# Enzymology of Nitrogen Fixation

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The incredibly strong (triple) bond in $N_2$ makes this reaction very difficult to carry out efficiently.
In fact, nitrogenase consumes ~16 moles of ATP for every molecule of $N_2$ it reduces to $NH_3$, which makes it one of the most energy-expensive processes known in Nature.

Plant genomes lack any genes encoding this enzyme. which occurs only in prokaryotes (bacteria).

***Only occurs in certain prokaryotes (Bacteria)***

Even within the bacteria, only certain free-living bacteria (Klebsiella, Azospirillum, Azotobacter), blue-green bacteria (Anabaena) and a few symbiotic *Rhizobial* species are known nitrogen-fixers.

Another nitrogen-fixing association exists between an Actinomycete (*Frankia* spp.) and alder (*Alnus* spp.)

The enzyme **nitrogenase** catalyses the conversion of atmospheric, gaseous dinitrogen ($N_2$) and dihydrogen ($H_2$) to ammonia ($NH_3$), as shown in the chemical equation below:

$$\mathbf{N_2 + 3\,H_2 \Rightarrow 2\,NH_3}$$

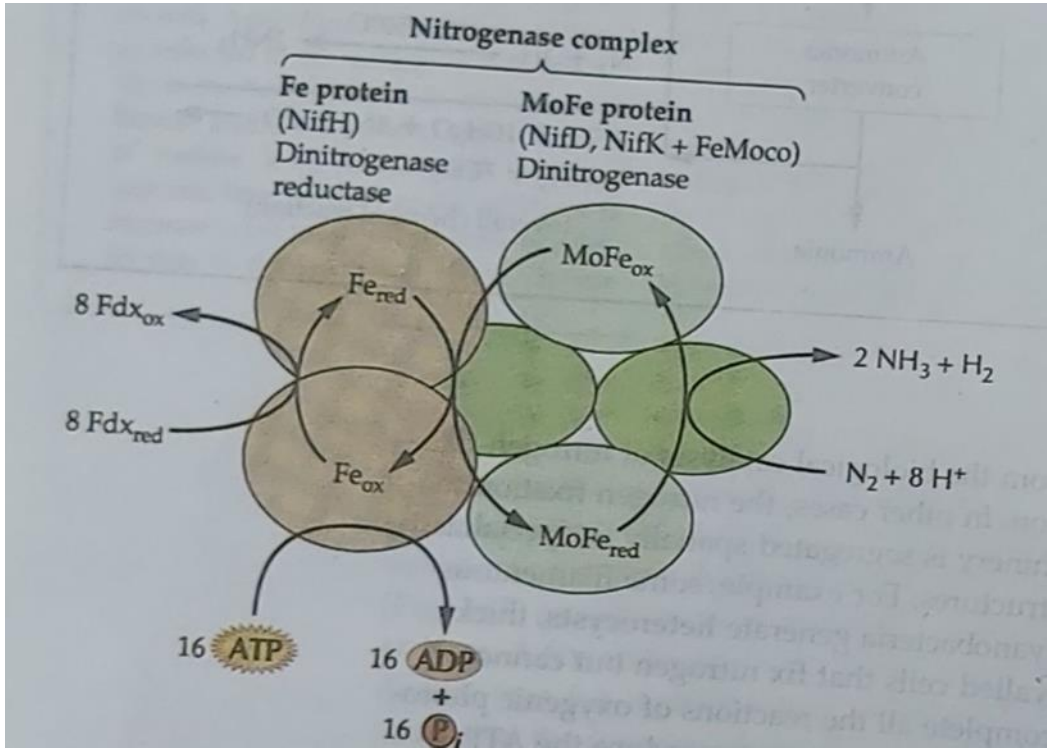
**Biological nitrogen fixation is the reduction of atmospheric nitrogen gas ($N_2$) to ammonium ions ($NH_4^+$) by the oxygen-sensitive enzyme, nitrogenase. Reducing power is provided by NAPH/ferredoxin, via an Fe/Mo centre.**

The reaction seems simple enough and the atmosphere is 78% $N_2$, so <u>why is this enzyme so important</u>?

Table 16.3 Substrates and products of nitrogenase

| Substrate | Common name | Product(s) |
|---|---|---|
| $N_2$ | Dinitrogen | $NH_3$ (ammonia) |
| $H^+$ | Hydrogen ion | $H_2$ (hydrogen gas) |
| $N_2O$ | Nitrous oxide | $N_2$, $H_2O$ |
| $CN^-$ | Cyanide | $NH_3$, $CH_4$ (methane) |
| $CH_3NC$ | Methyl isocyanide | $CH_3NH_2$ (methylamine), $CH_4$ |
| $N_3^-$ | Azide | $N_2$, $NH_3$ |
| $C_2H_2$ | Acetylene | $C_2H_4$ (ethylene), $C_2H_6$ (ethane) |
| $H_2NCN$ | Cyanamide | $NH_3$, $CH_3NH_2$ |
| $C_3H_4$ | Cyclopropene | $C_3H_6$ (cyclopropane) |
| $CH_2N_2$ | Dazirine | $NH_3$, $CH_3NH_2$ |

# Nitrogenase Complex- *Two protein components:*



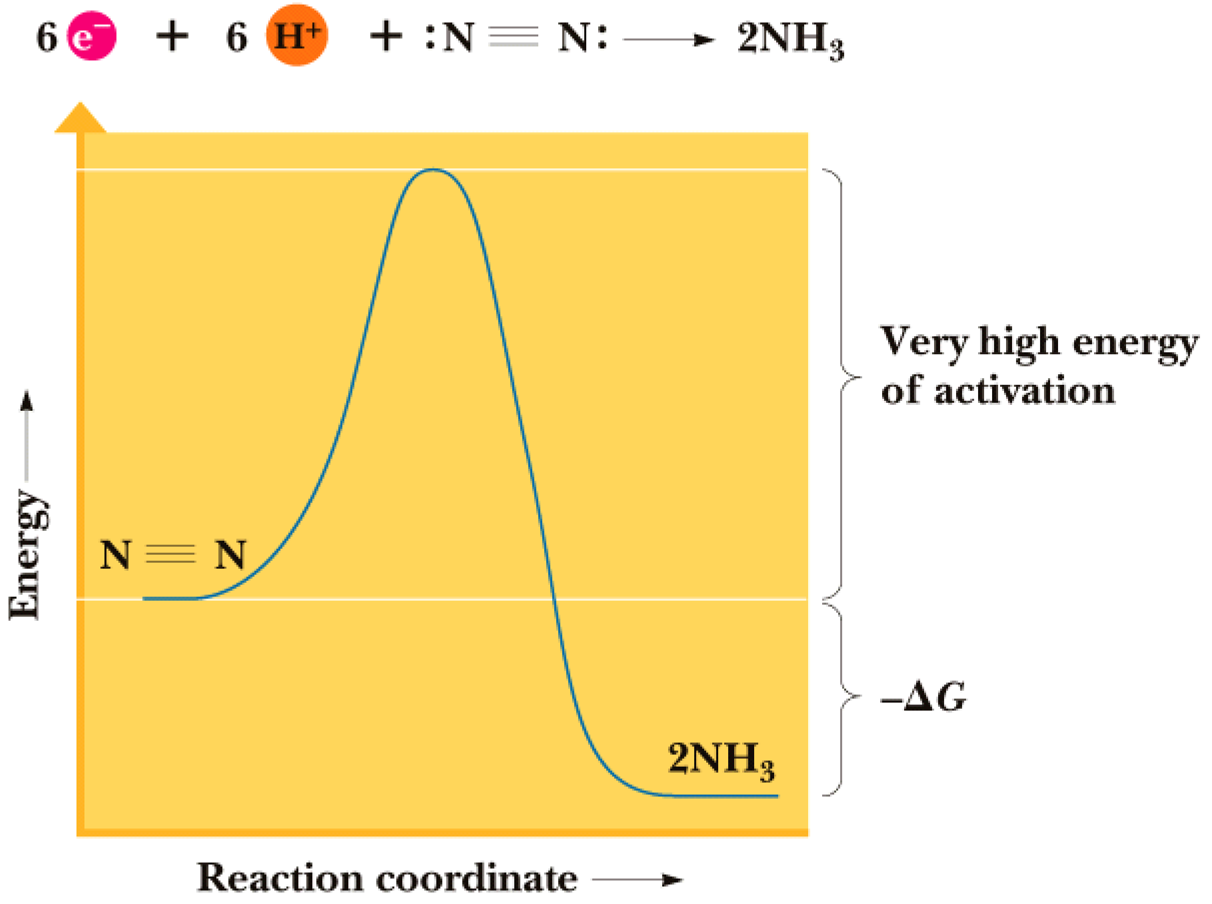

- Nitrogenase reductase
- Nitrogenase

# Nitrogenase Complex

## *Nitrogenase reductase*

- Nitrogenase reductase is a 60 kD homodimer with a single 4Fe-4S cluster
- Very oxygen-sensitive
- Binds MgATP
- 4ATP required per pair of electrons transferred
- Reduction of $N_2$ to $2NH_3$ + $H_2$ requires 4 pairs of electrons, so **16 ATP are consumed per $N_2$**

Garrett & Grisham: Biochemistry, 2/e
Figure 26.4

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# Why should nitrogenase need ATP???

- $N_2$ reduction to ammonia is thermodynamically favorable
- However, the activation barrier for breaking the N-N triple bond is enormous
- **16 ATP** provide the needed activation energy

# Nitrogenase

***A 220 kD heterotetramer***

- Each molecule of enzyme contains 2 Mo, 32 Fe, 30 equivalents of acid-labile sulfide (FeS clusters, etc)
- Four 4Fe-4S clusters plus two FeMoCo, an iron-molybdenum cofactor
- Nitrogenase is **slow - 12 $e^-$ pairs per second, i.e., only three molecules of $N_2$ per second**

$$\mathrm{N2} \xrightleftharpoons{8e^- + 8H^+} \mathrm{2NH3 + H2}$$

$$\mathrm{N2} \xrightarrow{2e^- + 2H^+} \mathrm{NH{=}NH} \xrightarrow{2e^- + 2H^+} \mathrm{H2N{-}NH2} \xrightarrow{2e^- + 2H^+} \mathrm{2NH3 + H2}$$

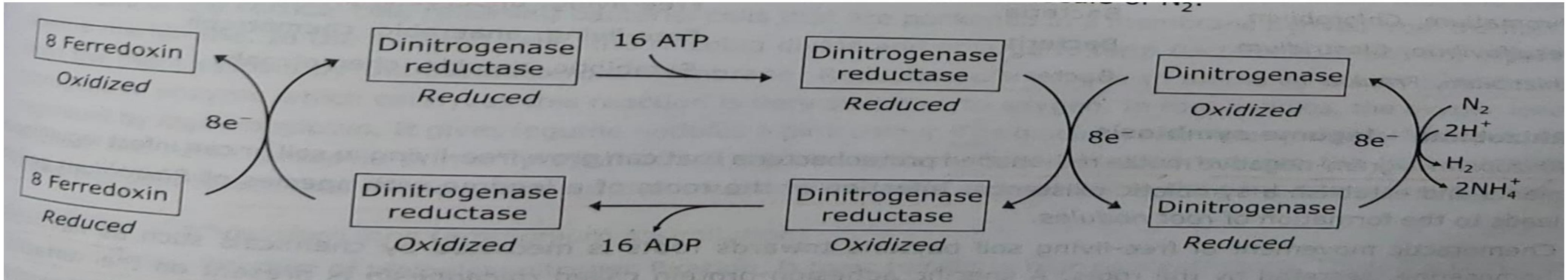


**Figure 3.12** Nitrogen fixation by nitrogenase complex.

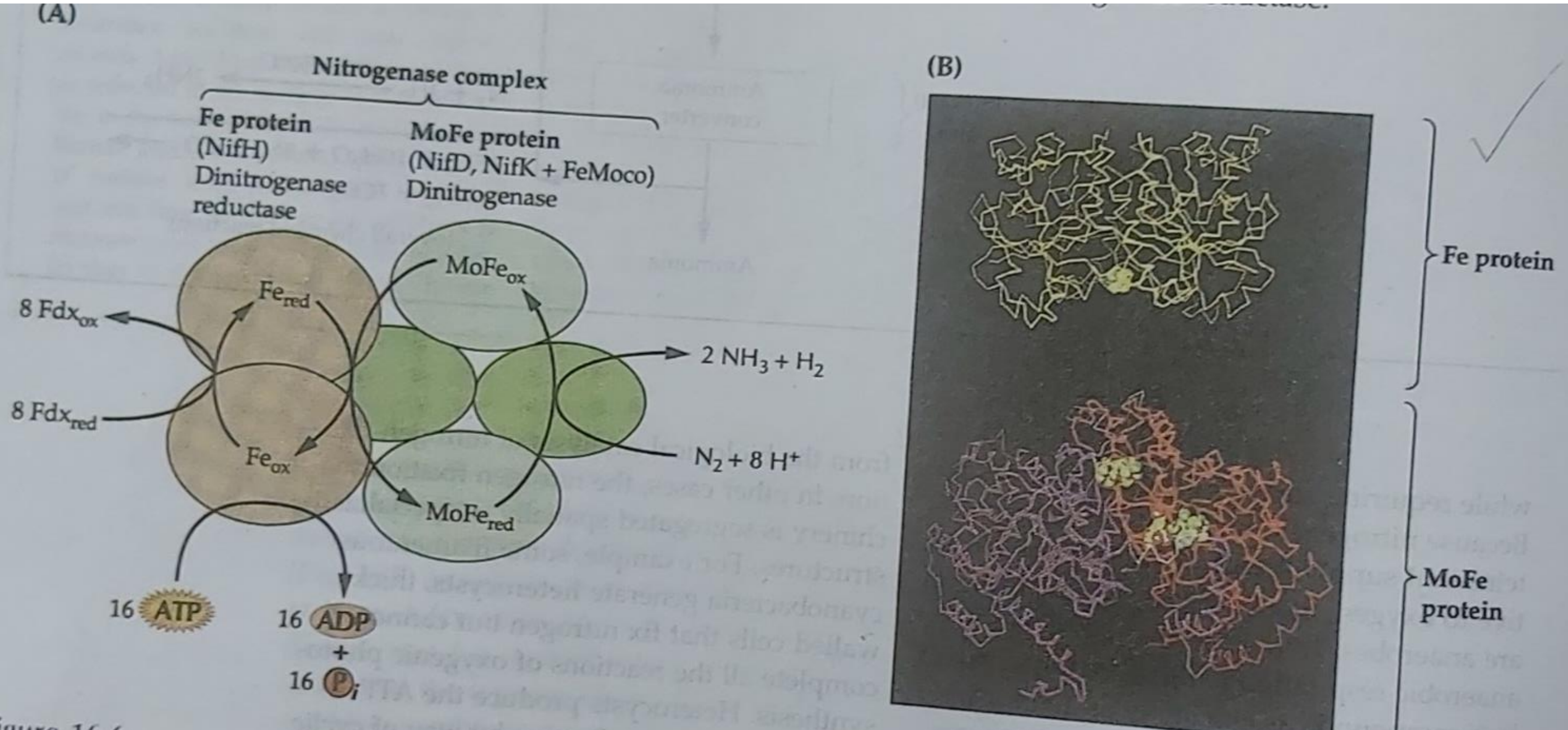


Figure 16.6

(A) Schematic diagram of the nitrogenase complex, showing the flow of reducing power and substrates in enzymatic nitrogen fixation. The Fe-protein, encoded by *nifH*, accepts electrons from a carrier, e.g., ferredoxin, flavodoxin, or another redox-active species of similar potential. The identity of the carrier varies, depending on the biological system involved. The Fe-protein transfers single electrons at very low potential to the MoFe-protein, accompanied by net hydrolysis of ATP. The MoFe-protein, an $\alpha_2\beta_2$ heterotetramer of subunits encoded by *nifD* and *nifK*, accepts electrons and binds $H^+$ ions and $N_2$ gas in a stepwise cycle, ultimately leading to the production of $H_2$ and ammonia. (B) Docking of the nitrogenase FE protein dimer (yellow) with half of the nitrogenase MoFe protein (red, nifD; purple, nifH). A 4Fe:4S cluster is associated with the Fe protein. The P cluster is near the nifD/nifH interface. FeMoCo (green) is mostly associated with nifD.

Thank you!