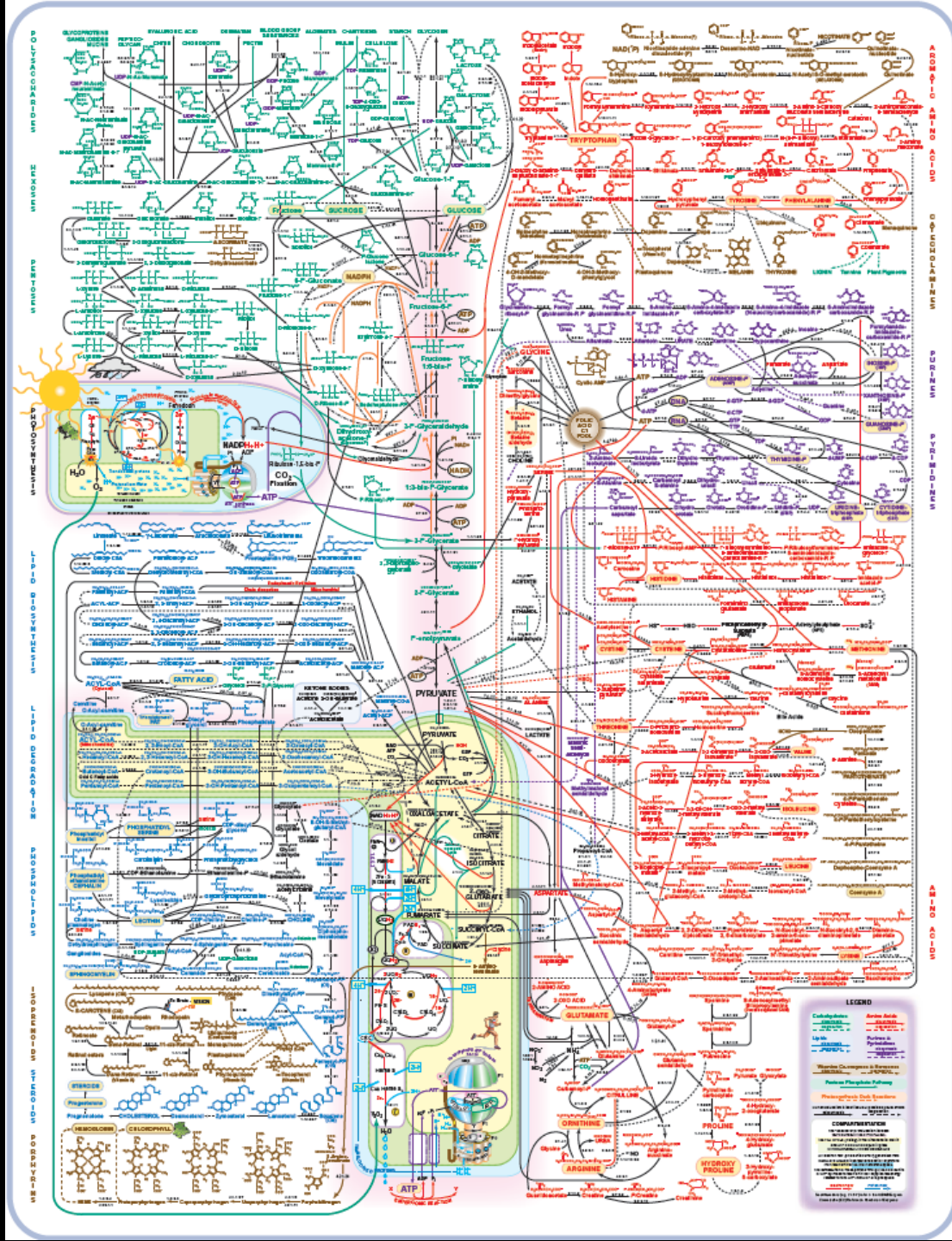


A Solar Biofuel-Producing Nanodevice that Outperforms Natural Photosynthesis

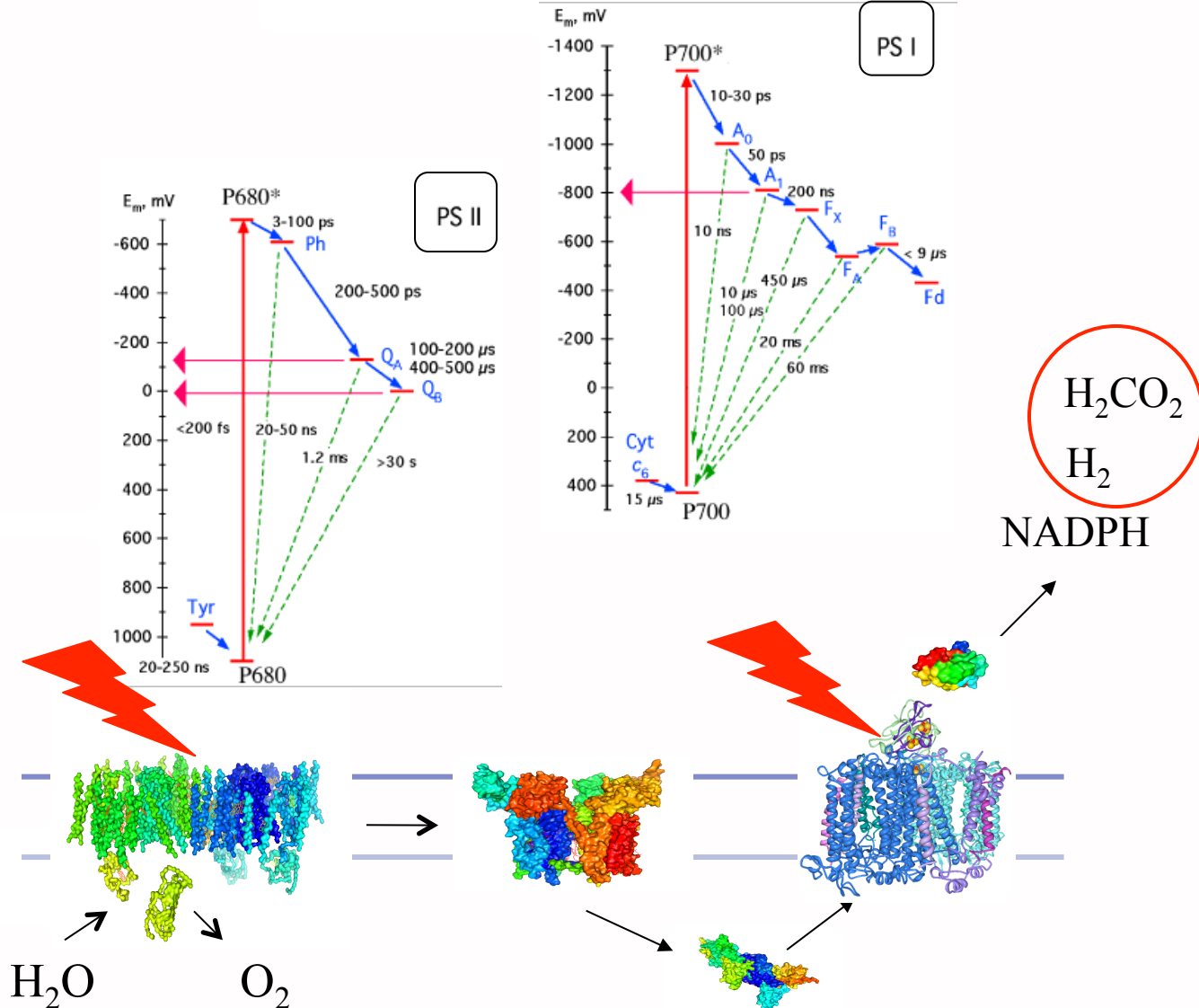


Sunday, December 18, 2011

John H. Golbeck
Department of Chemistry
Department of Biochemistry and Molecular Biology
The Pennsylvania State University
University Park, PA

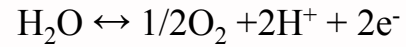


Oxygenic Photosynthesis

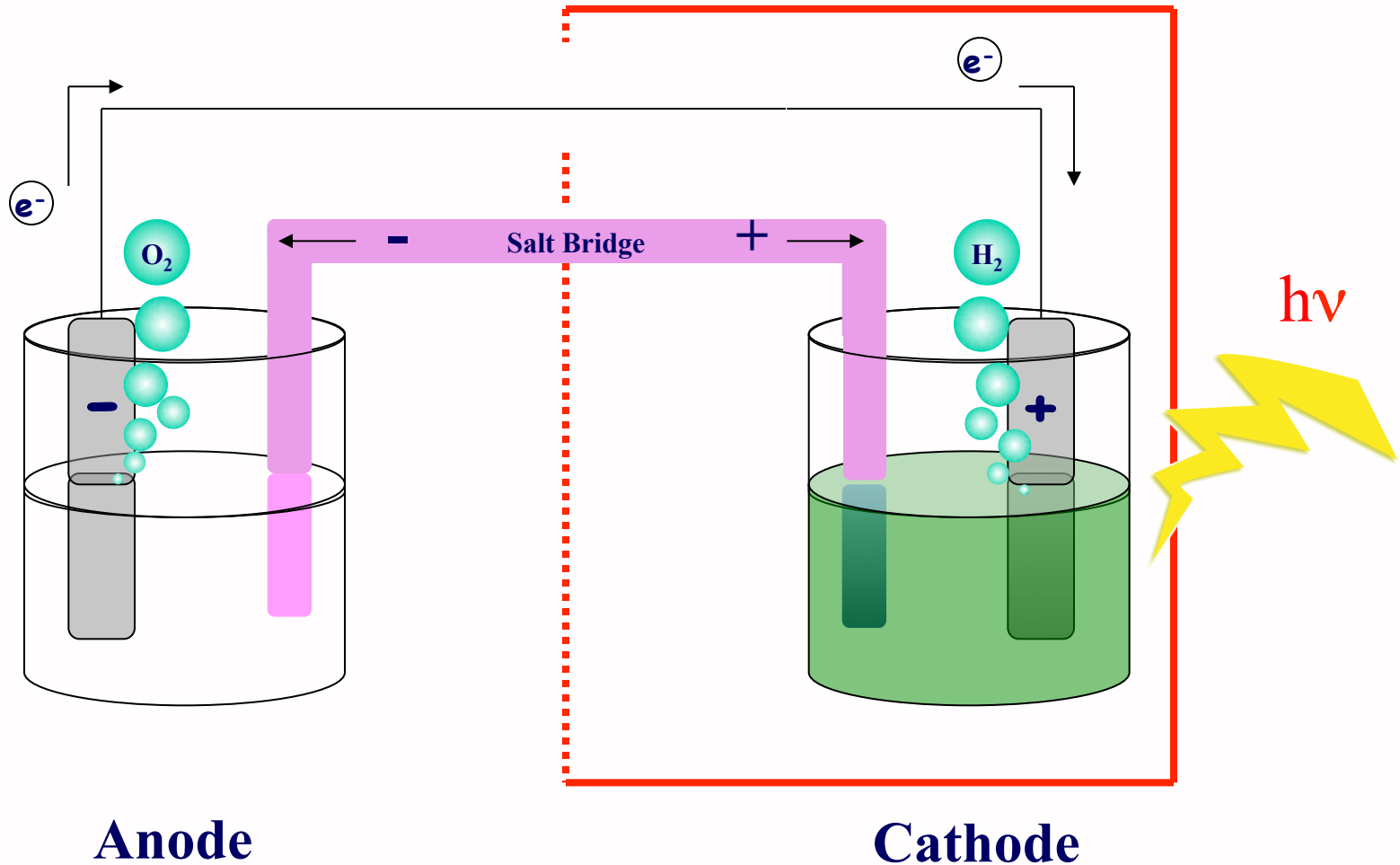
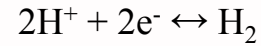


Light-Driven Electrolysis

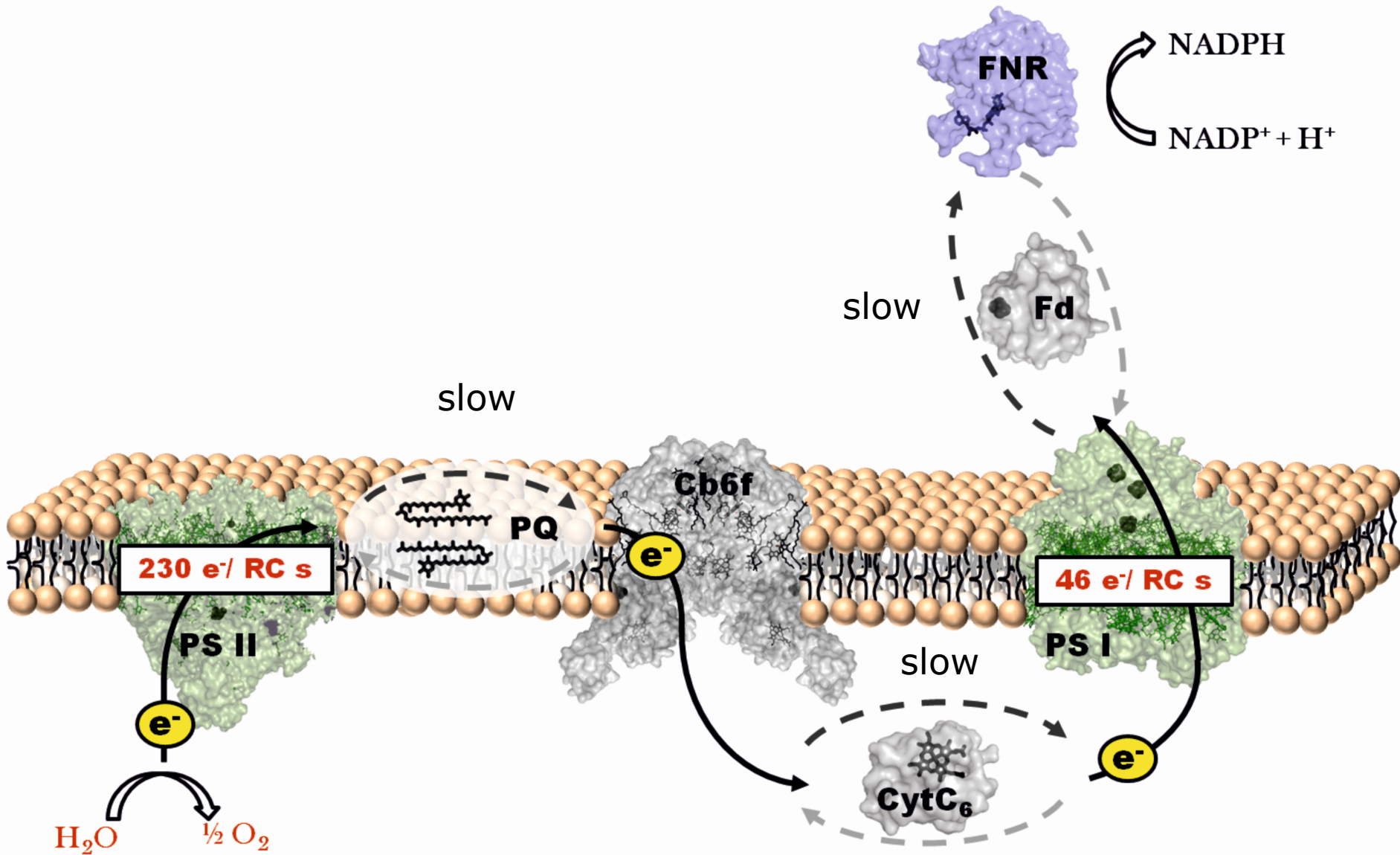
Anodic half reaction:

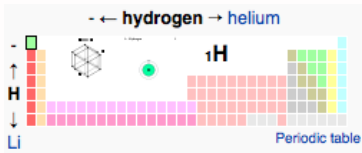


Cathodic half reaction:



Diffusion-Mediated Steps in Natural Photosynthesis



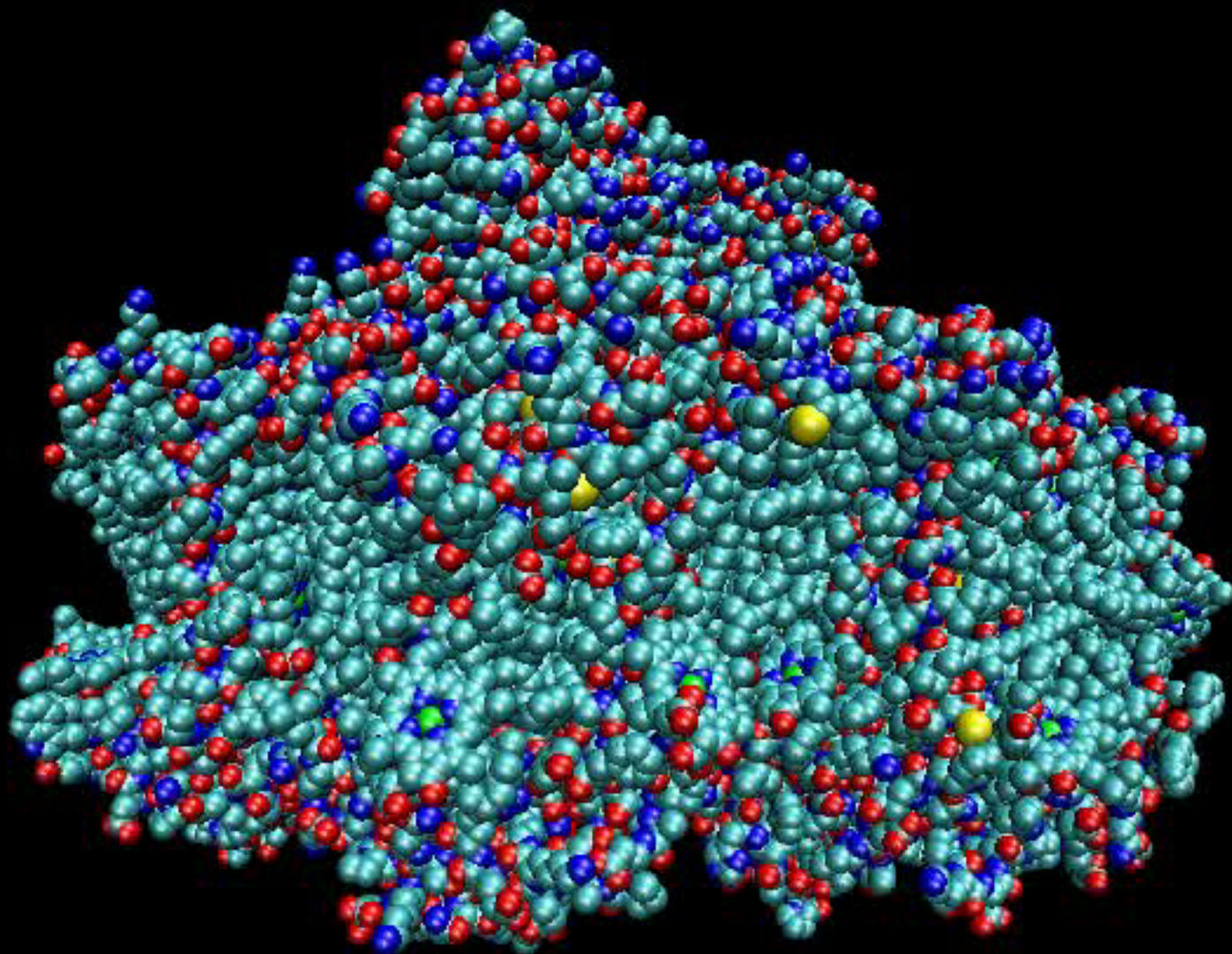


Hydrogen as a Primary Fuel

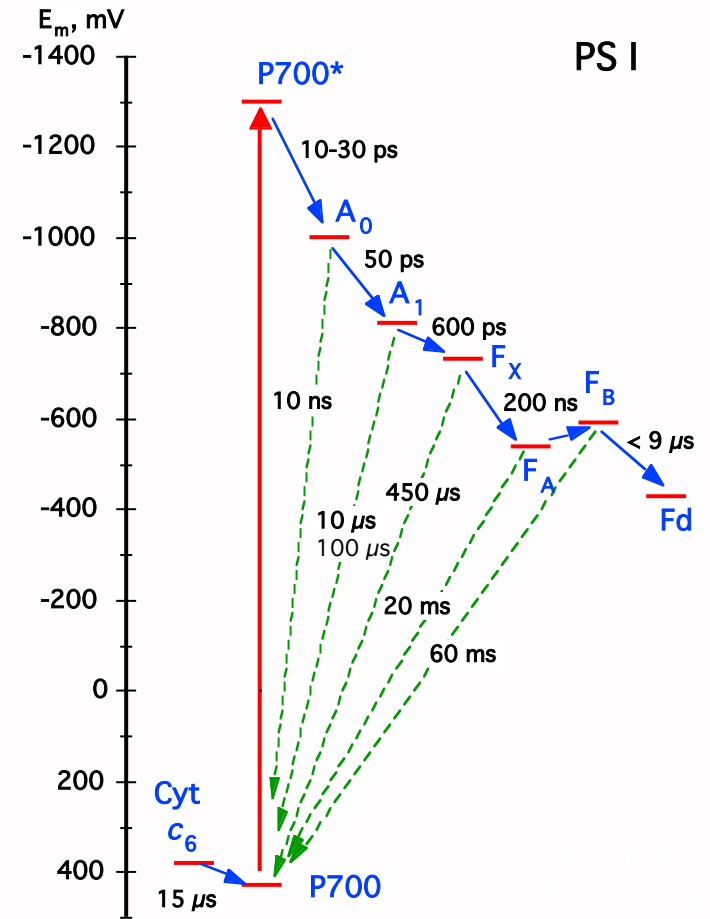
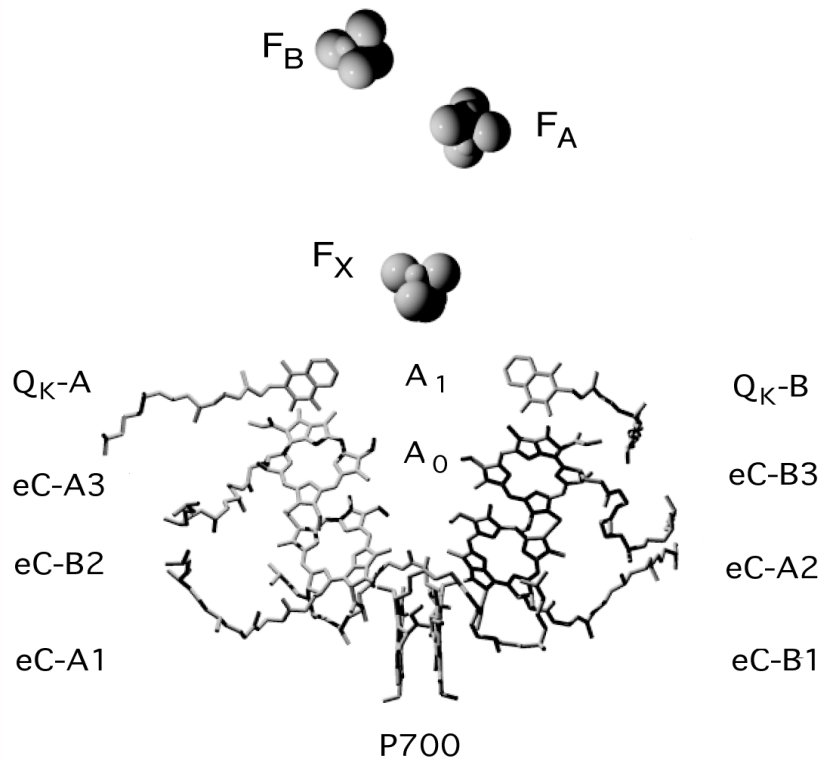
Upside

- Clean, i.e. no CO₂ emissions: $\text{H}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}$
- High energy value per unit weight:

hydrogen	143.0 MJ kg ⁻¹
(gasoline)	46.4 MJ kg ⁻¹



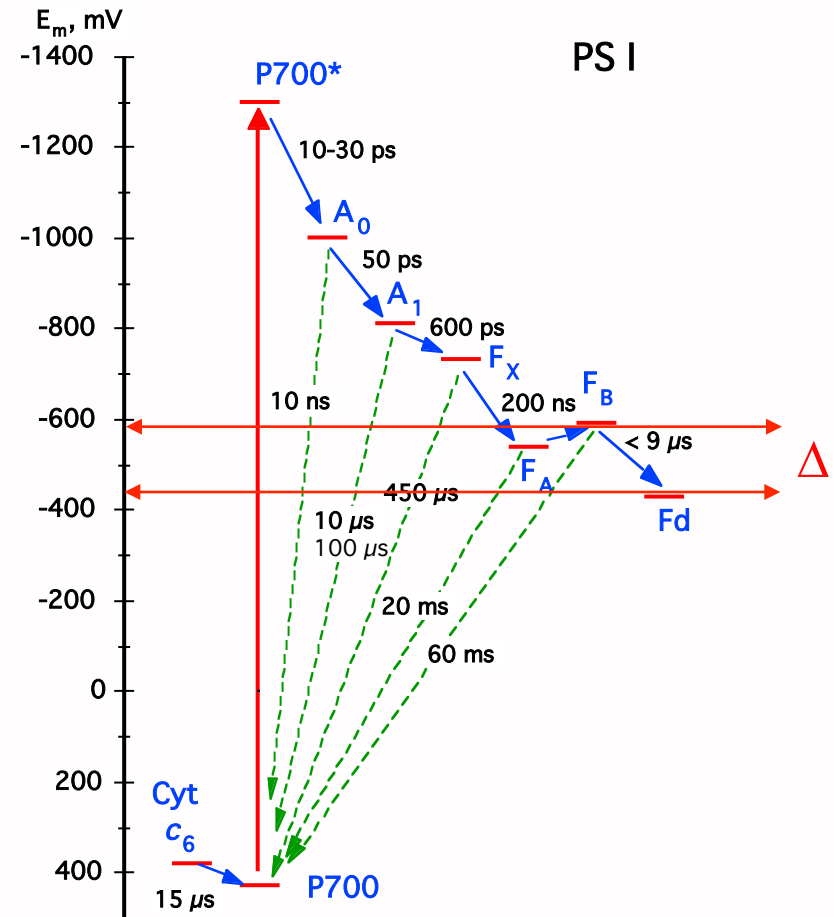
Photosystem I as the Photosensitizer



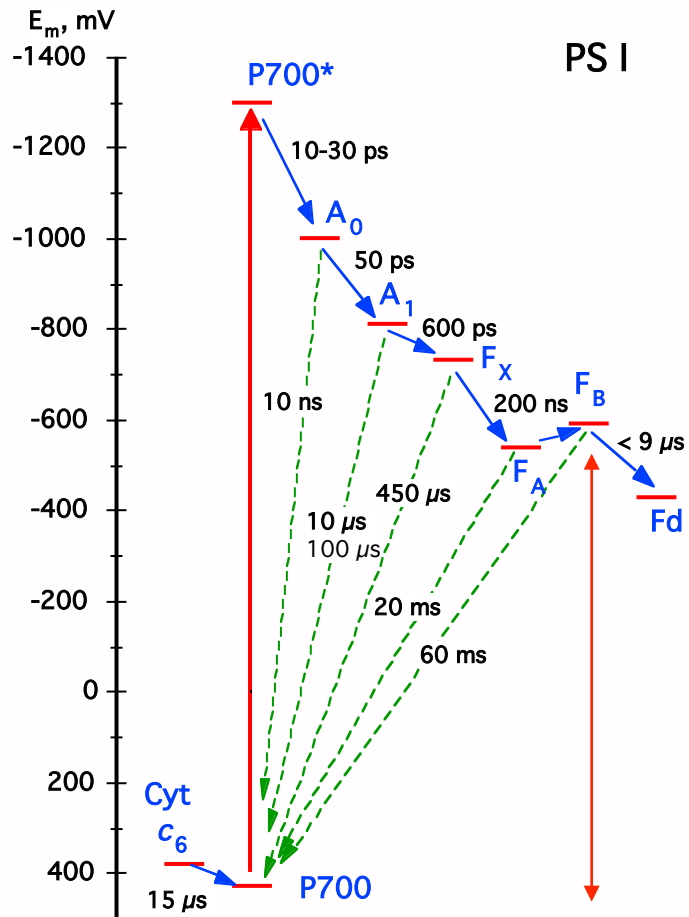
Favorable Energetics for H₂ Generation

The F_B cluster has a pH-independent redox potential of -580 mV

This is 160 mV more reducing than the half-cell potential of the H⁺/H₂ couple: $2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$ at pH 7.0

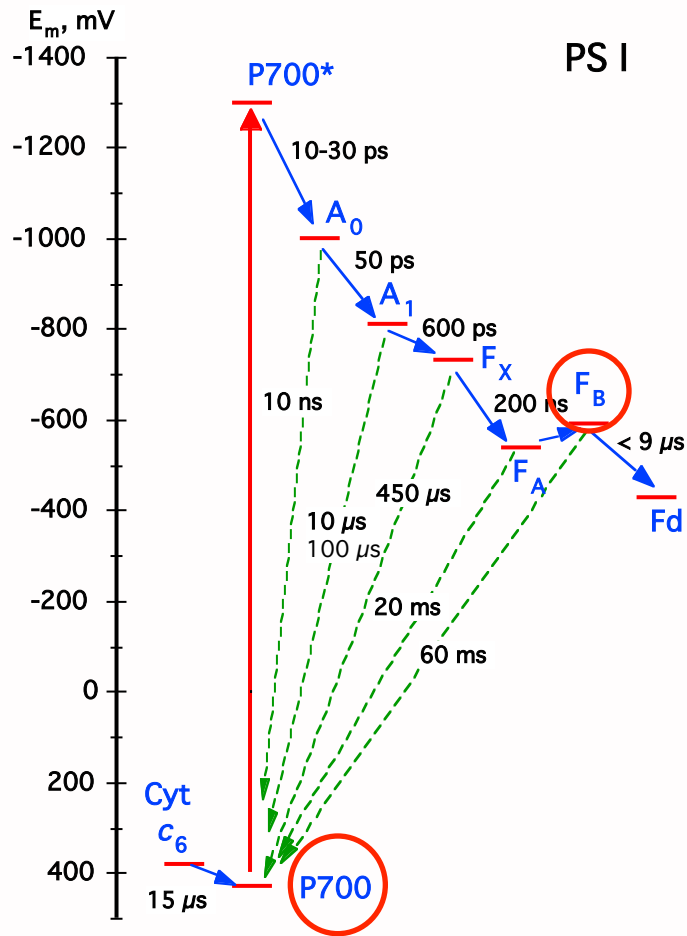


High Photon Conversion Efficiency



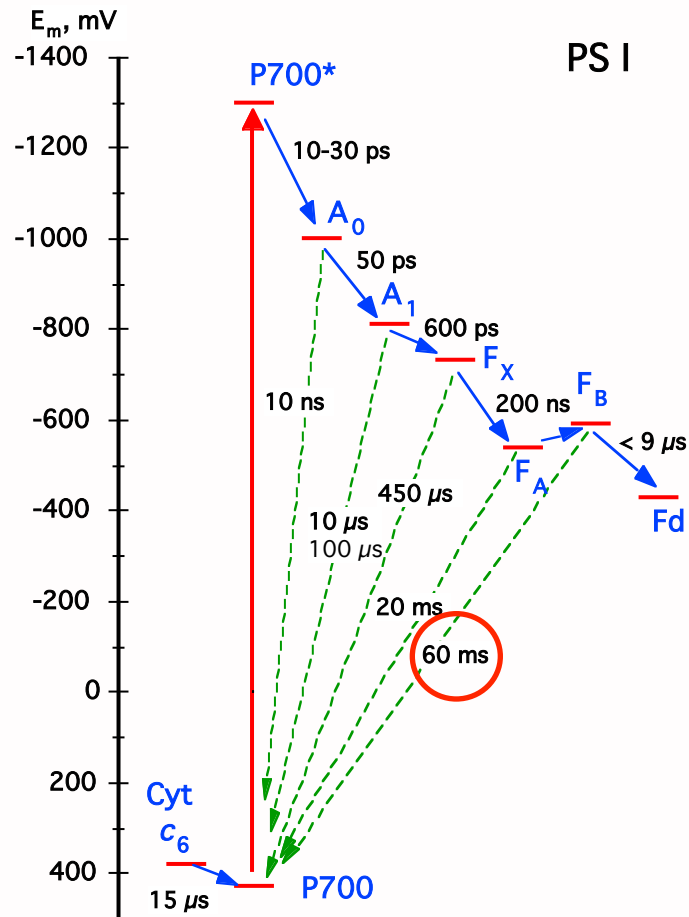
The 1.01 V in the charge-separated state $P_{700}^+F_B^-$ represents a **59% conversion efficiency for a red photon**, and a **38% conversion efficiency for a blue photon**

High Quantum Yield



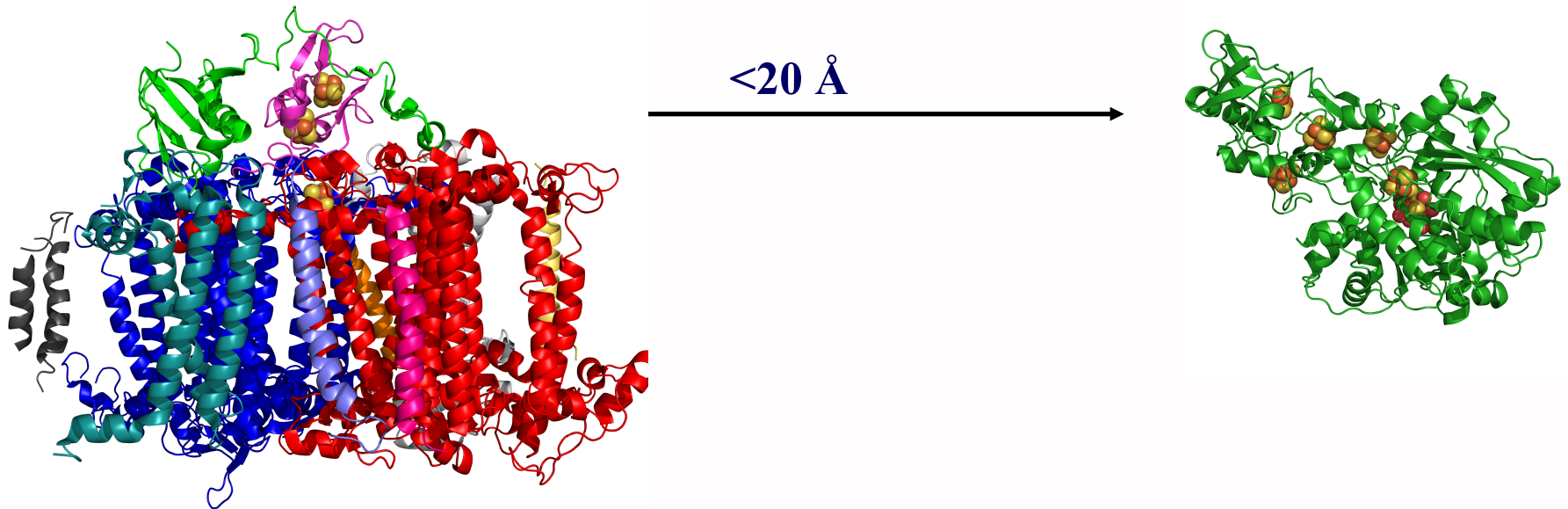
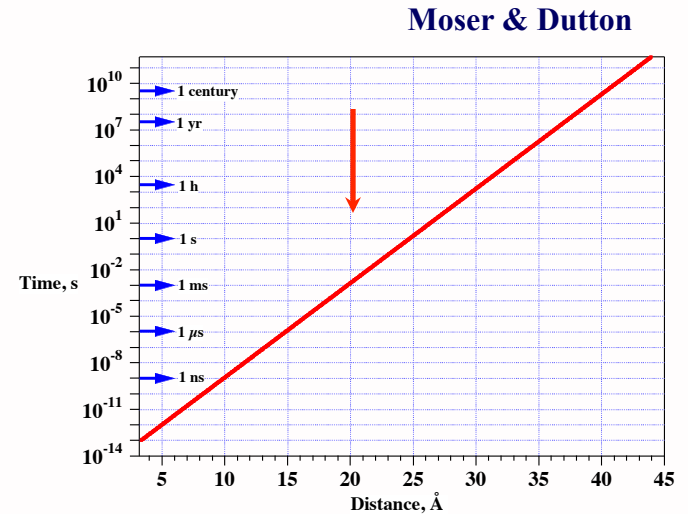
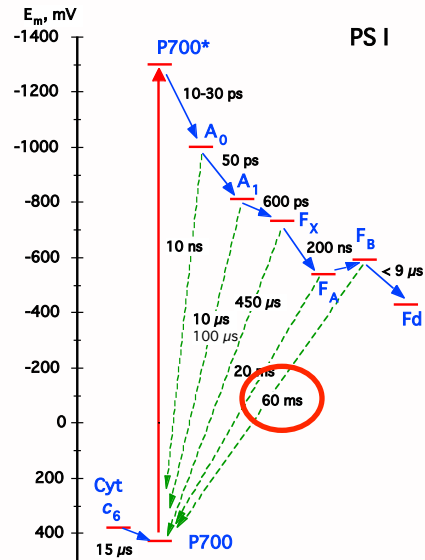
Regardless of wavelength, nearly every photon that is absorbed by PS I antenna is processed into the charge separated state $P_{700}^+ F_B^-$

Stable Charge-Separated State

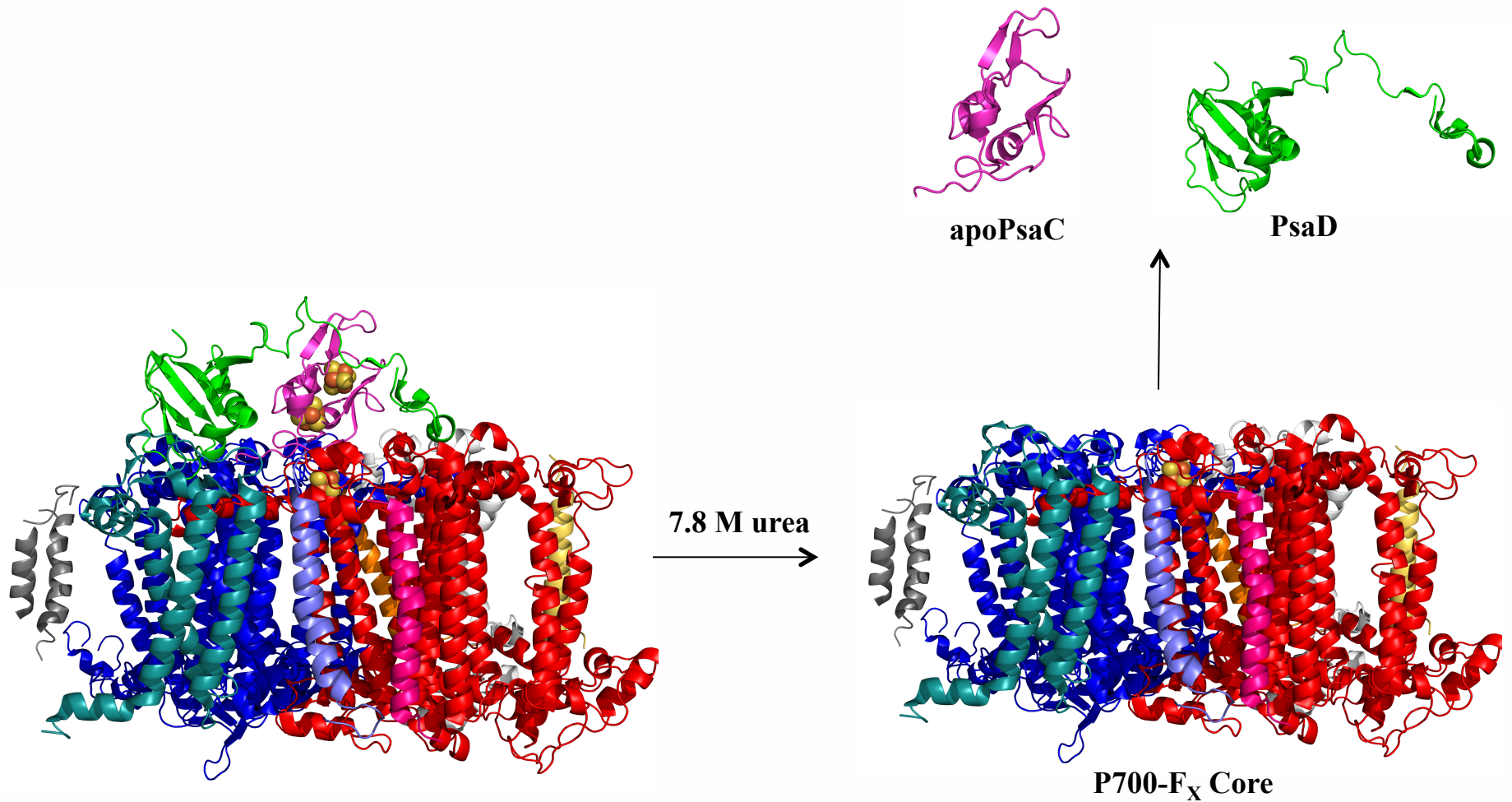


The charge-separated state is stable for ~60 ms, which is sufficient time to remove the low potential electron to perform useful work

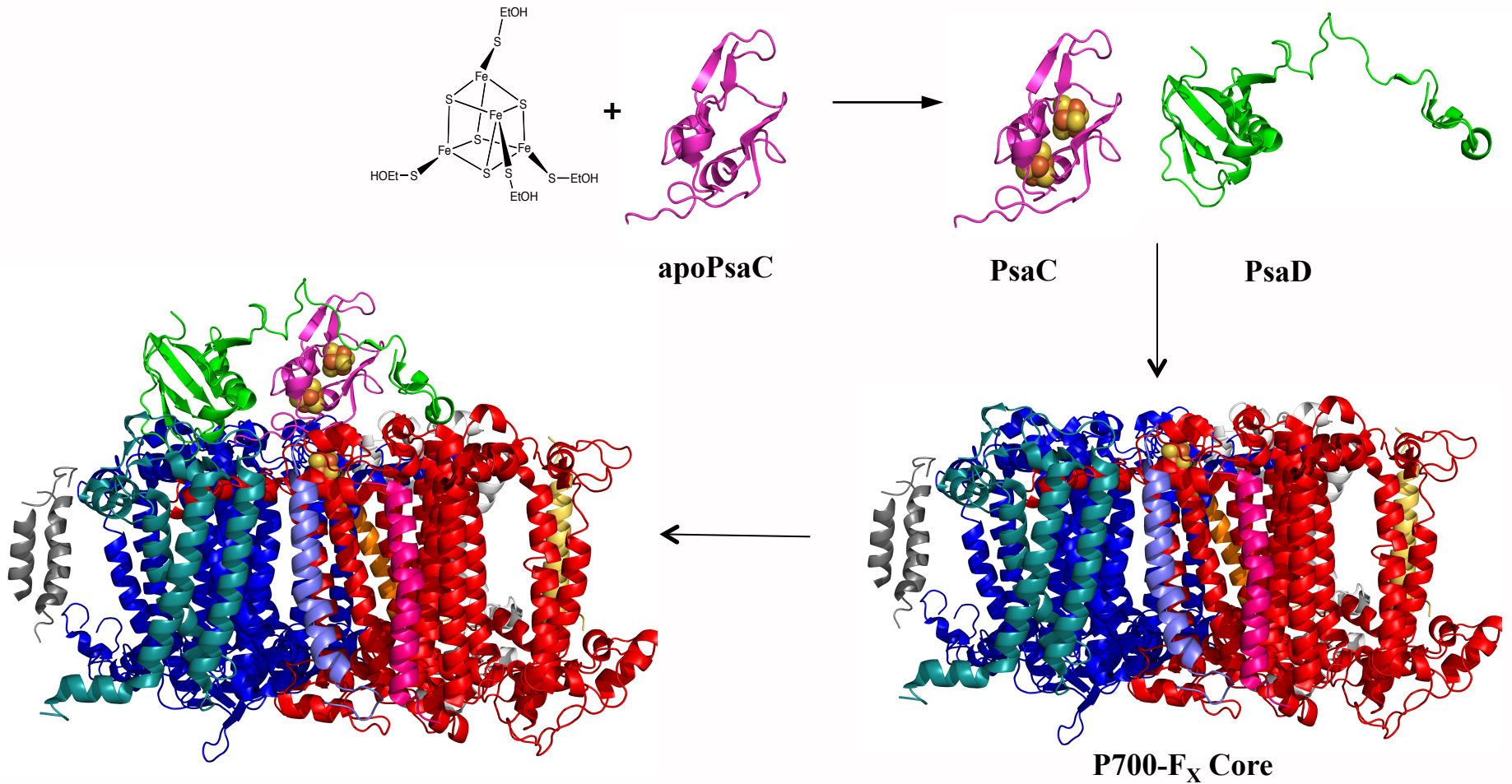
Coupling PS I to the H₂ase



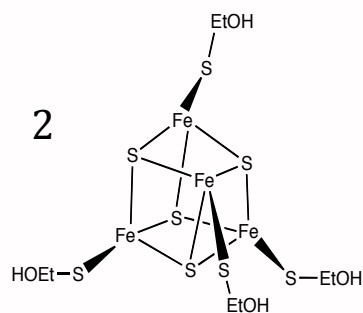
Resolve Photosystem I



Reconstitute Photosystem I



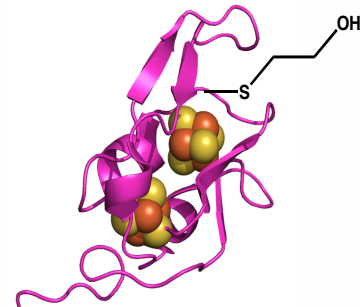
Reconstitute C13S Mutant of PsaC



+



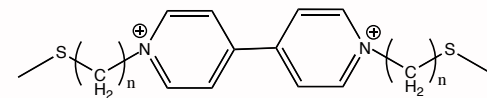
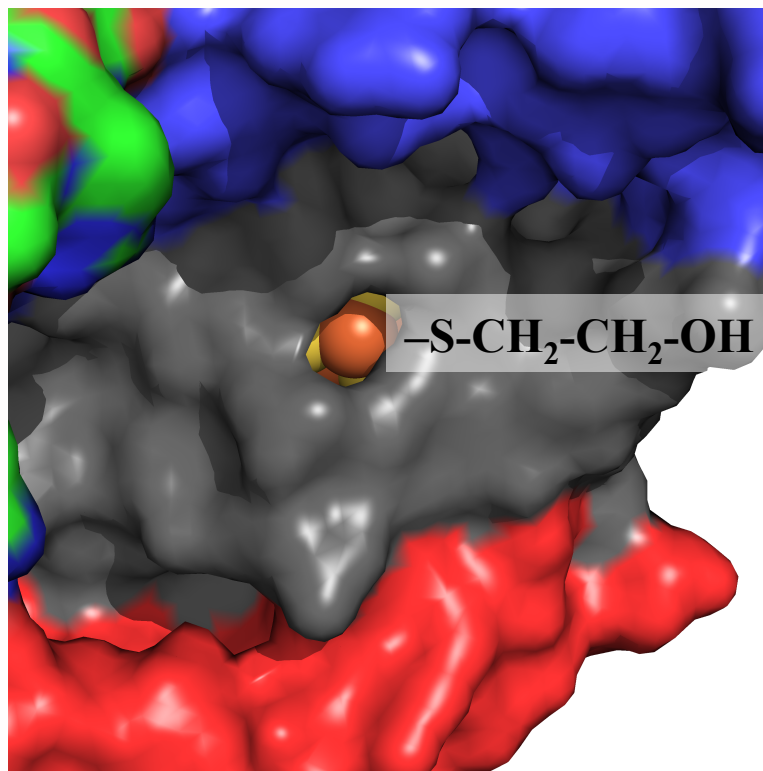
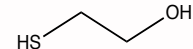
Apo- C13G PsaC



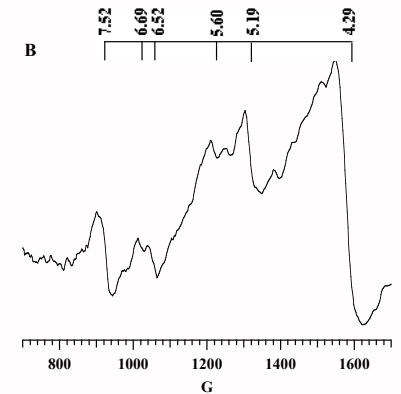
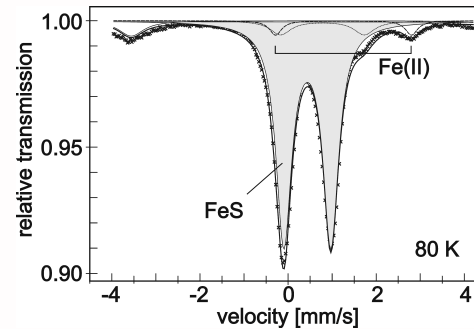
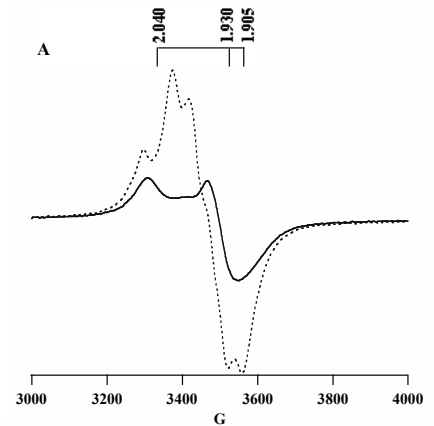
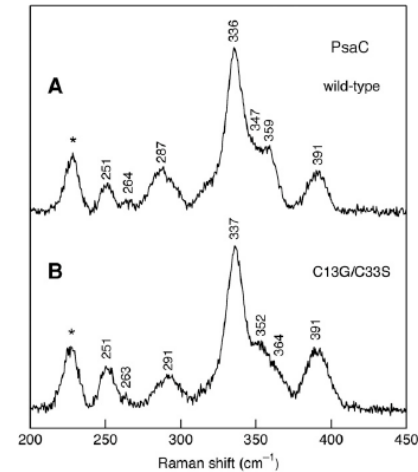
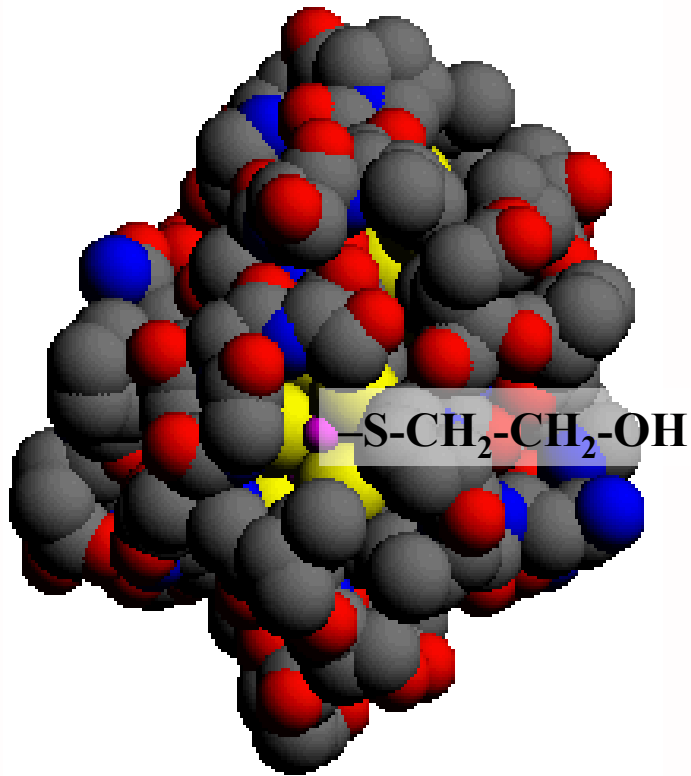
Holo-C13G PsaC

+

7



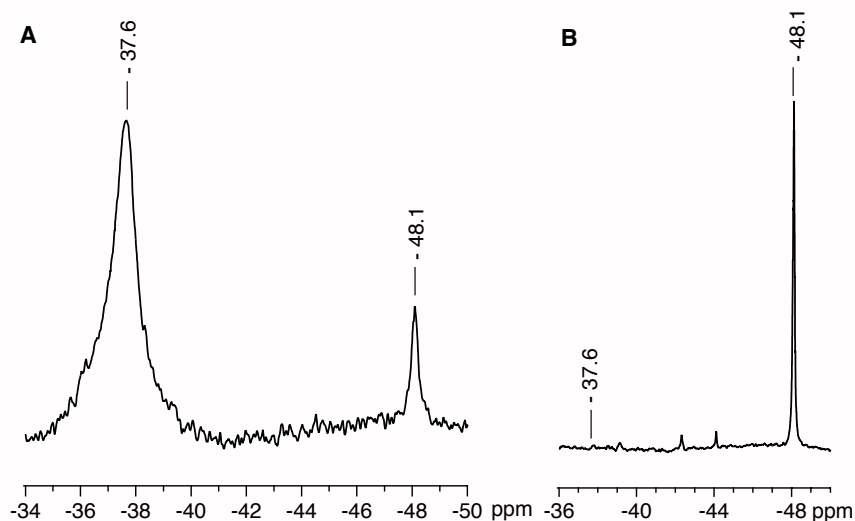
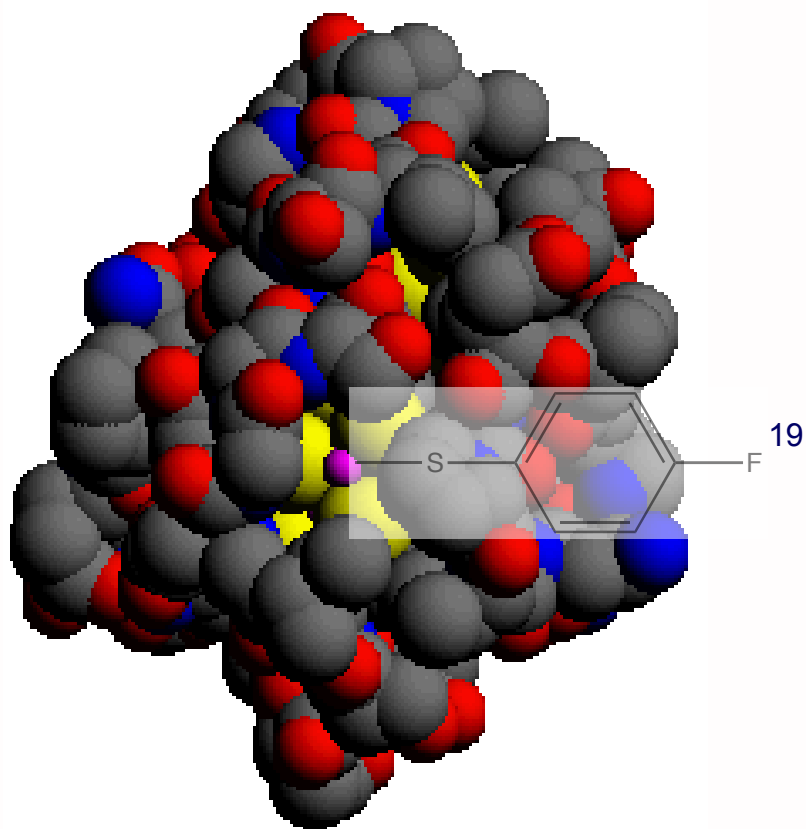
Verify C13S Variant of PsaC



2 [4Fe-4S]

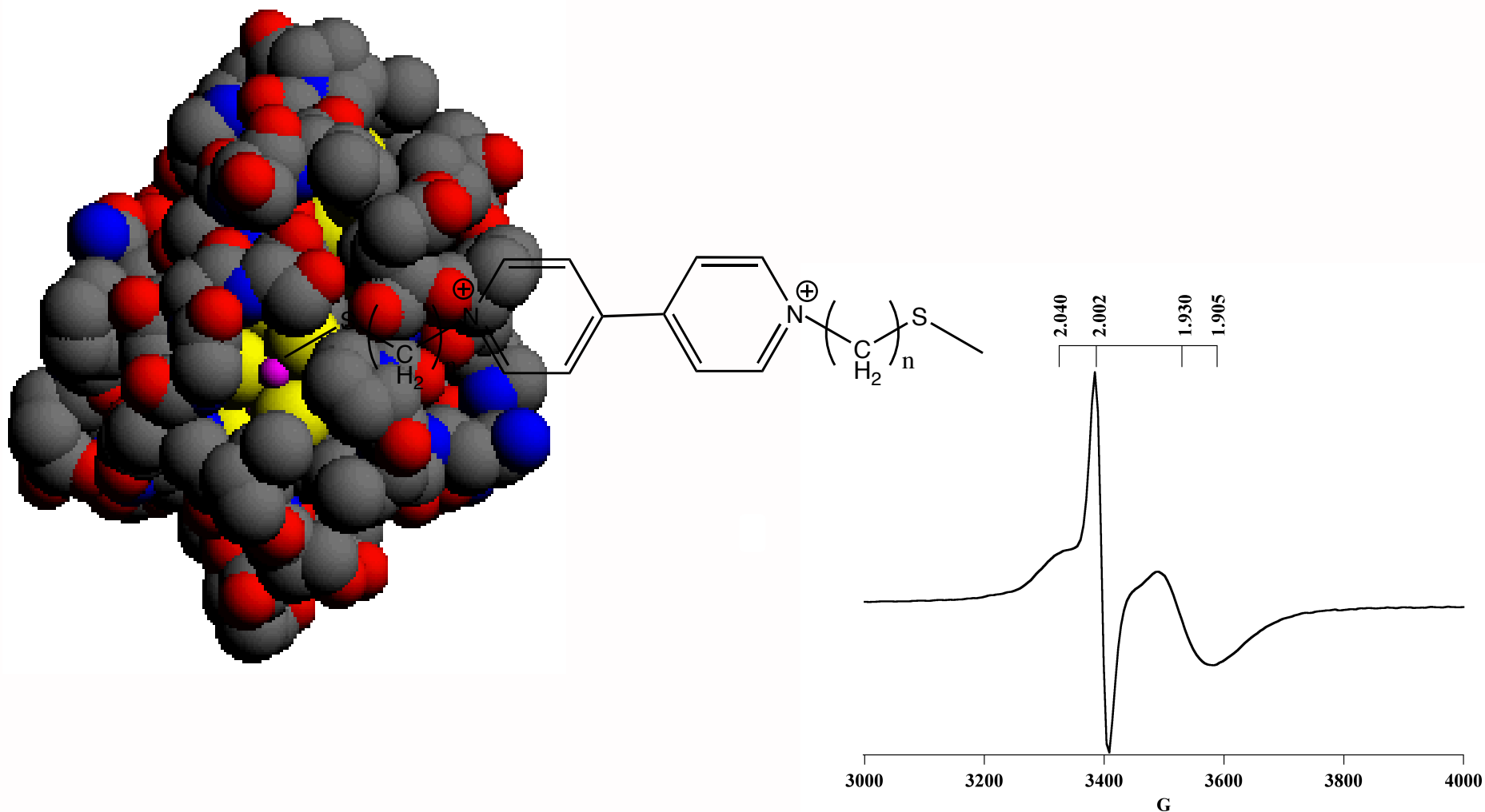
$S = 1/2, 3/2$

NMR Proof of Rescue Ligand

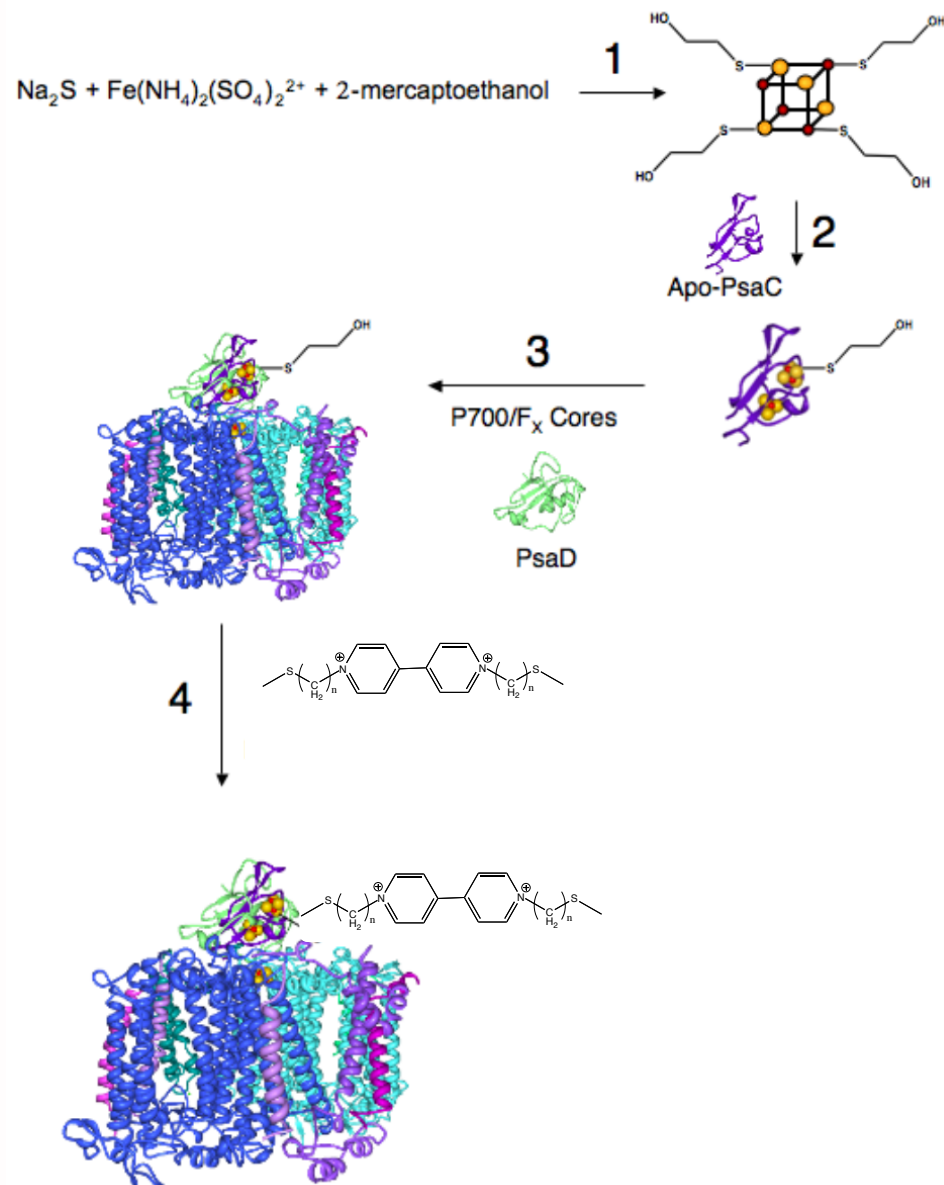


p-fluorothiophenol

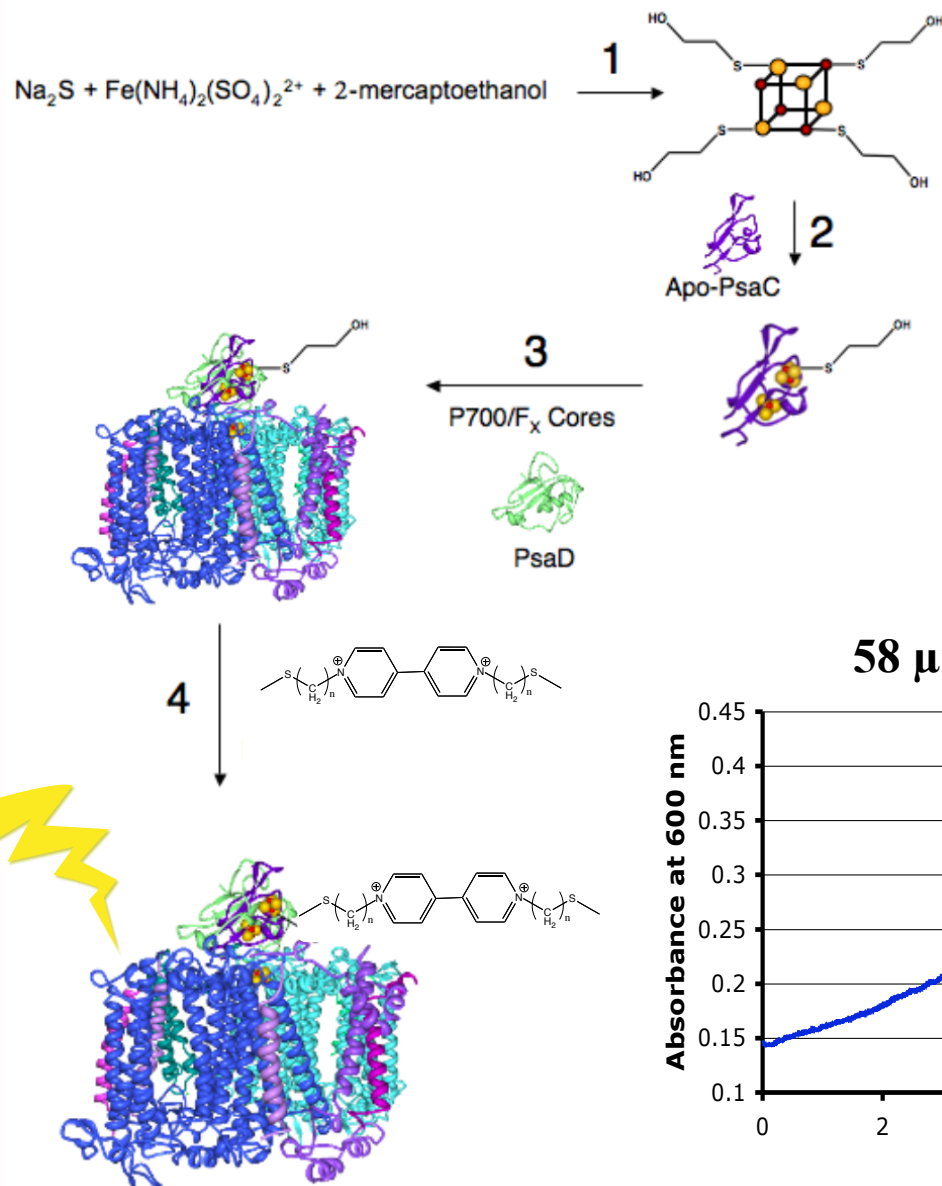
Tether-substituted 4,4'-Dipyridinium



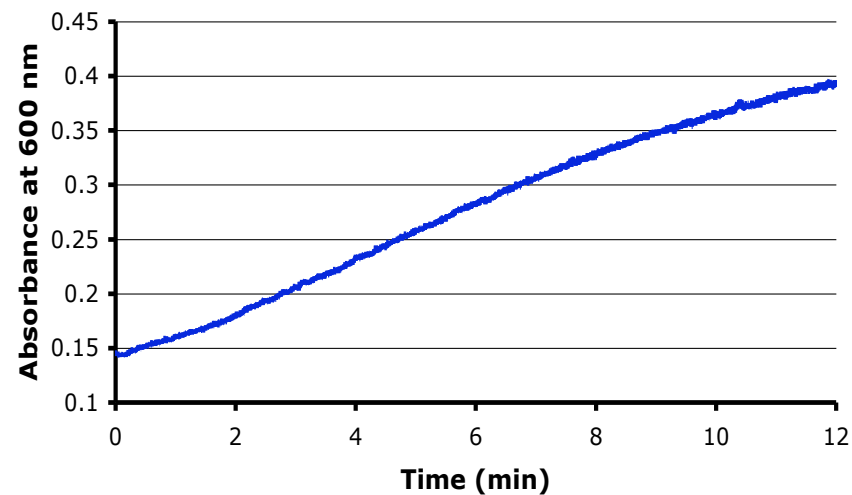
Rebuilding Photosystem I



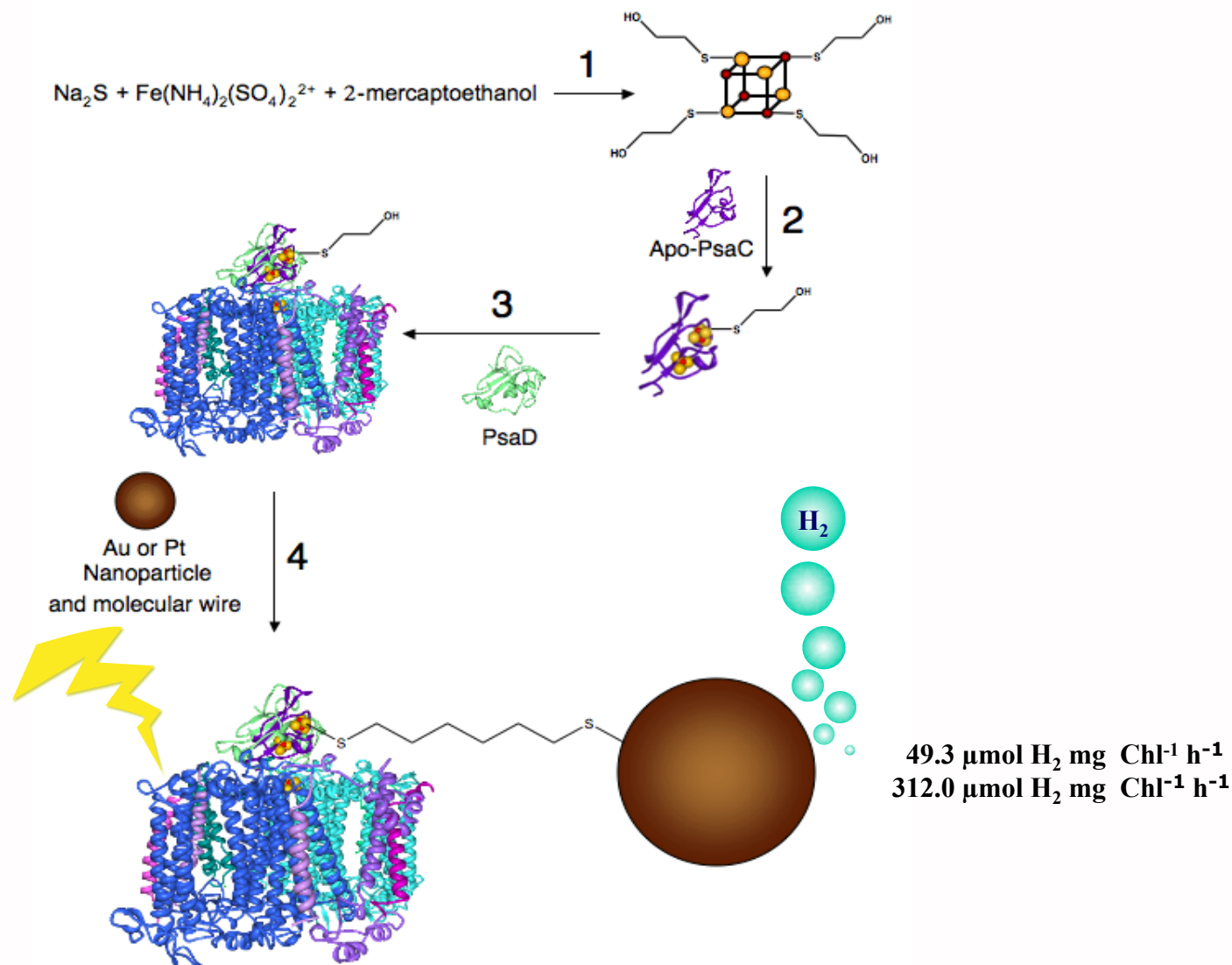
Single Electron Reduction



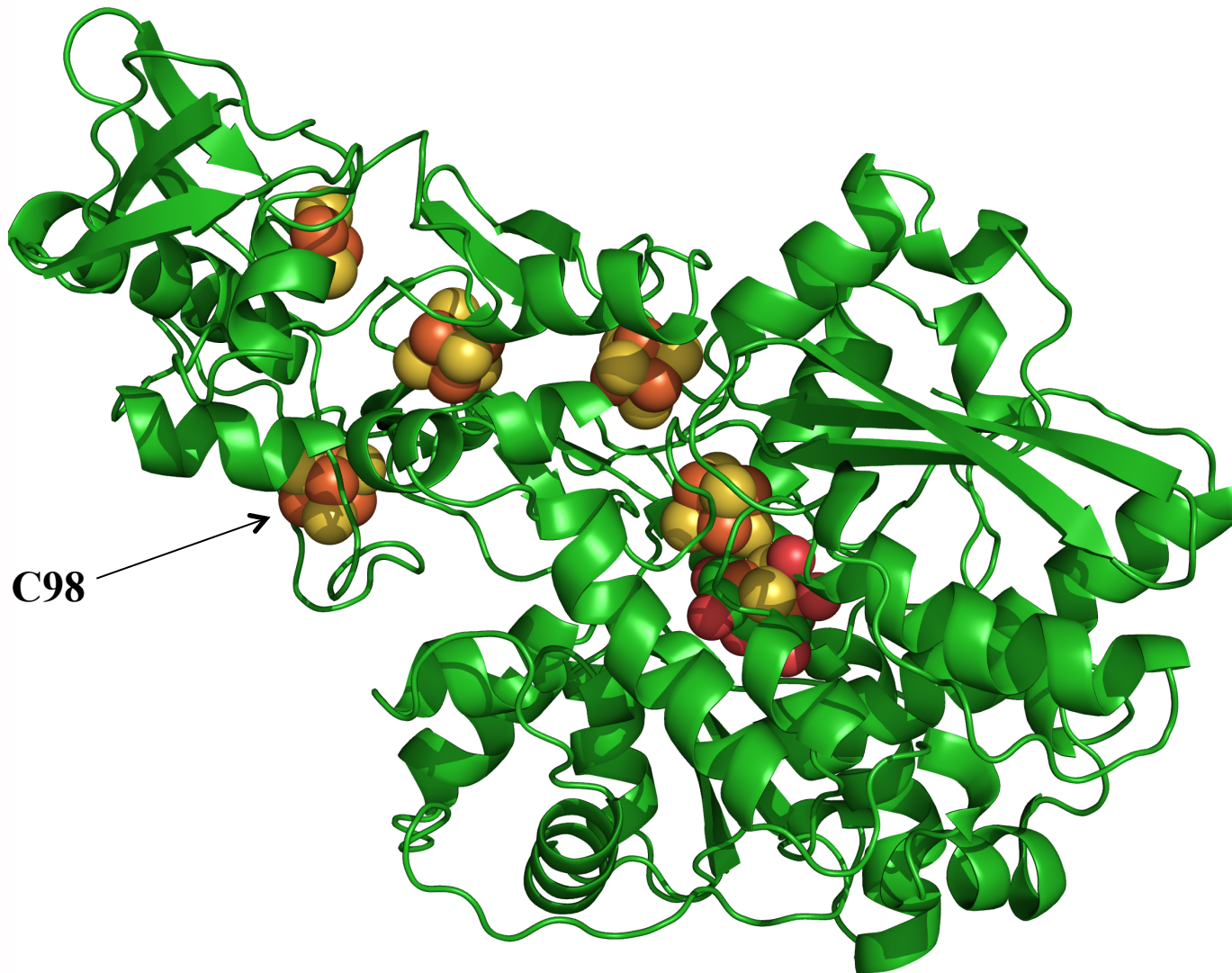
58 μmol viologen $\text{mg Chl}^{-1} \text{h}^{-1}$



PSI-Tether-Pt Nanoparticle

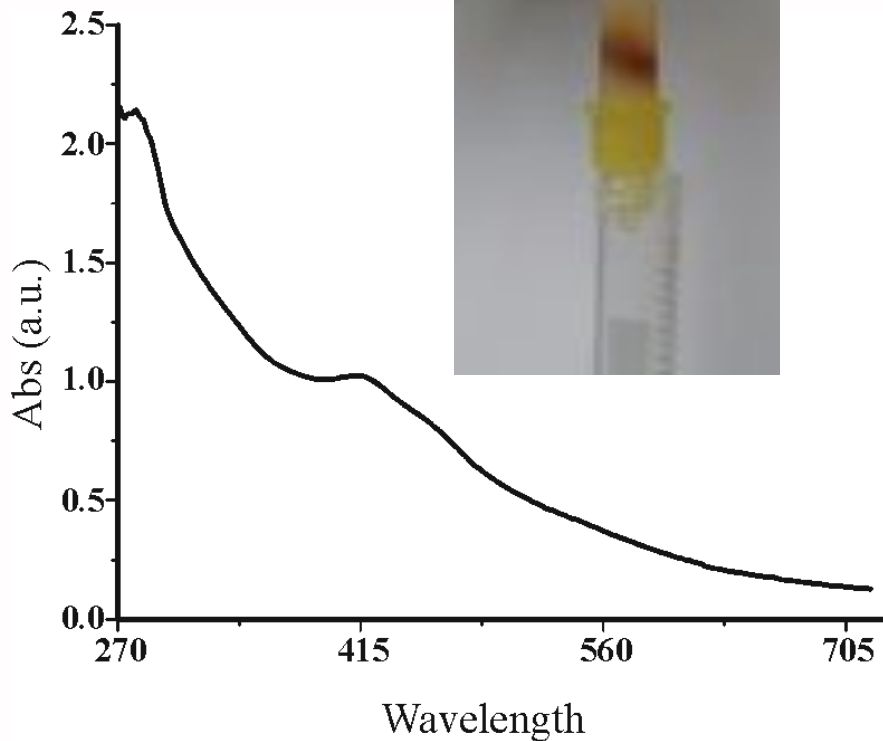


Clostridium acetobutylicum C98G Mutant

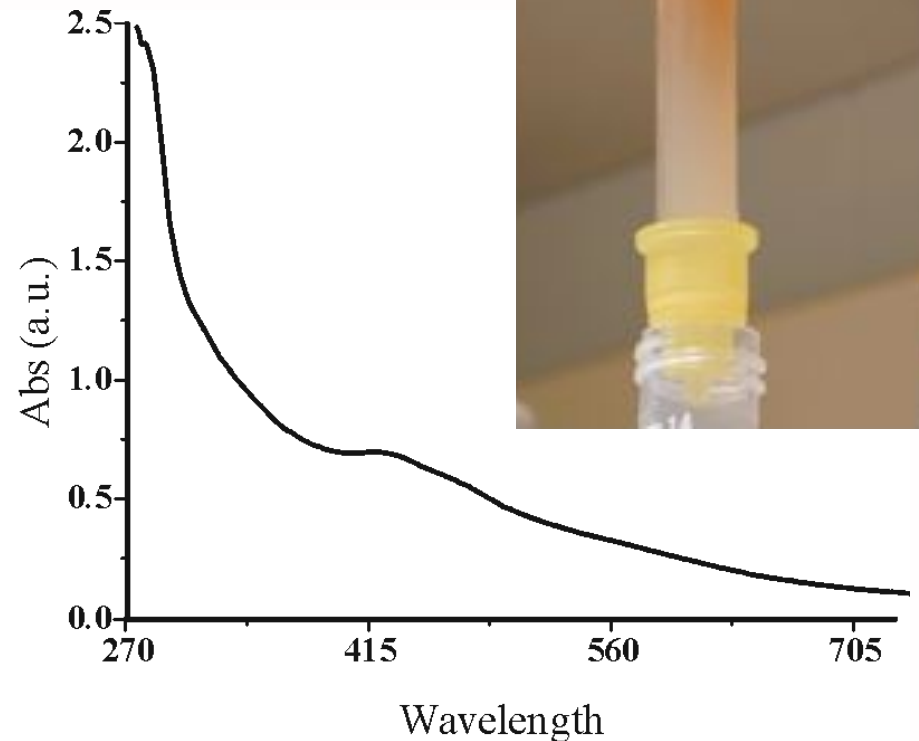


Cys₉₈->Gly Mutant Expressed in *Escherichia coli*

HydA



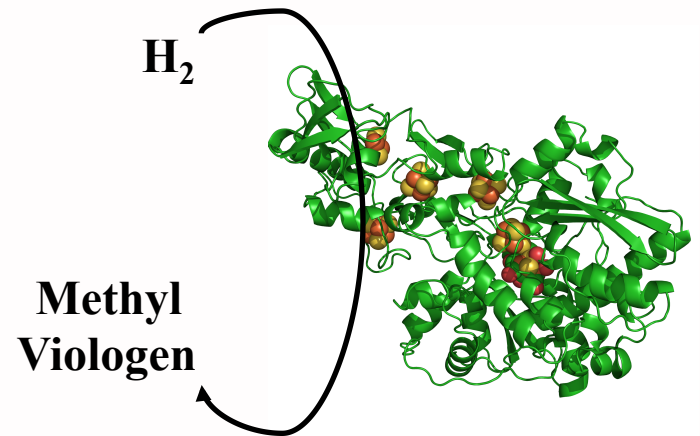
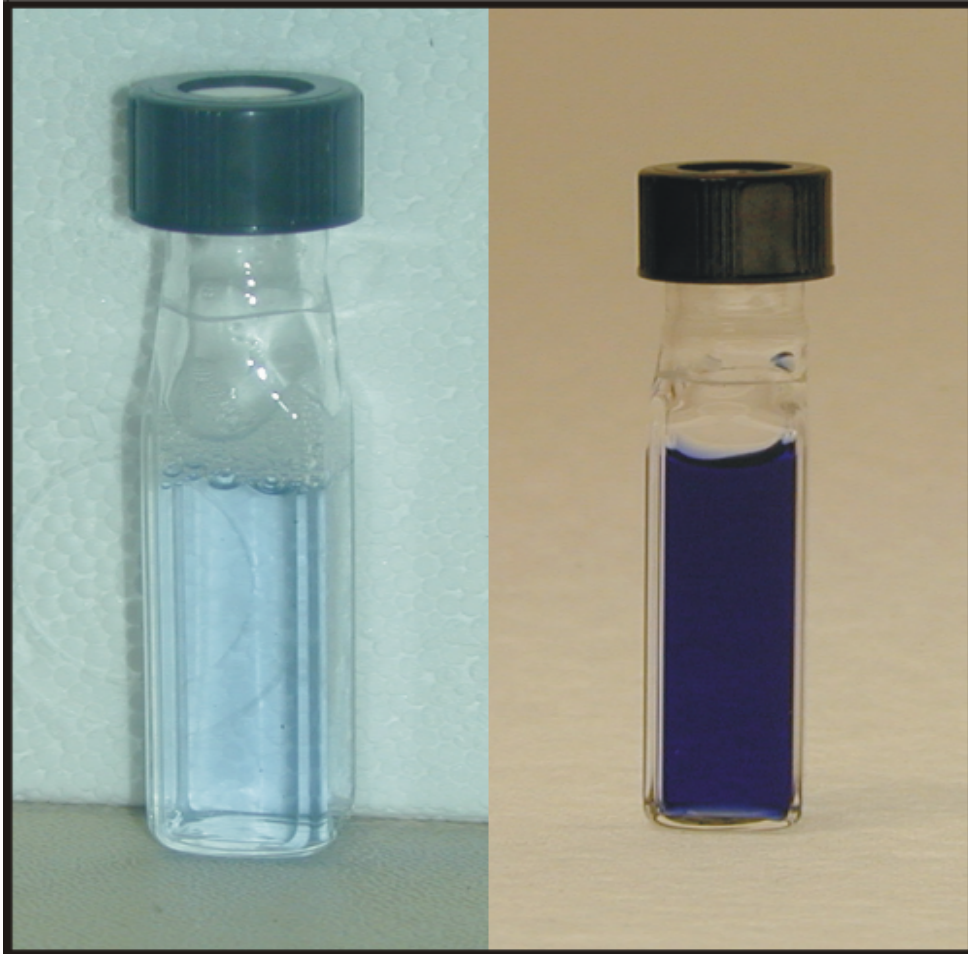
Cys₉₈->Gly
Mutant



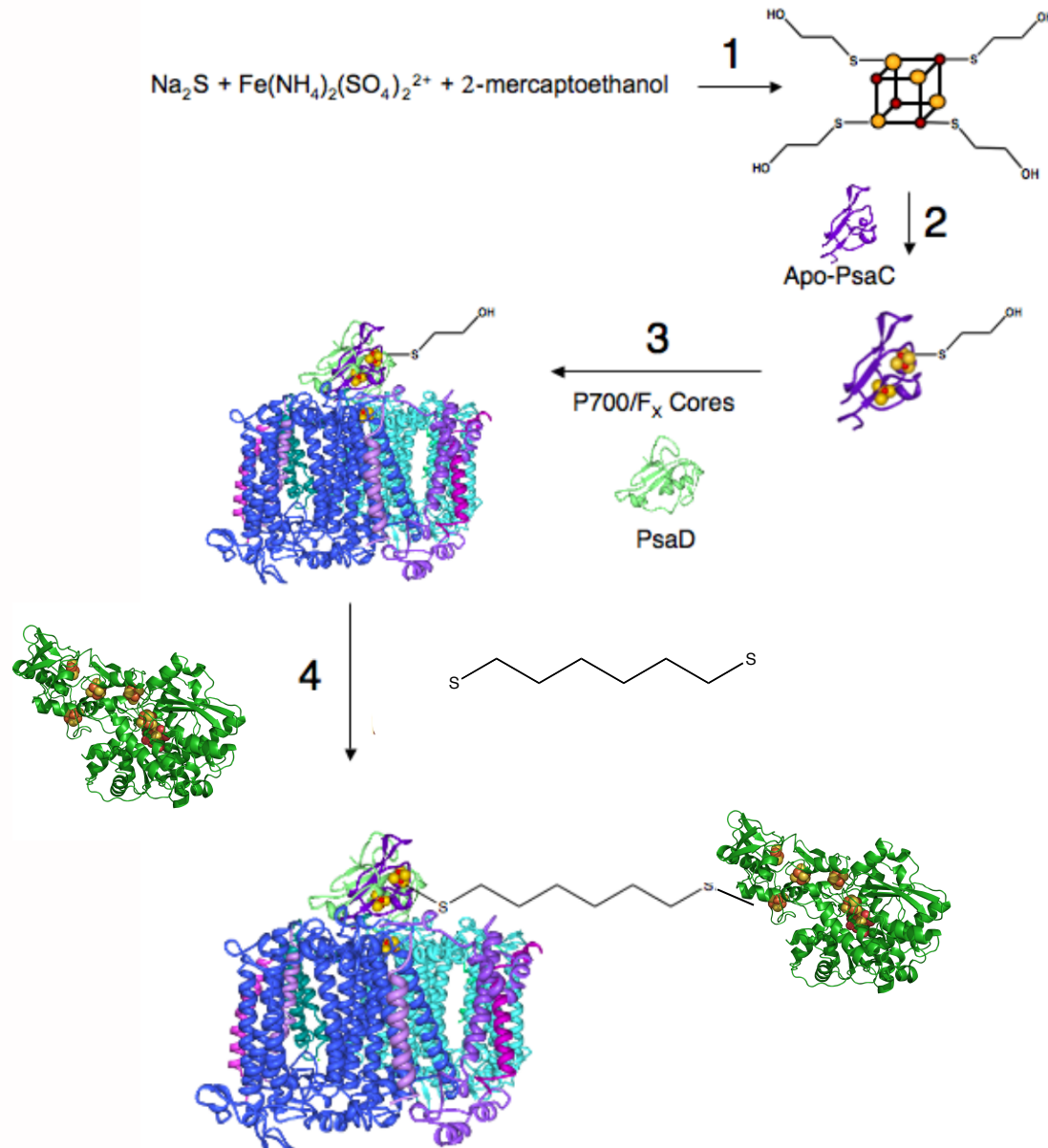
Activity of Cys₉₈->Gly Mutant

30 min

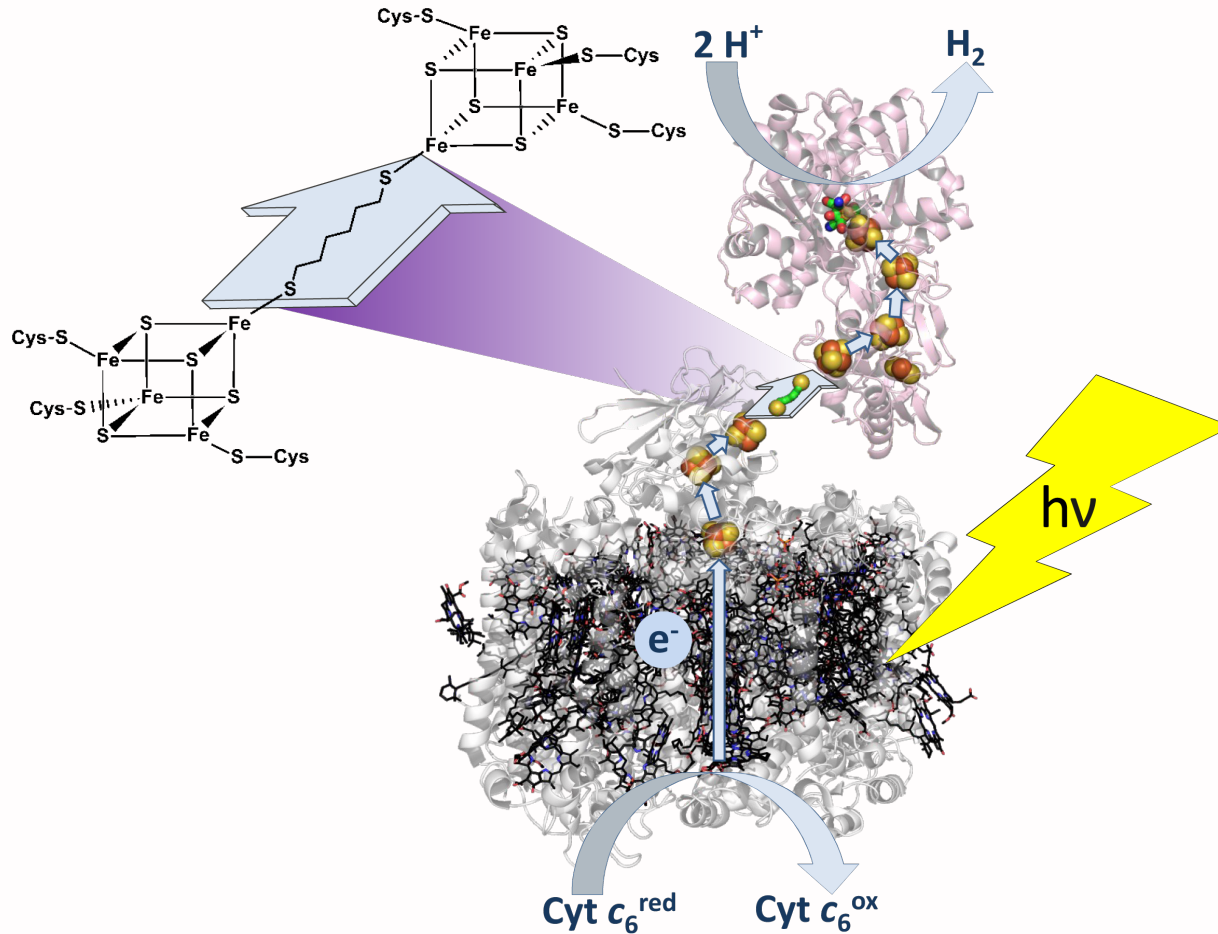
6 hr



Photosystem I-molecular wire-H₂ase



Photosystem I-molecular wire-H₂ase

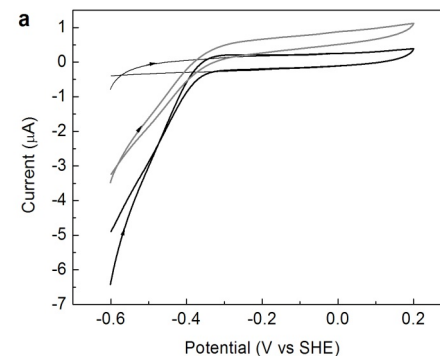


$3.9\ \mu\text{mol}\ H_2\ \text{mg}\ \text{Chl}^{-1}\ \text{hr}^{-1}$
(HydA expressed in *E. coli*)

H₂ase Expressed in *Clostridium acetobutylicum*

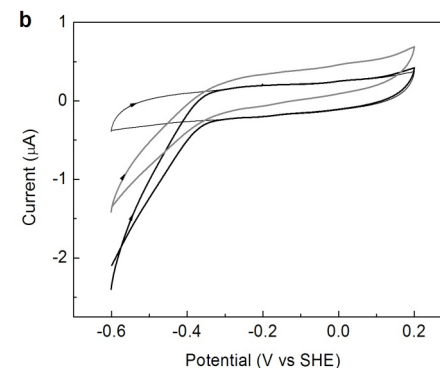
- Cys 97 changed to Gly, expressed in *Escherichia coli*

FeFe H ₂ ase	mol H ₂ mol H ₂ ase ⁻¹ s ⁻¹
HydA1	80.6
C97G HydA1	11.5

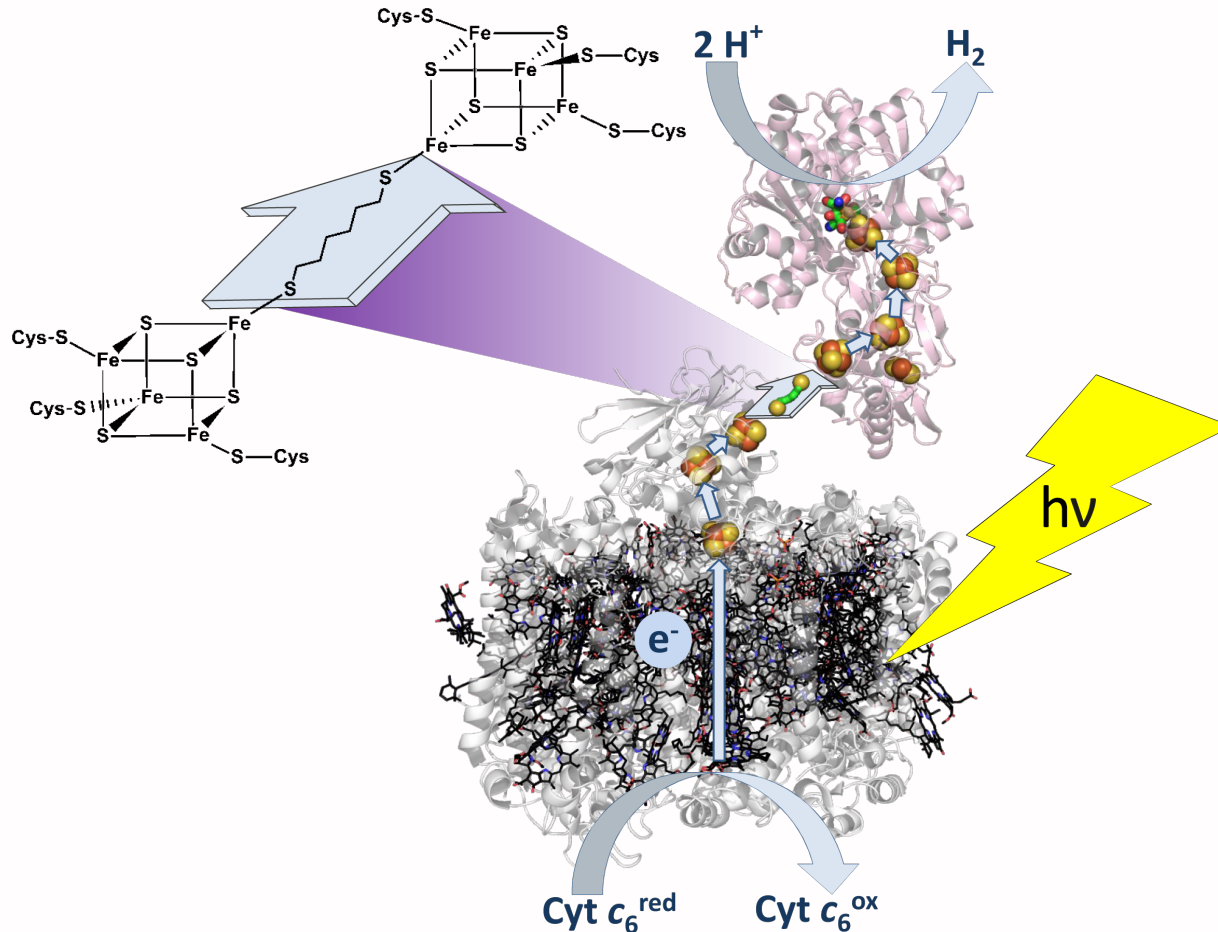


- Cys 97 changed to Gly, expressed in *C. acetobutylicum*

FeFe H ₂ ase	mol H ₂ mol H ₂ ase ⁻¹ s ⁻¹
C97G HydA1	138.7



Photosystem I-molecular wire-H₂ase



$3.9\ \mu\text{mol}\ H_2\ \text{mg}\ \text{Chl}^{-1}\ \text{hr}^{-1}$
(HydA expressed in *E. coli*)

$30.3\ \mu\text{mol}\ H_2\ \text{mg}\ \text{Chl}^{-1}\ \text{hr}^{-1}$
(HydA expressed in *C. a.*)

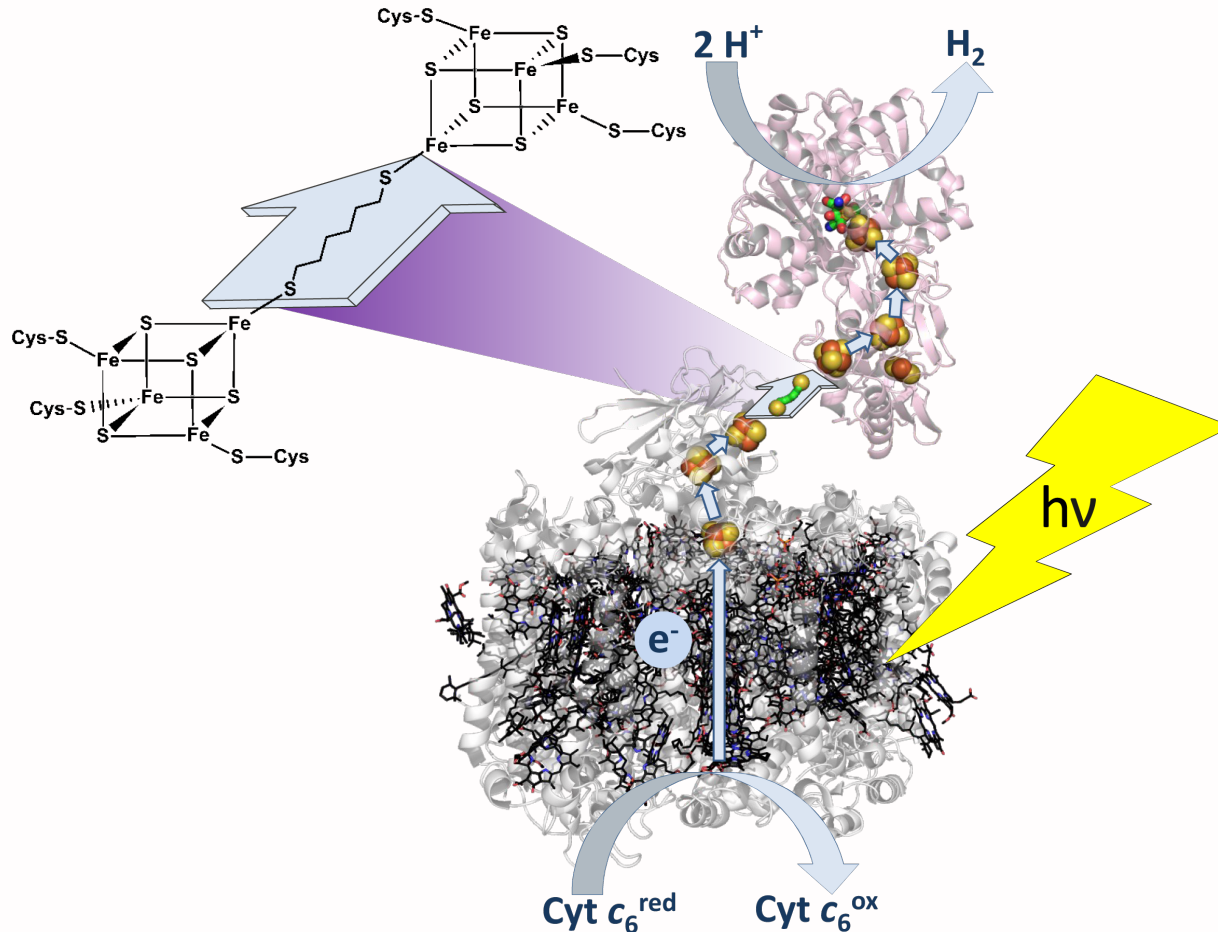
Effect of Buffer and pH on H₂ Evolution

pH	Buffer (50 mM)	Rate of H ₂ Production ($\mu\text{mol H}_2 \text{ mg Chl}^{-1} \text{ h}^{-1}$)	Electron Throughput ($e^- \text{ PS I}^{-1} \text{ s}^{-1}$)
8.3	Tris-HCl	202 ± 122	10.1 ± 6.1
	Tricine	1336 ± 425	66.8 ± 21.3
7.0	Na-Phosphate	1357 ± 21	67.9 ± 1.1
6.5	Tris-HCl	1020 ± 101	51.0 ± 5.1
	PIPES	1130 ± 204	56.5 ± 10.2
	Na-phosphate	1538 ± 147	76.9 ± 7.4
6.0	Na-phosphate	1428 ± 174	71.4 ± 8.7

Effect of Tether Length

Molecular Wire	Rate of H ₂ Production ($\mu\text{mol H}_2 \text{ mg Chl}^{-1} \text{ h}^{-1}$)	Electron Throughput ($e^- \text{ PS I}^{-1} \text{ s}^{-1}$)
1,6-hexanedithiol	1538 ± 147	76.9 ± 7.4
1, 8-octanedithiol	2198 ± 457	110.0 ± 22.9
1,10-decanedithiol	1199 ± 78	60.0 ± 3.9

Photosystem I-molecular wire-H₂ase

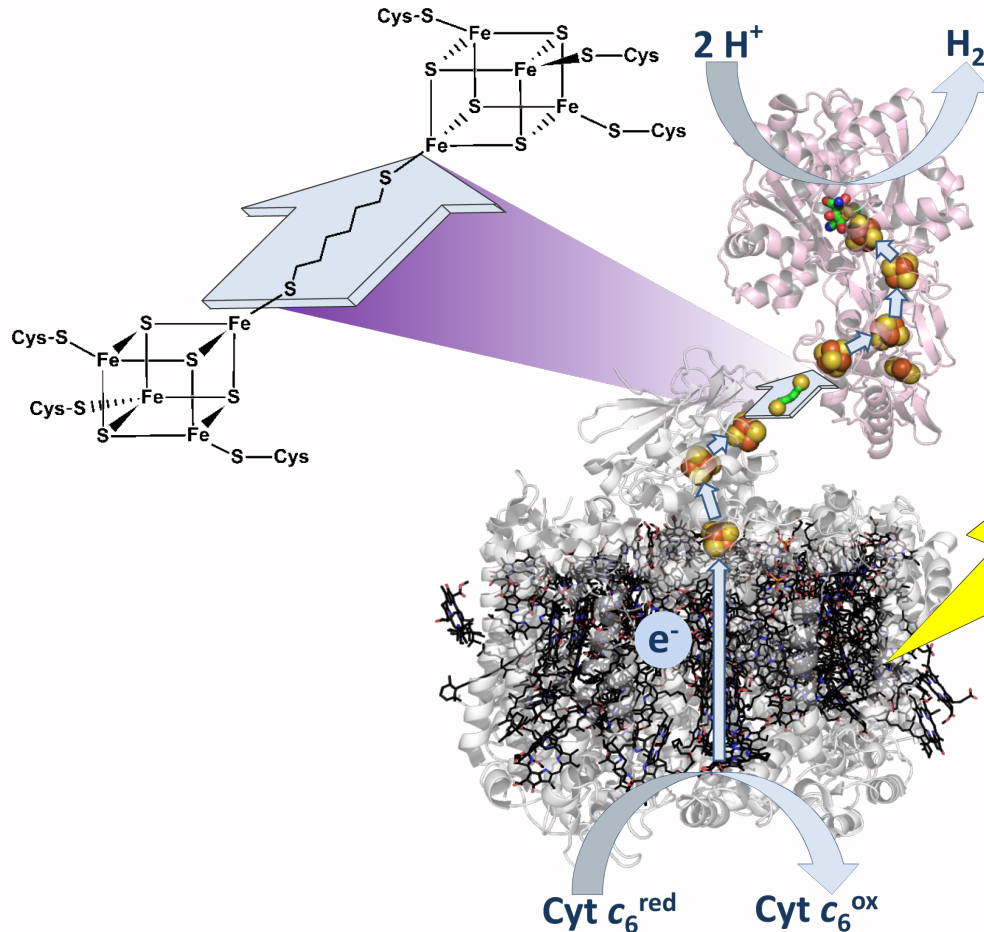


3.9 $\mu\text{mol H}_2 \text{ mg Chl}^{-1} \text{ hr}^{-1}$
(HydA expressed in *E. coli*)

30.3 $\mu\text{mol H}_2 \text{ mg Chl}^{-1} \text{ hr}^{-1}$
(HydA expressed in *C. a.*)

2850 $\mu\text{mol H}_2 \text{ mg Chl}^{-1} \text{ hr}^{-1}$
(x-linked Cyt c_6 , 20 mM KCl,
1,8-octanedithiol wire,
phosphate buffer at pH 6.5)

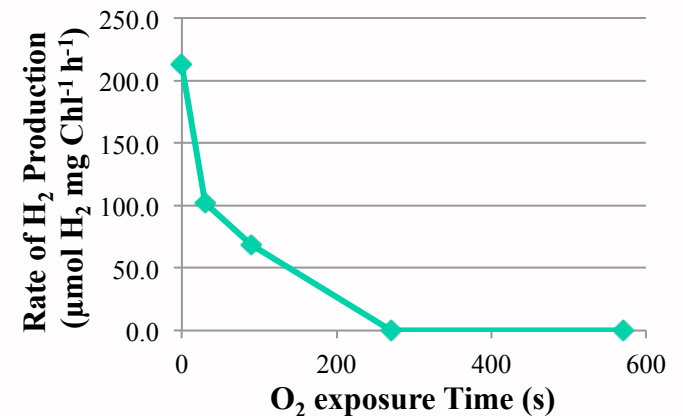
Photosystem I-molecular wire-H₂ase



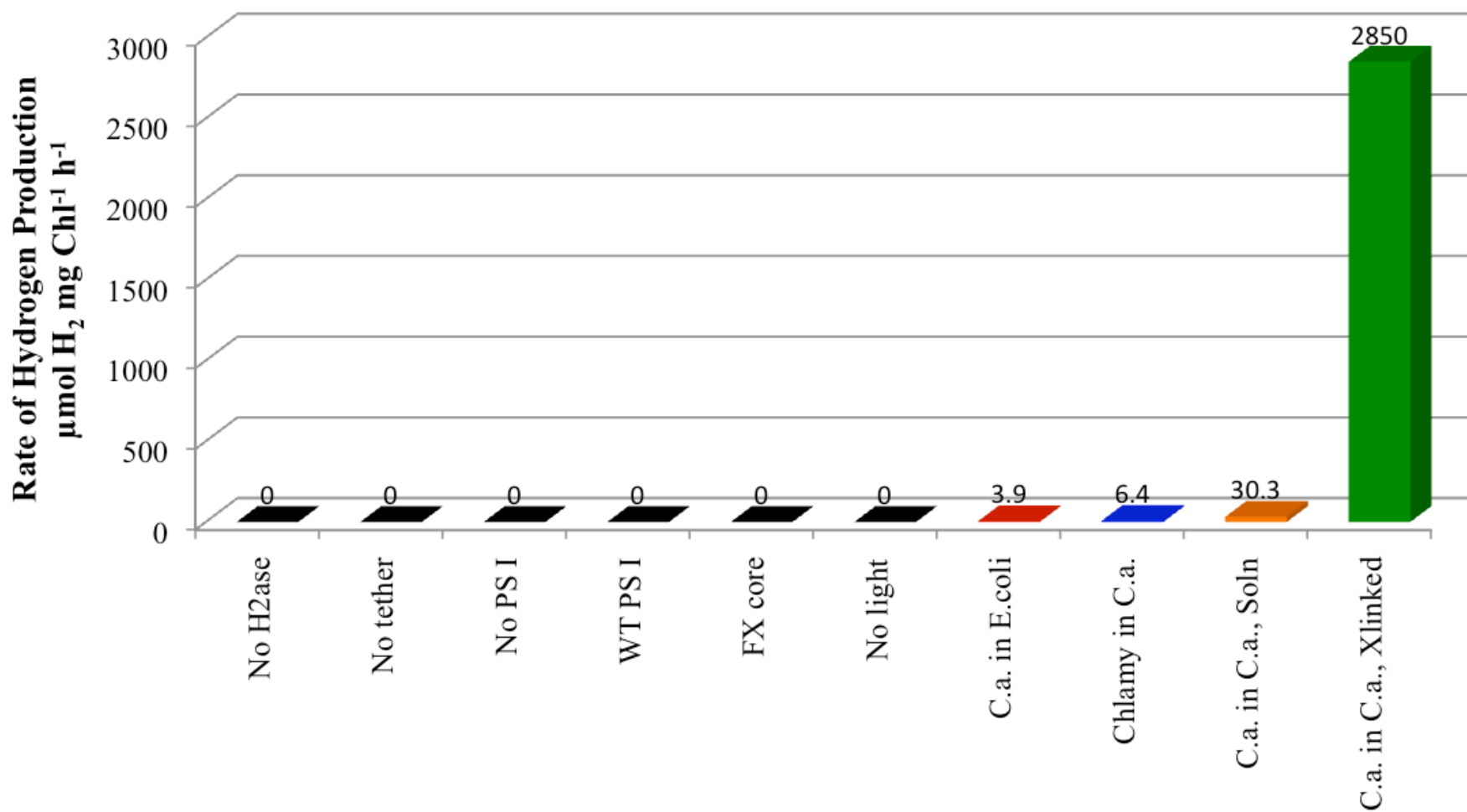
3.9 $\mu\text{mol } H_2 \text{ mg Chl}^{-1} \text{ hr}^{-1}$
(HydA expressed in *E. coli*)

30.3 $\mu\text{mol } H_2 \text{ mg Chl}^{-1} \text{ hr}^{-1}$
(HydA expressed in *C. a.*)

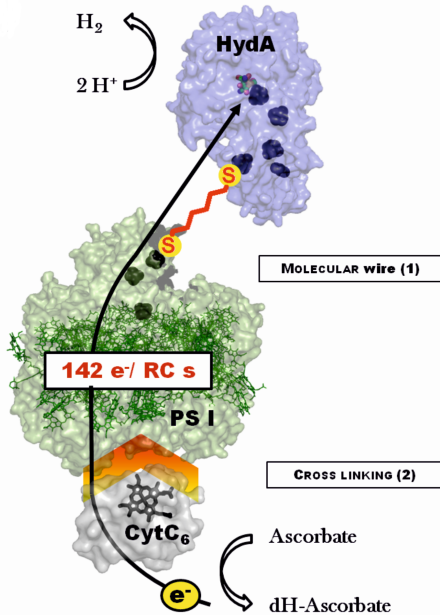
2850 $\mu\text{mol } H_2 \text{ mg Chl}^{-1} \text{ hr}^{-1}$
(x-linked Cyt c_6 , 20 mM KCl,
1,8-octanedithiol wire,
phosphate buffer at pH 6.5)



Control Experiments

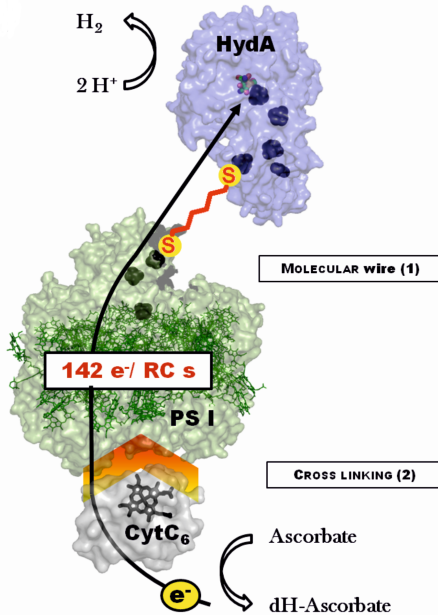


Surpassing Natural Photosynthesis

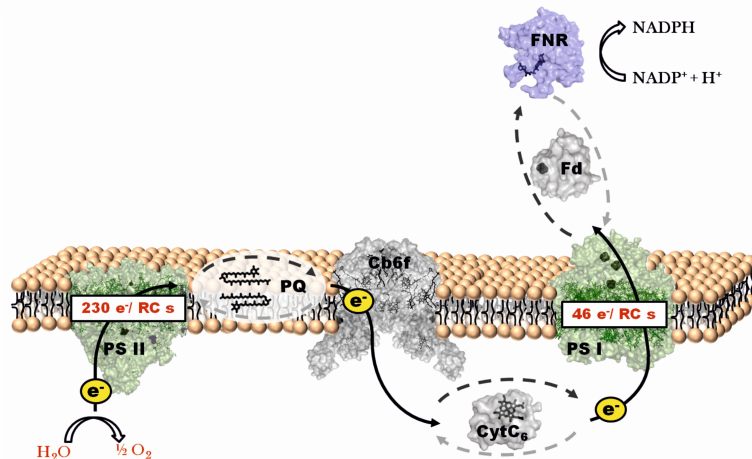


PS I—molecular wire—[FeFe]-H₂ase nanoconstruct evolves H₂ at a rate of 2850 $\mu\text{moles mg Chl}^{-1} \text{ h}^{-1}$, which is equivalent to **142 e⁻ PS I⁻¹ s⁻¹**

Surpassing Natural Photosynthesis

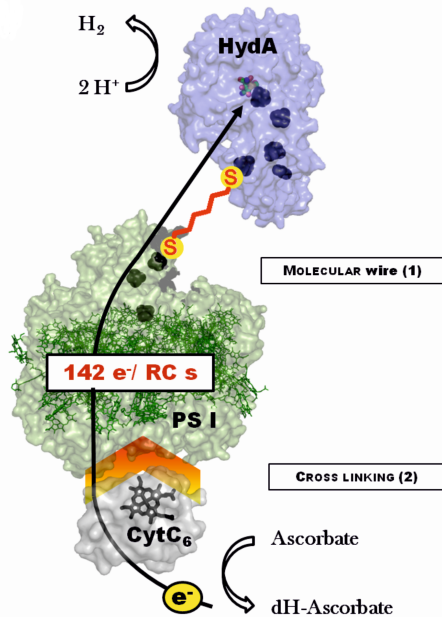


PS I—molecular wire—[FeFe]-H₂ase nanoconstruct evolves H₂ at a rate of 2850 $\mu\text{moles mg Chl}^{-1} \text{ h}^{-1}$, which is equivalent to **142 e⁻ PS I⁻¹ s⁻¹**



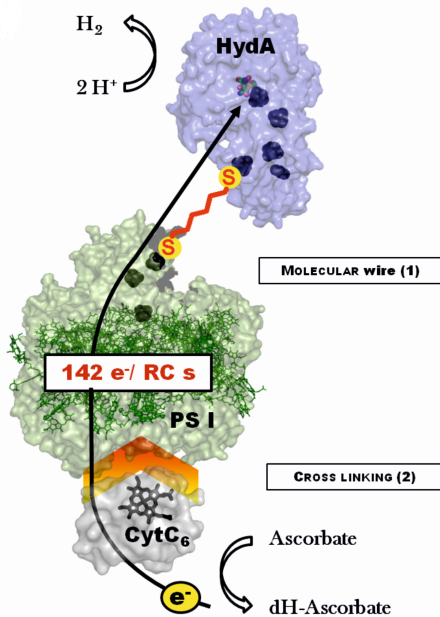
Cyanobacteria evolve O₂ at a rate of 400 $\mu\text{moles mg Chl}^{-1} \text{ h}^{-1}$, which is equivalent to **46 e⁻ PS I⁻¹ s⁻¹**, assuming a PS I to PS II ratio of 1.8 as occurs in *Synechocystis* sp. PCC 6803

Putting this into Perspective.....

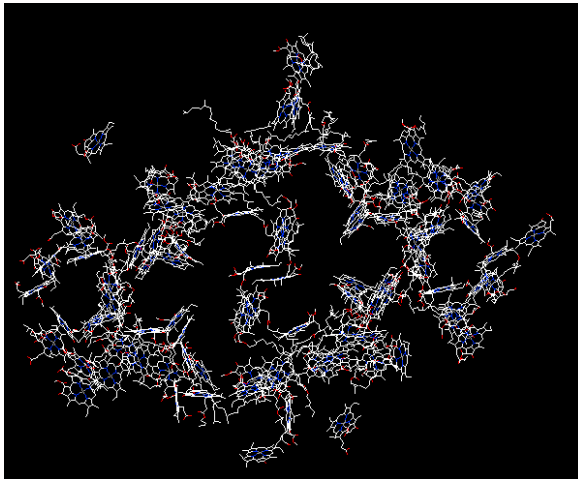


PS I—molecular wire—[FeFe]-H₂ase nanoconstruct evolves H₂ at a rate of 2850 $\mu\text{moles mg Chl}^{-1} \text{ h}^{-1}$, which is equivalent to **72 H₂ PS I⁻¹ s⁻¹**

Putting this into Perspective.....

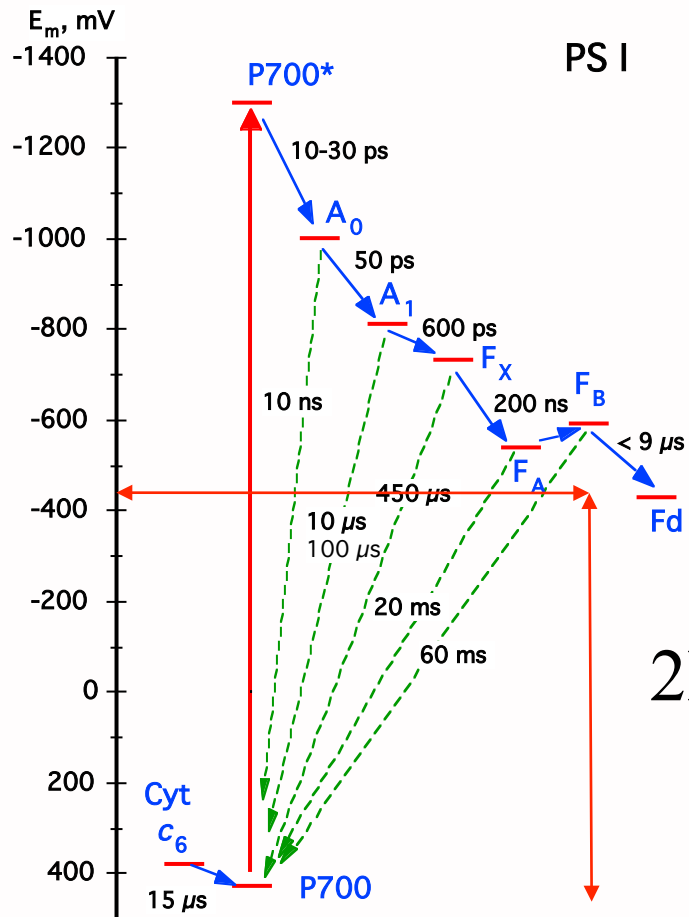


PS I—molecular wire—[FeFe]-H₂ase nanoconstruct evolves H₂ at a rate of 2850 $\mu\text{moles mg Chl}^{-1} \text{ h}^{-1}$, which is equivalent to **72 H₂ PS I⁻¹ s⁻¹**



Because there are 100 Chl *a* molecules per P700, 500 photons are absorbed per second in full sunlight, a maximum rate of production of **250 H₂ PS I⁻¹ s⁻¹** maybe possible

High Photon Conversion Efficiency



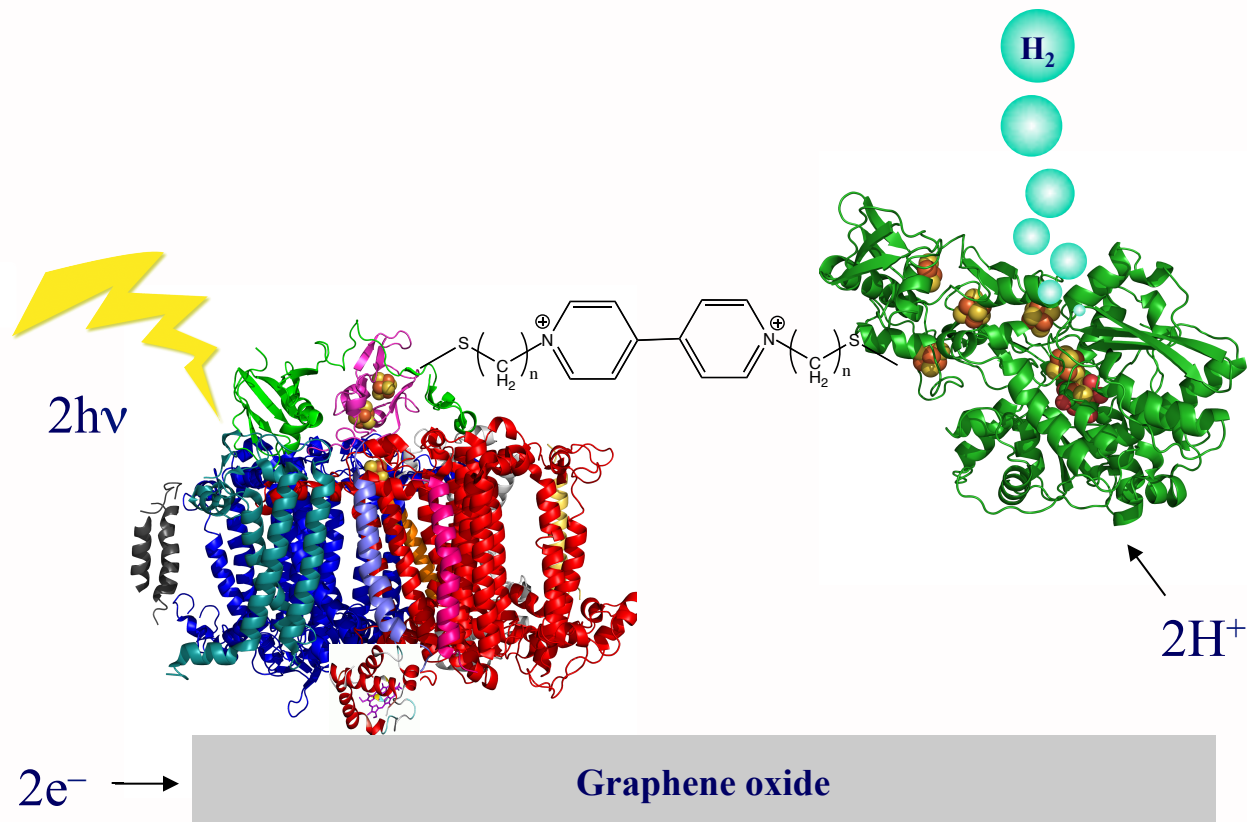
The 0.84 V stored in H₂ relative to P₇₀₀/P₇₀₀⁺ represents a **49% conversion efficiency for a red photon**, and a **32% conversion efficiency for a blue photon**



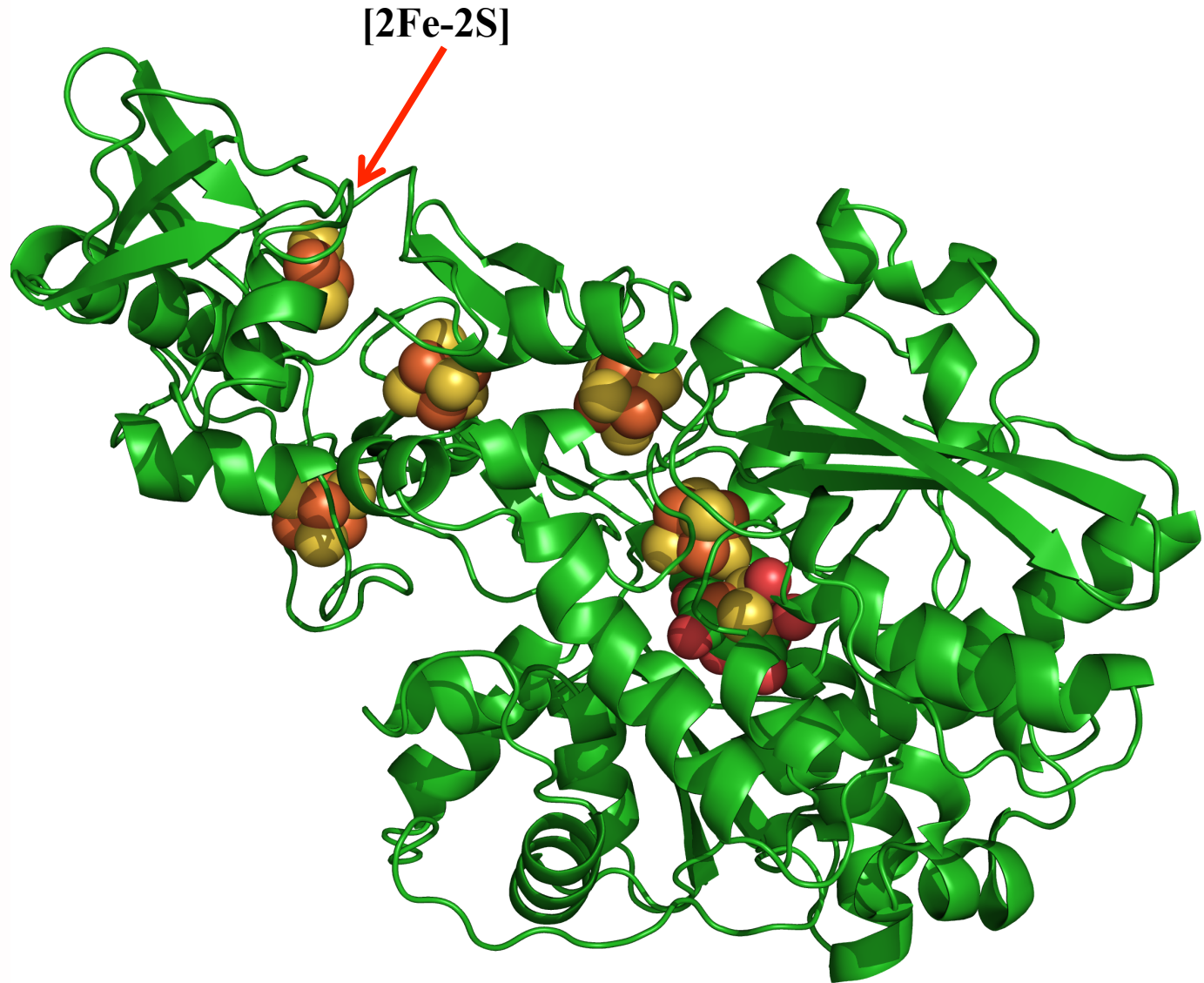
Comparative Rates of H₂ Evolution

Relevant characteristics	Electron mediator Donor side	H ₂ catalyst	Rate $\mu\text{mol H}_2 \text{ h}^{-1}$ mg Chl^{-1}	Reference
platinized thylakoids	native	Pt	0.1	(Lee et al. 1994) (6)
platinized PSI	plastocyanin	Pt	0.35	(Millsaps et al. 2001) (7)
platinized PSI	plastocyanin	Pt	0.03	(Evans et al. 2004) (8)
platinized PSI cross-linked to plastocyanin	plastocyanin	Pt	0.09	(Evans et al. 2004) (8)
PSI-wire-platinum	DCPIP	Pt	9.6	(Grimme et al. 2008) (4)
PSI-wire-platinum	Cyt c₆	Pt	49.3	(Grimme et al. 2008) (4)
platinized PSI	Cyt c ₆	Pt	5.5	(Iwuchukwu et al. 2009) (9)
PSI-molecular wire-platinum cross-linked to plastocyanin	X-linked plastocyanin	Pt	312	(Grimme et al. 2009) (5)
chloroplasts, ferredoxin, H ₂ ase in solution	native	H ₂ ase	94	(Rao et al. 1978) (10)
chloroplasts, ferredoxin, H ₂ ase in solution	native	H ₂ ase	400	(Krasnovksy et al. 1980) (11)
chloroplasts, methyl viologen, H ₂ ase in solution	native	H ₂ ase	40	(Haverkamp et al. 1995) (12)
PSI, H ₂ ase in solution	dithiothreitol ascorbate	H ₂ ase	1.8	(McTavish 1998) (13)
PSI-[NiFe]H ₂ ase genetic fusion	TMPD	H ₂ ase	0.58	(Ihara et al. 2006) (14)
PSI-wire-[FeFe]H₂ase	Cyt c₆	H₂ase	3.9	(Lubner et al. 2010a) (1)
PSI-wire-[FeFe]H₂ase	Cyt c₆	H₂ase	30.3	(Lubner et al. 2010b) (2)
PSI-[NiFe]H ₂ ase genetic fusion on Au electrode	PMS	H ₂ ase	2800 ²	(Krassen et al. 2009b) (15)
PSI-wire-[FeFe]H₂ase	X-linked Cyt c₆	H₂ase	2850	(Lubner et al. in preparation)

Photoelectrochemical Cathodic Half-Cell

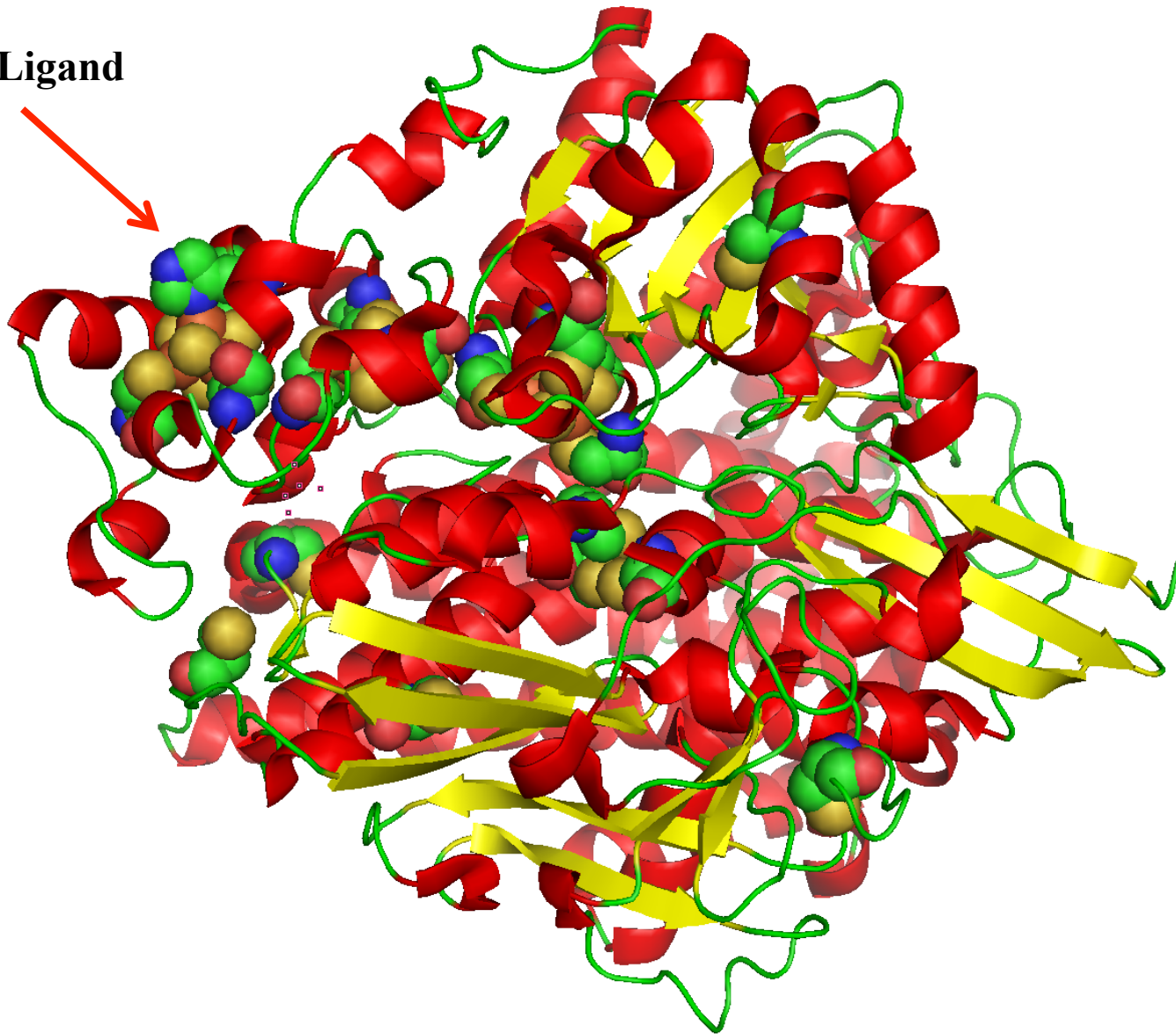


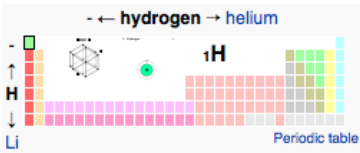
Can this Technique be Adapted to [2Fe-2S] Clusters?



Coupling to Oxygen-Tolerant [Ni-Fe]-H₂ase

His Ligand





Hydrogen as a Primary Fuel

Upside

- Clean, i.e. no CO₂ emissions: $\text{H}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}$
- High energy value per unit weight:

hydrogen	143.0 MJ kg ⁻¹
(gasoline)	46.4 MJ kg ⁻¹

Downside

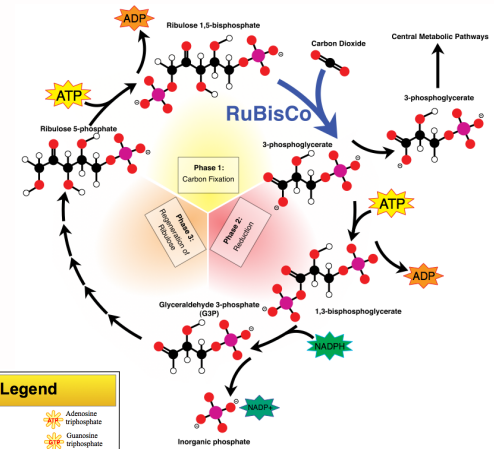
- Low energy density per unit volume:

gas	10.8 x 10 ⁻³ MJ l ⁻¹
compressed 700 Bar	5.6 MJ l ⁻¹
liquid	10.1 MJ l ⁻¹
(gasoline)	34.2 MJ l ⁻¹
- Solar H₂ must be collected in gaseous state over a large 2-dimensional flat surface
- Solar H₂ must be transported to site of utilization, hence energy consumed in compression, liquefaction, or transportation of metal hydride

Biological Carbon Dioxide Fixation

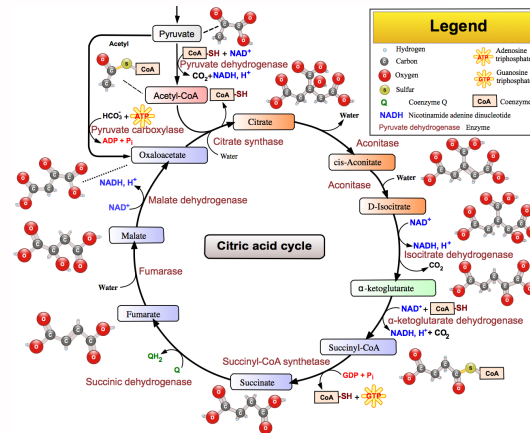
1. Reductive pentose phosphate cycle (Calvin-Benson Cycle)

Plants, cyanobacteria



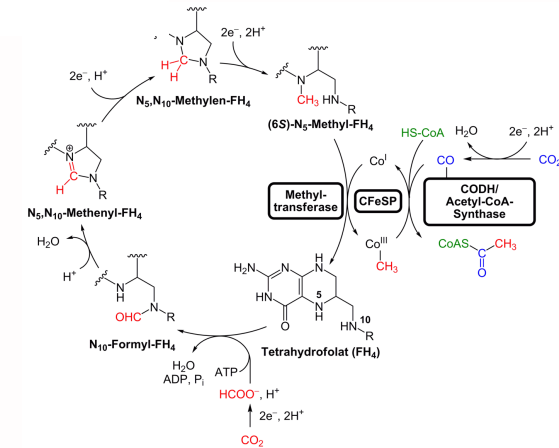
2. Reductive tricarboxylic acid cycle

Chlorobium limicola



3. Reductive acetyl Co-A pathway

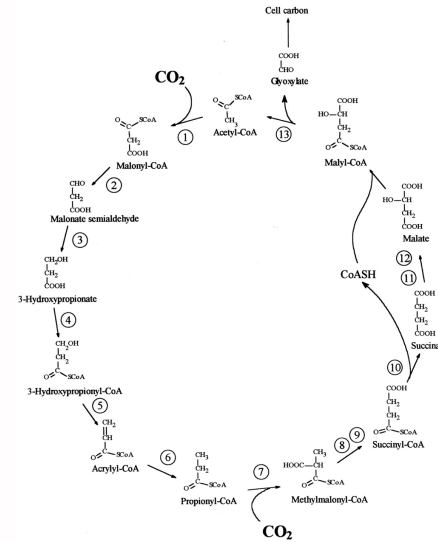
Methanogens



Biological Carbon Dioxide Fixation

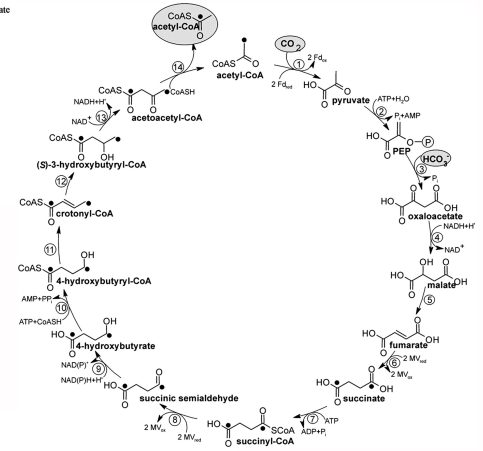
4. 3-Hydroxypropionate cycle

Chloroflexus aurantiacus



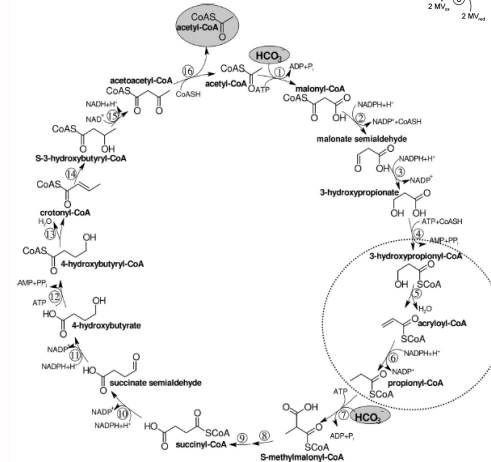
5. Dicarboxylate/4-hydroxybutyrate cycle

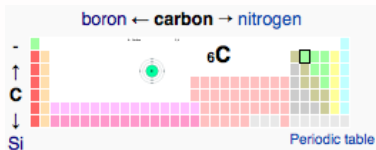
Ignicoccus hospitalis



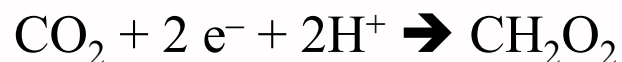
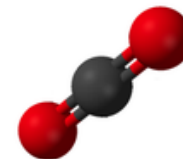
6.3-Hydroxypropionate/4-hydroxybutyrate cycle

Metallosphaera sedula

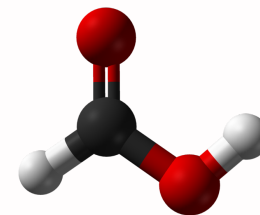




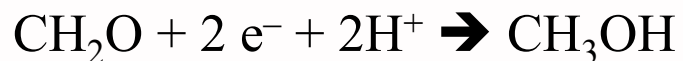
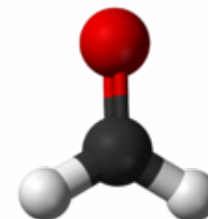
Reduction of Carbon Dioxide



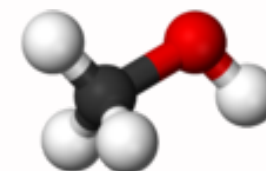
Formic Acid



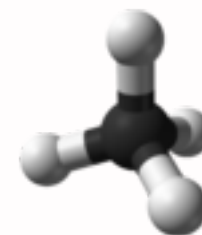
Formaldehyde

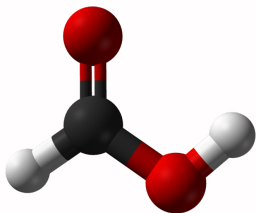


Methanol



Methane





Formic Acid as a Fuel

Upside

- No *net* CO₂ emissions: $\text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2\text{CO}_2$
- Biological standard (pH 7.0) midpoint potential $E_{\text{m}}^{0'} = -420 \text{ mV}$
- Dense liquid (1.22 g cm⁻³) with MP of 8.4° C, a BP of 100.8° C and a pK_a of 3.74.
- Solar formate can be collected by ion exchange over a large 2-dimensional flat surface

Downside

- Difficult to activate CO₂
- Energy density of formic acid (2086 Wh/l) is 46% that of neat methanol (4609 Wh/l)

Direct Formic Acid Fuel Cell

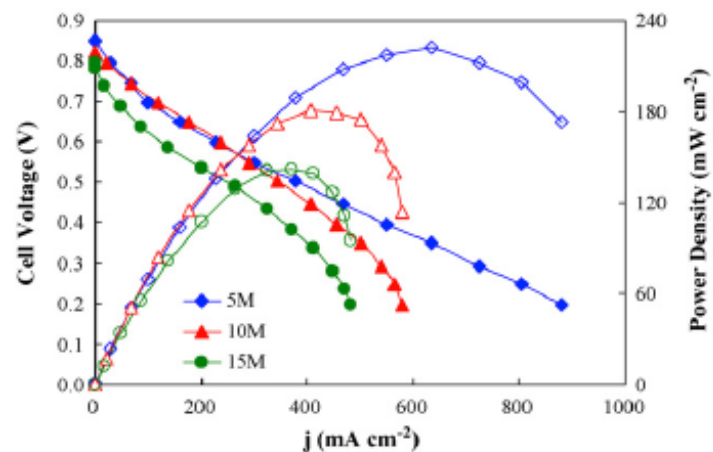
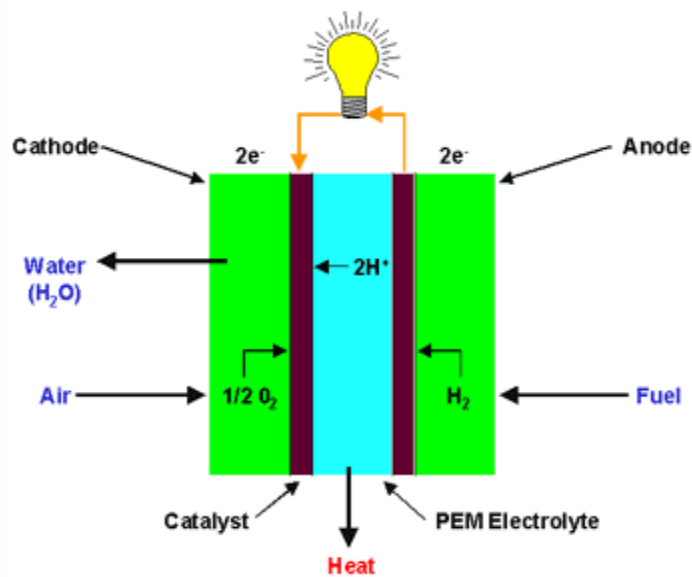
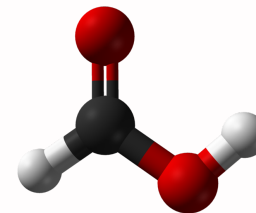
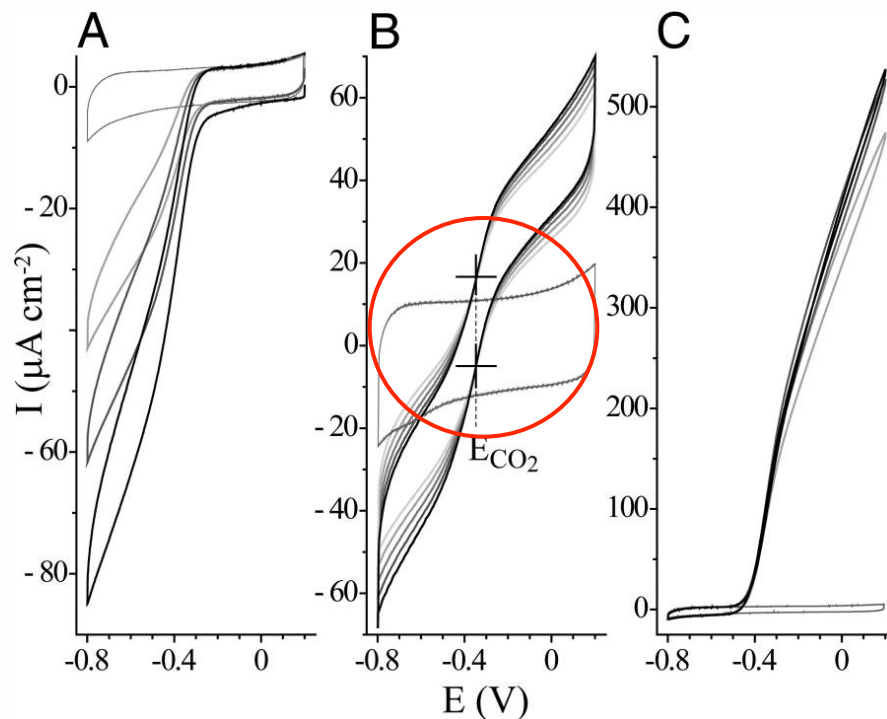
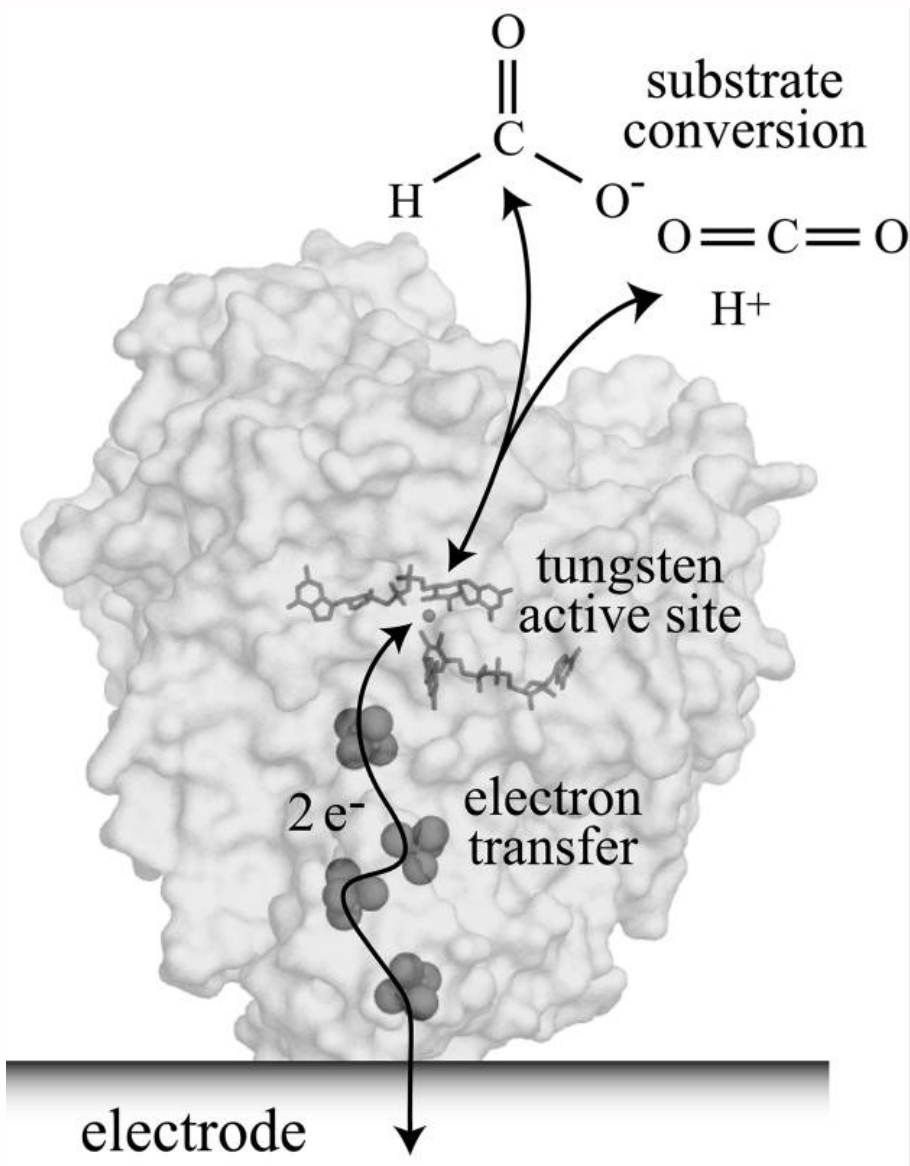


Fig. 5. Cell voltage and power density were plotted as a function of the current density using 5, 10 and 15 M formic acids. The formic acids and air were fed to the anode at a flow rate of 1 ml min⁻¹ and 400 sccm, respectively, at 30 °C. The dry air was used without applying any backpressure.

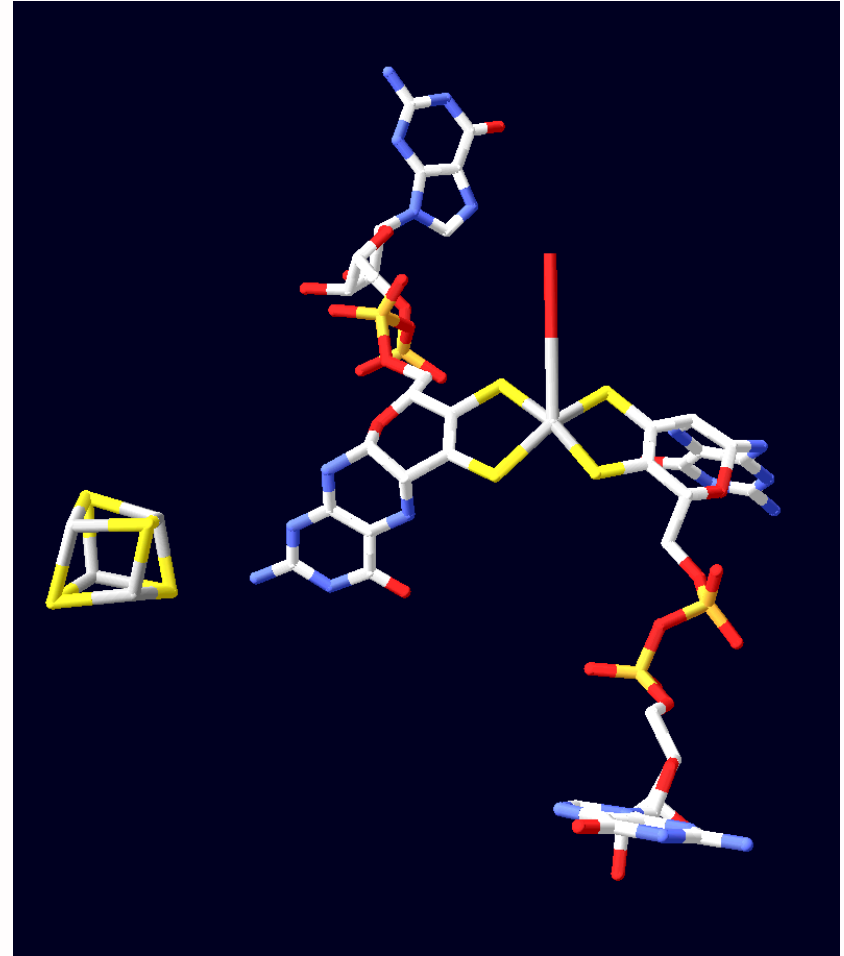
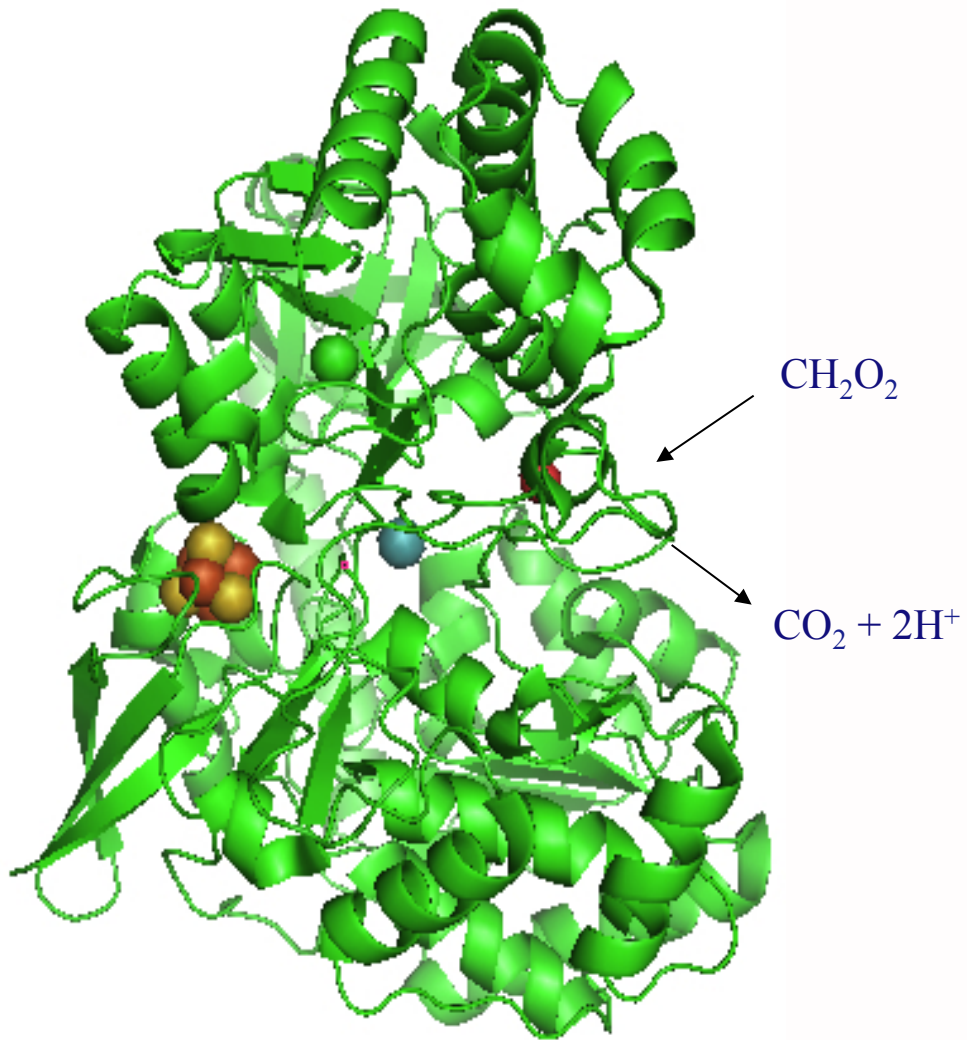
Maximum 6 M formic acid vs 1 M methanol translates to 2.5-fold greater power density per unit weight in a direct formic acid fuel cell

Formic Acid Dehydrogenase



Reda, T., Plugge, C. M., Abram, N. J., and Hirst, J. (2008) Reversible interconversion of carbon dioxide and formate by an electroactive enzyme, *Proc Natl Acad Sci U S A* 105, 10654-10658.

Formic Acid Dehydrogenase



Two Prerequisites

- **More reducing potential desirable on PS I for better rates**

$E_m^{0'} = -420 \text{ mV}$, but this is at pH 7.0 and 1 atmosphere of CO_2

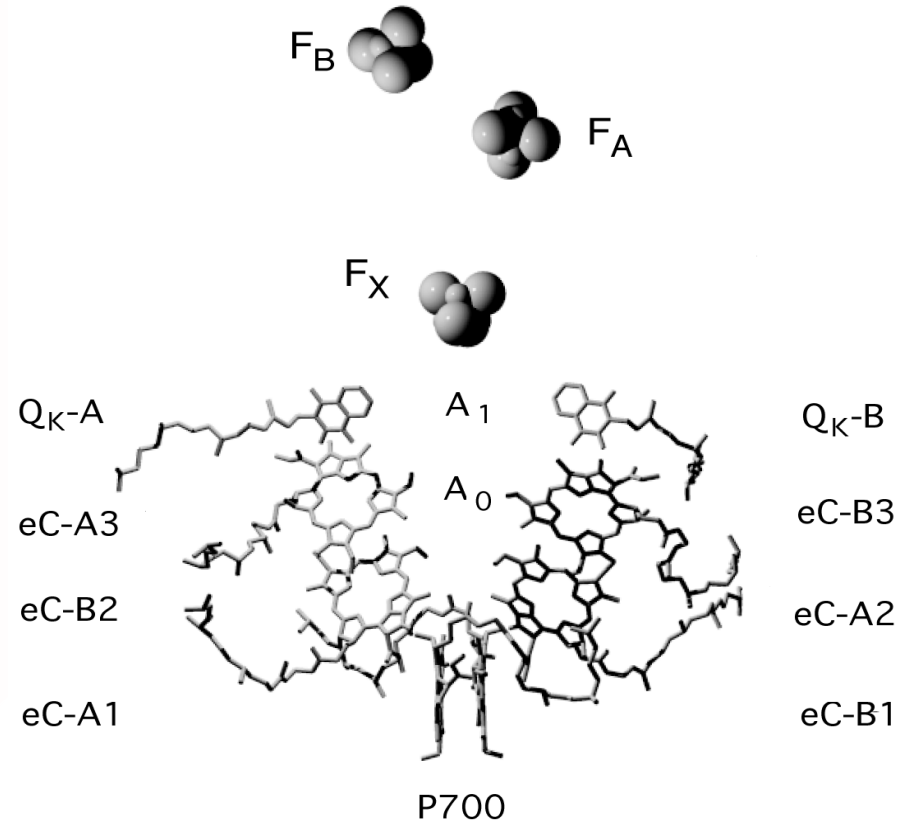
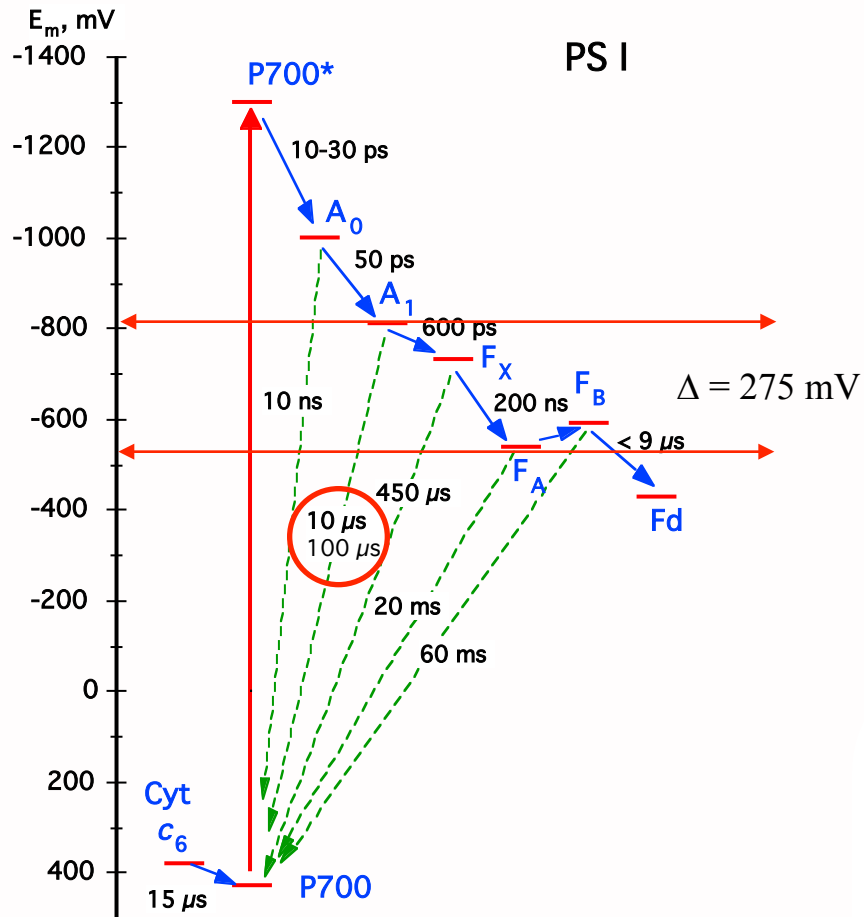
Atmospheric CO_2 is 388 ppm, so $E_m' = -524 \text{ mV}$ would be required

- **Cysteine -> Glycine variant of surface-located [4Fe-4S] cluster**

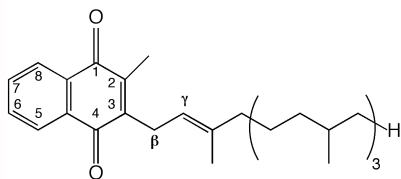
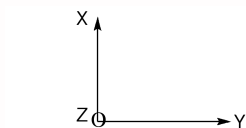
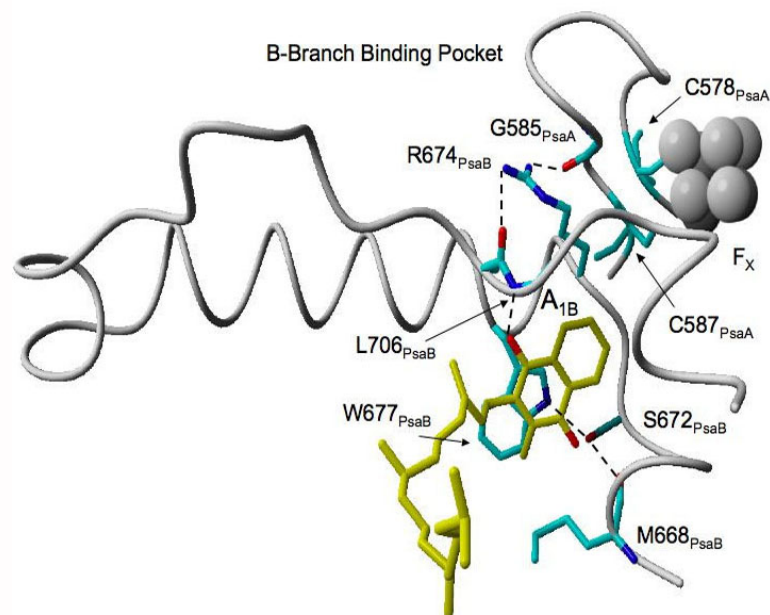
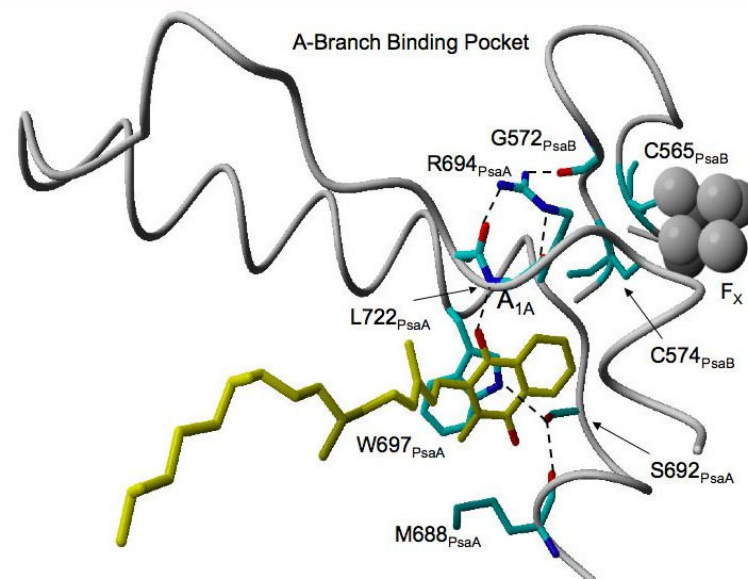
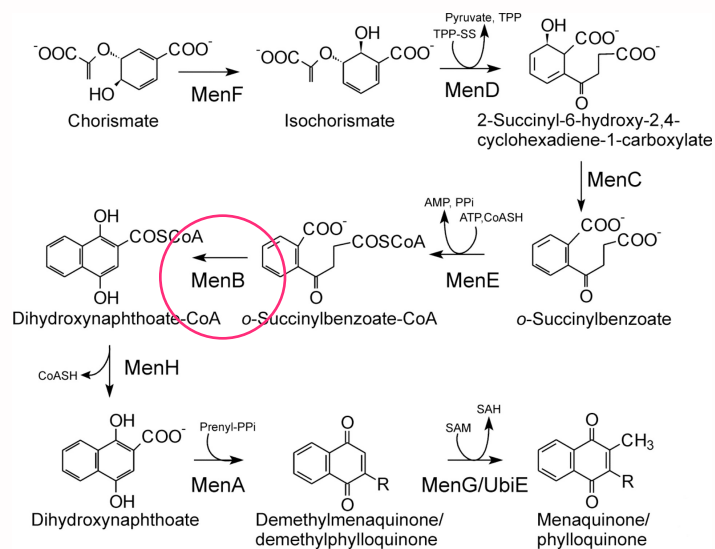
Use simpler formic acid dehydrogenase from *Escherichia coli*

Point of attachment of the tether at surface-located **Cys11 or Cys42**

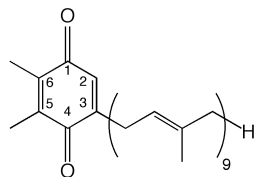
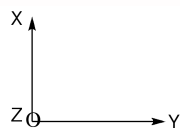
Extract the Electron at Q_K -A or Q_K -B



Use *menB* Mutant for Quinone Replacement

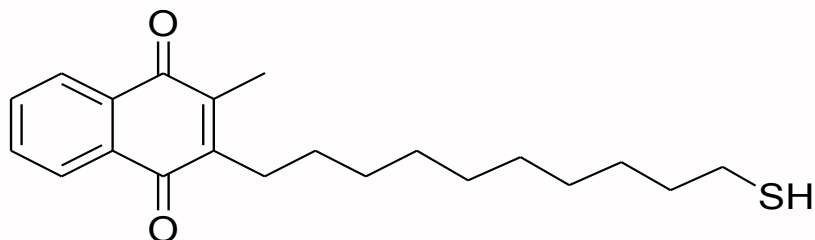


Phylloquinone
C₃₁H₄₆O₂
M : 450.35 g/mol

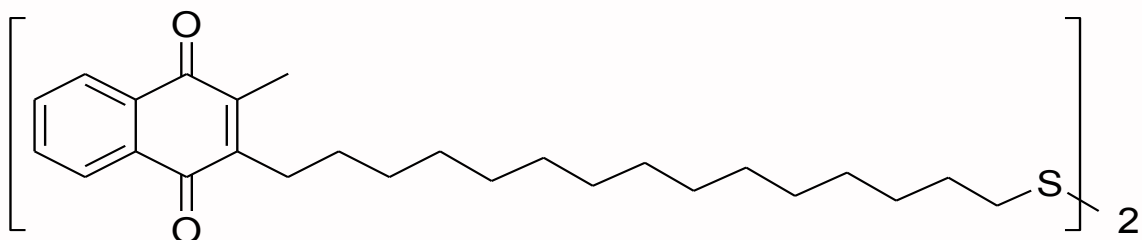


Plastoquinone 9
C₅₃H₈₀O₂
M : 748.64 g/mol

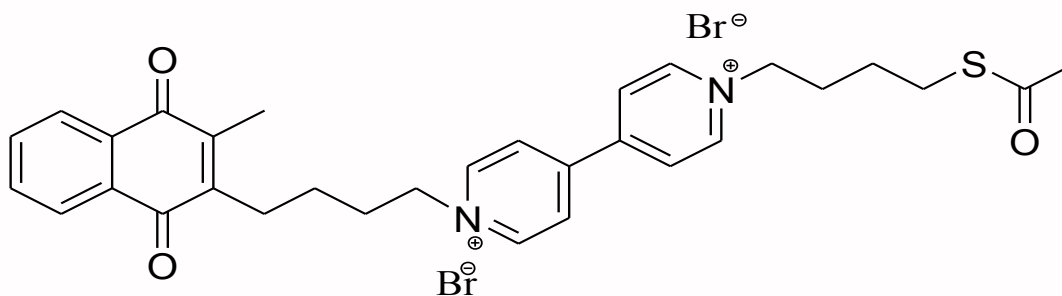
Naphthoquinone-Based Wires



2-(10-Mercapto-decyl)-3-methyl-[1,4]naphthoquinone

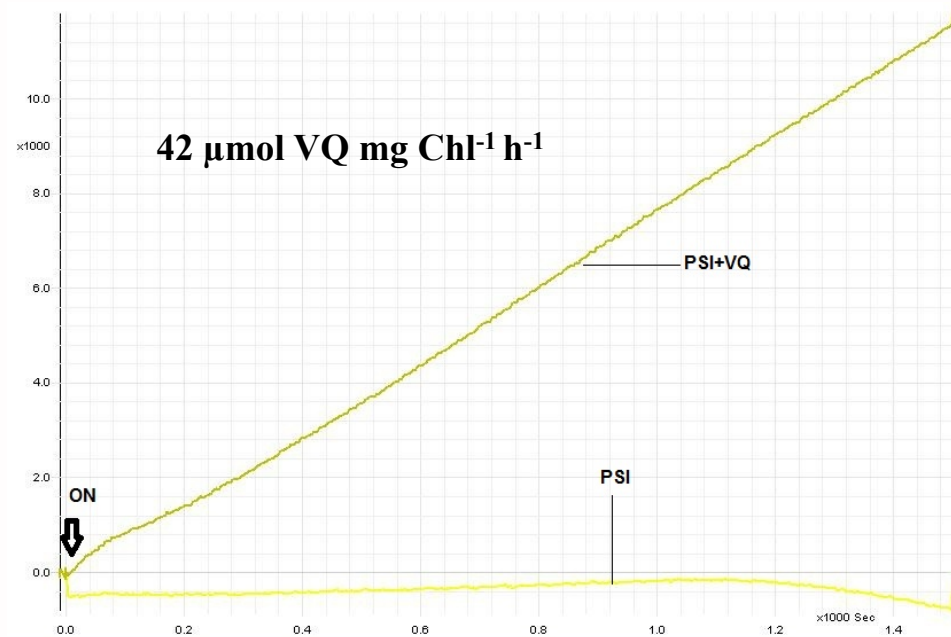
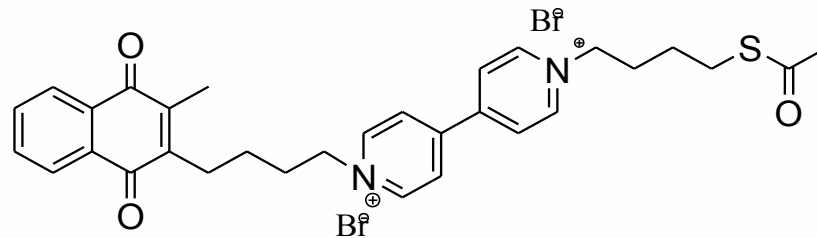
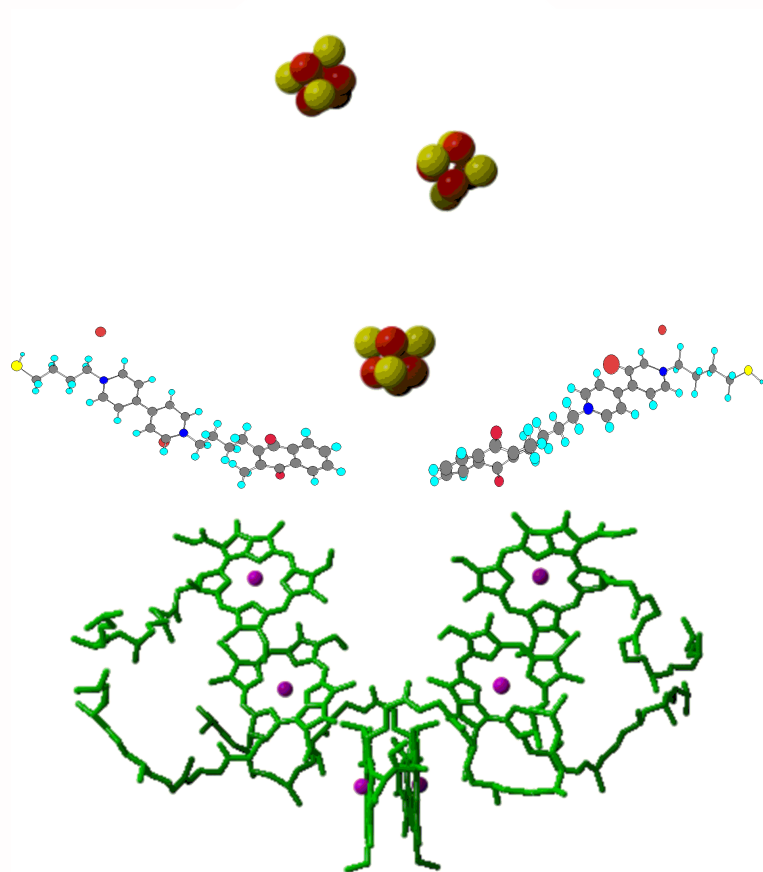


2-(15-Mercapto-pentadecyl)-3-methyl-[1,4]naphthoquinone



bipyridiniumquinone

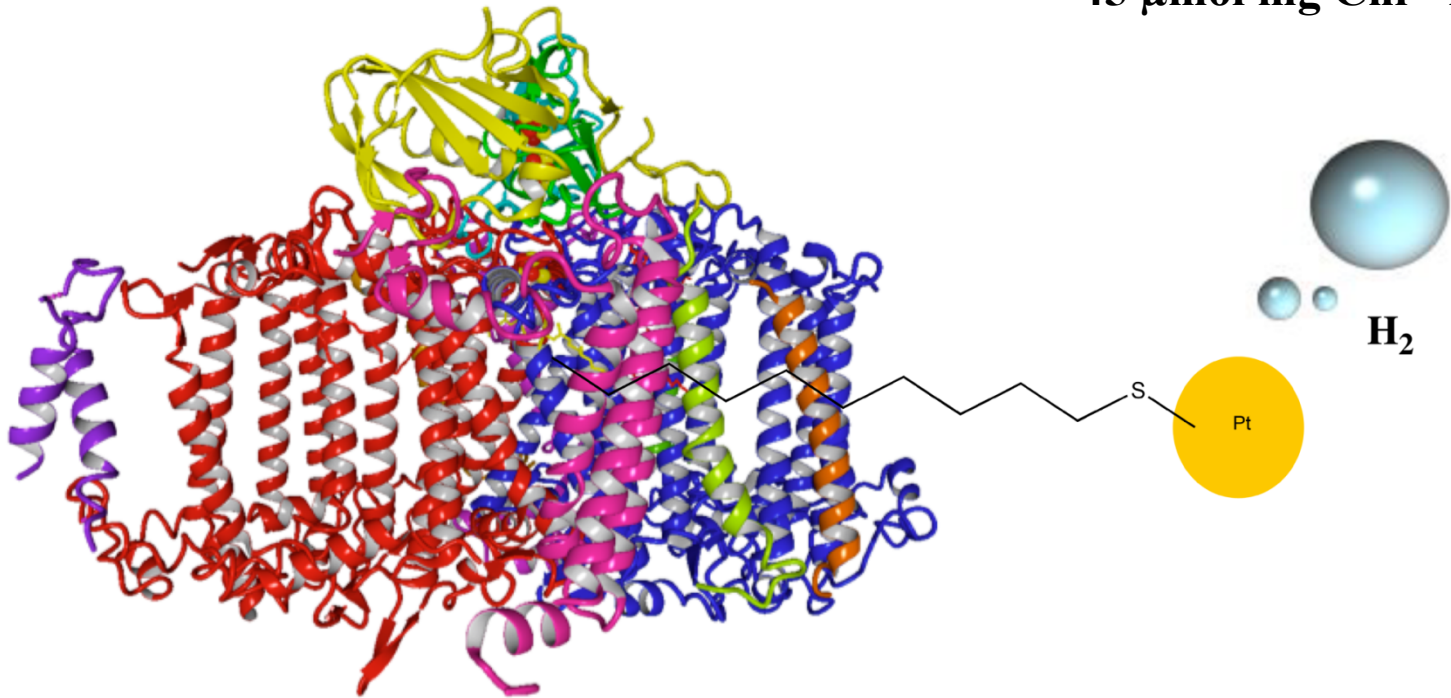
Proof of Concept: Viologen Reduction as Assay



Proof of Concept: Hydrogen as Assay

- Incubate Pt nanoparticles and C₁₅ molecular wire
- Add *menB26* and stir 16-24 hours in the dark at 298 K

45 $\mu\text{mol mg Chl}^{-1} \text{ h}^{-1}$

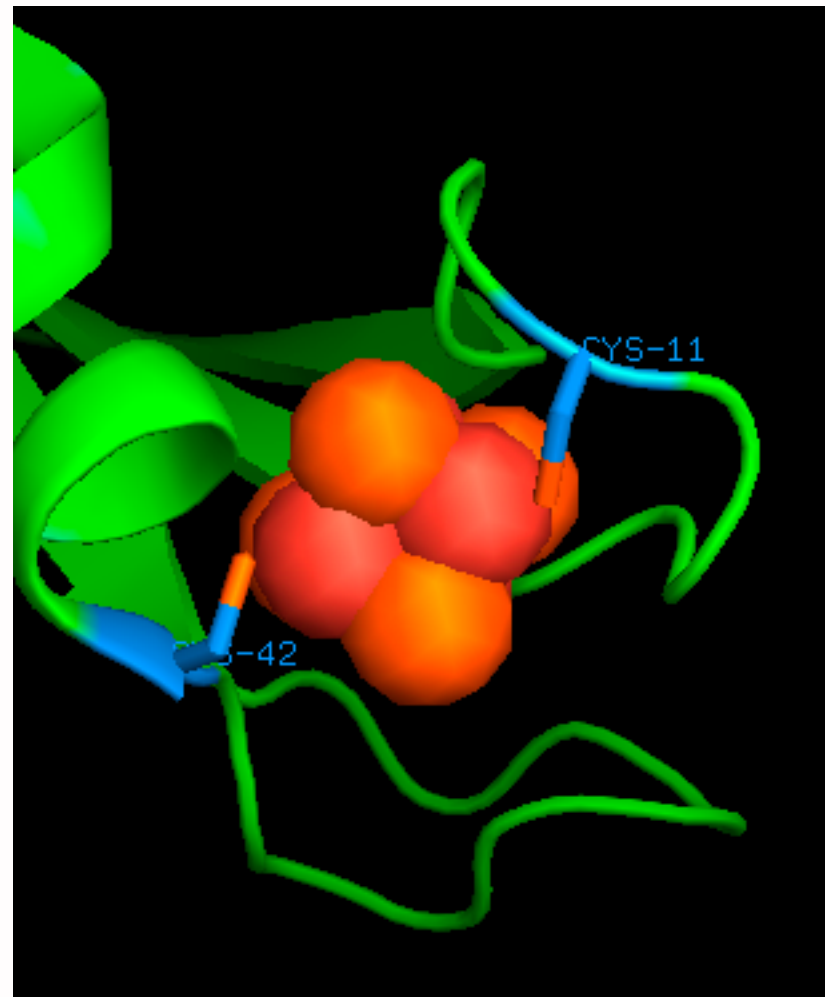


Cys11Gly and Cys42Gly Variants of FDH

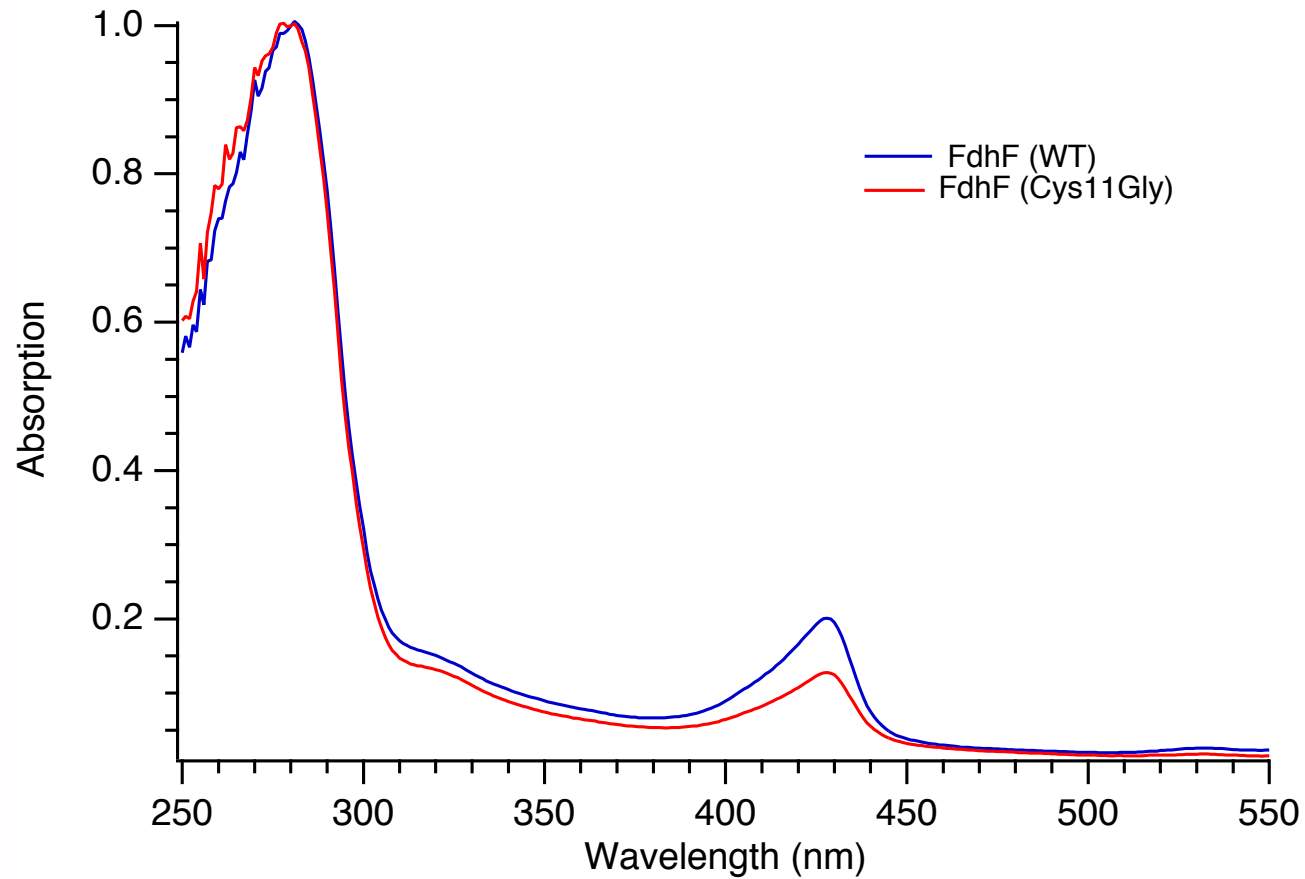
Goal: to replace a surface-located Cys that serves as a ligand to the [4Fe-4S] cluster to serve as the attachment point of the molecular wire

Method: Site-Directed Mutagenesis to generate the FdhF C11G and C42G mutants

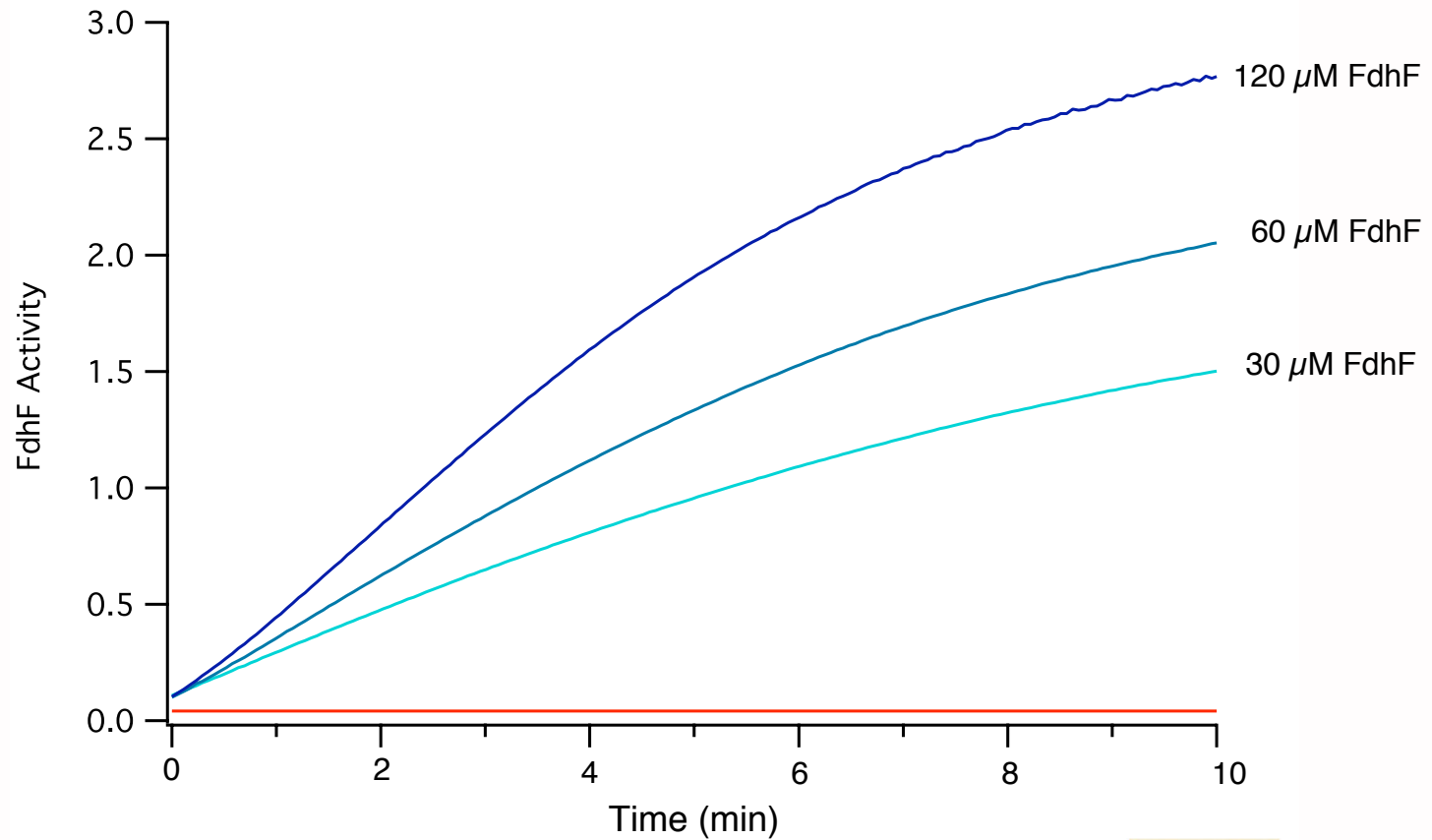
Constructs: two expression vectors (pDuctFdhF-SelC and pMALc4XFdhF) have been used for mutagenesis.



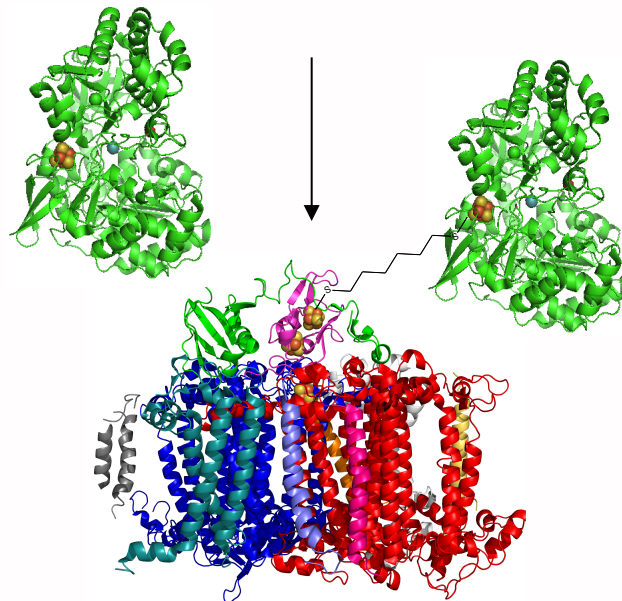
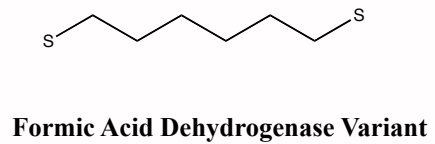
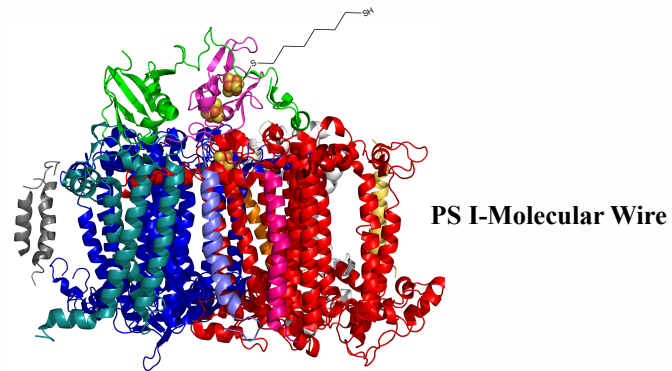
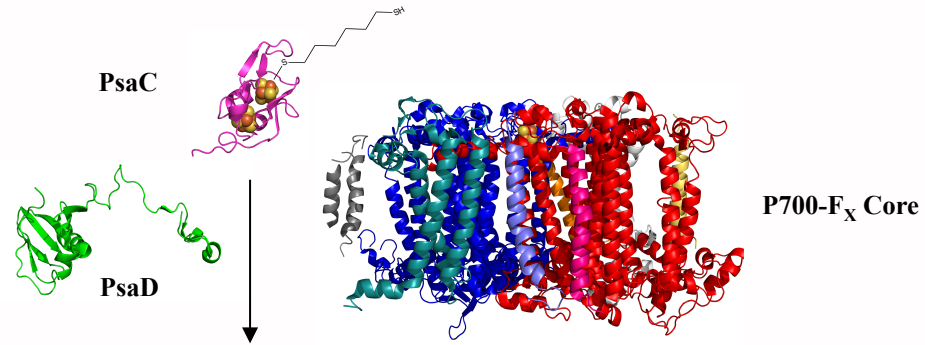
Absorption Spectra of Cys11Gly Variant of FDH



Activity Assay of Formic Acid Dehydrogenase

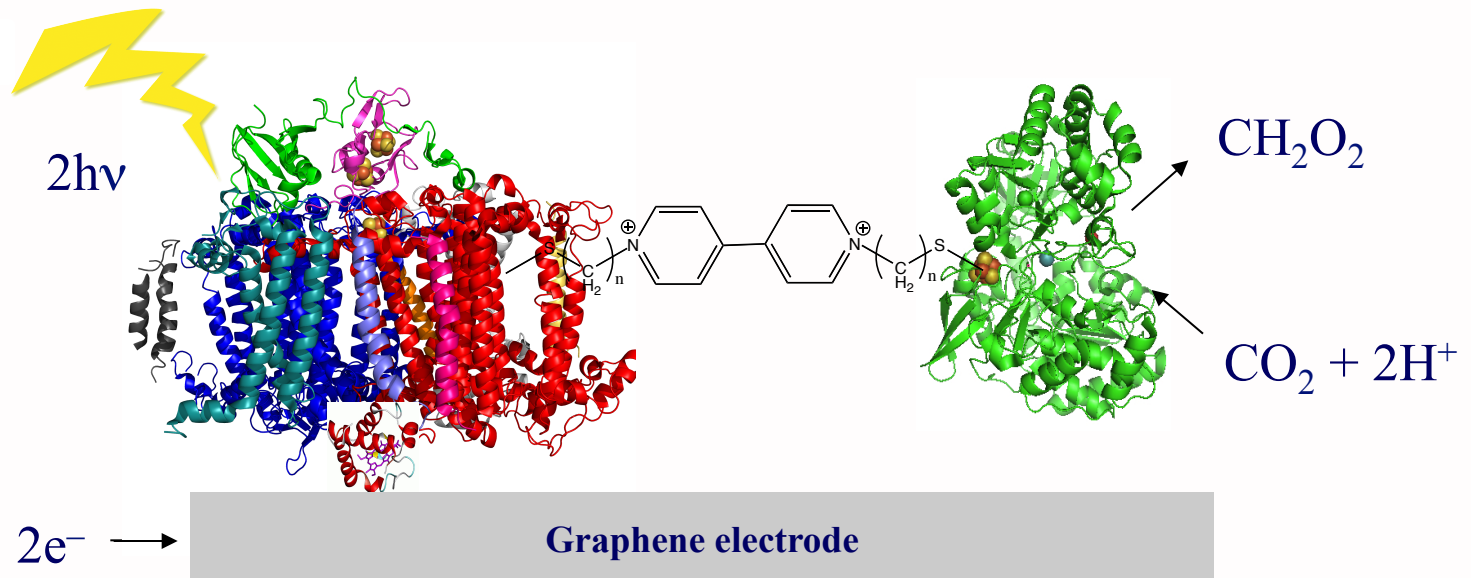


PS I-wire-FDH

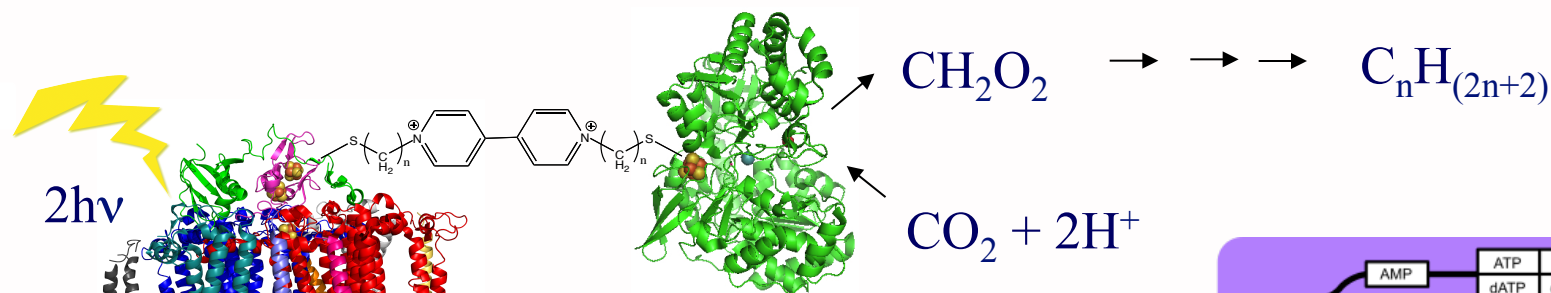


Project Goal

To use sunlight to drive the fixation of carbon dioxide into formic acid using a formic acid dehydrogenase enzyme tethered to Photosystem I



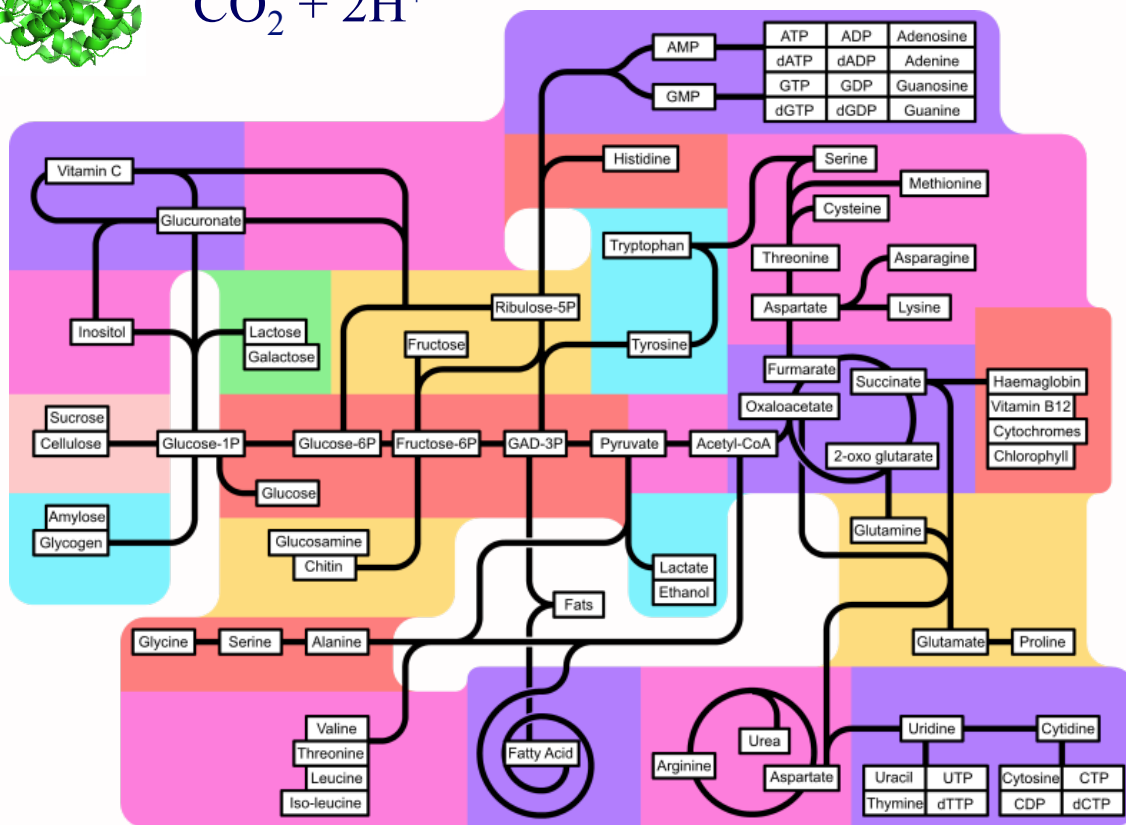
Biological Formate into Alkanes



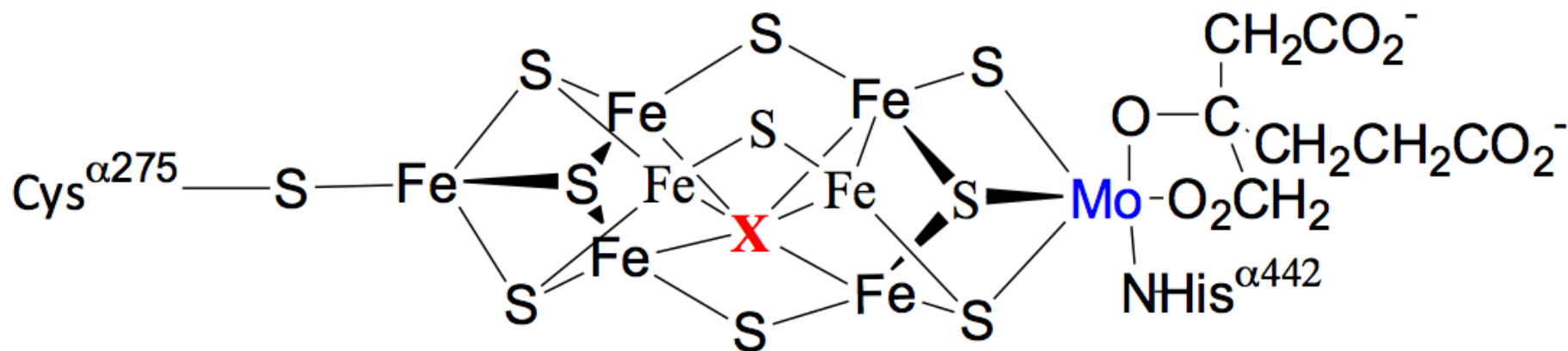
Oxygen Sensitivity

In vivo Incorporation

Pathway Discovery



Reduction of CO to Propane, Butane, Pentane



Summary

- Ability to connect redox centers of proteins with a molecular wire
- PS I hard wired to [FeFe]H₂ase evolves H₂ in the light
- Elimination of diffusional chemistry enhances rates 2.5-fold
- Future work: oxygen stable enzymes, *in vivo* tethering

Thank You for Your Attention

