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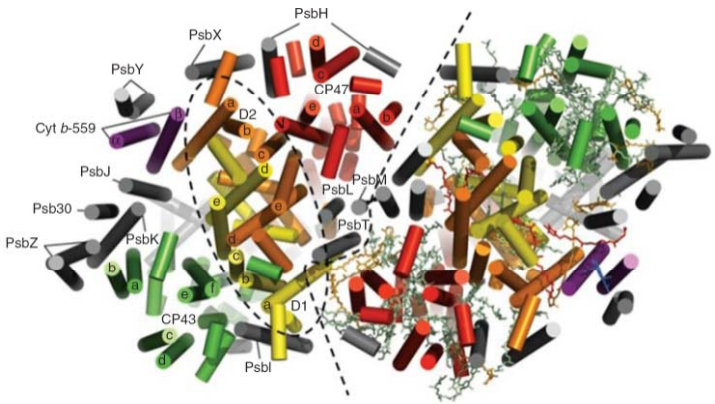
Beijing, China

Imperial College London

Isolation and characterisation of Photosystem II assembly complexes from the cyanobacterium *Synechocystis* sp. PCC 6803

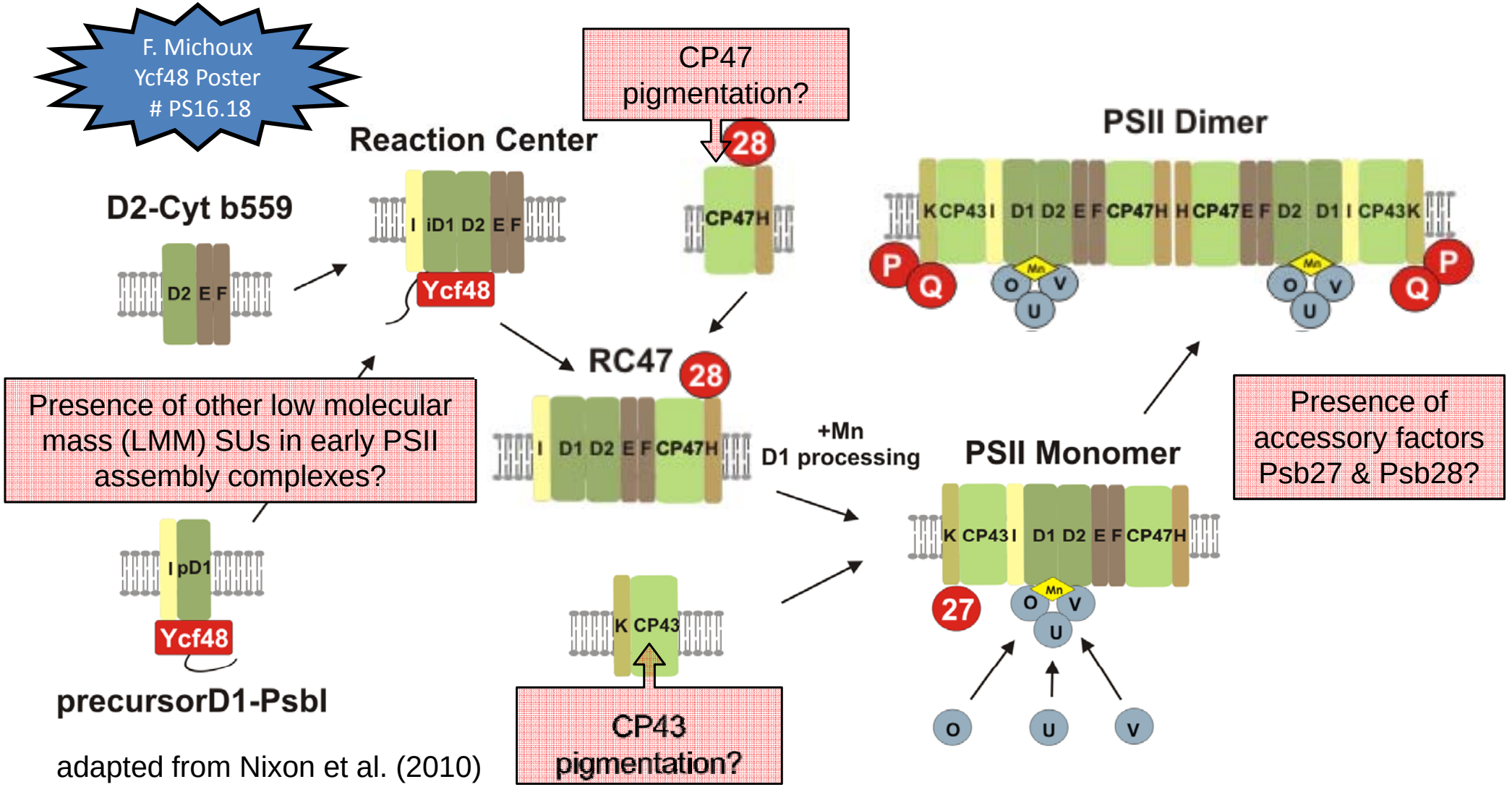
Marko Boehm



Introduction

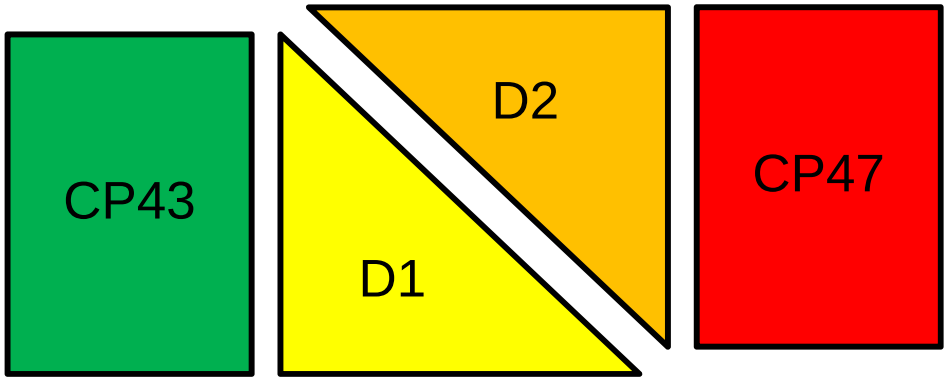
Cyanobacterial photosystem II (PSII) consists of more than 20 subunits.

High-resolution structural information is available (3.5 Å Ferreira et al., 2004; 2.9 Å Guskov et al., 2009), but the molecular details of the PSII protein complex assembly are still largely unclear.



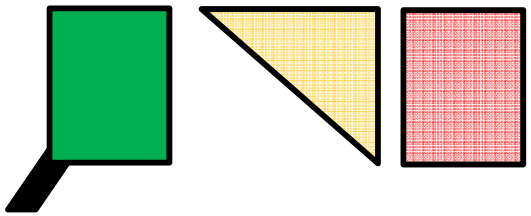
adapted from Nixon et al. (2010)

Mutant generation strategy

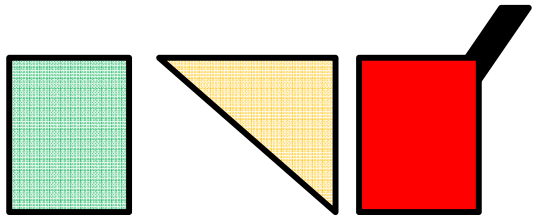


Generation of His-tagged PSII complexes
in mutants that are blocked during PSII assembly.

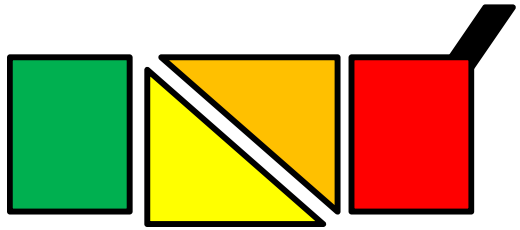
CP43-His
isolated from
ΔD1,
CP43-HT



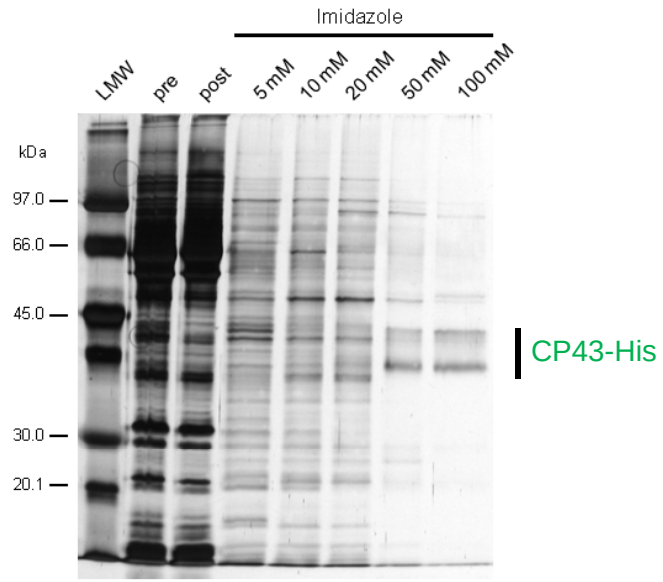
CP47-His
isolated from
ΔD1,
CP47-HT



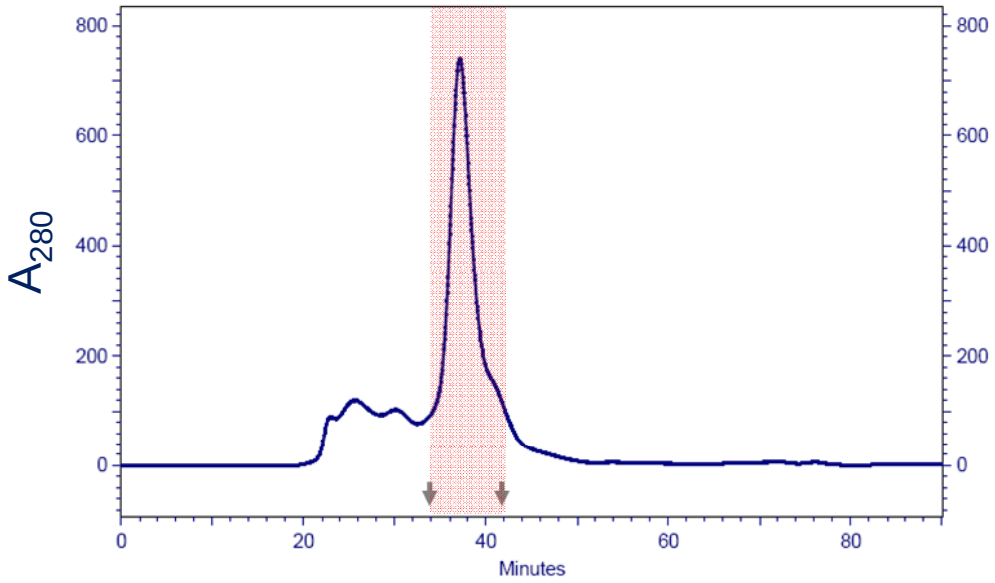
RC47-His
isolated from
ΔCP43,
CP47-HT



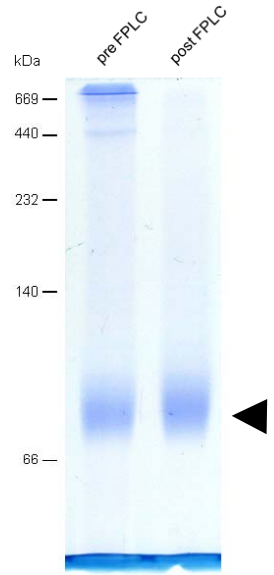
Isolation of CP43-His and CP47-His



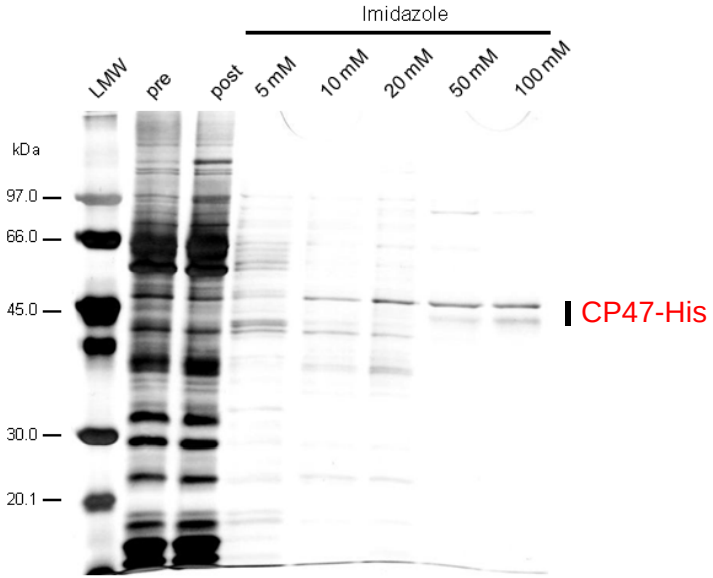
1. His-tag purification

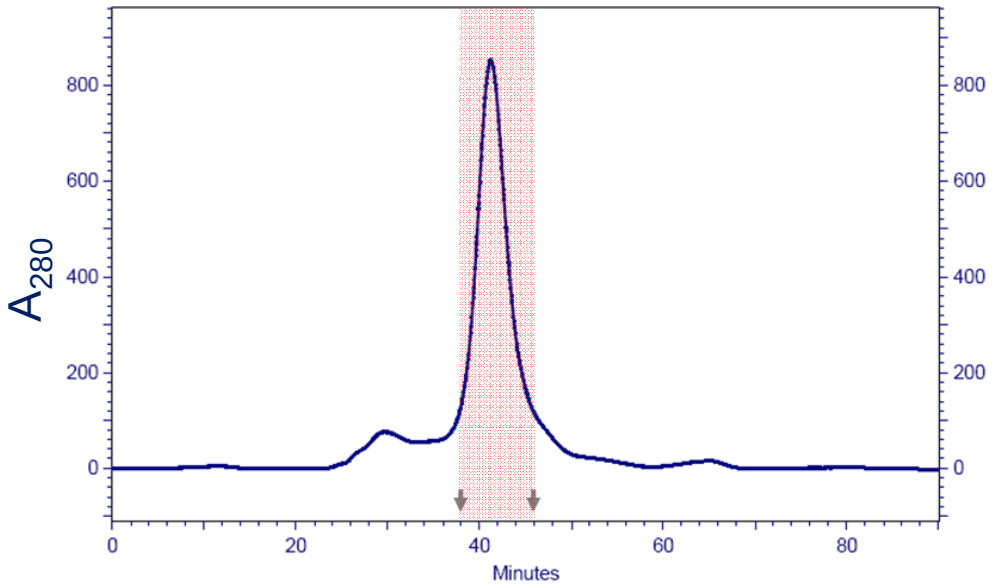


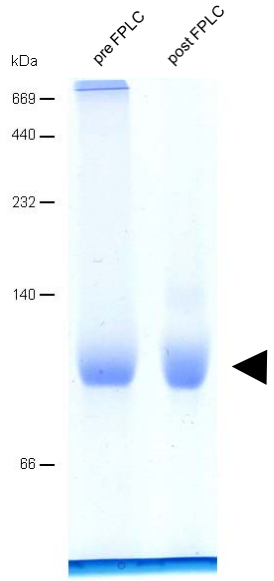
2. FPLC size exclusion



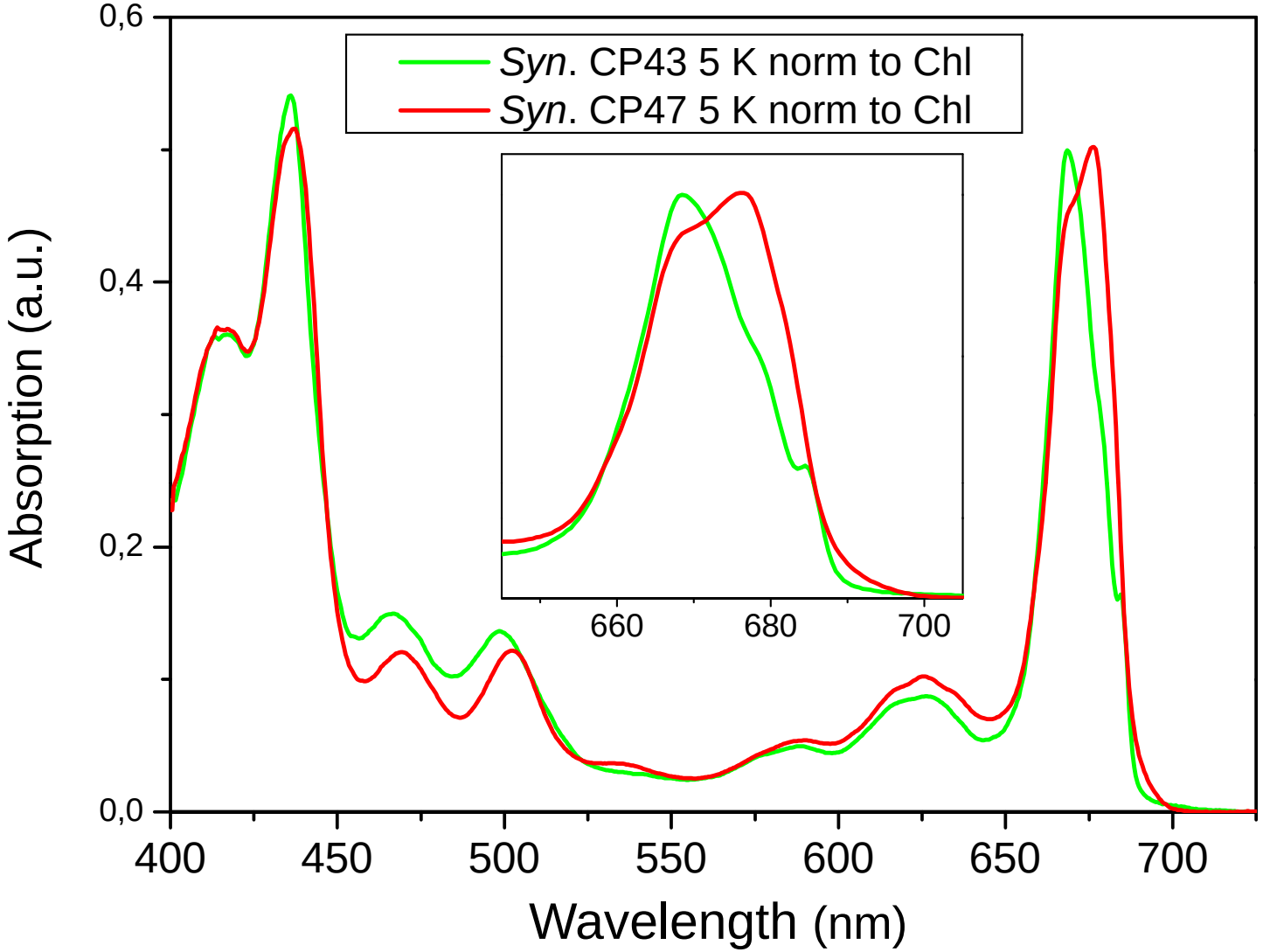
3. BN PAGE





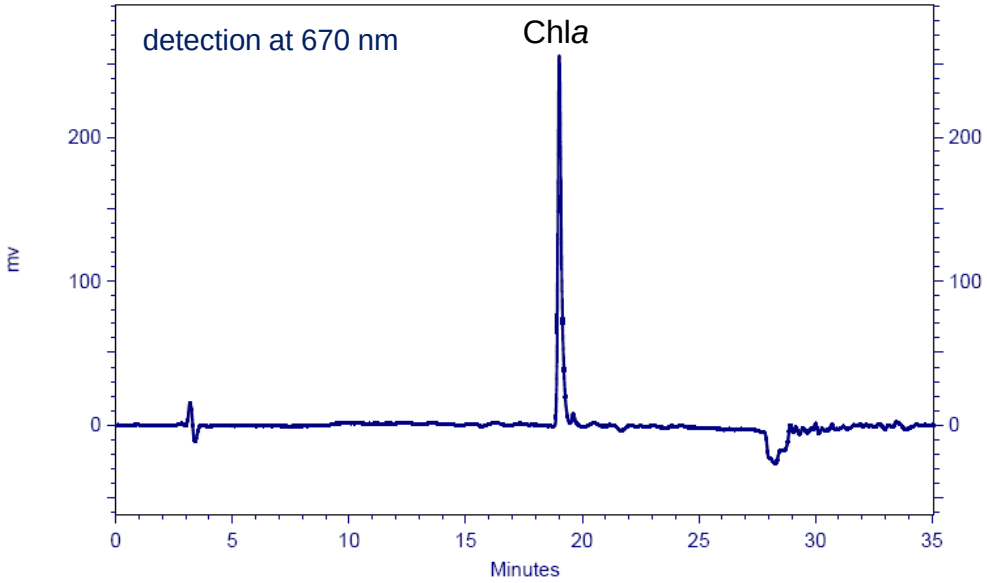


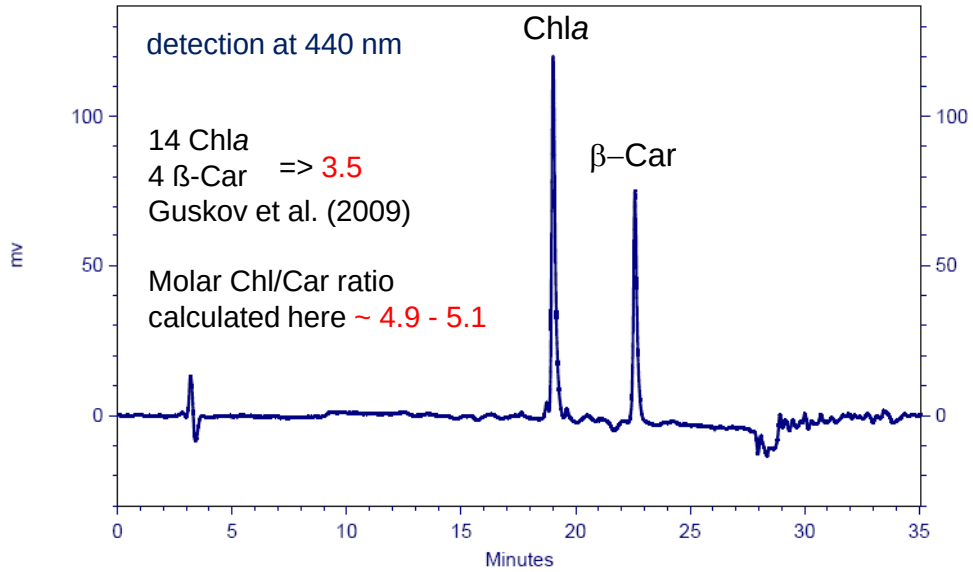
Unassembled CP43 and CP47 contain pigments



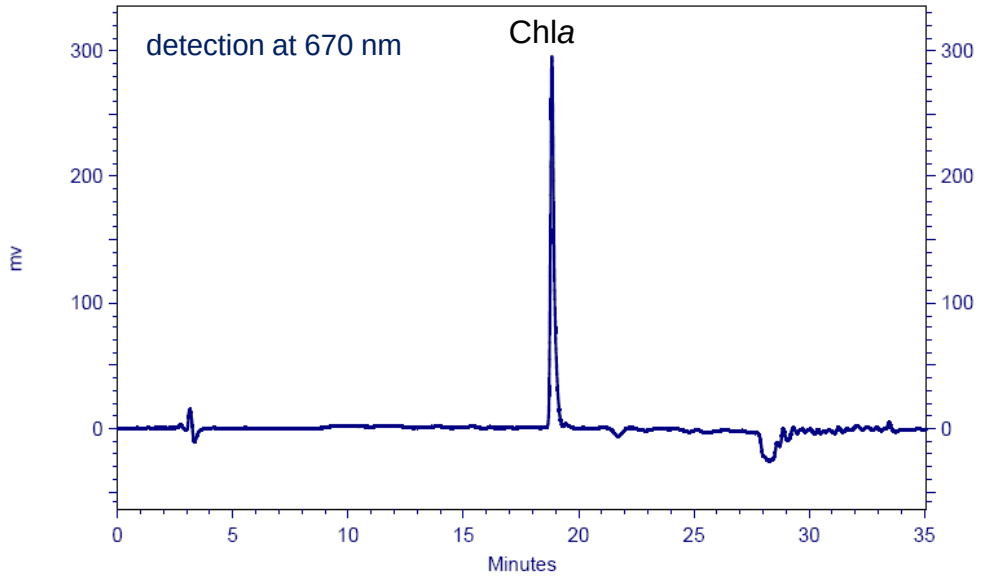
Assessing the Chl/Car ratio

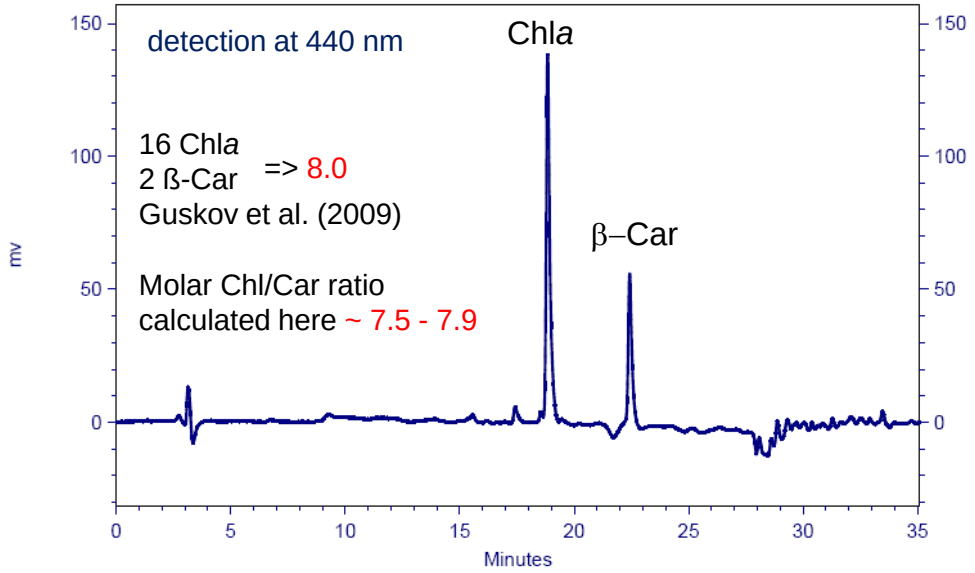
CP43





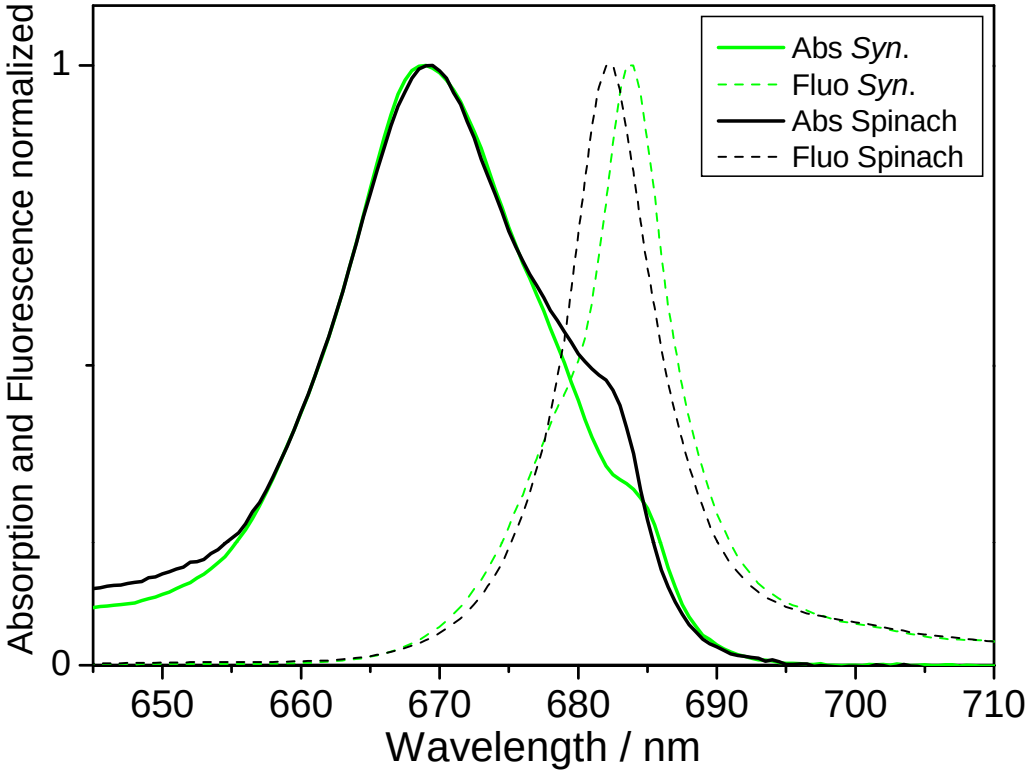
CP47



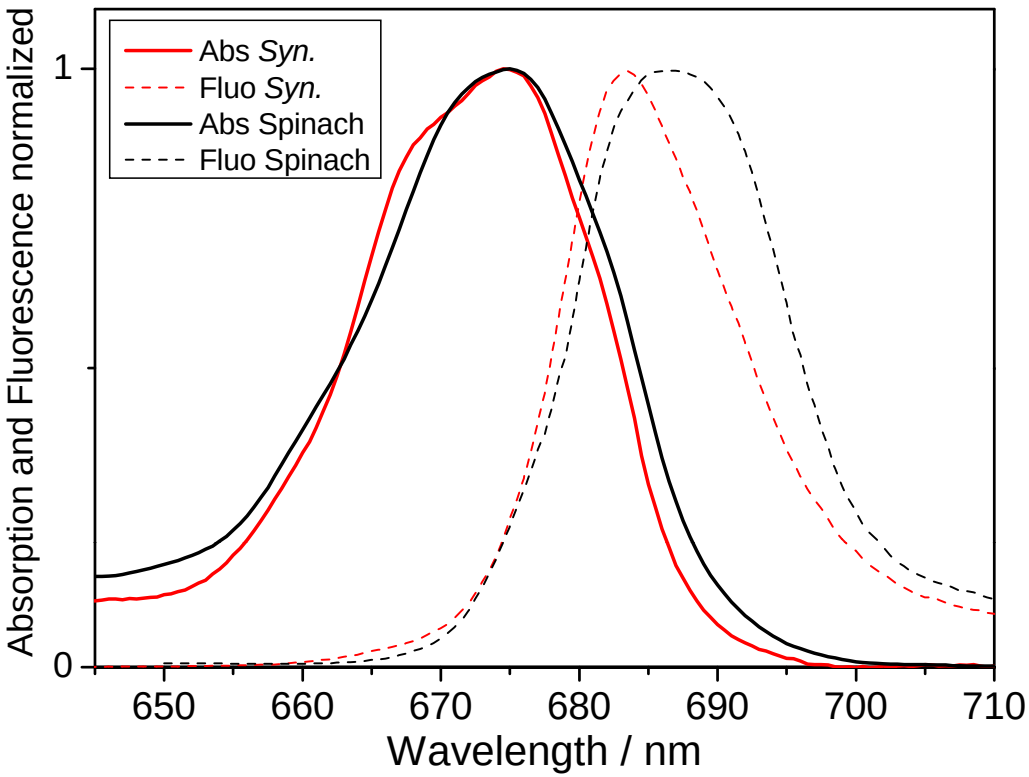


CP43 and CP47: *Synechocystis* vs Spinach

The fluorescence spectra provide further evidence that the pigments are protein-bound.



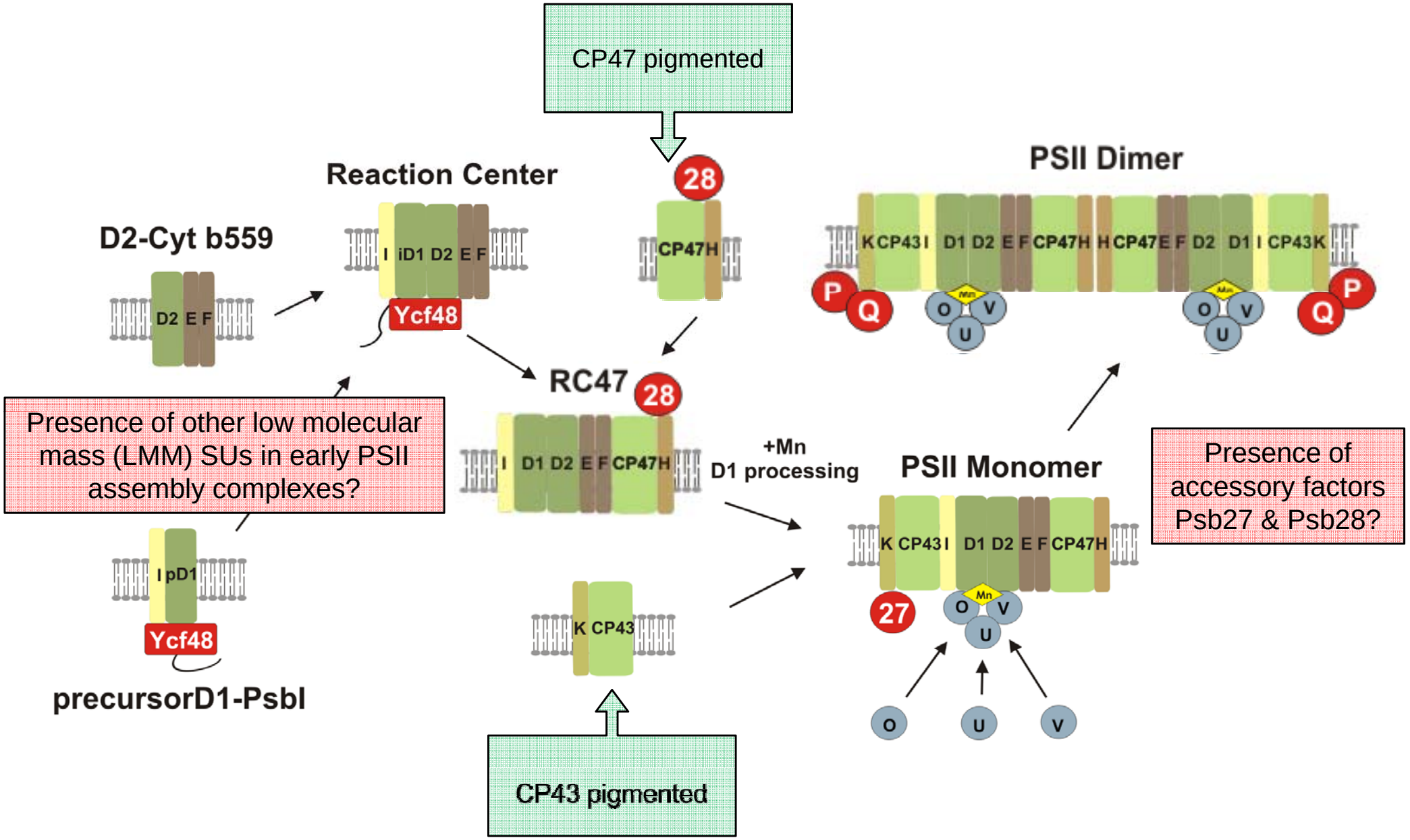
CP43 *Synechocystis* vs Spinach
2 nm red shift
Fluoresence Quantum Yield (FQY):
FQY (*Syn*) = ~ ¾ FQY (Spinach)



CP47 *Synechocystis* vs Spinach
1 – 3 nm blue shift
Fluoresence Quantum Yield (FQY):
FQY (*Syn*) = ~ ½ FQY (Spinach)

Overall, the spectroscopic properties of the inner antenna proteins CP43 and CP47 appear to be similar, but not identical, between *Synechocystis* and Spinach.

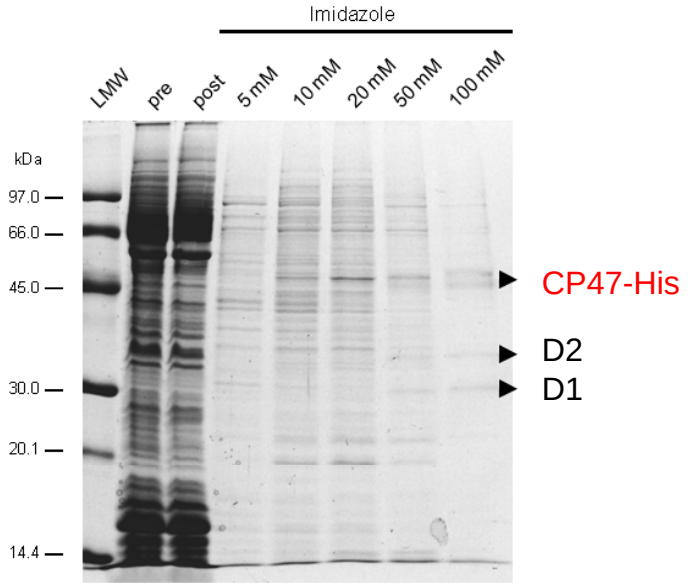
Conclusions I

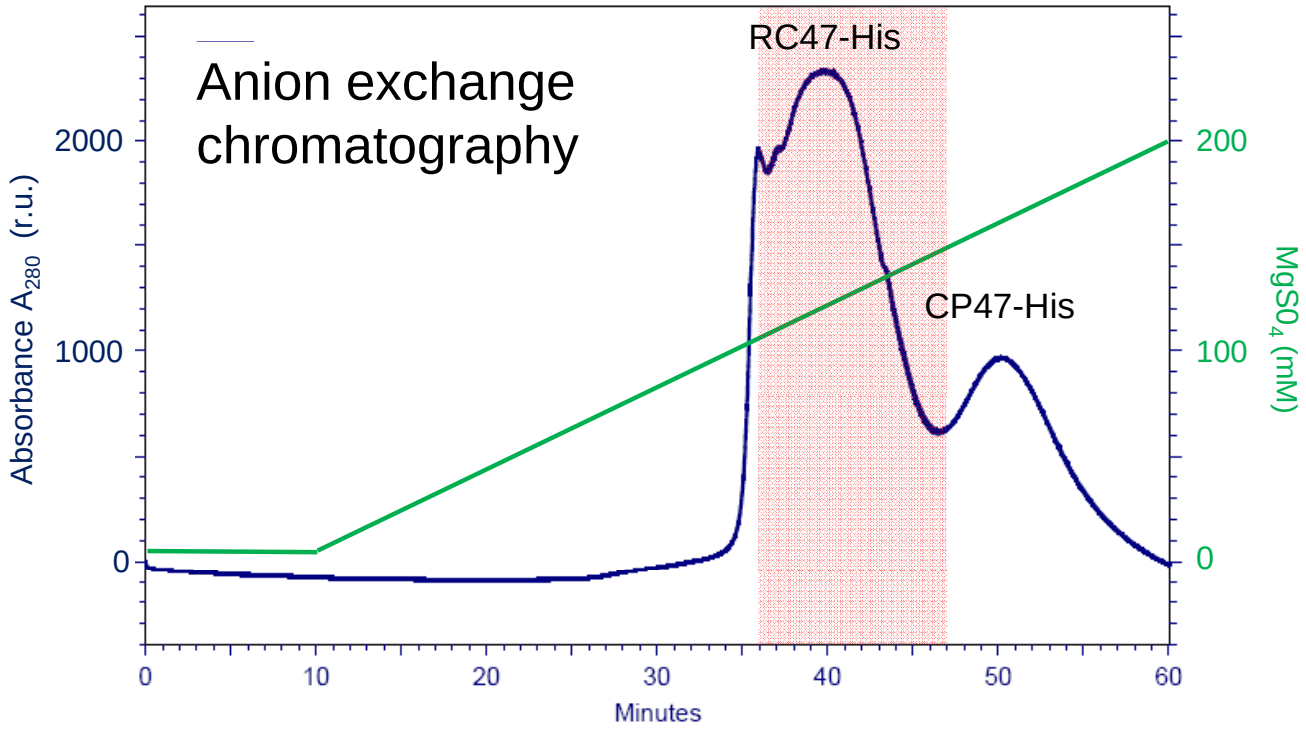


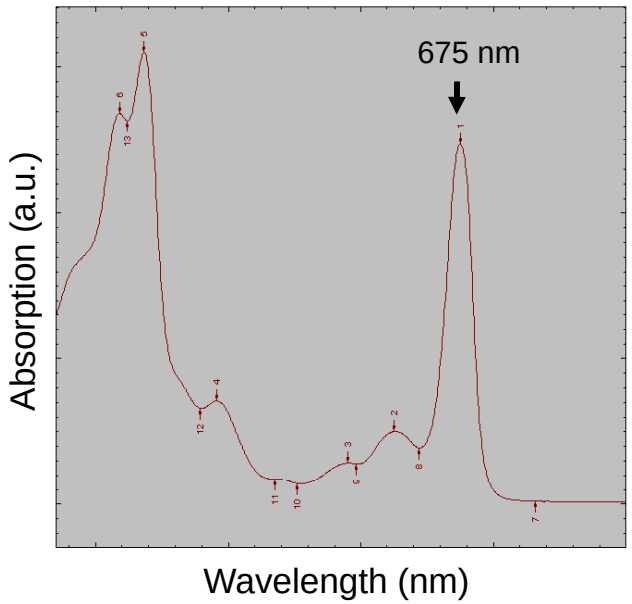
Isolation of RC47-His

Earlier work by Roegner et al. (1991):

- RC47 isolated from a CP43-less mutant.
- RC47 preparation was photochemically active, forming Y_z^+ / Q_A^-
- Presence of LMM SUs was not investigated.

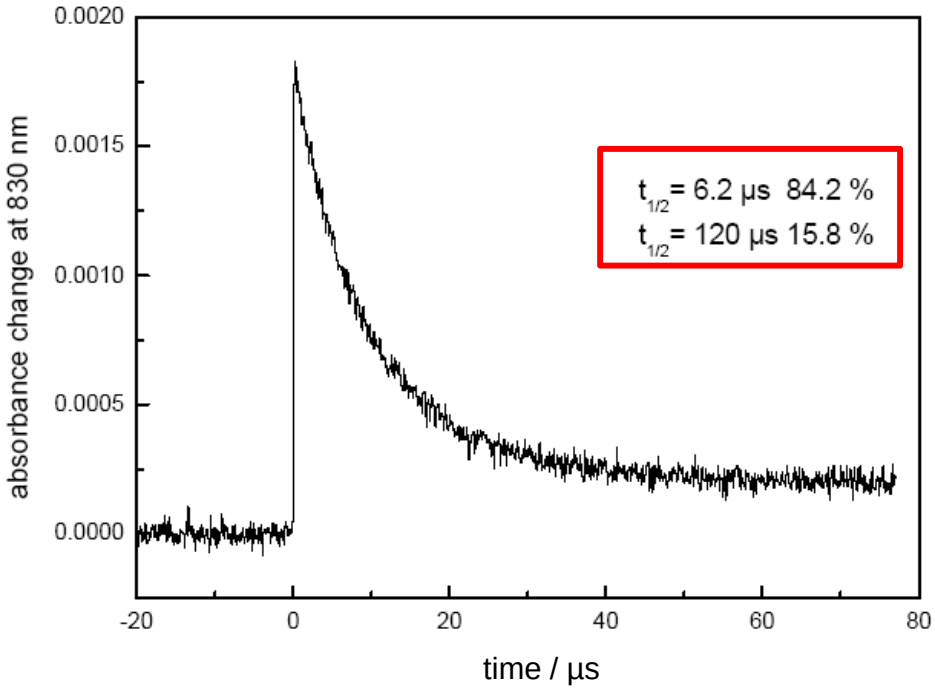




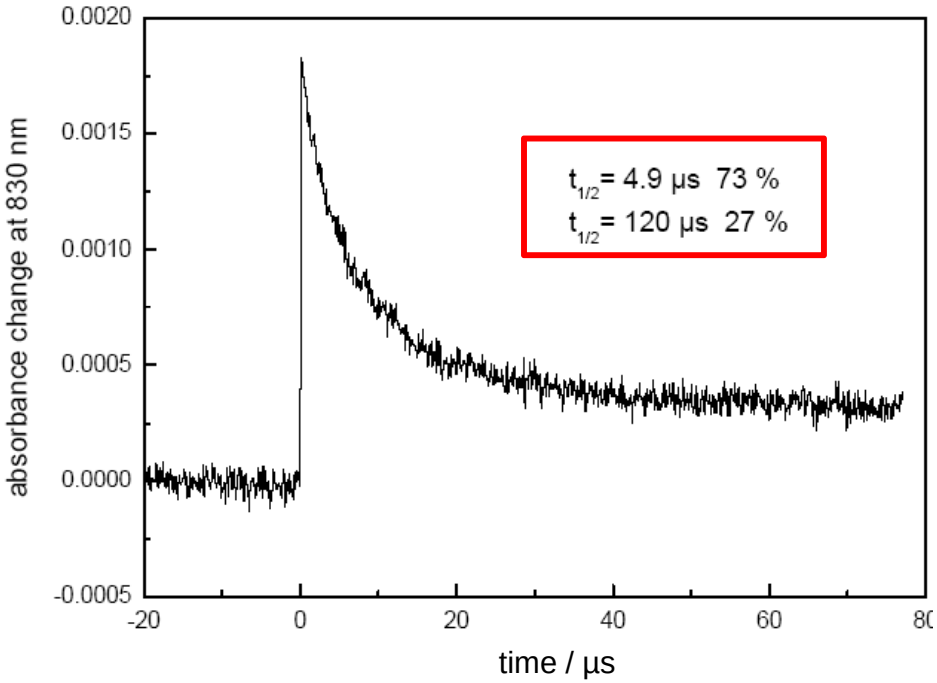


RC47-His is photochemically active

RC47-His



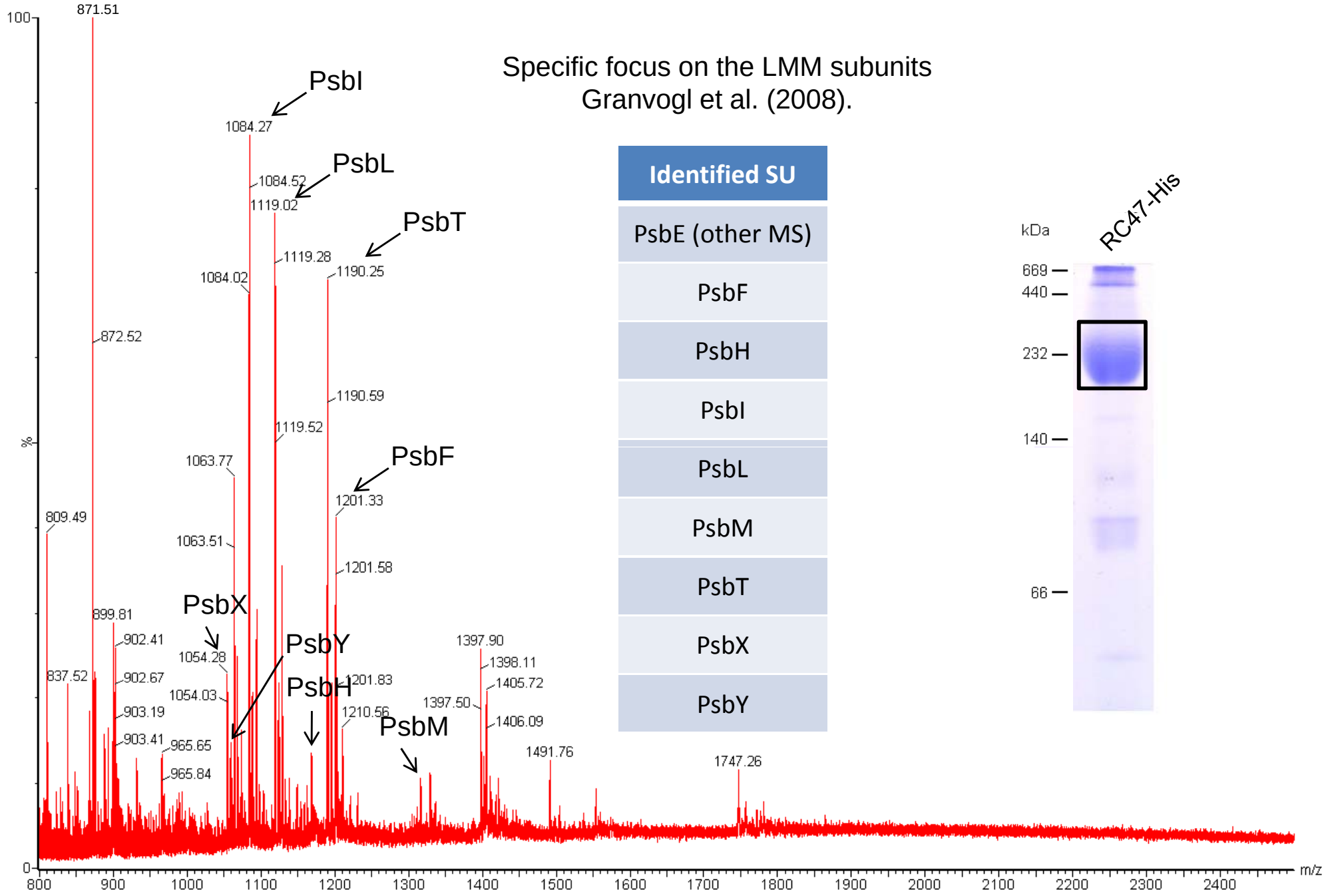
PSII-His
(non-O_2 evolving)



The kinetics of the absorbance decay at 830 nm are indicative of electron transfer from Tyr_z to $P680^+$ and were found to be similar for both RC47-His and non-oxygen evolving PSII complexes.

The absorbance decay in oxygen-evolving PSII dimers typically occur on the ns timescale ($t_{1/2}$ = 20 ns 49.3 %; $t_{1/2}$ = 200 ns 24.5 %; $t_{1/2}$ n.d. 26.2 %; data not shown).

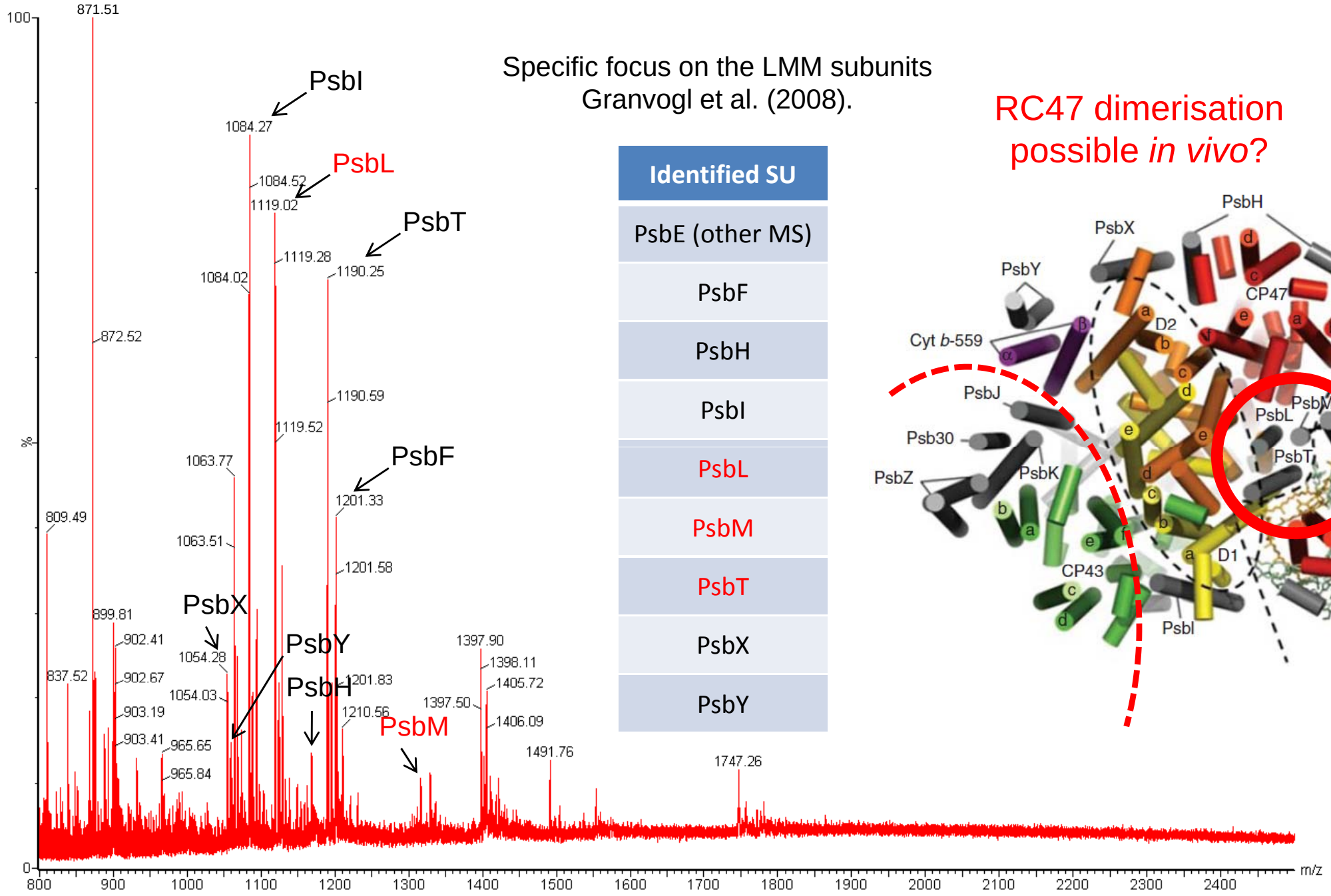
Identification of LMM SUs in RC47-His



Specific focus on the LMM subunits
Granvogl et al. (2008).

Identified SU
PsbE (other MS)
PsbF
PsbH
PsbI
PsbL
PsbM
PsbT
PsbX
PsbY

Identification of LMM SUs in RC47-His

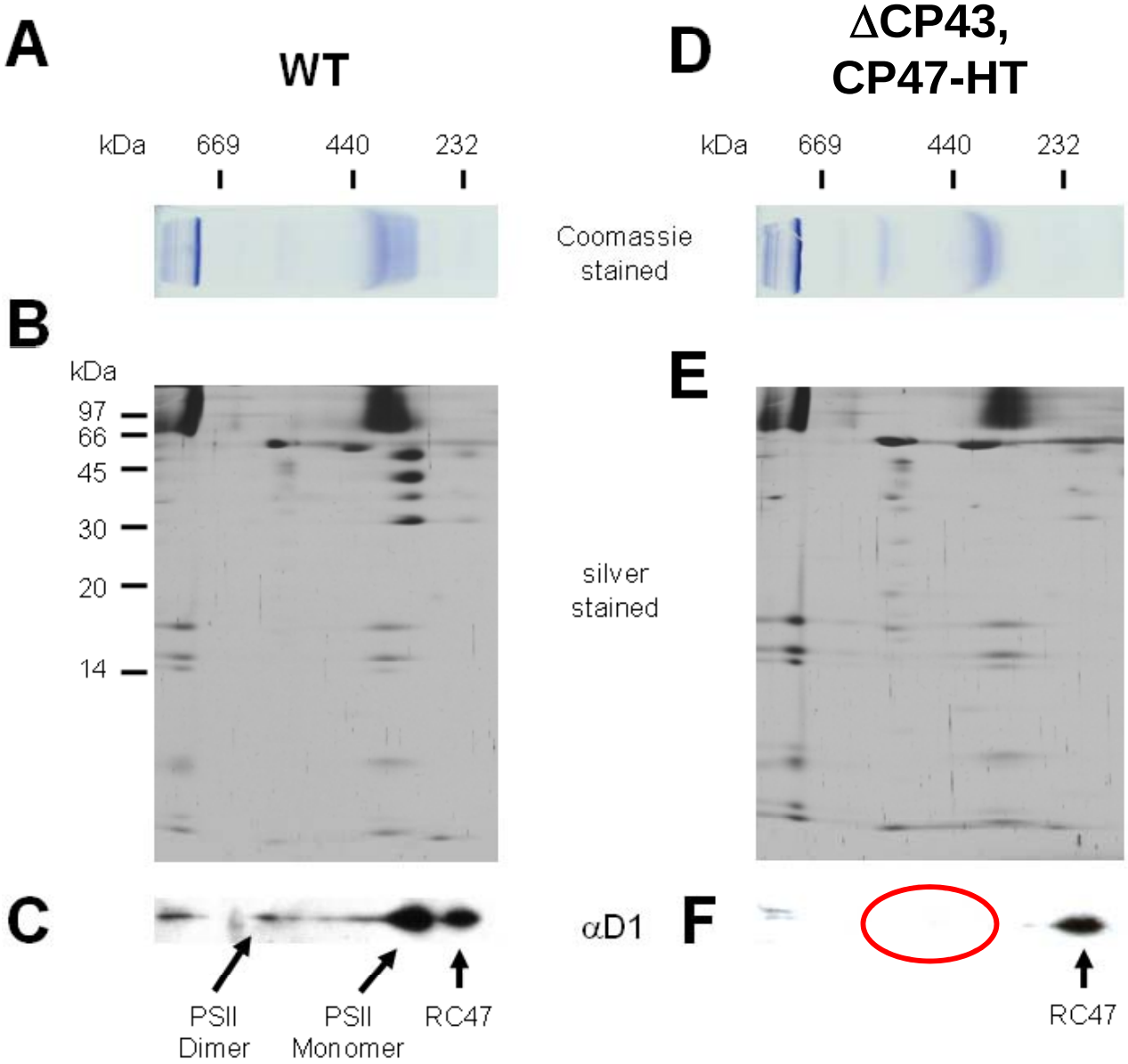


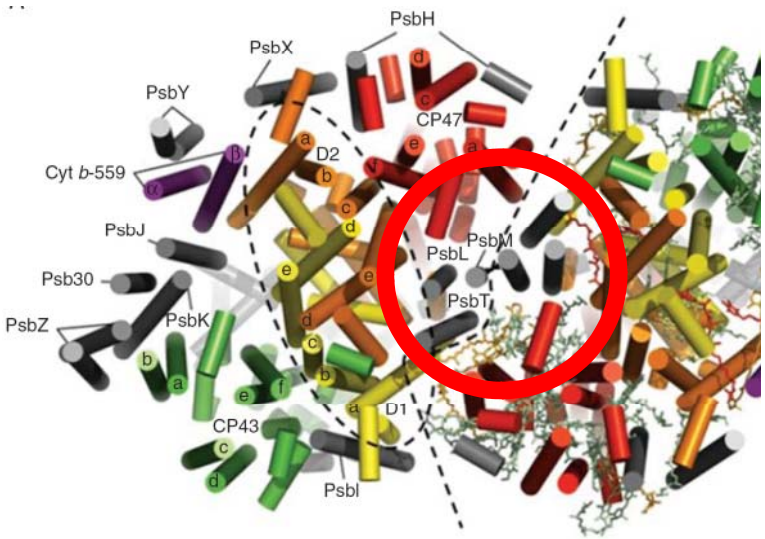
Specific focus on the LMM subunits
Granvogl et al. (2008).

Identified SU
PsbE (other MS)
PsbF
PsbH
PsbI
PsbL
PsbM
PsbT
PsbX
PsbY

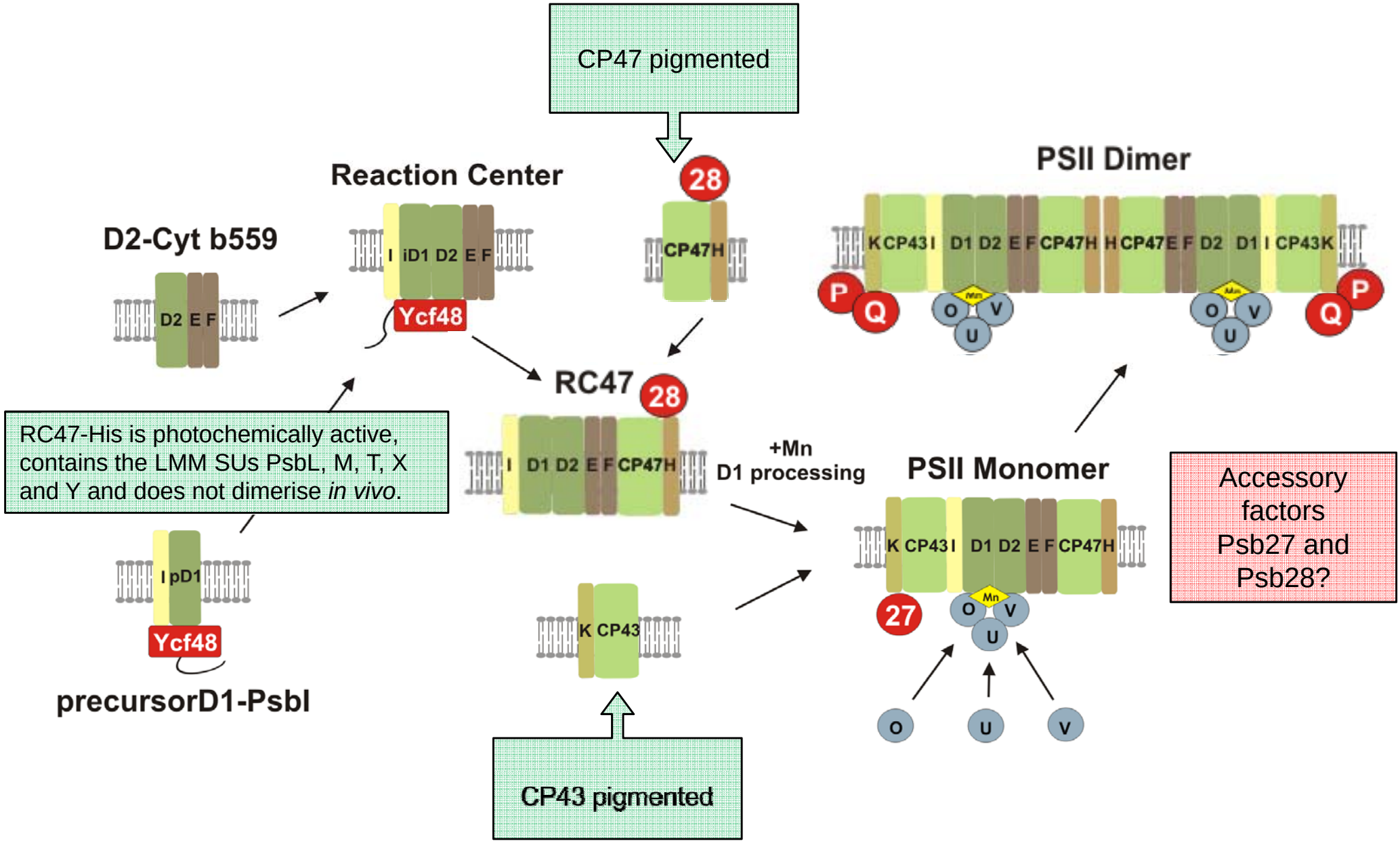
RC47 dimerisation possible *in vivo*?

RC47-His does not seem to dimerise *in vivo*

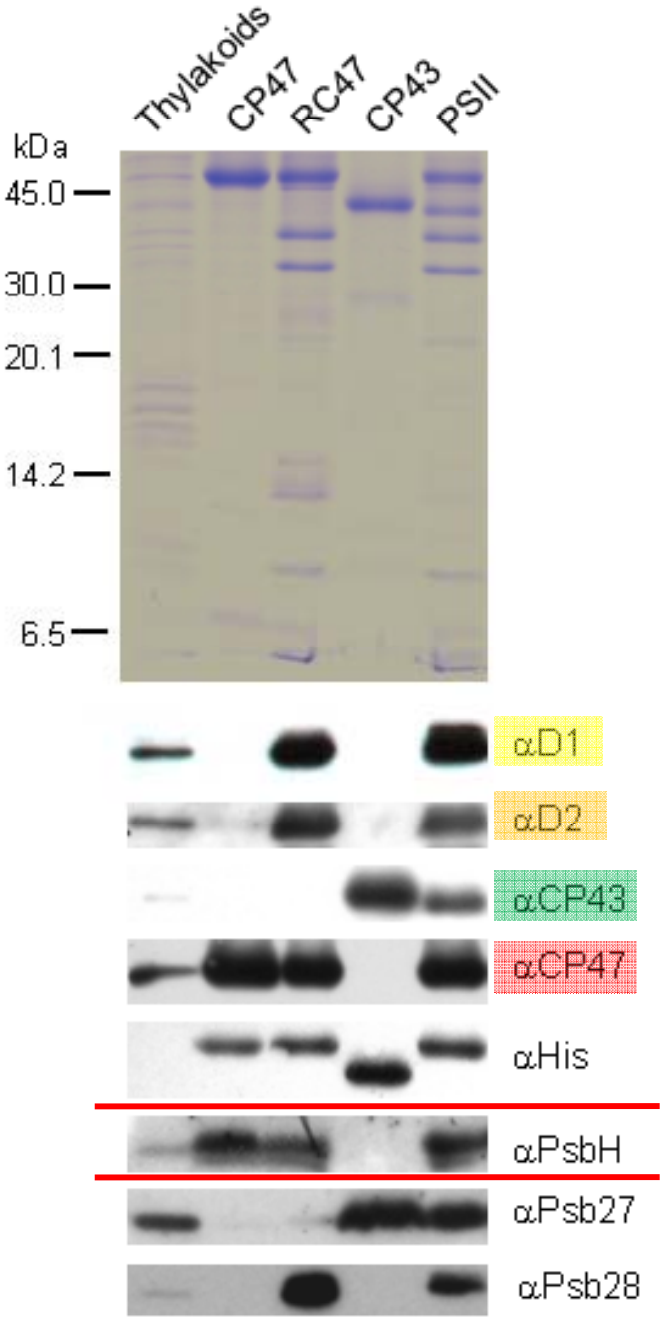


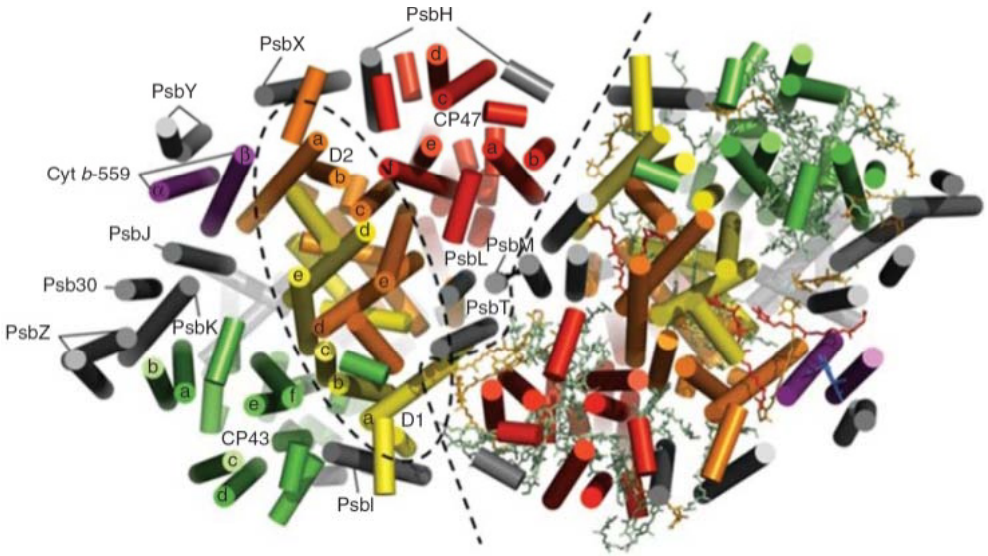


Conclusions II



Co-purification of LMM SUs and accessory factors





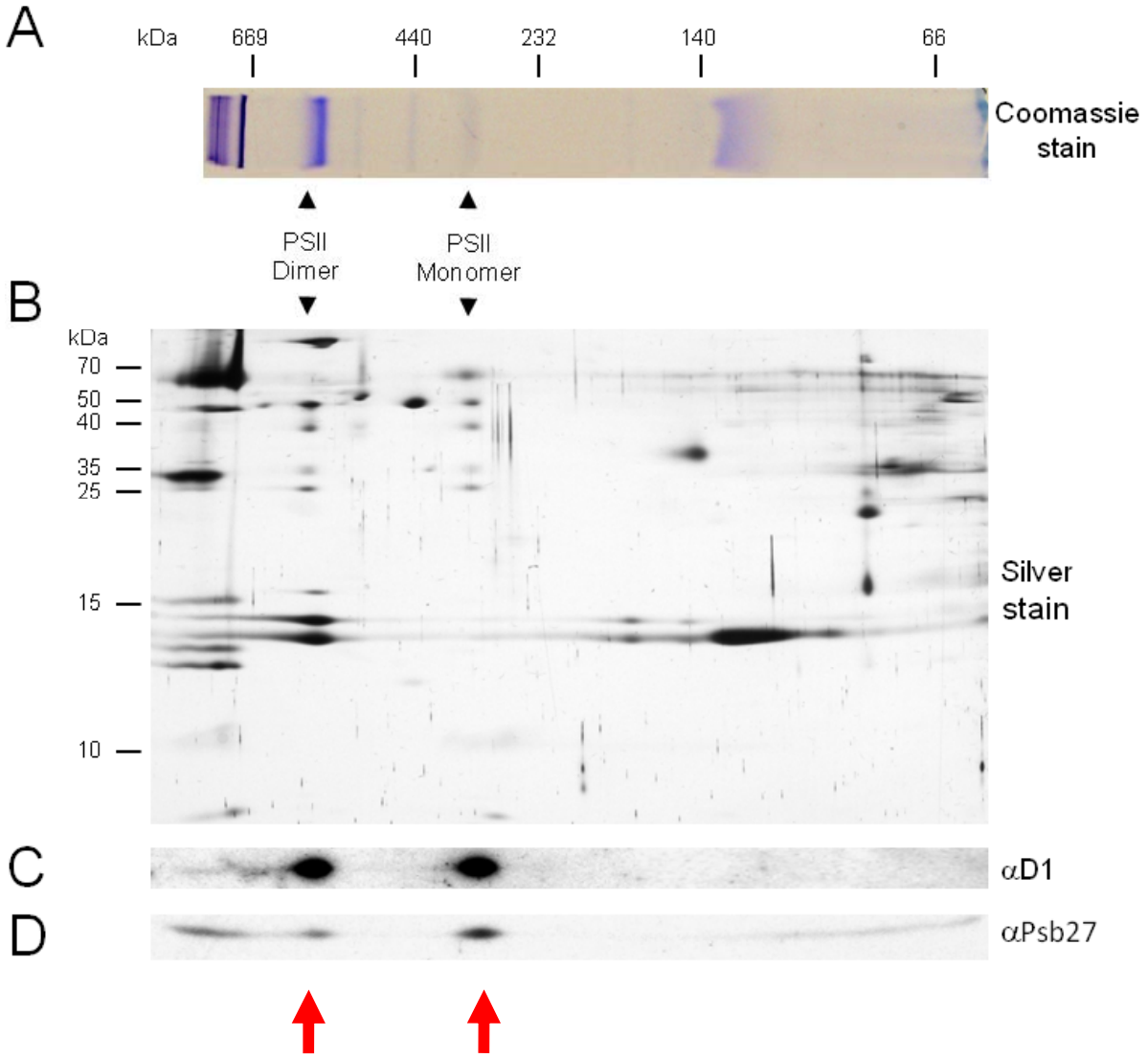
Reaction center core subunits

LMM subunit

Accessory factors

The immunoblot results for Psb27 and Psb28 could also be confirmed by mass spectrometry on BN PAGE gel bands.

Psb27 is localised in both PSII monomers and dimers



Psb27 has previously been reported to be present in inactive PSII monomers (Nowaczyk et al., 2006).

We performed a 2D BN/SDS PAGE analysis of *T. elongatus* thylakoid membranes followed by immunoblotting.

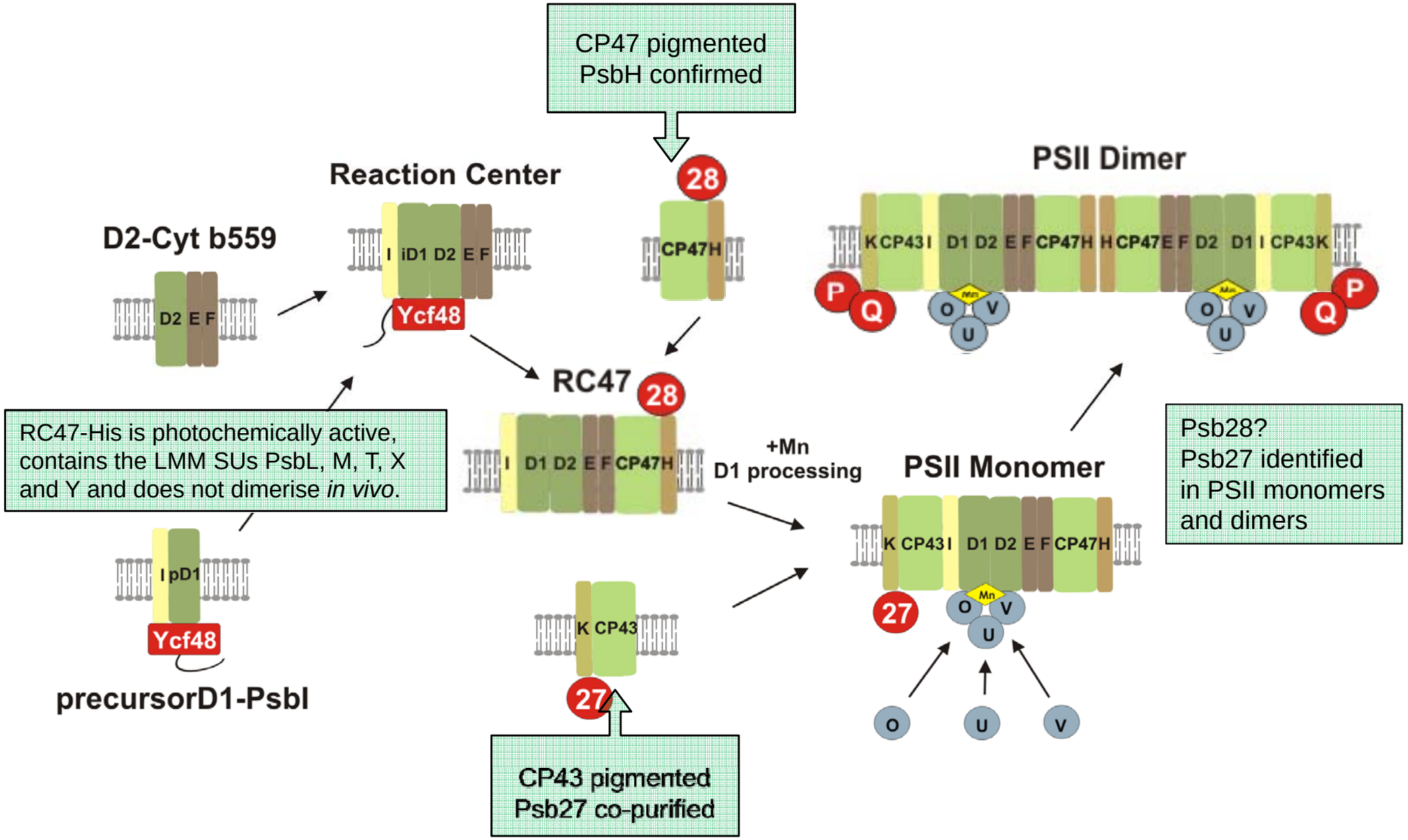
Psb27 seems to be present in PSII monomers and dimers.

The protein does not appear to be present in stoichiometric amounts and could be part of a specific subpopulation of PSII dimers.

It is unclear whether the Psb27 containing PSII complexes identified in this analysis are oxygen evolving.

F. Michoux
Psb27 Poster
PS16.7

Conclusions III



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BBSRC bioscience for the future