Carbon sequestration and hydrogen production by higher plants and algae to combat global warming

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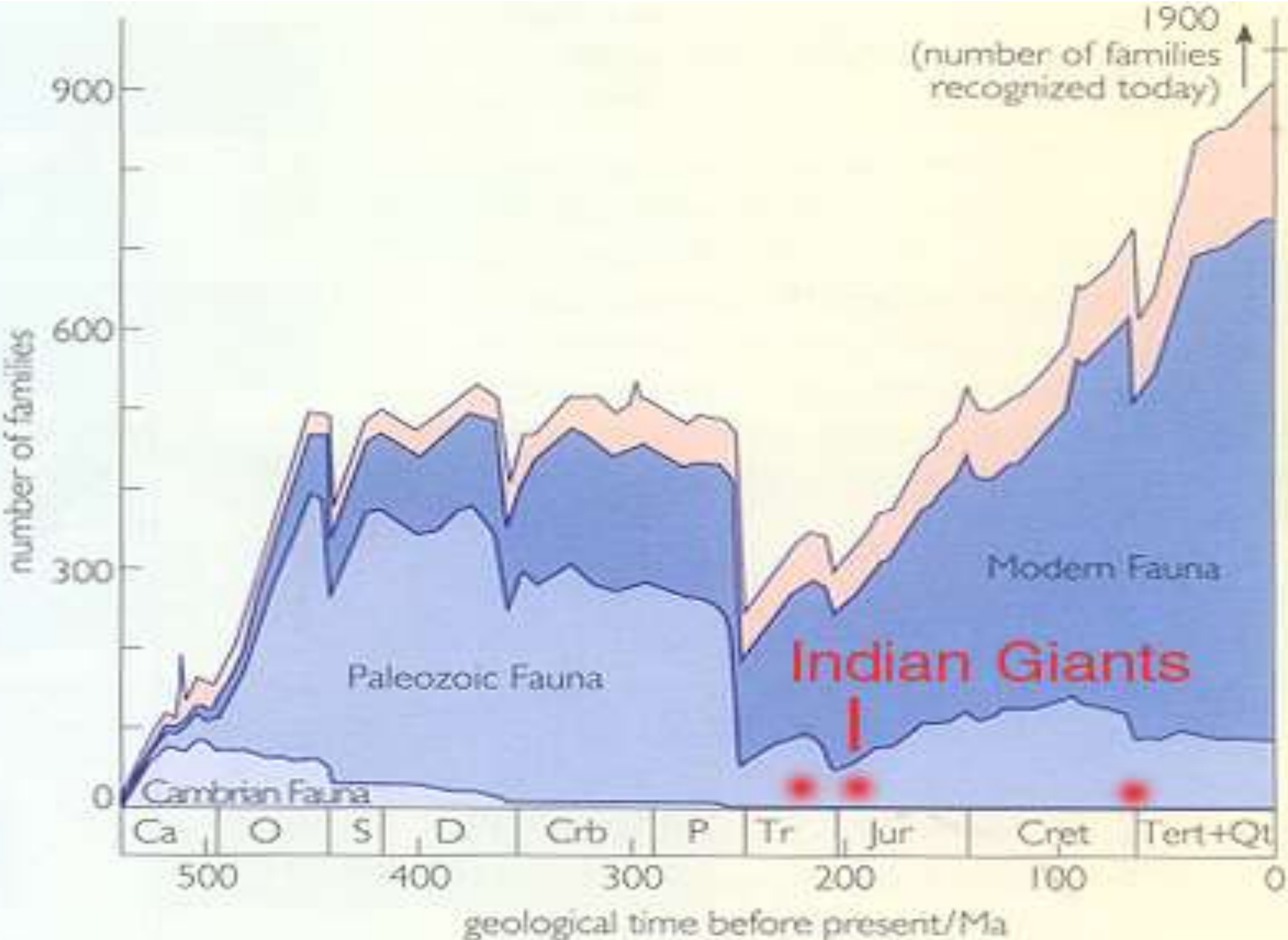
TRIGGERS FOR BIODIVERSITY

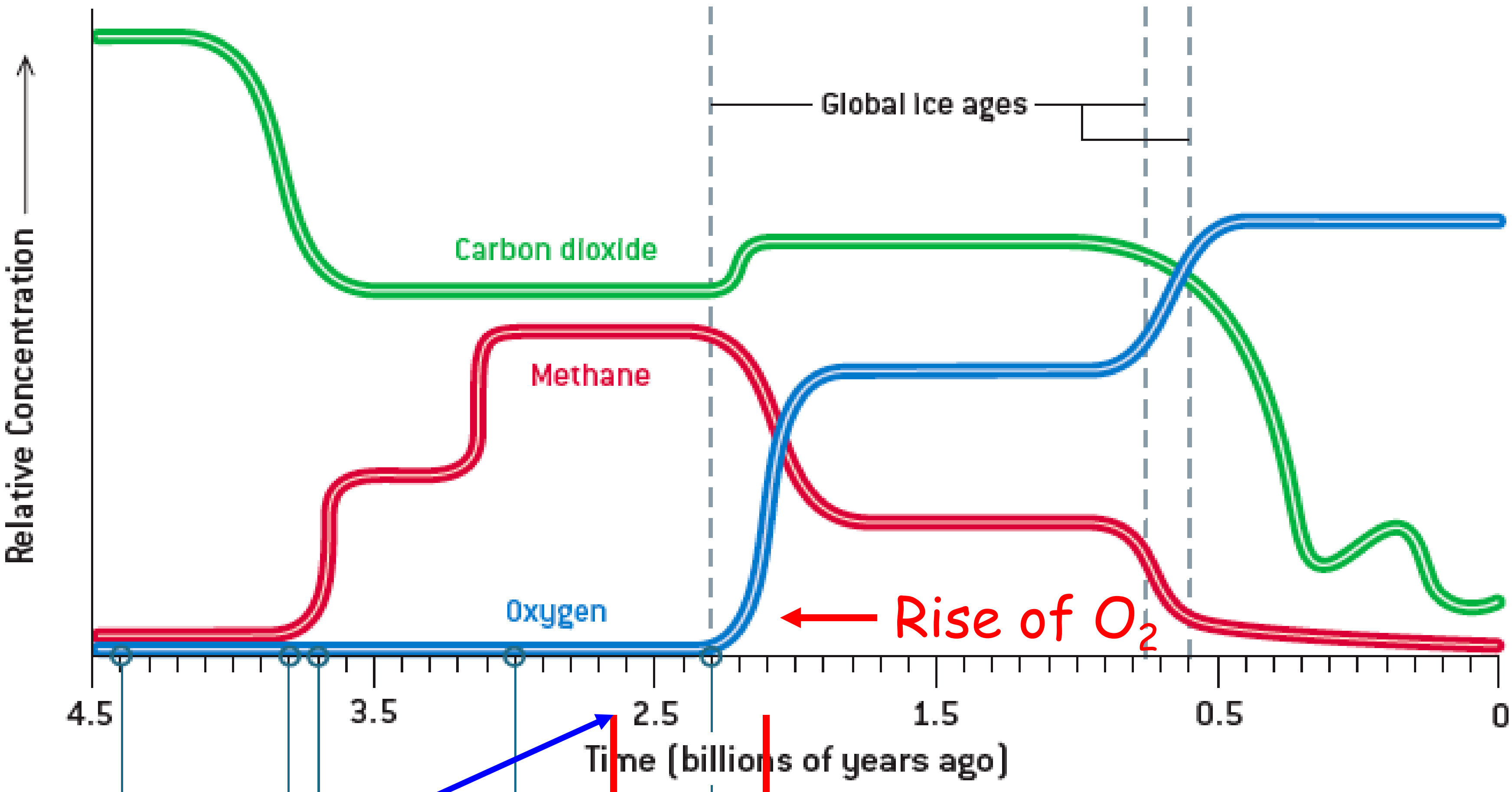
- Greenhouse Earth
- Oxygen metabolism & photosynthesis
- Competition & selection forces: evolution
- Increase & decrease of niches: Permissive Ecology
- Extinction Events

Relative to the geological past, how high is biodiversity today?



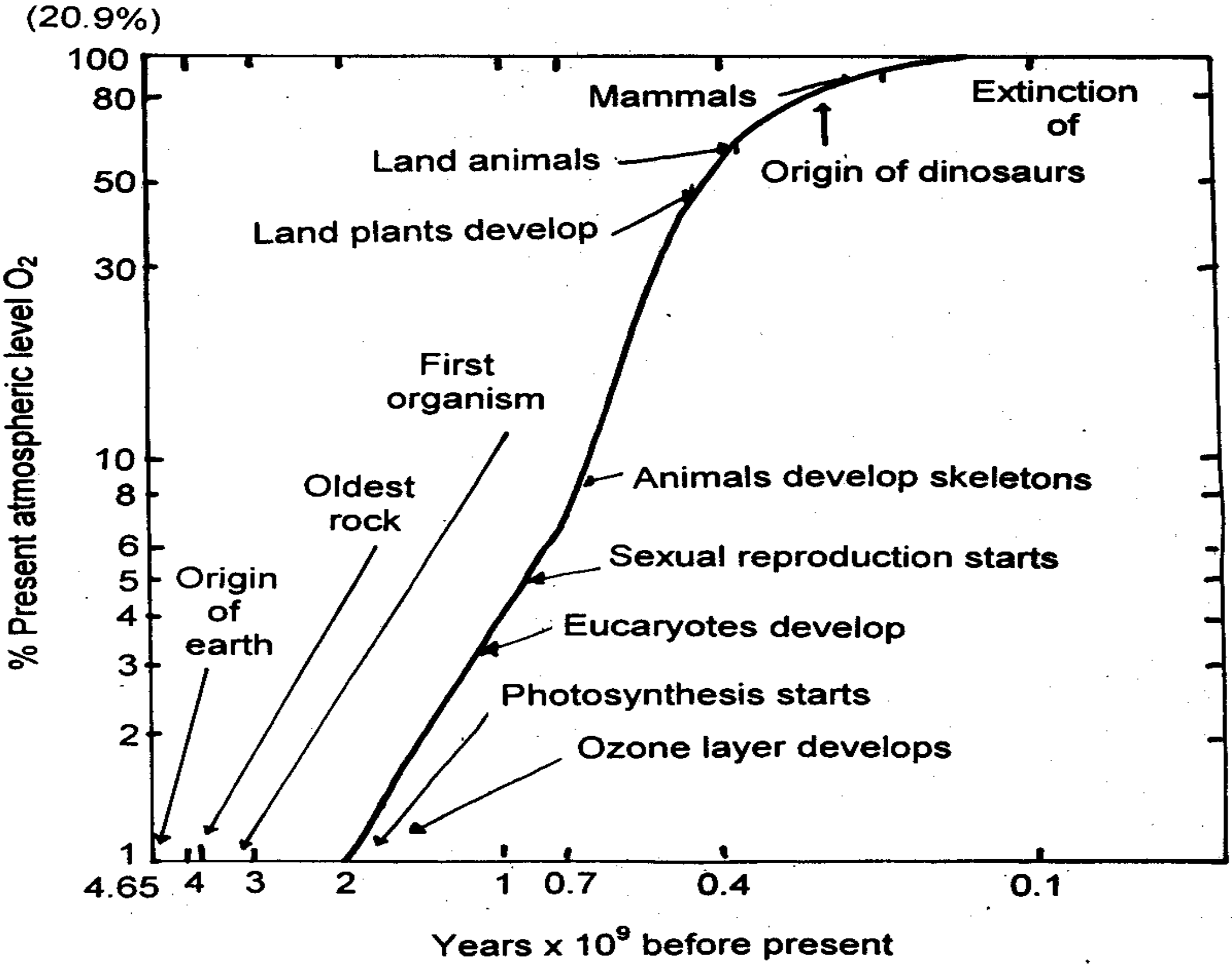
1900
(number of families
recognized today)
900
600
300
0
number of families
Modern Fauna
Indian Giants
Paleozoic Fauna
Cambrian Fauna
Ca
O
S
D
Crb
P
Tr
Jur
Cret
Tert+Qt
500
400
300
200
100
0
geological time before present/Ma

The Rise of Oxygen

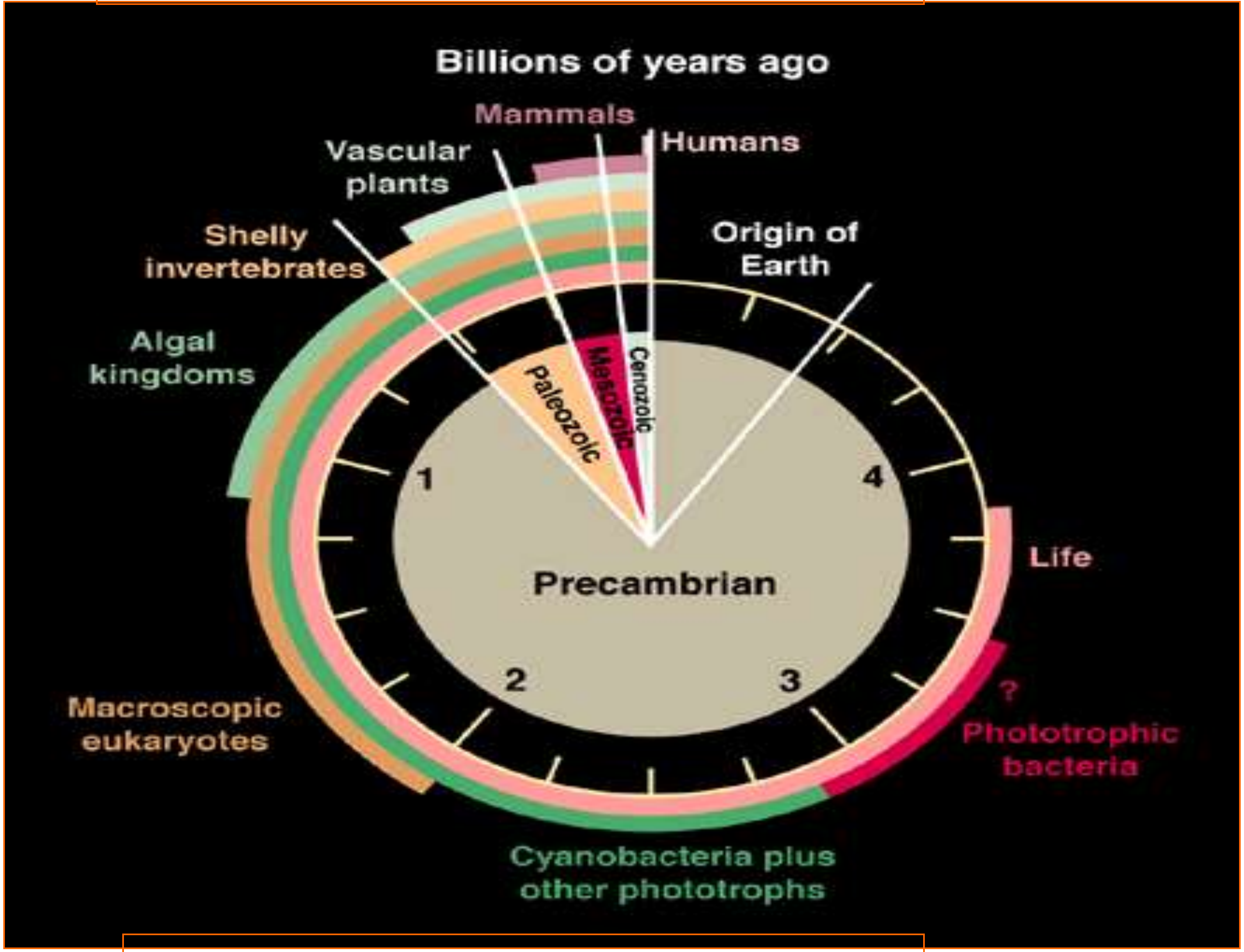


Biomarkers for cyanobacteria and eukaryotes?

Origin of Oxygenic Photosynthesis?

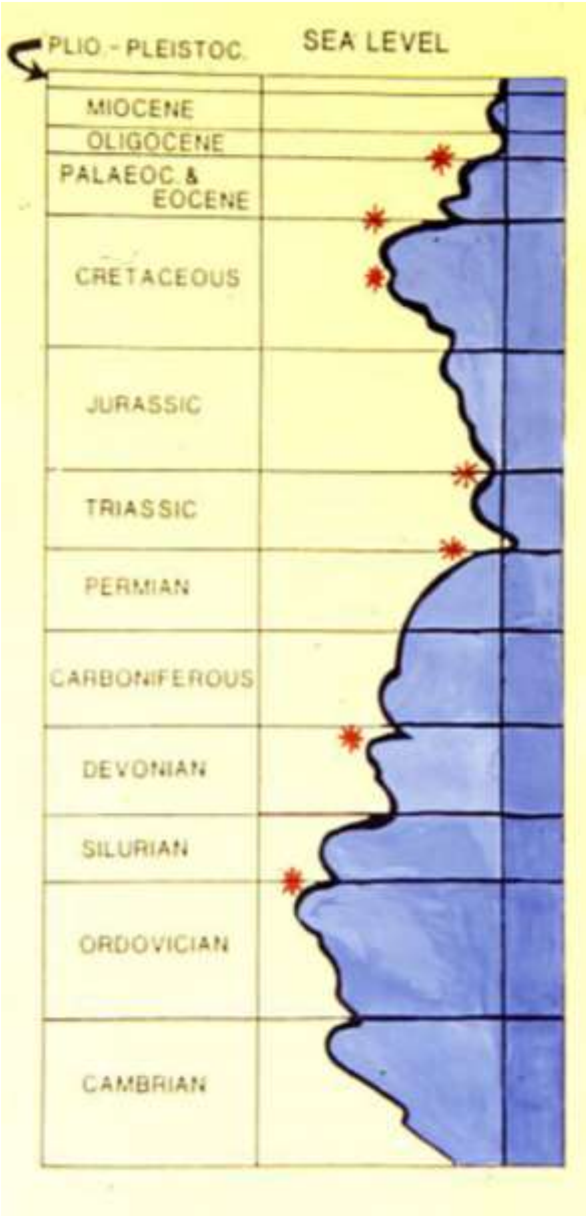

(20.9%)
100
80
50
30
10
8
6
5
4
3
2
1
% Present atmospheric level O_2
4.65
4
3
2
1
0.7
0.4
0.1
Years x 10^9 before present
Mammals
Extinction of
Origin of dinosaurs
Land animals
Land plants develop
First organism
Oldest rock
Origin of earth
Animals develop skeletons
Sexual reproduction starts
Eucaryotes develop
Photosynthesis starts
Ozone layer develops

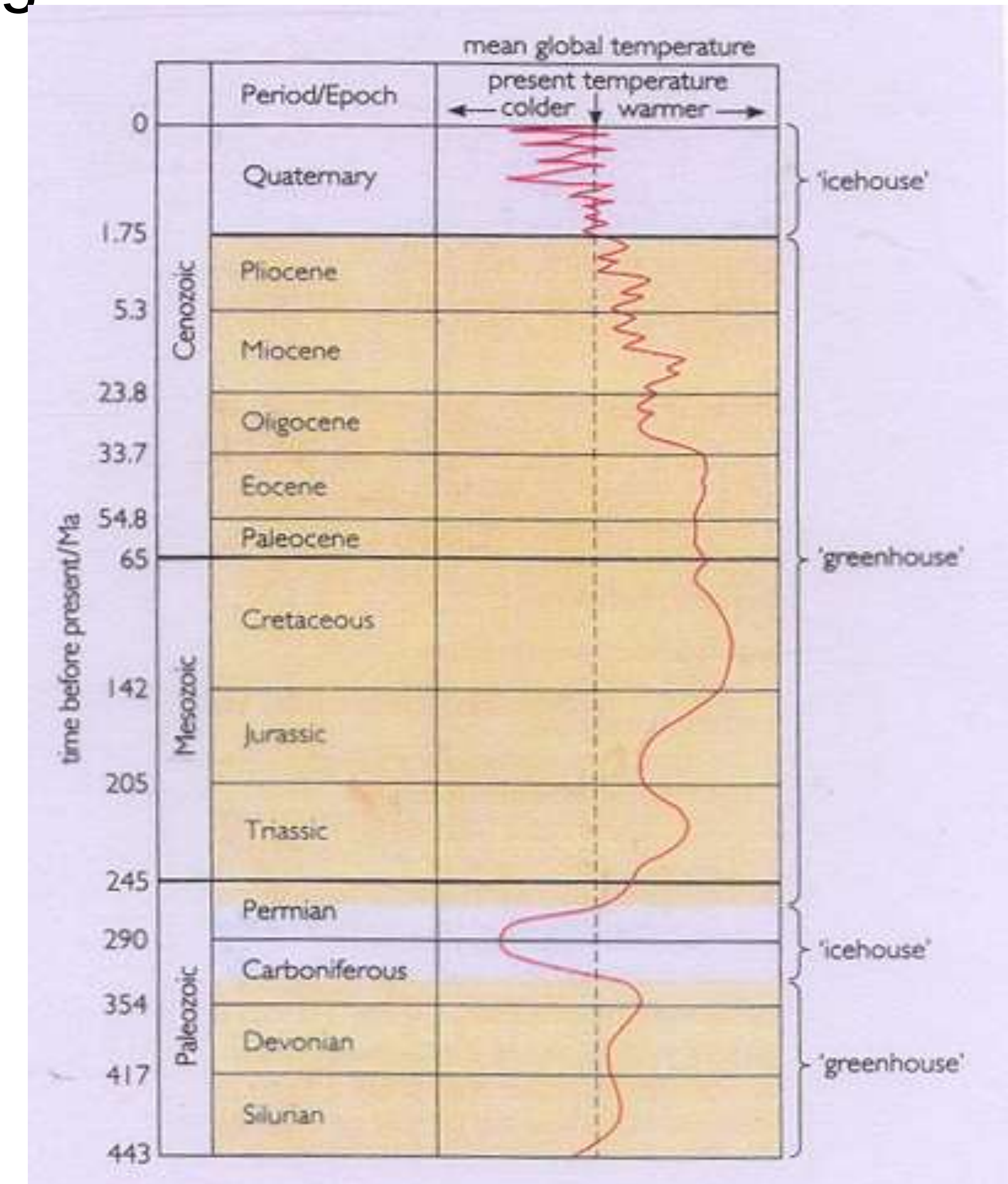
Earth's Biological Clock

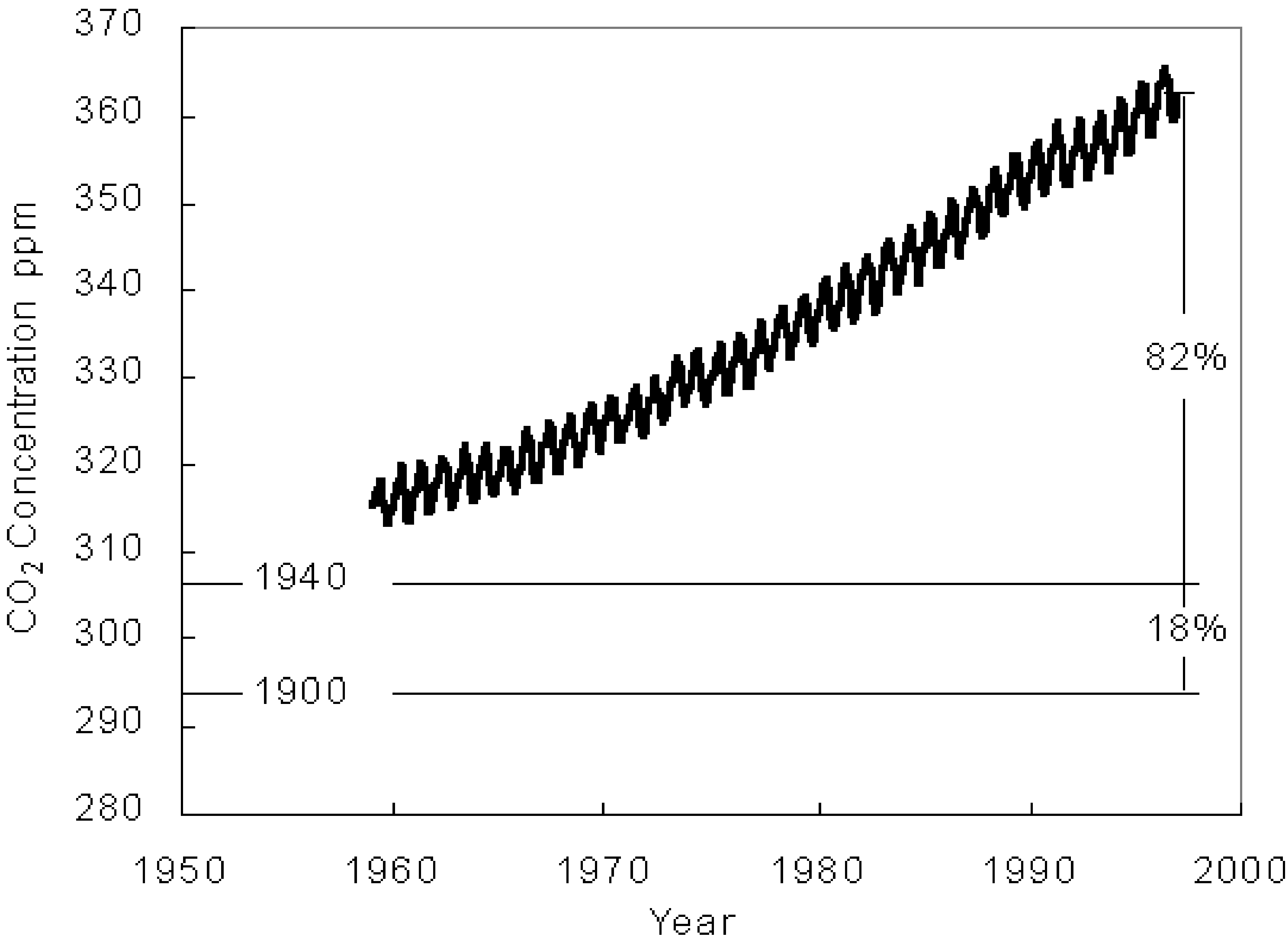


D. De Marais, Science (2000) 289, 1703

Icehouse /Greenhouse, sea level Fluctuations through time

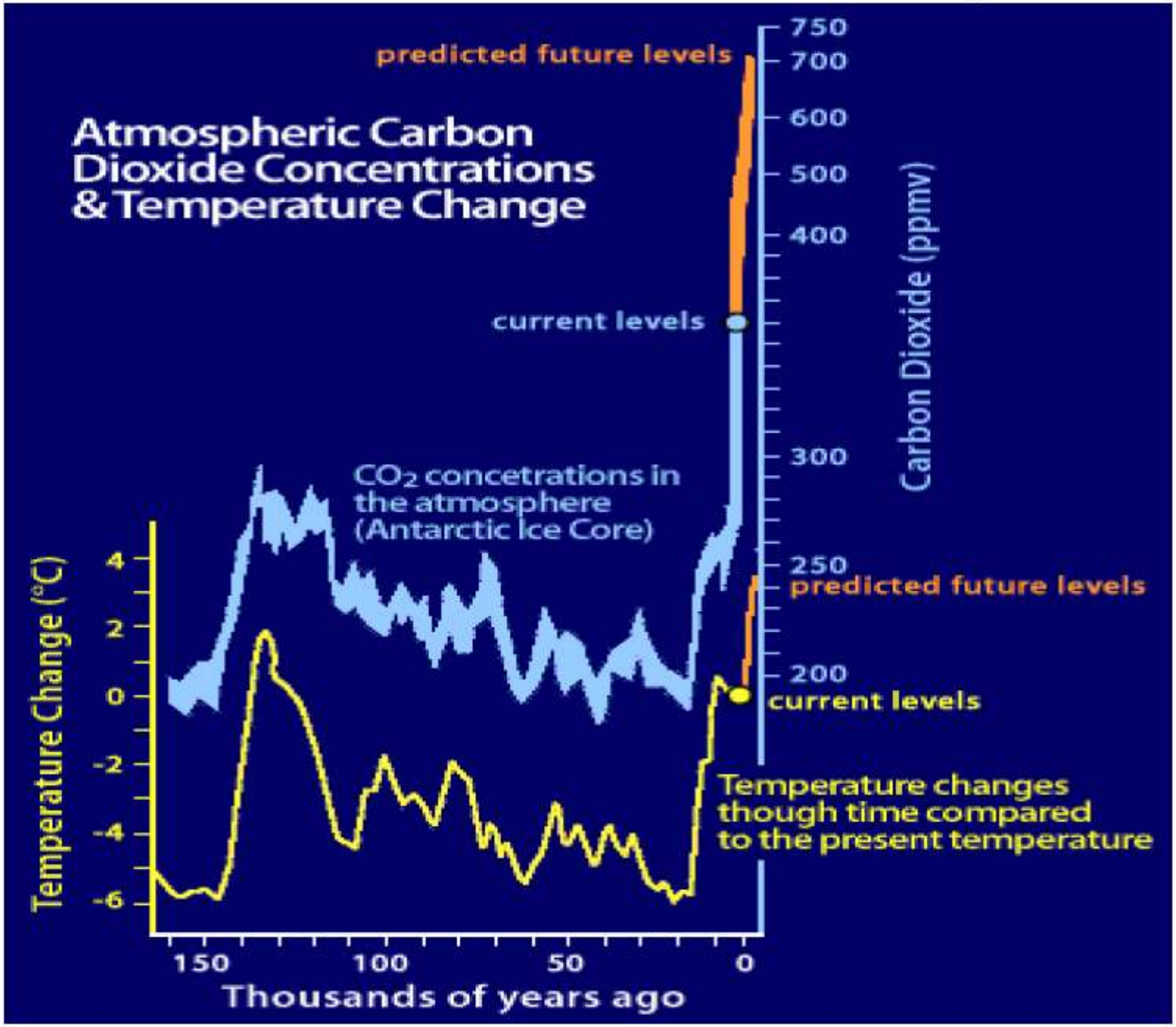




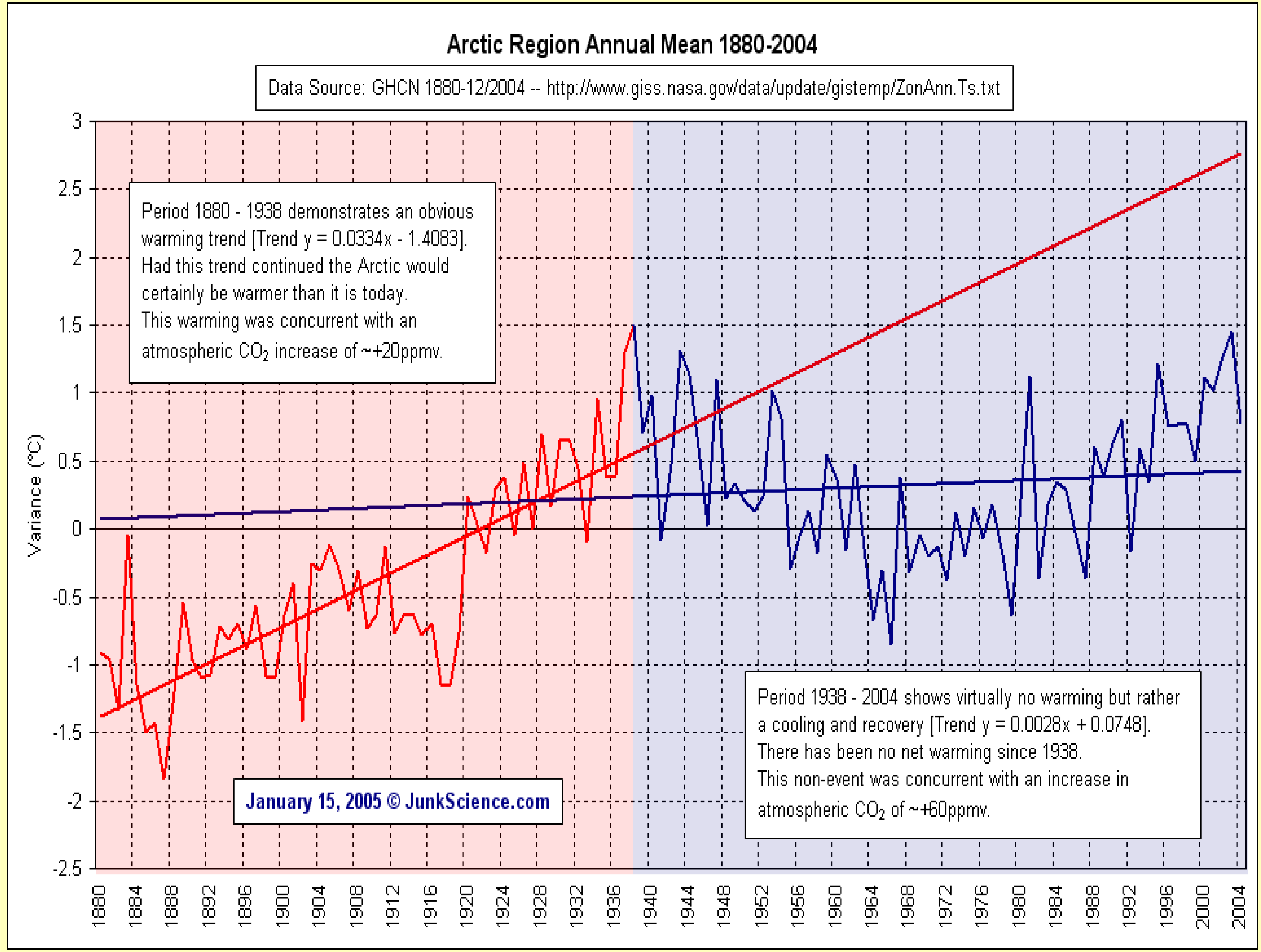


Increase in atmospheric CO2 concentrations in parts per million by volume

Past, present and future atmospheric CO_2 concentration

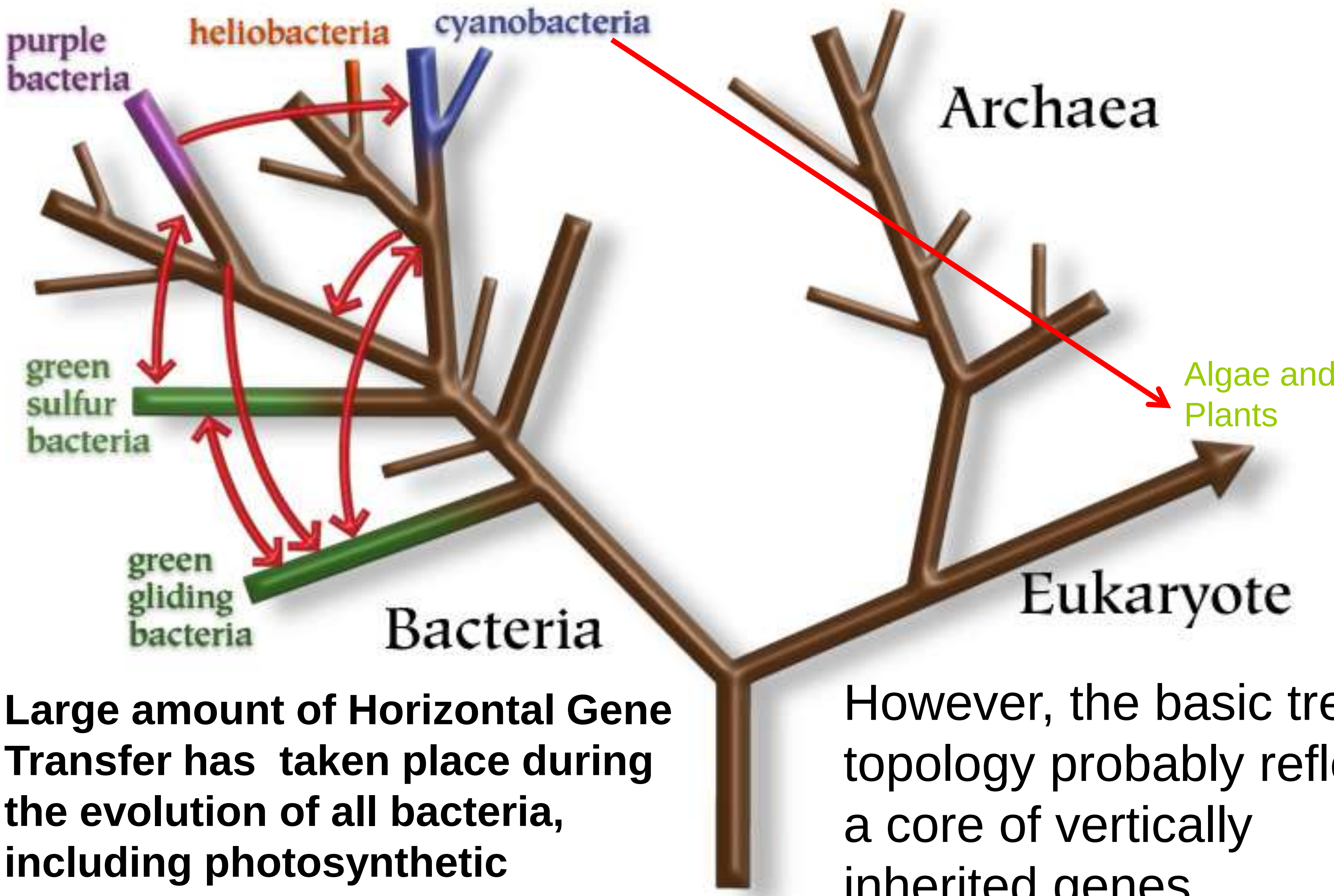


(Source : IPCC Third Assessment report, 2001)


Arctic Region Annual Mean 1880-2004
Data Source: GHCN 1880-12/2004 -- http://www.giss.nasa.gov/data/update/gistemp/ZonAnn.Ts.txt
Period 1880 - 1938 demonstrates an obvious warming trend [Trend y = 0.0334x - 1.4083]. Had this trend continued the Arctic would certainly be warmer than it is today. This warming was concurrent with an atmospheric CO_2 increase of ~+20ppmv.
Period 1938 - 2004 shows virtually no warming but rather a cooling and recovery [Trend y = 0.0028x + 0.0748]. There has been no net warming since 1938. This non-event was concurrent with an increase in atmospheric CO_2 of ~+60ppmv.
January 15, 2005 © JunkScience.com
Variance (°C)
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
1880
1884
1888
1892
1896
1900
1904
1908
1912
1916
1920
1924
1928
1932
1936
1940
1944
1948
1952
1956
1960
1964
1968
1972
1976
1980
1984
1988
1992
1996
2000
2004

Iceberg splitting, NASA

Universal Tree of Life

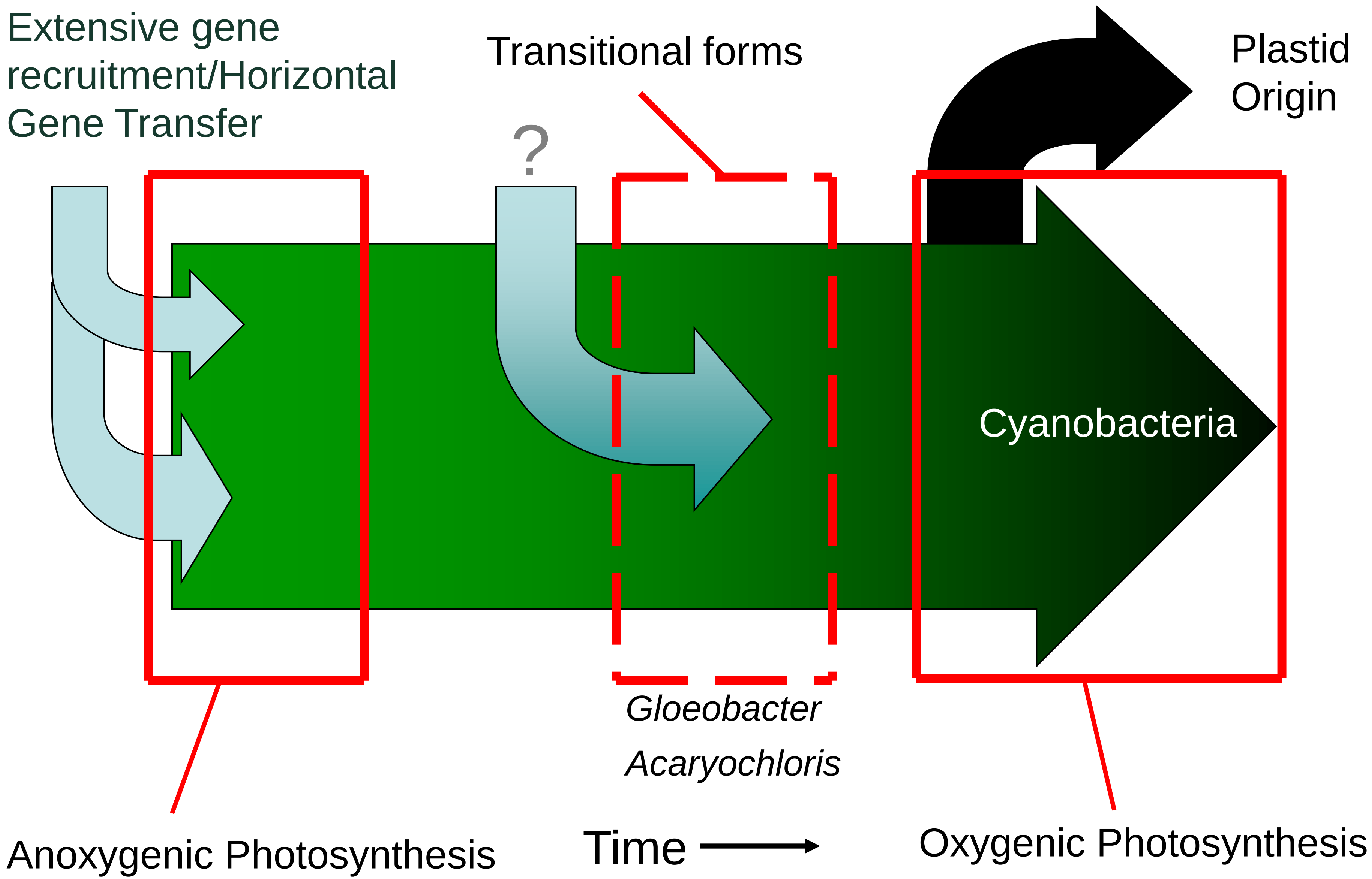


Large amount of Horizontal Gene Transfer has taken place during the evolution of all bacteria, including photosynthetic prokaryotes

However, the basic tree topology probably reflects a core of vertically inherited genes

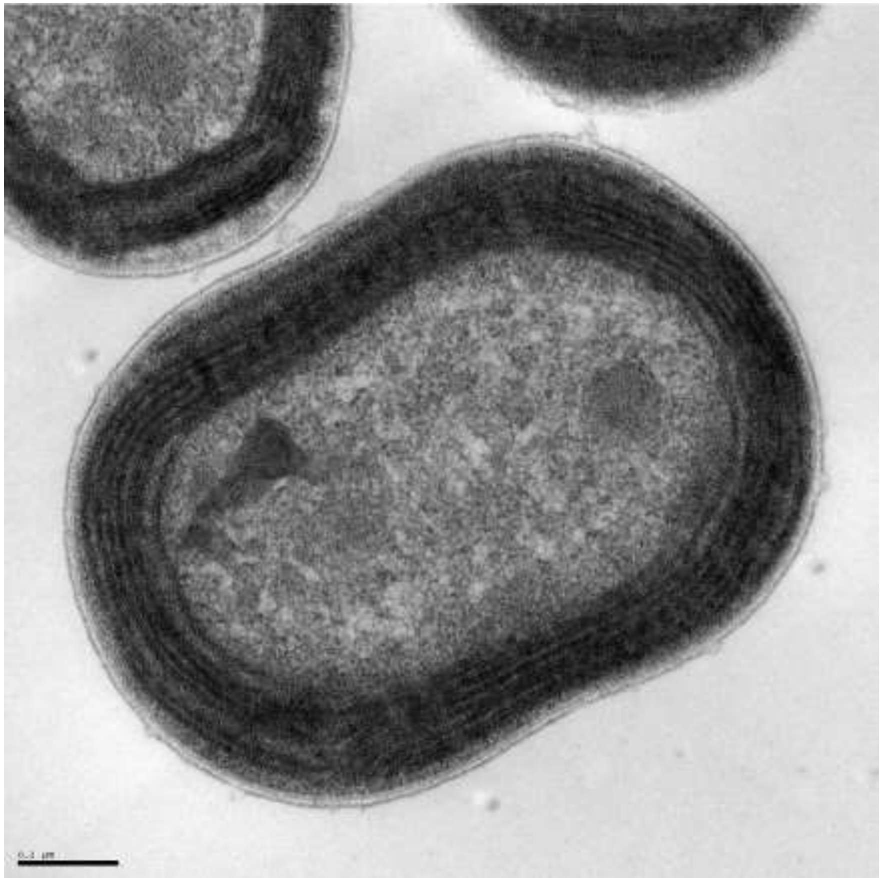
Inauguration of the Plaque, Photo by Govindjee, 2007

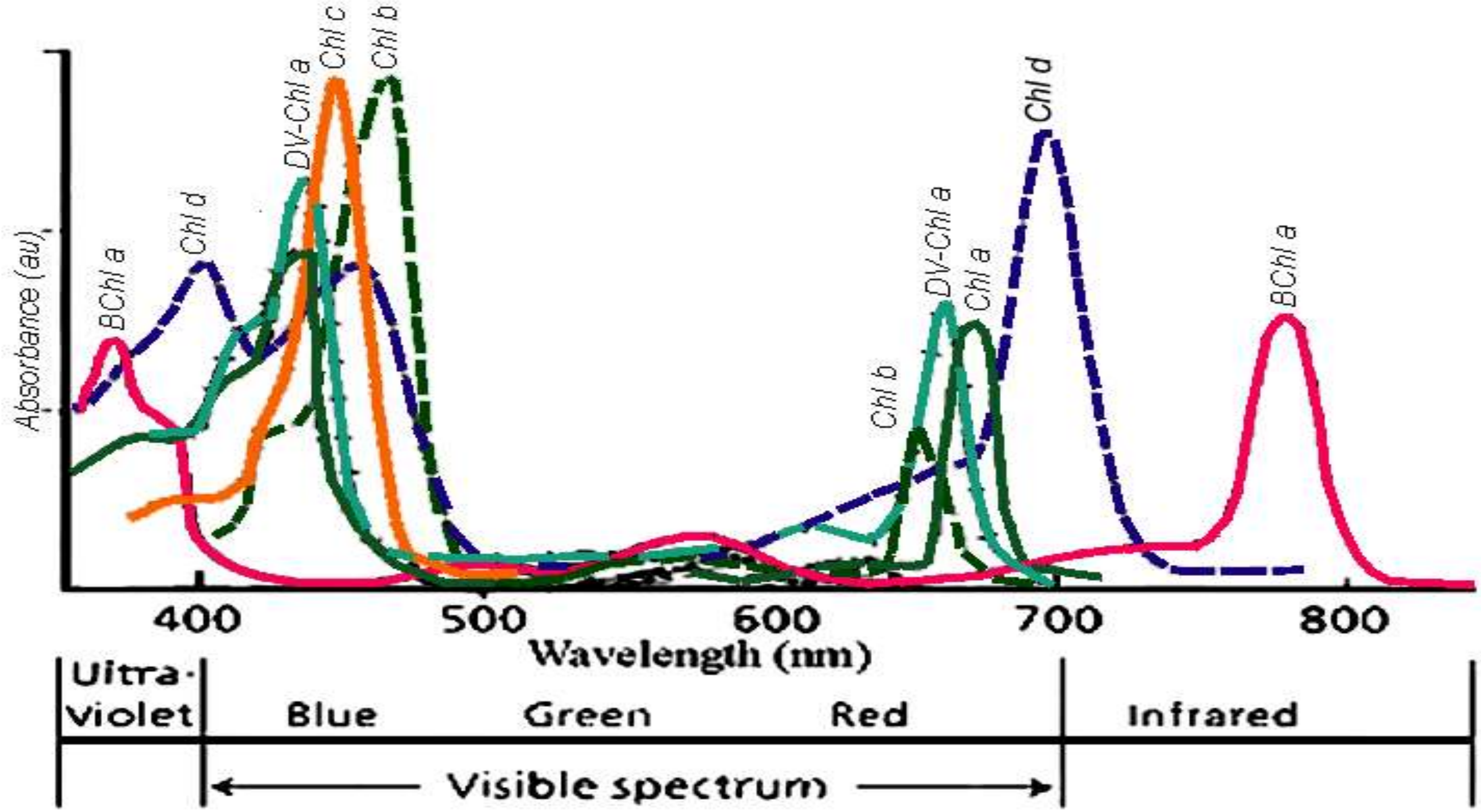
Transition to Oxygenic Photosynthesis



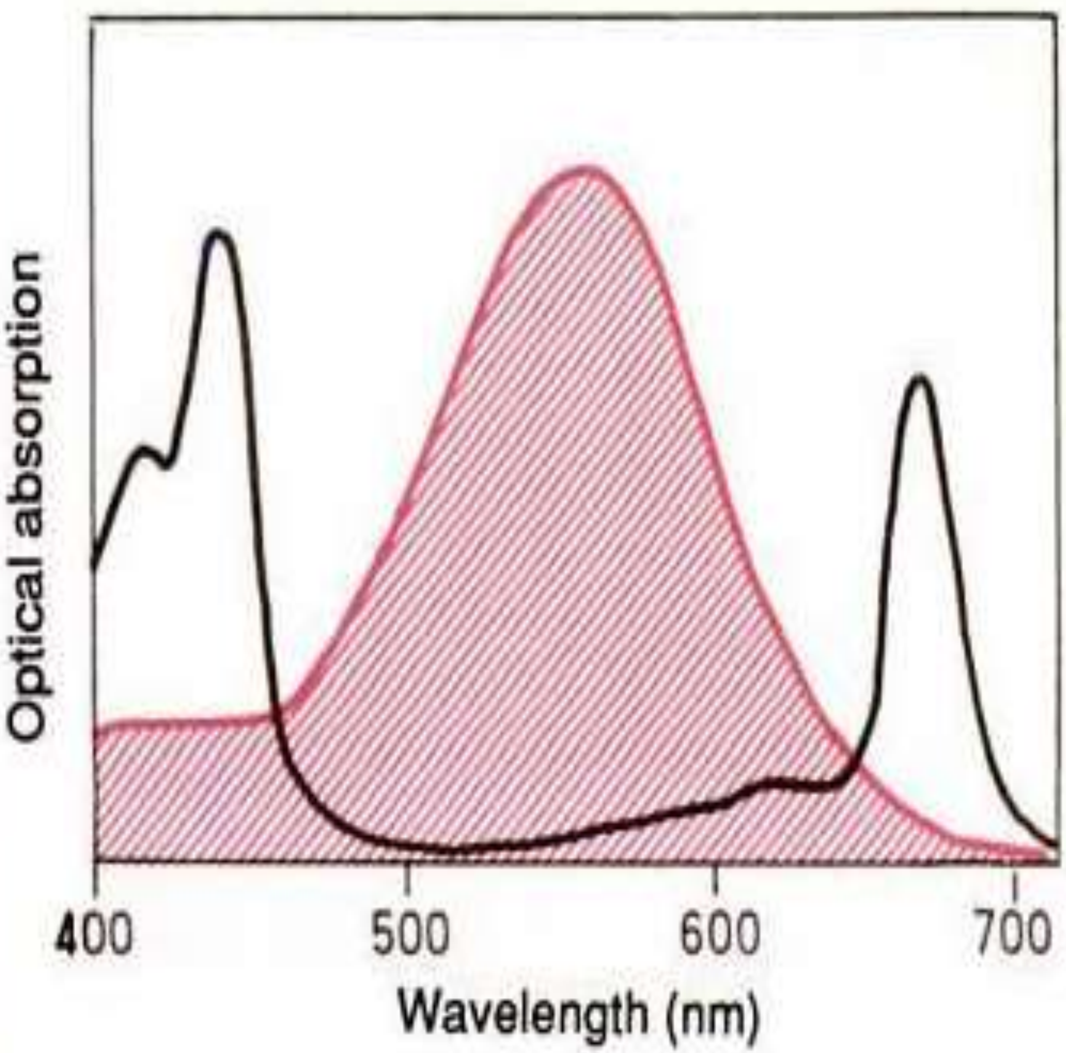
Acaryochloris marina

- Discovered in 1996 by Miyashita et al.
- Isolated from the Western Pacific Ocean
- Contains chlorophyll *d* as major photopigment
- May represent transitional form between anoxygenic and oxygenic photosynthesis
- Some other Chl *d* organisms have been discovered in 2005 (Miller et al. 2005)





Absorbance (au)
BChl a
Chl d
DV-Chl a
Chl c
Chl b
Chl d
Chl b
DV-Chl a
Chl a
BChl a
400
500
600
700
800
Wavelength (nm)
Ultra-
Violet
Blue
Green
Red
Infrared
Visible spectrum



The absorption spectrum of chlorophyll a *superimposed on the absorption spectrum of membranes containing bacteriorhodopsin (shaded). Chlorophyll's absorption peaks fit neatly on either side of bacteriorhodopsin's*

Structure of Bacteriorhodopsin

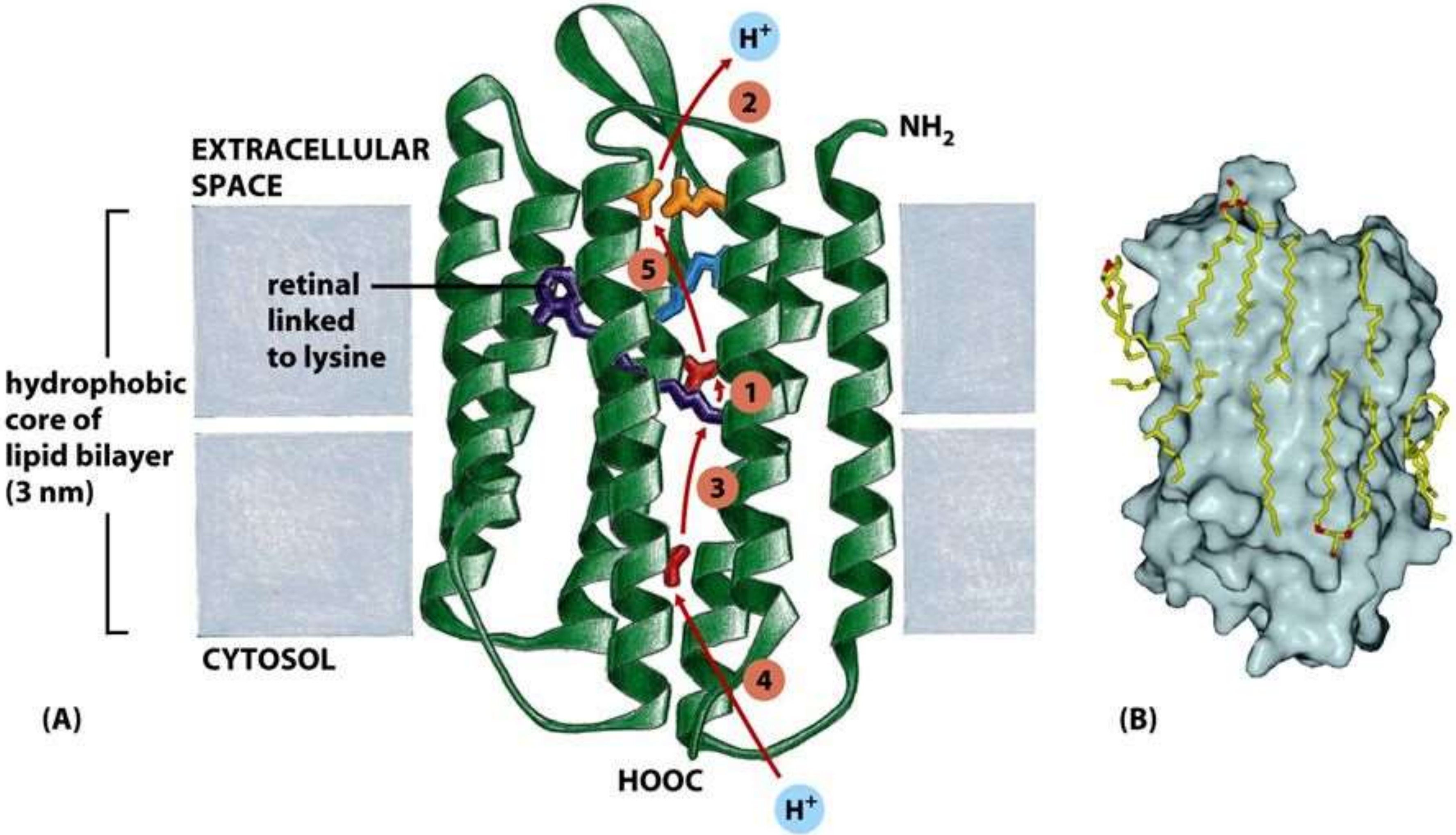
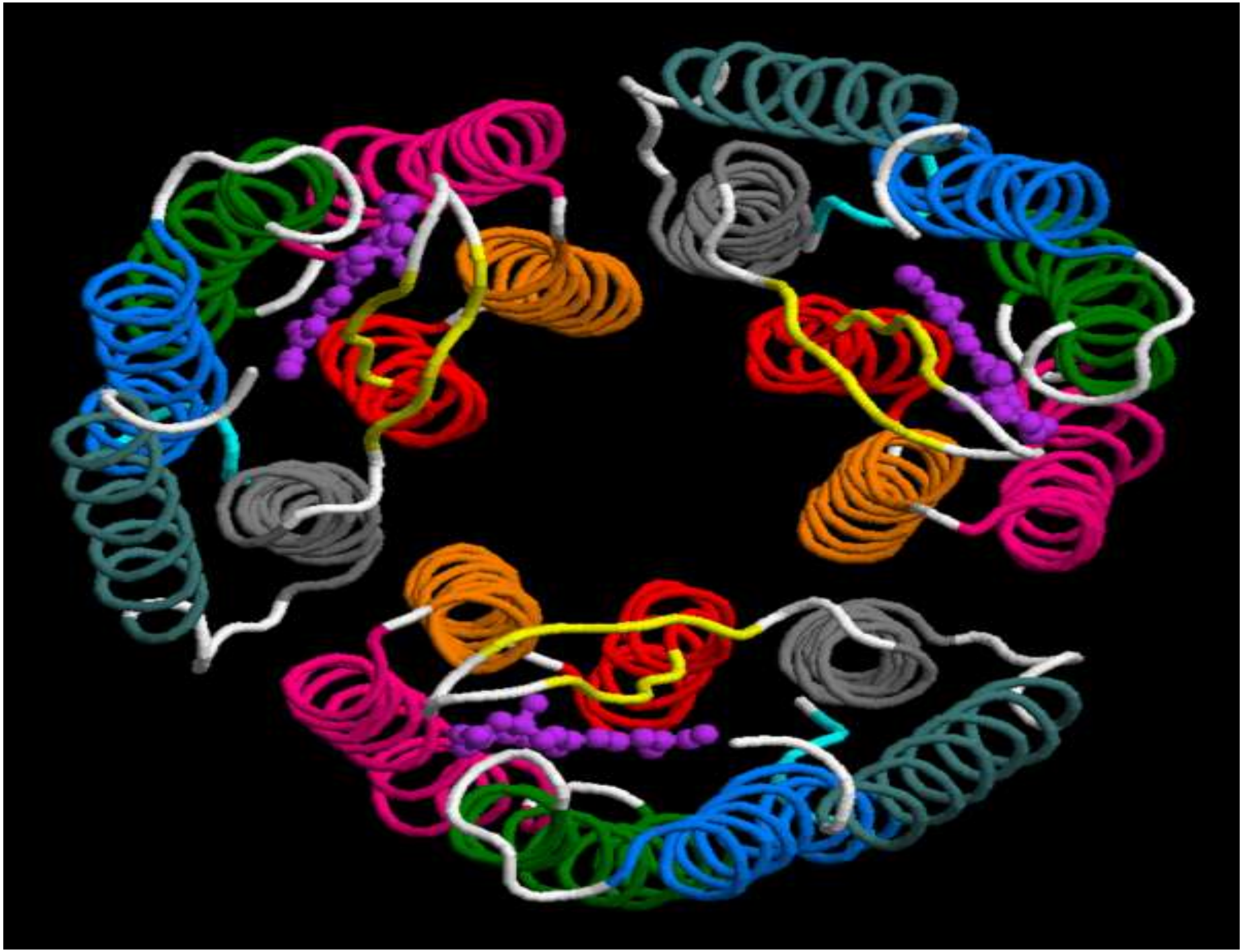
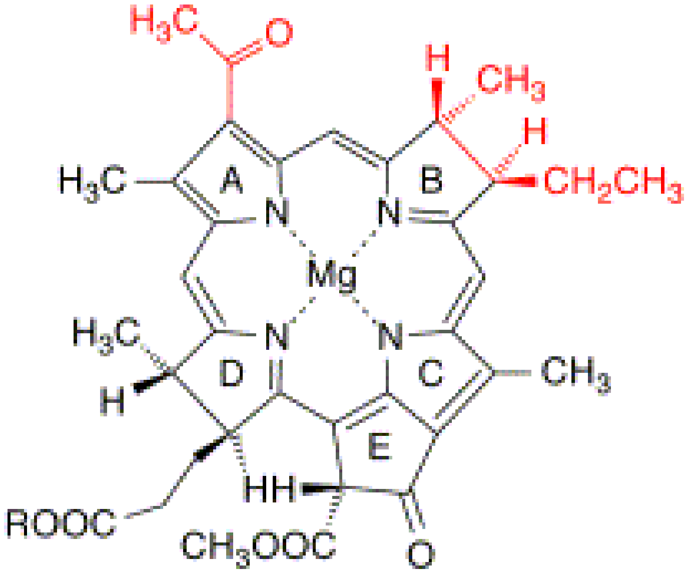


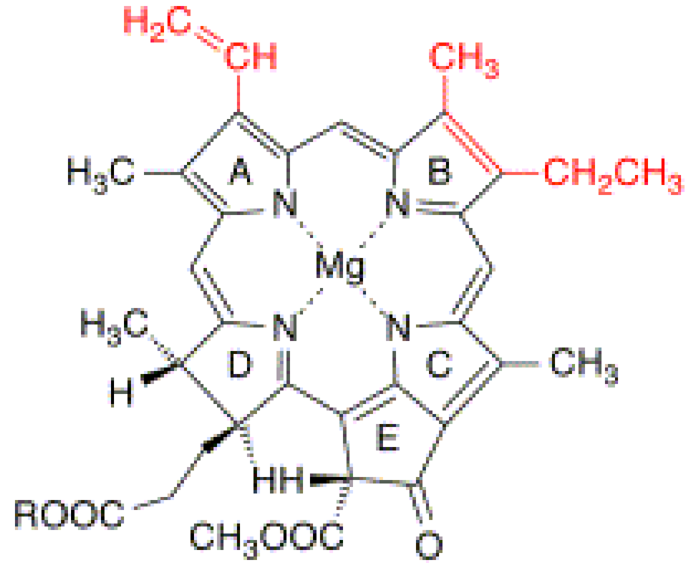
Figure 10-33 *Molecular Biology of the Cell* (© Garland Science 2008)



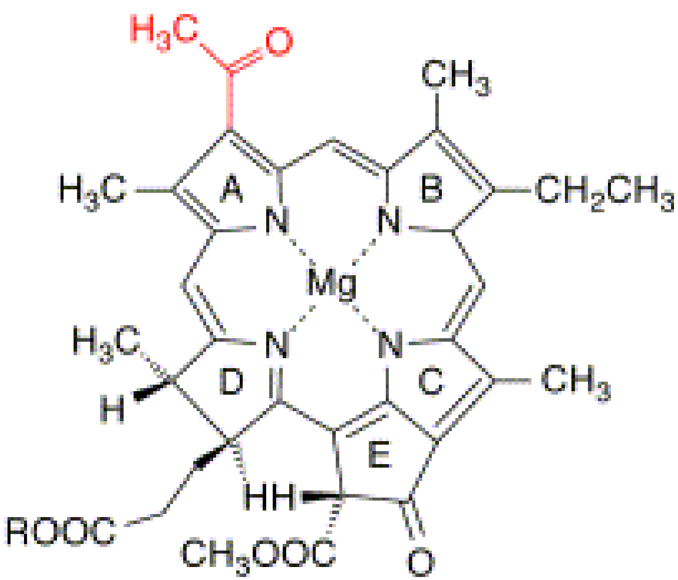
Bacteriorhodopsin trimer from Archea Halobacterium



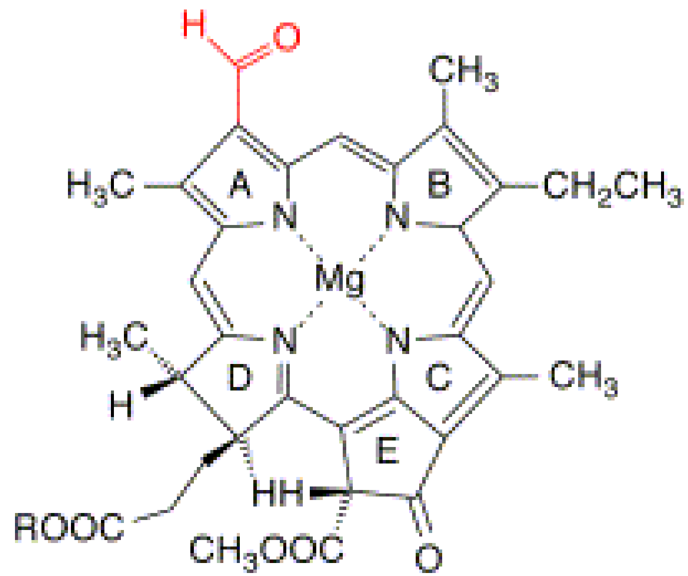
(a) Bacteriochlorophyll *a*



(b) Chlorophyll *a*

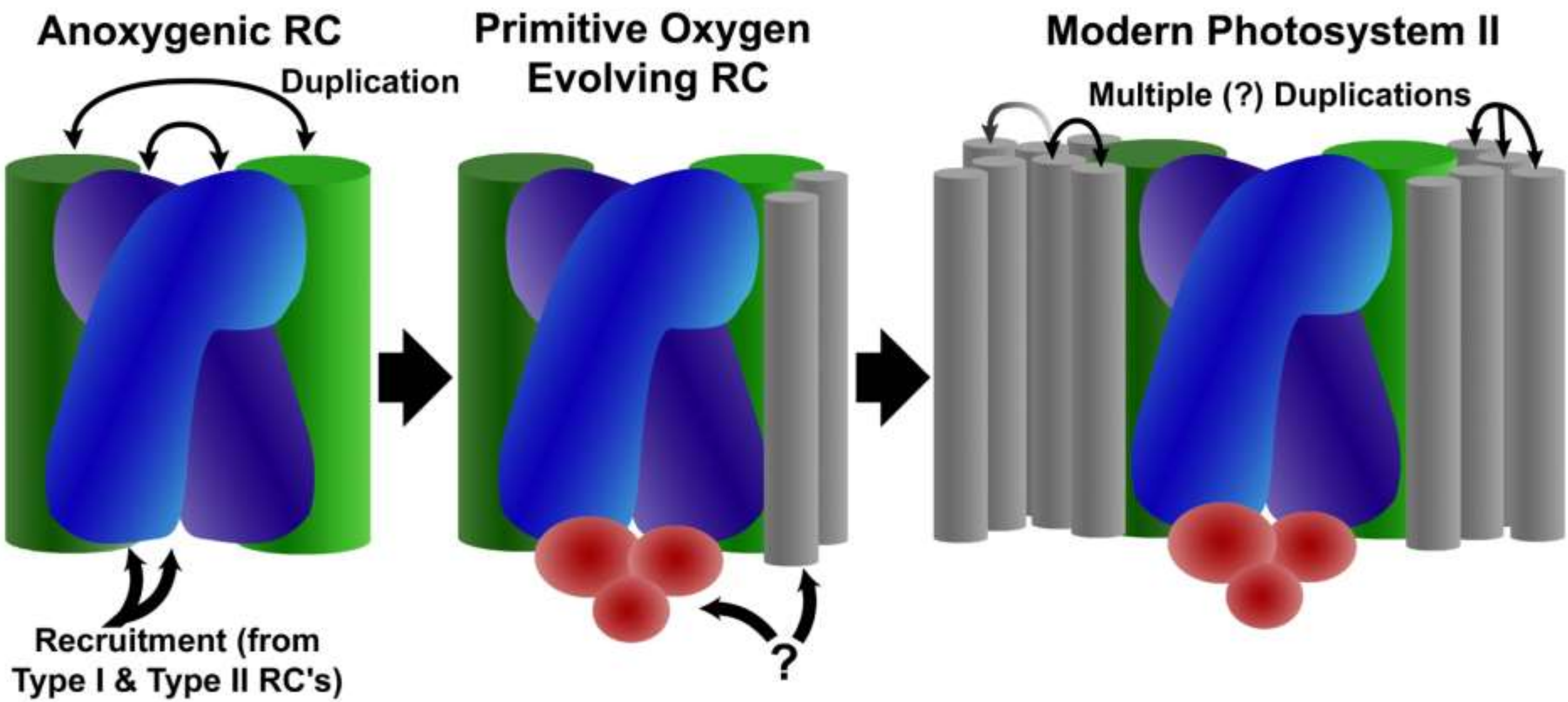


(c) 3-Acetyl-chlorophyll *a*



(d) Chlorophyll *d*

Evolution of Photosystem II Protein Complement



The evolution of Photosystem II proteins has been partially by gene recruitment and partially by gene duplication, but most of the proteins are of unknown origin and have no known homologs in any other organisms

Increase in carbon dioxide concentration should result in a stimulation in photosynthetic carbon fixation of between 30 and 50%, primarily due to a reduction in photorespiration as the ribulose 1·5-bisphosphate carboxylase/ oxygenase (Rubisco) carboxylation reaction is favoured in these conditions.

However, many plant species grown at elevated [CO_2] do not have increased photosynthesis and growth to the level of 30-50%.

It is substantially less than these figures. This is probably because plants at elevated CO_2 exhibit an acclimatory down-regulation, decreasing photosynthetic potential, particularly with long-term growth in elevated [CO_2].

This acclimatory response is often correlated with increased carbohydrate levels together with reductions in total nitrogen and Rubisco activity.

Therefore, it is essential to understand how perennial tree species acclimate themselves to high CO_2 environment after years of exposure to the elevated green house gas.

NO_3^- absorption from soil and its subsequent utilization cannot keep pace with increased photosynthesis at high CO_2 resulting in reduced protein contents and increased Carbohydrate contents of plants. This would lead to seed grains having increased starch and decreased protein.

This challenge needs to be addressed.

Free Air Carbon dioxide enrichment (FACE) facility built in the campus of Jawaharlal Nehru University. Mustard (*Brassica*) plants are grown inside two FACE Rings maintained at elevated CO_2 (600 ppm)

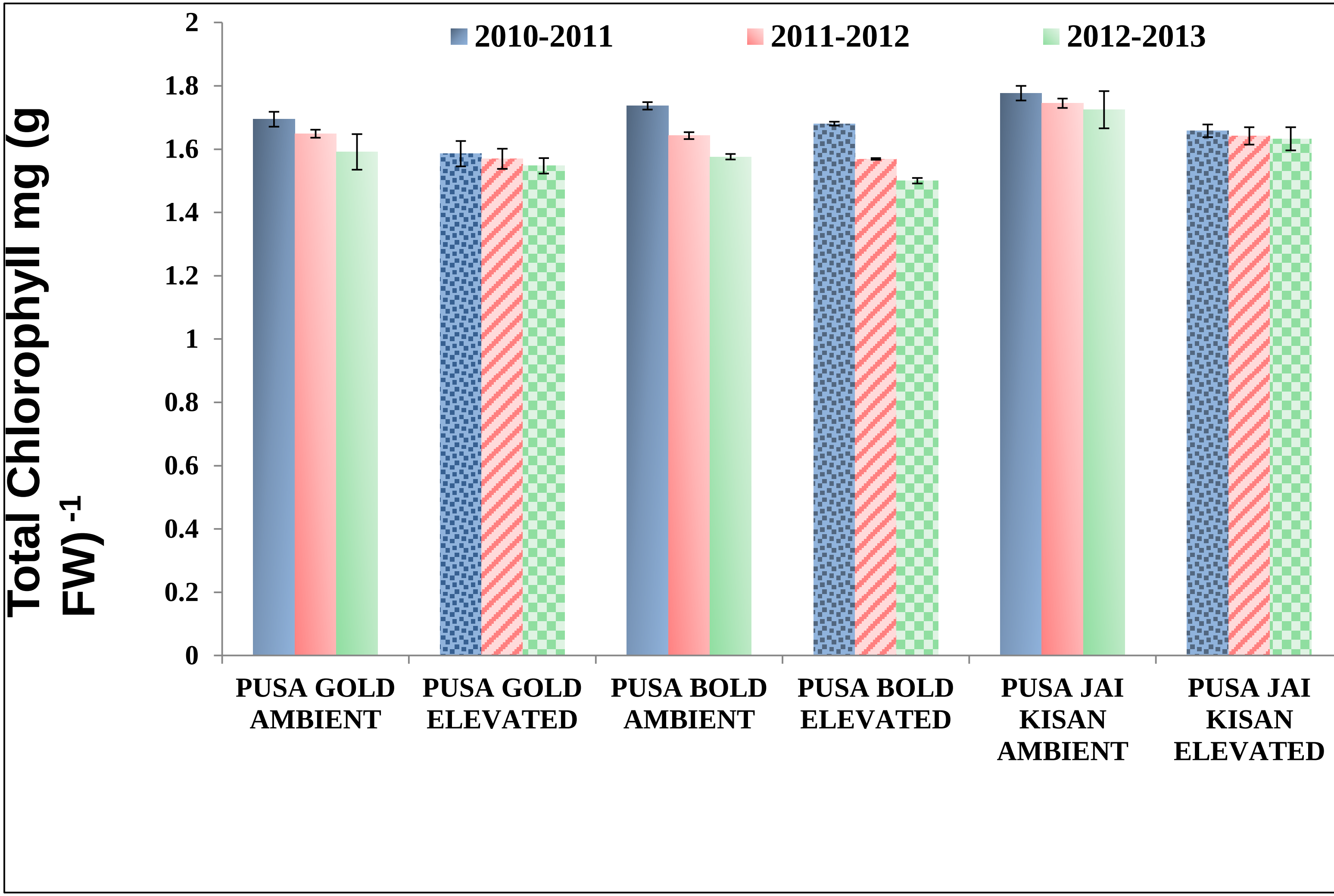


Fig 1. Total chlorophyll content of *Brassica campestris* cv. Pusa Gold, *Brassica juncea* cv. Pusa Bold and Pusa Jaikisan grown in ambient carbondioxide (385 μmol mol^{-1}) and elevated carbondioxide (585 μmol mol^{-1}) in three different years . Each data point is an average of six

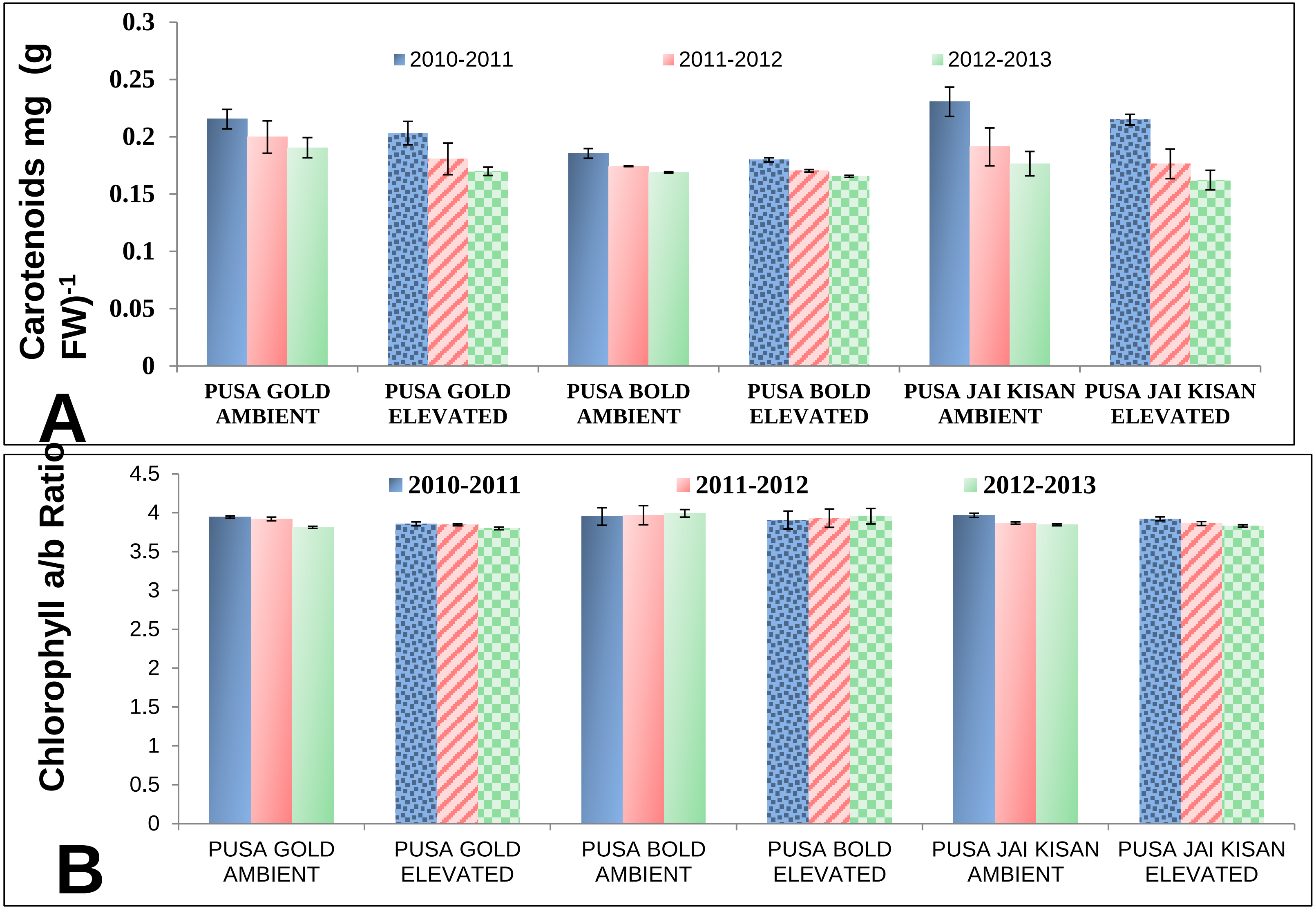


Fig 2. Carotenoids content (A) and chlorophyll a/b ratio (B) of *Brassica campestris* cv. Pusa Gold, *Brassica juncea* cv. Pusa Bold and Pusa Jaikisan grown in ambient carbondioxide (385 ppm) and elevated carbondioxide (585 ppm) in three different years .

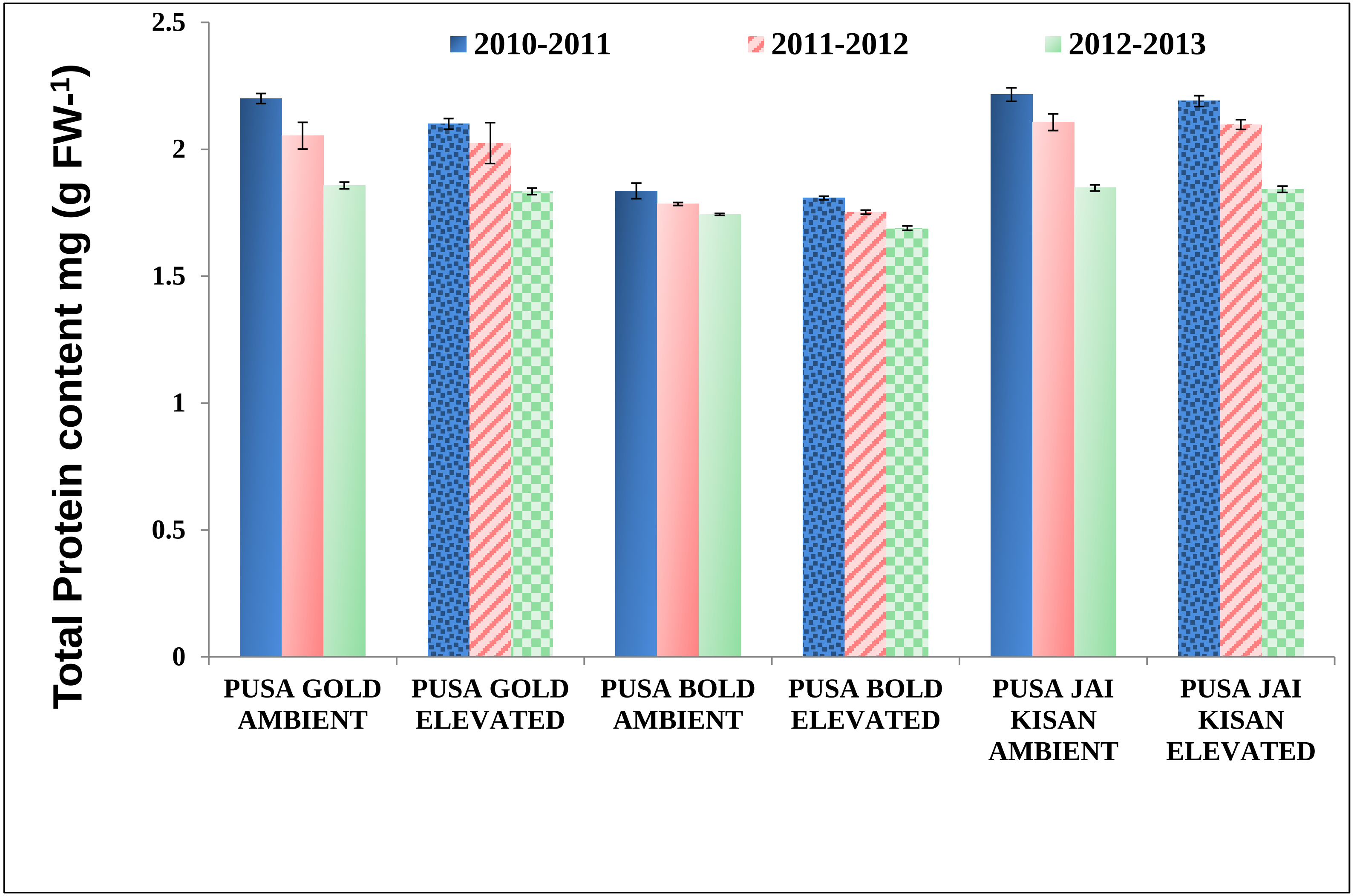


Fig 3.Total Protein Content of *Brassica campestris* cv. Pusa Gold, *Brassica juncea* cv. Pusa Bold and Pusa Jaikisan grown in ambient carbondioxide (385 ppm) and elevated carbondioxide (585 ppm) in three different growing seasons . Each data point is an average of six

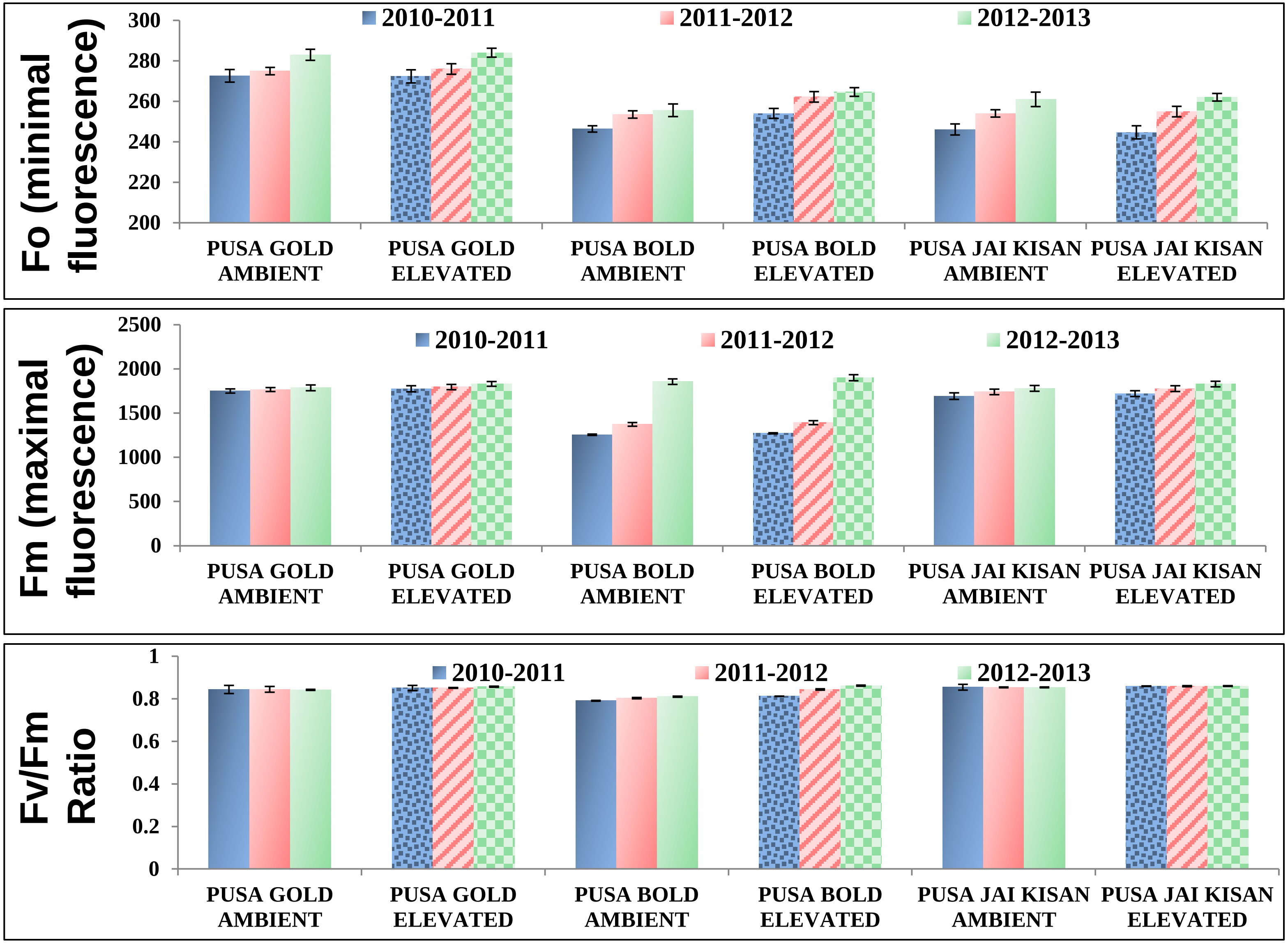


Fig 4. Fo, Fm and Fv/Fm in the leaves of Pusa Gold, Pusa Bold and Pusa Jaikisan in ambient (385 ppm) and enriched CO_2 concentrations (585 ppm).

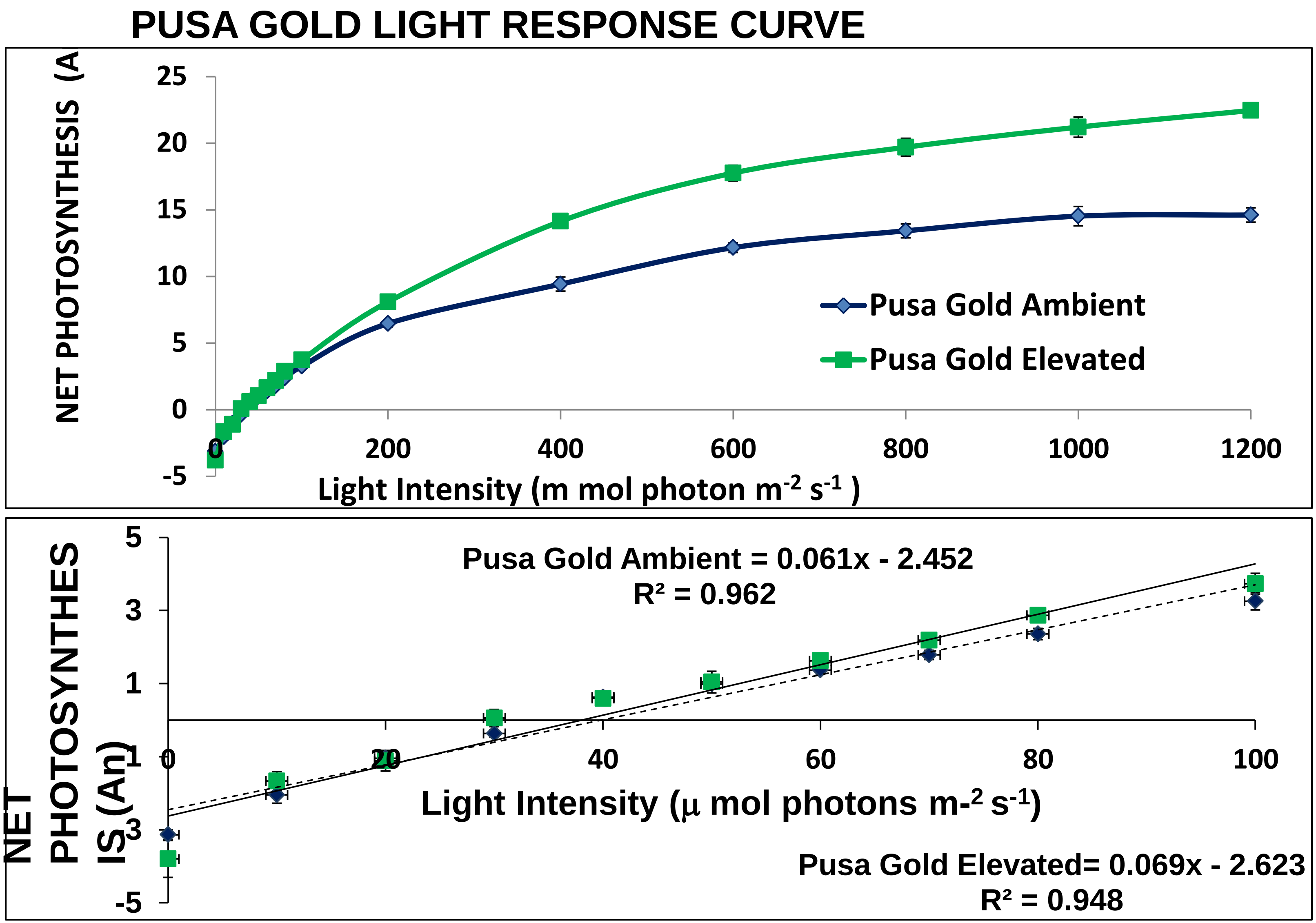


Fig. 13 Photosynthesis (net CO_2 assimilation rate) light response curves and quantum yield of attached leaves of *Brassica campestris* (Pusa Gold) plants grown in ambient and elevated CO_2 concentrations. A, Net CO_2 assimilation rates of attached leaves of *Brassica campestris* (Pusa Gold) plants were monitored by IRGA (Licor 6400-XT portable photosynthetic system) in ambient and elevated CO_2 at different light intensities. Light response curves were measured upto 1200 μmol of photons m^{-2} s^{-1} at 25^0C. B, Relative quantum yield of CO_2 fixation by leaves from *Brassica juncea* (Pusa Jaikisan) plants grown in ambient and elevated CO_2. Quantum yield was measured from the above photosynthetic rate after the IRGA chamber reached to a steady-state. Light intensity curves at limiting light intensities i.e., upto 100 μmol of photons m^{-2} s^{-1}; the slopes of these curves provide relative quantum yield of CO_2 fixation by leaves. Leaves were pre-exposed for 15 minutes at 700 μmol photons m^{-2} s^{-1} prior to CO_2 assimilation measurement. These experiments were done thrice with similar results Each data point is the average of six replicates and the error bar represents

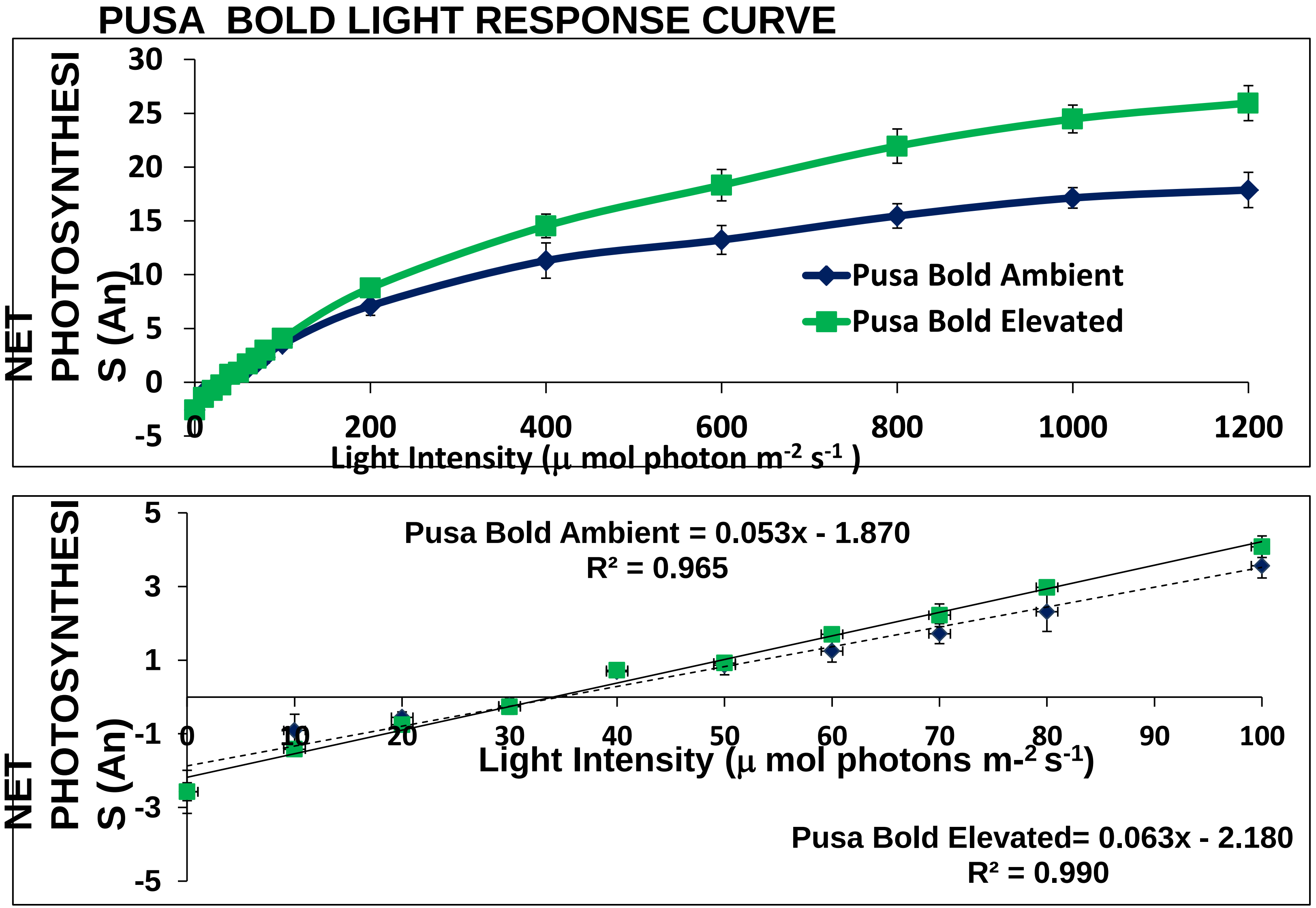
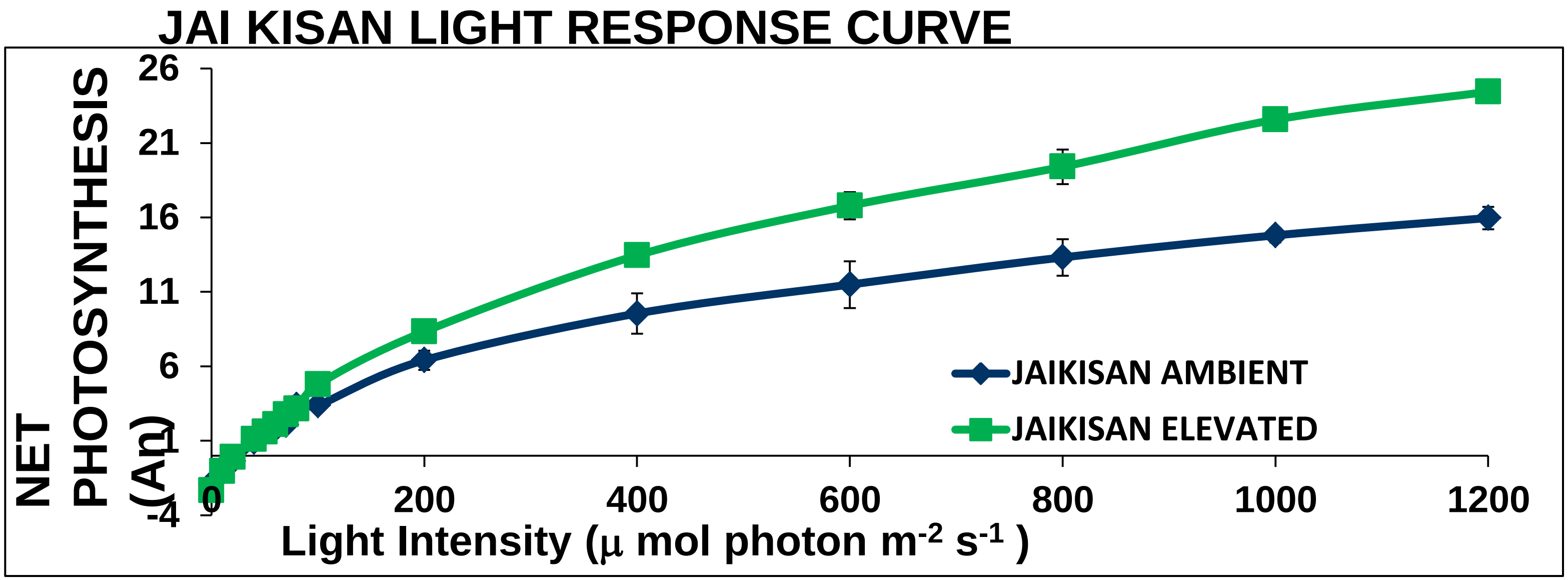


Fig. 14 Photosynthesis (net CO_2 assimilation rate) light response curves and quantum yield of attached leaves of *Brassica juncea* (Pusa Bold) plants grown in ambient and elevated CO_2 concentrations. A, Net CO_2 assimilation rates of attached leaves of *Brassica campestris* (Pusa Gold) plants were monitored by IRGA (Licor 6400-XT portable photosynthetic system) in ambient and elevated CO_2 at different light intensities. Light response curves were measured upto 1200 µmol of photons m^{-2} s^{-1} at 25^0C. B, Relative quantum yield of CO_2 fixation by leaves from *Brassica juncea* (Pusa Jaikisan) plants grown in ambient and elevated CO_2. Quantum yield was measured from the above photosynthetic rate after the IRGA chamber reached to a steady-state. Light intensity curves at limiting light intensities i.e., upto 100 µmol of photons m^{-2} s^{-1}; the slopes of these curves provide relative quantum yield of CO_2 fixation by leaves. Leaves were pre-exposed for 15 minutes at 700 µmol photons m^{-2} s^{-1} prior to CO_2 assimilation measurement. These experiments were done thrice with similar results Each data



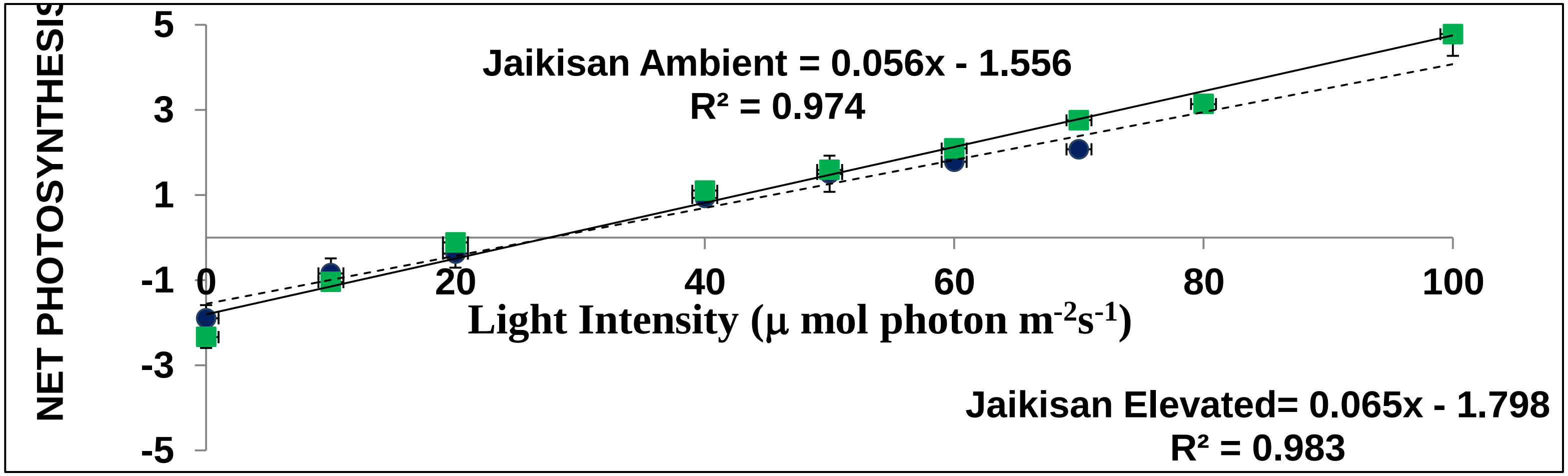


Fig. 15 Photosynthesis (net CO_2 assimilation rate) light response curves and quantum yield of attached leaves of *Brassica juncea* (Pusa Jaikisan) plants grown in ambient and elevated CO_2 concentrations. A, Net CO_2 assimilation rates of attached leaves of *Brassica campestris* (Pusa Gold) plants were monitored by IRGA (Licor 6400-XT portable photosynthetic system) in ambient and elevated CO_2 at different light intensities. Light response curves were measured upto 1200 μmol of photons m^{-2} s^{-1} at 25^0C. B, Relative quantum yield of CO_2 fixation by leaves from *Brassica juncea* (Pusa Jaikisan) plants grown in ambient and elevated CO_2. Quantum yield was measured from the above photosynthetic rate after the IRGA chamber reached to a steady-state. Light intensity curves at limiting light intensities i.e., upto 100 μmol of photons m^{-2} s^{-1}; the slopes of these curves provide relative quantum yield of CO_2 fixation by leaves. Leaves were pre-exposed for 15 minutes at 700 μmol photons m^{-2} s^{-1} prior to CO_2 assimilation measurement. These experiments were done thrice with similar results Each data point is the average of six replicates

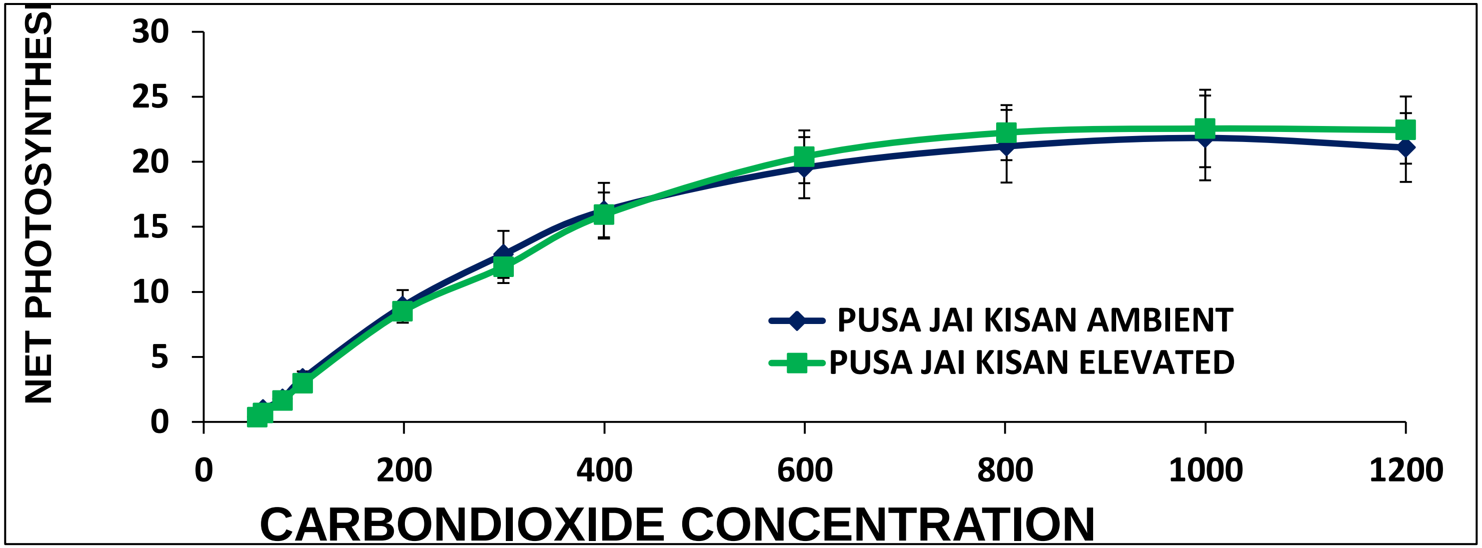


Fig. 17. The response of the net photosynthesis (An) to increasing carbon dioxide concentrations in Pusa Jaikisan. Ea

	PUSA GOLD AMBIENT	PUSA GOLD ELEVATED	PUSA BOLD AMBIENT	PUSA BOLD ELEVATED	PUSA JAIKISAN AMBIENT	PUSA JAIKISAN ELEVATED
Vcmax	73.65	74.72	74.88	73.63	66.60	65.80
Jmax	155.17	161.14	149.90	155.00	137.90	146.04

Table 1. Vcmax and Jmax values of of *Brassica campestris cv.* Pusa Gold *and Brassica juncea* cv. Pusa Bold and Pusa Jaikisan plants grown in ambient (385 ppm) and elevated CO_2 concentration (585 ppm)

RESPIRATION RATE

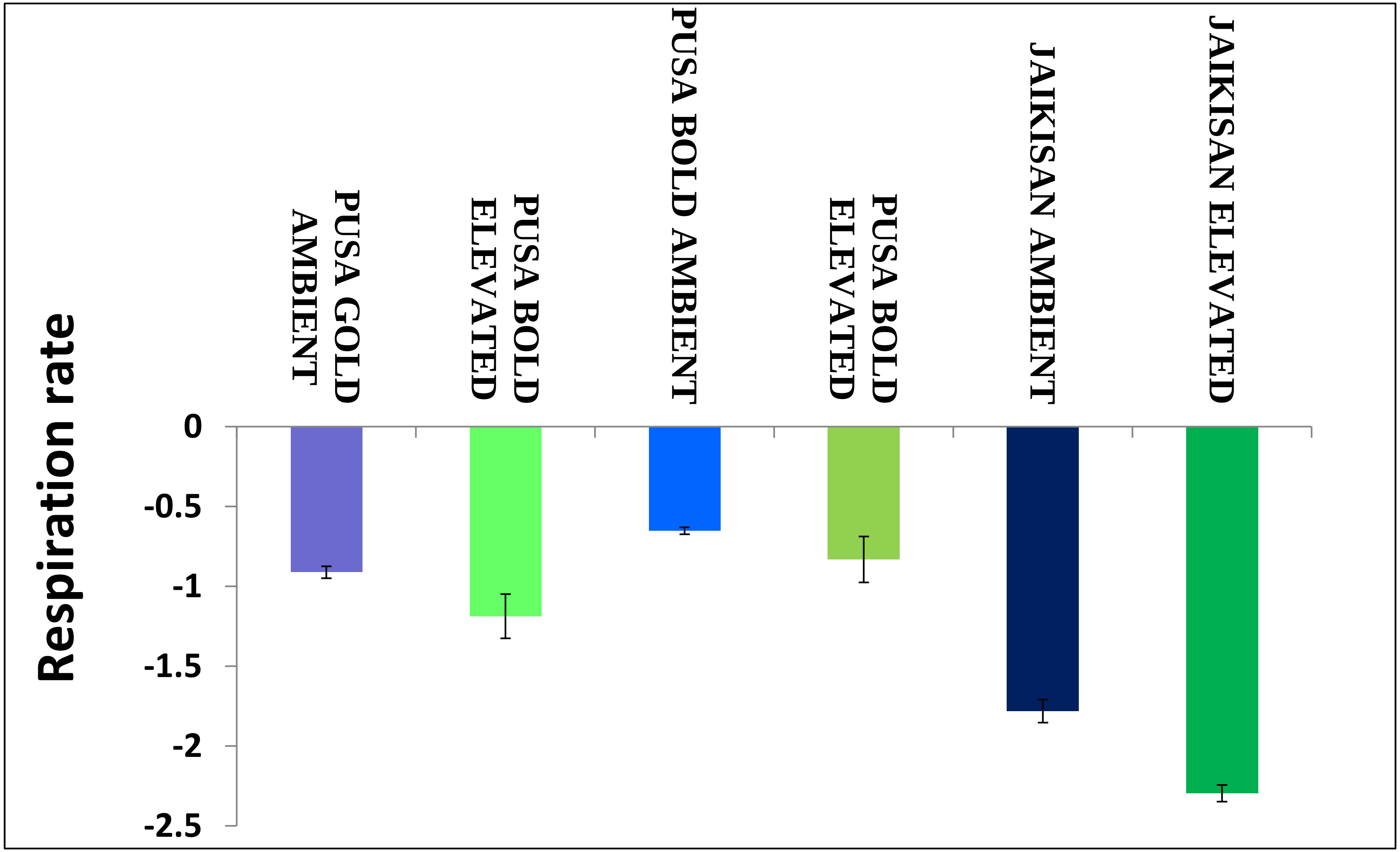


Fig 18. Respiration rate of all the three cultivars of Brassica grown in ambient an
elevated carbondioxide.

PUSA GOLD AMBIENT **PUSA GOLD ELEVATED**

Fig. 23 *Brassica campestris* cv Pusa Gold grown in (A) ambient CO_2 (385 ppm) or (B) elevated CO2 (585 ppm) inside the FACE ring. The Plants grown in elevated CO_2 had larger number of leaves, larger

PUSA BOLD AMBIENT **PUSA BOLD ELEVATED**

Fig. 24 *Brassica juncea* cv Pusa Bold grown in (A) ambient CO_2 (385 ppm) or (B) elevated CO2 (585 ppm) inside the FACE ring. The Plants grown in elevated CO_2 had larger number of leaves, larger roots and higher biomass.

PUSA JAIKISAN AMBIENT **PUSA JAIKISAN ELEVATED**

Fig. 25 *Brassica juncea* cv Pusa Jaikisan grown in (A) ambient CO_2 (385 ppm) or (B) elevated CO2 (585 ppm) inside the FACE ring. The

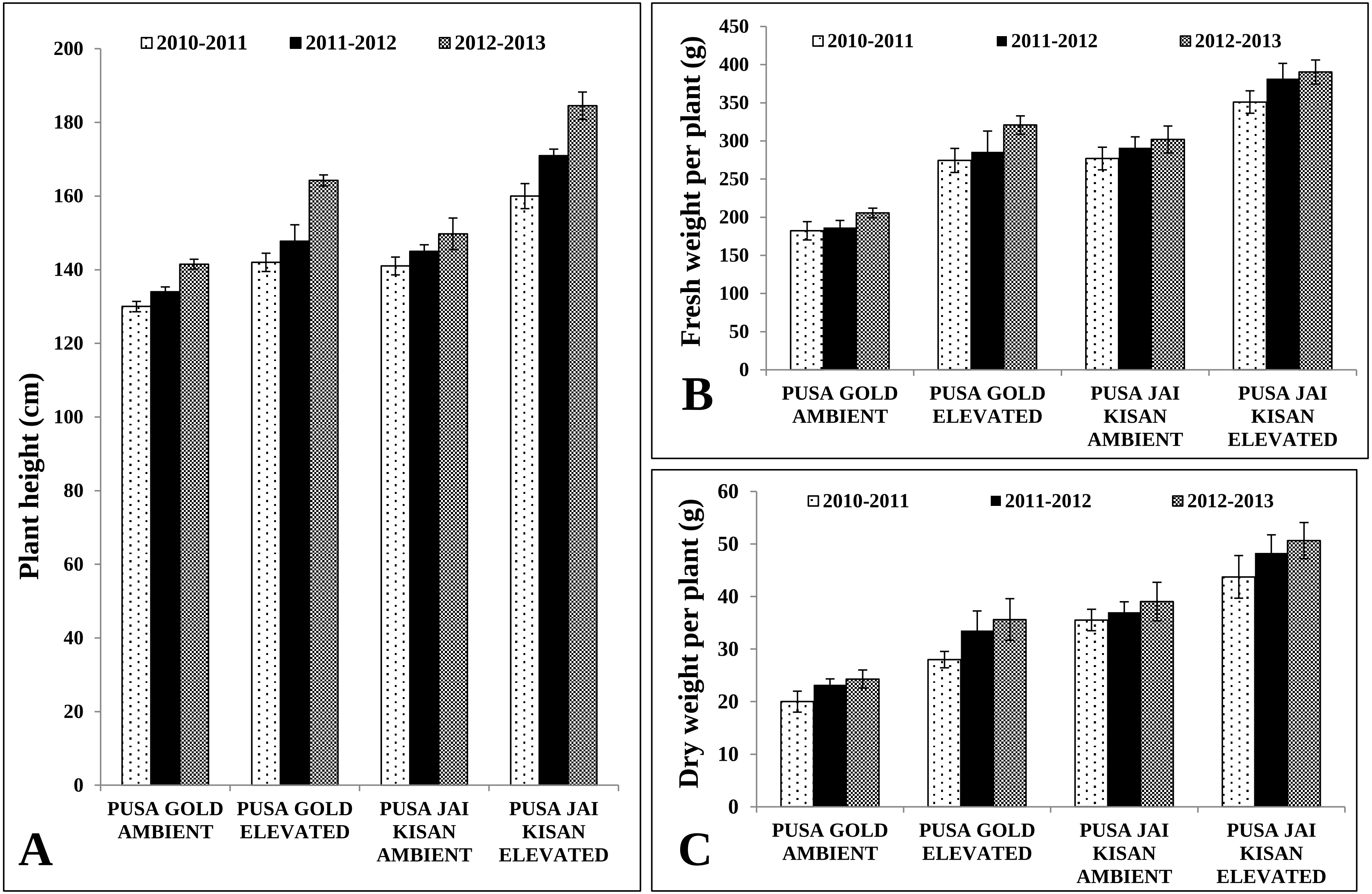


Figure. 10 Plant height (A), Fresh weight per plant (C) and Dry weight per plant (D) of *Brassica campestris* (Pusa Gold), *Brassica juncea (*Pusa Jai Kisan) leaves grown in ambient and elevated CO_2 (585 μmol mol^{-1}) in three different growing seasons. Each data point is the average of six replicates and the error bar represents SE. Asterisks indicate significant differences determined by t test (P < 0.05, **P<0.001).***

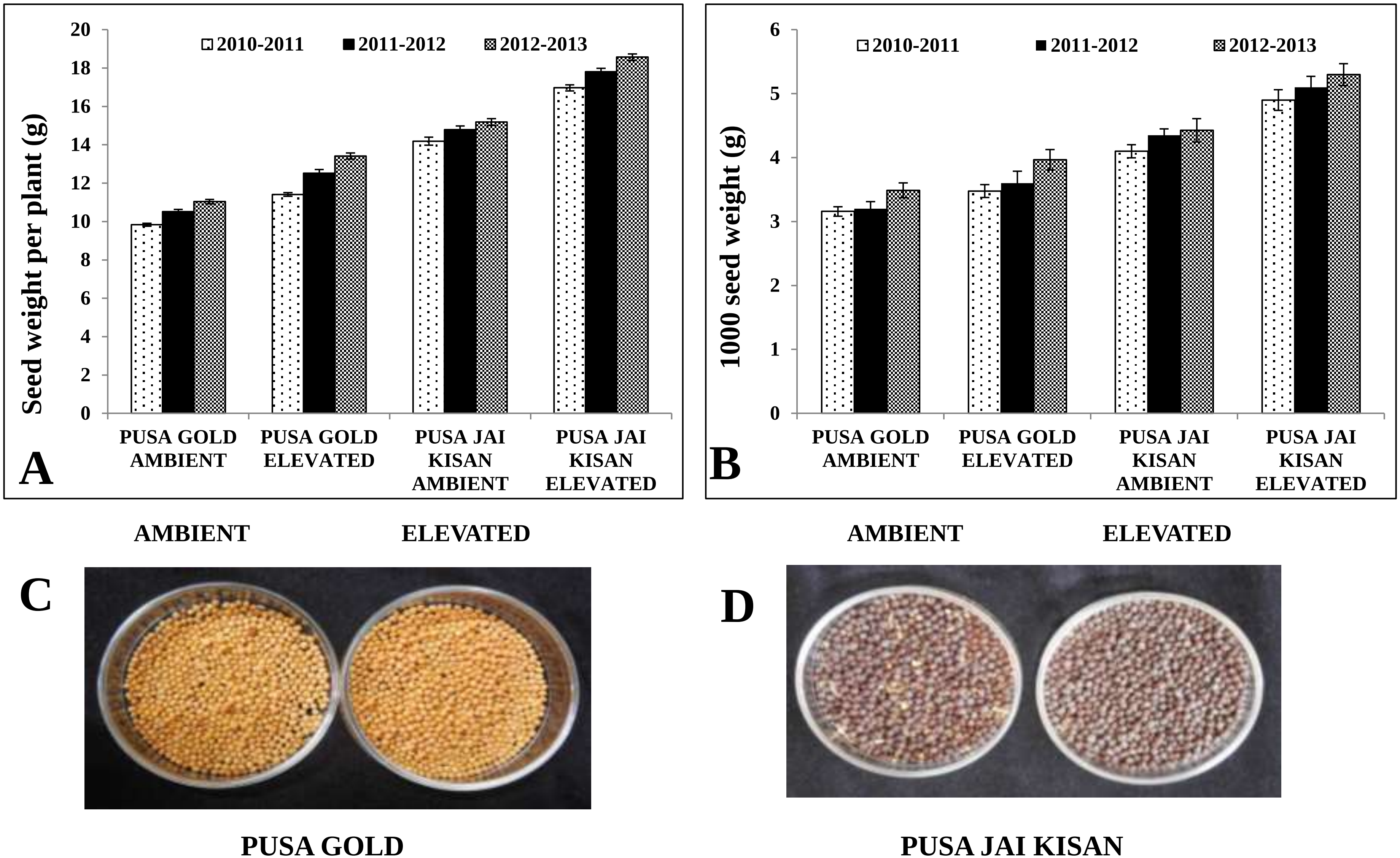


Figure. 11 Seed weight per plant (A), 1000 seed weight (B), seed morphology (C;D) of *Brassica campestris* (Pusa Gold), *Brassica juncea (*Pusa Jai Kisan) leaves grown in ambient and elevated CO_2 (585 µmol mol^{-1}) in three different growing seasons. Each data point is the average of fifty replicates and the error bar represents SE. Asterisks indicate significant differences determined by t test (*$P < 0.05$, **$P<0.001$).*

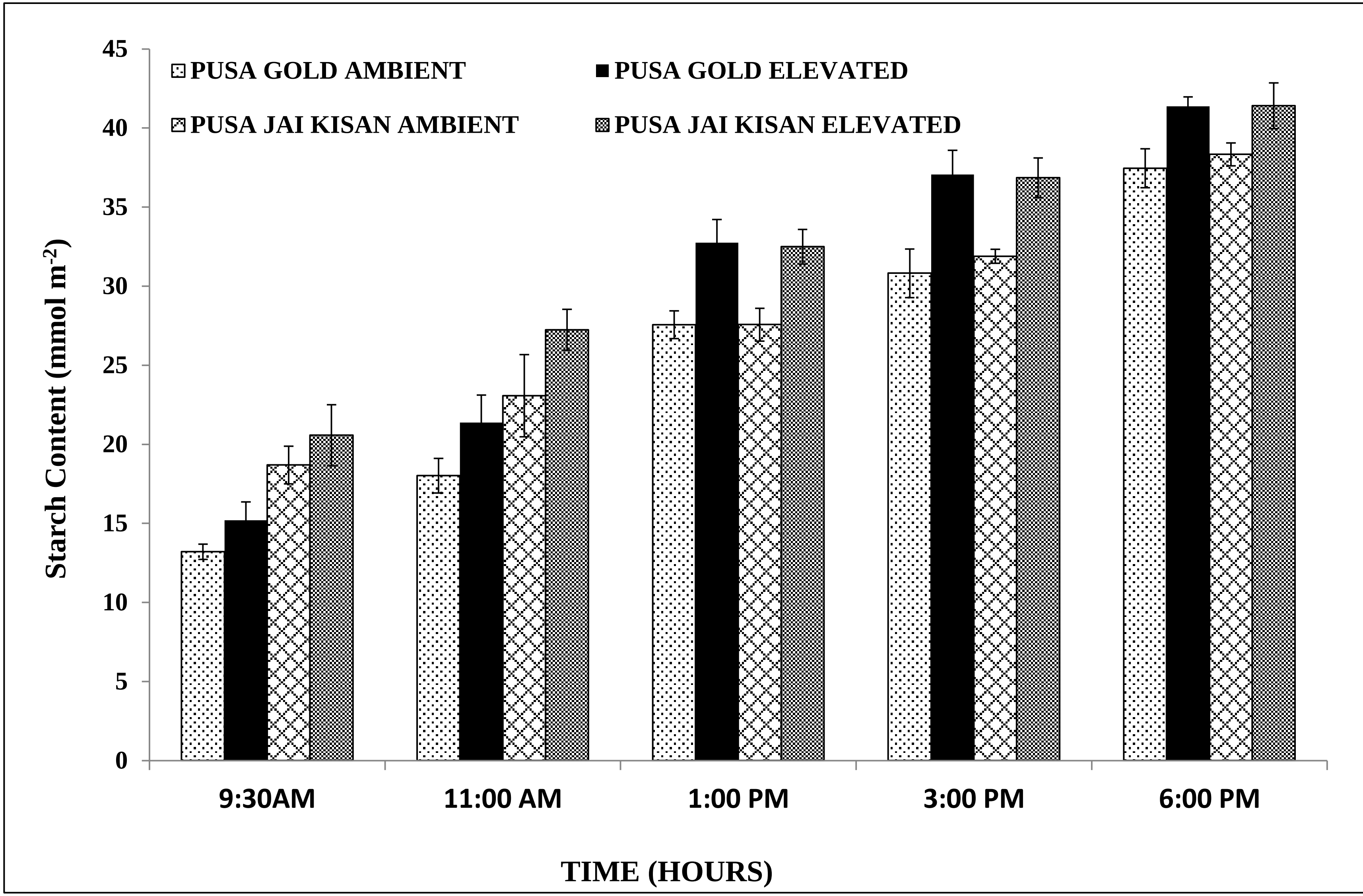


Figure 12. The diurnal measurement of Starch content from morning to evening, of *Brassica campestris* (Pusa Gold), *Brassica juncea (*Pusa Jai Kisan) leaves grown in ambient and elevated CO_2 (585 µmol mol^{-1}) . Each data point is the average of six replicates and the error bar represents SE. Asterisks indicate significant differences determined by t test (*$P < 0.05$, **$P<0.001$).

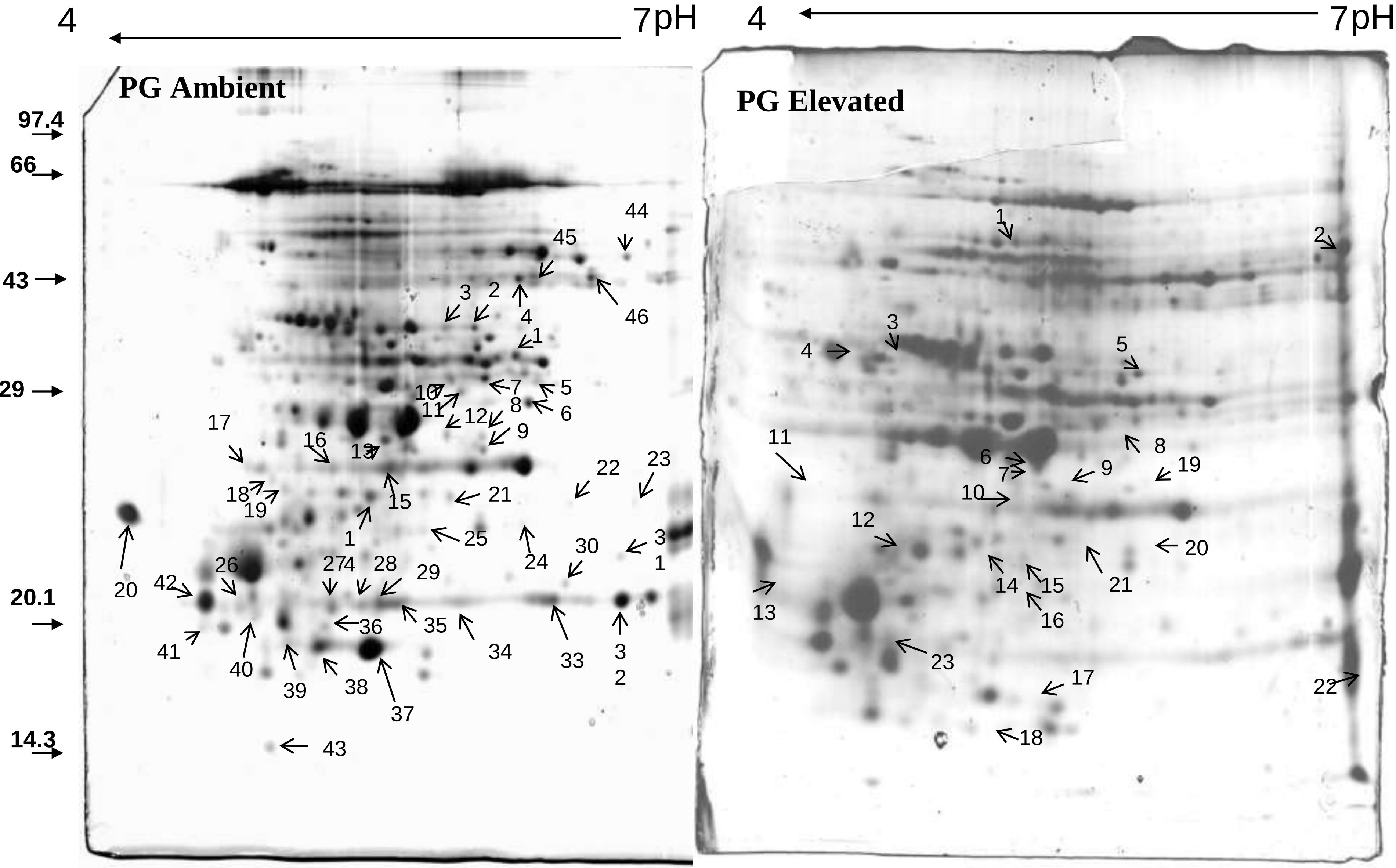


Fig. 13. 2-D gel of PEG fractionated soluble proteins of ambient (left panel) and elevated (right panel) Brassica, *Brassica campestris* (Pusa Gold) leaves grown in ambient and elevated CO_2 (585 µmol mol^{-1}). 2-D gel was run with 800 µg of protein and Coomassie Brilliant Blue (CBB) stained.

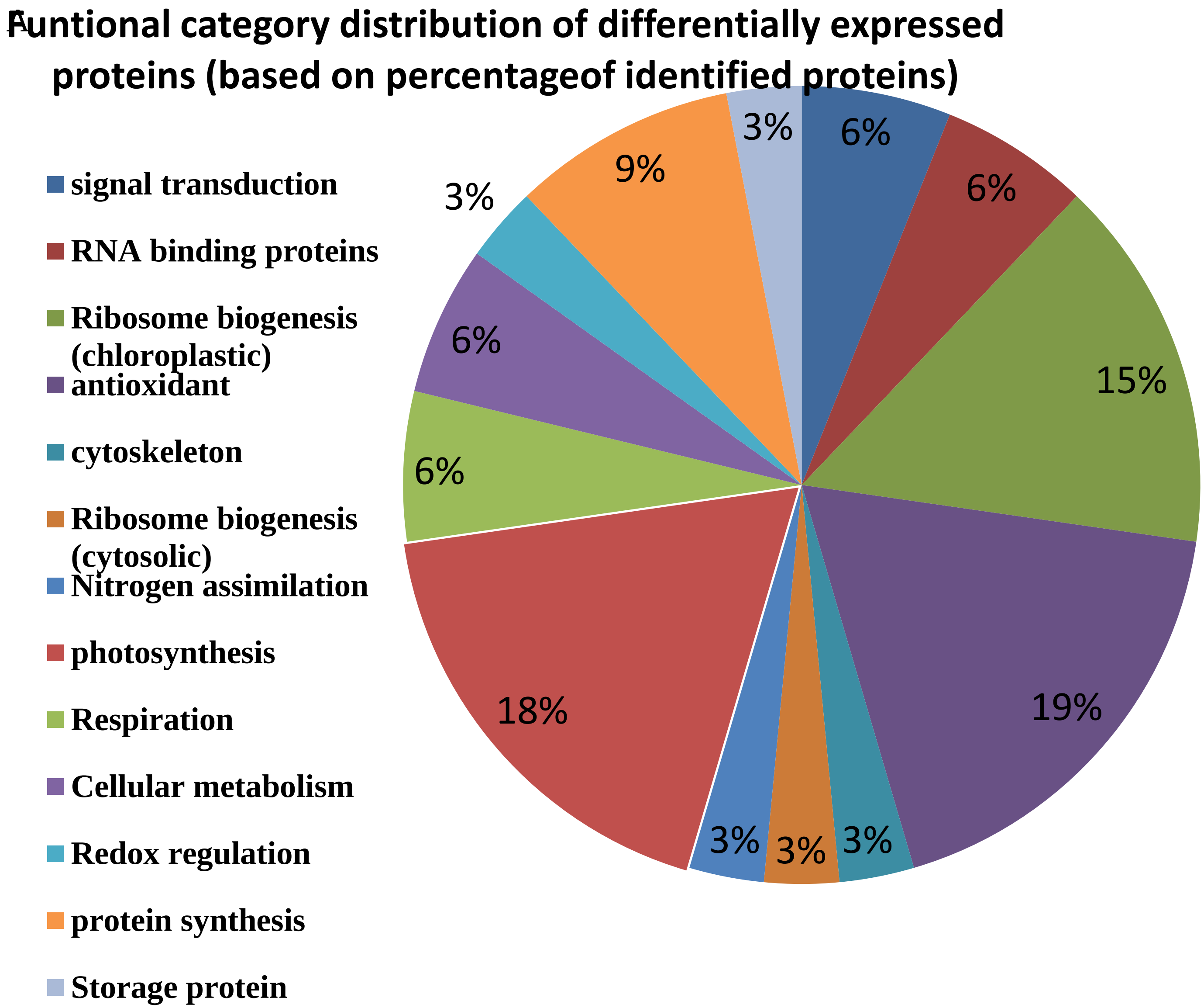


Fig. .. Functional category distribution of differentially expressed proteins in 2 D electrophoresis gel of PEG fractionated soluble proteins of ambient-and elevated -CO_2- grown Pusa Gold.

Increase in ocean temperature will release the dissolved CO_2 to the atmosphere further increasing global warming.

The temperature on sea surface will percolate down to the ocean floor resulting in rise of sea level up to 30 meters by the end of this millenium (year 3000).

Therefore, it is essential to generate crop plants Tolerant to high temperature and water logging especially for coastal region.

How to combat elevated CO_2

- Reduction in green house gas emission
- Increased CO_2 fixation by plants especially by reforestation program
- Plantation of fast-growing trees i.e. Poplar (*Populus deltoides*) to have long-term carbon sequestration

*Plantation of tree species of mangrove vegetation in the sea coast for carbon sequestration and conservation of soil

As world population is increasing, the demand on Agricultural land is also increasing.

A country like India cannot afford to loose agricultural land for generation of bioethanol i.e. from sugar cane.

Therefore we should look into the sea rather than land mass for generation of bioethanol.

INDIA'S POSITION

Large coast line 7000 km

National Coordinated Program for large scale cultivation and utilization of 3-4 taxa having both domestic and international market.

Gracilaria verrucosa

Kappaphycus alvarezii

Sargassum sp.

Porphyra sp.

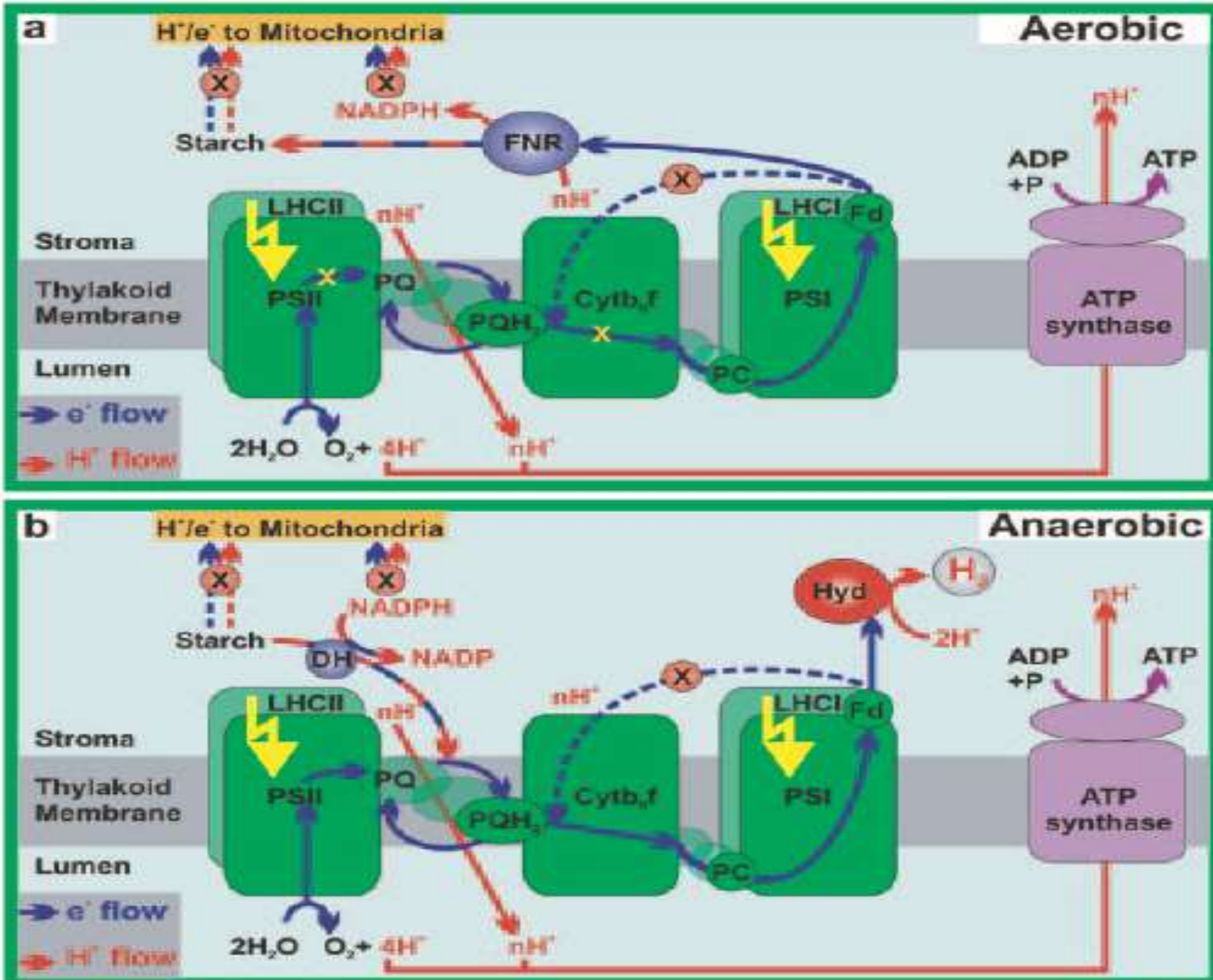
BIOLOGICALLY SUSTAINABLE HYDROGEN PRODUCTION

The development of new systems to produce zero CO_2 emission fuels for the future is one of the greatest challenges facing our society.

A select group of photosynthetic organisms have evolved the ability to harness the huge solar energy resource to drive H_2 fuel production from H_2O.

Hydrogenase under anaerobic conditions essentially acts as a H^+/e^- release valve by recombining H^+ from the medium and e^- from reduced ferredoxin to produce H_2 gas that is excreted from the cell by the reaction

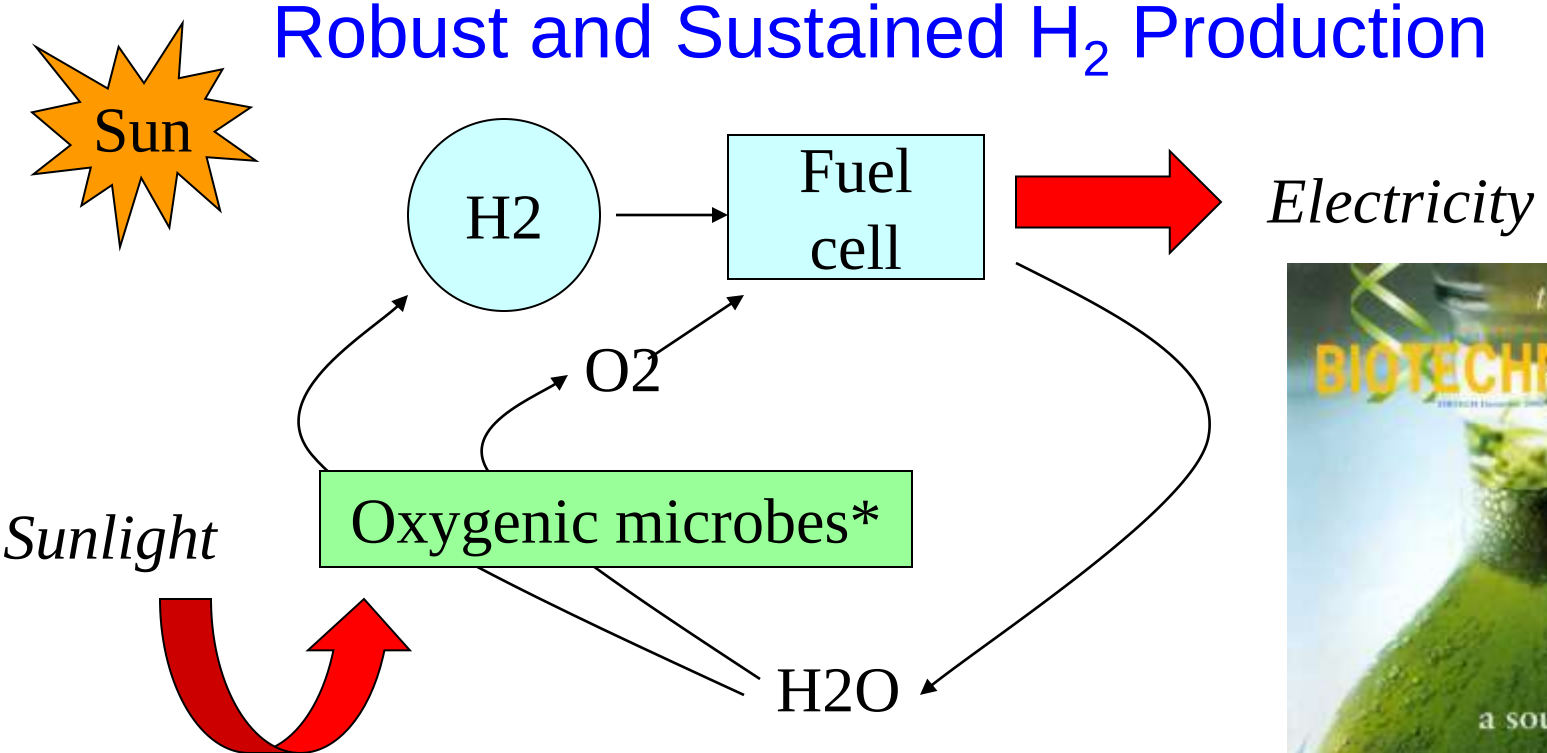
$$2H^+ + 2Fd^- \xrightarrow{\text{hydrogenase}} H_2 + Fd$$

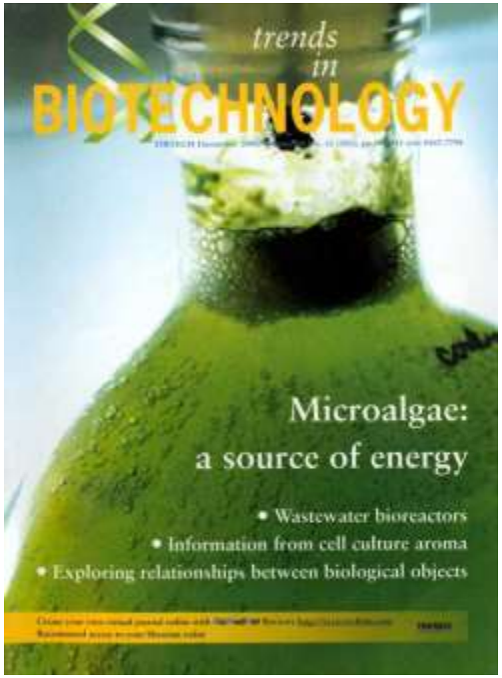


a
H+/e- to Mitochondria
Aerobic
NADPH
Starch
FNR
nH+
ADP
+P
ATP
LHCII
LHCI
Fd
Stroma
Thylakoid
Membrane
PSII
PQ
PQH2
Cytb6f
PSI
ATP
synthase
Lumen
PC
e- flow
H+ flow
2H2O
O2+ 4H+
nH+
b
H+/e- to Mitochondria
Anaerobic
Hyd
H2
2H+
NADPH
Starch
DH
NADP
ADP
+P
ATP
nH+
LHCII
LHCI
Fd
Stroma
Thylakoid
Membrane
PSII
PQ
PQH2
Cytb6f
PSI
ATP
synthase
Lumen
PC
e- flow
H+ flow
2H2O
O2+ 4H+
nH+

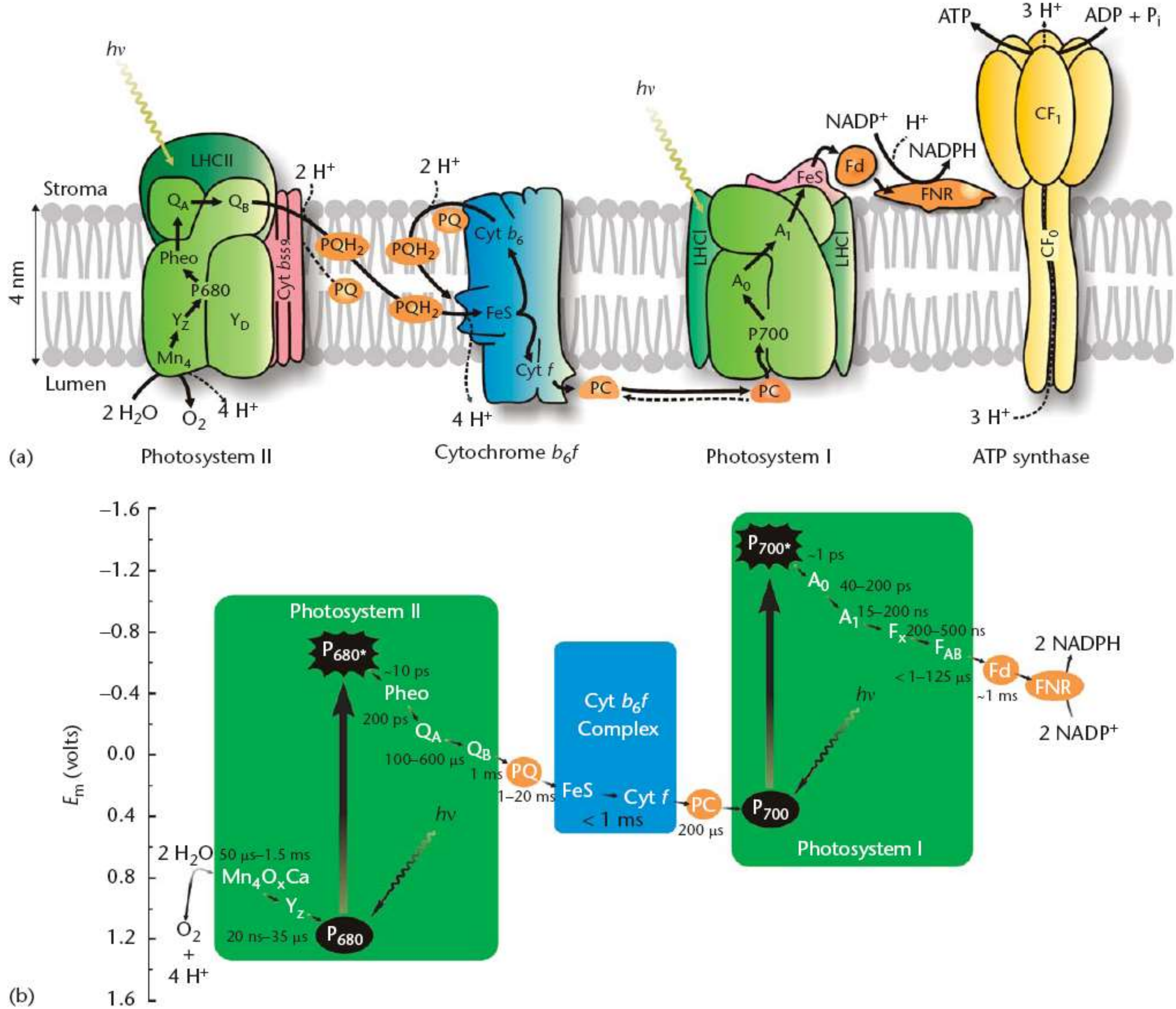
Vision for Renewable Biohydrogen Production/Utilization

Robust and Sustained H_2 Production

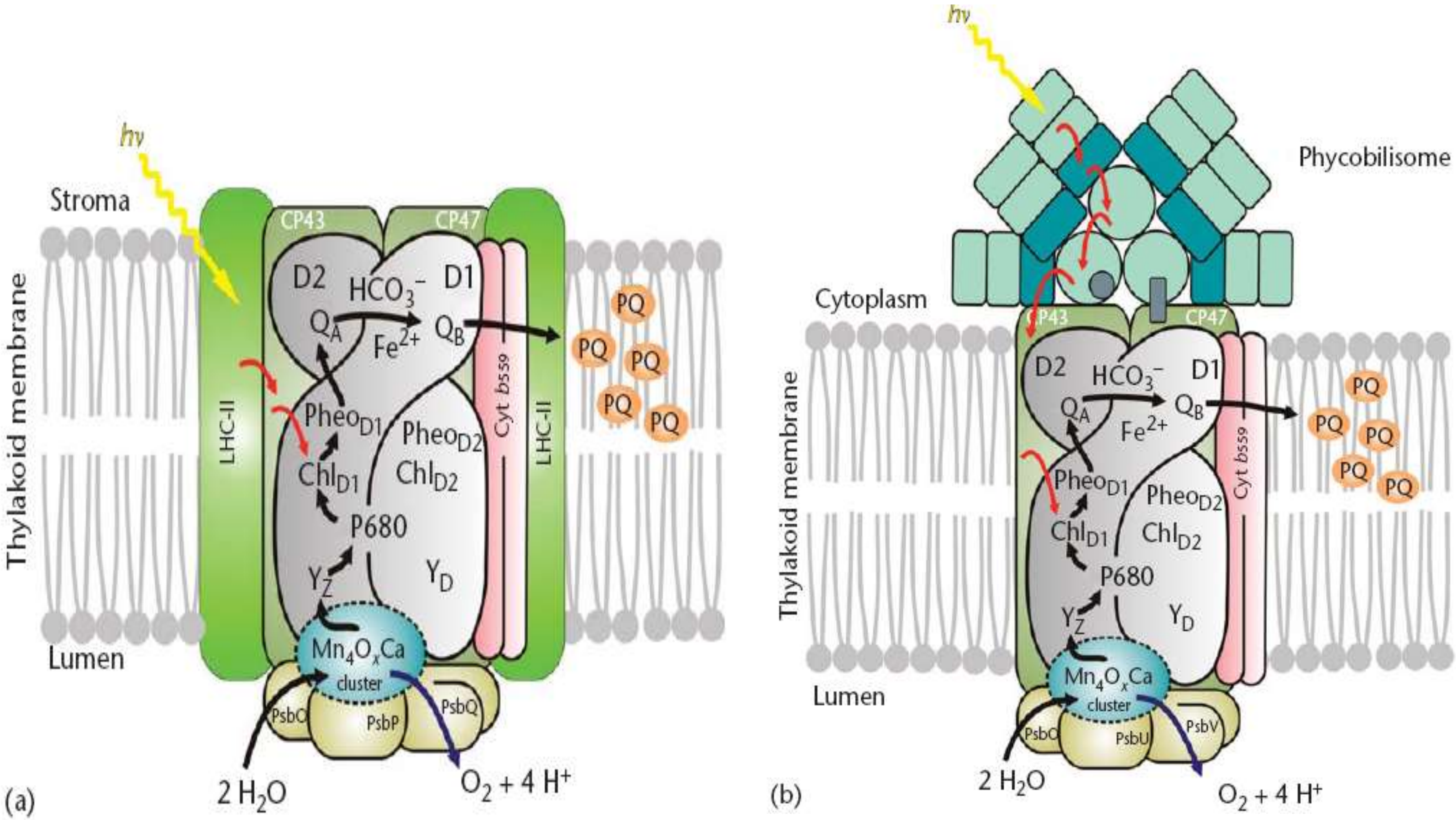




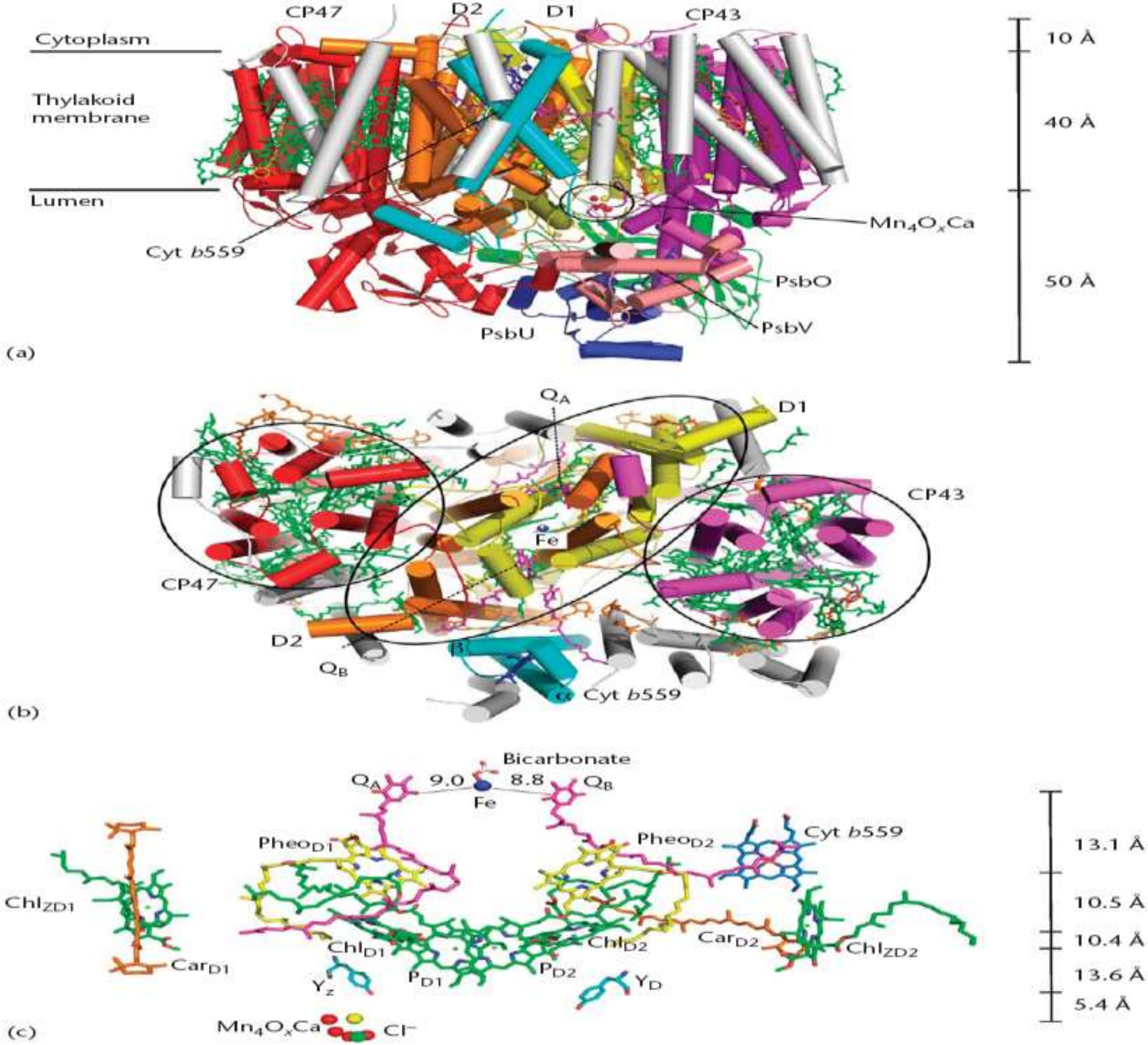
*Algae and cyanobacteria



hν
LHCII
Stroma
Lumen
4 nm
Q_A
Q_B
Pheo
P680
Y_Z
Y_D
Mn_4
Cyt b_{559}
2 H^+
2 H_2O
O_2
4 H^+
PQ
PQH_2
Cyt b_6
FeS
Cyt f
PC
LHCI
A_0
A_1
P700
FeS
Fd
FNR
$NADP^+$
H^+
NADPH
ATP
3 H^+
ADP + P_i
CF_1
CF_0
(a)
Photosystem II
Cytochrome b_6f
Photosystem I
ATP synthase
E_m (volts)
−1.6
−1.2
−0.8
−0.4
0.0
0.4
0.8
1.2
1.6
Photosystem II
P_{680*}
~10 ps
Pheo
200 ps
Q_A
Q_B
100–600 μs
1 ms
PQ
1–20 ms
2 H_2O
50 μs–1.5 ms
Mn_4O_xCa
Y_Z
20 ns–35 μs
P_{680}
O_2
+
4 H^+
Cyt b_6f
Complex
FeS
Cyt f
< 1 ms
PC
200 μs
P_{700*}
~1 ps
A_0
40–200 ps
A_1
15–200 ns
F_x
200–500 ns
F_{AB}
< 1–125 μs
Fd
~1 ms
FNR
2 NADPH
2 $NADP^+$
P_{700}
Photosystem I
(b)

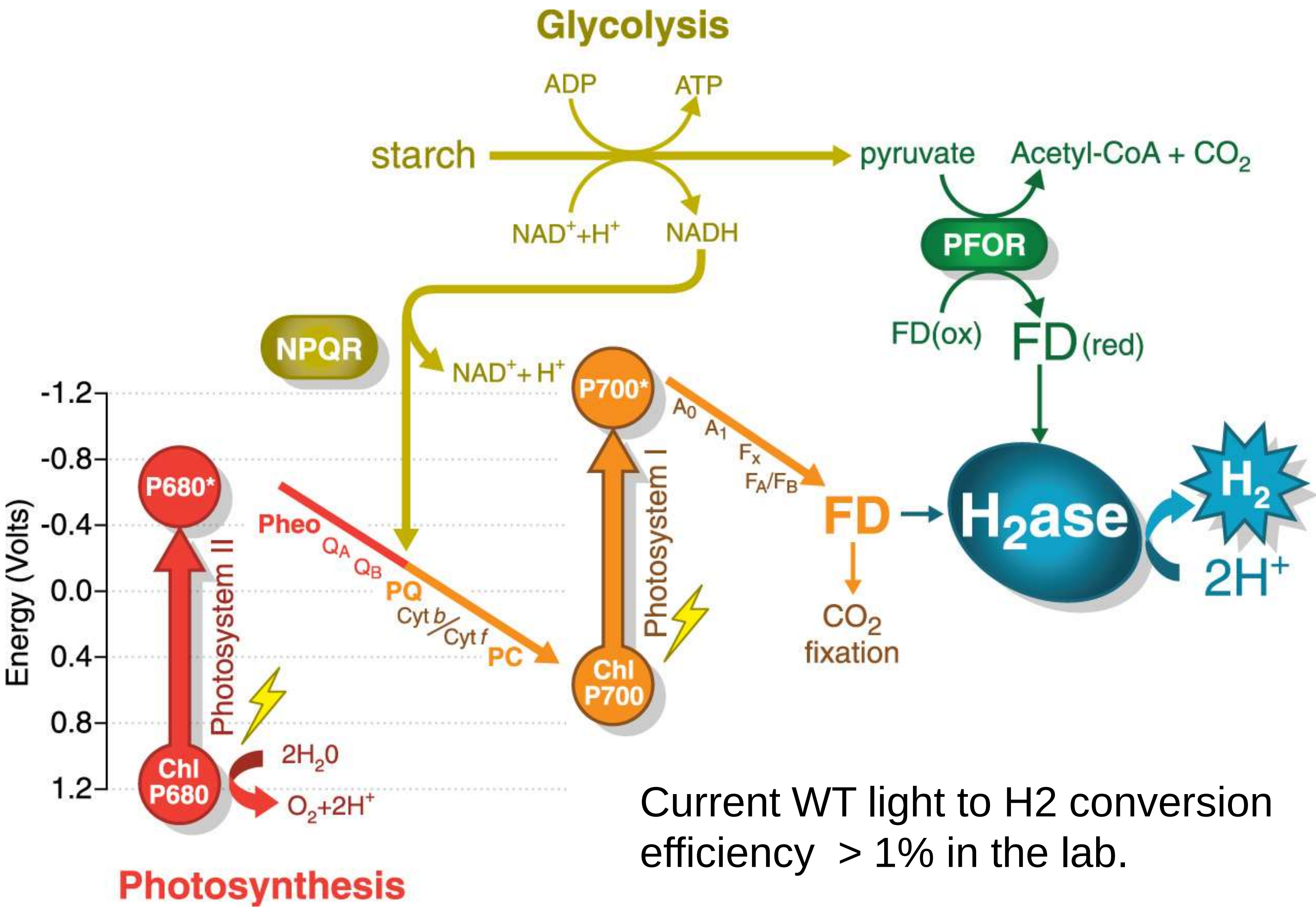


hν
Stroma
CP43
CP47
Thylakoid membrane
LHC-II
D2
HCO_3^-
D1
Q_A
Fe^{2+}
Q_B
PQ
$Pheo_{D1}$
$Pheo_{D2}$
Chl_{D1}
Chl_{D2}
P680
Y_Z
Y_D
Cyt b_{559}
Lumen
Mn_4O_xCa cluster
PsbO
PsbP
PsbQ
$2\ H_2O$
$O_2 + 4\ H^+$
(a)
hν
Phycobilisome
Cytoplasm
CP43
CP47
Thylakoid membrane
D2
HCO_3^-
D1
Q_A
Fe^{2+}
Q_B
PQ
$Pheo_{D1}$
$Pheo_{D2}$
Chl_{D1}
Chl_{D2}
P680
Y_Z
Y_D
Cyt b_{559}
Lumen
Mn_4O_xCa cluster
PsbO
PsbU
PsbV
$2\ H_2O$
$O_2 + 4\ H^+$
(b)



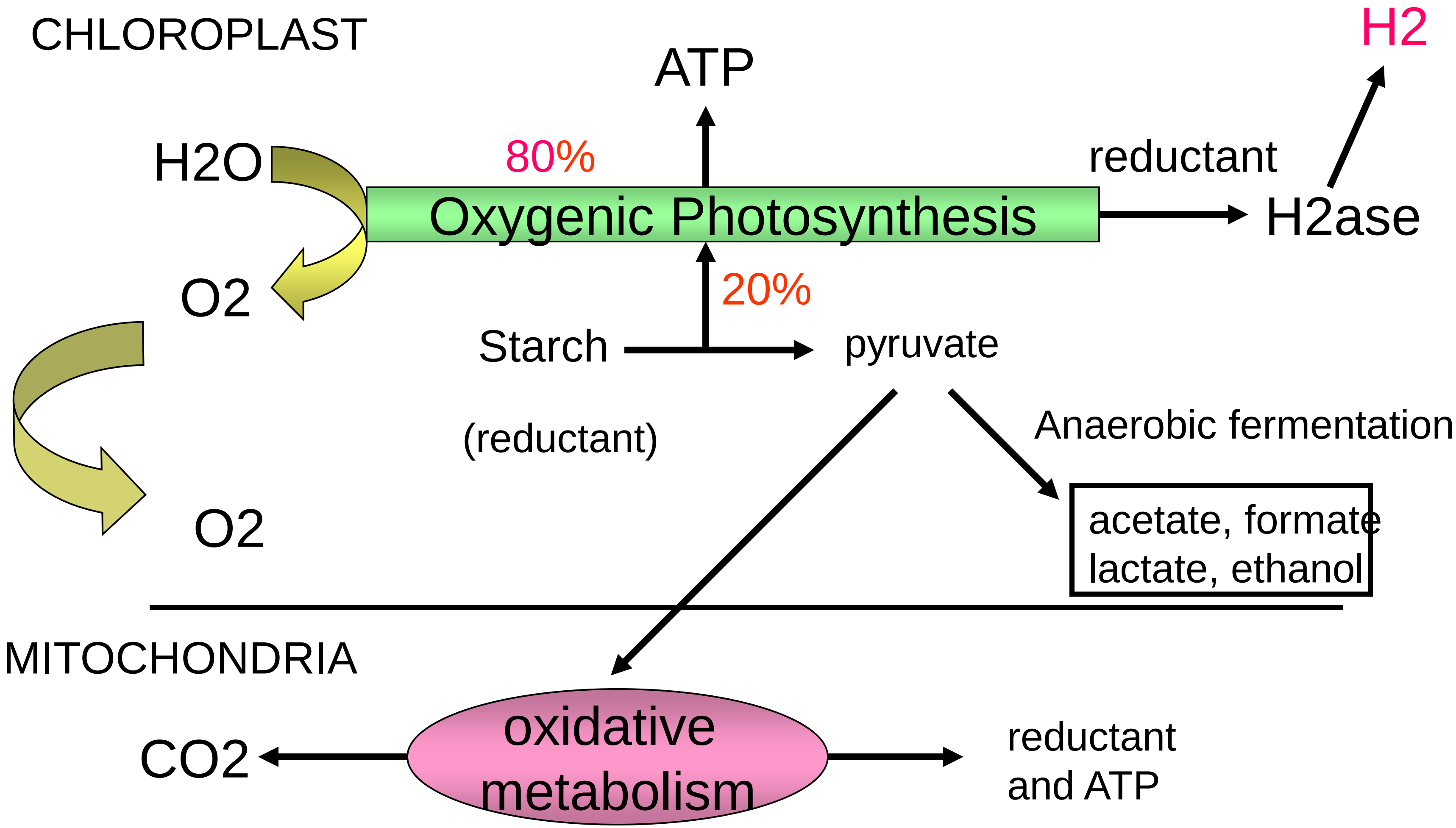
CP47
D2
D1
CP43
Cytoplasm
Thylakoid membrane
Lumen
Cyt b559
Mn4OxCa
PsbO
PsbV
PsbU
10 Å
40 Å
50 Å
(a)
QA
D1
CP43
Fe
CP47
D2
QB
β
α
Cyt b559
(b)
Bicarbonate
QA
9.0
8.8
QB
Fe
PheoD1
PheoD2
Cyt b559
ChlZD1
ChlD1
ChlD2
CarD2
ChlZD2
CarD1
YZ
PD1
PD2
YD
Mn4OxCa
Cl−
13.1 Å
10.5 Å
10.4 Å
13.6 Å
5.4 Å
(c)

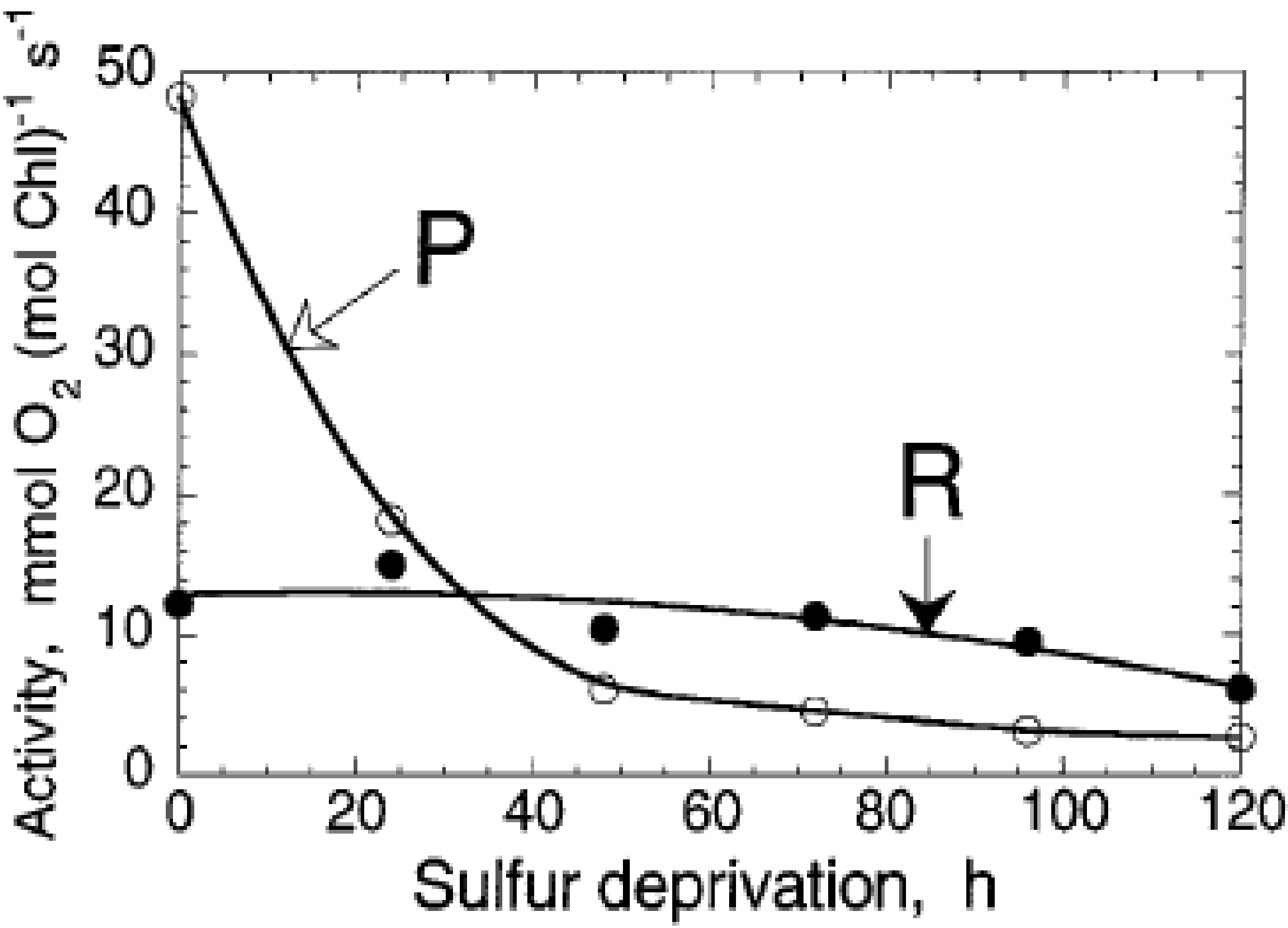
Chlamydomonas reinhardtii H2 Metabolic Pathways



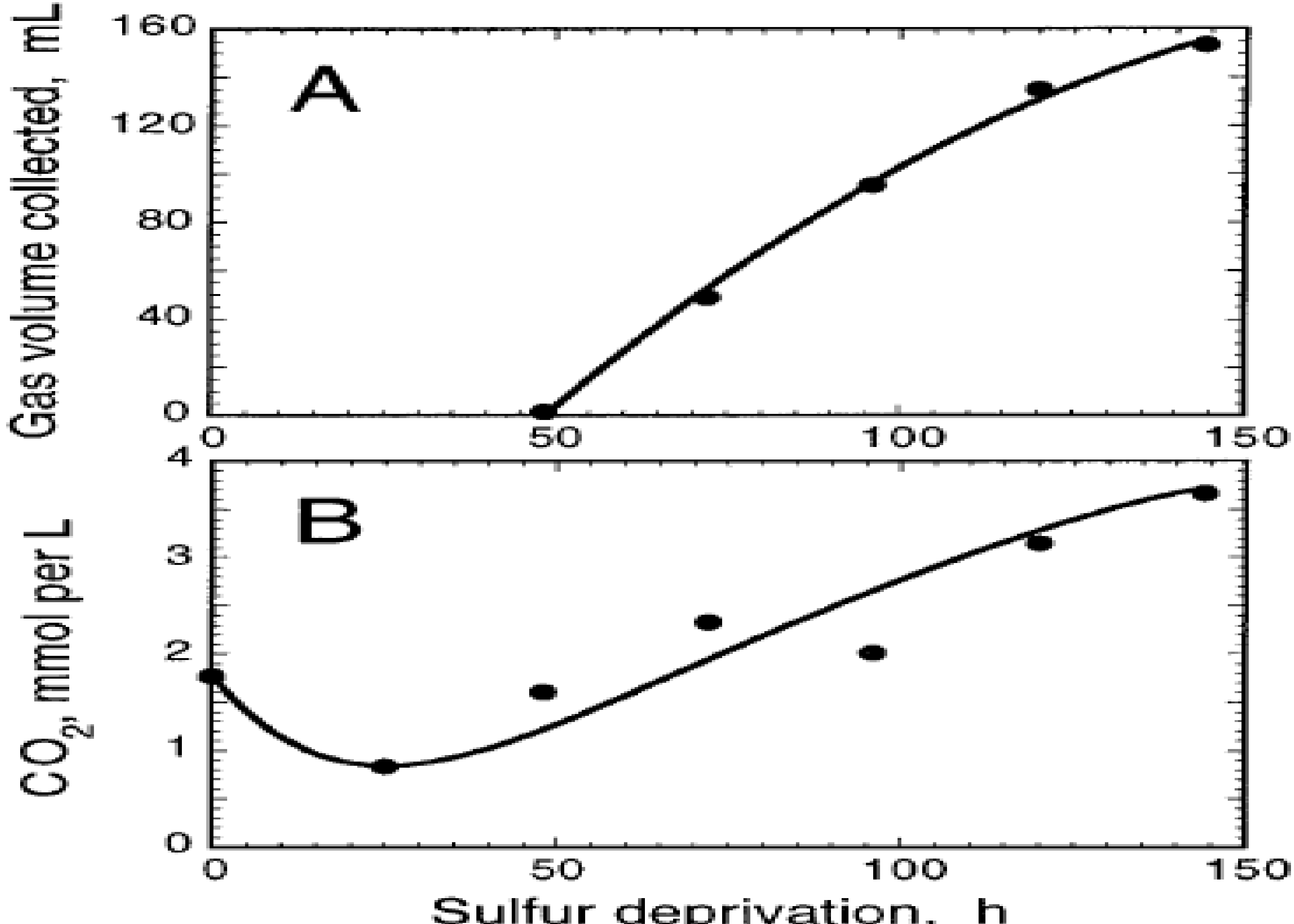
Current WT light to H2 conversion efficiency > 1% in the lab.

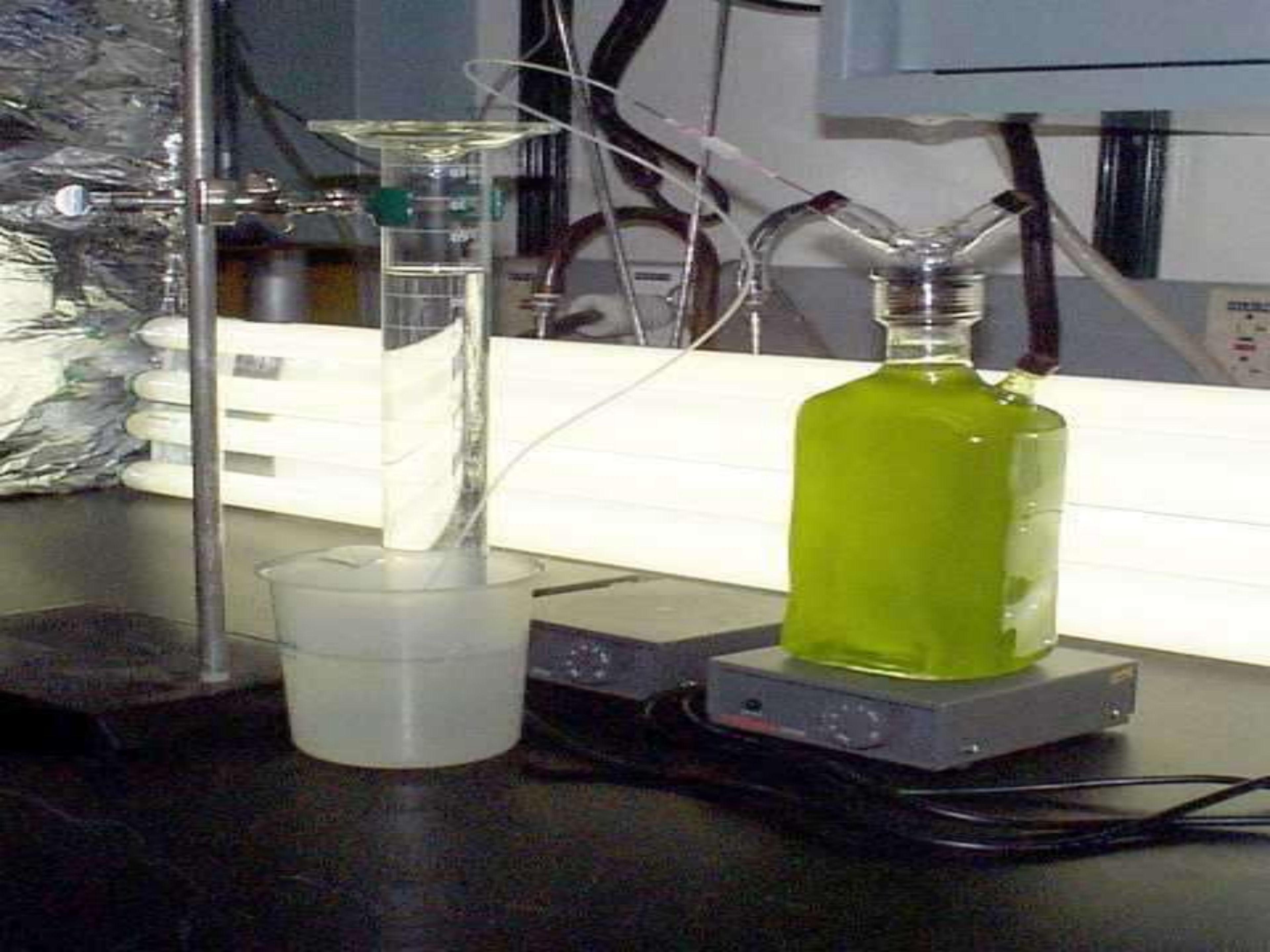
Co-occurrence of Aerobic Photosynthesis, Anaerobic Fermentation and Respiration





Activity, mmol O_2 (mol Chl)$^{-1}$ s^{-1}
50
40
30
20
10
0
P
R
0
20
40
60
80
100
120
Sulfur deprivation, h


A
B
Gas volume collected, mL
CO_2, mmol per L
Sulfur deprivation, h
0
40
80
120
160
1
2
3
4
50
100
150



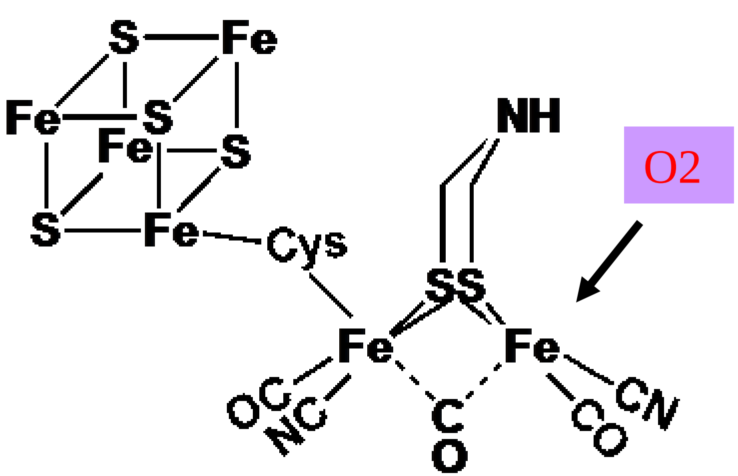
Immediate Challenges

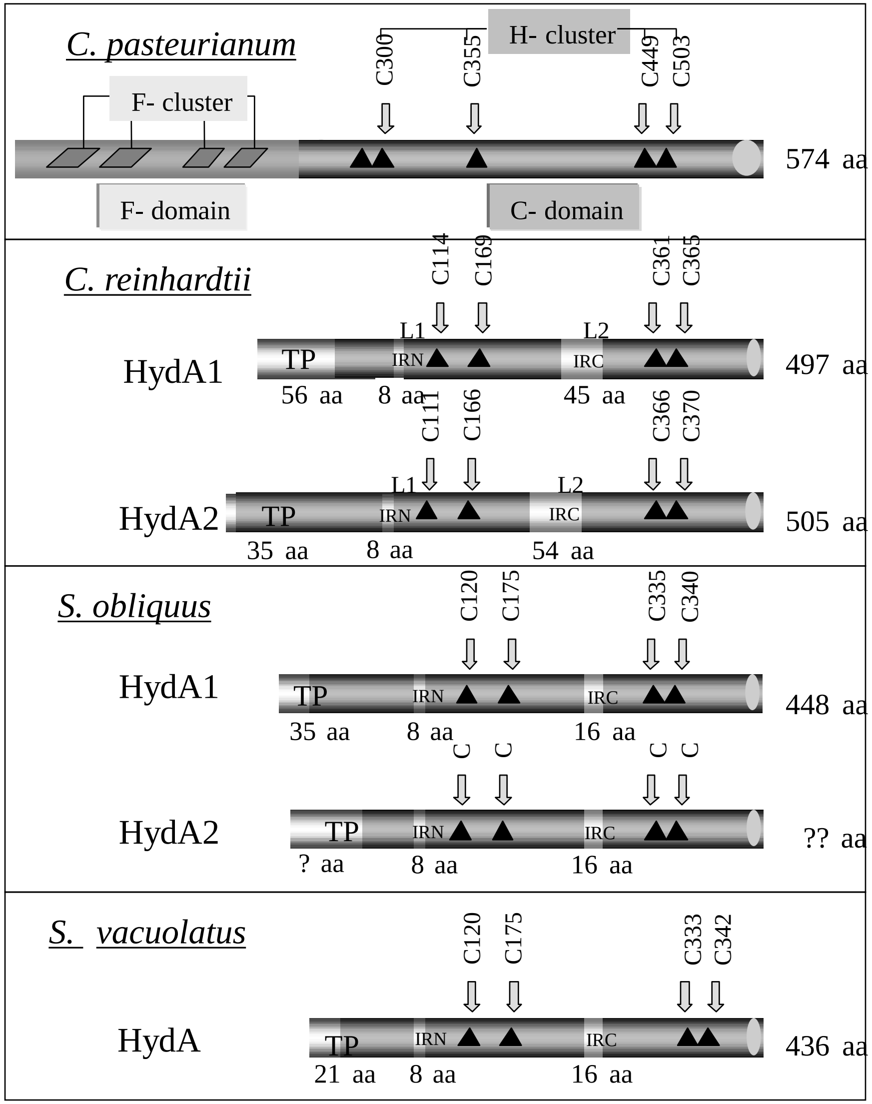
Can we (a) develop an aerobic system that can utilize the full potential of photosynthesis by addressing the hydrogenase O_2-sensitivity problem or

(b) is improving the H_2-production rates of our anaerobic sulfur-deprived system the best we can do?

Structure of Algal Hydrogenases

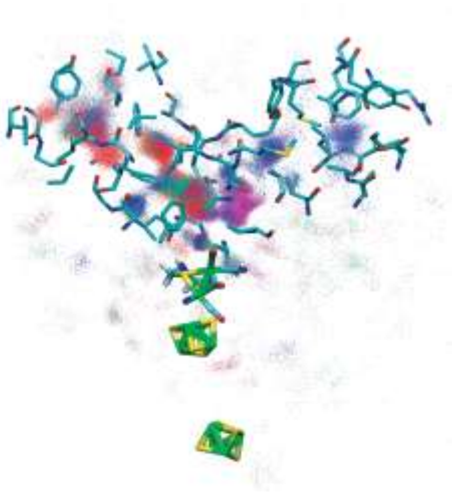
Two [FeFe]-hydrogenases have been discovered in *Chlamydomonas*



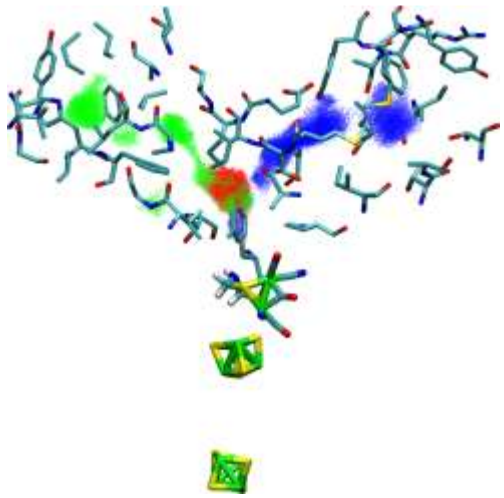


Increasing the O2 Tolerance of Algal Hydrogenase

Molecular dynamics modeling of gas diffusion in an [FeFe]-hydrogenase indicated two well-defined pathways for O_2 diffusion through a series of dynamic cavities and multiple pathways for H_2 diffusion.

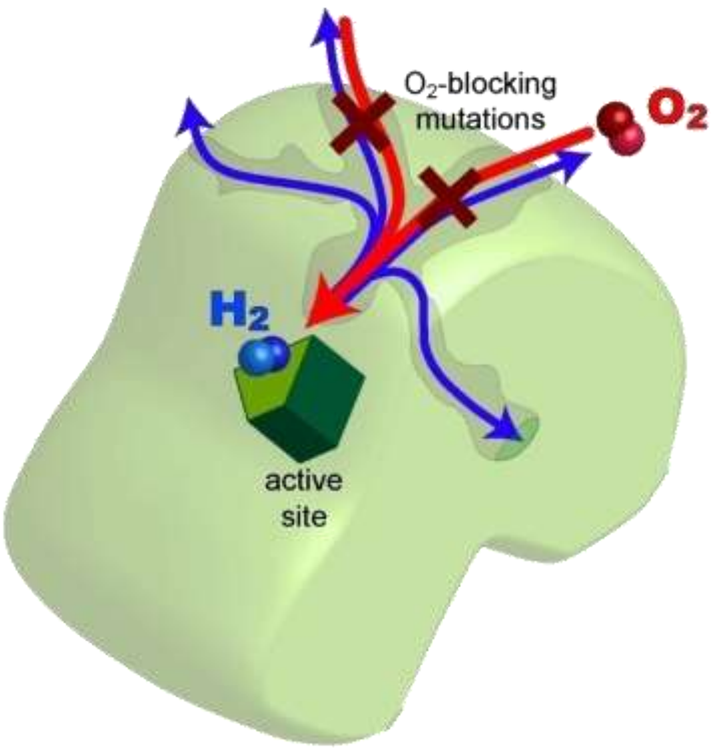


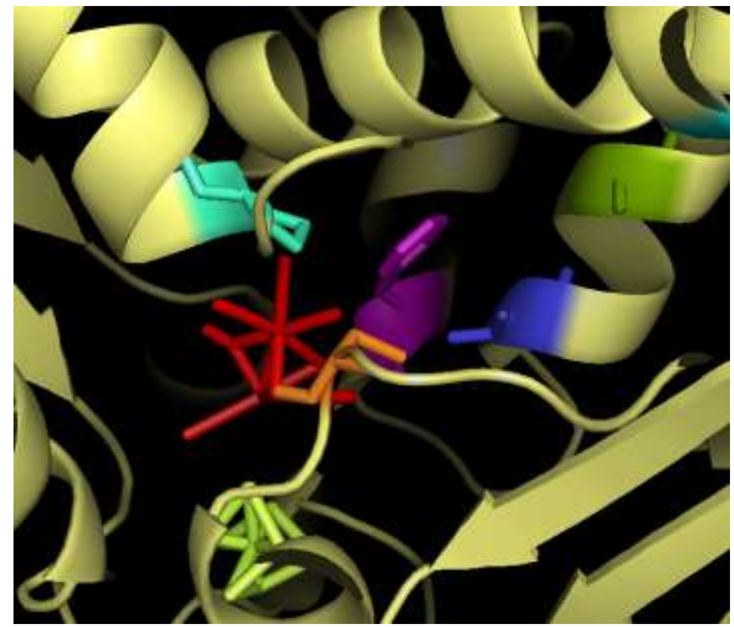
H2 Pathways



O2 Pathways

Molecular Engineering O2 Tolerance into the Hydrogenase

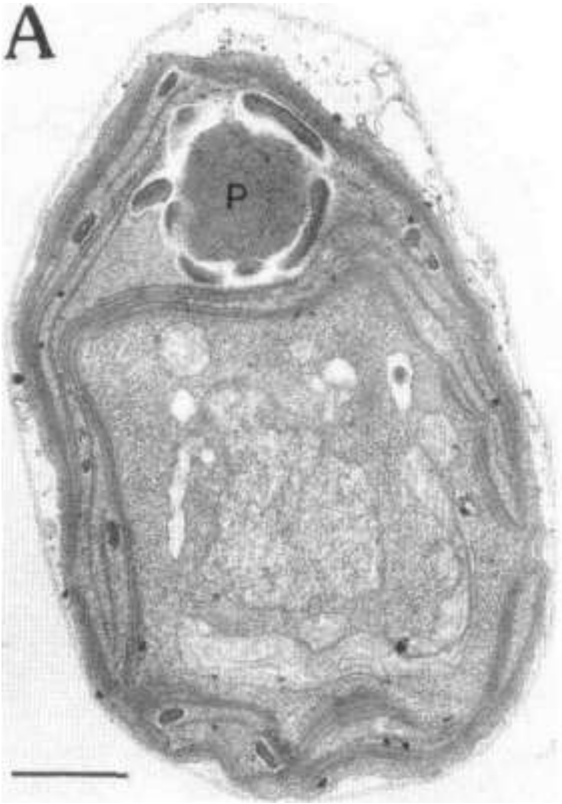




Engineering efforts focused in the area of the high energy barrier. Larger amino acids were substituted for, sterically hindering access of O2 to the catalytic site.

Previously Known Algal Genes Associated Directly with H_2 Photoproduction

- The hydrogenase structural genes (*HydA1* and *HydA2*).
- The hydrogenase assembly genes (*HydEF* and *HydG*).
- Starch metabolism genes (*Sta7*).
- Light-harvesting genes (*Lhc*).
- Sulfate permease (*SulP;* controls sulfate uptake into the algal chloroplast).



CONCLUSIONS

* To better understand photosynthetic, growth and productivity responses of crop plants, especially perennial tree species to elevated CO2 and higher temperature in FACE environment.

- To find a mechanism to restore the protein content of seed grains at high CO_2
- Plantation of fast growing tree species i.e., poplar for long term carbon sequestration
- Plantation of tree species mangrove in the coastal regions
- Generation of bioethanol from sea sources i.e., marine algae
- Photosynthetic generation of a zero CO_2 emitting fuel, H_2, from water by fresh water and marine algae

Thank You