

Thylakoid membrane heterogeneity, proteins trafficking and its role in photosynthetic function

Radek Kaňa et al.

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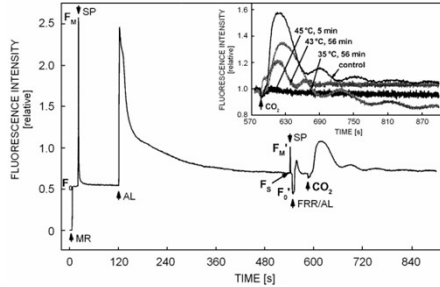


PLANTS AND ALGAE IN THE FREQUENCY DOMAIN
Český Krumlov, Czech Republic
Aug.25-29, 2025

MOTIVATION:

PAST

TIME/FREQUENCY DOMAIN:



Lazar et al. PHOTOSYNTHETICA 43 (1): 13-27, 2005

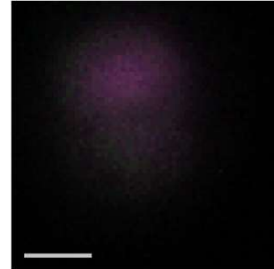
- DAMPED Chl a fluorescence oscillations in barley INDUCED at 2 % CO₂ under heat stress.
- High temperature (>45 °C) stops oscillations due to NADPH limitation.
- Model:
 - Rubisco controls period;
 - NADPH controls amplitude.

How does proteins organization/mobility in TM affect photosynthetic function???

PRESENT

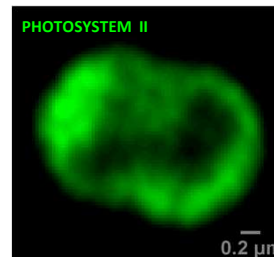
ROLE of SPATIAL HETEROGENEITY

PSI/PSII distribution



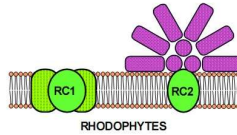
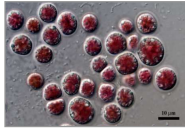
Synechocystis sp PCC6803

PROTEINS TRAFFICING



Question: How does proteins organization/mobility affect photosynthetic function?

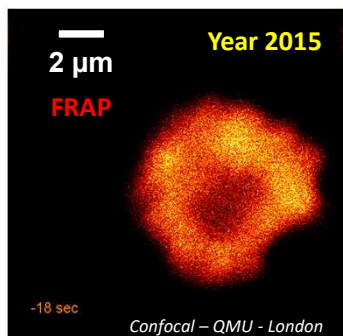
CASE studies : Phycobilisomes mobility in RED ALGAE, CYANOBACTERIA



METHODS – Proteins mobility:

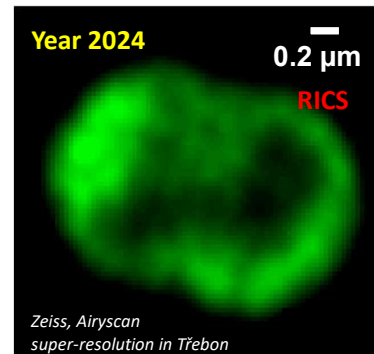
FRAP (Fluorescence Recovery After Photobleaching) – Semi-microscopic

FCS or RICS (Raster Image Correlation Microscopy) – pixel by pixel (~100 nm resolution)



Plant Physiology[®], August 2014, Vol. 165, pp. 1618–1631,

Proteins MOBILITY
Is affected by
HETEROGENEITY
↓
**MICRODOMAINS
+ NANODOMAINS**



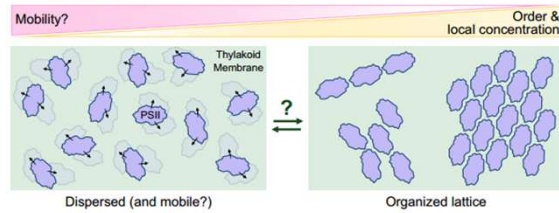
Scope of the TALK:

MOTIVATION: How could be “*membrane heterogeneity*” & “*proteins mobility*” linked to photosynthetic function, proteins activity (NPQ, electron transport, etc...)

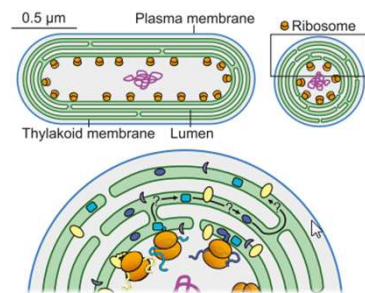
OUTPUT for MODELING: kinetic parameters (*rates*); spatial parameters (*sizes of microdomains*), proteins *co-localization*), proteins localization vs function, proteins *stability vs mobility*

OPEN QUESTIONS in the field:

1. How fluid are PROTEINS IN thylakoid membranes



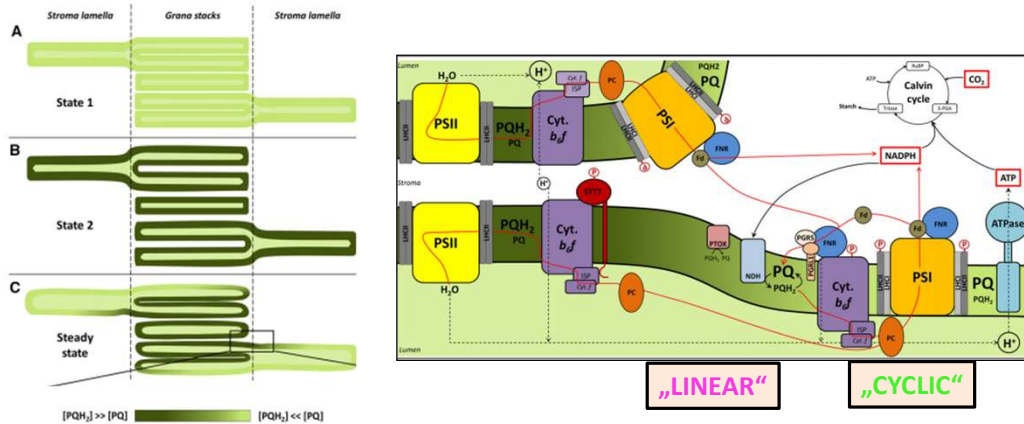
2. LOCALIZATION of proteins and their FUNCTION



CASE EXAMPLE: Linear vs cyclic el. flow

2. LOCALIZATION of proteins VS proteins FUNCTION

CASE EXAMPLE: Role of GRANA/STROMA architecture for **Cyclic/Linear** electron flows



How does organization and localization of PROTEINS
(PSI/PSII/NDH/Cytb6f) affects CYCLIC and LINEAR FLOW

Fig. 2 Schematic representation of thylakoid ultrastructure in various conditions. A Oxidizing, low light, "state 1" conditions: the PQ pool is evenly oxidized (membrane colored in light green) as compared to the poised state. The thylakoid membranes are appressed. B Reducing, low light, "state 2" conditions: the PQ pool is uniformly reduced (membrane colored in dark green) as compared to the poised state. The thylakoid membranes are no longer appressed due to the high level of protein phosphorylation by active Stt7 kinase. A large proportion of phospho-LHCII proteins migrate toward PSI in the lamellae. C "Steady-state" continuous illumination, between state 1 and state 2. The redox state of plastoquinones is heterogeneous due to diffusion limited to microdomains. More quinols would be found around PSII complexes in grana stacks (dark green), whereas stroma lamellae, more distant from PSII and containing PTOX, would retain some significant amounts of PQ (light green). The ultrastructure and lateral heterogeneity of the thylakoid membrane is destabilized (protein phosphorylations and redistribution). High-light conditions also create a strong influx of protons into the lumen which induce a swelling of the thylakoids. D Zoom in the grana margins shows that the cyt b6 f complex is distributed homogeneously in the thylakoid membrane although it can be phosphorylated and migrate to regions lacking PSII. These membrane microdomains likely retaining oxidized quinones in high light may catalyze cyclic electron flow more efficiently. Red arrows electron transport; dashed lines proton

influx and efflux
Photosynth Res
123

EVOLUTIONARY CONTEXT – PSI/PSII

3. SPECIES variability – difference in PROTEINS HETEROGENEITY - PSI/PSII SEPARATION

PLANTS/GREEN ALGAE

**Grana vs Stroma
ARCHITECTURE for PSI-PSII**
DONE

RED ALGAE

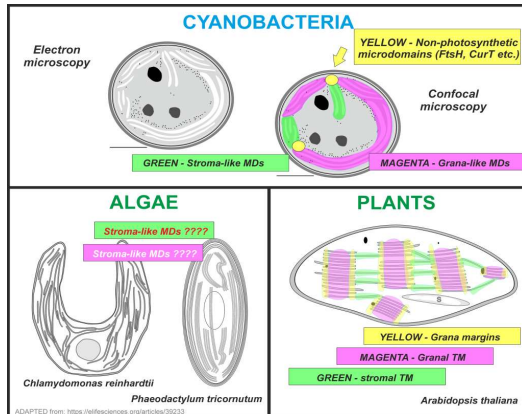
OPEN QUESTION:
PSI/PSII spill over, other??
- NOT yet

„RED CLADE“ (e.g. diatoms)

PSI/PSII - spill over
Other mechanism ?????
- NOT yet

CYANOBACTERIA

**Grana vs Stroma LIKE
MICRODOMAINS for PSI-PSII**
- RECENTLY DONE



STRUCTURE



FUNCTION

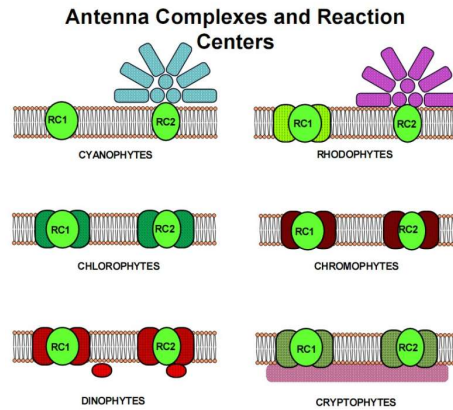


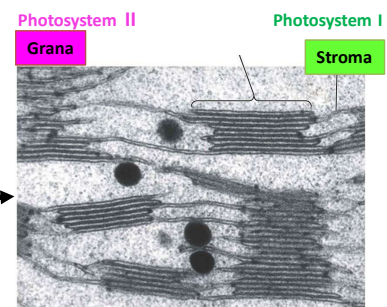
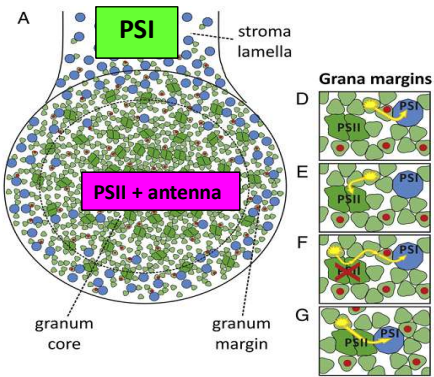
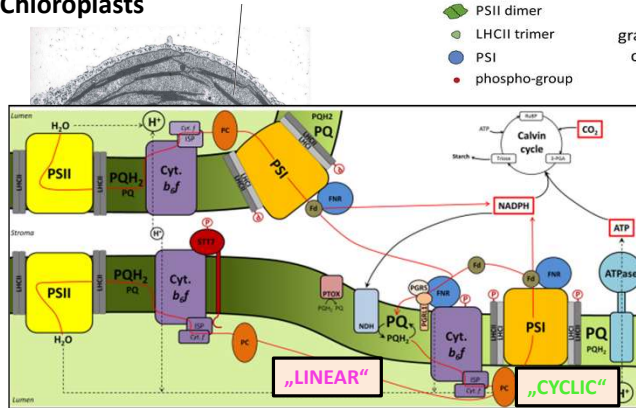
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influx and efflux
Photosynth Res
123

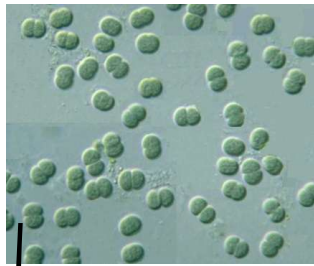
Higher PLANTS thylakoids



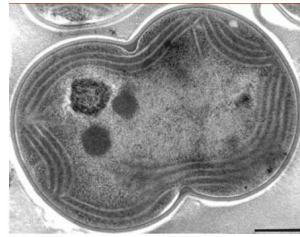
Chloroplasts



DATA: CYANOBACTERIA



NO chloroplast
NO membranes stacking



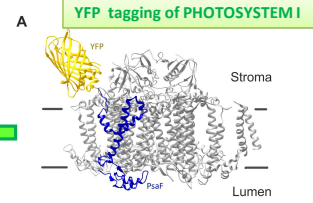
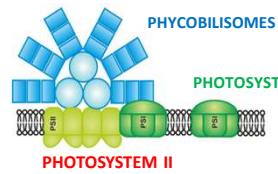
BBA - Bioenergetics 1860 (2019) 148053

Pigment-protein complexes are organized into stable microdomains in cyanobacterial thylakoids

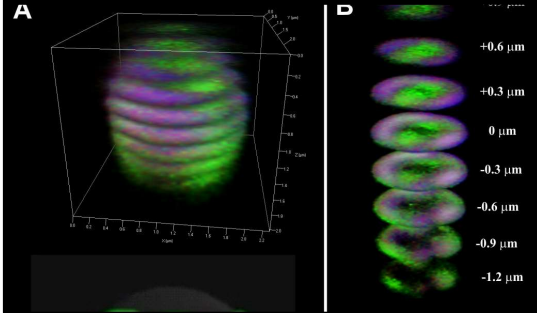
A. Strašková¹, G. Steinbach^{1,2}, G. Konert, E. Kotabová, J. Komenda, M. Tichý, R. Kaňa*

¹Institute of Microbiology, Czech Academy of Sciences, Centre Algaech, Novotná 237, 279 81 Třebíč, Czech Republic

BBA - Bioenergetics



YES – GRANA-STROMA like heterogeneity in cyanobacteria

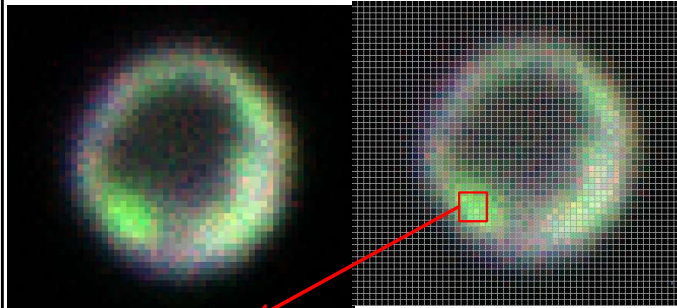


Grana-like
MICRODOMAINS
More PSII+PBS

STROMA - like
MICRODOMAINS
More PSI

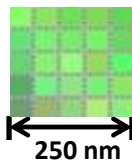
MICRODOMAINS in cyanobacteria

3 channel imaging *in vivo*

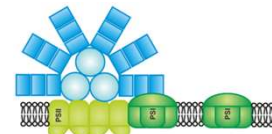


Resolution: 250 nm

Membrane heterogeneity
based on the **RGB** color

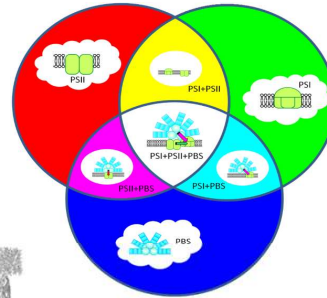


MAGENTA – dominant PSII+PBS
GREEN – dominant PSI
WHITE – intermixed



PHOTOSYSTEM II - Red:
PHOTOSYSTEM I - Green
PHYCOBILISOMES - PBS – Blue

7 BASIC COMBINATIONS



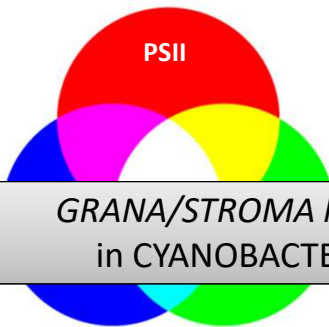
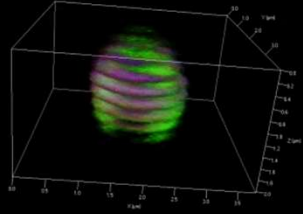
BBA - Bioenergetics 1860 (2019) 148053

BBA - Bioenergetics

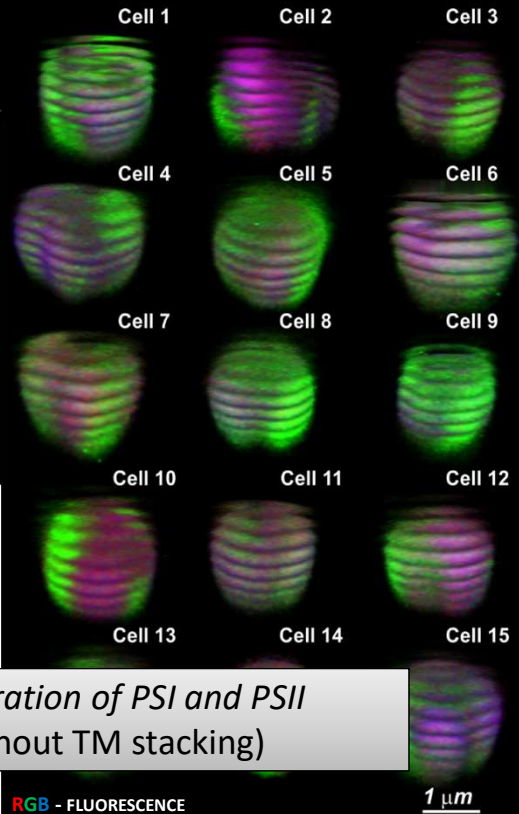
Pigment-protein complexes are organized into stable microdomains in cyanobacterial thylakoids

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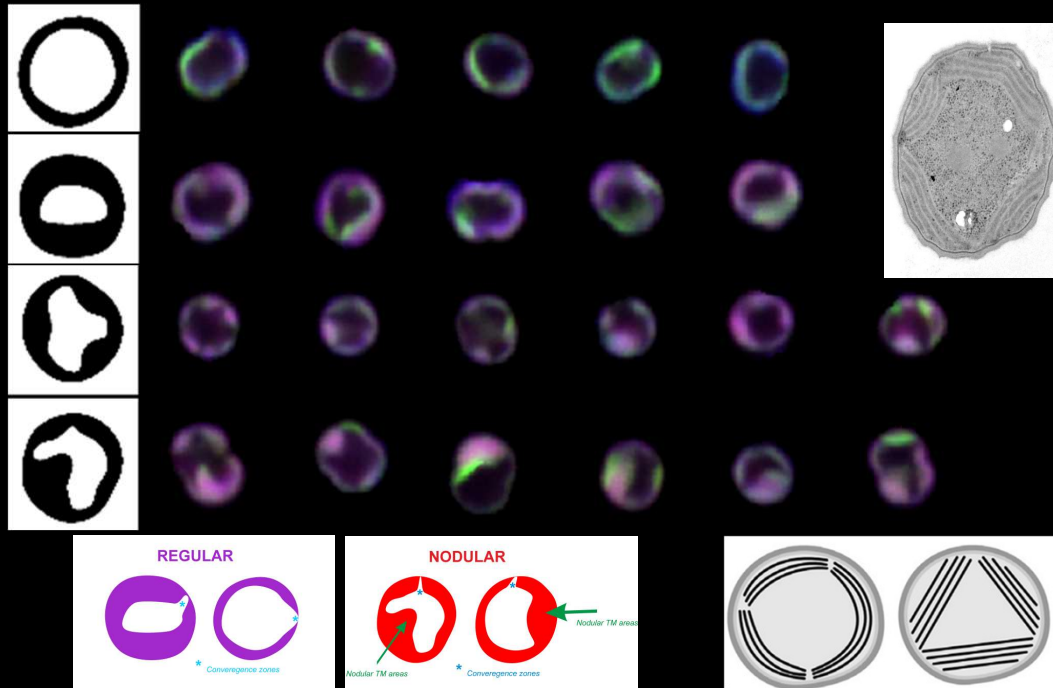
MICRODOMAINS



*GRANA/STROMA like separation of PSI and PSII
in CYANOBACTERIA (without TM stacking)*



CELL to CELL HETEROGENEITY in MDs



Konert et al. in preparation

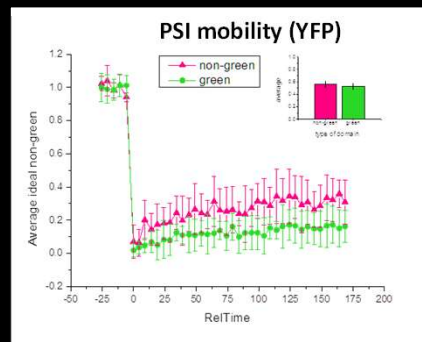
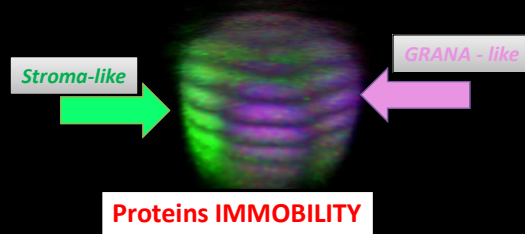
PROTEINS MOBILITY in THYLAKOIDS

DOES THE MOBILITY OF
PHOTOSYSTEMS AFFECT
THEIR SEPARATION???

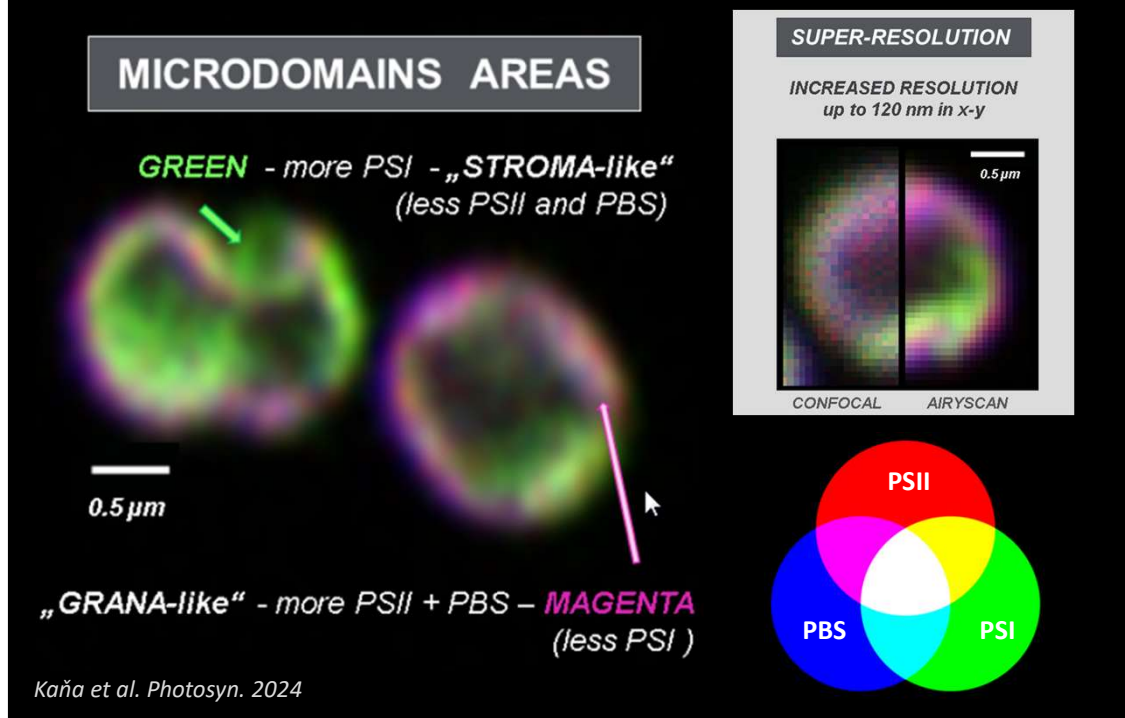


*Microdomains are stable
in the scale of minutes*

DIFFERENT MICRODOMAINS



NEXT STEP – SUPER-RESOLUTION



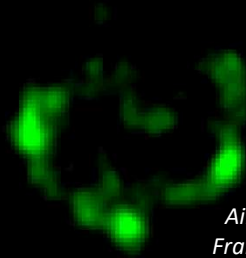
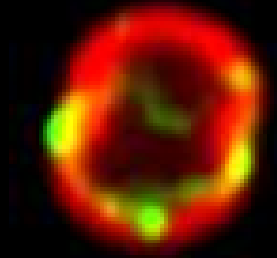
https://mdpi-res.com/d_attachment/photonics/photonics-04-00041/article_deploy/photonics-04-00041-v3.pdf?version=1502087017

Heterogeneous proteins trafficking

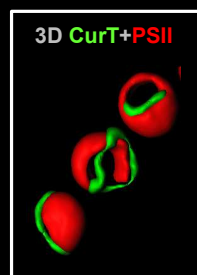
[1] Non-pigmented proteins TRAFFICKING

CHL
CurT-TFP

200 nm

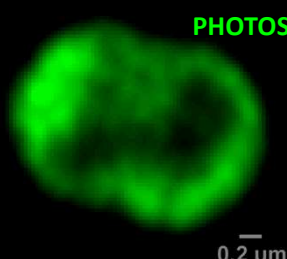


Airyscan det.
Frame - 600 ms



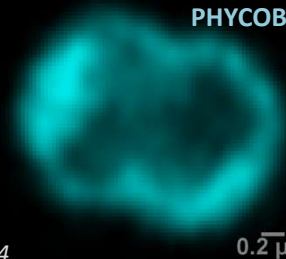
[2] Photosystems AND Phycobilisomes TRAFFICKING

PHOTOSYSTEM II



0.2 μm

PHYCOBILISOMES

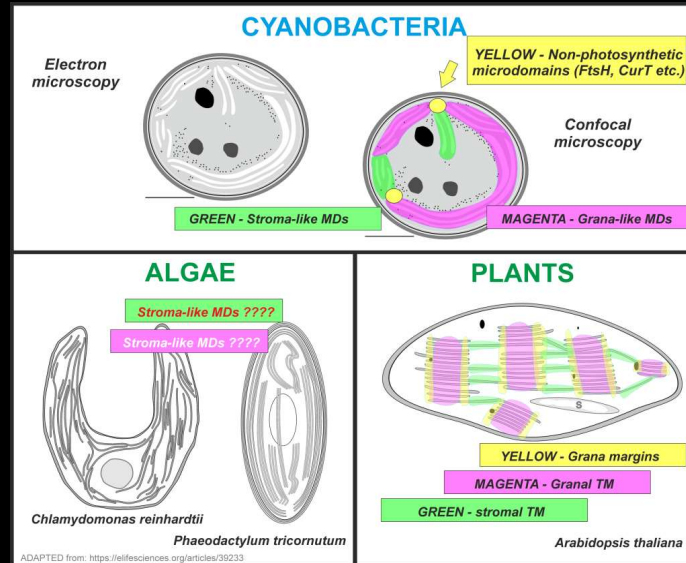


0.2 μm

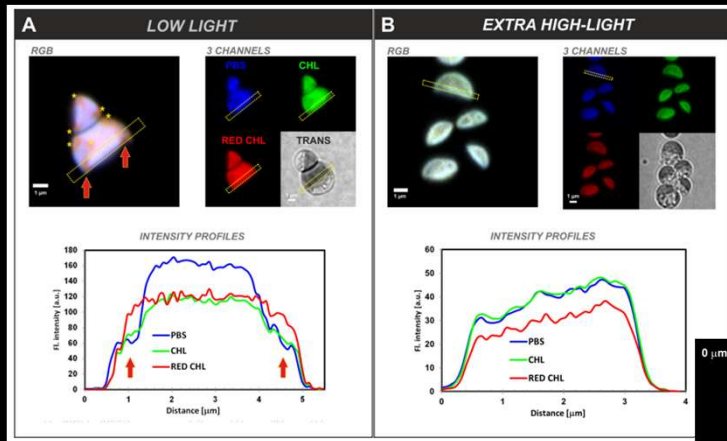
Kaňa et al. Photosyn. 2024

Fli – curt - OK 25 x --- GIF - C2-CurT-TFP NL d S02 AS_TFPkin_2 Aver Vyrez B-2
Na emku 220629

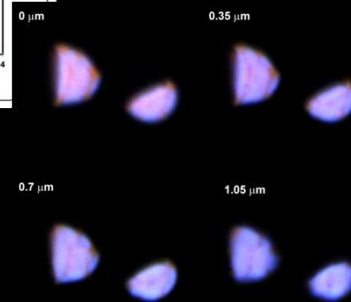
Are MICRODOMAINS Specific ONLY for Cyanobacteria?



RED ALGAE – *C. merolae*



ABRAM et al. 2025, submitted



CONCLUSIONS and questions:

(in the context of DREAM project)

1. SPATIAL HETEROGENEITY in PHOTOSYNTHETIC FUNCTION

Microdomains represents stable **100 - 500 nm** areas in TM with different proteins composition
- e.g. *PSI-PSII* separation

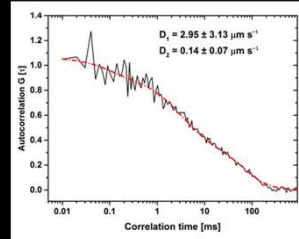


How does **SIZE of MICRODOMAINS** + **SPECIFIC PROTEINS trafficking** affect FREQUENCY DOMAIN ANALYSES???

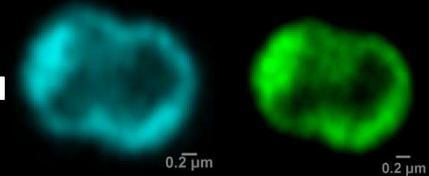
2. MOBILITY OF PROTEINS

There is a continuous trafficking of proteins on nanoscale level

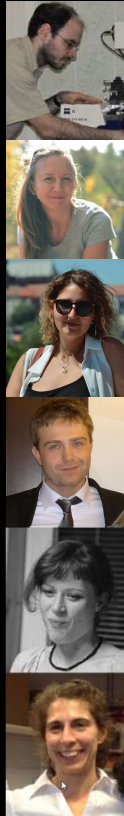
FREE proteins - $D_{PetC1} = 0.9 - 1.5 \mu m s^{-2}$ (FCS)



BOUND COMPLEXES - $D_{PSII} = 0.001-0.001 \mu m s^{-2}$ (FRAP)



Acknowledgements



*Bára Šedivá
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Petra Skotnicová
Vendula Krynická
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Ondřej Prášil
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Myriam Canonico
Adéla Kaňová
Edel Semanat
Gábor Steinbach
Amelie Schober
and many others...*



*Joanna Kargul – Univ. of Warsaw
György Vámosi – Debrecen*



Thank you for your attention