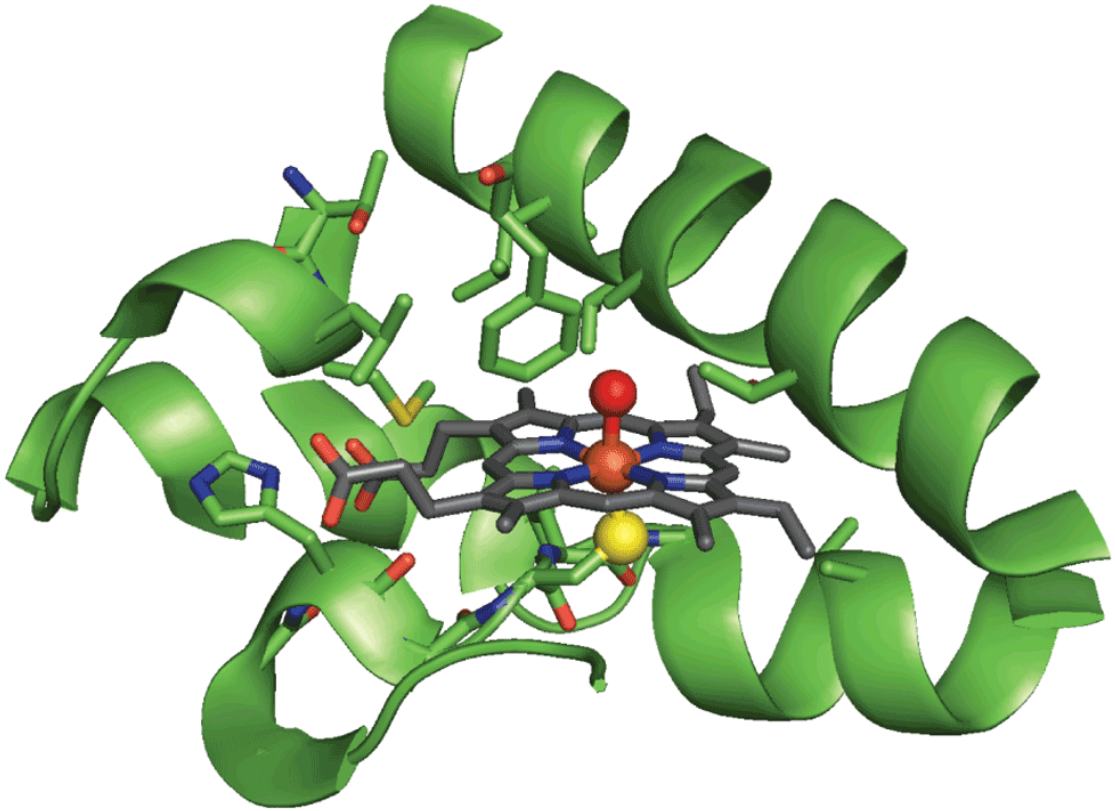
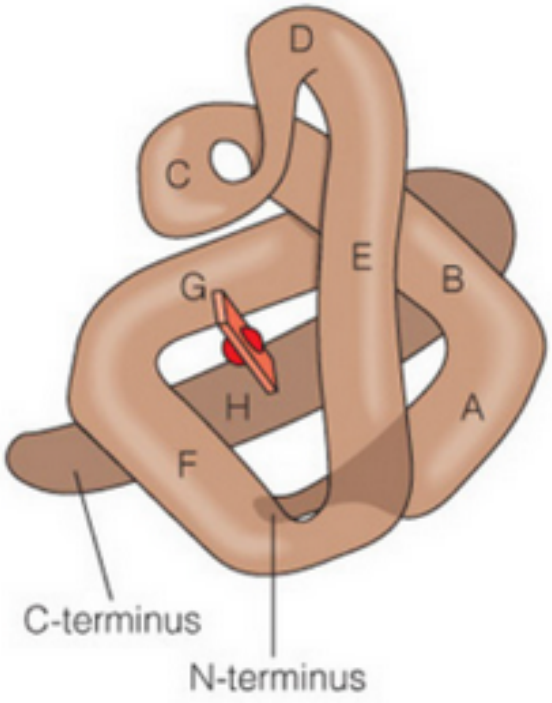
Heme Synthesis & Degradation

Dr Piyush B. Tailor
Professor
Department of Biochemistry
Govt. Medical college
Bhavnagar

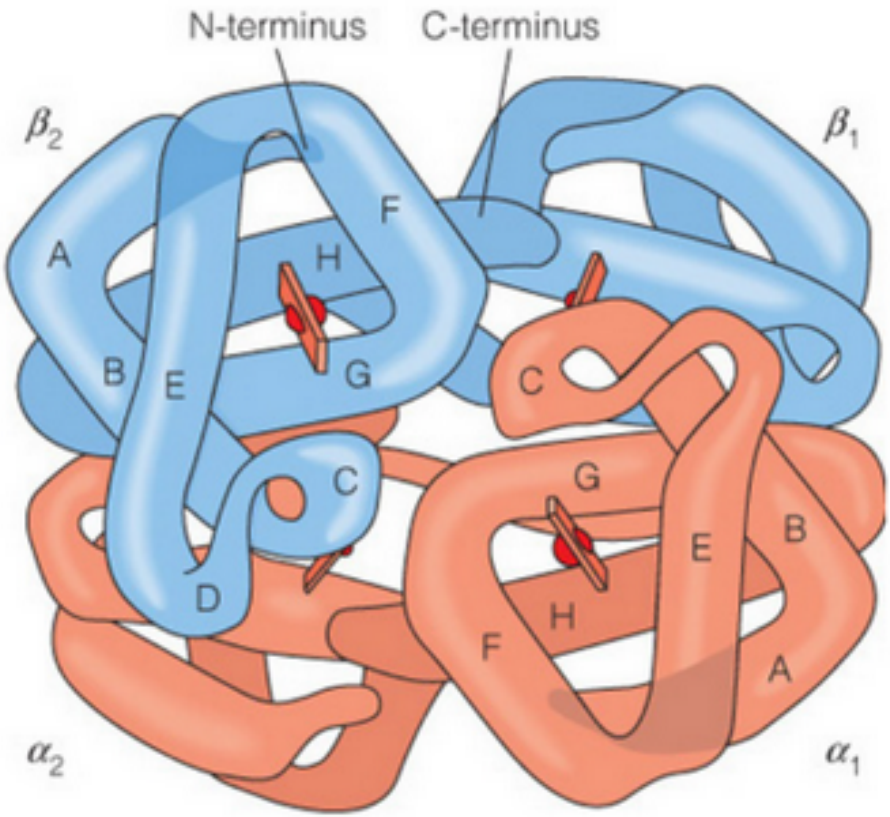
HEME-CONTAINING PROTEINS

- Hemoglobin
- Myoglobin
- Cytochromes
- Catalase
- Peroxidases

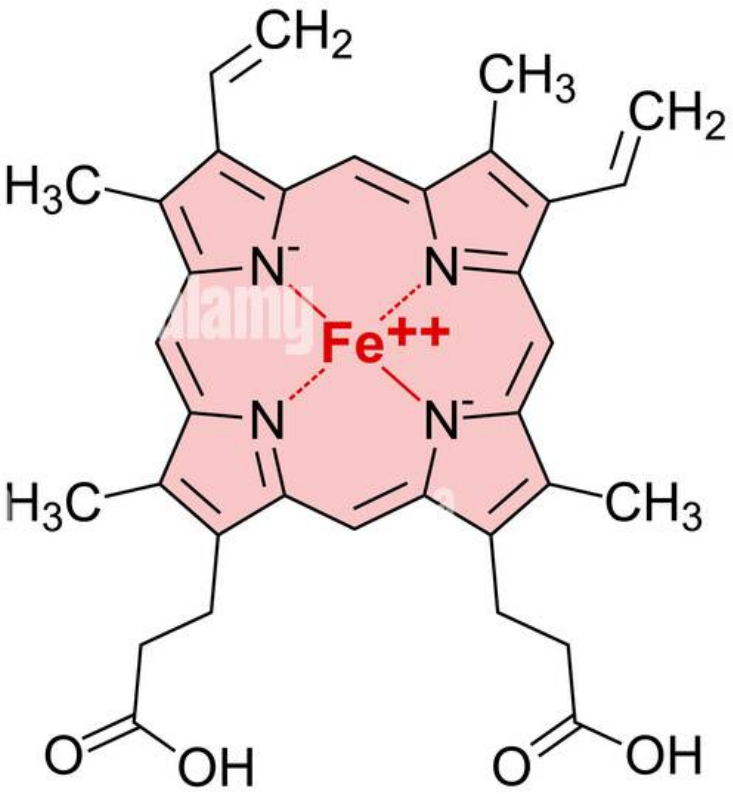




Myoglobin

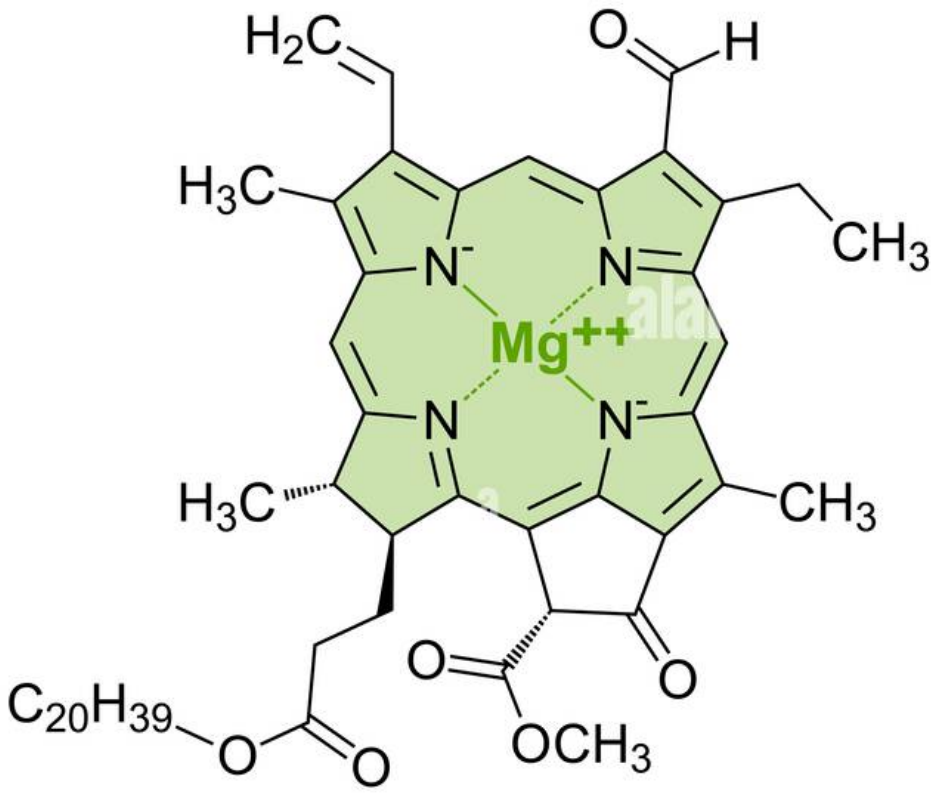


Hemoglobin



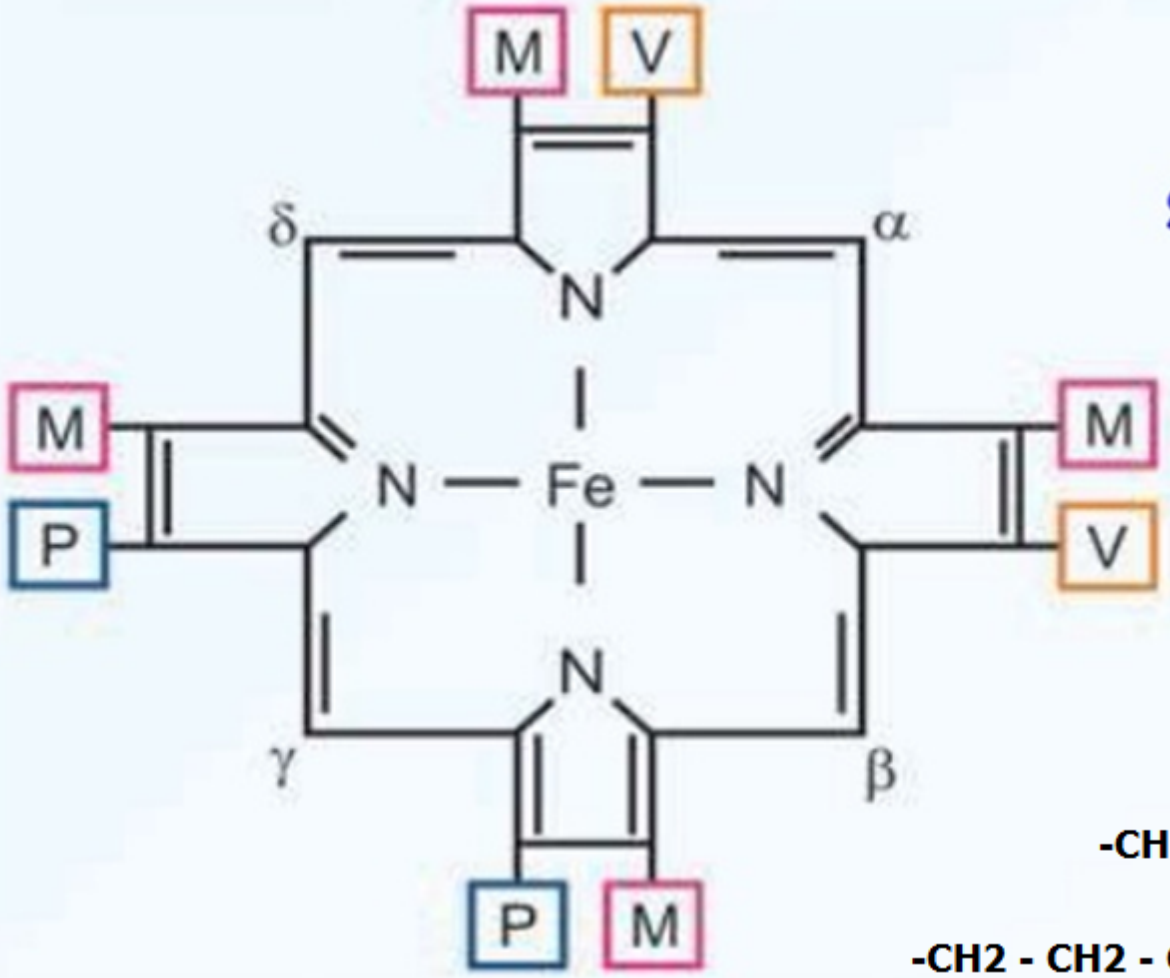
Heme B

Red blood pigment precursor



Chlorophyll *b*

Green pigment in land plants

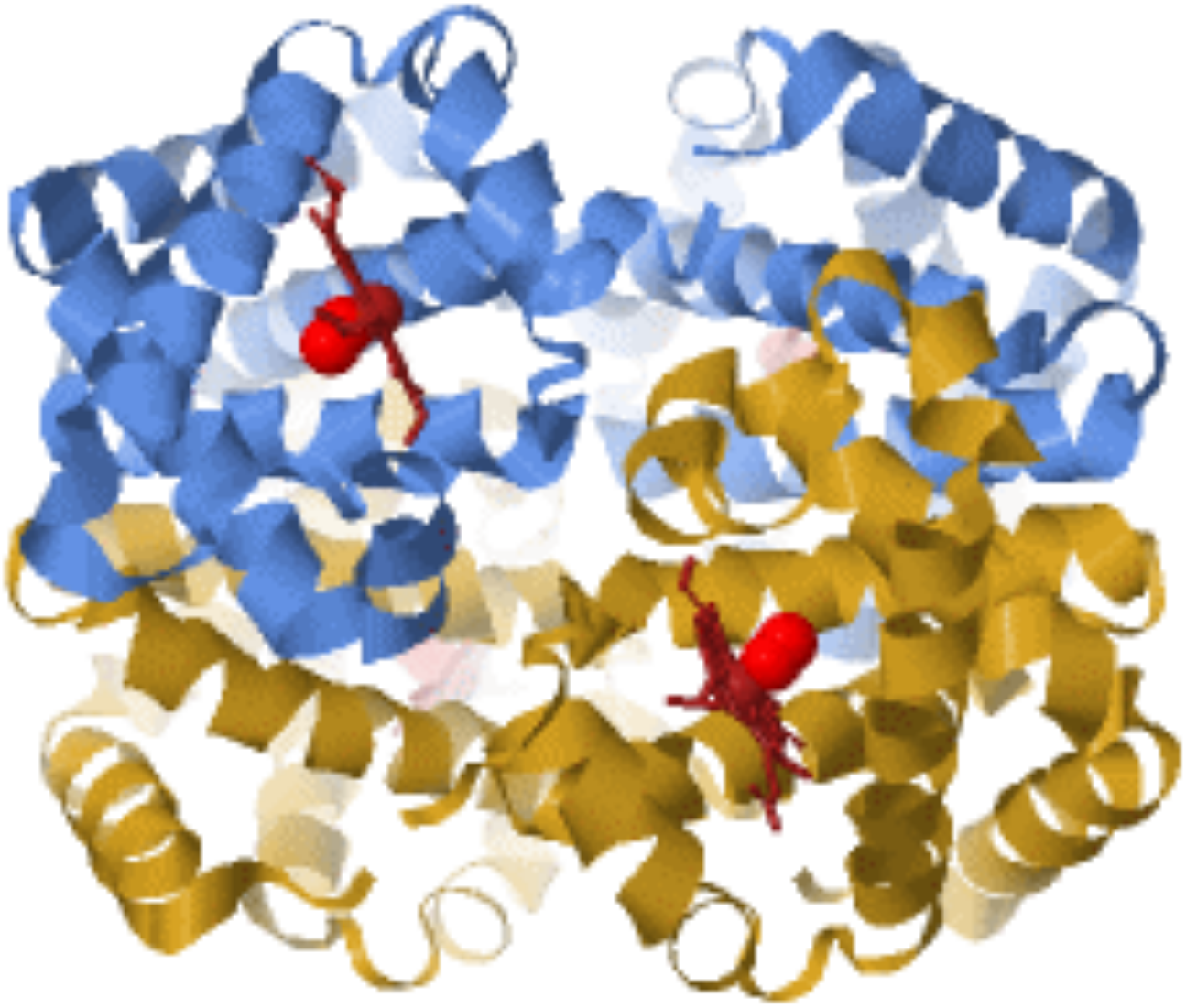


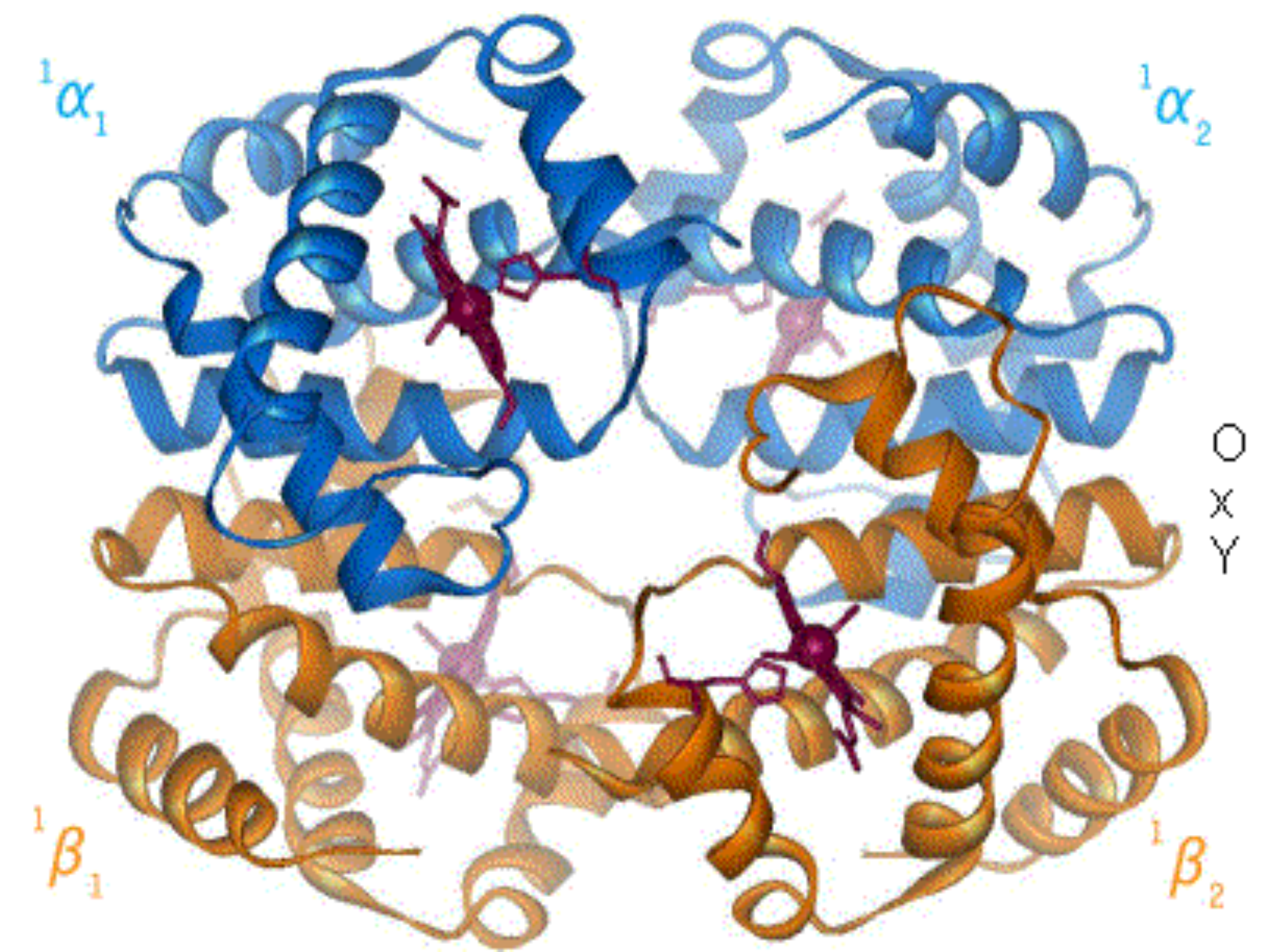
Structure of heme

-CH3 M = Methyl

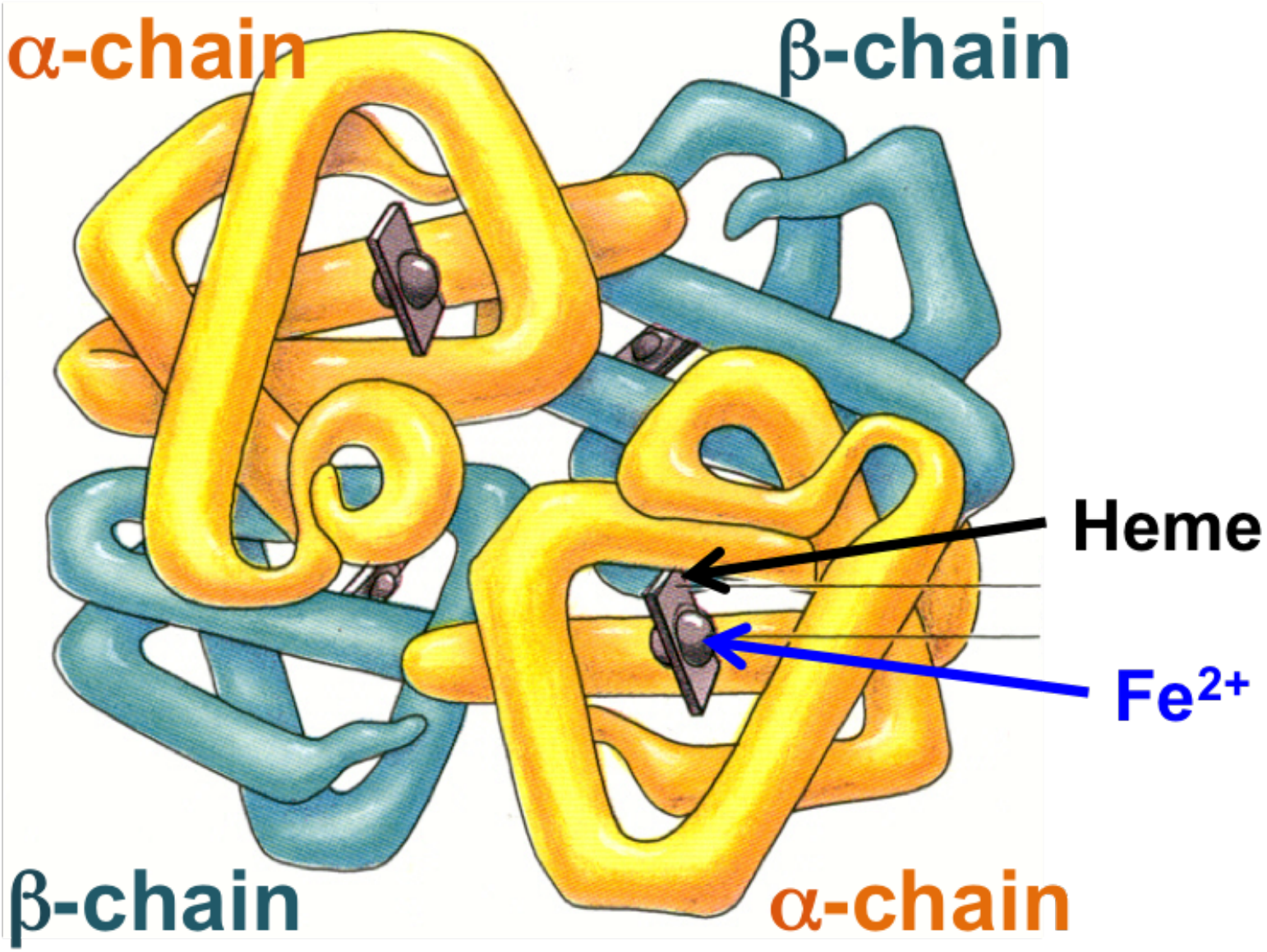
-CH=CH2 V = Vinyl

-CH2 - CH2 - COOH P = Propionyl

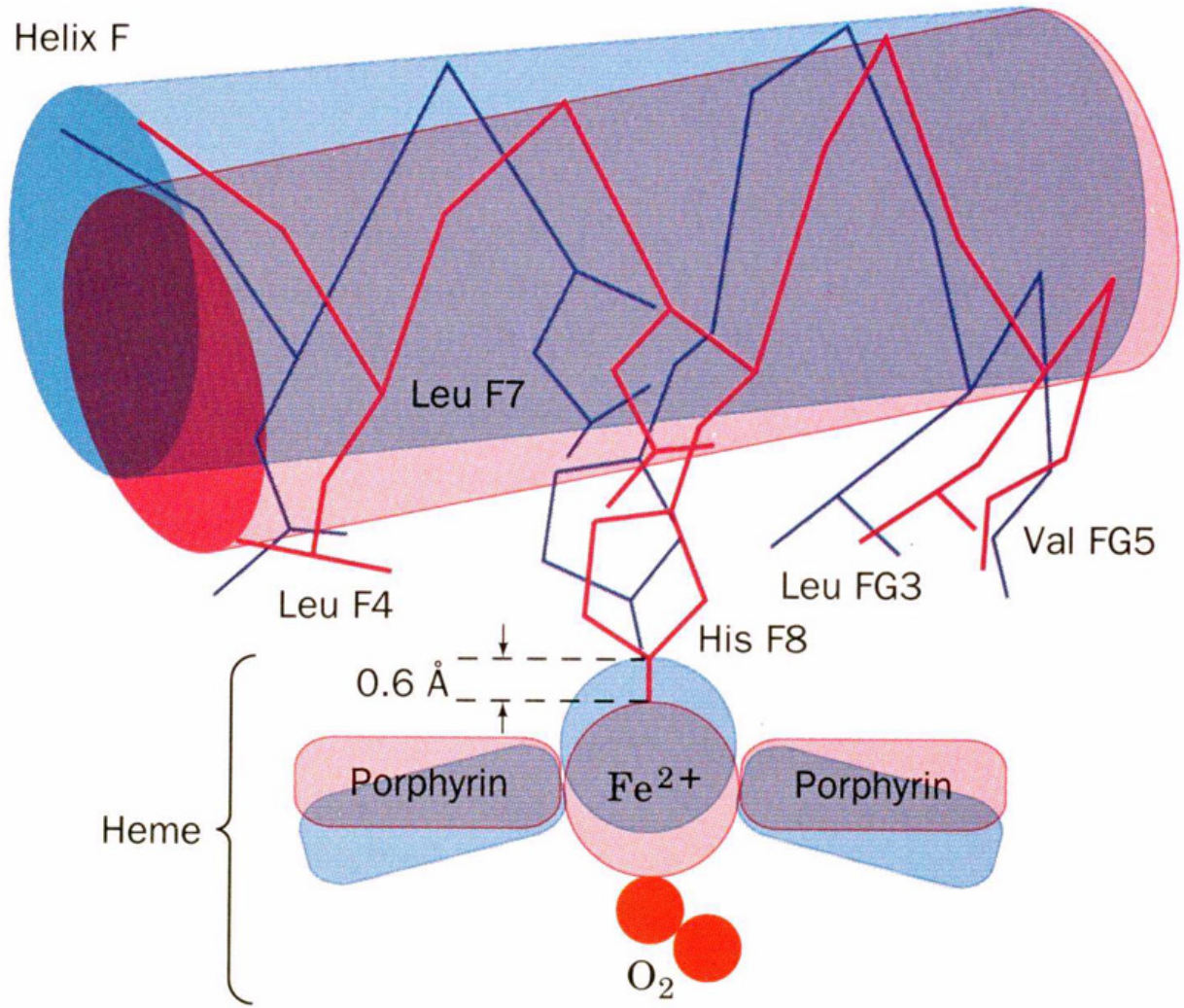

Jmol

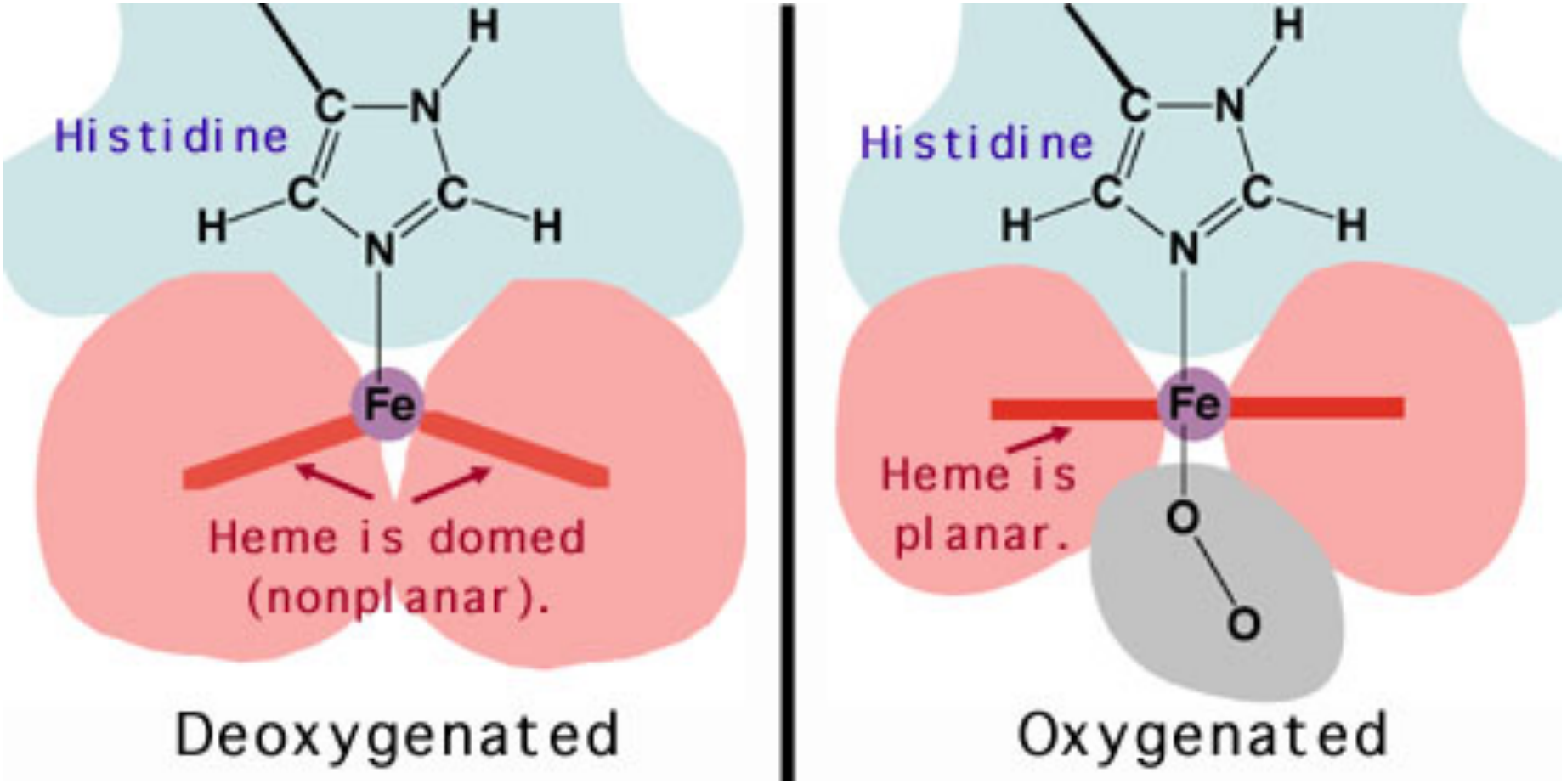

$^1\alpha_1$
$^1\alpha_2$
O
X
Y
$^1\beta_1$
$^1\beta_2$

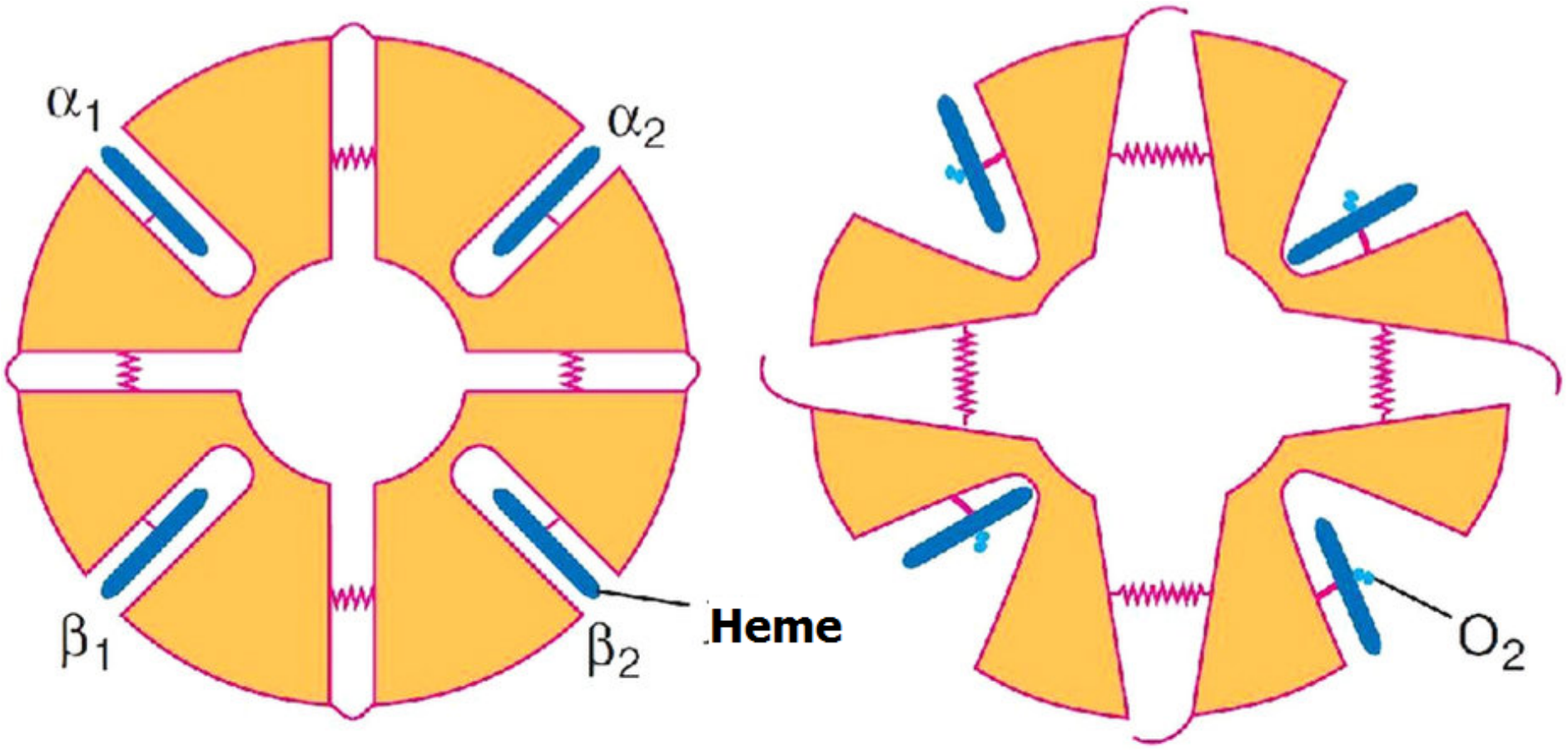
Haemoglobin Structure



O_2 and heme changes

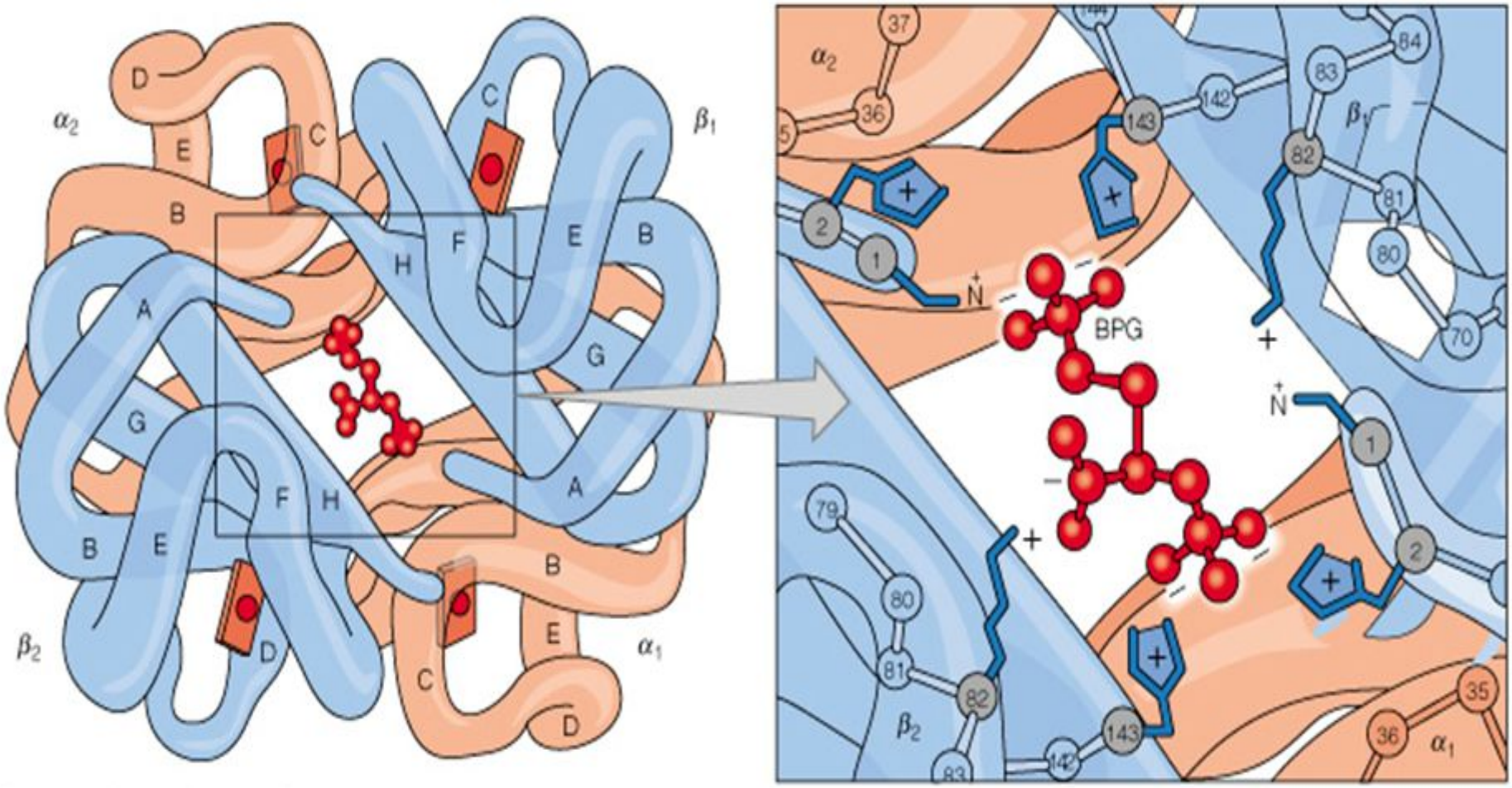



Histidine
H
C
N
C
C
H
H
N
Fe
Heme is domed
(nonplanar).
Deoxygenated
Histidine
H
C
N
C
C
H
H
N
Fe
Heme is
planar.
O
O
Oxygenated



T (Tight) Form **R (Relax) Form**

2-3 BPG = Effect on Heme



2 3 BPG Effect on Hb F

- Beta Chain is Replace by Gamma Chain
- Histidine is replace by Serine
- Positive Charge is replace by Serine
- Positive charge ???? Decrease / Increase ?
- Attraction to 2-3 BPG, Decrease / Increase ?
- Will Haemoglobin remain in Tight / Relax form?
- HbF affinity for Oxygen, Decrease / Increase ?

2 3 BPG Effect on Hb F

- Beta Chain is Replace by Gamma Chain
- Histidine is replace by Serine
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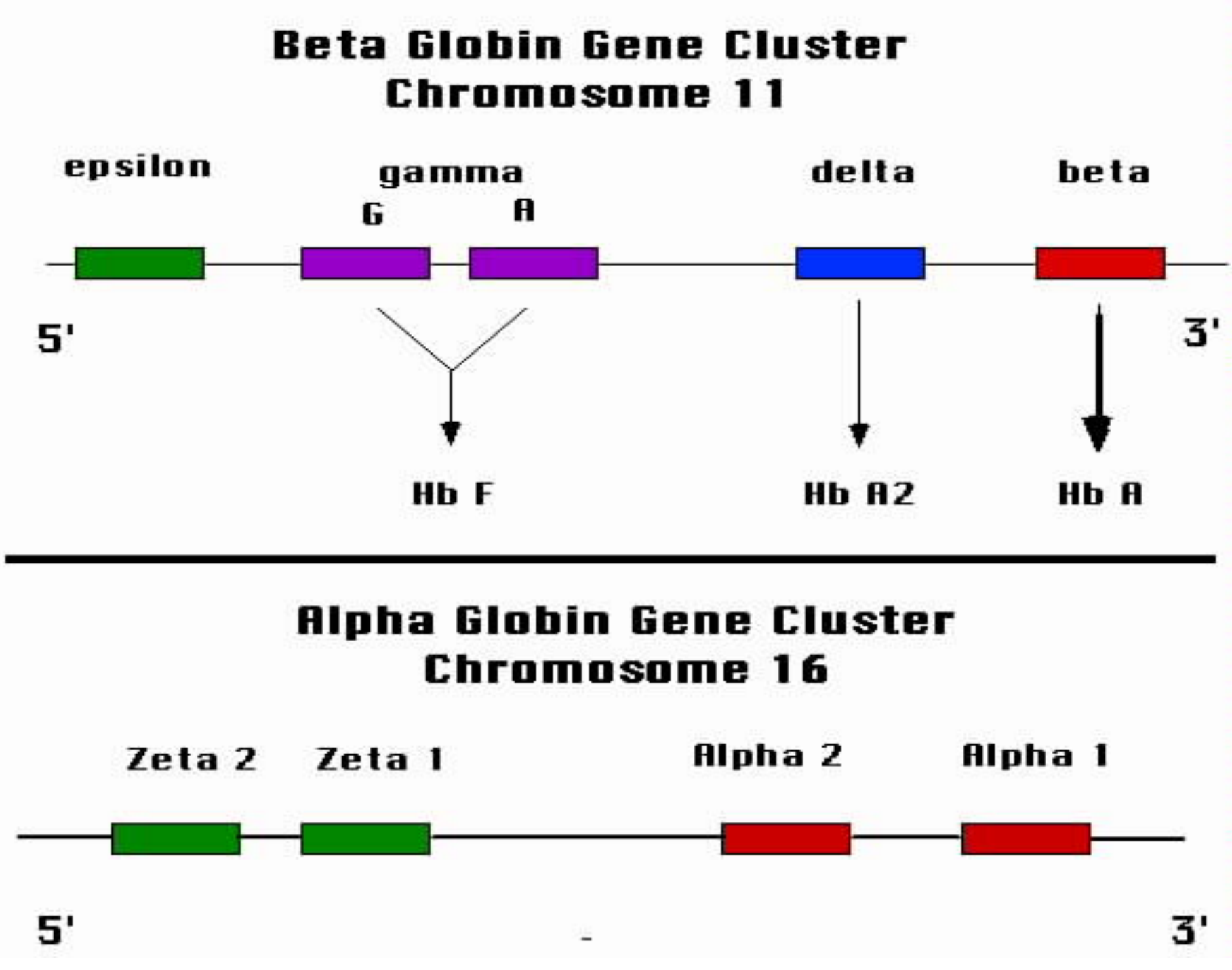
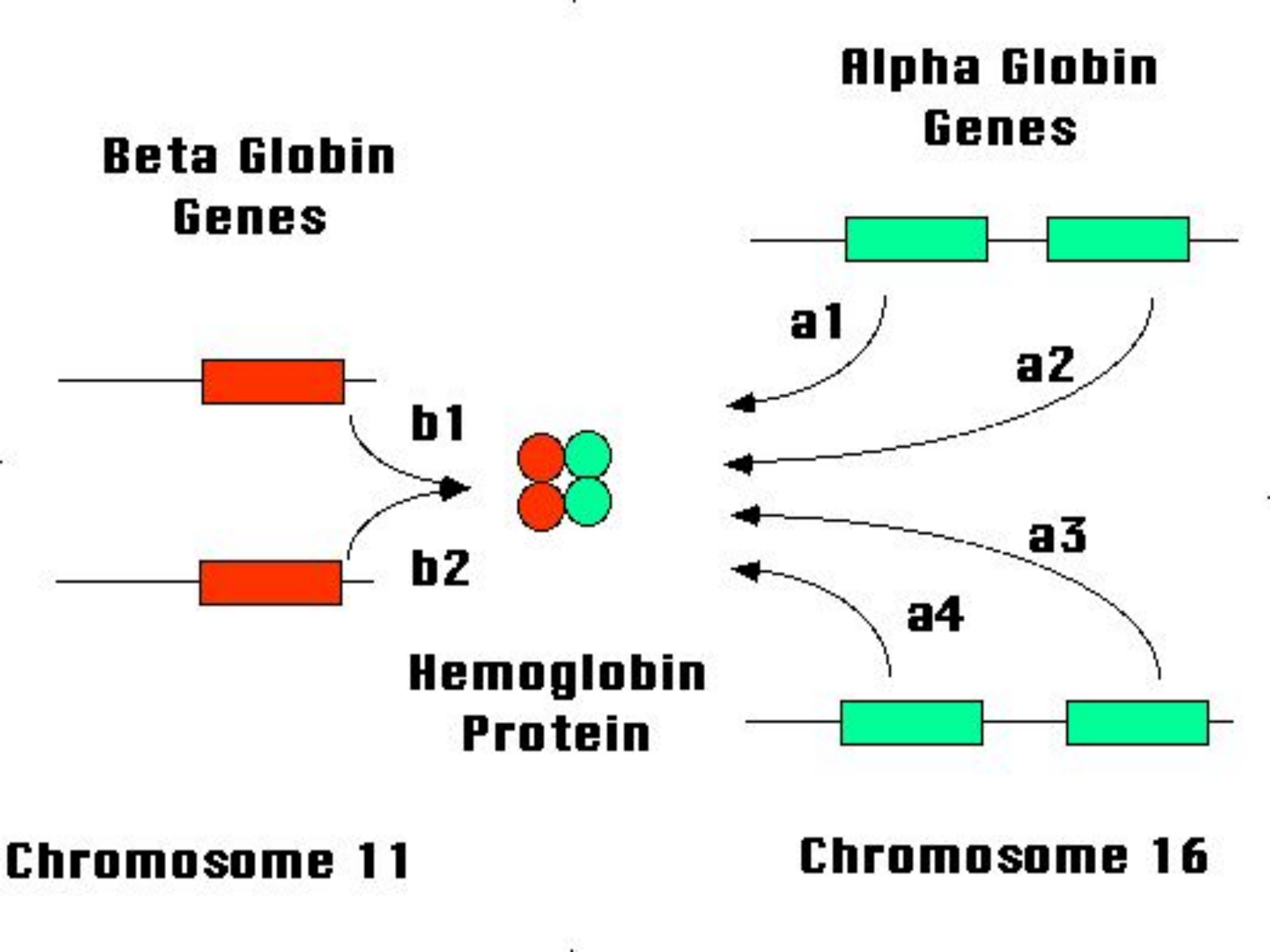
- Positive charge ???? Decrease / ~~Increase~~
- Attraction to 2-3 BPG, Decrease / ~~Increase~~
- Will Haemoglobin remain in Tight / ~~Relax~~ form
- HbF affinity for Oxygen, ~~Decrease~~ / Increase

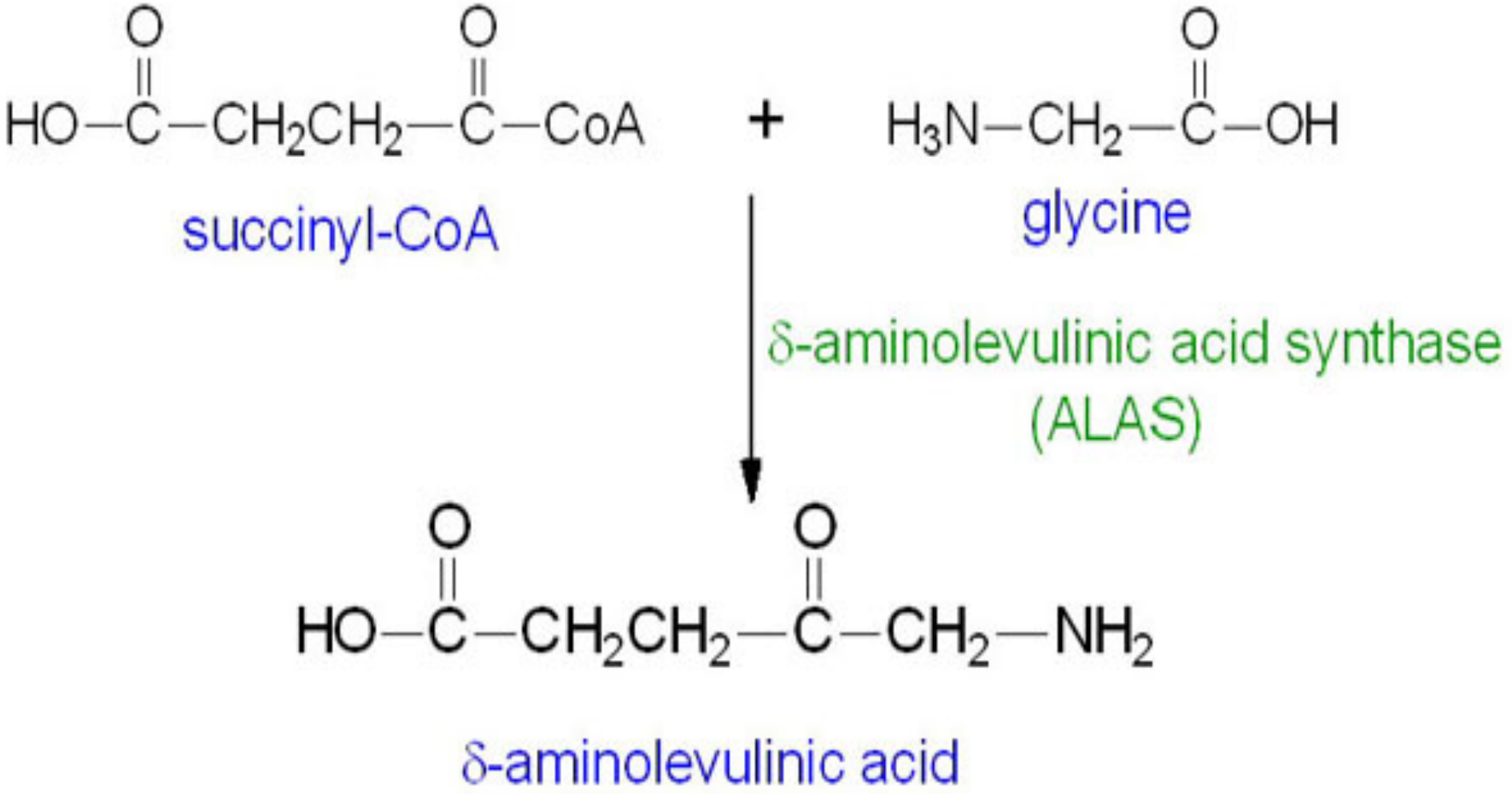

Beta Globin Gene Cluster
Chromosome 11
epsilon
gamma
G
A
delta
beta
5'
3'
Hb F
Hb A2
Hb A
Alpha Globin Gene Cluster
Chromosome 16
Zeta 2
Zeta 1
Alpha 2
Alpha 1
5'
3'

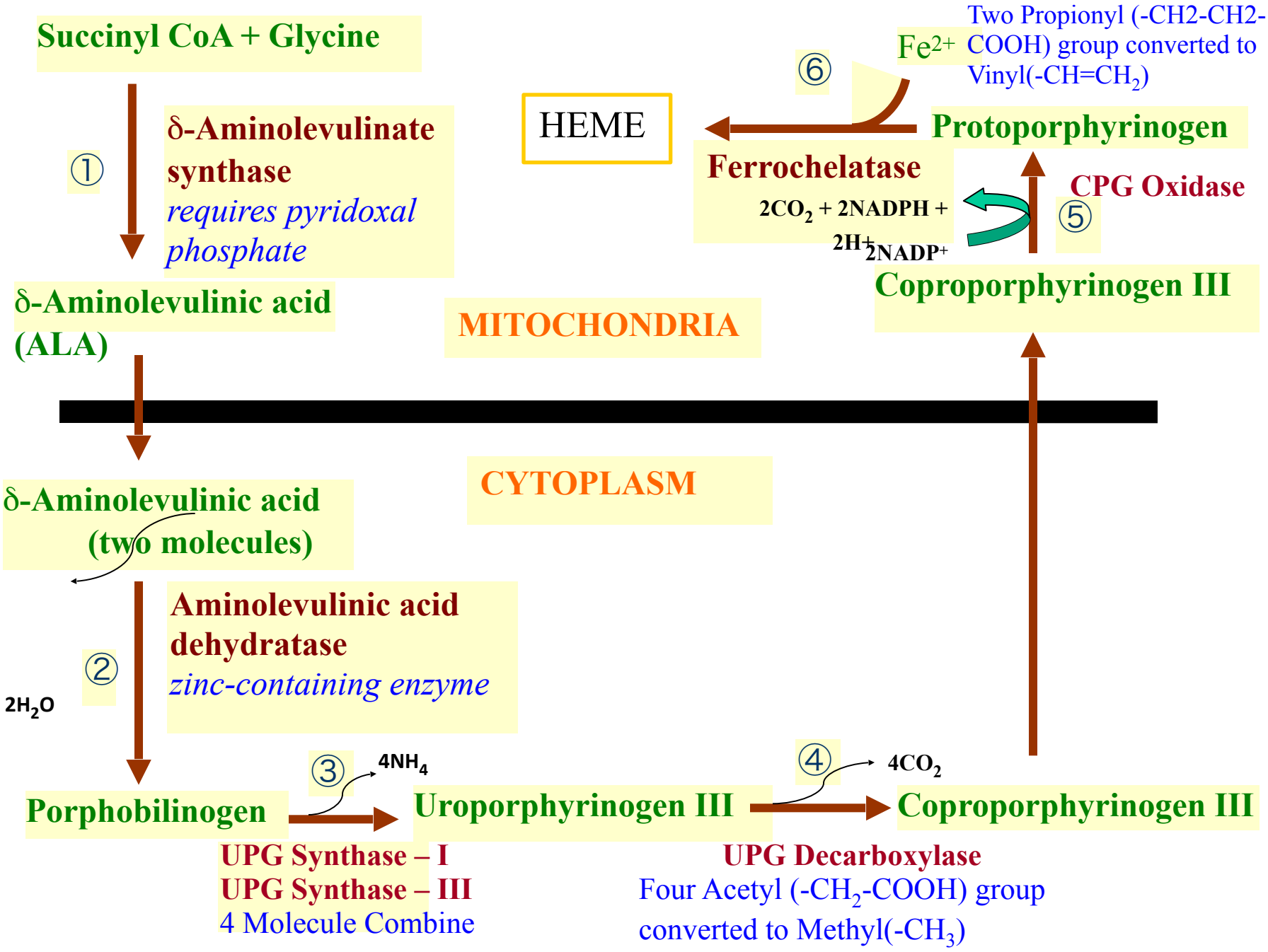

Alpha Globin Genes
Beta Globin Genes
a1
a2
a3
a4
b1
b2
Hemoglobin Protein
Chromosome 11
Chromosome 16

STRUCTURE OF HEME

- 4 Pyrrole rings linked together by Methenyl bridges = PORPHYRIN
- Porphyrin + Ferrous ion (Fe^{+2}) = HEME
- Pyrrole rings named = I , II, III & IV
- Bridges named = α , β , γ & δ
- Substitution denoted as = 1 to 8
- If Substitution group have a symmetrical arrangement (1,3,5,7 & 2,4,6,8) = Series I
- If Substitution group have a asymmetrical arrangement (1,3,5,8 & 2,4,6,7) = Series III (Predominant in biological system)
- Substitution group = Propionyl ($-CH_2-CH_2-COOH$)
  = Acetyl ($-CH_2-COOH$)
  = Methyl ($-CH_3$)
  = Vinyl ($-CH=CH_2$)

Heme Synthesis (First Step)




Succinyl CoA + Glycine
①
δ-Aminolevulinate synthase
requires pyridoxal phosphate
δ-Aminolevulinic acid (ALA)
MITOCHONDRIA
CYTOPLASM
δ-Aminolevulinic acid (two molecules)
②
2H2O
Aminolevulinic acid dehydratase
zinc-containing enzyme
Porphobilinogen
③
4NH4
Uroporphyrinogen III
UPG Synthase – I
UPG Synthase – III
4 Molecule Combine
④
4CO2
Coproporphyrinogen III
UPG Decarboxylase
Four Acetyl (-CH2-COOH) group converted to Methyl(-CH3)
⑤
CPG Oxidase
2CO2 + 2NADPH + 2H+
2NADP+
Coproporphyrinogen III
Ferrochelatase
⑥
Fe2+
Two Propionyl (-CH2-CH2-COOH) group converted to Vinyl(-CH=CH2)
Protoporphyrinogen
HEME

Regulation of Heme and Globin Synthesis

- ALA synthase has **two Iso – Enzymes.**
  - Erythroid = X chromosome (**Not Repress** by Heme)
  - Non Erythroid = On 3rd chromosome
- **Repression** of Gene for ALA synthase .
  - High Heme
  - High Glucose
    - High Catabolite Repressor
    - Repression of ALA synthase

- Free Heme = Stimulation of Globin synthesis
- Excess Heme = Fe+2 is oxidised to Fe+3 (Hemetin)

Regulation of Heme and Globin Synthesis

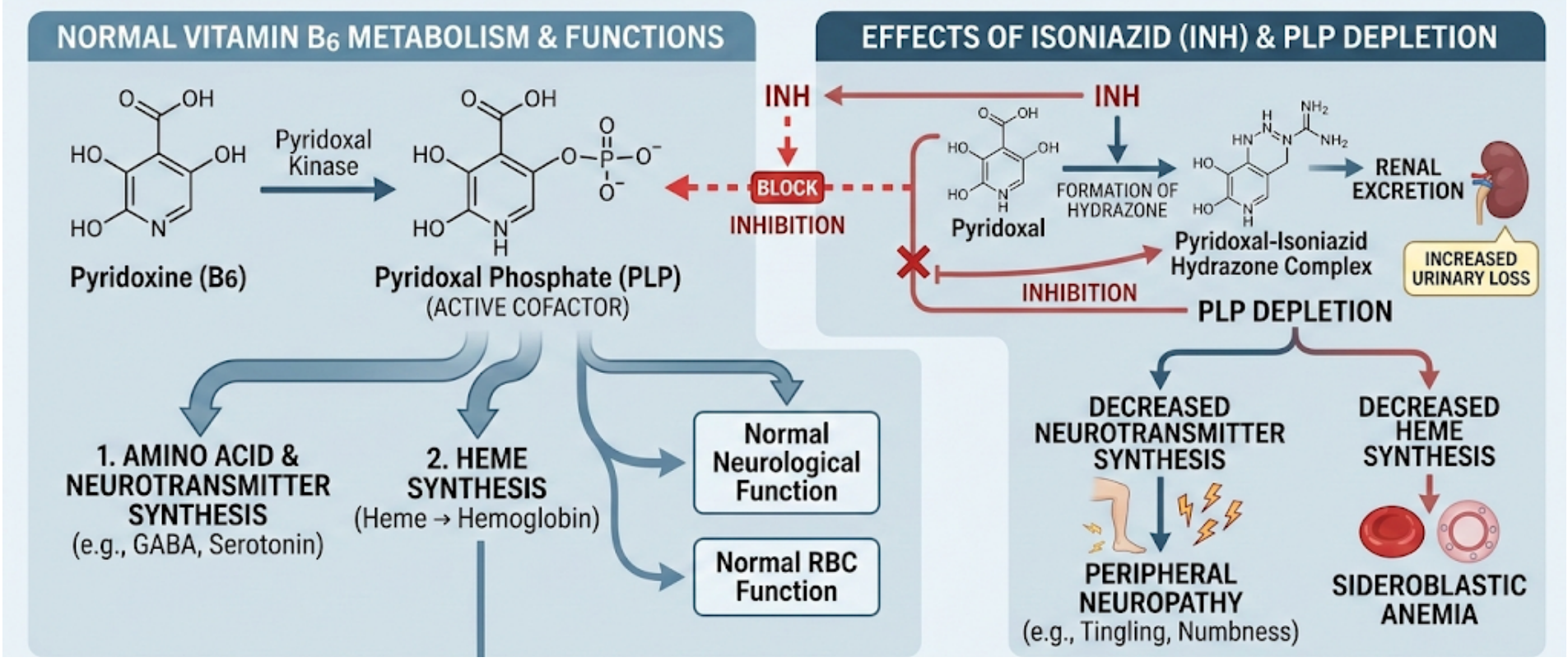
Drug effect on Regulation

- **Barbiturates**
  - Require Cytochrome P -450 for their metabolism.
  - For Cytochrome P - 450 synthesis - Heme is require
  - So Heme synthesis is stimulated
  - Induction of Enzyme
    - ALA Synthase

Regulation of Heme and Globin Synthesis

Drug effect on Regulation

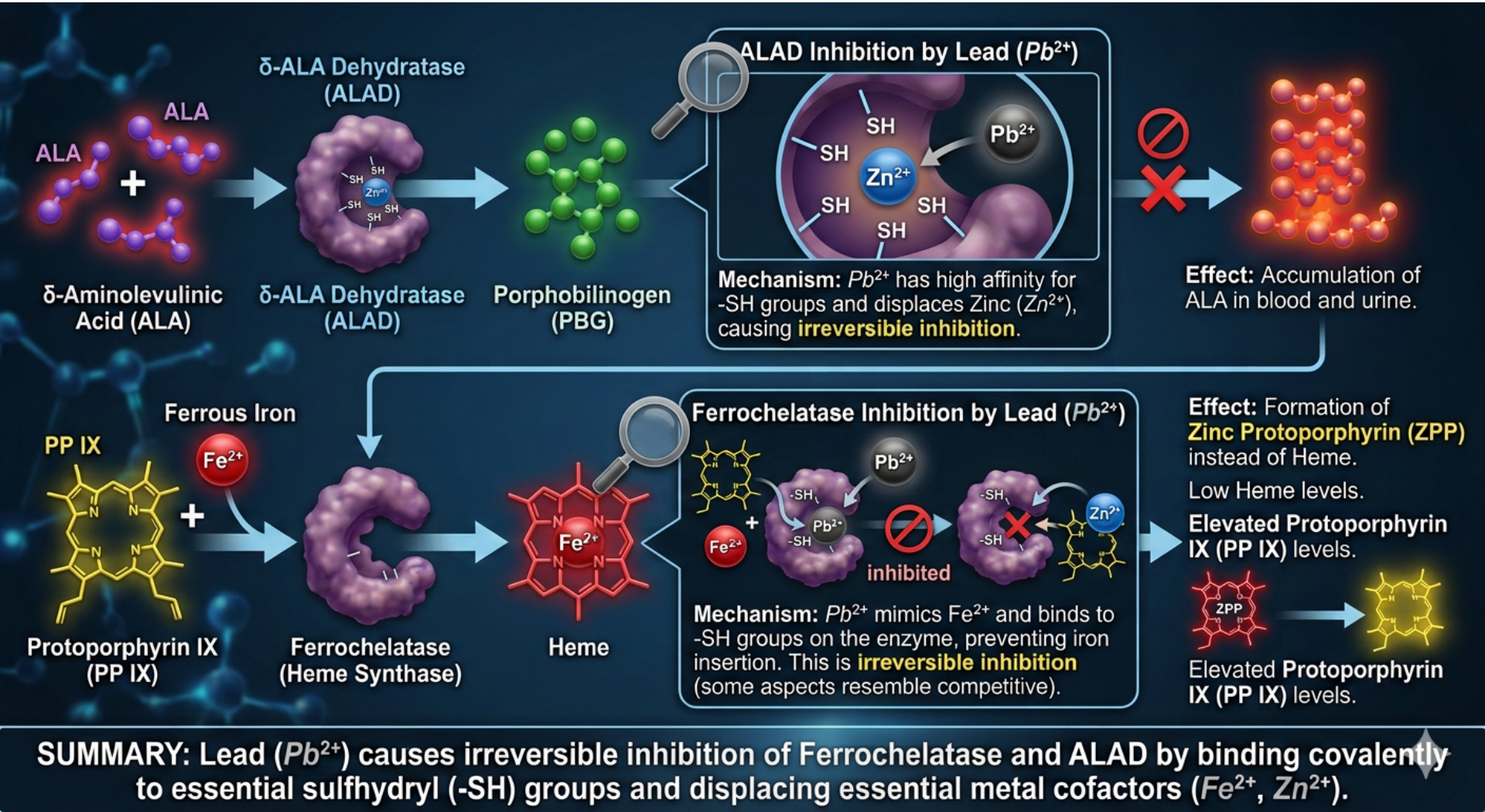
- **Isoniazide (INH)**
  - Used in Treatment of Tuberculosis
  - Decrease availability of Pyridoxal phosphate.
  - Decrease activity of ALA Synthase
  - Decrease Heme synthesis



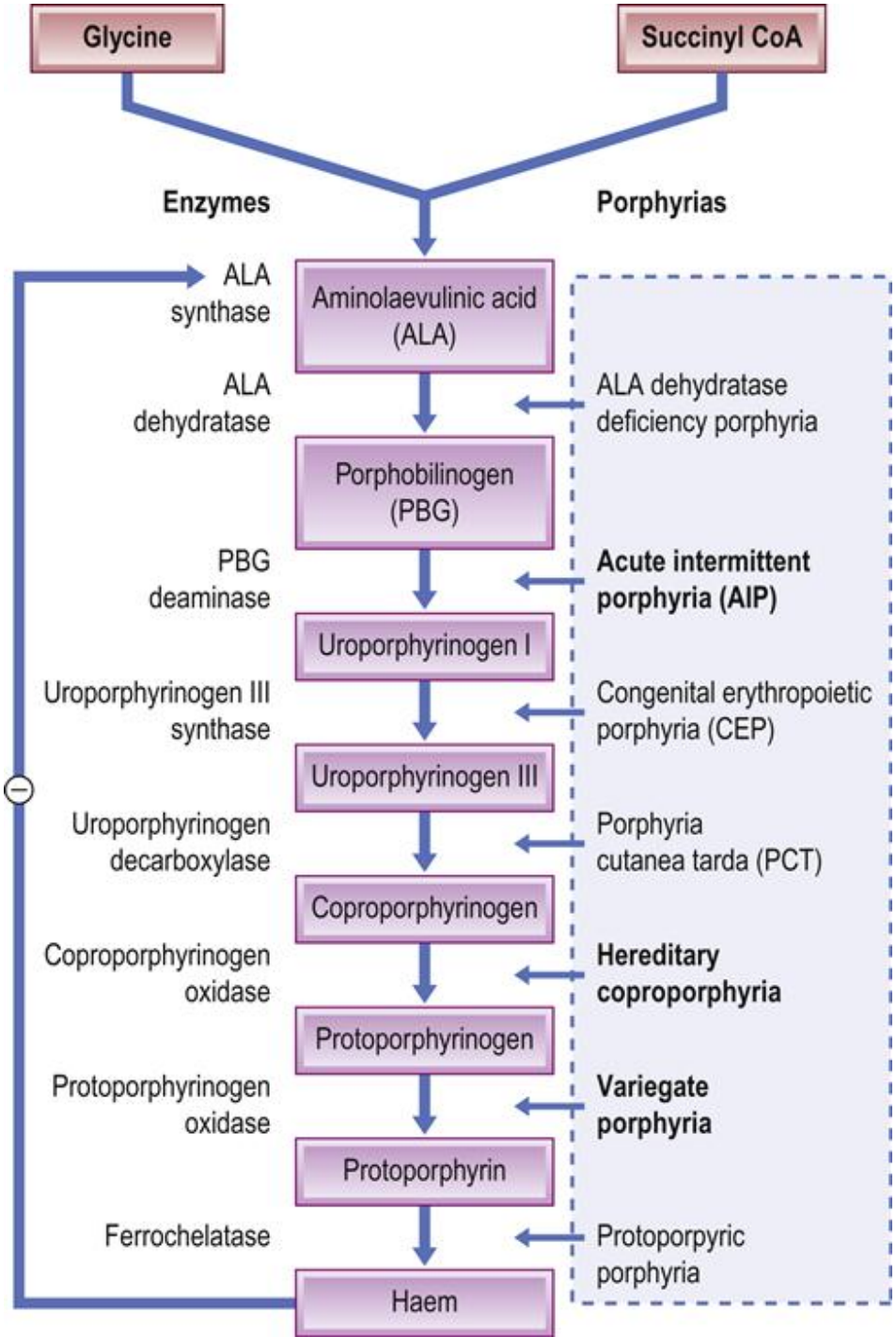
Regulation of Heme and Globin Synthesis

Drug effect on Regulation

- **Lead**
  - Non-Competitive - Irreversible inhibition
  - Inhibit ALA dehydratase enzyme.
  - Inhibit Ferrochelatase (Heme Synthase)enzyme.



SUMMARY: Lead (Pb^{2+}) causes irreversible inhibition of Ferrochelatase and ALAD by binding covalently to essential sulfhydryl (-SH) groups and displacing essential metal cofactors (Fe^{2+}, Zn^{2+}).


Glycine
Succinyl CoA
Enzymes
Porphyrias
ALA synthase
Aminolaevulinic acid (ALA)
ALA dehydratase
ALA dehydratase deficiency porphyria
Porphobilinogen (PBG)
PBG deaminase
Acute intermittent porphyria (AIP)
Uroporphyrinogen I
Uroporphyrinogen III synthase
Congenital erythropoietic porphyria (CEP)
Uroporphyrinogen III
Uroporphyrinogen decarboxylase
Porphyria cutanea tarda (PCT)
Coproporphyrinogen
Coproporphyrinogen oxidase
Hereditary coproporphyria
Protoporphyrinogen
Protoporphyrinogen oxidase
Variegate porphyria
Protoporphyrin
Ferrochelatase
Protoporpyric porphyria
Haem

Acute Intermittent Porphyria

- Autosomal dominant trait
- ***Deficiency of UPG – I synthase*** (***Porphobilinogen De-aminase***)
- Thus increase activity of UPG – III synthase.
- Increase level of ALA & PBG (Porphobilinogen)
- Due to Photo-Oxidation, PBG converted into Porphyrin.
- Most commonly, "***Acute Abdominal Pain***".
- Neurological manifestration
    - Sensory – Motor disturbances, Confusion, Mania
- ***Not Photosensitive sign***
- Female Sex hormone =Stimulate ALA synthase
    - AIP is more severe during menstruation.
    - AIP is less severe before menarche & after menopause.
- Attack is precipitated by Starvation
- Means Glucose helps to relieve attack.

Congenital Erythropoietic Porphyria

- Autosomal recessive trait
- ***Deficiency of UPG – III synthase*** ,
- Thus increase activity of UPG – I synthase.
- Increase level of Porphyrin – I (***Photosensitive***)
- Makes urine dark red colour.
- Porphyrin absorb light at 400 nm
- Emit intense Red light (Reactive Oxygen Species = Free Radical).
- ***"Mimic leprosy"***
  - Dermatitis
  - Facial deformoty (monkey facies)
  - Mutation of Nose, Ear & Cartilage

	Acute Intermittent Porphyria (Hepatic)	Congenital Erythropoietic Porphyria (Erythropoietic)
Primary Defect Zone	Hepatocyte (Cytosol)	Erythroid Cell (Mitochondria/Cytosol)
Enzyme Deficient	PBG Deaminase / Uroporphyrinogen I Synthase	Uroporphyrinogen III Synthase
Major Accumulation	Delta-Aminolevulinic acid (ALA) & Porphobilinogen (PBG)	Uroporphyrin I & Coproporphyrin I
Pathology	ALA is Neurotoxic	Photosensitive (ROS generation)
Clinical Features	Severe abdominal pain Neurological symptoms Psychiatric attacks	Blistering Photosensitivity Scarring Erythrodontia
Photosensitivity	No	Yes, Severe

Billirubin Synthesis (Heme Degradation)

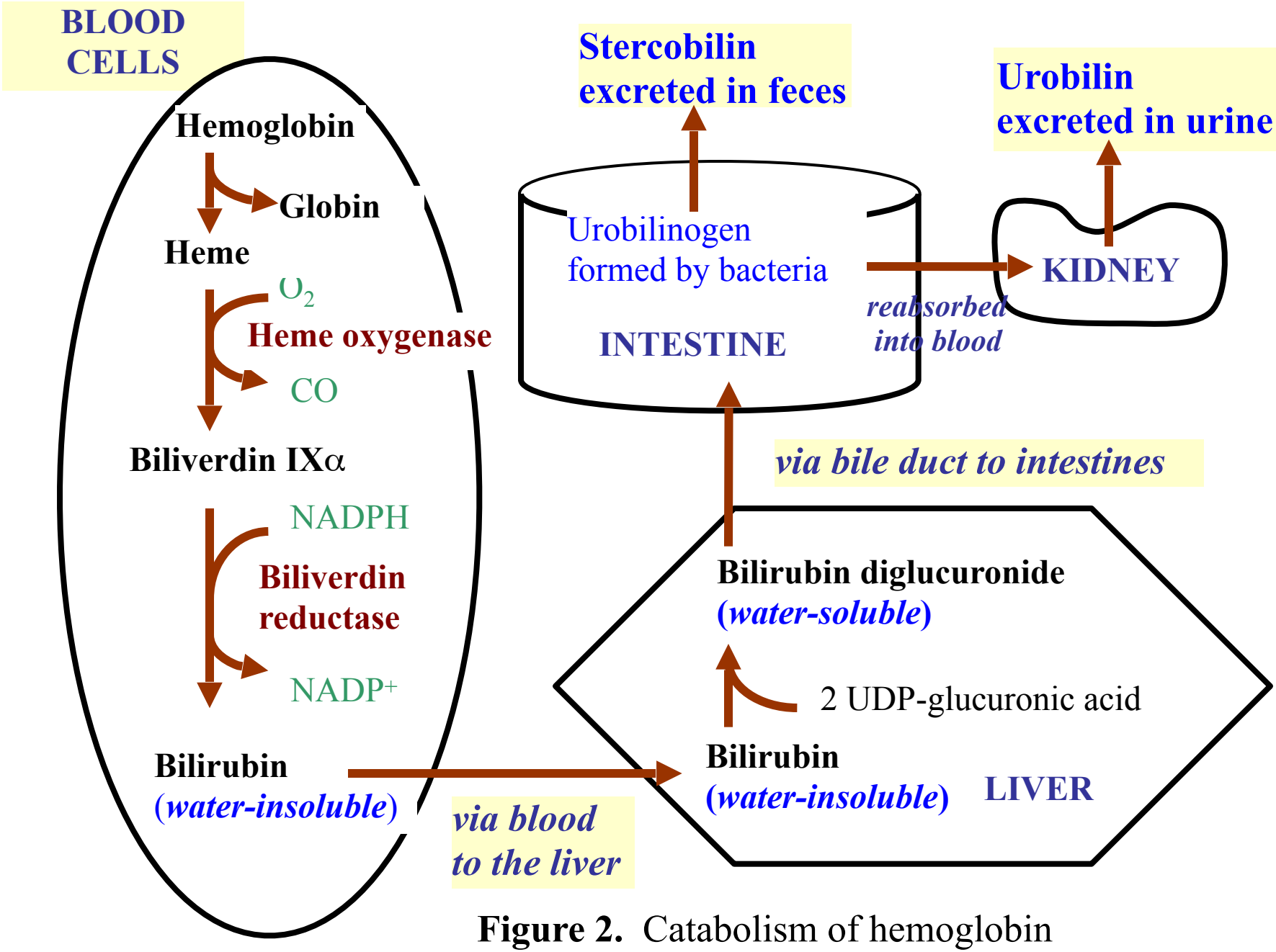
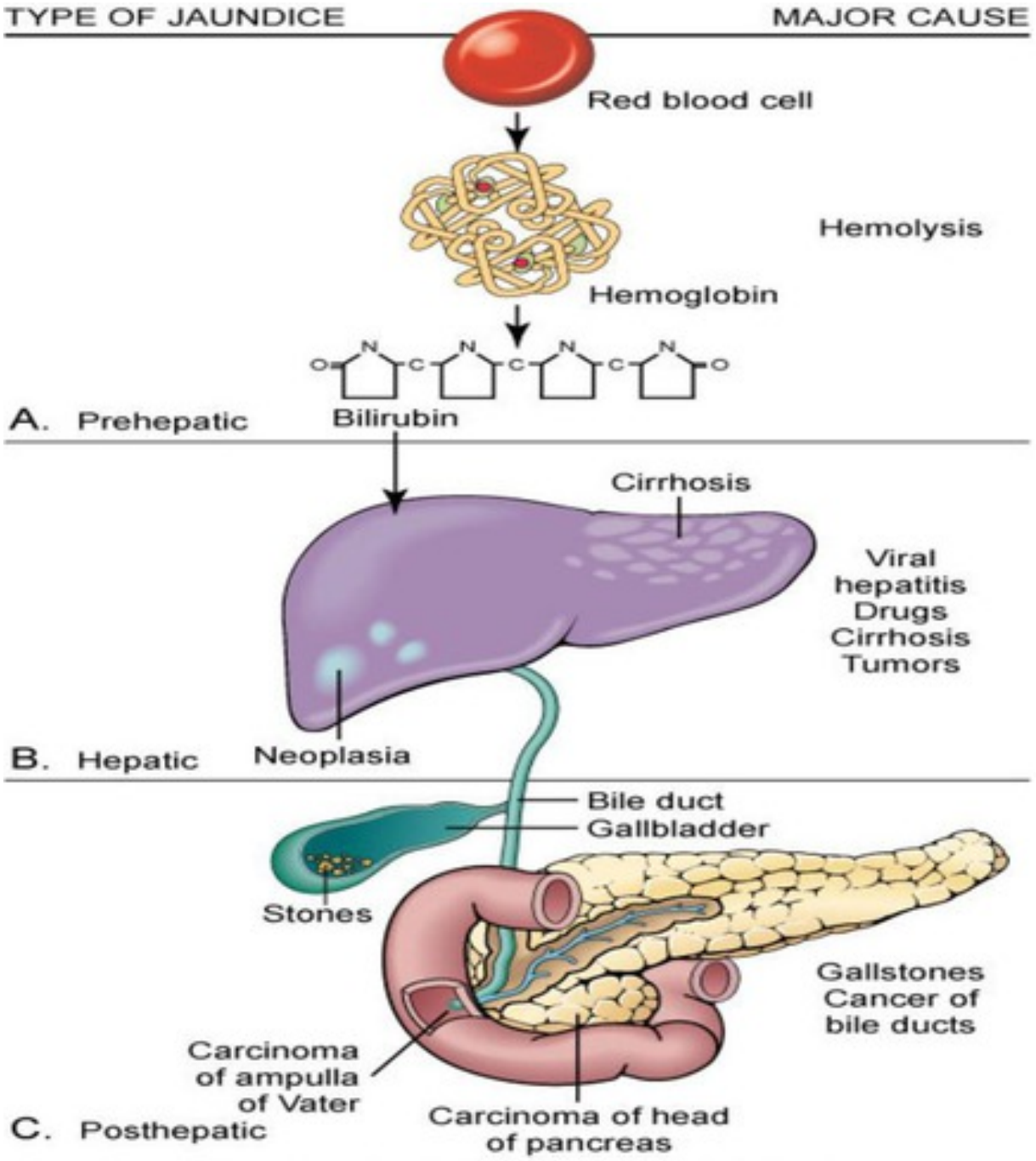


Figure 2. Catabolism of hemoglobin

- Which enzyme is require to convert unconjugated billirubin to conjugated billirubin?
- What are the disease related to this enzyme deficiency?
- What is diffirence between diglucoronic acid and mono-glucoronic acid



Type & Cause of Jaundice

➢ **Pre-hepatic Jaundice**

- ✓ Neonatal (Physiological) Jaundice
- ✓ Malaria
- ✓ G 6 PD deficiency
- ✓ Thalassaemia
- ✓ Sickle cell disease
- ✓ Mis-match Blood Transfusion
- ✓ Auto-immune

➢ **Intra-Hepatic Jaundice**

- ✓ Acute Viral hepatitis
- ✓ Alcohol Cirrhosis
- ✓ Cirrhosis of Liver
- ✓ Primray Biliary Cirrhosis,
- ✓ Haemochromatosis
- ✓ Wilson Disease
- ✓ Alpha-1 antitrypsin deficiency
- ✓ Drug induce – Quinine Group, NSAID, Chemotherapeutic drugs

Ø **Post Hepatic Jaundice**

ü Gall Bladder - Common Bile Duct - Pancreatic duct Stone

ü Gall Bladder - Hepatic – Pancreatic – Duodenal Carcinoma

Features	Pre-hepatic Heamolytic	Hepatic Hepatocellular	Post-hepatic Obstructive
Blood Examination			
Total Billirubin	↑↑	↑↑	↑↑
Direct Billirubin	Normal	↑	↑↑
Indirect Billirubin	↑↑	↑	Normal
ALT	Normal	↑ ↑	Normal
Alkaline phosphatase	Normal	Normal / ↑	↑ ↑
Urine Examination			
Bile Pigment	Normal	Normal / ↑	↑ ↑
Urobillinogen	↑ ↑	Normal / Absent	Absent
Bile Salt	Normal	Normal / ↑	↑ ↑
Stool Examination	Normal	Normal	Clay Colour
Specific Investigation	Haemoglobin, LDH	Liver Function Test	USG Abdomen

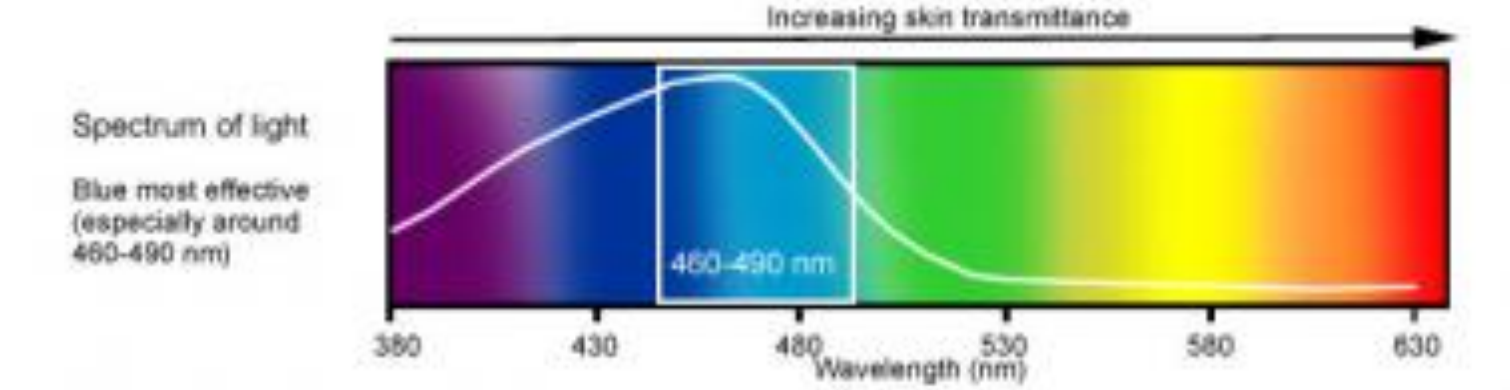
Genetic Disorders of Bilirubin Metabolism

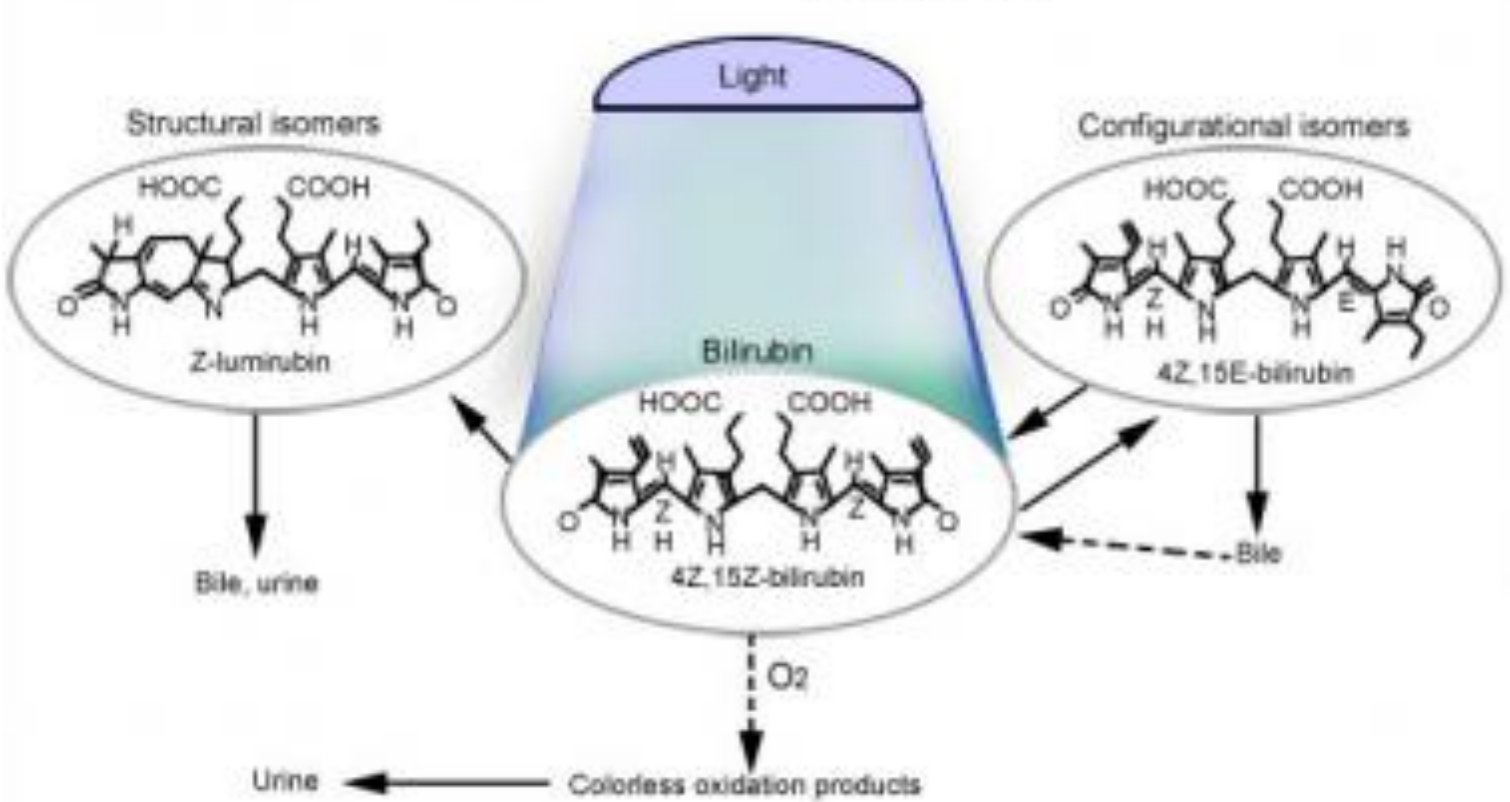
Name	Defect	Level of Serum Billirubin
Crigler-Najjar syndrome Type I	Complete deficiency of UDP-glucuronyltransferase	20 mg% Indirect Bilirubin
Crigler-Najjar syndrome Type II	Decrease (less than 10 %) activity of UDP-glucuronyltransferase	15 – 20 mg % Indirect Bilirubin
Gilberts syndrome	Decrease (Approx. 30 %) activity of UDP-glucuronyltransferase	1.4 - 5.0 mg % Indirect Bilirubin
Dubin-Johnson syndrome	Defect in transport of conjugated bilirubin from hepatocyte to biliary system	Direct Bilirubin

Role of Photopherapy

- Convert Bilirubin into Water Soluble Isomer
- So Excreted
- Normal bilirubin (4Z,15Z-bilirubin)
- After Exposer to Photopherapy (430 – 490 nm)
- 2 isomer forms of bilirubin
  - Structural = Z-lumirubin = Irreversible.
  - Configurational = 4Z,15 *E* –bilirubin = Reversible.
- Both are Less lipophilic than normal bilirubin
- Excreted into bile without Conjugation in the liver.

Role of Phototherapy





Phototherapy

Role of Phenobarbitone

- Induce Enzyme production
- Increase UDP-Glucoronate transferase Enzyme
- Increase Conjugation of Billirubin
- Excretion of Billirubin
- **Not useful in Criggler-Najar Syndrome Type – I**