Electron transfers are key steps in many enzymatic reactions involving the oxidation or reduction of a bound substrate. Relevant examples include cytochrome c oxidase ($\text{O}_2 \rightarrow 2\text{H}_2\text{O}$) and nitrogenase ($\text{N}_2 \rightarrow 2\text{NH}_3$). To reinforce the claim that electron-transfer steps are of widespread importance, several other redox systems, representative of diverse metabolic processes, will be mentioned here.

Xanthine oxidase (275 kDa; $\alpha_2$ dimer) catalyzes the two-electron oxidation\(^{37-39}\) of xanthine to uric acid (Equation 6.7).

\[
\text{xanthine} + \text{H}_2\text{O} \rightarrow \text{uric acid} + 2e^- + 2\text{H}^+
\]

This enzyme, which plays a prominent role in the biodegradation of purines, is the target of drugs administered to patients suffering from gout (joint inflammation, due to precipitation of sodium urate). Figure 6.17 displays the cofactors in a subunit: a Mo-pterin, termed MoCo; two [2Fe-2S] centers; and one FAD. The binuclear iron-sulfur sites serve to shuttle electrons between the reduced substrate ($\text{XH}$) and $\text{O}_2$.

*Figure 6.17 - Representation of the cofactors in one subunit of xanthine oxidase.*
The first step in the biosynthesis of DNA involves the reduction of ribonucleotides (Equation 6.8) catalyzed by ribonucleotide reductase. The \textit{E. coli} enzyme is an \(\alpha_{2} \beta_{2}\) tetramer composed of a B1 protein (160 kDa) and a B2 protein (78 kDa). The B1 protein (a dimer) contains redox-active di-thiol groups, binding sites for ribonucleotide substrates, and regulatory binding sites for nucleotide diphosphates. Protein B2, also a dimer, possesses a phenolate radical (Tyr-122) that is stabilized by an antiferromagnetically coupled binuclear iron center (Figure 6.18). This radical is essential for enzyme activity, and is \(\sim 10 \text{ Å}\) from the protein-B1/protein-B2 interface. Hence it cannot directly participate in an H-atom abstraction from the substrate (bound to protein B1). Instead, the x-ray structure of the B2 protein suggests that a long-range electron transfer from the Tyr radical to a residue (perhaps Trp-48) on the B1 protein is operative during enzyme turnover.

\[ \text{ribonucleotide reductase} \]

\[ \text{Figure 6.18 - Schematic of the binuclear iron center and Tyr-122 radical in the B2 protein of E. coli ribonucleotide reductase.} \]

Most of the presently known metal-containing mono- and dioxygenases are multicomponent, requiring the involvement of additional proteins (electron transferases) to shuttle electrons from a common biological reductant (usually NADH or NADPH) to the metallooxynase. Cytochrome P-450, whose substrate oxidation chemistry was discussed in detail in Chapter 5, serves as an excellent example. Figure 5.10 presented a catalytic cycle for cytochrome P-450-dependent hydroxylations that begins with substrate (RH) binding to the ferric enzyme (RH is camphor for \textit{Pseudomonas putida} cytochrome P-450). To hydroxylate the camphor substrate, the monoxygenase must be reduced via the electron-transport chain in Equation (6.9).
The ferredoxin reductase receives two electrons from NADH and passes them on, one at at time, to putidaredoxin, a [2Fe-2S] iron-sulfur protein. Thus, two single-electron-transfer steps from reduced putidaredoxin to cytochrome P-450 are required to complete one enzyme turnover.

The activity of the enzyme appears to be regulated at the first reduction step. In a 1:1 putidaredoxin-cytochrome P-450 complex, the reduction potential of putidaredoxin is -196 mV, but that of cytochrome P-450 is -340 mV in the absence of camphor; reduction of the cytochrome P-450 is thus thermodynamically unfavorable (k ~ 0.22 s\(^{-1}\)). Upon binding camphor, the reduction potential of cytochrome P-450 shifts to -173 mV, and the electron-transfer rate in the protein complex accordingly increases to 41 s\(^{-1}\). "Costly" reducing equivalents are not wasted, and there are no appreciable amounts of noxious oxygen-reduction products when substrate is not present.

In the third step, molecular oxygen binds to the camphor adduct of ferrous cytochrome P-450. This species, in the presence of reduced putidaredoxin, accepts a second electron, and catalyzes the hydroxylation of the bound camphor substrate. The turnover rate for the entire catalytic cycle is 10-20 s\(^{-1}\), and the second electron-transfer step appears to be rate-determining.

The bulk of the interest in electron-transfer reactions of redox proteins has been directed toward questions dealing with long-range electron transfer and the nature of protein-protein complexes whose structures are optimized for rapid intramolecular electron transfer. Before we undertake a discussion of these issues, it is worth noting that studies of the reactions of redox proteins at electrodes are attracting increasing attention. Direct electron transfer between a variety of redox proteins and electrode surfaces has been achieved. Potential applications include the design of substrate-specific biosensors, the development of biofuel cells, and electrochemical syntheses. An interesting application of bioelectrochemical technology is the oxidation of p-cresol to p-hydroxybenzaldehyde (Figure 6.19).

![Figure 6.19 - Enzyme-catalyzed electrochemical oxidation of p-cresol to p-hydroxybenzaldehyde. AZ is azurin, and ENZ is p-cresol methylhydroxylase.](image-url)