# Photosynthetic Hydrogen Production: Computational & Experimental Results are Indicative that Evolutionary Mutants May Allow for Commercial Viability

S. Plummer, R. Chapas, M. Plummer

This document appeared in

Detlef Stolten, Thomas Grube (Eds.):
18th World Hydrogen Energy Conference 2010 - WHEC 2010
Parallel Sessions Book 2: Hydrogen Production Technologies – Part 1
Proceedings of the WHEC, May 16.-21. 2010, Essen
Schriften des Forschungszentrums Jülich / Energy & Environment, Vol. 78-2
Institute of Energy Research - Fuel Cells (IEF-3)
Forschungszentrum Jülich GmbH, Zentralbibliothek, Verlag, 2010
ISBN: 978-3-89336-652-1

# Photosynthetic Hydrogen Production: Computational & Experimental Results are Indicative that Evolutionary Mutants May Allow for Commercial Viability

**Scott Plummer**, **Richard Chapas**, $H_2$OPE Biofuels Inc., USA
**Mark Plummer**, MPr&d LLC, USA

Carbon dioxide and other greenhouse gases are rising to alarming levels in our atmosphere. An economy and a transportation infrastructure based on molecular hydrogen and fuel cells could positively affect global climate change. Generation of molecular hydrogen as a biofuel, i.e. generation of hydrogen via photosynthetic algae (*Chlamydomonas reinhardtii*) would allow for a cheap and renewable energy source. However, the enzyme responsible for hydrogen gas generation (hydrogenase) has a short half-life and is extremely sensitive to oxygen.

One approach toward solving these problems is through directed evolution whereby mutations are introduced into the DNA of the native hydrogenase. Directed evolution is a technique that mimics natural evolution in that multiple mutations are created and tested for enhanced traits. Albeit on a shorter timescale, the proteins with evolved mutations are submitted to repeat cycles of evolutionary pressure.

In this talk, we will present a computational model and the corresponding experimental results, which indicate the near-term feasibility of commercial biohydrogen production. Specifically, we will present a computational model, based on our most promising mutant hydrogenases, that suggests that hydrogenase mutants can produce 40-760X more hydrogen than the wild-type hydrogenase. Ultimately, a mutant with increased hydrogen production will facilitate a practical method of producing hydrogen for use as a commercial fuel source.

The modeling portion of this work had dual objectives. The first was to see if the folding of a hydrogenase protein (575 amino acids) could be more efficiently modeled using electrostatic potential surfaces (EPS) along with molecular dynamics (MD) versus using MD only. The basis of the modeling is that a surface of constant electrostatic potential can be used as a measure of the effort needed to share or transfer an electron from one region of a molecule to another region. And, that electron sharing in hydrogenases should affect their folding mechanisms and kinetics.

It is also proposed that positive and negative EPS regions can be quickly folded and matched by variation of bond angles between regions followed by MD structuring and energy minimization. This approach should minimize computational time for modeling folding kinetics and mechanisms over that required if only MD calculations were used. Lastly, it is proposed that the ratio of water accessible volume to water accessible surface area can be used as a correlation parameter to follow the extent of folding.

MD only calculations showed that folding starts at both ends of the entire hydrogenase mutant and works its way toward the middle. The center point for this folding is the amino

acid 267. Hence to obtain shorter computation times, the rest of the folding work dealt with the 267-575 portion of this mutant. This region shows the greatest diversity of positive (green) and negative (pink) EPS regions in the initial α-helical structure as shown in Figure 1.

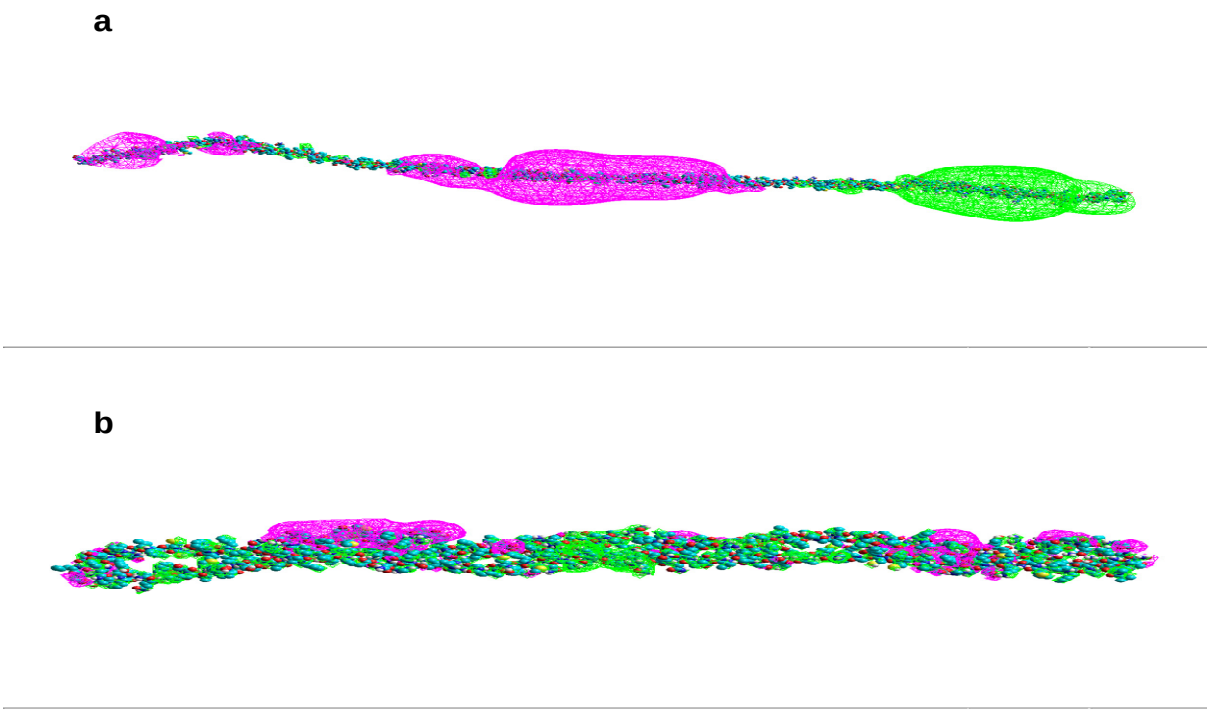


**Figure 1: Modeling: Electrostatic potential surfaces.**
**a) Structure of a typical mutant hydrogenase (green indicates a region of positive electrostatic potential and pink indicates a negative electrostatic potential surface); b) Structure of hydrogenase after one folding iteration.**

Protein folding kinetics has been shown to exhibit two folding regions. First, the protein quickly folds from a linear state into a globular structure. Next, the globular structure folds slowly to the final tertiary state. The results from this work support these literature findings. That is, a correlation between RMS error and the water accessible volume to surface area ratio was found to exhibit two straight-line segments of considerable different slopes. It was concluded that these separate straight-line segments represent the fast and slow folding regions. Single correlations of this type were obtained for all folding temperatures and times studied.

Based on the above results, the EPS concept was tested for predicting the hydrogen productions of hydrogenase mutants using their unfolded α-helical structure. The ratio of total positive EPS area to total negative EPS area was evaluated as the correlation parameter. As demonstrated by the above modeling results, folding is affected by long-range electrostatic forces. Those mutants exhibiting a certain EPS ratio would fold correctly to yield the maximum hydrogen production; an EPS of zero indicates no positive EPS regions and an infinite ratio indicates no negative EPS regions. The best fit to this data was obtained with the Log Normal equation yielding a Chi squared error of 1.0, wherein the

maximum hydrogen production would be 758 times that obtained with the control hydrogenase – Figure 2.

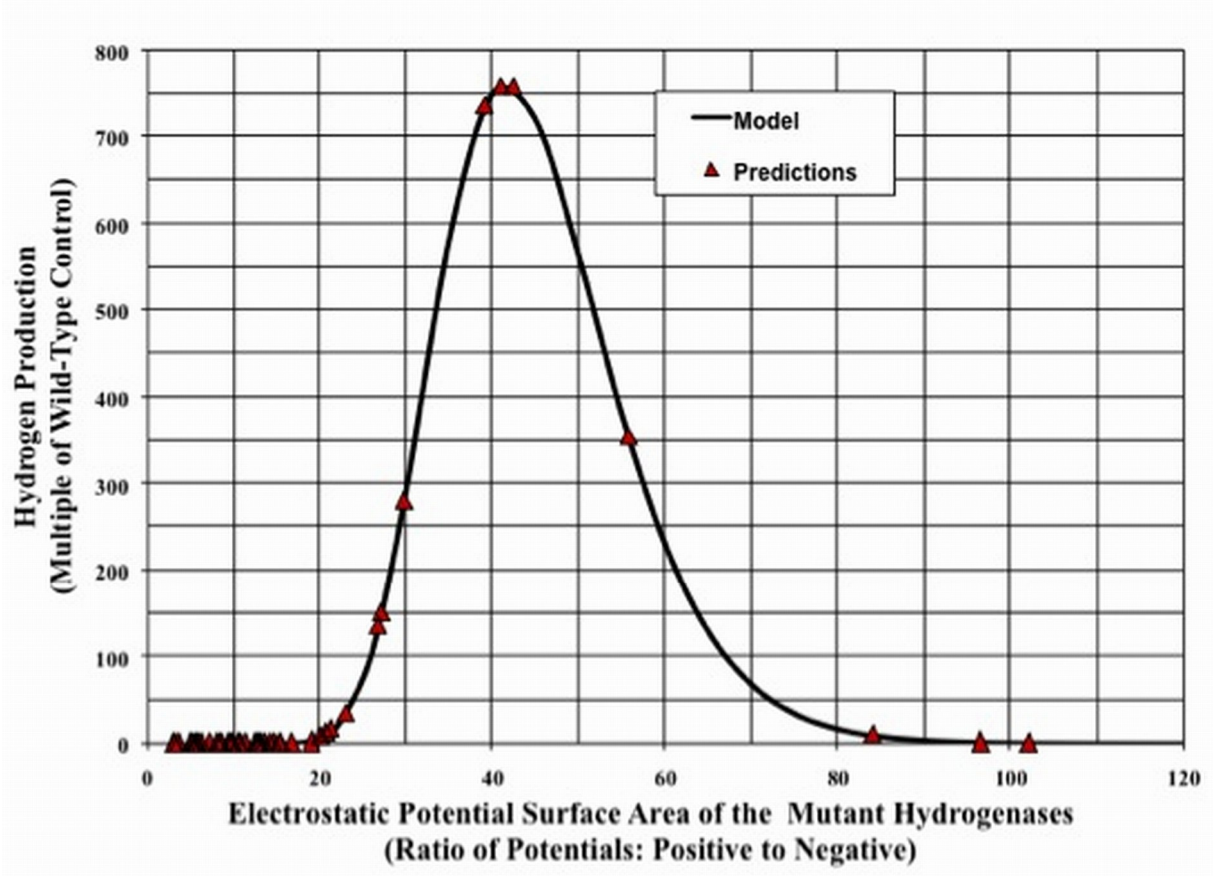


**Figure 2: Measured and predicted hydrogen productions versus ratio of positive to negative EPS of unfolded structures.**

Based on the above results, 70 of the 256 mutants produced in the experimental work were evaluated using the developed model. Fifteen of these mutants have predicted hydrogen productions in the range of 1.4 to 758 times that for the wild type control.

## References

[1] Plummer, M.A., Plummer, S.M., “Modeling the Folding and Hydrogen Production of Clostridium acetobutylicum and Clostridium saccharobutylicum Mutants using Electrostatic Potential Surfaces and Molecular Dynamics”, Computational Biology and Chemistry, 2009, 33, 404-407.

[2] Plummer, S., “Photosynthetic Hydrogen Production from the Green Alga Chlamydomonas reinhardtii”. Doctoral Thesis at the Colorado School of Mines, Department of Chemistry, 2008, 1-134.