

Chapter 9

Isolation and Spectral Characterization of Chlorophyll-Protein Complexes of Chloroplast Thylakoid Membranes

William E. Dietrich, Jr.

Department of Biology
Indiana University of Pennsylvania
975 Oakland Ave.
Indiana, PA 15705-1090
dietrich@iup.edu
(724) 357-2625

Bill Dietrich is a Professor of Biology and Biochemistry. He presently teaches Cell Biology for nursing and allied health professions students, Botany for biology majors, and Biochemistry for biochemistry majors. He is co-founder of the IUP undergraduate B.S. in Biochemistry program. He is working on a cell biology textbook for Allied Health Professions students. His research interests include chlorophyll-proteins of cyanobacteria and the proteins of paper wasp nests.

Reprinted From: Dietrich, W. E. jr. 2003. Isolation and spectral characterization of chlorophyll-protein complexes of chloroplast thylakoid membranes. Pages 131-144, in Tested studies for laboratory teaching, Volume 24 (M. A. O'Donnell, Editor). Proceedings of the 24th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 334 pages.

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Contents

Introduction.....	132
Materials	133
Student Outline	134
Notes for the Instructor	137
Literature Cited.....	141
Acknowledgments.....	141
Appendix A. Flow Chart.....	142
Appendix B. Chlorophyll-Protein Complexes: a brief review....	143

Introduction

In the undergraduate biology laboratory, labs on photosynthesis ordinarily fall into a small number of categories:

1. measurement of the overall process of photosynthesis by carbon dioxide uptake or oxygen evolution
2. measurement of the Hill reaction (the light driven reduction of an exogenous dye with electrons from water) in isolated chloroplasts
3. pigment analysis by thin-layer or paper chromatography

Our overall goal is to introduce modern molecular techniques into the teaching of plant science at IUP. We are doing this at three levels in three courses:

- Introductory Botany (BIOL 210), a requirement for all biology majors (and usually taken by sophomores), is part of the Diversity Core of the Biology curriculum. It is an elective for biochemistry majors. The “green gel” lab presented here is part of this course. Additionally, we use PCR and Koch’s Postulates to identify *Agrobacterium* as the causative agent of sunflower tumors.
- Plant Physiology (BIOL 350/550) is an elective for both biology and biochemistry majors. We use a reporter gene to investigate gene expression, and plant tissue culture to transform a reporter gene into plant tissue.
- Laboratory Methods (BIOL 499) is a requirement for biochemistry majors and an elective for biology majors. Here we are actually introducing plant science to the lab methods curriculum. We perform *in vitro* translation of a plant virus and analyze it using Western Blots and perform a physical and restriction analysis of an amplified target gene.

Our Botany course has no distinction between lecture periods and lab periods. This lab is performed as part of the unit on photosynthesis. It is performed by groups of eight students (two groups of four) with the instructor demonstrating and “cheering them on”. We use Spinach as the prime example that we know will work, and plants grown at high and low light intensities to show the plasticity of the photosynthetic apparatus.

Prior to the lab, the instructor sets up the gel apparatus with gels. The lab is set up in a flow pattern with stations for each procedure since we have only one refrigerated centrifuge and

spectrophotometer. The electrophoresis and cutting out gel bands is done at the students' tables in groups of four.

At the beginning of the lab period the instructor briefly reminds the students of relevant lecture material, outlines the procedures, and gives safety instructions. With everyone looking on, the instructor demonstrates the procedures first and then allows the students to take over.

After the electrophoresis is completed, the instructor selects one group's gel and places it on an overhead projector for discussion and comparison. The lab continues with the measurement of absorption spectra of gel bands. The spectra are discussed during a later class period.

In our Botany course this exercise, as described here, is the goal. More advanced classes can use this as the starting point. Examination of the electrophoretic pattern and absorbance spectra of leaves subjected to various environmental perturbations can make a multi-week project. Advanced biochemistry labs can subject each band to polypeptide analysis using the LaemmLi system. Further, the Photosystem I complexes contain an active P₇₀₀ which can be detected by oxidized-minus-reduced or light-minus-dark difference spectra.

Materials

Equipment

- Blender
- Refrigerated (4°C) centrifuge with head to accept 50-mL plastic tubes
- Plastic or glass funnel
- Vortex or Glass or teflon homogenizer
- Micropipets
- Spectrophotometer and glass cuvettes
- Vertical PAGE mini-gel apparatus – with gel
- Power supply – 100 VDC

For each student group:

- One ice bucket & ice
- Two 50-mL plastic centrifuge tubes
- One glass or plastic stir rod
- One 10-mL graduated cylinder

Supplies

- Miracloth (Calbiochem #475855)
- Microfuge & tubes
- Micropipet tips

Plant Material

- Fresh, dark green leaves

Solutions

- Grinding Buffer: 50mM NaOH-Tricine (Sigma # T-5816), pH 8.0, 0.4 M sucrose, 10 mM NaCl, 10 mM MgCl₂
- Wash Buffer: 25 mM NaOH-Tricine, pH 8.0, mM Na₂-EDTA
- Extraction Buffer *minus* SDS: 6.2 mM Tris (Tris base, Sigma # T-6066), 48 mM glycine, 10% glycerol (This buffer need not have its pH adjusted.)
- absolute acetone
- 10% w/v Sodium Dodecyl Sulfate (also sodium lauryl sulfate, Sigma # L-4390)
- Electrophoresis Buffer: 6.2 mM Tris, 48 mM glycine, 1% SDS (Dilute from 10X stock solution). This buffer may be tinted light pink with phenol red or pale blue with bromphenol blue.

Student Outline

Introduction

The photosynthetic apparatus that harvests photons, carries out photosynthetic electron transport, splits water, creates the chemiosmotic proton motive force, and makes ATP is found as part of the thylakoid membranes of the chloroplast. Photosynthetic pigments are found within groups of proteins called chlorophyll-protein complexes (CP's). Photosystem I and Photosystem II are found in different complexes. The Photosystem I complex is the largest and has only chlorophyll *a*. Photosystem II is associated with at least three complexes containing both chlorophyll *a* and *b*. Carotenoids, β -carotene, and xanthophylls are associated with both sets of complexes.

The *in vivo* absorption spectrum of the chloroplast differs from the spectra of photosynthetic pigments extracted into organic solvents. This is due to the fact that the pigments -- chlorophylls and carotenoids -- are very sensitive to their environment, and this is exhibited in their absorption spectra. Although the chlorophylls and carotenoids are lipid materials, they are not found in the lipid bilayer portion of the thylakoid membrane, but are sequestered within integral membrane protein molecules called chlorophyll proteins. This special environment creates a number of subpopulations of chlorophyll *a* and *b* and of carotenoids, resulting in absorption spectra that are quite complex. The subpopulations of chlorophylls serve to make each photosystem act as an "energy funnel," directing excitation energy to the reaction center, which is always the subpopulation of chlorophyll with the longest absorption wavelength.

The observation/isolation of the CP's is accomplished by solubilizing the hydrophobic complexes of the chloroplast membranes using a detergent, sodium dodecyl sulfate (SDS). The process requires five steps:

1. isolation of photosynthetic membranes from leaves by centrifugation
2. spectrophotometric estimation of the chlorophyll content of the isolated membranes
3. solubilization of the membranes with a buffered detergent solution
4. electrophoresis of the solubilized membranes on a polyacrylamide "green" gel
5. measurement of the absorption spectra of the isolated complexes.

A. Isolation of photosynthetic membranes

DO QUICKLY AND AT ICE TEMPERATURE

1. Derib leaves of spinach or some other dark green vegetable.
2. Wash the leaves in cold, running water; blot dry with paper toweling.
3. Tear the leaves into one-centimeter squares. Store in a plastic bag in the refrigerator until needed.
4. Place a small amount (handful - about 10 g fresh weight) of the washed leaf pieces into an *ice-cold* blender vessel.
5. Add about 50 mL (exact volume not critical) of *ice-cold* 'Grinding Buffer'.
6. Blend twice for 5 seconds each with a 30-sec. interval in between. (If you have time, save a few mL of this crude homogenate. View with the microscope.)
7. Pour through a funnel lined with 2 layers of Miracloth® directly into a plastic centrifuge tube. (Save a few mL for later microscopic viewing.)

BE SURE TO BALANCE THE CENTRIFUGE BEFORE STARTING IT!

8. Centrifuge at 3000 x g for 1 minute.
9. Quickly, but gently, pour off and discard the deep green supernatant. Retain the loose, deep green pellet of intact chloroplasts.
10. Resuspend the pellet in *ice-cold* 'Wash Buffer' by vortex or homogenizer.
11. Centrifuge at 30,000 x g for 10 min. (*Be sure to balance the centrifuge before starting it!*)
12. Discard the almost colorless supernatant and resuspend the green pellet of thylakoid membranes in a few (about 3) mL of *ice-cold* 'Extraction Buffer *minus* SDS' using a glass rod and vortex or *ice-cold* homogenizer. Be sure that the membranes are evenly suspended – no clumps.
13. Quickly measure the total volume of the membrane suspension using a small graduated cylinder.
14. Store the membranes on ice for further steps.

B. Measurement of the total chlorophyll of the membrane suspension

15. Pipet 50 μ L of the resuspended membranes into a microfuge tube.
16. Bring volume to 200 μ L with water, *i.e.*, add 150 μ L of water. [Note: If your membrane suspension is very dilute you will need to use more than 50 μ l. Compensate by adding less water.]
17. Pipet 1.0 mL of acetone into the tube.
18. Cap and vortex.
19. Place in a microfuge. (*Be sure to balance the centrifuge before starting it!*)
20. Centrifuge at full speed for 3 minutes.
21. Carefully pour into a *glass* cuvette in such a way as not to disturb the pellet.
22. Measure the absorbance at 652 nm using water or acetone as the blank.
23. Calculate the total amount of chlorophyll in the entire membrane sample (from A.12) as follows:

$$\sum \text{mg of chlorophyll} = 0.033 \cdot [A_{652}] \cdot \left[\frac{\sum \text{mL membrane suspension}}{\text{mL used for chlorophyll assay}} \right]$$

C. Solubilization of Membranes

DO QUICKLY AND AT ICE TEMPERATURE

24. Pipet 10% SDS into the resuspended membrane suspension (from step 12). Use 0.1 mL (100 μ L) for each mg of chlorophyll in the membrane suspension, *e.g.*, if you have 5.0 mg of chlorophyll, pipet 0.5 mL of SDS.
25. Quickly but thoroughly vortex or homogenize. Try to keep foam to a minimum.
26. Pour into an ice-cold plastic centrifuge tube.
27. Centrifuge at 40,000 x g for 5 min at 4°C. (*Be sure to balance the centrifuge before starting it!*)

28. Save the supernatant and discard the gray-green pellet. (Of what is the pellet composed? How would you test for pellet composition? Why is the supernatant transparent?)

D. Electrophoresis

29. Following the directions of your instructor and *WEARING GLOVES*, assemble the electrophoresis gel apparatus using a 5% polyacrylamide gel made in electrophoresis buffer (previously prepared).
30. Decide which membrane sample is to go into each well. Prepare a written “lane map.”
31. Using a Pasteur pipet or a micropipet, carefully fill the wells of the polyacrylamide gel with the green membrane solution.

ELECTROPHORESIS USES DIRECT CURRENT. DC VOLTAGE IS VERY DANGEROUS. FOLLOW YOUR INSTRUCTOR'S DIRECTIONS CAREFULLY.

32. Connect the leads to the power supply.
33. Plug in the power supply, turn on the voltage and run the gel at 100 V DC.
34. Watch the gel and allow it to run until the individual bands are resolved enough to cut apart. This should take no more than 30 minutes.
35. Turn off and unplug the power supply.
36. Disconnect the leads from the gel apparatus.
37. While wearing gloves, remove the gel from the apparatus.

E. Absorption Spectra

38. Place the gel horizontally and observe the number, placement and shade of color of each gel band.
39. Number each band from top to bottom.

WEAR GLOVES FOR THE FOLLOWING STEPS

40. Disassemble the gel as demonstrated by the instructor.
41. Using a single edge razor blade cut each band out of the gel. Use a “chopping” motion rather than a “sawing” motion.
42. Place each gel band between two small pieces of colorless plastic and record the absorption spectra. Your instructor will help with the spectrophotometer. A similar piece of plastic can be used as a blank. Follow the directions of your instructor.

Observations

1. Sketch the gel and label each band by number and describe its color.
2. From the absorption spectra of each band record the following:
 - a. The wavelengths of the blue and red maxima
 - b. The ratio of the height of the blue peak to the height of the red peak
 - c. The half-band width (Δ wavelength at the A that is half the maximum) is a measure of the breadth of an absorbance band. Do this for the red peak of each complex. For example, if the red peak has an absorbance of 0.15, measure the span of nanometers of the peak where the absorbance is 0.075.

Questions

1. Judging from the absorbance spectra, which complex has the most chlorophyll *a*?
2. Can you conclude which complex contains PS I? PS II?
3. After consulting your textbook, devise an experiment that would confirm your identification of the PS I- and PS II-containing complexes.
4. The photosynthetic apparatus is developmentally quite plastic. What environmental parameters could you vary in order to alter the sizes or relative abundance of the complexes?
5. Does the electrophoretic gel contain colorless proteins as well as the green complexes? If it does, how would you detect them? Speculate on the identity of the colorless proteins.

Notes for the Instructor

A. Isolation of photosynthetic membranes

- Market Spinach works best but Swiss Chard, Bok Choy, Pea, Tobacco, Corn and Beet will also work well. In fact, an advanced class might try a number of different species or growth conditions of the same species. Try to avoid tough leaves, leaves with high oil content, very fuzzy, succulent or waxy leaves.
- The solutions can be made in batches big enough to freeze for later use.
- Use a Osterizer or Waring blender with a low volume container if possible (37 – 110 mL container available from Fisher #14-509-18B). A mortar and pestle without sand will also work but the yields are very low. Actually, you can chew up the leaves and spit them out into the funnel.
- ***BE SURE EVERYTHING IS COLD. WHEN IN DOUBT PUT IT IN AN ICE BUCKET!***
- Any plastic centrifuge tube that can withstand 40,000 x g is fine: polyethylene, polypropylene or polycarbonate.
- Miracloth® is available from Calbiochem, catalogue # 475855. You also can use 4 - 8 layers of pre-wetted cheesecloth or a layer of pre-wetted white flannel cloth with the nap side upward; neither is as effective as Miracloth.
- After filtration save a few mLs for viewing under the microscope.
- Be sure that the crude, whole chloroplast pellet is well homogenized in the Wash Buffer with no clumps; the efficiency of the osmotic breakage of the chloroplasts depends on it. It is best

to use a 10–30 mL homogenizer with a glass or Teflon pestle (Ten-Broeck, or Potter-Elvehjm available from Fisher, VWR or Kontes) to achieve the best contact with the Wash Buffer.

- We find that 30-mL Corex tubes in a rubber shield work best for the last centrifugation (step 11). Any plastic centrifuge tube used in previous steps will also work.
- Resuspending the membranes (step 12) in a small volume works best. Using a homogenizer (see above) works very well for this step. Repeated pipetting works also.

B. Measurement of the total chlorophyll of the membrane suspension

- We find that using a Spec 20 for the chlorophyll estimation is convenient. If you intend to use a Spec 20 you will have to *double* all volumes for the chlorophyll assay.
- For the measurement of the chlorophyll concentration in 80% acetone, *most plastic cuvettes cannot be used*. Certain plastic cuvettes *are* resistant to acetone, *e.g.*, BrandTech UV-Transparent Disposable Cuvets (#759150). While quartz cuvettes work just fine, they are expensive; we prefer to use glass, semi-micro (1.5 mL) cuvettes.
- The chlorophyll calculation (step 23) is based on Arnon's (1949) equations.

C. Solubilization of Membranes

- 10% SDS crystallizes at refrigerator temperatures. Let it sit at room temperature to “melt” before pipetting it.
- Using a homogenizer (see above) after adding the SDS works very well.
- Ask the students to note that the finished suspension is now transparent. Why?
- Have the students observe the pellet. It consists mostly of starch. Stain it with I₂/KI.

D. Electrophoresis

Gels: Any vertical PAGE outfit will work. Use what you have. We use the 8 X 10 cm mini-gel format in the old Hoefer SE-200 (“Mighty Small”) single gel apparatus. This gives each student group their own gel to watch. Because we run the absorbance spectra in the gel, we use 1.5 mm thick gels. Thinner gels, 0.75 mm and 0.5mm, can be used but they are not as useful for direct spectra. Use glass gel plates, instead of alumina, so that you can put the gel directly on an overhead projector and discuss the results with your students.

Gels can be cast individually or in a multi-caster; we cast gels for both semesters in the fall. The directions in Markwell are very explicit. Polyacrylamide gels are made as follows being aware that acrylamide, bis-acrylamide and TEMED are all toxic – HANDLE WITH CARE:

(100 mL recipe)

10X Electrophoresis Buffer.....	10 mL
gel (20% acrylamide, 0.5% N,N'-methylenebisacrylamide).....	25 mL
distilled water.....	36.3 mL
10% fresh ammonium persulfate.....	1.25 mL
TEMED (N,N,N,N'-Tetramethylethylenediamine).....	50µl

Gels can be stored in a refrigerator for a considerable length of time (couple of months) if wrapped in two layers of plastic film and then placed in a Zip-Loc bag with a wet paper towel. Mold will eventually grow on the paper towel with no effect on performance of the gels.

Commercially prepared gels are not suitable since they use Tris/Tris-HCl as the buffer system in the gel itself. Chloride ions cause denaturation. I have tried pre-electrophoresing commercially

prepared gels using the Tris-glycine electrode buffer for 30 minutes prior to use with very mixed results.

Agarose Alternative to PAGE: A 4% agarose (Sigma cat. # A6013, low end agarose) gel in Electrophoresis Buffer will also sieve these complexes adequately. We have used 50 X 75 mm mini-gels. Melting the agarose requires some extra care because of the viscosity of 4% agarose and foam due to the presence of SDS. Be sure that the agarose melts completely. If using a microwave oven, be aware that the solution will foam considerably; using a boiling water bath to melt the agarose is preferred. The top surface of the gel, once cast, will have tiny bubbles. Cast the gel when it is very hot so that it flows well. The wells in horizontal mini-gels are pretty small compared to vertical PAGE gels but the gel patterns can be easily discerned and viewed on an overhead projector. Make a very thick gel if possible. While agarose is not optically clear, absorbance spectra can be measured with appropriate reference material (non-green portion of the gel) or the gel can be eluted.

Power Supply: We use the old BRL Model 100. Almost any electrophoresis power supply that can generate 50 -100 V DC can be used. You can even use five 9-volt batteries linked in series (45 V DC).

- You can use narrow-tipped Pasteur pipets and a light touch instead of a micropipettor to load the gels. Try the "The Graduate" by Life Technologies (Cat.# 100245-066) available from Carolina Biological Supply.
- The temperature of electrophoresis is usually room temperature. Greater stability is gained if run in the cold. This can be attained in a refrigerator or cold room. One can also pour fresh, ice-cold buffer into the gel apparatus just prior to loading.

General Note: This is one of the few labs where students can actually see electrophoresis happening; it is gratifying to some students for that alone. There is no marker dye needed since the complexes themselves are colored. The time of electrophoresis is not critical; you do not have to run until the free pigment reaches the bottom. The complexes are not that stable so heating of the gel and time tend to cause a greater amount of free pigment liberation and spreading out of the bands. So, the shorter the electrophoresis time, the better.

A good measure of degree of native conformation of the complexes is found in the quantity of detergent-solubilized chlorophyll in the free pigment zone (bottom most) of the gel. The less the better. An excellent prep should have almost no chlorophyll in the free pigment zone; there will be carotenoid.

The pattern of green bands and their apparent molecular weights can be found in Markwell *et.al.* (1978).

E. Absorption Spectra

- Viewing the gel on a light box is the place that class discussion of the results can begin. This is why glass gel plates are best.
- A flat, thin spatula and a water bottle are useful for getting the gel out from between the plates in one piece.
- Chop each band out of the gel with a single-edge razor blade. Put the slices into a microfuge tube to keep them from drying out.

- For spectra, we hold each gel slice between two pieces of colorless, transparent Plexiglass. These pieces measure 12 mm x 50 mm and are 1.5 mm thick. This size will fit nicely into standard 1-cm cuvette holders. We join the pieces by tape at one end so that they don't become separated. Center the gel slice in the narrow dimension but close to the bottom. Move the sandwich up and down until the A_{680} is maximal.

There are several other options for doing the gel band absorption spectra depending on the geometry of the sample compartment of your scanning spectrophotometer:

- Poke each slice into a 1.0-mL plastic semi-microcuvette.
- The Eppendorf Uvette cuvet sounds interesting.
- Stick each gel slice onto a microscope slide.
- Squash each slice and put into a microcuvette.
- Let each slice stand overnight in a microfuge tube bathed in a small volume of Extraction Buffer Minus SDS, at 4°C. This will elute most of the complex. Run spectra as usual for a solution.

The absorbance spectra of each of the complexes can be found in Markwell *et al.* (1978).

Further Pigment Analysis

Classical chromatographic pigment analysis of the complexes can be done by extracting the pigments into Petroleum Ether followed by thin-layer chromatography on Silica Gel plates. Elute the complexes from the gel overnight into a small volume of Extraction Buffer Minus SDS and then extract the liquid. Since the amount of total pigment is low, be sure that the extraction is performed in dim light. A small amount of $MgCO_3$ in the petroleum ether will help prevent pheophytinization of the chlorophyll. Concentrate the extract by evaporation *in vacuo* or with a stream of nitrogen gas.

Further Analysis of the proteins

Polypeptide analysis by the SDS denaturing gel system of LaemmLi does not work very well with these proteins. A modified 8–15% gradient LaemmLi gel containing a 3–6 M urea gradient works best. Complex denaturation in gel slices can be achieved in 6% SDS, 0.1 M dithiothreitol at 60°C for one hour. Since the polypeptides do not strongly stain with CoomassieBlue, be sure to fix gels with 5% sulfosalicylic acid before staining.

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(This reference gives explicit directions on making and running gels in this system, called MARS, and the system used by Anderson. Other chapters in this volume give explicit directions on isolating photosynthetic membranes and the chlorophyll quantitation.)
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Acknowledgment

Partial support for this work was provided by the National Science Foundation's Course, Curriculum and Laboratory Improvement Program under grant DUE-0088013.

APPENDIX B

Chlorophyll-Protein Complexes: a brief review

Photosynthesis uses sunlight energy to synthesize organic compounds, primarily carbohydrate, and to reduce some inorganic ions. This process supports most autotrophs and most heterotrophs on the planet with organic nutrients and oxygen. Photosynthesis takes place in the chloroplast, a semi-autonomous organelle possessing some genes on a plastome, and the means to express those genes. Other genes are nuclear; the pre-proteins must be imported into the chloroplast. Chloroplasts have a structure that includes an outer and inner membrane (the chloroplast envelope) wrapping the entire organelle, and a complex inner thylakoid system. This latter system is composed of hollow discs, each enclosing a space called the lumen, or thylakoid space. Thylakoids are often stacked into grana, which are often connected to one another by stroma thylakoids. All of the photosynthetic pigments are localized in the thylakoid membranes.

The process of photosynthesis can be divided into two parts: (1) the conversion of sunlight energy into metabolically-useable forms – the so-called “light” reactions, which include oxygen production, photoreduction of NADP^+ and photophosphorylation and (2) the fixation and subsequent reduction of atmospheric carbon dioxide – the “dark” reactions. This division of the process into two parts is reflected in the locations of these two processes. Conversion of light energy into metabolically available forms (ATP and NADPH) takes place in the thylakoid membrane system, including the lumen, while carbon dioxide metabolism occurs in the stroma, the liquid outside the thylakoids.

The “light” reactions of photosynthesis use sunlight energy to drive the reduction of NADP^+ by electrons from water and to create a proton motive force (pmf) across the thylakoid membrane. The resulting products are oxygen (oxidation product of water), NADPH and ATP by chemiosmotic coupling (photophosphorylation). Light is absorbed by thylakoid membrane pigments: chlorophylls *a* and *b*, β -carotene and xanthophylls. Upon absorption of a photon, the pigment is raised to an excited singlet state. The excited state is relaxed in one of four ways: internal conversion to heat, fluorescence, excitation energy transfer (EET) to an adjacent pigment molecule, and the loss of an electron to an adjacent electron acceptor. This latter, charge separation reaction is the primary photochemical reaction of photosynthesis whereby the energy of the photon is stored as a separation of charge between an oxidized pigment and a reduced acceptor molecule. Which of these four fates for the absorbed quantum actually occurs depends upon its probability relative to the lifetime of the excited state. Fluorescence occurs in a nanosecond, but charge separation or excitation energy transfer occurs hundreds to thousands of times more rapidly in the thylakoid membrane. The close orientation of adjacent pigment molecules favors EET, and the close orientation of an electron acceptor to a reaction center chlorophyll favors charge separation.

The functional unit of photosynthetic light reactions is the photosystem, of which there are two: Photosystem I (PS I) and Photosystem II (PS II). PS I accepts electrons from the photosynthetic electron transport chain, and uses a photon to raise its energy so that it can reduce the protein ferredoxin. PS II reduces the electron donor of the photosynthetic electron transport chain and accepts electrons from water, producing oxygen. Both photosystems are composed of a number of light-harvesting chlorophyll protein complexes surrounding (or so it is thought) a reaction center chlorophyll protein complex. Only the reaction center complex can undertake the charge separation reaction. The light-harvesting complexes of PS I contain only chlorophyll *a*, while those of PS II contain both chlorophylls *a* and *b*. Light-harvesting complexes absorb light energy and pass it down a chain of chlorophylls of decreasing excited state energy to the reaction center.

I tell my students, “proteins are the workers of the cell.” This is no less true for the light reactions of photosynthesis. All of the photosynthetic pigments of thylakoid membranes are sequestered in protein molecules; the protein molecules are bound together into complexes. A protein molecule accomplishes the orientation of light-harvesting pigments in such a way as to favor EET within the protein and between the light-harvesting protein and the reaction center complex. Further, the orientation of an electron acceptor close to the reaction center chlorophyll is accomplished within a protein molecule. When you view the photosystems in this way, you see that photosynthetic pigments are the functional equivalents of enzyme prosthetic groups. Also, chlorophyll-proteins are specific, being divided into reaction centers and light-harvesting molecules. For further reading see Green & Durnford (1996).

The reaction center complexes of the photosystems must also interact with other components of the thylakoid membrane. PS II reaction center must be able to accept electrons from the complex that extracts electrons from water, and to donate electrons to plastoquinone in the lipid phase of the membrane. PS I must be able to accept electrons from plastocyanin and to donate them to ferredoxin. Further, each reaction center must be oriented in the membrane so as to cause the transport of protons into the thylakoid lumen, creating the pmf for photophosphorylation.

Each chlorophyll protein complex in the thylakoid is a structure composed of several different polypeptides, not all of which contain pigments. Unpigmented proteins may serve as anchors for peripheral proteins and tethers holding the

complexes together in the fluid membrane environment, as well as functions not yet discovered. The following two tables, from Malkin & Niyogi (2000), present the subunit composition of the core complexes of the two photosystems.

Table 1: Polypeptide subunits of the PS1 core complex

Protein	Gene	Location of Gene	Mol. mass (kDa)	Function
Hydrophobic subunits				
PsaA	<i>psaA</i>	C	83	Reaction center protein
PsaB	<i>psaB</i>	C	82	Reaction center protein
PsaG	<i>psaG</i>	N	11	Unknown
PsaI	<i>psaI</i>	C	4	Unknown
PsaJ, PsaM	<i>psaJ, psaM</i>	C	5,3.5	Unknown
PsaK, PsaL	<i>psaK, psaL</i>	N	8.4, 18	Unknown
Hydrophilic subunits				
Stromal orientation				
PsaC	<i>psaC</i>	C	9	Fe-S apoprotein
PsaD	<i>psaD</i>	N	18	Ferredoxin docking
PsaE	<i>psaE</i>	N	10	Cyclic electron transport
PsaH	<i>psaH</i>	N	10	LHC-1 linker
Luminal orientation				
PsaF	<i>psaF</i>	N	17	PC docking
PsaN	<i>psaN</i>	N	10	unknown

Table 2: Protein subunits of the PS2 core complex

Protein	Gene	Location of gene	Mol. mass (kDa)	Function
Hydrophobic subunits				
D1	<i>psbA</i>	C	32	Reaction center protein
D2	<i>psbB</i>	C	34	Reaction center protein
CP47	<i>psbC</i>	C	51	Antenna binding
CP43	<i>psbD</i>	C	43	Antenna binding
Cytb559				
A subunit	<i>psbE</i>	C	9	Unknown
B subunit	<i>pabF</i>	C	4	Unknown
PsbH-PsbBN	<i>psbH-psbN</i>	C	3.8-10	Unknown
22kDa	<i>psbS</i>	N	22	Photoprotection
Hydrophilic subunits				
33kDa	<i>psbO</i>	N	33	Oxygen evolution
23kDa	<i>psbP</i>	N	23	Oxygen evolution
17kDa	<i>psbQ</i>	N	17	Oxygen evolution
10kDa	<i>psbR</i>	N	10	Unknown

The *modus operandi* of much of the research leading to the elucidation of structure and function of the thylakoid membrane pigment complexes has a familiar ring. Isolation of mutants with impaired photosynthesis, followed by analysis of the functional impairment and isolation of the complexes by electrophoresis, followed by electrophoretic analysis of polypeptide composition of the complexes.

Chlorophyll protein complexes also play a role in maintaining an energy balance between Photosystems I and II. Since PS I absorbs, on average, longer wavelength photons than does PS II, it is possible to activate one system more than the other. The resulting “piling up” or “draining out” of electrons from the photosynthetic electron transport creates an imbalance of photoreduction and photophosphorylation. Altering the lateral heterogeneity of light-harvesting complexes of PS II (LHC II) controls this imbalance. Reduction of plastoquinone activates a protein kinase which phosphorylates LHC II’s causing them to repel one another because of their similar negative charges. The phosphorylated LHC II’s migrate laterally in the membrane away from the PSII core, effectively decreasing the antenna size of the photosystem.