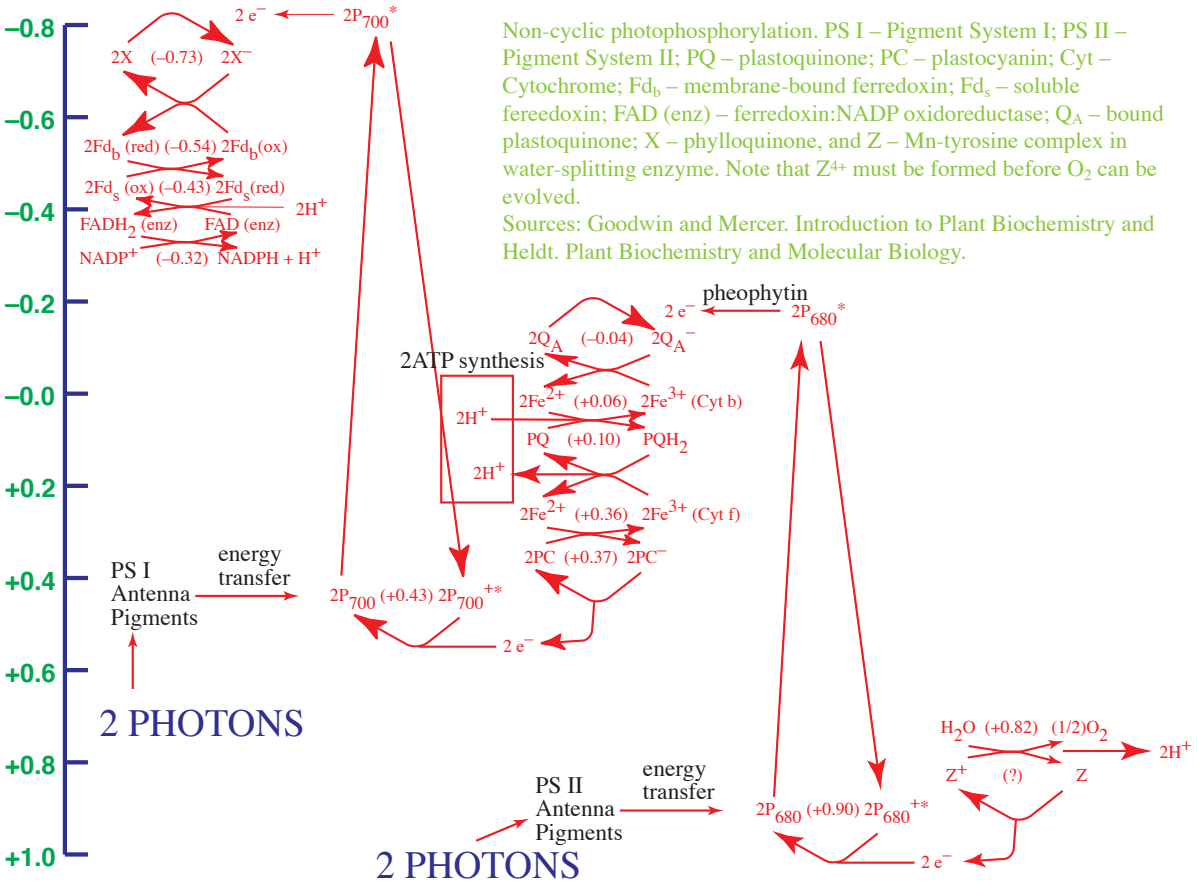


Non-cyclic photophosphorylation. PS I – Pigment System I; PS II – Pigment System II; PQ – plastoquinone; PC – plastocyanin; Cyt – Cytochrome; Fd_b – membrane-bound ferredoxin; Fd_s – soluble fereedoxin; FAD (enz) – ferredoxin:NADP oxidoreductase; Q_A – bound plastoquinone; X – phylloquinone, and Z – Mn-tyrosine complex in water-splitting enzyme. Note that Z^{4+} must be formed before O_2 can be evolved.

Sources: Goodwin and Mercer. Introduction to Plant Biochemistry and Heldt. Plant Biochemistry and Molecular Biology.

Photosynthesis
SC/BIOL 4160

LIGHT and ELECTRONS

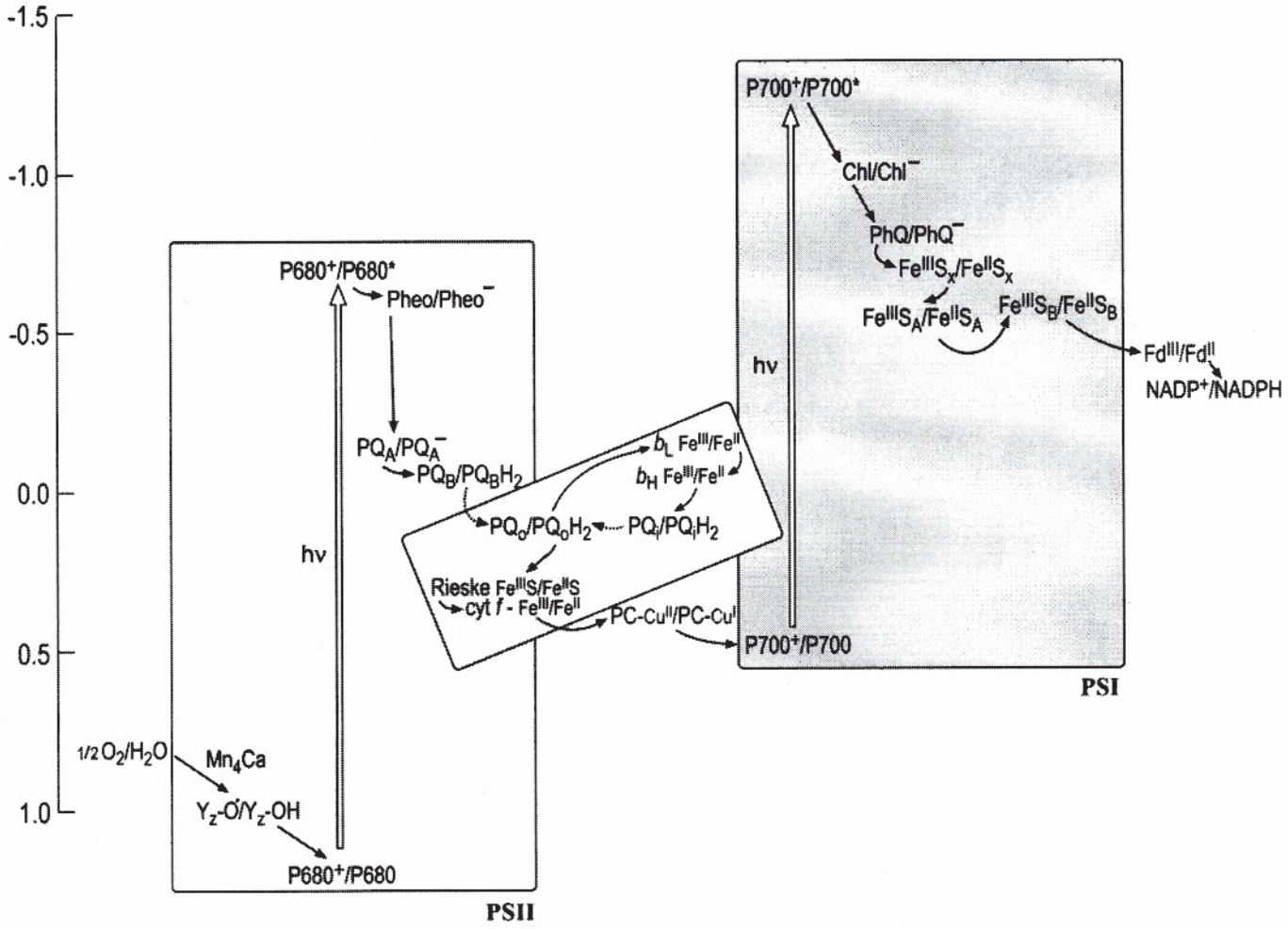


Figure 1. An Updated Z-Scheme Describing Photosynthetic Electron Transfer. Each carrier is shown in both its oxidized and reduced form to facilitate the readers' understanding of the sequence of steps, in which each carrier accepts an electron from a donor to become reduced and is reoxidized as it gives an electron to an acceptor. The midpoint potentials of the carriers in this version of the scheme have been updated to reflect the recent literature but are yet only approximate, owing in part to artistic aesthetics and in part to the intrinsic difficulty of estimating these numbers. In the cytochrome b6f complex (shown in pink to distinguish it as a heme-containing complex), the donor (reduced plastoquinol PQH2) provides two electrons: one is transferred through the Rieske FeS protein and cytochrome f to plastocyanin (or cytochrome c6) to photosystem I, while the other is transferred through the b-hemes to a bound quinone on the stromal side. The dashed arrow to and from PQH2 distinguishes diffusion of the redox carrier from the solid arrows that signify electron transfer. The conversion from the ground to the excited state (indicated with an open vertical arrow) occurs upon absorption of a photon. Some of the carriers appear to have obscure names (e.g., Z in PSII and A0 and A1 in PSI) and these have a historical origin in that the carriers had been identified as spectroscopic signals long before their chemical identities were known. In this scheme, A0 is indicated as Chl and A1 as PhQ to lead the reader away from obscurity. Redding and van der Est (2005) have suggested a specific nomenclature for the electron transfer cofactors in PSI.

Source: Sabeeha Merchant and Michael R. Sawaya (2005) The light reactions: A guide to recent acquisitions for the picture gallery. Plant Cell 17:648–663.

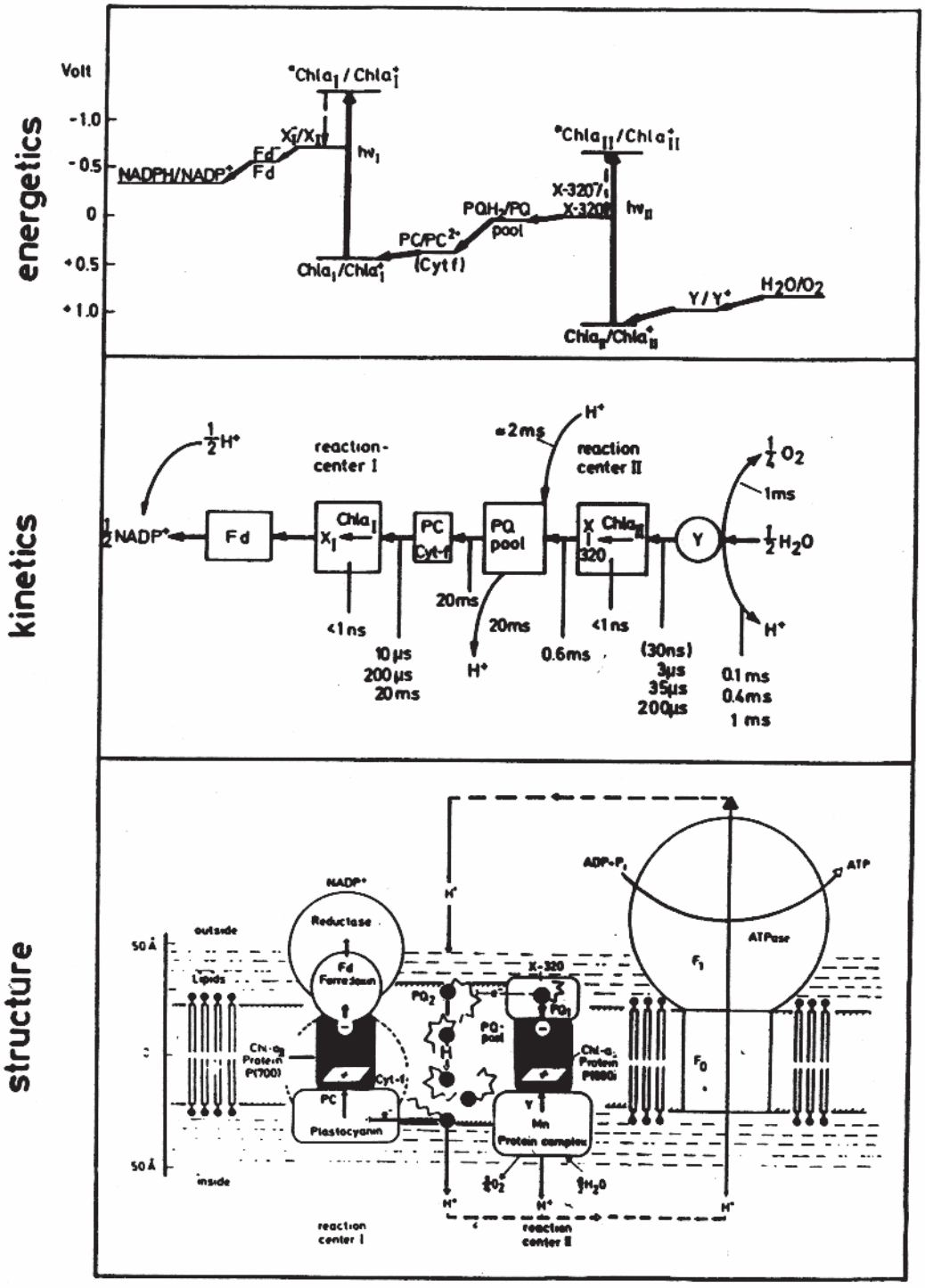


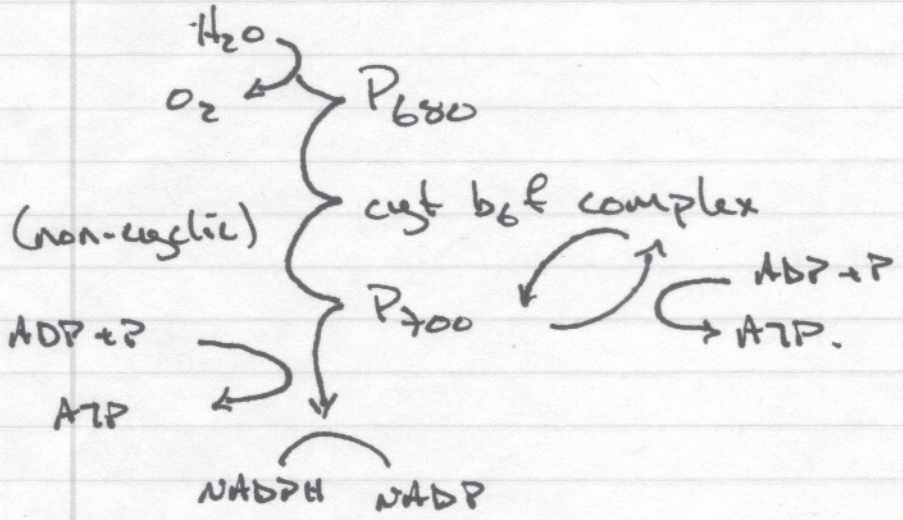
Fig. 1 Scheme of energetics, kinetics, vectors, and organizations within the functional membrane of photosynthesis (1).

NADPH & ATP PRODUCTION

So far, we have explored light & its absorption, then, its utilization in photochemistry in the reaction centers.

The next step, still part of the LIGHT REACTIONS of photosynthesis is the production of NADPH and ATP. These compounds supply reducing equivalents and "high energy" phosphate bonds will be utilized in the DARK REACTIONS of photosynthesis: CO_2 fixation.

Although we have focussed on P_{680} (PS II), and P_{700} (PS I), there is another complex which mediates electron transport (cyt b_6f) making three in toto



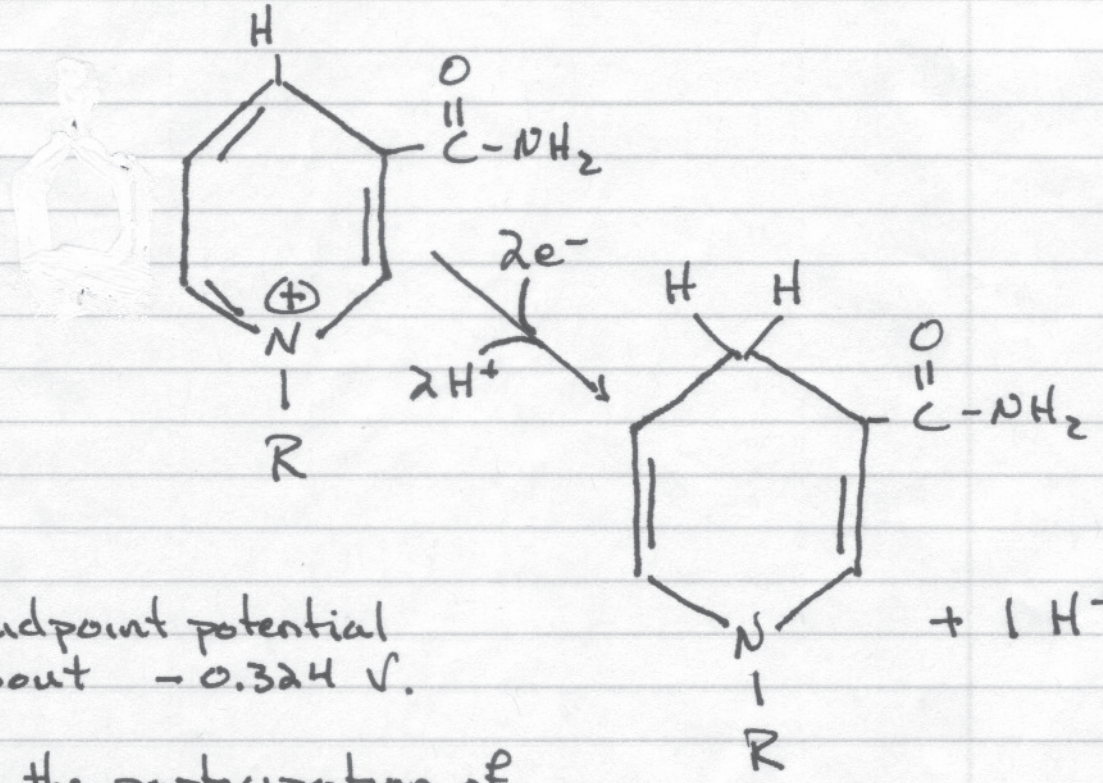
All three contribute to the e^- transfers which produce the ΔH^+ gradient required for ATP production.

e⁻ & H⁺ TRANSFER MECHANISMS

Introduction to the players.

NAD⁺ & NADP are very similar in structure, although they are specific to particular metabolic processes. NADP functions in chloroplasts, NAD functions in mitochondria

The nicotinamide ring is the functional component:



The midpoint potential is about −0.324 V.

Notice the participation of H^+ in the redox reaction.
Thus, the midpoint potential will be pH-dependent.

Structure of NADPH (and NADH)[1]

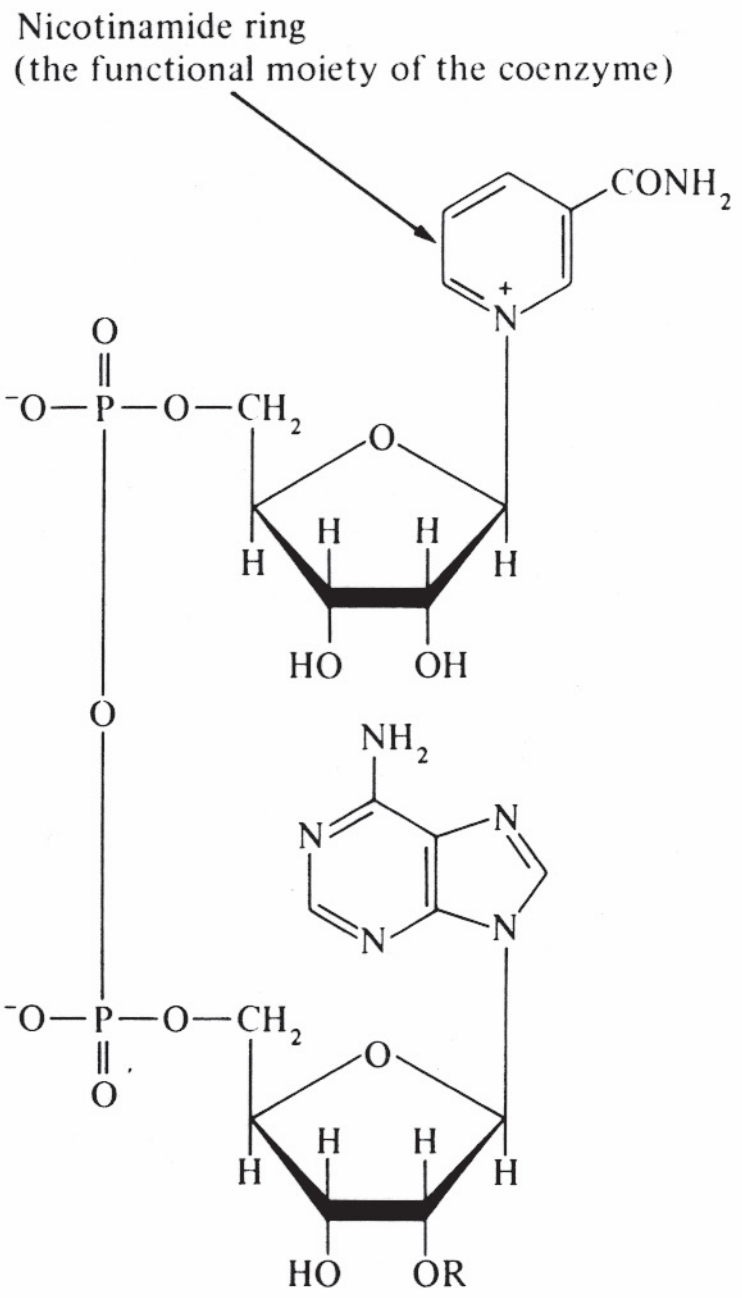
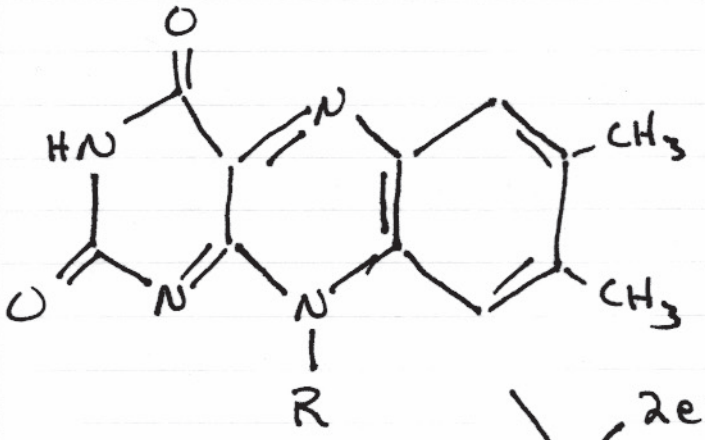


Fig. 6.7. Structure of the pyridine nucleotides; NAD^+, R = H; $NADP^+$, $R = PO_3^{2-}$.

[1] Source: TW Goodwin and EI Mercer 1990 Introduction to Plant Biochemistry. Pergamon Press. page 172

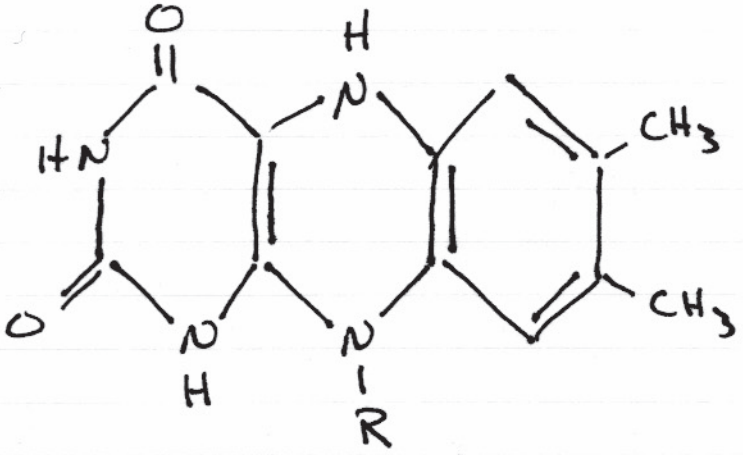
Flavins, which would be covalently bound to proteins also participate in redox reactions in photosynthesis. Specifically, as a cofactor for the enzyme ferredoxin-NADP reductase which produces NADPH.



FAD

$2e^-$ (in two steps of $1e^-$, followed by $1e^-$ & $2H^+$)

$2H^+$



$FADH_2$

The midpoint potential, overall, is about -0.32 V.
Again it will be pH dependent since H^+ participate in the redox reaction.

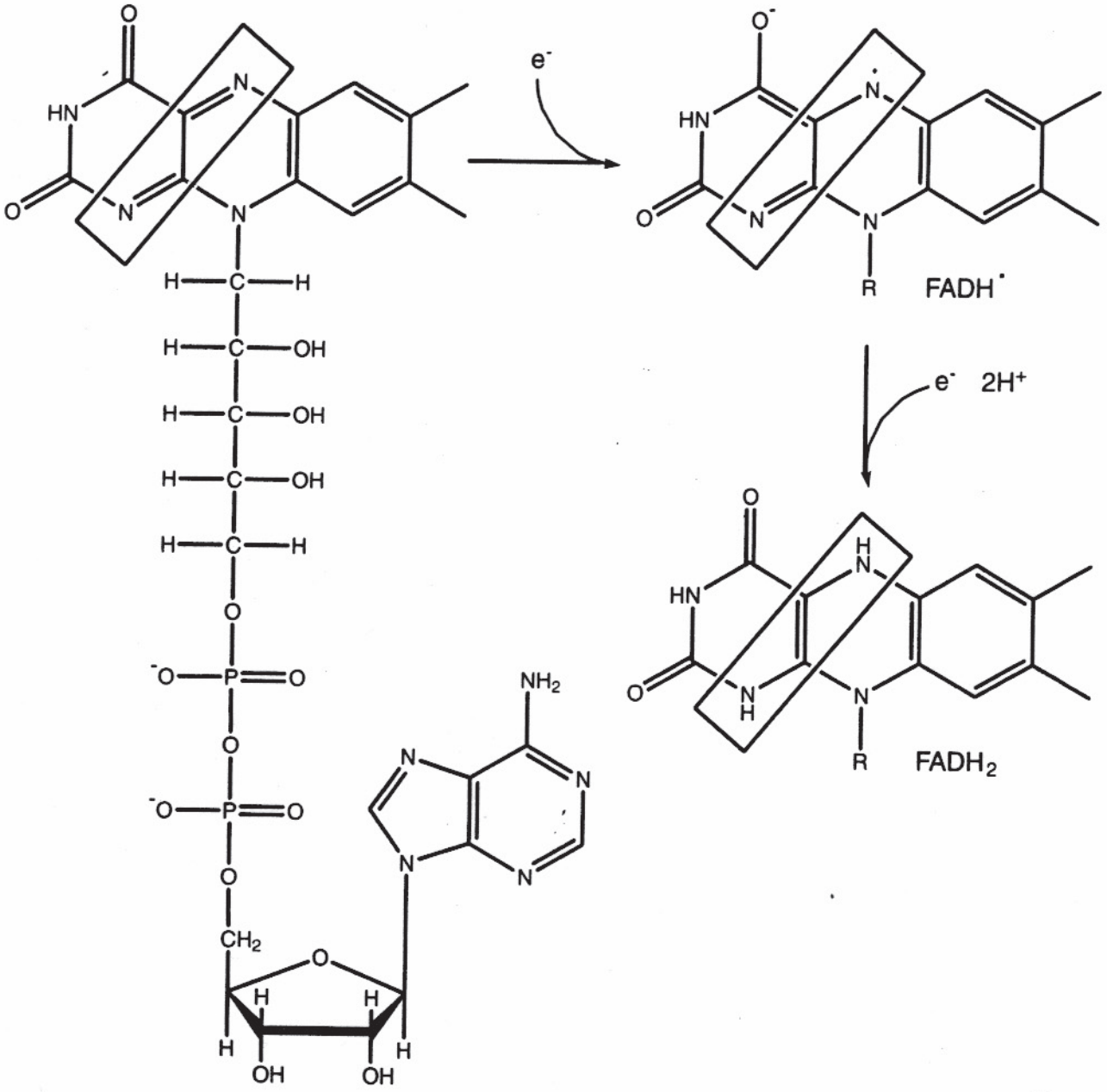
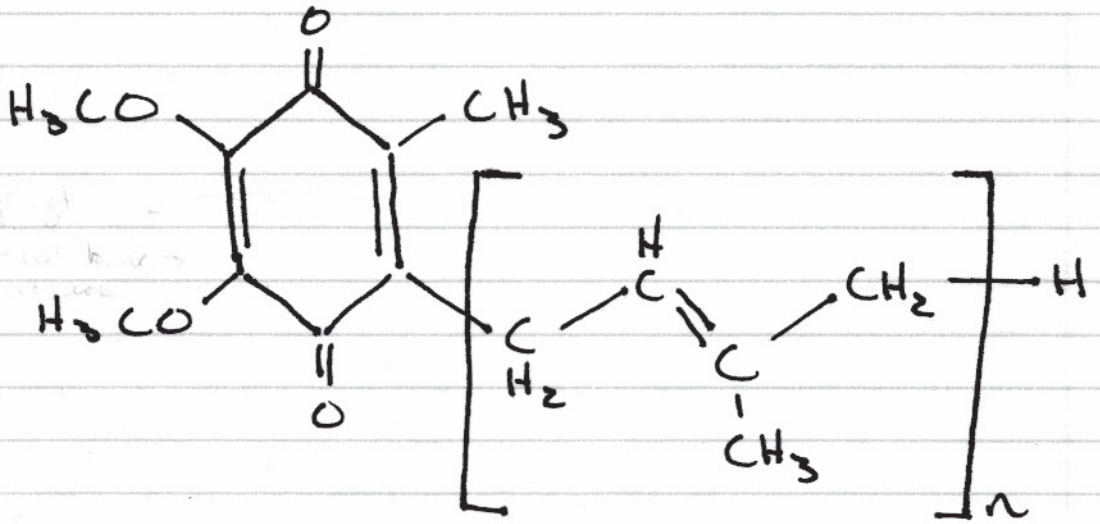


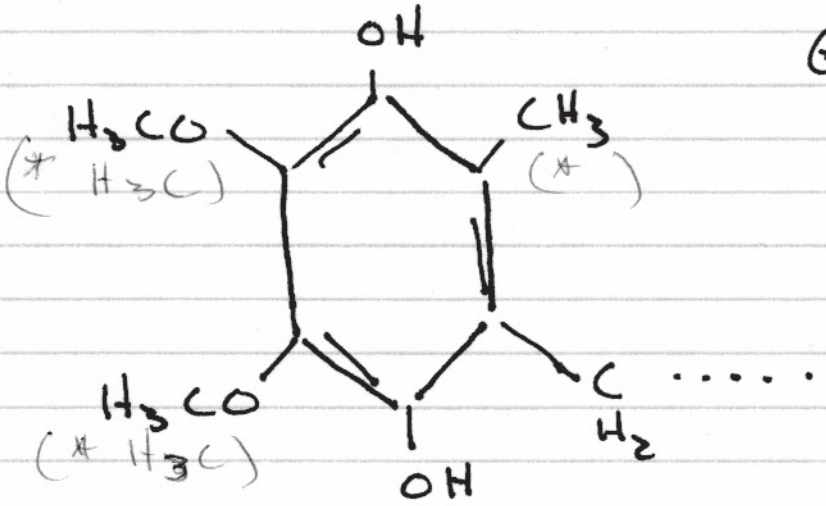
Figure 7.12 Structure of flavin adenine dinucleotide (FAD), the cofactor for ferredoxin-NADP reductase. The oxidized quinone (FAD), partially reduced semiquinone ($FADH^{\bullet}$) and fully reduced hydroquinone ($FADH_2$) forms are shown. The redox-active portion of the isoalloxazine ring is boxed.

Ubiquinone shuttles e^- & H^+ equivalents:



$2H^+$ $2e^-$

isoprene unit. n = 9 or 10 in higher plants

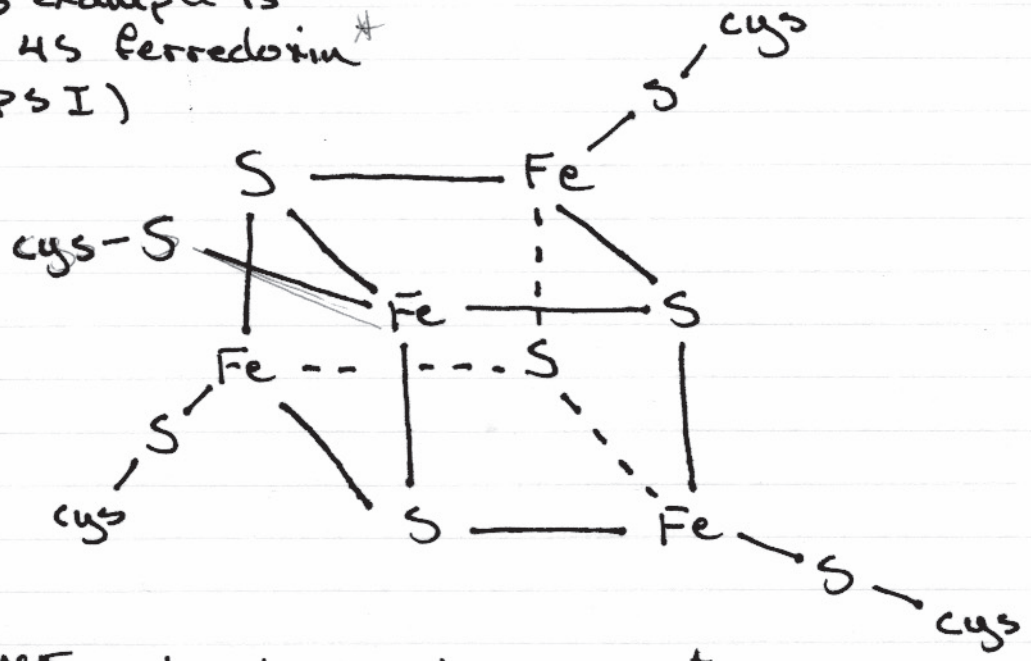


(*) note bene: there are chemical variants of the quinone-compounds. The plastoquinone variant is noted (*)

The midpoint potential is about 0.0 V

There are a variety of iron-sulfur centers which function in redox reactions involving only e^-, not H^+.

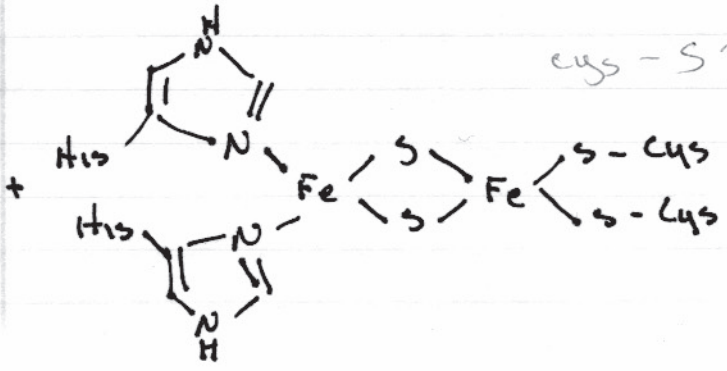
This example is
4 Fe 4S ferredoxin*
(in PS I)

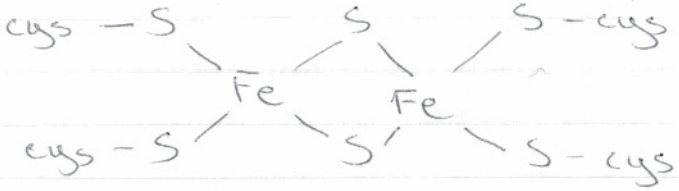


~~Simpler~~ other structures also occur*
The midpoint potentials of iron-sulfur centers vary over a wide range ($u = -0.43$ to $+0.36$ V)

*for example Rieske Fe-S protein cofactor in which histidines bond to the Fe^+

*2 Fe 2S ferredoxin





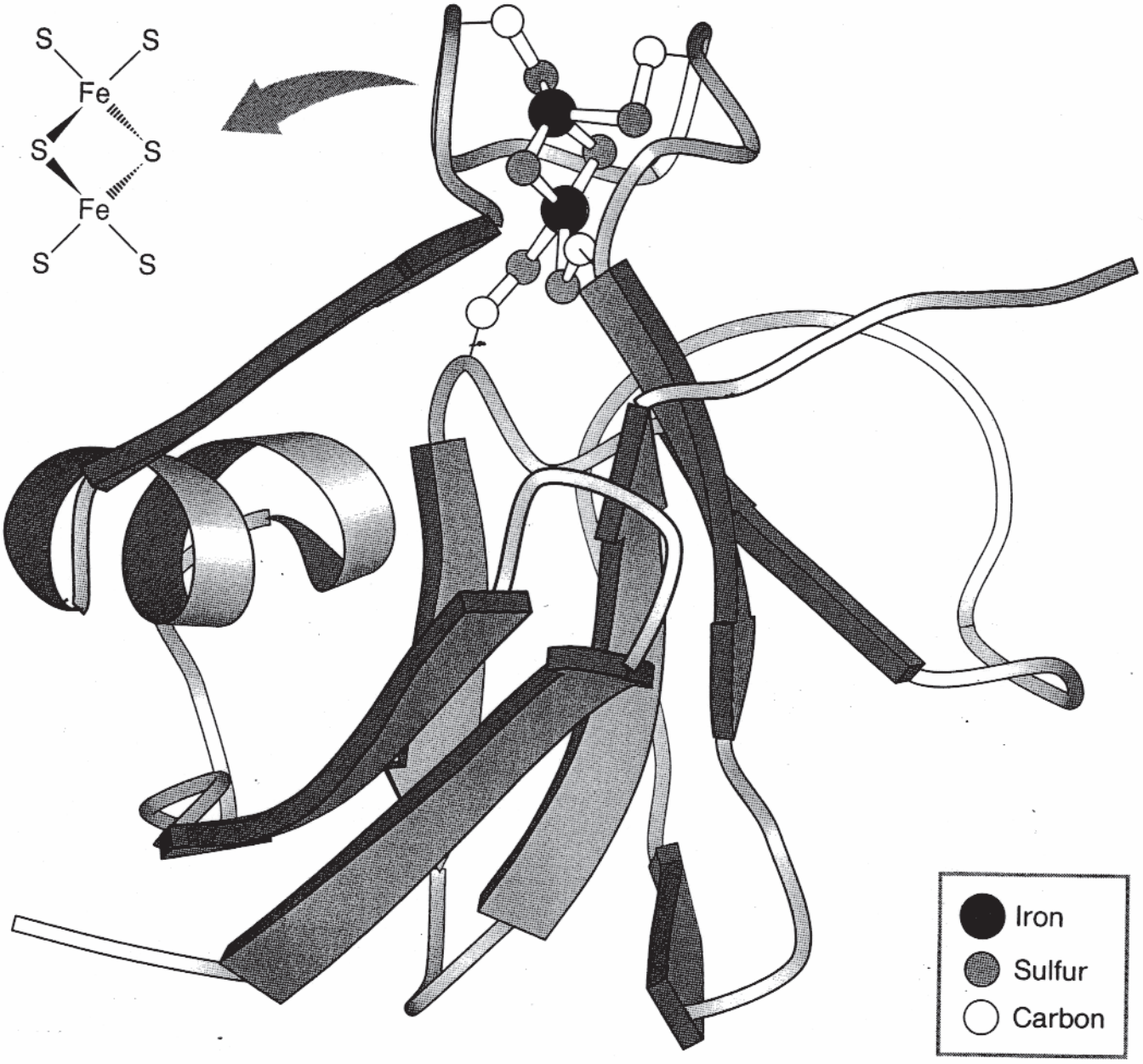
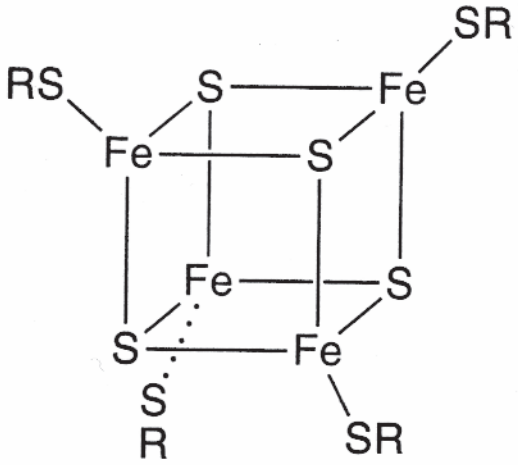


Figure 5.9
Structure of *S. platensis* ferredoxin and its 2Fe-2S cluster.



Finally, hemes function in e⁻ transfers.

This are cyclic tetrapyrroles, not unlike chlorophylls, but with Fe rather than Mg.

These are the functional moieties of the cytochromes. They transfer only 1e⁻ at a time.

Midpoint potentials will vary

Historically, they have been very useful in studies of e⁻ transfer in both photosynthesis and in mitochondrial respiration because of the change in absorbance spectra reduced versus oxidized.

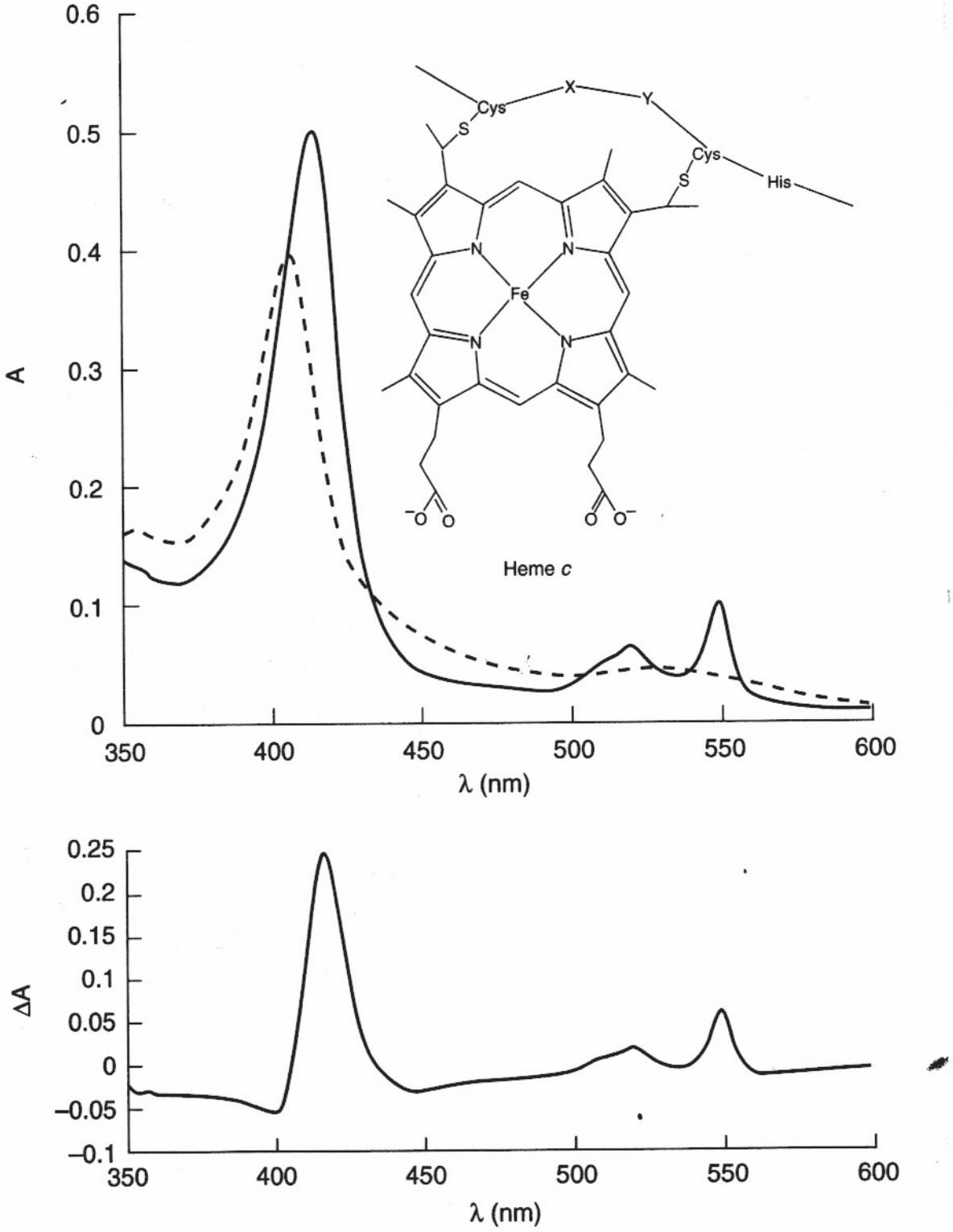
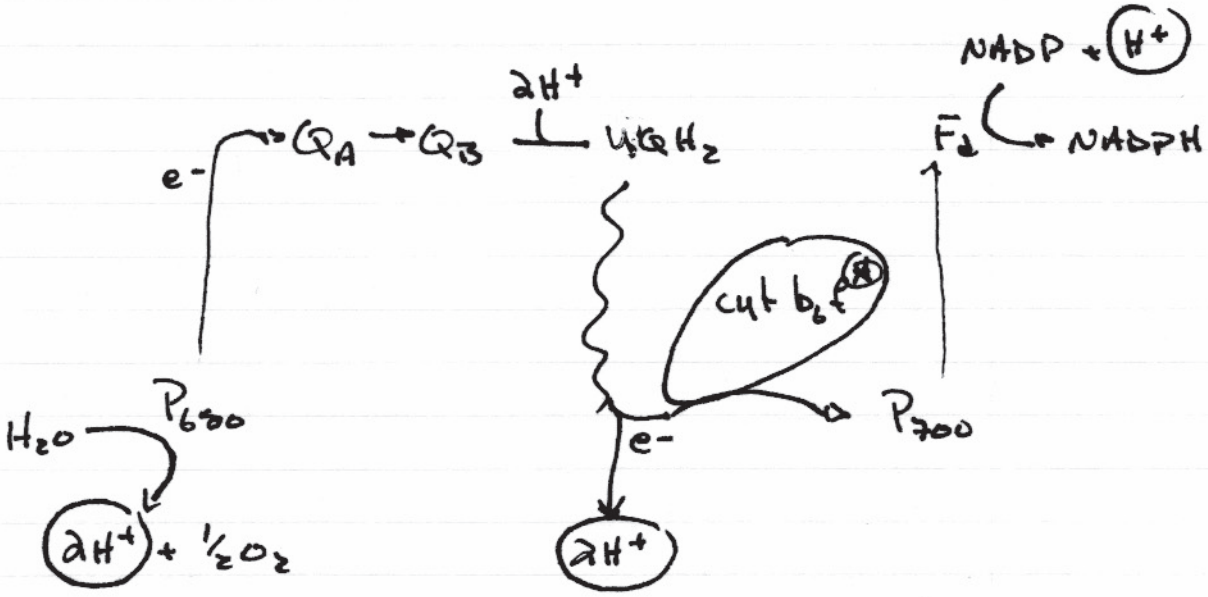
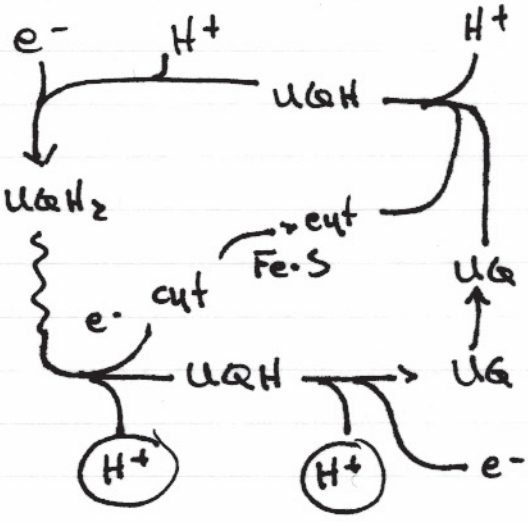


Figure 7.1 Absorption spectrum of oxidized (dashed line) and reduced (solid line) cytochrome *c*, with the structure of the heme cofactor for *c*-type cytochromes. The heme group is covalently attached to the protein via two thioether linkages to cysteine residues. The lower panel shows the reduced-minus-oxidized difference spectrum.



(*) The cyt b_6f complex plays a role in H^+ pumping by ubiquinol (or plastoquinol [PQ]) via the 'so-called' Q-cycle which accounts for a stoichiometry of $2H^+/e^-$.

A generalized scheme:



in which 1 e- gets re-cycled, resulting in $2H^+$ per e-

The net result is the formation of a gradient of H^+'s. Vectorially, the H^+ are pumped into the thylakoidal lumen.

CHEMIOSMOTIC THEORY and the COUPLING FACTOR.

There is a long history behind the eventual understanding that a gradient of H^+ was coupled to the purely biochemical ATP synthesis in photosynthesis.

Laboratory exercises on light-induced ATP formation and delta pH gradient-induced ATP formation are direct evidence in support of this startling conclusion.

Energetically, the two processes are equivalent:

The H^+ gradient is comprised of a ΔpH and a $\Delta\Psi$ - an electrical potential difference

$$\Delta\mu_{H^+} = 2.303\,RT\,\Delta pH + F\Delta\Psi.$$

- conversion since ΔpH is $\log_{10}$
- gas constant
- temperature (°K)
- Faraday constant.

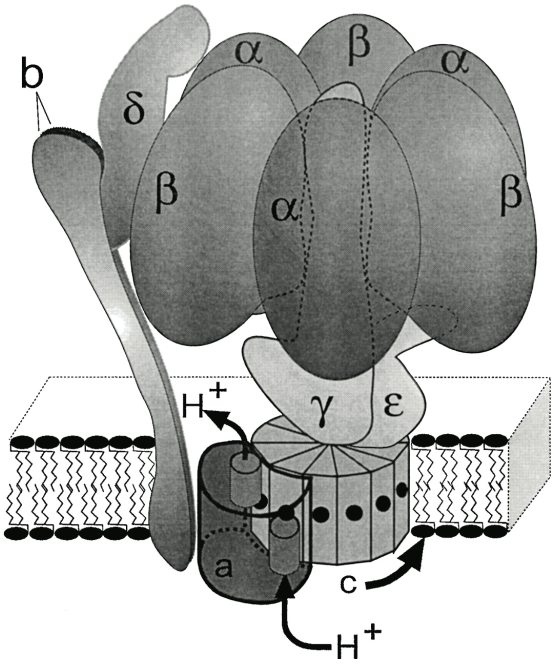
A ΔpH of 3 pH units & a 100 mV potential difference are equivalent to ~ 6 kcal/mole

The energy of ATP:

$$\Delta G = \Delta G^\circ + 2.303\,RT \log_{10} \frac{[ADP][P_i]}{[ATP]}$$

will vary depending on $[Mg^{2+}]$ and pH.

The structure of the ATP synthase has similarities to the flagellar motor, especially evidence that rotation of the c complex in the membrane cause conformational change that are transduced into ATP synthesis from ADP and phosphate.



Schematic subunit arrangement of the Escherichia coli F_0F_1 ATP synthase and the proposed proton pathway in F_0. The $\alpha_3\beta_3$ hexamer containing the catalytic sites in each of the β subunits surrounds the rotor shaft made up of the γ subunit coiled-coil. The rest of the proposed rotor consists of the ε and c subunits. The stator $\alpha_3\beta_3$ and a subunits are connected by the δ and two b subunits. The 10–12 c subunits are believed to be arranged in a ring, with subunit a on the side. Proton transport is mediated between the a and c subunits. As shown, the protons enter from the periplasmic space (right half-channel) to protonate the c subunit, and the protonated c subunit rotates counterclockwise until it meets the cytoplasmic-facing half-channel (left), where the protons are released. Nakamoto RK, CJ Ketchum and MK Al-Shawi (1999) Rotation coupling in the F_0F_1 ATP synthase. Annual Review of Biophysics and Biomolecular Structure. 28:205–234.

$$ADP + P_i + mH^+_{outside} \longleftrightarrow ATP + mH^+_{inside}$$

Energetic equivalence of the chemical potential and Gibbs free energy at equilibrium:

$$\Delta G_{total} = n \bullet \Delta\mu_{H^+} + \Delta G_{ATP} = 0$$

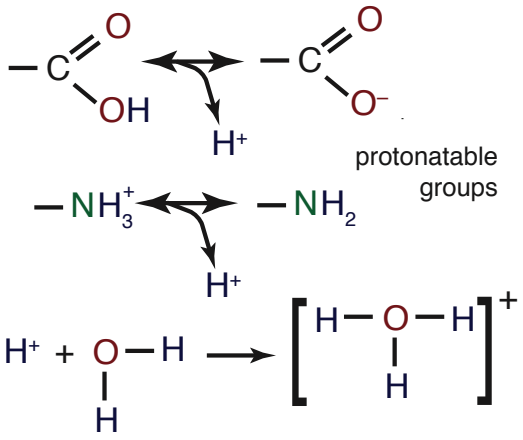
$$n \bullet \left(RT \ln\left(\frac{a^{inside}_{H^+}}{a^{outside}_{H^+}}\right) + F\Delta\Psi\right) + \Delta G^o_{ATP} + RT \ln\left(\frac{[ATP]}{[ADP][P_i]}\right) = 0$$

The equilibrium energy is determined by solving for $\Delta\Psi$

(ΔG^o_{ATP} and H^+ activities can be determined experimentally, as can [ATP], [ADP] and $[P_i]$).

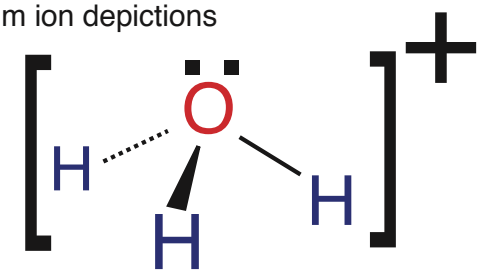
Biochemists identified the role of the proton motive force in ATP synthesis and bacterial motility over a period of time extending from the 1950's through the 1990's. Much of this scientific history is embedded in introductory and advanced biochemistry textbooks in various forms.

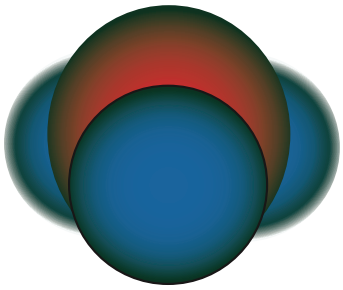
It is, however, highly unlikely that the proton (H^+, a positively charged hydrogen ion) could be the transported ion. It is very small, with a correspondingly high charge density, difficult to polarize, and unlikely to exist for more than a brief moment in aqueous biological environs, where there are so many other molecules it can interact with (especially water molecules, H_2O, but also ionizable groups such as carboxyls, aminos and phosphates). If it did react with H_20, the product would be the hydronium ion H_3O^+.



This is important in the context of flagellar rotation (or ATP synthesis), because the mechanisms using a naked proton H^+ would be different from those involving H_3O^+. The hydrogen ion would tend to pass from one ionizable group to the next (often called a proton wire). This could result in conformational changes that cause a torsional strain and eventually rotation. The H_3O^+ would have to pass through a pore structure[1]. Now, the actual mechanism could involve a combination of both proton wire and hydronium ion pore, but in fact this is not the case. The reason for such certainty is that certain bacteria rely upon Na^+ motive force, rather than H^+ motive force for synthesis of ATP and flagellar rotation[2]. Unlike the naked proton H^+, the Na^+ ion will not react with ionizable groups by forming a covalent bond.

Hydronium ion depictions





[1]Source: Boyer, PD (1988) Bioenergetic coupling to proton motive force: Should we be considering hydronium ion coordination and not group protonation? Trends in Biochemical Science 13:5–7.

[2]Source: Dimroth P (1995) On the way towards the Na^+-binding site within the F_1F_o ATPase of *Propionigenium modestum*. Biochem. Soc. Trans. 23:770–775.

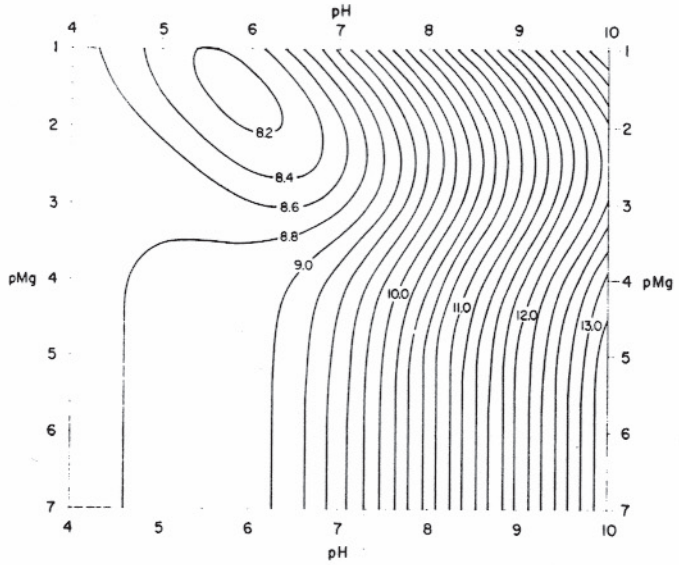


FIG. 5. Contour map of $-\Delta G^{0\prime}$, in kilocalories per mole, for the hydrolysis of ATP to ADP and P_i at 25° and 0.2 ionic strength. The *contour lines* are at intervals of 0.2 kcal $mole^{-1}$.

Alberty RA 1968 Effect of pH and metal ion concentration on the equilibrium hydrolysis of adenosine triphosphate to adenosine diphosphate. Journal of Biological Chemistry 243:1337-1343.

VALUE OF $\Delta G°$ FOR THE HYDROLYSIS OF ATP

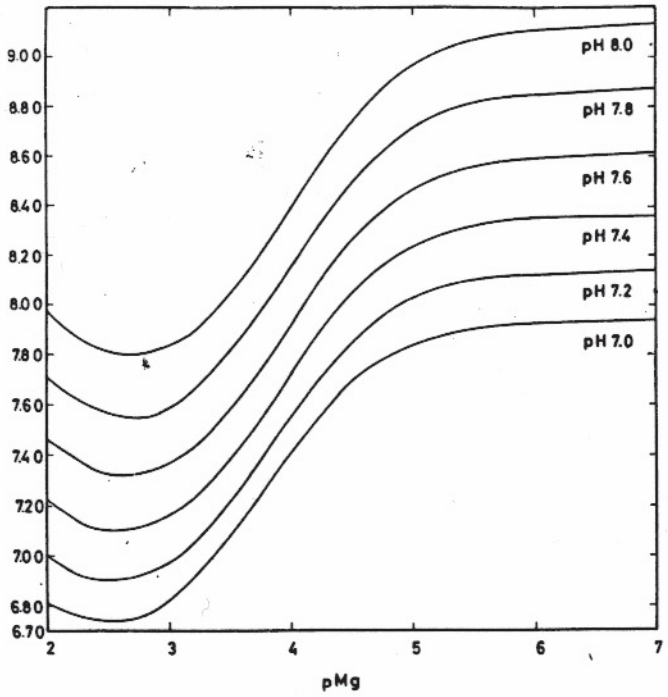


Fig. 3. $\Delta G°_{obs}$ as function of pMg at I 0.20, 25 °C and various pH values.

Rosing J and EC Slater 1972 The value of ΔG^{o} for the hydrolysis of ATP. Biochimica Biophysica Acta 267:275-290.

The overall reaction is

$$ADP + P_i + nH^+_o \rightleftharpoons ATP + nH^+_c$$

At equilibrium:

$$\Delta G_{TOT} = n \cdot \Delta\mu_{H^+} + \Delta G_{ATP} = 0$$

much depends upon how many H^+ are required per ATP. This remains unclear. 3 H^+ has been the commonly accepted value.

The enzyme responsible for coupling the vectorial H^+ gradient to synthesis of ATP from ADP + P_i is the ATP synthetase.

It is made up of CF_1 (which resides outside the membrane and CF_o (which resides within the membrane.

CF_1 subunits	no.	CF_o subunits	
α	·3	I (b)	·1
β	·3	II (b')	·1
γ	·1	III (c)	·10-14
δ	·1	IV (a)	·1
ε	·1		

The α & β subunits bind the ADP, P_i & ATP

The c subunits function as a 'channel' for H^+ movement through the membrane.

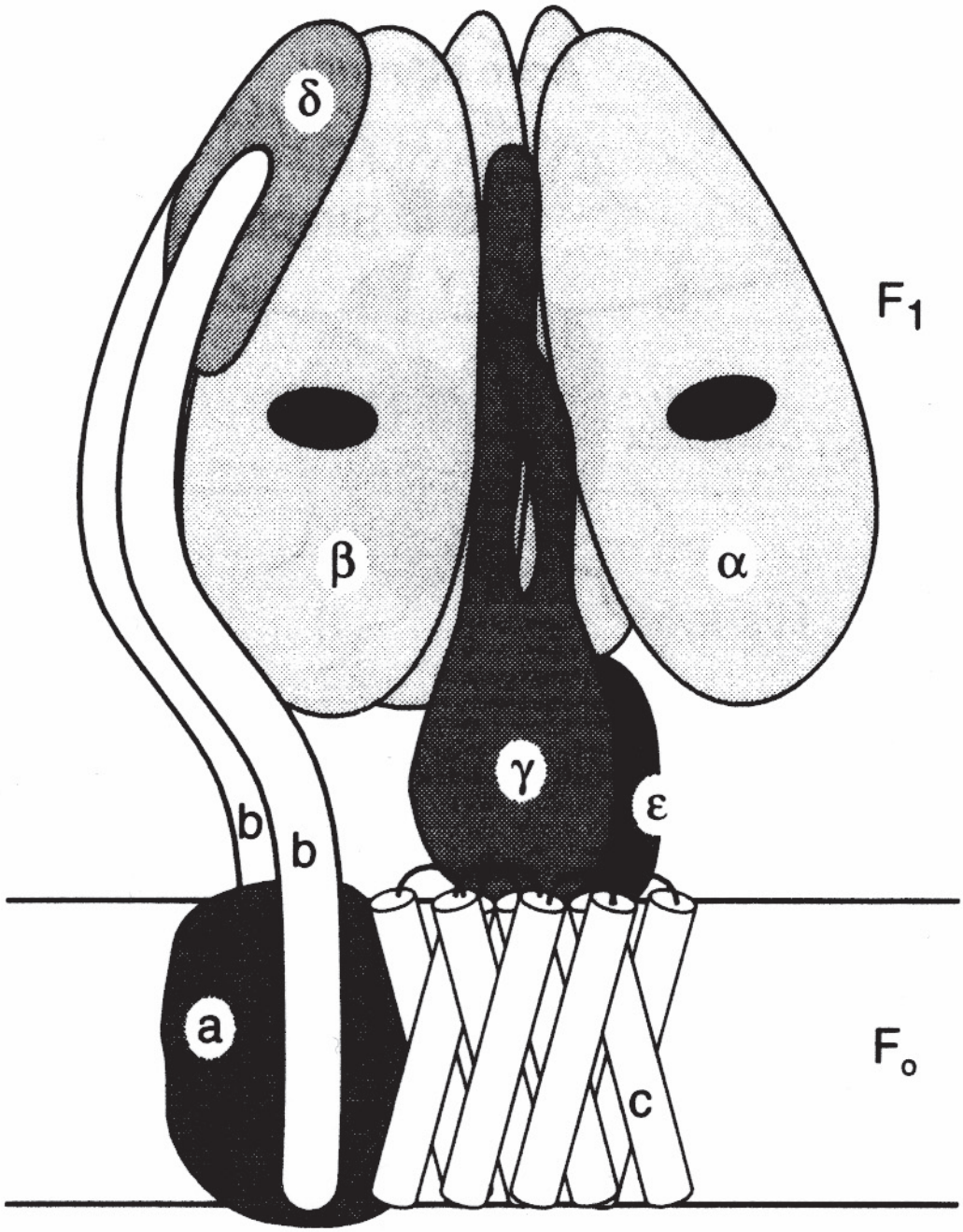


Figure 8.4 Structure of the ATP synthase enzyme. The F_1 and F_o portions of the enzyme are shown. Figure reproduced from Junge *et al.* (1997) with permission from Elsevier Science.

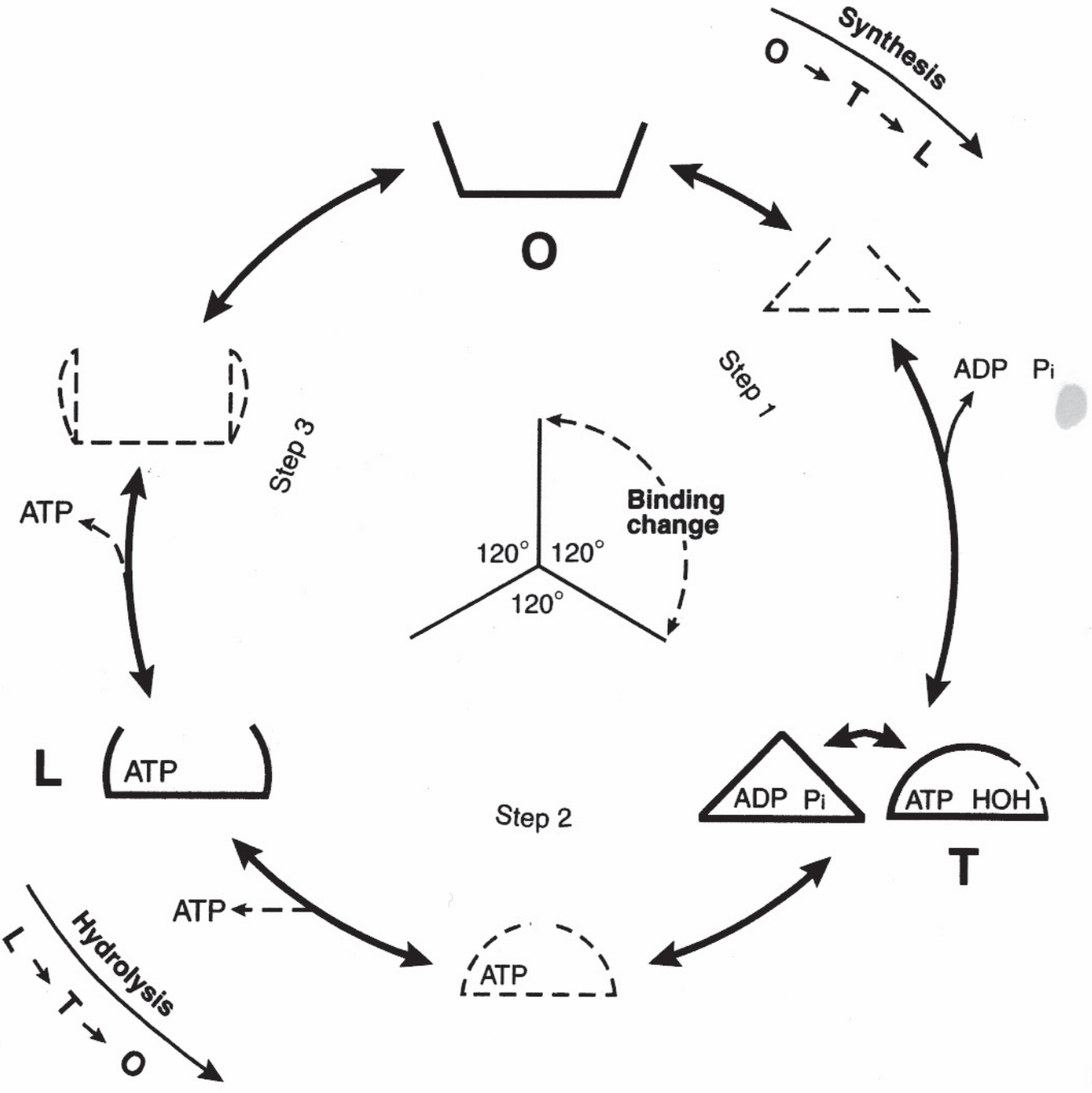
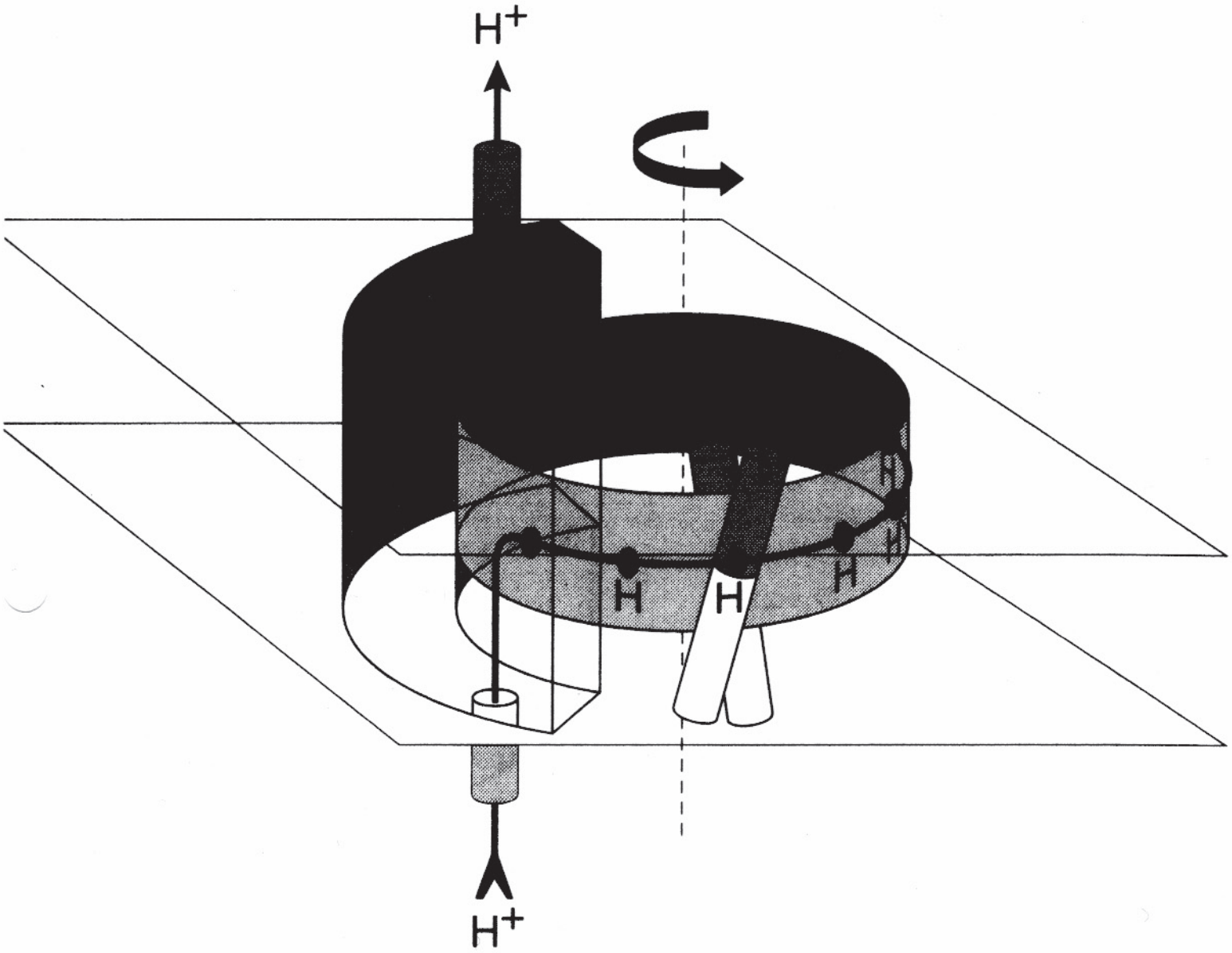


Figure 8.6 Binding change mechanism of ATP synthesis proposed by Boyer. The enzyme has three catalytic nucleotide binding sites, which cycle between the tight, loose and open conformations. The conformational changes induced by the rotary motion of the γ subunit cause the enzyme to change its affinity for the nucleotide. Figure reproduced from Boyer (2000) with permission from Elsevier Science.



8.7 Proposed pathway of proton translocation through the F_o portion of the ATP synthase. reproduced from Junge *et al.* (1997) with permission from Elsevier Science.

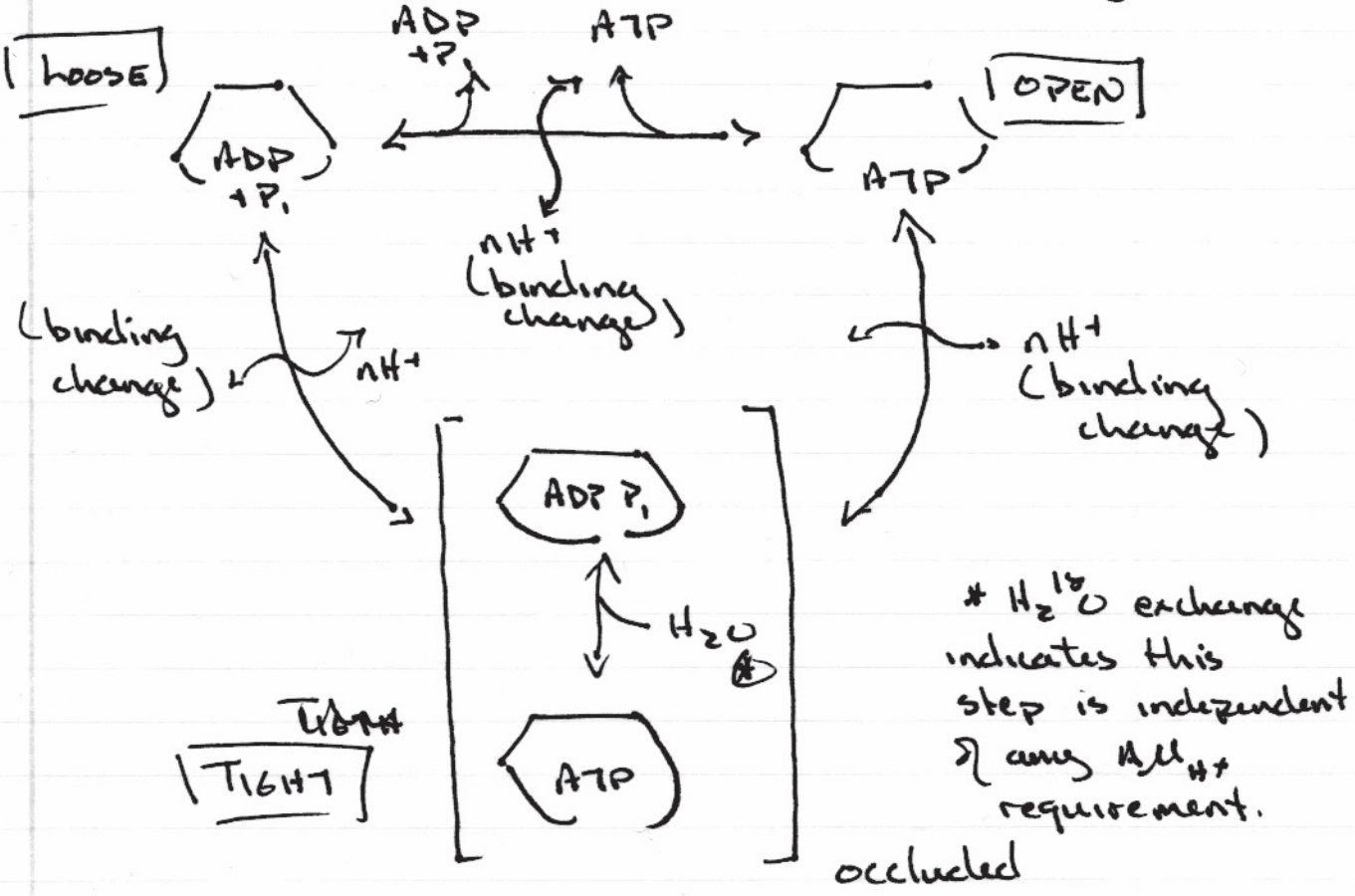
One caveat that is important to note because of the implications for mechanism.

In some halobacteria, the ATP synthetase couples a Na^+ gradient to ATP synthesis, rather than a H^+ ~ . (Dimroth and others).

Although H^+ move through the e- transfer chain as bonafide "naked protons", in the ATP synthetase, it is an hydronium ion H_3O^+ which is used.

(An H_3O^+ bears some physical resemblance to Na^+.)

The kinetic mechanism involves three binding sites:



The idea is that the three β subunits exist in

	β_T	"tight"
,	β_L	"loose"
&	β_O	"open"

conformations

Energy input is required to release ADP + P_i, or ATP but not form ADP + P_i → ATP bond formation.

Experiments in which the F_1 was bound to a slide, decorated with a fluorescent filament.* Then, after ATP addition, rotation observed is now part of biochemical dogma

* on the γ (gamma) subunit

It is sensible, since it is known that bacteria flagella rotate due to a rotating "motor" energized by a $\Delta\mu_{H^+}$ (or, $\Delta\mu_{Na^+}$).

The net result is ATP synthesis.