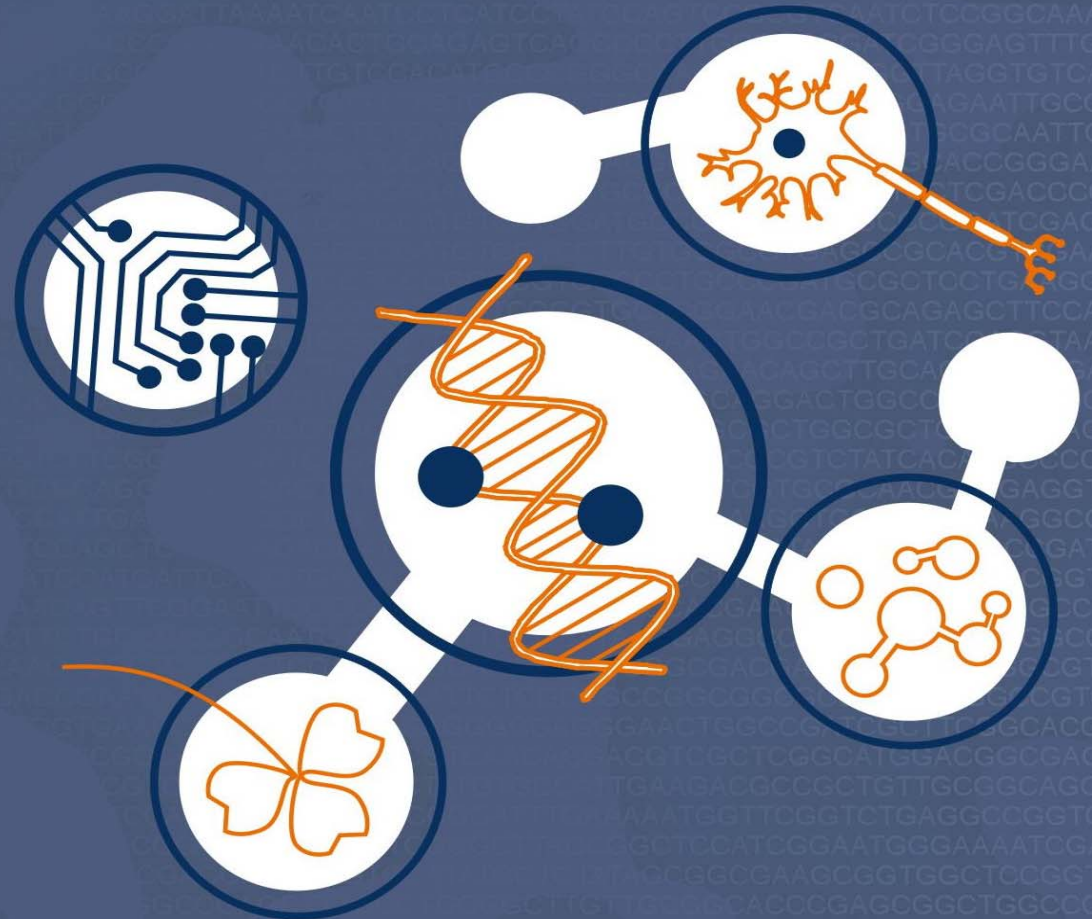


# Novel Approaches in Biohydrogen Research



BAYGEN Institute, Bay Zoltán Foundation for Applied

Research leader: Gergely Maróti PhD

e-mail: [marotig@baygen.hu](mailto:marotig@baygen.hu)

# Prospects of BioEnergy

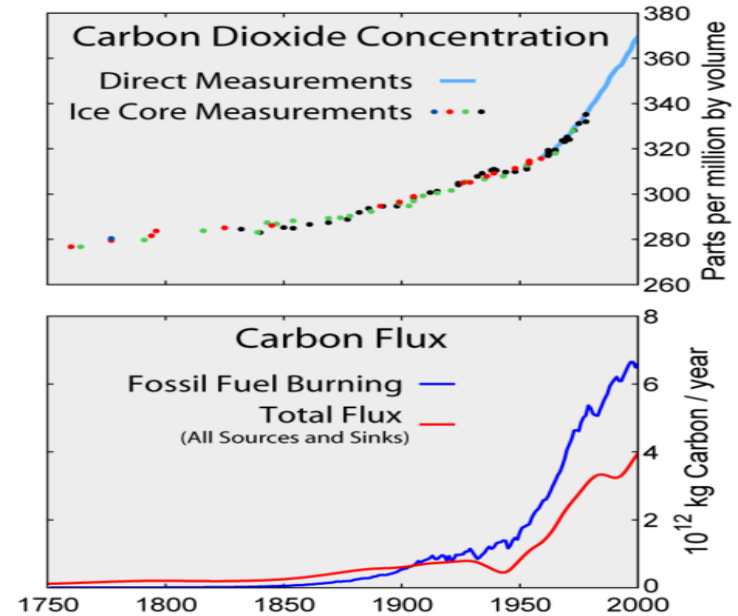
Fossil fuel' burning impacts on Earth



Permanent seeking for clean energy carriers



Bioenergy is one candidate



**BioEnergy** is one kind of Renewable energies (wind, hydro, solar, etc. )

**BioEnergy:** Any kind of energy produced from biomass (can be electricity, heat, cooling, etc.)

Solar Energy is expensive to harvest directly, it is often converted to **BioEnergy** first

**Bioethanol** (potato, corn, sugar cane) - solar based

**Biodiesel** (soybean, oilseed-rape) - solar based

**Main Types of Bioenergy:** **Biohydrogen** (direct, indirect) – solar based

**Biogas** (landfill waste, wastewater, manure) – indirectly solar based

**Others** (wood combustion, biobutanol, biomethanol, algae-fuel, etc.)

# Two Basic Strategies for Biohydrogen Production

## Enzymatic – *in vitro*

hydrogenase

### Important enzyme features

- high catalytic activity
- toward H<sub>2</sub> production
- stability under the conditions of usage
  - heat, oxygen

electricity  
redox dyes  
redox cofactors



Fuel cells

## Whole cell – *in vivo*

hydrogenase vs. nitrogenase

- high catalytic activity
- toward H<sub>2</sub> production
- stability under the conditions of growth
  - oxygen, proteases

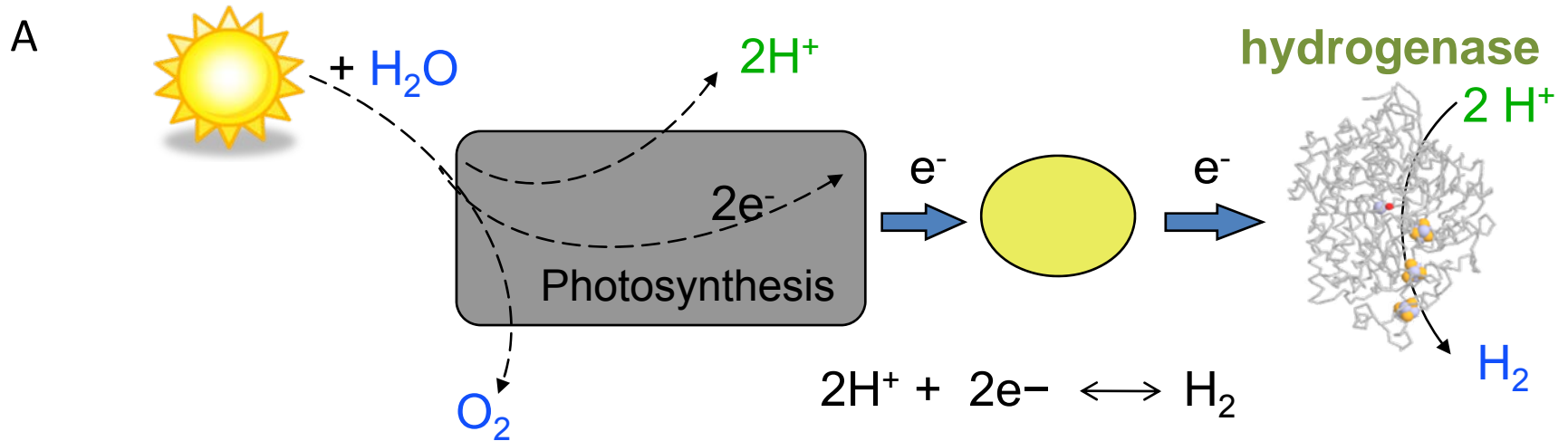
metabolic network links  
links to other bioenergetic processes  
*in vivo* roles



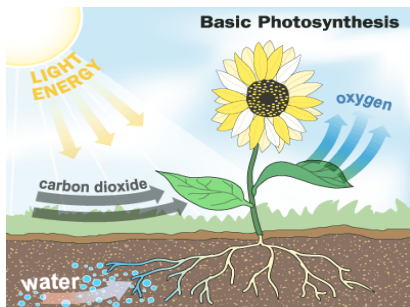
Bioreactors

e<sup>-</sup> sources

# Key Elements: Hydrogenase, Nitrogenase Enzymes



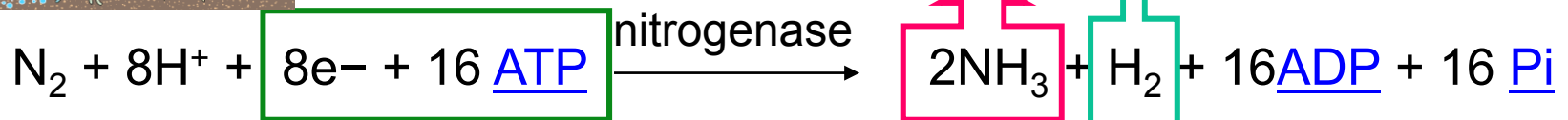
B



Nitrogen fixation

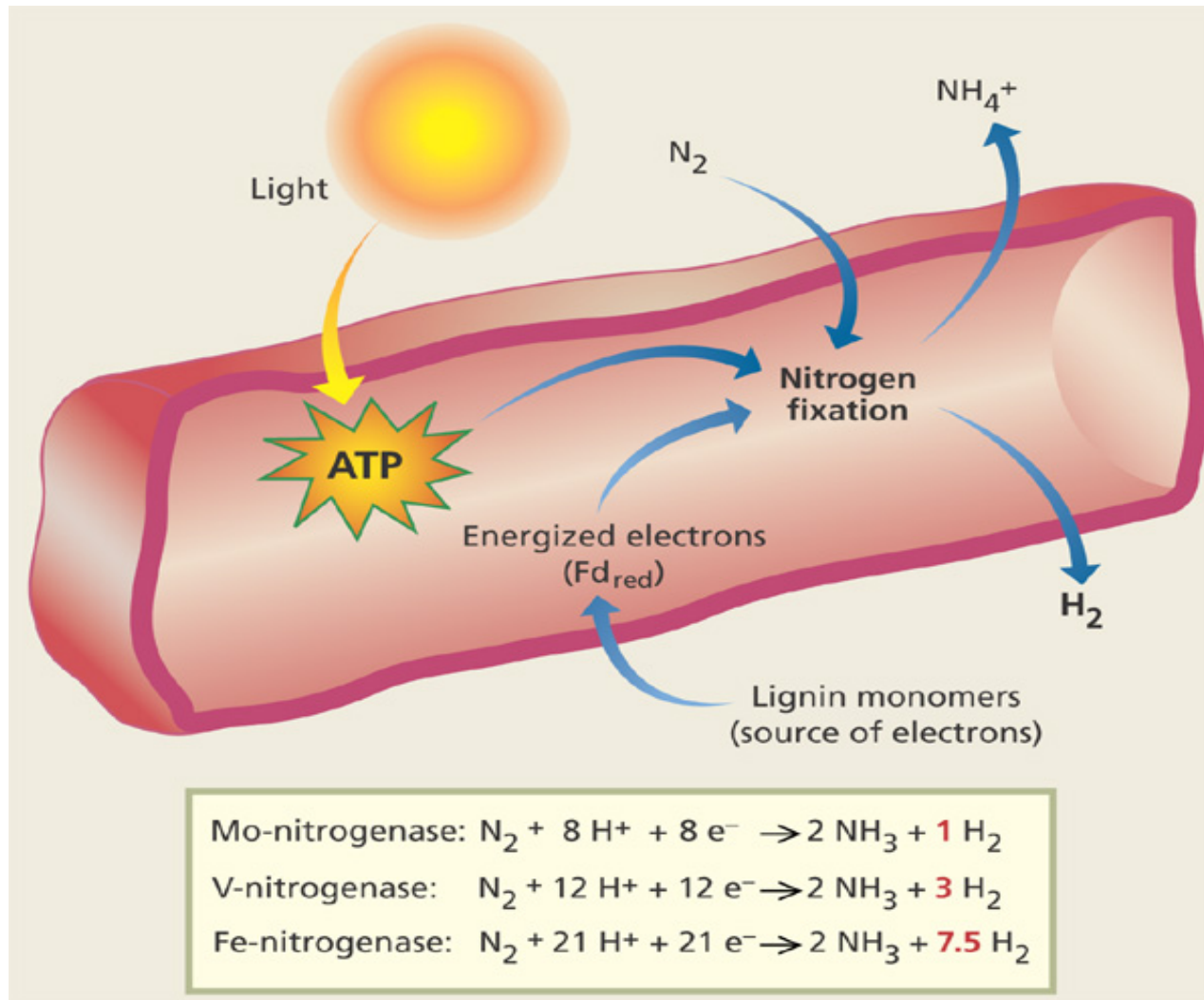
Nitrogen source for plants

photosynthesis



ANR-NKTH project: Focus on Nitrogenase-mediated biohydrogen production

# Nitrogenase-based Light Energy Conversion



Source: Nature

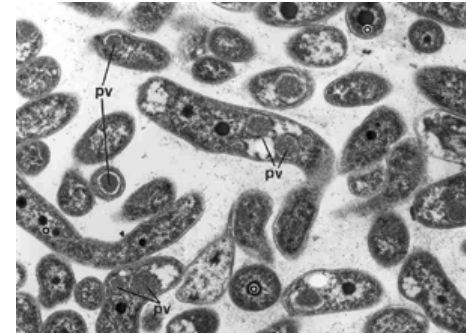
Principle: Solar energy conversion using photosynthetic microbes

# Nitrogenase-based Light Energy Conversion

## Model organisms:

Bradyrhizobium ORS278  
Bradyrhizobium BTAi1  
*Rhodopseudomonas palustris*  
*Thiocapsa roseopersicina*

Photosynthetic  
microbes



Bradyrhizobium sp.

Bradyrhizobium ORS278  
Bradyrhizobium BTAi1

Symbiotic and Free living  
(symbionts of genus *Aeschynomene* – aquatic legume)

*Rhodopseudomonas palustris*  
*Thiocapsa roseopersicina*

Free-living  
purple bacteria



Rhodopseudomonas sp.



Thiocapsa sp.

All harbour nitrogenase and hydrogenase enzyme(s)

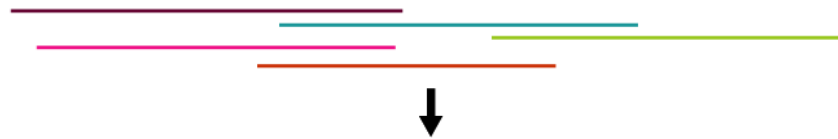
# Metagenomic Approach

Using NGS (new generation sequencer)

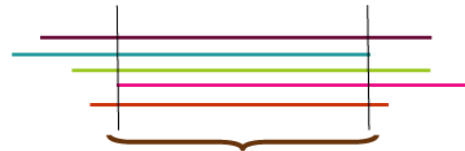
## Identification of novel enzymes (here H<sub>2</sub>ases, N<sub>2</sub>ases)

Environmental sampling (water, soil)  
Create genomic database (translated)  
Build Hidden Markov Models (HMM)

Collect proteins (same functions) to be in the “seed”



Generate and trim multiple alignment



Region of good alignment and closest similarity

Run HMM algorithm:  
Computes statistical probabilities  
for amino acid patterns in the seed

Search new model against  
all proteins in the database

Select candidate enzymes

Express and test the enzymes in various hosts

# Competence fields

- Environmental sampling for identification of novel enzymes, strains
- Characterization of hydrogen producing microorganisms
- Establishment of recombinant *in vivo* hydrogen producing systems
- Technology development for improved photobiohydrogen production
- Genetic, molecular-level modifications, mutagenesis studies