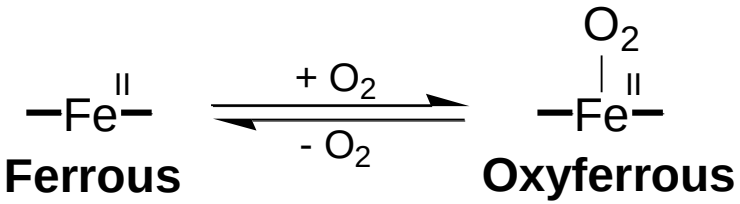
The Dehaloperoxidase Paradox:
How can one structure provide different functions?

NC State University

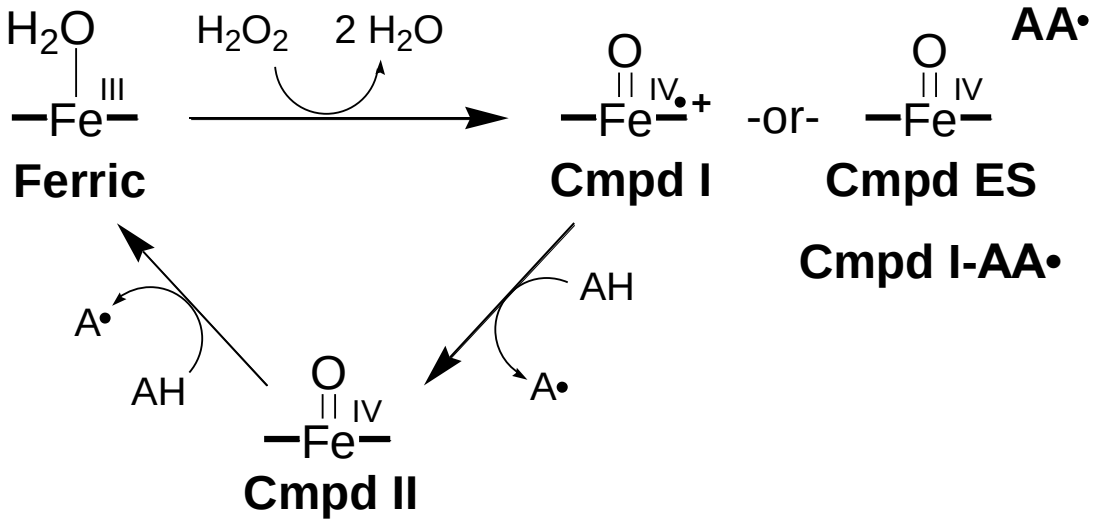
Raleigh, North Carolina

The Dehaloperoxidase Paradox

- O_2-Transport
  - Reversible O_2-binding is mediated by only a ferrous heme



- Peroxidase Activity
  - Ferric resting state; oxyferrous is inactive



Two major functions related to oxygen in living organisms

Transport: requires that the O_2 molecule bind reversibly to a metal (Fe or Cu).

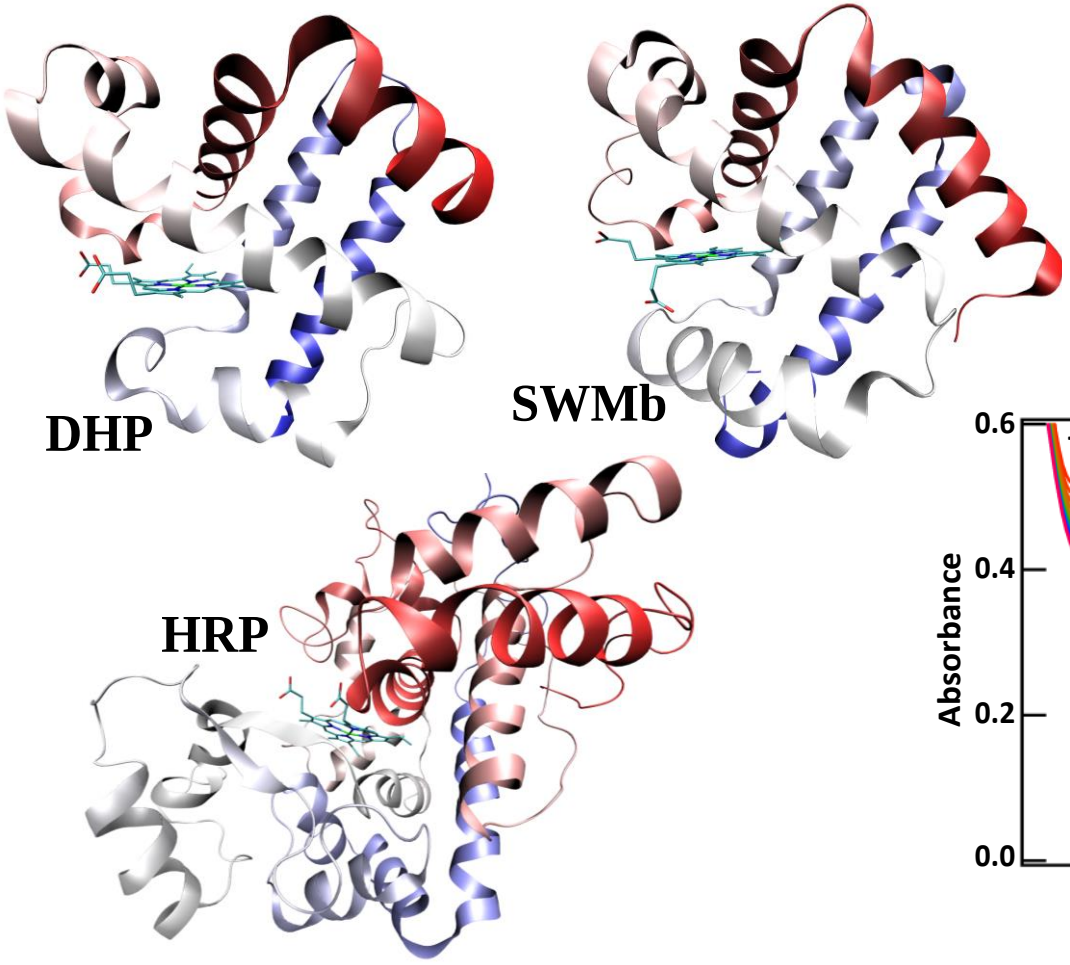
Activation: requires that the O-O bond of the O_2 molecule is cleaved leading to chemical change.

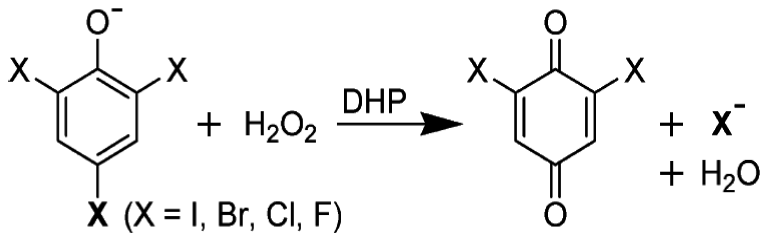
These two functions are normally thought to be mutually exclusive.

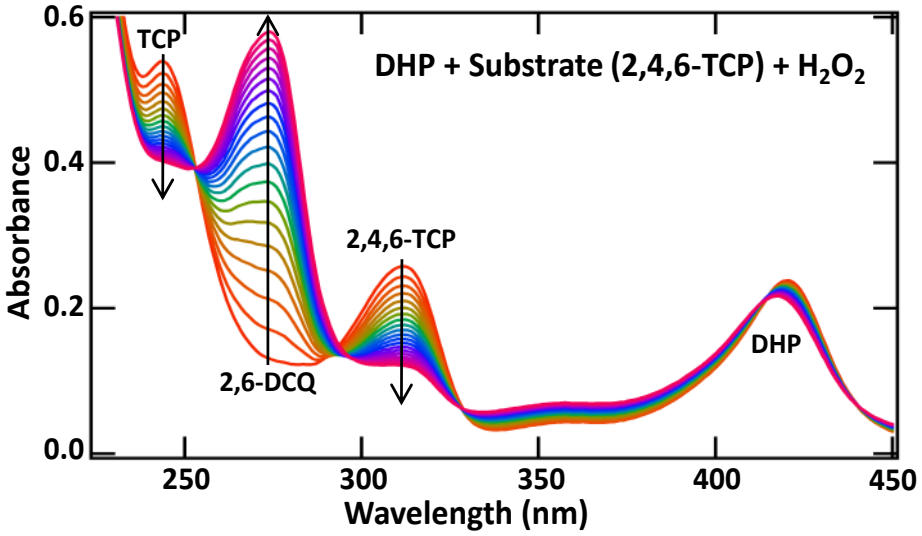
Belyea et al. Biochemistry 2005, 44, 15637
Franzen et al. BBA 2007, 1774, 121
Feducia et al. Biochemistry, 2009, 48, 995
Zhao et al. J. Phys. Chem. B (2012), 116, 12065
Zhao et al. J. Phys. Chem. B (2013), 117, 8301

Chen et al. J. Biol. Chem. (1996) 271., 10515
Osborne et al. BBRC (2004) *324*, 1194
Du et al. Biochemistry (2010) 49, 6404
Davydov et al. JACS (2010) 132, 14495
Wang et al. Biochemistry, (2013), 52, 6203

DHP has a globin structure and functions as both a hemoglobin and a peroxidase



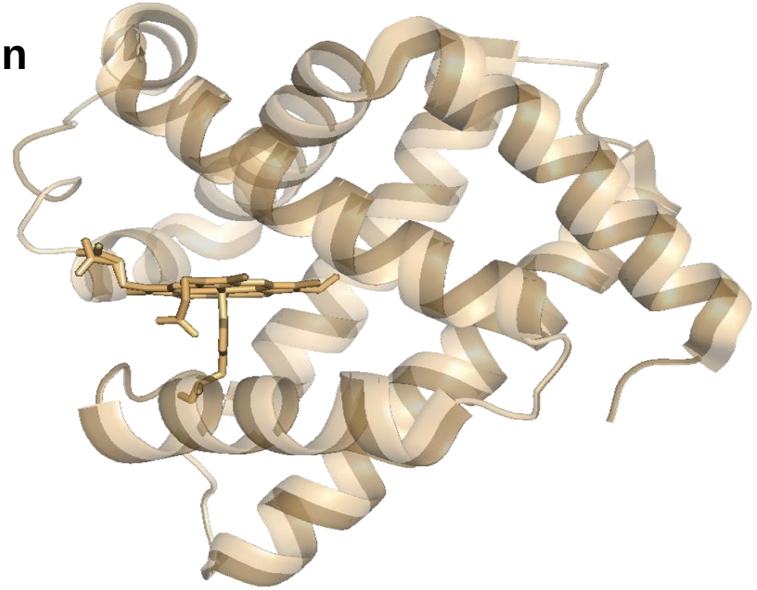


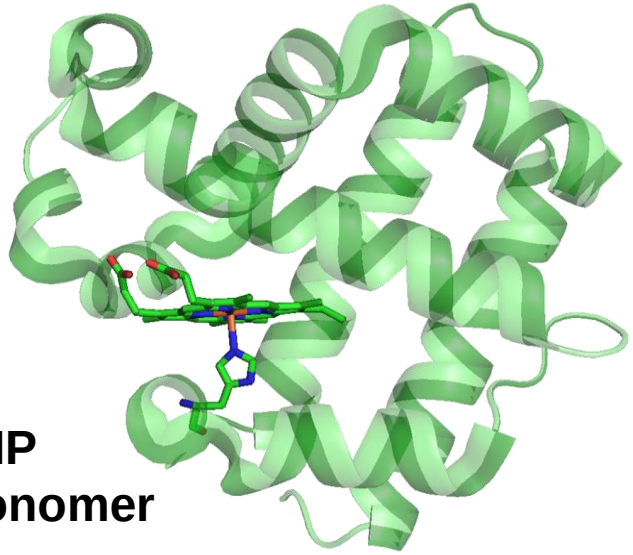


Recent work suggests other functions are possible.

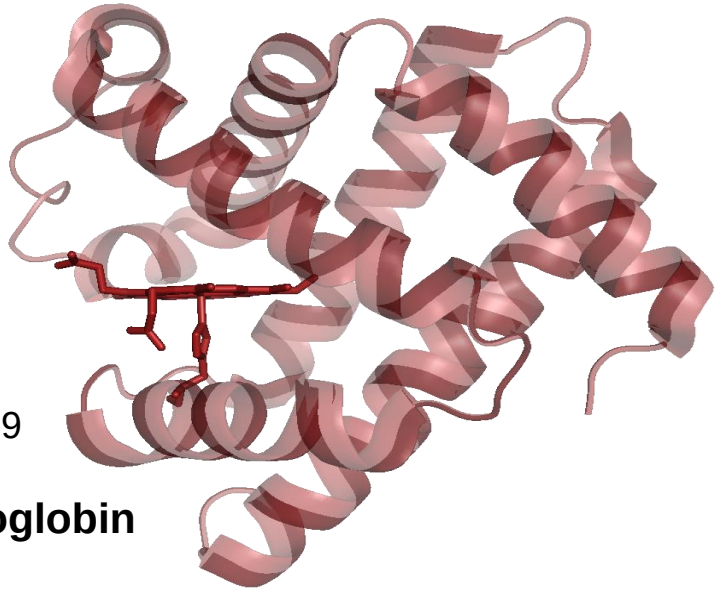
DHP monomer has a globin fold and function

Horse Heart Myoglobin





DHP monomer



Sperm Whale Myoglobin

DHP was first discovered as an Oxygen storage protein in *A. ornata*

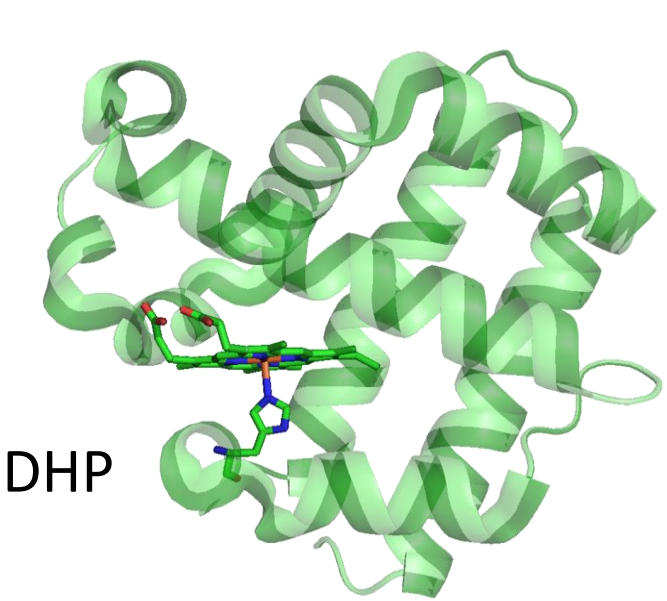
Bonaventura et al. *Comp. Biochem. Phys.* 1977, *56A*, 179

DHP has a natural peroxidase function

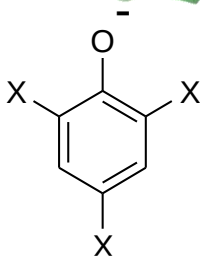
Engineered globin peroxidases

Mauk group

Watanabe group



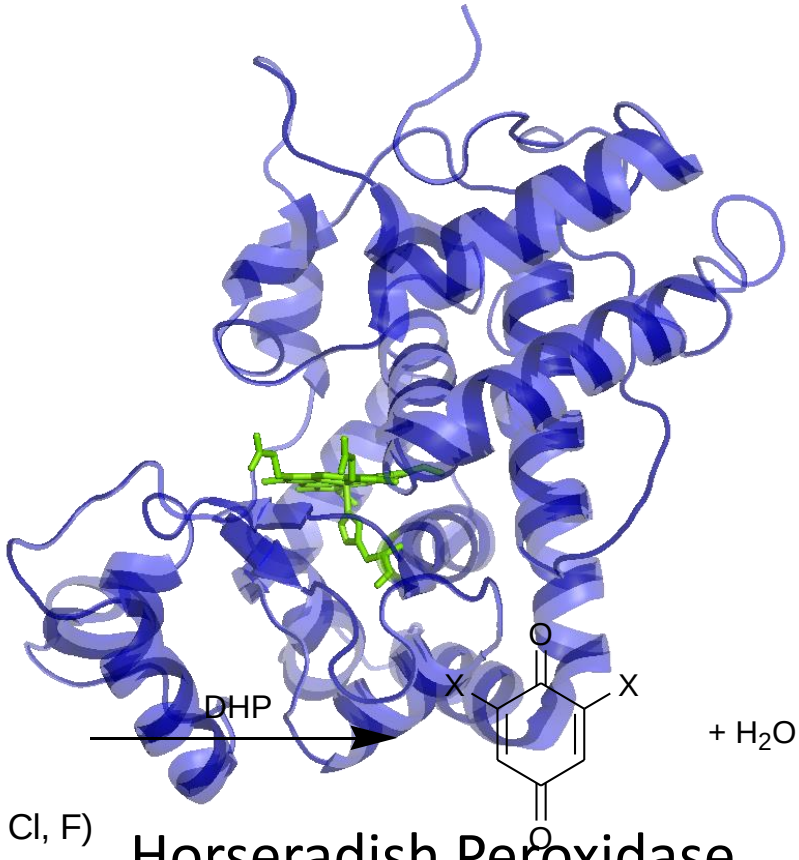
DHP



Trihalogenated Phenol

+ H_2O_2

(X = I, Br, Cl, F)



Horseradish Peroxidase

+ H_2O + X^-

Dihalogenated Quinone

Two unique features of DHP

Enlarged (or flexible) distal pocket: permits binding of a range of substrates including some very large aromatic molecules.

High reduction potential: permits function of "shifted" peroxidase cycle and other non-standard chemistries Such as peroxygenase, or sulfide oxidase.

How do these features expand the repertoire of catalytic functions?

Anomalous redox potential of DHP

DHP

E_0 = +221 mV

Globins

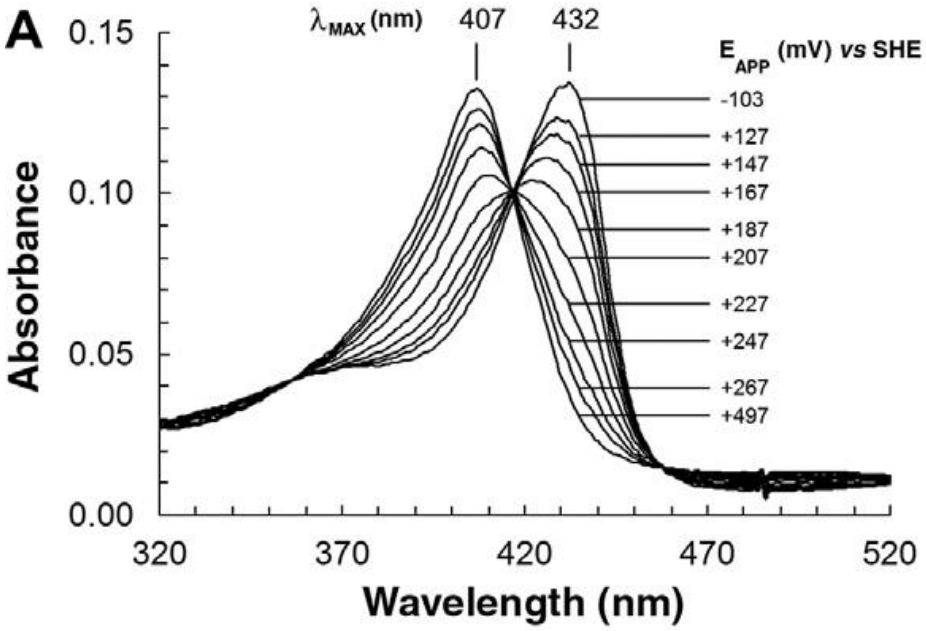
E_0 = +50 mV

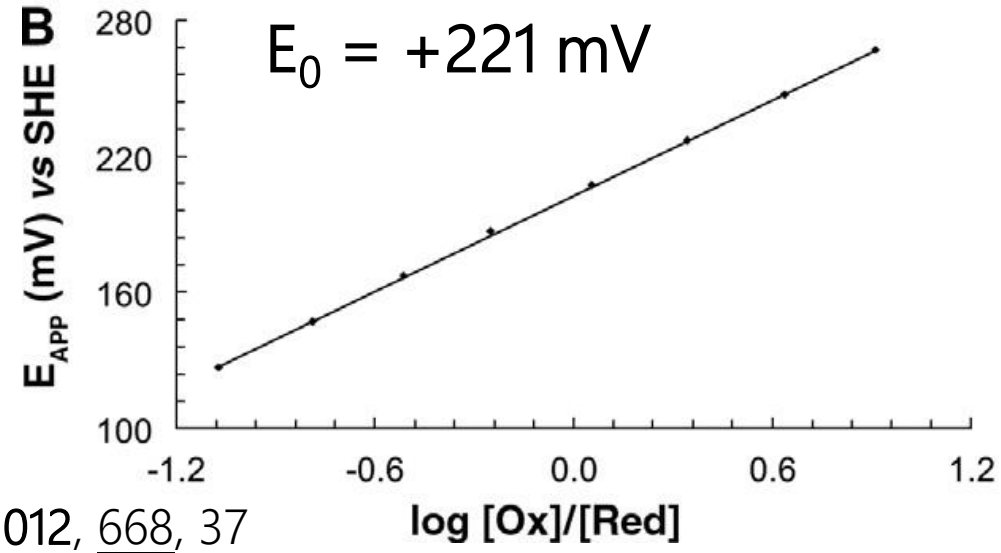
Peroxidases

E_0 = -270 mV

Fe^{2+}

Fe^{3+}

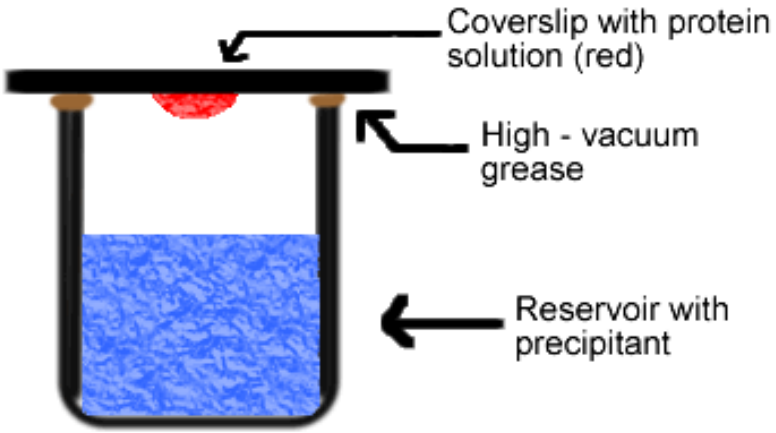




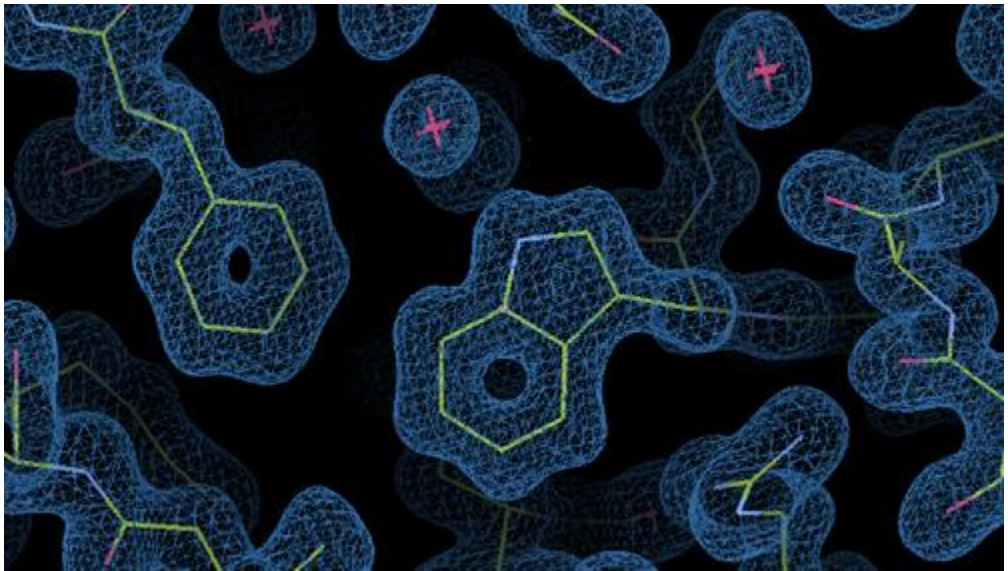
E_0 = +221 mV

D'Antonio et al. J. Electroanal. Chem. **2012**, 668, 37

Protein Crystallography



Crystallized protein is used to determine the protein's 3-D structure via X-ray diffraction

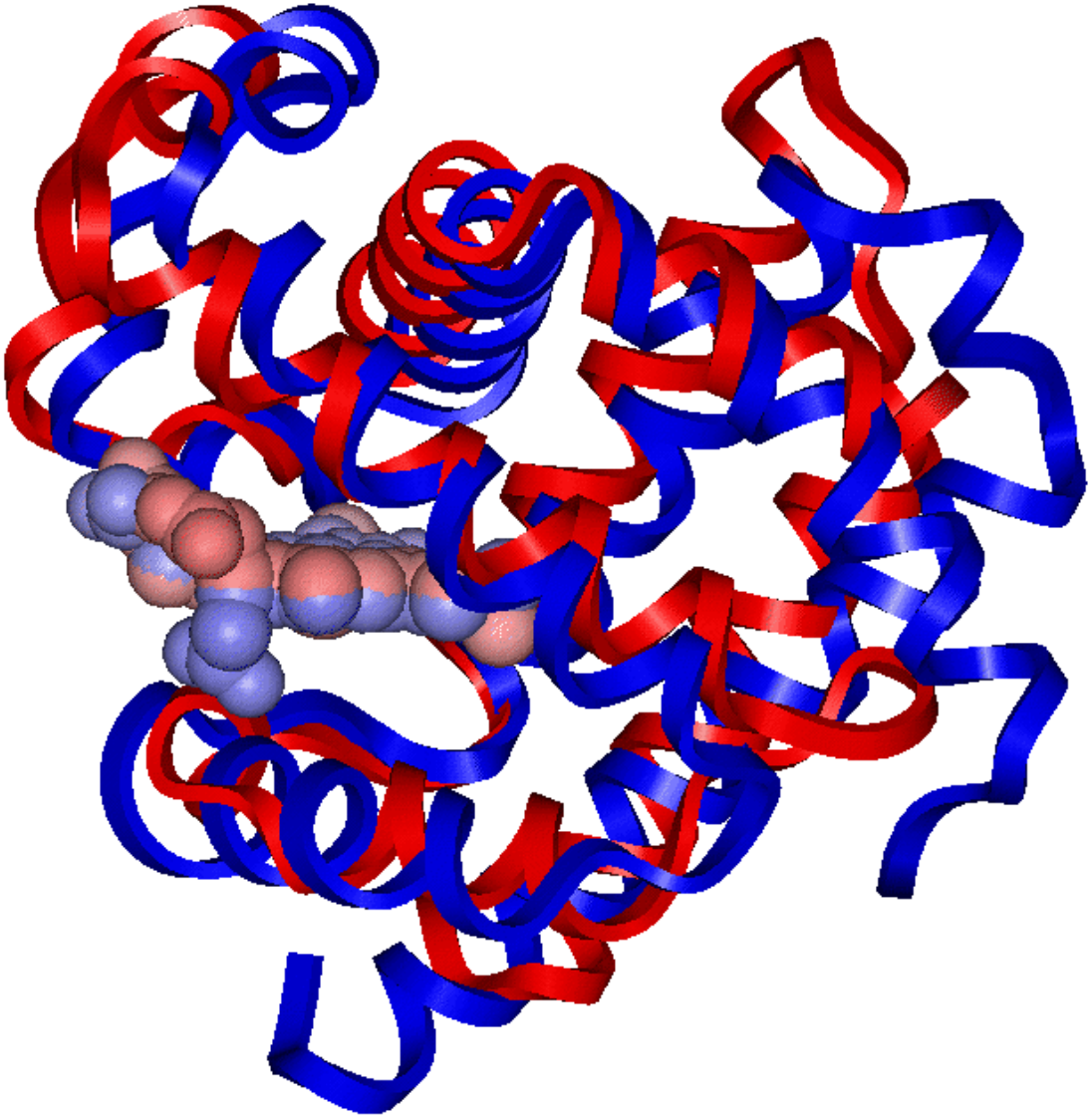


Data collection at the Advanced Photon Source – Argonne Natl. Lab.

- Tunable X-rays
- 16 published structures
- >70 structures solved
- Time-resolved X-ray experiment

De Serrano et al. Acta Cryst. D **2007**, 63, 1098-1101 D'Antonio et al. Peptide Sci A
Chen et al. Acta Cryst. D **2009**, 65, 34-40 D'Antonio et al. Biochemistry
de Serrano Acta Cryst. D, **2010**, 66, 529-538
Thompson et l. Biophys. J. **2010**, 99, 1586-1599

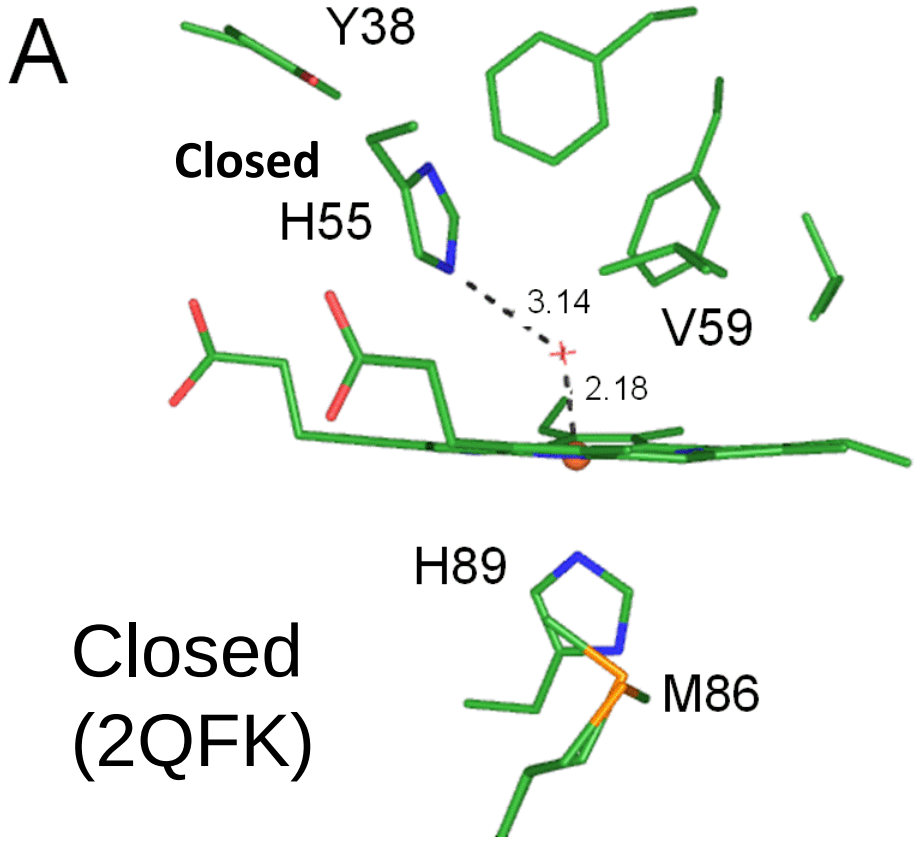
Comparison of DHP and Mb Structures



Mb

DHP

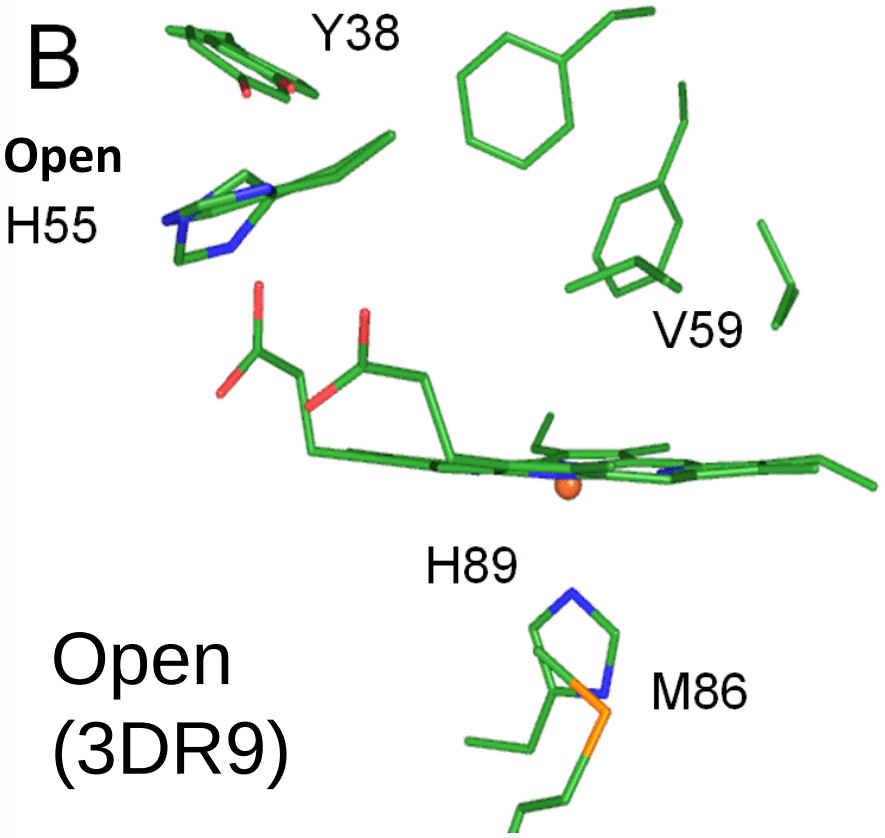
X-ray crystal structures at 100 K



Closed (2QFK)

- **Ferric Metaquo DHP**
- **Closed Histidine**
- **Hydrogen Bond to H_20**
- **6-Coordinate High Spin**

de Serrano et al. Acta Cryst. D **2007**, 63, 1094-1101



Open (3DR9)

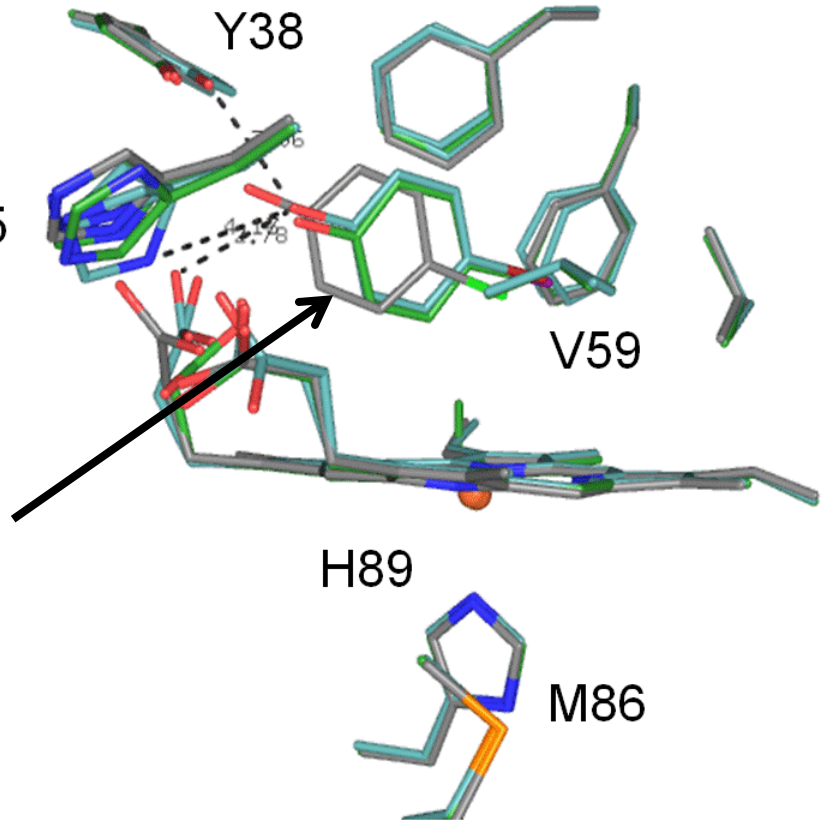
- **Deoxyferrous DHP**
- **Open Histidine**
- **No H_20 bound**
- **5-Coordinate High Spin**

Chen et al. Acta Cryst. D **2009**, 65, 34-40

Inhibitor bound structures

Open Conformation

4-halophenol bound in the distal pocket

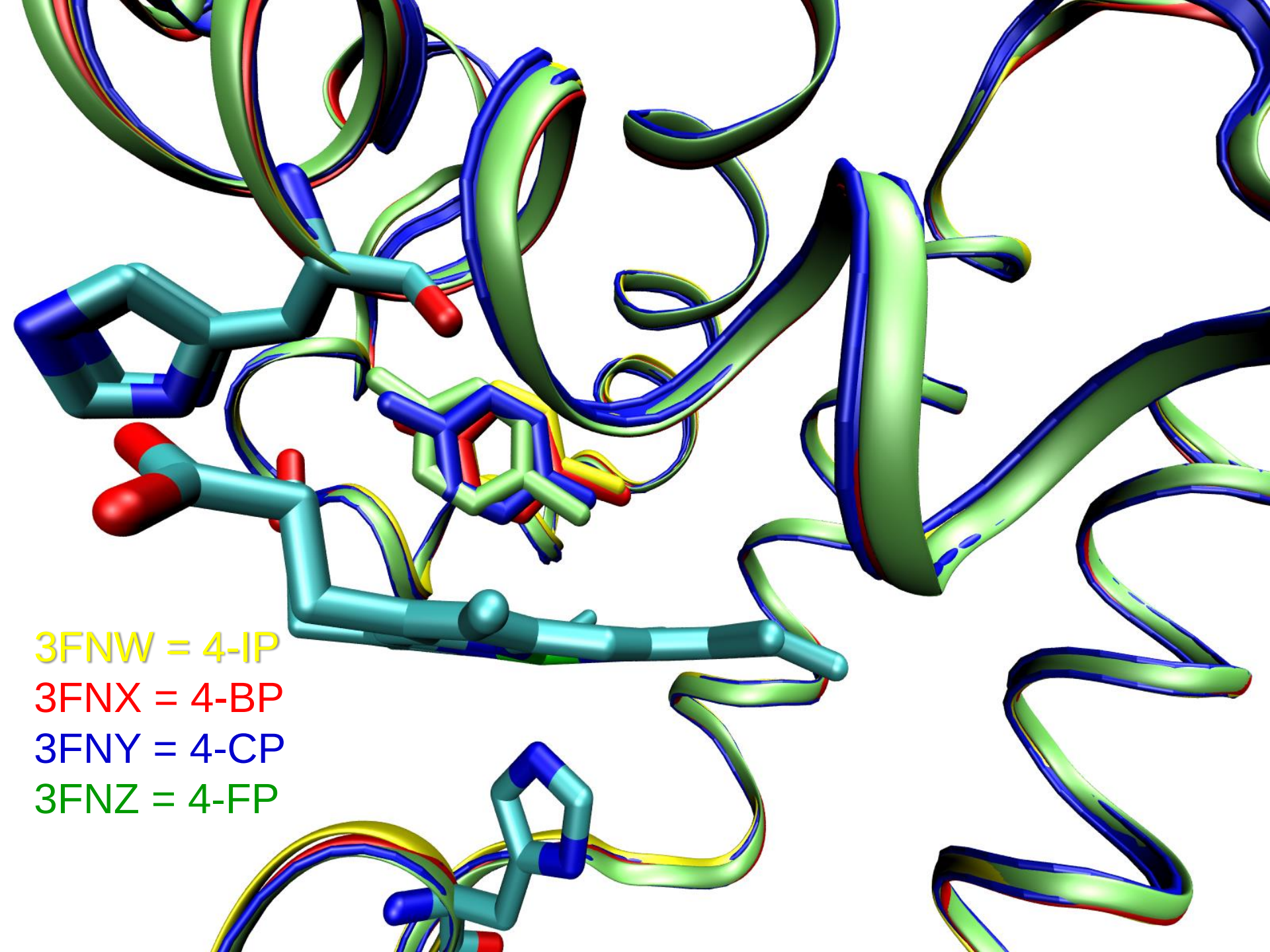


PDB

3FNW = 4-IP
3FNX = 4-BP
3FNY = 4-CP
3FNZ = 4-FP

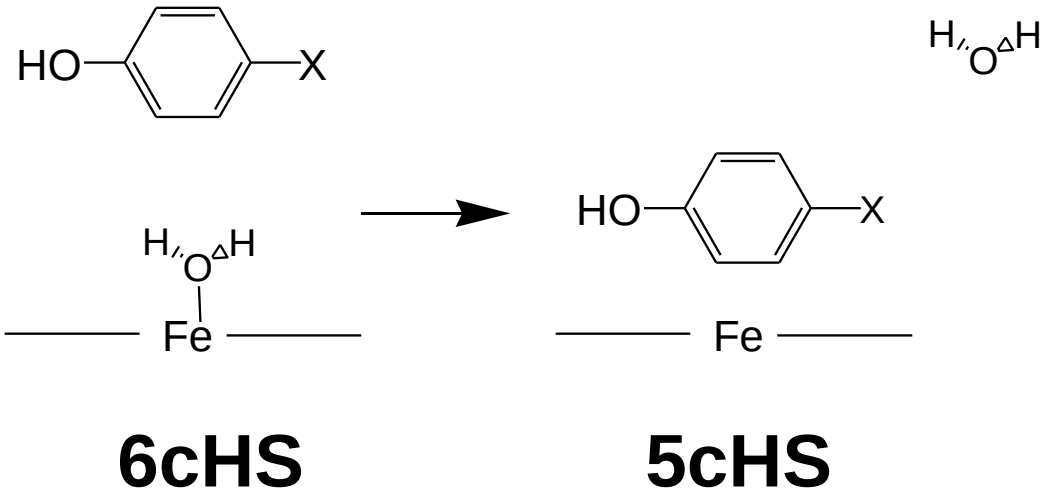
Spectroscopic and structural studies of DHP support binding of 4-halophenols in the pocket

LaCount et al. (2000) *J. Biol. Chem. 275*, 18712
Smirnova et al. JACS 2008, 130, 2128
Nienhaus et al. J. Phys. Chem. B 2006, 110, 13264
Nienhaus et al. Biochemistry, 2008, 47, 12985

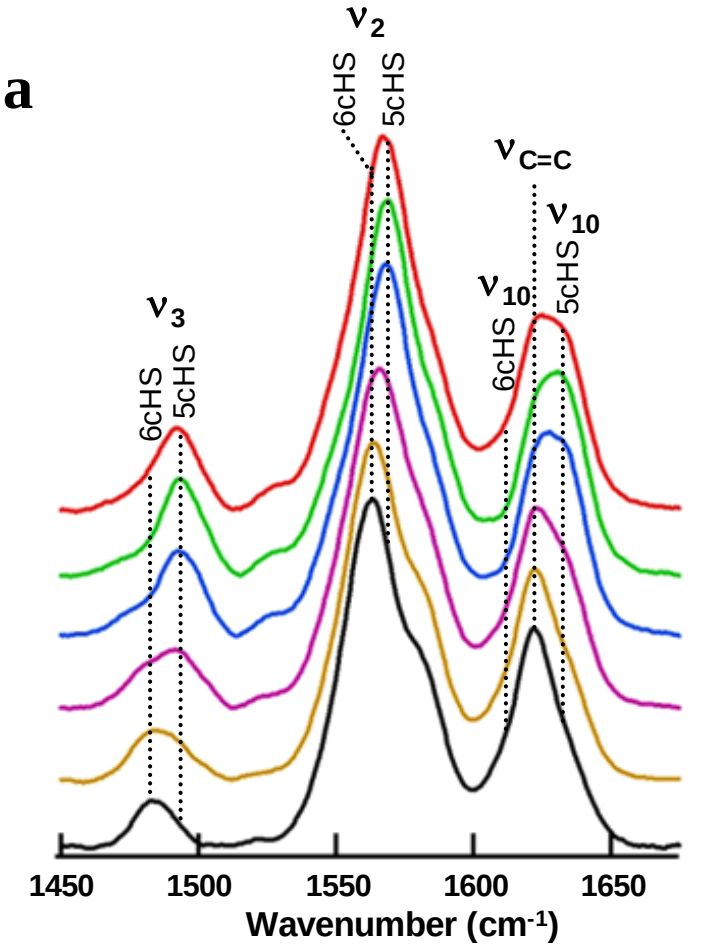

3FNW = 4-IP
3FNX = 4-BP
3FNY = 4-CP
3FNZ = 4-FP

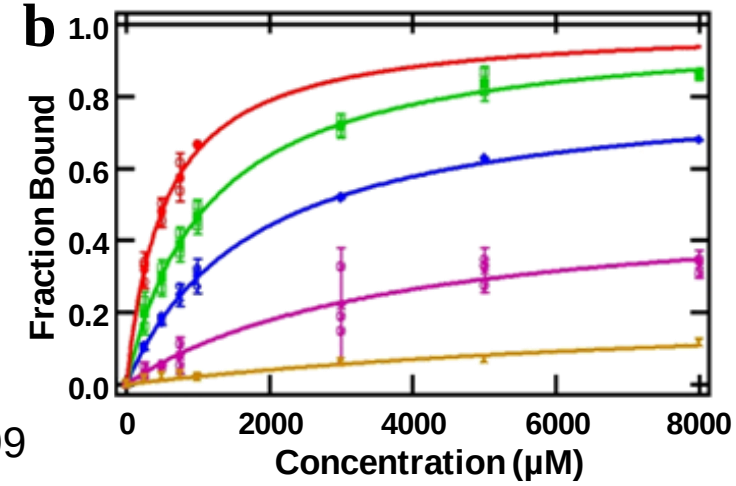
Raman probe of binding in the internal binding site

Different modes of binding are observed in the core size marker modes of the resonance Raman spectrum.
(X = I > Br > Cl > F > H)

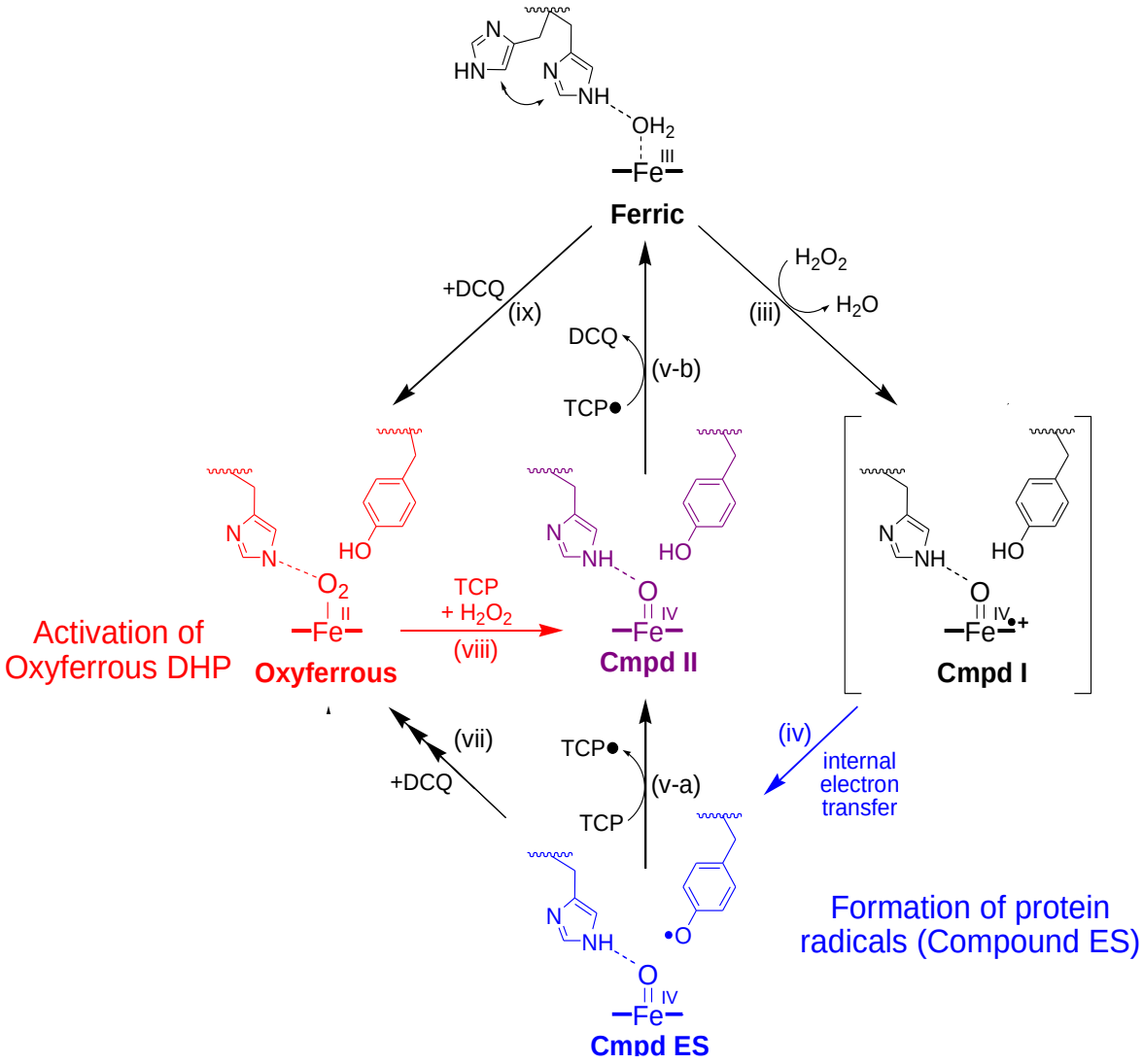


6cHS **5cHS**

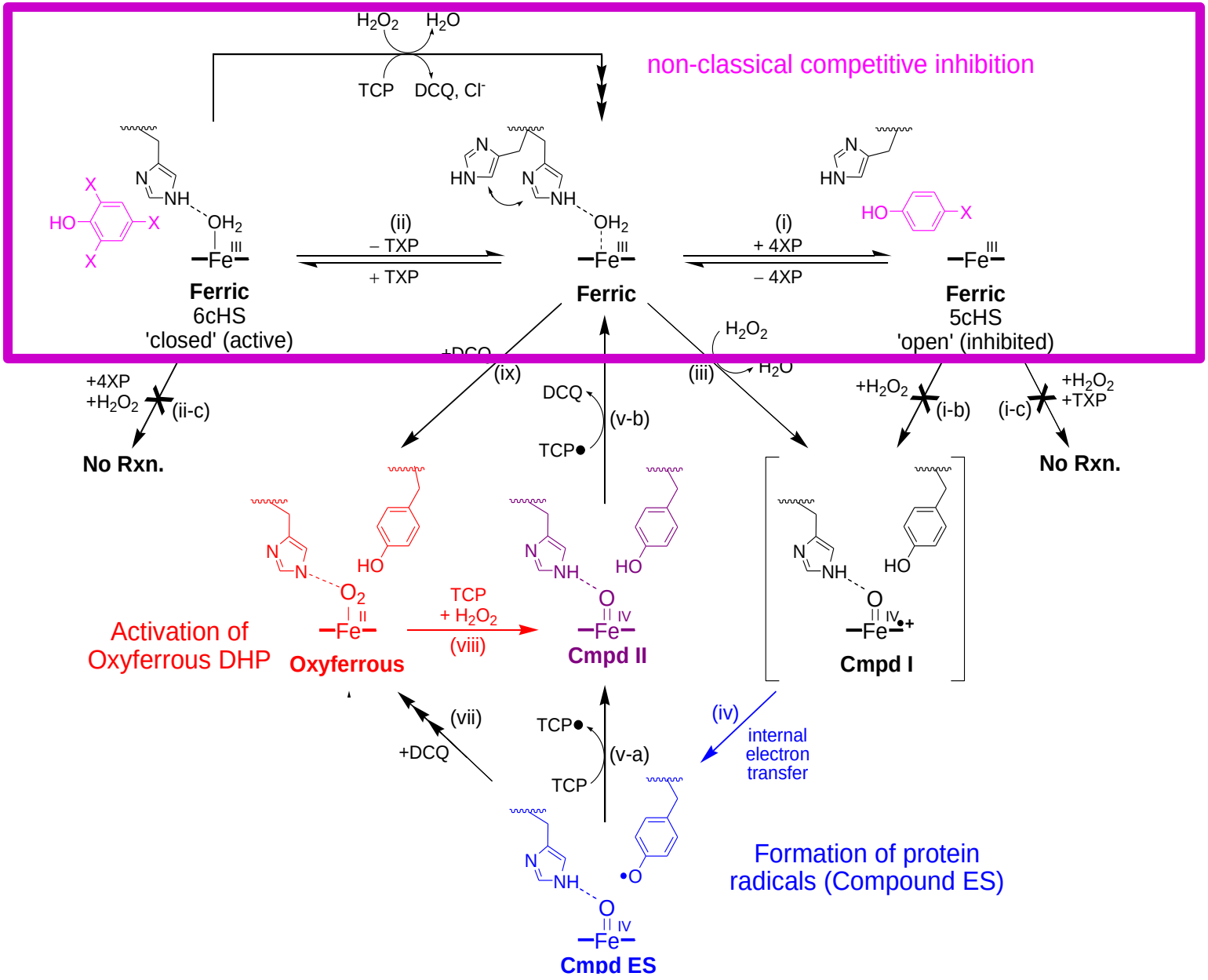


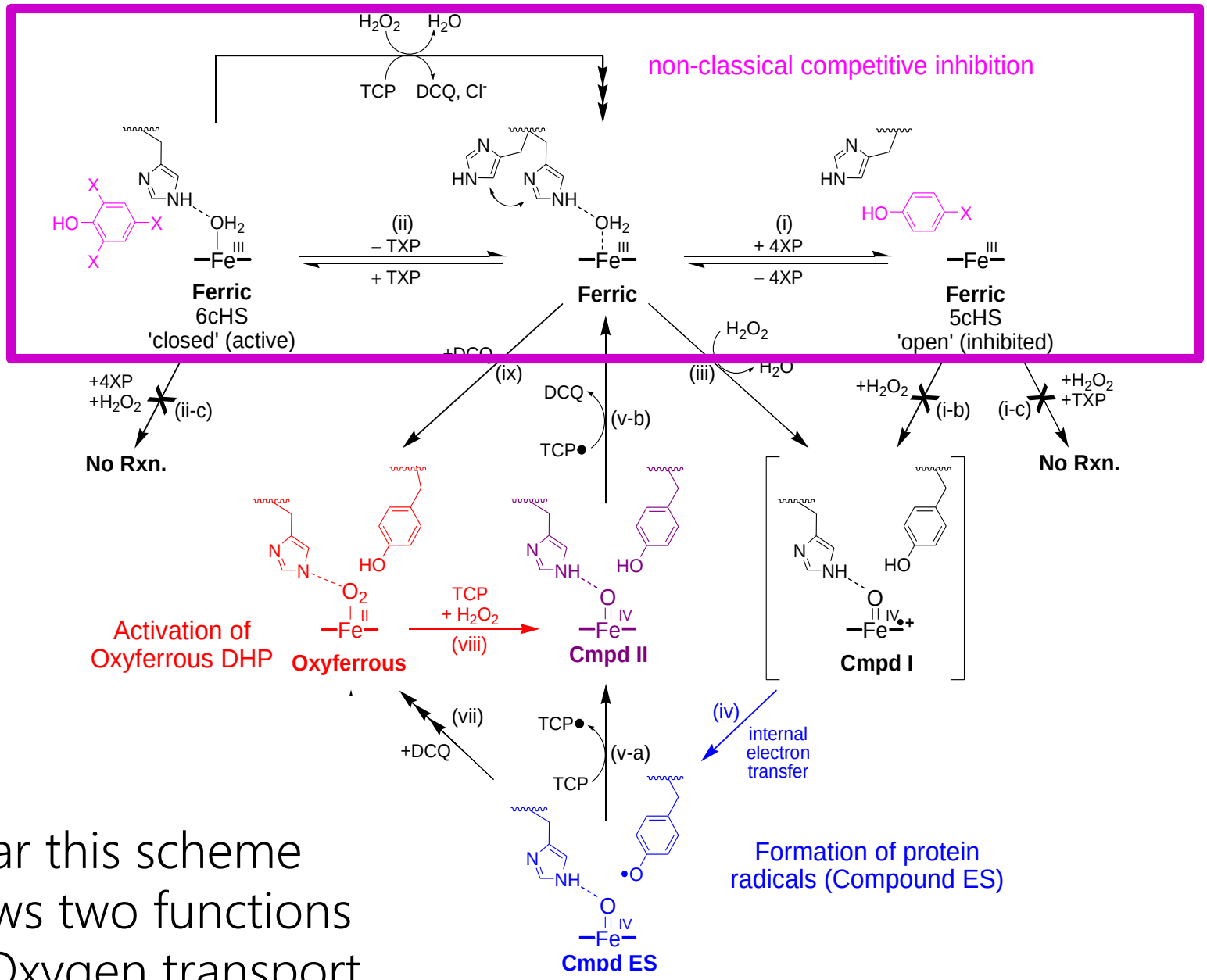


Thompson, Franzen et al. Biophys. J. (2010), 99, 1586-1599



Franzen, Thompson and Ghiladi, BBA (2012) 1842, 578-588


non-classical competitive inhibition
H_2O_2
H_2O
TCP
DCQ, Cl⁻
(ii)
– TXP
+ TXP
(i)
+ 4XP
– 4XP
Ferric
6cHS
'closed' (active)
Ferric
Ferric
5cHS
'open' (inhibited)
+4XP
+H_2O_2
(ii-c)
No Rxn.
+DCQ
(ix)
DCQ
(v-b)
TCP●
H_2O_2
(iii)
H_2O
+H_2O_2
(i-b)
(i-c)
+H_2O_2
+TXP
No Rxn.
Activation of
Oxyferrous DHP
Oxyferrous
TCP
+ H_2O_2
(viii)
Cmpd II
Cmpd I
(vii)
+DCQ
TCP●
(v-a)
TCP
(iv)
internal
electron
transfer
Formation of protein
radicals (Compound ES)
Cmpd ES



So far this scheme
Shows two functions

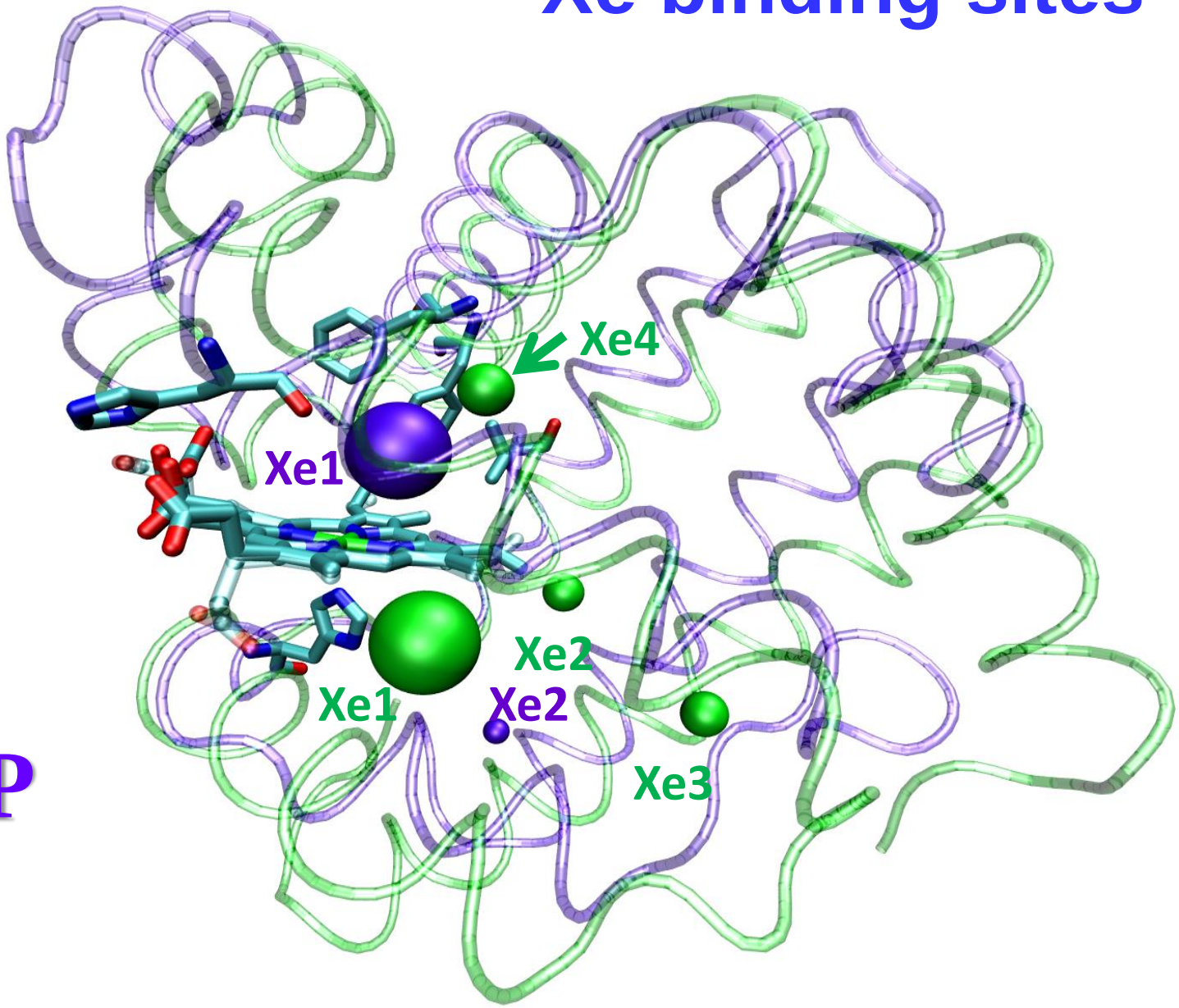
1. Oxygen transport
2. Peroxidase catalysis

Hypothesis: Autooxidation is not physiologically relevant for DHP

Oxidized forms of DHP may form during a catalytic cycle. Reductase may be needed for this reason, but not because of (auto)oxidation by O_2.

There is no need for complicated regulation of the distal pocket such as observed in most hemoglobins or myoglobins.

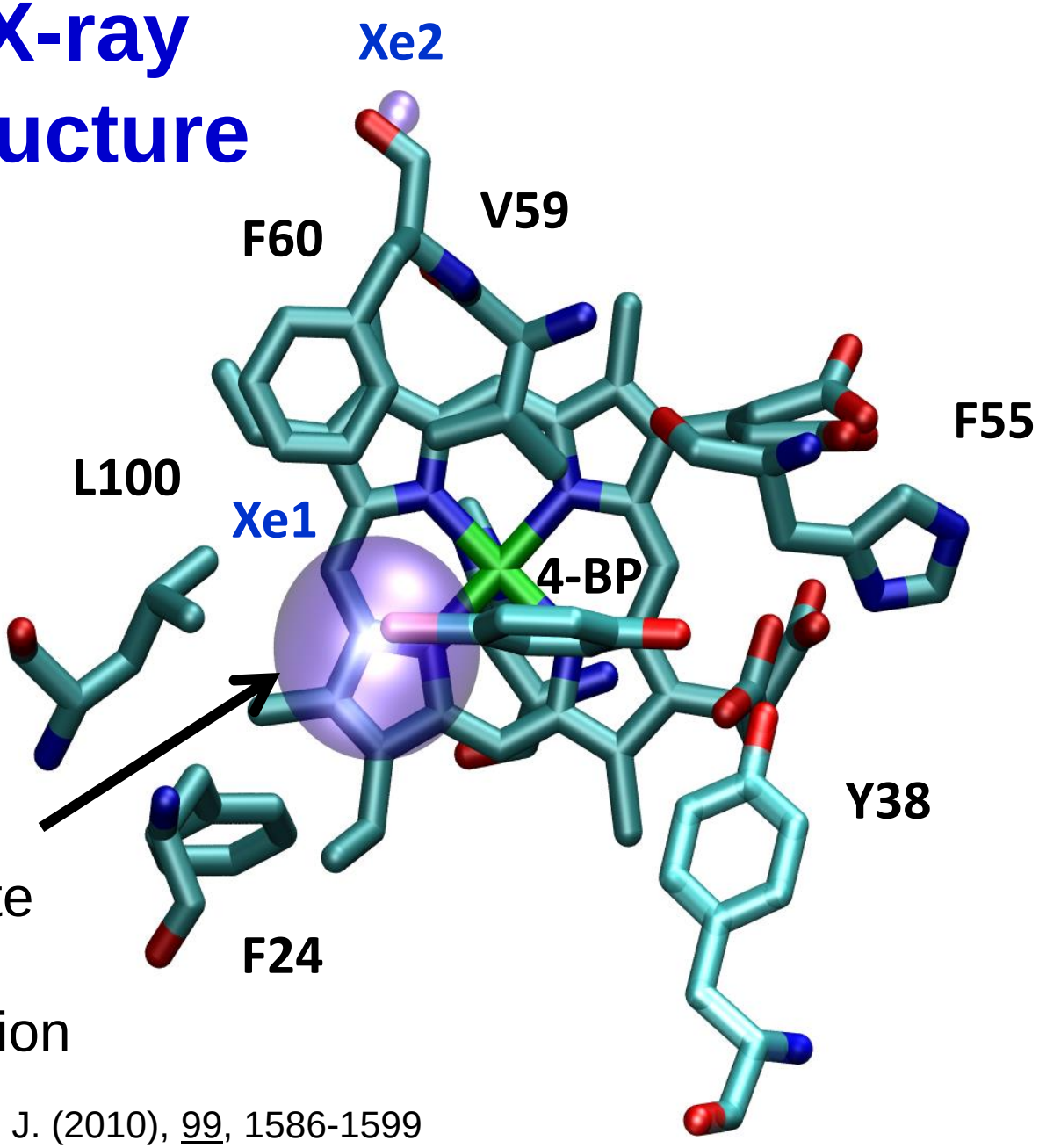
Xe binding sites



DHP

Mb

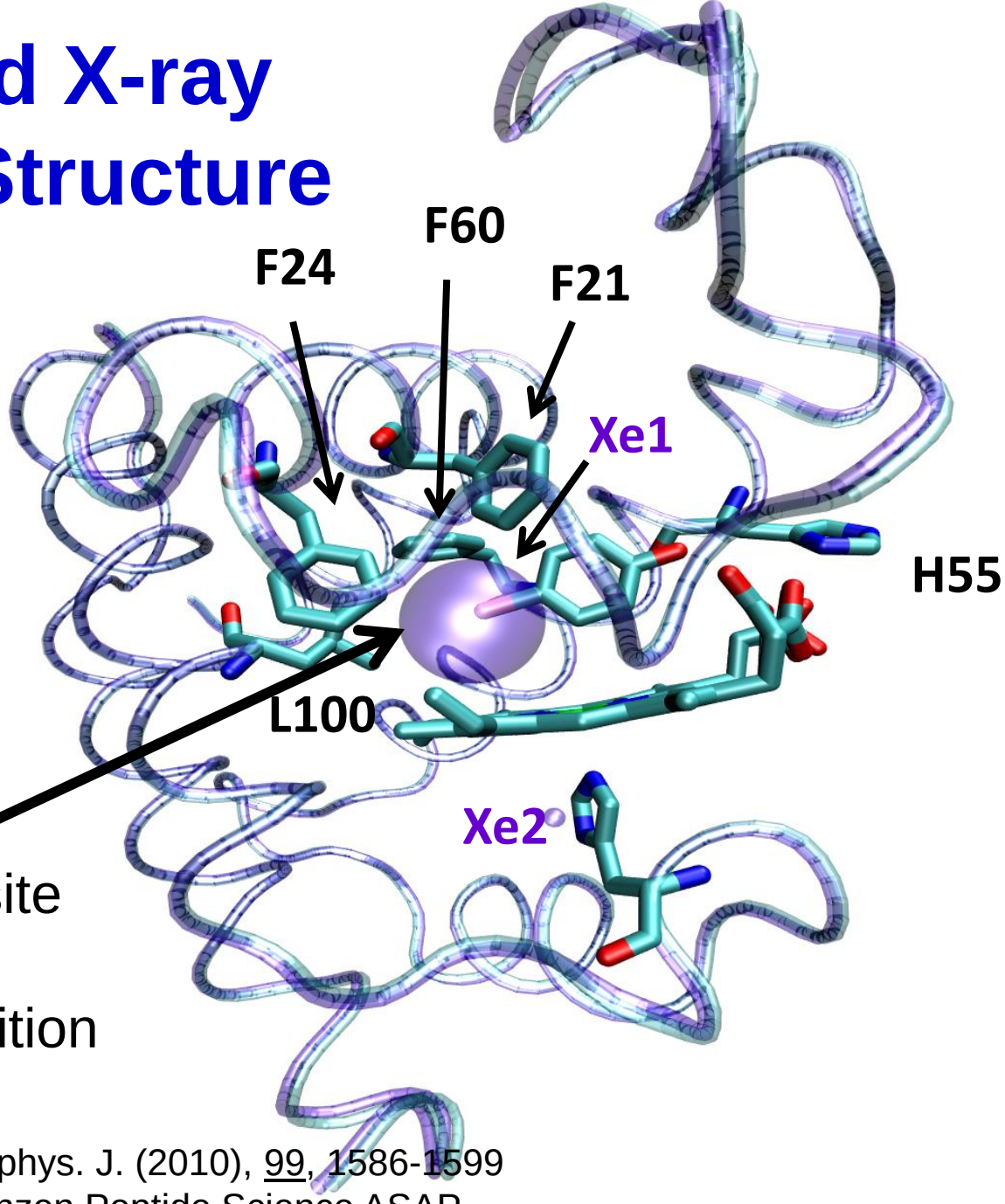
Xe-bound X-ray Crystal Structure



Overlay of
Xe binding site
and
inhibitor position

Thompson et al. Biophys. J. (2010), 99, 1586-1599
De Serrano and Franzen Peptide Science ASAP

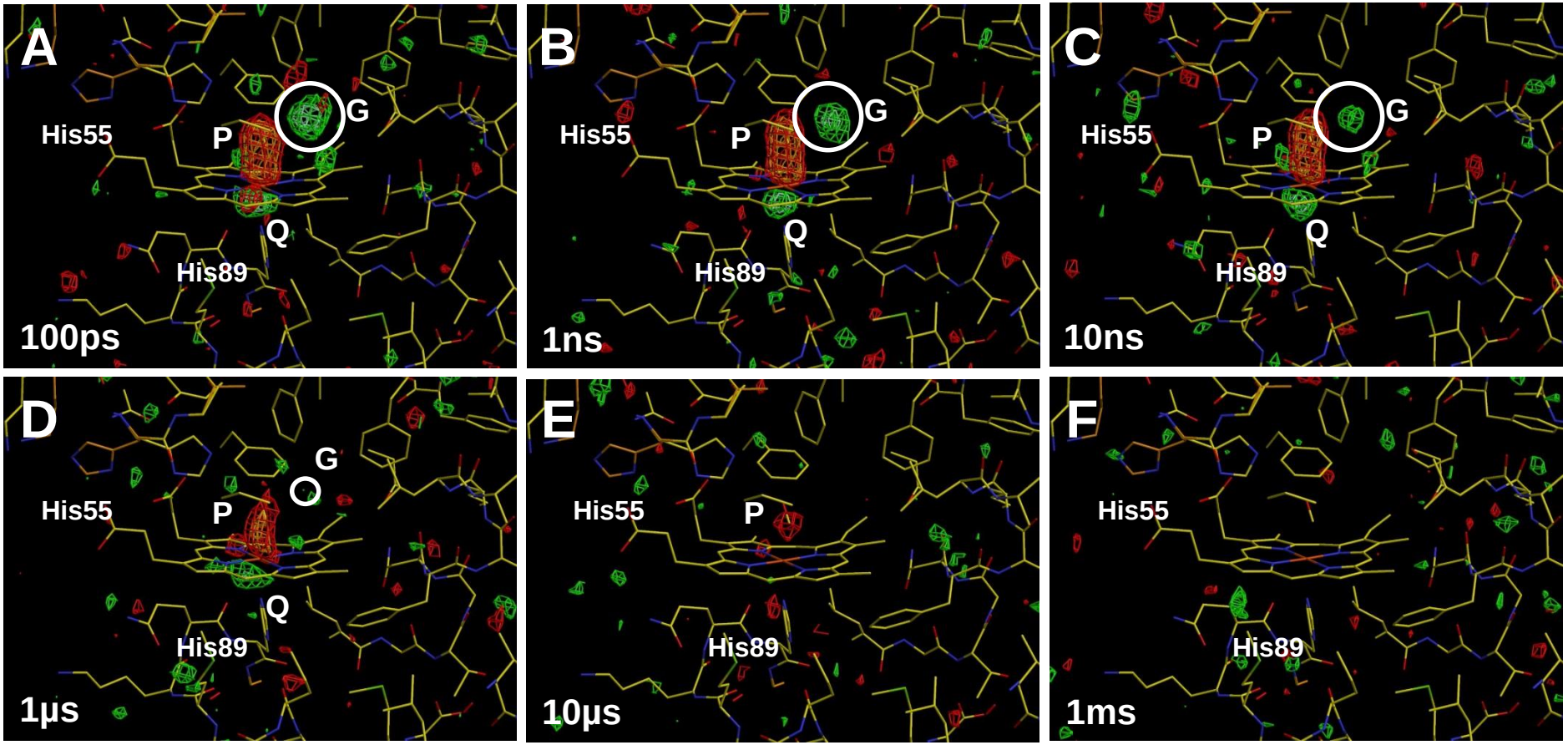
Xe-bound X-ray Crystal Structure



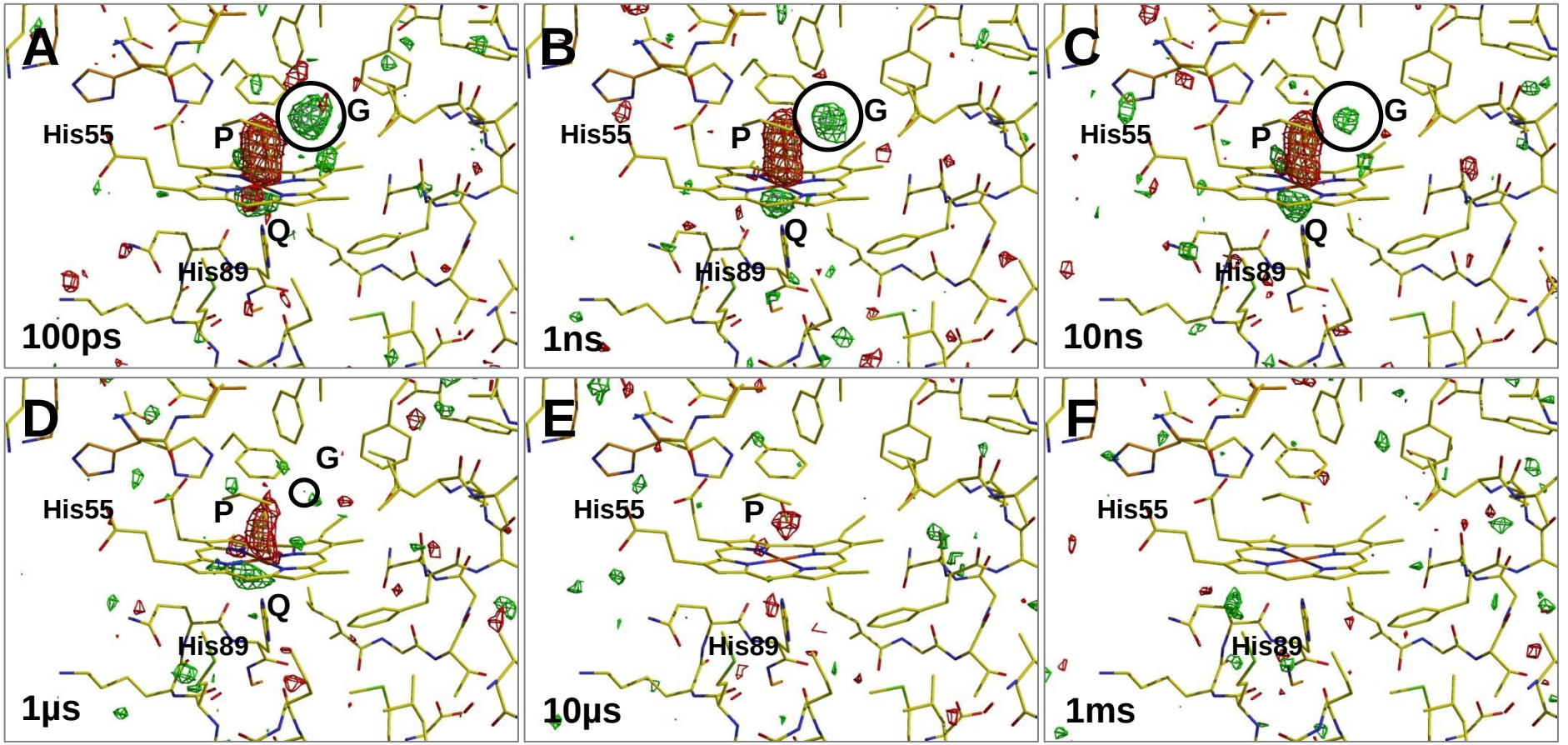
Overlay of
Xe binding site
and
inhibitor position

Thompson et al. Biophys. J. (2010), 99, 1586-1599
De Serrano and Franzen Peptide Science ASAP

Time-resolved X-ray crystallography confirms the enlarged distal pocket

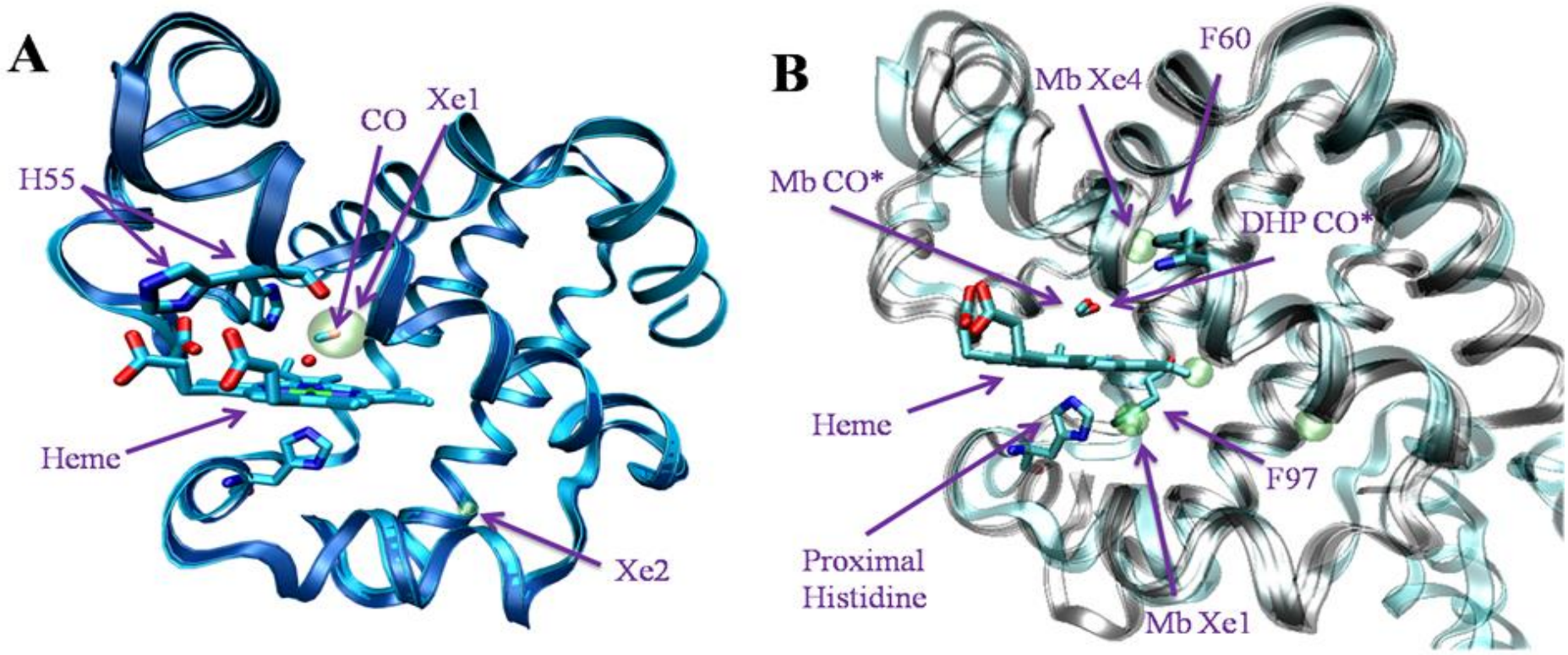


Time-resolved X-ray crystallography confirms the enlarged distal pocket



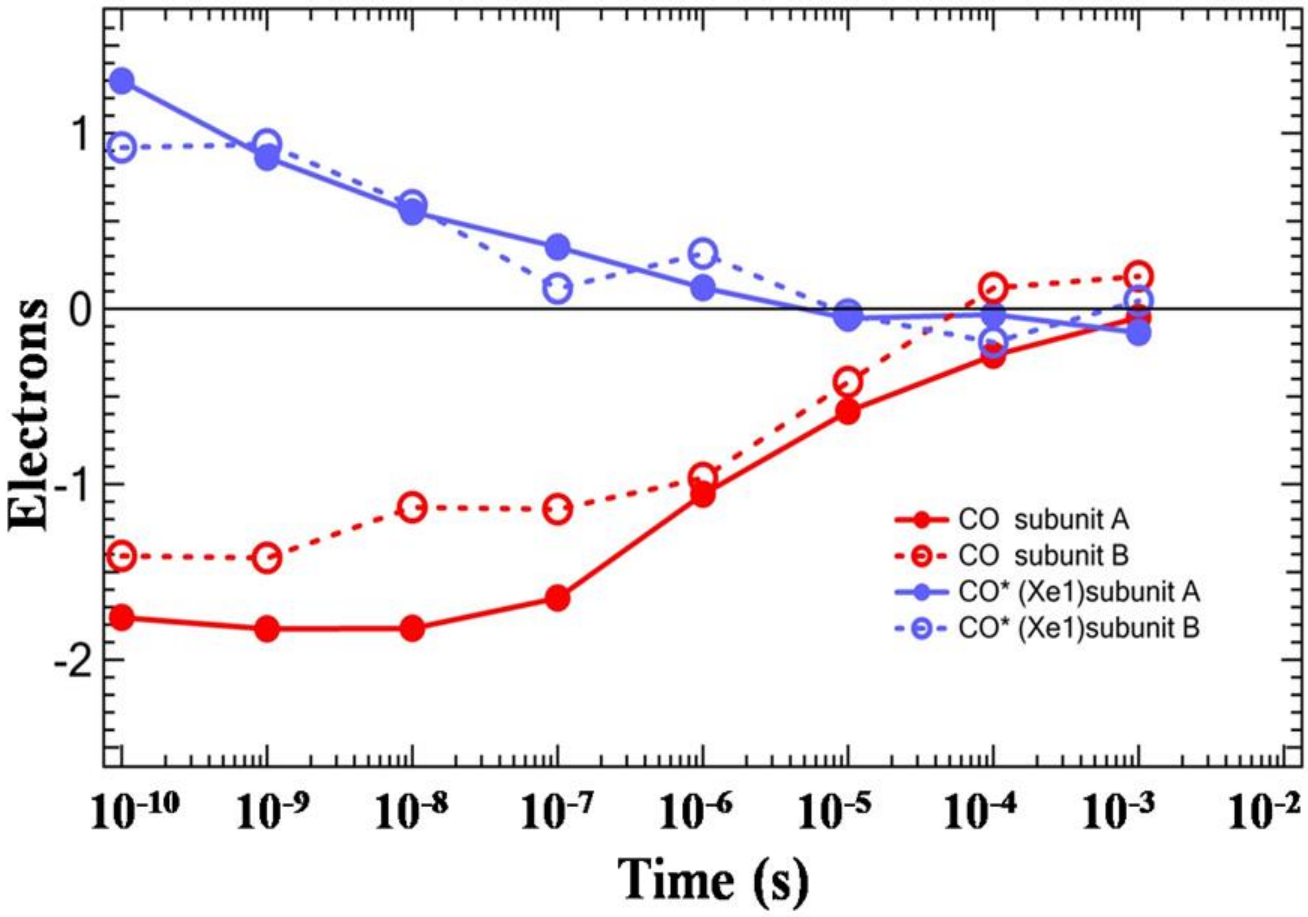
Zhao et al. Biochemistry (2013) 52, 2427

Initially CO moves to the primary Xe binding site



The single site for of diatomic ligand binding in DHP can be contrasted with the more complex series of sites in Sperm Whale myoglobin, studied by many groups using time-resolved X-ray.

CO escapes from the distal pocket in the crystalline form



Zhao et al. Biochemistry (2013) 52, 2427

Distal pocket of DHP permits Free entry and exit of CO (and O_2)

The CO ligand moves immediately to the Xe binding site in DHP. This is NOT the same as the "docking" site seen in other time-resolved X-ray structures.

The docking site is closer to the heme Fe

CO must push other amino acids away and is trapped by them.

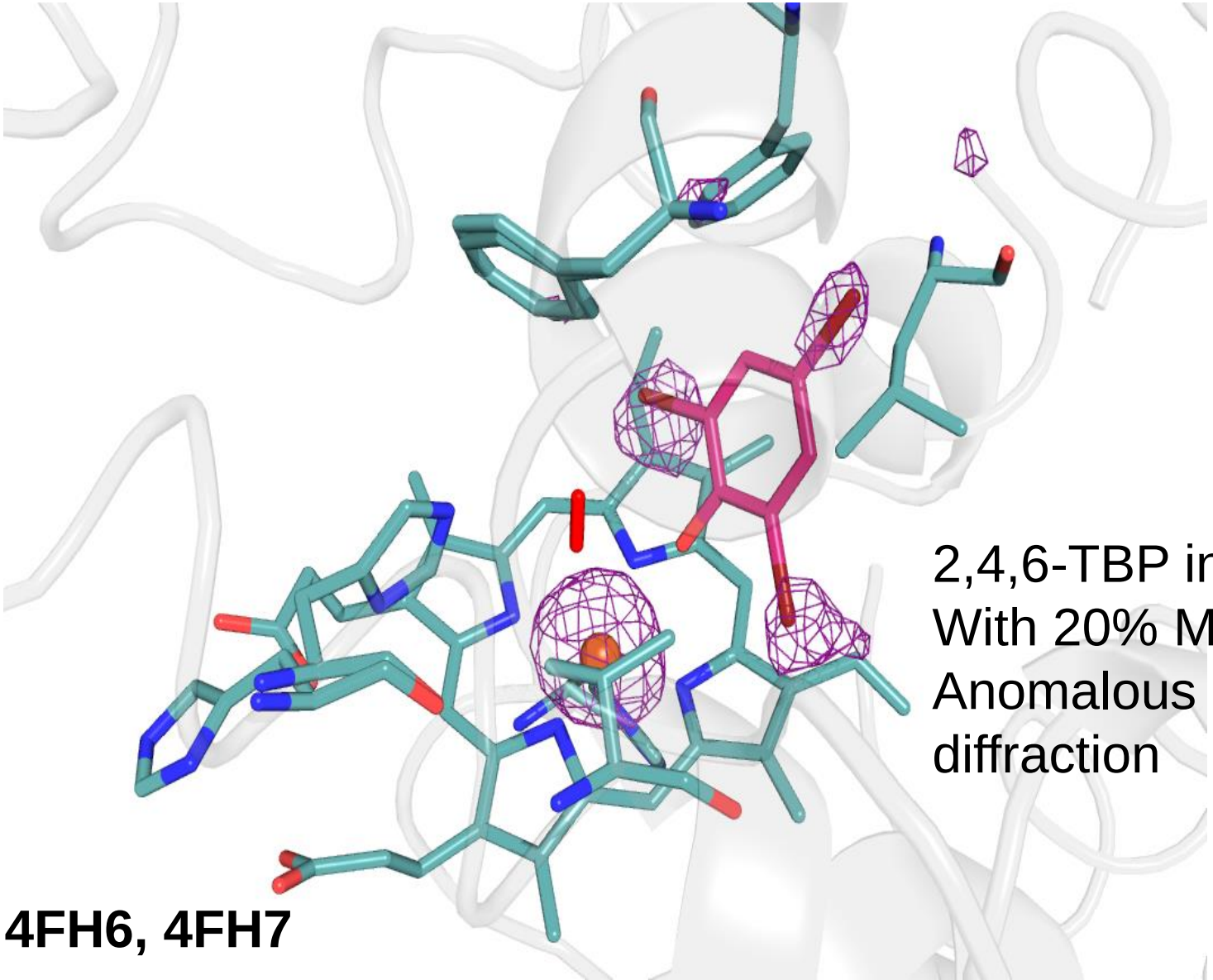
CO does not escape from the protein, but moves to Xe sites and then recombines.

Hypothesis : Substrate binding can trigger switching between functions

Promiscuity in DHP may be related to the diversity of brominated (and chlorinated) molecules in the environment.

The different fates of molecules depends on specific chemistry. For example, 4-bromophenol radicals Lead to polymerization and therefore they do not form (4-BP is an inhibitor). 2,4,6-tribromophenol radicals Lead to quinone formation, which is favorable so this Chemistry occurs. Oxidation of 2,4-dibromophenol By O-atom transfer is favorable. It should also bind. We can measure competitive binding equilibria.

Internal substrate binding site

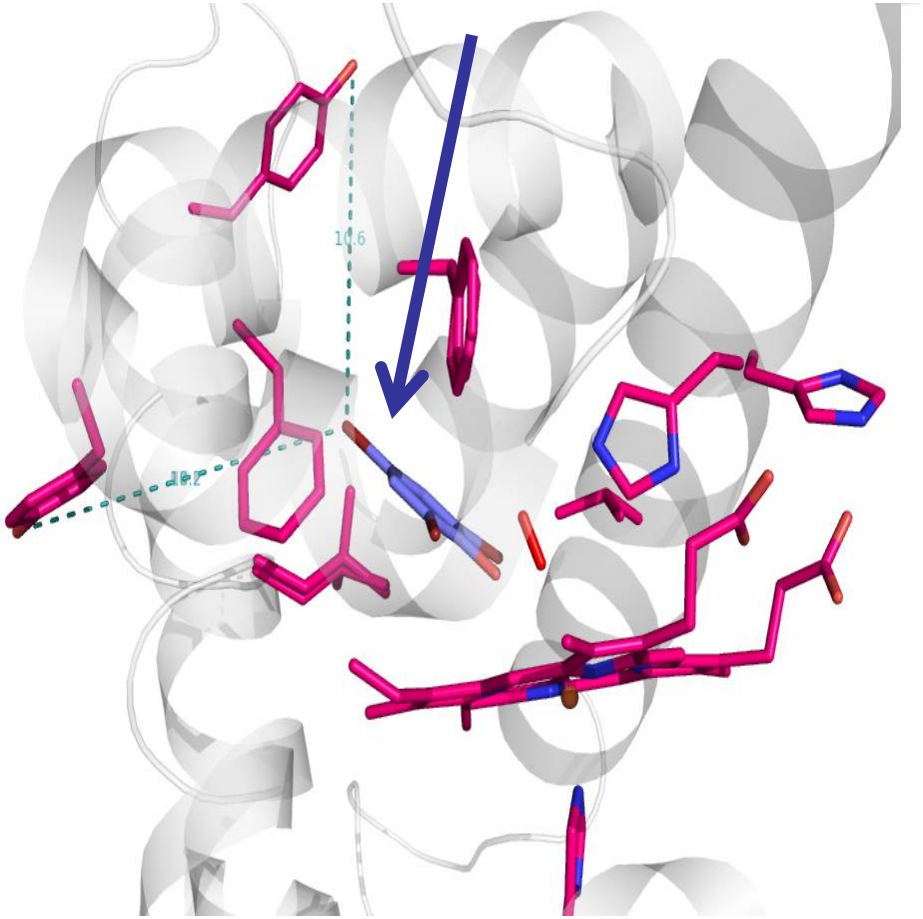


2,4,6-TBP infused
With 20% MeOH
Anomalous
diffraction

PDB: 4FH6, 4FH7

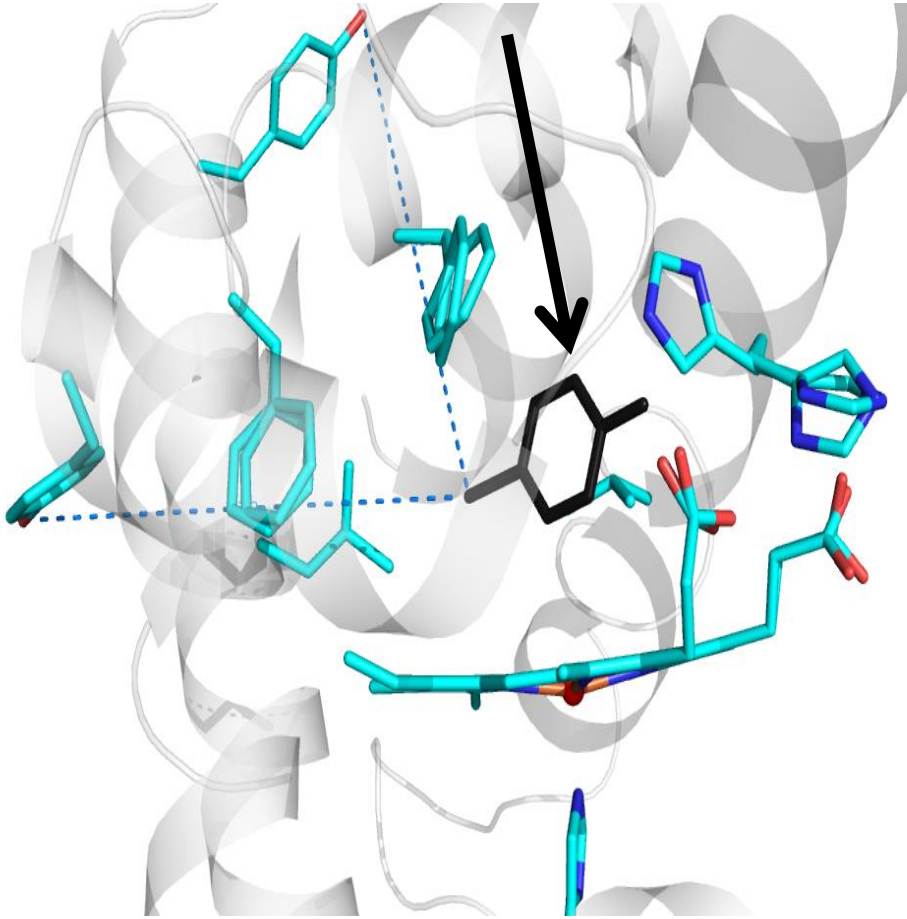
Comparison of substrate and inhibitor binding sites

Substrate



PDB: 4FH6, 4FH7

Inhibitor



PDB: 1EWA 3LB1-4