Utilizing Photosynthetic Complexes for Solar Energy Conversion: Building a Bio-generator

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Acknowledgements

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The Driving Force for our Research
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| Tyres | Schwalbe Big Ben 55-559/26 x 2.15 |
| Rims | Sun Ringle Track 26* |
| Pedals | Wellgo Co98 Blk |

### H2 System

| Weight of fuel cell system | 3.7 kg |
| Max. working pressure of cylinder | 340 bar |
| Storage capacity | 33 gr H2, corresponding to 1,000 Wh |
| Range per cylinder filling | > 100 km |
| Fuelling time | 1 – 6 min |
| Fuel cell lifetime | 5 years |
| Fuel cell efficiency | ~ 50% |
| Buffer battery | 60 Wh |
Photosynthesis for solar energy conversion
Present and Future Technologies

- Biomass (combustion)
- Biofuels (combustion)
- Artificial photosynthesis (organic/inorganic compounds, heavy/rare metal compounds)
- Photosynthetic components (isolation of complexes, chemical tethering of complexes to electrochemical system)
The Oxygenic Photosynthetic membrane

20-200 mA/mg chlorophyll at full activity
1 mg chlorophyll = ~ 1 leaf
Oxygegenic Photosynthesis provides the strongest oxidant (PSII) and most negative reductant (PSI) in Nature

$H_2$ production @ -0.55 V vs. Ag/AgCl (pH=6)

Z diagram of the electron transport in the photosynthesis process
Photosynthesis works well from low to high light intensities – giant antennas with dynamic EET control.
The photosynthetic apparatus is “sealed” within compartments and performed in membranes.
Green machines for harnessing photosynthesis for solar energy conversion to hydrogen

Light

H_2O → Photosynthetic membranes

Electron carriers

Electrical current or reducing power

Photosynthetic membranes

H_2, O_2
Prerequisites for the engineering of a useful bio-generator

1. A Photoautotrophic organism – so that it can be grown cheaply in large quantities.
3. No expensive, polluting isolation or synthetic steps.
4. Stability for a useful amount of time.
Our original idea – shuttling electrons from a mutated PSII with a protein mediator

Goal #1: Identification of mutation site

Goal #2: Site directed mutagenesis creates photoautotrophic mutants with near-normal growth and photosynthetic activity rates

Goal #3: Membranes from mutants reduce cytochrome c in the presence of DCMU (inhibitor) and are fully active

Goal #4: Electron transfer to cytochrome c protects PSII activity from photoinhibition

Larom S. et al., PNAS (2010)
Our original idea – cyt. c shuttles electrons from PSII

Larom et al. Photosynthesis Research 2015
Photocurrent generation is greater in the absence of cytochrome c.

Dependence of photocurrent in BPEC -1 on Cyt c concentration. Photo-chronoampermetric measurement was performed to Glu membranes in the presence of DCMU with the addition of 0, 0.5 or 1mg/ml cytochrome c. Insert: The photocurrent decreases linearly with cyt c concentration.

Larom et al. Photosynthesis Research 2015
Gently treated cyanobacteria generates the maximum and the fastest photo-current

Syn
- Growth media

OsSyn
- Osmotic Shock

iSyn
- Microfluidizer

mSyn
- French-press

No treatment or too strong treatment $\Rightarrow$ no current

Saper et al. under review 2018
Low pressure microfluidic treatment does not fully break the cell membranes

- Syn: Growth media
- OsSyn: Osmotic Shock
- iSyn: Microfluidizer
- mSyn: French press

λExcitation:
- PBS: 633nm
- Chl: 458nm

λEmission:
- > 650nm

Image descriptions:
- Syn: Growth media
- OsSyn: Osmotic Shock
- iSyn: Microfluidizer
- mSyn: French press
iSyn are living and multiplying cells.

(A) Growth curve of Syn (green), iSyn (black) and iSyn that were illuminated for 30 min in the BEPC (blue), in liquid medium was examined by the absorption at 750nm. The vertical lines show the standard deviation values (n=5). (B) Colony formation test for Syn, iSyn and photosynthetic membranes (mSyn). Syn, iSyn and mSyn were plated at serial dilutions on agar plate containing growth medium. The equivalent amount of cells or membranes containing 1 ng of chlorophyll was plated in the lanes marked 1.
Major electron source is the respiratory system - carbohydrates oxidation

Photosynthesis

O₂ H₂O

PSII OEC

P680

QA

QB

DCMU

Cyt. b₆f complex

Cyt. c₆

PSI

P700

ferredoxin

NADPH

Glycolysis

Glucose

G3P

Acetyl coA

TCA cycle

Oxidative Phosphorylation

NADH

FADH₂

PSII

PSI

DCMU

O₂

FADH₂

NADPH

O₂

H₂O
A soluble diffusive mediator smaller than 3 kD is secreted

Figure 4: iSyn electron transfer to the BPEC is via a diffusive endogenous mediator. (A) CV in the light for buffer (black) and the iSyn (blue) separated by a 3 kD membrane on the graphite electrode. (B) CV of supernatant fraction from centrifuged iSyn cells separated by filtration (see SI Methods for details). Filtrate (solid blue line), retentate (dashed blue) and buffer (black). (C) Illustration of the scanning electrochemical microscopy set up. iSyn settled on the working electrode (WE) electrode. The counter electrode (CE) is platinum, and the reference electrode (RE) a Ag/AgCl/3M KCl. The tip is a carbon-based microelectrode with a mixture of bilirubin oxidase (BOD) embedded in an Os-complex modified polymer matrix. All electrodes are connected to the bipotentiostat. (D) CA of the iSyn (blue) and the BOD tip current (red) measuring the oxidation of the mediator at a distance of 30 μm from the graphite electrode.
Electron transfer via a diffusive mediator smaller than 3 kD
The mediator release is light induced
The mediator is probably a quinone or a flavin derivative

Hydrogen is evolved on the cathode at a lower voltage than in water electrolysis.

(A) Schematic drawing of the working set up for the hydrogen evolution measurements. The CA was measured at 50 mV (vs. Ag/AgCl/3M NaCl) which corresponds to a voltage of 650 mV between the anode and cathode. (B) Simultaneous CA measurement of photocurrent (blue) and GC measurement of hydrogen production (red) measured as a function of time for the iSyn at 50 mV (vs. Ag/AgCl/3M NaCl). (C) Schematic drawing of the electron flow from carbohydrates (internal or external) via the respiratory, light driven activity of PSI, and an endogenous diffusive mediator to form hydrogen gas. The multiple electron transfer steps between the PQ and PSI are shown as multiple curved arrows for simplicity.
Plant thylakoids require a mediated electron transfer - ferricyanide

Photosystem 2  \( b_{6f} \) complex  Photosystem 1  ATP synthase

Electrode

Roy Pinhassi  Dan Kallmann  Gadiel Saper
Plant membranes provide high currents and H₂ in BPEC with 0.5V bias.

Ferricyanide mediates currents of up to 0.5mA/cm² (100mg chlorophyll). Membranes are not physically attached to electrode and can be easily replaced for extended use.

Photocurrent, measured at an electrode potential of 0.5VAg/AgCl, as a function of time during exposure to solar-simulated light. The inset shows the long-term photocurrent stability in high (3 mM, full line) and low (0.3 mM, dashed line) Fe(III)CN concentrations. The arrows indicate light turn on (up) and off (down) points.

Batch mode of operation wherein damaged thylakoids were replaced with fresh ones every 10 min. All the other cell components were reused.

Scanning electron microscopy image of the spinach membranes on the surface of a FTO coated glass electrode.

Pinhassi et al. Nature Communications 2016
Plant membranes provide high currents and \( \text{H}_2 \) in a stand alone BPEC.

Photocurrent (black curve) and anode (blue squares) and cathode (red triangles) potentials as a function of the applied bias between the anode and cathode. The dashed line represents a potential of 0V RHE.

A tandem stand-alone \( \text{H}_2 \) evolution BPEC with PV absorbing IR.

Hydrogen evolution at the cathode in the presence (red squares) or absence (black diamonds) of the herbicide DCMU.

Pinhassi et al. Nature Communications 2016
PQ pool is the electron donor to Fe(III)CN.
Summary: Electron transfer to the electrode from plants and from cyanobacteria

**Plants**
- Simple preparation of the spinach thylakoids
- Demand for an exogenous mediator
- High potential (300 mV vs. Ag/AgCl)
- Current of 0.5 mA*cm^-2
- Short life-time
- Electron source: water
- Electron extraction site: PQ pool – cyt B₆F complex
- FTO and PV cell enable stand alone BPEC without added bias

**Cyanobacteria**
- Simple preparation of the cells
- Transfer of electrons utilizing an endogenous mediator
- Low potential (50 mV vs. Ag/AgCl). Requires small added bias for H₂ production.
- Current of 31 µA*cm^-2
- Live cells: current provided for extended life-time.
- Electron source: carbohydrates
- Electrons flow from PSI to the electrode (auto-mediation)
- Dark current – utilize as bio-energy storage system.
Where would we use such a BPEC?

- Off the grid
- At home
- Mars?
Thank you for keeping our world a better place!