2009 DOE Hydrogen Program

Montana Palladium Research Initiative:

Use of Biological Materials and Biologically Inspired Materials for H_2 Catalysis

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and
Center for Bioinspired Nanomaterials

MONTANA STATE UNIVERSITY

DOE Project ID#: PDP_19_Peters

This presentation does not contain any proprietary, confidential,
or otherwise restricted information

Overview

Timeline

- Start - Aug. 2006
- End - Dec. 2009

Budget

- Total project funding
  $1,303,041
  - DOE $1,031,433

Barriers addressed

- Stability/Durability
- Oxygen Sensitivity
- Electron Donors
- Coupling

Partners

- Montana State University

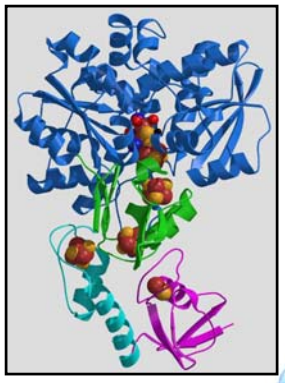
MONTANA STATE UNIVERSITY

DOE Project ID#: PDP19

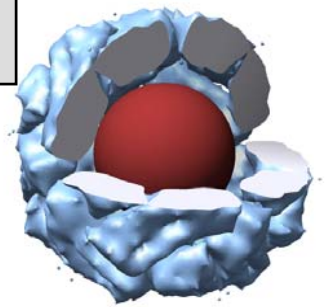
Approaches

Couple Different Catalyst Systems for Light Driven Hydrogen Generation

Biological catalysts (Hydrogenases)



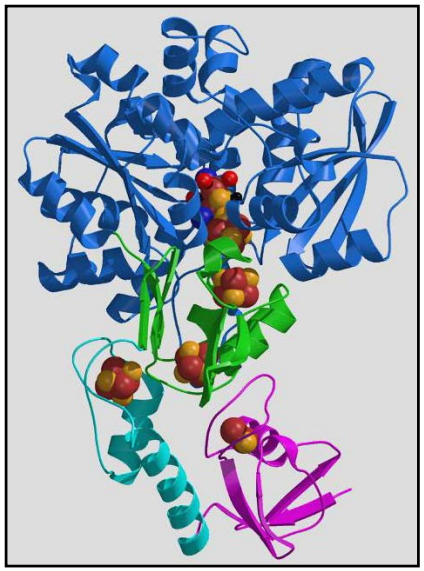
Nanoparticle biomimetic catalysts



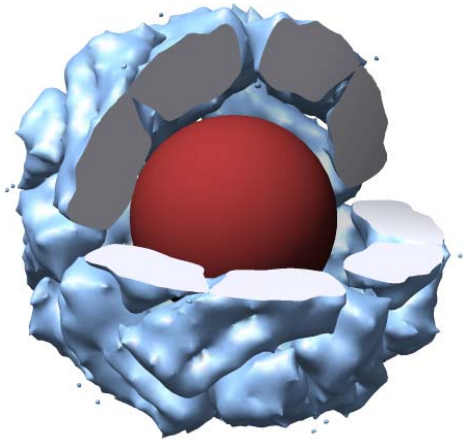
Objectives Addressed

1. Immobilize hydrogenase in gels
2. Determine basis of hydrogenase stability
3. Improve conductivity, mass transfer, and hydrogen production in gels
4. Biomimetic hydrogen production catalyst synthesis
5. Photocatalyst synthesis
6. Coupling catalysts to electrode surfaces
7. H_2 production device fabrication

Approach:
Biological and Biomimetic Catalysts for H_2 production

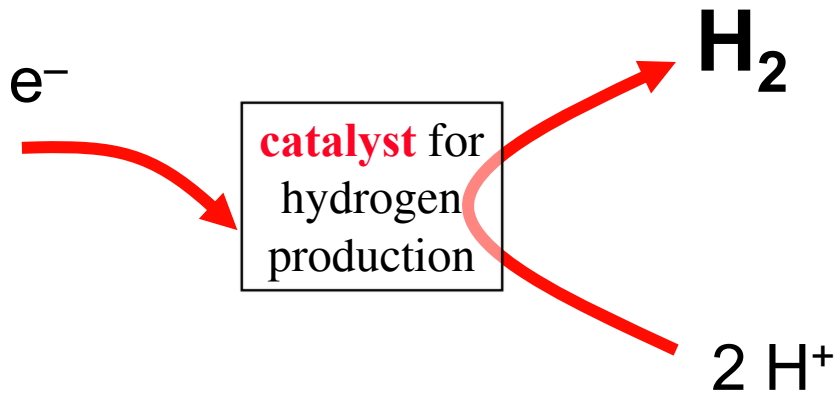


Hydrogenase Enzymes
(protein architecture protecting
Metal sulfide active site)



Protein encapsulated
nano-catalyst

Coupled Reactions to Generate Hydrogen



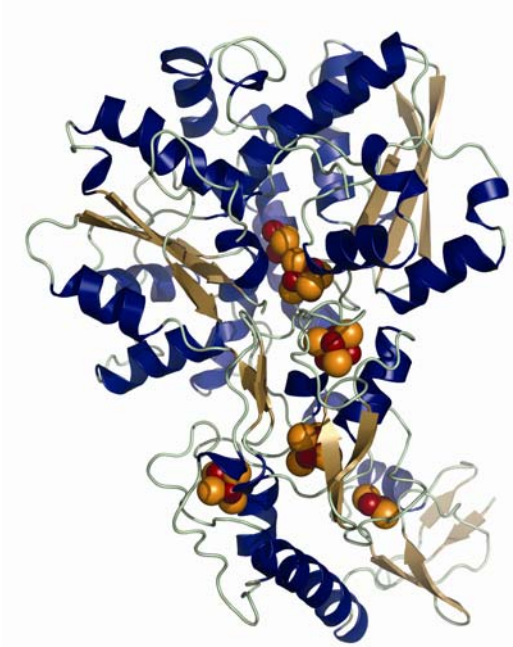
GOAL: use **biological catalysts** and develop **biomimetic catalysts** with a variety of sacrificial electron donors or electrochemical source of e^- to produce H_2

Issues and Barriers: Catalyst Stability

- Durability – shelf life
- Reusability
- Product based Inhibition
- Oxygen tolerance / resistance
- Susceptibility to proteolytic inactivation
- Optimization – electron transfer, pH, ionic strength, mediators

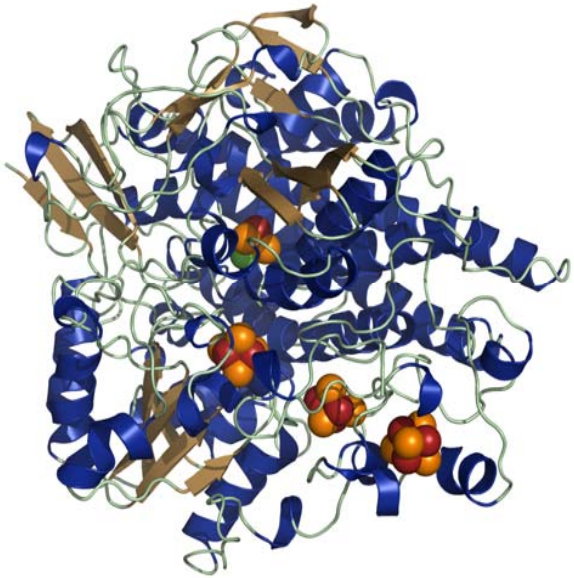
Hydrogenases: Highly evolved finely tuned catalysts for *hydrogen oxidation and proton reduction (hydrogen production)*

C. pasteurianum



$$H_2 \leftrightarrows 2H^+ + 2\ e^-$$

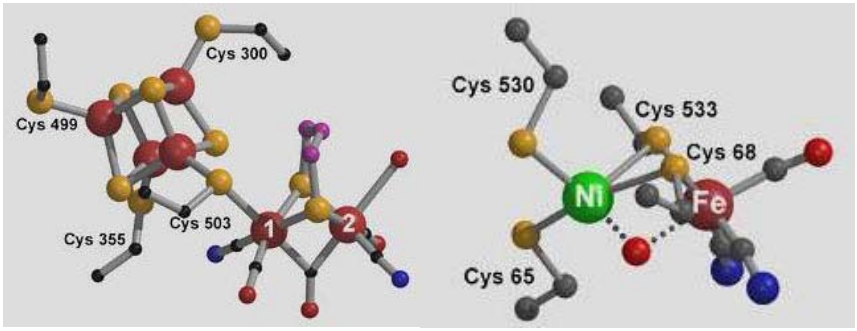
Desulfovibrio gigas



Cellular location

Membrane Associated
Soluble
Periplasmic
Cytoplasmic

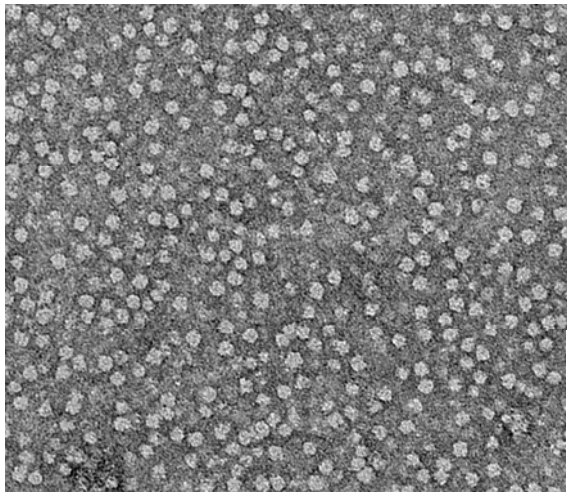
“H Cluster” **NiFe Cluster**



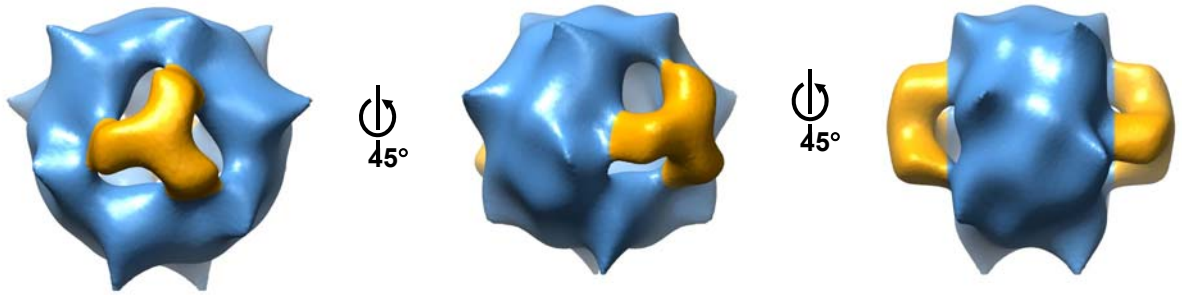
Microorganisms:

hydrogen, acetate-grown, methanogenic, green, purple, cyanobacteria; algae; fungus.

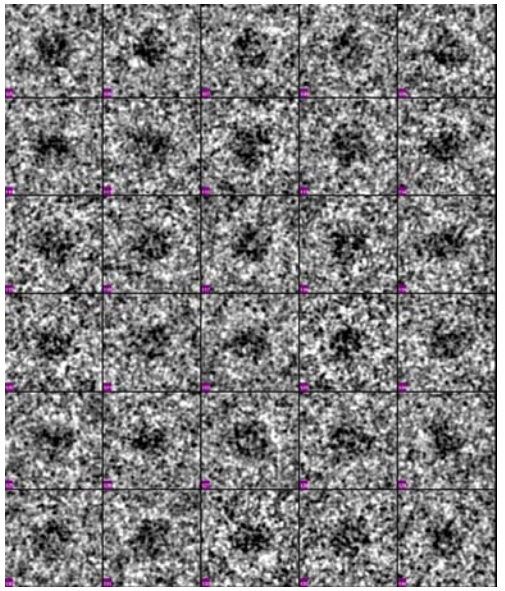
Stable NiFe hydrogenase from purple sulfur bacteria forms supermolecular structures



Electron microphotograph of hydrogenase complexes from *T. roseopersicina* negatively stained with 2% uranyl acetate



Cryo reconstruction of hydrogenase from *T. roseopersicina* at ~33 Å.



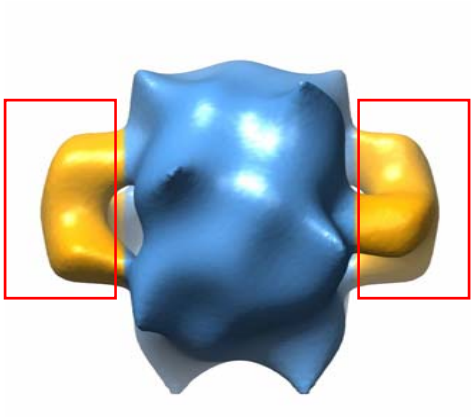
Properties	***Thiocapsa roseopersicina***
Large subunit	64kDa
Small subunit	34kDa
Temperature optimum	**80ºC**
Stability to Oxygen	**stable**

Structural studies indicate a role for C-termini in the stability and super molecular complex formation of hydrogenase

A

```
      1         10        20        30
T.r.  AALEQAKRPSVIWLSFQECTGCTESLTRSH
D.r.    LTAKKRPSVVYLHNAECTGCSESVLRTV
                40        50        60
T.r.  APTLEDLILDVISLDYHHTLQAAAGDAAEH
D.r.  DPYVDELILDVISMDYHETLMAGAGHAVEE
                70        80        90
T.r.  AREQAMAANPGEYLVIVDGSIPGPDSNPGY
D.r.  ALHEAI---KGDFVCVIEGGIPMGDGGY-W
               100       110       120
T.r.  STVAGHSNYAMLMETVENAAAVIAVGTCAT
D.r.  GKVGGRNMYDICAEVAPKAKAVIAIGTCAT
               130       140       150
T.r.  FGGLPGANPNPTGAMSVMDLVKDKPV--IN
D.r.  YGGVQAAKPNPTGTVGVNEALGKLGVKAIN
               160       170       180
T.r.  VSGCPPIPMVITGVIAHYLTFGRLPELDAY
D.r.  IAGCPPNPMNFVGTVVHLLTKG-MPELDKQ
               190       200       210
T.r.  NRPMAFFGQSIHDRCYRRPFYDKGLFAKTF
D.r.  GRPVMFFGETVHDNCPRLKHFEAGEFATSF
               220       230       240
T.r.  DDEGARLGWCLYELGCKGPTTYNACATMRW
D.r.  GSPEAKKGYCLYELGCKGPDTYNNCPKQLF
               250       260       270
T.r.  NDGTSWPVEAGHPCLGCSEPRFWDA-GGFY
D.r.  NQ-VNWPVQAGHPCIACSEPNFWDLYSPFY
               280       290       300
T.r.  NTVSVPTSASGVNVLAAGAAGAIVGGAVAA
D.r.  SA
               310       320       325
T.r.  LAKKQTKTAVAHRQPVTVEELEAKL
D.r.
```

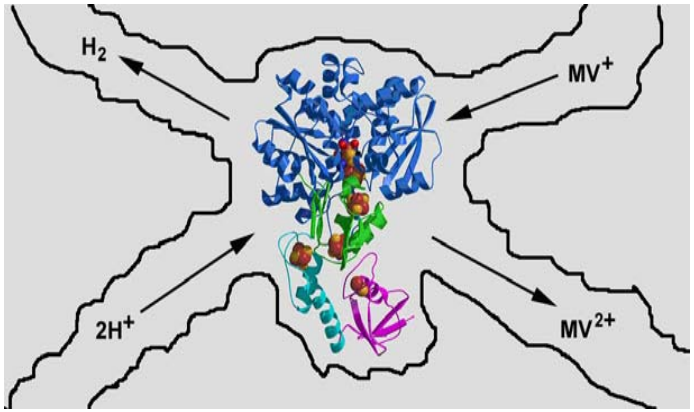
B



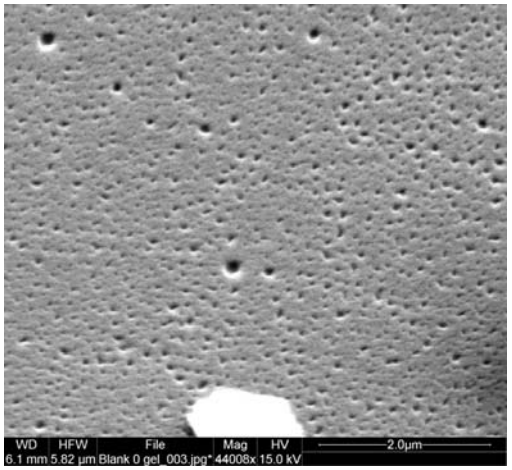
Sequence alignment of small subunits of stable hydrogenase from *T. roseopersicina* (*T. r.)* and *D. gigas* (*D. g.*) (A) and cryoEM m (B). In the red box the C-termini residues, which could be involve in the cap formation are shown.

With Liang Tang – University of Kansas

Encapsulation of purified active hydrogenases in tetramethyl ortho silicate gels



- Nanoscopic encapsulation;
- Immobilization of unaltered enzyme
- “Heterogeneous material”



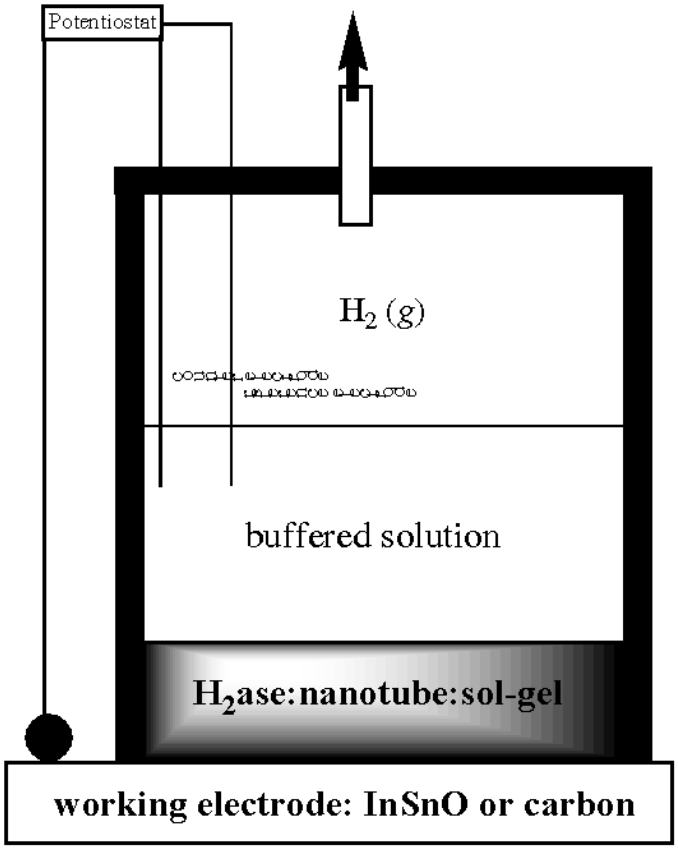
Screening electron microscopy photographs of sol-gel

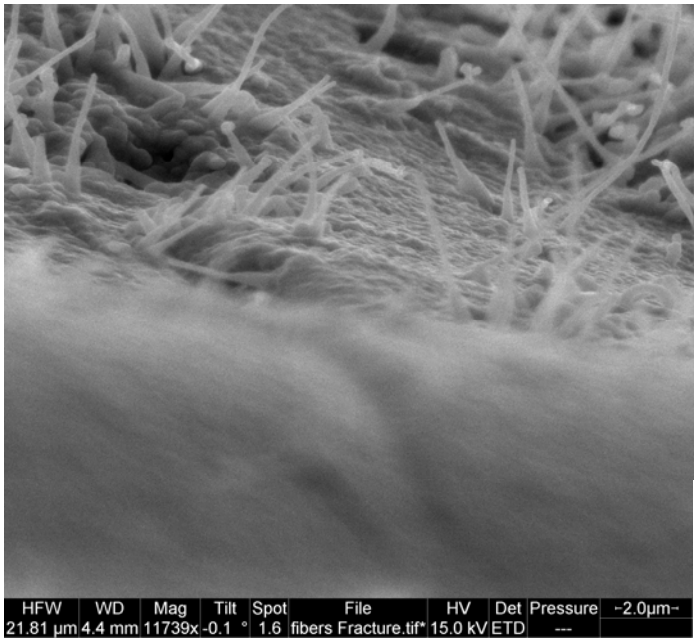
Recovery of hydrogenase activity* encapsulated in Sol-Gel

Hydrogenase	Solution	Gel	Solution/Gel (%)
C. pasterianum (extract)	12550	7581	60.4±16
L. modestogalophilus	9150	6175	67.5±9
T. roseopersicina	12600	8834	70.1±3

•Activity measure at 25º C indicated in nmol/min/mg protein. Values represent average rate over a four-hour period.

Carbon nano tubes incorporated into Sol Gels





Scanning electron microscopy photographs of sol-gel in presence of carbon nanotubes

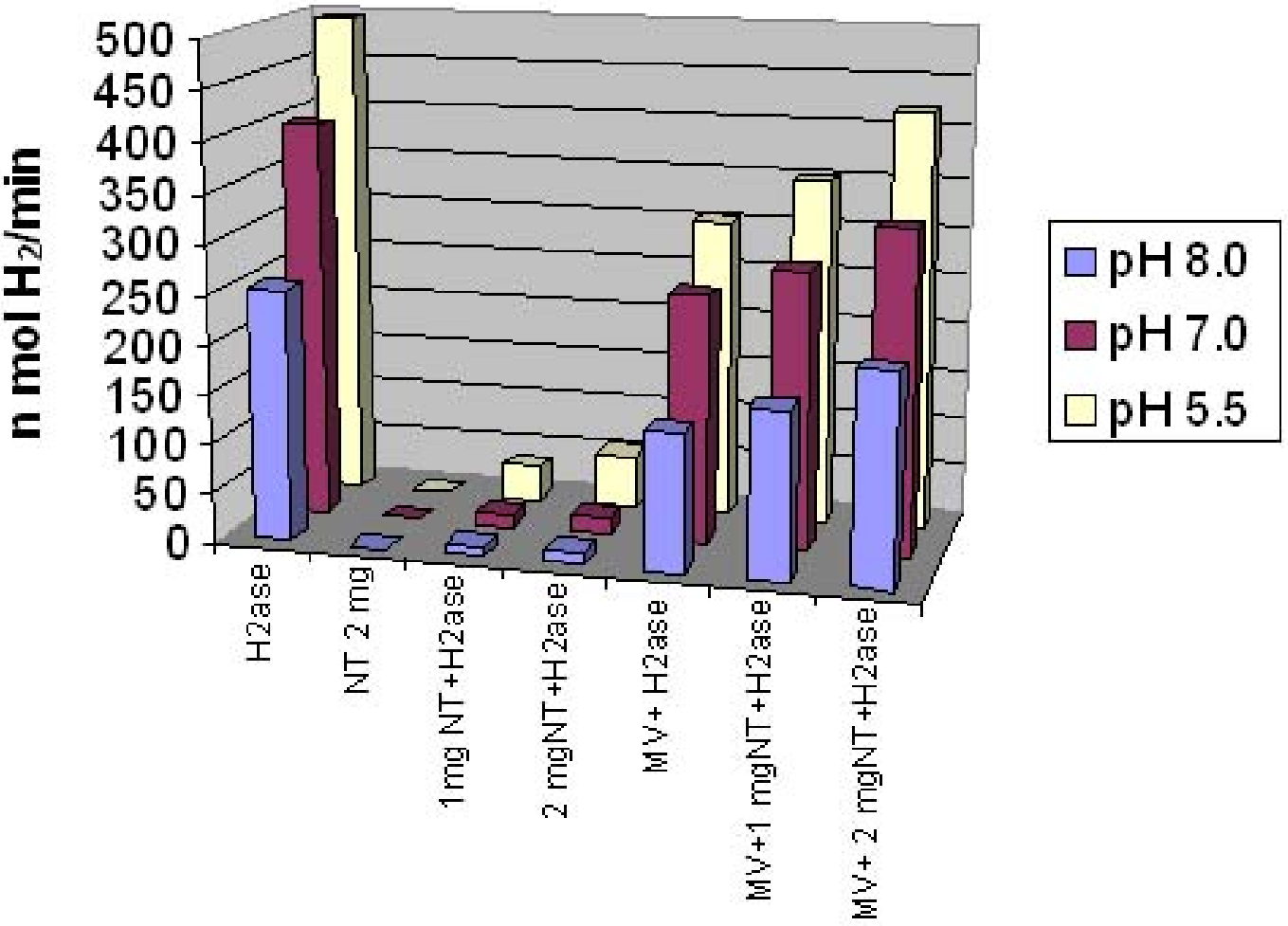
→ Enhance electron transfer

→ Facilitate electron transfer between immobilized mediators and hydrogenase

→ Facilitate electron transfer between electrodes and hydrogenase in devices

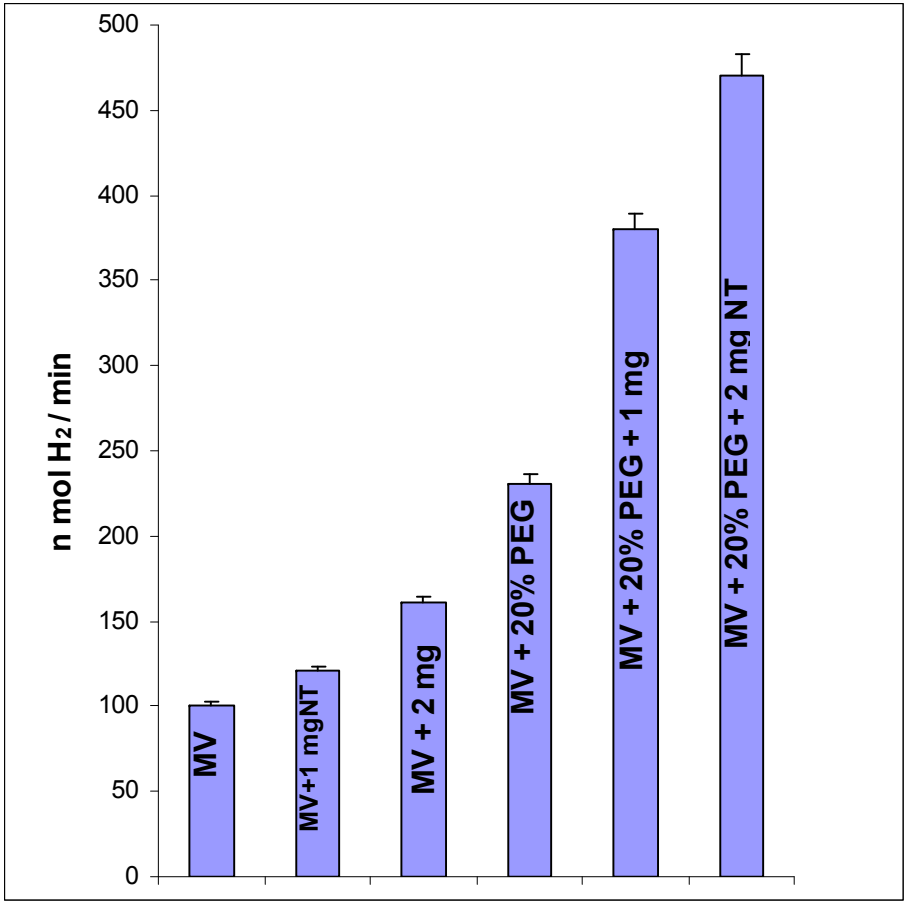
Formation of electro active matrix by encapsulation of the hydrogenase with multiwall carbon nanotubes

Hydrogen production by the silica gel matrix containing hydrogenase (H2ase), carbon nano tubes (NT) and methyl viologen (MV)



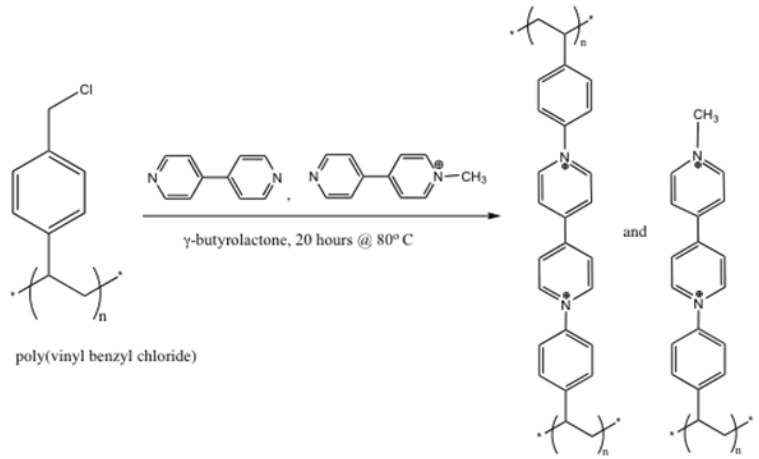
Hydrogenase activity highest in gels with hydrogenase and carbon nanotubes coencapsulated and activity is observed without addition of the redox mediator methyl viologen

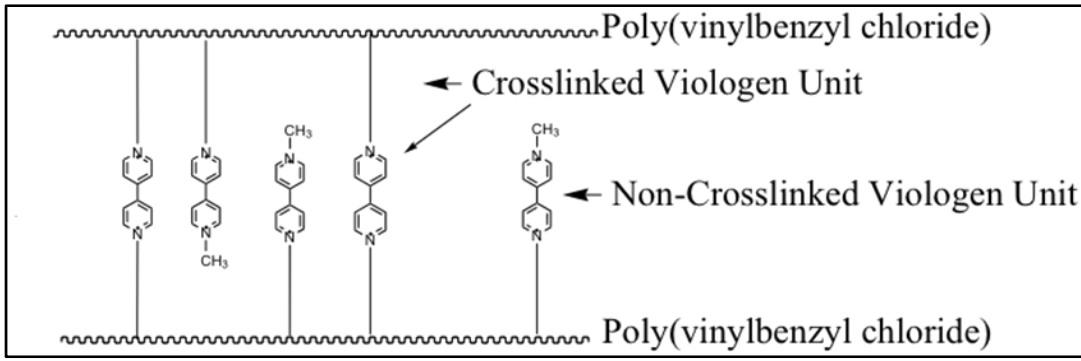
The addition of polyethylene glycol (PEG) to the sol gel enhances the hydrogen production by encapsulated hydrogenase



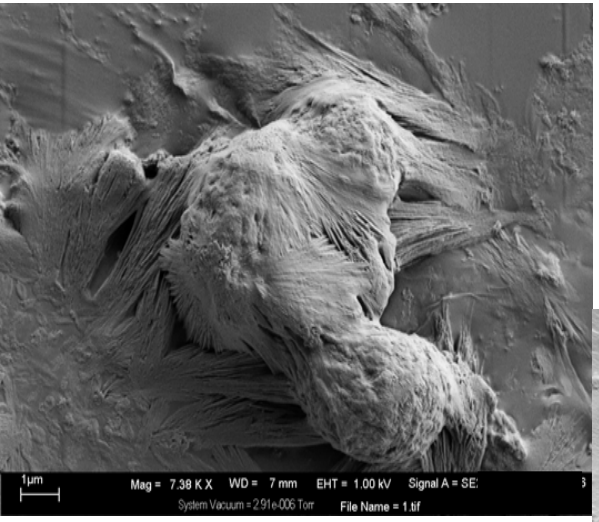
Poly anions and cations modulate the pore size in gels and mass transfer

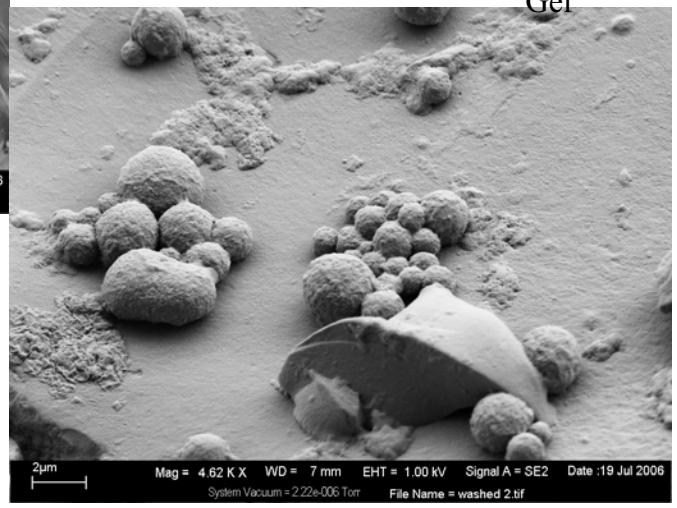
Controlled synthesis of electroactive polymer gels – controlling protein adsorption, mass transfer, and conductivity

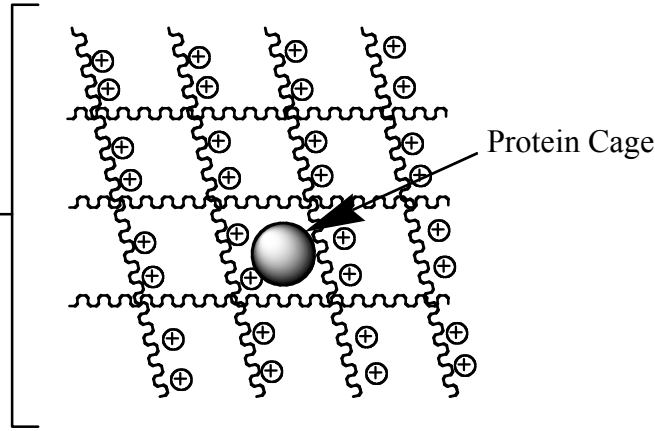




Electrostatic incorporation of protein catalysts

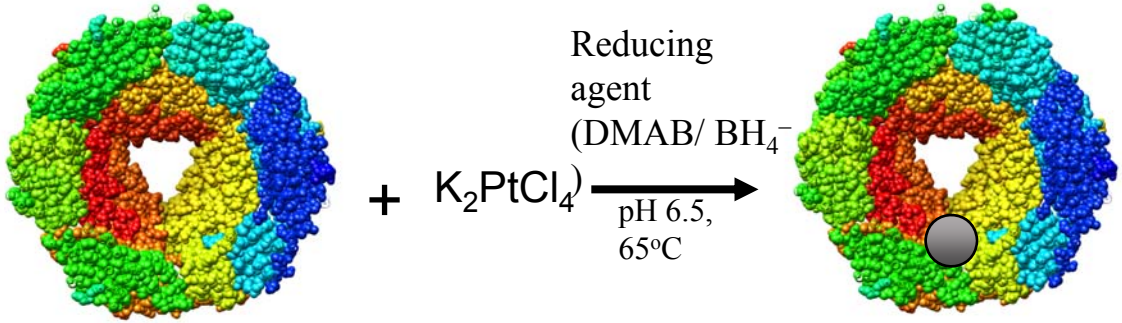


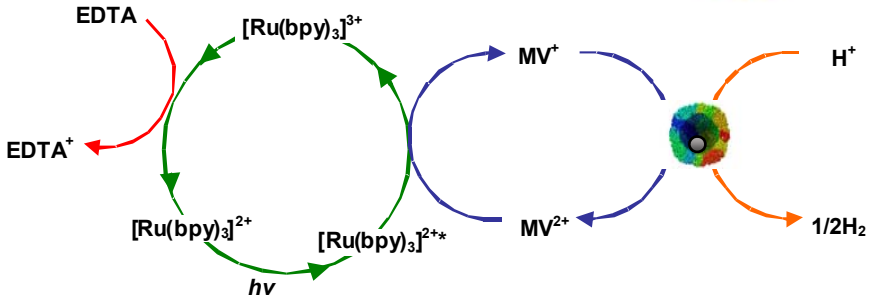


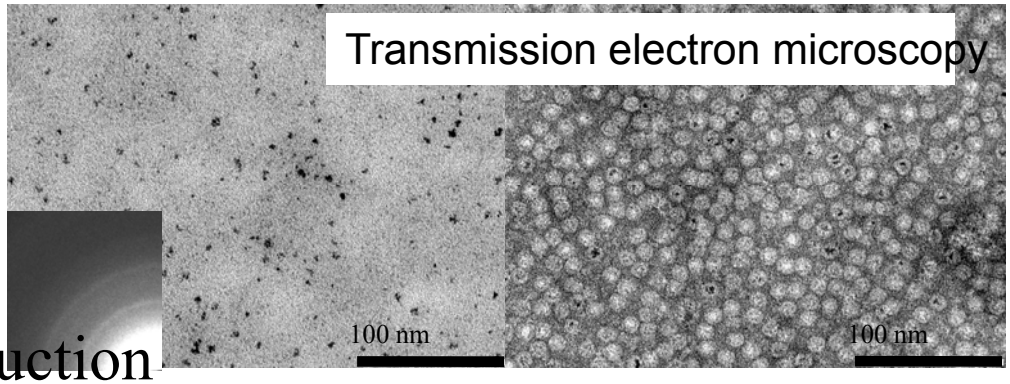


SEM of polymer gels

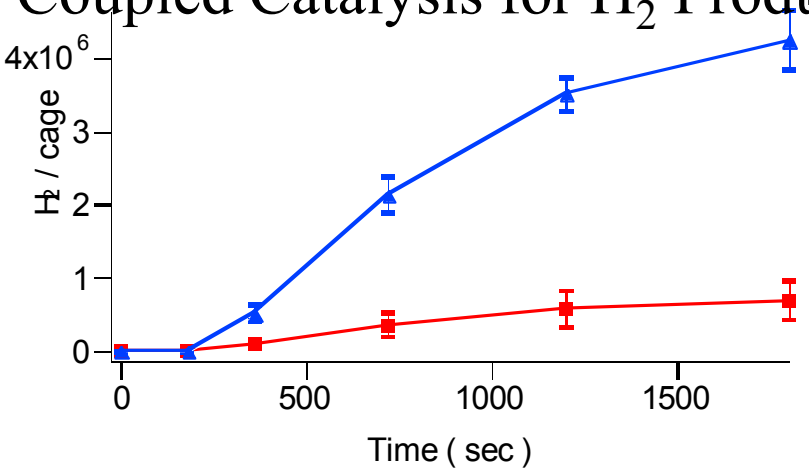
Biomimetic Catalysts - Synthesis of Pt^0 Encapsulated Within a Protein Cage Architecture







Coupled Catalysis for H_2 Production

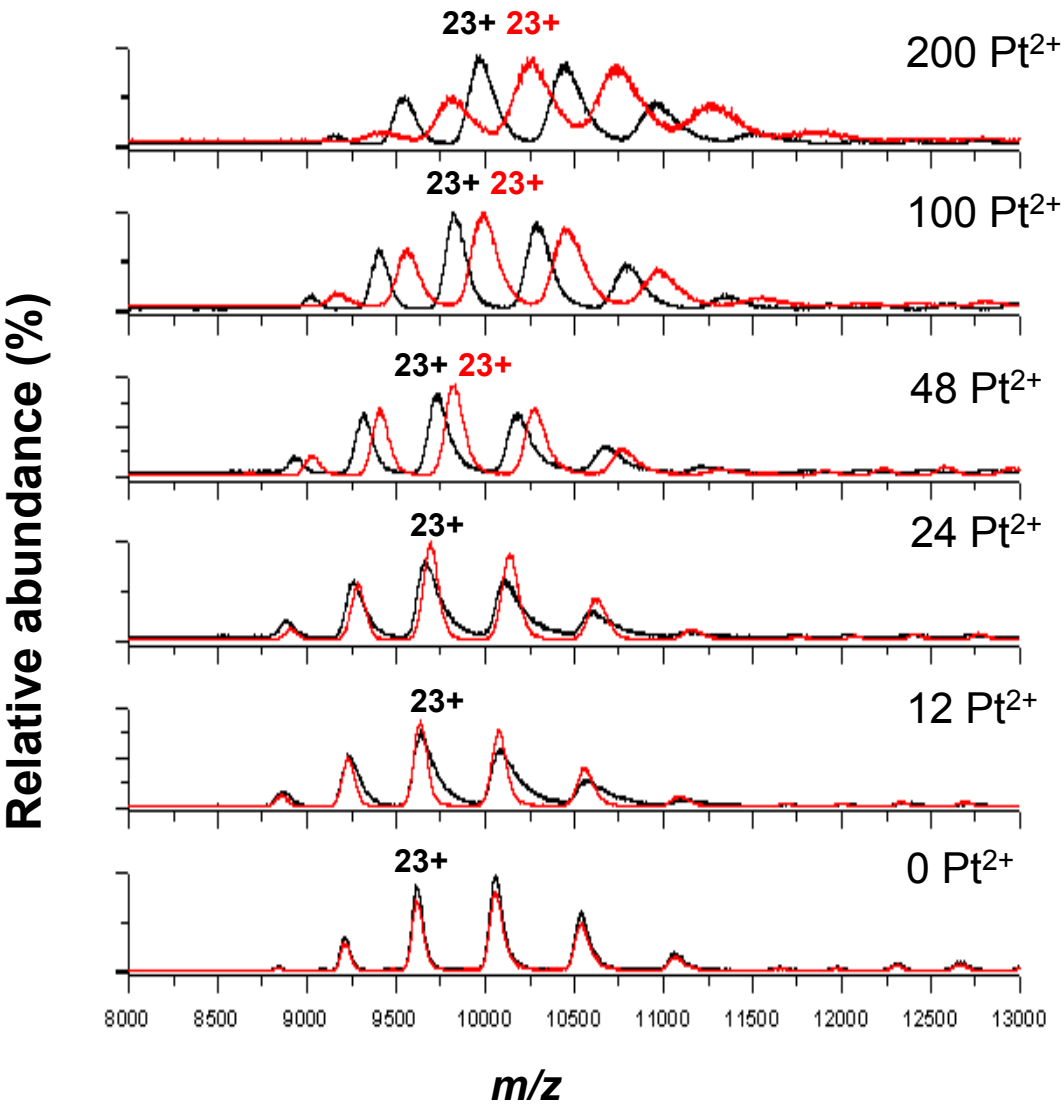


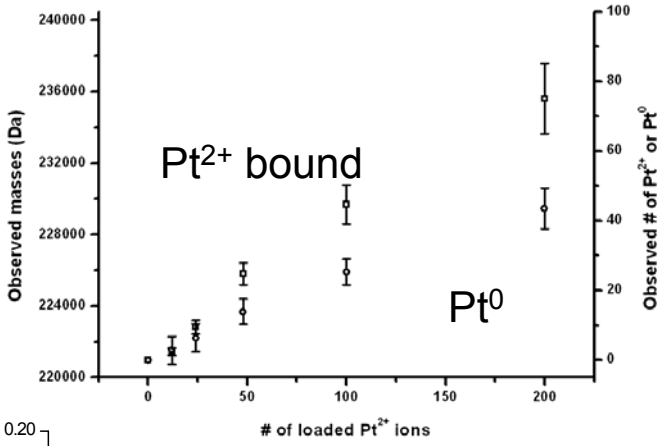
Initial rates (Pt): 4.47×10^3 H_2/sec/Hsp

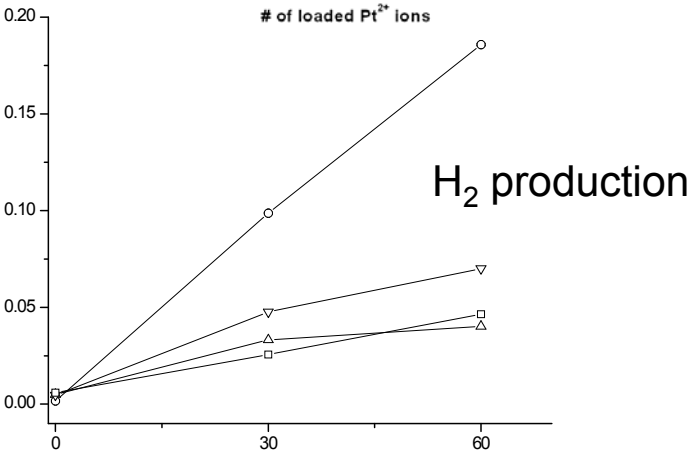
1.5×10^4 H_2/sec/ferritin

(Hydrogenase => $6 \times 10^3 H_2$/sec/hydrogenase)

Control of Pt cluster size (monitored by NCMS) correlation between activity and cluster size – defining the minimum catalytic cluster

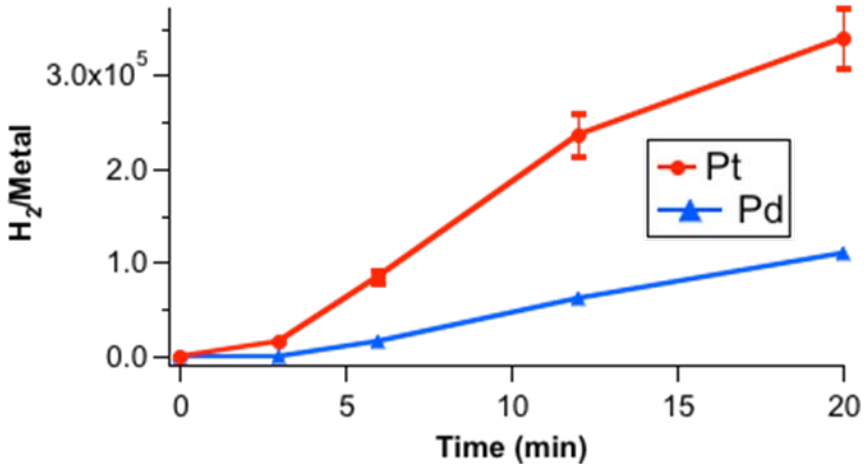


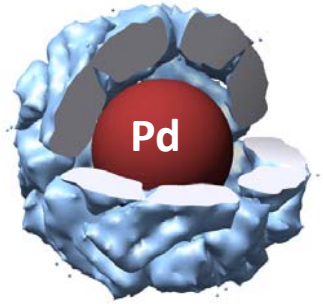




Mass spectra Pt^{2+} bound (black) and Pt^{0} (red) cages - loading of Pt^{2+} (0, 12, 24, 48, 100, and 200 Pt/cage).
Charge state 23+ are shown

Moving beyond Pt…
Pd Nanoparticles encapsulated with Ferritin as H_2 Catalysts



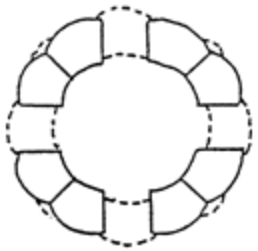


Schematic of Pd encapsulated within Ferritin

Pd particles show significantly lower activity than Pt

Stable to 80 ºC
Oxygen insensitive

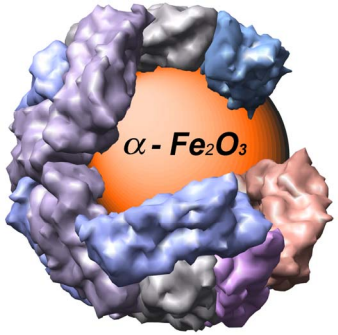
Photocatalyst synthesis - Hematite (α-Fe_2O_3) in ferritin



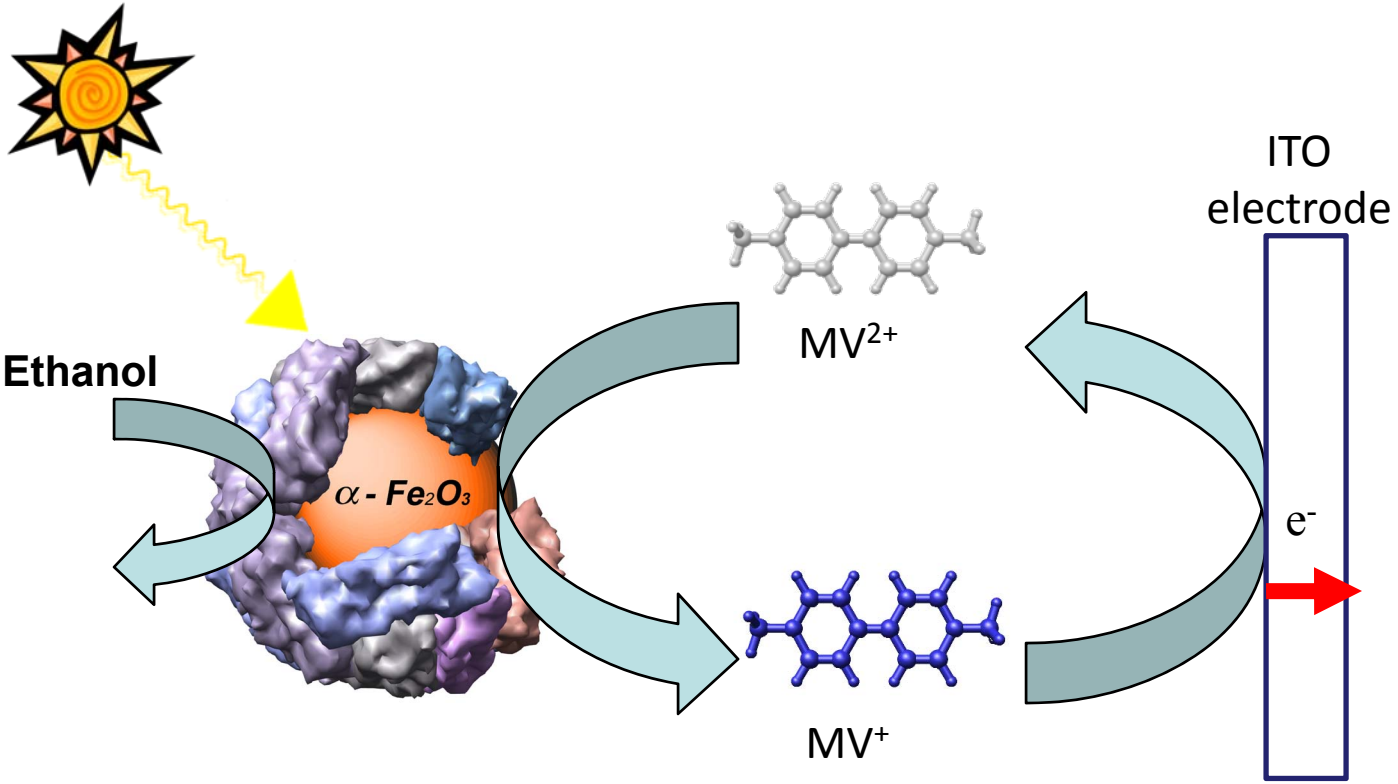
Apo-Ferritin

Fe^{2+}

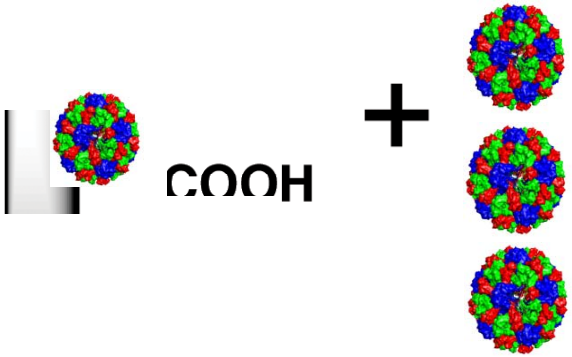
Controlled oxidation

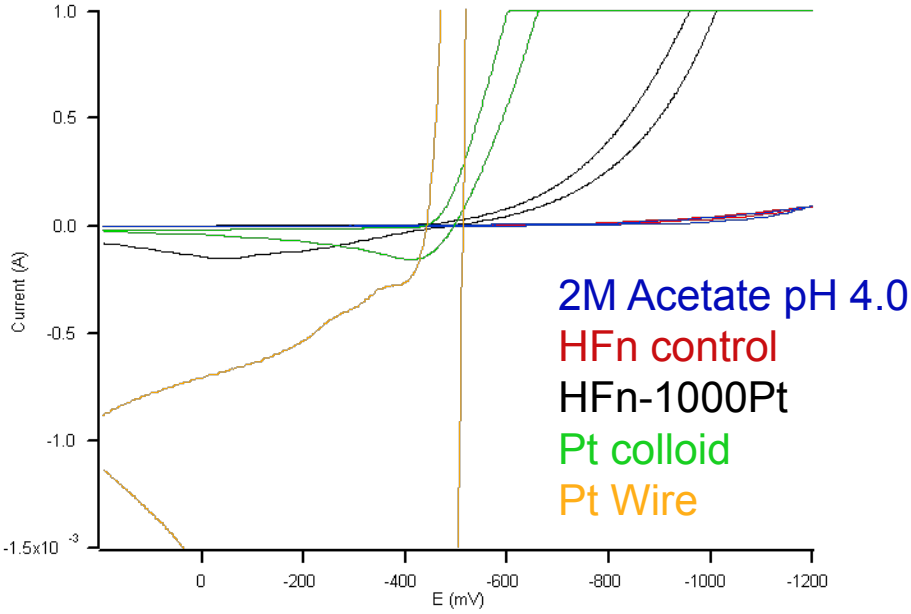


α-Fe_2O_3 is a stable, visible band-gap semiconductor

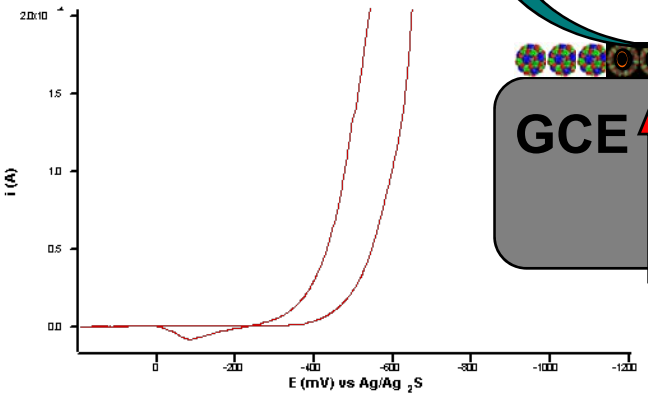


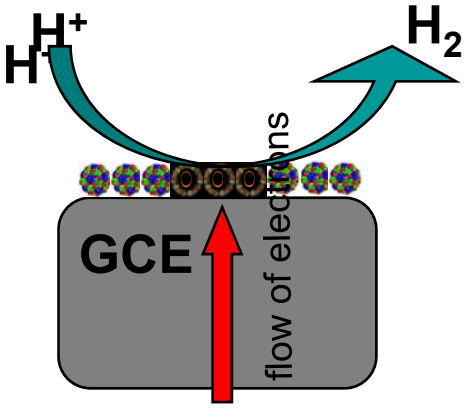
Attachment of catalysts to electrode surfaces – Cyclic voltammetry to probe e⁻ transfer to catalysts





Protein shell requires an overpotential of ~200mV compared to naked Pt colloid

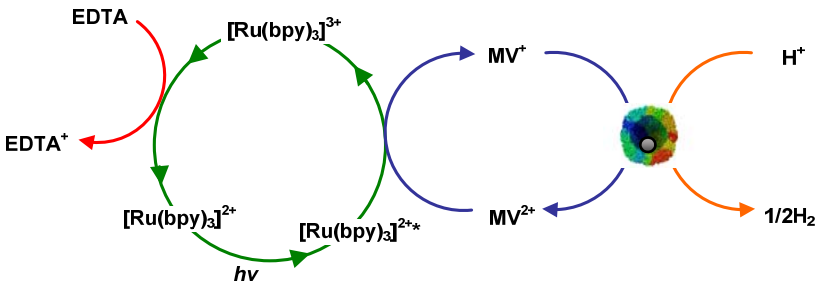


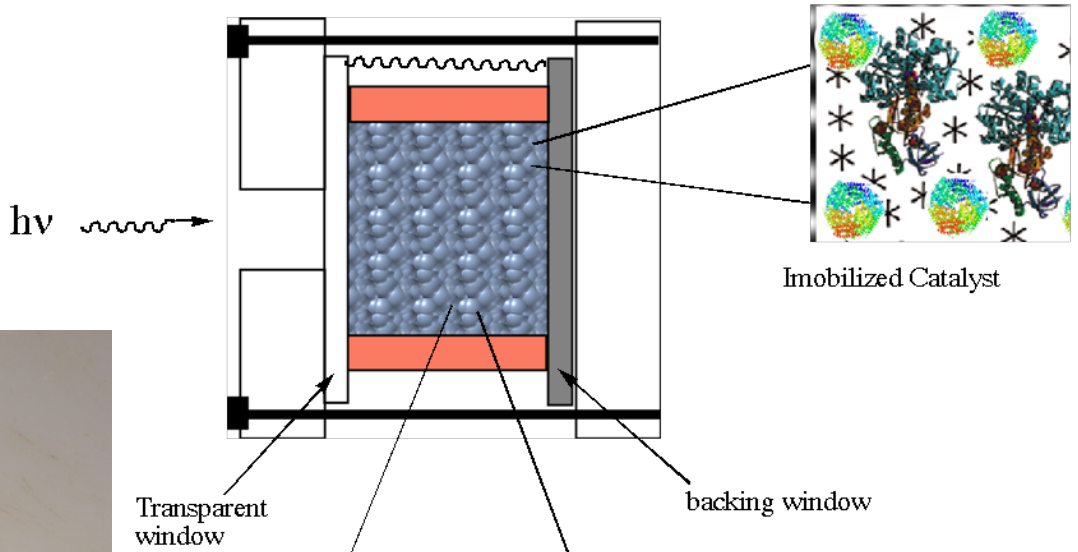


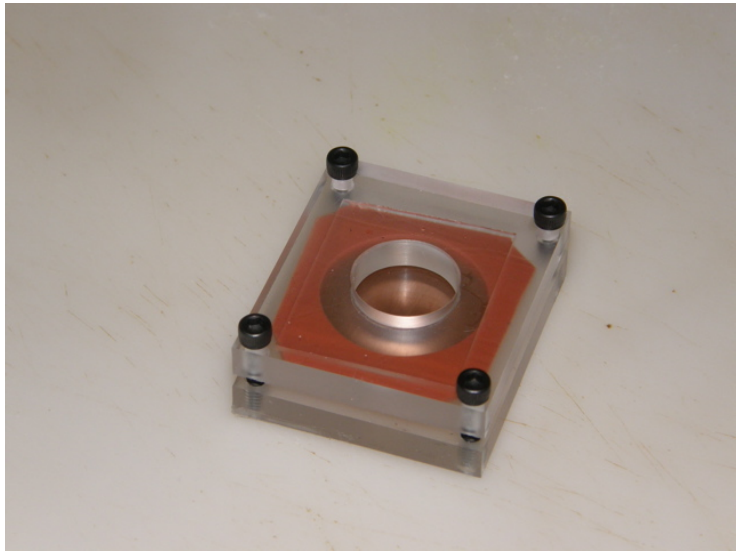
Attachment of MoS_x - protein cage to GCE - H_2 production

Design and fabrication of prototype devices

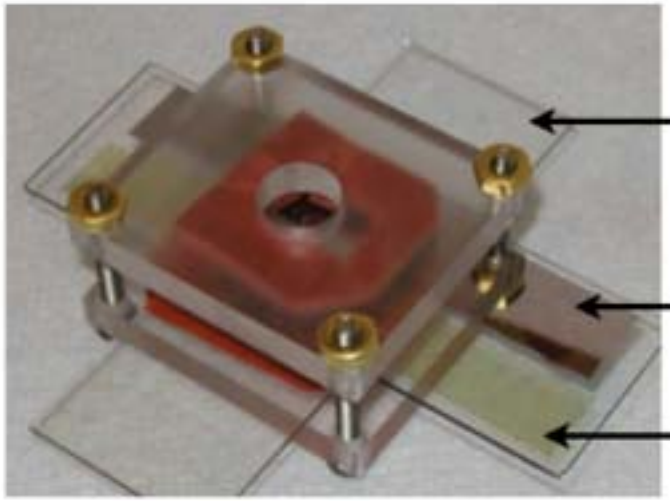
Based initially on the solution assay







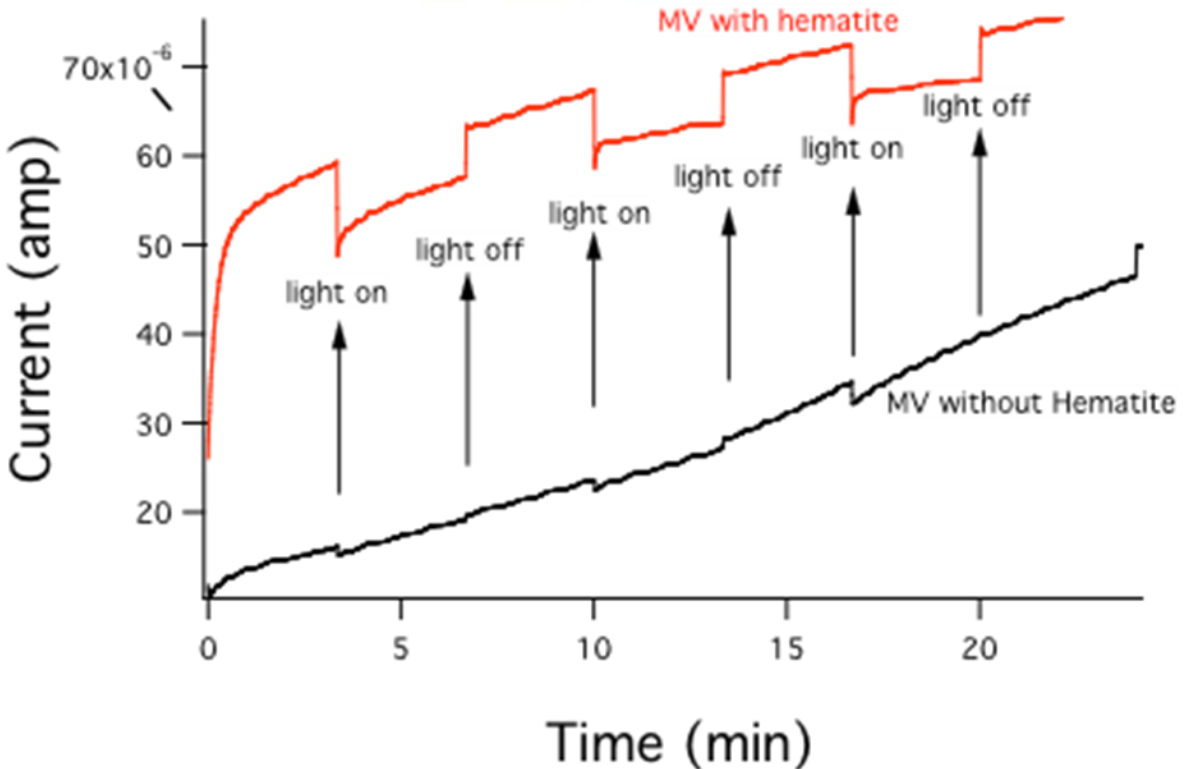
Testing - Measuring a Photocurrent with α-Fe_2O_3 Ferritin



Working electrode:
ITO coated glass

Reference electrode:
Ag strip

Counter electrode:
ITO strip



100 mV bias voltage

Summary

1) Hydrogenase can be immobilized in gels and retain activity
2) C-termini contributes to stability of stable hydrogenases
3) Carbon nanotubes enhance performance of hydrogenase/catalyst in gels
4) Pore size in gels can be controlled effecting mass transfer and hydrogenase/catalyst activity
5) Pt and Pd hydrogen production catalysts can be synthesized using biological templates
6) Photocataysts can be synthesized using biological templates
7) Catalysts can be attached to conducting surfaces
8) Prototype device has been fabricated and initial testing is underway

Future Work

1) Device testing and optimization
2) Establish benchmarks for hydrogen production efficiency
3) Evaluate hydrogen production efficiency (electrochemical, photochemical, chemical reducing equivalents)
4) Evaluate device for durability and sustained H_2 production