PHOTOSYNTHESE

IM WELTRAUM

Gerichtete Evolution photosynthetischer Reaktionen:

neue Strategien zur beschleunigten Anpassung

an Streßsituationen

Why photosynthetic microalgae in Space?

On Earth, algae create

- **over half of the current oxygen**
- **remove ca. half of the greenhouse CO_2**

In Space, algae might provide

- **an oxygenic-atmosphere**
- **clean water**
- **edible biomass**
- **anti-oxidants**
- **pharmaceuticals**

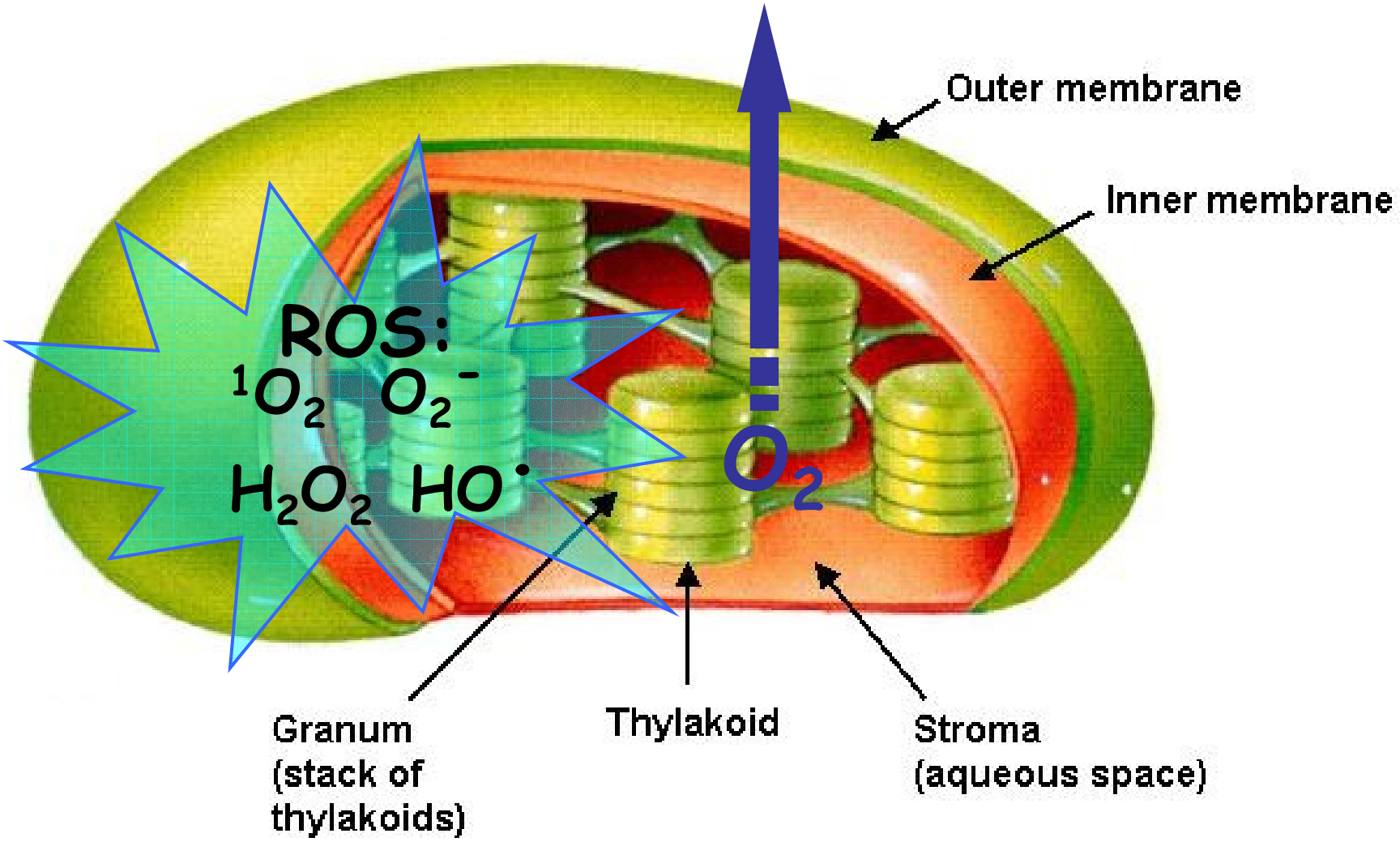
Exposure to Space Radiation can damage:

- DNA
- Proteins
- Pigments
- Lipids

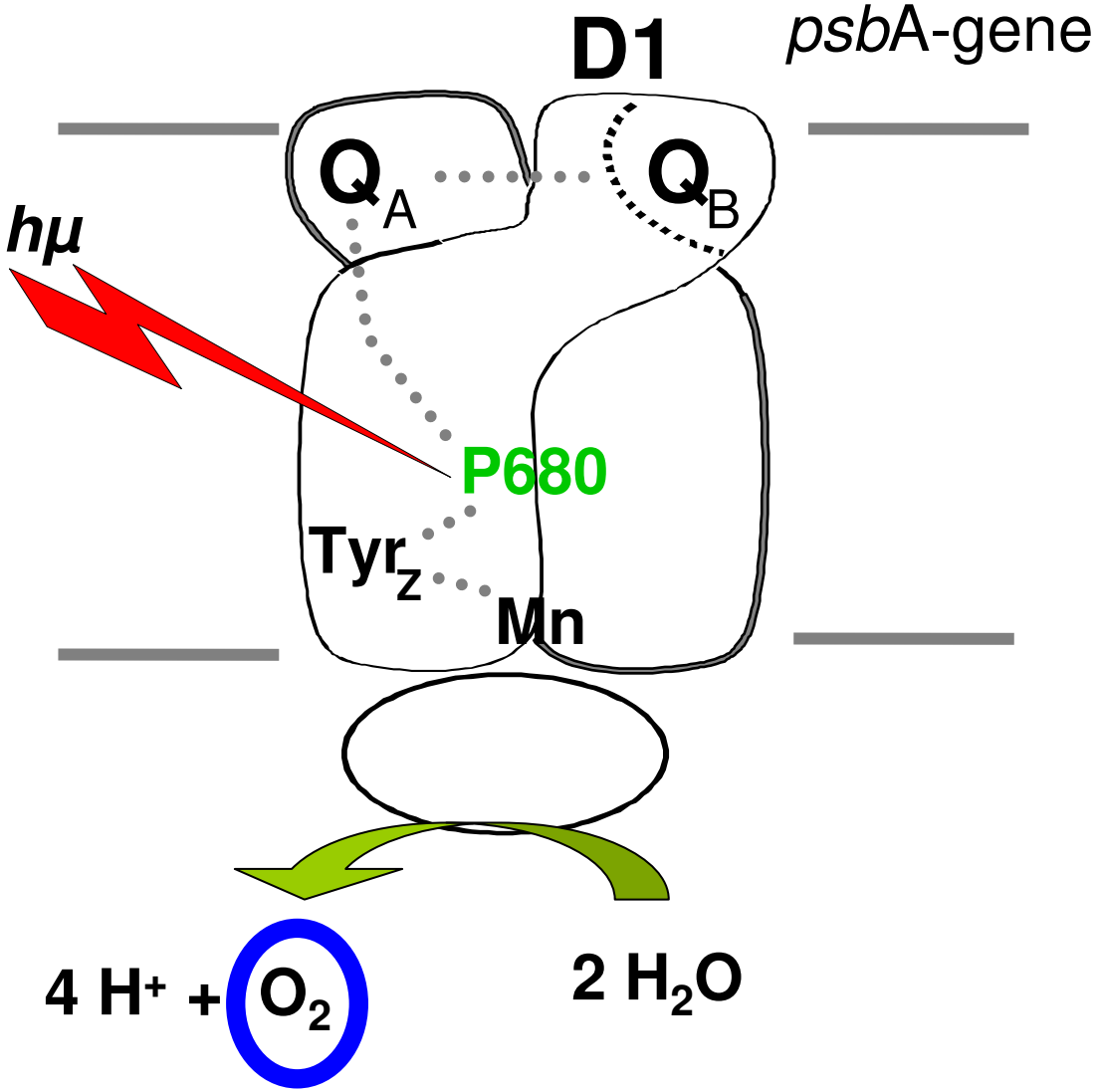
In photosynthesis, one important target for radiation damage is photosystem II

Radiation generates reactive oxygen species (ROS), thus destroying the D1 subunit of PSII

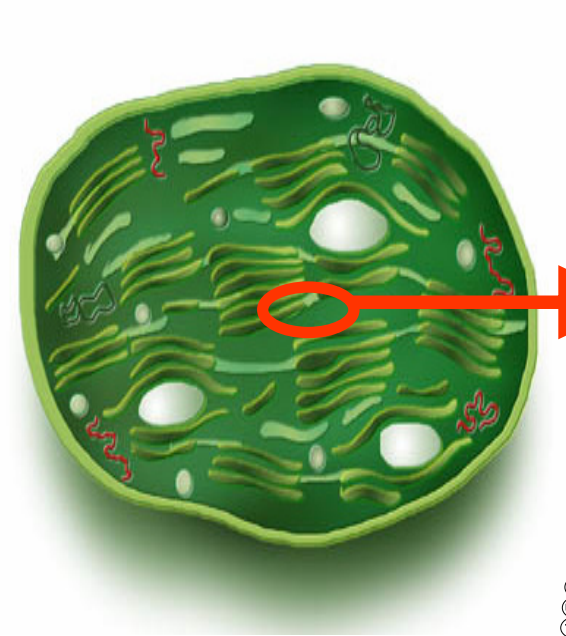
Chloroplast structure



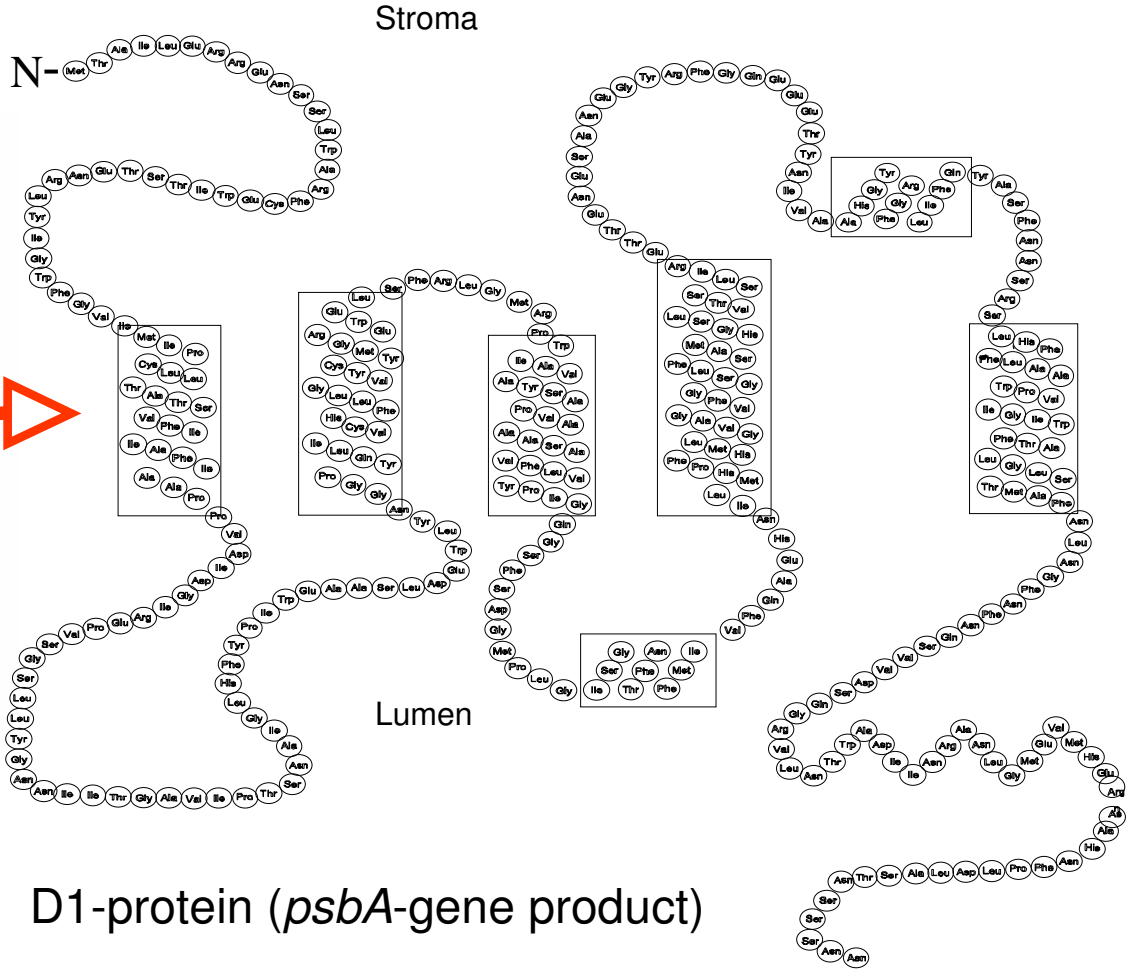
Photosystem II: site of oxygen evolution for earth's atmosphere



D1-subunit of photosystem II



Chloroplast

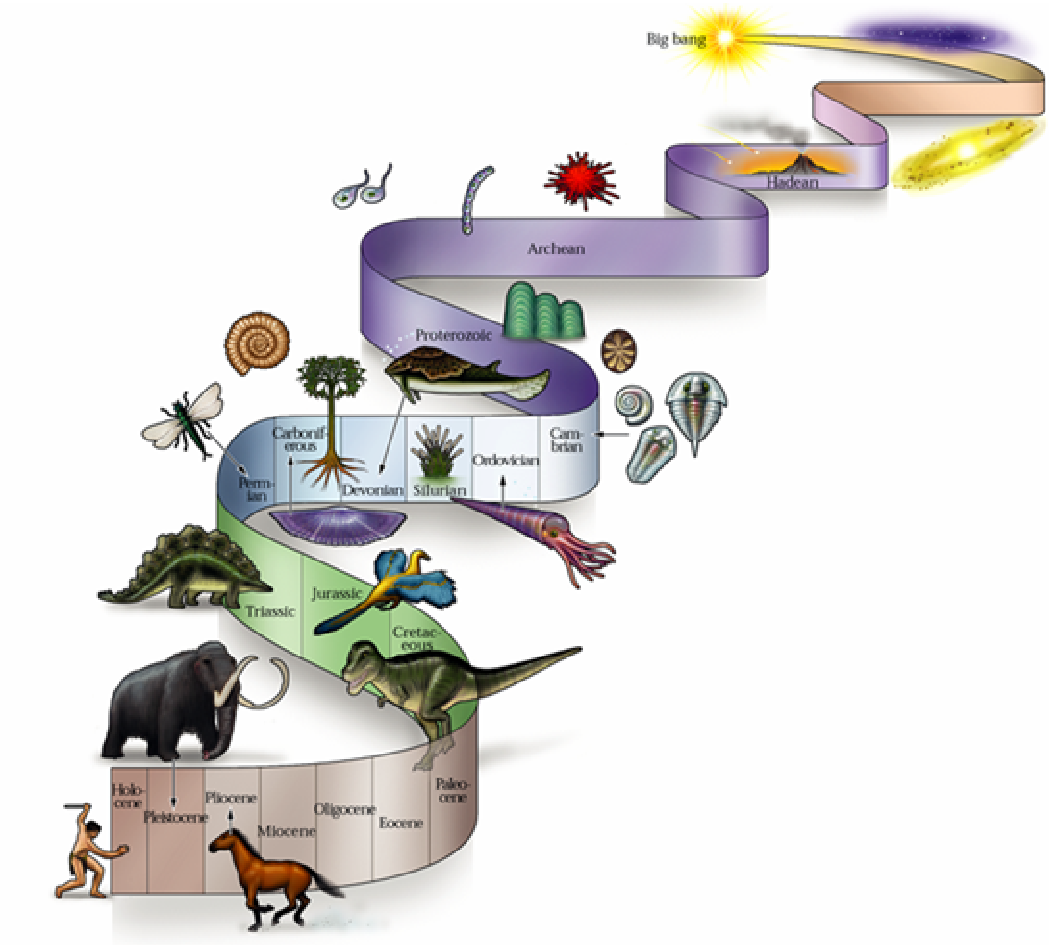


D1-protein (*psbA*-gene product)

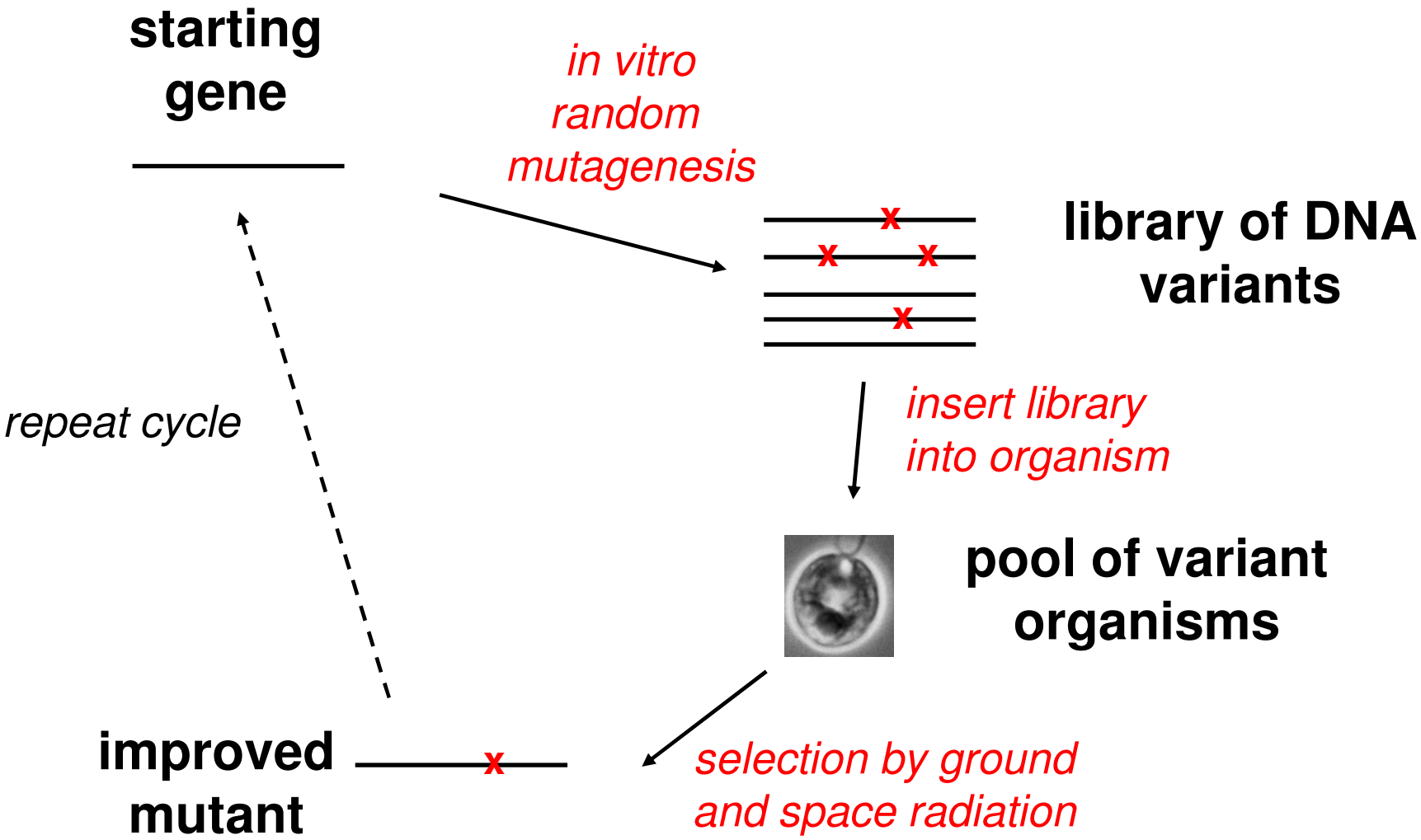
Microalgae are not adapted to conditions in spacecrafts and planetary environments

What can we do using existing algae and modern techniques to obtain better adapted strains?

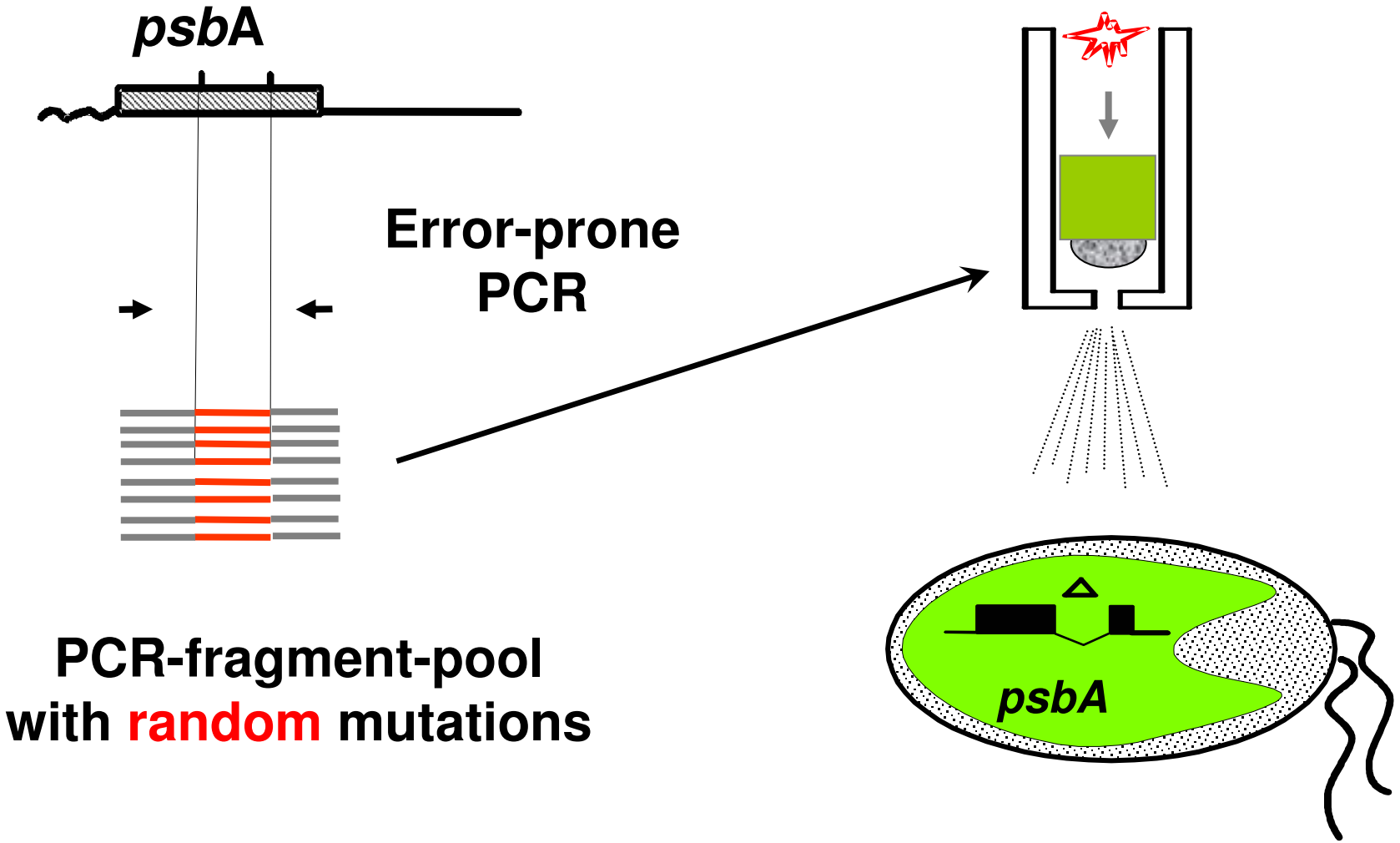
Natural evolution takes millions of years…



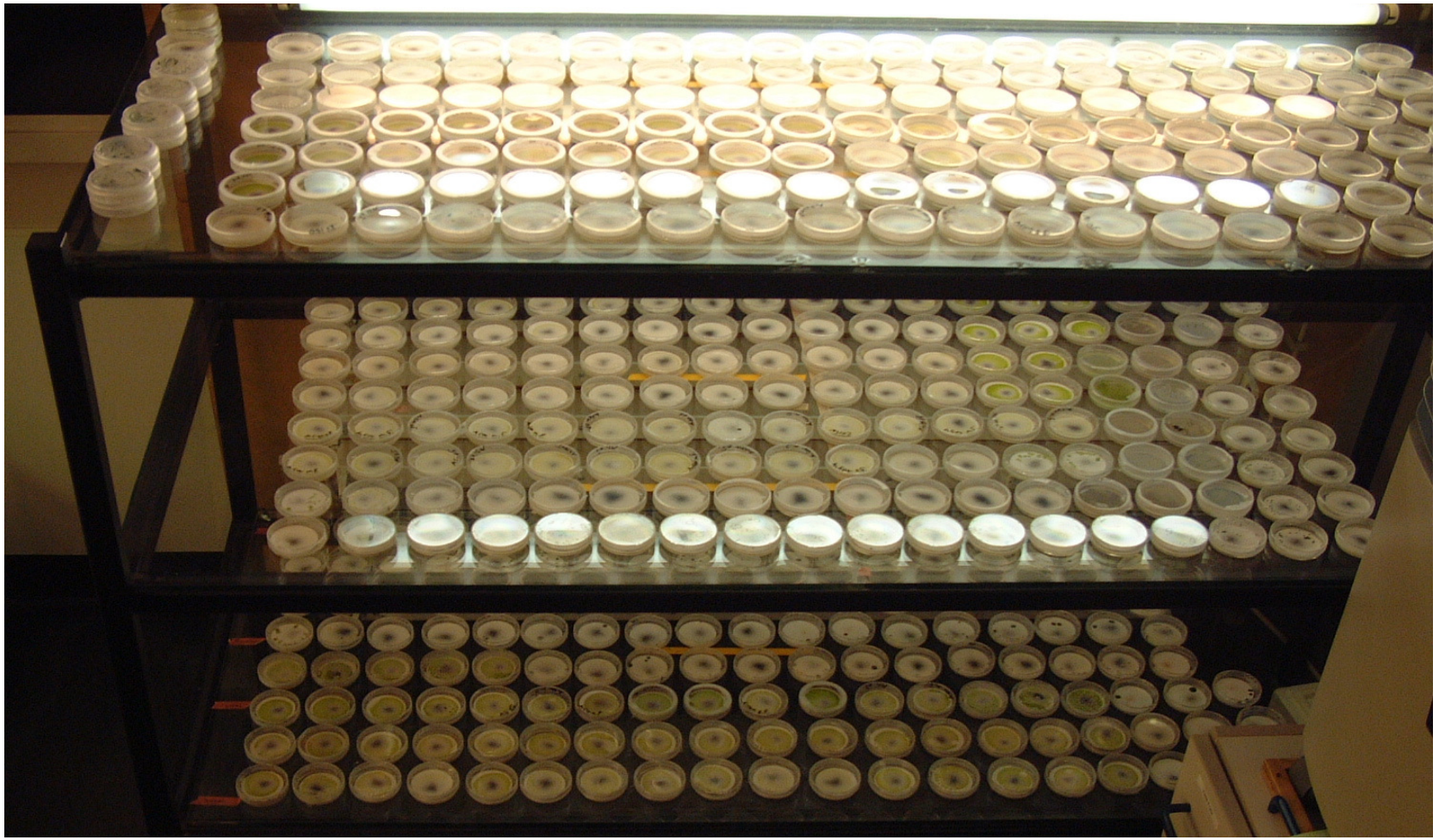
Directed evolution takes weeks…



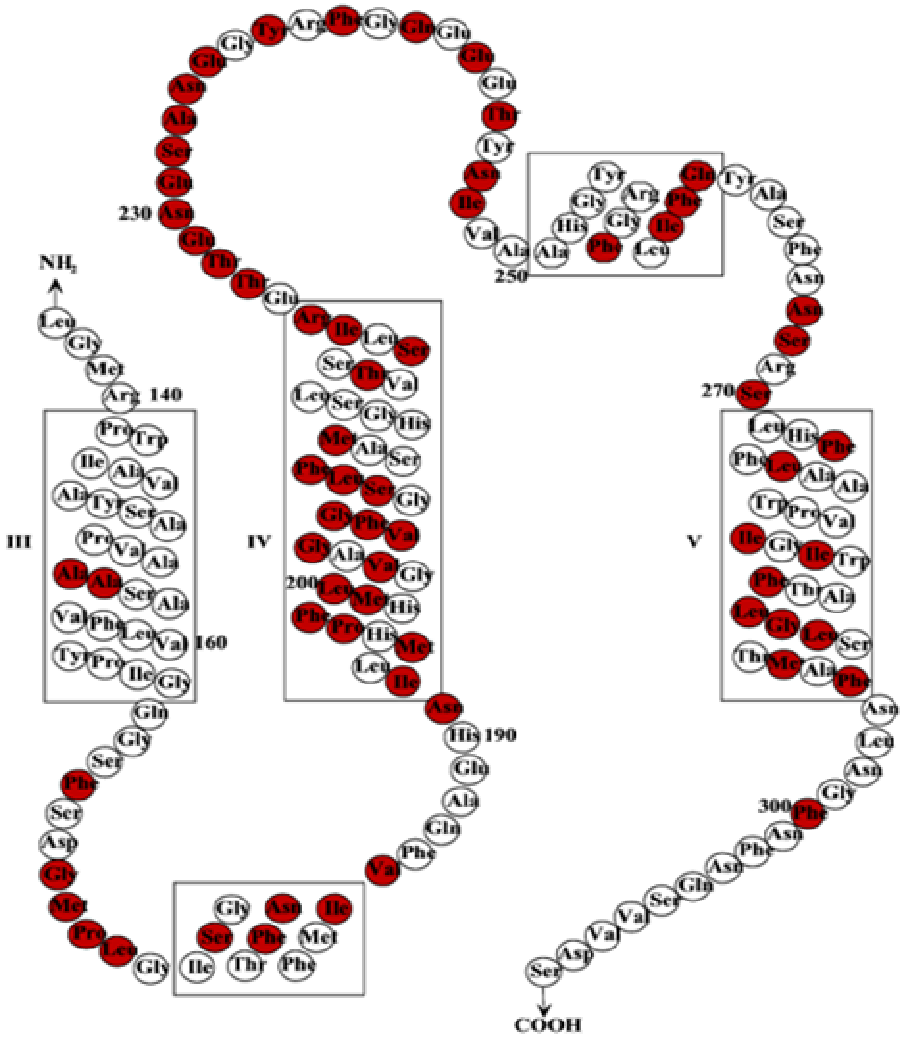
*psb*A mutagenesis with PCR-fragments and transformation



Chlamydomonas transformants to be analysed

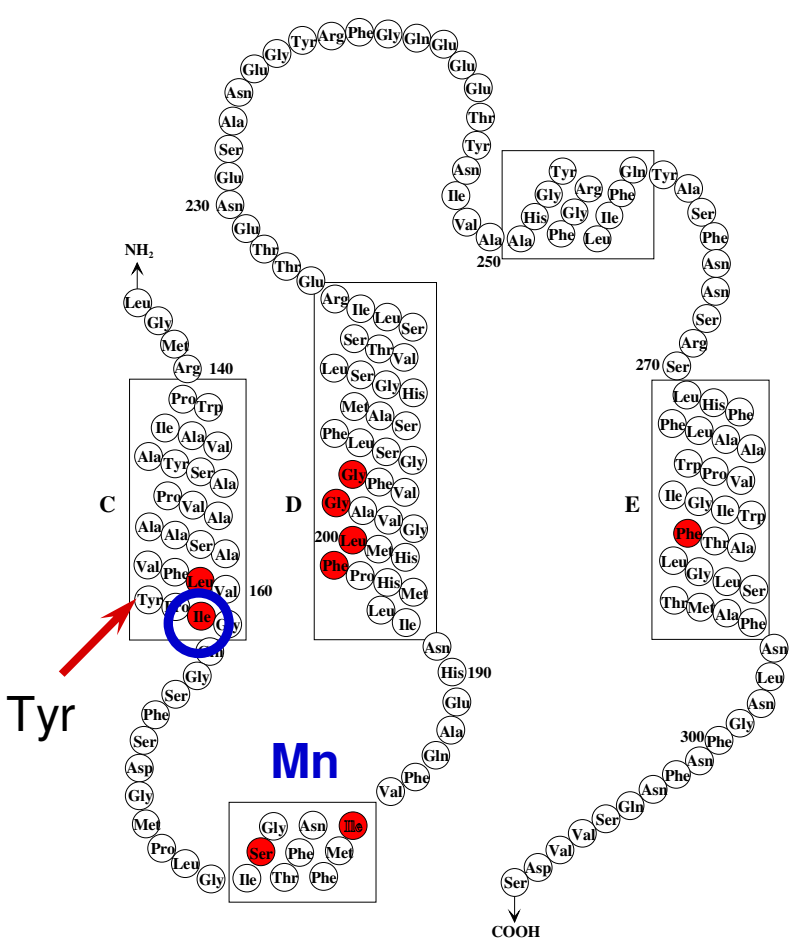


Overview of positions modified in D1 by random mutagenesis

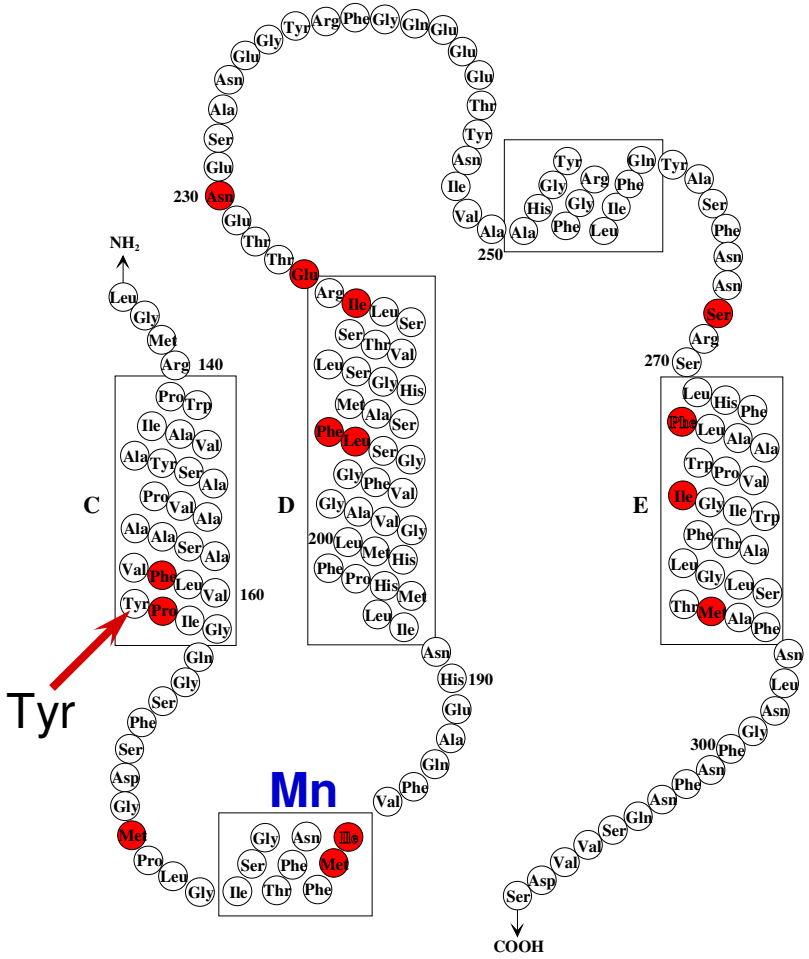


- ca. 2000 transformants were obtained
- 100 were analysed
- within these, 66 modified amino acids (in red) were detected
- this is only a fraction of mutants which can be obtained !!!

Survivors of neutron/proton bombardment (ground experiment)



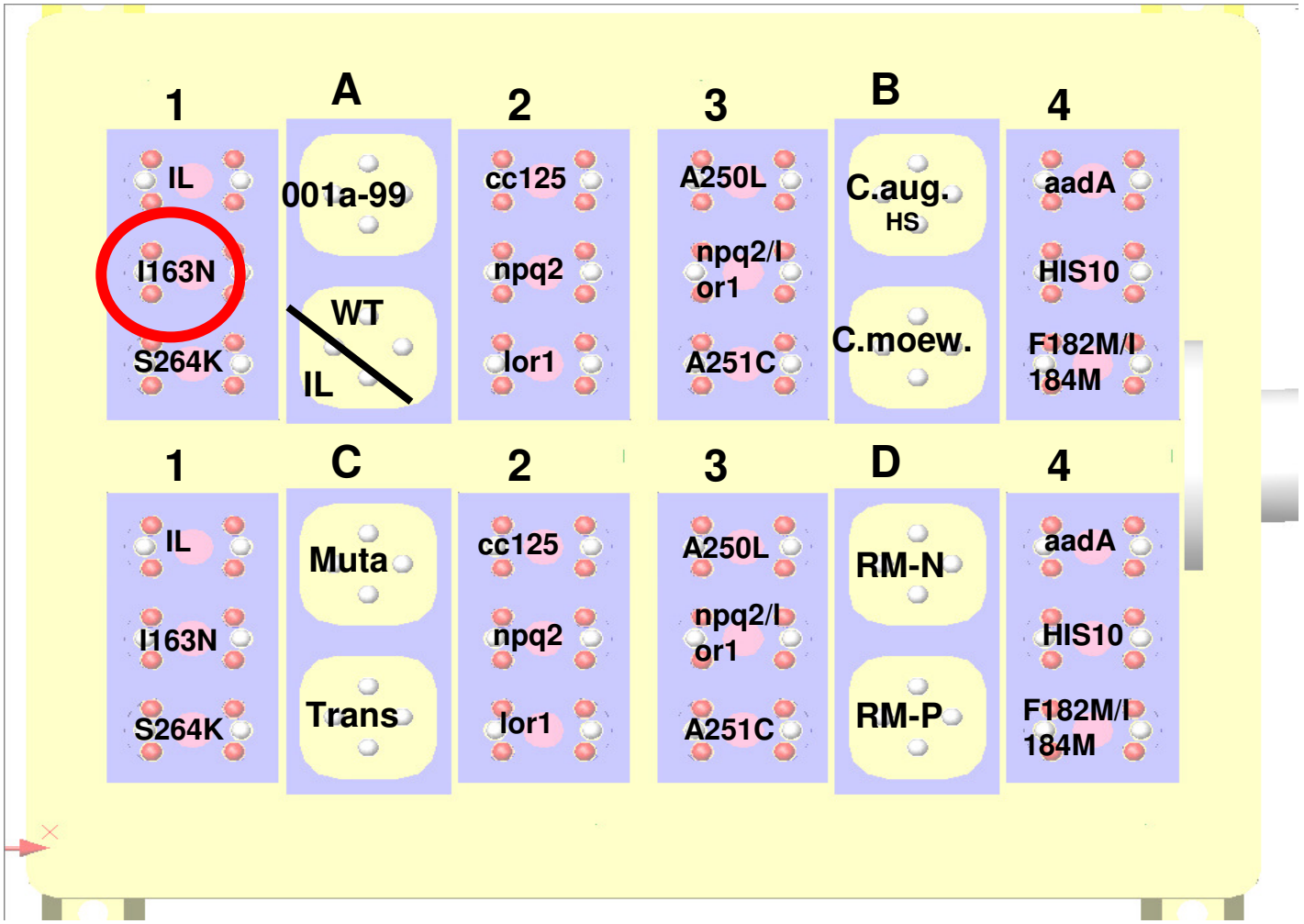
neutron exposure: 14 MeV



proton exposure: 27 MeV

more than 30 colonies were sequenced

Photo II modul in Foton-M3



Daily trend in Fv/Fm

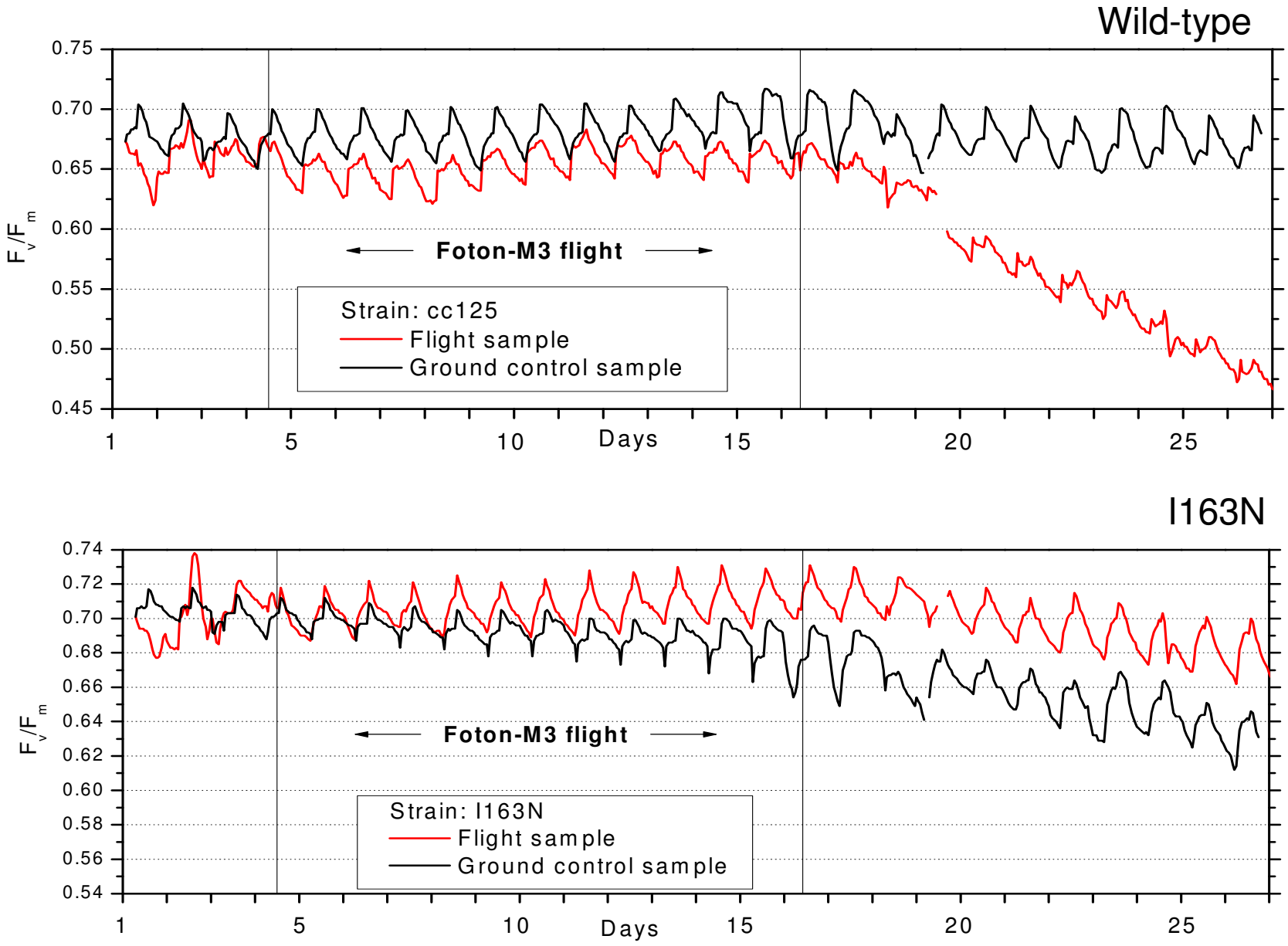
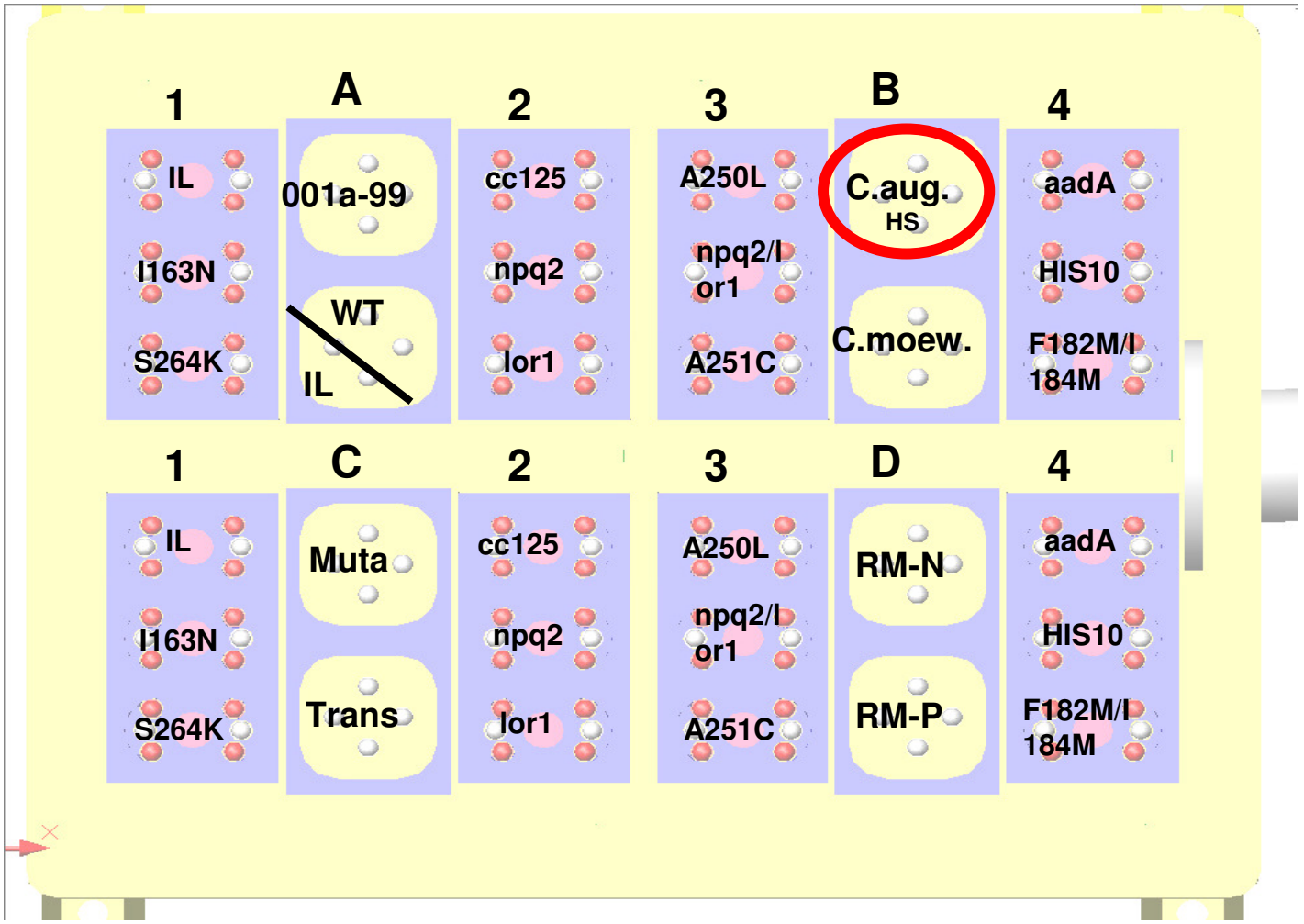


Photo II modul in Foton-M3

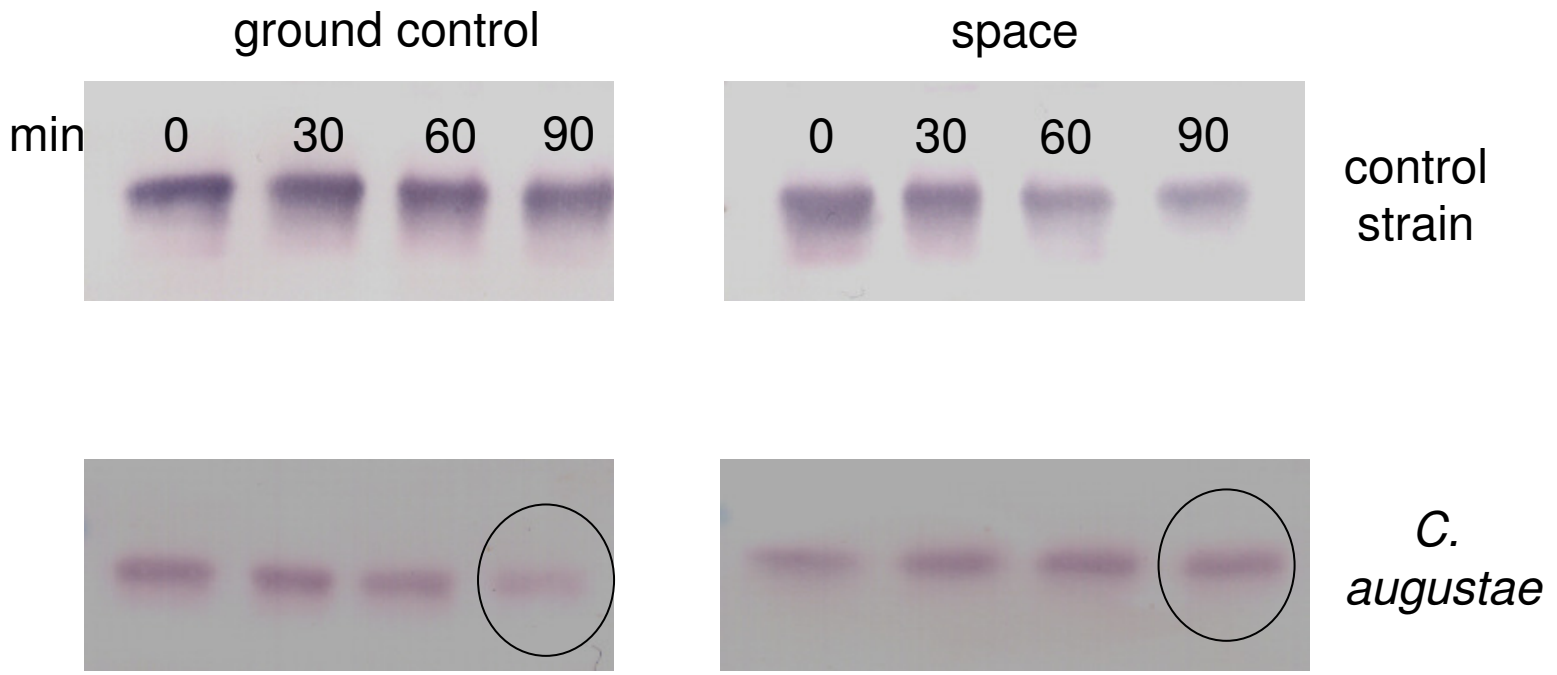


FOTON-M3 experiment: Fv/Fm-data

sample	start	end / ground control	end / space samples
Scenedesmus	0,76	0,73	0,67
***C.r.* wt**	0,76	0,74	0,74
***C.r.* IL**	0,78	0,74	0,72
C. augustae	0,40	0,33	0,57
C. moewusii	0,64	0,45	0,69
Muta	0,76	0,75	0,73
Trans	0,67	0,71	0,67
RM-N	0,76	0,68	0,67
RM-P	0,72	0,65	0,67

start: (07.09.2007) two days after integration of algae in the module in the lab in Halle
end: (02.10.2007) algae samples back in our lab (14.09.-26.09.2007)

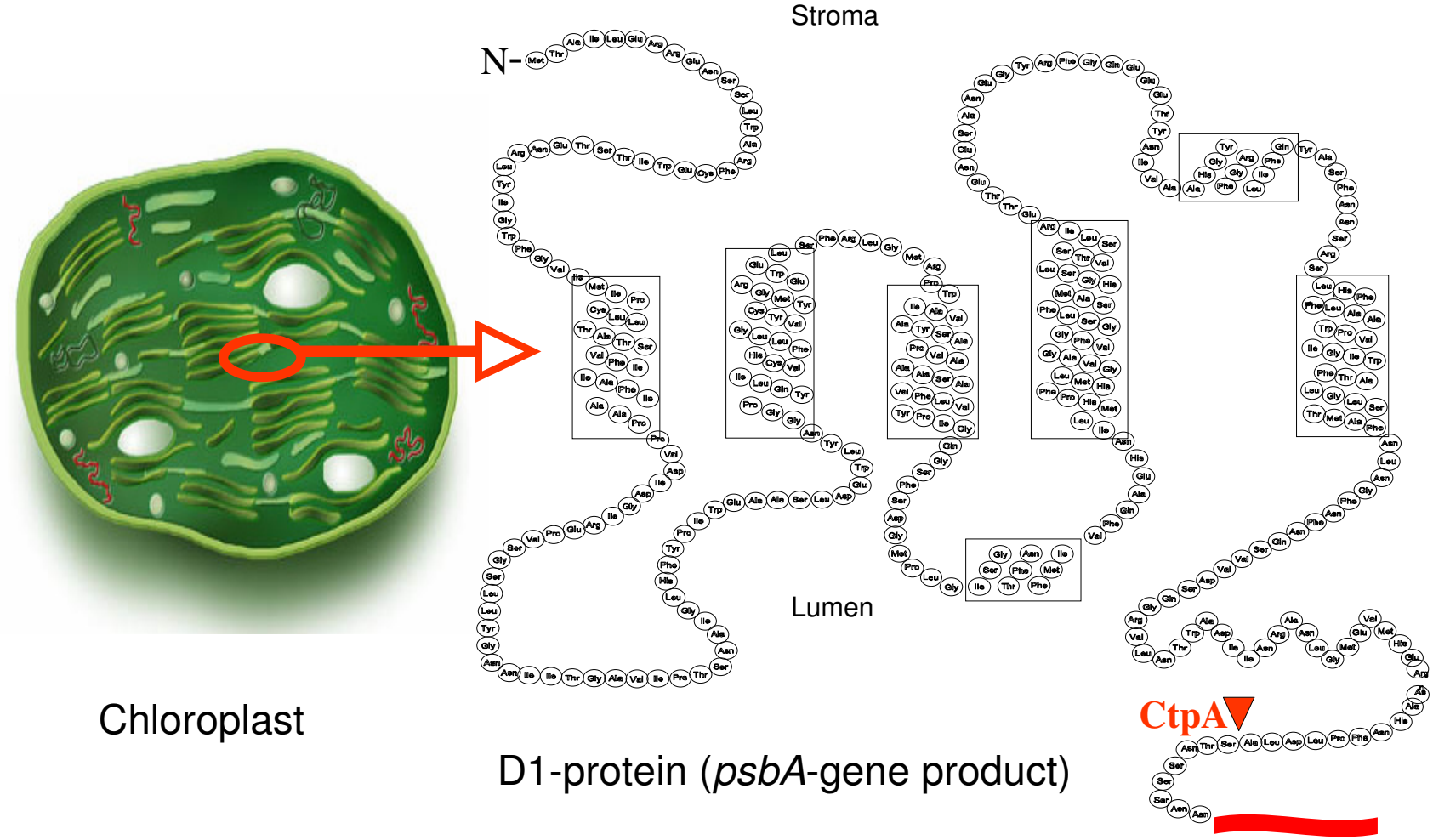
D1-stability in high light



D1-protein comparison:
C. reinhardtii – C. augustae

```
C.reinhardtii      MTAILERRENS-SLWARFCEWITSTENRLYIGWFGVIMIPCLLTATSVFIIAFIAAPPVD 59
C.augustae         MTAVINKQDVSTSLWARFCEWVTSTENRIYVGWFGTIMFPTLLTATSVYIIAFVAAPPVD 60
                   ***:::::: * *********:******:*:****.**:* *******:****:******

C.reinhardtii      IDGIREPVSGSLLYGNNIITGAVIPTSNAIGLHFYPIWEAASLDEWLYNGGPYQLIVCHF 119
C.augustae         IDGIREPVSGSLLYGNNIISGAVIPTSNAIGLHFYPIWEAASLDEWLYNGGPYQMIVCHF 120
                   ********************:************************************:*****

C.reinhardtii      LLGVYCYMGREWELSFRLGMRPWIAVAYSAPVAAASAVFLVYPIGQGSFSDGMPLGISGT 179
C.augustae         FIGICAYMGREWELSYRLGMRPWICVAYSAPVAAATAVFIIYPIGQGSFSDGMPLGISGT 180
                   ::*: .*********:********.**********:***::******************

C.reinhardtii      FNFMIVFQAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETTENESANEGYRF 239
C.augustae         FNFMIVFQAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETTENESANAGYKF 240
                   ************************************************** **:*

C.reinhardtii      GQEEETYNIVAAHGYFGRLIFQYASFNNSRSLHFFLAAWPVIGIWFTALGLSTMAFNLNG 299
C.augustae         GQEEETYNIVAAHGYFGRLIFQYASFNNSRSLHFFLAAWPVIGIWFTALGISTMAFNLNG 300
                   ************************************************:*********

C.reinhardtii      FNFNQSVVDSQGRVLNTWADIINRANLGMEVMHERNAHNFPLDLASTNSSSNN- 352
C.augustae         FNFNQSVVDSQGRVLNTWADIINRANLGMEVMHERNAHNFPLDLASVEAPSVNA 354
                   ********************************************.::.* *
```

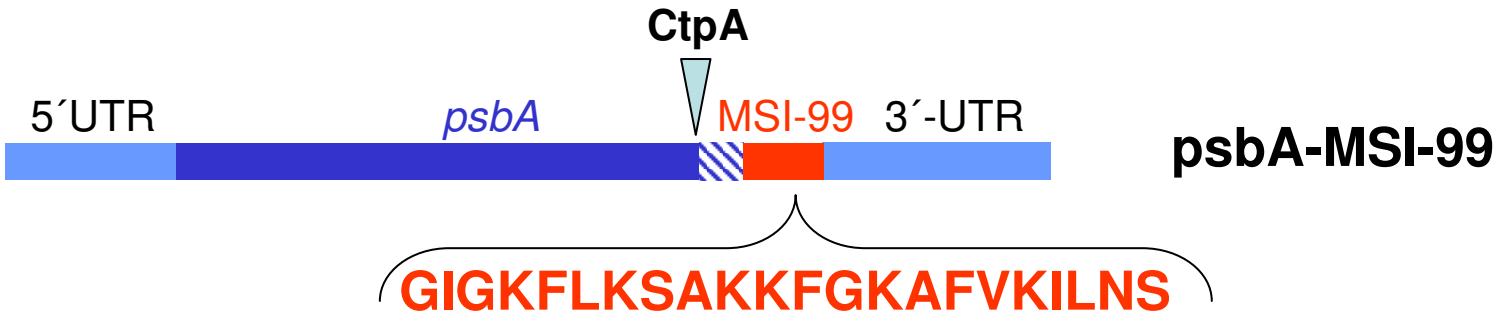
D1-mediated expression of foreign proteins: a novel expression system

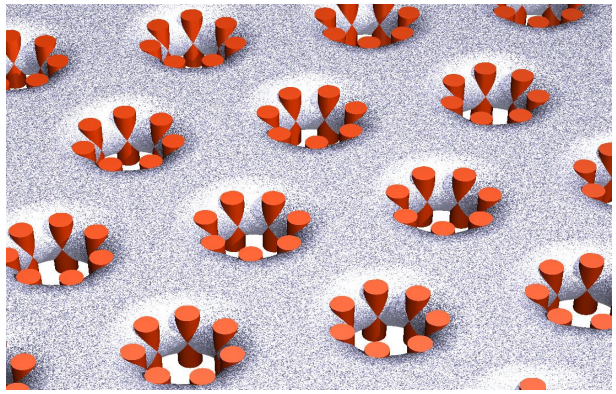


Chloroplast

D1-protein (*psbA*-gene product)

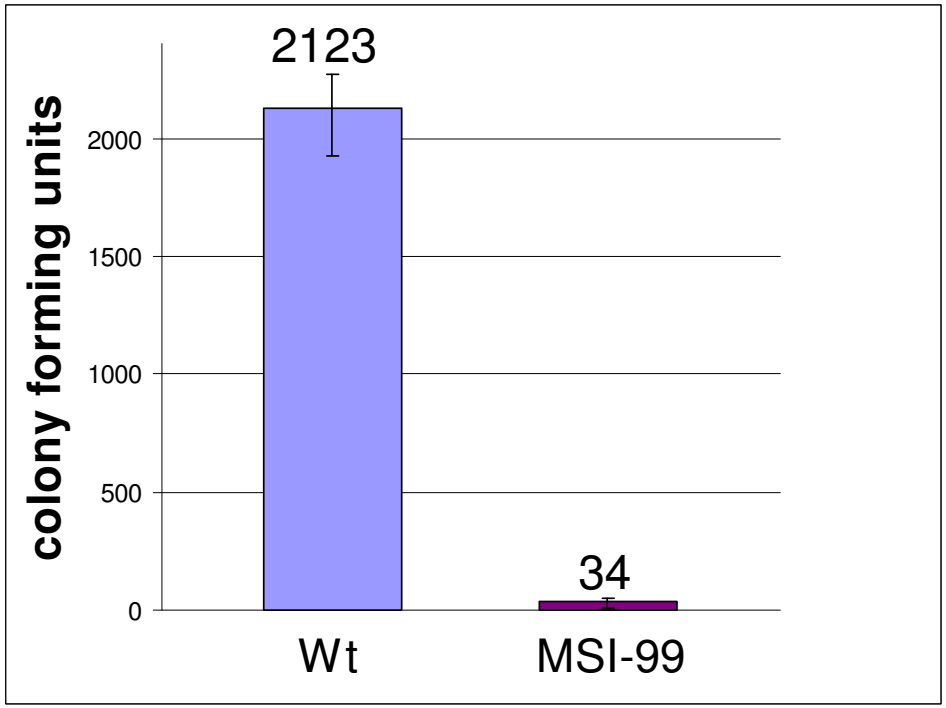
First evidence for the expression of an antimicrobial peptide in the novel strain psbA-MSI-99

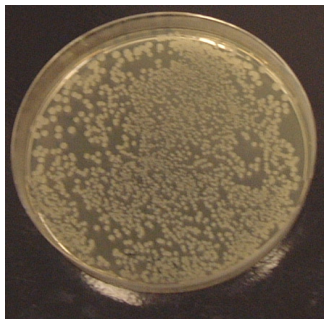


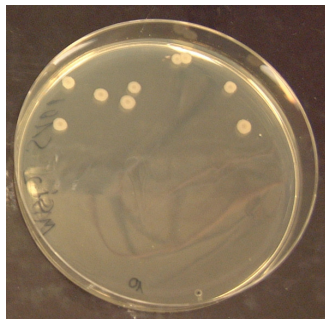


Magainin analogs induce pores into membranes with negatively charged phospholipid headgroups

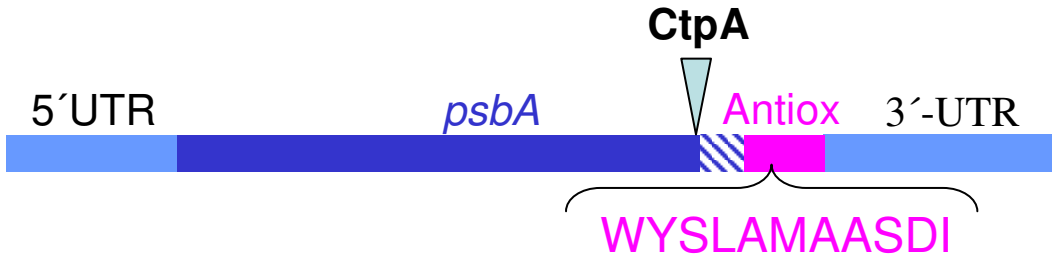
Effect of algal extracts on *Bacillus subtilis*



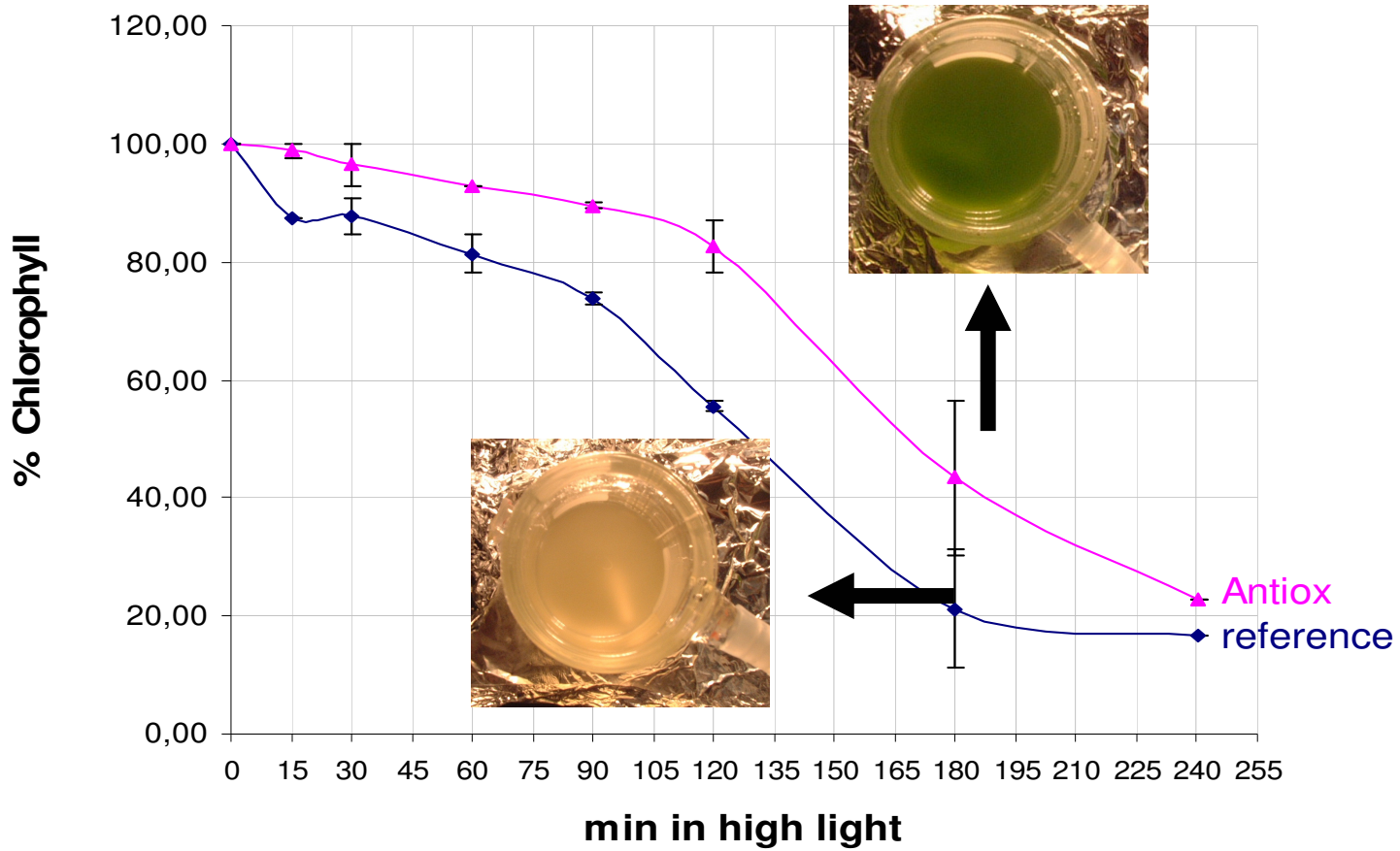




First evidence for the expression of an antioxidative peptide in the novel strain psbA-Antiox



***psbA*-Antiox**



Thanks to

DLR and ESA

Ivo Bertalan and Dirk Fischer (MLU Halle)

Maria Teresa Giardi & coworkers (CNR, Rome)

Conclusion I

- Neutron/proton bombardment of randomly mutated cells reveal mutations in a D1 region near the redox-active Tyrosin 161 and the Mn-binding site: since these regions could be hotspots for ROS generation, the new mutations could act by reducing this effect

- Mutants at position 163 were generated independently by bombardment with neutrons in 2005 (I163N) and 2007 (I163T / I224F), indicating a particular role of the Tyr161-proximity in protection

- Mutant I163N appeared to be more stable than wild type in space experiment

Conclusions II

- The snow algae *Chlamydomonas augustae* grew better after space exposure as opposed to ground control; this was supported by higher D1-stability under high light conditions and better photosystem II-activity

- As mutant pools survived inside Foton M3, selection conditions were not strong enough; for increasing the selection pressure, pools should definitely positioned outside protecting shields

Work in progress

- Mutant I163N is a good candidate for a more tolerant strain; however we cannot exclude that other mutations somewhere in the genome are responsible for tolerance.

  To exclude this, we are generating a strain which contains this mutation by site-directed mutagenesis. If this mutant has the same properties like that obtained from neutron bombardment, that specific point mutation is responsible for tolerance.

PHOTO II and Liulin-Photo hourly measurements

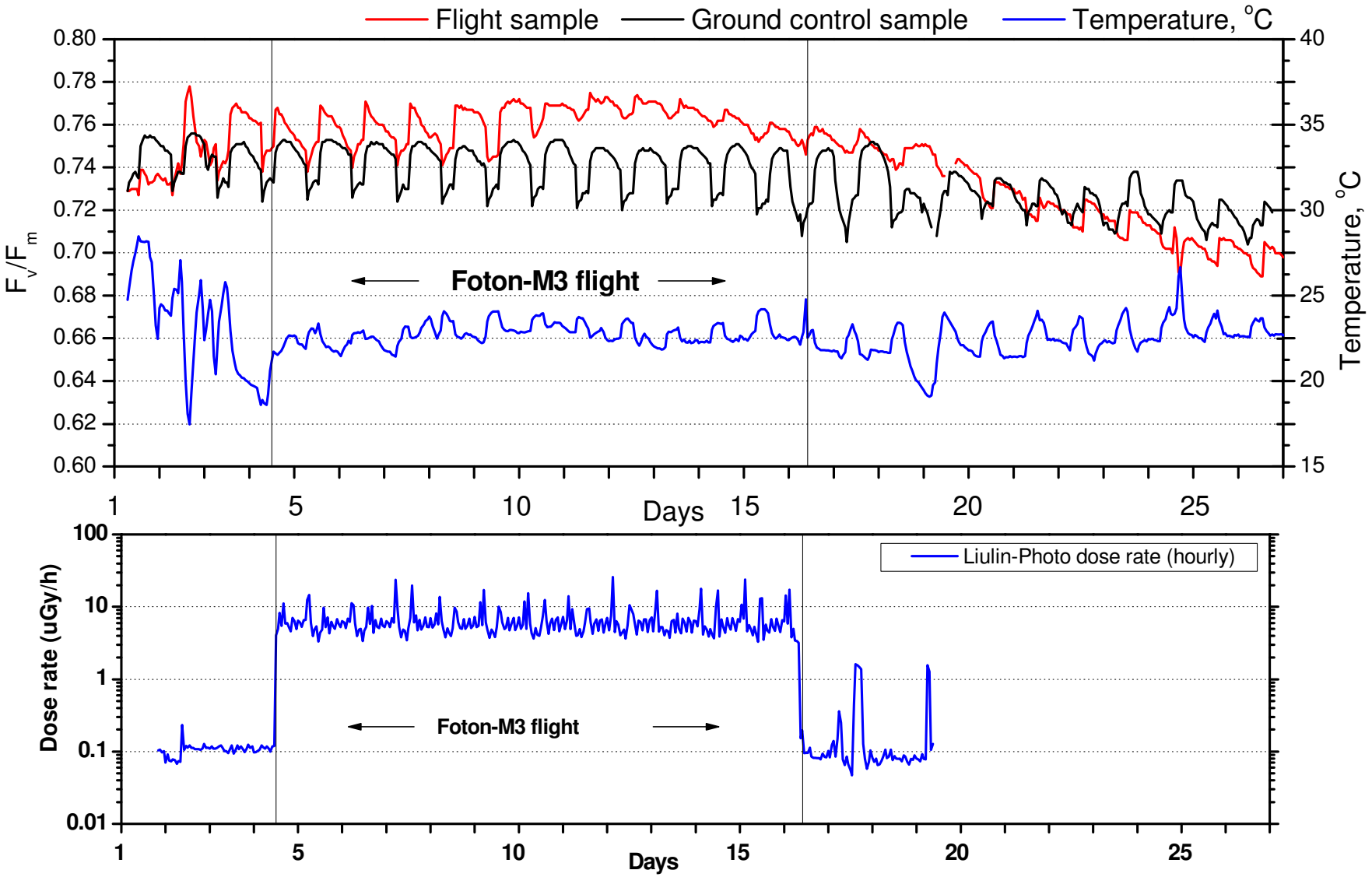
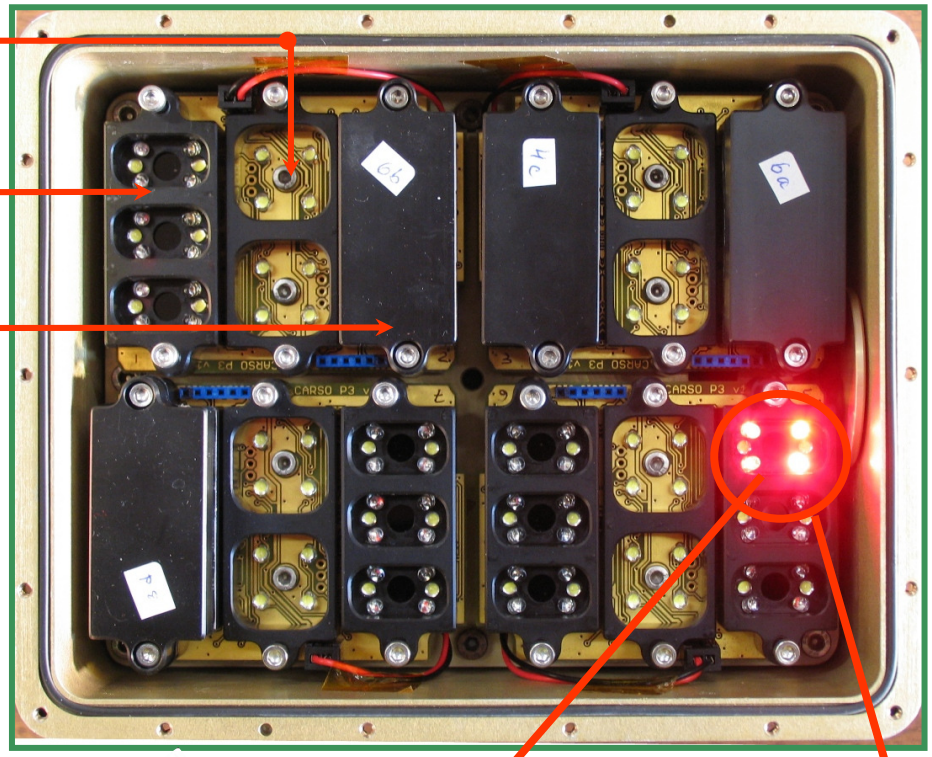


PHOTO II biosensor – working mode

Survival cell, only with white LEDs

Survival and measuring cell, with red and white LEDs

Survival and measuring cell with multicell on the top



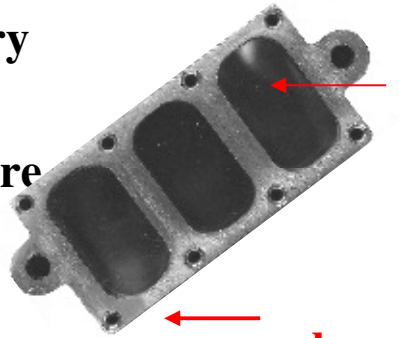
red LEDs – pulse with peak at 660 nm and duration 6 s

white LEDs – actinic light 7/17h light/dark period

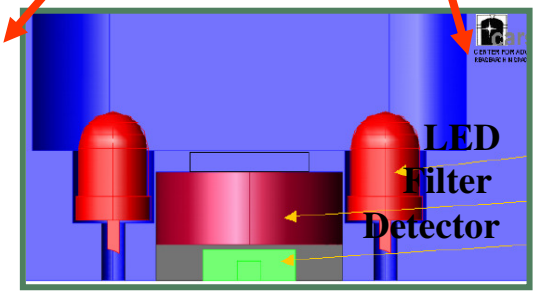
fluorescence measurement every hour

a sensor provided a temperature value for each fluorescence measurement

the flash memory allowed 21 days continuous working.



multicell



chamber, algae on agar