Enzyme mechanisms

Biophysical Chemistry 1, Fall 2010

Hemoglobin and allosteric interactions

Serine proteases

Cytochrome P450

Cytochrome c oxidase

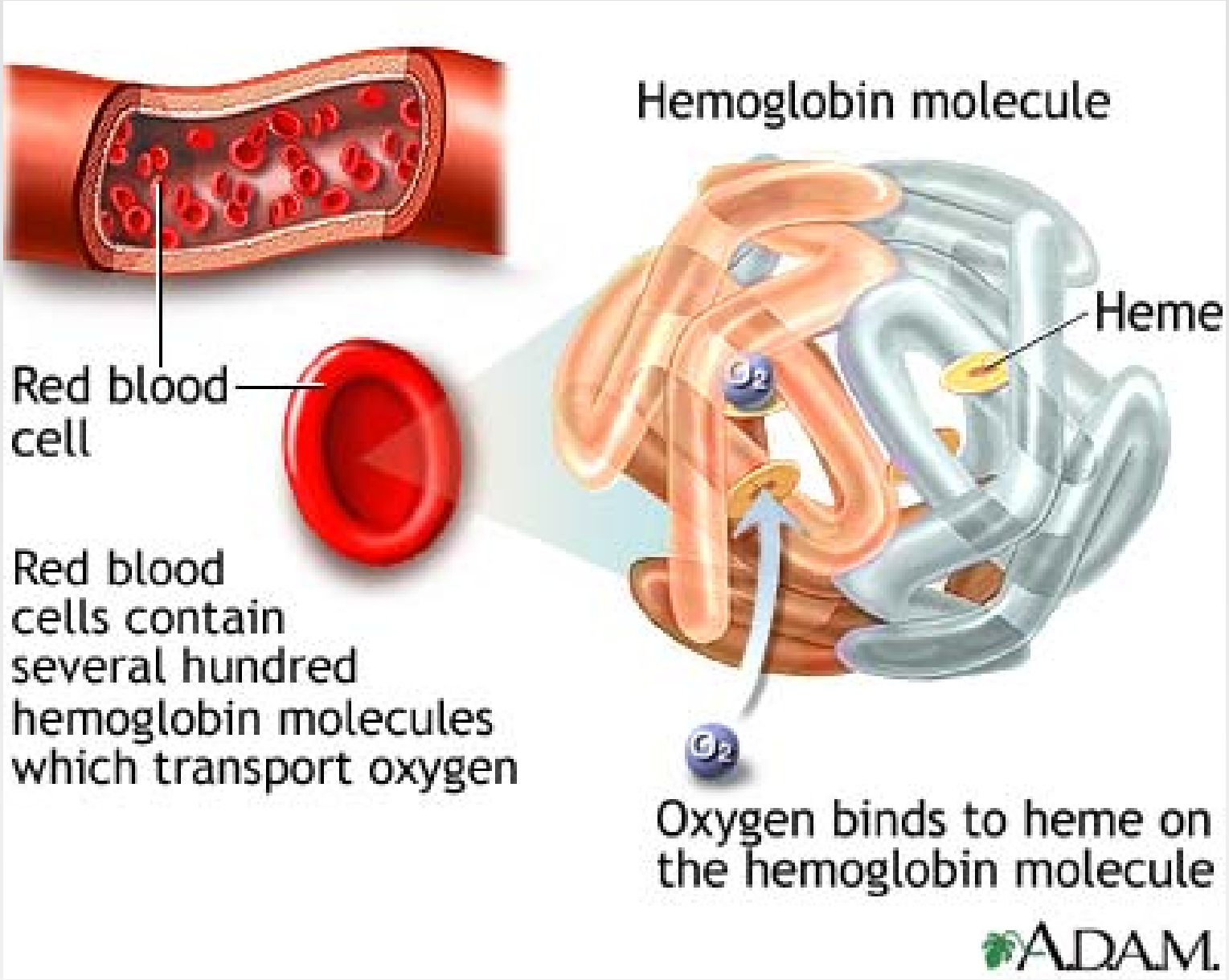
Reading assignment: Chap. 5

Hemoglobin, blood and blood substitutes

- Chemistry and biochemistry of oxygen transport
- Practice and limitations of blood transfusions
- Two major ideas for red-blood-cell-free substitutes:
  - Perfluorcarbon (PFC)-based (“un-natural”?)
  - Hemoblobin-based (“natural”)
- How these might be used, why progress has been so slow

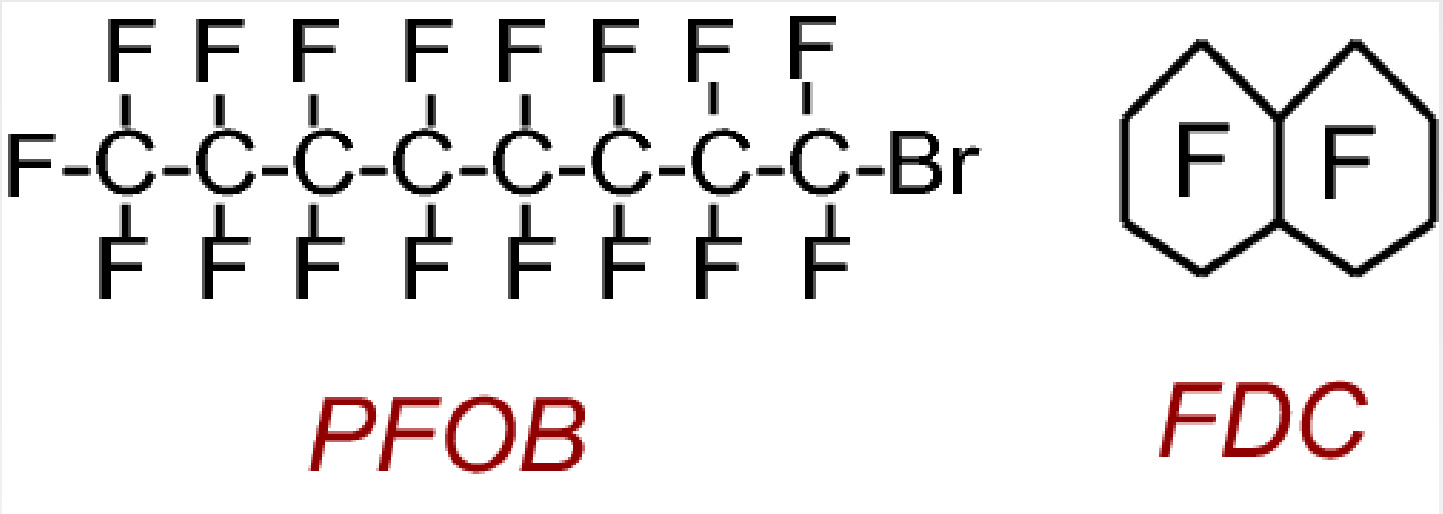
“IANAP”

Overview of oxygen transport

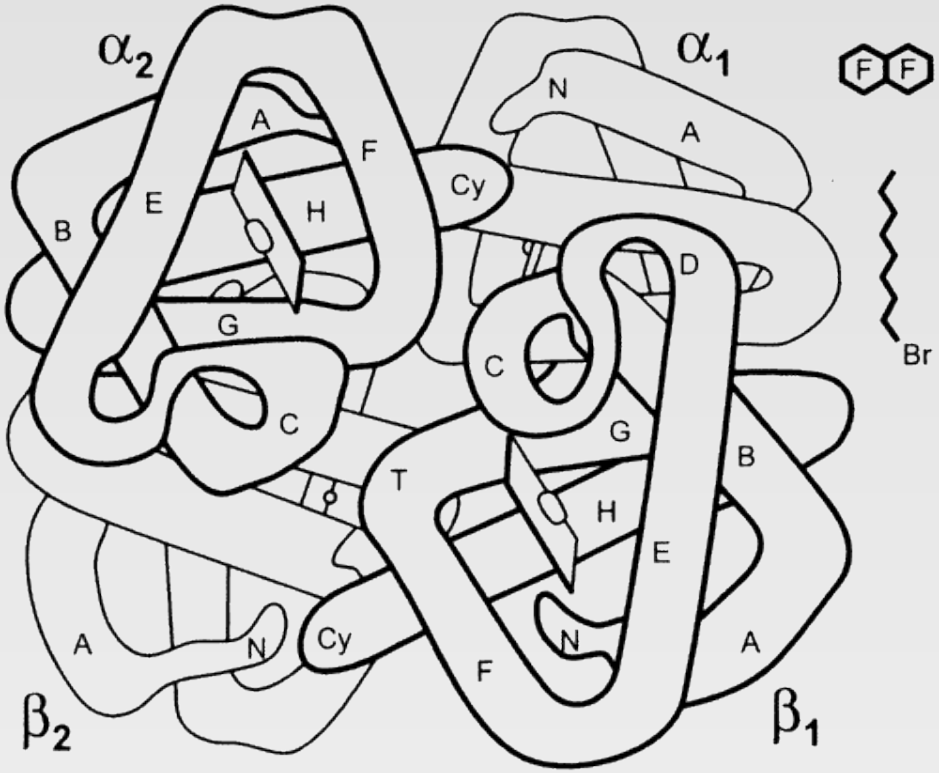


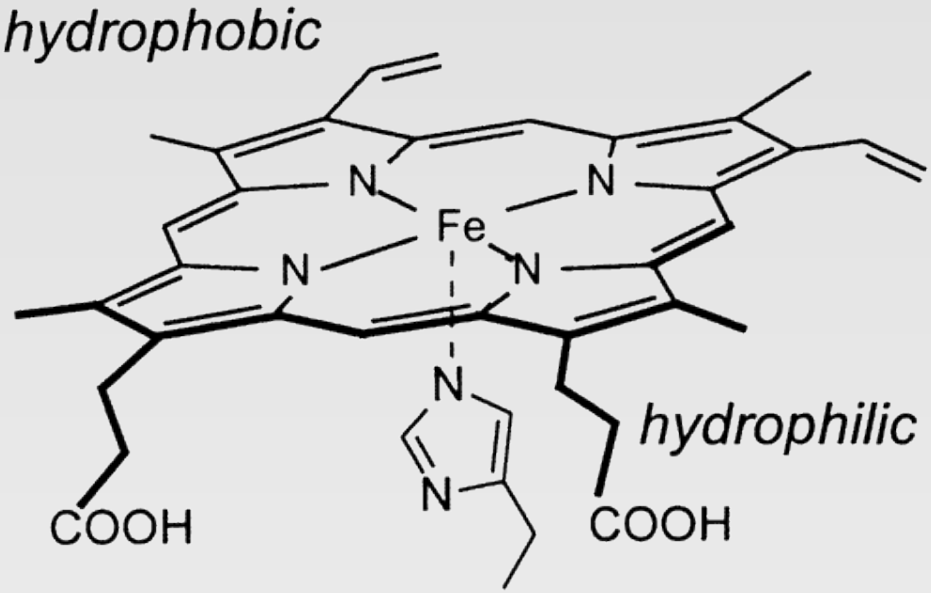
A tale of some very simple molecules...

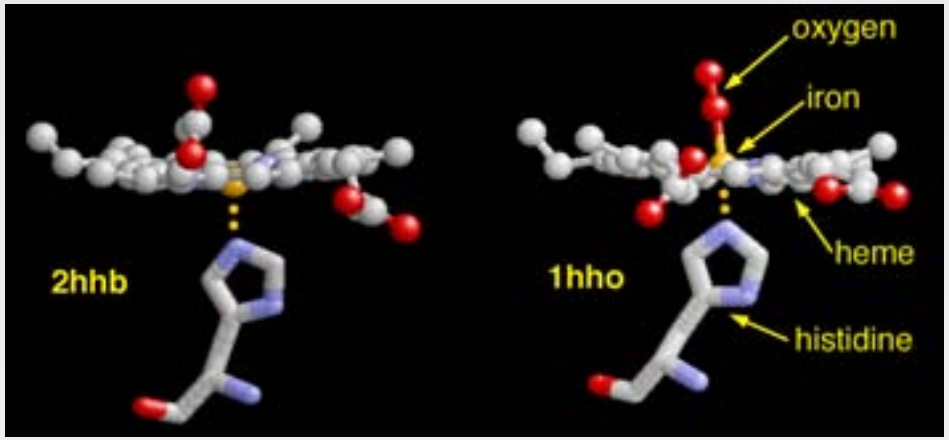
- O_2: (di-)oxygen (20% of air)
- N_2: (di-)nitrogen (80% of air)
- CO: carbon monoxide (competes with O_2 in hemoglobin)
- CO_2: carbon dioxide (by-product of respiration)
- NO: nitric oxide (control of blood pressure and vasoconstriction)
- $C_{10}F_{18}$: “FDC” (main component of *fluosol*)
- $C_8F_{17}Br$: “PFOB” (most promising perfluorocarbon)



... and a much more complex one







Dangers and nuisances of conventional transfusions

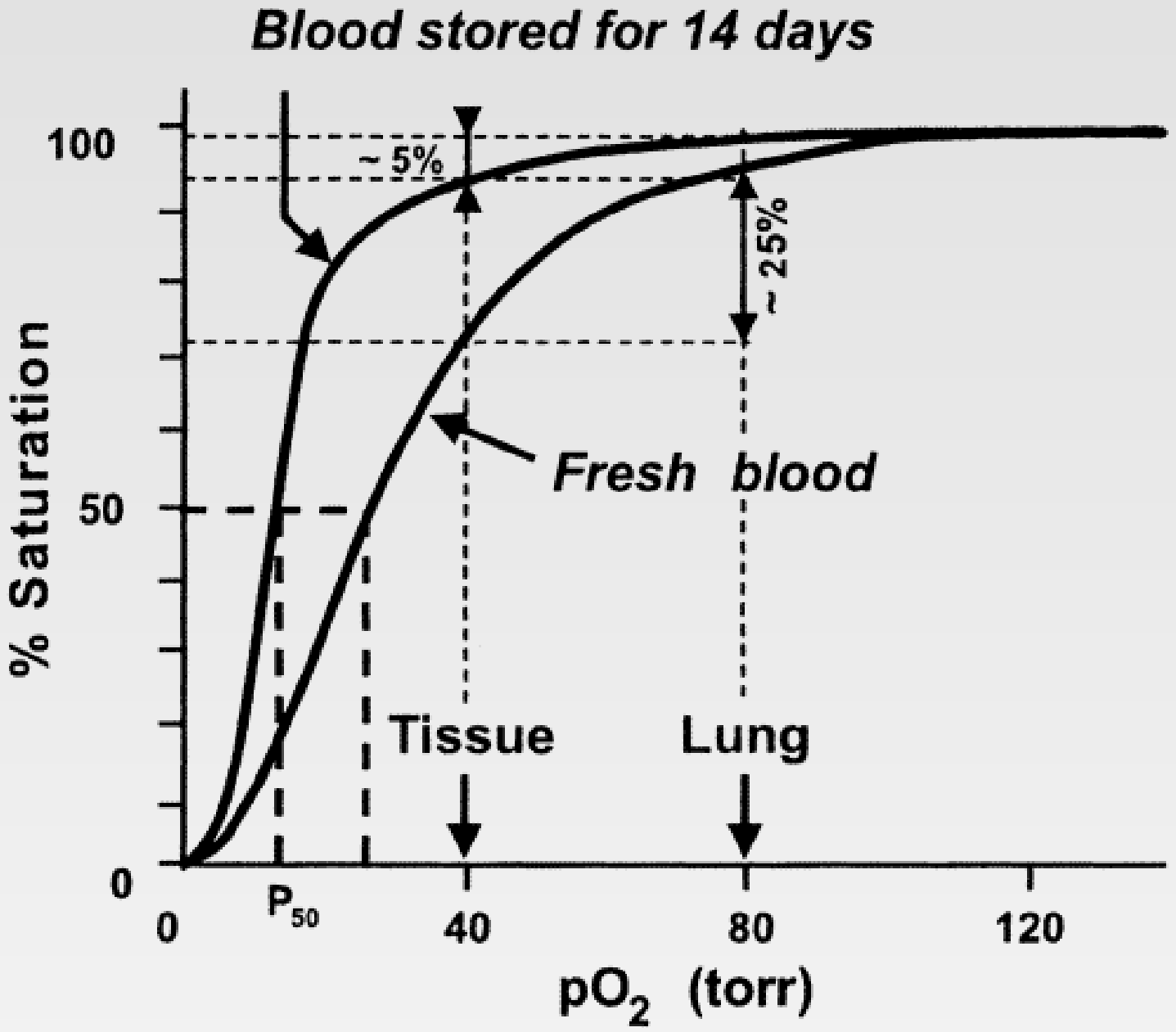
Dangers

- mild allergic reactions (1 in 30)
- acute respiratory distress (1 in 5000)
- hepatitis (1 in 30,000 to 100,000) [was 1 in 4 prior to 1965]
- HIV-AIDS (1 in 500,000)
- administrative errors (1 in 20,000)
- immune system suppresion

Nuisances

- Blood must be screened and typed (ABO)
- Very limited shelf life (2-4 weeks)
- Not immediately effective in delivering oxygen (lack of DPG)
- Potential and real shortages

Stored blood quickly becomes outdated



Foreseeable benefits of an oxygen carrier

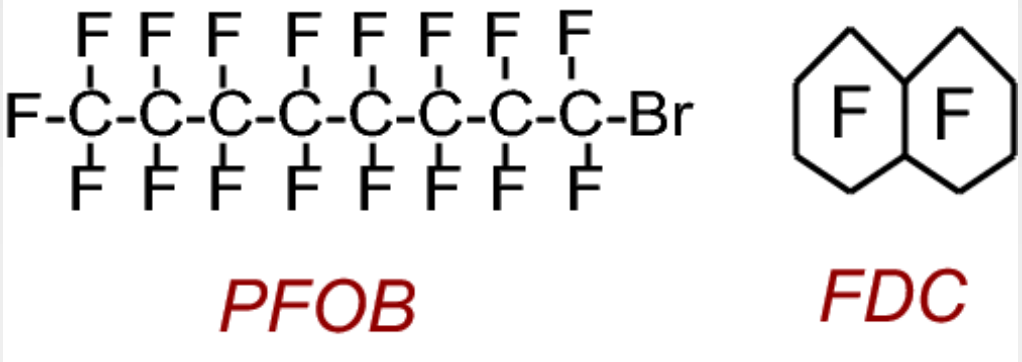
- Emergency–trauma
- Cardiopulmonary bypass surgery
  - ANH: (extract blood before surgery, replace it afterwards)–red blood cells not exposed to bypass circuitry and pump
  - dissolution of air bubbles that lead to neurological dysfunction (50% incidence, severe incidence 6%)
- tissue and organ storage
- getting oxygen inside solid tumors
- simplified blood banking

Perfluorocarbons

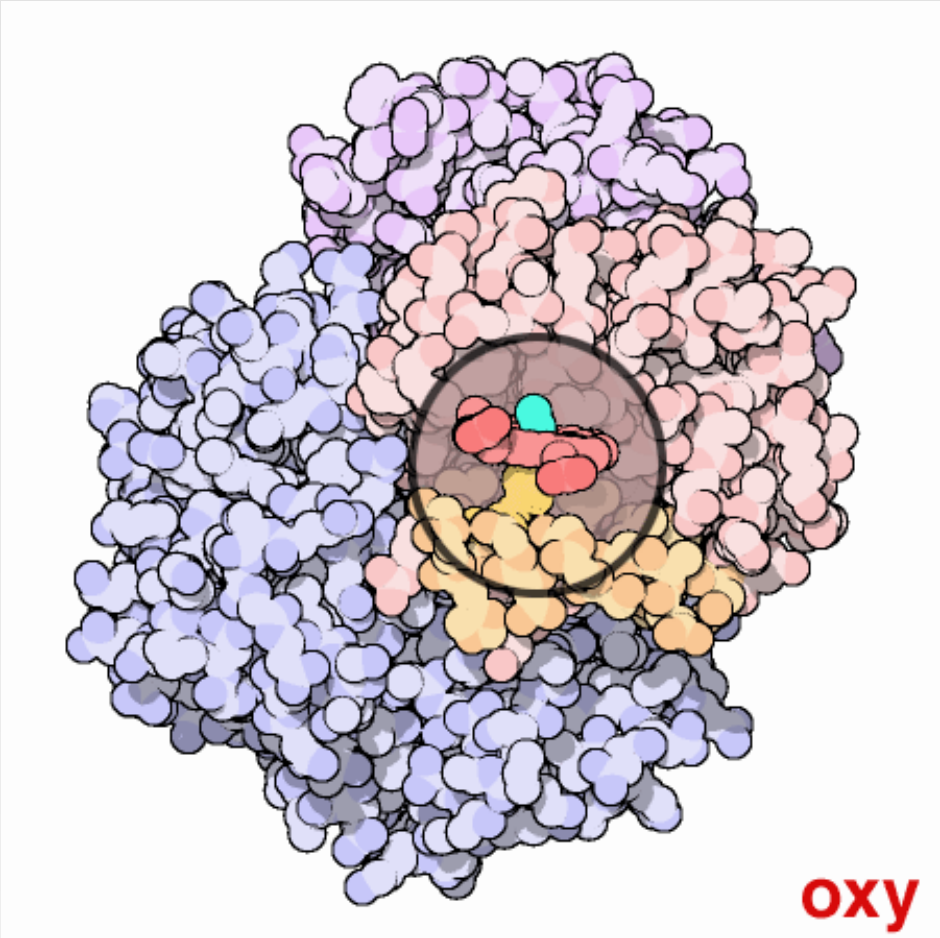
- Among the most inert materials known
  - not immuogenic
  - can be heat sterilized
  - can be manufactured in large quantities
- Must be formulated into emulsions with surfactants
  - most common surfactants are egg-yolk phospholipids
- oxygen solubility is 20 times greater than plasma
- extent of oxygen delivery is regulated by simply controlling pO_2
- PFCs are not metabolized, eventually excreted through the lungs with expired air

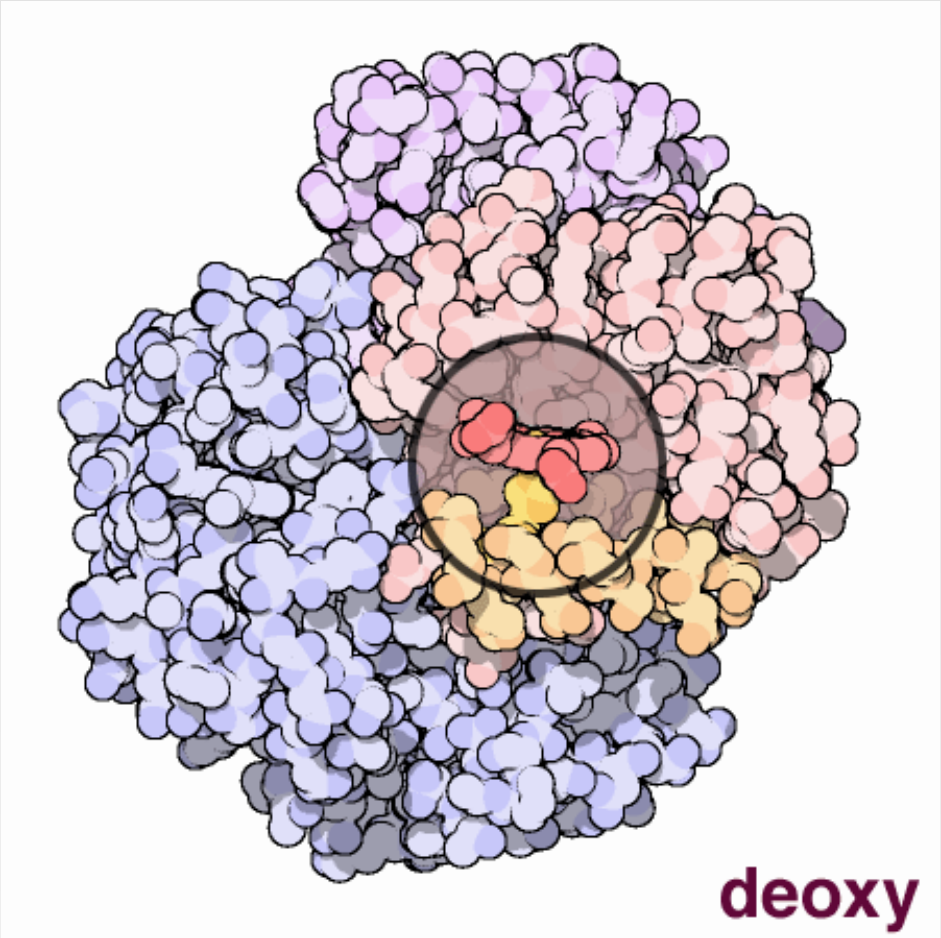
A brief history of fluorocarbon emulsions

- 1966: Clark & Gollan show a mouse could live with oxygen-saturated PFC
- 1970s: Green Cross creates *Fluosol*, emulsion of FDC
  - difficulty to formulate the emulsion
  - excessively long retention times (2.5 years in animals)
  - was used for a time for angioplasty, halted in 1994
- 2000s: Alliance and Baxter Healthcare create PFOB-based *Oxygent* now in phase II and phase III human trials

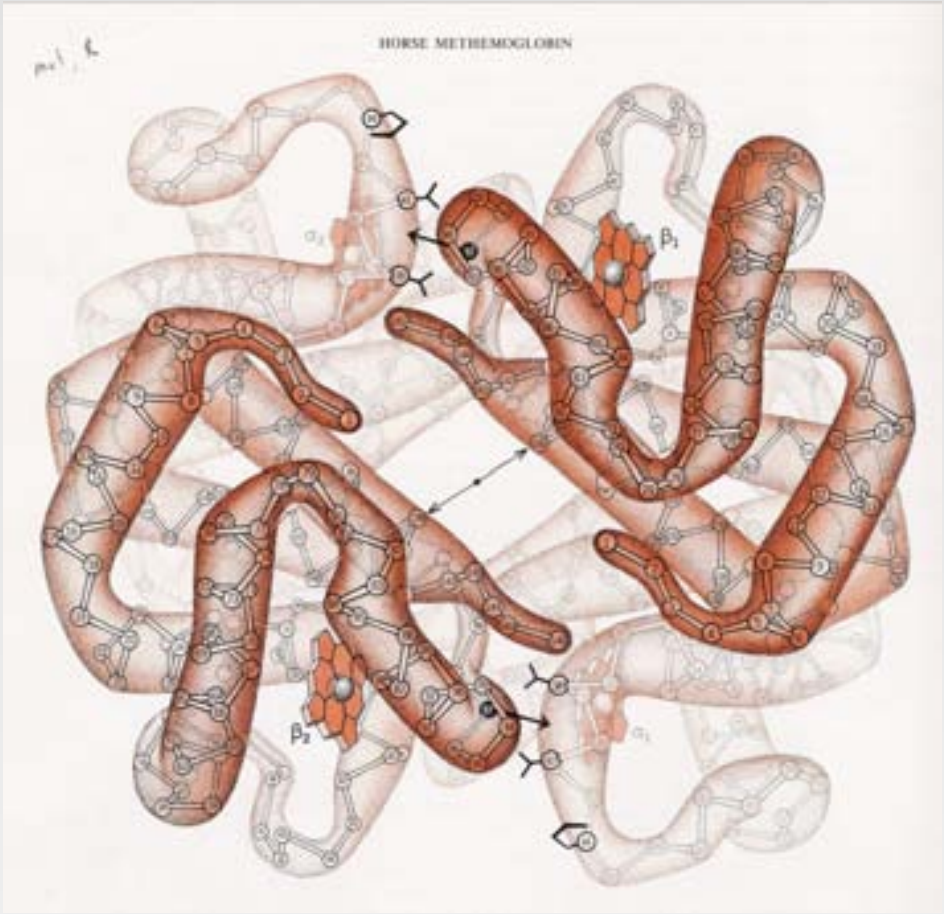


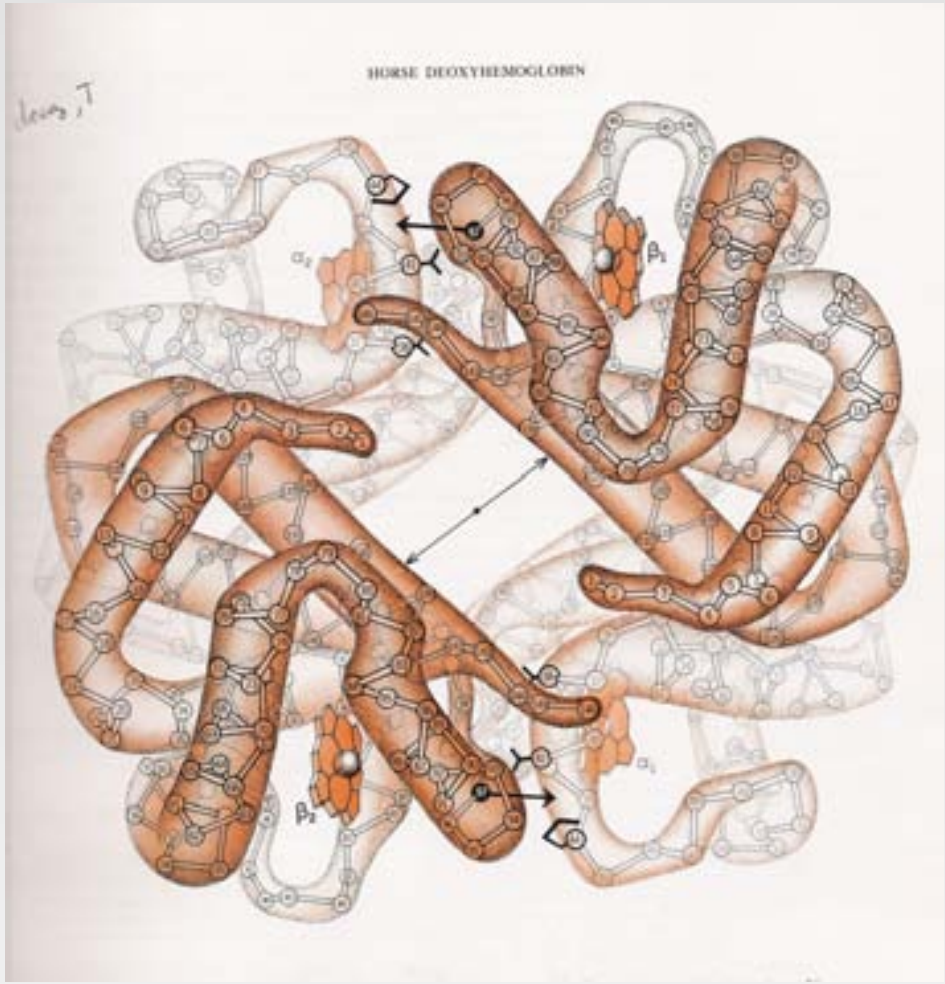
Hemoglobin as a shape-shifter



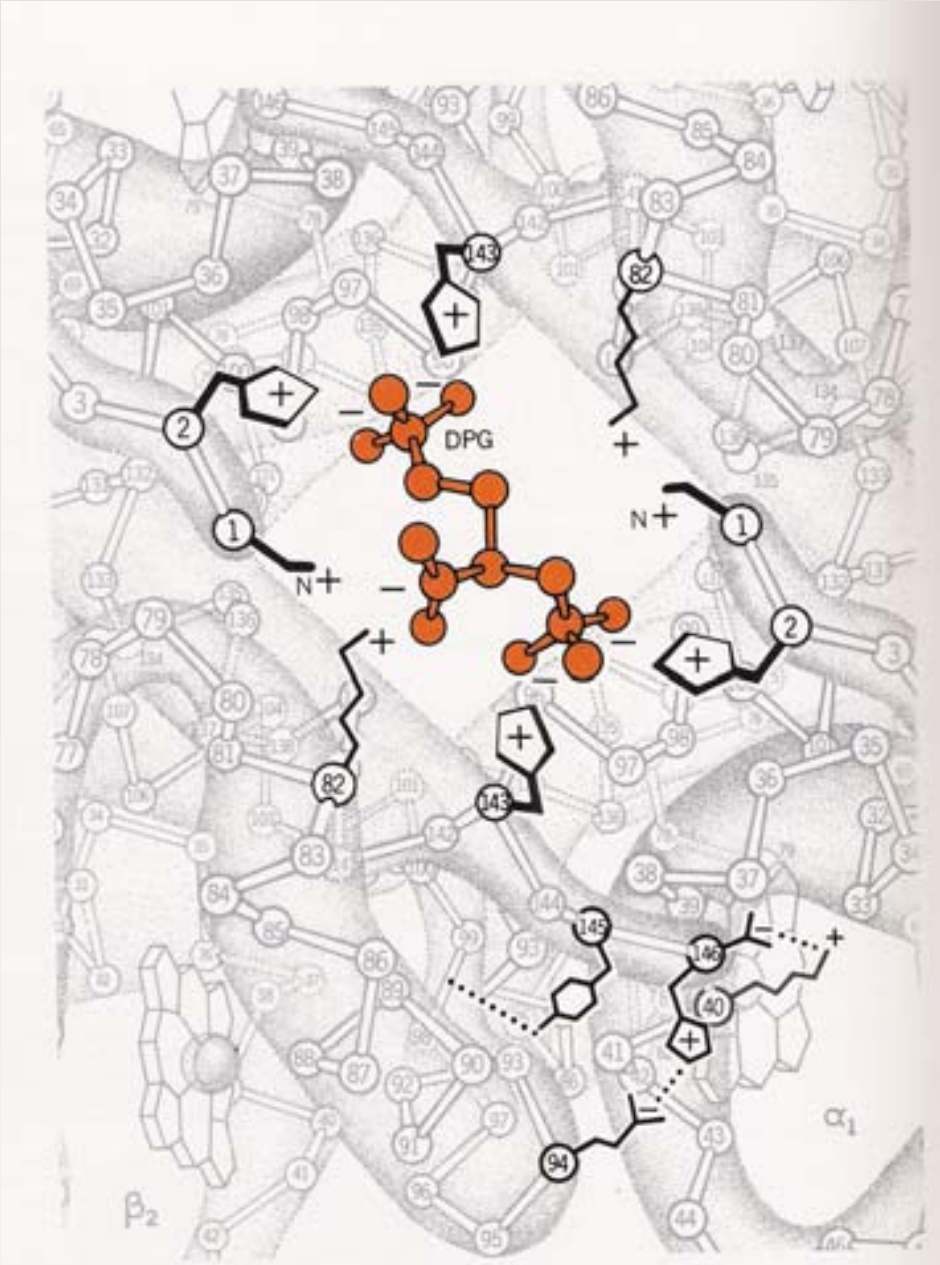


Regulation of affinity by phosphates



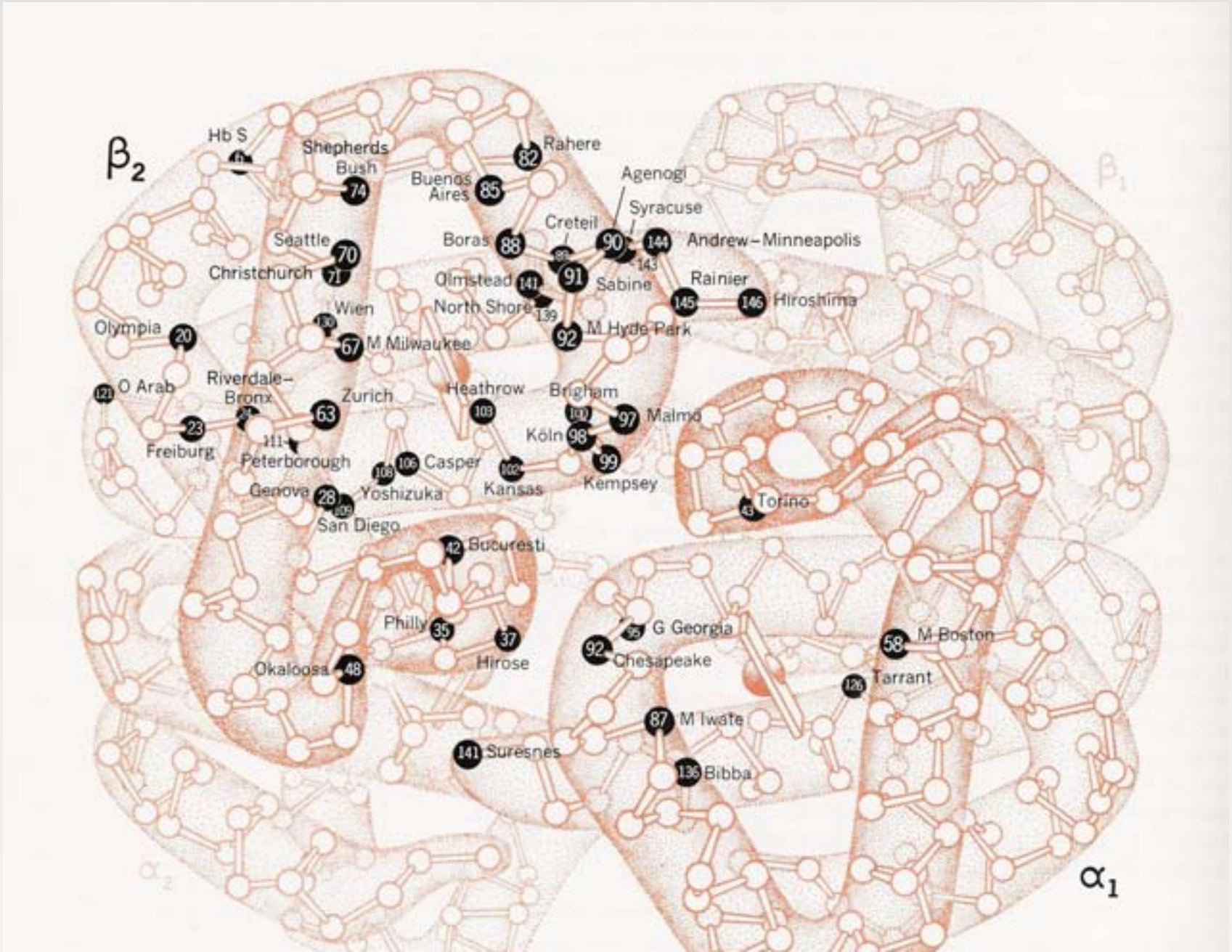


DPG binds only in the bigger central cavity



- DPG binds only to the deoxy form of hemoglobin
- The deoxy form has lower affinity for oxygen
- $\Rightarrow$DPG lowers oxygen affinity
- DPG is in red blood cells but not in the bloodstream itself
- $\Rightarrow$a red-blood-cell free hemoglobin needs some other way to lower oxygen affinity

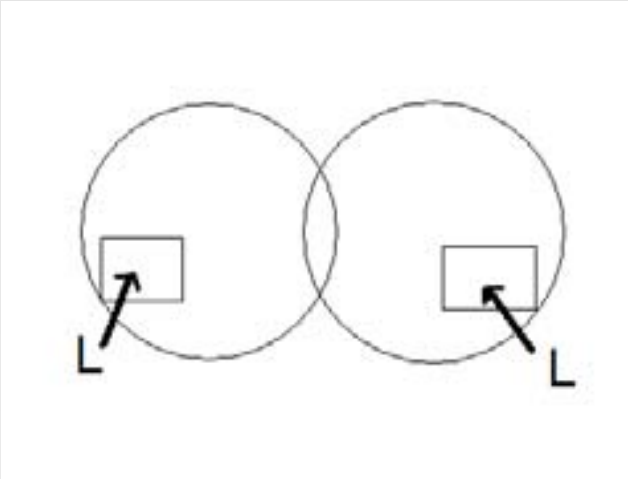
“Mutant” hemoglobins offer ideas



Main challenges for hemoglobin based substitutes

- protein itself too small and is rapidly excreted through the kidneys
  - $\Rightarrow$stabilize the tetramer form relative to dimers (*e.g.* by mutations)
  - $\Rightarrow$chemically cross link into oligomers
- oxygen affinity is too high in the absence of DPG
  - $\Rightarrow$search for modified hemoglobins with lower affinity (Hb Presbyterian)
- adverse vasoconstriction and blood pressure effects
  - understand more fully how NO interacts with hemoglobin
- difficulty and expense of producing modified hemoglobins
  - bacterial toxins contaminate proteins produced in *E. coli*
  - expression in transgenic pigs, or in yeast, is still quite expensive

Simple model of allostery



$$[P] = 1$$

$$\frac{[P_\alpha]}{[P][L]} = k$$

$$[P_\alpha] = k[L]; \quad [P_\beta] = k[L]$$

$$\frac{[P_{\alpha\beta}]}{[P_\alpha][L]} = k \Rightarrow [P_{\alpha\beta}] = k^2[L]^2$$

Note the the partition function is just the sum of the relative populations (concentrations) of all species:

$$Q = 1 + 2k[L] + k^2[L]^2 = q_0 + q_1\lambda + q_2\lambda^2 = \sum_{i=0}^{N} q_i\lambda^i$$

More on allostery

Now, compute the fraction of binding sites that contain ligands:

$$\overline{y} = \frac{\binom{0}{2}1 + \binom{1}{2}2k\lambda + \binom{2}{2}k^2\lambda^2}{1 + 2k\lambda + k^2\lambda^2} = \frac{1}{2}\frac{\sum i q_i \lambda^i}{\sum q_i \lambda^i}$$

$$= \frac{\lambda}{2}\frac{\sum i q_i \lambda^{i-1}}{\sum q_i \lambda^i} = \frac{\lambda}{2}\frac{\partial \ln Q}{\partial \lambda}$$

$$\boxed{\overline{y} = \frac{\lambda}{N}\frac{\partial \ln Q}{\partial \lambda} = \frac{1}{N}\frac{\partial \ln Q}{\partial \ln \lambda}}$$

Since this is *uncoupled* binding:

$$Q = 1 + 2k\lambda + k^2\lambda^2 = (1 + k\lambda)^2$$

See Onufriev, Case, Ullmannn, *Biochemistry* **40**, 3413 (2001) for a generalization.

The Henderson-Hasselbach equation

$$\begin{aligned} AH &\Leftrightarrow A^- + H^+ \\ Q &= 1 + k\lambda \end{aligned}$$

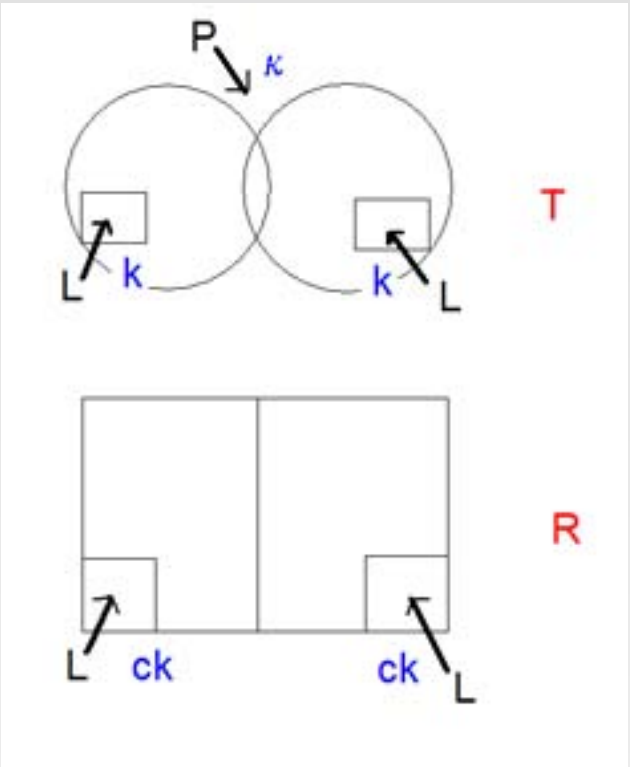
$$\overline{y} = \lambda \frac{\partial \ln Q}{\partial \lambda} = \frac{\lambda k}{1 + \lambda k}$$

Now, $k = 10^{pK_a}$ and $\lambda = 10^{-pH}$; hence:

$$\overline{y} = \frac{10^{pK_a - pH}}{1 + 10^{pK_a - pH}}$$

This yields the usual sigmoidal binding curve you learned about in high school.

Hemoglobin-like model



$$\frac{[T^P]}{[T][P]} = \kappa; \quad \mu \equiv [P]$$

$$\frac{[R]}{[T]} = L$$

λ	T	T^P	R
0	1	$\mu\kappa$	L
1	$k\lambda$	$\mu\kappa k\lambda$	$Lck\lambda$
1	$k\lambda$	$\mu\kappa k\lambda$	$Lck\lambda$
2	$k^2\lambda^2$	$\mu\kappa k^2\lambda^2$	$Lc^2k^2\lambda^2$

$$Q = (1 + k\lambda)^2(1 + \mu\kappa) + L(1 + ck\lambda)^2$$

No phosphate

$$
\begin{aligned}
\bar{y} &= \frac{1}{2}\frac{\partial \ln Q}{\partial \ln \lambda} = \frac{\lambda}{2Q}\left(\frac{\partial Q}{\partial \lambda}\right) \\
&= \frac{\lambda}{2Q}\frac{\partial}{\partial \lambda}\left[(1+k\lambda)^2 + L(1+ck\lambda)^2\right] \\
&= \frac{\lambda}{2Q}\left[2(1+k\lambda)k + 2L(1+ck\lambda)ck\right] \\
&= \frac{(1+k\lambda)k\lambda + L(1+ck\lambda)ck\lambda}{(1+k\lambda)^2 + L(1+ck\lambda)^2}
\end{aligned}
$$

If $L = 0$, get simple non-cooperative binding; for $L < 1$ and $c > 1$ (that is, T state is favored in the absence of ligand, but the R state has a higher affinity), get "hemoglobin-like" cooperative binding.
When $\mu > 0$, get a linkage between $\overline{y_L}$ and $\overline{y_P}$.

Linkage relations

$$N\overline{y_L} = \frac{\partial \ln Q}{\partial \ln \lambda}; \quad M\overline{y_P} = \frac{\partial \ln Q}{\partial \ln \mu}$$

$$\begin{aligned} d(\ln Q) &= \frac{\partial \ln Q}{\partial \ln \lambda} d\ln\lambda + \frac{\partial \ln Q}{\partial \ln \mu} d\ln\mu \\ &= N\overline{y_L} d\ln\lambda + M\overline{y_P} d\ln\mu \end{aligned}$$

or (see pp. 25-26 in Slater):

$$\boxed{\left(\frac{\partial \ln \lambda}{\partial \ln \mu}\right)_{y_P} = -\frac{M}{N}\left(\frac{\partial \overline{y_P}}{\partial \overline{y_L}}\right)}$$

Let $P = H^+$ and L=O_2 and $N = 4$:

$$\left(\frac{\partial \log[O_2]}{\partial pH}\right)_{\overline{y_{O2}}} = \frac{M}{4}\left(\frac{\partial \overline{y_{H+}}}{\partial \overline{y_{O_2}}}\right)_{pH} \simeq H^+_{deoxy} - H^+_{oxy}$$

An example of some literature analysis

Analysis of Cooperativity in Hemoglobin. Valency Hybrids, Oxidation, and Methemoglobin Replacement Reactions†

Attila Szabo and Martin Karplus*

ABSTRACT: An allosteric model proposed previously for structure–function relations in hemoglobin is applied to the analysis of low- and high-spin valency hybrids. By assuming that the low-spin oxidized chains have the tertiary structure of oxygenated chains while the high-spin oxidized chains have a tertiary structure intermediate between that of deoxygenated and oxygenated chains, the model parameters associated with the different valency hybrids can be obtained, and their equilibrium properties can be estimated. The hybrid results are used also to provide an interpretation of methemoglobin and its ligand replacement reactions and of the oxidation–reduction equilibrium of normal hemoglobin. For the various systems studied, it is found that the effects of pH and 2,3-diphosphoglycerate are in agreement with the model.

To understand the mechanism of cooperative ligand binding by the hemoglobin tetramer, it is not sufficient to know the structure and properties of the completely deoxygenated (Hb) and fully oxygenated ($Hb(O_2)_4$) species. Information about the intermediates ($Hb(O_2)$, $Hb(O_2)_2$, $Hb(O_2)_3$) that occur in the course of the oxygenation reaction is required. Such knowledge is difficult to obtain in a highly cooperative system like hemoglobin because the equilibrium concentra-

† From the Institut de Biologie Physico-Chimique, Université de Paris VI, Paris 5e, France, the MRC Laboratory of Molecular Biology, Cambridge CG 2 2QH, England, and the Laboratoire de Chimie Théorique, Université de Paris VII, Paris 5e, France. *Received June 25, 1974*. Supported in part by grants from the National Science Foundation (GP36104X) and the National Institutes of Health (EY00062). A. Szabo was supported by a fellowship from the National Research Council of Canada.

* Address correspondence to Department of Chemistry, Harvard University, Cambridge, Mass. 02138.

This math you've already seen!

(1) Thermodynamic Description and the Allosteric Model

The equilibrium of a macromolecule M with N binding sites for a ligand X at concentration (activity) λ can be described by a generating function (Szabo and Karplus, 1972) defined as:

$$\Xi_N(\lambda) = \sum_{s=0}^{N} \lambda^s A_s \tag{1}$$

where A_s is the macroscopic equilibrium constant for binding of s ligands:

$$\mathrm{M} + s\mathrm{X} \rightleftharpoons \mathrm{MX}_s \tag{2}$$

The utility of the generating function, Ξ_N, lies in the fact that each term $\lambda^s A_s$, $s = 0, 1, \ldots N$, is proportional to the probability that s ligands are bound. Thus, the fractional saturation, $\langle y \rangle$, of the macromolecule with ligand is given by:

$$\langle y \rangle = \frac{\langle s \rangle}{N} = \frac{\sum_{s=0}^{N} s\lambda^s A_s}{\sum_{s=0}^{N} \lambda^s A_s} = \frac{\lambda}{N} \frac{\partial}{\partial \lambda} [\ln \Xi_N(\lambda)] \tag{3}$$

Plot curves as a function of parameters

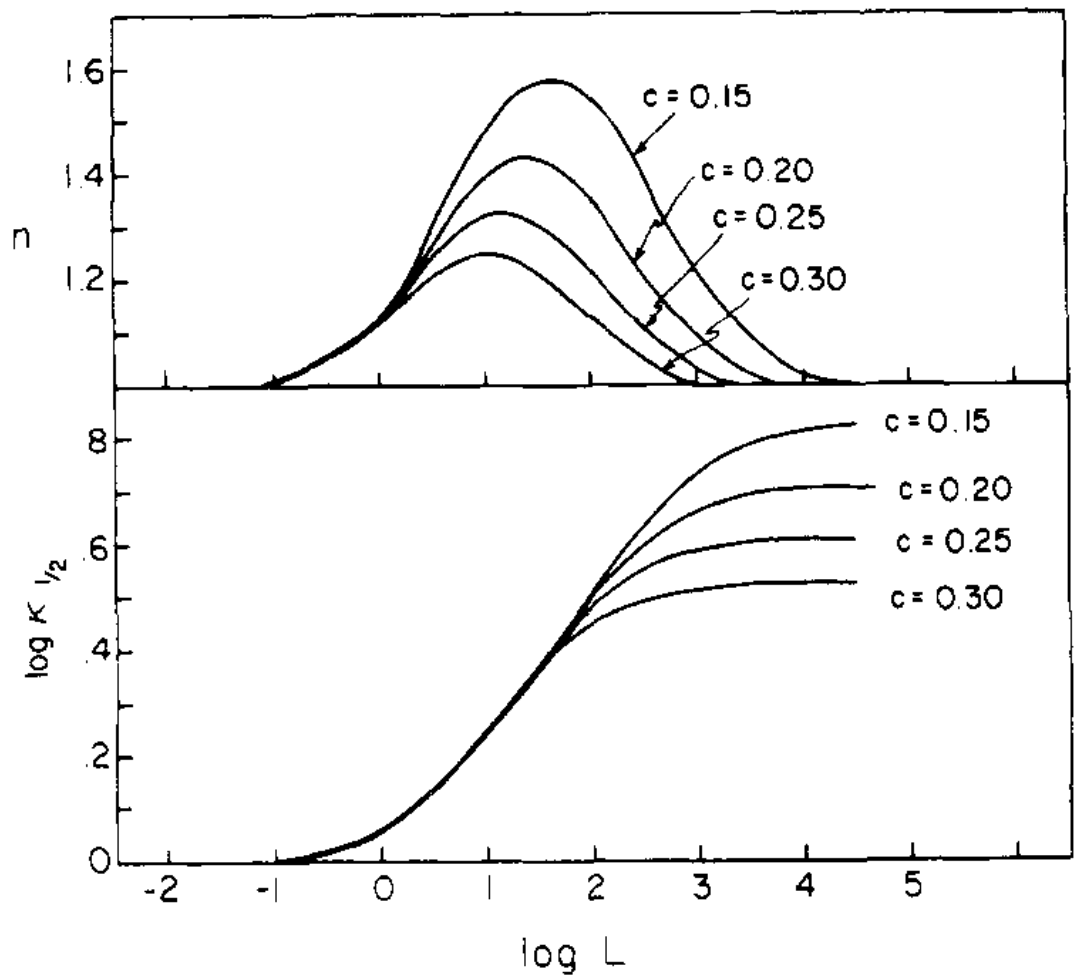
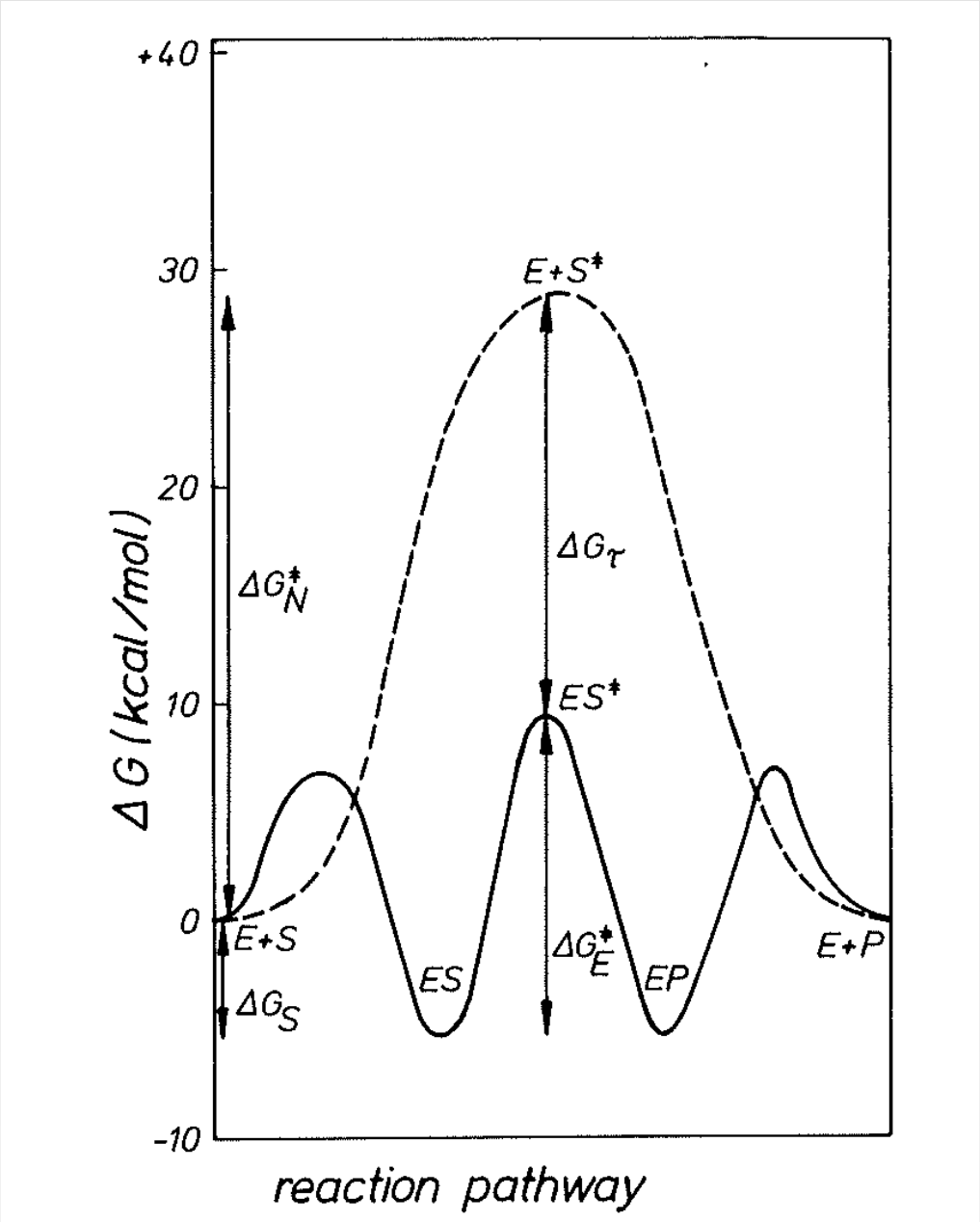
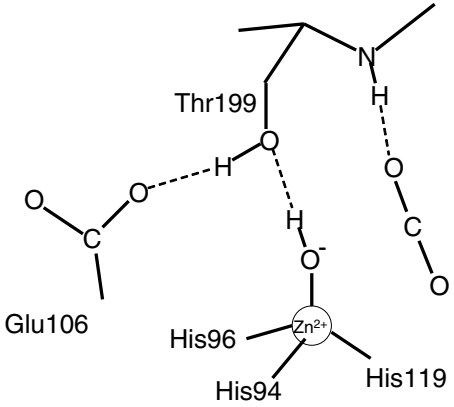


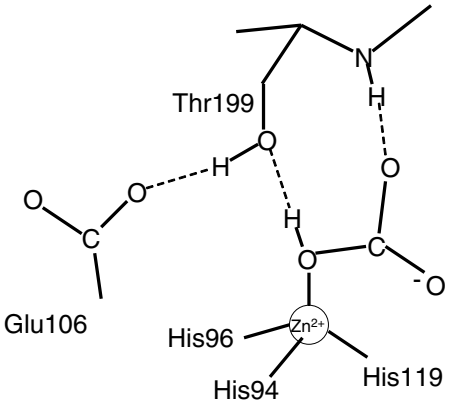
FIGURE 3: Allosteric model for ligand replacement reactions; Hill coefficient n and scaled affinity $\log \kappa_{1/2}$ vs. $\log L$ for $c = 0.15$, 0.20, 0.25, and 0.30.

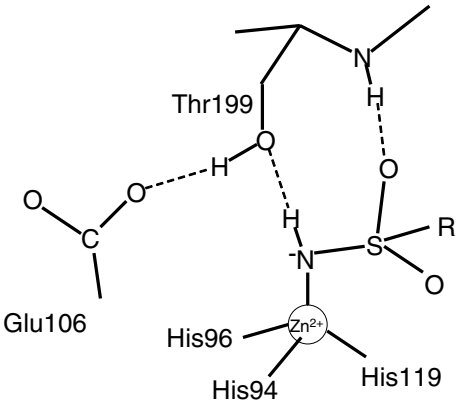
Basic ideas of catalysis

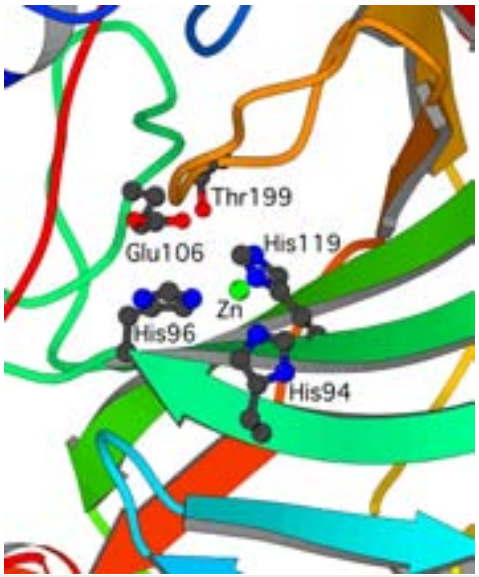


Carbonic anhydrase









Ribonucleotide reductase

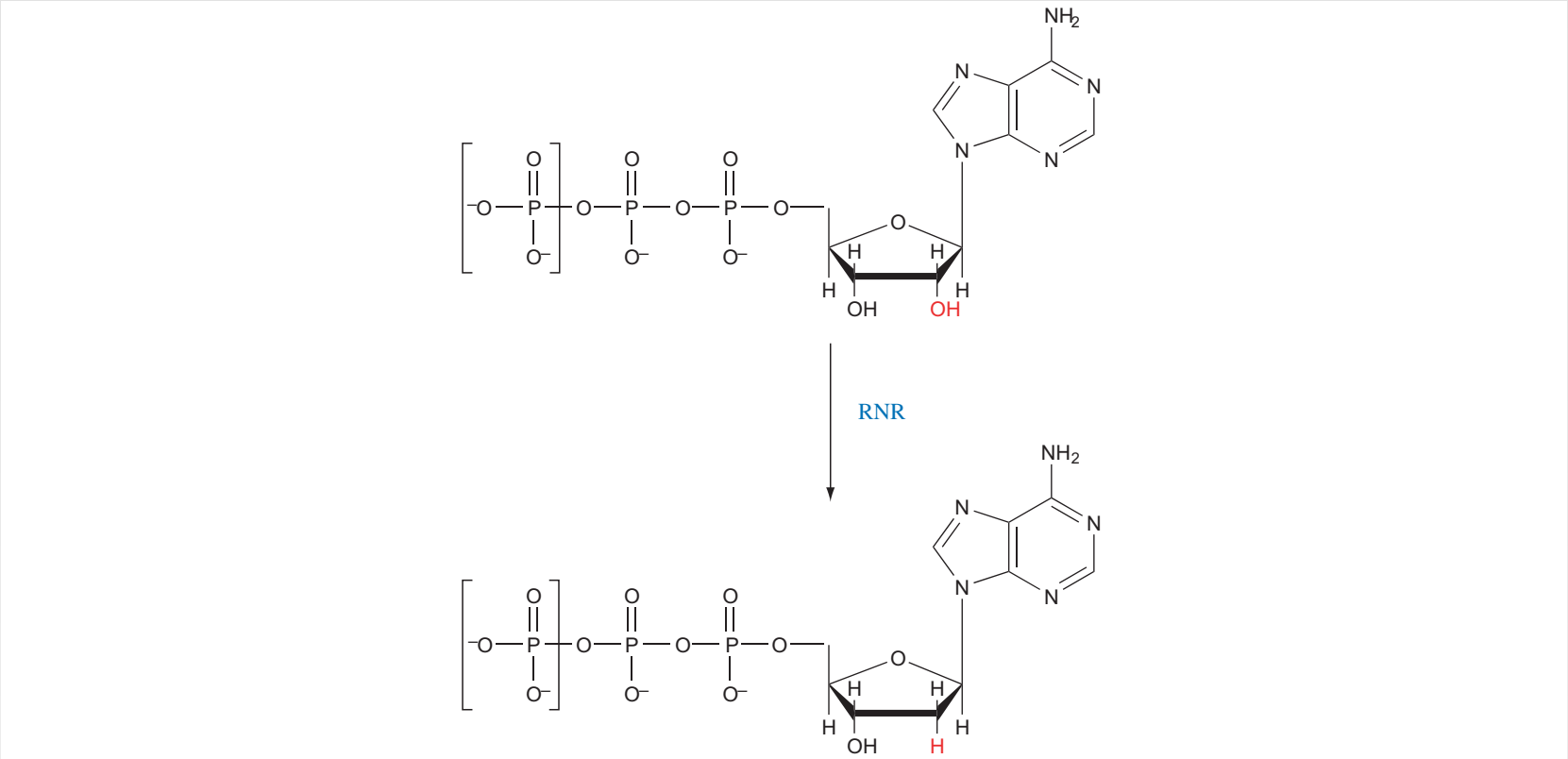
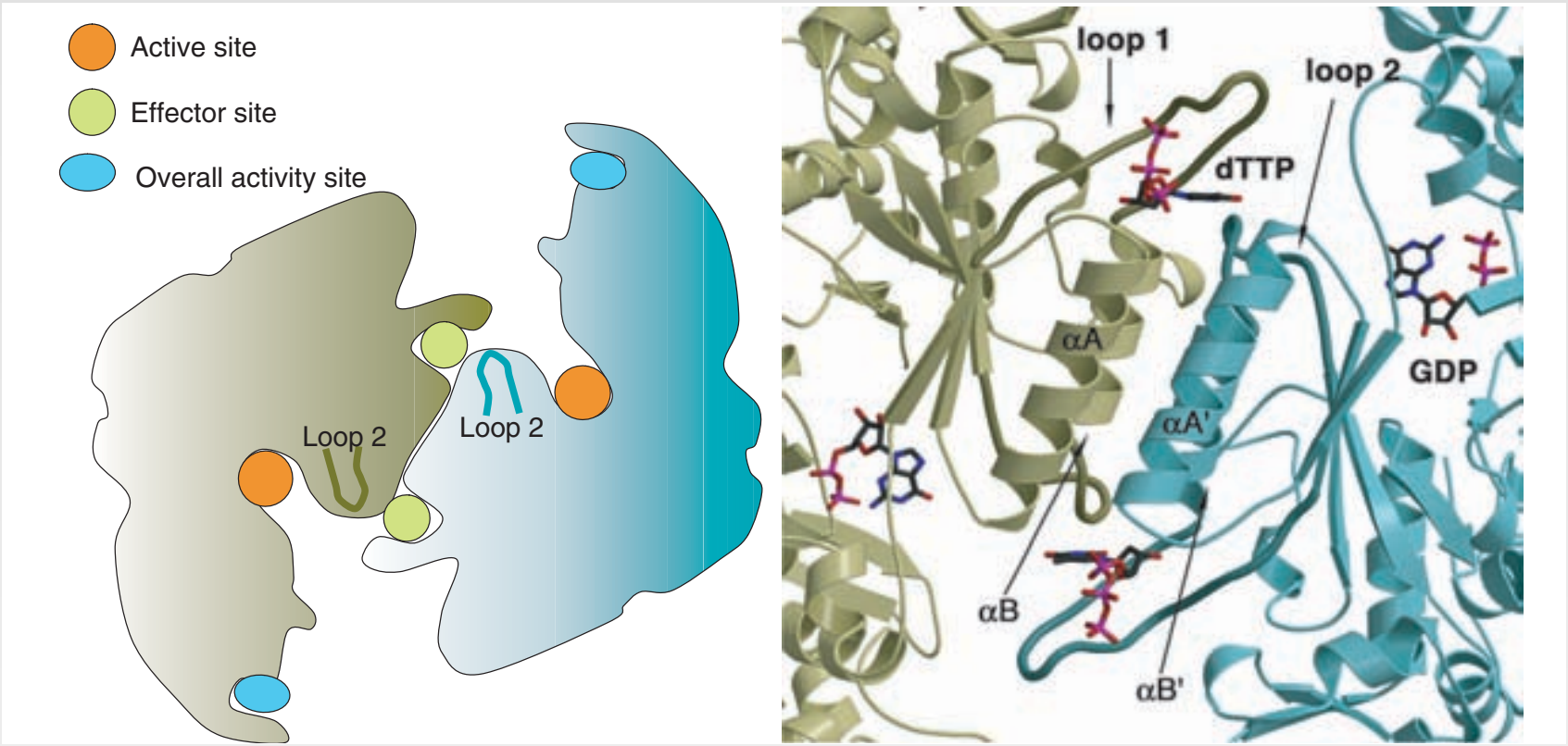
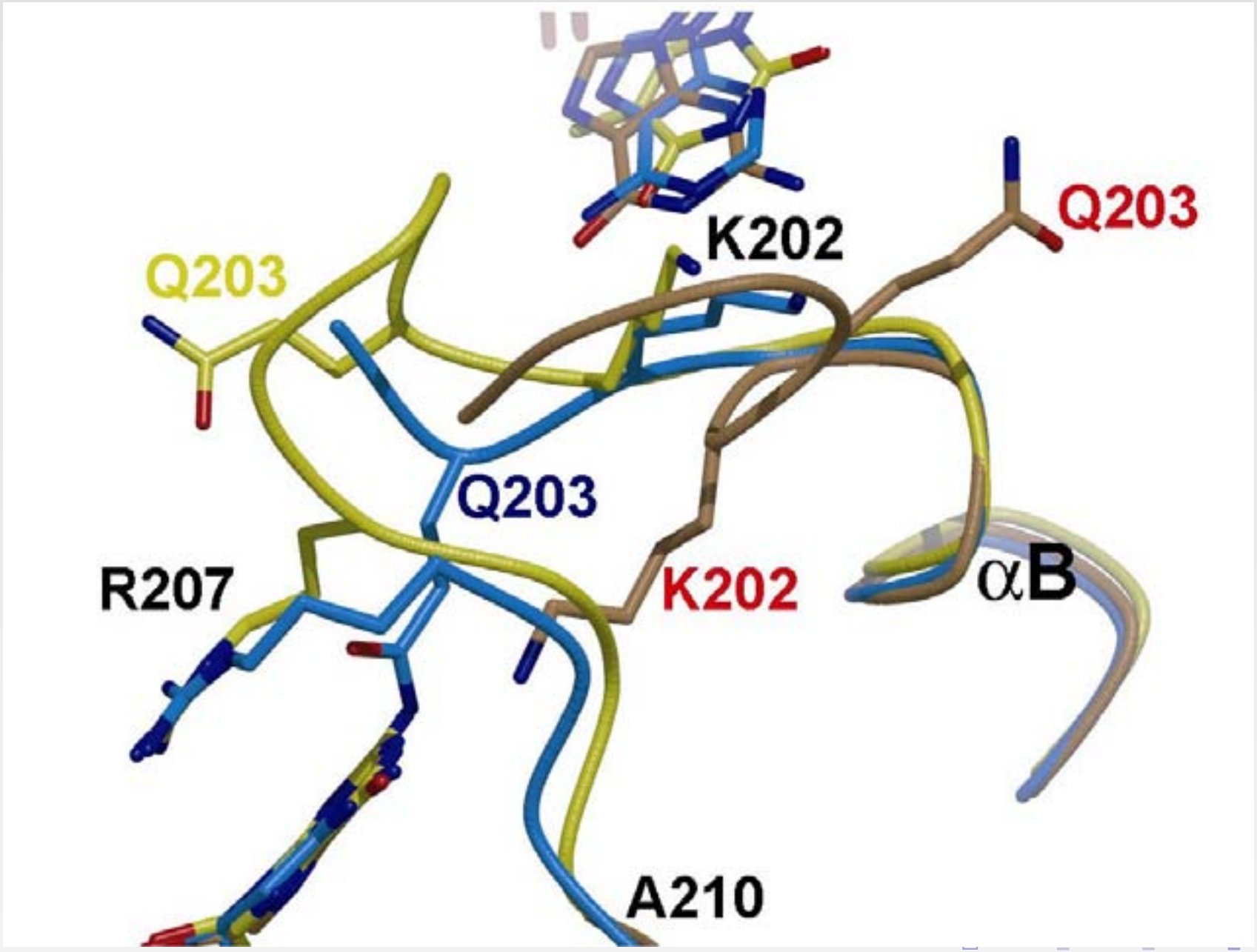


FIGURE 5.3 ■ The reaction catalyzed by ribonucleotide reductase (RNR). The 2′ hydroxyl group of the ribose of ATP is replaced by a hydrogen atom to become dATP. (Illustration kindly provided by Derek Logan.)

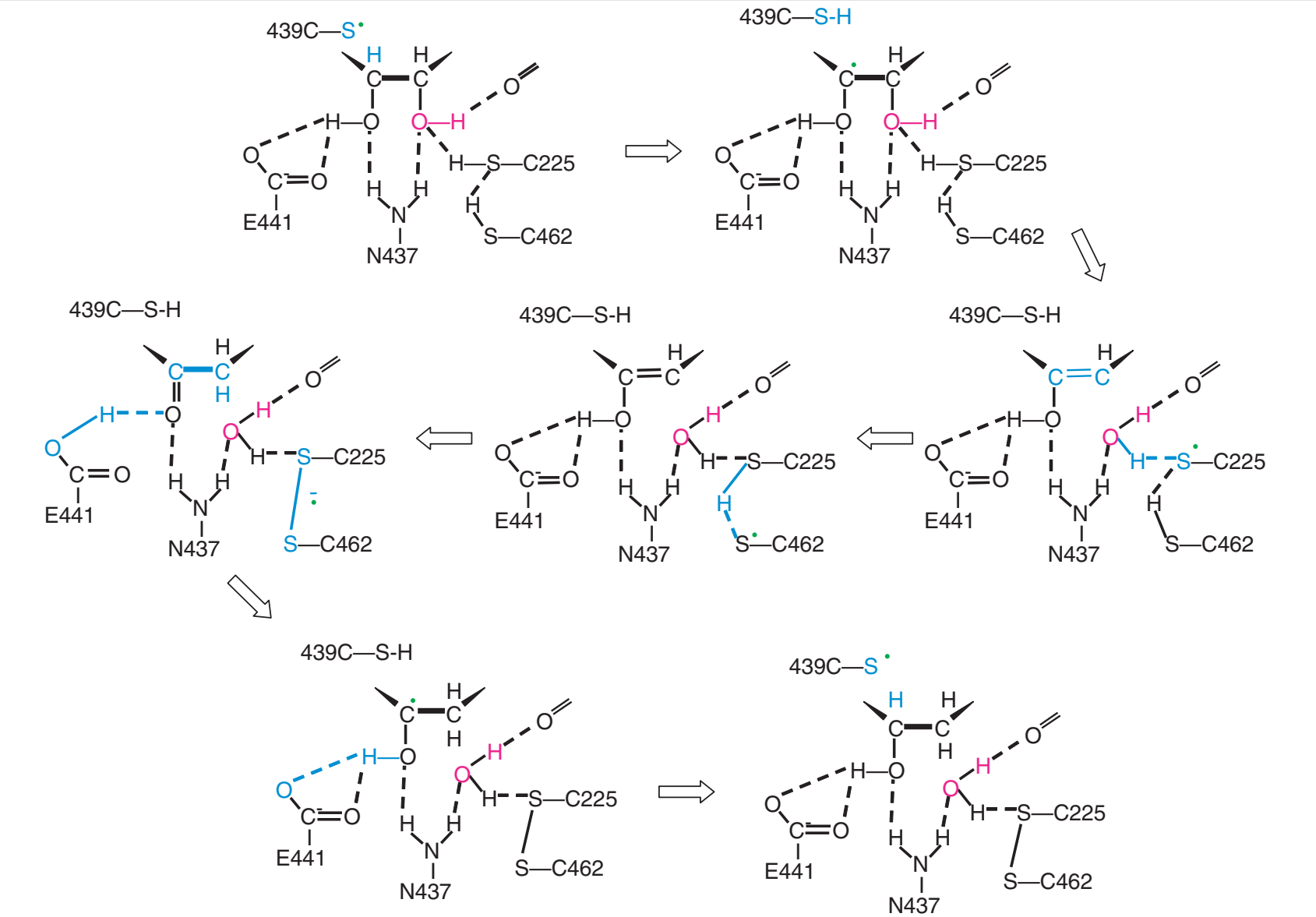
Ribonucleotide reductase



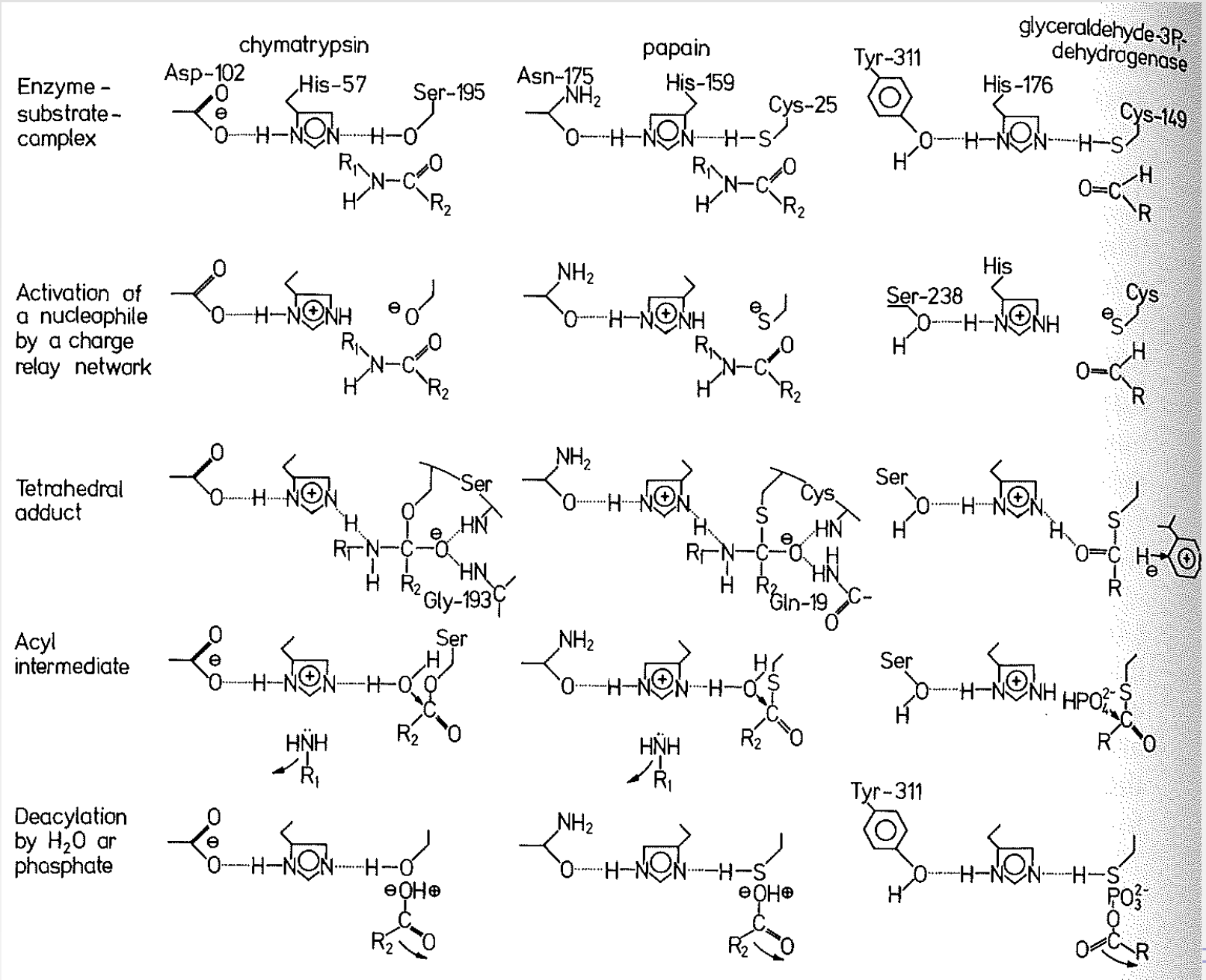
Ribonucleotide reductase



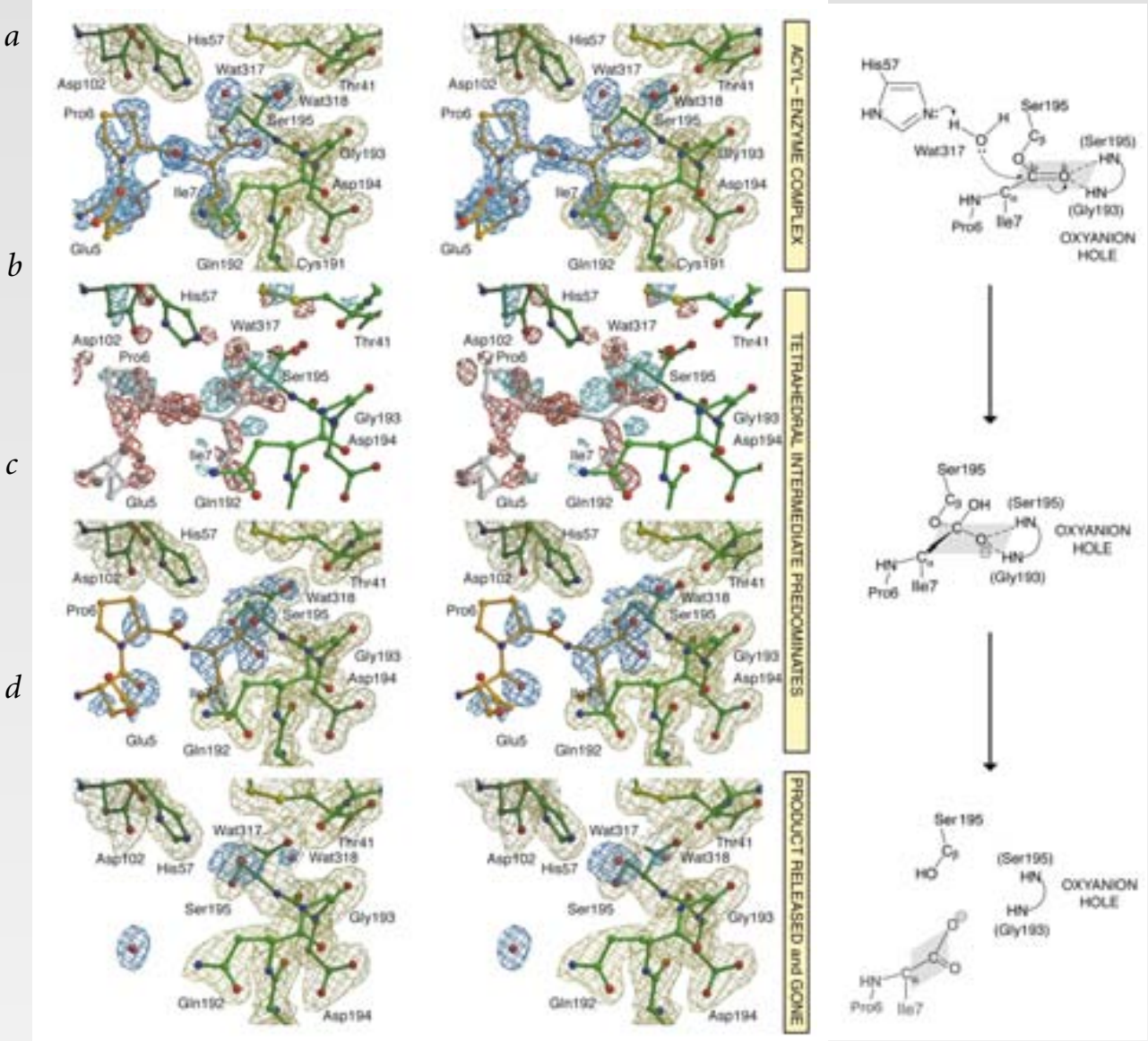
Ribonucleotide reductase



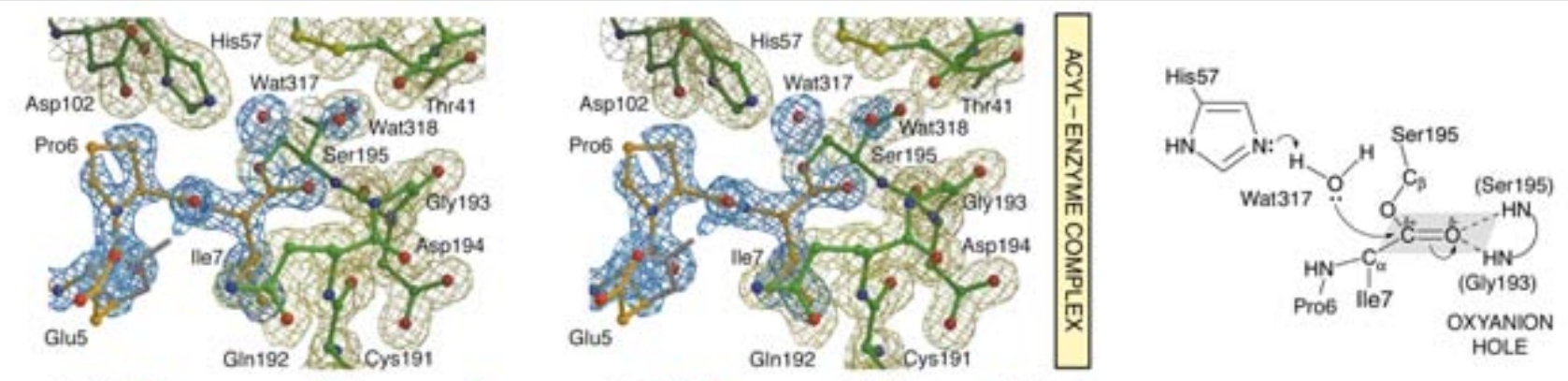
Mechanisms of serine protease hydrolysis



Intermediates in serine proteases can be characterized

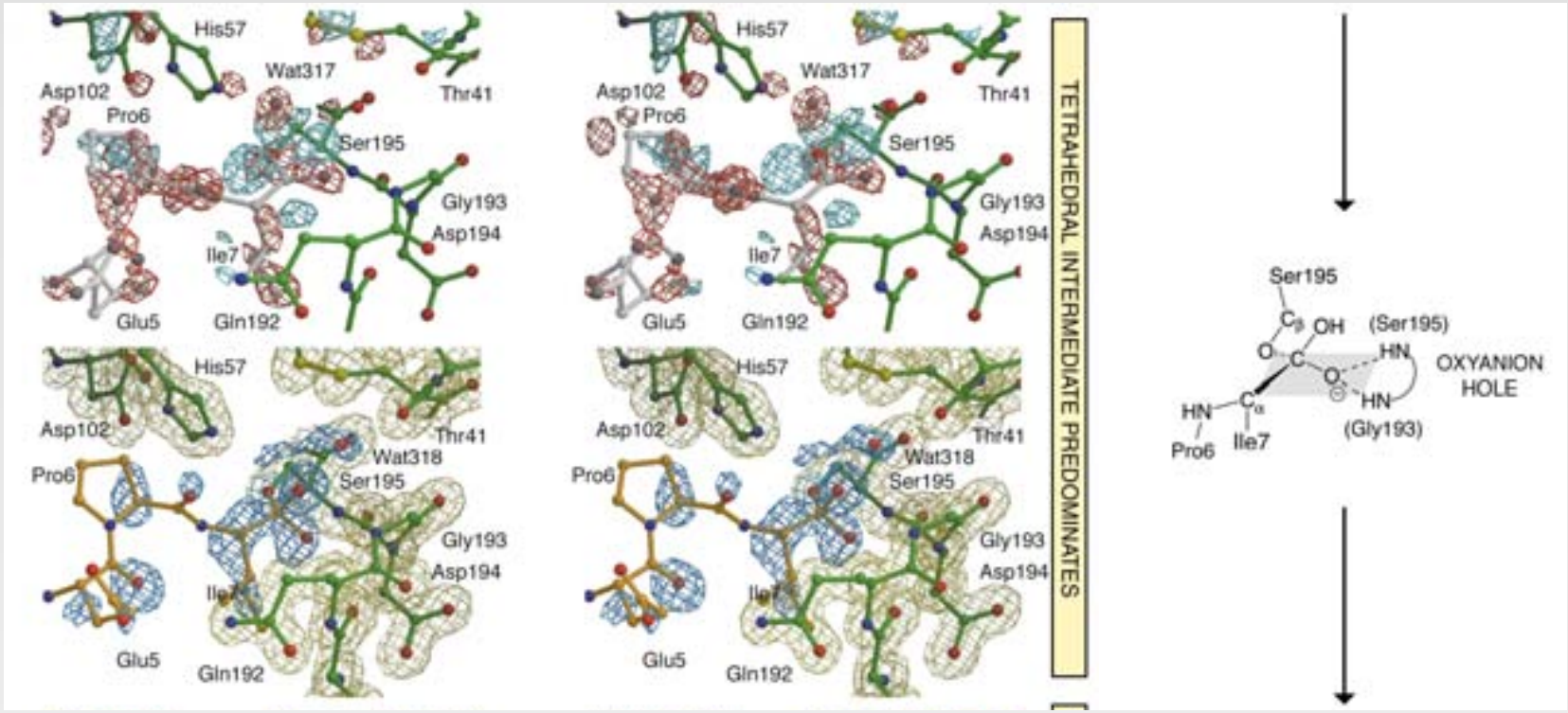


Intermediates in serine proteases

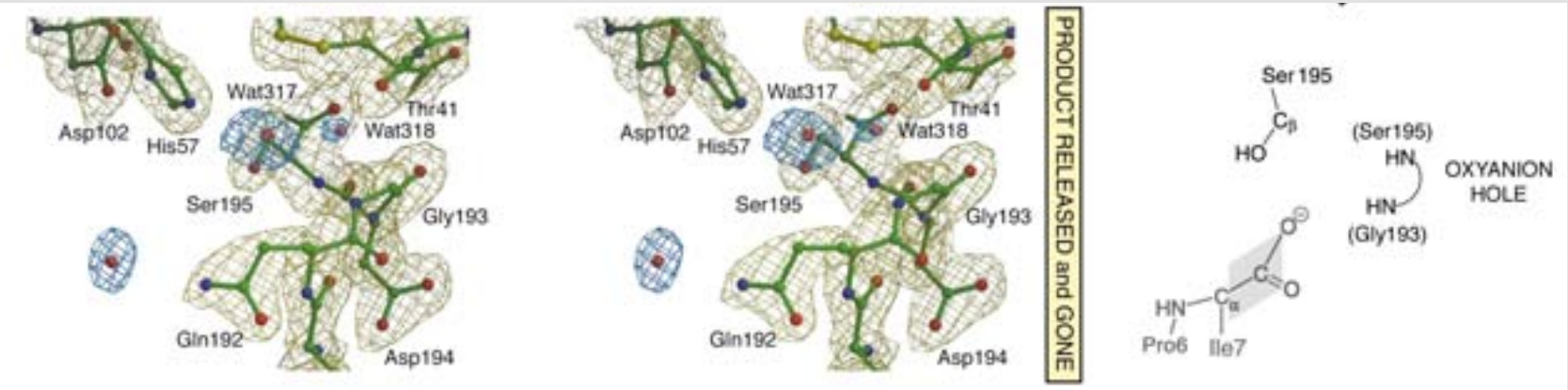


Wilmouth *et al., Nature Struct. Biol.* **8,** 689 (2001)

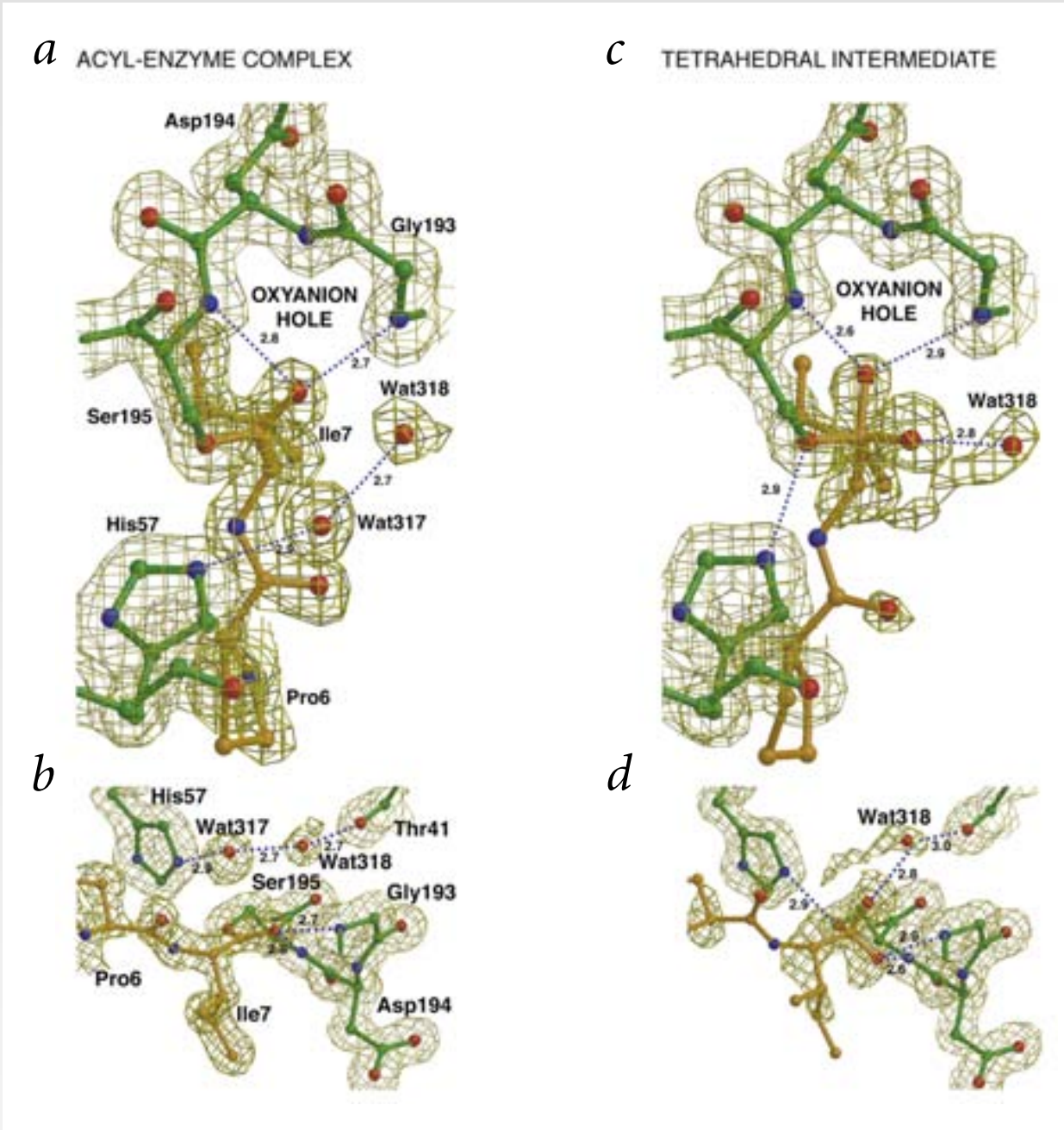
Intermediates in serine proteases



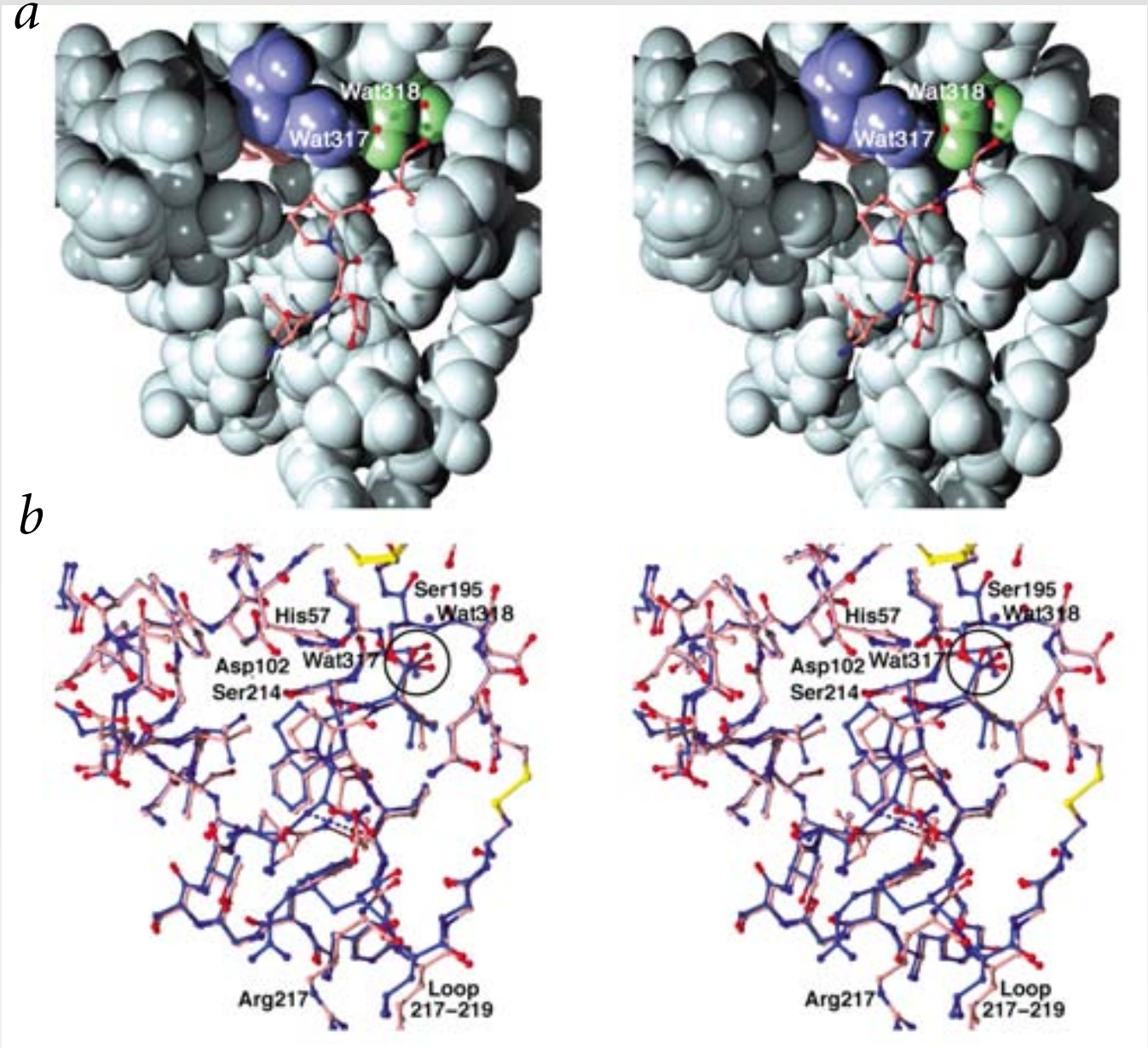
Intermediates in serine proteases



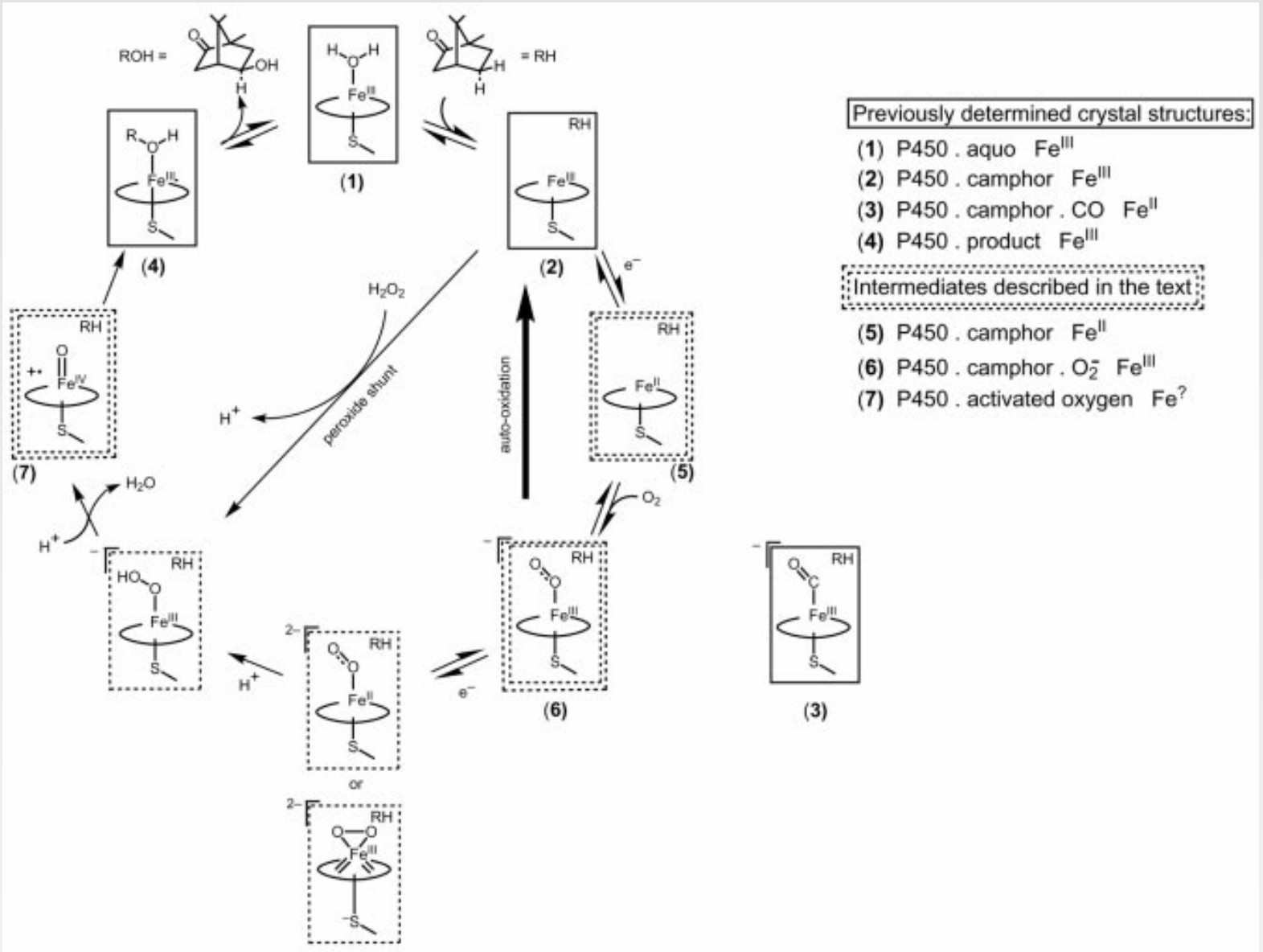
Intermediates in serine proteases



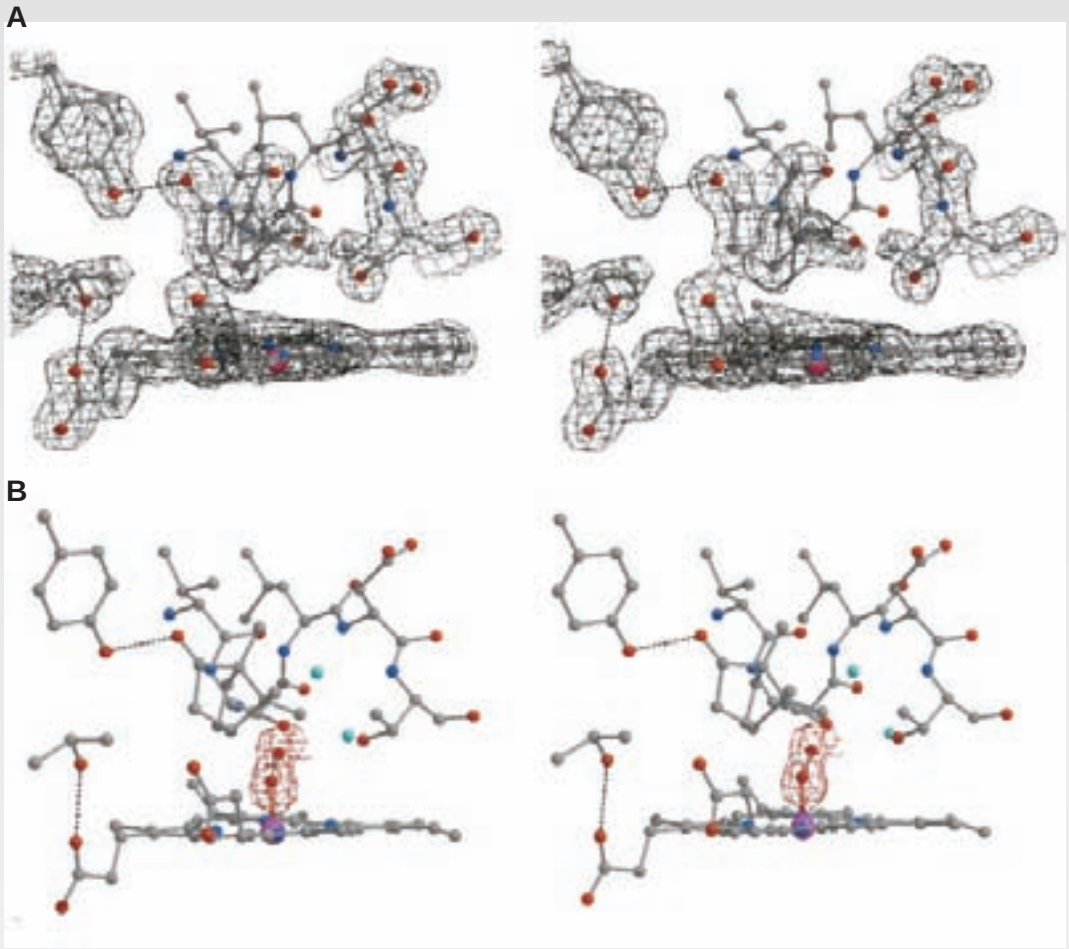
Intermediates in serine proteases



Cytochrome P450 hydroxylates many substrates



Again, intermediates can be structurally characterized



Schlichting *et al., Science* **287,** 1615 (2000)

Catalysis by cytochrome P450

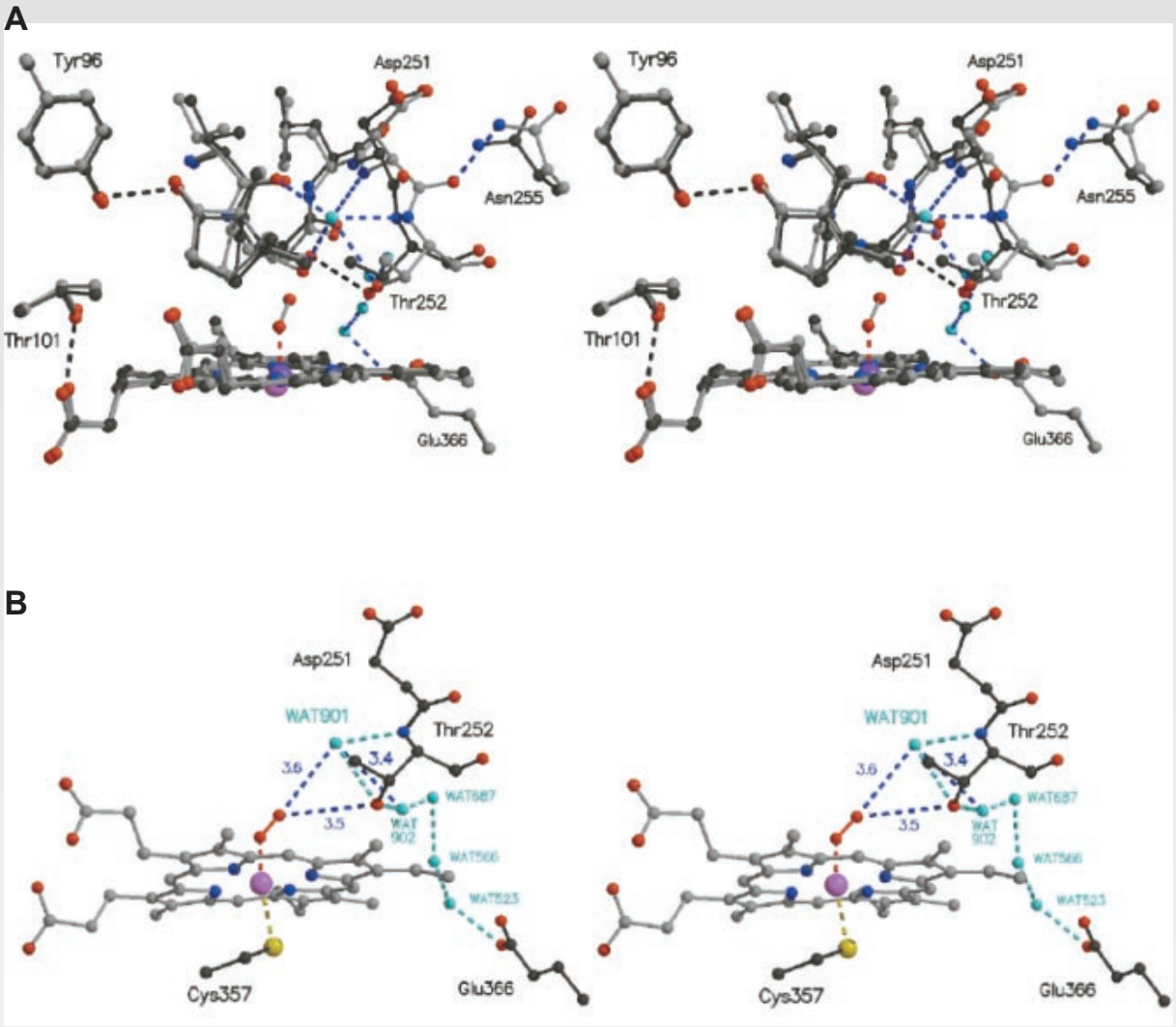
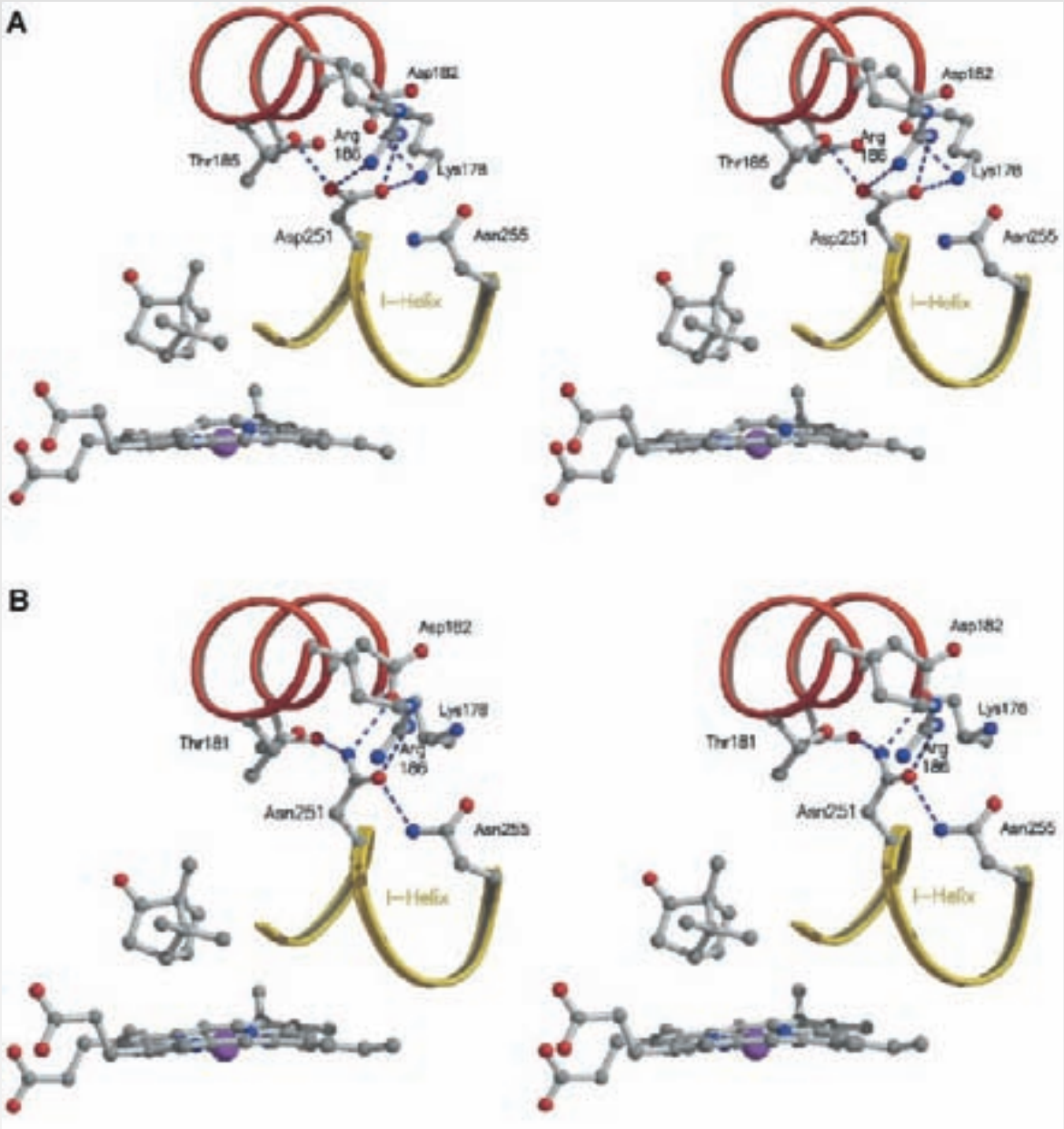
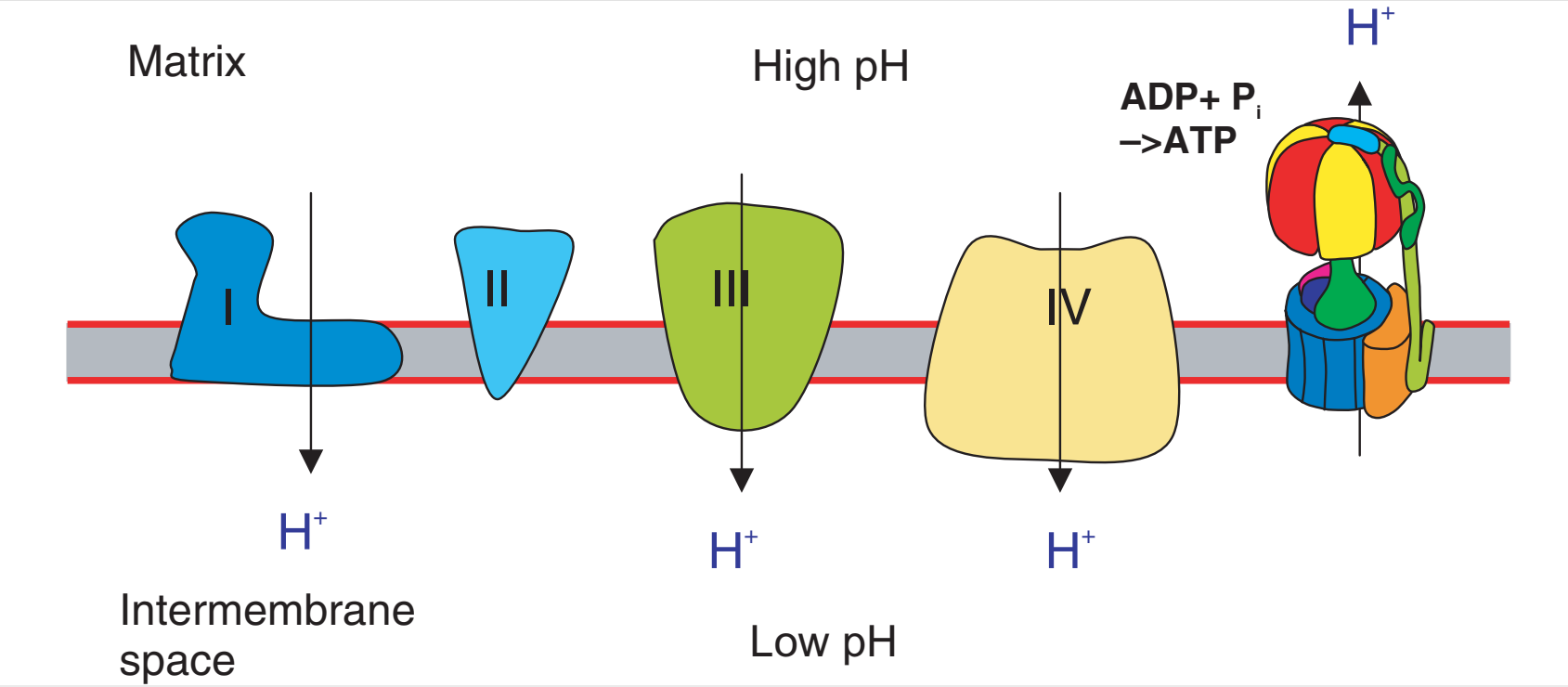


Fig. 4. (**A**) Stereoview of comparison of the camphor complexes of ferrous (dark gray and dark blue water molecules) and ferrous dioxygen-bound (light gray and cyan water molecules) P450. Upon oxygen binding, camphor is displaced, two new water molecules bind, the backbone carbonyl group of Asp^{251} flips, and the backbone amide of Thr^{252} rotates as does its side chain. (**B**) The interactions of the two new water molecules and the water chain extending from the first new water molecule to Glu^{366}. Figures were generated with Bobscript (34) and Raster 3D (35).

Catalysis by cytochrome P450

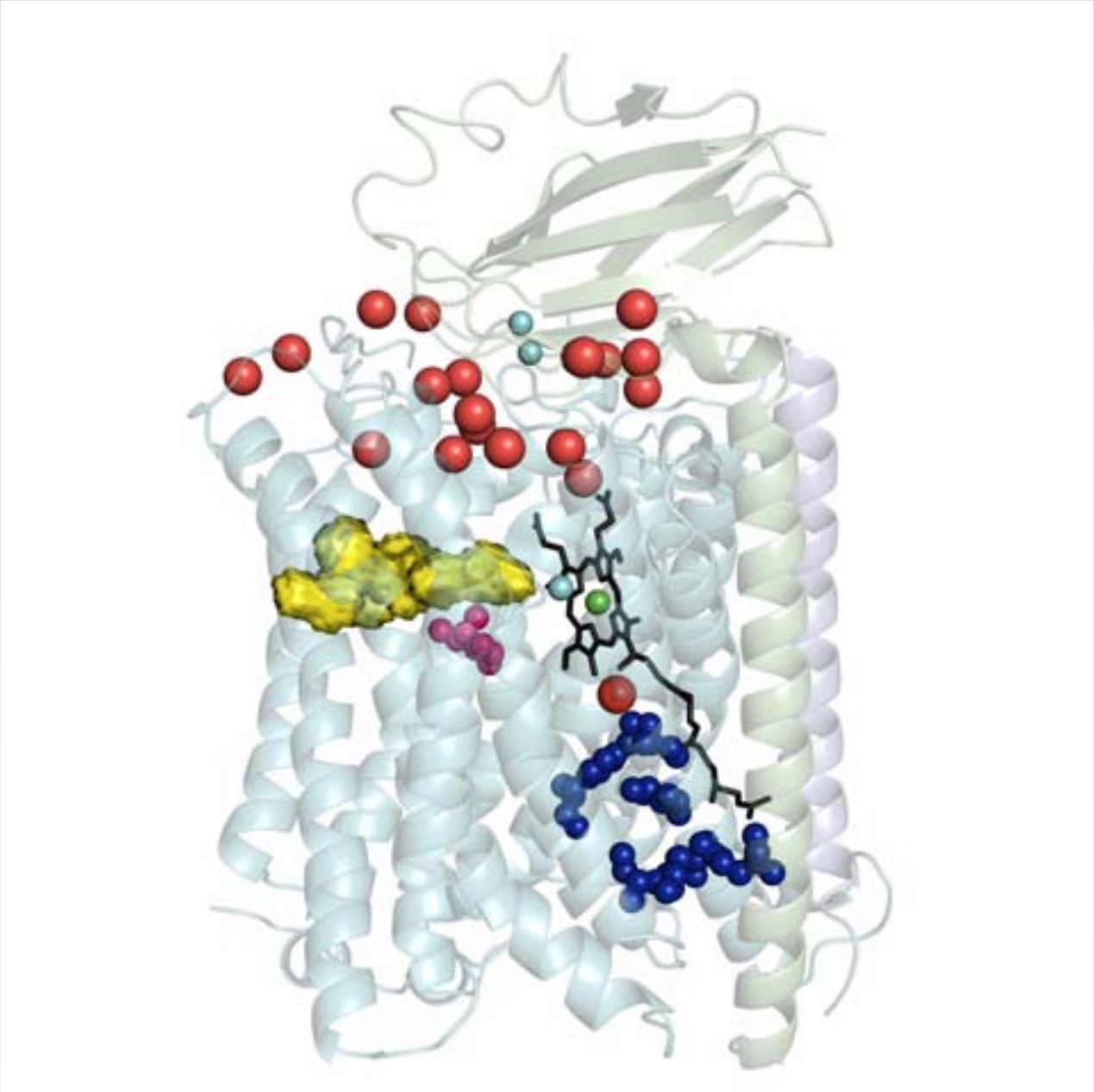


Respiration, again

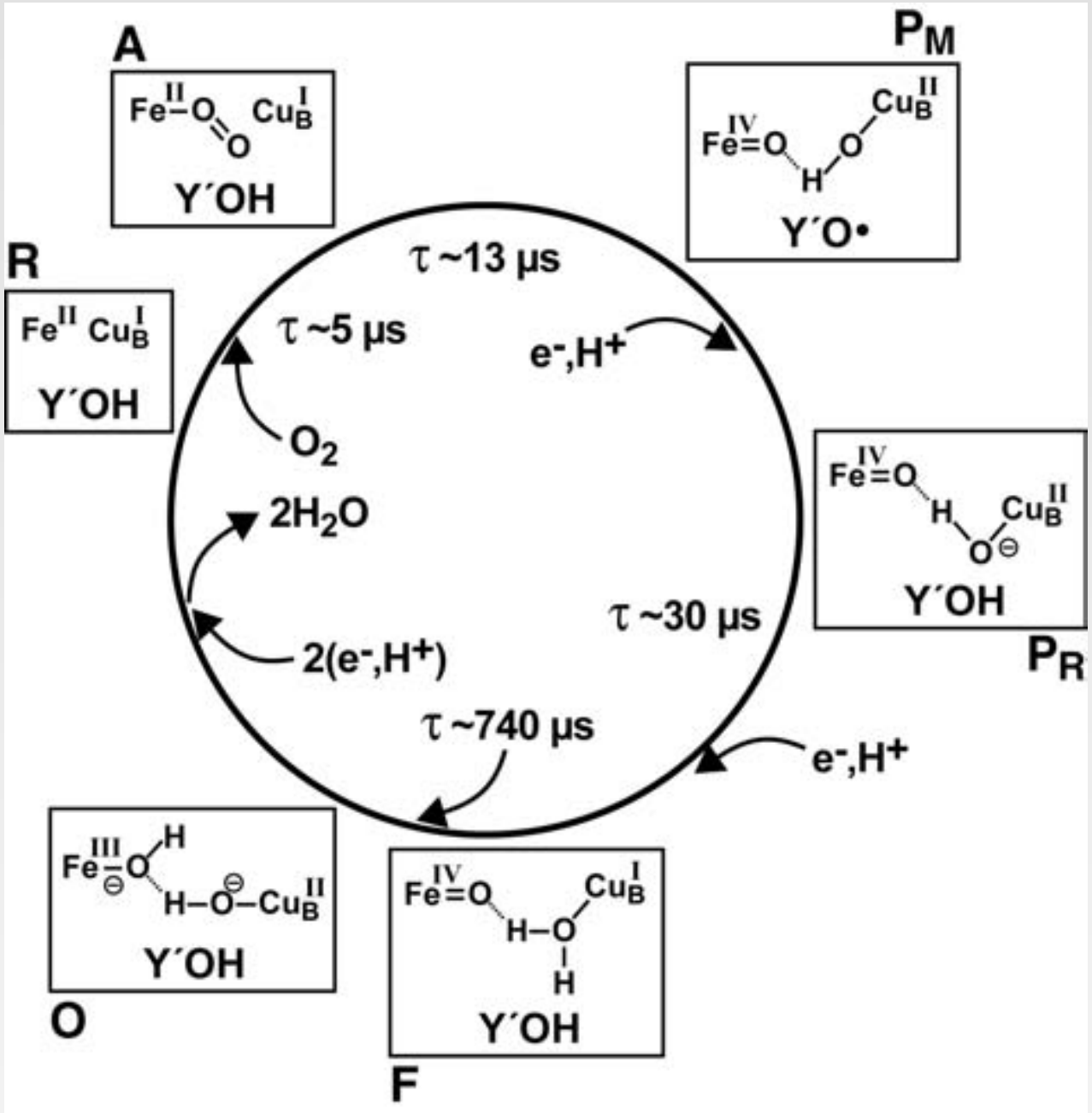


Fee, Case, Noodleman, *J. Am. Chem. Soc.* **130,** 15002 (2008)

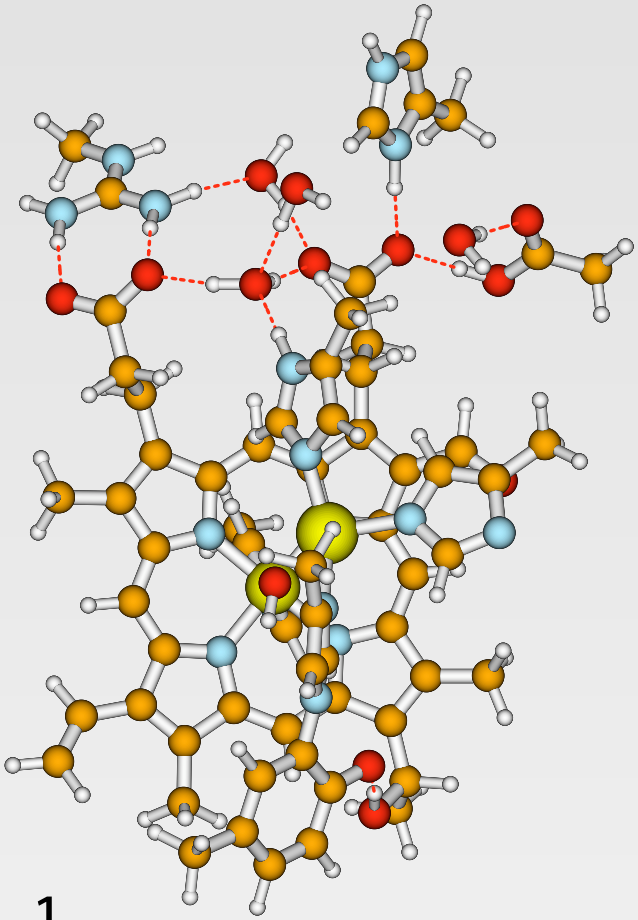
Cytochrome c oxidase shuttles electrons and protons



Catalysis by cytochrome c oxidase

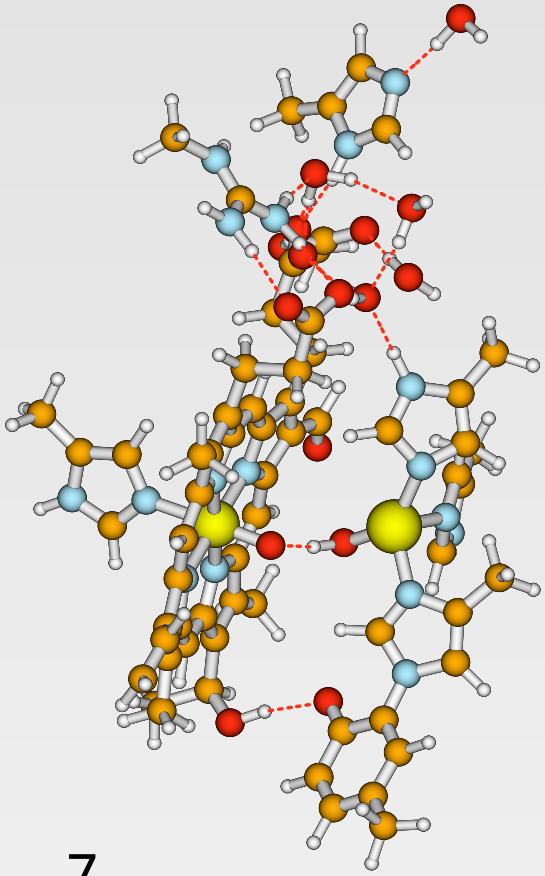


Here are some key (proposed) intermediates



1

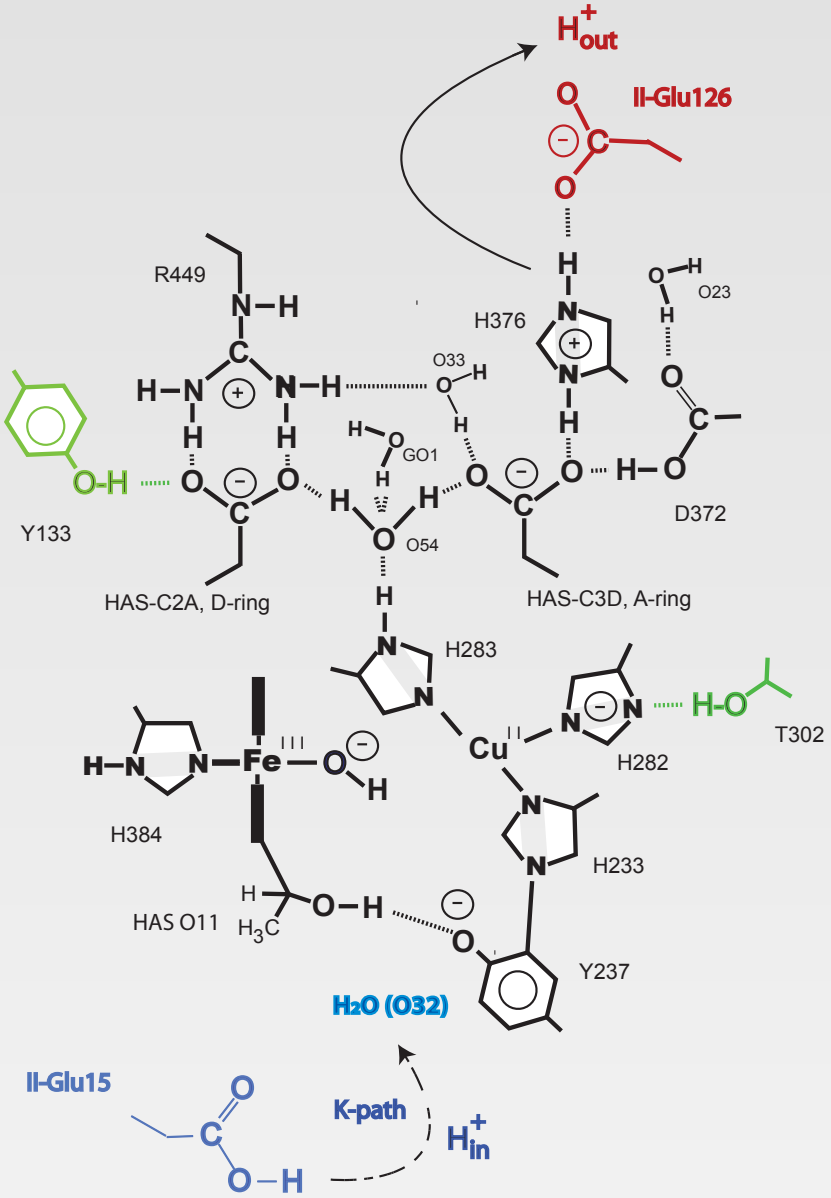
Fe-OH



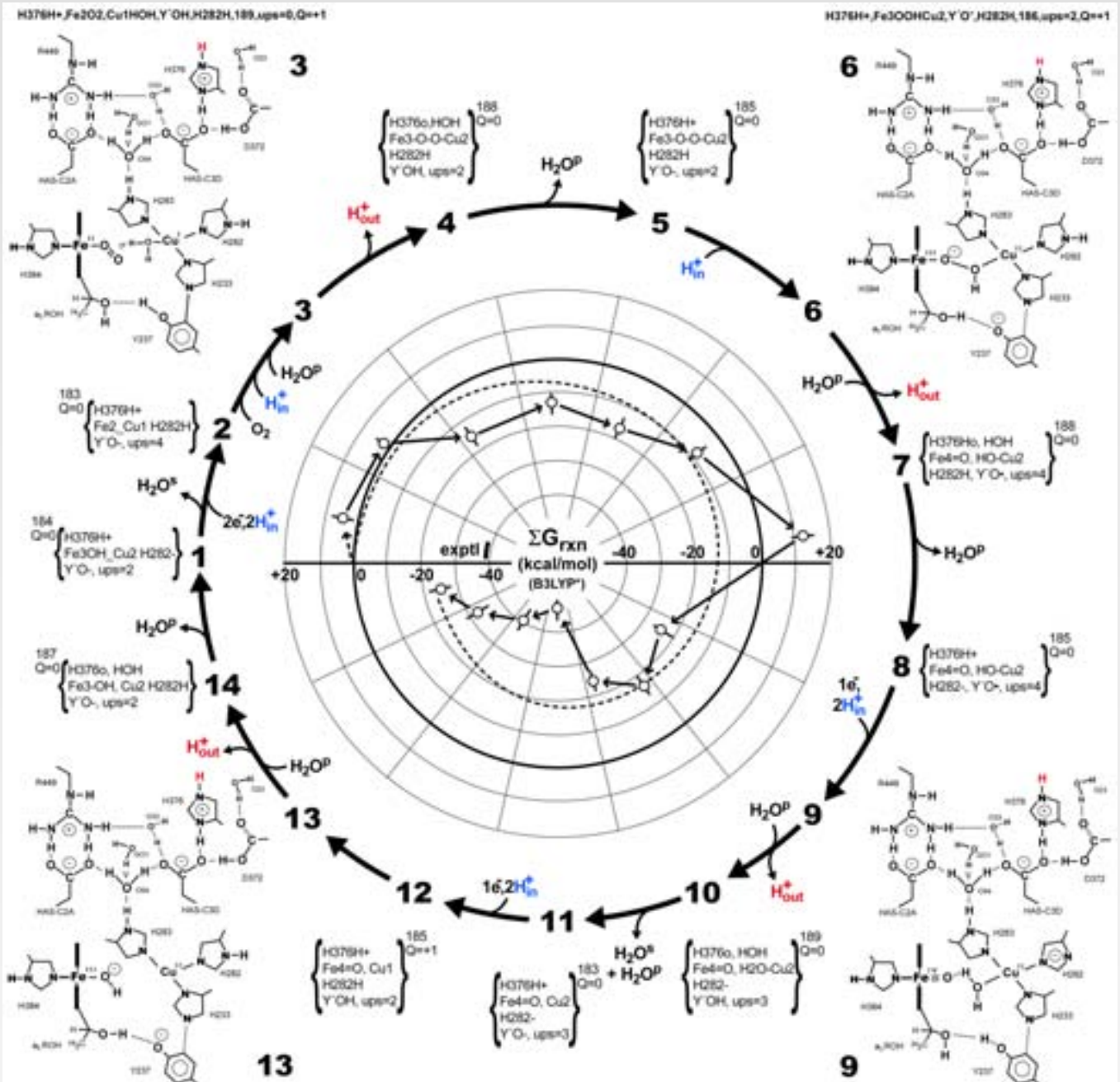
7

Fe=O, Cu-OH

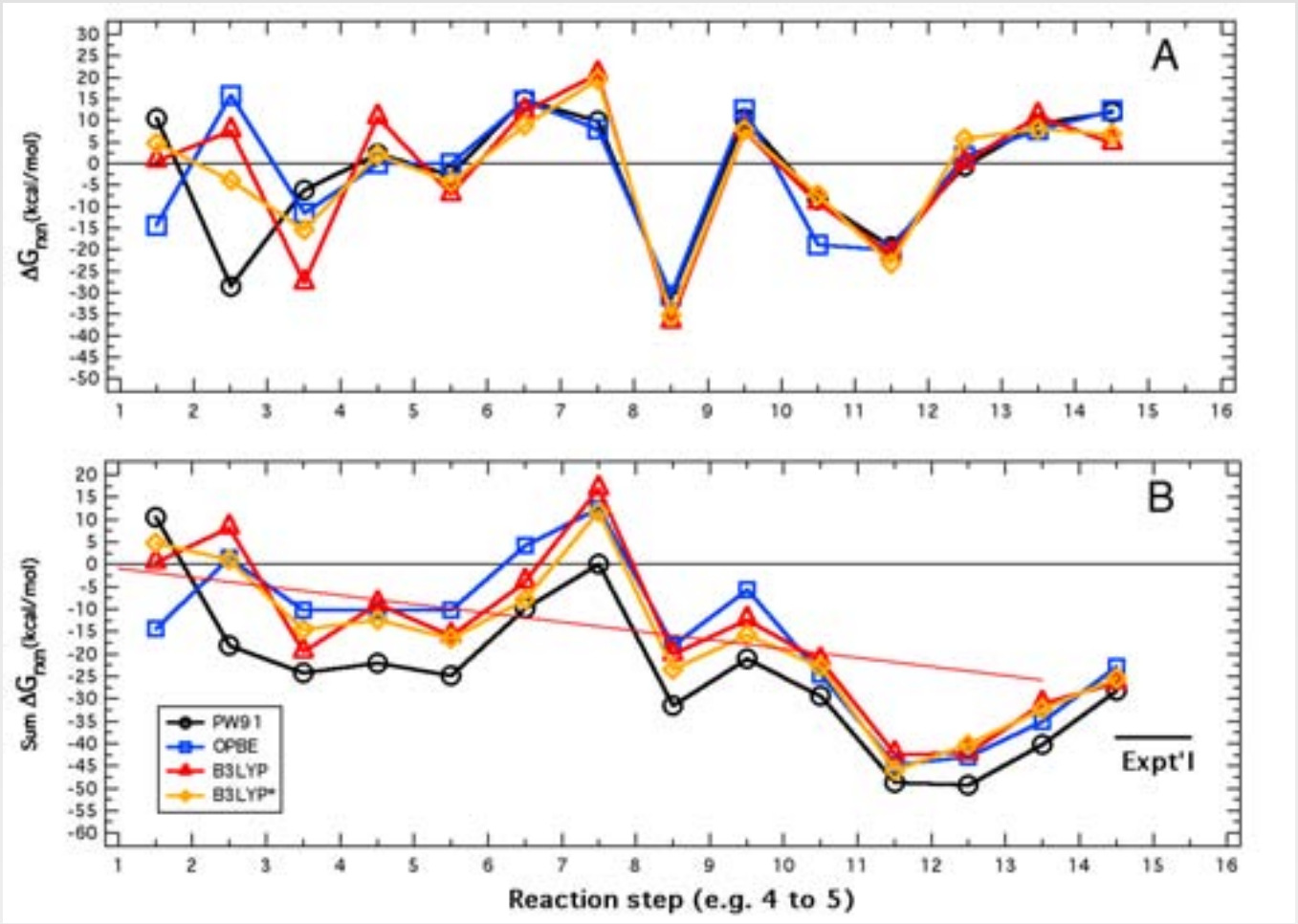
Proton pumping pathways are partially known



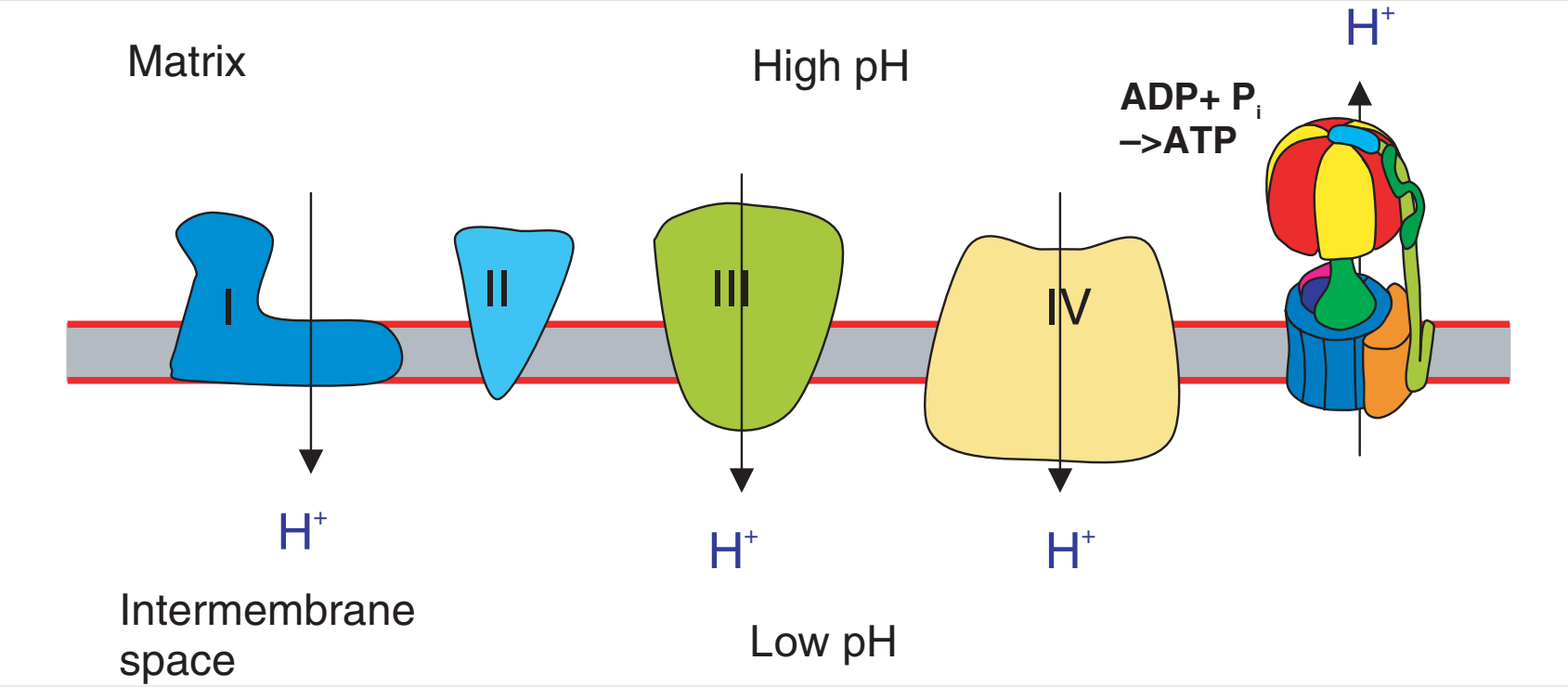
The complete mechanism is probably very complex



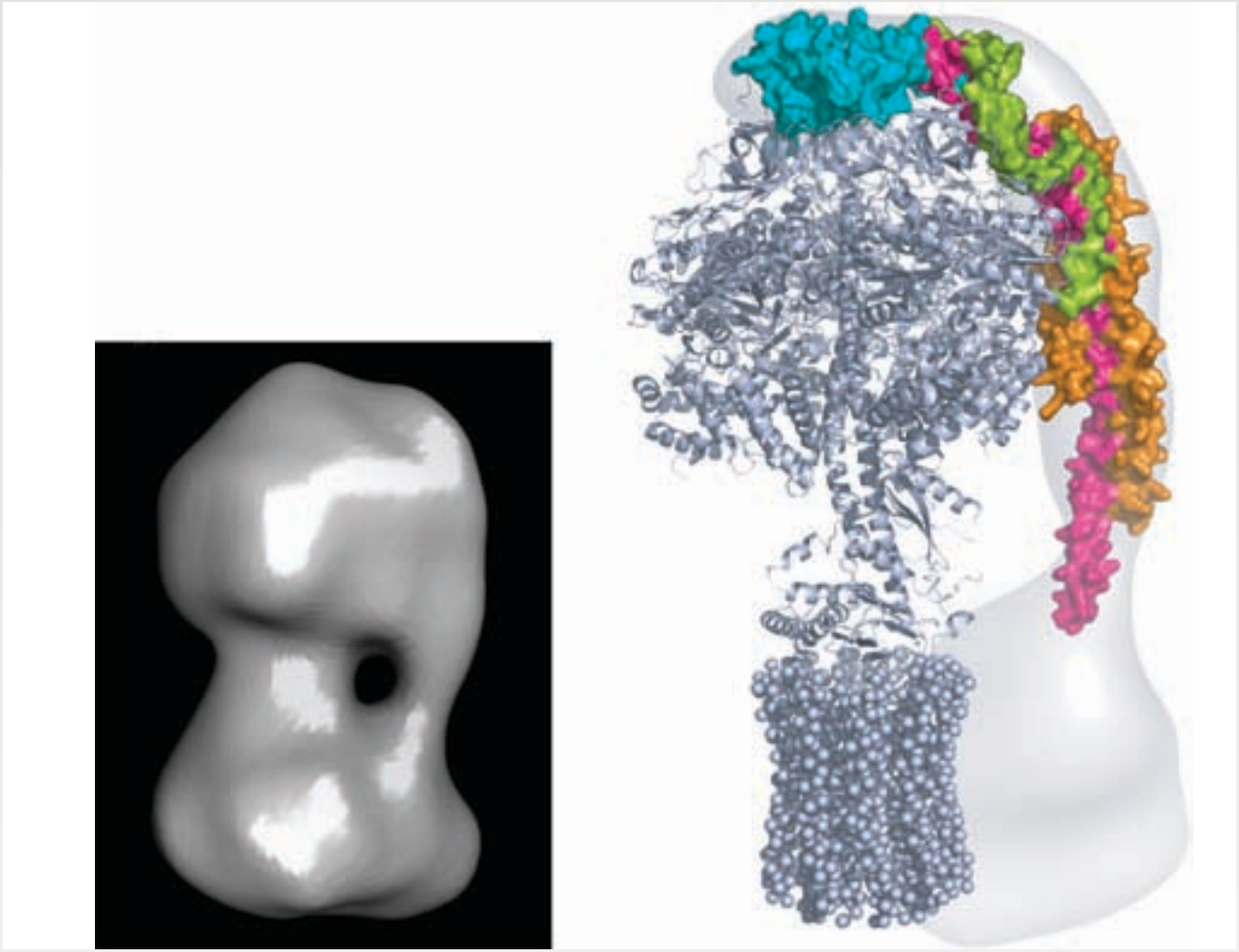
Thermodynamic analysis of this pathway



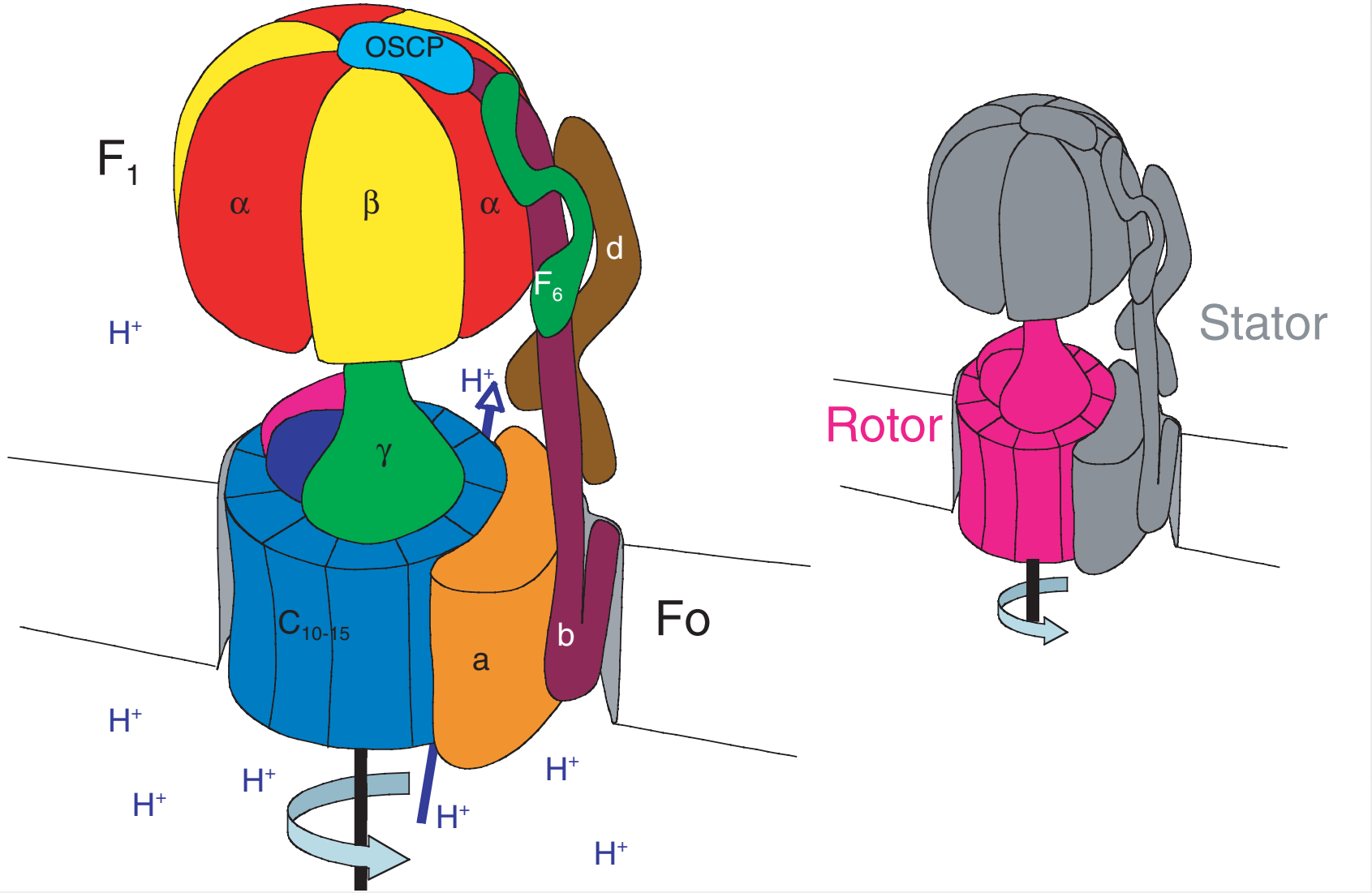
Respiration, again



ATP synthase at an atomic level



ATP synthase at a cartoon level



ATP synthase mechanism cartoon

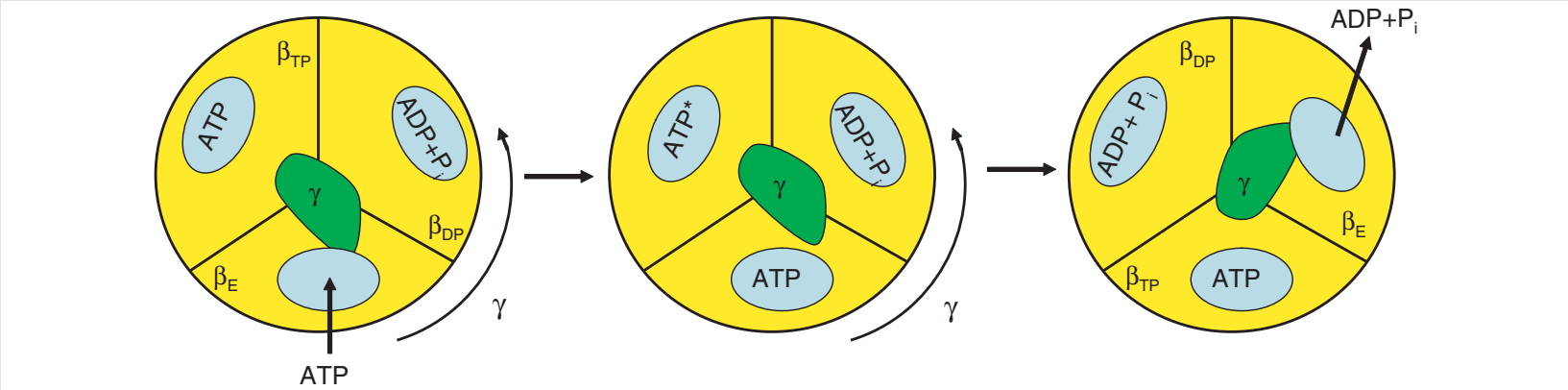


FIGURE 5.17 ■ The hydrolytic mechanism of ATP synthase initially proposed by Boyer and subsequently modified according to crystallographic results. The three β subunits are shown in three different conformations during the functional cycle. The β_E subunit is empty, β_{DP} contains an already hydrolyzed ATP molecule, and β_{TP} has an ATP molecule bound. When ATP binds to β_E, the γ subunit rotates with respect to the β subunits. This leads to an activation of the ATP in the former β_{TP} subunit. When the ADP and inorganic phosphate (P_i) is released, the continued rotation of the γ subunit leads to the open conformation β_E. ATP synthesis proceeds in the opposite direction, driven by the proton gradient across the membrane.

ATP synthase mechanism cartoon