_4^+ + \text{H}_2$
sulfite reductase	$\text{SO}_3^{2-} + 7 \text{ H}^+ + 6 \text{ e}^- \rightleftharpoons \text{HS}^- + 3 \text{ H}_2\text{O}$
aldehyde oxidase	$\text{R-CHO} + 2 \text{ OH}^- \rightleftharpoons \text{R-COOH} + \text{H}_2\text{O} + 2 \text{ e}^-$
xanthine oxidase	
NADP oxidoreductase	$\text{NADP}^+ + \text{H}^+ + 2 \text{ e}^- \rightleftharpoons \text{NADPH}$

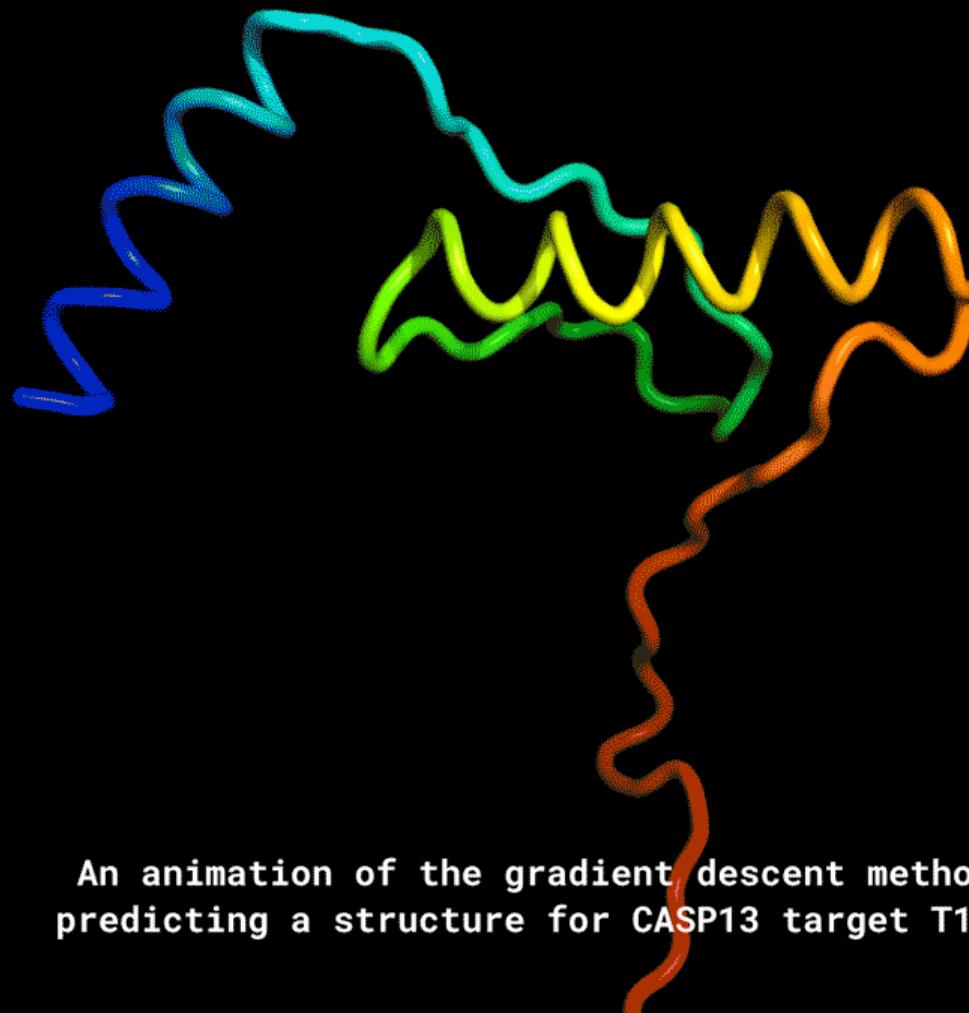
# **Folding (Structure)**

Many proteins adopt a unique, **native structure**: compact & folded state. Whereas, *intrinsically disorder proteins* (IDPs) are recently found responsible for e.g. cellular signaling.

The globular protein has a **well-defined hydrophobic core** with **restricted fluctuations**. While, surface residues are more flexible and may be highly disordered.



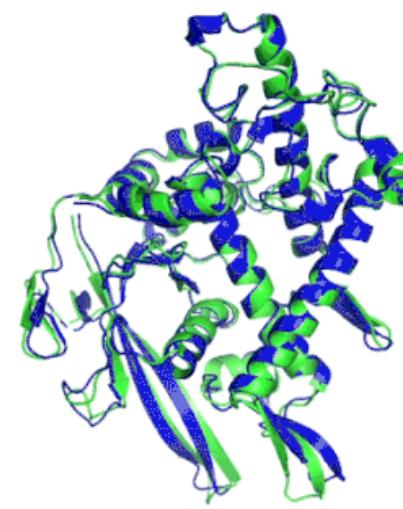
# AlphaFold: Using AI for scientific discovery



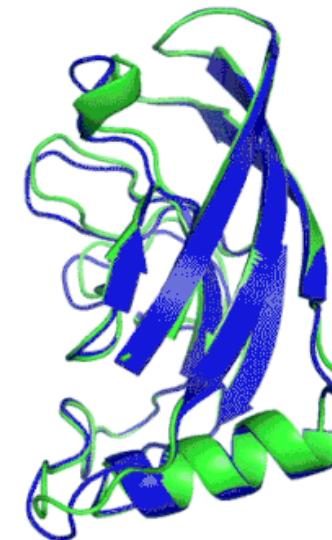
An animation of the gradient descent method  
predicting a structure for CASP13 target T1008

NEWS • 30 NOVEMBER 2020

# 'It will change everything': DeepMind's AI makes gigantic leap in solving protein structures



**T1037 / 6vr4**  
90.7 GDT  
(RNA polymerase domain)

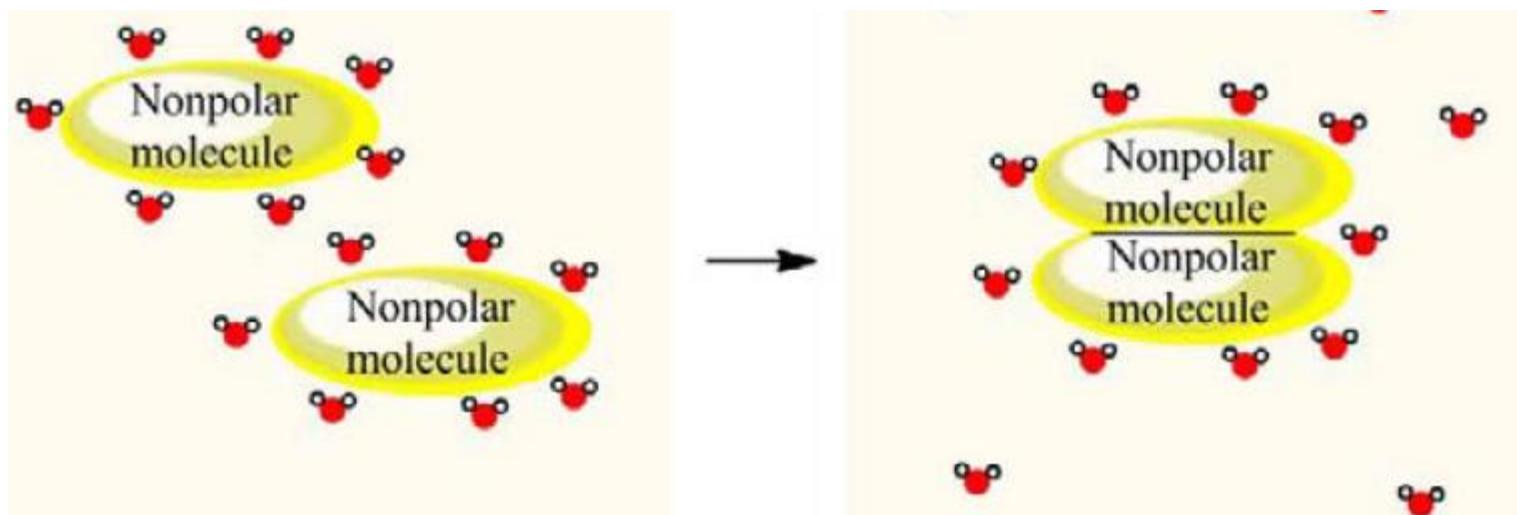


**T1049 / 6y4f**  
93.3 GDT  
(adhesin tip)

- Experimental result
- Computational prediction

**Enthalpy & entropy** contribute to the **stability of the protein** (a small overall free-energy change of only a few KJ/mol) for the folding. **Difference solvation** effect in the folded & unfolded states is also essential (e.g. hydrophobic effect in water).

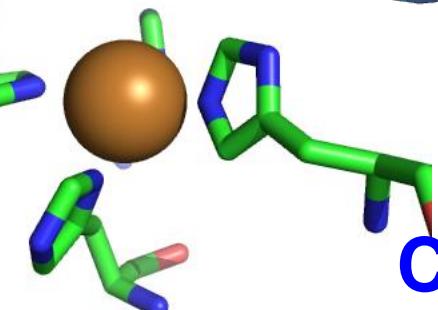
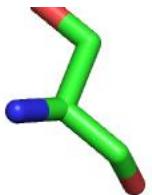
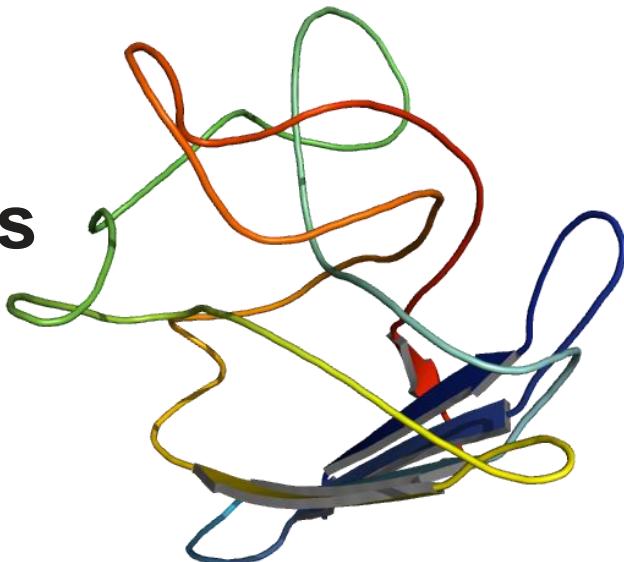
- **Enthalpy:** (1) all non-bonding interactions in the folded state, (2) formation of disulfide bonds, & (3) coordination of protein ligands to metal.
- **Entropy:** loss in degrees of freedom in the folded protein.



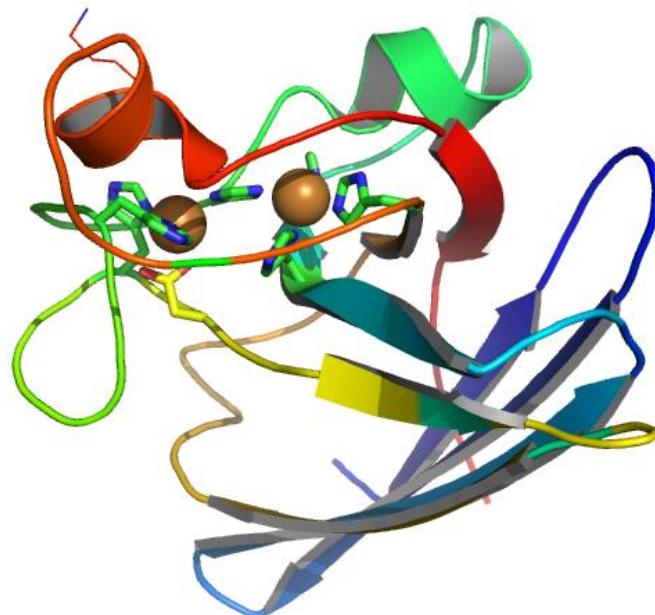
# Effect of Metal on some protein structures

1. Metal ions can maintain the correct arrangement of the overall structure in some proteins, e.g. Cu,Zn SOD.

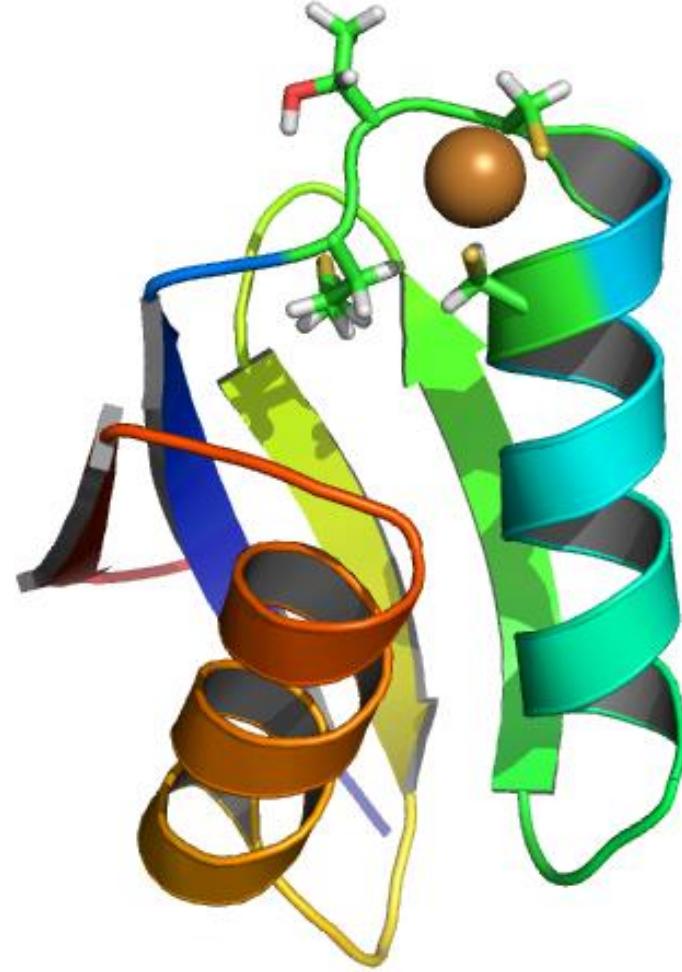
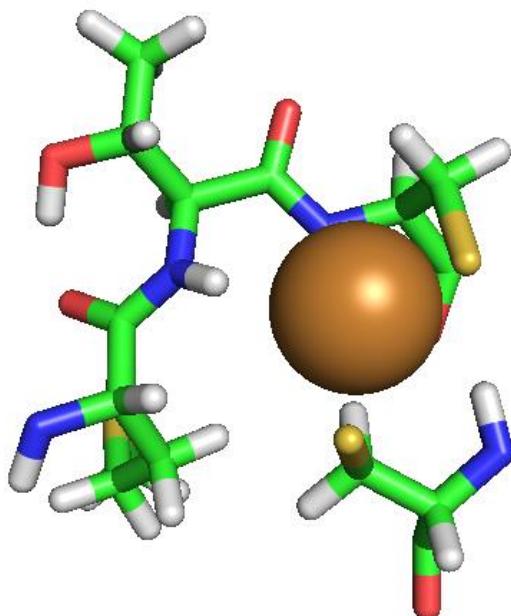
No metals



Cu,Zn SOD  
(Superoxide  
Dismutase)



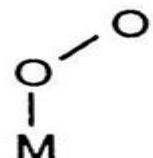
2. The removal of the metal ion **may** introduce some **disorder** at its **binding site** only, but the **whole structure** is essentially **maintained** in **some** proteins, e.g. the Cu transport protein Ccc2 (with a well-defined hydrophobic core).



**Cu transport protein  
Ccc2**

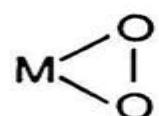
# **Transport & Storage**

# Coordination Modes of O<sub>2</sub> to Transition Metals



η<sup>1</sup>

end-on



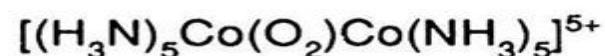
η<sup>2</sup>

side-on



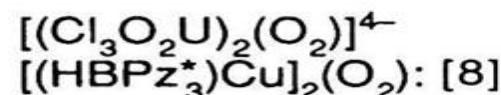
μ-η<sup>1</sup>:η<sup>1</sup>

end-on bridging



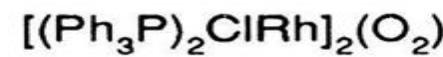
μ-η<sup>2</sup>:η<sup>2</sup>

side-on bridging



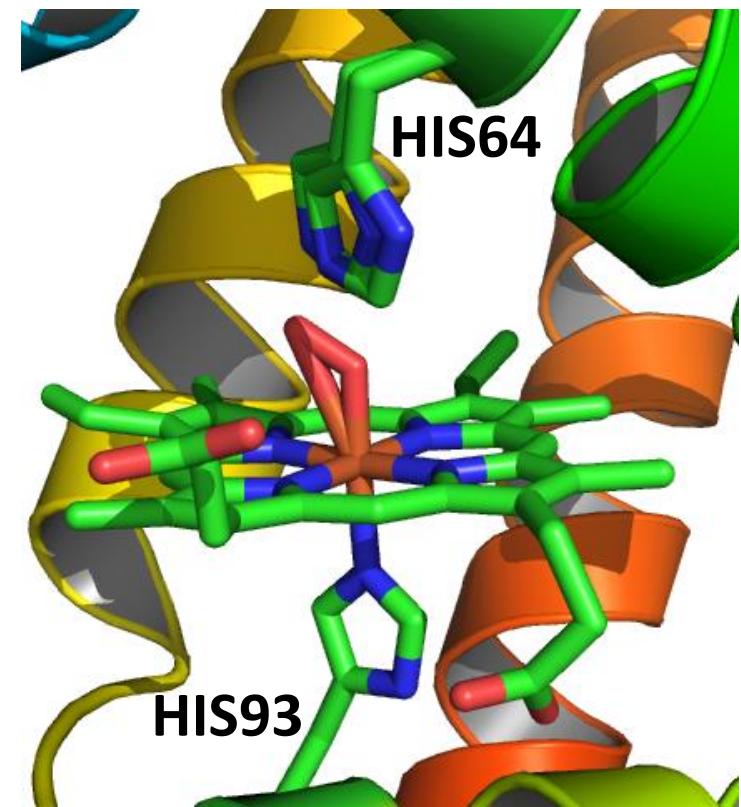
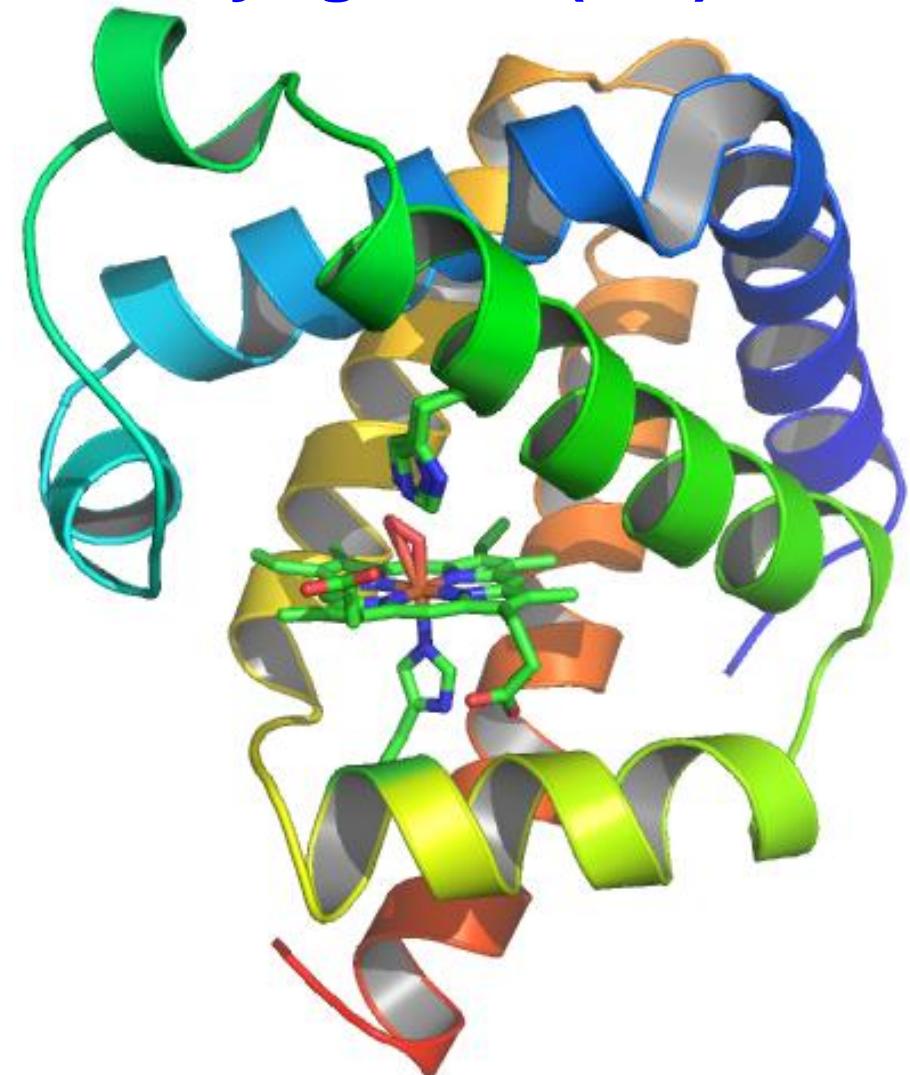
μ-η<sup>1</sup>:η<sup>2</sup>

end-on/side-on  
bridging



# Transport & Storage of O<sub>2</sub> by Heme-containing Proteins:

## 1. Myoglobin (Mb): Storage of O<sub>2</sub>



**One unit only**

**The O<sub>2</sub> binding site close to the protein surface**

# Database: Structures of Biological Macromolecules

- The Protein Data Bank (PDB, <http://www.rcsb.org>): Archive for 3D structures (187,170 deposited structure, 22 Feb 2022) of biological macromolecules
- Established in 1971 at Brookhaven National Lab

TAXONOMY	REFINEMENT RESOLUTION	POLYMER ENTITY TYPE
<input type="checkbox"/> Eukaryota (102060)	<input type="checkbox"/> < 0.5 (2)	<input type="checkbox"/> Protein (183212)
<input type="checkbox"/> Bacteria (64614)	<input type="checkbox"/> 0.5 - 1.0 (863)	<input type="checkbox"/> DNA (8819)
<input type="checkbox"/> Riboviria (10567)	<input type="checkbox"/> 1.0 - 1.5 (15707)	<input type="checkbox"/> RNA (5882)
<input type="checkbox"/> other sequences (6460)	<input type="checkbox"/> 1.5 - 2.0 (55656)	<input type="checkbox"/> NA-hybrid (222)
<input type="checkbox"/> Archaea (5558)	<input type="checkbox"/> 2.0 - 2.5 (50725)	<input type="checkbox"/> Other (5)
<input type="checkbox"/> Duplodnaviria (2702)	<input type="checkbox"/> 2.5 - 3.0 (29223)	
<input type="checkbox"/> Varidnaviria (611)	<input type="checkbox"/> 3.0 - 3.5 (12279)	
<input type="checkbox"/> Monodnaviria (509)	<input type="checkbox"/> 3.5 - 4.0 (4554)	
<input type="checkbox"/> unclassified sequences (378)	<input type="checkbox"/> 4.0 - 4.5 (1748)	
<input type="checkbox"/> Naldaviricetes (48)	<input type="checkbox"/> > 4.5 (2816)	
<a href="#">More...</a>		
REFINEMENT RESOLUTION	EXPERIMENTAL METHOD	ENZYME CLASSIFICATION
	<input type="checkbox"/> X-RAY DIFFRACTION (163234)	<input type="checkbox"/> Hydrolases (40635)
	<input type="checkbox"/> SOLUTION NMR (13545)	<input type="checkbox"/> Transferases (35013)
	<input type="checkbox"/> ELECTRON MICROSCOPY (9927)	<input type="checkbox"/> Oxidoreductases (16850)
	<input type="checkbox"/> NEUTRON DIFFRACTION (195)	<input type="checkbox"/> Lyases (8104)
	<input type="checkbox"/> ELECTRON CRYSTALLOGRAPHY (174)	<input type="checkbox"/> Isomerase (4040)
	<input type="checkbox"/> SOLID-STATE NMR (144)	<input type="checkbox"/> Ligases (3402)
	<input type="checkbox"/> SOLUTION SCATTERING (73)	<input type="checkbox"/> Translocases (1335)
	<input type="checkbox"/> FIBER DIFFRACTION (39)	
	<input type="checkbox"/> POWDER DIFFRACTION (20)	
	<input type="checkbox"/> EPR (8)	

# Crystal Structure of Sperm Whale Oxy-Myoglobin

- PDB ID: **1A6M**
- Use a text editor to open 1A6M.pdb file:

HEADER OXYGEN TRANSPORT 26-FEB-98 1A6M

TITLE OXY-MYOGLOBIN, ATOMIC RESOLUTION

COMPND 2 MOLECULE: MYOGLOBIN;

COMPND 3 CHAIN: A

SOURCE 3 ORGANISM\_COMMON: SPERM WHALE;

KEYWDS HEME PROTEIN, MODEL COMPOUNDS, OXYGEN STORAGE,

LIGAND

KEYWDS 2 BINDING GEOMETRY, CONFORMATIONAL SUBSTATES,  
OXYGEN TRANSPORT

EXPDTA X-RAY DIFFRACTION

AUTHOR

J.VOJTECHOVSKY,K.CHU,J.BERENDZEN,R.M.SWEET,I.SCHLICHTING

REMARK 2 RESOLUTION. 1.00 ANGSTROMS.

REMARK 200 TEMPERATURE (KELVIN) : 100

REMARK 200 PH : 7.0

SEQRES 1 A 151 VAL LEU SER GLU GLY GLU TRP GLN LEU VAL LEU HIS VAL  
SEQRES 2 A 151 TRP ALA LYS VAL GLU ALA ASP VAL ALA GLY HIS GLY GLN  
SEQRES 3 A 151 ASP ILE LEU ILE ARG LEU PHE LYS SER HIS PRO GLU THR  
SEQRES 4 A 151 LEU GLU LYS PHE ASP ARG PHE LYS HIS LEU LYS THR GLU  
SEQRES 5 A 151 ALA GLU MET LYS ALA SER GLU ASP LEU LYS LYS HIS GLY  
SEQRES 6 A 151 VAL THR VAL LEU THR ALA LEU GLY ALA ILE LEU LYS LYS  
SEQRES 7 A 151 LYS GLY HIS HIS GLU ALA GLU LEU LYS PRO LEU ALA GLN  
SEQRES 8 A 151 SER HIS ALA THR LYS HIS LYS ILE PRO ILE LYS TYR LEU  
SEQRES 9 A 151 GLU PHE ILE SER GLU ALA ILE ILE HIS VAL LEU HIS SER  
SEQRES 10 A 151 ARG HIS PRO GLY ASP PHE GLY ALA ASP ALA GLN GLY ALA  
SEQRES 11 A 151 MET ASN LYS ALA LEU GLU LEU PHE ARG LYS ASP ILE ALA  
SEQRES 12 A 151 ALA LYS TYR LYS GLU LEU GLY TYR

HETNAM SO4 SULFATE ION

HETNAM HEM PROTOPORPHYRIN IX CONTAINING FE

HETNAM OXY OXYGEN MOLECULE

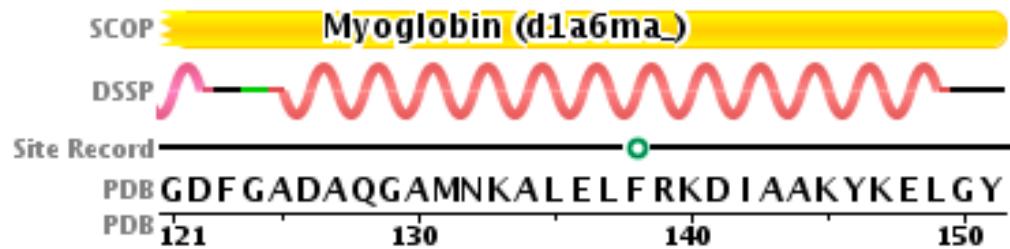
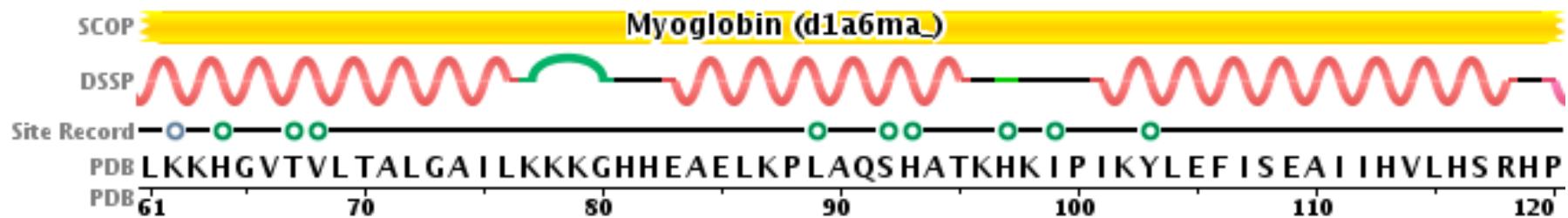
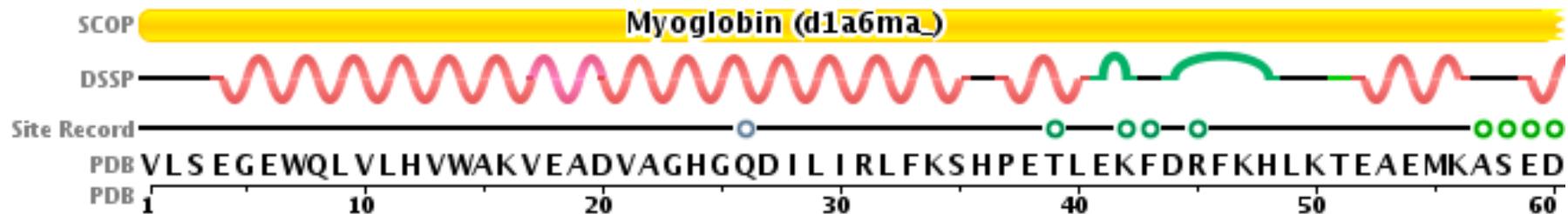
HETSYN HEM HEME

FORMUL 2 SO4 2(O4 S 2-)

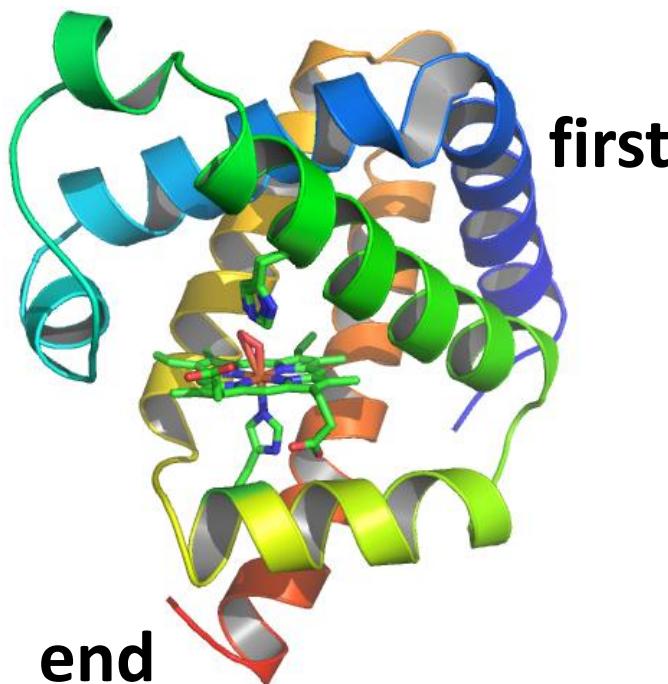
FORMUL 4 HEM C34 H32 FE N4 O4

FORMUL 5 OXY O2

FORMUL 6 HOH \*186(H2 O)



151 residues



#### Site Record Legend

- BINDING SITE FOR RESIDUE SO4 A 156 (SOFTWARE)
- BINDING SITE FOR RESIDUE HEM A 154 (SOFTWARE)
- BINDING SITE FOR RESIDUE SO4 A 155 (SOFTWARE)

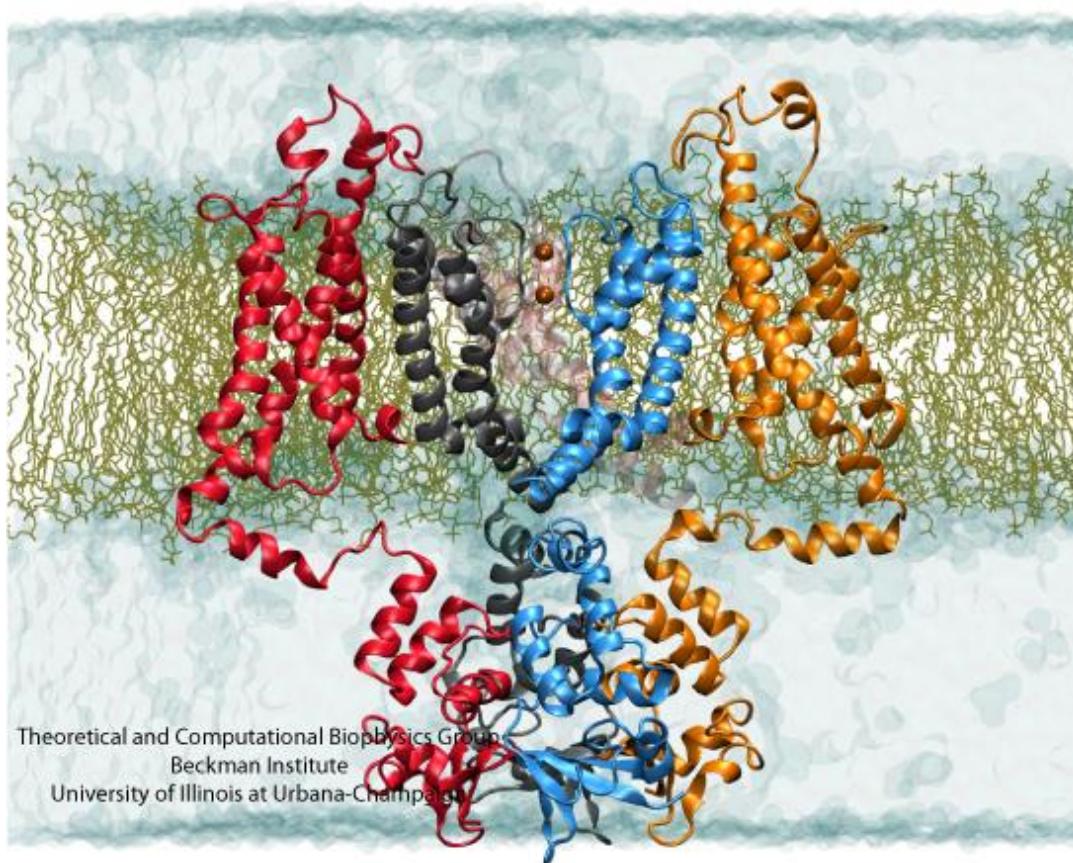
#### DSSP Legend

- T: turn
- empty: no secondary structure assigned
- G: 3/10-helix
- S: bend
- H: alpha helix

# Tool: ‘See’ Crystal Structures of the Oxy-Mb

## One of the Most Popular Free Software to Visualize Biomolecules:

- PyMOL (originally developed by Warren Lyford DeLano)



PyMOL

# 76 The PyMOL Molecular Graphics System

File Edit Build Movie Display Setting Scene Mouse Wizard Plugin

Help

nal Product - Copyright (C) 2010 Schrodinger, LLC.  
Executable Build is restricted to full-time students  
while engaged in educational activities. All other  
use of a PyMOL Maintenance and/or Support Subscription.  
<http://www.pymol.org/funding.html> for more information and  
1.org when you are ready to purchase a Subscription.

ild integrates and extends Open-Source PyMOL 1.3.

Open...

Save Session

Save Session As...

Save Molecule...

Save Image As

Save Movie As

Log...

Resume...

Append...

Close Log

## 1. Load the PDB structure

Reset Zoom Orient Draw Ray  
Unpick Deselect Rock Get View  
|< < Stop Play > >| MClear  
Command Builder  
Rebuild Abort

e Only

all

A S H L C

HEADER OXYGEN TRANSPORT 26-FEB-98 1A6M  
TITLE OXY-MYOGLOBIN, ATOMIC RESOLUTION  
COMPND MOL\_ID: 1;  
COMPND 2 MOLECULE: MYOGLOBIN;  
COMPND 3 CHAIN: A  
ObjectMolecule: Read secondary structure assignments.  
ObjectMolecule: Read crystal symmetry information.  
Symmetry: Found 2 symmetry operators.  
CmdLoad: "E:/Comput Chem/1A6M.pdb" loaded as "1A6M".

PyMOL>

Reset Zoom Orient Draw Ray  
Unpick Deselect Rock Get View  
|< < Stop Play > >| MClear  
Command Builder  
Rebuild Abort

PyMOL Viewer

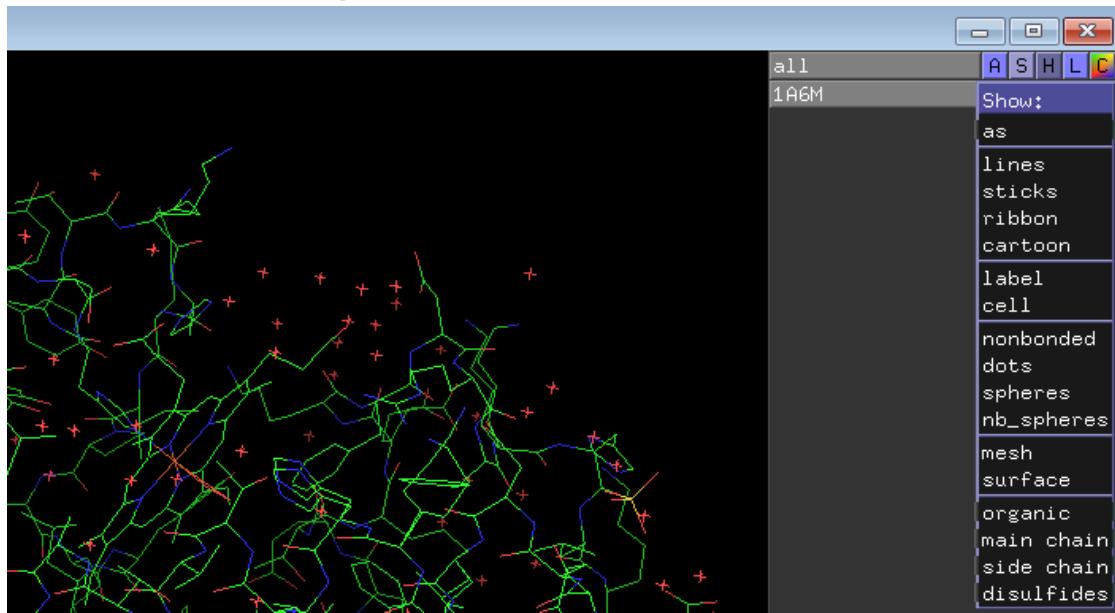
For Educational Use Only

all  
1A6M  
A S H L C



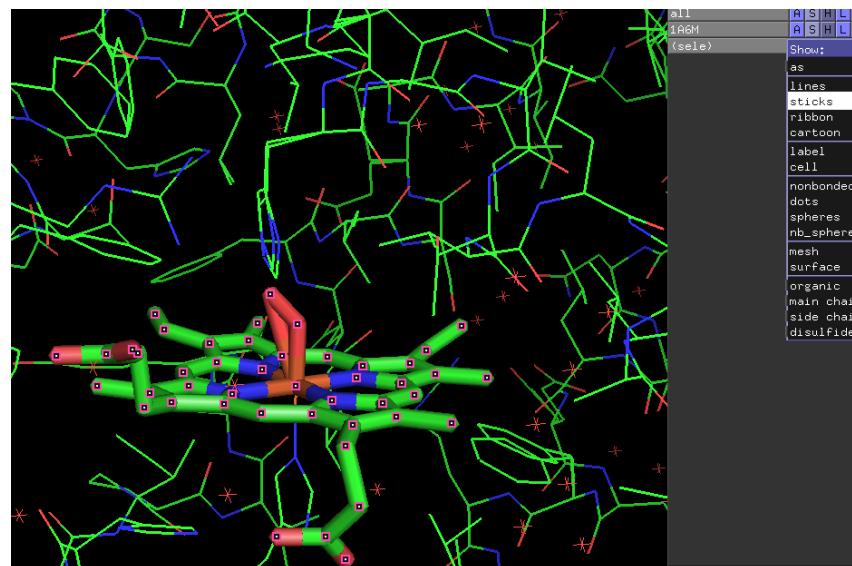
## 2. Change orientation of the structure: a. Rotate: left click; b. Scale: right click; c. Translation: middle click

### 3. Change representation of the structure:

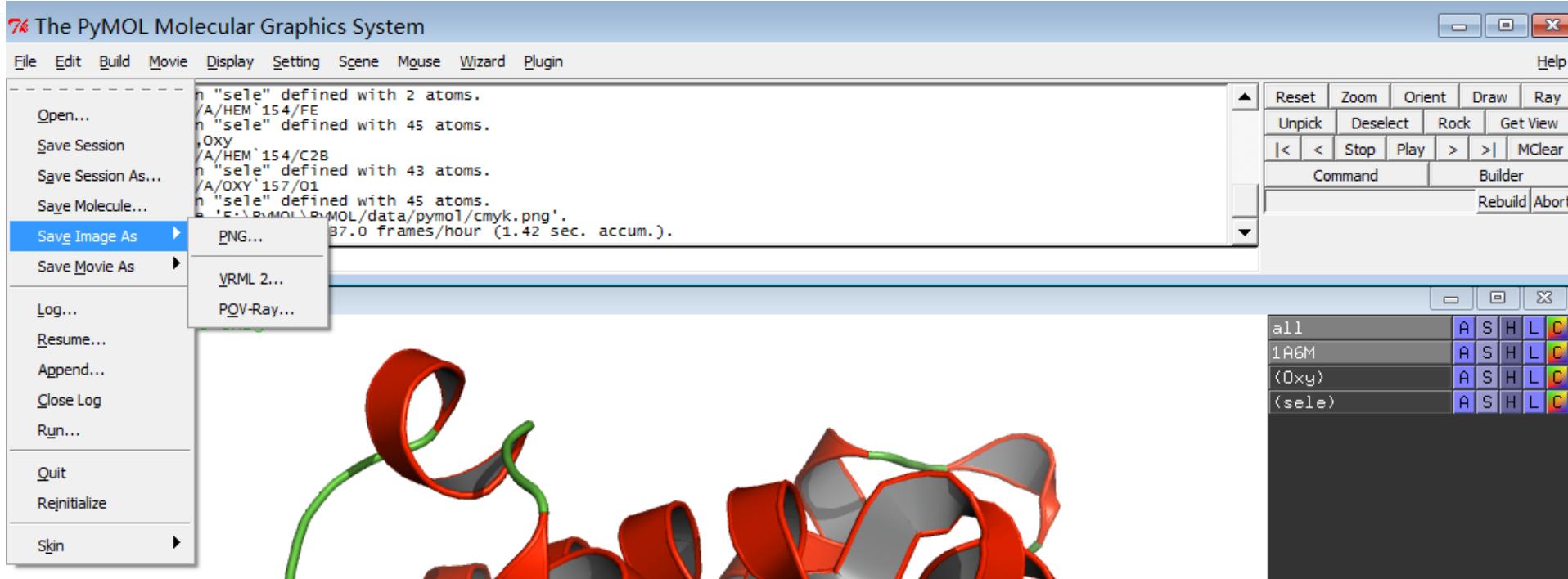


*By default, the structure is shown in lines form*

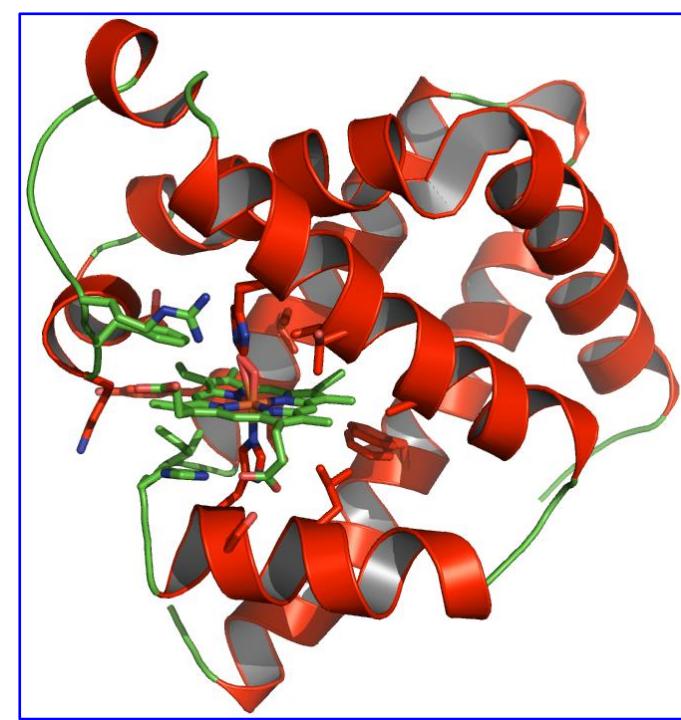
#### The active-site oxy-myoglobin



**Click “HEM” & “OXY” \*select “sticks” model**



**4. Make a beautiful image of structure (select “cartoon” model): Ray**



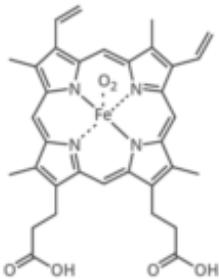
# O<sub>2</sub> Carriers: Hemoglobin, Hemerythrin & Hemocyanin



*Red*

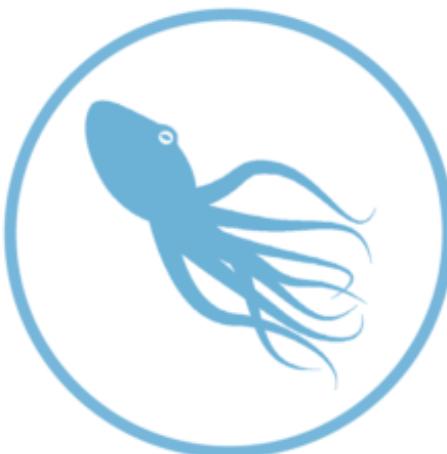
HUMANS AND THE MAJORITY OF OTHER VERTEBRATES

## HAEMOGLOBIN



HAEMOGLOBIN  
(oxygenated form)

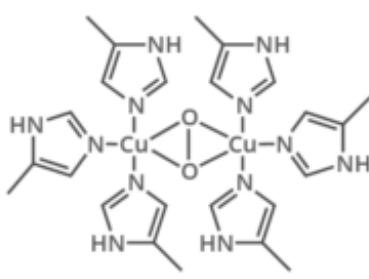
Haemoglobin is a protein found in blood, built up from subunits called 'haems'. These subunits contain iron, and their structure gives blood its red colour when oxygenated. Deoxygenated blood is a deep red colour - not blue!



*Blue*

SPIDERS, CRUSTACEANS, SOME MOLLUSCS, OCTOPUSES & SQUID

## HAEMOCYANIN



HAEMOCYANIN  
(oxygenated form)

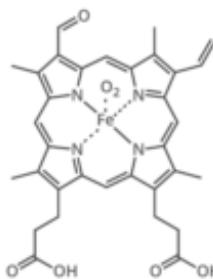
Unlike haemoglobin, which is bound to red blood cells, haemocyanin floats free in the blood. Haemocyanin contains copper instead of iron. When deoxygenated, the blood is colourless, but when oxygenated, it gives a blue colouration.



*Green*

SOME SEGMENTED WORMS, SOME LEECHES, & SOME MARINE WORMS

## CHLOROCRUORIN



CHLOROCRUORIN  
(oxygenated form)

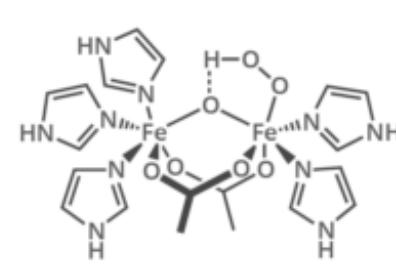
Chemically similar to haemoglobin; the blood of some species contains both haemoglobin & chlorocruorin. Light green when deoxygenated, it is green when oxygenated, although when more concentrated it appears light red.



*Violet*

MARINE WORMS INCLUDING PEANUT WORMS, PENIS WORMS & BRACHIOPODS

## HAEMORYTHRIN

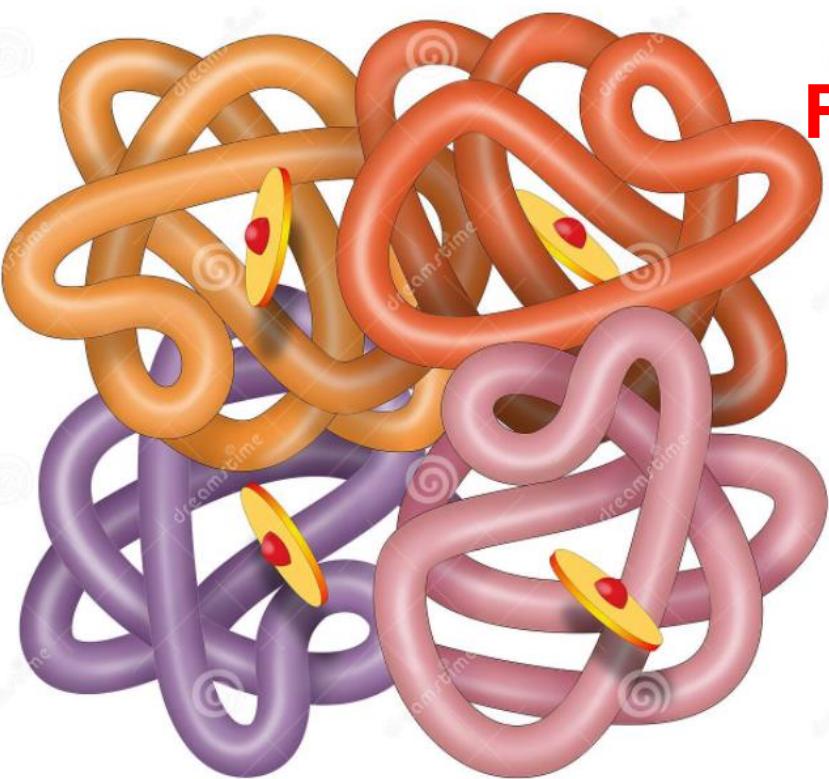
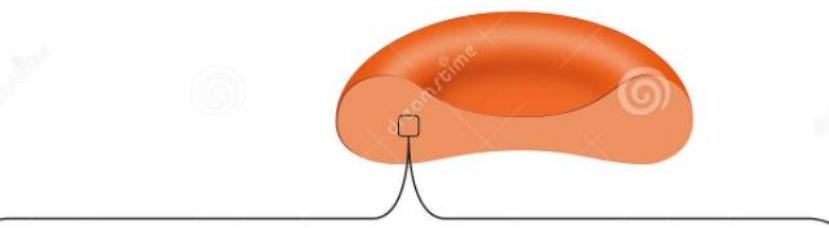


HAEMORYTHRIN  
(oxygenated form)

Haemerythrin is only 1/4 as efficient at oxygen transport when compared to haemoglobin. In the deoxygenated state, haemerythrin is colourless, but it imparts a violet-pink colour when oxygenated.

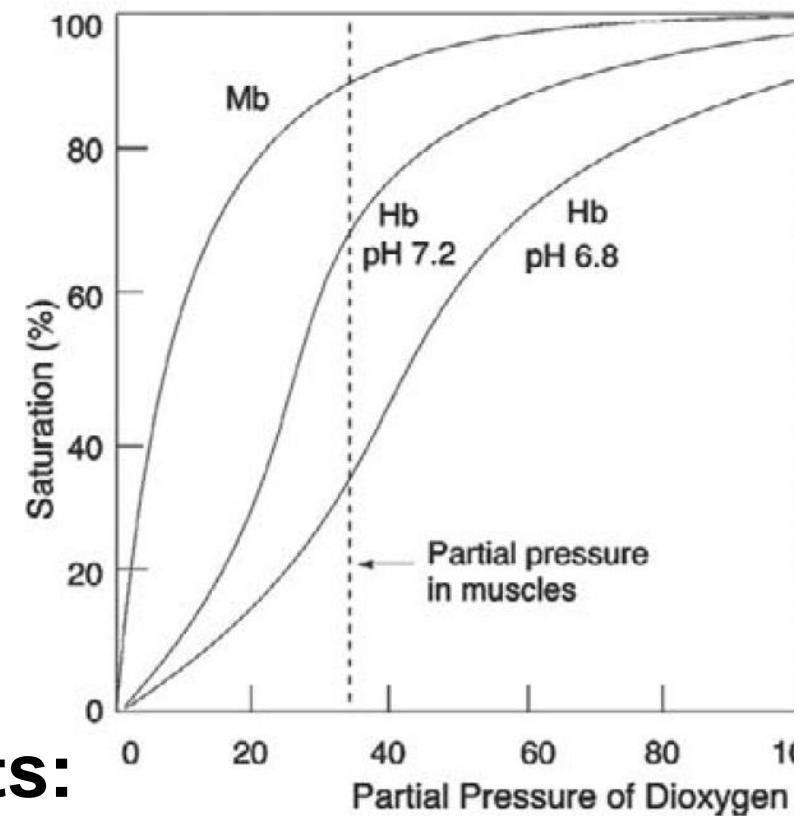
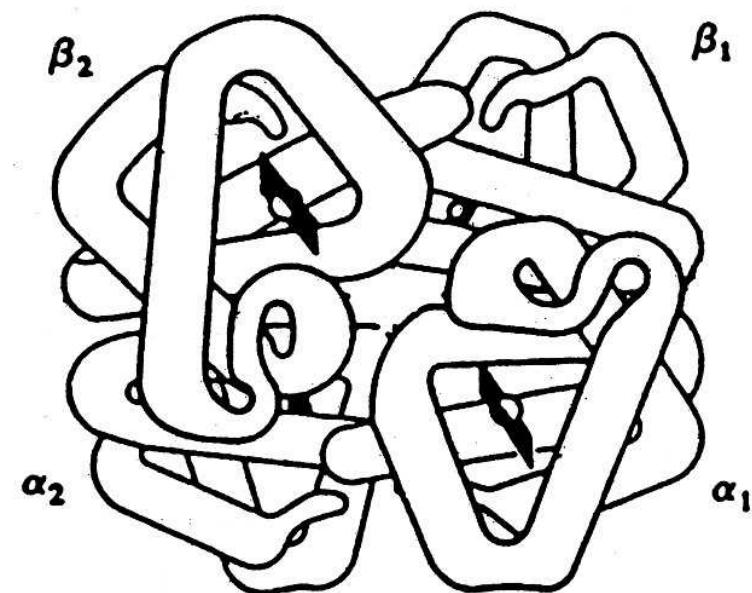
# Transport of $O_2$ by Heme-containing Proteins:

## 2. Hemoglobin (Hb): Transport of $O_2$ ; Cooperativity $O_2$ binding

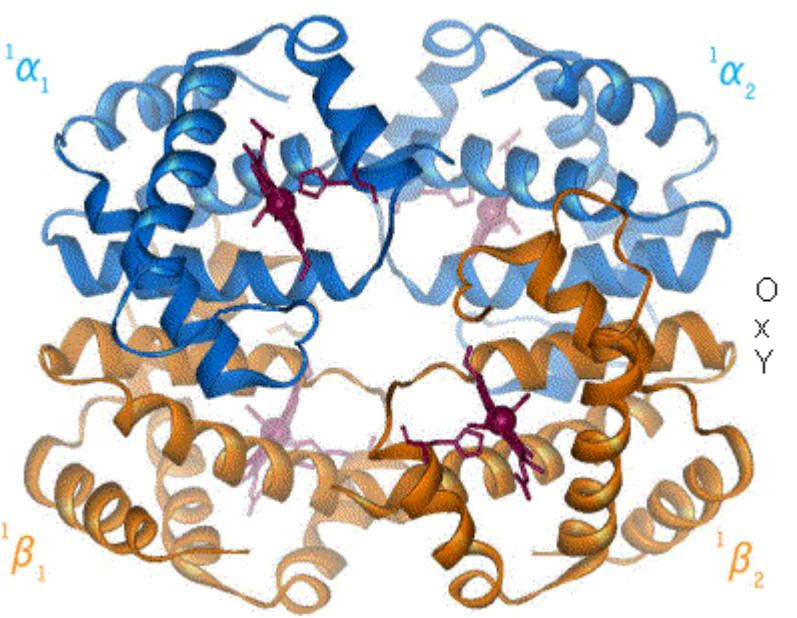
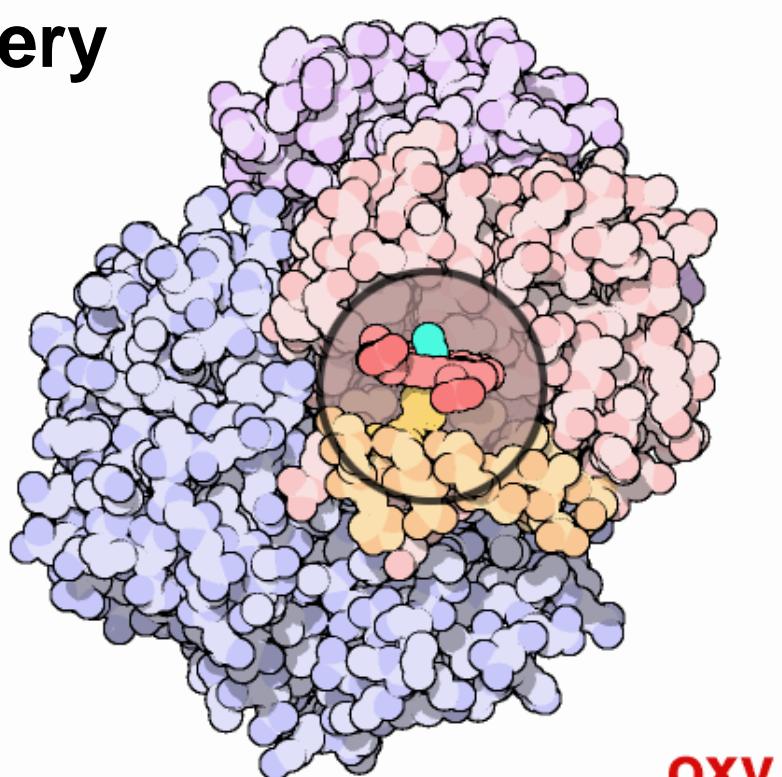
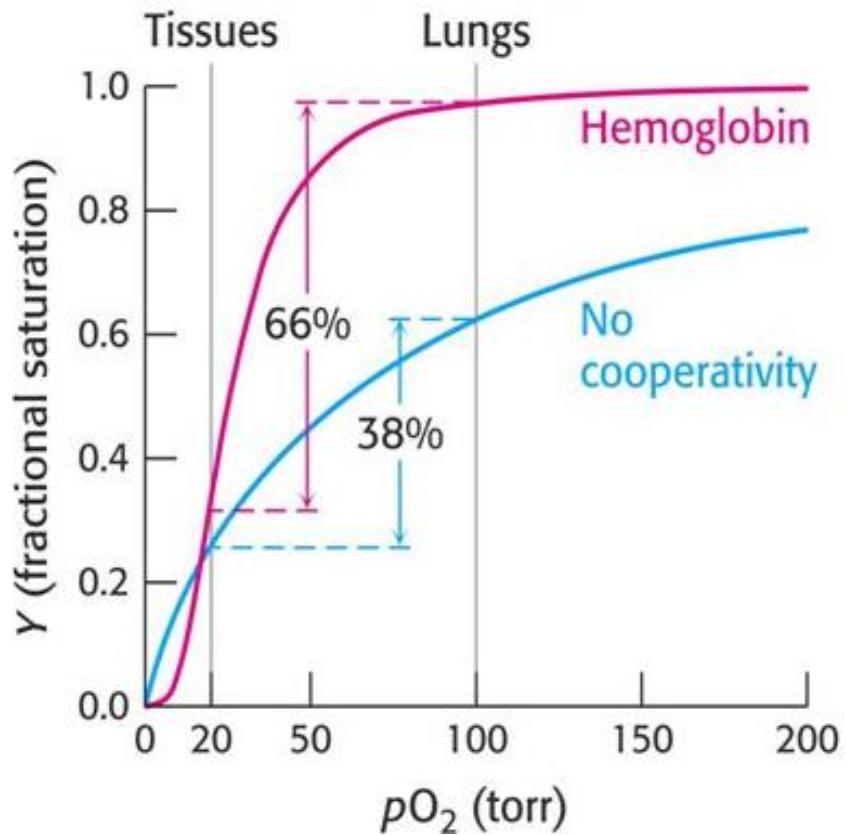


Four units:

$\alpha_2\beta_2$



# Cooperativity O<sub>2</sub> binding: Allostery

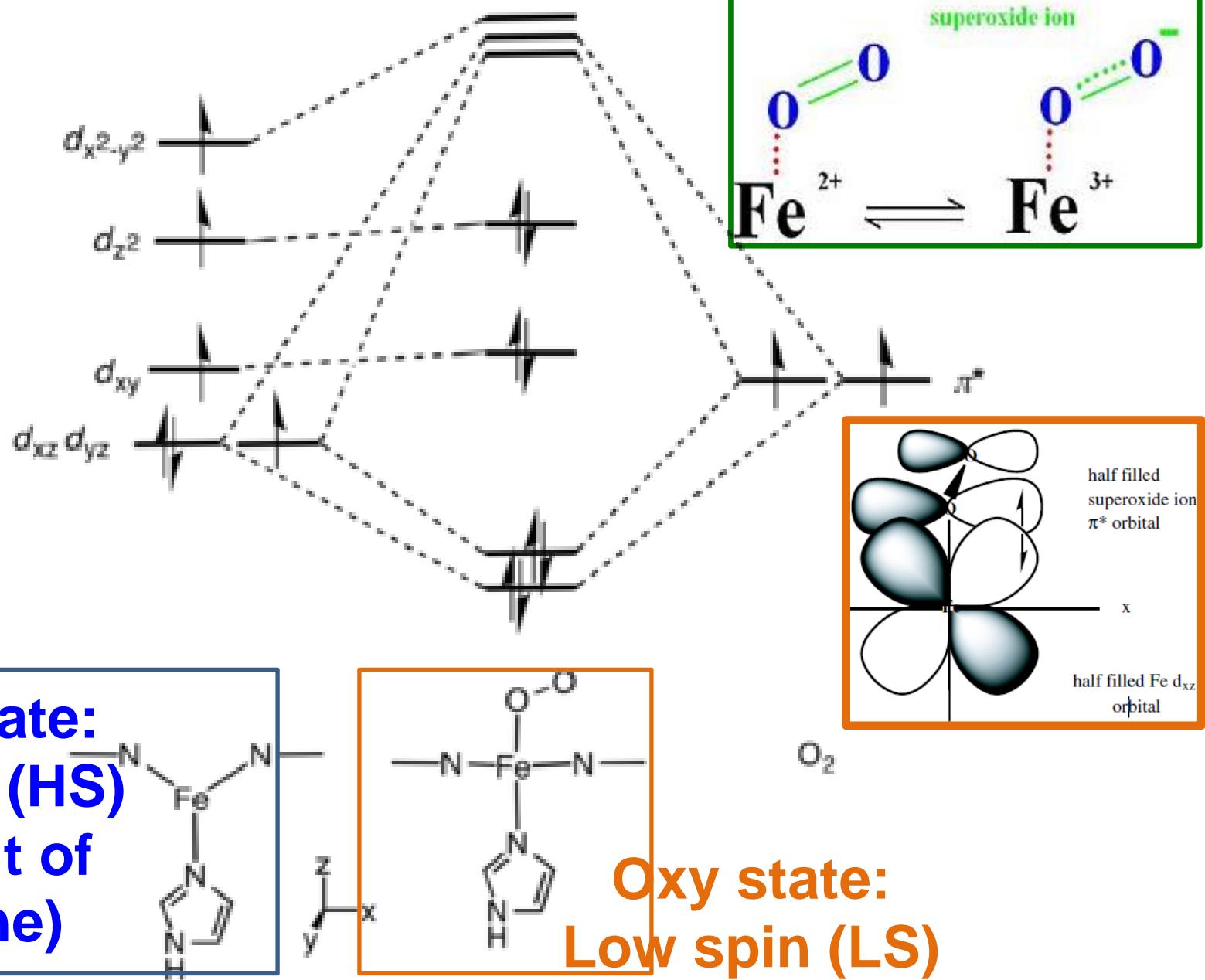


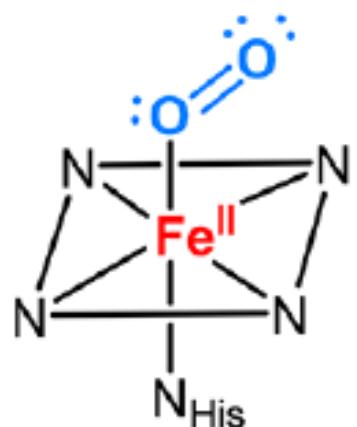
Stronger O<sub>2</sub> binding when more O<sub>2</sub> binds to hemes in a Hb.

**Deoxy-Hb (T state):** low binding

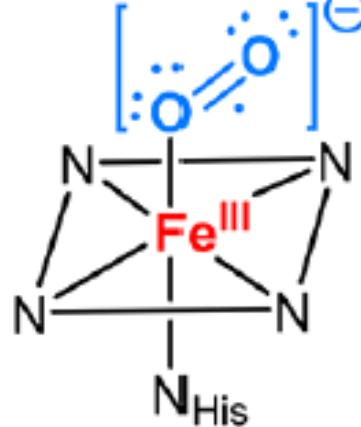
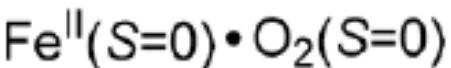
**Oxy-Hb (R state):** high binding

# Schematic key molecular orbitals of $\pi(\text{Fe-O}_2)$ Bonding

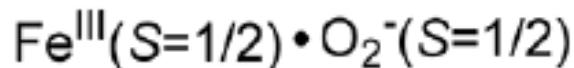




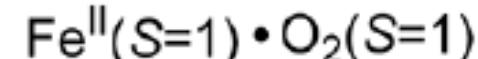
**Pauling**



**Weiss**

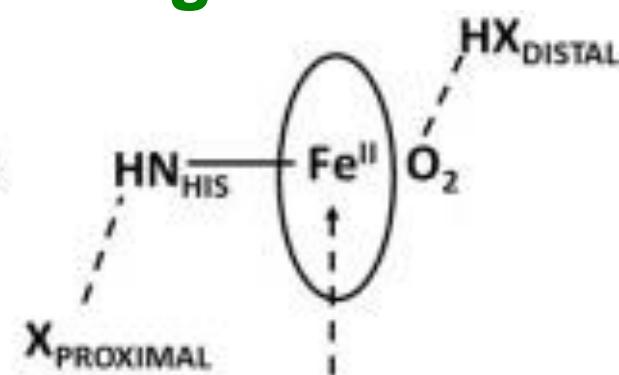
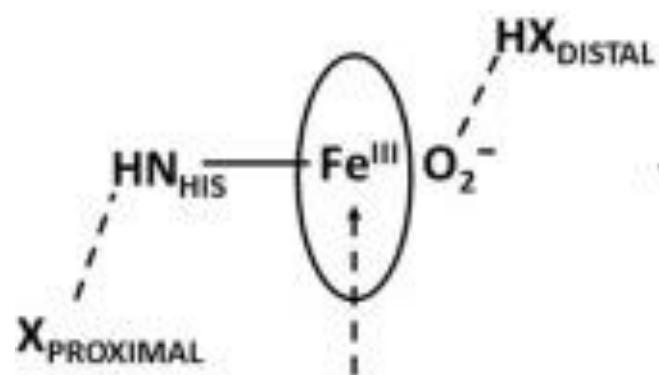


**McClure-Goddard**



**Weiss**

**Pauling & McClure**



**Back-donation to  $\pi^*(\text{O}_2)$ :** Stronger

**H-bond (distal):** Stronger

**O₂ affinity:** Higher

**O-O bond:** Longer

Weaker

Weaker

Lower

Shorter

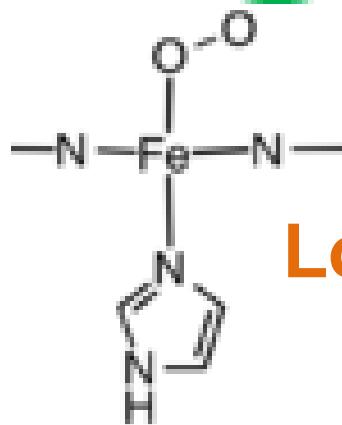
# Spin crossing for the O<sub>2</sub> coordination

HS

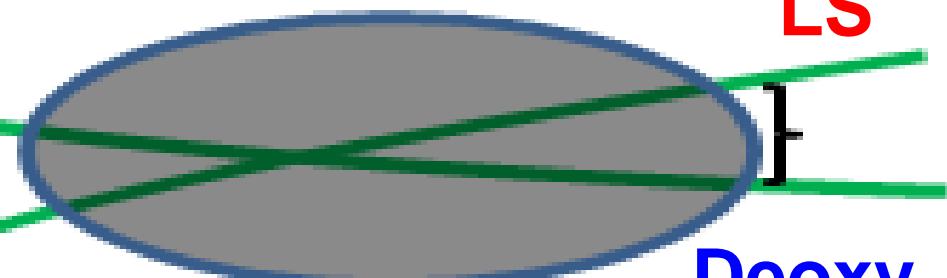
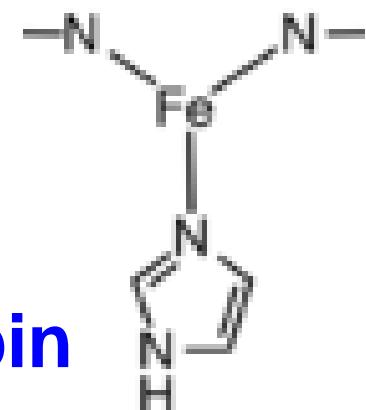
Kepp, Coord. Chem.  
Rev, 2017, ASAP

LS

Oxy state:  
LS



High spin  
(HS)



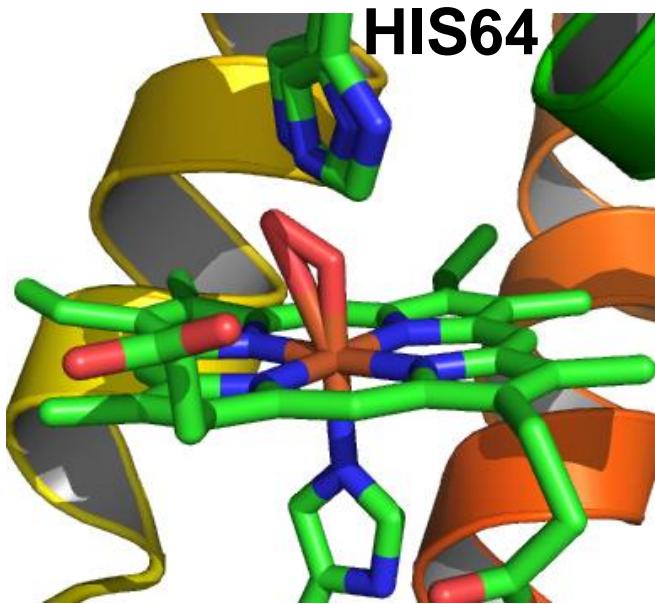
# **O<sub>2</sub>/CO Binding Discrimination (Fe(II))**

Stronger CO binding affinity to heme than O<sub>2</sub> without Mb and with Mb, but the Mb protein enhances the relative binding of O<sub>2</sub>:

In **free solution**, CO could bind to **heme** > 20,000 times stronger **than O<sub>2</sub>**.

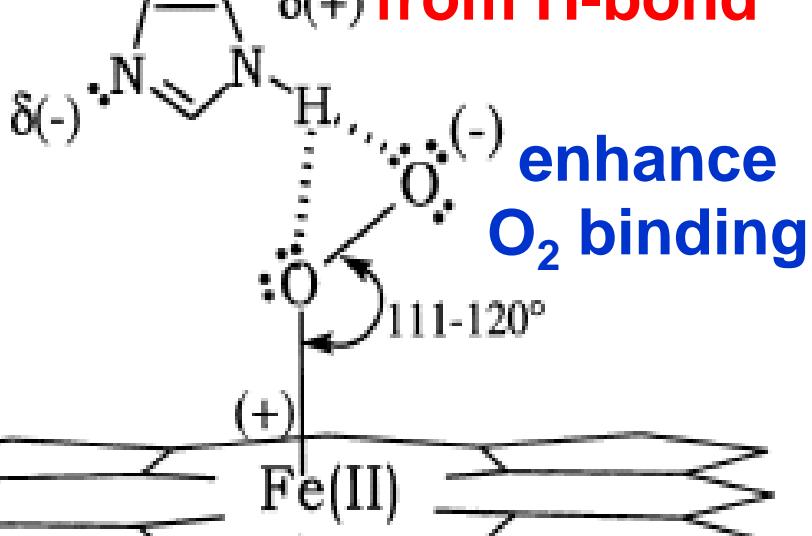
However, when the **heme** is bound to **Mb**, CO could binds ~200 times stronger **than O<sub>2</sub>**.

**How the proteins enhance the binding of O<sub>2</sub>?**



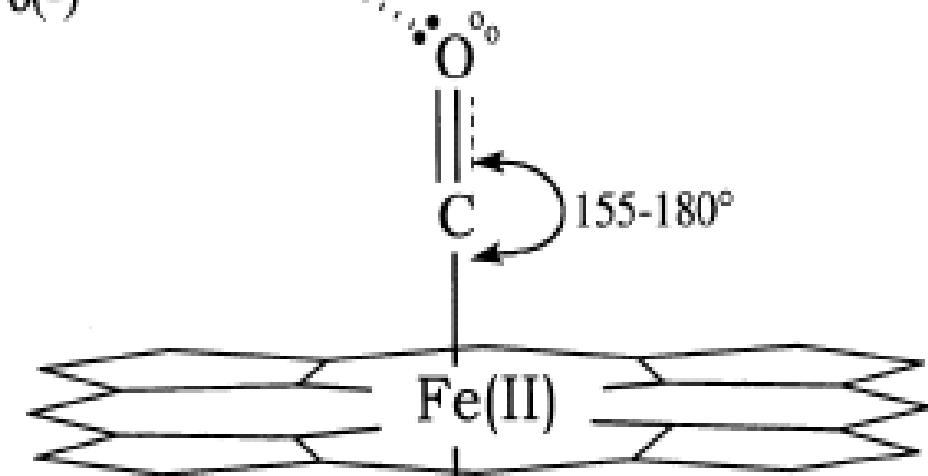
$\text{His}^{64}(\text{E7})$

**Stronger  
stabilization  
from H-bond**

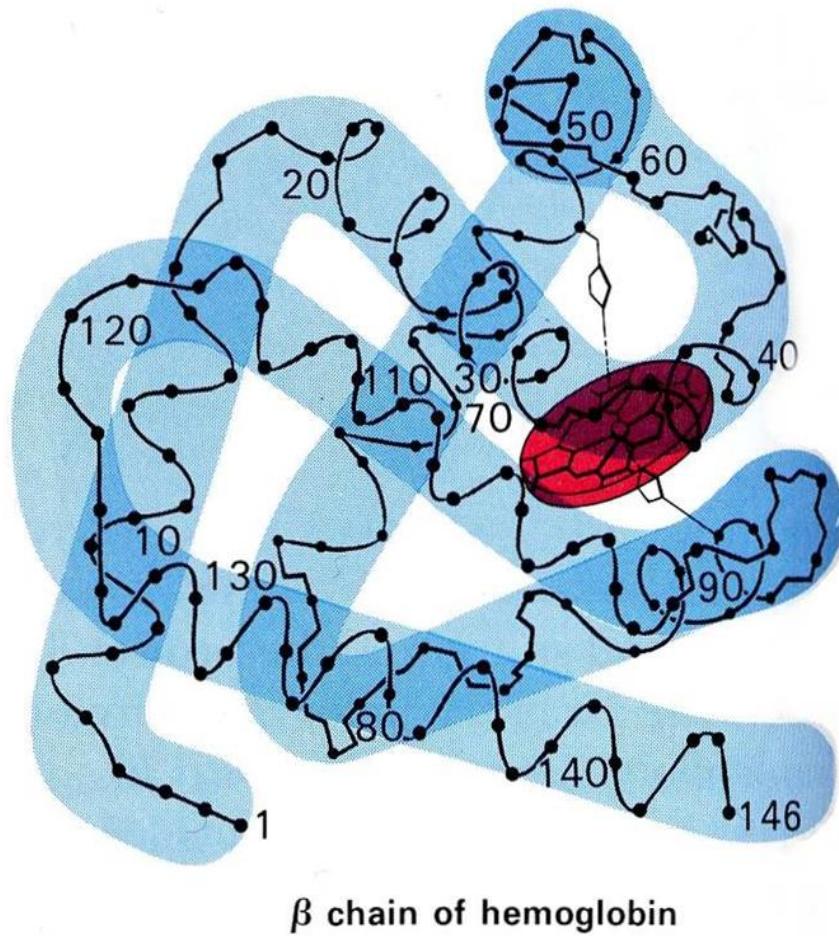
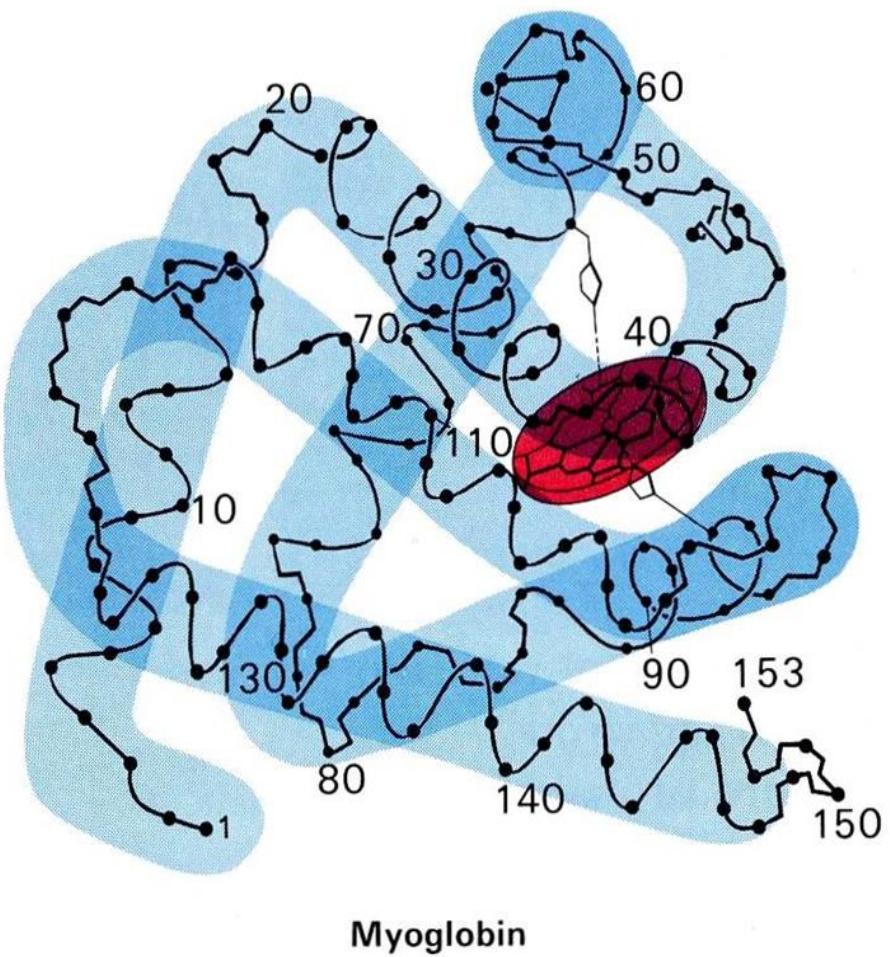


$\text{His}^{64}(\text{E7})$

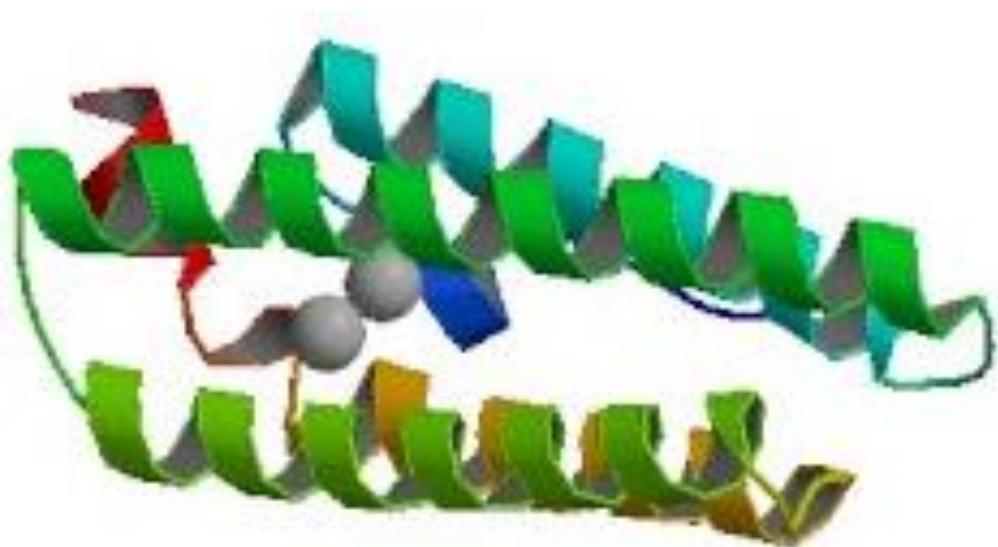
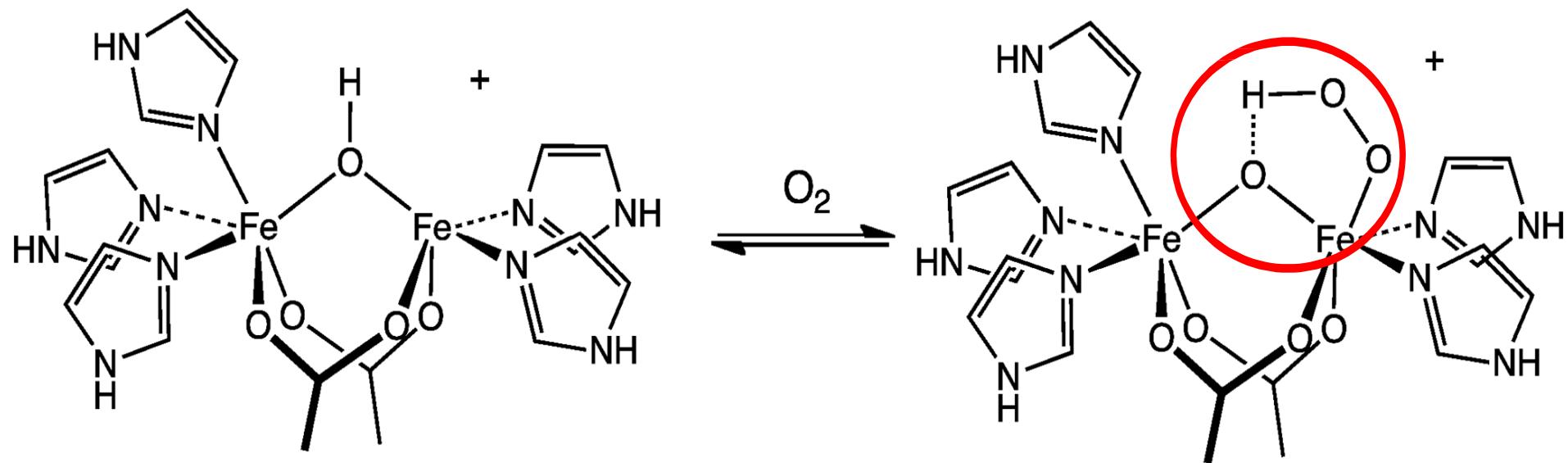
**Less stabilization  
(+ possibly steric  
repulsion)**



# Similar conformation of the O<sub>2</sub> binding site

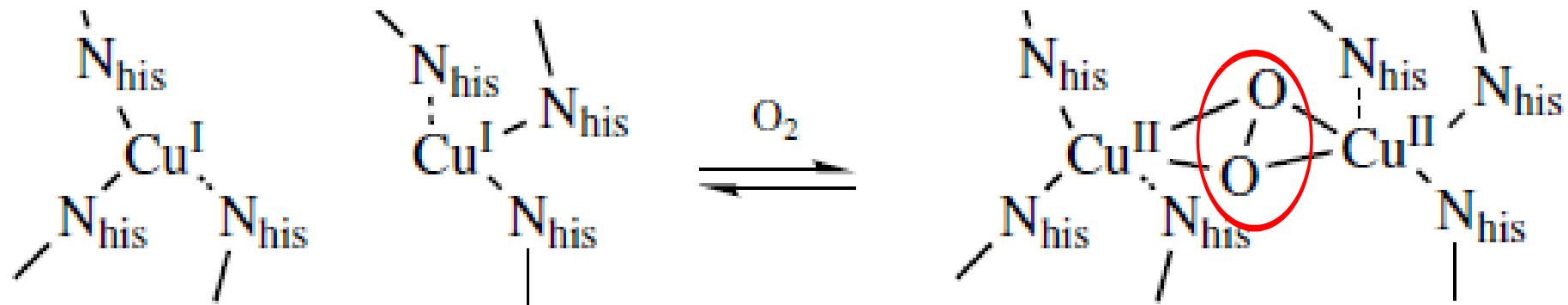


# $O_2$ Carrier by Non-Heme-containing Proteins: Hemerythrin (Hr)



No stabilization from hydrogen-bond with the distal His as in Mb/Hb, but H-bond from the **ligated OH**.

# $O_2$ Carrier by Cu-containing Proteins: Hemocyanin



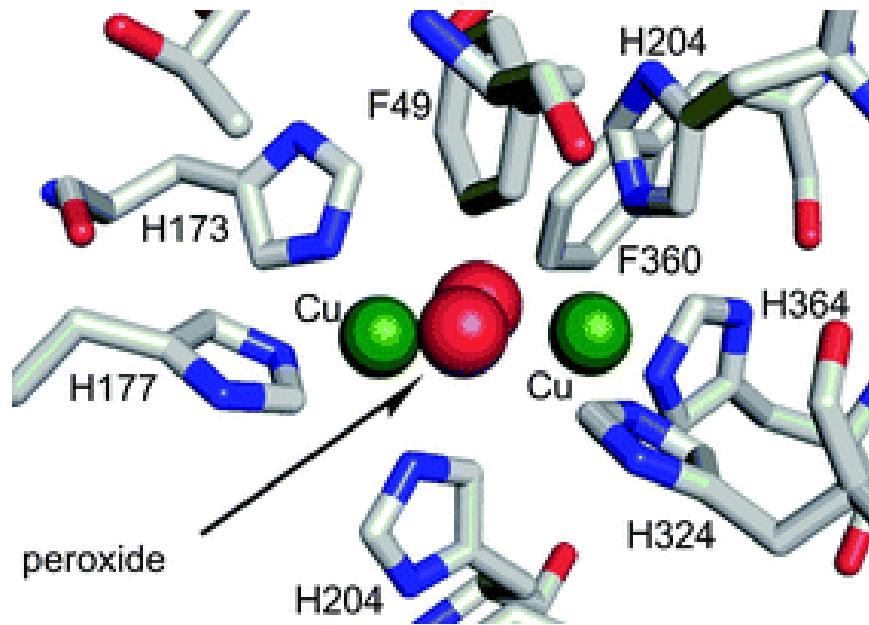
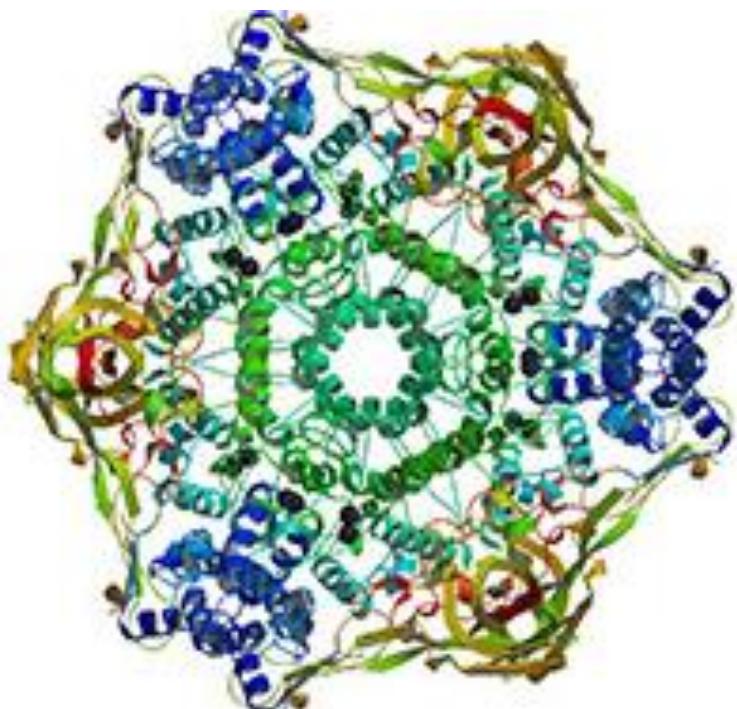
Deoxyhemocyanin colorless

$Cu \dots Cu = 4.6 \text{ \AA}$

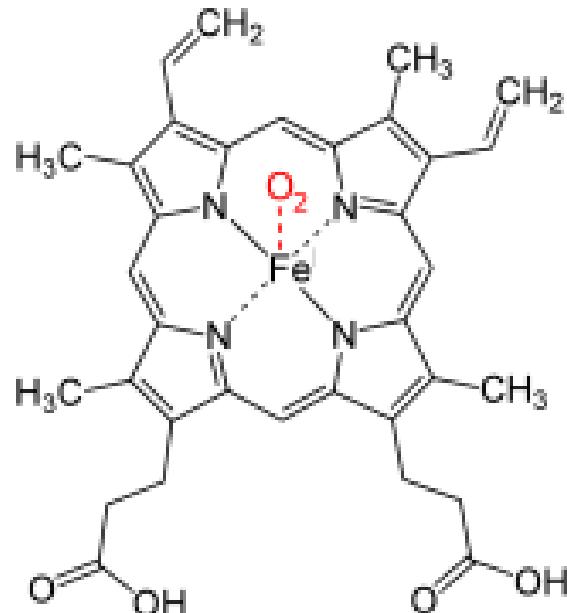
Oxyhemocyanin blue

$Cu \dots Cu = 3.6 \text{ \AA}$

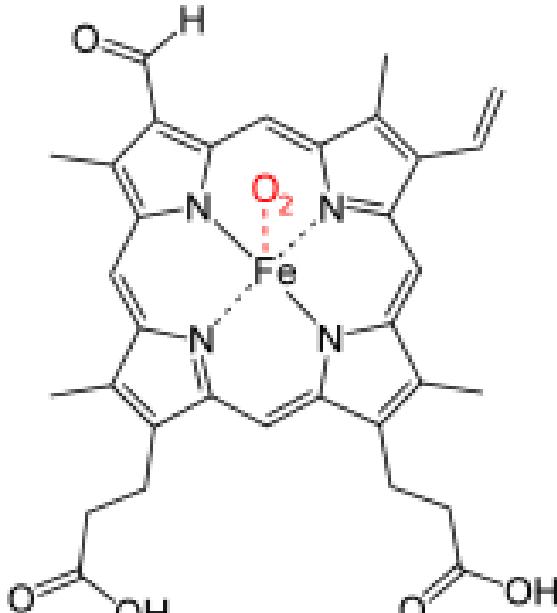
$\mu\text{-}\eta^2\text{-}\eta^2$  Coordination



# Hemoglobin, Hemerythrin & Hemocyanin

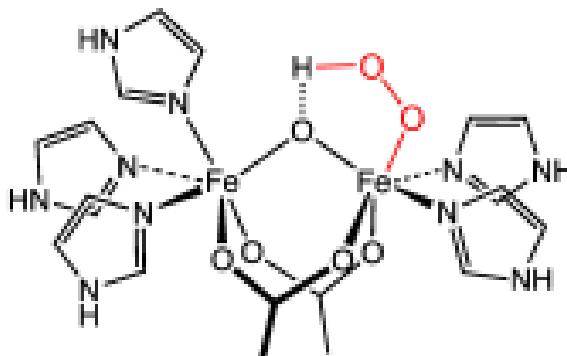


**Hemoglobin**

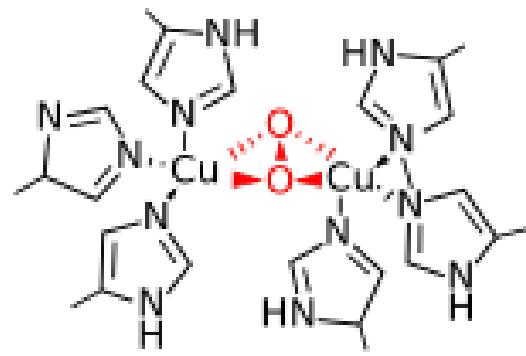


**Chlorocruorin**

*Nature uses  
**different ways** to  
bind/store  $O_2$*



**Hemerythrin**



**Hemocyanin**

# Transport & Storage of Metals

Average Relative Abundance of Selected Elements in the Earth's Crust,  
Sea Water, Mammalian Blood Plasma, and in Mammalian Cells or Tissue

Element	Crust (ppm)	Sea Water ( $\mu M$ )	Blood Plasma ( $\mu M$ )	Cell/Tissue <sup>a</sup> ( $\mu M$ )
Ca	$4 \times 10^4$	$1 \times 10^4$	$2 \times 10^3$	$1 \times 10^3$
Cd	0.2	$1 \times 10^{-3}$		
Co	25	$2 \times 10^{-5}$	$2.5 \times 10^{-5}$	
Cu	55	$4 \times 10^{-3}$	8–24	$\sim 68$
Fe	$5 \times 10^4$	$1 \times 10^{-3}$	22	0.001–10
K	$3 \times 10^4$	$1 \times 10^4$	$4 \times 10^3$	$1.5 \times 10^5$
Mg	$2 \times 10^4$	$5 \times 10^4$	500	$9 \times 10^3$
Mn	950	$5 \times 10^{-4}$	0.1	180
Mo	1.5	0.1		$5 \times 10^{-3}$
Na	$3 \times 10^4$	$5 \times 10^5$	$1 \times 10^5$	$1 \times 10^4$
Ni	75	$8 \times 10^{-3}$	0.04	2
V	135	0.03	0.07	0.5–30
W	1.5	$5 \times 10^{-3}$		
Zn	70	0.01	17	180

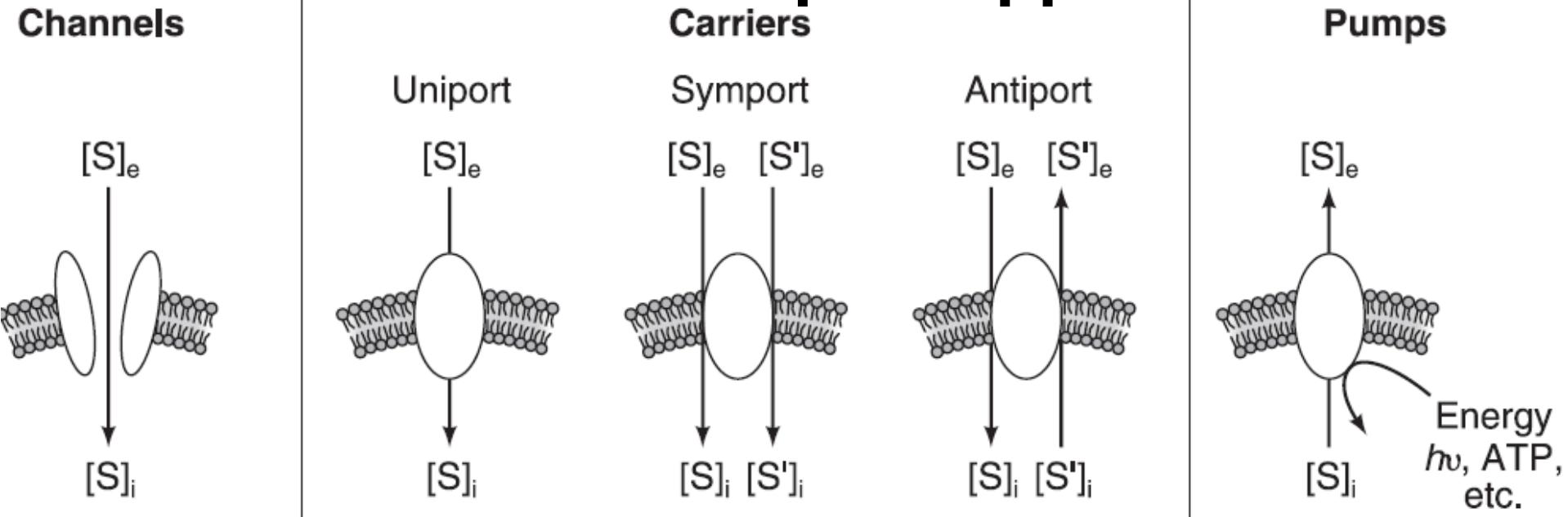
# Metal ion transport/uptake

**Sufficient amounts** of metal ions is important for biological functions, but **excess metals** (e.g. Fe, Cu) can be **toxic** (e.g. redox reactions to form reactive oxygen species (ROS), which damage DNA, lipids & proteins).

**Transporters** facilitate **selective movement** of ions across the barrier, responsible for the **proper distribution of metal ions** within whole organisms & individual cells.

Properties of metals can influence uptake/transport, e.g. redox (oxidation state), hydrolysis, solubility, chelation of the free ion, and ligand exchange rates.

# 3 Common Transport Approaches

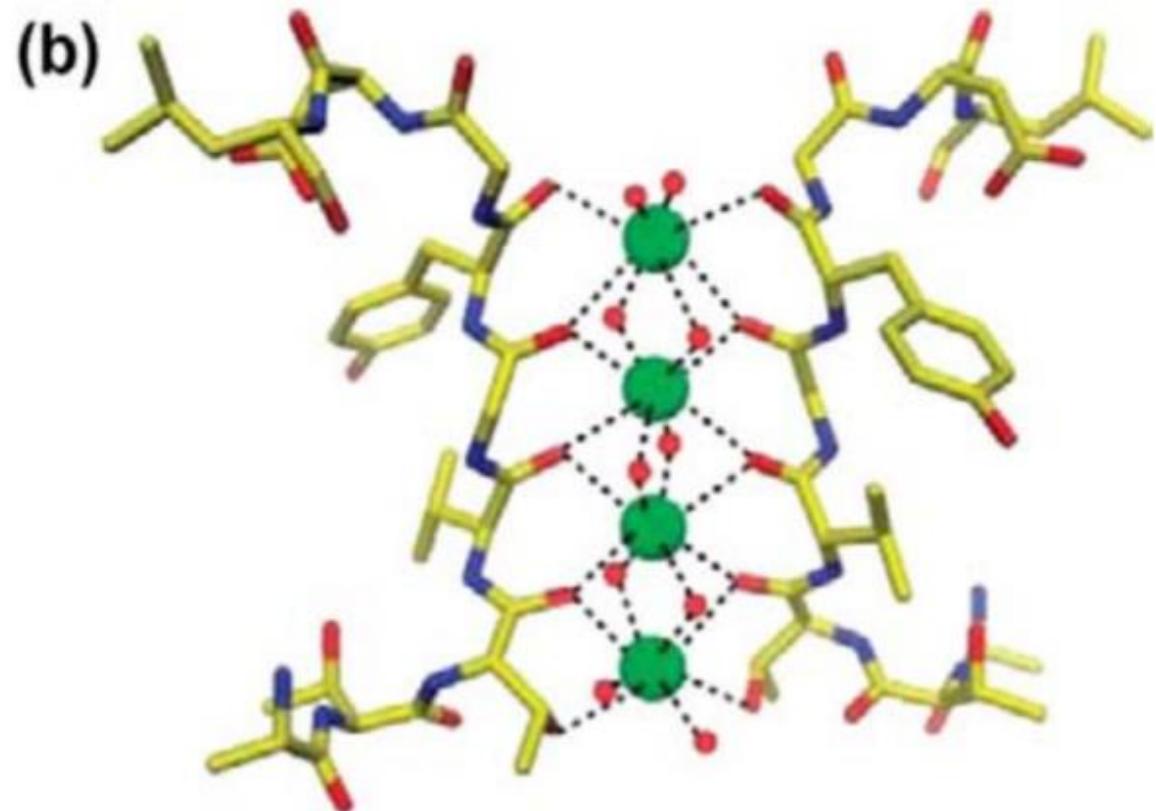
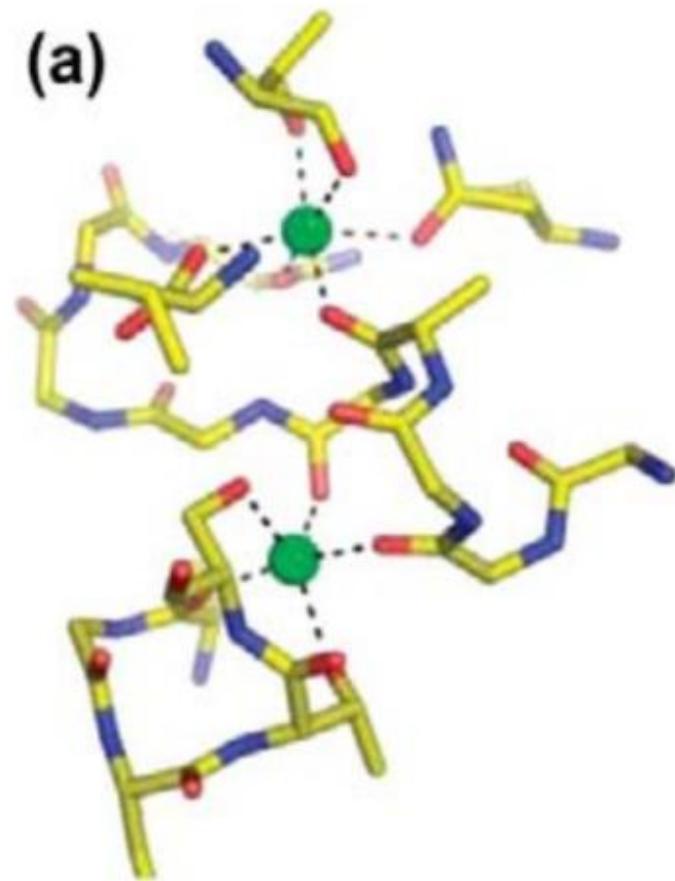


**Carriers:** bind their substrate(s), undergo a **conformational change**, & then *release* the substrate on the opposite side.

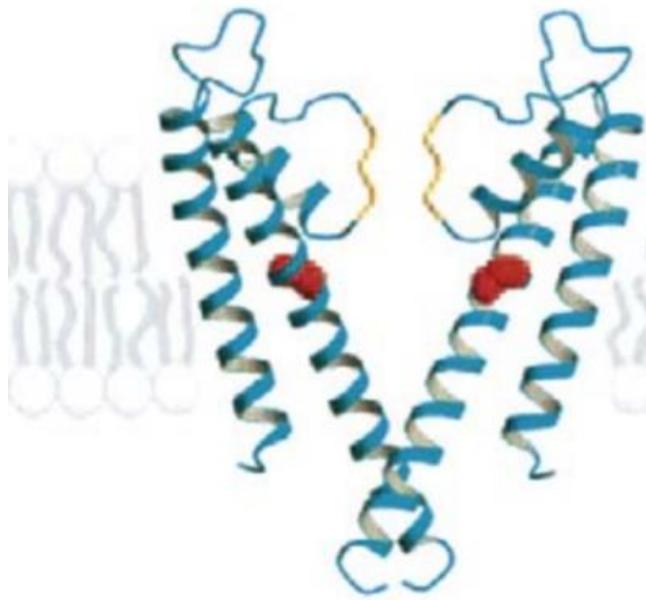
**Channels:** proteins form pores in the membrane and allow the movement of the substrate across the membrane **by diffusion**.

**Pumps:** **use energy** from hydrolysis of ATP or other energy sources (e.g., light) to provide the energy for transport (**active transport**).

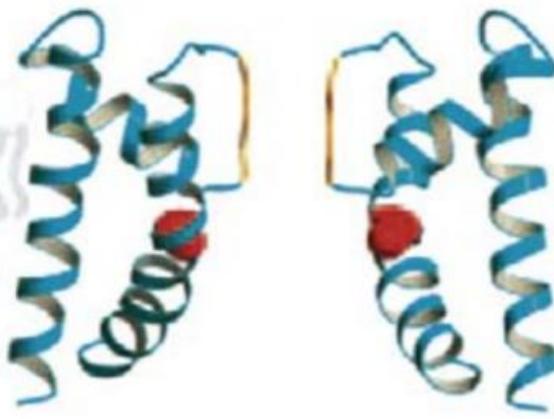
# Transport of Na & K



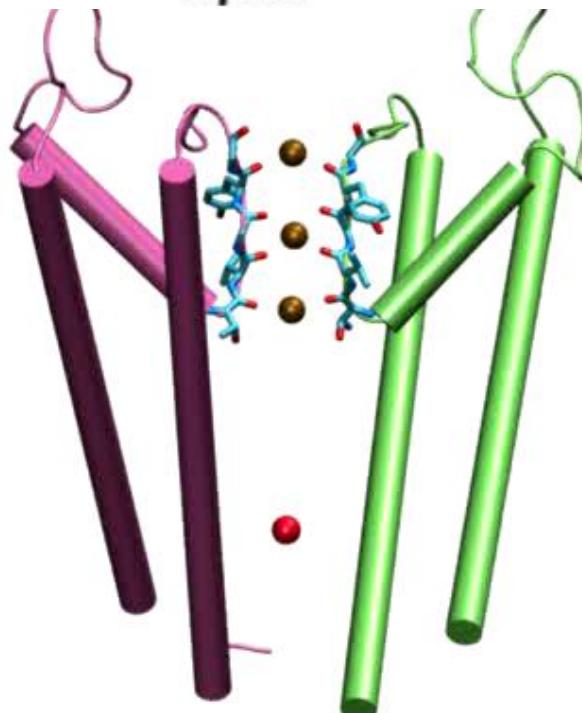
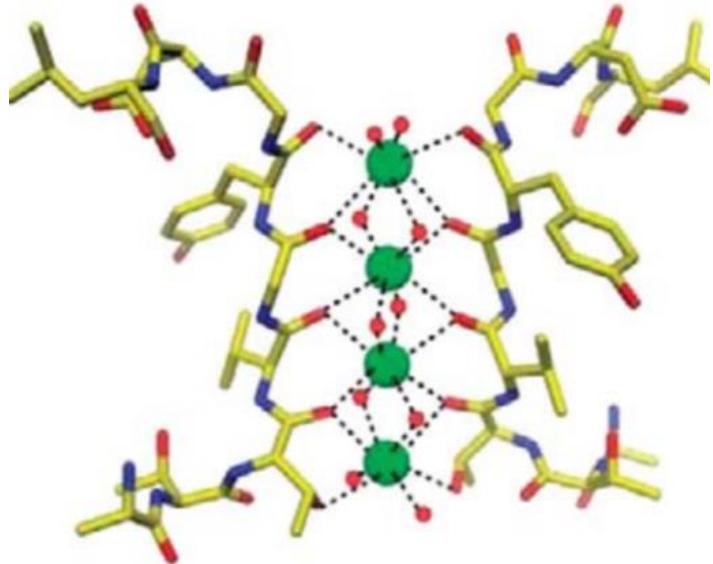
(a) Two **Na binding sites** in the LeuT Na-dependent pump. (b) Four **K binding sites** in the KcsA K channel.



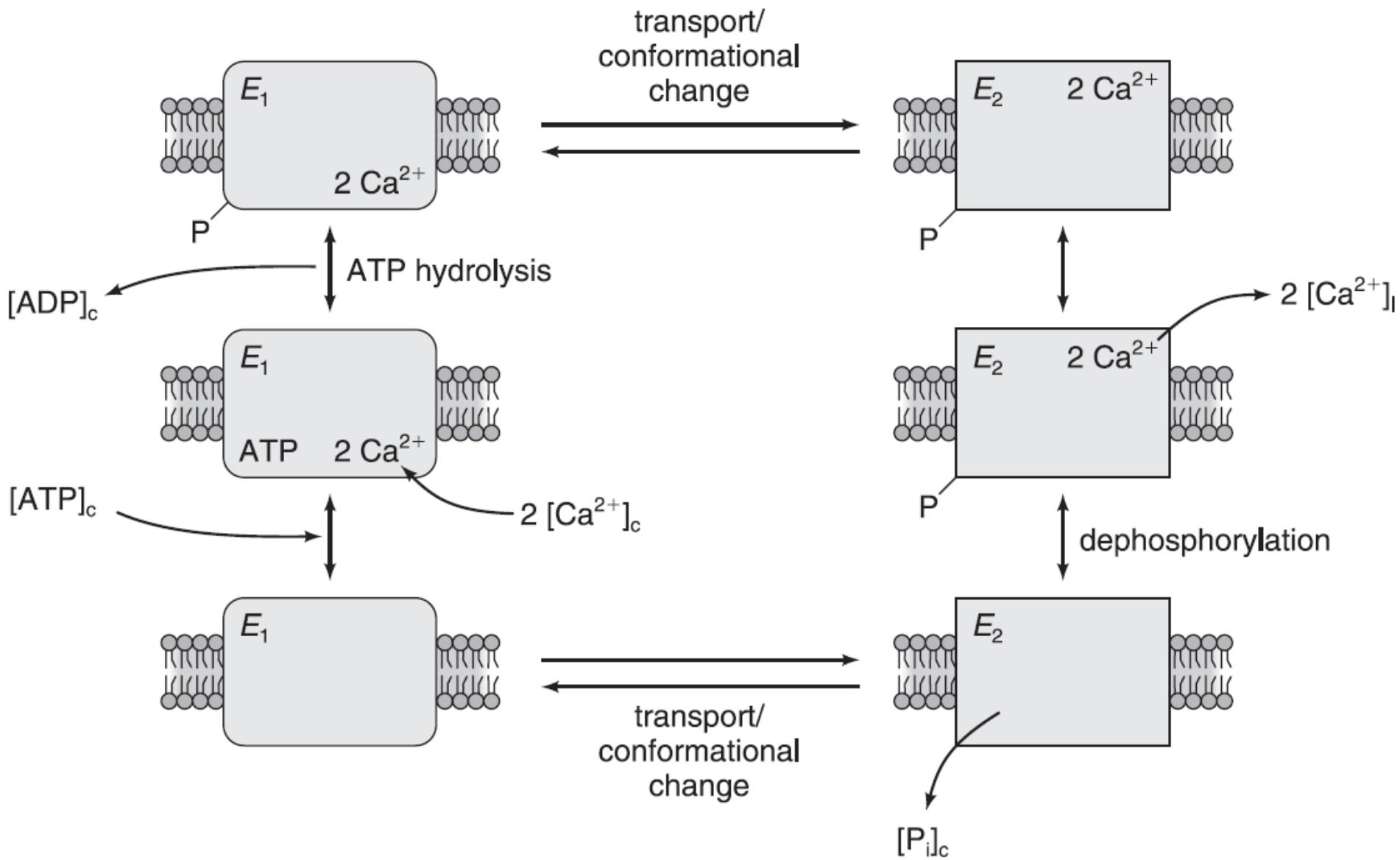
KcsA K<sup>+</sup> channel  
"closed"



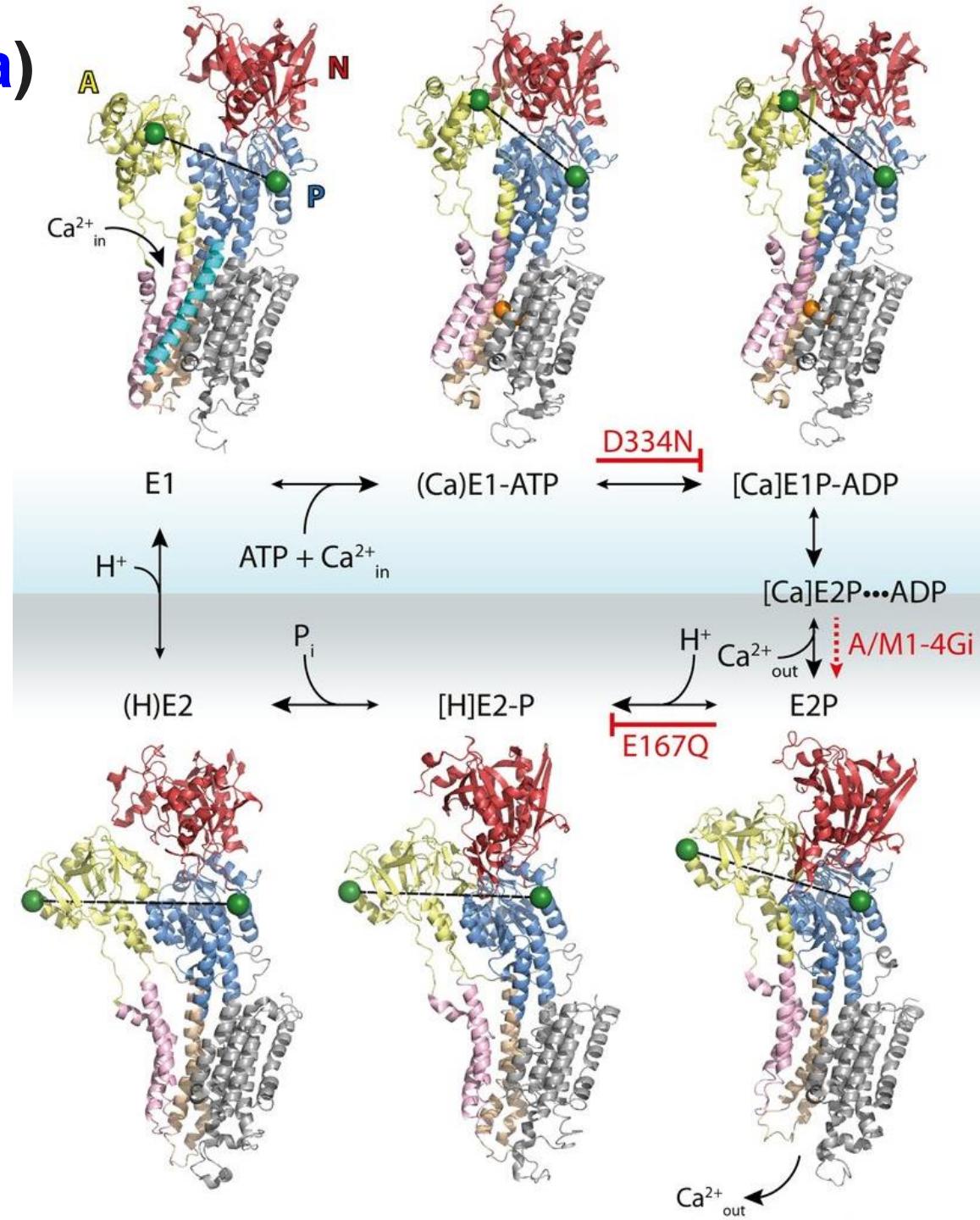
MthK K<sup>+</sup> channel  
"open"



# Ca-ATPase (Ca)



# Ca-ATPase (Ca)



# Storage/transport of Fe

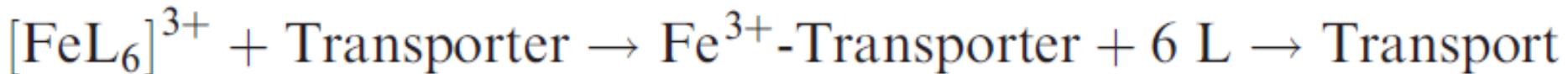


$$K_{\text{sp}} = [\text{Fe}^{3+}][\text{OH}^-]^3 \approx 10^{-38} \text{ M}$$

$$[\text{Fe}^{3+}] = 10^{-38} / [\text{OH}^-]^3$$

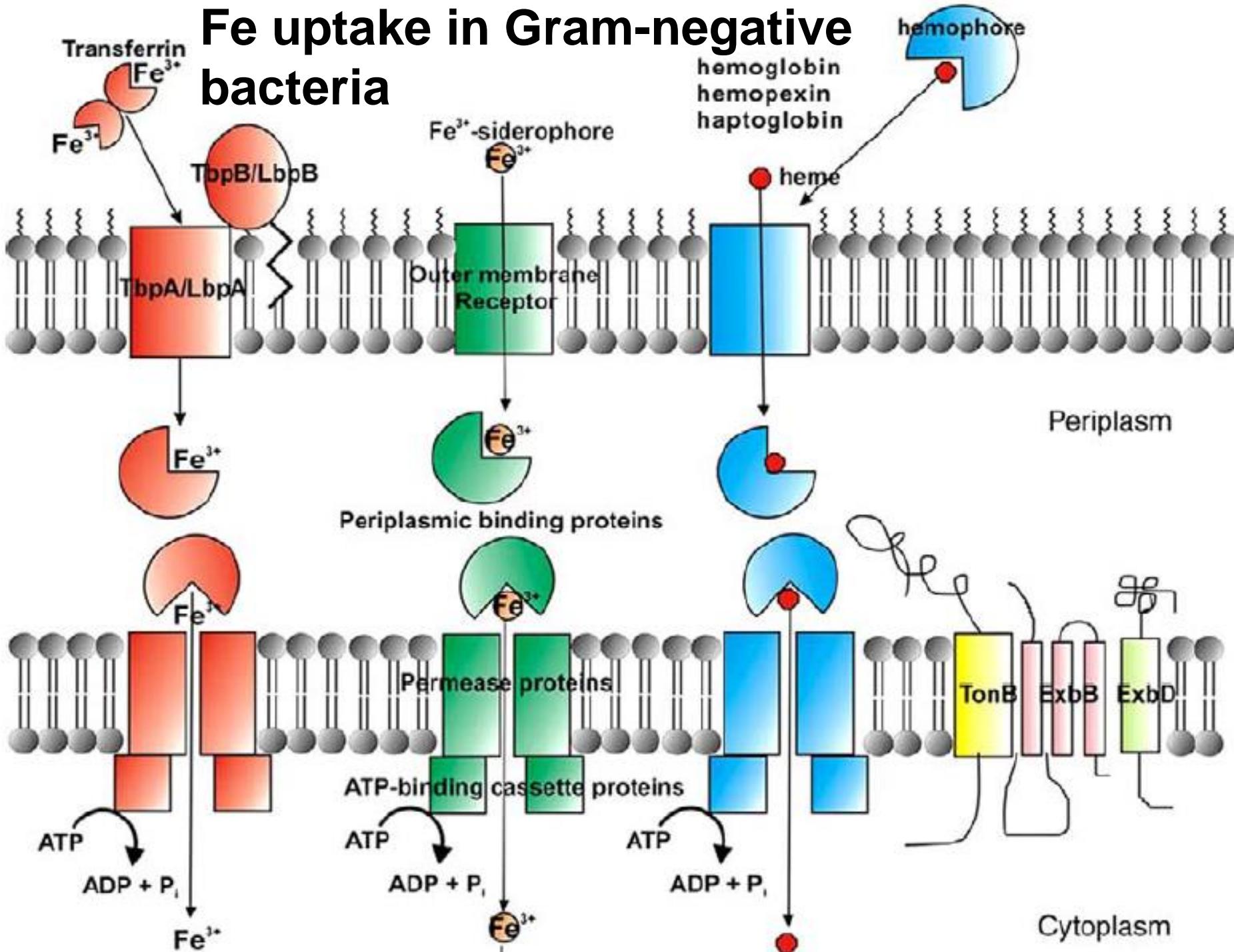
$$\text{At pH 7.0, } [\text{Fe}^{3+}] = 10^{-38} / (10^{-7})^3 = 10^{-17} \text{ M}$$

- Oxidation of Fe(II) to Fe(III) usually undergoes.
- **Free Fe(III) ion is insoluble** at physiological pH in aqueous solutions.



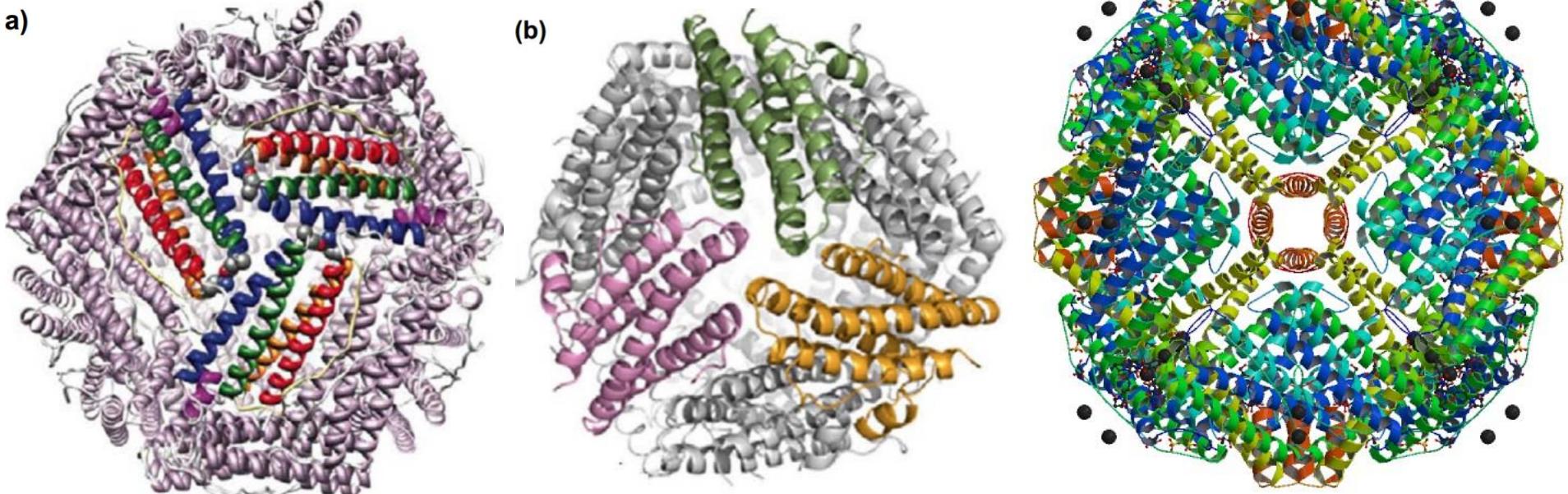
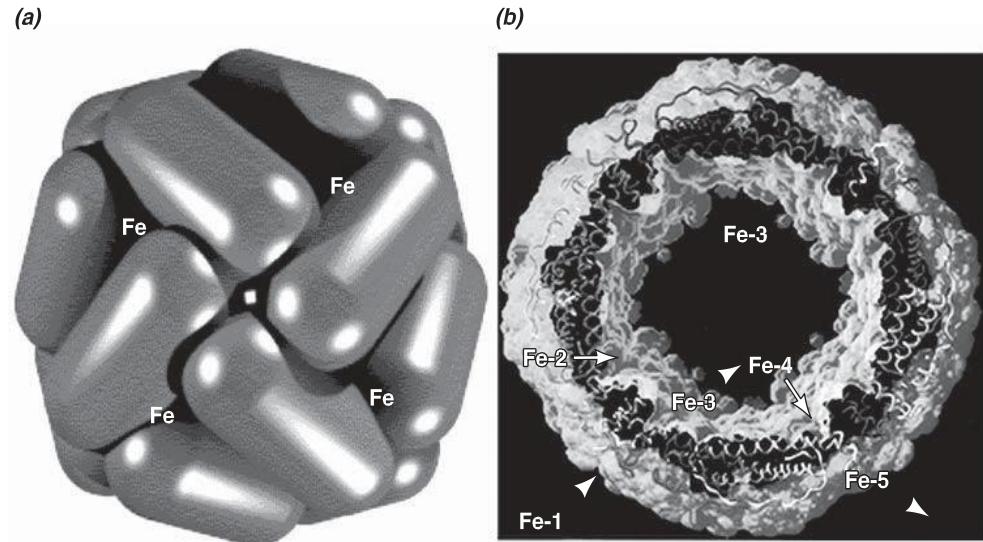
- **Soluble Fe<sup>3+</sup>** can exist at much higher concentrations if it is **bound to proteins or chelators**, e.g. citrate.

# Fe uptake in Gram-negative bacteria

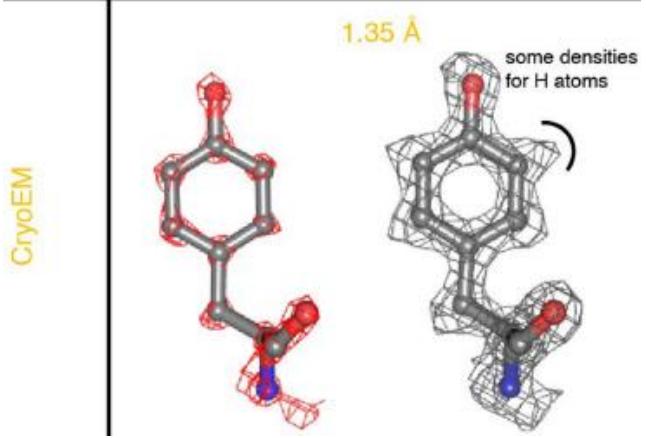
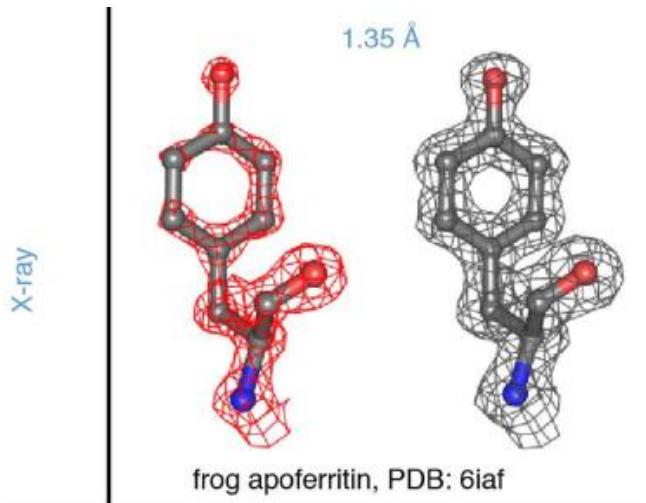
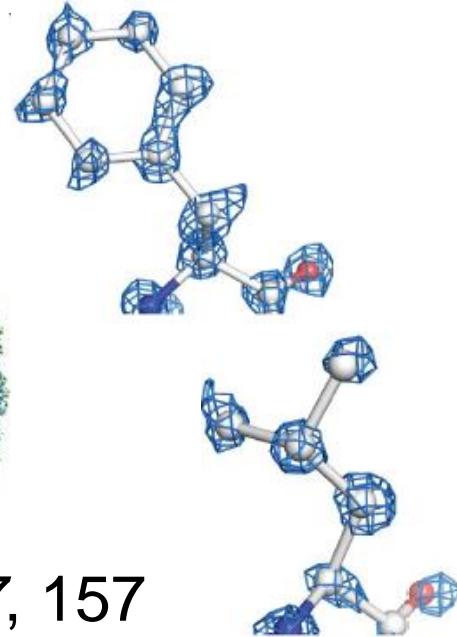
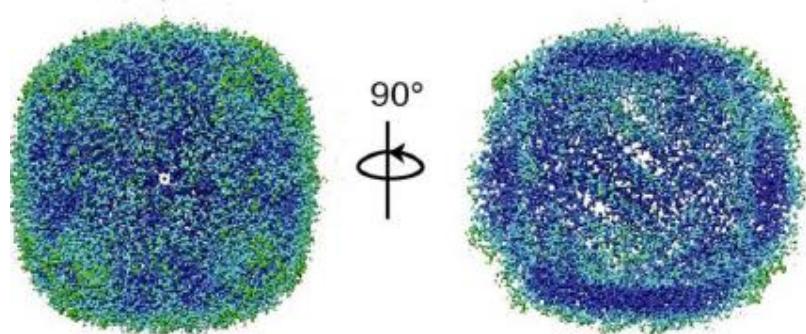
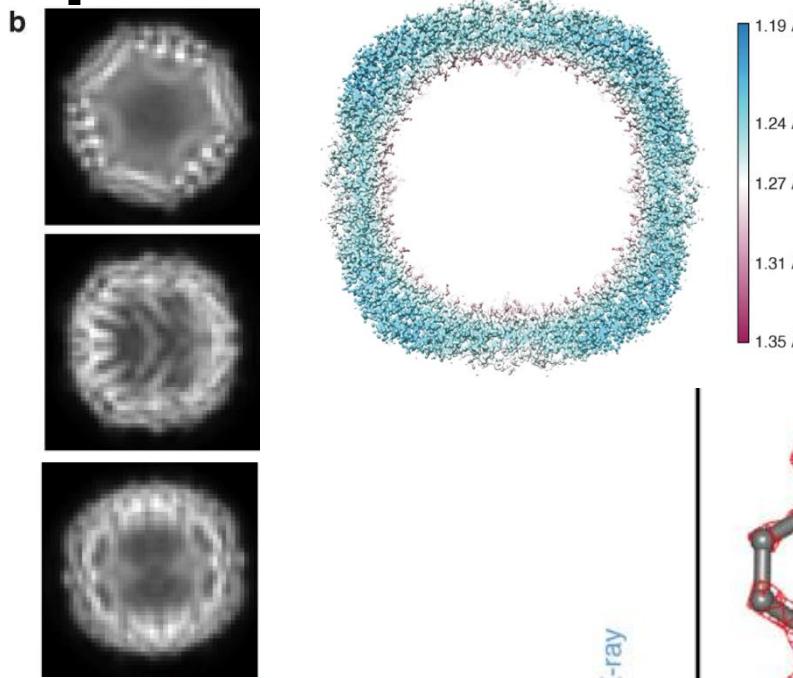
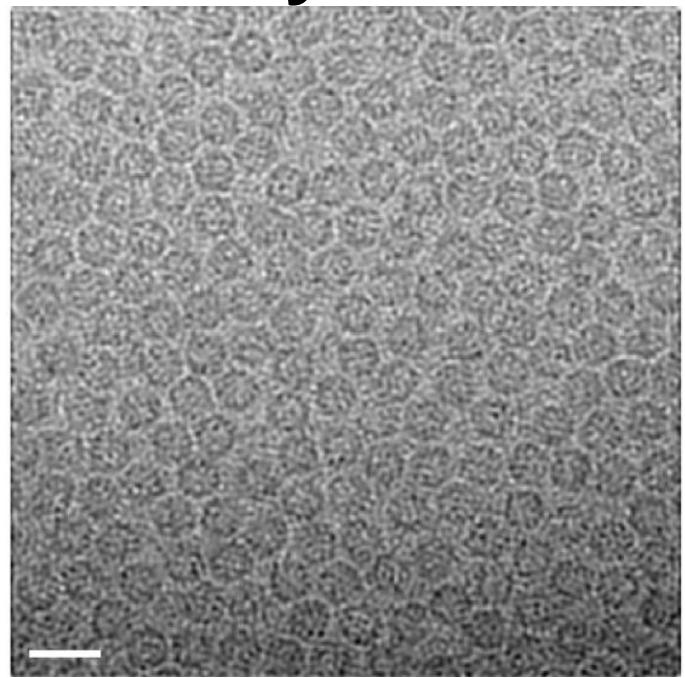


# Storage of Fe: Ferritin

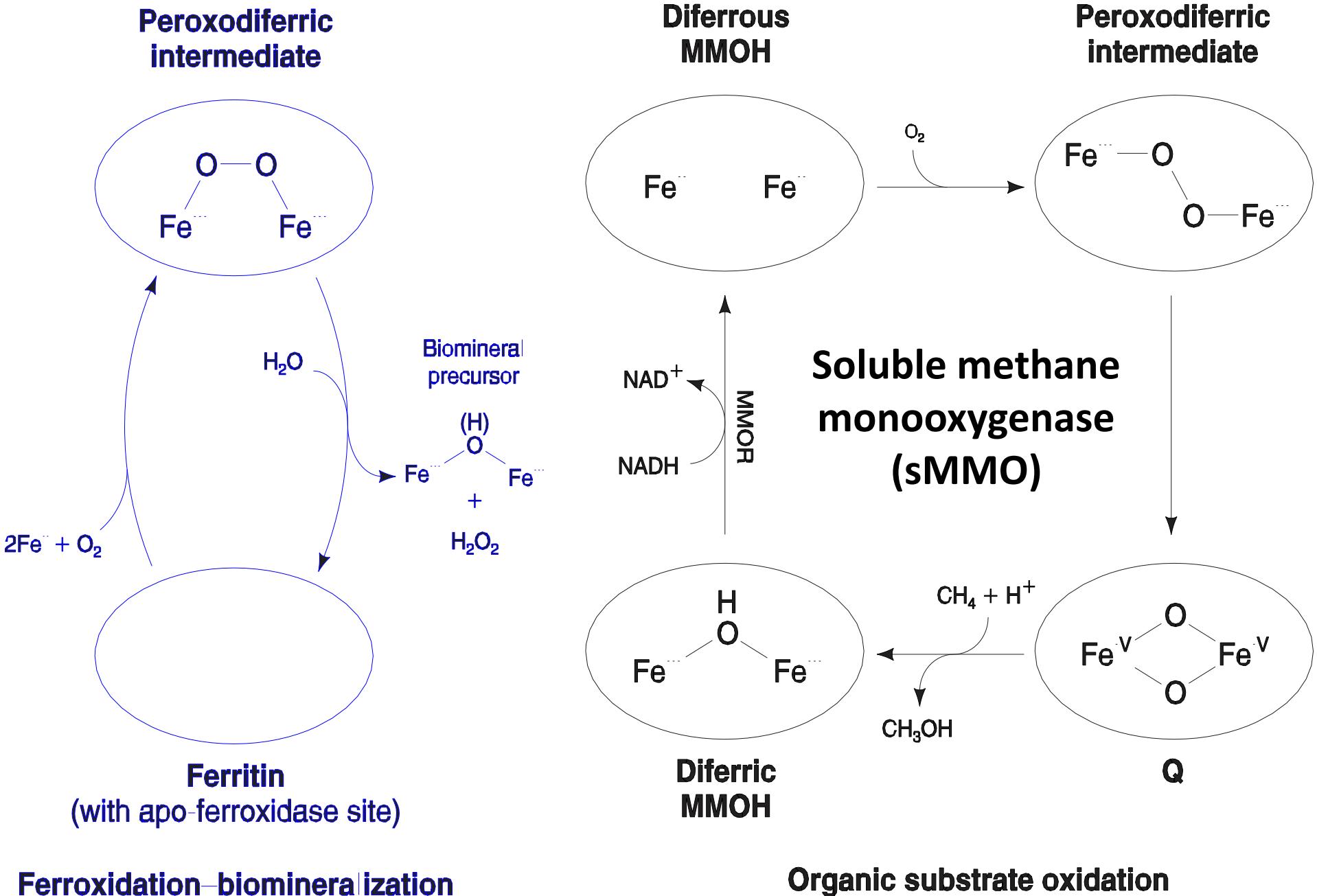
- A very **soluble** protein in plants, animals, & bacteria.
- High symmetry (with 24 polypeptide subunits).
- Hollow sphere inside the proteins (inner diameter: ~7 nm).



# Cryo-EM for apoferritin (1.22 & 1.25 Å)



# Active-site Structure Ferritin



$\sim 36\text{--}2200$  Hydrated  $\text{Fe}^{2+}$  +  $\sim 18\text{--}1100$   $\text{O}_2$

→  $\sim 18\text{--}1100$  hydrated  $[\text{Fe}^{\text{III}}\text{--O--O--Fe}^{\text{III}}]$

$\sim 18\text{--}1100$  Hydrated  $[\text{Fe}^{\text{III}}\text{--O--O--Fe}^{\text{III}}]$

→  $\sim 36\text{--}2200$  hydrated  $\text{Fe}^{3+}$  +  $\sim 18\text{--}1100$   $\text{H}_2\text{O}_2$

$\sim 36\text{--}2200$  Hydrated  $\text{Fe}^{3+}$  →  $[\text{Fe}_2\text{O}_3 \cdot (\text{H}_2\text{O})_x]_{\sim 18\text{--}1100}$

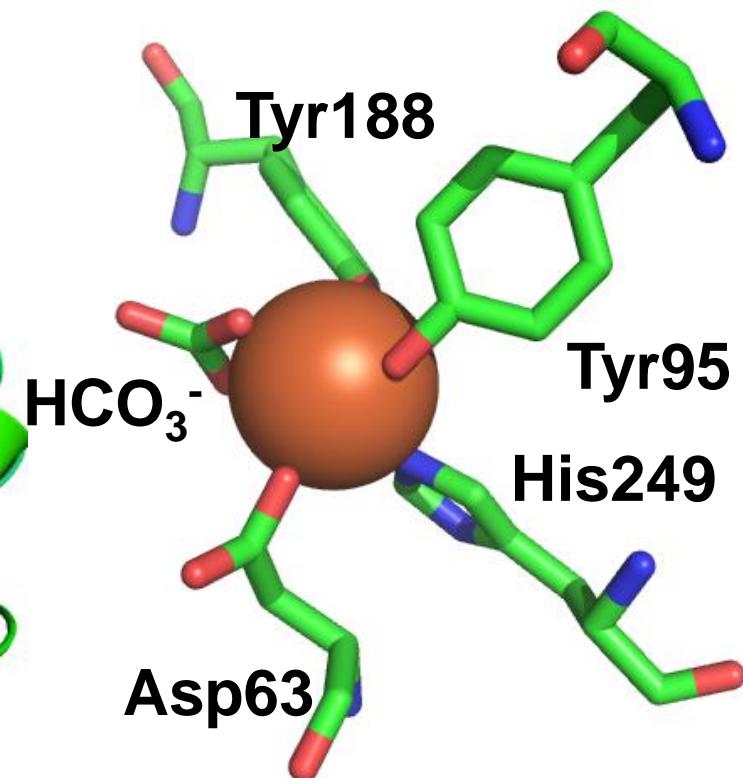
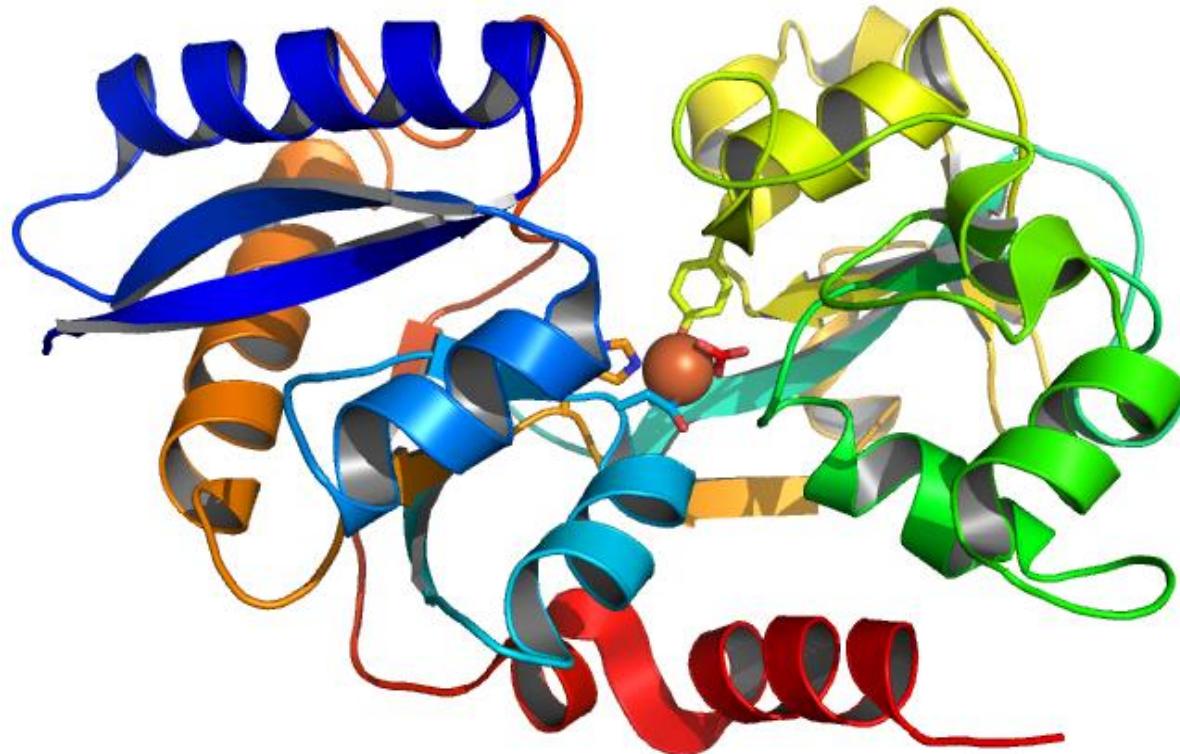
+  $\sim 54\text{--}3300$  hydrated  $\text{H}^+$

Ferritins use the protein cavity as a nano-reactor for the reaction of aqueous oxygen and iron that forms the iron mineral (rust).

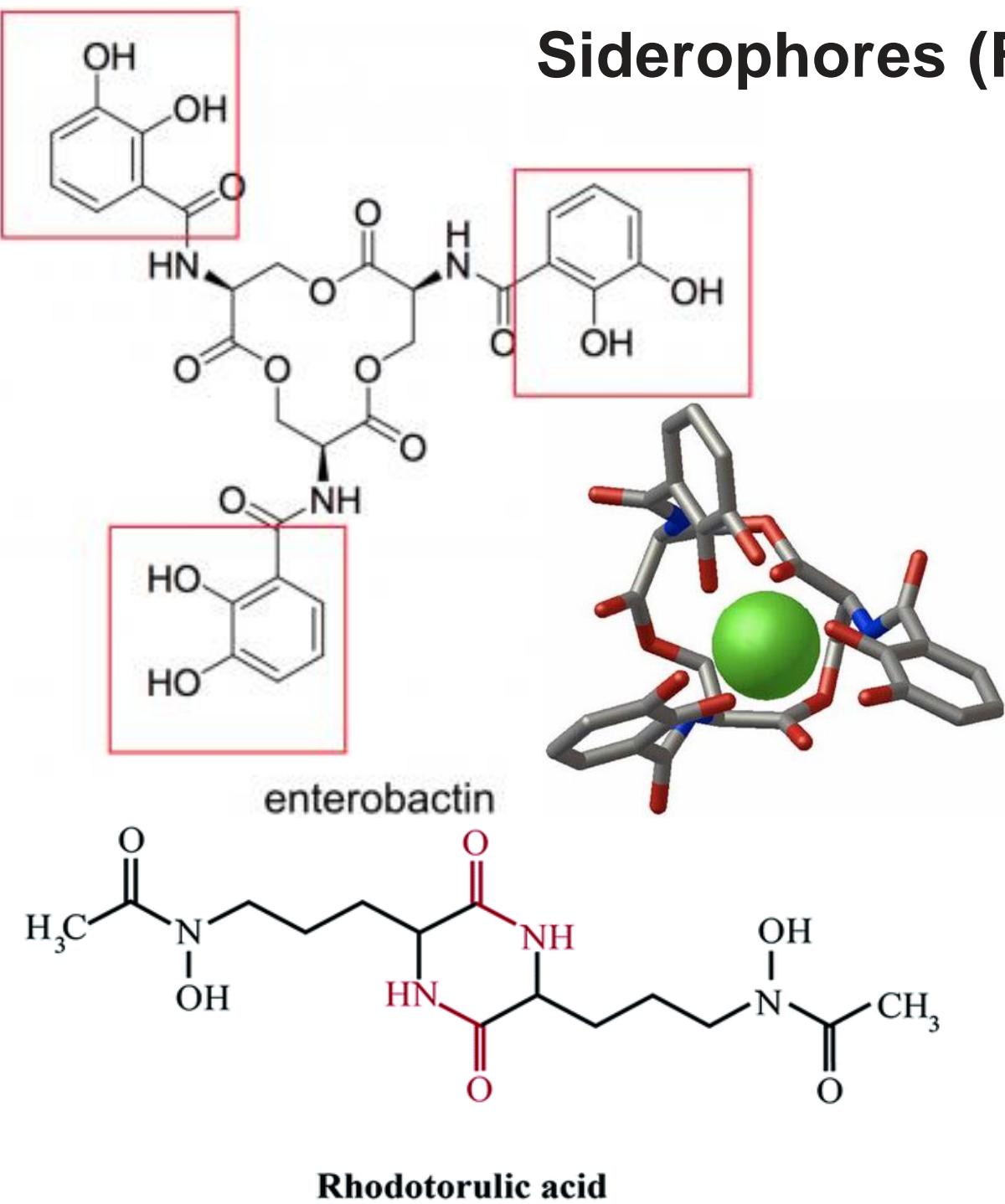
# Transport of Fe: Transferrin

- Uptake of Fe(III) and  $\text{HCO}_3^-$
- Not very specific ( $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ...)
- **Release of Fe(III) by reduction to Fe(II) and binding by porphyrine**

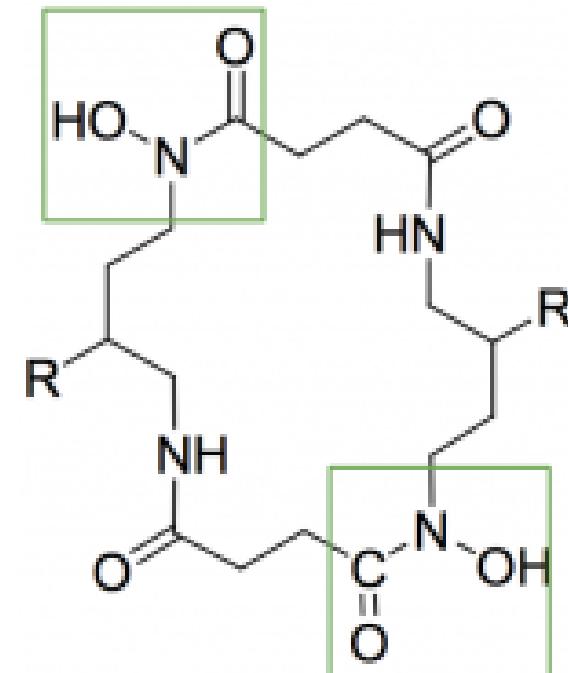
## Human Serum Transferrin



# Siderophores (Fe)

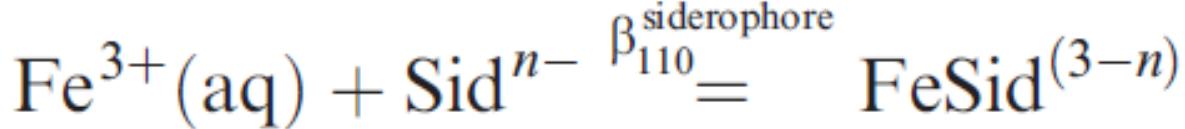


Bacteria & other microorganisms can produce siderophores to solubilize and **take up Fe(III).**



Rhodotorulic acid

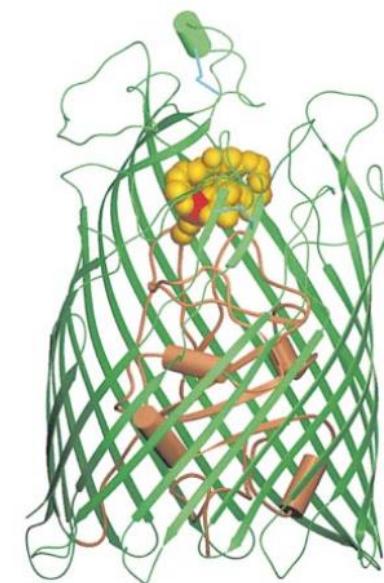
alcaligin



$$\beta_{110}^{\text{siderophore}} = \frac{[\text{FeSid}^{(3-n)}]}{[\text{Fe}^{3+}(\text{aq})][\text{Sid}^{n-}]}$$

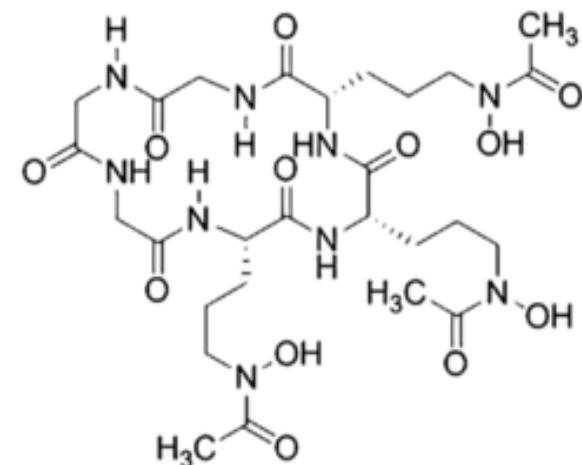
$(\beta_{mlh}$  for the formation of  $M_mL_lH_h$ , where M is metal, L is ligand, and H is proton)

Siderophore	$\log \beta_{110}$	pM
Enterobactin	49	35.5
Desferrioxamine B	30.6	26.6
Ferrichrome	29.07	25.2
Aerobactin	22.5	23.3
Rhodotorulic acid	21.55	21.8
	$\log \beta_{230} 62.2$	
Alcaligin	23.5	23.0



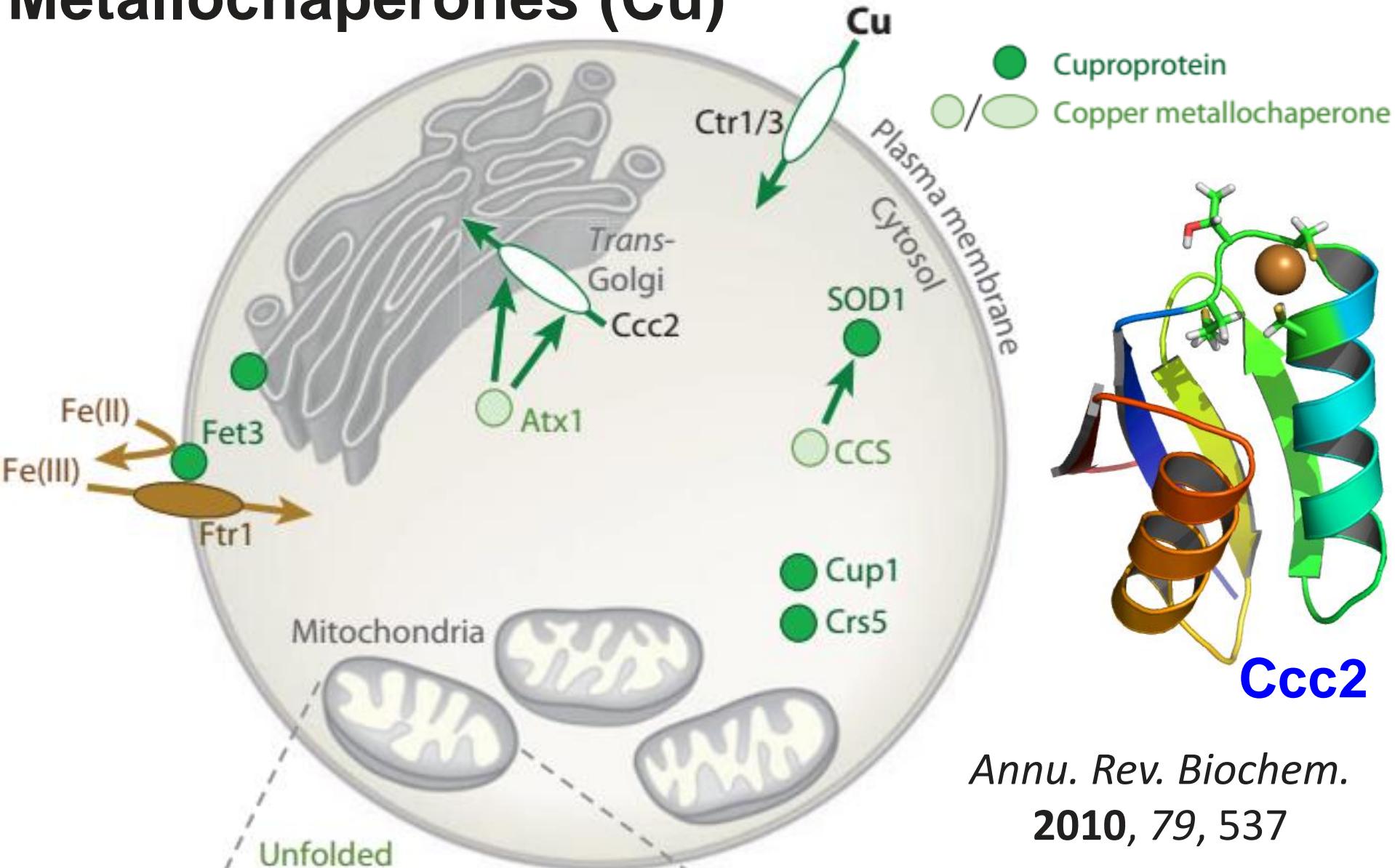
**Ferric hydroxamate receptor with ferrichrome (bottom)**

(Cell 1998, 95, 771)



**Very high binding**

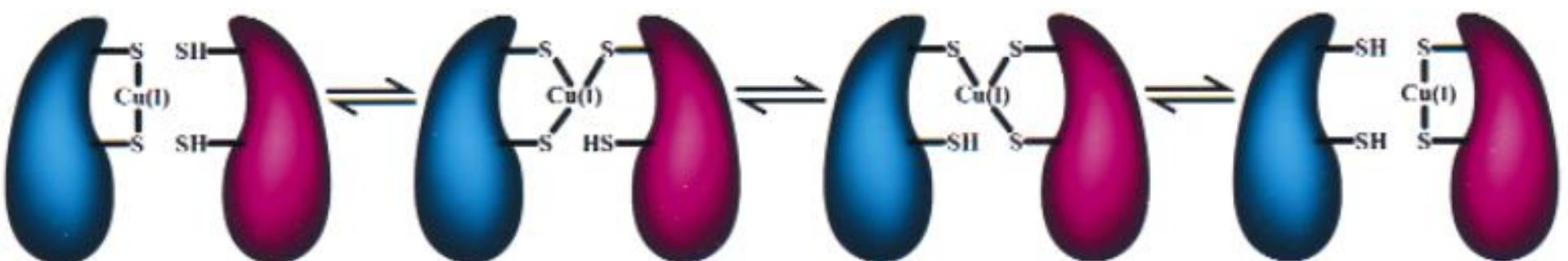
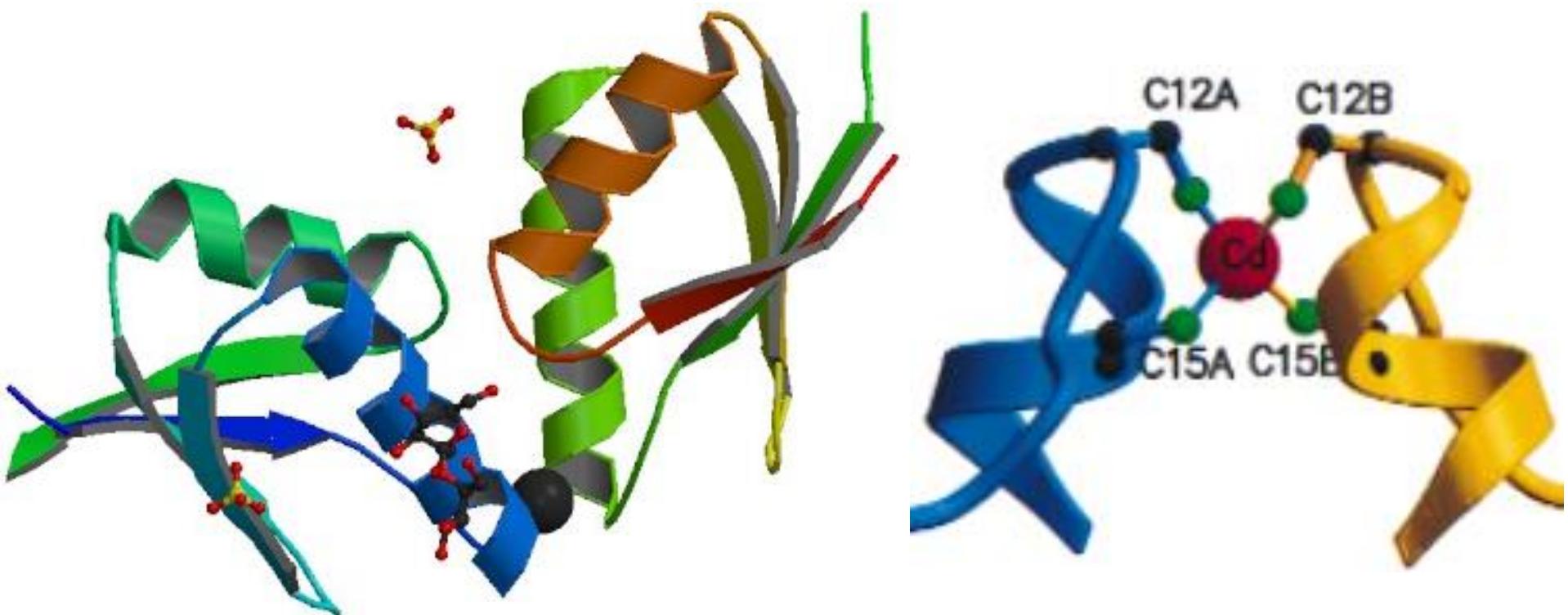
# Metallochaperones (Cu)



Ann. Rev. Biochem.  
2010, 79, 537

**Cu** specific soluble transport proteins, so-called **copper chaperones** (e.g. Atx1), deliver Cu.

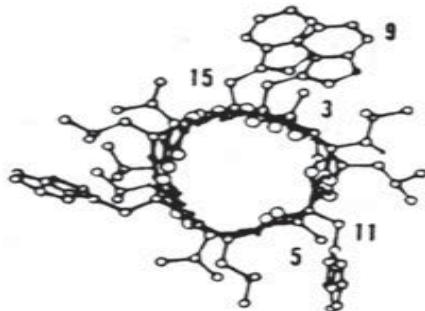
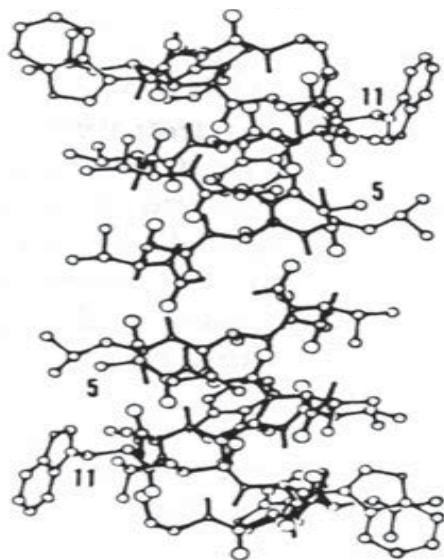
# Crystal Structure of Cu Chaperone protein (Hah1)



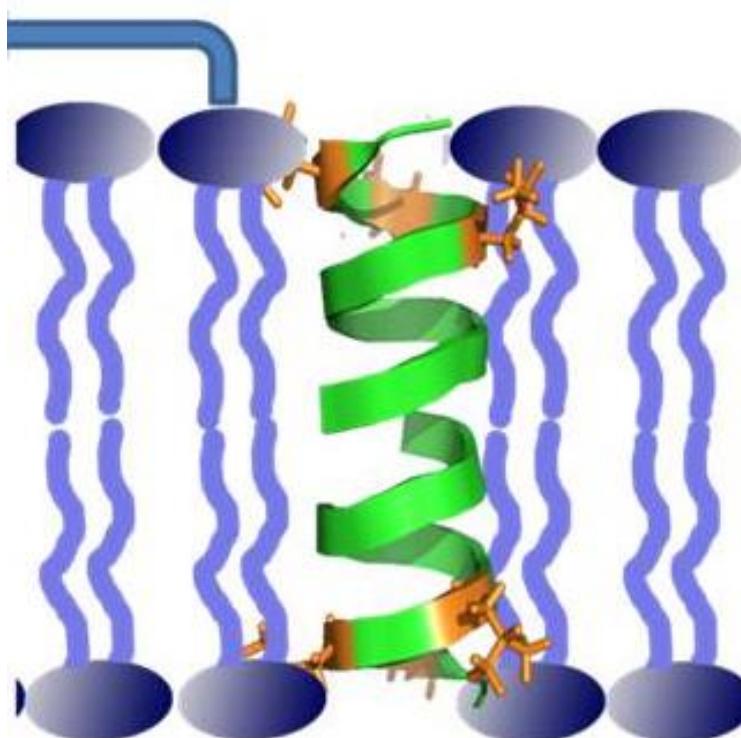
# Gramicidin channel (Ionophores, e.g. Zn)

Formyl-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-  
1    2    3    4    5    6    7    8  
L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-NHCH<sub>2</sub>CH<sub>2</sub>OH  
9    10    11    12    13    14    15

b



an oligopeptide comprised of 15 alternating L- & D-amino acids.



# Key Summary

**Biological ligands for coordination to metals:**  
Generally, **heteroatoms** of **biological molecules** (e.g. proteins, DNA/RNA, cofactor) as potential **ligands** can coordinate to metal: **coordination modes** depends on ligands, metal(s), biological molecules.

**Coordination to metal** can **sometimes maintain the correct overall (folded)** structure, but **sometimes only affect its binding site** in **some** biological molecules.

**Storage & Transport** of small molecules (e.g. O<sub>2</sub> and metal ions) by some bioinorganic systems can keep **sufficient amounts/distribution** (not too many or few) of the molecules for various biological functionals.

**Thank You for Your  
Attention!  
Any Questions?**