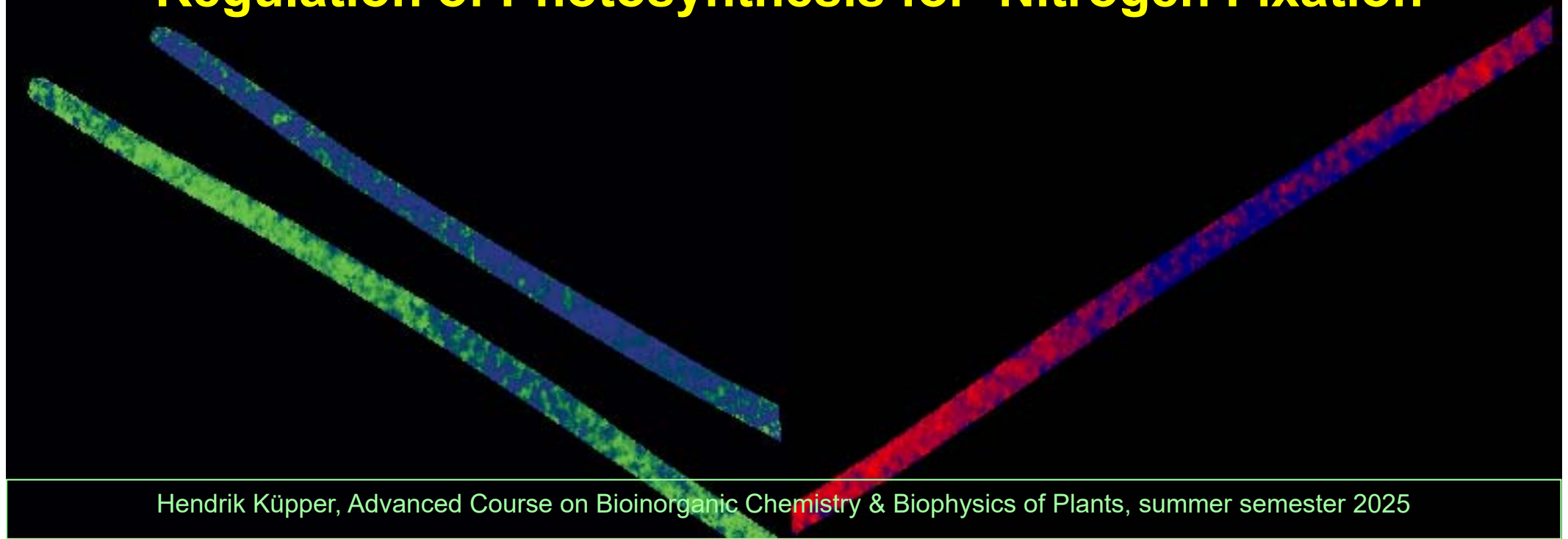


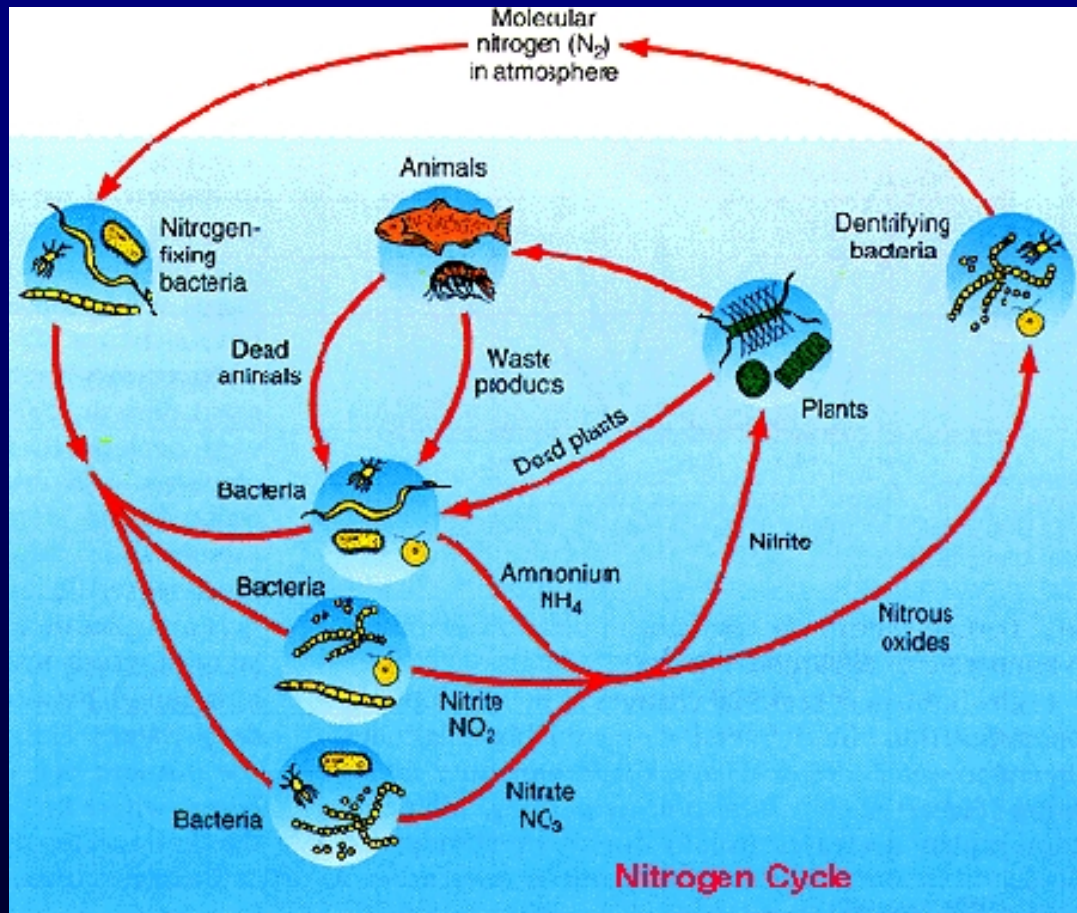
Nitrogen Fixation and Regulation of Photosynthesis for Nitrogen Fixation



**Part I:
Nitrogen fixation**

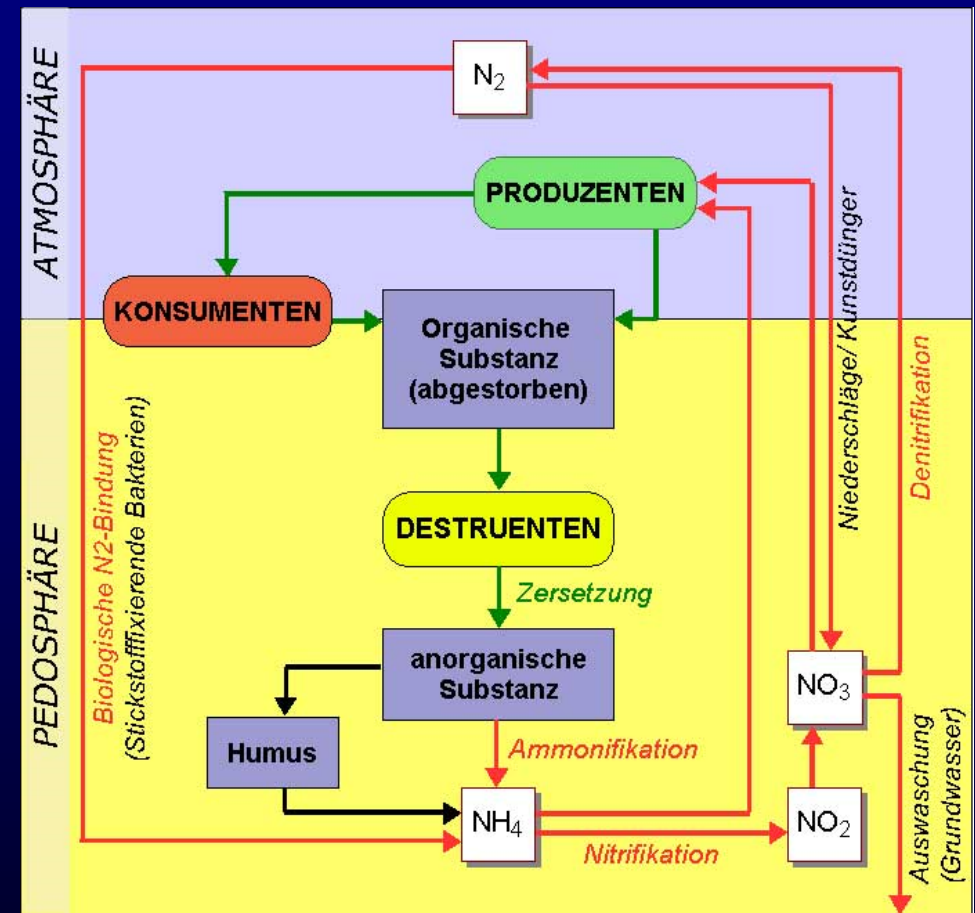
Nitrogen cycle (I): biological processes

Im Wasser



von: www.oceanography.geol.ucsb.edu

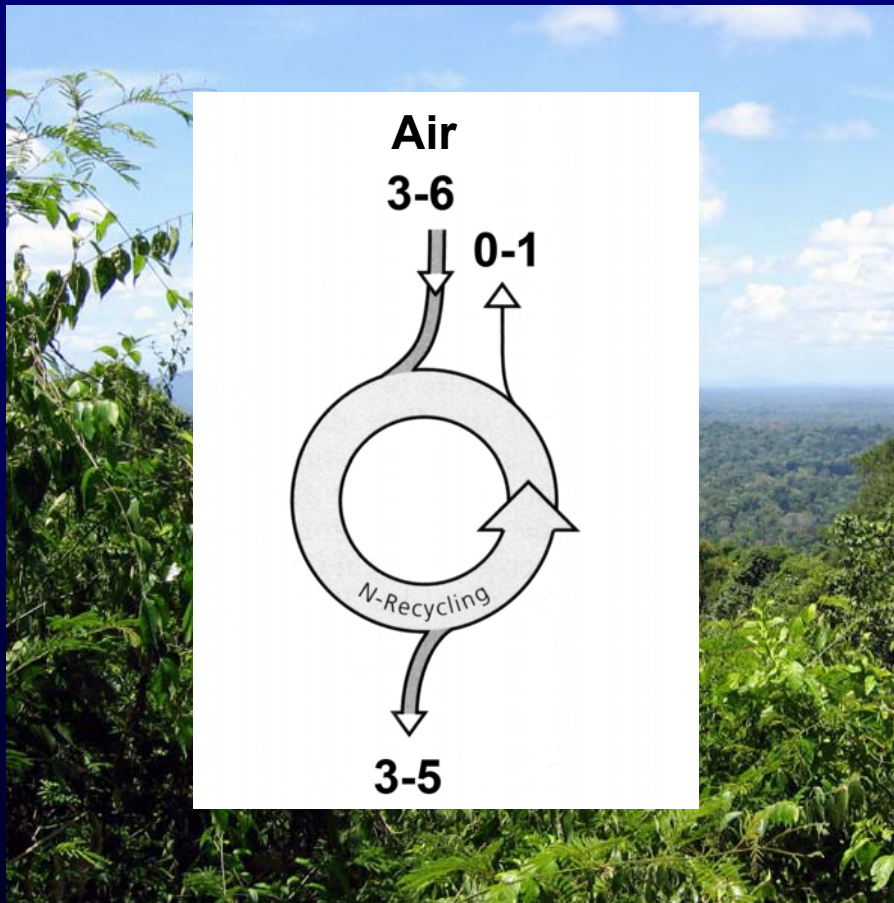
An Land



von: www.hypersoil.uni-muenster.de

Nitrogen cycle (II): natural vs. anthropogenic processes

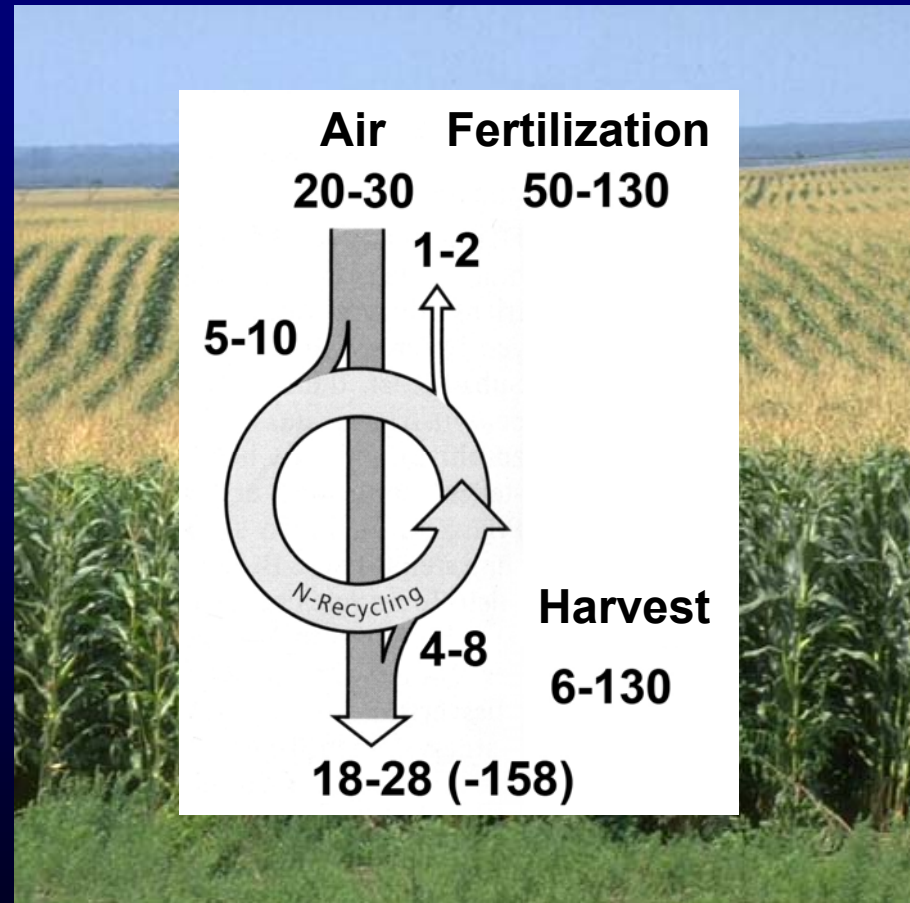
Lightnings, biol. N₂-fixation



NO₃⁻, Herbivores, Erosion

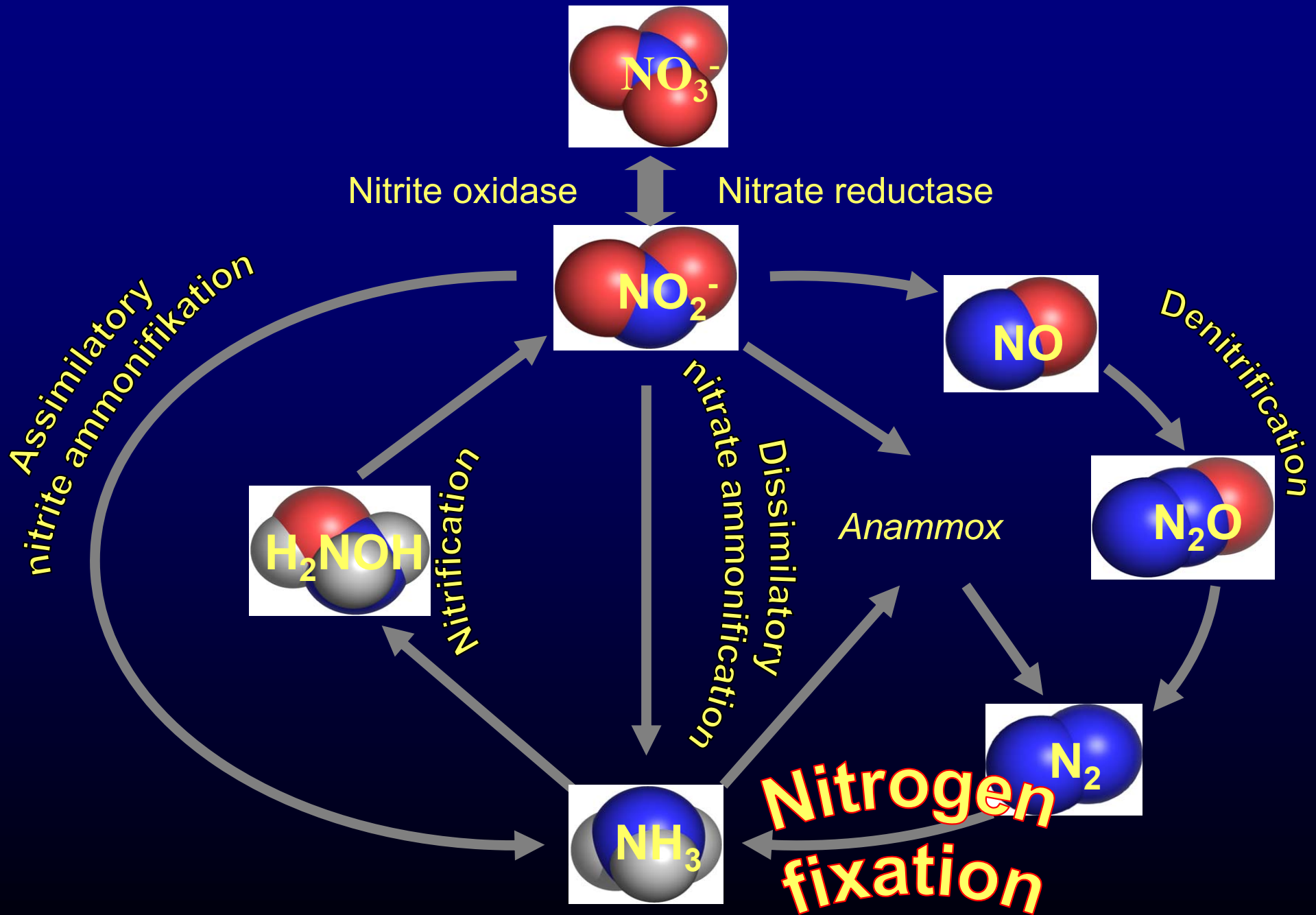
NO_x, organ. / mineral. Fertilizers

kg N / (ha * Jahr)

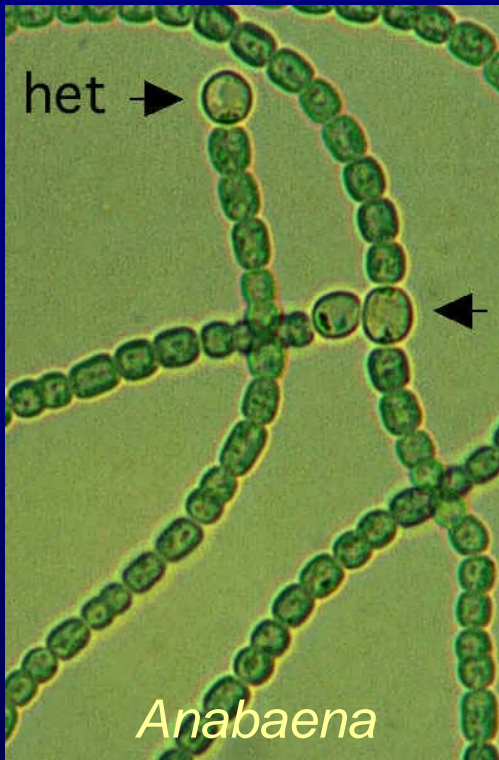


NO₃⁻, Harvest, Erosion

Nitrogen cycle (III): intermediate steps



Organisms involved in nitrogen fixation



Anabaena



Trichodesmium



Gloeotheca



Azotobacter



Azolla



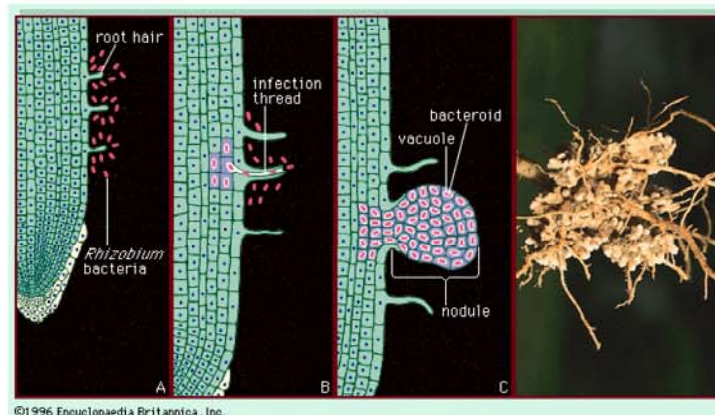
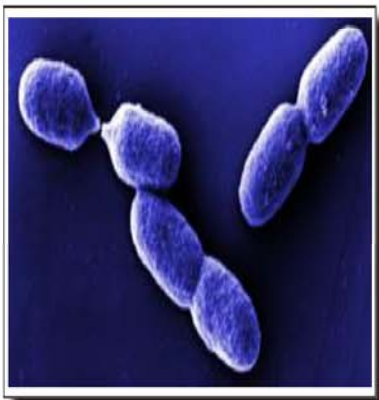
Rhizobien

Biological N₂ Fixation

Microorganisms can do the job under „normal conditions“ (T, P)

- free living soil bacteria, e.g. *Azotobacter vinelandii*
- Cyanobacteria with specialized cells, e.g. *Anabaena sp.*, *Nostoc sp.*)
- *Rhizobia* which live in special plant organelles (root noudles)

The process, however, is costly. Plants have to deliver up to 25% of their photosynthetically produced ATP to N₂ fixing bacteria in the root nodules.

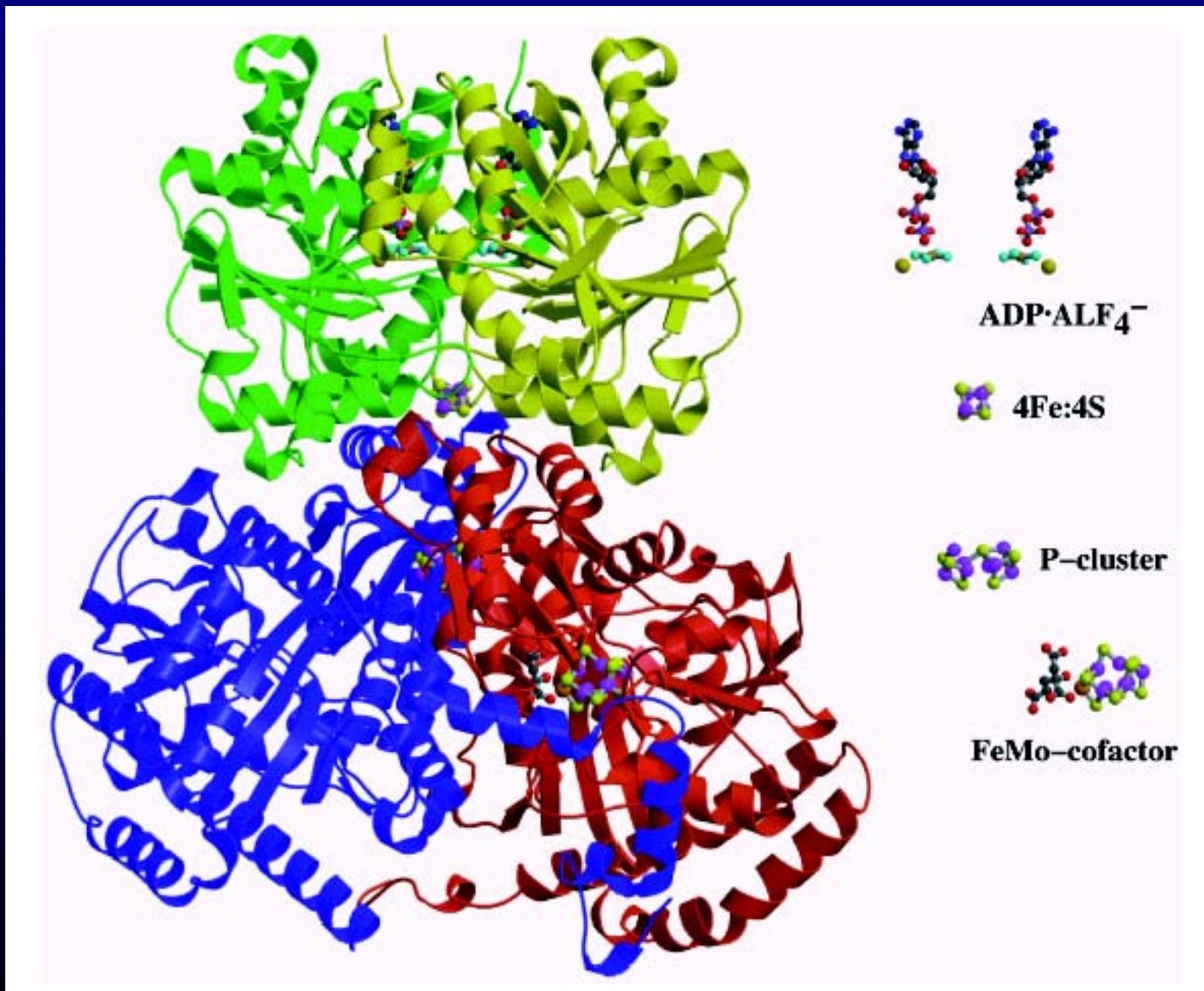


Note: N₂ Fixation is an anaerobic process

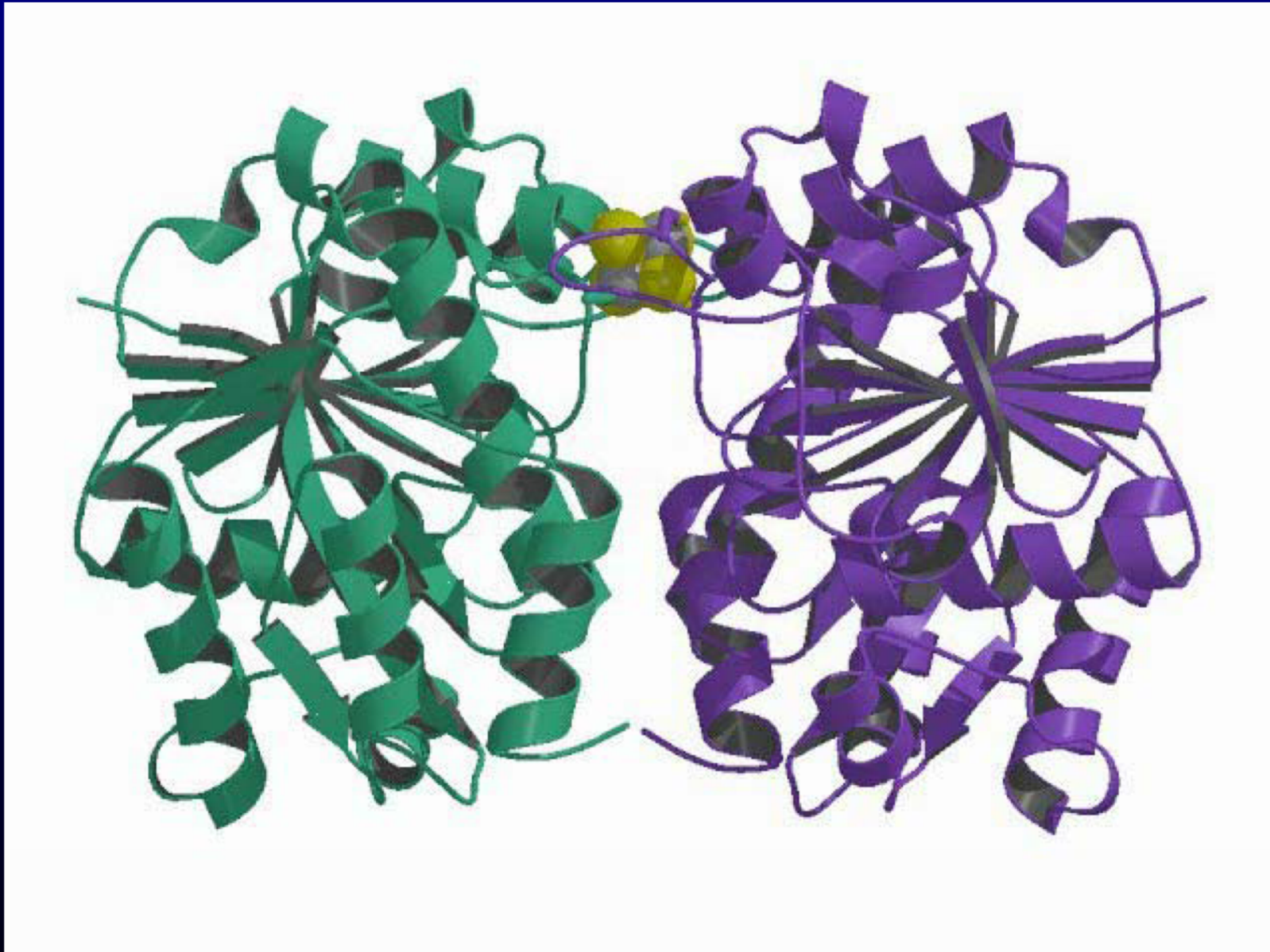
Total equation of biological nitrogen fixation



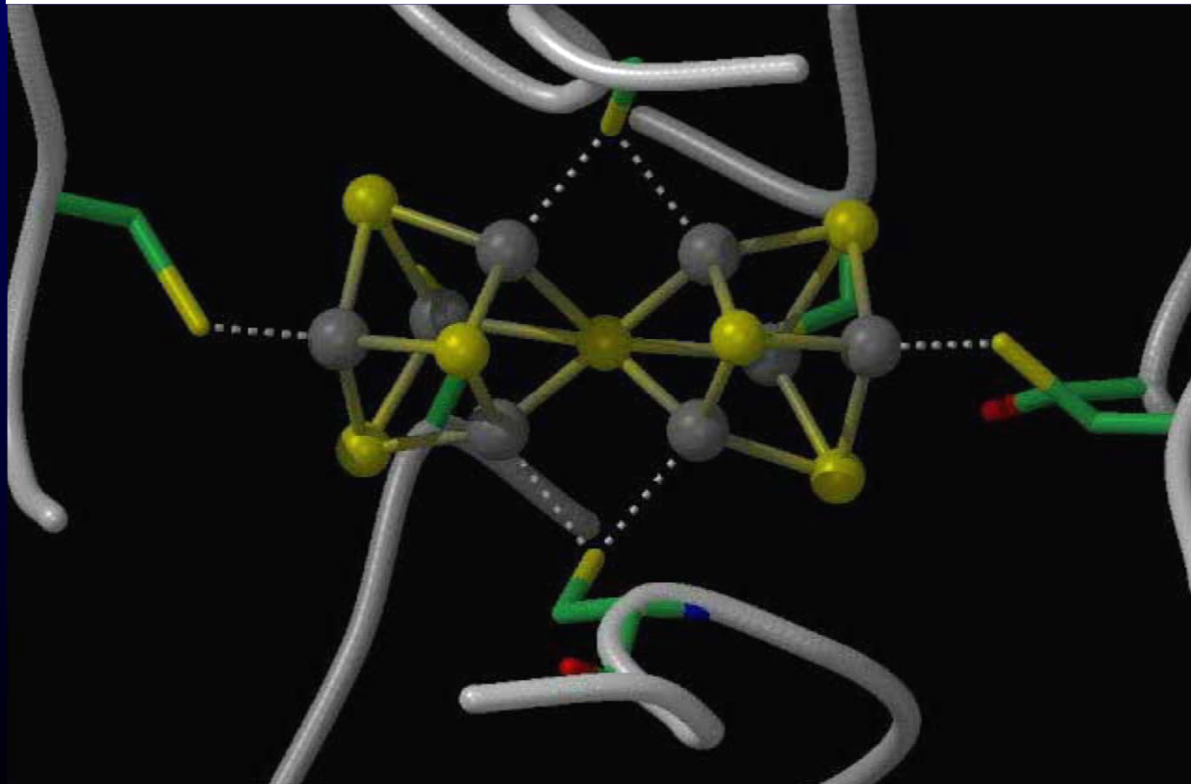
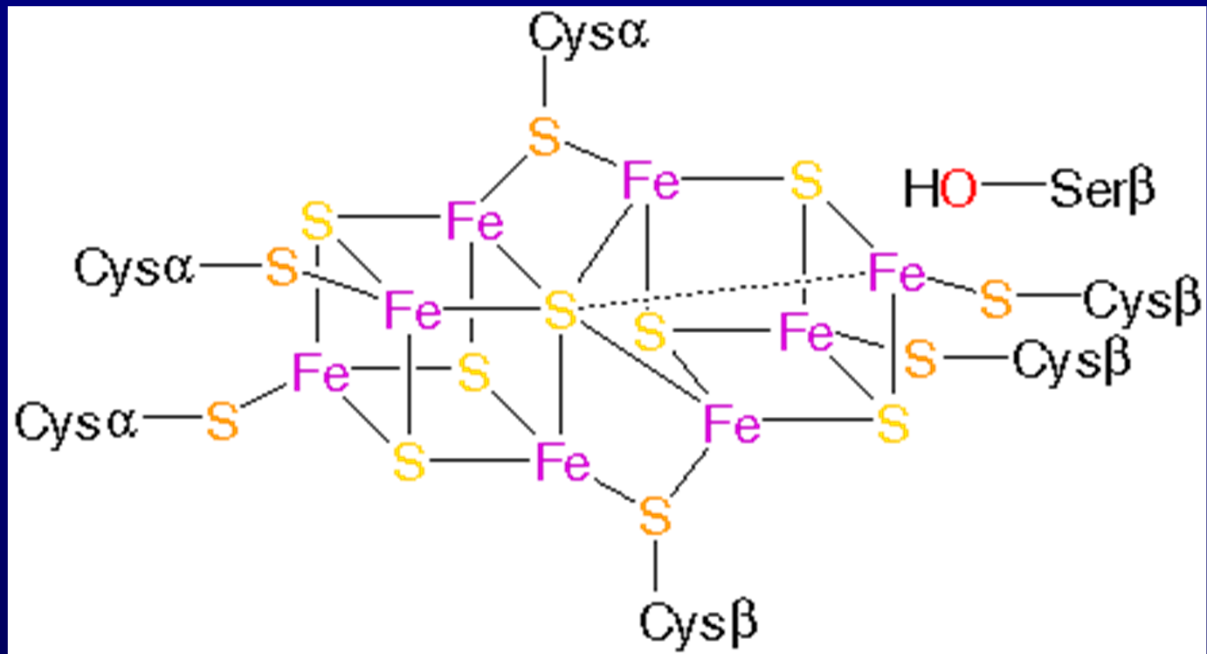
Mechanism of biological nitrogen fixation: nitrogenase of cyanobacteria



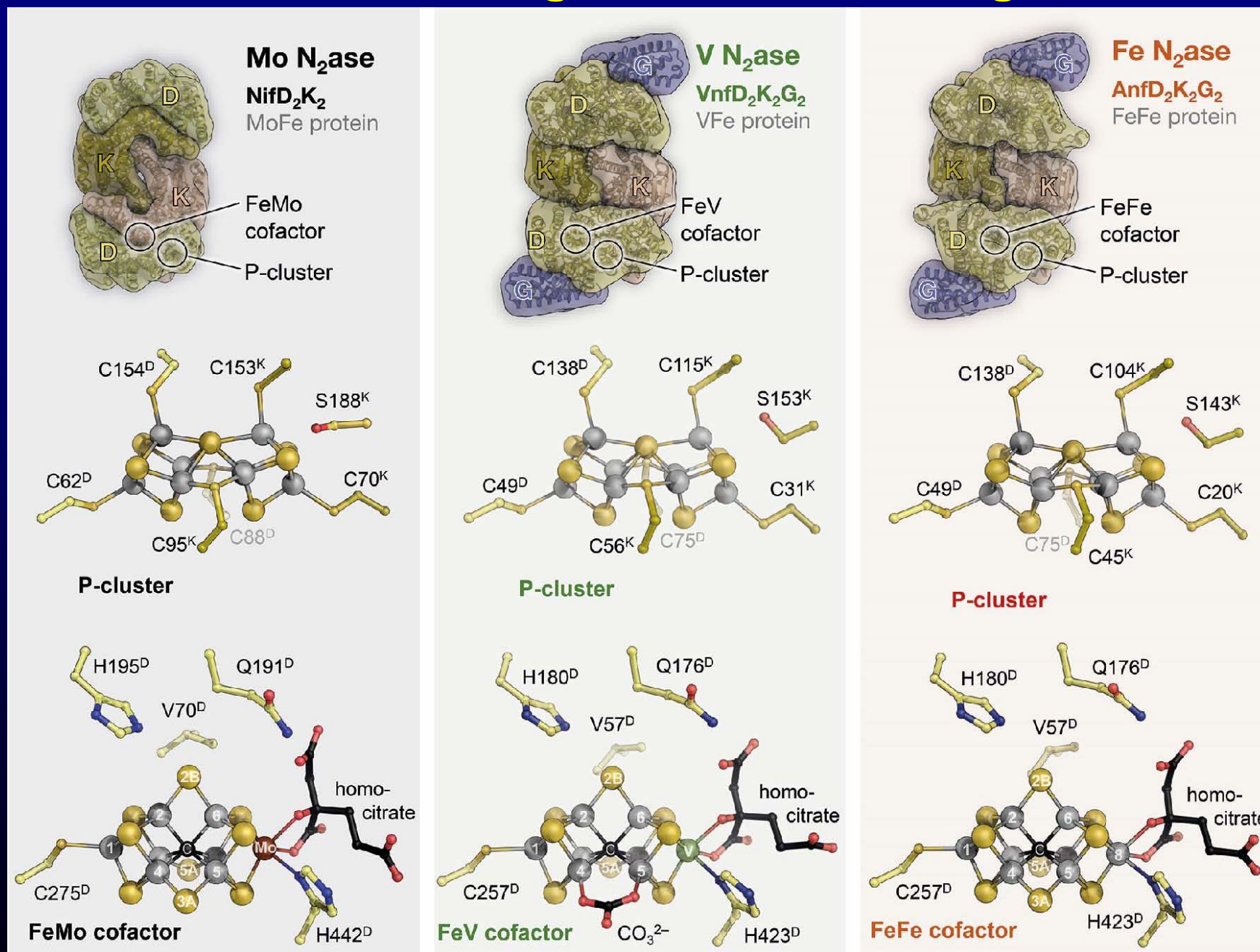
Small subunit: The iron protein



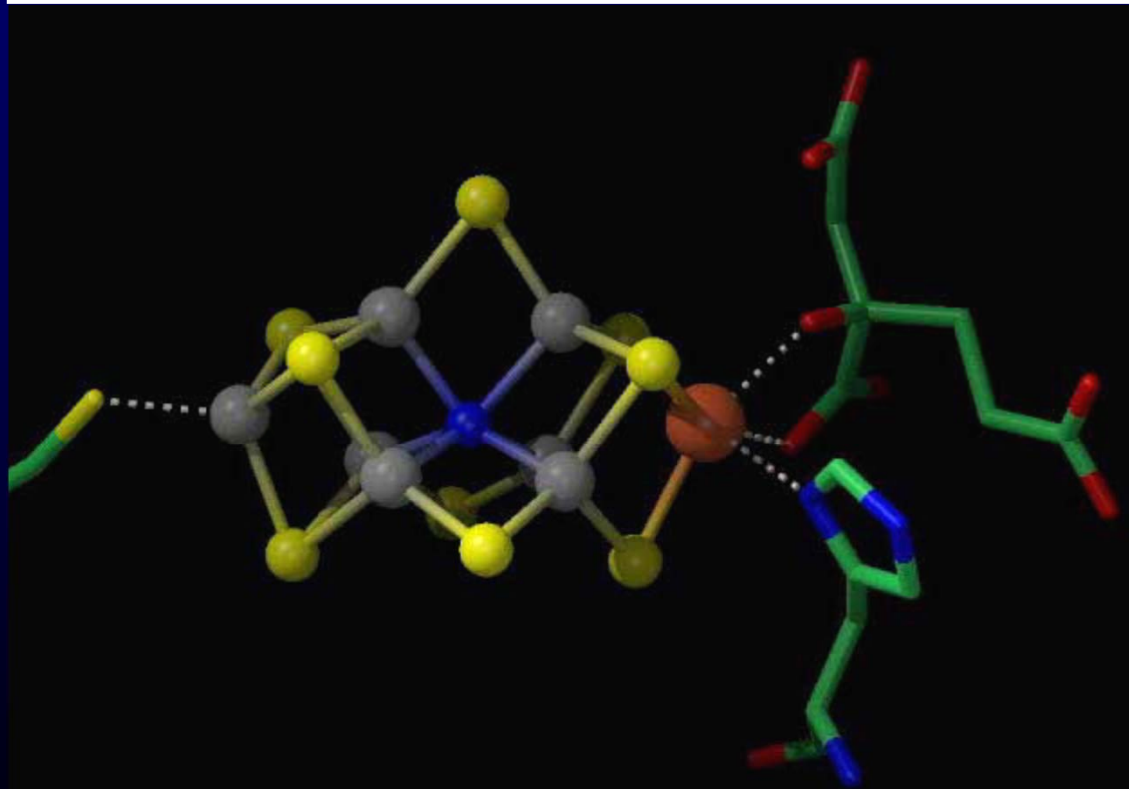
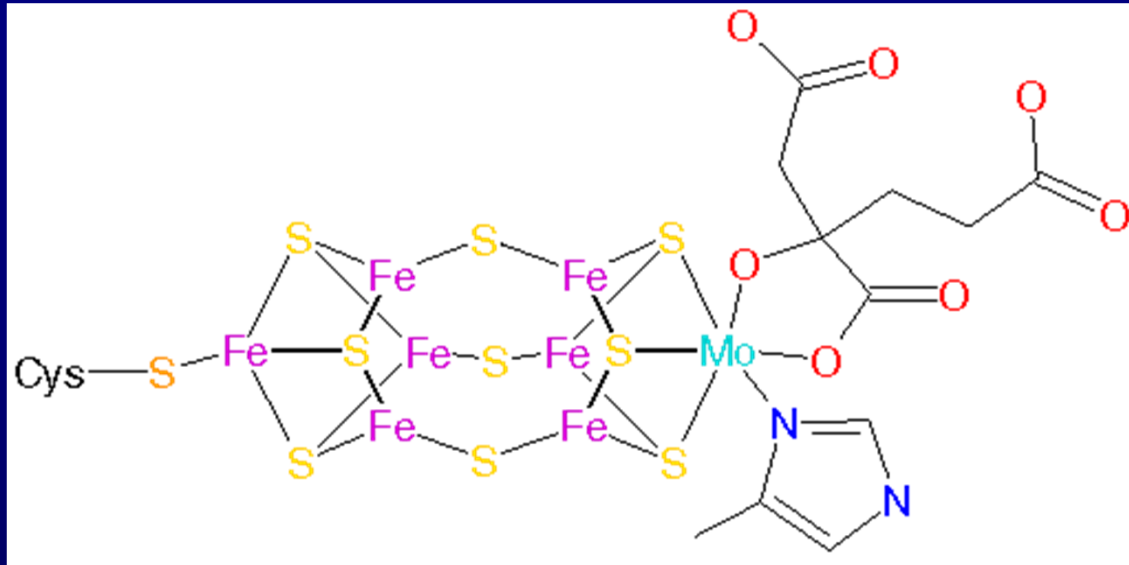
Nitrogenase: Large subunit, P-Cluster



Mechanism of biological nitrogen fixation: variants of the large subunit of nitrogenase

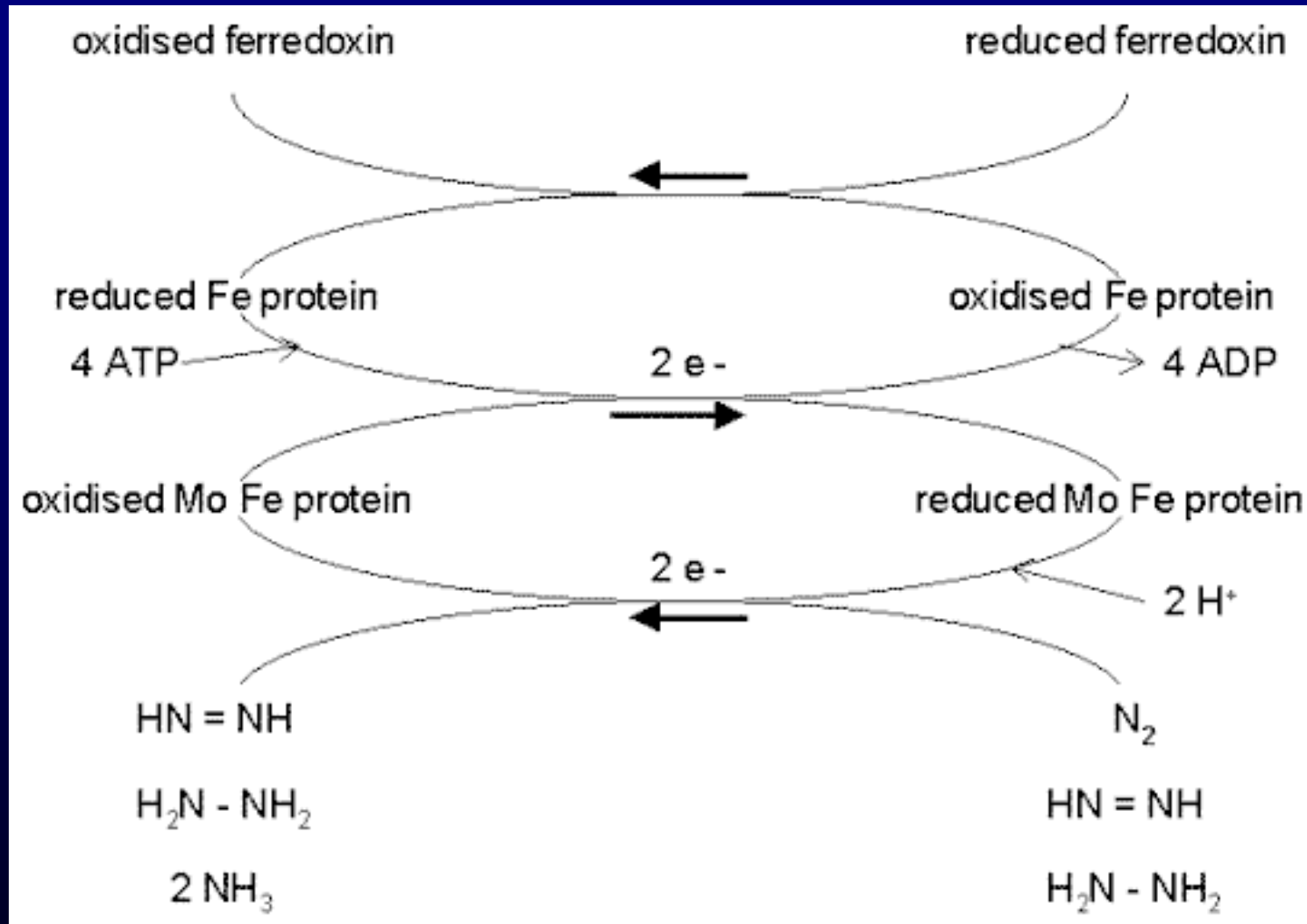


Classical FeMo-nitrogenase: Nitrogenase: M-Cluster

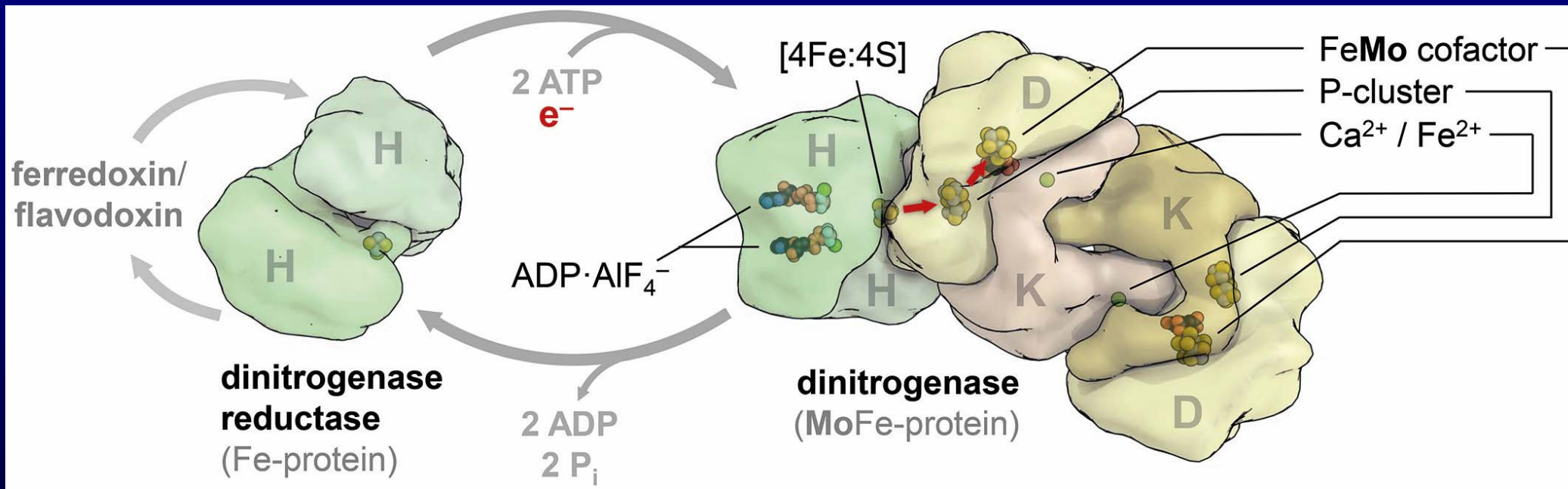


Structure of Mayer and Smith, 1999, 1.6 Å resolution: discovery of the "central nitrogen", meanwhile re-identified as central carbon (Spatzal Thomas; Aksoyoglu Muege; Zhang Limei; et al. 2011, Science 334; Lancaster Kyle M.; Roemelt Michael; Ettenhuber Patrick; et al., 2011, Science 334)

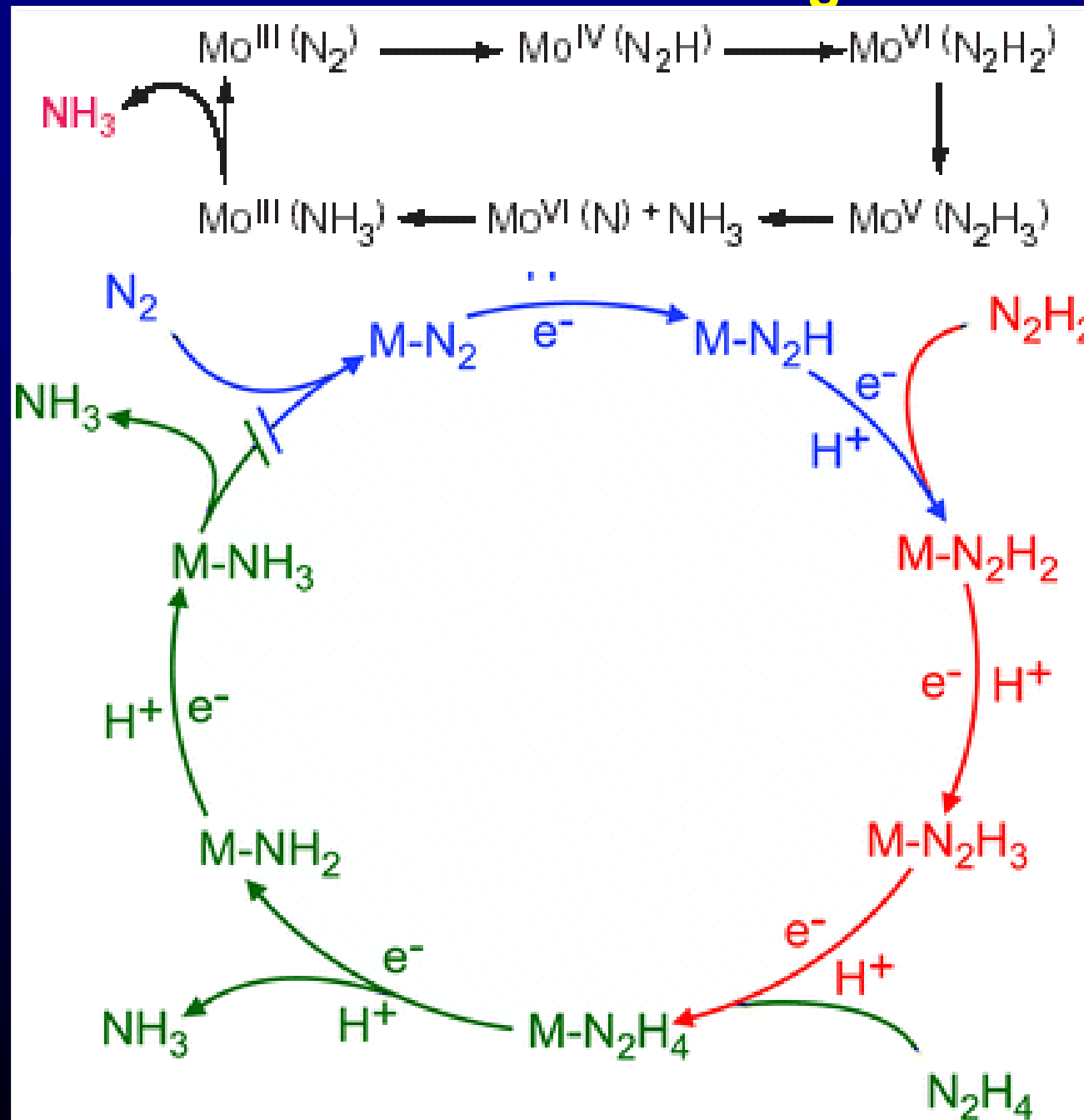
Nitrogenase: Mechanism of nitrogen reduction



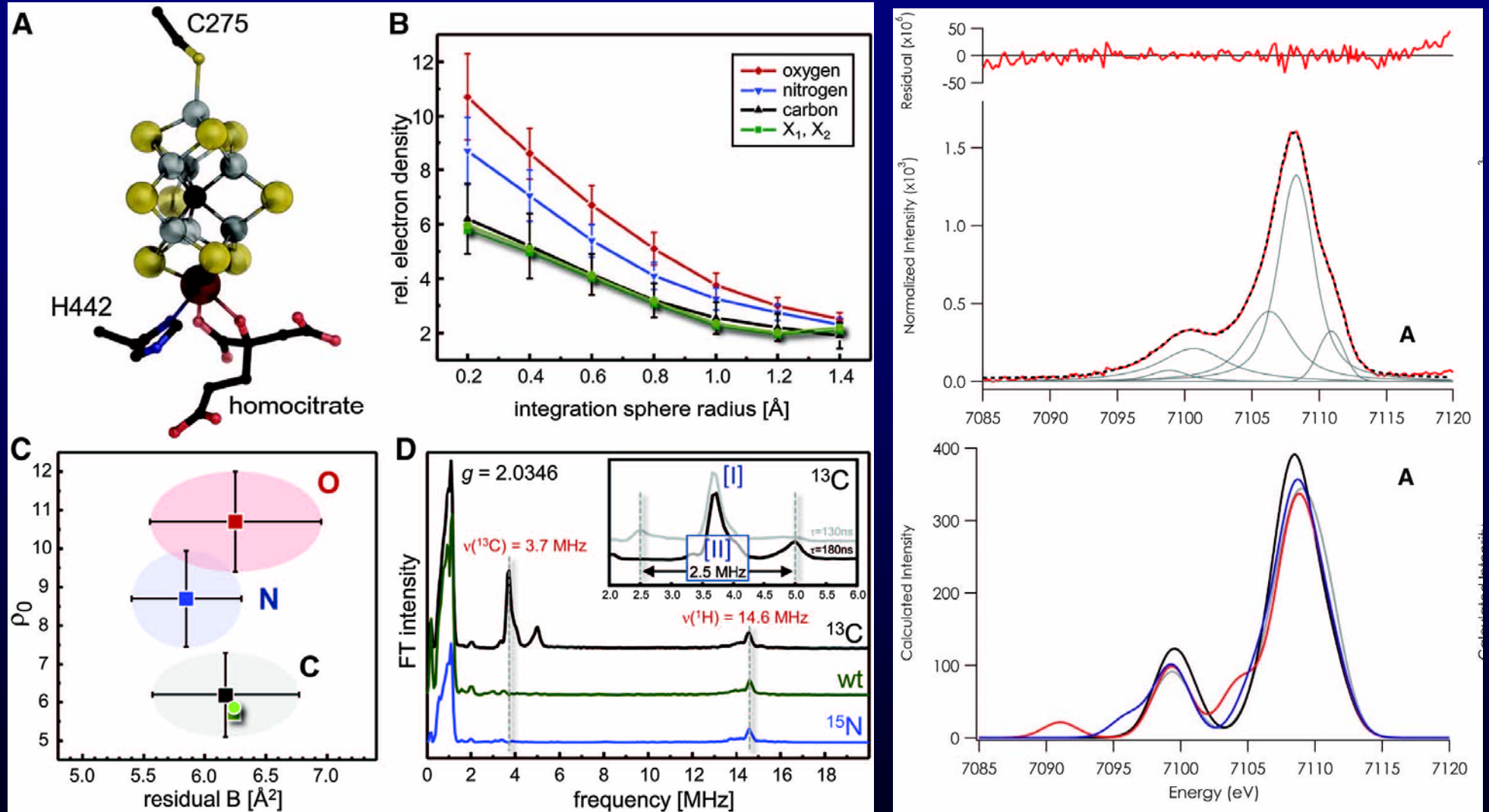
Nitrogenase: Interaction of components



Nitrogenase: putative intermediates of nitrogen reduction



Nitrogenase: central C

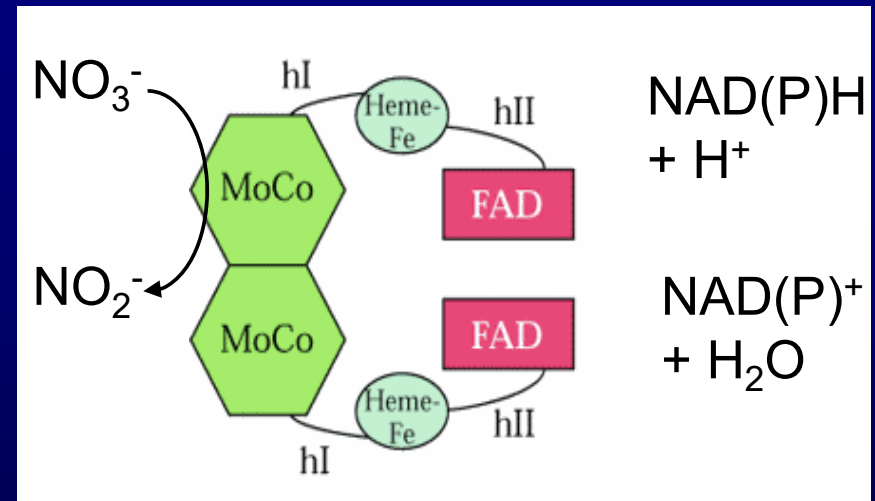


Nitrate reduction

Nitrate reductase in the cytoplasm



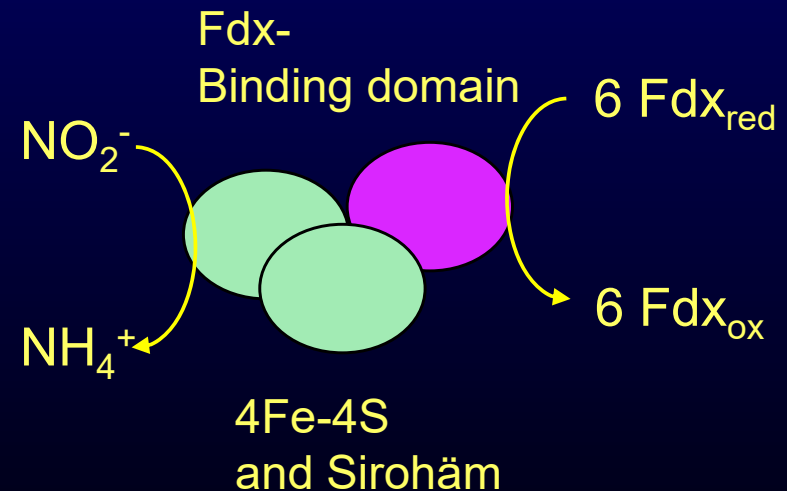
Mo as essential cofactor



Nitrite reductase in the chloroplast stroma

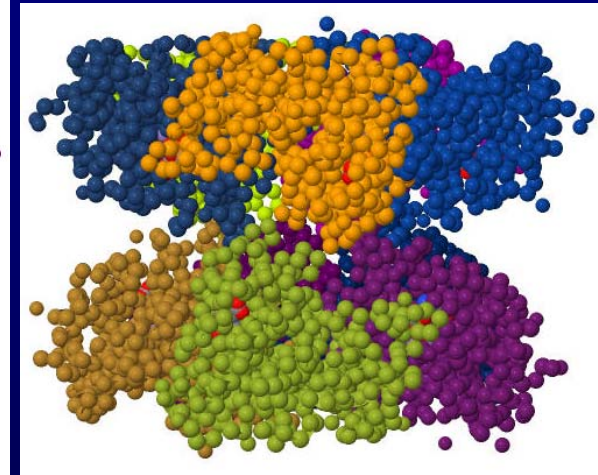
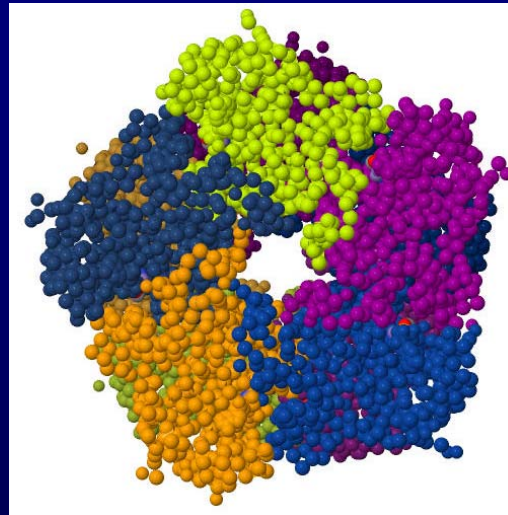
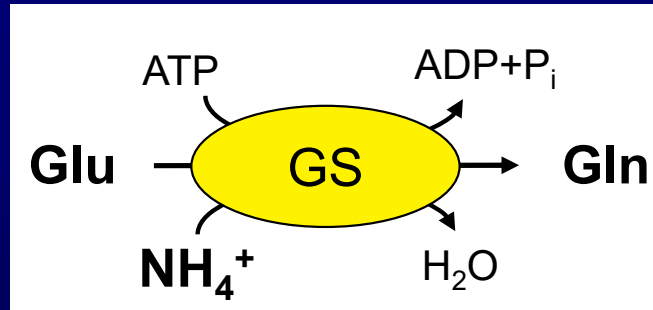


Receives energy directly from the linear electron transport of photosynthesis



Ammonium assimilation

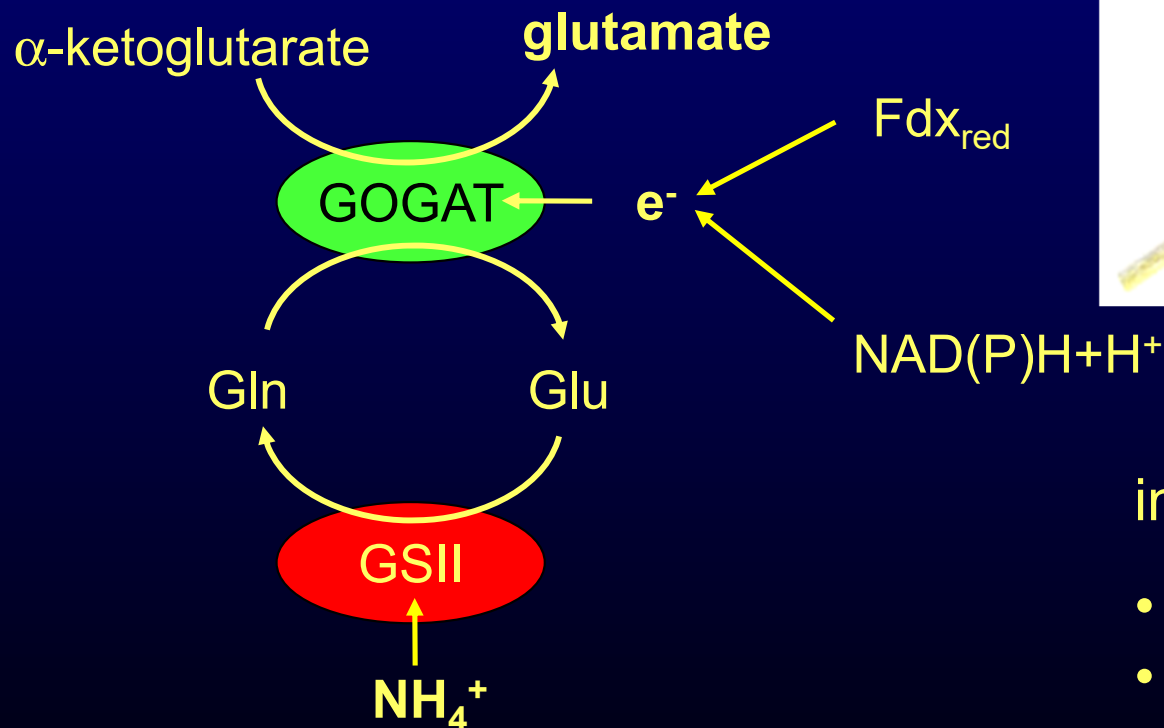
Glutamine-Synthetase



- Decamer with 10 reaction centres at the contact points of the subunits
- Mn as essential cofactor
- GSII in the chloroplast stroma → primary assimilation
- GSI in the cytoplasm → recycling
- Inhibition by phosphinothrizin (Glufosinate, Basta®)

Glutamate synthase = GOGAT

- **Glutamine oxoglutarate aminotransferase**
- in plastids
- main pathway of N-Assimilation
- active site: 3Fe4S-cluster combined with flavin mononucleotide (FMN)



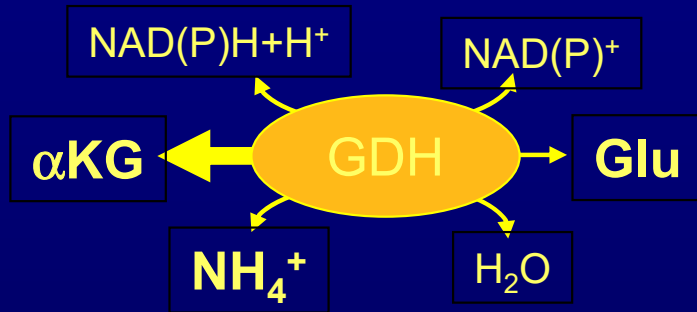
in green **leaves**:

- Primary assimilation
- Photorespiration

in **roots** and cotyledons:

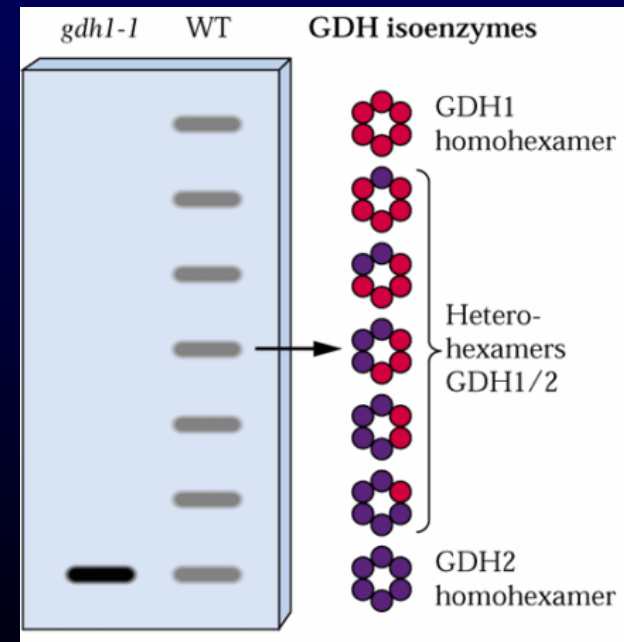
- primary assimilation,
- amino acid recycling

Glutamate dehydrogenase

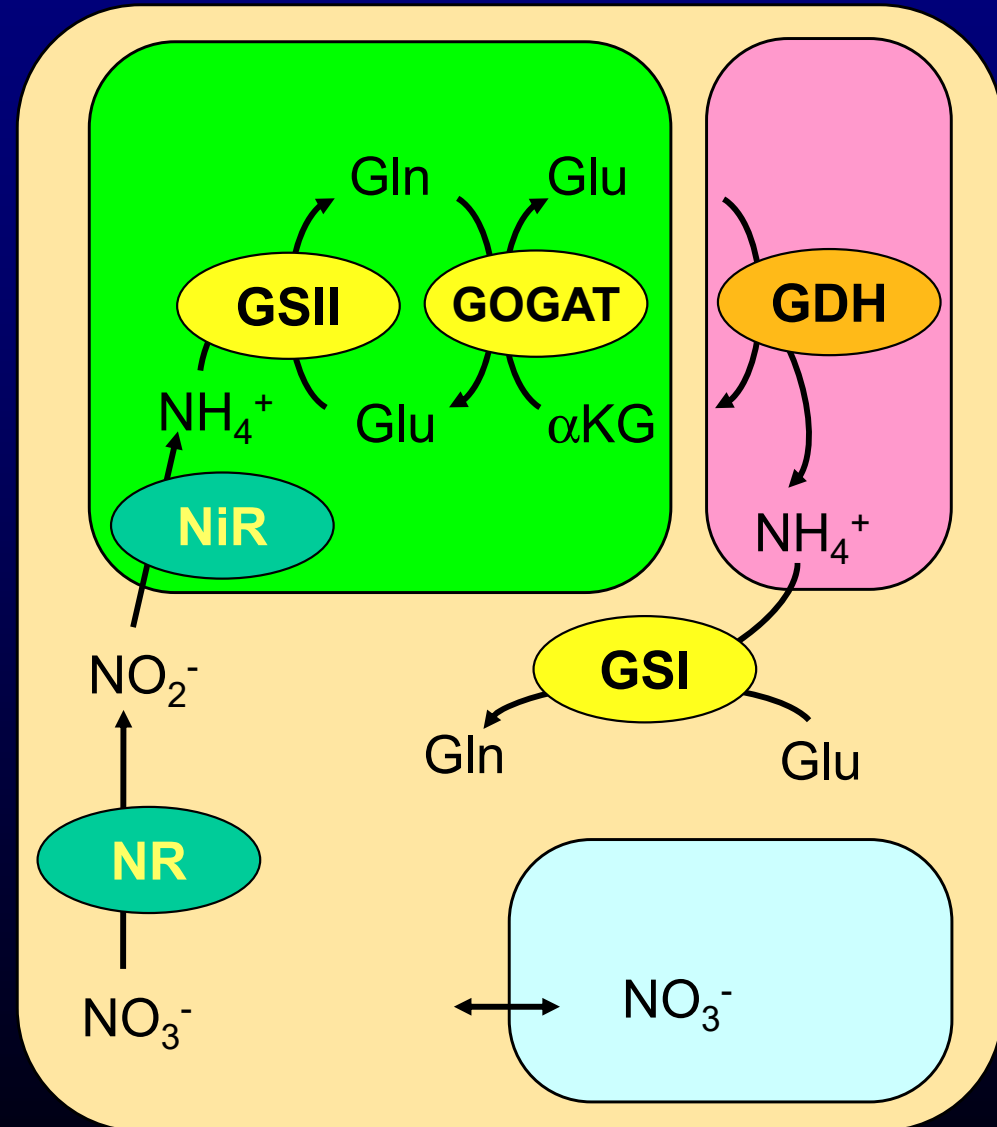
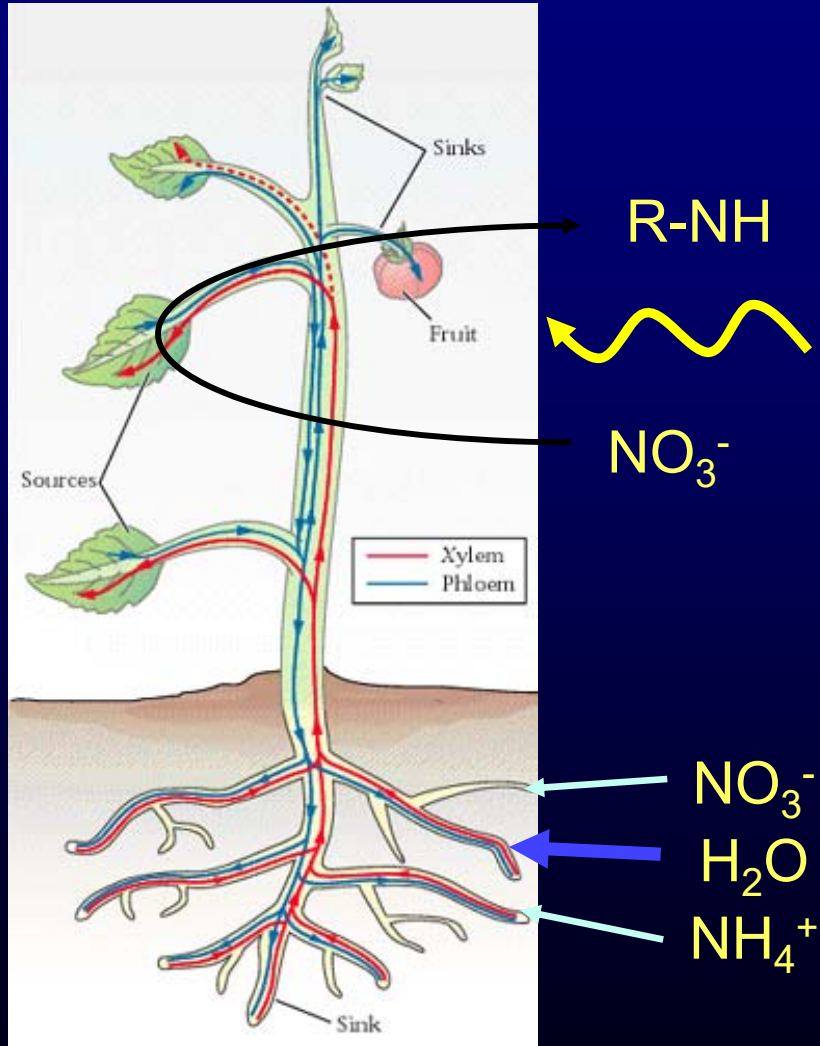


- low affinity for NH₄⁺
- predominantly degradation of glutamate
- Cu (and Co ?) as cofactors

- in mitochondria
- 3 isoforms
- GDH1 and GDH2 form homo- or hetero- hexamers

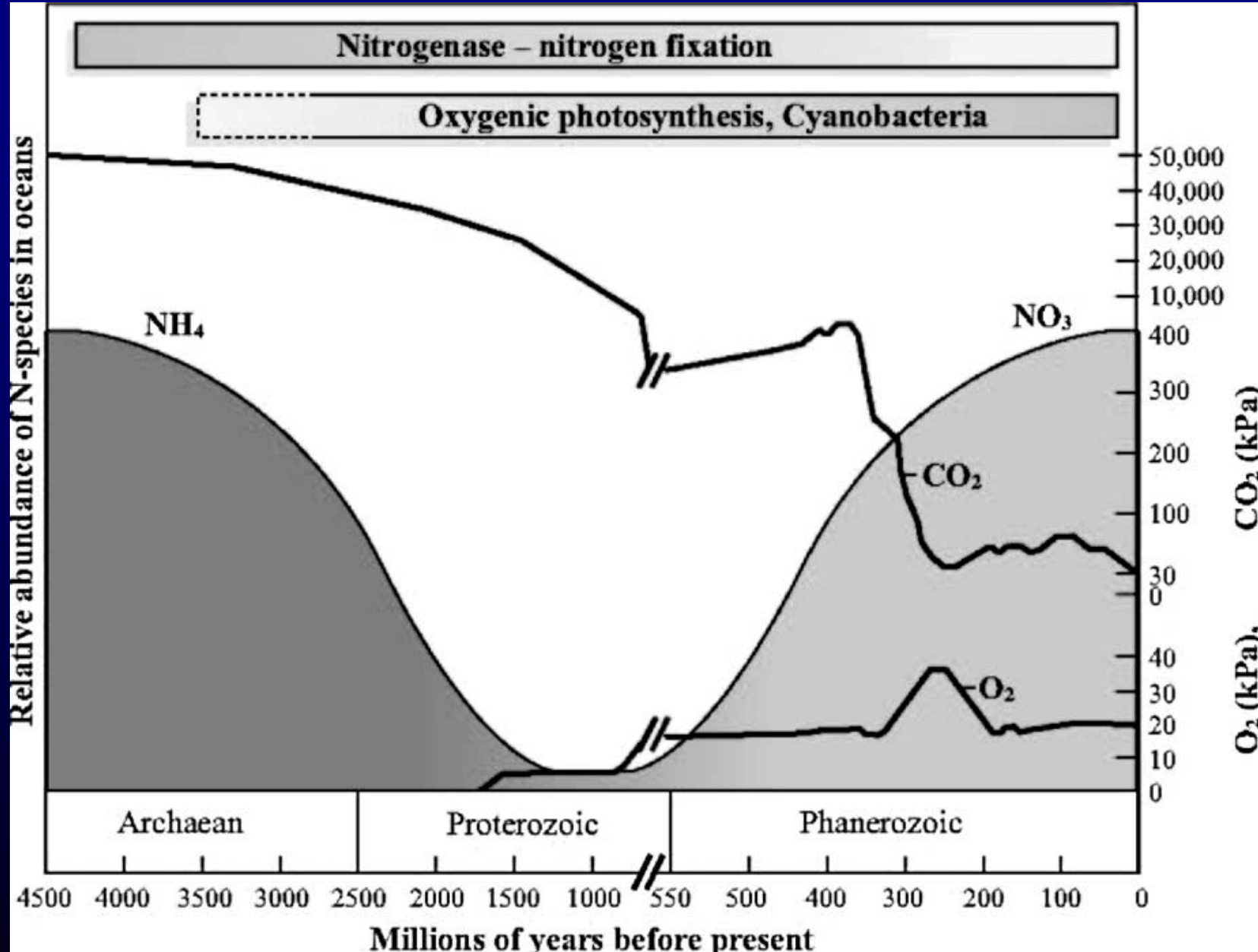


Summary: Nitrogen assimilation in plants

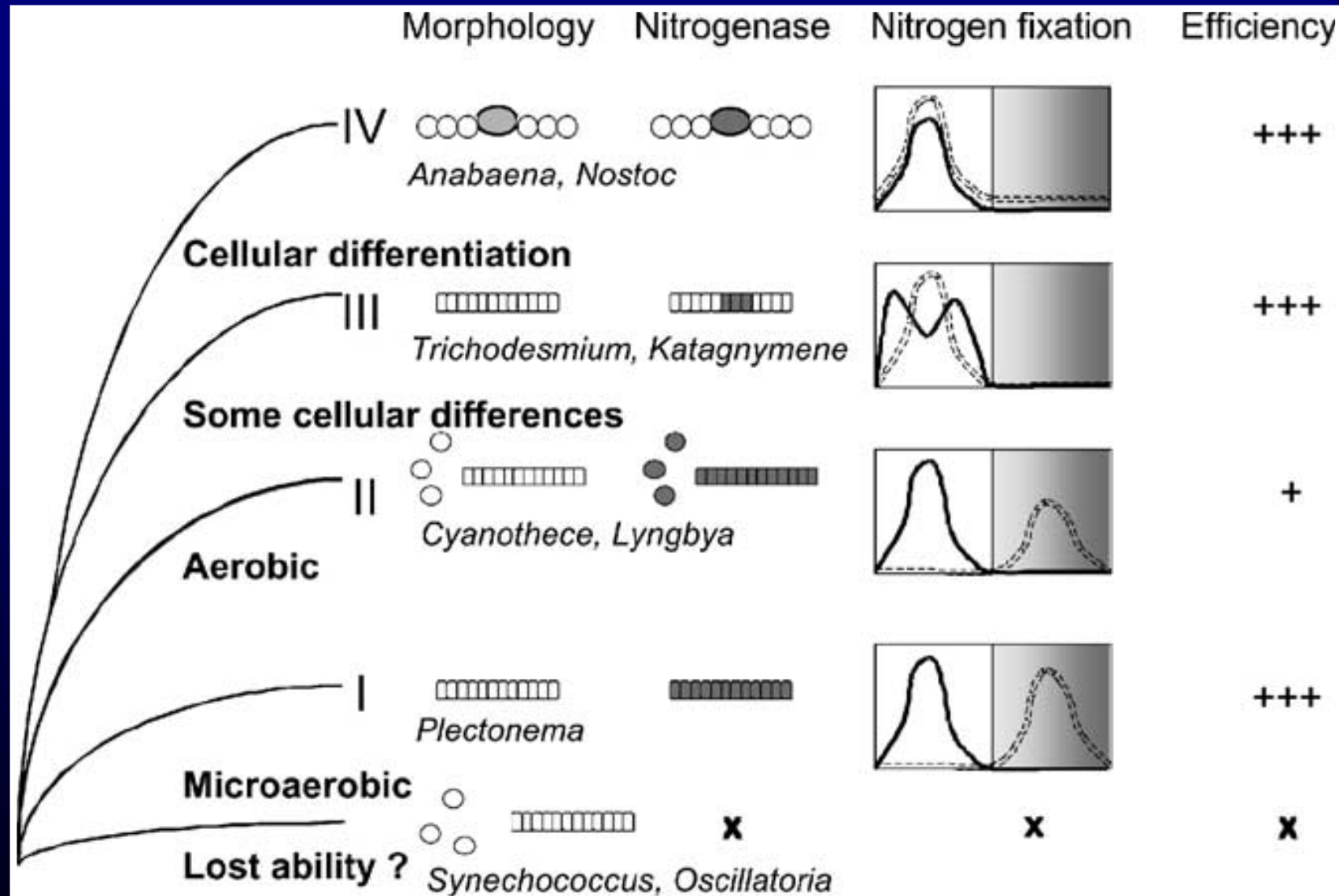


Part II:
Regulation of photosynthesis for nitrogen fixation

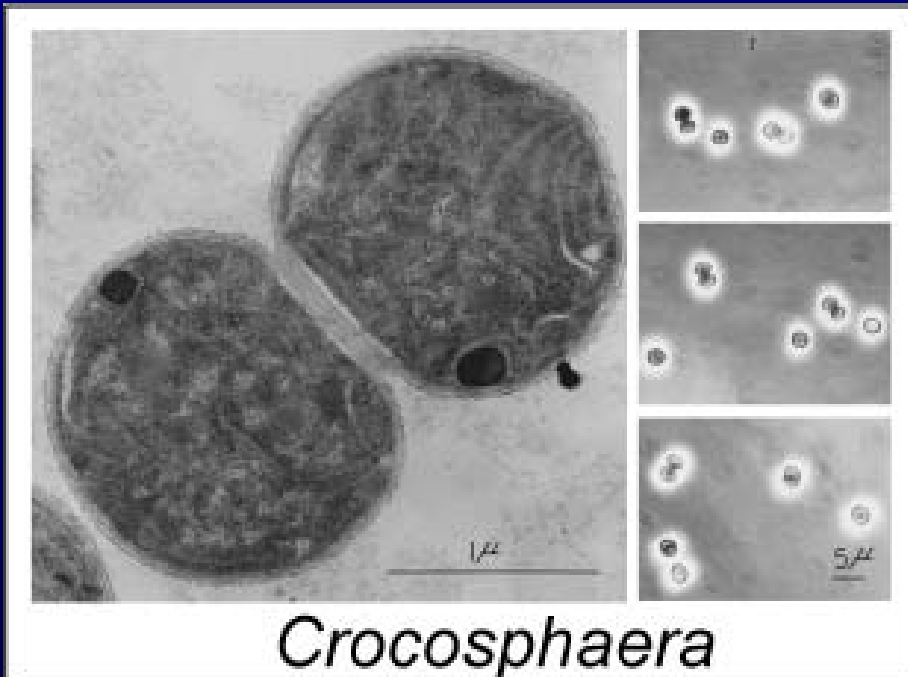
Evolution of biological nitrogen fixation in comparison to photosynthesis



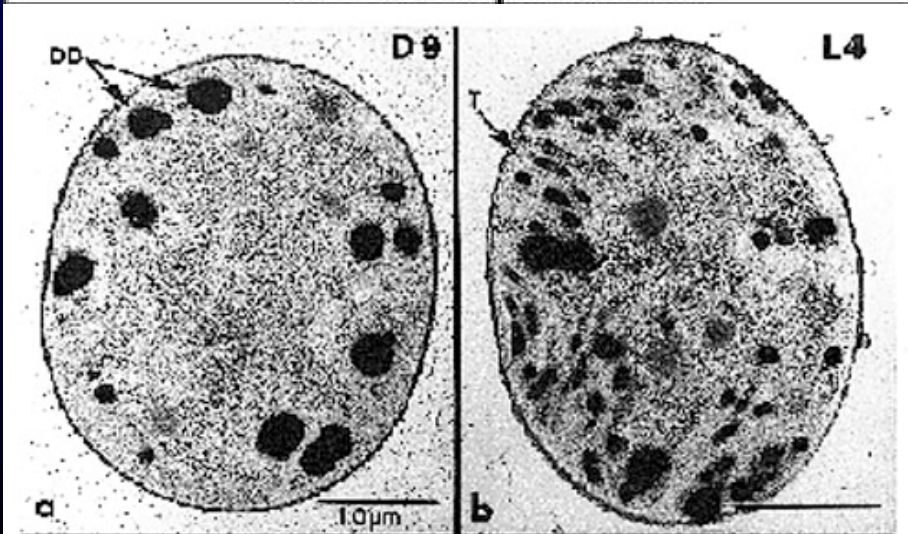
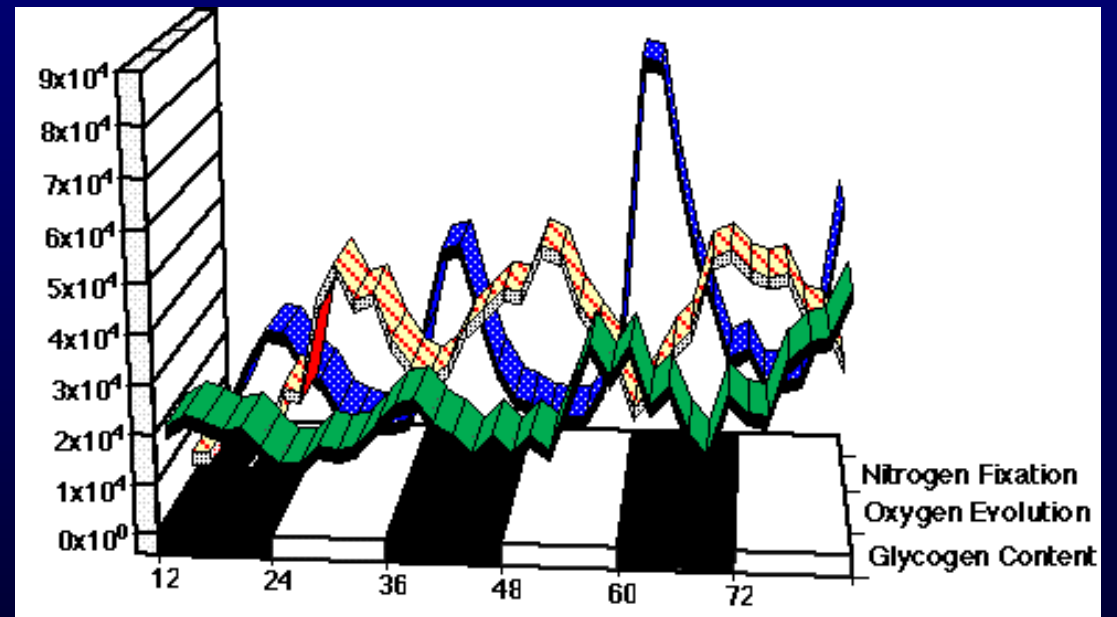
Strategies of photosynthesis regulation for nitrogen fixation in cyanobacteria



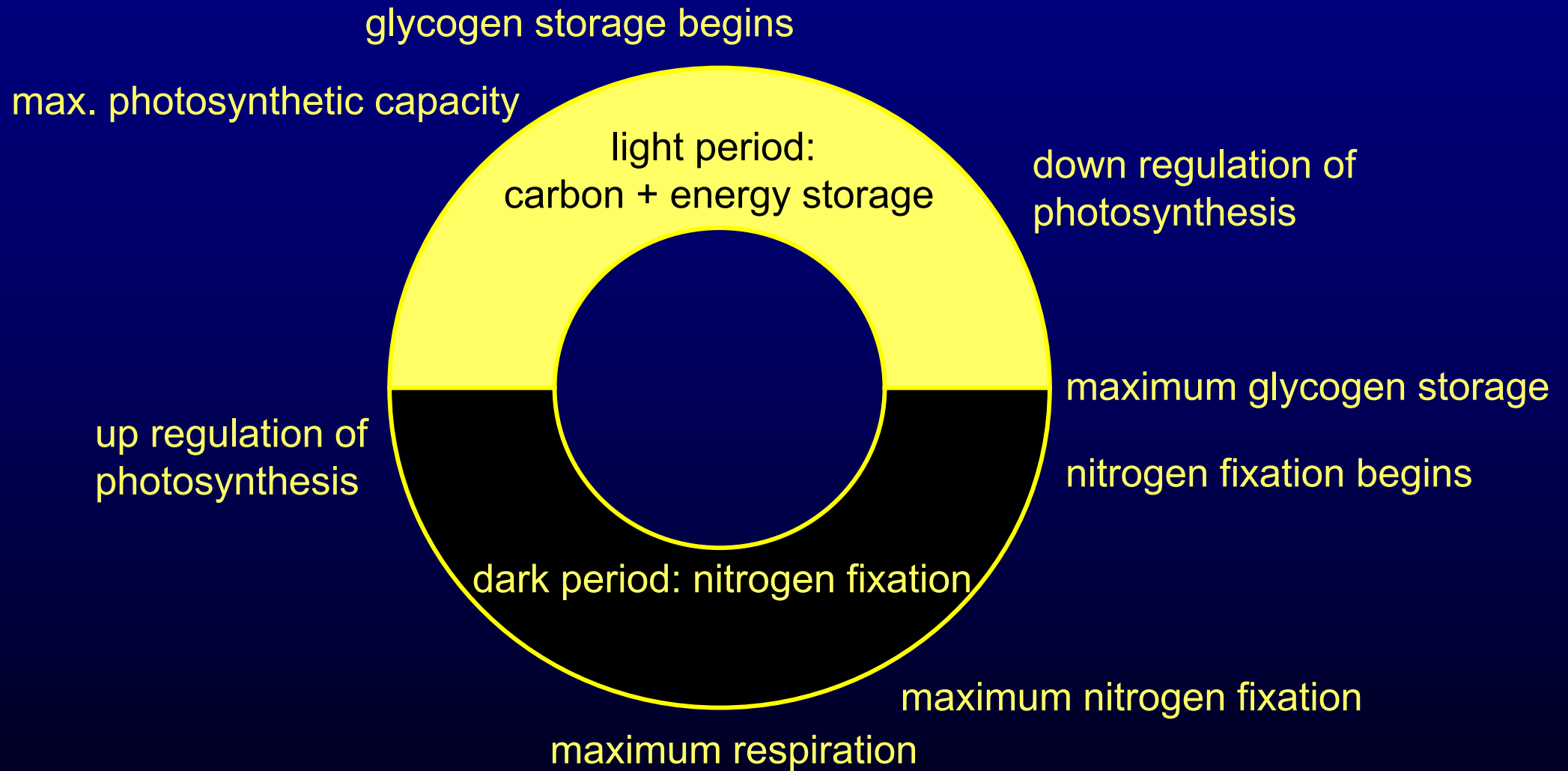
Unicellular cyanobacteria



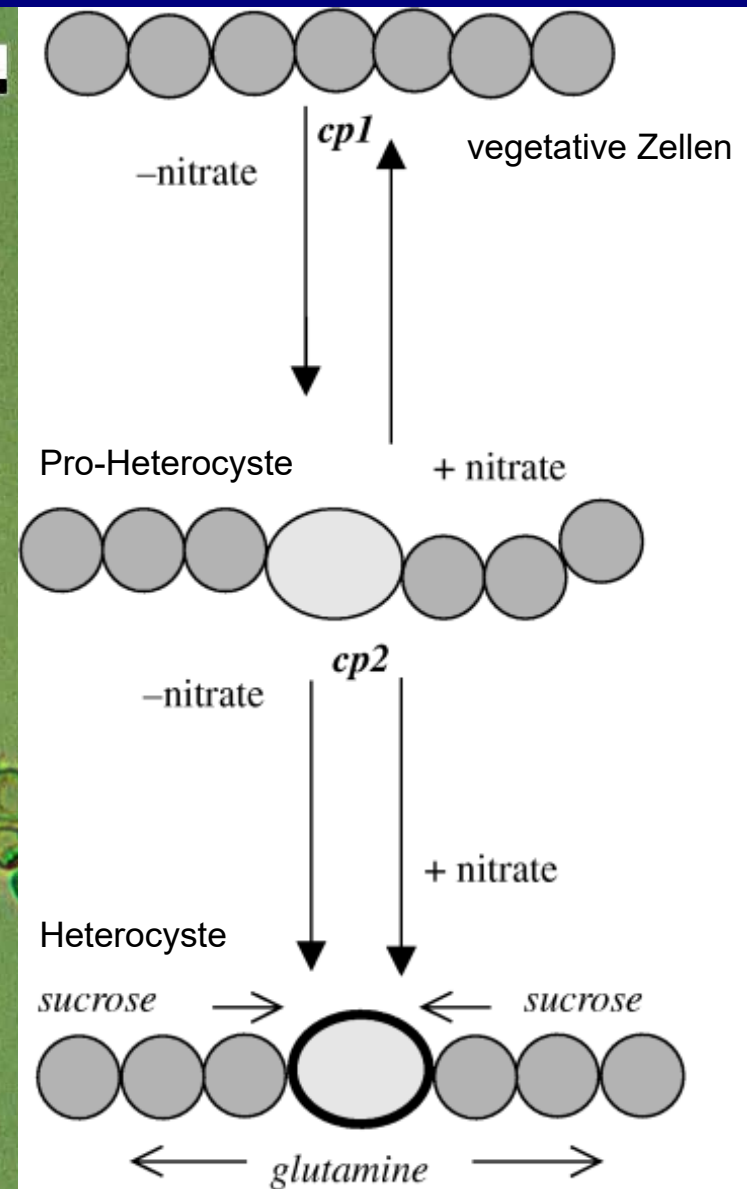
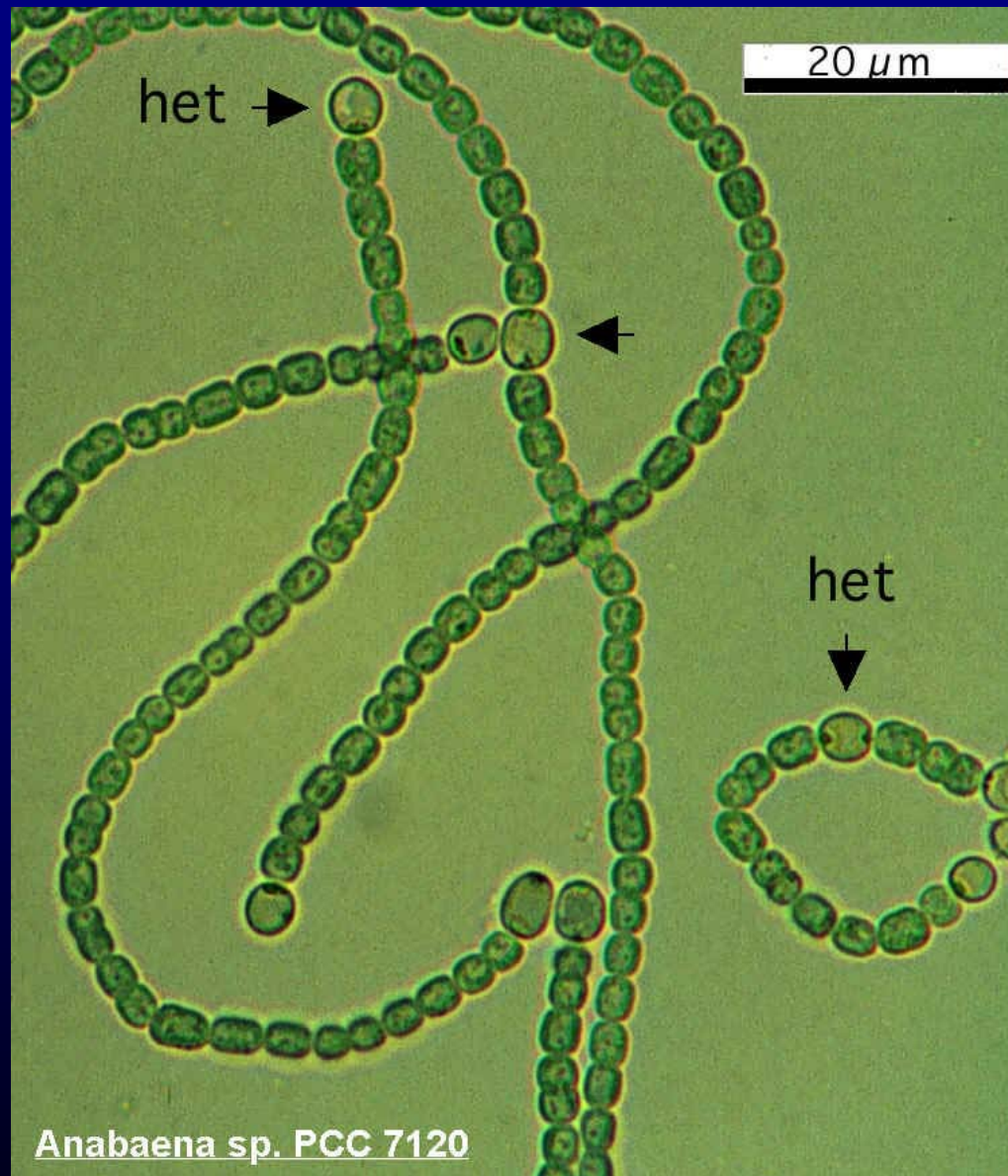
Crocosphaera



Regulation of photosynthesis for nitrogen fixation in unicellular cyanobacteria (II)



Heterocyst forming cyanobacteria

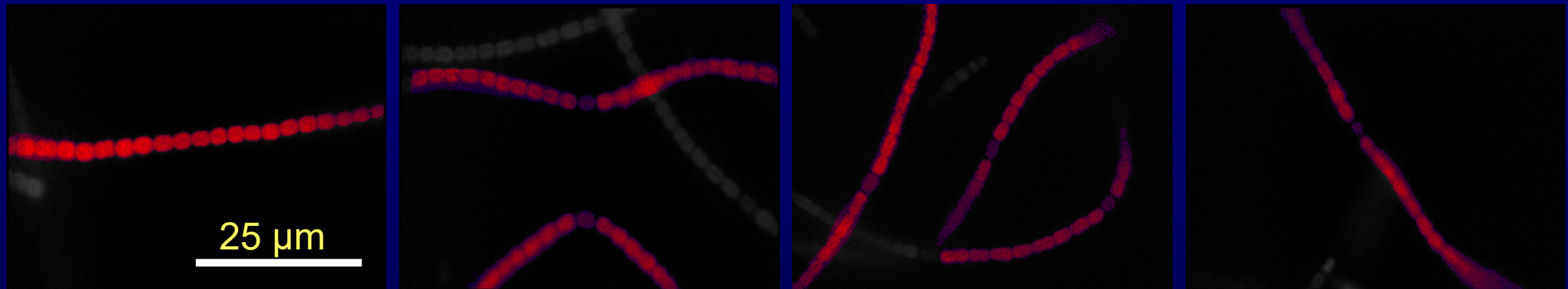


From: Culture service of Instituto de Bioquímica Vegetal y Fotosíntesis, Sevilla, Spain

From: El-Shehawy et al 2003 Physiol Plant 119 (1), 49-55

Heterocyst differentiation: distribution maps of chlorophyll fluorescence kinetic parameters

Maximal fluorescence quantum yield (F_m)

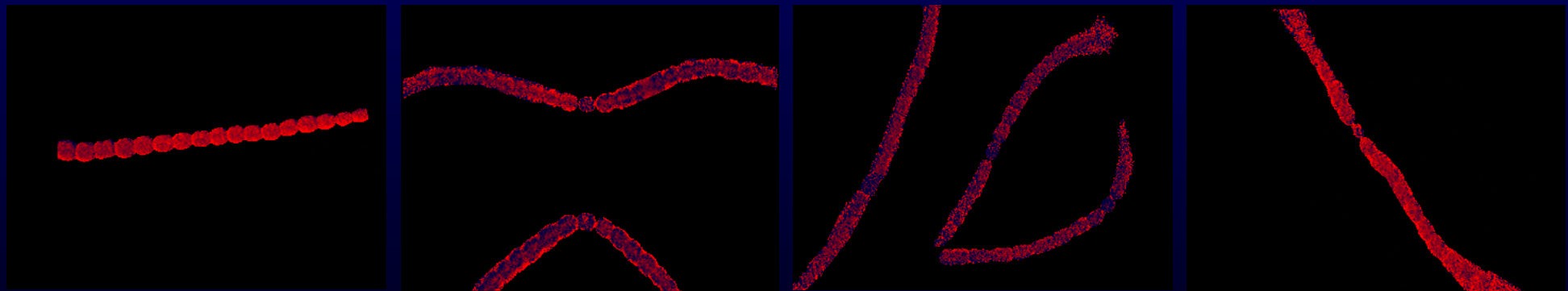


13 h

36 h

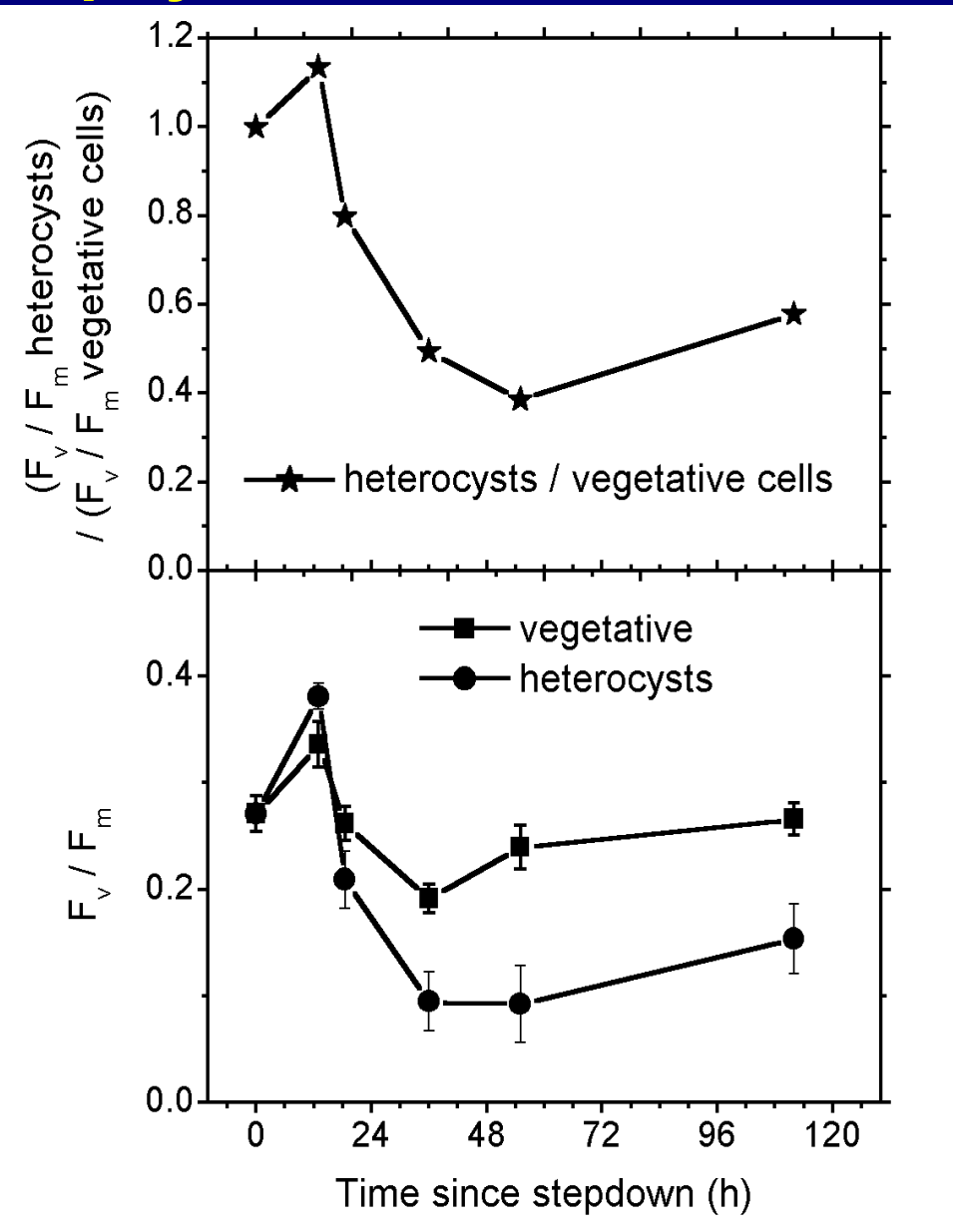
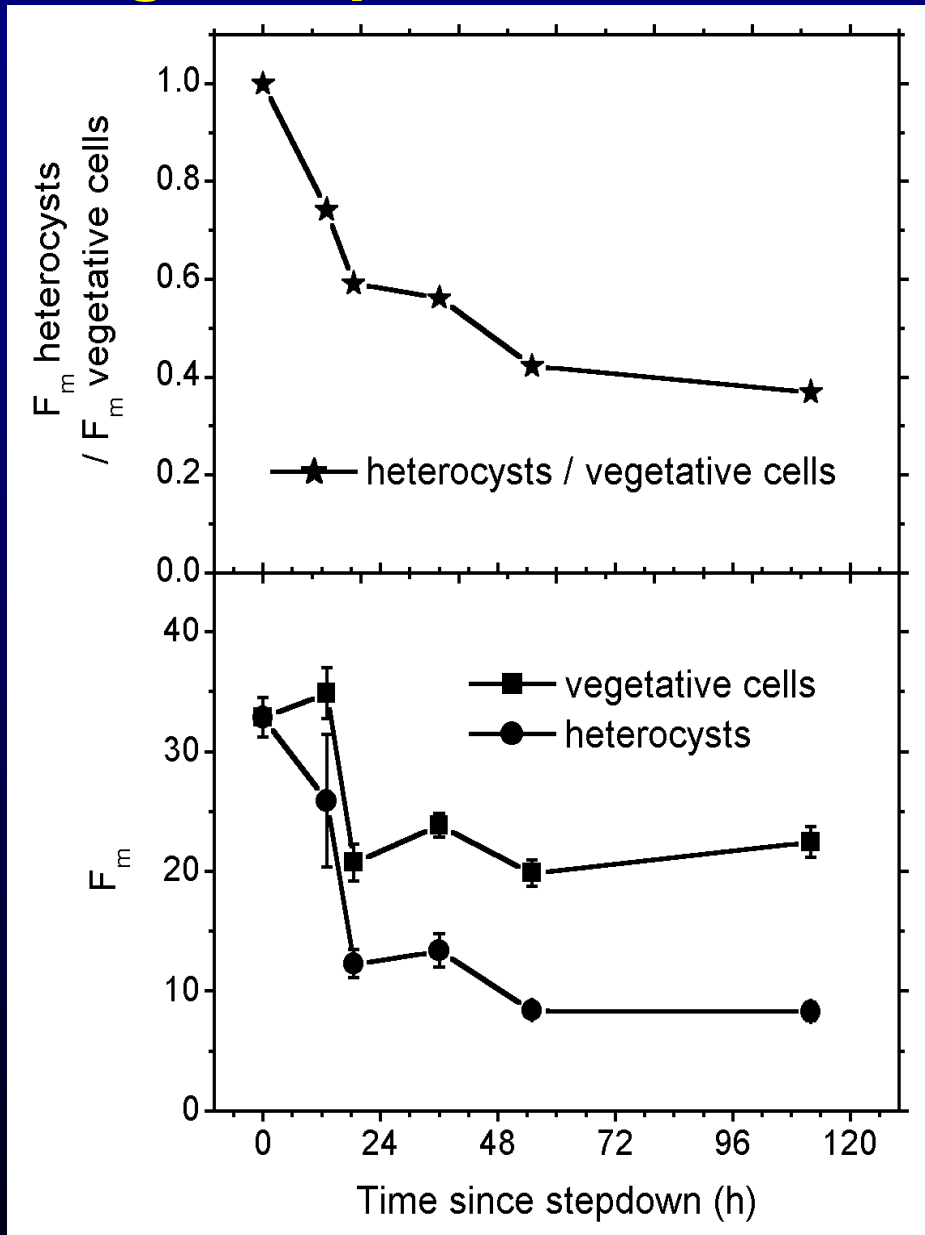
55 h

112 h



Photosystem II activity (F_v/F_m)

Heterocyst differentiation: changes in parameters of chlorophyll fluorescence kinetics



Trichodesmium:

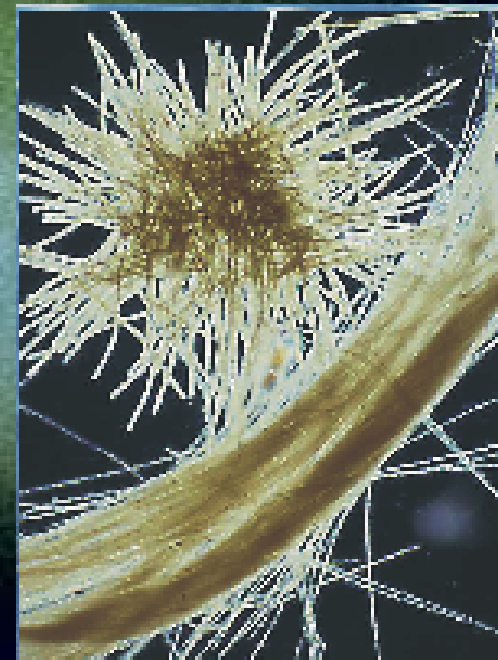
**anoxygenic photosynthesis energizing nitrogen fixation
in the same cells during the photoperiod**

Trichodesmium

- Marine filamentous, non-heterocystous cyanobacteria
- contribution of *Trichodesmium* to marine N₂ fixation: 30-50%
- Nitrogen fixation is confined to the photoperiod and occurs simultaneously with oxygenic photosynthesis.
- How nitrogenase is protected from damage by photosynthetically produced O₂ has remained an enigma.



Surface blooms in the
Arafura Sea



Colonies – tuft and puff
formation

Trichodesmium

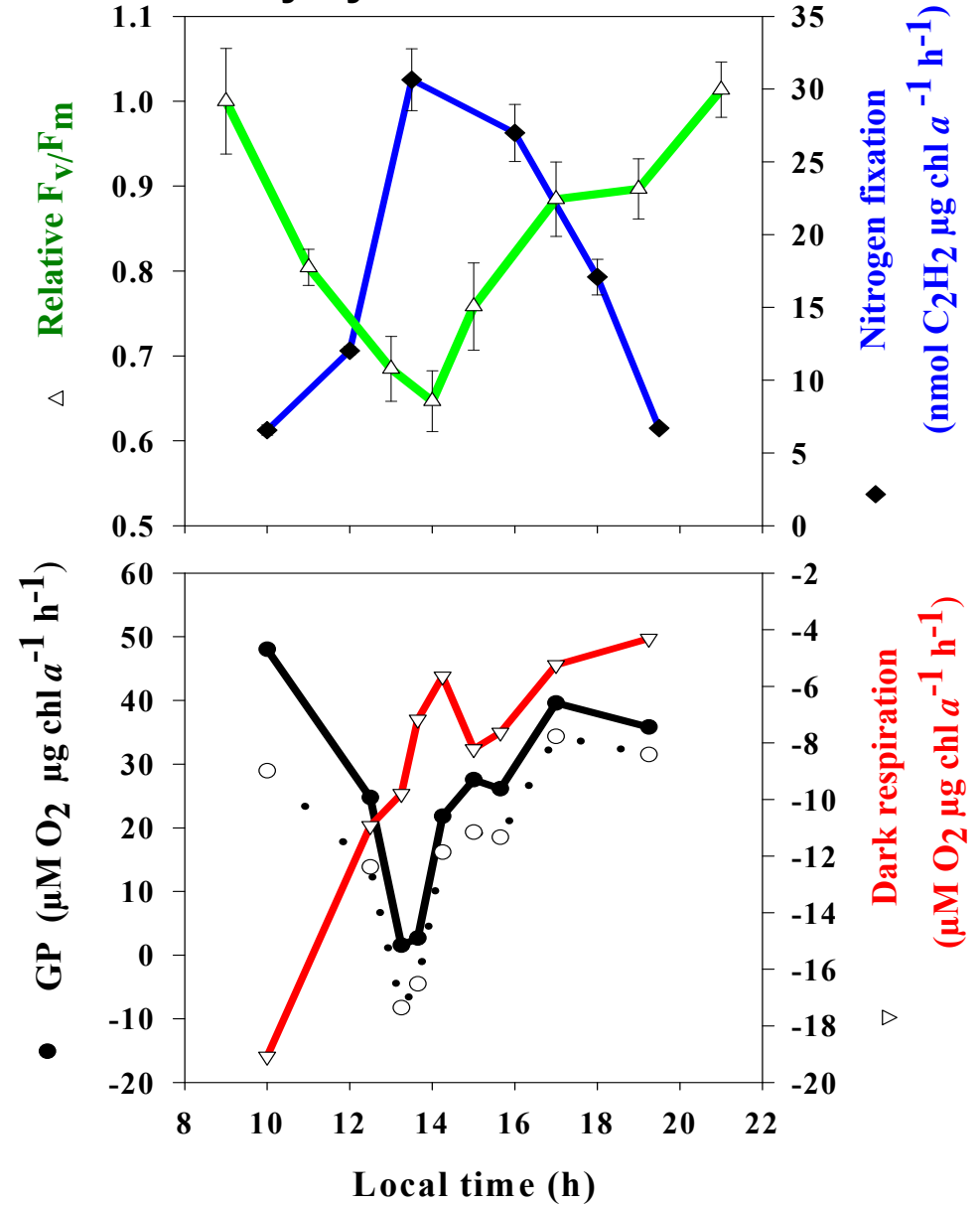
Trichodesmium-bloom



colonies: "Tuft" and "Puff"

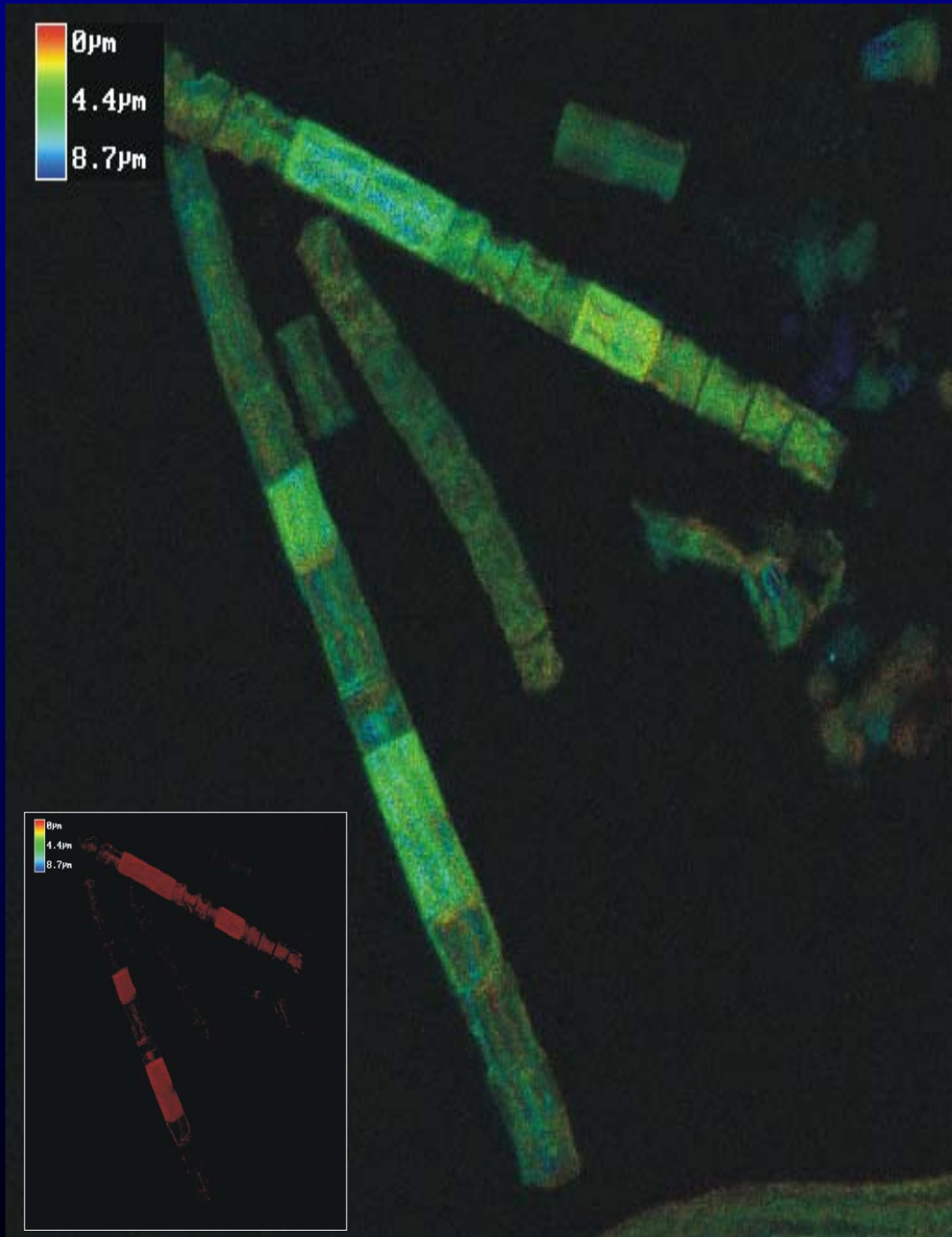


Activity cycle of *Trichodesmium*



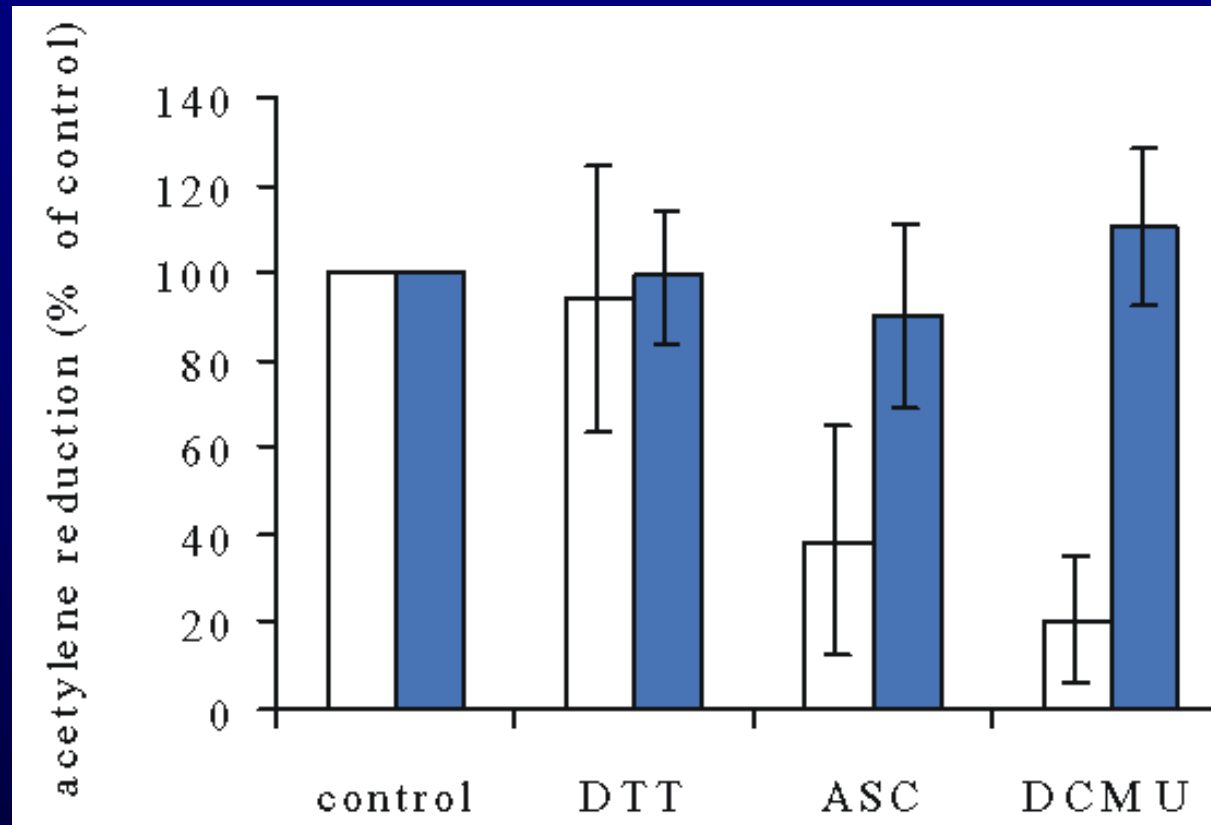
Berman-Frank I, Lundgren P, Chen Yi-B, Küpper H, Kolber Z, Bergman B, Falkowski P (2001) Science 294, 1534-1537

Co-Localisation of nitrogenase and PSII in *Trichodesmium*



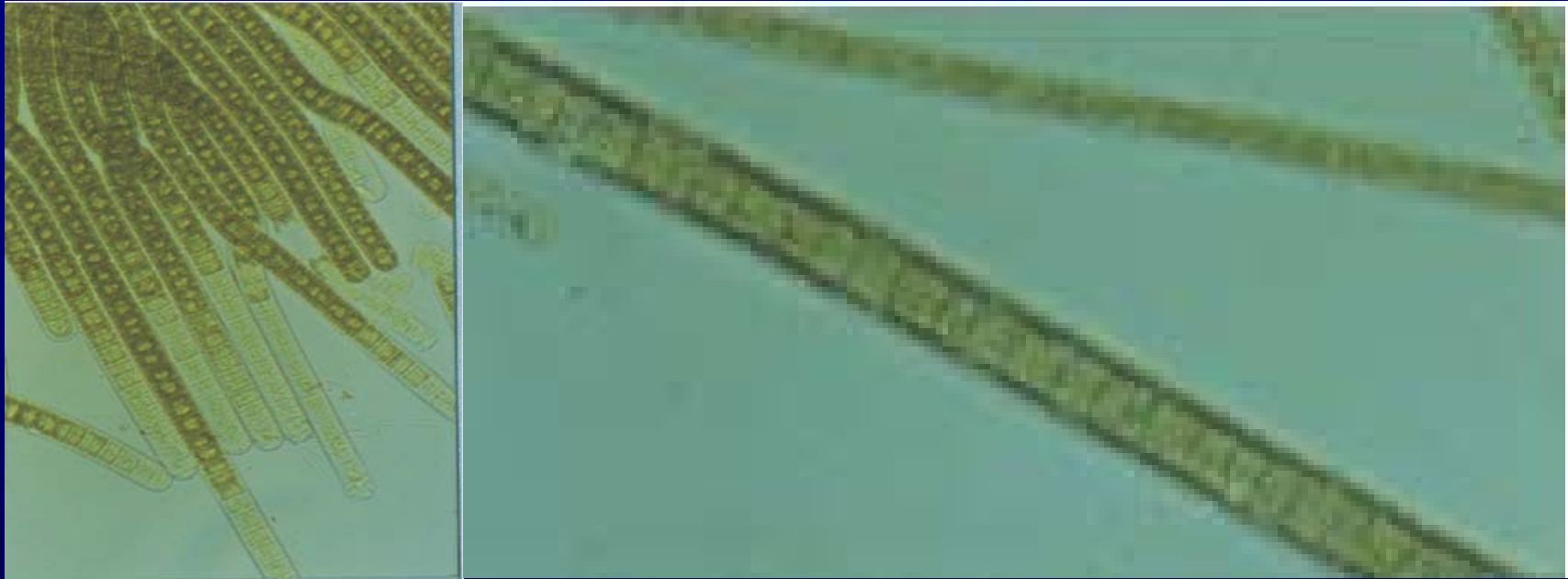
D1 protein (green) and Nitrogenase (rot).
Big picture: Overlay, small picture: only
nitrogenase (Immunostain)

Inhibitor-Tests: Need of PSII-activity for nitrogen fixation in *Trichodesmium*



Influence of DCMU (10 μ M), ascorbic acid (100 μ M), and DTT (100 μ M) were tested for cultures incubated under aerobic (white columns) and anaerobic (blue columns) conditions. Changes in nitrogenase activity as measured by acetylene reduction.

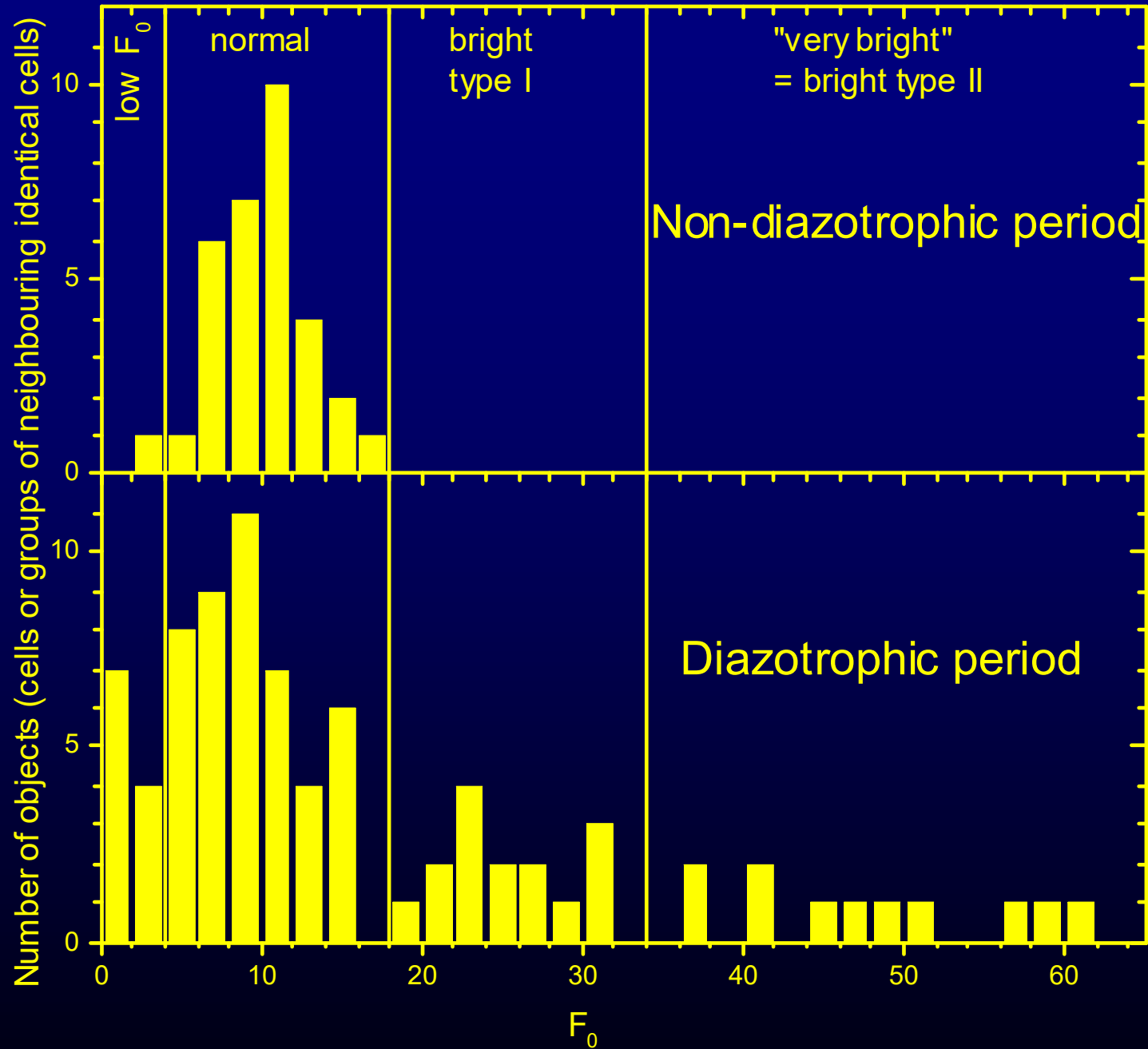
Proof of Mehler-reaction during nitrogen fixation



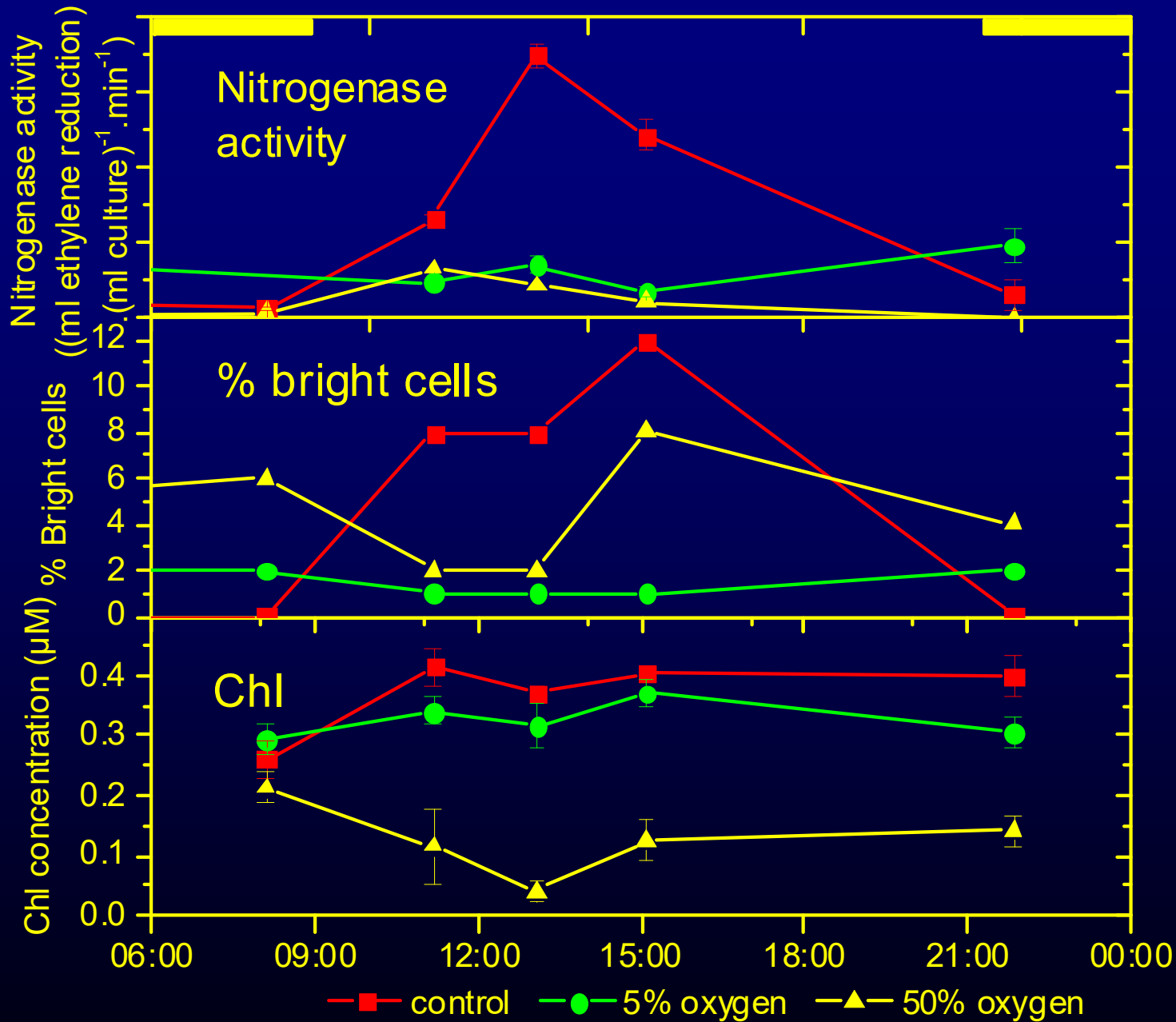
Staining with DAB (Diaminobenzochinon) shows intracellular distribution of H_2O_2 as brown stain in all cells



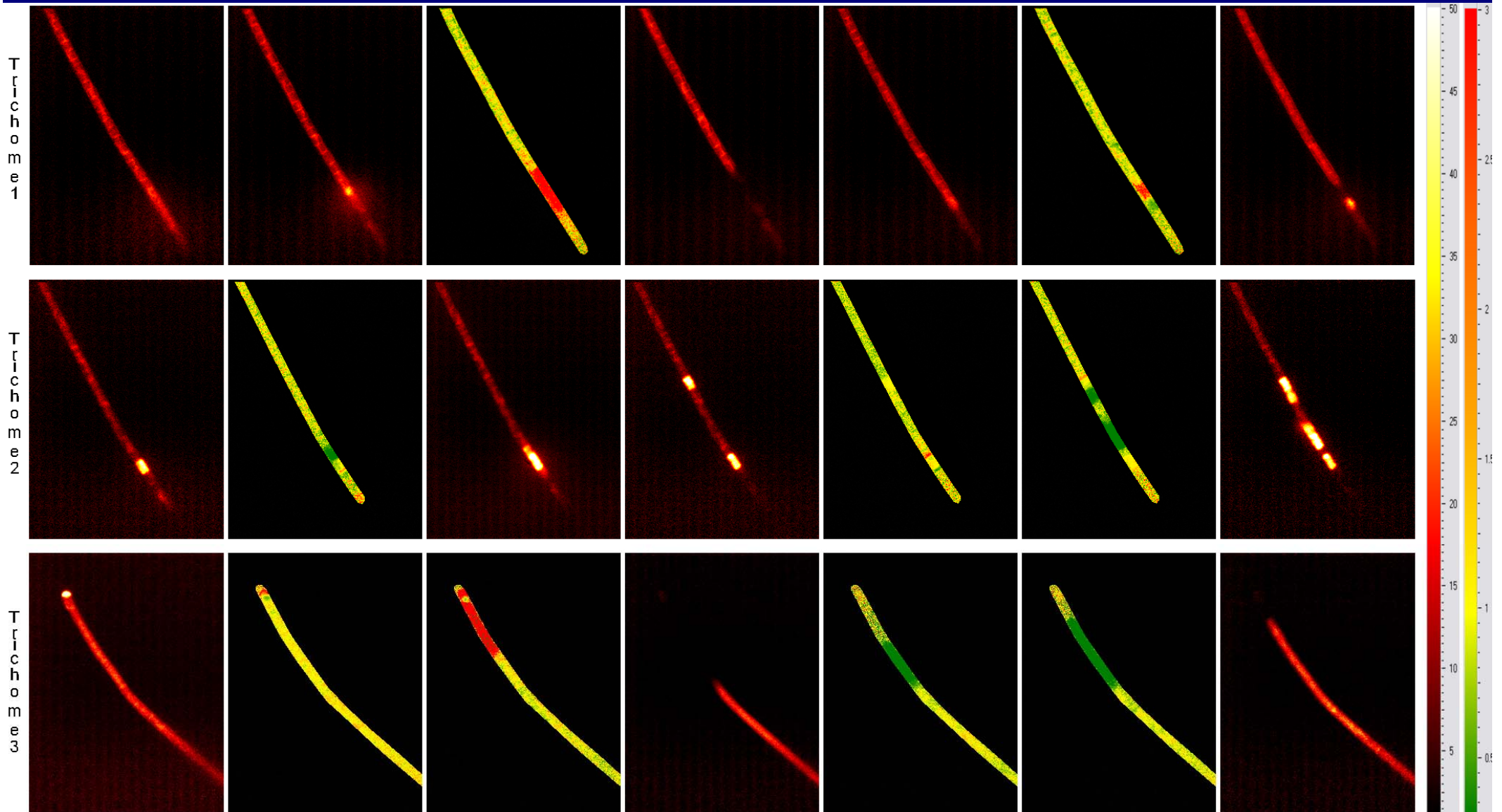
Diurnal cycle of activity: Distribution of F_0 values

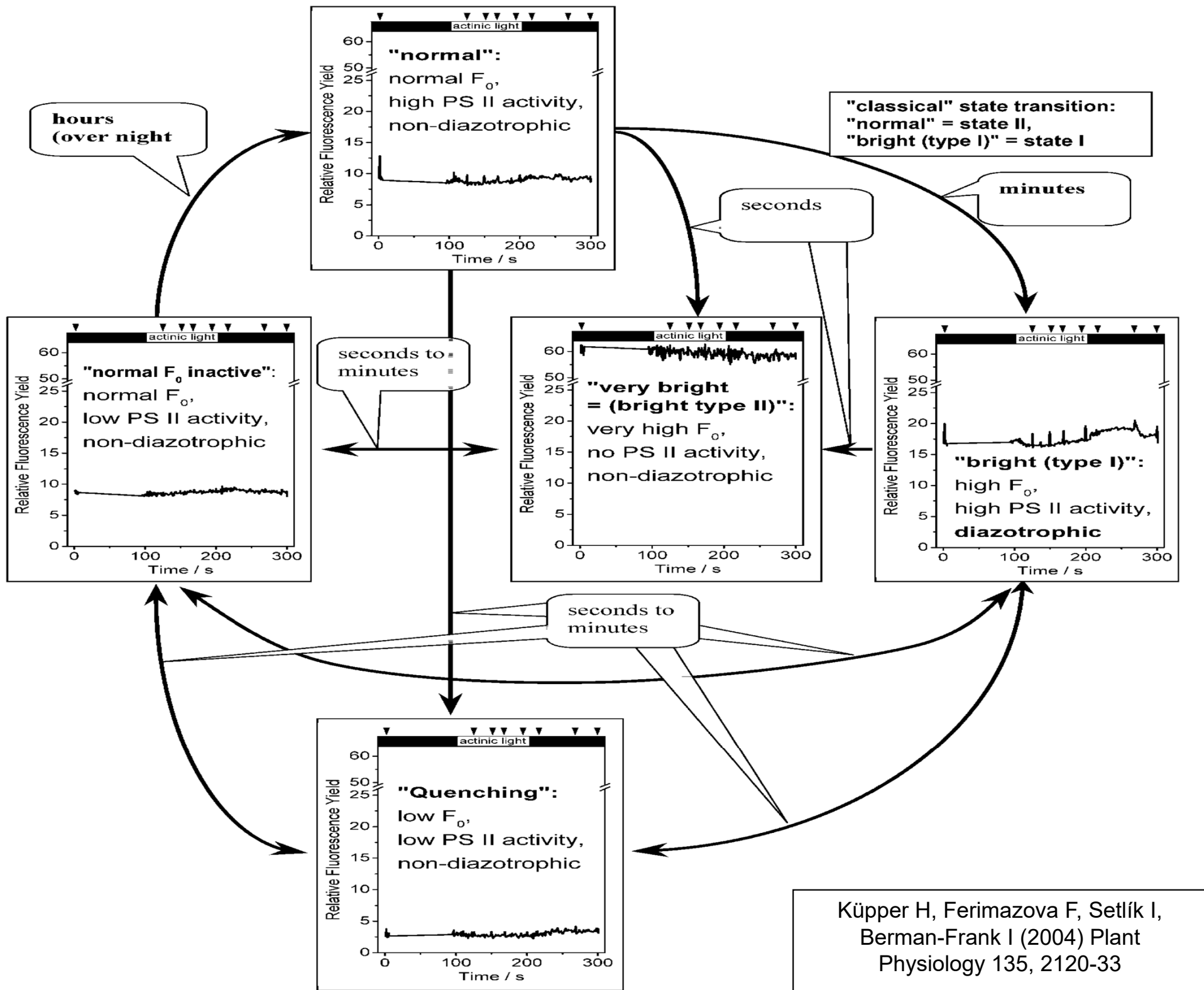


Diurnal cycle of activity: correlation between bright cells, pigment content and nitrogenase activity

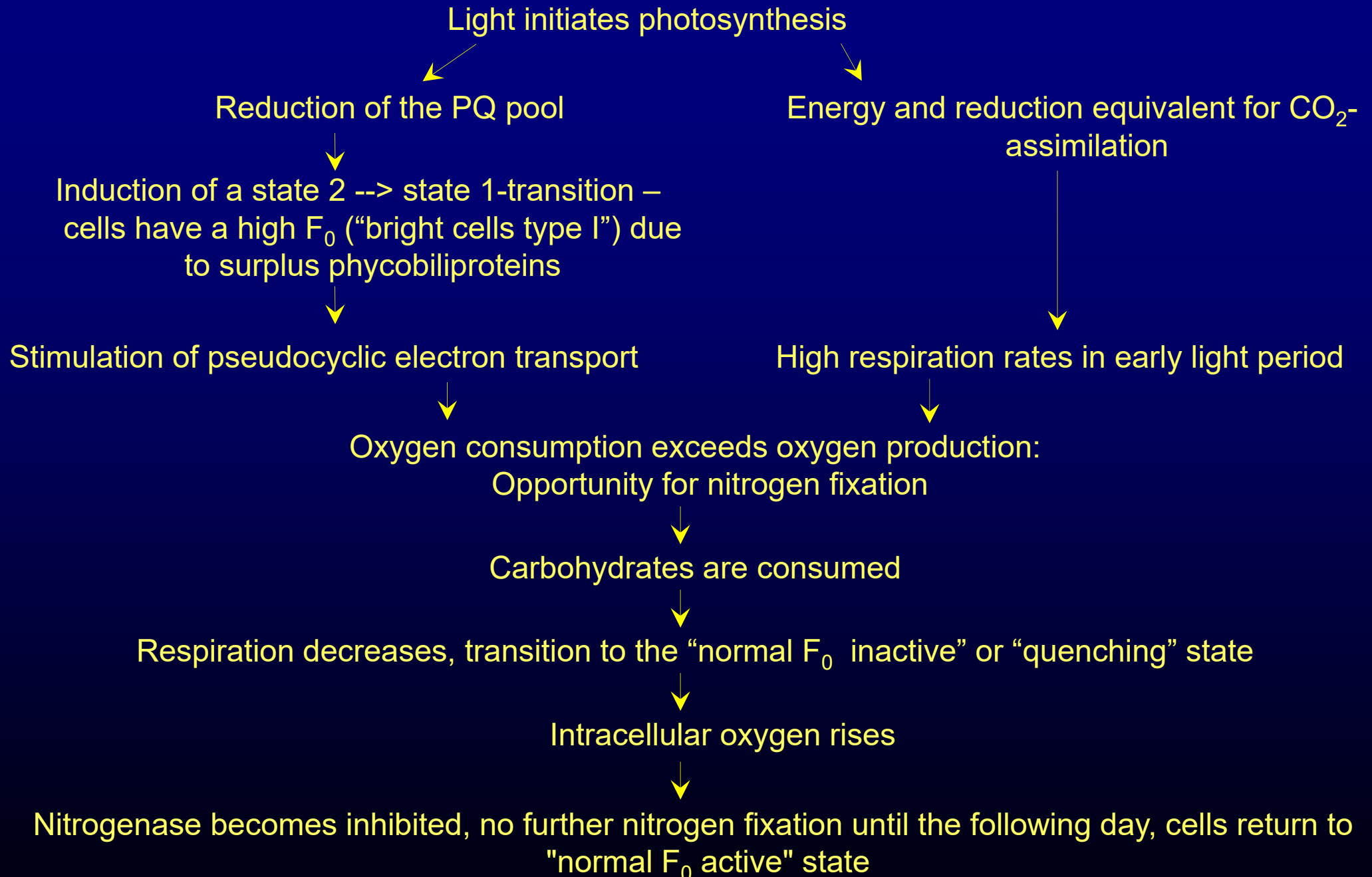


PSII-activity *Trichodesmium*: reversibility of changes in fluorescence yield





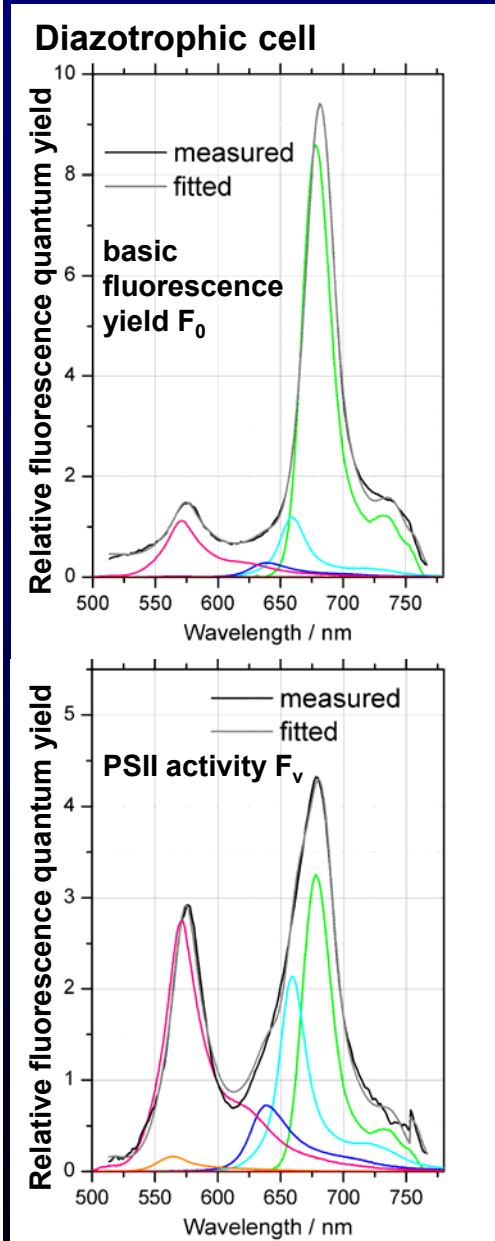
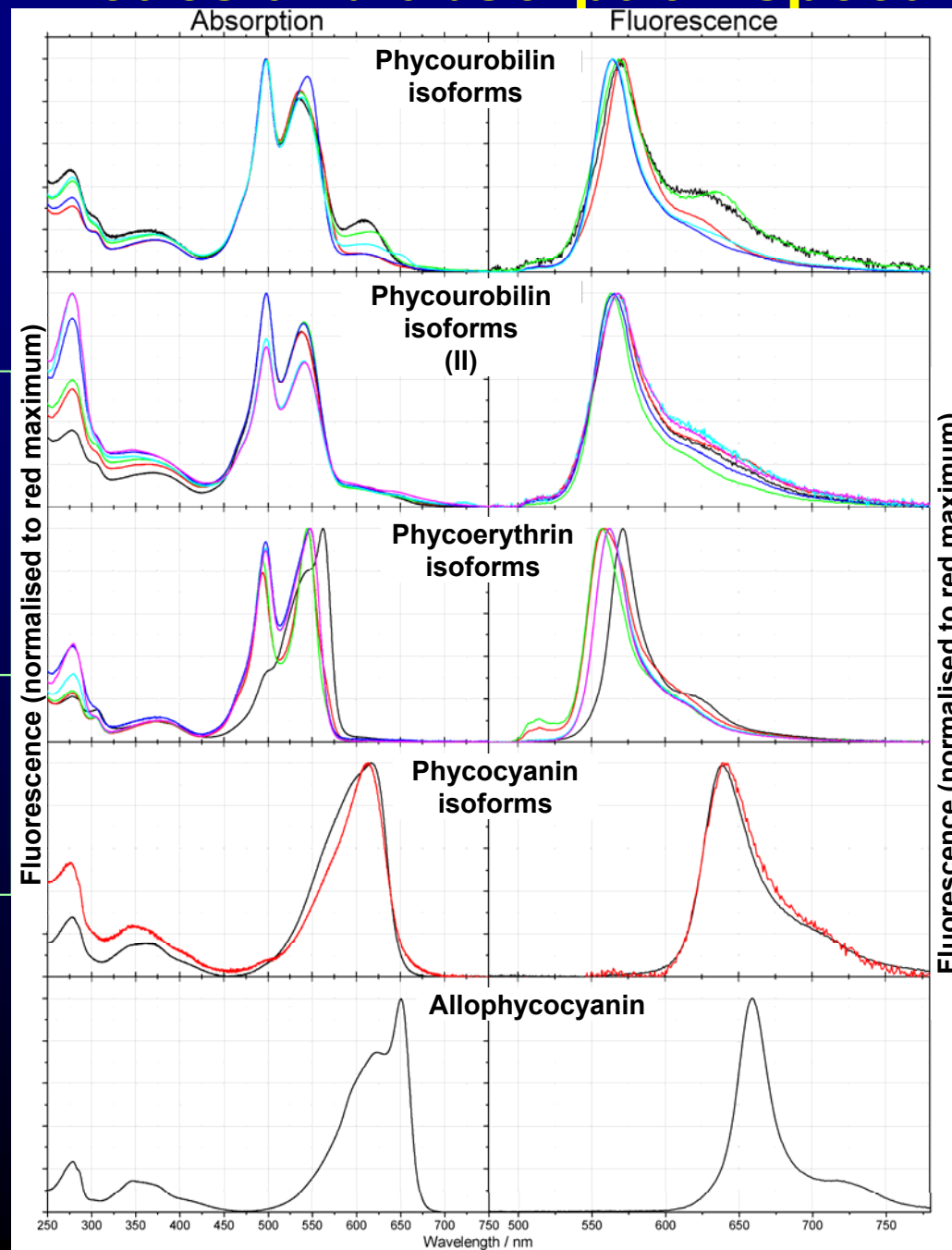
Hypothesis about the regulation of photosynthesis for nitrogen fixation in *Trichodesmium*



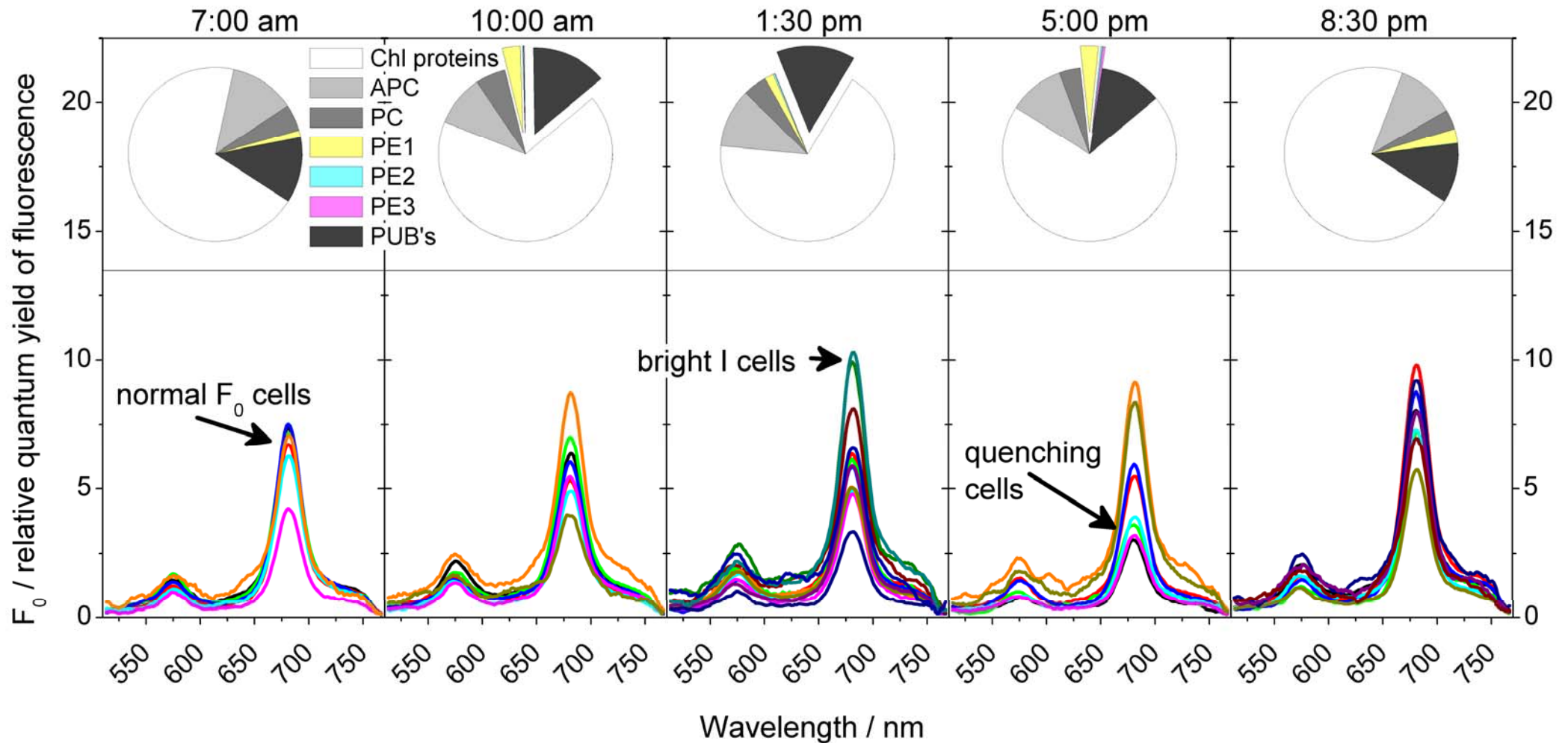
Purification of *Trichodesmium* phycobiliproteins for deconvoluting spectrally resolved *in vivo* fluorescence kinetics and absorption spectra

Phycobiliprotein purification + characterisation: Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) *Biochim. Biophys. Acta (Bioenergetics)* 1787, 155-167

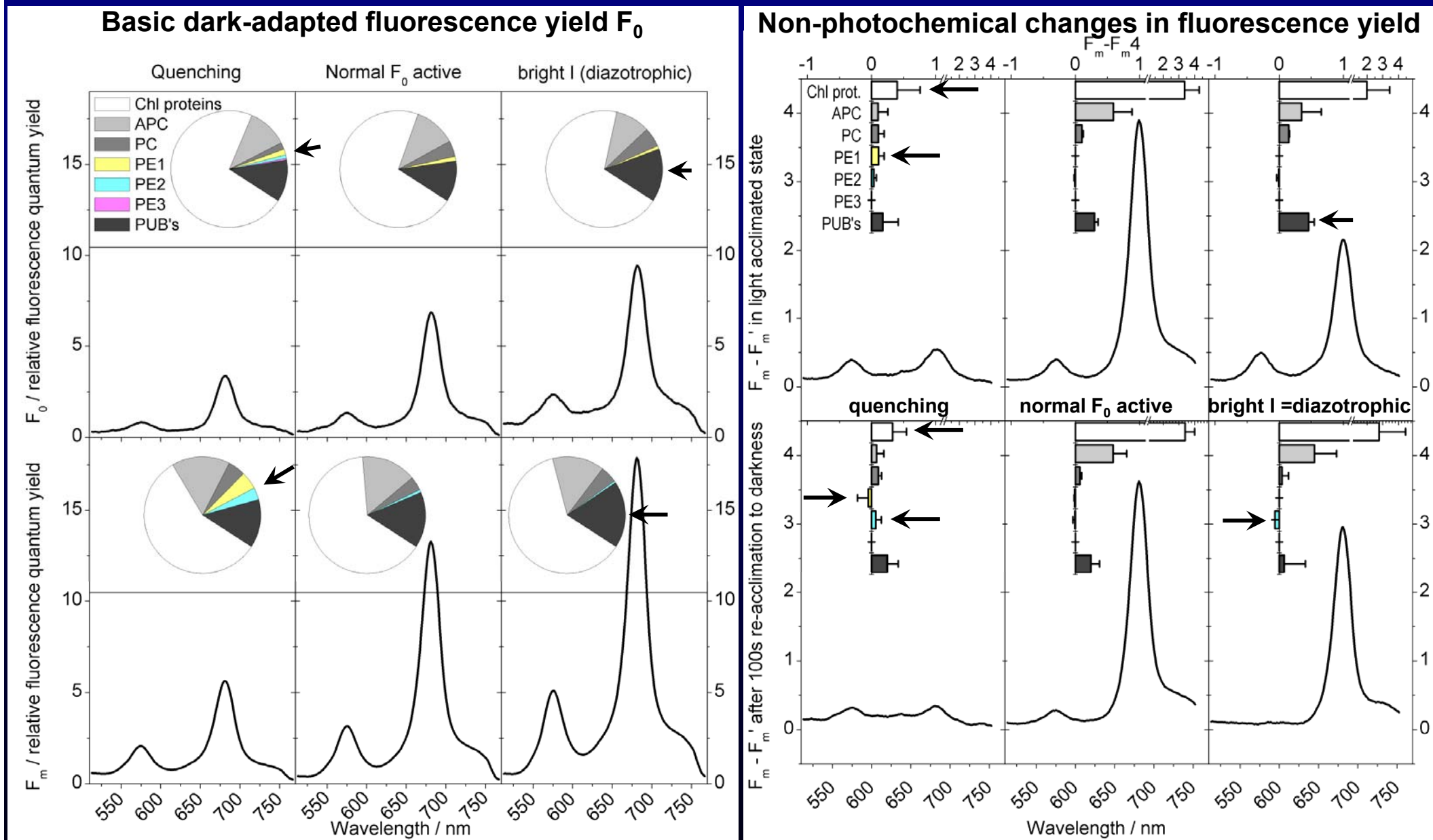
Method of deconvolution: Küpper H, Seibert S, Aravind P (2007) *Analytical Chemistry* 79, 7611-7627



Deconvolution of spectrally resolved *in vivo* fluorescence kinetics shows reversible coupling of individual phycobiliproteins

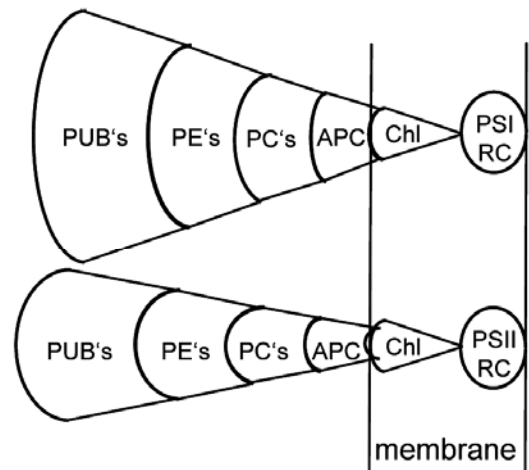


Deconvolution of spectrally resolved *in vivo* fluorescence kinetics shows reversible coupling of individual phycobiliproteins

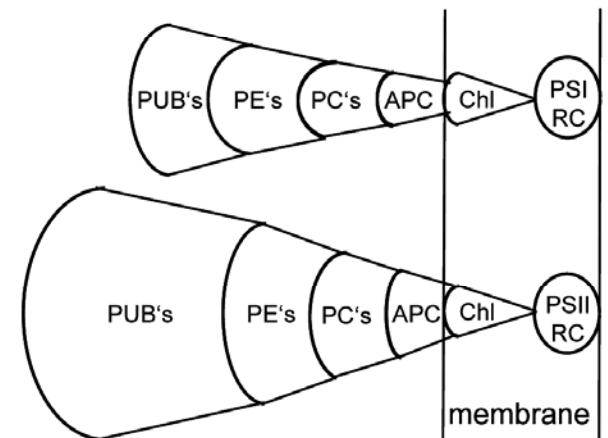


Reversible coupling of individual phycobiliproteins...

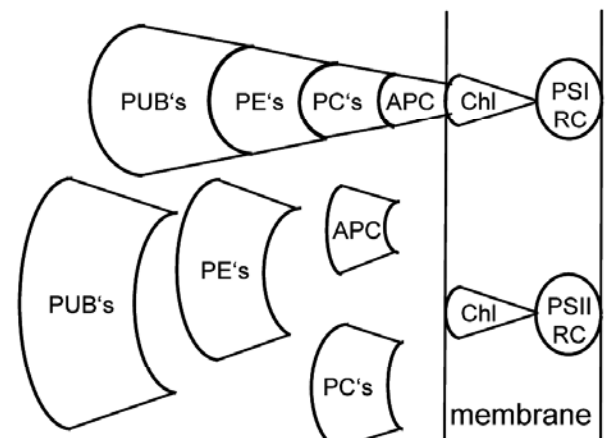
...as a basis for diazotrophic photosynthesis



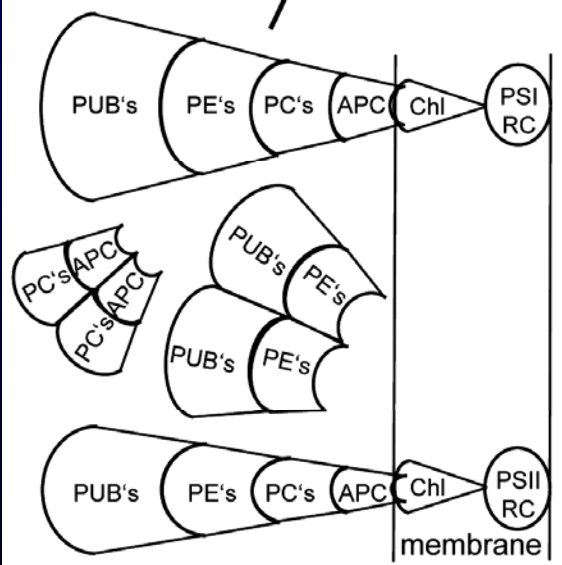
normal non-diazotrophic
(large phycobiliprotein antenna coupled to PSI)



bright I → diazotrophic
(large PUB antenna coupled to PSII)



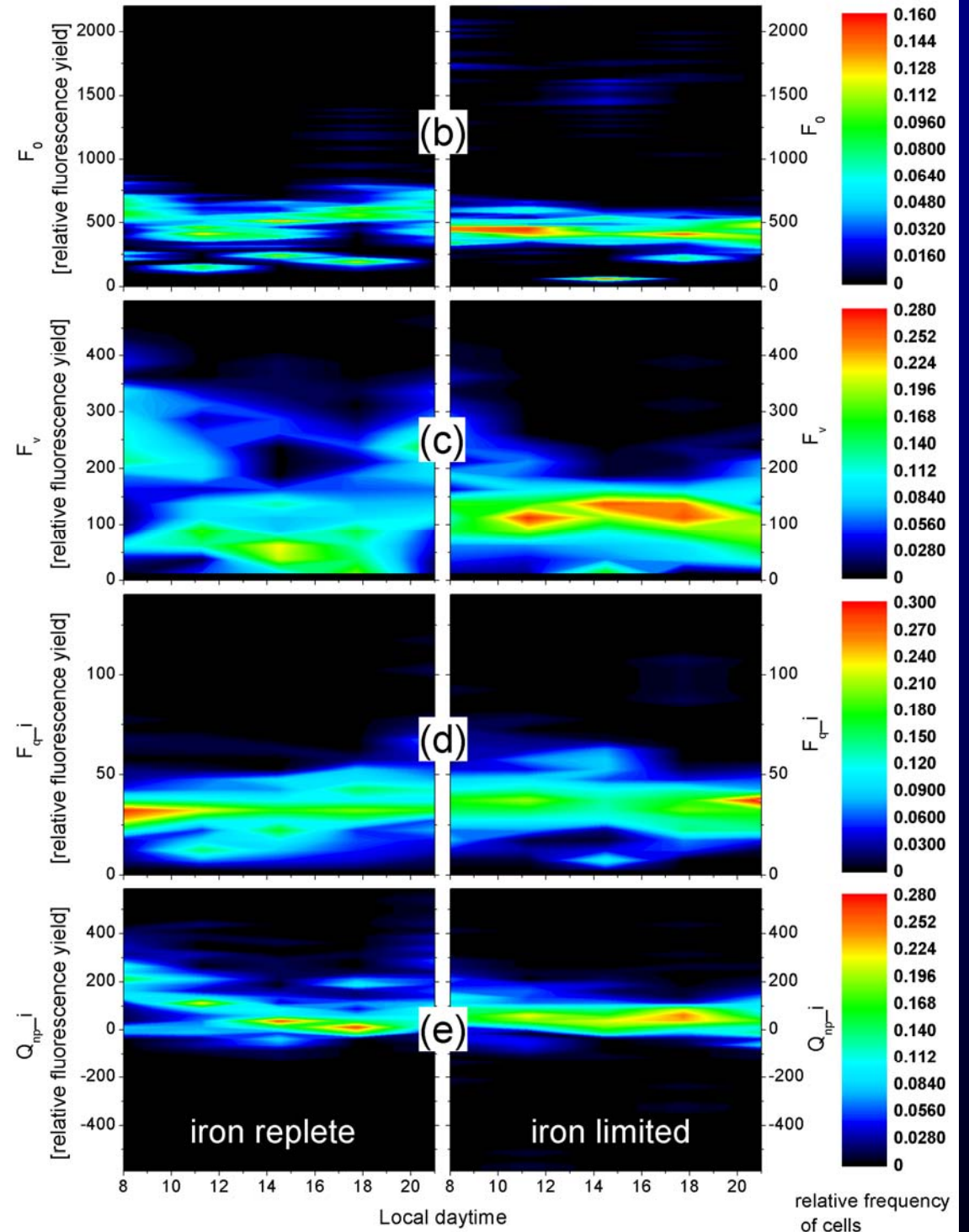
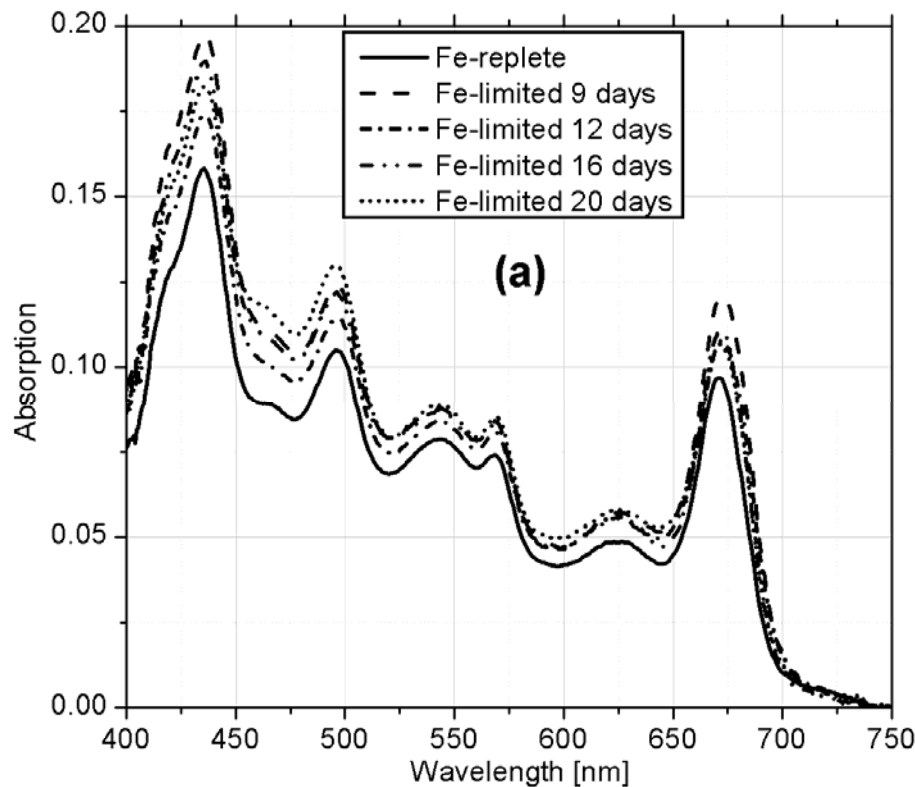
bright II („very bright“)
(uncoupled phycobiliproteins)



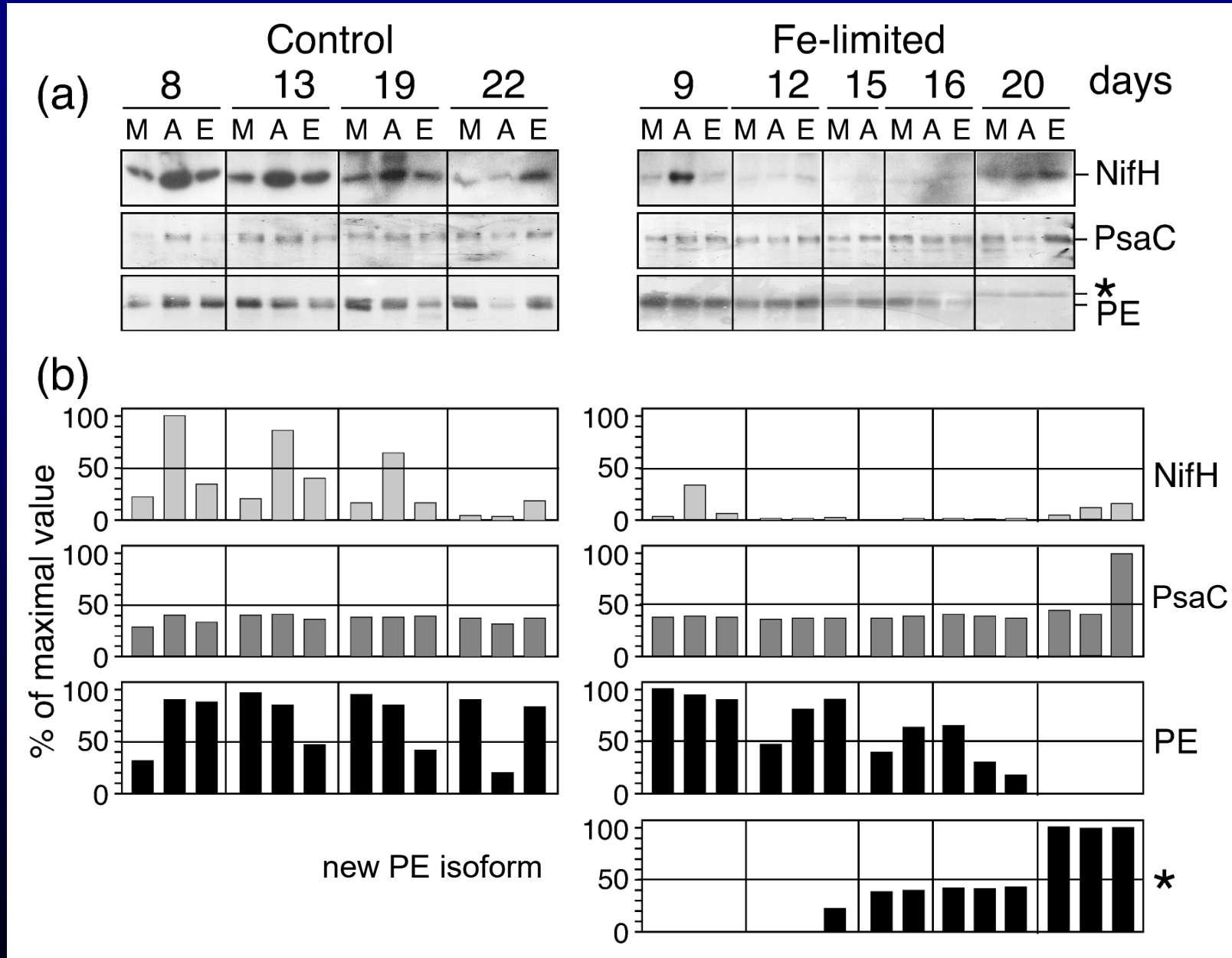
low fluorescence (quenching)
(unknown quenching arrangement, probably phycobiliprotein aggregation)

Küpper H, Andresen E, Wiegert S, Šimek M, Leitenmaier B, Šetlík I (2009) Biochim. Biophys. Acta (Bioenergetics) 1787, 155-167

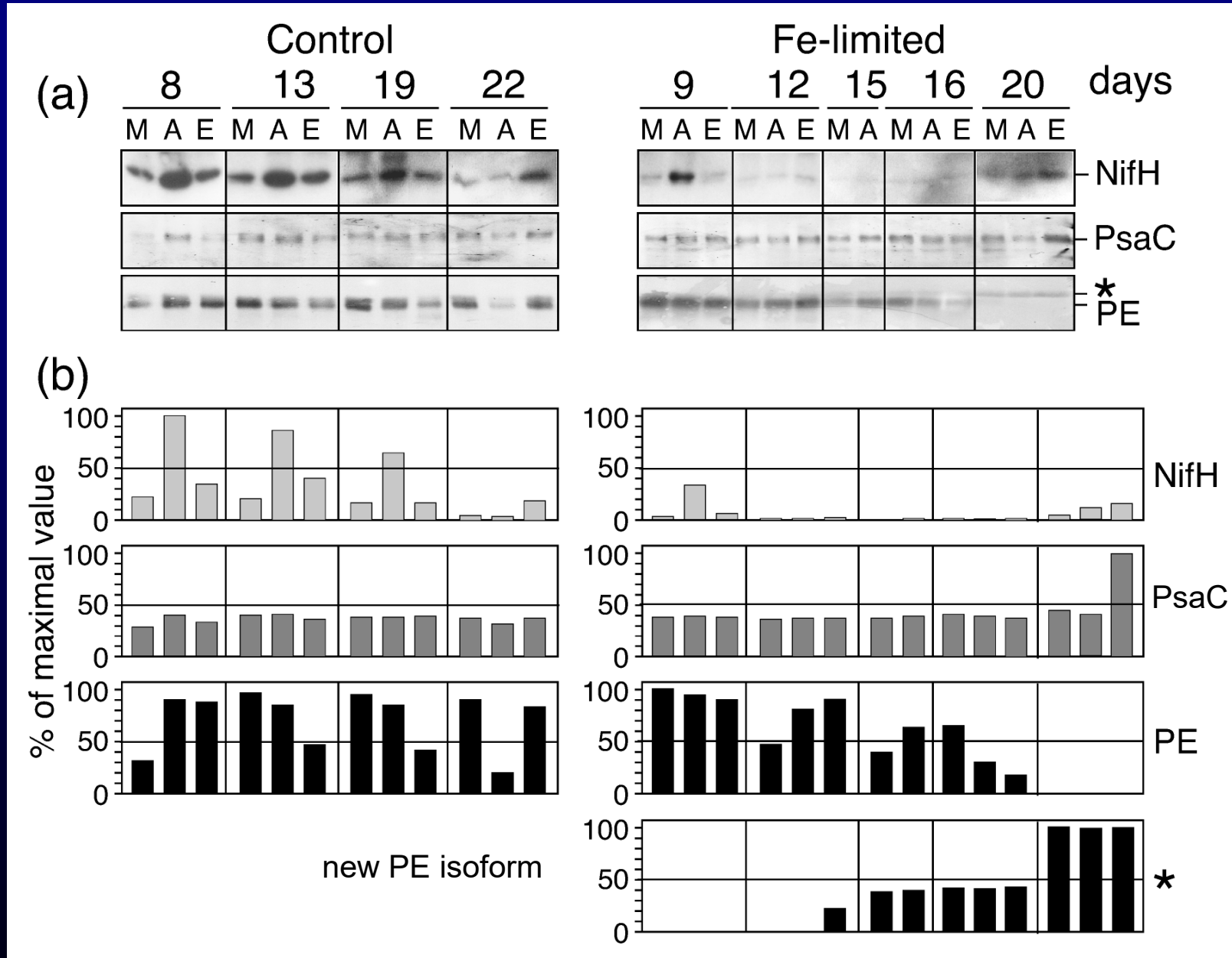
Iron limitation: photosynthetic components remain active...



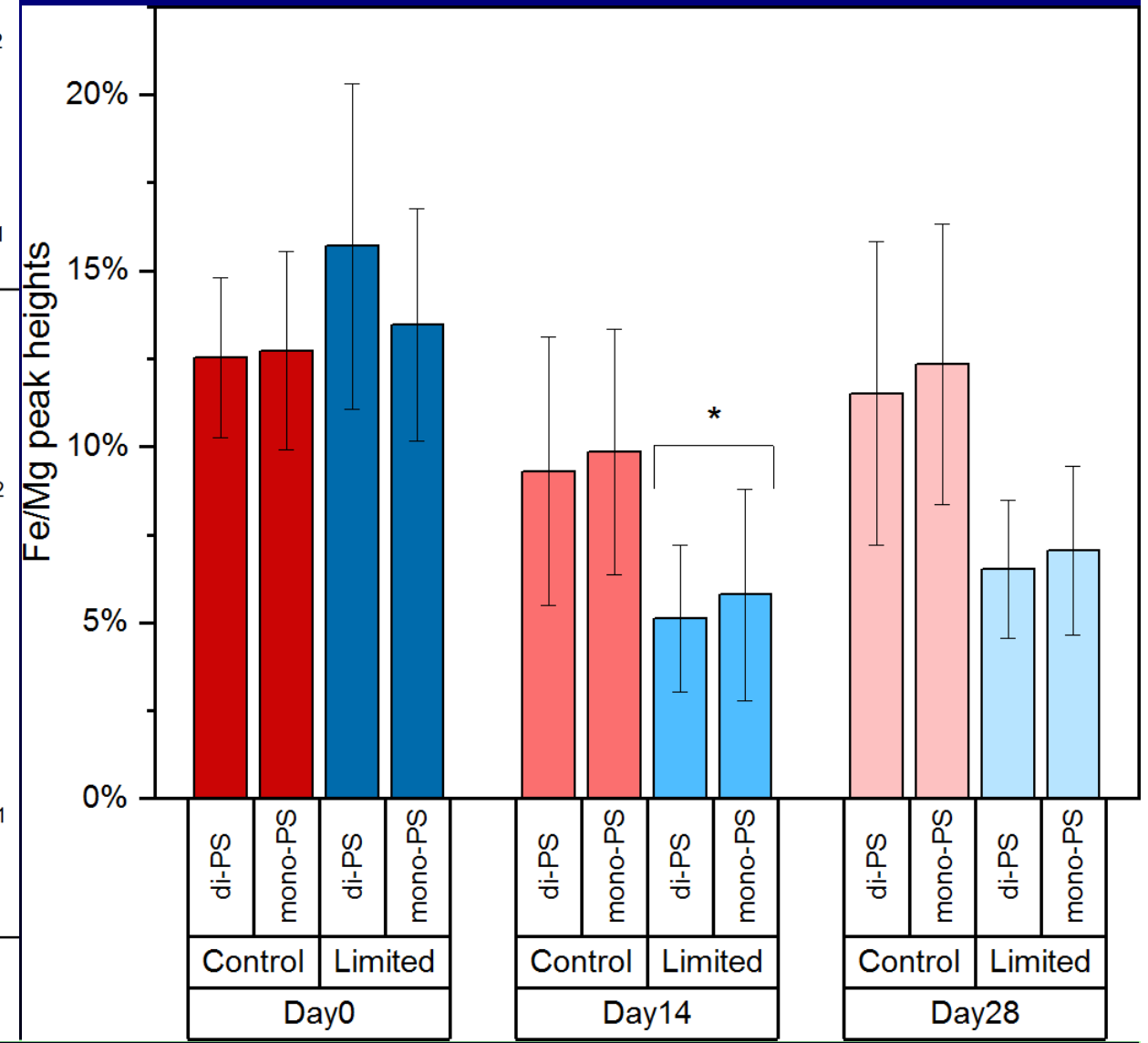
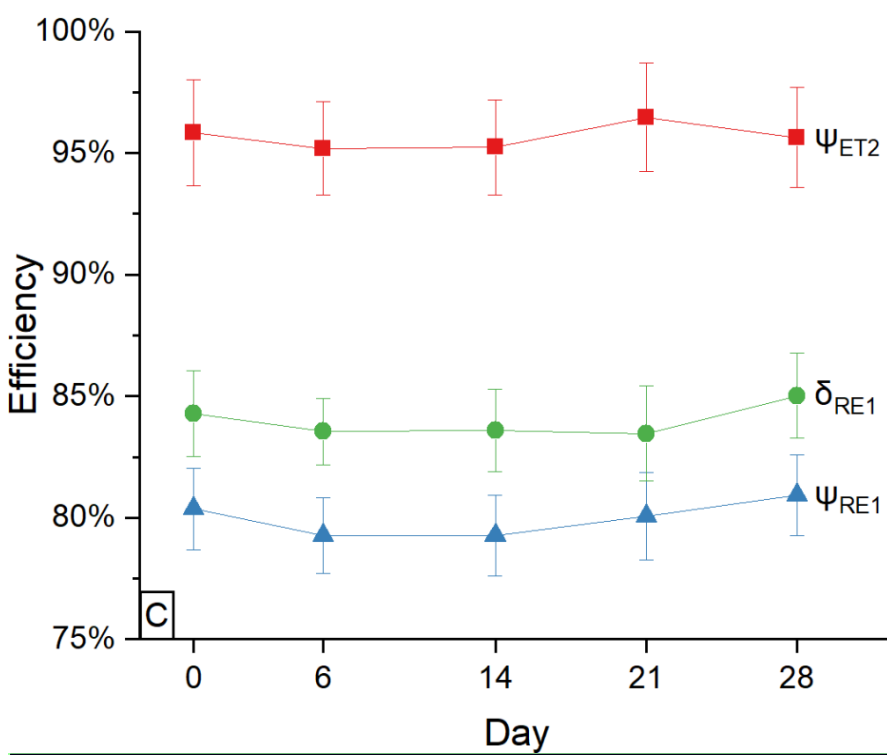
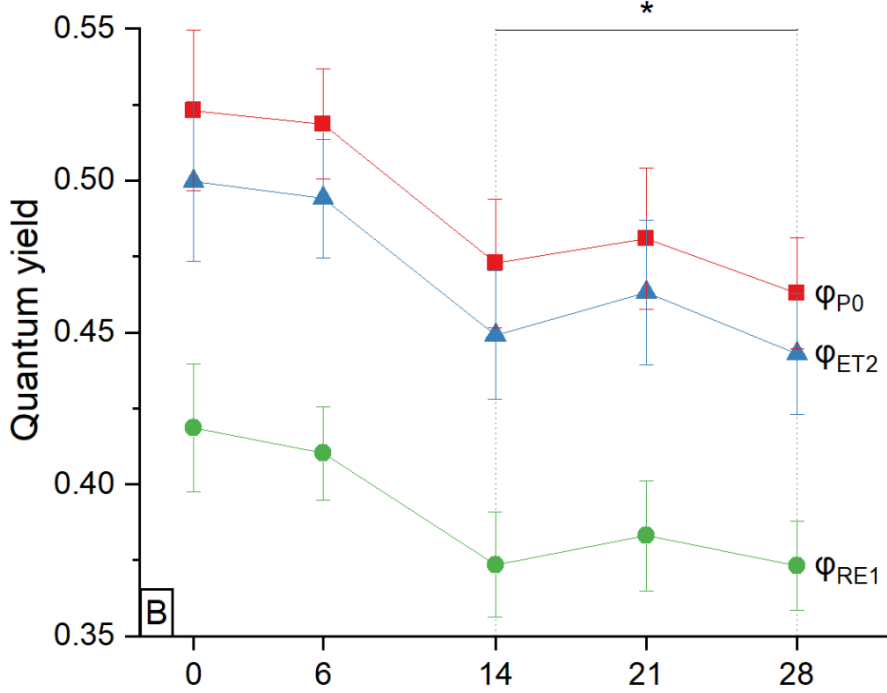
Iron limitation: rescue of photosynthetic components... ...by sacrificing nitrogenase



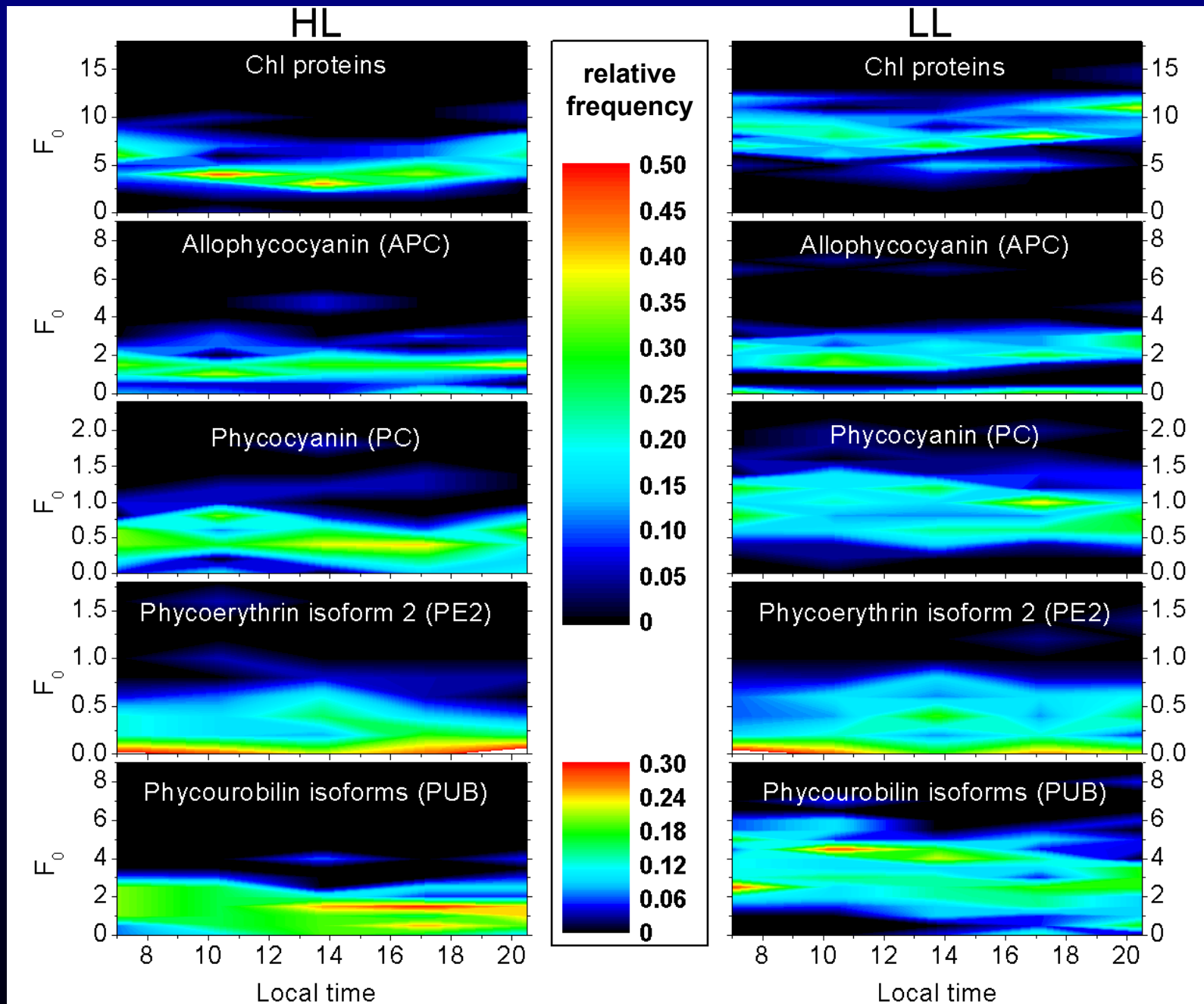
Iron limitation: rescue of photosynthetic components... ...by sacrificing nitrogenase



Iron limitation: PSII yield decrease and re-adjustment of PSII vs PSI ratio



Light limitation: acclimation by reversible phycobiliprotein coupling: basic fluorescence yield F_0



Conclusions

- For *Trichodesmium*, photosystem II activity is essential for providing energy for nitrogen fixation.
- Regulation of PSII activity for nitrogen fixation is achieved mainly by quickly reversible (un)coupling of individual phycobiliproteins.
- The nitrogen fixing activity state is characterised by a particularly large PSII-associated antenna, which is achieved mainly by coupling of additional units of phycourobilin (PUB) isoforms.
- Therefore, acclimation to light limitation (“low light stress”) involves enhanced synthesis mainly of phycourobilin, which is then mainly coupled to PSII. Synthesis and levels of other photosynthetic components decrease.
- Because of their vital importance, when adverse conditions require a choice, *Trichodesmium* in contrast to other cyanobacteria does not sacrifice its phycobilisomes, but rather its nitrogenase.
- Stress leads to expression of alternative phycobiliprotein isoforms

**All slides of my lectures can be downloaded
from my workgroup homepage**

Biology Centre CAS → Institute of Plant Molecular Biology → Departments
→ Department of Plant Biophysics and Biochemistry,
or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html