

Peroxidases and Hemoglobins: Oxidation of halogenated phenols

NC State University

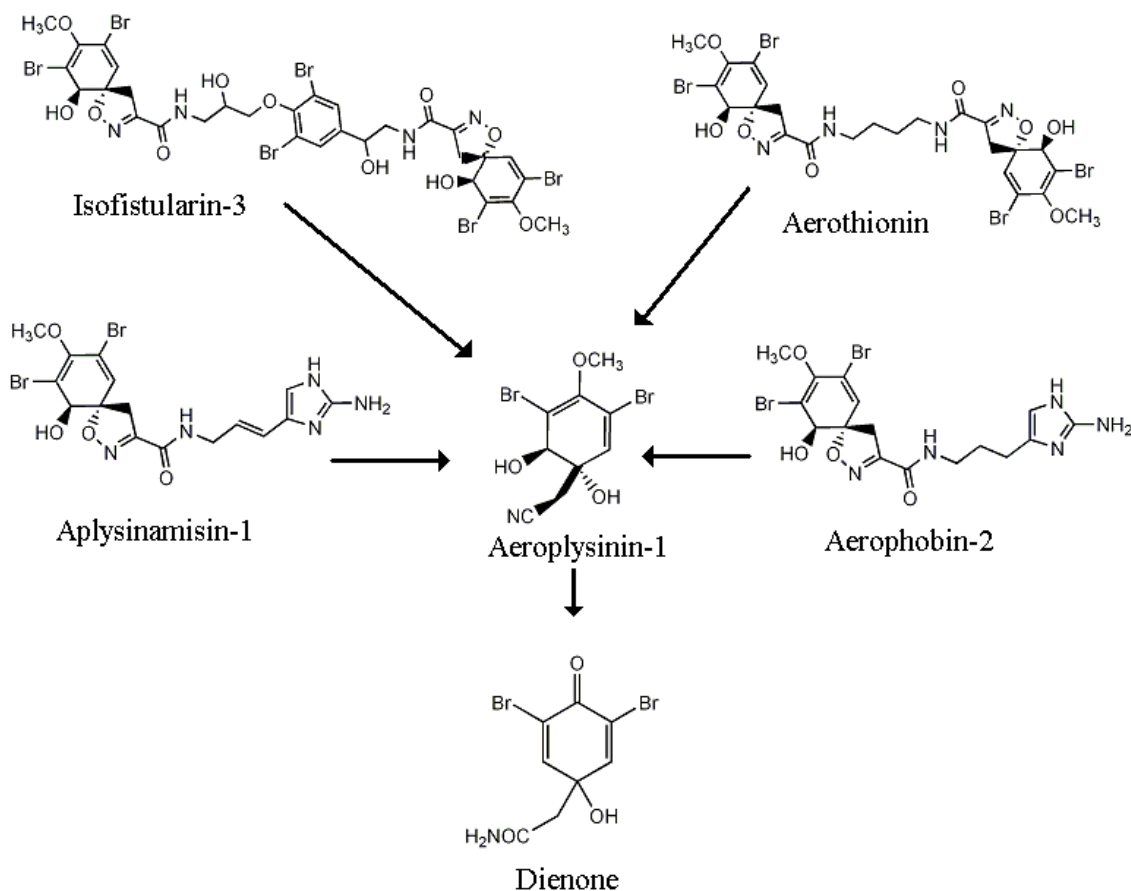
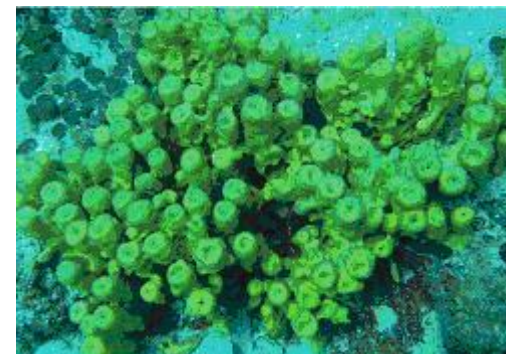
Why does the beach sometimes smell bad?



You can't blame it all on people.

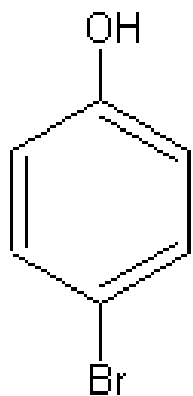
Protective Molecules Containing Bromine

The sponge metabolites of Mediterranean sponge *Aplysina aerophoba* provide an example for a wound-induced bioconversion of defensive metabolites.

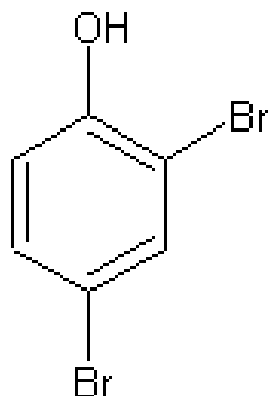


Common Organobromines from Marine Worms

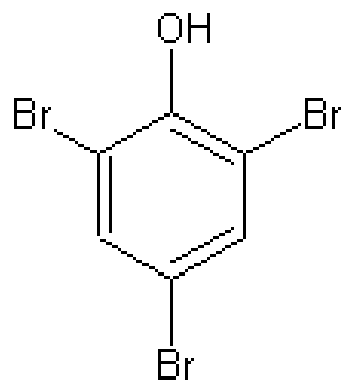
- Organobromines secreted by polychaetes discovered in 1960's.
- 2,6-dibromophenol (2,6-DBP) and 2,4,6-tribromopyrrole (2,4,6-TBP) were shown to have a deterrent effects against a wide variety of taxa.
- Fungicidal, bactericidal, ascaricidal, and molluscicidal effects.



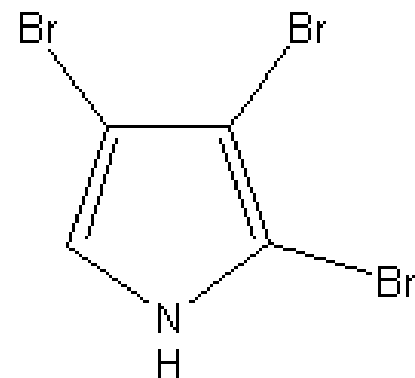
4 brom ophenol



2,4 brom ophenol



2,4,6- brom ophenol



2,3,4- bromopyrrole

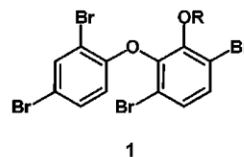
Brominated Compounds Produced by Algae

- Polybrominated diphenyl ethers (PBDE) found in mussels and birds in the Baltic sea.

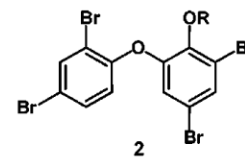
- Originate in red algae.

- Similar compounds found in green algae in Japan.

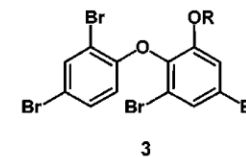
Chlorophyta (green algae)
 Phaetophyta (brown algae)
 Rhodophyta (red algae)



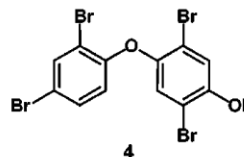
1
 R=H: 6'-OH-BDE49^S
 R=CH₃: 6'-MeO-BDE49^S



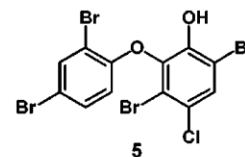
2
 R=H: 2'-OH-BDE68^{a, m, S}
 R=CH₃: 2'-MeO-BDE68^{a, m, S}



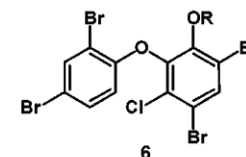
3
 R=H: 6-OH-BDE47^{a, m, S}
 R=CH₃: 6-MeO-BDE47^{a, m, S}



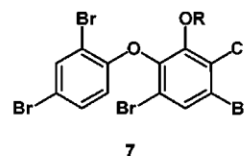
4
 4'-OH-BDE49^S



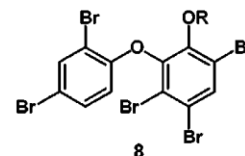
5
 3'-Cl-6'-OH-BDE49^S



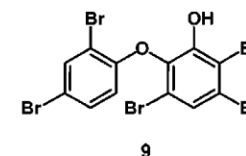
6
 R=H: 6'-Cl-2'-OH-BDE68^{m, S}
 R=CH₃: 6'-Cl-2'-MeO-BDE68^S



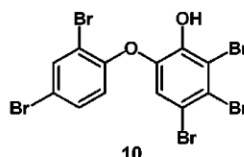
7
 R=H: 5-Cl-6-OH-BDE47^S
 R=CH₃: 5-Cl-6-MeO-BDE47^S



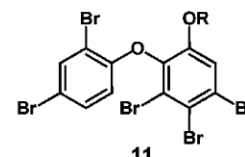
8
 R=H: 6-OH-BDE90^{a, m, S}
 R=CH₃: 6-MeO-BDE90^S



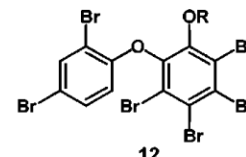
9
 6-OH-BDE99^{a, m, S}



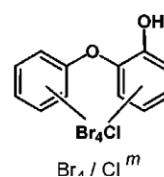
10
 2-OH-BDE123^{a, m}



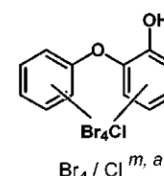
11
 R=H: 6-OH-BDE85^{a, m}
 R=CH₃: 6-MeO-BDE85^{a, m}



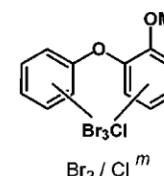
12
 R=H: 6-OH-BDE137^{a, m}
 R=CH₃: 6-MeO-BDE137^{a, m}



Br₄Cl^m



Br₄Cl^{m, a}



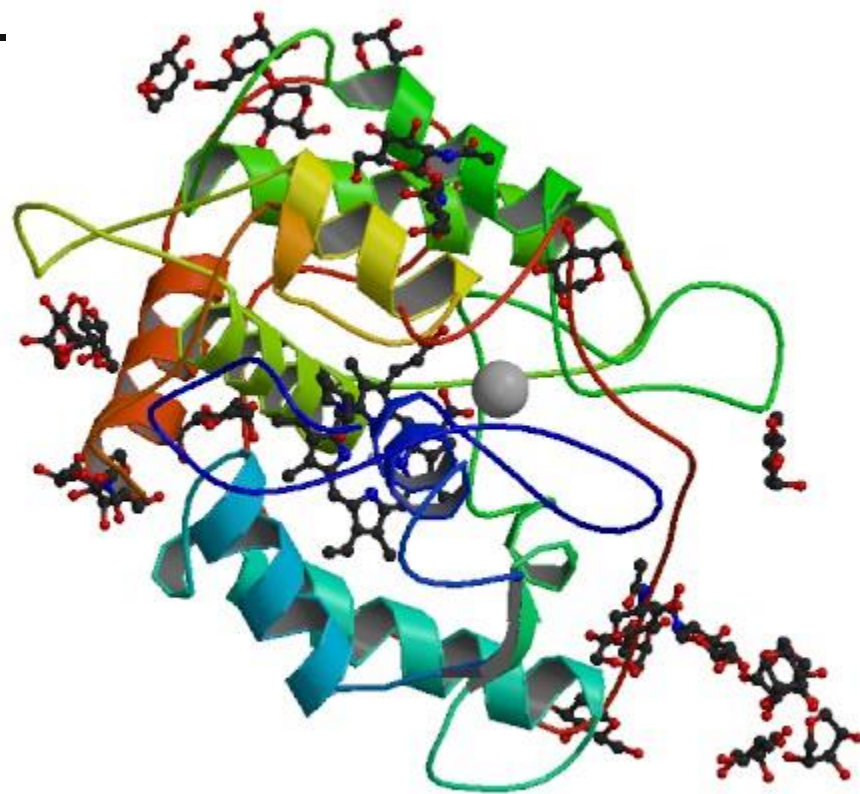
Br₃Cl^m

Example of a halogenating enzyme: Heme Haloperoxidases: CPO from *C. fumago*

Heme-thiolate haloperoxidases most versatile biocatalysts and similar to fungal peroxidases, cytochrome P450 monooxygenases, and catalases.

Chloroperoxidase (CPO) of *Caldariomyces fumago* is best best known.

Non-specific chlorination, bromination, and iodation of a variety of electrophilic organic substrates via hypohalous acid as halogenating agent.

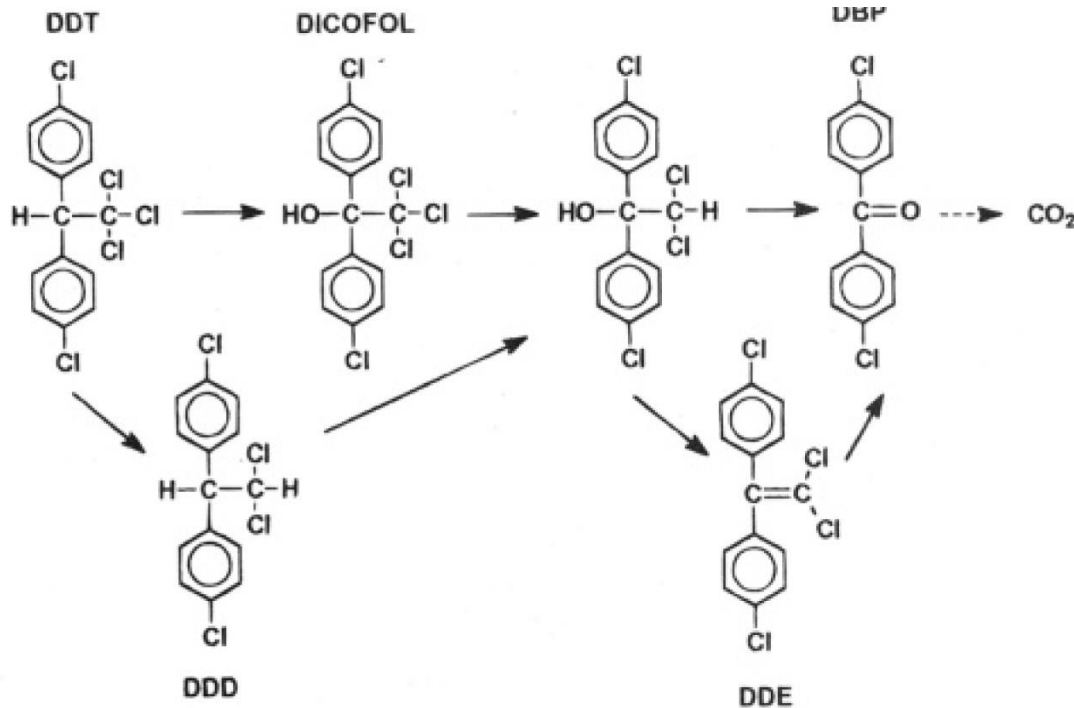


Welcome to the bromine flats



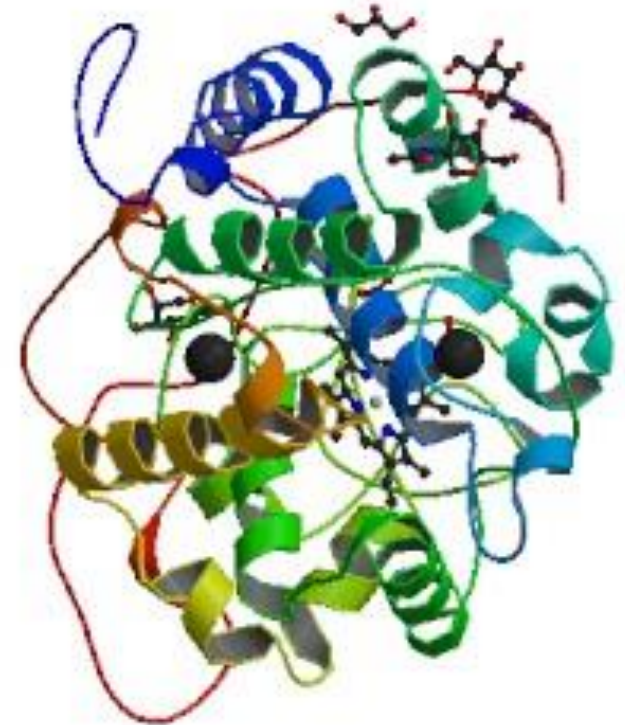
Who cleans up the mess?

Example of a dehalogenating enzyme: White rot fungus P450

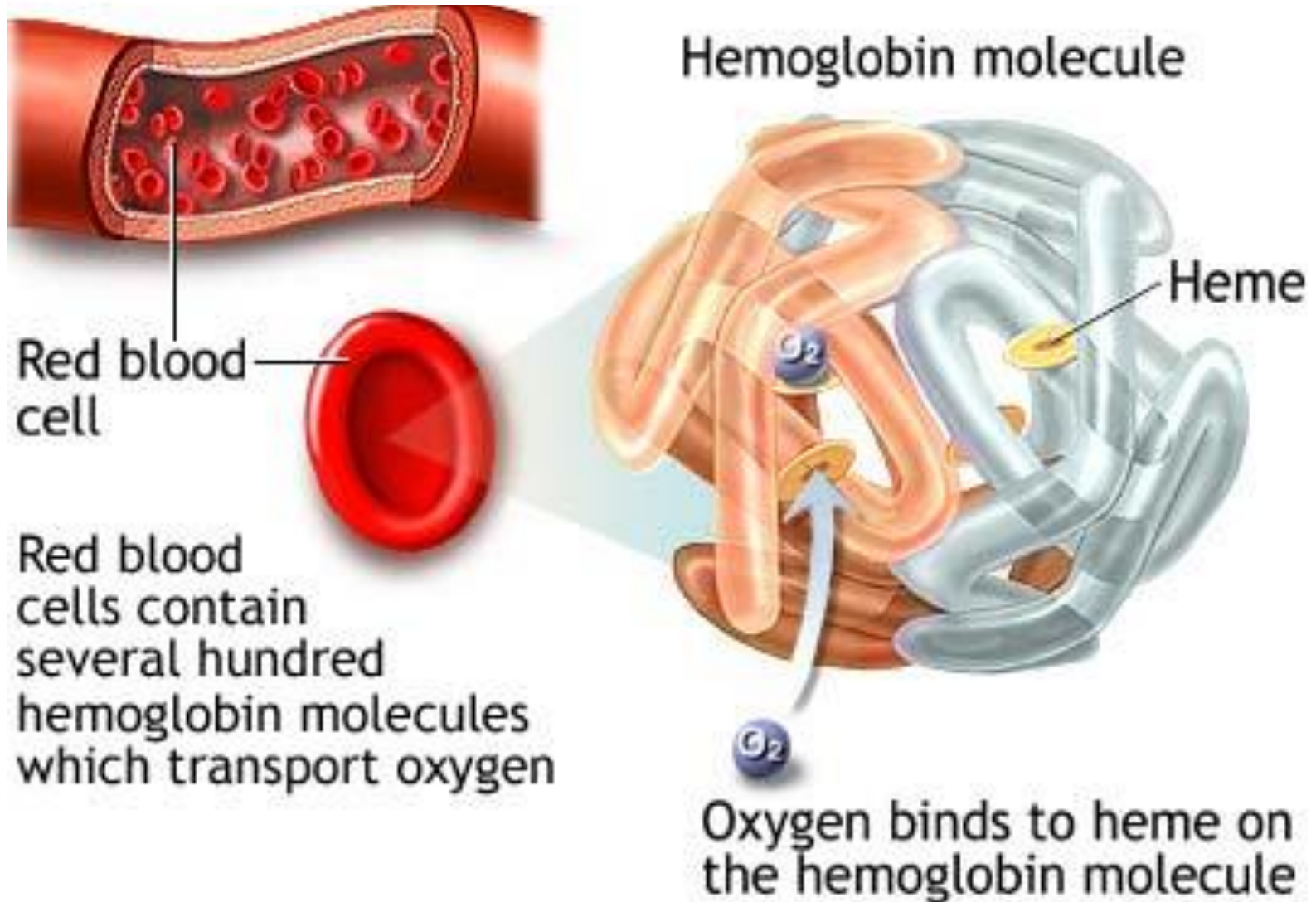


The ligninolytic system shows a high degree of non-specificity and oxidizes many compounds including DDT.

The white-rot fungi produce a specialized group of peroxidases, that catalyzes the degradation of the complex plant polymer lignin.



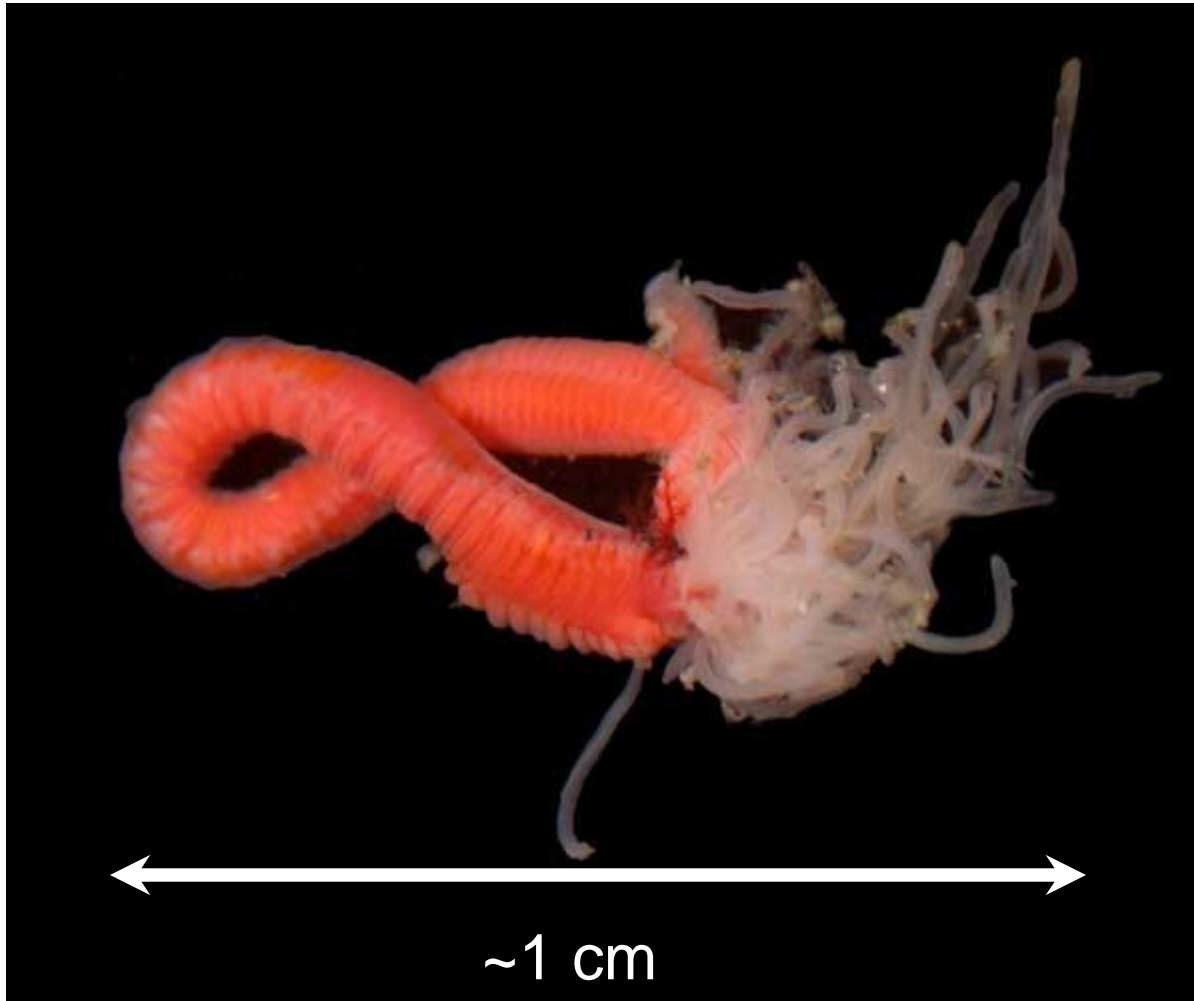
What does hemoglobin have to do with dehalogenation?



Dehaloperoxidase:
A Unique Enzyme Isolated
From *Amphitrite ornata*

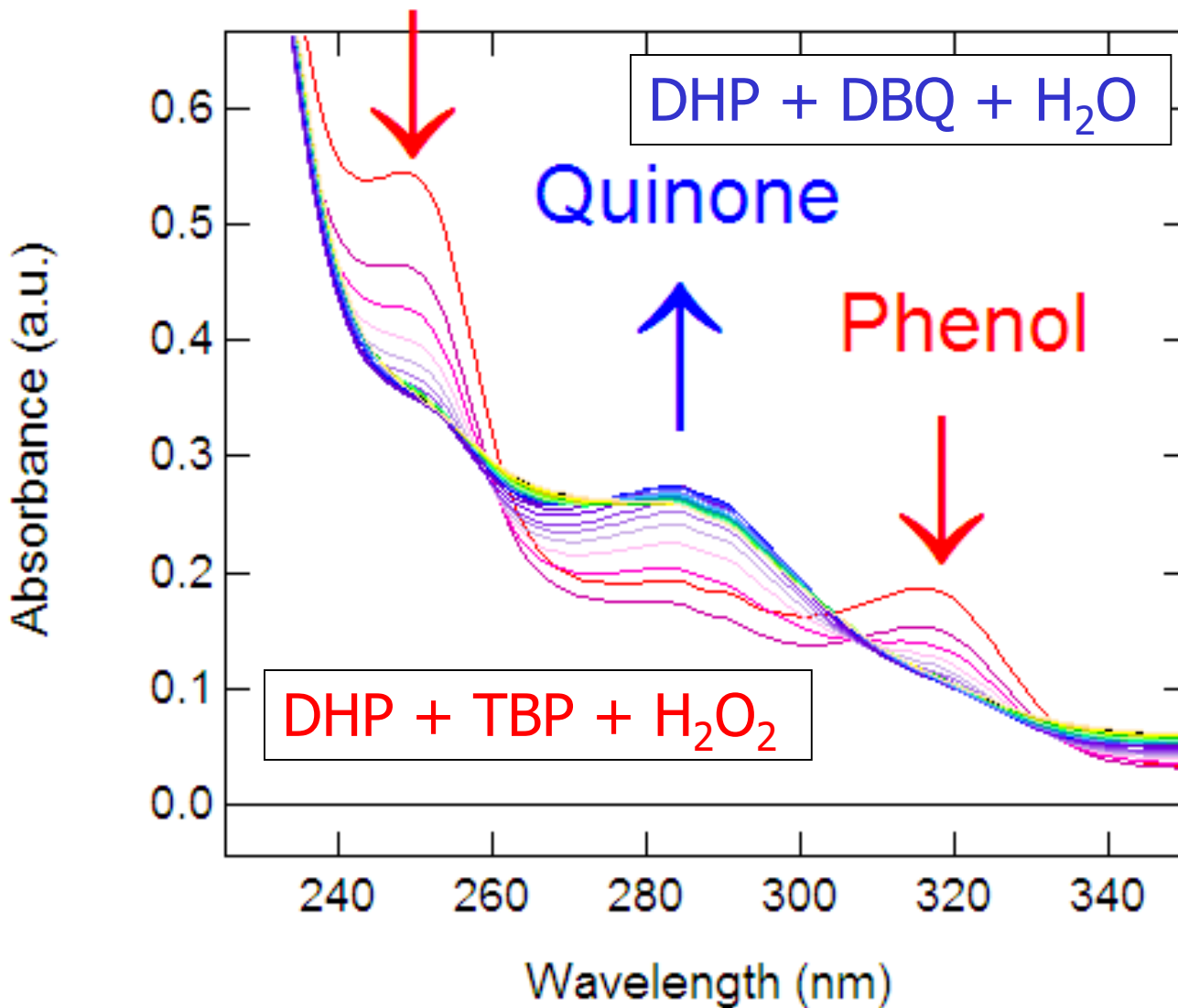
NC State University

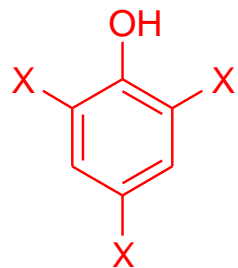
Amphitrite ornata



A terebellid polychaete

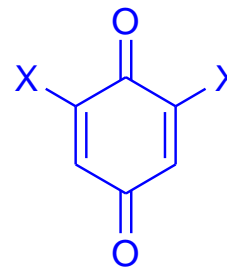
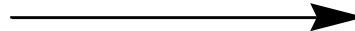
DHP oxidizes tribromophenol





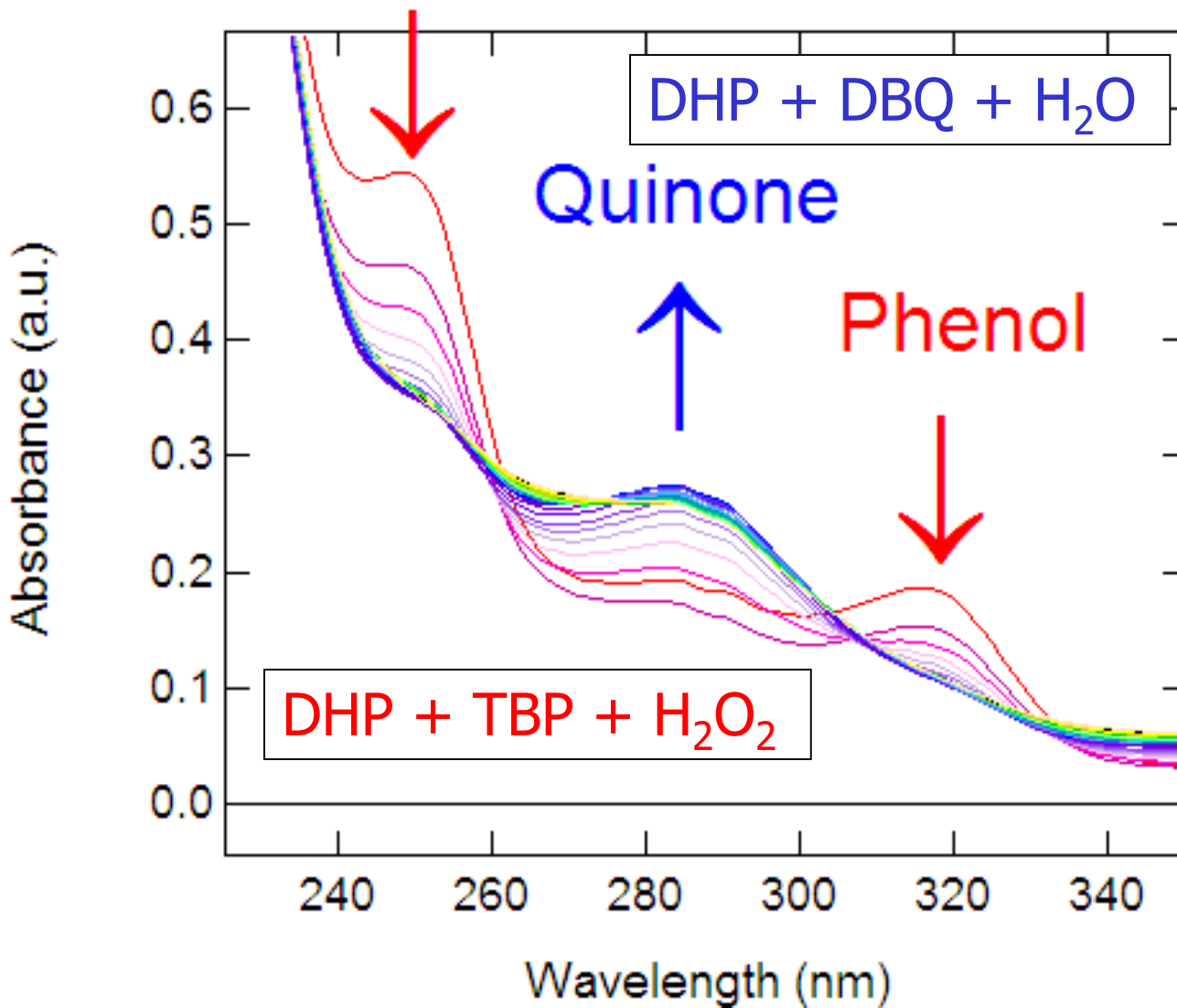
+ H₂O₂

DHP



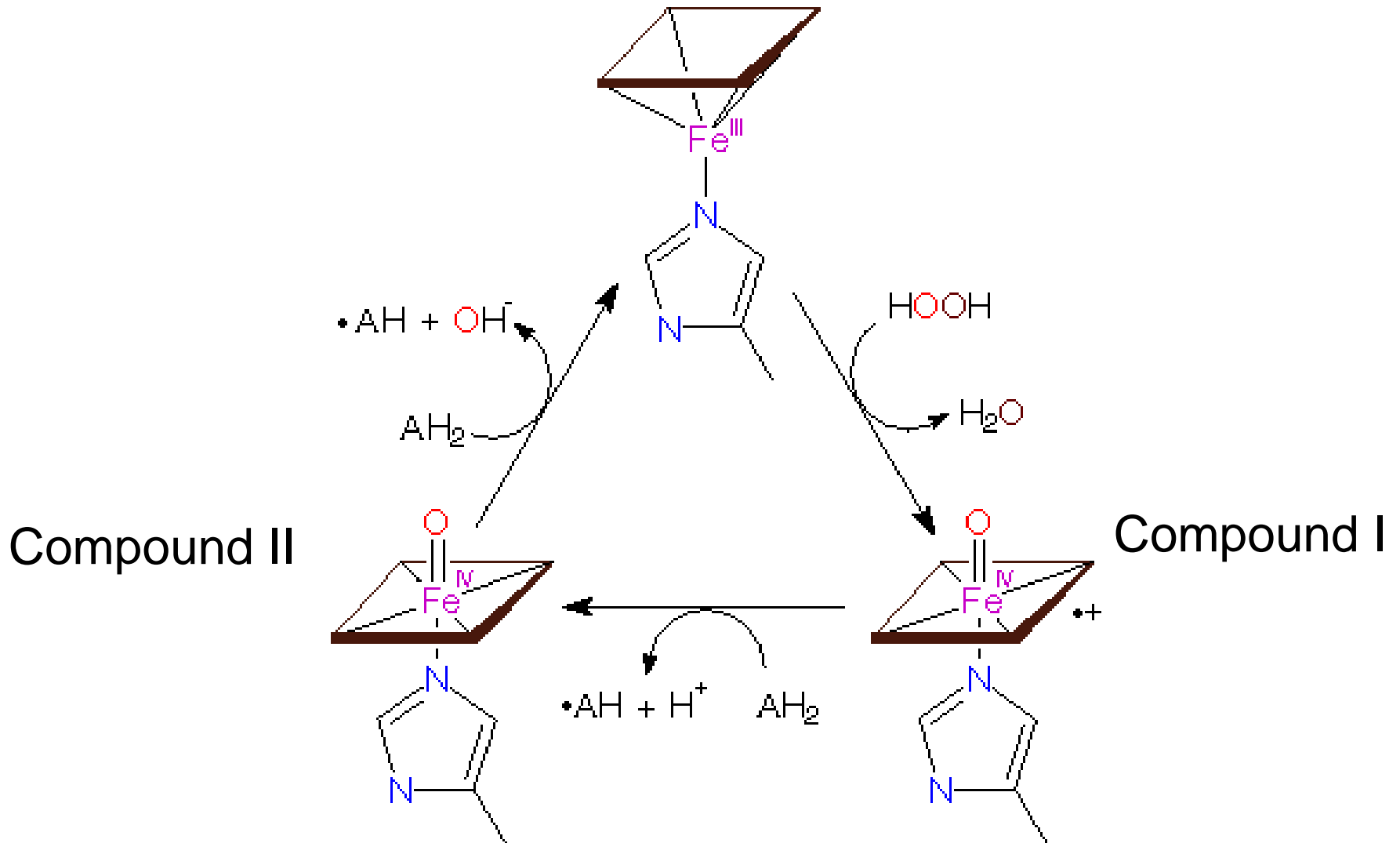
+ H₂O + H⁺X⁻

(X = I, Br, Cl, F)

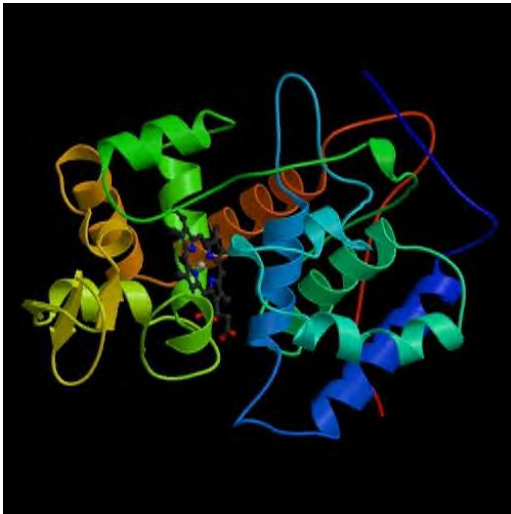


Peroxidase Mechanism

Resting Enzyme



Many Peroxidases belong to the Cytochrome c Peroxidase family



PDB: 1A2F

Cytochrome c Peroxidase (CCP)

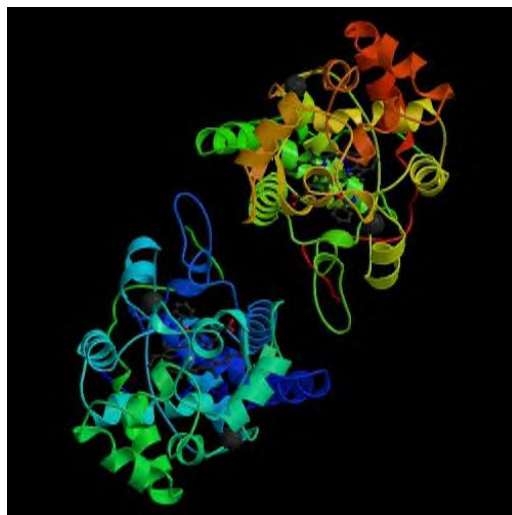
Class: All α proteins

Superfamily:

Heme peroxidases

Family: CCP-like

Goodin and McCree
Scripps Institute



PDB: 2ATJ

Horseradish Peroxidase (HRP)

Class: All α proteins

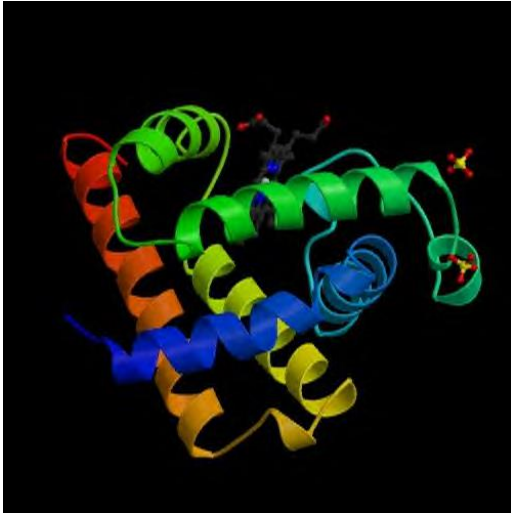
Superfamily:

Heme peroxidases

Family: CCP-like

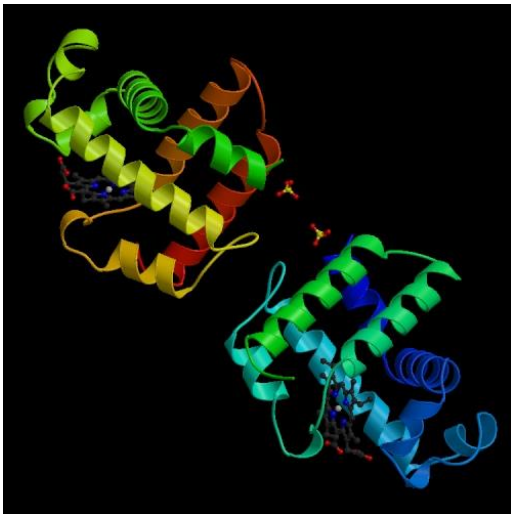
Hendrickson et al.
Biochemistry (1998)
37, 8054

Dehaloperoxidase is a peroxidase that belongs to the globin family



PDB: 1A6G
Myoglobin (Mb)
Class: All α proteins
Superfamily:
 Globin-like
Family: Globins

Vojtechovsky,
Berendzen,
Schlichting



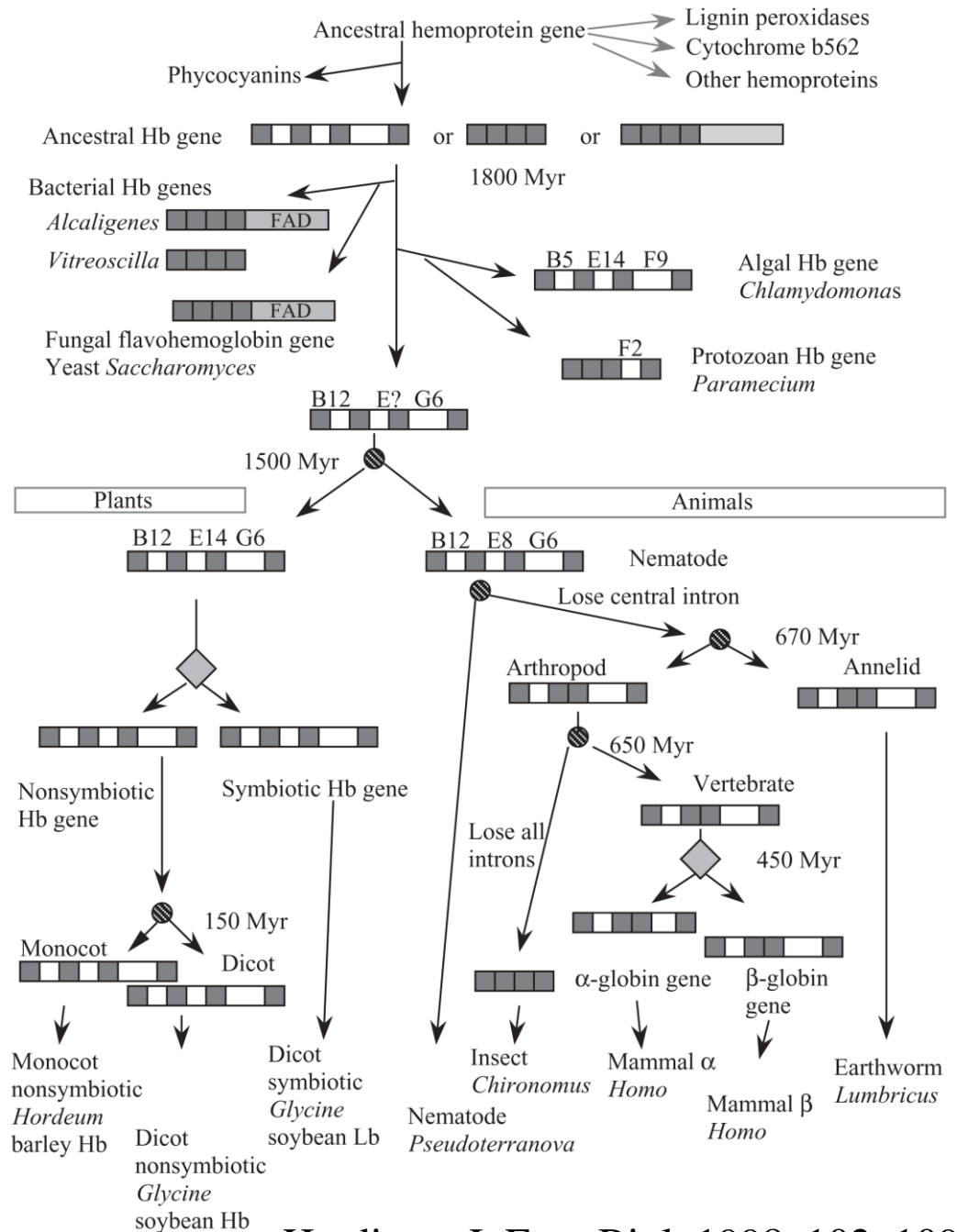
PDB: 1EW6
Dehaloperoxidase (DHP)
Class: All α proteins
Superfamily:
 Globin-like
Family: Globins

Lebioda et al.
J.Biol.Chem. 275
18712 (2000)

Globins and Peroxidases diverged 1.8 billion years ago

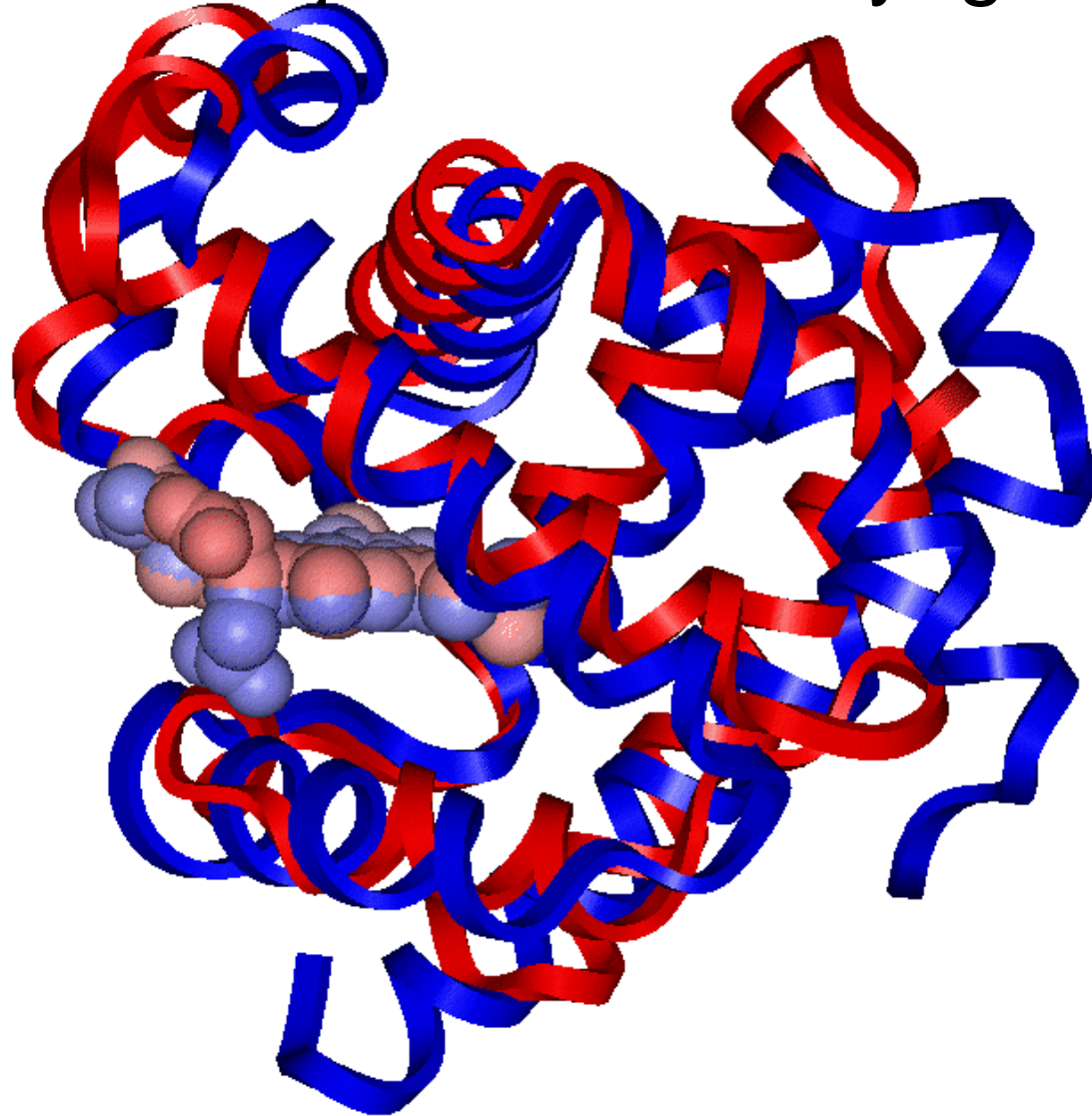
Implicit meaning:
Ancestral protein was both a hemoglobin and a peroxidase

Terrebellid polychaetes do not figure in the scheme



Comparison of DHP and SWMb Structures

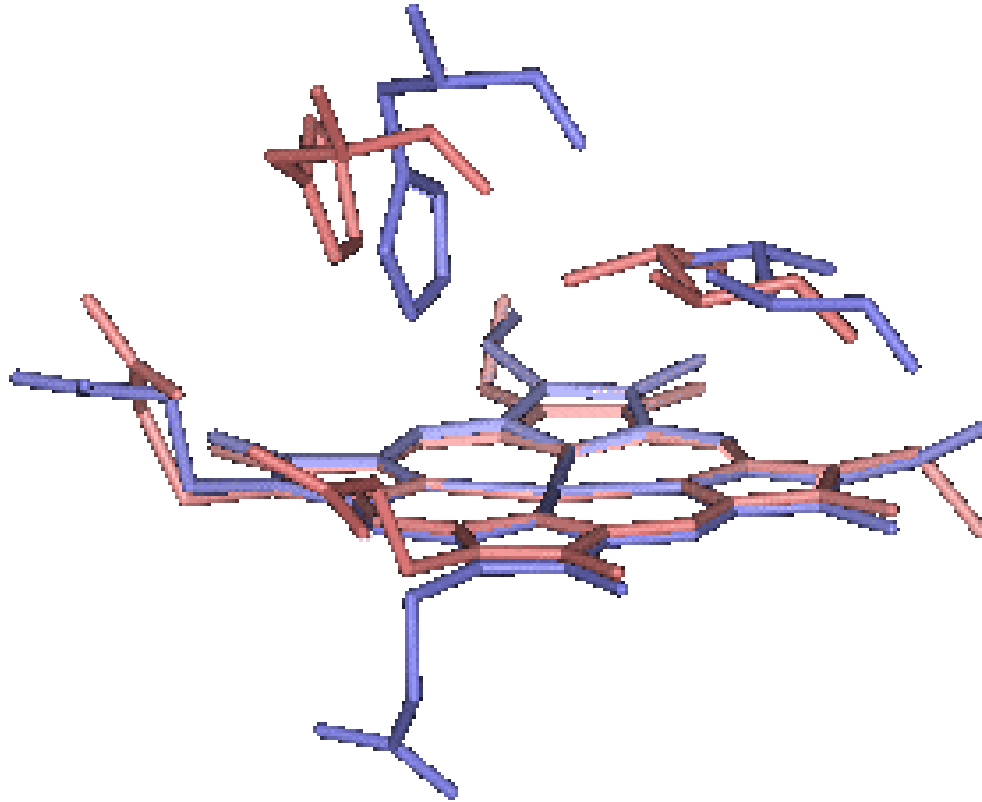
SWMb = Sperm Whale Myoglobin



SWMb

DHP

Overlay of active sites



Mb

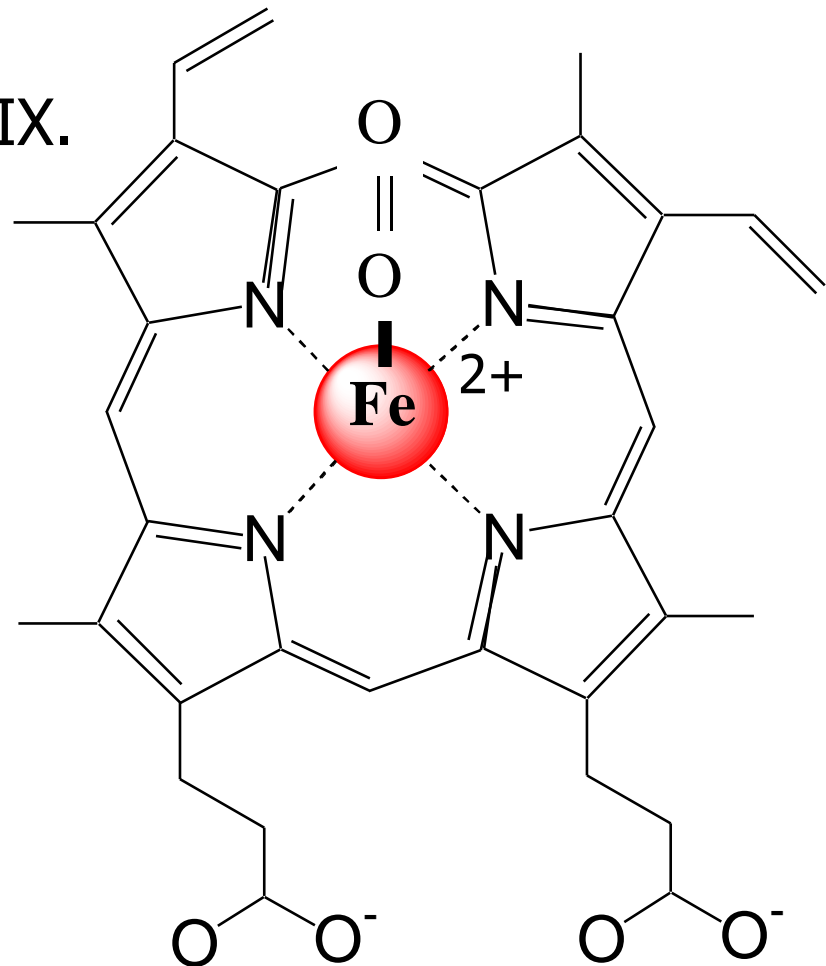
DHP

Globins have ferrous iron and bind O₂

Heme is iron protoporphyrin IX.

Functional aspects in Mb

1. Discrimination against CO binding.
2. O₂ is the physiologically relevant ligand, but it can oxidize iron (autooxidation).

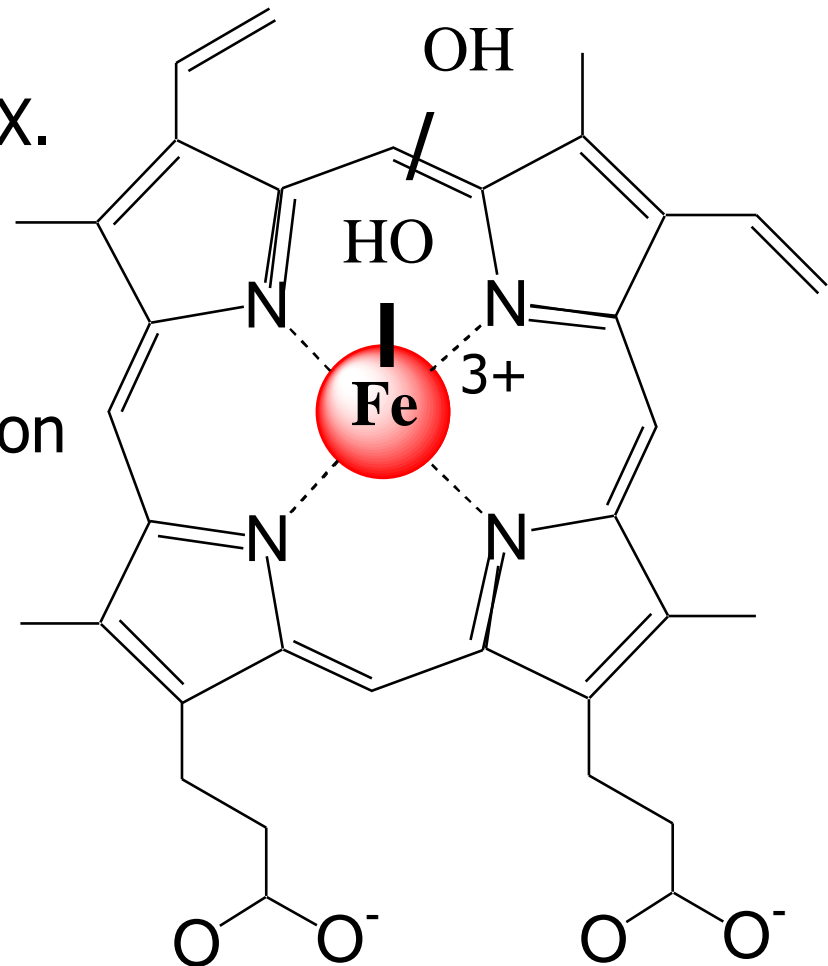


Peroxidases have ferric iron and bind H_2O_2

Heme is iron protoporphyrin IX.

Functional aspects in HRP

1. Activation involves formation of compounds I and II.
2. Edge electron transfer to substrate.



DHP oxidation state may switch during peroxidase function

How can a protein be both
and globin and a
peroxidase?

What is the trigger?

Substrate binding must
be the key.

