The four major complexes of the electron transport chain operate independently in the inner mitochondrial membrane. Each of the complexes is an aggregate of proteins that are held firmly together by noncovalent forces. There is no experimental evidence that the four complexes associate with one another in the membrane. Each of these complexes has its own rate of lateral diffusion through the bilipid membrane which shows that the complexes do not move together. Kinetic studies of reconstituted electron transport systems support the theory of four independent complexes.

The model for the electron transport system is shown above. Four independent complexes are transferring electrons through the mobile electron carriers of CoQ and cytochrome c. CoQH₂ is produced by both complex I and complex II and delivers the electron to complex III via the Q-cycle. Complex III reduces cytochrome c which is a water soluble electron carrier located in the intermembrane space of the mitochondria. The reduced cytochrome c carries the electrons to complex IV which transfers the electrons to molecular oxygen. The process of electron transfer is coupled to transporting protons from the matrix to the intermembrane space of the mitochondria. This generates a chemical potential and an electrostatic potential. This potential energy is used to drive the synthesis of ATP.

Complex I: \[ \text{NADH} + 5\text{H}^+ + \text{Q} \rightarrow \text{NAD}^+ + \text{QH}_2 + 4\text{H}^+_p \]
Complex II: \[ \text{FADH}_2 + \text{Q} \rightarrow \text{FAD} + \text{QH}_2 \]
Complex III: \[ \text{QH}_2 + 2\text{H}^+_N + 2\text{Cyt c (Fe}^{3+}) \rightarrow 2\text{Cyt c (Fe}^{2+}) + \text{Q} + 4\text{H}^+_p \]
Complex IV: \[ 4\text{Cyt c (Fe}^{2+}) + 8\text{H}^+_N + \text{O}_2 \rightarrow 4\text{Cyt c (Fe}^{3+}) + 4\text{H}^+_p + 2\text{H}_2\text{O} \]

Overall reaction beginning with NADH:
\[ \text{NADH} + 11\text{H}^+_N + \frac{1}{2}\text{O}_2 \rightarrow \text{NAD}^+ + 10\text{H}^+_p + \text{H}_2\text{O} (10\text{H}^+_p/2\text{e}^-) \]
Overall reaction beginning with FADH$_2$:
FADH$_2$ + 6H$^+$ + $\frac{1}{2}$O$_2$ $\rightarrow$ FAD + 6H$^+_p$ + H$_2$O (6H$^+_p$/2e$^-$)

NAD$^+$ + H$^+$ + $\frac{1}{2}$O$_2$ $\rightarrow$ NAD$^+$ + H$_2$O
NAD$^+$ + 2e$^-$ + H$^+$ $\rightarrow$ NADH \hspace{1cm} E'' = -0.315 V
$\frac{1}{2}$O$_2$+ 2e$^-$ + 2H$^+$ $\rightarrow$ H$_2$O \hspace{1cm} E'' = +0.816 V
$\Delta$E'' = +0.816 V - (-0.315 V) = 1.136 V
$\Delta$G'' = -nFE'' = -219 kJ/mol

FADH$_2$+ $\frac{1}{2}$O$_2$ $\rightarrow$ FAD + H$_2$O
FAD + 2e$^-$ + H$^+$ $\rightarrow$ FADH$_2$ \hspace{1cm} E'' $\approx$ 0.000 V
$\frac{1}{2}$O$_2$+ 2e$^-$ + 2H$^+$ $\rightarrow$ H$_2$O \hspace{1cm} E'' = +0.816 V
$\Delta$E'' = +0.816 V - (0 V) = 0.816 V
$\Delta$G'' = -nFE'' = -157.5 kJ/mol

Most of the free energy from the transfer of electrons from NADH or FADH$_2$ to molecular oxygen has been used to pump electrons out of the matrix across the inner mitochondrial membrane into the intermembrane space. Recall from BICH410 the electrochemical energy inherent in this difference of proton concentration.

$\Delta$G = RTln(C$_2$/C$_1$) + ZF$\Delta$ψ

Where C$_2$/C$_1$ is the concentration ratio for the ion being transported, Z is the absolute value of the ions electrical charge which is 1 for a proton. And $\Delta$ψ is the transmembrane electrical potential measured in volts.

When one talks about hydrogen ion concentrations, one usually talks in terms of pH = $-\log[H^+]$.
The log function (base 10) and the natural log function (base e) are related by the following relationship. 
Ln(x) = 2.303Log(x)

$\Delta$G = 2.303RTlog([H$^+_p$]/[H$^+_N$]) + ZF$\Delta$ψ
$\Delta$G = 2.303RT(log [H$^+_p$] - log[H$^+_N$]) + ZF$\Delta$ψ
$\Delta$G = 2.303RT(-pH$_p$ + pH$_N$) + ZF$\Delta$ψ
$\Delta$G = 2.303RT(pH$_N$ -pH$_p$) + ZF$\Delta$ψ

let $\Delta$pH = (pH$_N$ -pH$_p$)
$\Delta$G = 2.303RT$\Delta$pH + ZF$\Delta$ψ

Z = 1; F = 96.4 kJ/mol•V
$\Delta$G = 2.303RT$\Delta$pH + 96.4 kJ/mol•V $\Delta$ψ

This free energy is called proton-motive force.

Actively respiring mitochondria have: $\Delta$pH = 0.75 pH units; $\Delta$ψ $\approx$ 0.17 V; T=298 °K, R = 8.3145 kJ/mol•°K
$\Delta$G = 2.303(2.478kJ/mol)(0.75) + 96.4 kJ/mol•V (0.17 V) = 20.6 kJ/mol

This is the free energy required to pump a proton across the inner mitochondrial membrane under respiring conditions.

To pump 10 protons across the membrane $\Delta$G = 206 kJ
For NADH (10H$^+_p$/2e$^-$) $\Delta$G'' = -219 kJ
All but 13 kJ/mol of the free energy is used to actively transport the protons across the membrane.
Similarly for FADH$_2$ ($6H^+_p/2e^-$) $\Delta G^\circ = -157.5$ kJ/mol
To pump six protons across the membrane $\Delta G = 6 \times 20.6$ kJ = 124 kJ.
All but 33.5 kJ of the free energy is used to actively transport the protons across the membrane.

**ATP Synthase**

How is the concentration gradient of protons across the inner mitochondrial membrane used to generate ATP? The proton motive force drives the synthesis of ATP as protons flow back from the intermembrane space to the matrix of the mitochondria. The protons are channeled through an enzyme call ATP synthase which catalyzes the following reaction.  
$ADP + Pi + nH^+_p \rightarrow ATP + H_2O + nH^+_N.$  

Within the mitochondrial membrane, there is a complex of proteins that carries out ATP synthesis called ATP synthase or $F_1F_0$-ATPase (named for the reverse reaction it catalyzes). ATP synthase is composed of two principle complexes, the $F_1$ unit which catalyzes the synthesis of ATP. This $F_1$ unit is associated with an integral membrane protein aggregate, the $F_0$ unit. The $F_0$ unit forms a transmembrane pore through which protons are channeled to drive ATP synthesis. The $F_1$ unit is composed of five polypeptide chains $\alpha, \beta, \gamma, \delta, \varepsilon$ which a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$. The $\alpha$ and $\beta$ subunits are homologous to each other, each subunit contains an ATP binding site. The catalytic sites are located in the 3 $\beta$ subunits.

The Structure of ATP synthase.

Shown in Figure (b) is a side view of the $F_1$ unit. It contains 3 $\alpha$ subunits and 3 $\beta$ subunits arranged like the segments of an orange. The $\gamma$ subunit forms a shaft. (c) is the top view of the $F_1$ unit. The single $\gamma$ subunit associates primarily with one of the $\alpha\beta$ pairs forcing each of the $\beta$ subunits into a different conformation. One $\beta$ subunit has an ADP bound, the next $\beta$-subunit contains ATP the next is empty. This difference in nucleotide binding among the three $\beta$ subunits is critical to the mechanism of the complex.
Shown to the far left is the side view of the F₁F₀ structure. The F₁ complex is in purple and grey and the F₀ complex is shown is shades of yellow and red.

To the immediate left is ATP synthase viewed from the P-face towards the N face.

The F₀ subunit is composed of three subunits denoted a, b and c in stoichiometry of ab₂c₁₀₋₁₂. The c subunits are the alpha helices that span the membrane with a small extending out into the matrix side of the membrane.

The c subunits are arranged into two concentric circles with a 55 Å diameter. The ring of c-subunits forms a rotor that turns with respect to the α-subunit.

The 2 b-subunits of F₀ complex associate firmly with the α and β subunits of F₁ holding them fixed relative to the membrane. In the membrane embedded cylinder (subunit c) of F₀ is attached the shaft composed of the γ and ε subunits of F₁. As protons flow through the membrane from the P side to the N side through the F₀ channel the c subunits turn which in turn turns the embedded shaft which rotates causing the β-subunits to change conformation as the γ subunit turns.

In the presence of a proton gradient, ATP synthase catalyzes the following reaction:
ADP + Pi + E → [E•ADP•Pi] → [E•ATP] → E + ATP

In the absence of a proton gradient, there is no net synthesis of ATP, but ATP synthase catalyzes the exchange of the hydroxyl groups of inorganic phosphate with the aqueous solvent as shown by the incorporation of O\textsuperscript{18} water into phosphate shown to the left.

This shows that ATP synthesis does not require the input of energy, but the release of the newly synthesized ATP does require energy. The movement of protons through the F\textsubscript{0} channel causes the γ subunit to rotate which drives a conformational change in the structure of the β-subunit resulting in the binding of substrates (ADP and Pi) and the release of the product ATP.

The F\textsubscript{1} complex of ATP Synthase has three interacting conformations and three conformationally distinct active sites. The Open conformation is inactive and has a low affinity for ligands. The L form has a loose affinity for ADP and Pi and also inactive. The tight conformation is active and has a high affinity for ADP and Pi. The synthesis of ATP is initiated by the binding of ADP and Pi to an open L site. In the next step, an proton driven conformational change converts the L conformation into the T conformation and simultaneously converts the O form to the L form and the T form to the O form. In the third step ATP is synthesized at the T site and ATP is released from the O site.

This cycle continues over and over.