Photosystem Localization and Pigment Turnover in Cyanobacteria

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ASU Arizona State University

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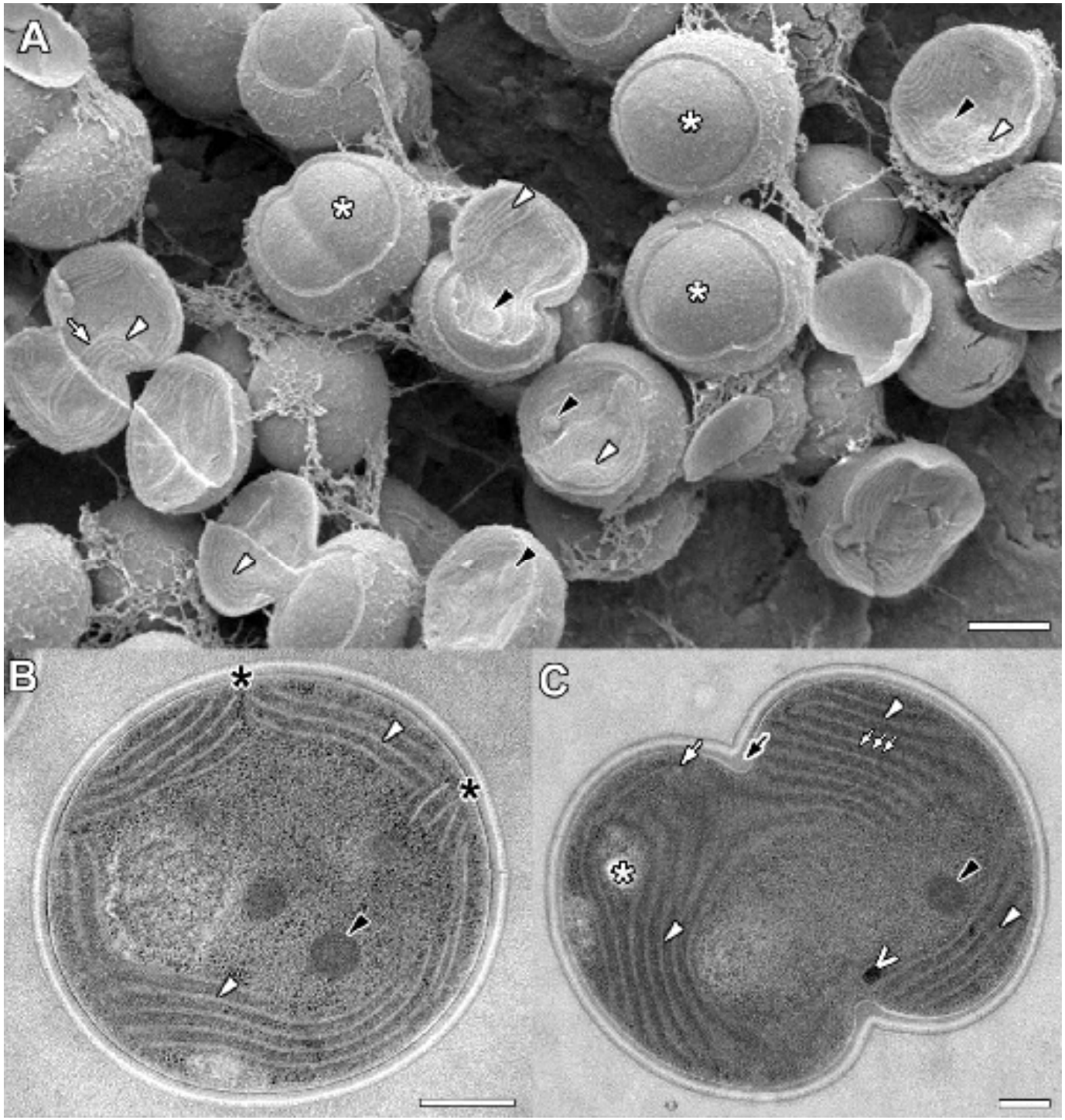
Sandia National Laboratories



Synechocystis:

- Multiple thylakoid layers
- Thylakoids mostly peripheral
- Are all thylakoids equal in their pigment composition?

Localization of pigments by means of fluorescence in *Synechocystis*

Complex because:

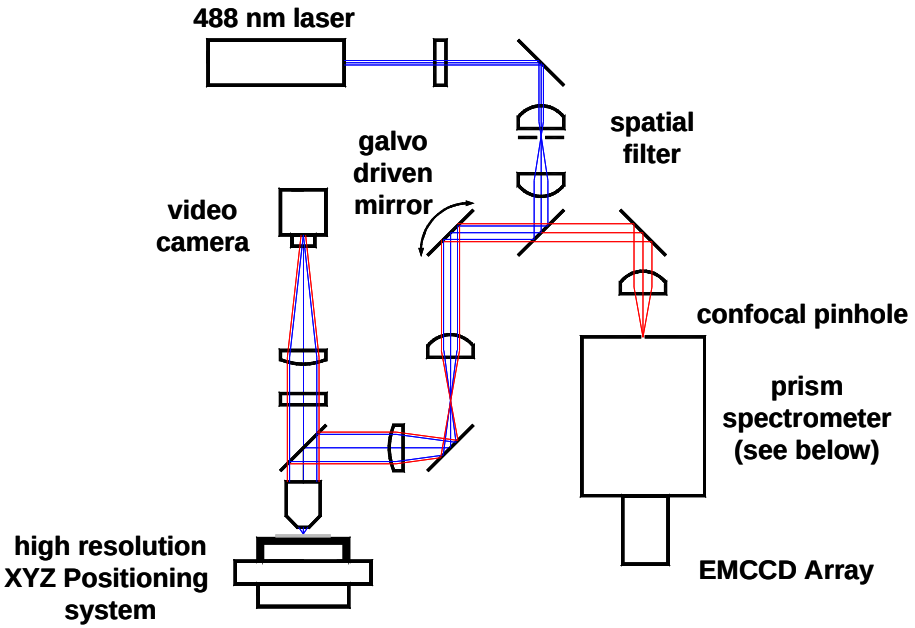
- Many different overlapping spectra (phycocyanin, allophycocyanin, chlorophyll, etc.)
- Small cells
- 3D pigment localization

Solution:

- Hyperspectral confocal fluorescence imaging: fluorescence spectra of 3D pixels are measured and individual spectra are resolved using spatial variation in fluorescence spectra of pixels

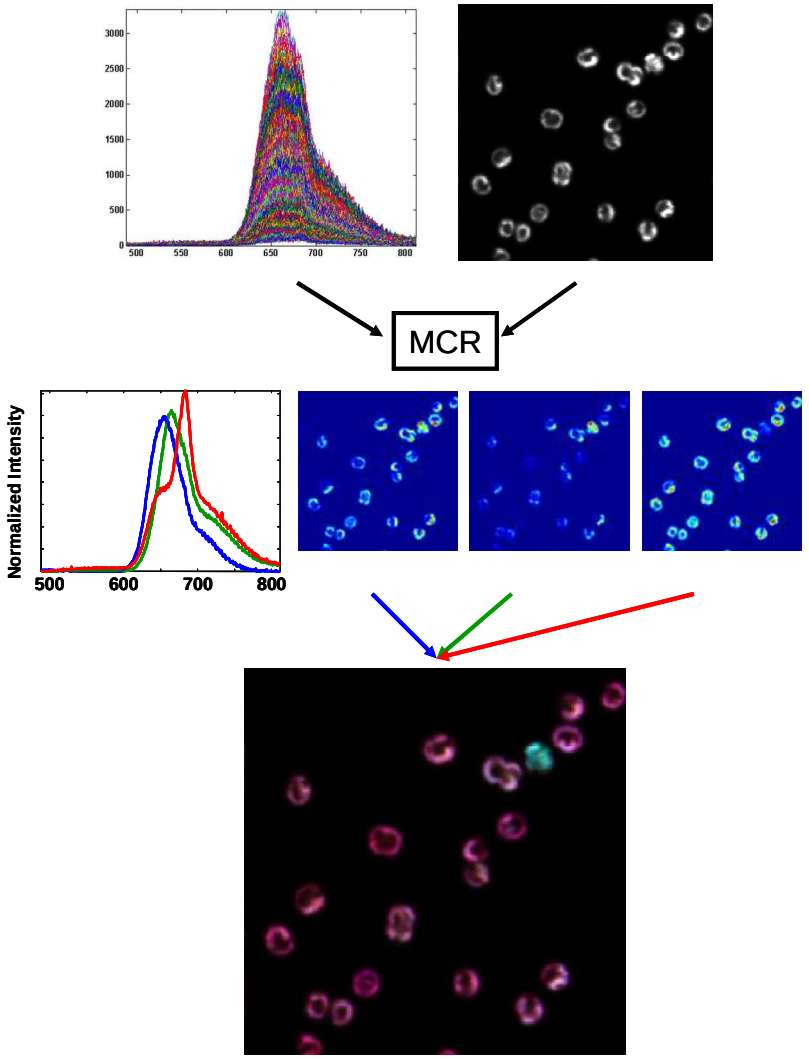
Hyperspectral imager

Optical Layout

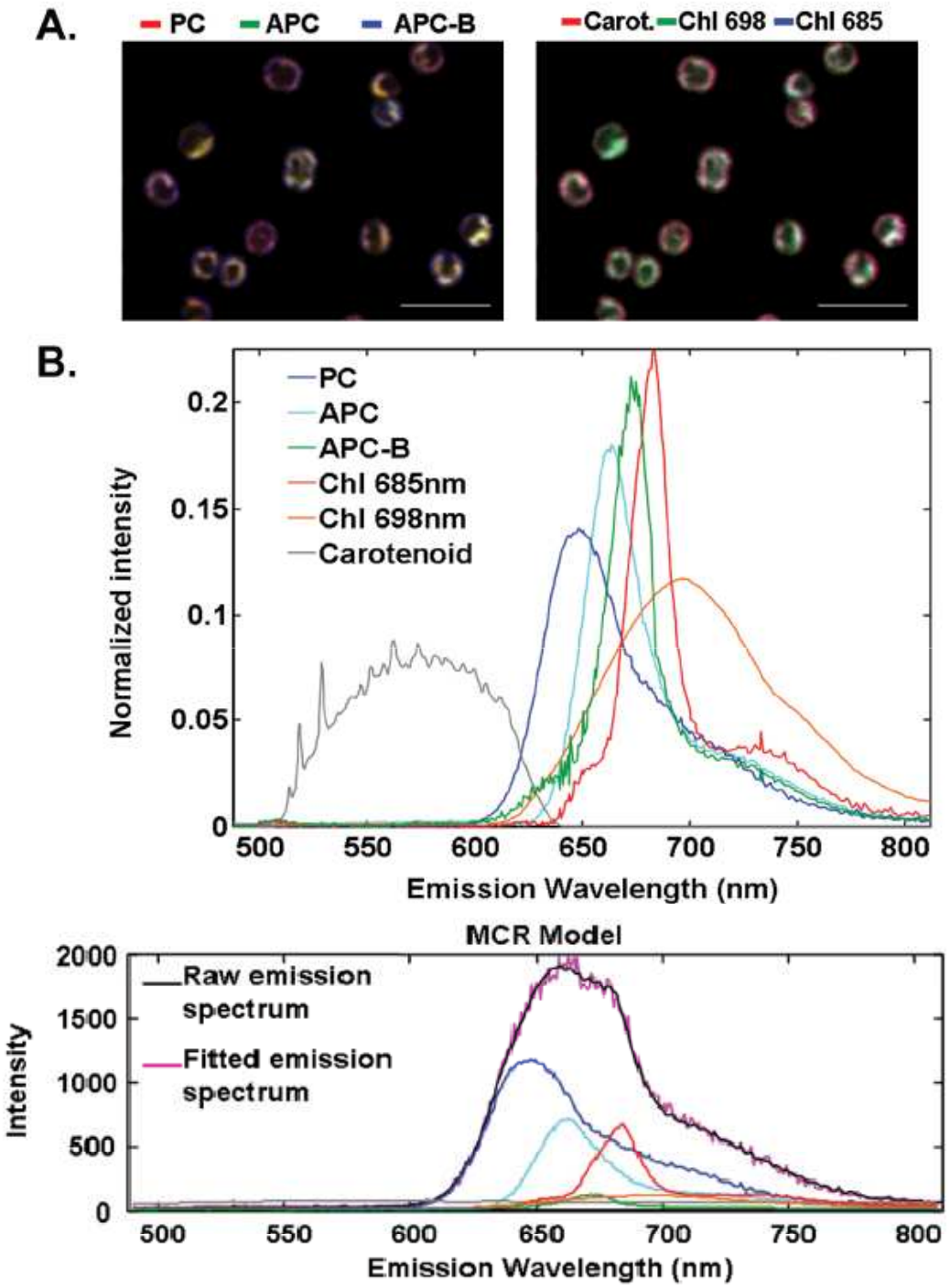


Resolution:
diffraction-limited (~250 x 250 x 600 nm)

individual spectra



MCR: multivariate curve resolution



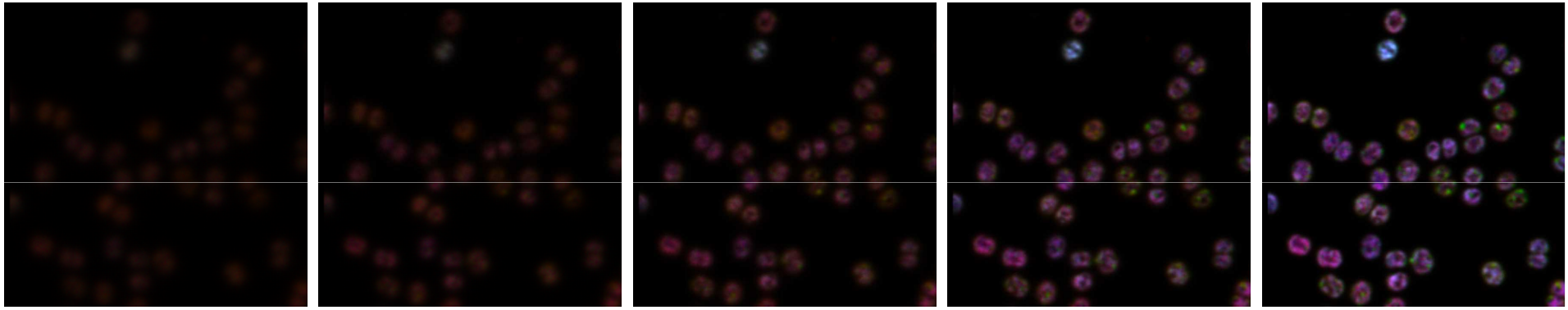
Bottom line regarding wild type:

1. Six components:
   - phycocyanin
   - allophycocyanin
   - L_{cm}/allophycocyanin-B
   - chlorophyll (PS I)
   - chlorophyll (PS II)
   - carotenoids (!!)
2. Carotenoid spectra enhanced by Resonance Raman peaks
3. Excellent fit to the data
4. Encourages a detailed characterization in wild type and mutants

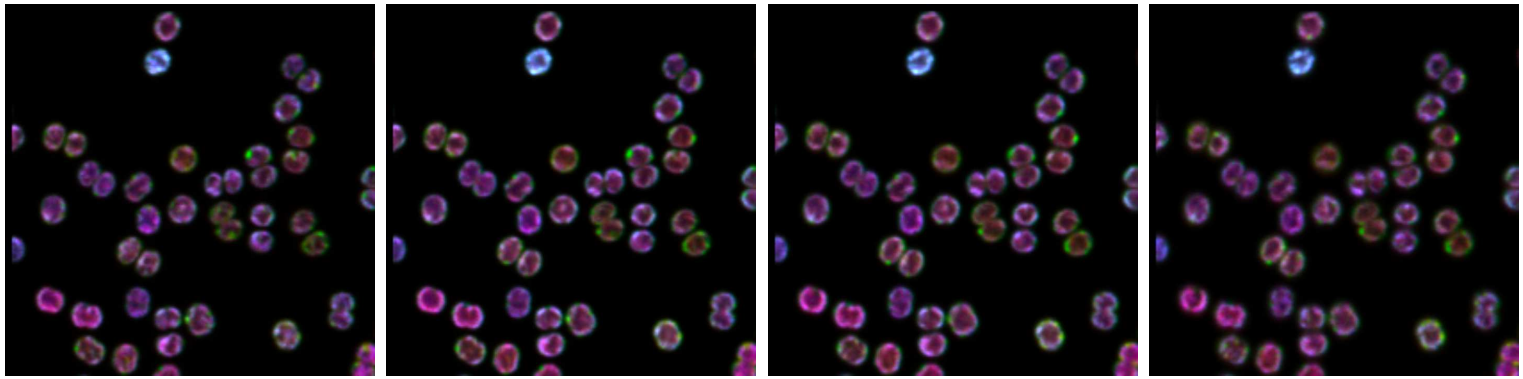
Hyperspectral imaging data in three dimensions (or even in four dimensions: the fourth one being time)

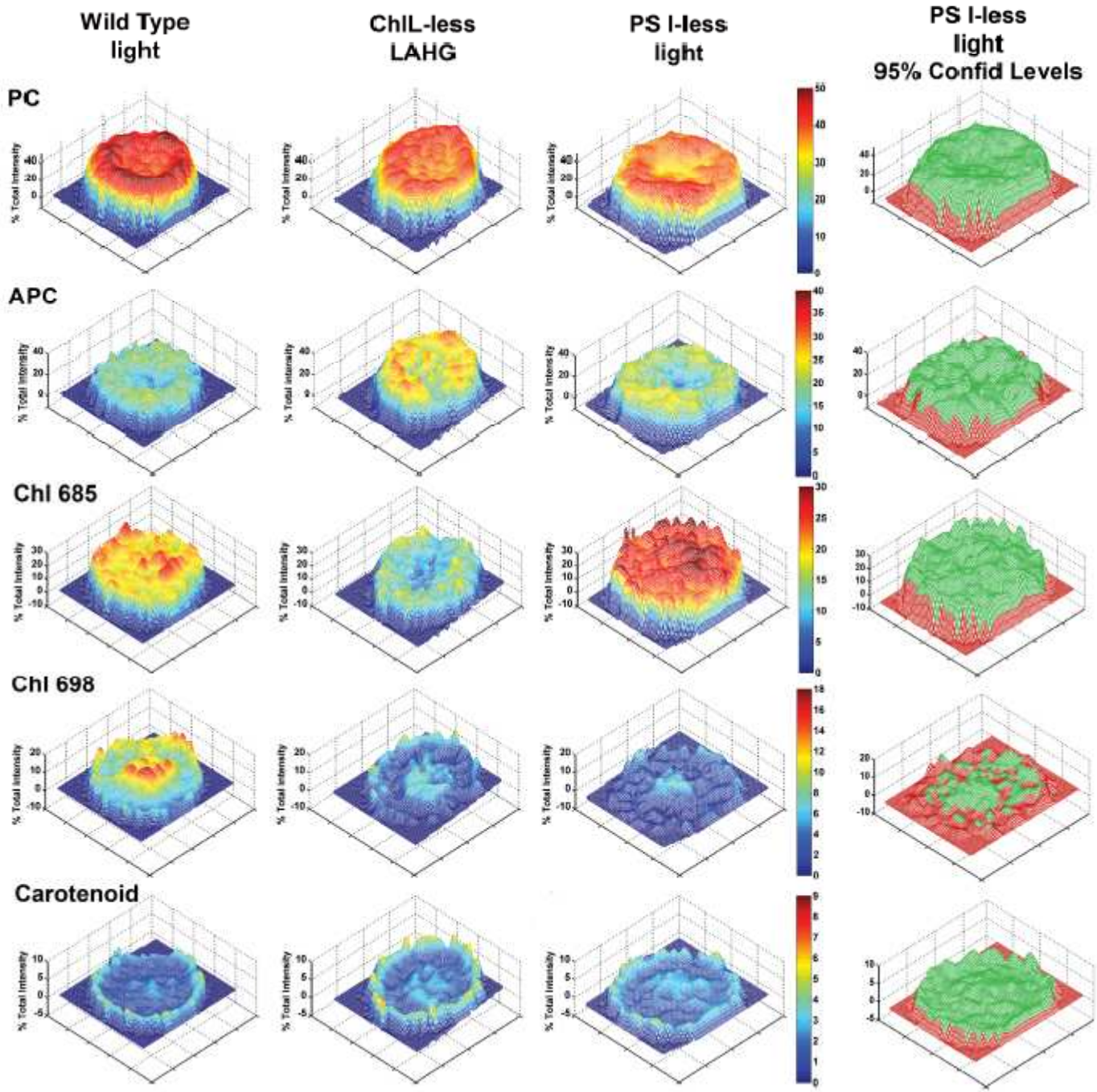
“3-D”(scan through in Z direction; false-color representation of the fluorescence components)

Slice 1 Slice 2 Slice 3 Slice 4 Slice 5



Slice 6 Slice 7 Slice 8 Slice 9



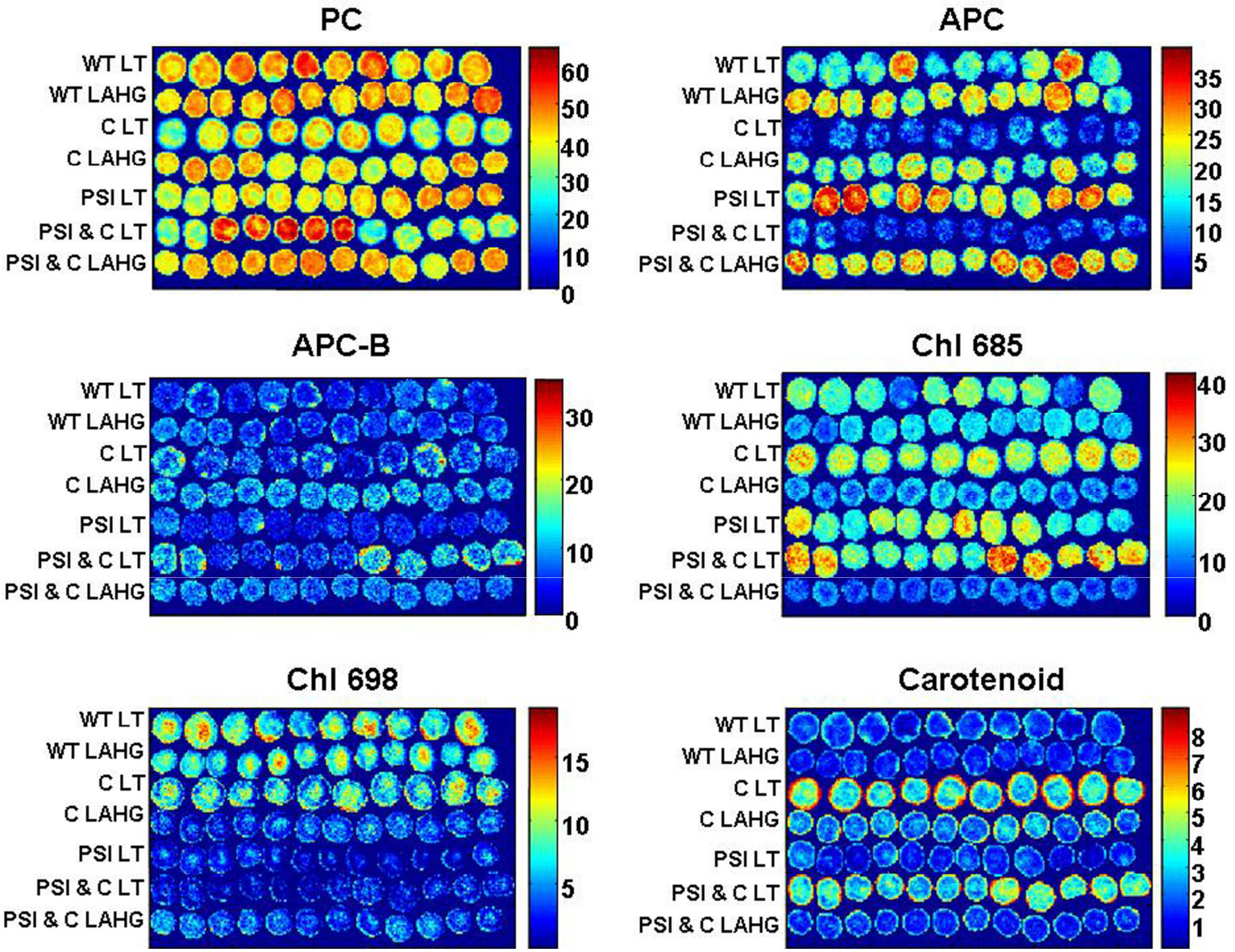


Total fluorescence in each pixel is taken as 100%, and contributions of the various components are plotted.

- Phycocyanin fluorescence is more intense around the periphery; Chl698 is more toward the center; Chl685 is evenly distributed
- Chl698 is PS I-related
- Carotenoids are primarily in the cell wall

So far, so good, but:

- How reproducible are the results between cells from one mutant strain relative to another strain or wild type?
- Can we get independent confirmation of the apparent heterogeneity in pigment distribution?
- Are there potential biophysical issues (scatter, self-absorption) that may affect the results obtained?



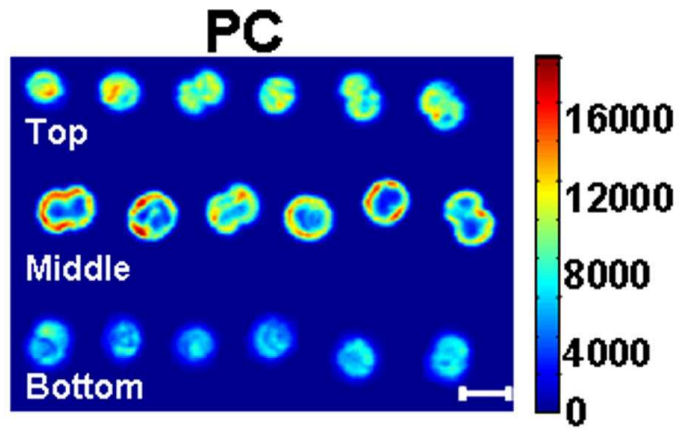
C: ChlL-less (no new Chl in dark)

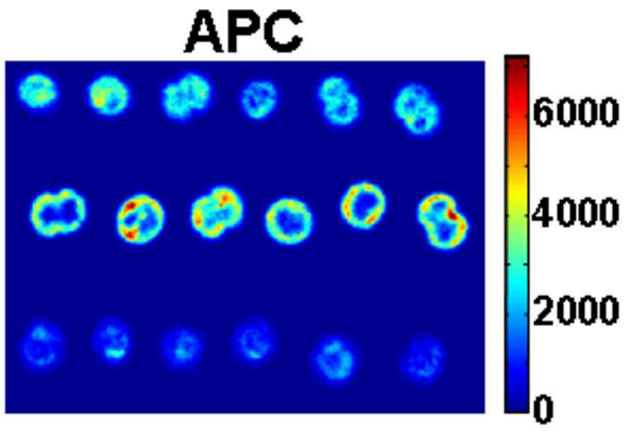
PSI: PS I-less

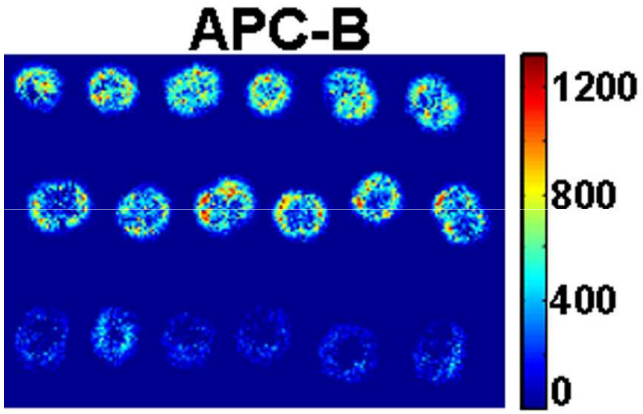
LT: light

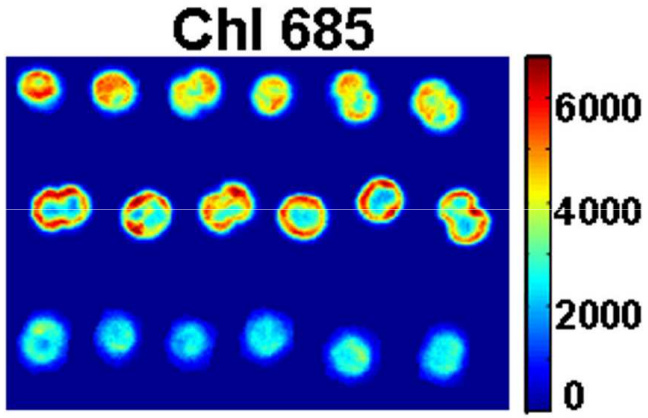
LAHG: light-activated heterotrophic growth

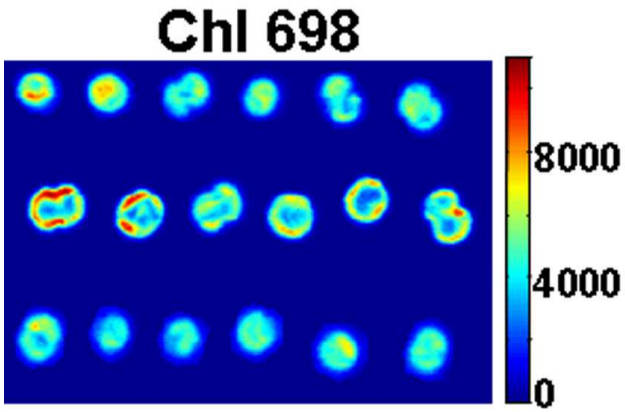
Bottom line: Good reproducibility of relative intensity and distribution of components within a strain or growth condition, and significant variation between strains / growth conditions

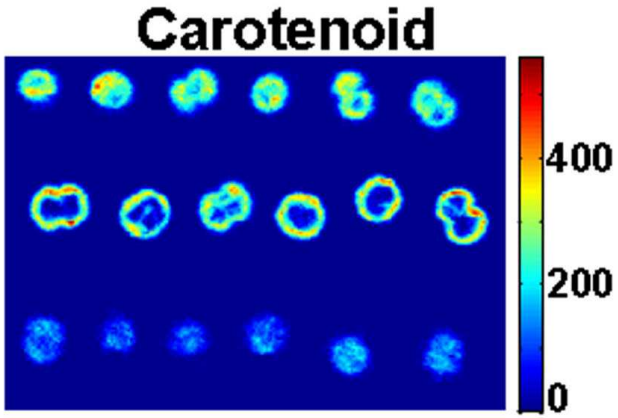












Make a hyperspectral image of the top, middle and bottom of a cell: images consistent with particularly the phycobilins being around the periphery of the cell.

Scattering by cells contributes (bottom less bright than top), but little or no self-absorption as the pure spectra of top and bottom are identical.

So, this is how we interpret the data:

- There is heterogeneity in pigment distribution in cells; assignment backed up with mutants
- Phycobilisomes more prevalent (but by no means exclusively) around the periphery
- Chl685 (PS II chlorophyll, among others) fairly evenly distributed among thylakoids
- Chl698 (PS I chlorophyll) more prevalent (again, not exclusively) in the “inside” thylakoids
- Carotenoids prevalent in the cell wall, but also some in thylakoid membranes
- Interpretation/speculation: PS I-dependent cyclic electron flow may be prevalent in the inner thylakoids; linear electron flow may be more prevalent in the outer layers of the thylakoids

Now a different angle: the chlorophylls

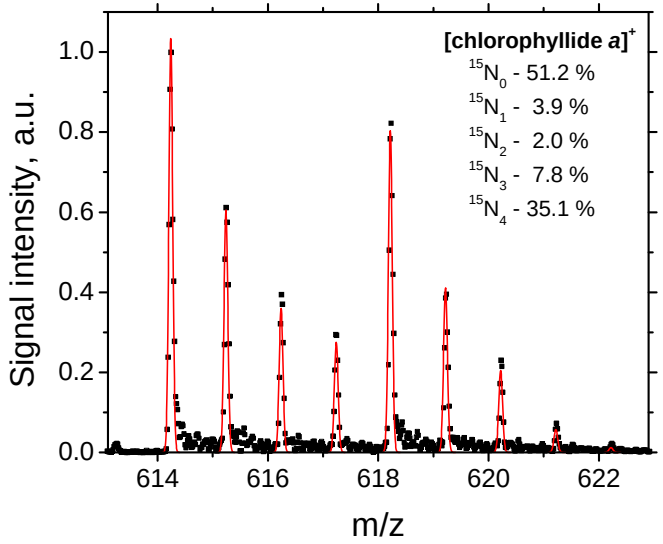
A quick reminder:

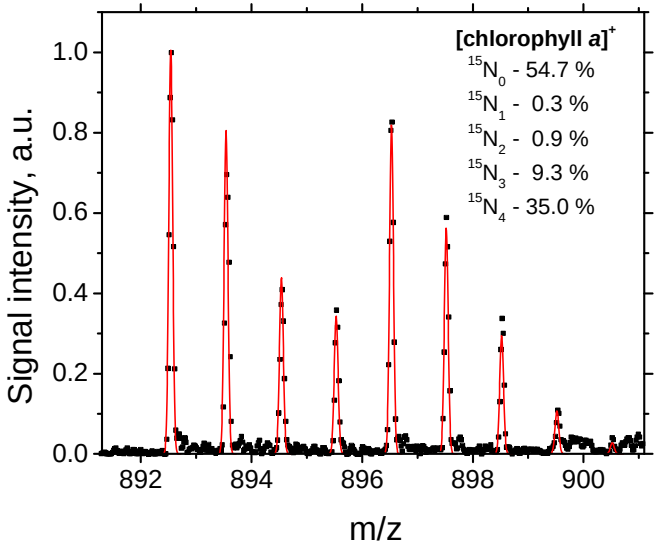
- What happens to chlorophylls when chlorophyll-binding proteins (such as D1, D2, CP47 or CP43) turn over? Are they degraded or reused? And how?
- Results of a couple of years ago: Chlorophylls live for 10 days or so, even at reasonably high light intensity, and photosystem II proteins live for just minutes or hours under those conditions.
- Question: How is chlorophyll in PS II recycled?
- Short answer: Think SCPs (Small Cab-like Proteins)

Chlorophyll biosynthesis and degradation kinetics

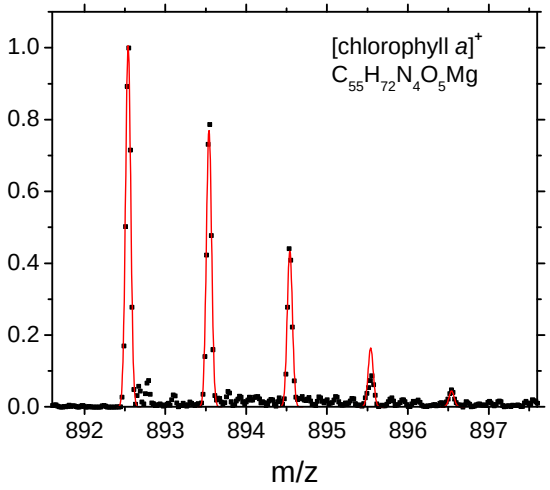
- Use pulse labeling with ^{15}N-labeled nitrogen sources, and determine chlorophyll mass distribution at different incubation times:

with ^{15}N





unlabeled



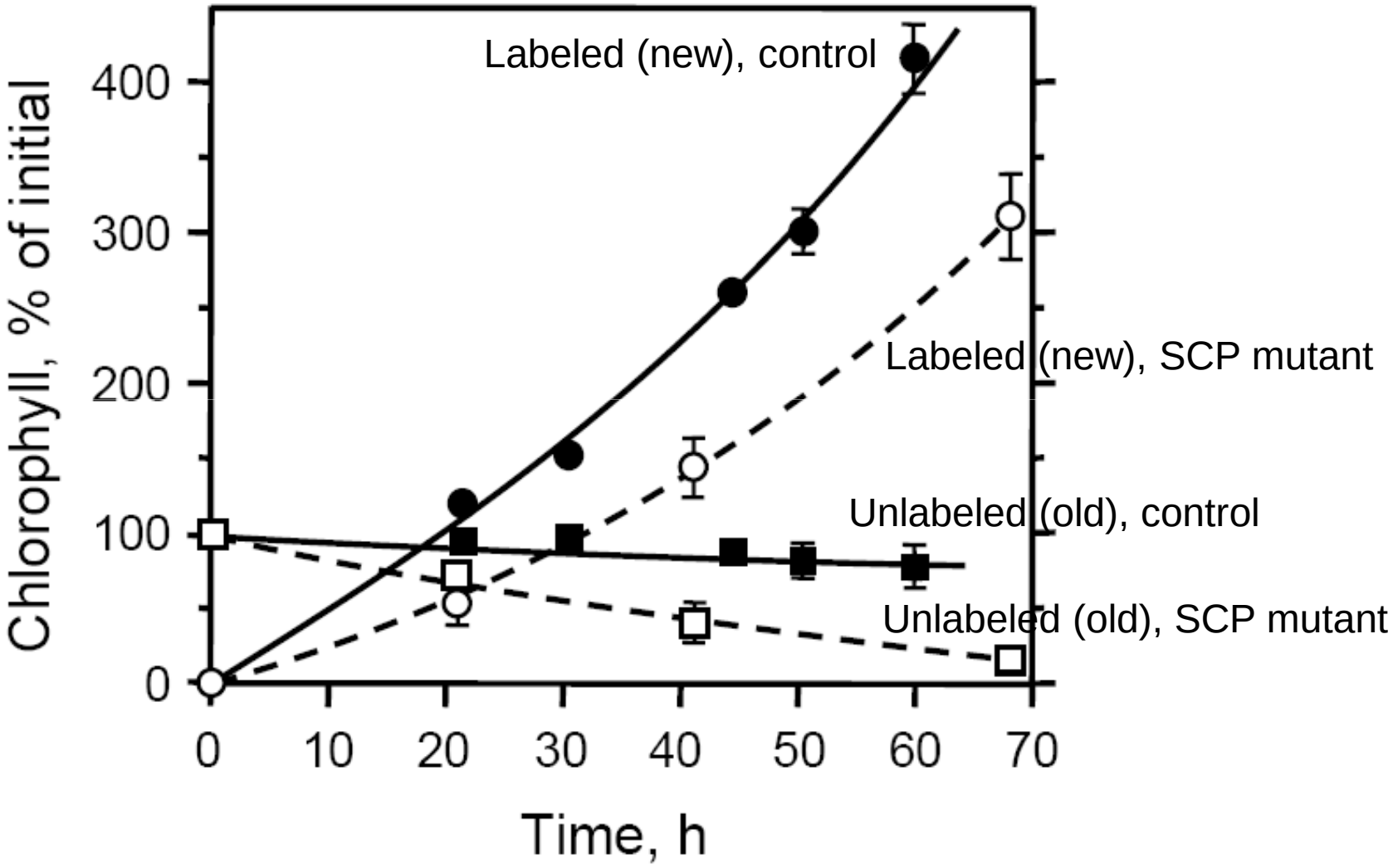
(chlorophyllide is chlorophyll without the phytol tail)

(see Vavilin et al., *Biochim. Biophys. Acta* 1708 (2005) 91-101)

SCPs (particularly ScpB and ScpE) are important for PS II but not PS I-associated chlorophyll stability

Strain	Growth light intensity, µmol m^{-2} s^{-1}	Chloro-phyll half-life time, $t_{1/2}$, h	Cell doubling time, h	Chloro-phyll, µg Chl/mL/ OD_{730}	Chlorophyll synth rate, f_{Chl}, % of WT
PS II-less	45	>200	20.4±1.8	2.15±0.12	68
PS II-less/*scp*ABCDE$^-$	45	>200	18.3±3.0	1.68±0.08	59
PS I-less	2-4	***161±25***	21.5±3.3	0.48±0.03	15
PS I-less/*scp*B$^-$	2-4	***64±3***	26.8±2.3	0.43±0.03	13
PS I-less/*scp*BC$^-$	2-4	65±4	25.5±2.6	0.44±0.04	14
PS I-less/*scp*BCD$^-$	2-4	71±10	27.1±3.1	0.41±0.02	12
PS I-less/*scp*ACD$^-$	2-4	***113±19***	20.6±1.1	0.44±0.03	15
PS I-less/*scp*ACDE$^-$	2-4	***46±7***	22.9±3.5	0.33±0.03	13
PS I-less/*scp*ABCD$^-$	2-4	***48±4***	21.4±1.0	0.39±0.05	16
PS I-less/*scp*ABCDE$^-$	2-4	***32±5***	53.5±5.4	0.13±0.02	4

Drastic effects of SCPs (Small Cab-like Proteins) on chlorophyll lifetimes in PS I-less strains



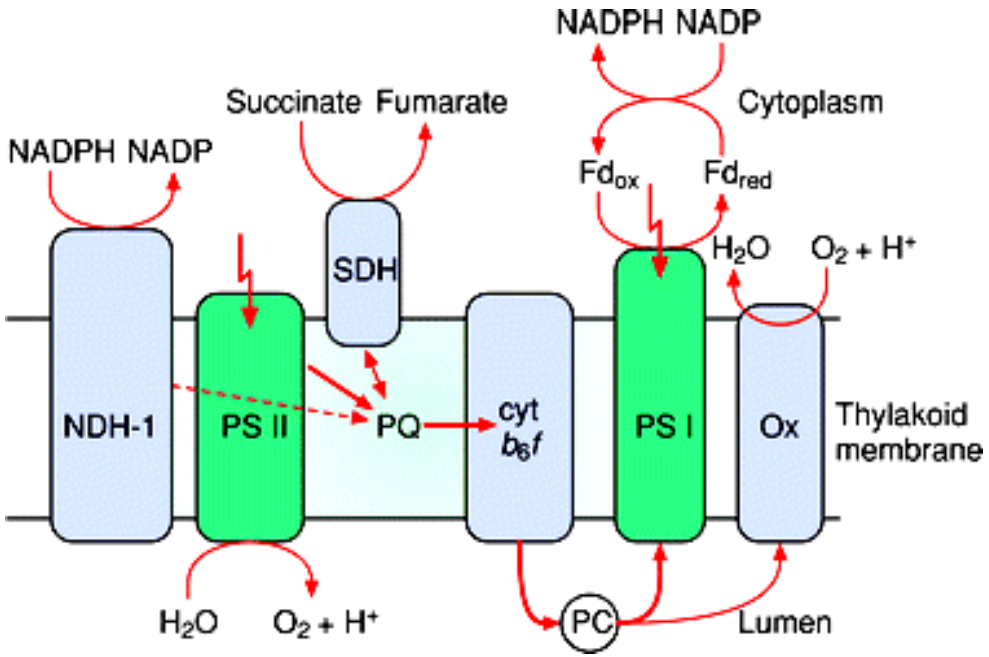
Photosystem II

- 40 chlorophylls, 2 pheophytins, 10 carotenoids (mostly β-carotene) per monomer
- about two dozen protein subunits, 0.4 MDa per monomer
- D1, a chlorophyll-binding protein in the center of photosystem II, turns over rapidly (<1 h) at high light intensity

The PS I/PS II stoichiometry in *Synechocystis* is > 3/1, so <10% of the chlorophylls in the wild type is associated with PS II

Photosystem I

- 120 chlorophylls per monomer
- carotenoids
- about a dozen protein subunits, 0.3 MDa per monomer
- the photosystem is rather stable even at high light intensity



Sequence alignment of five SCPs from *Synechocystis* and C-terminal region of LHCII protein from pea

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                                                    A1   A2   A4
LHCII YRIAGGPLGEVVDPLYPGGSFD PLGLADDPEA FAELKVKELK NGRLAMFSMF GFFVQAIVTG KGPLENLADH...
ScpA  VMDSLNDPPCT FETVPHPKKN MKMYPQERWE WGLTTAAEVW NGRLAMLGFI ALLV.ELISG QGPLHFVGLL...
ScpB  .......... .......... ...MNNENSK FGFTAFAENW NGRLAMIGFS SALILELVSG QGVLHFFGIL*
ScpE  .......... ...MSEELQP NQTPVQEDPK FGFNNYAEKL NGRAAMVGFL LILVIEYFTN QGVLAWLGLR*
ScpC  MTTRGFRLDQD NRLNNFAIEP EVYVDSSVQA .GWTKYAEKM NGRFAMIGFA SLLIMEVVTG HGVIGWLNSL*
ScpD  MTSRGFRLDQD NRLNNFAIEP PVYVDSSVQA .GWTEYAEKM NGRFAMIGFV SLLAMEVITG HGIVGWLLSL*
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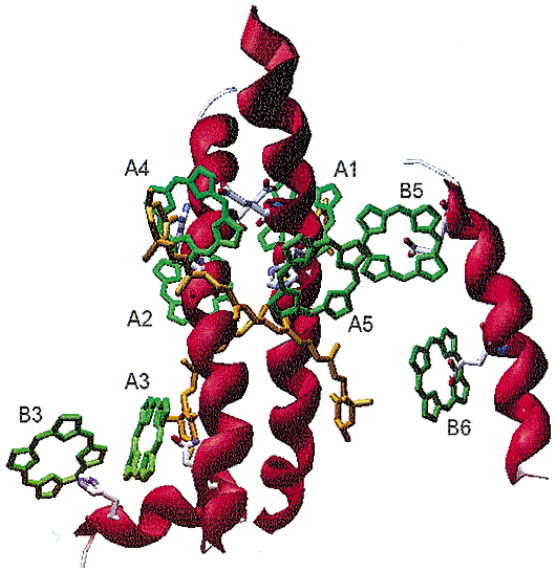
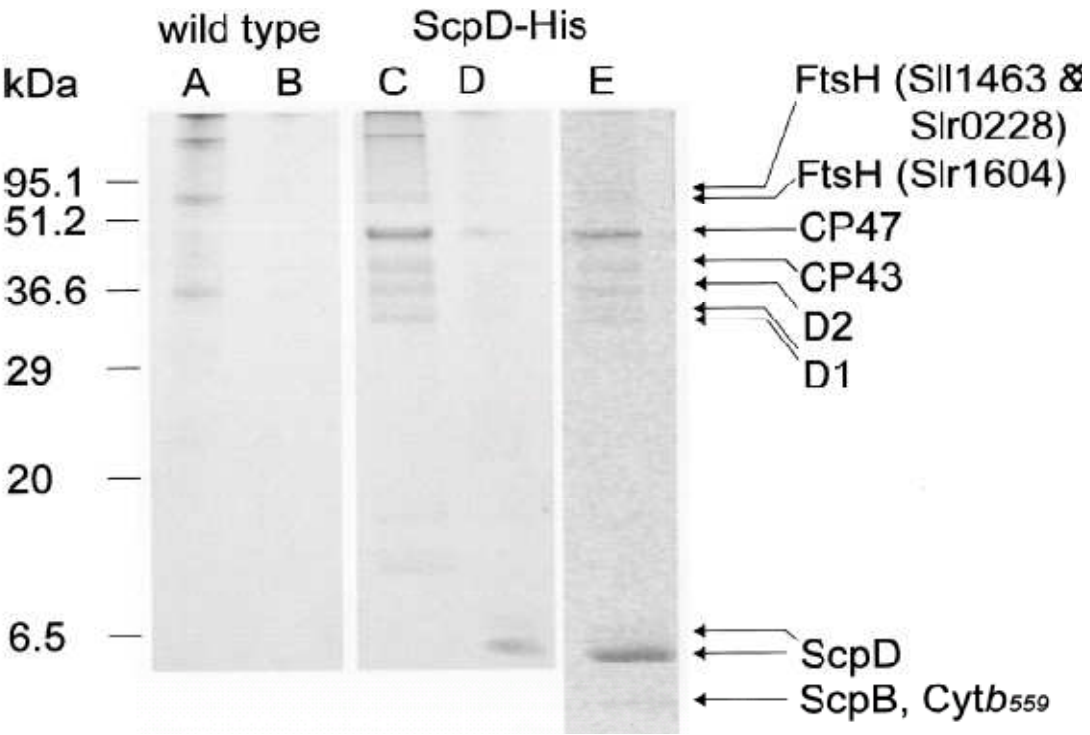
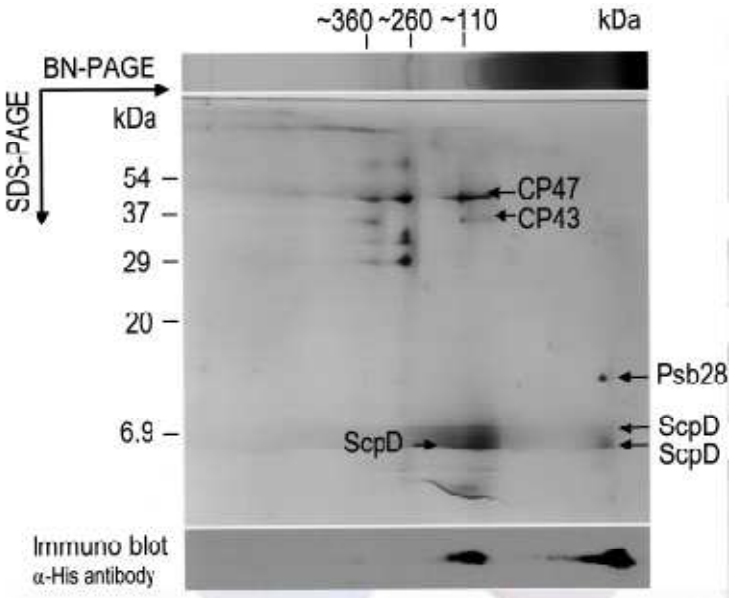


Where are SCPs located? Label ScpD with an N-terminal His tag

Structural model of LHCII (from Simonetto et al. (1999) Biochemistry 38: 12974-12983).

ScpD associates with PS II, esp. CP47





(more maltoside)

TABLE 1

Mass spectrometry identification of proteins apparently forming a complex with ScpD-His (data shown in Fig. 1)

Experimental/theoretical mass	ORF[a]	Gene product	Mascot search and score[b]	Sequence coverage	r.m.s. error
				%	*ppm*
95.1/76.8 kDa	slr0798	Hypothetical protein	SQ 193	35	14
88.1/74.3 kDa	sll1021	Hypothetical protein	PMF 249	44	7
76.7/68.1 kDa	sll1463	FtsH	SQ 190	32	14
76.7/68.4 kDa	slr0228	FtsH	PMF 150	39	17
70.8/67.1 kDa	slr1604	FtsH	PMF 83	24	23
47.3/55.8 kDa	slr0906	CP47	SQ 228	39	15
47.3/52.4 kDa	slr0909	Hypothetical protein	SQ 142	40	11
39.8/51.8 kDa	sll0851	CP43	SQ 143	17	25
36.7/39.4 kDa	sll0849/slr0927	D2	SQ 157	22	15
36.7/35.6 kDa	slr1128	Hypothetical protein	PMF 173	51	13
34.3/39.6 kDa	sll1867	D1	SQ 78	13	11
33.5/39.6 kDa	sll1867	D1	SQ 132	20	10
6.9/7.7 kDa	ssr2595	ScpD	SQ 112	50	13
6.0/7.7 kDa	ssr2595	ScpD	SQ 131	50	32
4.8/7.7 kDa	ssl1633	ScpB	MIS 105	18	
4.8/4.8 kDa	smr0006	Cytochrome b_{559}	MIS 109	38	

[a] ORF, open reading frame; r.m.s., root mean square; SQ, sequence query including peptide mass fingerprint (PMF) and MS/MS ion search (MIS) data.
[b] Identified by a search in the NCBInr Database.

(Yao et al., JBC 2007)

Working hypothesis: ScpD/C temporarily stores chlorophyll when PS II is being repaired

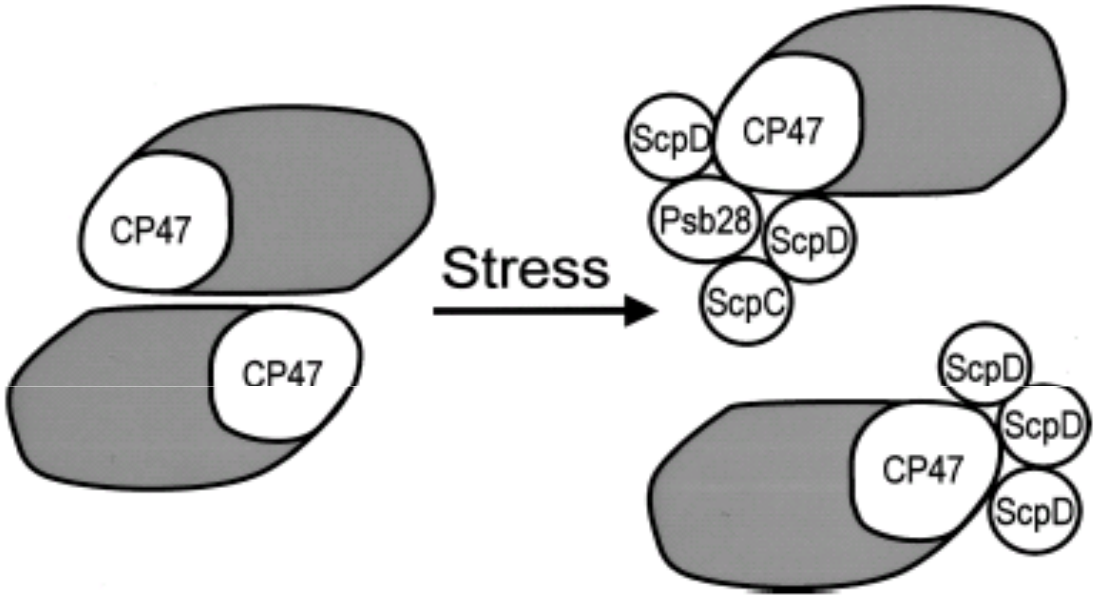
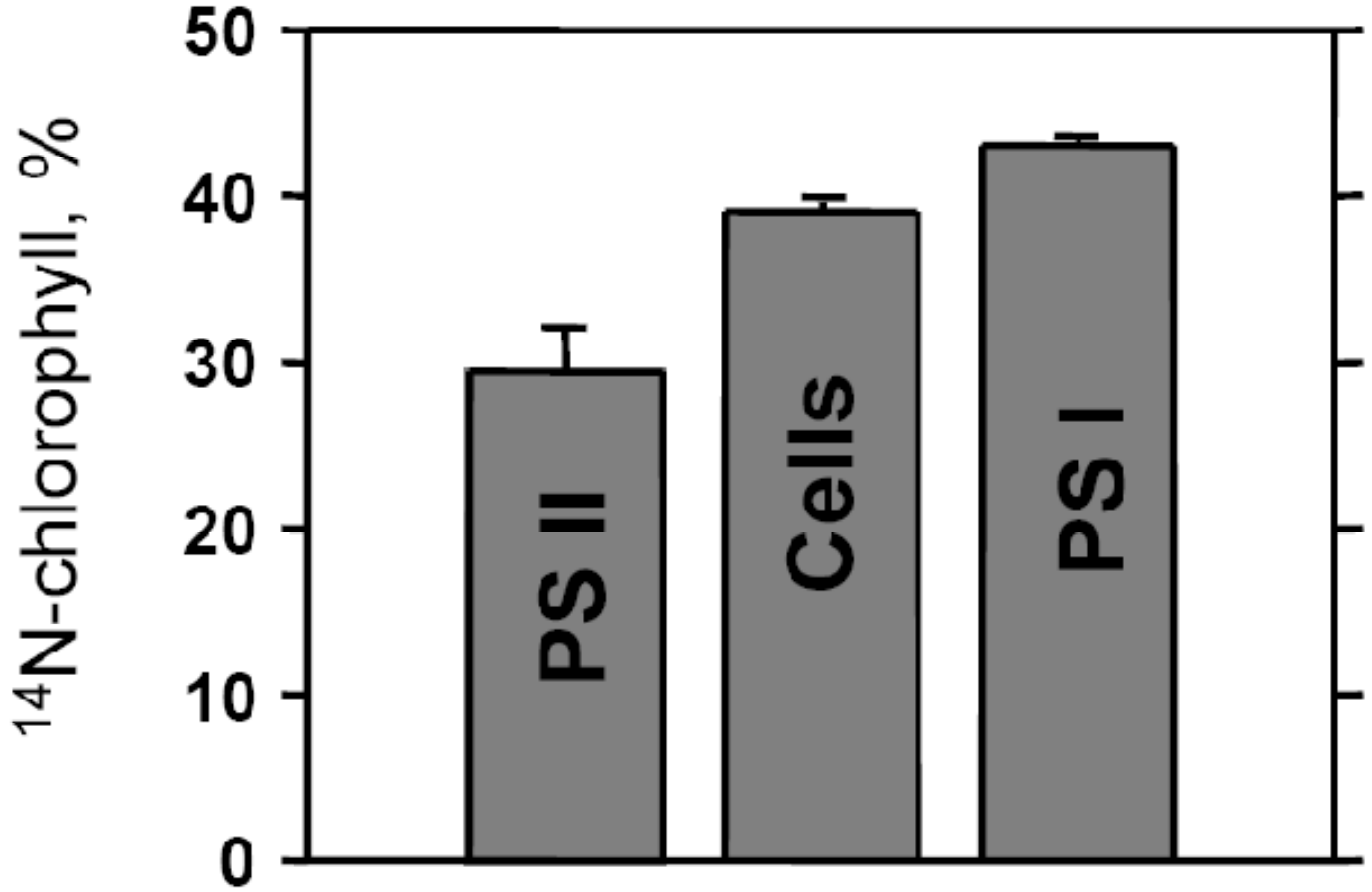


FIGURE 7. **Model of ScpD binding to PSII.** Stress conditions cause PSII monomerization. ScpD and probably also ScpC bind to the monomers and are located close to CP47. Substoichiometric Psb28 might stabilize the binding between CP47 and ScpC/ScpD.

(Yao et al., JBC 2007)

Chlorophyll is more stable in PS I than in PS II:

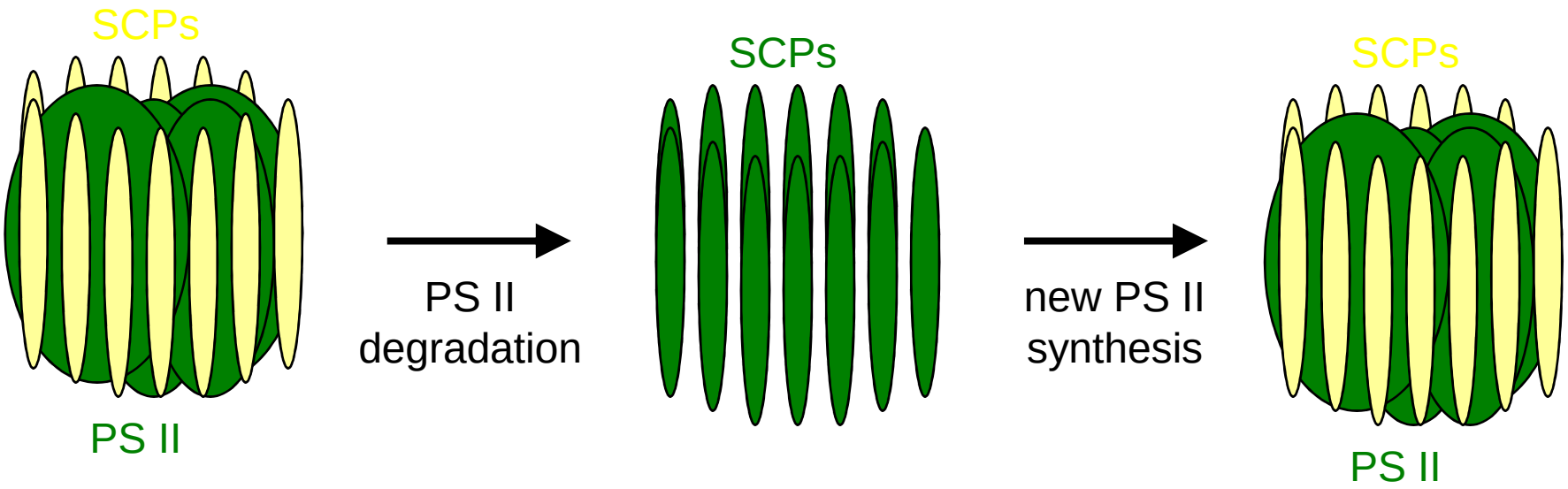
Grow cells for 14 hours after adding ^{15}N, and monitor the amount of labeled chlorophyll in the two photosystems: PS I chlorophyll barely turned over, but part of PS II chlorophylls was replaced.



Vavilin et al., JBC, 2007

Bottom line:

- PS II chlorophylls turn over more rapidly than PS I associated chlorophylls, and SCPs play a major role in stabilizing PS II-associated chlorophylls (Vavilin et al. (2007) JBC 282, 37660-37668)
- At least some of the SCPs are associated specifically with PS II (Promnares et al. (2006) JBC 281, 32705-32713; Yao et al. (2007) JBC 282, 267-276)
- No effect of SCPs on PS I chlorophyll lifetimes
- SCPs may serve as temporary binding sites for chlorophyll (near carotenoids?) as PS II is repaired; important for keeping chlorophyll from being toxic in the light in the presence of O_2



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- The “STINT SCP consortium”: Christiane Funk (Umea), coordinator

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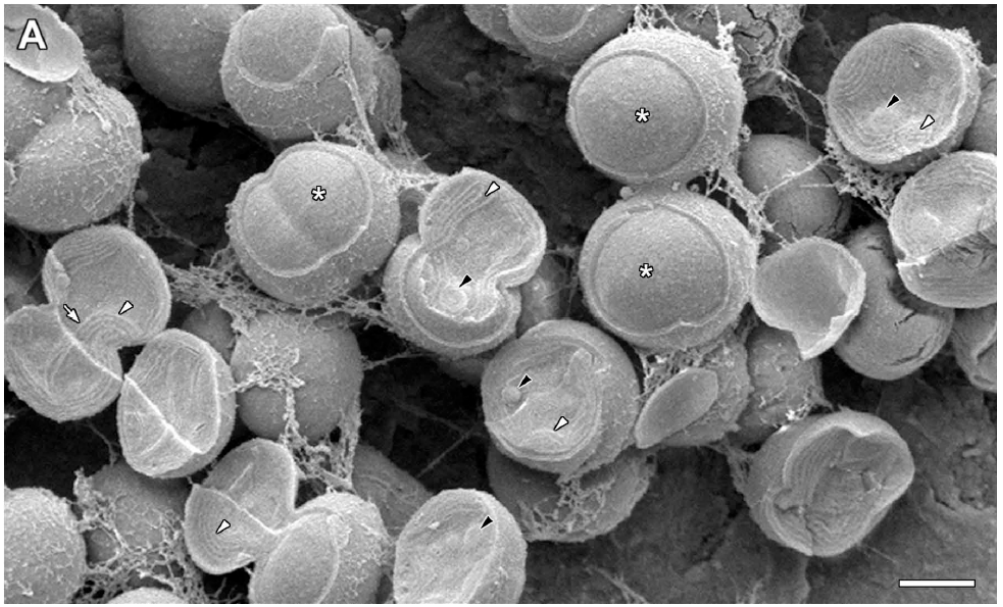
and *Synechocystis*



and last but not least:

Gordon Research Conferences

frontiers of science

2008 Photosynthesis Gordon Conference

When? June 22-27, 2008

Where? Mount Holyoke College, South Hadley, Massachusetts

For more info: Chair: Wim Vermaas (wim@asu.edu)

New: June 21-22, 2008, before the Gordon Conference, we'll have the Gordon-Kenan **Graduate Research Seminar on Photosynthesis and Bioenergy** (see http://www.grc.org/programs.aspx?year=2008&program=grad_photo). This seminar is specifically for graduate students and starting postdocs. Graduate Research Seminar participants are recommended to participate in the Photosynthesis Gordon Conference as well.