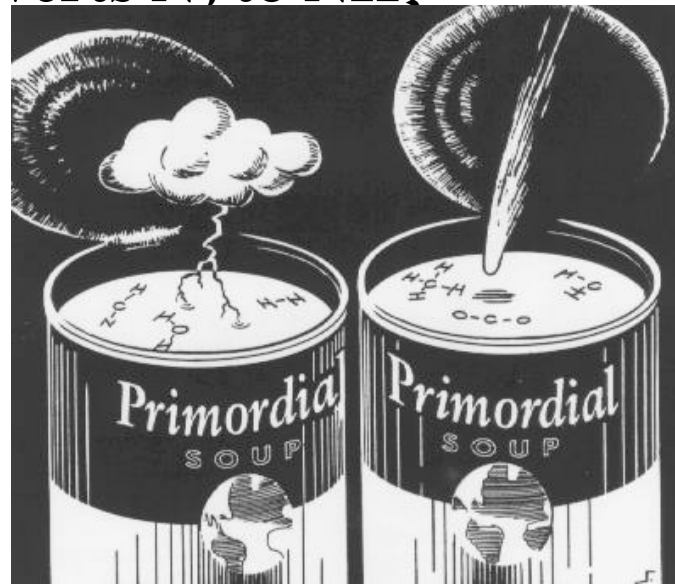


Nitrogenase

Like the story of Rubisco, the story of nitrogenase begins with the evolution of oxygenic phototrophs 3 billion years ago.

NH_3 was plentiful before that. Since then, most N in the atmosphere is in the form of N_2 .

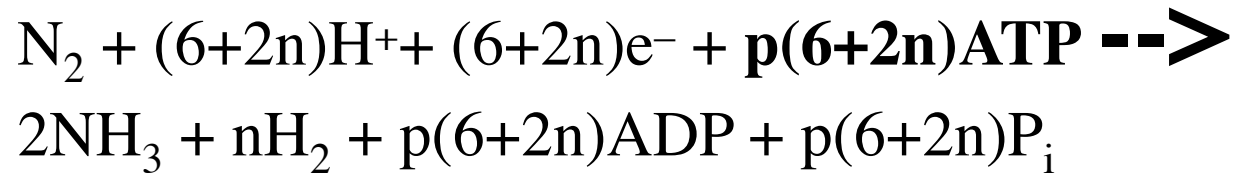
Nitrogenase converts N_2 to NH_3



The high cost of nitrogen fixation



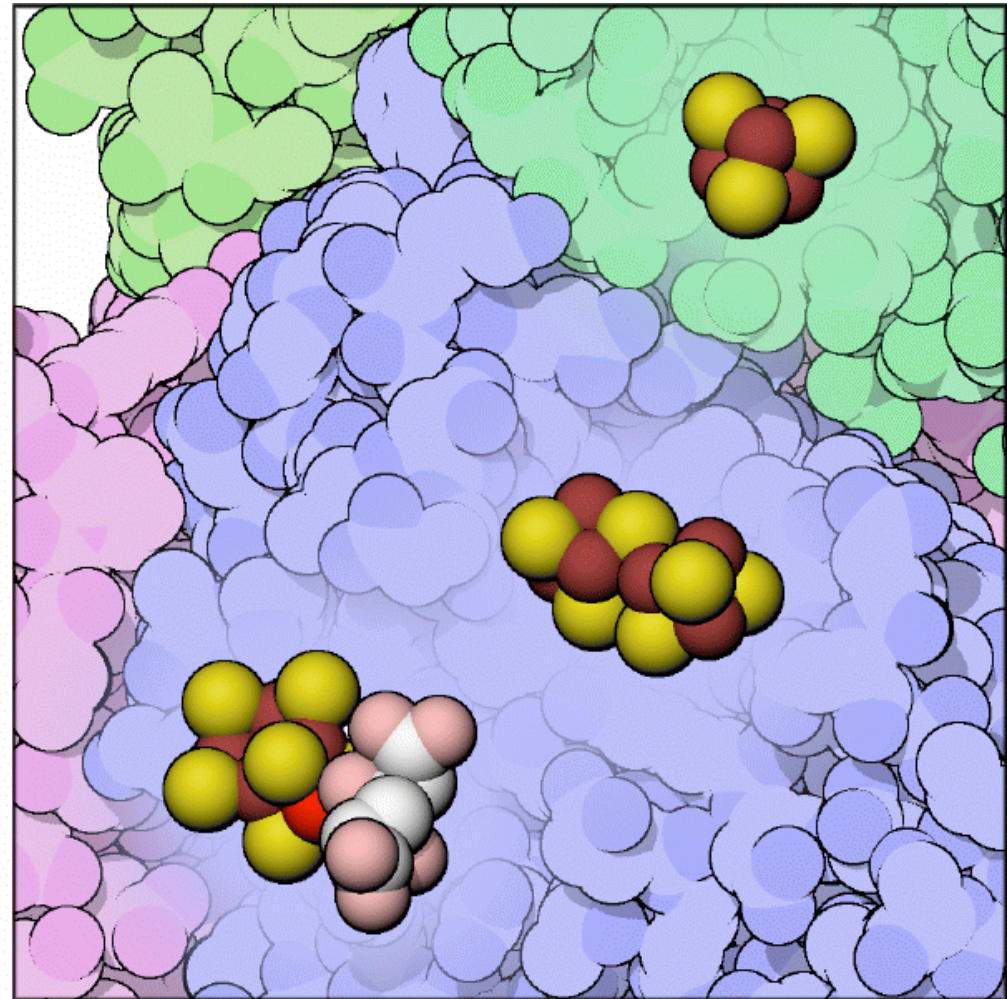
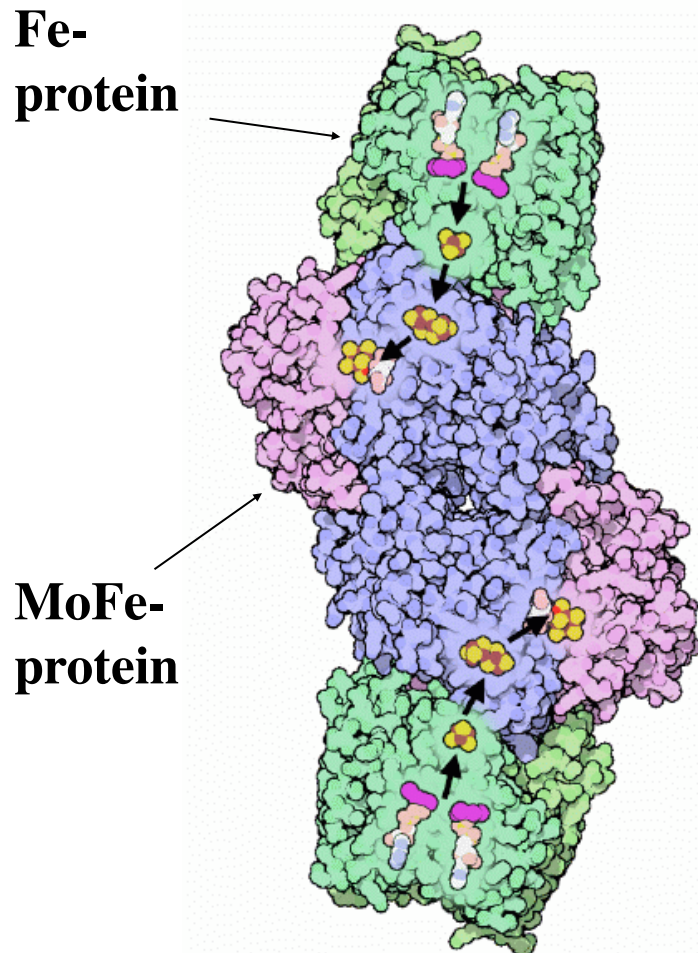
The stoichiometry of nitrogenase is still not completely known.



n=number of H₂ molecules formed (1 or 2, unknown)

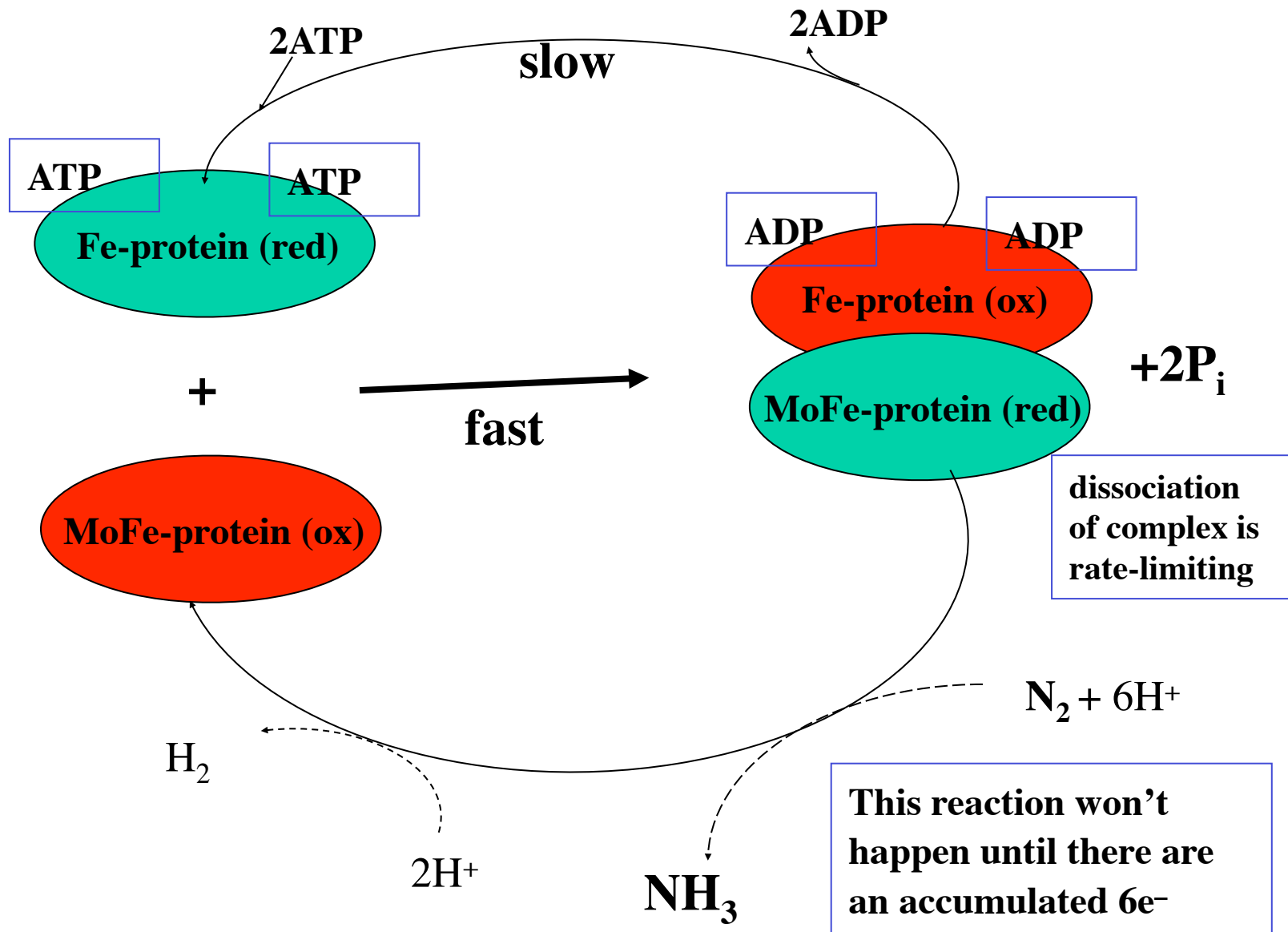
p=number of ATP required per electron (probably 2)

Nitrogenase is a hetero-tetramer. 2 Fe-proteins, 2 MoFe-proteins. With 3 Iron-containing clusters.



The Fe-protein cluster passes e- to the P-cluster, which passes them to the FeMo-cluster. e- accumulate at the FeMo-cluster, where the reduction of N₂ takes place.

Mechanism: simplified

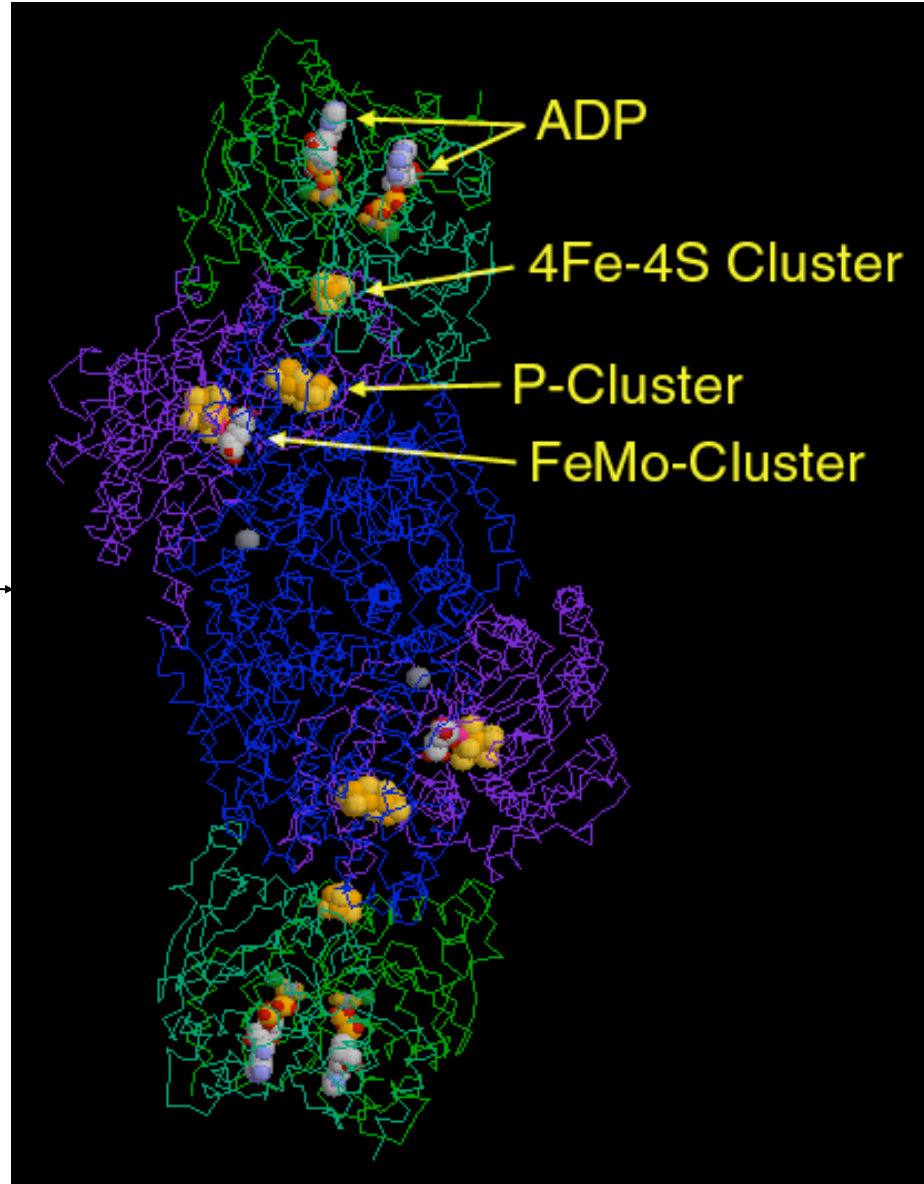


2 ATP bind to the Fe-protein before it binds to the MoFe-protein.

Fe-protein →

MoFe protein →

Fe-protein →



Guided tour of Nitrogenase (1N2C)

Open 1N2C.pdb in Jmol (www.pdb.org)

In Jmol open a console window (right-click) and type:

There are 8 chains in the asu. Which chains are related to which by non-crystallographic symmetry?

backbone

color chain

select 50 and alpha

label %c

set fontsize 20

color labels yellow

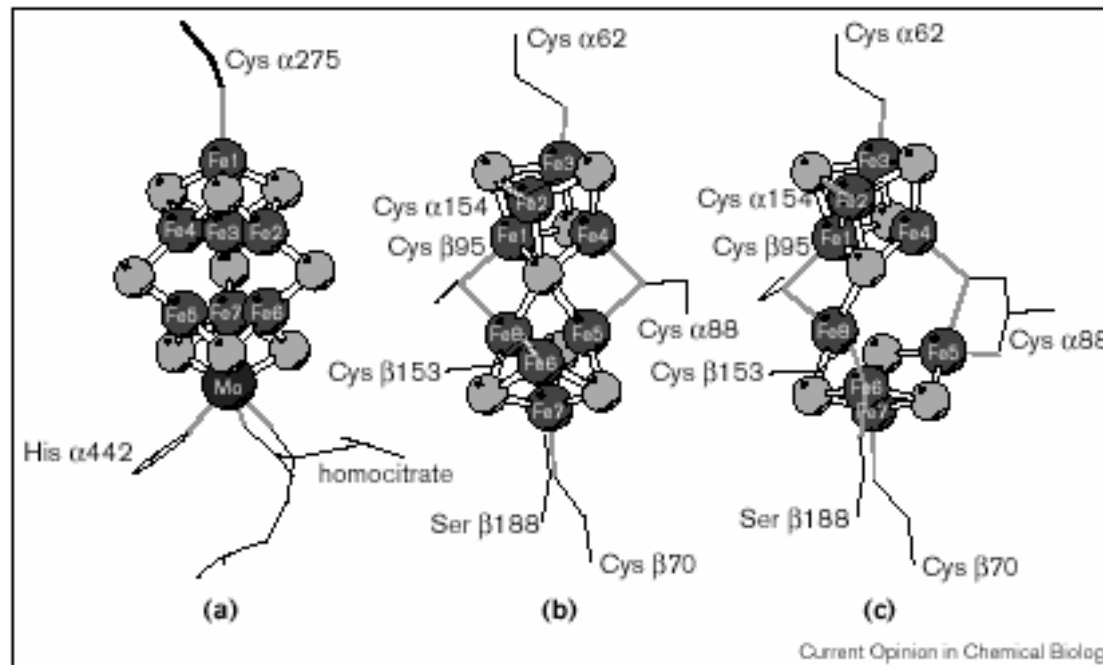
line up the molecule along the non-crystallographic 2-fold.

There are two clusters in the MoFe-protein

**FeMo
cluster**

**Reduced
P-cluster**

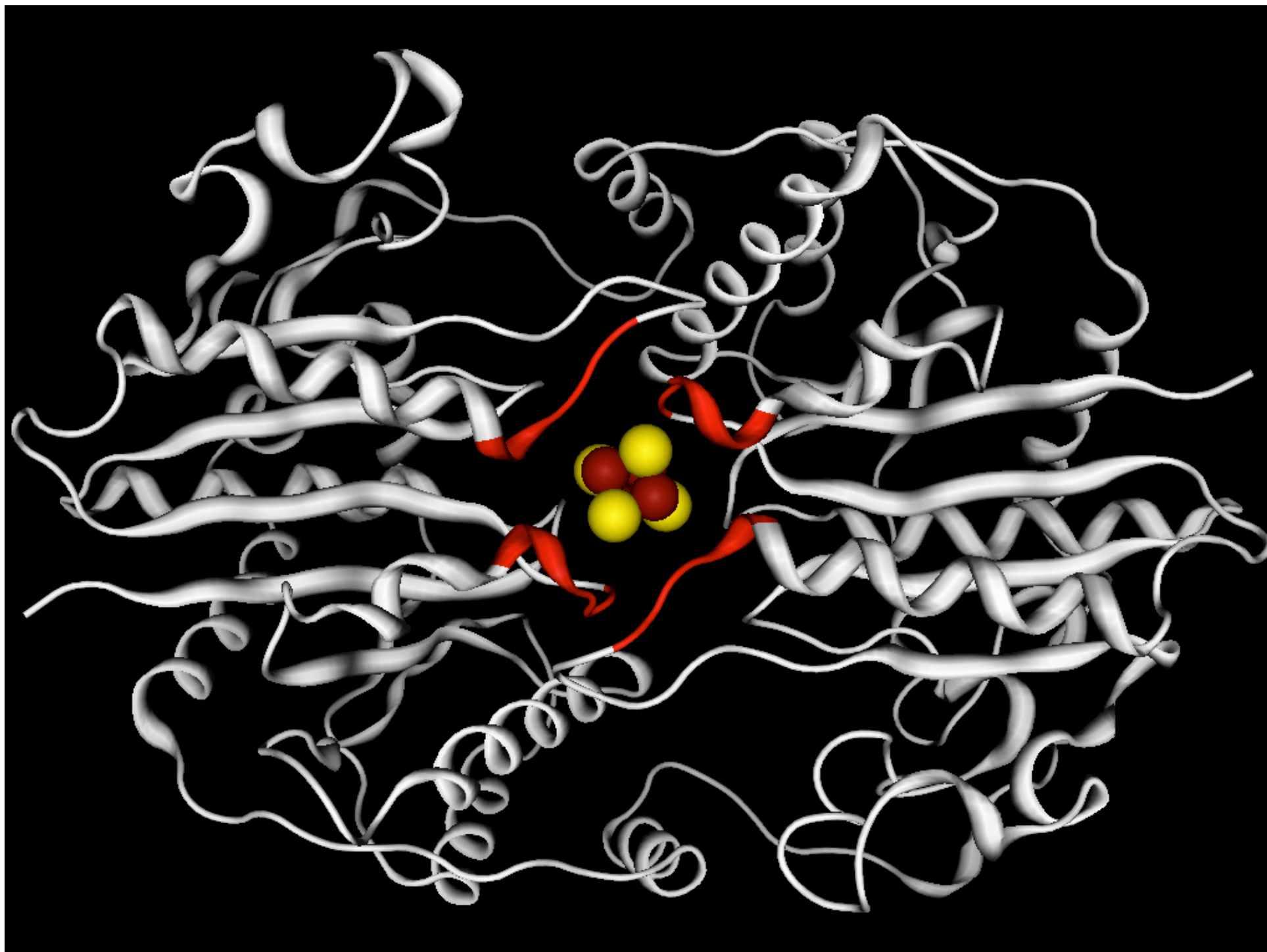
**Oxidized
P-cluster**

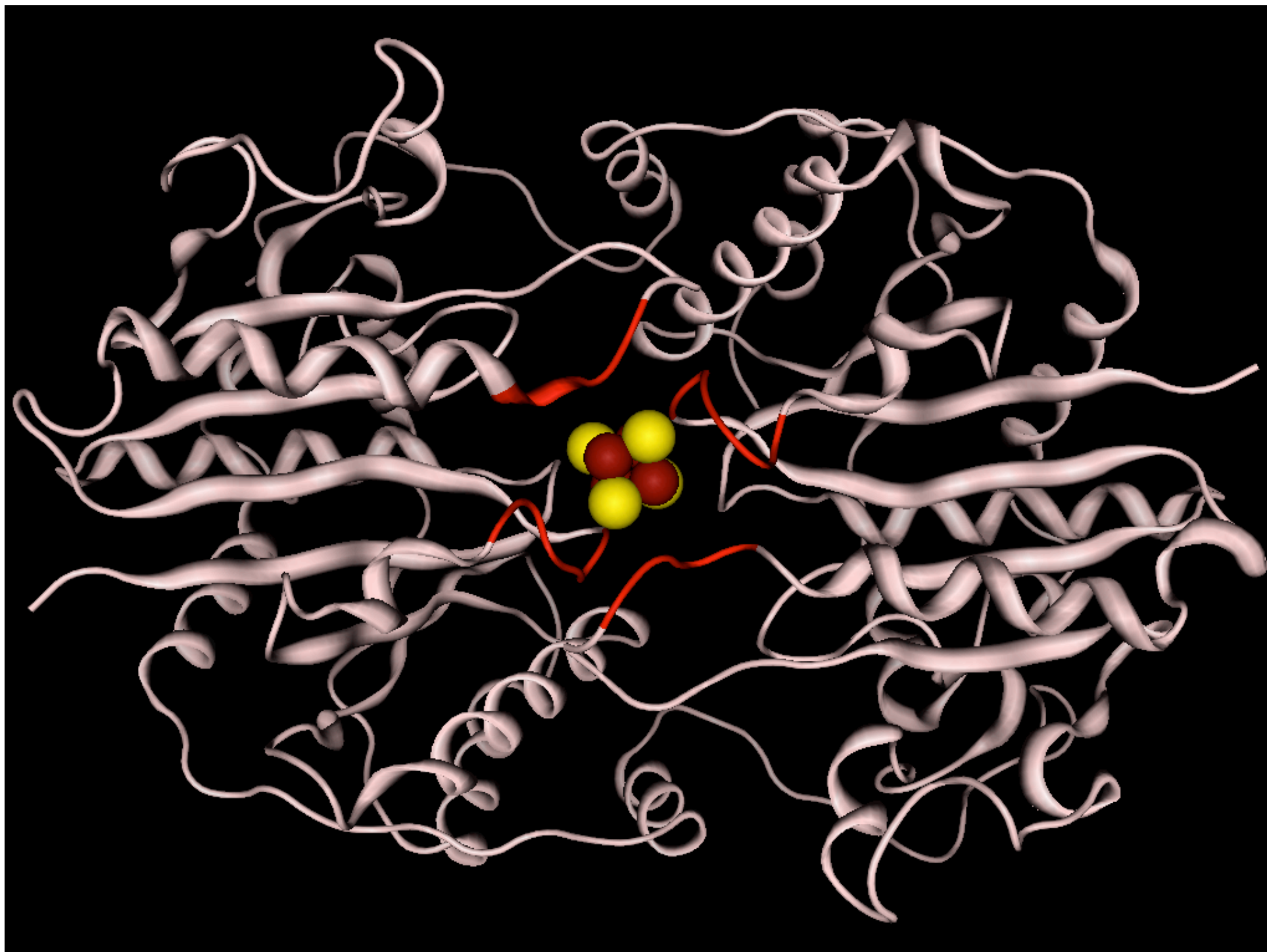


dithionite

A protein conformational change *might* favor *oxidized* over *reduced*, driving the reaction forward.

from Rees & Howard (2000) Current Opinion in Chemical Biology, 4(5):p559-566.





Many small molecules bind to the FeMo-cluster

| | | |
|----------------------------------|---------------------------|-------------------------------|
| O₂ | (molecular oxygen) | inactivates |
| CO | (carbon monoxide) | inhibits (competitive) |
| HCCH | (acetylene) | substrate |
| HCN | (cyanide) | substrate |
| N₃⁻ | (azide) | substrate |

N₂ binds with $K_M = 0.02$ atm

Interesting mutants of nitrogenase

His 195A --> Glu

Blocks N-fixation but allows reduction of acetylene.

Gly 69A --> Ser

Blocks reduction of acetylene, but allows N-fixation.

How do these mutations affect function?

restrict within (10., 195:A)

center selected

backbone off

wireframe 0.15

select 195:A and alpha

label %m%r

color labels yellow

what interacts with 195:A?

what would a E do?

Does Gly69A have a positive phi-angle?

labels off

select 68-69:A

wireframe 50

restrict selected

center selected

color cpk

Line up atoms 69:A n and 69:A ca to measure the phi angle. R-handed is positive.

Mutating G69 blocks reduction of acetylene, but allows N₂-fixation. Would mutating Gly69A to a Serine possibly change its conformation?

Guided tour of Nitrogenase

Draw a TOPS diagram of chain E.

**restrict :E
center selected
cartoon on
color structure**

Line the structure up with the beta sheet perpendicular to the screen. Ignore short helices (they are not really helices). Draw strands as up or down arrows and the helices as circles. Then draw connecting lines, to the middle if the connection is toward you, to the edge if the connection is away from you. Find the N-term. **Number the strands from N to C. Find the fold class from SCOP (scop.berkeley.edu). (goto “top of the heirarchy” then class 3, “alpha and beta proteins”)**

SCOP structural classification

Chain E is “3-layer”. When you have numbered the strands, look in SCOP for the “Fold” with the observed strand order (for example: 43125, etc). (cute trick: use the browser’s “search in page” function).

The strand order can be read from right-to-left or left-to-right. If a terminal strand is at the edge of the beta sheet it might be missing. Also, extra strands might be added at the C-term or N-term if it occurs at the sheet edge.

Write the fold name here _____

Guided tour of nitrogenase

Characterize the environment of the ADP and metal clusters.

select within (6., hetero) and (:A | :B | :E | :F)

restrict selected

Display -->ball and stick

color white

select selected and hetero

spacefill

color cpk

select within (6., hetero) and (:A | :B | :E | :F)

select selected and acidic

color red

select within (6., hetero) and (:A | :B | :E | :F)

select selected and basic

color blue

continued...

Guided tour of nitrogenase

Characterize the environment of the ADP and metal clusters.

select within (6., hetero) and (:A | :B | :E | :F)

select selected and polar and not basic and not acidic

color green

How would you characterize the binding sites? (check one for each het group)

| | mostly basic | mostly acidic | mostly polar | mixed charges | non- polar |
|------------|-------------------------|--------------------------|-------------------------|--------------------------|-----------------------|
| ADP | | | | | |
| FS4 | | | | | |
| CLF | | | | | |
| CFM | | | | | |
| CA | | | | | |