Light-Induced Water Splitting and Hydrogen Production in Nature: 
Blueprints for the Design of Chemical Catalysts
Chemical Energy Storage - Solar Fuel Production

Oxygenic Photosynthesis

- PS II
- ATP NADPH
- Rubisco
- CO₂ reduction
- Hydrogenase
- H₂ production
- "Biomimetics"
- 2 H₂O → 4 H⁺ + O₂↑

Biomimetic System

- Solar Fuel
- H₂-Cat
- 4H⁺
- Solar Fuel
- O₂-Cat
- Fuel cell
- 2 H₂O
- O₂, 4 H⁺

Molecular Concepts of Water Splitting: Nature’s Approach

Nicholas Cox, Wolfgang Lubitz, MPI for Chemical Energy Conversion, Mülheim/Ruhr
Hydrogen: Energy Carrier of the Future

Basic material for production of other energy carriers (e.g. methanol) other chemicals and other uses (e.g. ammonia)

Energy carrier

Production of Ammonia (Haber Bosch process)

Production of methanol

Food industry

Metallurgy

Steatochemistry

Refrigeration

Balloon gas

Welding technology

Electronics

Artificial production of diamonds

Glass industry
HYDROGEN PRODUCTION

1. **Hydrocarbons**
   Reformation of natural gas, coal

2. **Water electrolysis**
   Electricity from nuclear power or fossil resources
   Renewable energy (photovoltaics, wind ....)

3. **Thermal methods**
   Waste heat (nuclear power)
   Solar-Tower

4. **Biological / Biomimetic Methods**
   A. Modified natural systems (biophotolysis / photosynthesis)
   B. Semiartificial systems (enzymes on electrodes)
   C. Biomimetic systems (artificial photosynthesis, catalysis)
Photosynthesis: Structure and Function of Photosystem II and the Water Oxidizing Complex
**Oxygenic Photosynthesis**

**Equation:**

\[
\text{H}_2\text{O} + \text{CO}_2 \xrightarrow{\text{light, chlorophyll}} (\text{CH}_2\text{O}) + \text{O}_2 \uparrow
\]

- **Input:** Sunlight, water, carbon dioxide
- **Output:** Biomass, carbohydrates, oxygen

**Examples:**
- **Oxygen atmosphere:** 15 km
- **Ozone layer:** 50-80 km
- **Troposphere:** 0-15 km

**Products:**
- **Food:** Fruits, vegetables, grains
- **Raw materials:** Biomass, renewable
- **Fossil fuels:** Oil, coal, gas

**Additional Information:**
- **Chlorophyll**
- **H_2O**
- **CO_2**
- **O_2**
- **H_2**
- **O_3**
- **CH_2O**

**Diagram Key:**
- Sunlight
- Water
- Carbon dioxide
- Biomass
- Carbohydrates
- Oxygen
Photosynthesis: Light & Dark Reactions

Photosynthetic membrane

Light reactions

Antenna (Lhcb)

Stroma

Membrane

1/2 H₂O

1/4 O₂+H⁺

CO₂

H₂O

CO₂

O₂

Calvin cycle

CO₂ fixation to carbohydrates (CH₂O) enzyme Rubisco

dark reactions

NADPH

ATP

ADP+Pi

CF₁

PS II

Cyt b₆f

PS I

ATP synthase

CF₀
Electron Transport in Oxygenic Photosynthesis

**Z-Scheme**

**PS II**
- \( P680 \)
- \( \text{Pheo} \)
- \( Q_A \) to \( Q_B \): 300 ps
- \( 2H^+ \) to \( 2H^+ \): 3 ps
- \( P680 \) to \( P700 \): \( 90^\circ \)
- \( 2H^+ \) to \( \text{Q complex} \): \( < 300 \mu s \)
- \( \text{cyt b}_6f \) complex: \( < 1 \text{ ms} \)
- \( \text{cyt b}_6H \) and \( \text{cyt b}_6L \):
  - \( \text{cyt b}_6H \): \( < 1 \text{ ms} \)
  - \( \text{cyt b}_6L \): \( < 1 \text{ ms} \)
- \( \text{PC} \) to \( \text{PQ} \): \( < 300 \mu s \)

**PS I**
- \( \text{P700} \)
- \( A_0 \) to \( A_1 \): 30 ps
- \( F_A / F_B \) to \( F_d \): 800 ns
- \( F_d \) to FNR: 1-125 ms
- \( \frac{1}{2} \text{NADP}^+ + \frac{1}{2} \text{H}^+ \)

**Oxygenic Photosynthesis**
- \( 2 \text{H}_2\text{O} \) to \( \text{O}_2 + 4 \text{H}^+ \): 50 - 1200 \( \mu s \)
- \( \text{Mn}(\text{S}_\text{i}) \) to \( \text{Y} \): 20 - 250 ns
- \( \text{P680} \) to \( \text{P700} \): 3 ps
- \( \text{P700} \) to \( \text{P700} \): 300 ps
- \( \text{PC} \) to \( \text{PQ} \): 300 \( \mu s \)
- \( \text{P700} \) to \( \text{P700} \): 3 ps
X-Ray Crystallography: Structure of Photosystem II

Arrangement of water molecules

20 subunits
35 Chlorophylls
2 Pheophytins
11 Carotens
>20 lipids
2 plastoquinones
1 Fe, 2 hemes
Mn₄Ca-cluster

1300 water molecules in one monomer

T. vulcanus

Umena, Kawakami, Shen, Kamiya, Nature 473, 55-60 (2011)

Resolution 1.9 Å
X-Ray Crystallography: Structure of Photosystem II

Cofactor Arrangement: Reaction Center

Resolution 1.9 Å

Umema, Kawakami, Shen, Kamiya, Nature 473, 55-60 (2011)
Model Structures of the $\text{Mn}_4\text{O}_x\text{Ca}$ Cluster

X-Ray-Crystallography

3.5 Å Resolution (radiation damage)


X-Ray-Crystallography

2.9 Å Resolution (radiation damage)


X-Ray-Crystallography

1.9 Å Resolution („little“ radiation damage)

Umena et al., Nature (2011)
X-ray damage reduces the Mn$_4$(III$_2$IV$_2$) cluster (S$_1$ State) to Mn(II) and alters the geometric and electronic structure significantly.

Yano et al. 2005 PNAS
102, 12047-12052

Yachandra, Messinger et al. 2005
Radiation Damage in X-Ray Diffraction: Photoreduction

Photoreduction of higher metal oxidation states

Mn K-edge

A: 25% Mn(II);  3% total dose
B: 45% Mn(II); 15% total dose
C: 75% Mn(II); ~50% total dose

X-ray damage reduces the Mn$_4$(III$_2$IV$_2$) cluster (S$_1$ State) to Mn(II) and alters the geometric and electronic structure significantly

Yano et al. 2005 PNAS 102, 12047-12052
Imaging Biomolecules

Free Electron Laser
- $10^{12-13}$ photons
- 10 keV
- 10 fs pulse

particle injection

100 nm focus

Laue lens

$\sim 10^{21}$ W/cm$^2$

diffraction pattern

Avoid radiation damage (even at RT)

Explore time resolution

Structure of short-lived intermediates

Room temperature femtosecond X-ray diffraction of photosystem II microcrystals

Jan Kern\textsuperscript{ab}, Roberto Alonso-Mori\textsuperscript{b}, Julia Hellmich\textsuperscript{c}, Rosalie Tran\textsuperscript{a}, Johan Hattn\textsuperscript{e}, Hartawan Laksmono\textsuperscript{d}, Carina Glöckner\textsuperscript{c}, Nathaniel Echols\textsuperscript{a}, Raymond G. Sierra\textsuperscript{d}, Jonas Sellberg\textsuperscript{e,f}, Benedikt Lassalle-Kaiser\textsuperscript{a}, Richard J. Gildea\textsuperscript{g}, Pieter Glatzel\textsuperscript{g}, Ralf W. Grosse-Kunstleve\textsuperscript{b}, Matthew J. Latimer\textsuperscript{e}, Trevor A. McQueen\textsuperscript{b}, Dörte DiFiore\textsuperscript{b}, Alan R. Fry\textsuperscript{b}, Marc Messerschmidt\textsuperscript{b}, Alan Miahnahri\textsuperscript{b}, Donald W. Schafer\textsuperscript{b}, M. Marvin Seibert\textsuperscript{b}, Dimosthenis Sokaras\textsuperscript{a}, Tsu-Chien Weng\textsuperscript{a}, Petrus H. Zwart\textsuperscript{b}, William E. White\textsuperscript{b}, Paul D. Adams\textsuperscript{a}, Michael J. Bogan\textsuperscript{b,d}, Sébastien Boutet\textsuperscript{b}, Garth J. Williams\textsuperscript{b}, Johannes Messinger\textsuperscript{d}, Nicholas K. Sauter\textsuperscript{a}, Athina Zouni\textsuperscript{c}, Uwe Bergmann\textsuperscript{b,1}, Junko Yano\textsuperscript{a,1}, and Vittal K. Yachandra\textsuperscript{a,1}

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Edited by* Edward I. Solomon, Stanford University, Stanford, CA, and approved May 2, 2012 (received for review March 20, 2012)

Comparison of electron density computed from CXI data with SR data truncated to 6.5 Å resolution. Overview of the electron density (blue mesh) for one monomer of PS II computed from the CXI data (A) and from the truncated SR data (B). Protein is shown as cartoon in yellow; 2mFo-DFc electron density from the CXI data (C) or from the truncated SR data (D) is contoured at 1σ. View is along the membrane plane with cytoplasm at top. Electron density using a pulse width of 50 fs and a flux of $3.4 \times 10^{11}$ photons∕pulse at 9 keV. Resolutions of some highlighted Bragg spots are given in yellow and resolution at edges of the selected area of the detector are indicated by white dashed circles. The background was removed by subtracting the average image of 1,052 misses recorded directly before and after the crystal diffraction. (a) Enlarged view of an area in the top left corner of the diffraction pattern shown in B (marked by a blue box) to show highest resolution spots observed as orange sphere. The lumenal end of TMH c as well as helix cd and the C-terminal helix (eC) of D1 and the ef helix of CP43 are labeled. The 2mFo-DFc electron density map is contoured at 1σ (blue mesh); the difference density (mFo-DFc map) is shown at $±3σ$ (green mesh) and at $−3σ$ (red mesh).
Refinement of the OEC Structure

Upon geometry optimization O(5) relaxes to form Mn-Mn µ-oxo bridge.

Now Mn-Mn and Mn-O distances comparable with EXAFS.

Cooperation Frank Neese

Ames et al., JACS (2011)
PS II - Functional Model

**Activity Measurement:**
O$_2$ release via **Clark Electrode**

**Functional Test:**
O$_2$ release after 1-4 µs-light flashes:
**Joliot-Type Electrode**

Light

2 H$_2$O $\rightarrow$ O$_2$↑ + 4 H$^+$ + 4 e$^-$
Oxygen Evolution in PS II
induced by μs light flashes in spinach thylakoids

Activity Measurement:
O₂ release via Clark Electrode

Functional Test:
O₂ release after 1-4 μs-light flashes:
Joliot-Type Electrode

H₂O

O₂

S₀

S₁

S₂

S₃

S₄

5 state cycle

Kok, 1970

Joliot et al., 1969
thylakoids
pH 6.3, 4° C

Messinger et al., 1993

H₂O

H₂O
**Oxygen Evolution in PS II**

*induced by μs light flashes in spinach thylakoids*

**Activity Measurement:**

$O_2$ release via **Clark Electrode**

**Functional Test:**

$O_2$ release after 1-4 μs-light flashes:

**Joliot-Type Electrode**

---

**Kok, 1970**

---

**Messinger et al., 1993**

**Joliot et al., 1969**

**thylakoids**

pH 6.3, 4° C
Water Oxidation in Aqueous Solution and in PS II: Function of Water-Splitting Complex

Gibbs energy change / eV

aqueous solution

WOC

Messinger & Renger, 2008
1. Light-induced single electron transfer \((ps)\) coupled to 4-electron water oxidation process \((ms)\)-storage of oxidation equivalents

2. Redox leveling at the catalyst: small and equal steps!

3. Electron-proton coupling at the catalyst – charge balance

4. Interfacing: via a redox-active tyrosine \(Y_z\)

5. Water binding, orientation and O-O bond formation – catalysis mechanism

6. High oxidative power of P680\(^{\bullet\bullet}\) - protection

7. Chl triplets and singlet oxygen (D1 damage) - repair/replacement
Assembly and Repair: Replacement of D1 Protein in PS II

D1 half lifetime in the light 30 min!
Electronic Structure of the S-States: EPR

Mn(II)  S=5/2
Mn(III) S=4/2
Mn(IV) S=3/2

\[ \text{(Mn}^{\text{III}})_{3}\text{(Mn}^{\text{IV}}) \]

\[ \text{S}_{0} \]

\[ \text{H}^{+} \]

\[ \text{S}_{0} \]

\[ \text{S}_{1} \]

\[ \text{(Mn}^{\text{III}})_{2}\text{(Mn}^{\text{IV}})_{2} \]

\[ \text{S}_{2} \]

\[ \text{S}_{3} \]

\[ \text{(Mn}^{\text{III}})_{2}\text{(Mn}^{\text{IV}})_{3} \]

\[ \text{S}_{4} \]

\[ \text{(Mn}^{\text{IV}})_{4} \]

\[ \text{O}_{2} \]

\[ \text{H}_{2}\text{O} \]

\[ \text{H}_{2}\text{O} \]

\[ \text{H}^{+} \text{e}^{-} \]

\[ \text{H}^{+} \text{e}^{-} \]

\[ \text{e}^{-} \]

\[ \text{S}_{0} \]

\[ \text{S}_{1} \]

\[ \text{S}_{2} \]

\[ \text{S}_{3} \]

\[ \text{S}_{4} \]

S-states differ by one electron oxidation

I \(^{55}\text{Mn}\)=5/2 → Hyperfine Structure

Messinger et al./Ahrling et al. 1997

Dismukes and Siderer, 1981
Metal Centers and Radicals in Proteins

PELDOR-DEER

- Distance between electron spins
- Magnetic Field

ELDOR-detected NMR

- HFC of strongly coupled nuclei

Davies ENDOR

- HFC and NQC determination

Mims ENDOR

- HFC and NQC determination

Echo Envelope Modulation (ESEEM)

- ESE-detected EPR

HYSCORE

- Weakly coupled nuclei (hyperfine & quadrupole)

Field-Swept Pulse EPR

*High Frequency / High Field*

- FID-detected EPR

Electron-Nuclear-Nuclear Triple Resonance

- Signs and assignments

Pulse EPR & Multiresonance Techniques

EPR spectra, ns time resolution

Echo Envelope Modulation

- ESE-detected EPR

High Frequency / High Field

FID-detected EPR
There are no $^{55}$Mn ENDOR signals above ~180 MHz; all signals fall in the 60-180 MHz range.

$\text{Mn}^{\text{II}}$ not present in the S states.

The spin projection coefficients must all be approximately 1 ($\rho \sim 1$) (see also EPR simulation).

$\nu_H$, ENDOR intensity, a.u.

$\text{Q-Band}$

$\text{X-Band}$

$\text{Mn}_{\text{X}}$

$\text{Exp}$.

$\text{Sim}$.

$\text{Mn}_{\text{X}}$

$\text{EPR intensity, a.u.}$

$\text{width}$

$\text{Mn}^{\text{II}}$ not present in the S states

$\text{EPR intensity, a.u.}$

$\text{Mn}_{\text{X}}$

$\text{Mn}_{\text{A}}$

$\text{Mn}_{\text{B}}$

$\text{Mn}_{\text{C}}$

$\text{Mn}_{\text{D}}$

$\text{Exp}$

Simulations:

- 4 nuclei all
- 3 nuclei B,C,D
- 2 nuclei C,D

$g_\parallel = 1.96$

$g_\perp = 2.00$

$\text{Peloquin et al., JACS (2000)}$

$\text{Kulik et al., JACS (2005, 2007)}$
Electronic Structure: Multiline EPR and $^{55}$Mn ENDOR

Electronic Structure
Exchange Coupling Scheme

Oxidation States ($S_2$)

Structural Model ($S_2$)

Pantazis et al. PCCP (2009)
Cox, Rapatskiy et al. JACS (2011)
Ames et al. JACS (2011)
Lohmiller et al. JBC (2012)
All oxygens (i.e. possible substrates) EPR/NMR silent

[Chemical structures and labels indicating exchange sites and enrichment]

3x exchanged (in dark, S1 state) 1 hour 75% enrichment (final)
ENDOR versus ELDOR-detected NMR (EDNMR)

A) Davies ENDOR

B) ELDOR-detected NMR

$^{17}$O EDNMR vs. ENDOR

$W$-band (94 GHz)

Rapatskiy et al., J. Am. Chem. Soc. in press
Three $^{17}$O species are required to reproduce the entire envelope

1. Weakly coupled water (matrix) – Ca-W1/W3/W4  
2. Intermediary coupled water (Mn bound) – Mn$_{A4}$-OH (W2)  
3. Strongly coupled water ($\mu$-oxo bridge)
Rapid Dilution in H$_2^{17}$O; the same signal envelope is seen!

All three $^{17}$O species are seen after dilution in H$_2^{17}$O (1x) and rapid freezing (<15 s)

1. **Matrix** – Ca-W3/W4 and W1
2. **Mn bound** – Mn$_{A4}$-OH/OH$_2$
3. **μ-oxo bridge** – O5

---

**Graphical Representation**

- W2
- W1, W3, W4
- O4 or O5
- His332

**EDNMR amplitude**

- 15 s dilution
- PSII-Ca
- PSII-Sr

**Graphical Structure**

- W1, W3, W4, O4, O5, His332
- Mn$_{A4}$
- OH/OH$_2$
- μ-oxo bridge
- S$_2$
Possible Mechanism of Water Oxidation

I: Nucleophilic attack

\[
\begin{aligned}
\text{Mn}^{IV} &= O^* \\
\text{or} &
\text{Mn}^{V} &= O \\
\text{or} &
\text{H}_2\text{O-}\text{Ca} \\
\text{or} &
\text{HO-}\text{Ca}
\end{aligned}
\]

O5 + W3

* or O5 + W2

O5 + WX

II. Oxo/oxylation radial coupling
Why is O5 exchangeable on a seconds timescale?

- O5 is a μ-oxo bridge to the Ca (Ca tunes pKₐ of bound water(s))
- O5 has a flexible coordination sphere

'S = 4.1 state'
\( S_G = 5/2 \)

Simulation
\( g = 4.1 \)
\( g = 2.0 \)

X-band \( h\nu = 0.3 \text{ cm}^{-1} \)

'S = multiline state'
\( S_G = 1/2 \)

Neese Cooperation

Pantazis et al., Angew. Chem. (2012)
Lessons from Nature: A light-driven water splitting machine

1. **Choice of the catalyst**: earth-abundant metals (Mn, Ca) with optimized physicochemical properties and simple (bridging) ligands (O) forming a cage-like/dangler structure.

2. **Nuclearity of cluster**: 4 Mn for storage of oxidizing equivalents – charge accumulation: coupling of one-electron charge separation to four-electron water oxidation.

3. **Redox leveling** at the catalyst: avoiding high-energy intermediates during the reaction.

4. **Co-metal**: Ca for water binding/delivery.

5. Correct and precise sequential **substrate water binding** in juxtaposition at the metals, deprotonation and preparation for O-O bond formation.

6. Substrate water „integration“ in the cluster: active **participation of the catalyst**.

7. Proton coupled electron transfer **PCET**: stepwise H⁺ release for charge neutrality.

8. Efficient **interfacing** of ET chain and catalyst via Yz – lowering the overpotential.

9. **Smart Matrix**: correct binding Mn₄Ca-cluster, channels for educts & products, proton management; electrostatic environment, ET and catalyst dynamics, protection - all that providing **high efficiency** (TOF) of the catalyst.

10. **Self assembly** of the catalyst; efficient repair “self healing“ in case of damage – provides good **stability** and long **lifetime**.
Hydrogenases: Spectroscopy & Electrochemistry, Structure, Function and Oxygen Sensitivity
Two Main Classes of Hydrogenases

[**NiFe**] Hydrogenase

Volbeda et al., 1995
Higuchi et al., 1997

\[
\text{H}_2 \Leftrightarrow \text{H}^+ + \text{H}^+ \Leftrightarrow 2\text{H}^+ + 2\text{e}^-
\]

Desulfovibrio vulgaris Miyazaki F, PDB 1WUI

[FeFe] Hydrogenase

Peters et al., 1998
Nicolet et al., 1999

\[
\text{H}_2 \Leftrightarrow \text{H}^+ + \text{H}^+ \Leftrightarrow 2\text{H}^+ + 2\text{e}^-
\]

Desulfovibrio desulfuricans, PDB 1HFE

---

Ogata et al. (2005) Structure v13 pp. 1635-42
Biological Functions of Hydrogenases

[NiFe]-hydrogenases

- $\text{H}_2$ oxidation
  - Re-generation of reducing equivalents (SH)
  - Energy generation (MBH)
  - $\text{H}_2$ sensing (RH)

[FeFe]-hydrogenases

- $\text{H}_2$ evolution
  - Disposal of reducing equivalents

Activity:
- $10^2 - 10^3$ [µmol H$_2$/min* mg protein]
- $10^3 - 10^4$ [µmol H$_2$/min* mg protein]

$\text{H}_2$ uptake $\leftrightarrow$ evolution $\rightarrow \text{2 H}^+ + 2 \text{e}^-$
The most active [FeFe] hydrogenases produce 10,000 molecules $H_2$ per second at 30°C.

‘... one mole of such a hydrogenase could produce enough hydrogen to fill the Graf Zeppelin in 10 minutes......’

Initial Questions (AC)

- **intermediates** of the reaction cycle: **redox states** of active site and ET components.

- **Formal** oxidation and spin states of the metal ions
  **Electronic configurations** of the ground states.

- Identification and function of the **diatomic ligands** at the Fe and the bridging ligand X.

- **Intermediates** in the reaction cycle and their **geometry**

- **Binding site** of the substrate **hydrogen**.

- **Effect of light** on the hydrogenase intermediary states.

- Impact of the **protein surrounding**.

- Mechanism of **enzyme inhibition** (e.g. by O_2 or CO) and **oxygen tolerance**.

- **Mechanism of activation/deactivation** and reversible **hydrogen conversion** by hydrogenases

- **Structural basis** for enzyme activity (TOF) and lifetime (TON)
Desulfovibrio vulgaris Miyazaki F, PDB 1WUI

Desulfovibrio desulfuricans, PDB 1HFE
Hydrogenase Structure

\[
\text{H}^+ + \text{H}^- \rightleftharpoons \text{H}_2
\]

Structure of the active (hydrogen-carrying) intermediate

*Hyperfine Spectroscopy: ENDOR & ESEEM*

Distance between Ni and H\(^+\) is \(d = (1.7 \pm 0.1) \text{ Å}\) and \(d = (1.8 \pm 0.1) \text{ Å}\) between Fe and H\(^-\)

Ni-C (H/D exchange)

*Brecht et al., JACS 2003*
EPR and HYSCORE Spectra of Ni-C and Ni-L
H/D Exchanged NiFe-Hydrogenase RH of *R. eutropha*

ENDOR of Ni-L: H/D Exchanged, RH of *R. eutropha*

Ni-L2
Regulatory Hase

Pulse Q-band
$^1$H ENDOR
position $g_y$
$T = 10$ K

- H$_2$O/H$_2$, $h\nu$
- D$_2$O/D$_2$, $h\nu$
- Difference

Flores et al. unpublished
Hydrogen Conversion Mechanism of [NiFe]-Hydrogenases


Rauchfuss et al. 2009
Shafaat et al. 2012
Weber et al. 2012, JACS
Reaction Scheme for Standard [NiFe] Hydrogenases

[Diagram showing the reaction scheme with various states and reaction steps involving [NiFe] and [4Fe4S] clusters, including light-induced states, active states, and CO-inhibited states.]

[NiFe]- und [FeFe]-Hydrogenases

[NiFe] Hydrogenase

\[ \text{H}_2 \rightleftharpoons \text{H}^- + \text{H}^+ \rightleftharpoons 2\text{H}^+ + 2e^- \]

Volbeda et al., 1995
Higuchi et al., 1997

Desulfovibrio vulgaris Miyazaki F, PDB 1WUI

[FeFe] Hydrogenase

Peters et al., 1998
Nicolet et al., 1999

Desulfovibrio desulfuricans, PDB 1HFE
$^{14}$N in the dithiolate bridge: Implications for the $\text{H}_2$ splitting mechanism

$^{14}$N HYSCORE

$^{14}$N detected via pulse EPR

Silakov et al. *PCCP* (2009)

$H_{\text{ox}}$: Fe(I)$_p$Fe(II)$_d$

$p$

$H_{\text{red}}$: Fe(I)$_p$Fe(I)$_d$

$[4\text{Fe}4\text{S}]_\text{H}$

Cys

OC

NC

CO

CN

[2Fe3S](PMe₃)₂ complex, ¹⁴N & ¹⁵N HYSCORE

Ö Erdem, et al.  

Cooperation S. Ott (Uppsala)

<table>
<thead>
<tr>
<th></th>
<th>active site model compound</th>
<th>native system Hₐ₀ active site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aₙ (x, y, z) [MHz]</td>
<td>(0.3, 0.8, 0.7)</td>
<td>(1.0, 1.9, 1.4)</td>
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<tr>
<td>A iso [MHz]</td>
<td>0.6</td>
<td>1.43</td>
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<tr>
<td>K [MHz]</td>
<td>1.28</td>
<td>1.23</td>
</tr>
<tr>
<td>η</td>
<td>0.11</td>
<td>0.13</td>
</tr>
</tbody>
</table>
[FeFe] Hydrogenase: Proposed Catalytic Mechanism

H ox-CO

Fe I Fe II [CO]

2+ + CO

Fe I Fe II [ ]

H ox

Fe I Fe II [ H2]

2+ - H2

Fe I Fe I [ ]

H red

Fe I Fe I [ ]

2+ + H2

Fe I Fe I [ H+]

H sred

Fe I Fe I [ ]

2+ - H+ + H+

Fe I Fe I [ H+]

H

S

N

C

O

2+

Fe I Fe II [CO]

A. Adamska et al.  

Cooperation T. Happe (Bochum)
Essential components of the hydrogen producing active site of [FeFe] hydrogenase:
Design criteria for artificial hydrogenase catalysts:

Reaction: \[ \text{H}_2 \rightleftharpoons \text{H}^+ + \text{H}^+ \rightleftharpoons 2\text{H}^+ + 2\text{e}^- \]

A. **Metal center at optimal potential and spin state** (CN/CO ligands) to polarize \( \text{H}_2 \) for heterolytic splitting (open coordination site!) and accept hydride as ligand (hydride acceptor ability)

B. **Adjacent base** to accept proton (\( \text{H}^+ \) acceptor ability) in concert with metal

C. **Energetic matching** of metal and base acceptor reactions to ensure reversibility, avoid high energy intermediates and thereby achieve **higher rates** and **lower overpotentials** for both reactions

D. Provide efficient delivery of educts and transport of products (solvent; water; **smart matrix**) and efficient, optimized **electron transport chain**

E. Solve problem of **oxygen sensitivity**

F. Solve problem of degradation (**self-repair, self-healing, simplicity**)  

F. Integrate into a **device** for (sun) light harvesting, charge separation/transport, water oxidation catalysis – including a membrane system (\( \text{H}^+, \text{e}^-/\text{h}^+ \) movement)
A Synthetic Nickel Electrocatalyst with a Turnover Frequency Above 100,000 s\(^{-1}\) for H\(_2\) Production

Monte L. Helm,\(^{1,2}\*\) Michael P. Stewart,\(^1\) R. Morris Bullock,\(^1\†\) M. Rakowski DuBois,\(^1\) Daniel L. DuBois\(^1\†\)

[Diagram of chemical structures 1-6]
Reversible Electrocatalytic Production and Oxidation of Hydrogen at Low Overpotentials by a Functional Hydrogenase Mimic**

Stuart E. Smith, Jenny Y. Yang,* Daniel L. DuBois,

Previous studies in our laboratory have focused on the synthesis and catalytic performance of functional hydrogenase mimics. Our studies have focused on iron, cobalt, and nickel complexes with the diphosphine ligand \( \text{PR}_2\text{NR}_2 \) shown in Scheme 1, where \( R \) is the substituent on phosphorous, and \( R' \) is the substituent on nitrogen. From our experimental and theoretical studies on \([\text{Ni(PR}_2\text{NR}_2)_2]^{2+}\) derivatives, we have established how steric and electronic properties of the \( R \) and \( R' \) substituents affect the hydride acceptor and proton acceptor abilities of these complexes. These two quantities determine the free-energy of hydrogen addition to these complexes. The ability to vary these properties by varying substituents on the ligand has led to the design of catalysts that are biased towards hydrogen production or hydrogen oxidation by adjusting the free-energy for hydrogen addition to be either positive or negative, respectively.
Some Unsolved Problems:

1. Oxygen sensitivity of catalysts

2. Self-assembly & self-repair - long term stability

3. Fast rates TOF

4. Low overpotential

5. Interfacing (e.g. to electrodes) – development of linkers

6. Combination with water oxidizing catalysis

7. Combination with (sun) light induced processes (antenna, charge separation etc.)

8. Costs – materials, difficulty of synthesis
Oxygen Inhibition of Hydrogenases

[NiFe] Hydrogenase *D. vulgaris* MF

- E=+150 mV vs NHE
- 40°C, pH 6.0
- 2500 rpm, 1 bar H₂

- [O₂] = 15 µM

[NiFe] Hydrogenase *A. aeolicus*

- E=+150 mV vs NHE
- 40°C, pH 6.0
- 2500 rpm, 1 bar H₂

- [O₂] = 59 µM
- [O₂] = 108 µM
- [O₂] = 205 µM (sat at 40°C)
1. Narrower gas access channel

2. Blockage of active site by additional ligands (CN⁻)

3. Modification of redox potentials of the active site and the electron transport components (proximal 4Fe4S-cluster)
EPR Redox Titration of the FeS Clusters in *A. aeolicus*

4 distinct EPR species

- 4: -173 mV
- 3: +12 mV
- 2: +234 mV

Magnetic Field (mT)

Redox potential, mV vs NHE

- 4 e⁻ (I)
- 1 e⁻
- 1 e⁻

Pandelia et al., PNAS 2011

Redox potential, mV vs NHE

[NiFe] [Fe₄S₄] [Fe₃S₄] [Fe₄S₄]

- S(1/2)
- S(1/2)
- S(1/2)
- S(1/2)

[Green] [2+] [1+] [2+]
[Red] [1+] [0] [2+] [1+]
Proximal 4Fe4S-cluster in A. aeolicus

Electron transfer chain in standard enzymes

Sequence Alignment Showing Binding of the Proximal FeS Cluster

<table>
<thead>
<tr>
<th>A. vinosum</th>
<th>T. roseopersicina</th>
<th>D. vulgaris</th>
<th>D. gigas</th>
<th>D. desulfuricans</th>
<th>D. fructosovorans</th>
<th>D. baculatum</th>
<th>A. aeolicus</th>
<th>R. eutropha MBH</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAVIAVCTAAGGCACGPAPNPTGAMSVMDLVRD...KPVNPGECPPI</td>
<td>CKAVIASWCGASWCGQQARPPNPTPATPHHEVRD...KPIVKGCPCPI</td>
<td>AQAVIAVCTAAGGCACGPAPNPTGAMSVMDLVRD...KPVNPGECPPI</td>
<td>AKAVIATGGCGGACGPAPNPTGAMSVMDLVRD...KPVNPGECPPI</td>
<td>AKAVIATGGCGGACGPAPNPTGAMSVMDLVRD...KPVNPGECPPI</td>
<td>AKAVIATGGCGGACGPAPNPTGAMSVMDLVRD...KPVNPGECPPI</td>
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</tr>
</tbody>
</table>

Proximal 4Fe4S-cluster in A. aeolicus

[Fe₄S₄] distal

[Fe₃S₄] medial

[Fe₄S₄] proximal

[Fe₄S₄] site

Cys19
Cys17
Cys20
Cys149
Cys120
Cys115
Proximal [4Fe-3S] Cluster: Mössbauer Analysis

Reduced
\[ S_{\text{tot}} = 1/2 \]

Oxidized
\[ S_{\text{tot}} = 0 \]

Superoxidized
\[ S_{\text{tot}} = 1/2 \]

Pandelia et al. (2013)
PNAS
Hydrogenase Catalysis in the Presence of Oxygen

Hydrogenase

Oxygen Reductase Oxidase

H^+ \rightarrow e^- \rightarrow \text{H}_2 \text{O}

H^+ \rightarrow e^- \rightarrow \text{H}_2 \text{O}

\[ \text{Ni}^{III} \text{Fe}^{II} \text{S}_2 \text{S}_2 \text{H}^- \rightarrow \text{Ni}^{II} \text{Fe}^{II} \text{S}_2 \text{S}_2 \text{H}^+ \rightarrow \text{Ni}^{III} \text{Fe}^{II} \text{S}_2 \text{S}_2 \text{OH}^- \]

Proximal cluster [4Fe-3S]-6Cys

Acknowledgements

Hydrogenases

[FeFe] Hydrogenases and related model systems:

Rüdiger, Ogata, Kellers, Pandelia, Flores, van Gastel, Stein, Bill, Fichtner, Shafaat, Nishikawa, Weber

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Thank you for your attention!