# Design of a Solar Biohydrogen Production: Natural Systems as Blue Print for Producing "Clean" Energy

Matthias Rögner

## Background and aim of the project

Global environmental problems, a limited amount of and steadily increasing costs for fossil energy sources make hydrogen the most important energy carrier of the future. Fuel cell driven cars, the major user of hydrogen, are considered to appear in a few years in the market to solve the problem of exhaust fumes. Consequently, a rapidly growing demand for hydrogen is inevitable. As the production of hydrogen from the fossil fuels will be very limited new and unused resources are required. Especially renewable energy sources such as solar energy should be used to produce hydrogen in the future.

The aim of our project is the production of hydrogen from water and sunlight, as both of them are nearly everywhere available on earth and as the reaction of hydrogen with oxygen which produces the required energy – for instance in a fuel cell – results again in the production of water. While the splitting of water can also be achieved by a combination of solar cells with photovoltaics our approach is using photo-biological reactions for the following reasons: it is the direct conversion of solar energy using the most efficient process on earth – photosynthesis as realised in all green plants; and it is environmentally most acceptable as no $CO_2$ is released during its generation, but on the contrary $CO_2$ is used up in the natural process of photosynthesis in plants.

In order to produce hydrogen from water a two step reaction is required: The first step is the generation of electricity (charge separation) in combination with the splitting of water; in the natural process of photosynthesis – occurring in all green plants – this light-driven process yields protons ($H^+$), electrons (e-) and oxygen. The second step is the reduction of protons by electrons coming from the splitting of water in order to produce hydrogen, $H_2$; in nature this step is catalysed by the enzyme hydrogenase ($H_2$ase). The combination of both processes is shown in Figure 1 and is based on water as the source of electrons and the sun as a continuous source of free energy. This principle is guided by the idea that a cycle of water is the most effective and the least polluting cycle of any substance on earth.

## State of the art

Both the components of photosynthesis in plants and the enzyme hydrogenase are well known. Although some organisms such as uni-cellular cyanobacteria and green algae exist in which both processes, i.e. photosynthesis and hydrogen production co-exist (see Figure 2), there are some serious problems which prevent the efficient use of photosynthesis-powered hydrogen production in living cells:

■ Hydrogenases are usually extremely sensitive towards oxygen ■ The most active hydrogenases which can be found in green algae are also the most oxygen sensitive enzymes, i.e. in order to achieve a high yield of hydrogen production there should be extremely anaerobic conditions – a contradiction to water-splitting photosynthesis. ■ Although the primary reactions of photosynthesis occur with unsurpassed efficiency they are quite labile and light-sensitive; especially Photosystem 2, the water-splitting enzyme, survives only several hours if isolated from green plants like spinach.

## Strategy

In order to solve these problems our strategy is to isolate the most robust and active key components, i.e. Photosystem 2, Photosystem 1 and the hydrogenase, and arrange them in an appropriate artificial environment. Especially, the process of oxygen evolution (i.e. the water-splitting process) has to be separated physically from the oxygen-sensitive process of hydrogen production by the hydrogenase. This can be achieved by immobilising these components on surfaces in separate compartments – one with and one without oxygen (see Figure 3). The immobilisation on appropriate electrode surfaces and the anchoring in a bio-mimetic, stabilising environment should extend their lifetime. And connection of the electrodes in the different compartments should provide an electric photo-current from the water-splitting process in compartment 1 to the hydrogen production in compartment 2.

Photosystem 1 and 2 (PS1 and PS2 see Figure 2) are routinely purified in my group from the thermophilic cyanobacterium *Thermosynechococcus elongatus* which was originally isolated from the Beppu hot spring in Japan. Due to its thermo-tolerance the Photosystems of this organism are the most stable known up to now. They can be isolated on a preparative scale as this cyanobacterium can be grown in large scale photobiofermenters in our laboratory (see Figure 4). Recently, new hydrogenases of very high stability have been detected in the bacterium *Thiocapsa roseopersicina* which support our view that hydrogenases could be utilised in the future in industry substituting metallic catalysis. The very recent structure determination of PS1, PS2 and

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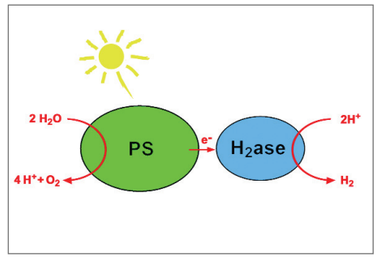


Figure 1 Combination of light-driven splitting of water by photo-synthesis (PS) and production of hydrogen ($H_2$) by hydrogenase ($H_2$ase).

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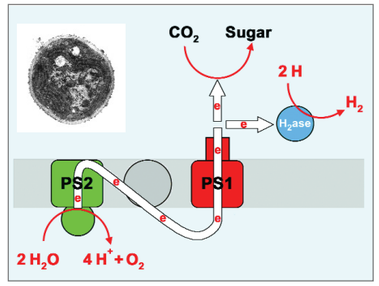


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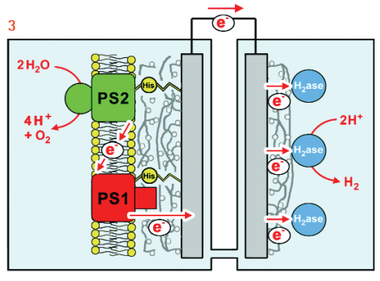


Figure 2 Cyanobacterial cell (Synechocystis PCC 6803, insert, with extended internal thylakoid membrane system) and scheme of its internal thylakoid membrane, harbouring the components of photosynthesis (Photosystem 1 and 2, i.e. PS1 and PS2) and hydrogen production (hydrogenase, i.e. $H_2$ase).

Figure 3 Model of a semi-artificial device for hydrogen production from water using the natural components Photosystem 1 (PS1), Photosystem 2 (PS2) and hydrogenase ($H_2$ase).

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various hydrogenases contribute considerably to a mechanistic understanding of these "nanomachines" on a molecular level and also enable the engineering of their function according to the requirements of our hydrogen-producing "device".

In summary, this system consists of several modules which are responsible for light harvesting (antenna-function), water-splitting (electron source), electron transfer and hydrogen production.

## Realisation

For the realisation of the device the following steps are of central importance:

1) The genetic engineering of Photosystems and hydrogenase for easier large-scale isolation and proper orientation on electrode surfaces.

2) The development of photobiofermenters for large scale fermentation of cyanobacteria and development of strategies for large scale purification of Photosystems and hydrogenases

3) Long-term stabilisation of all components (i.e. from minutes/ hours to days/ weeks).

4) Immobilisation of the Photosystems and the hydrogenase on solid surfaces, i.e. electrodes, and the evaluation of efficient native and artificial electron carriers.

For step 1) and 2) both PS1 and PS2 have been genetically modified and supplied with a tag which helps to orient them on the surface of gold electrodes. In parallel, large photobiofermenters have been developed which allow the growth of 25 litre cyanobacteria within three days.

For step 3), PS1 and PS2 could be stabilised either by artificial, thermostable lipids or by artificial polymers which were used instead of detergents.

For step 4) we use a chemically modified gold surface in order to enhance the specific and oriented binding of the Photosystems. With this set-up we succeeded recently in measuring a light-induced current of PS2 (Figure 5b) which was immobilised according to Figure 5a on the electrode surface. By varying the wavelength of light excitation we could show that this reaction is powered by chlorophyll (see Figure 5c). This is a major breakthrough for the realisation of the overall process. Before, our Japanese partners – the group of Dr. Miyake, Osaka – already succeeded in immobilising *Thiocapsa*-hydrogenase on electrode surfaces and in producing hydrogen gas by applying external voltage.

## The prospect for the bio-hydrogen project

Finally we should end up with a model device similar to Figure 3, in which PS1, PS2 and the hydrogenase are interconnected by appropriate electron carriers and in which the hydrogen production with electrons from water is realised. Such a model system could be the first step to fully artificial devices for hydrogen production on large scale using a bio-mimetic approach. It could also be the first step towards a natural, self-replicating engineered cell system as shown in Figure 2. In this case, the optimal components from different organisms have to be combined within one organism by molecular biology procedures. Although this process is difficult and time-consuming – especially the engineering of an oxygen-tolerant hydrogenase – it is not impossible. For the future, such a simultaneous production of hydrogen and oxygen from water – also called direct biophotolysis – would be the preferred process, because it is self-replicating and because such cells can be grown in large fermenters requiring just light, water, and some inorganic compounds. Which of the two strategies will finally be more successful is presently unknown. However, it is also very unlikely that one solution alone will satisfy the variety of all present and future requirements for energy. For this reason it is certainly reasonable to follow both strategies in order to finally contribute to a future hydrogen-based economy which is environmentally acceptable. Figure 6 summarises the "biological" strategy of this project and its future application showing the circuit of hydrogen, water and energy: In this scenario algal cells produce (bio-) hydrogen from water using sun energy; in parallel, atmospheric $CO_2$ is transformed into bio-mass which can also be used as additional energy source and/or food supplement. Biohydrogen produced by the algal cells provides energy in a fuel cell in a reaction with oxygen; the "waste" product of this reaction is water which in turn is the substrate for the algal cells. The combination of both processes may be an attractive model for regenerative energy without waste, involving self-reproducing organisms, water and sunlight.

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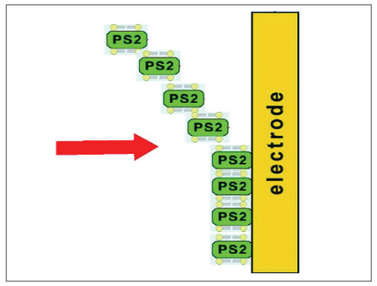


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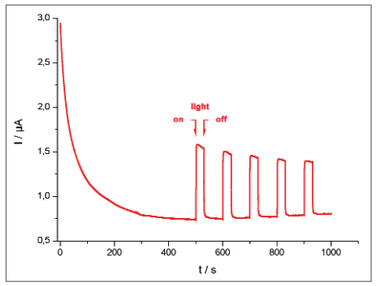


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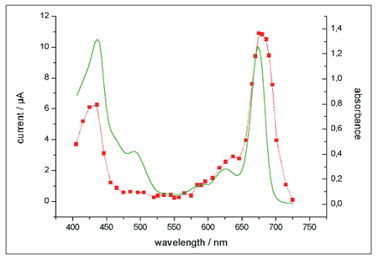


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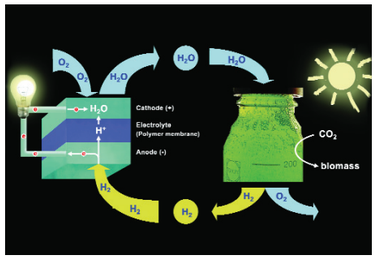


*Prof. Dr. Matthias Rögner, born in 1952, studied biology and japanology in Tübingen and received a PhD at the Technical University Berlin. From 1988 to 1990 he was Postdoc at DuPont in Wilmington, USA, and worked till 1995 at the Institute of Botany, University of Münster. Since 1996 he holds the Chair of Plant Biochemistry, Ruhr University Bochum.*

Figure 4
25 litre photobiofermenter for the mass production of cyanobacteria (the light device has been removed in order to see the cylinder with the algae).

Figure 5
a) Isolated Photosystem 2 (PS2) immobilised and oriented on gold electrode surface via Histidine-tags.
b) Light-induced photo-current of immobilised PS2 in dependence of time (with "white light" as light source).
c) Wavelength-dependence of the photo-current (red) in comparison with the absorption spectrum of chlorophyll (green).

Figure 6
Circuit of hydrogen and water involving a hydrogen-producing algal suspension (right) and a fuel cell (left) being powered by sun light and producing electricity.