Using XRF to Probe Protein Conformational Changes Governing Photoprotection in Cyanobacteria

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Pigments and Photoprotection

"The lifeblood of a photosynthetic organism is its
hip

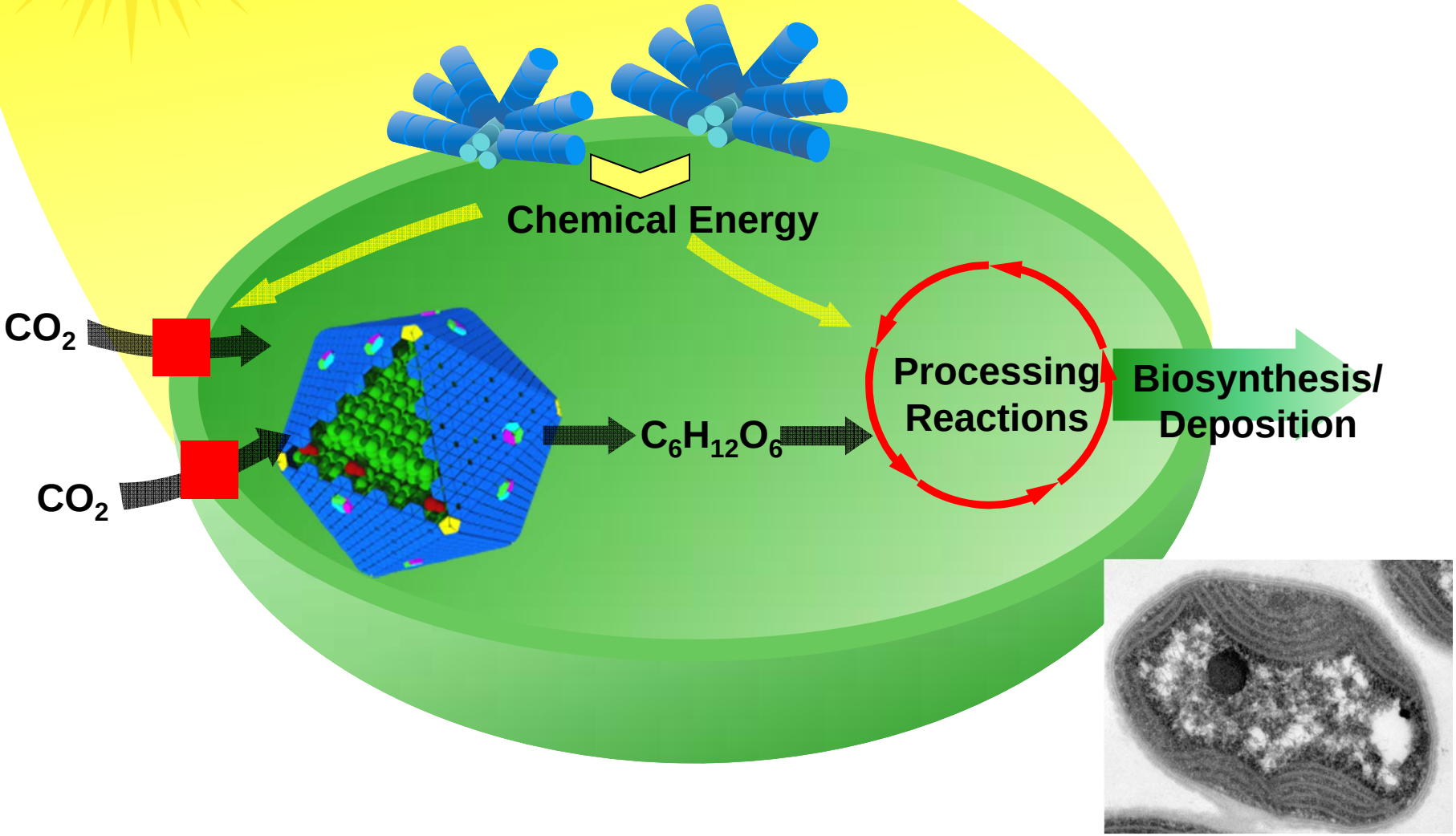
undant light harvesting
chlorophyll can be
l can lead to production
es (ROS)

accessory light harvesting
g Complexes, Peridinin-
horhodopsin, etc.). Also
tive functions in plants

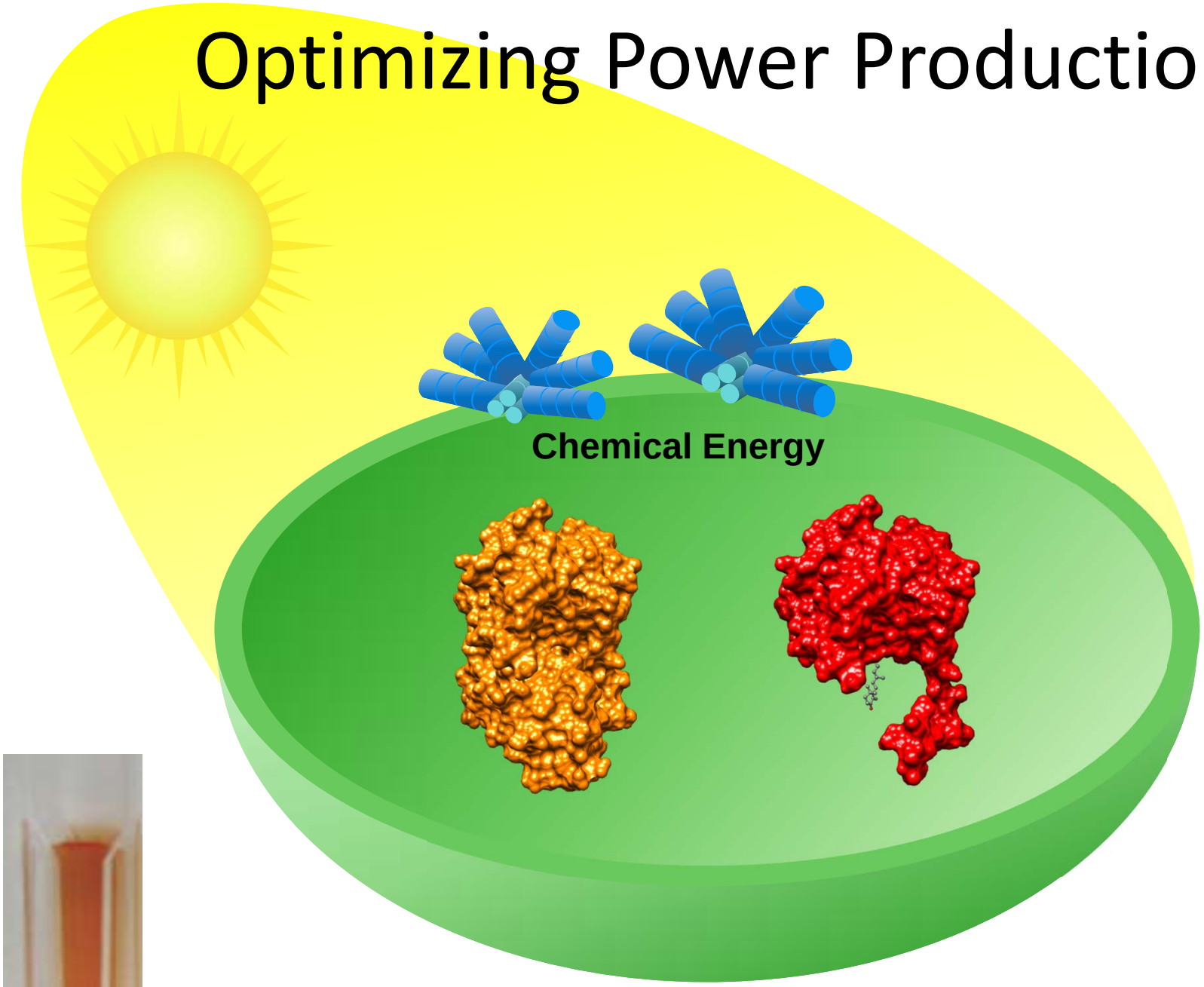
hylls

er in antenna systems in
fast and 'flexible' Non-photochemical quenching mechanisms—Engineering Motivation

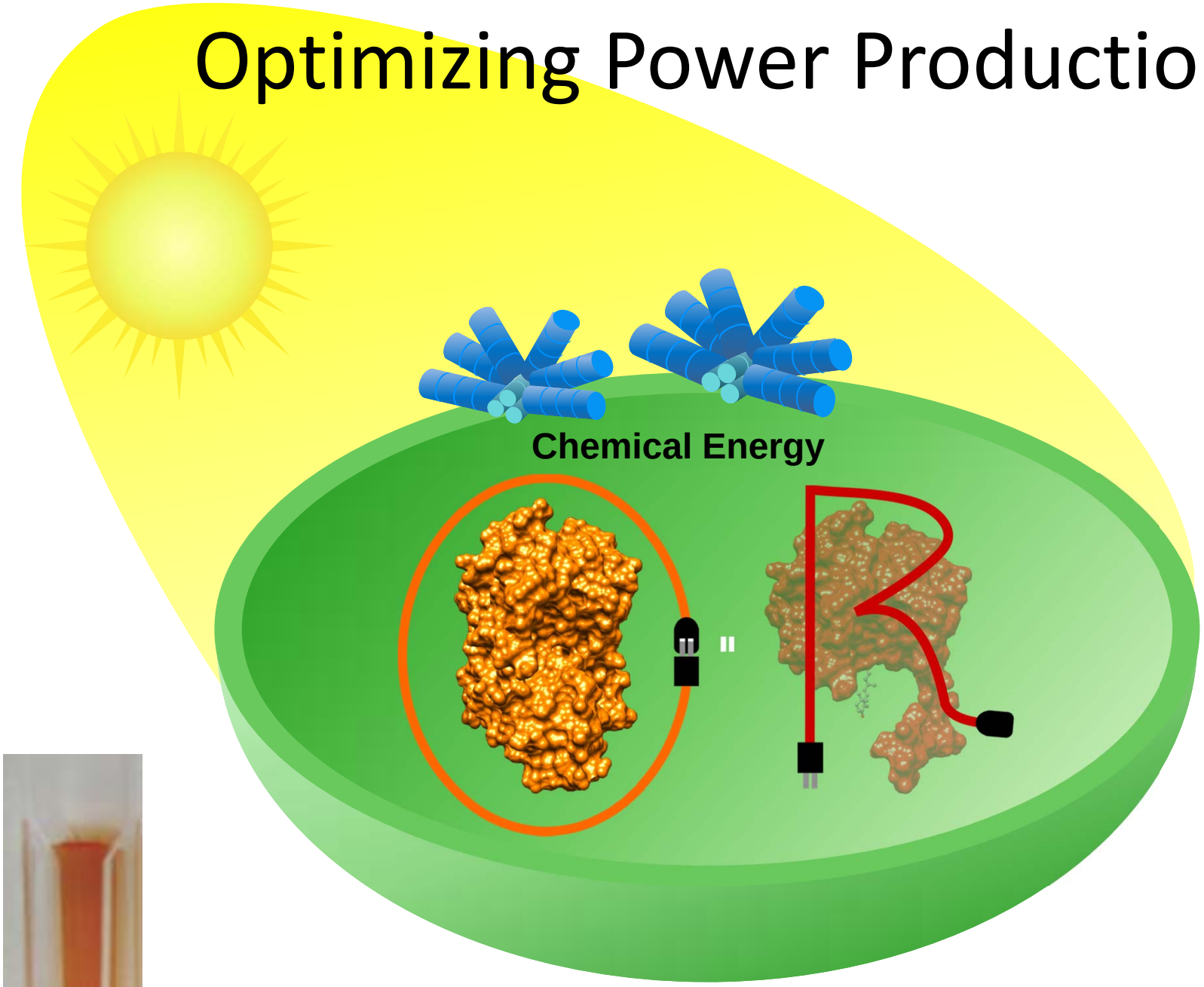
Modules in Cyanobacterial Photosynthesis

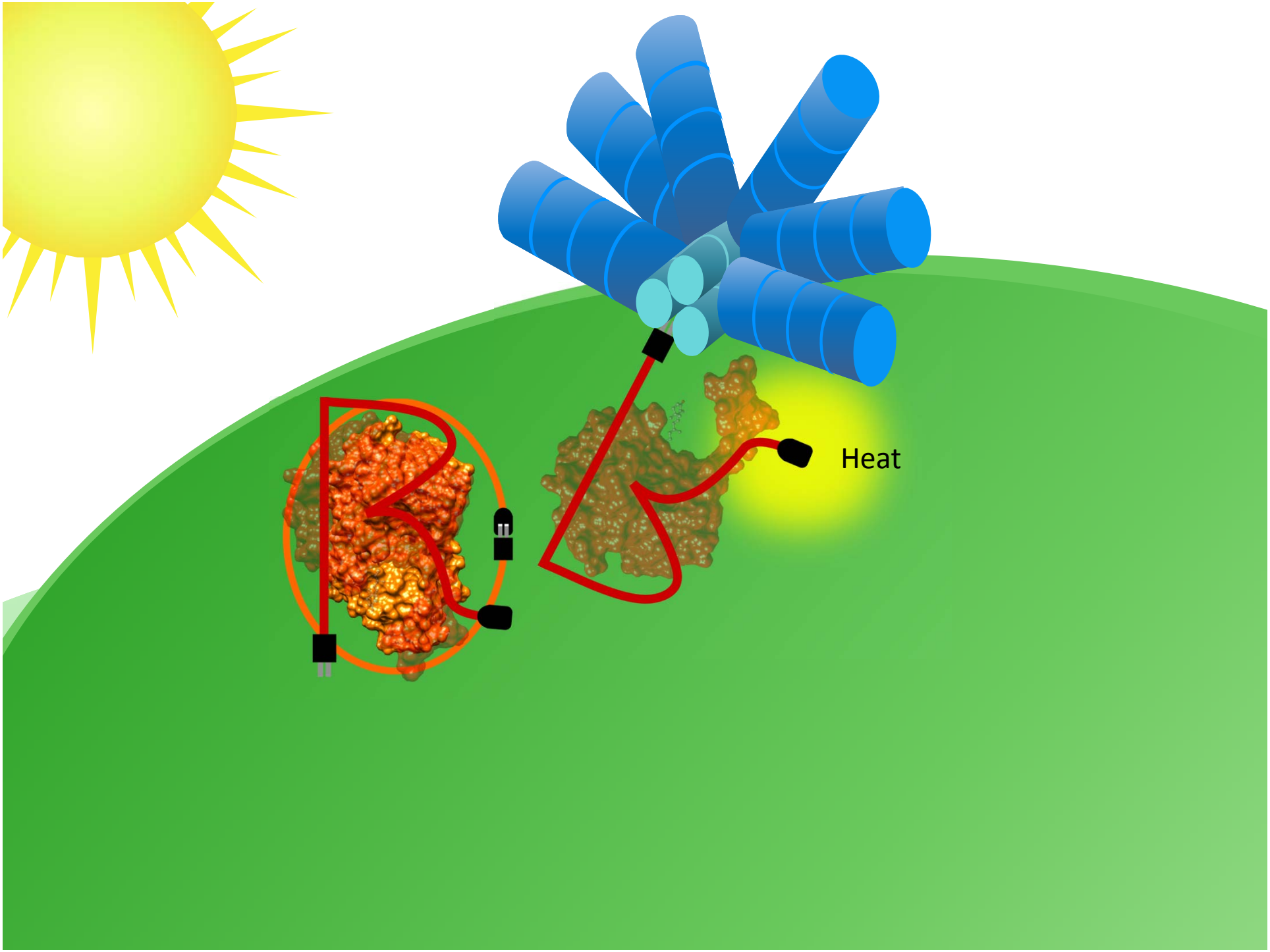


Optimizing Power Production

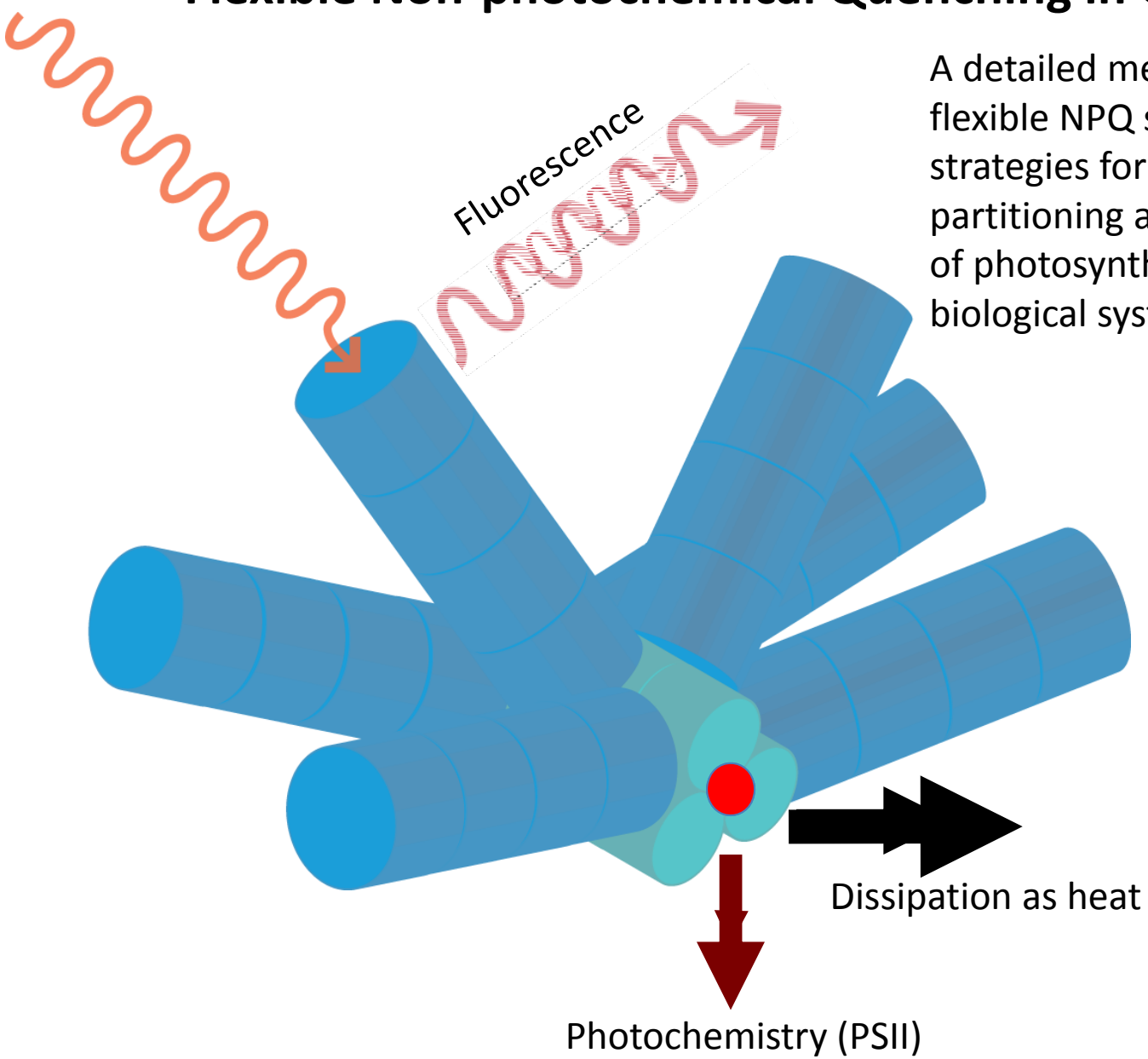


Optimizing Power Production




Heat

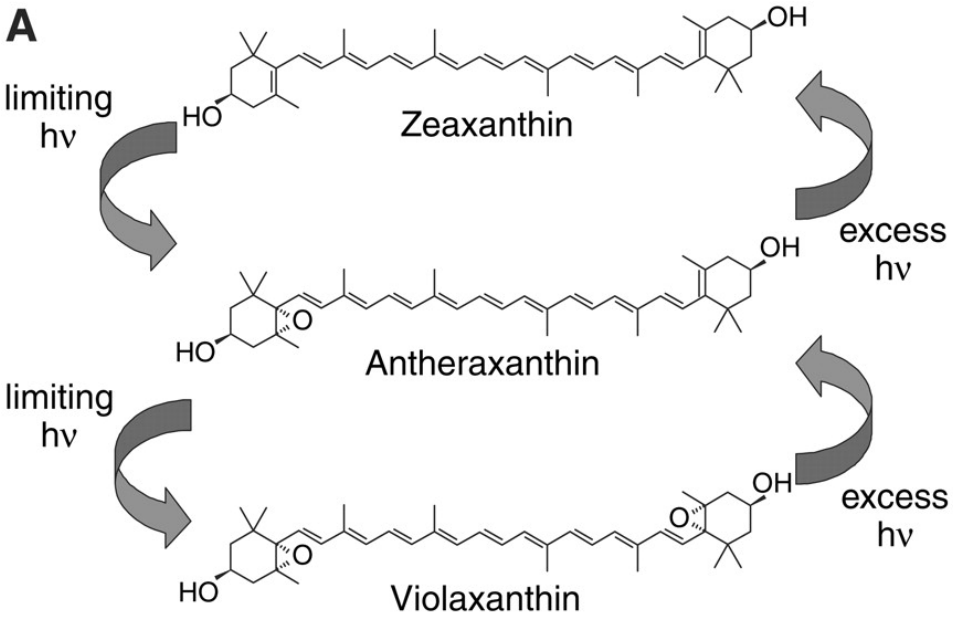
Flexible Non-photochemical Quenching in Cyanobacteria



A detailed mechanistic understanding of flexible NPQ should lead to guided strategies for optimizing its role in energy partitioning and increasing the efficiency of photosynthesis in engineered biological systems

Flexible Non-photochemical Quenching: A Comparison

Plants (LHC/PSBS)



The Xanthophyll Cycle

Reactions driven by “limiting” and “excess” light are enzyme catalyzed and drive conformational changes in transmembrane Light Harvesting Complex (LHC) proteins

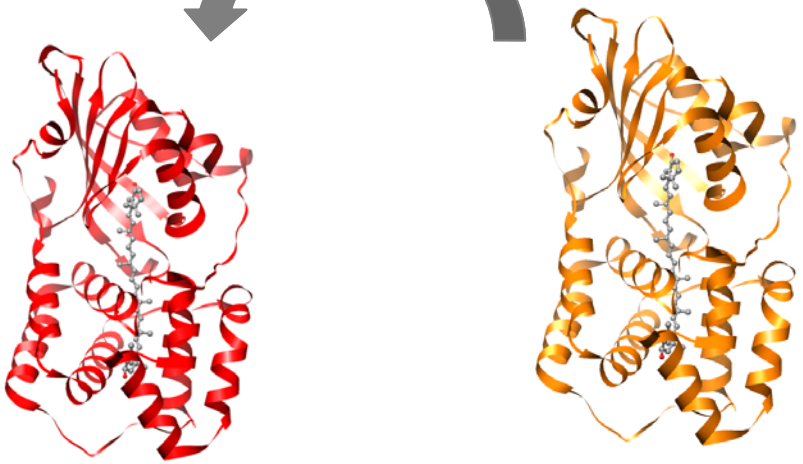
Flexible NPQ in plants also involves sensing of lumen pH by another protein, PSBS

Configurational change of carotenoid involved?

Figure Credit (Left): Holt et al., 2005, DOI: 10.1126/science.1105833

Cyanobacteria (OCP)

1 (light)



Quenching active OCP^R

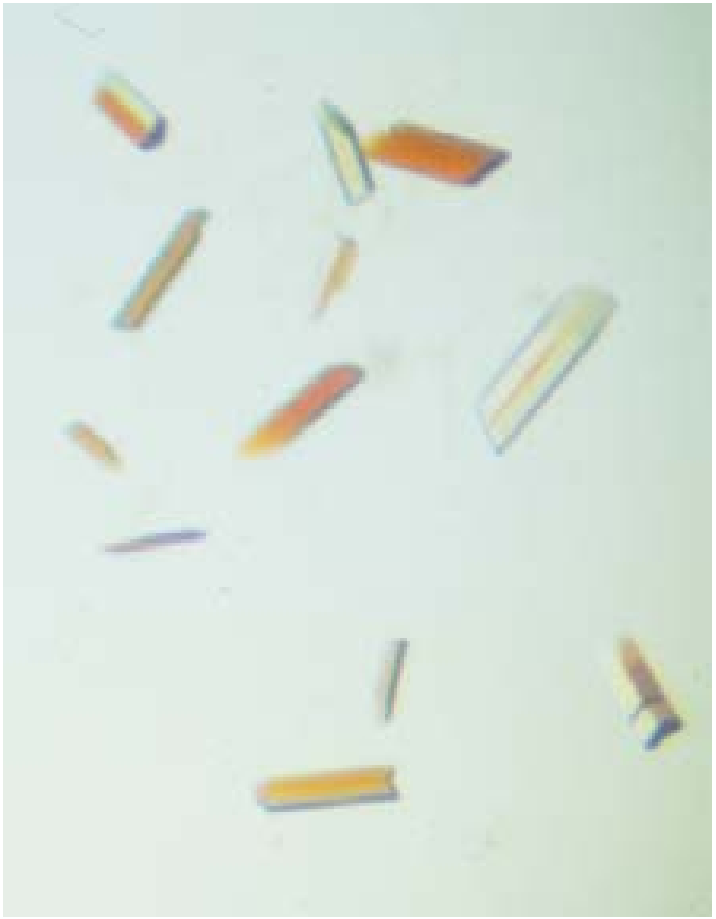
Photoactive OCP^O

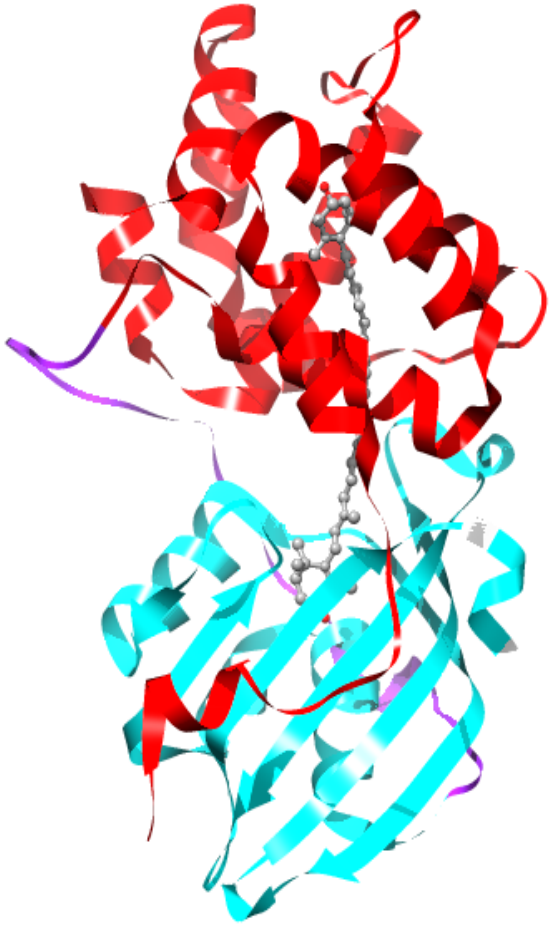
2 (FRP)

1.) what are the structural requirements for inducing NPQ_{cyano}? That is, what makes active OCP active? (OCPR/RCP)?

2.) OCP ideal model system—water soluble pigment binding protein

The OCP^O Structure





Kerfeld et al., *Structure* 2003
Wilson et al., *J. Biol Chem* 2010

Protein Structural Dynamics of full-length OCP: X-ray footprinting at LBNL (w/ Corie Ralson and Sayan Gupta)

Introduction to the technique:

1. Sampled pumped through glass capillary and x-rays generate hydroxyl radicals in solvent

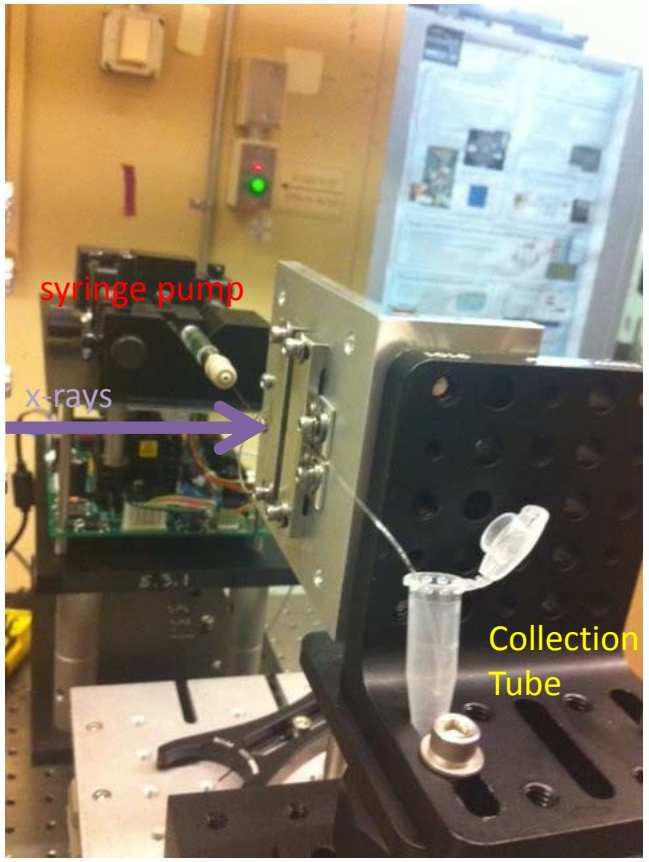
$$H_2O \xrightarrow{\text{x-rays}} \bullet OH$$

2. Radicals react with polypeptide; covalently/permanently labeling amino acids (more solvent accessibility = higher likelihood of oxidation)

Amino acids are labeled at different rates.

Cys > Met > Trp > Tyr > Phe > His > Leu > Ile …

3. Protein digested and oxidations subsequently detected w/ 2D-MS

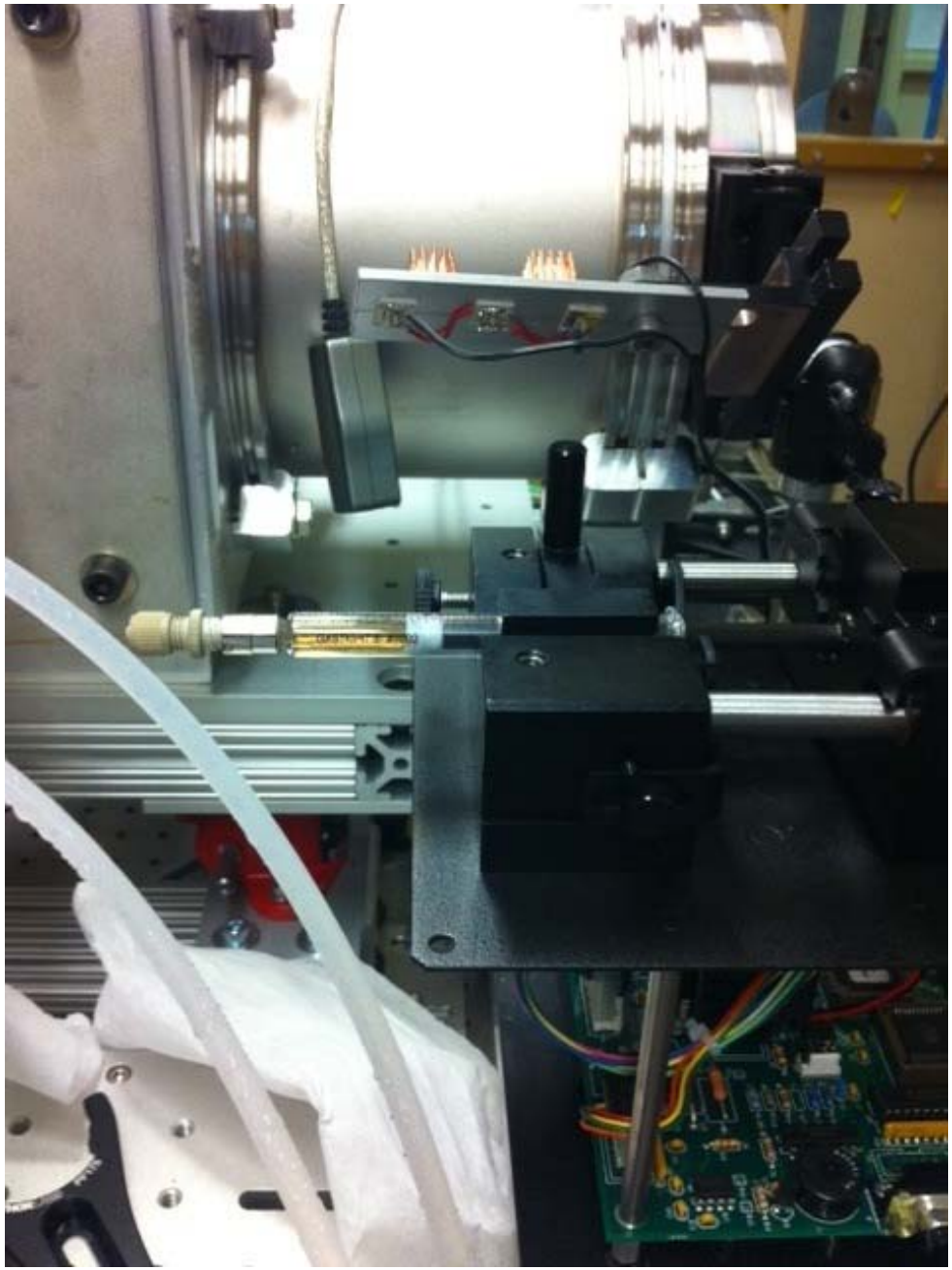


A simple application of x-ray footprinting: probing differential solvent accessibility in different forms of a protein (i.e. OCP^O vs OCP^R):

More exotic applications w/ x-ray hydrolysis: structural dynamics (incl. time resolved studies of protein folding or protein-protein interactions), *in vivo* footprinting, etc.

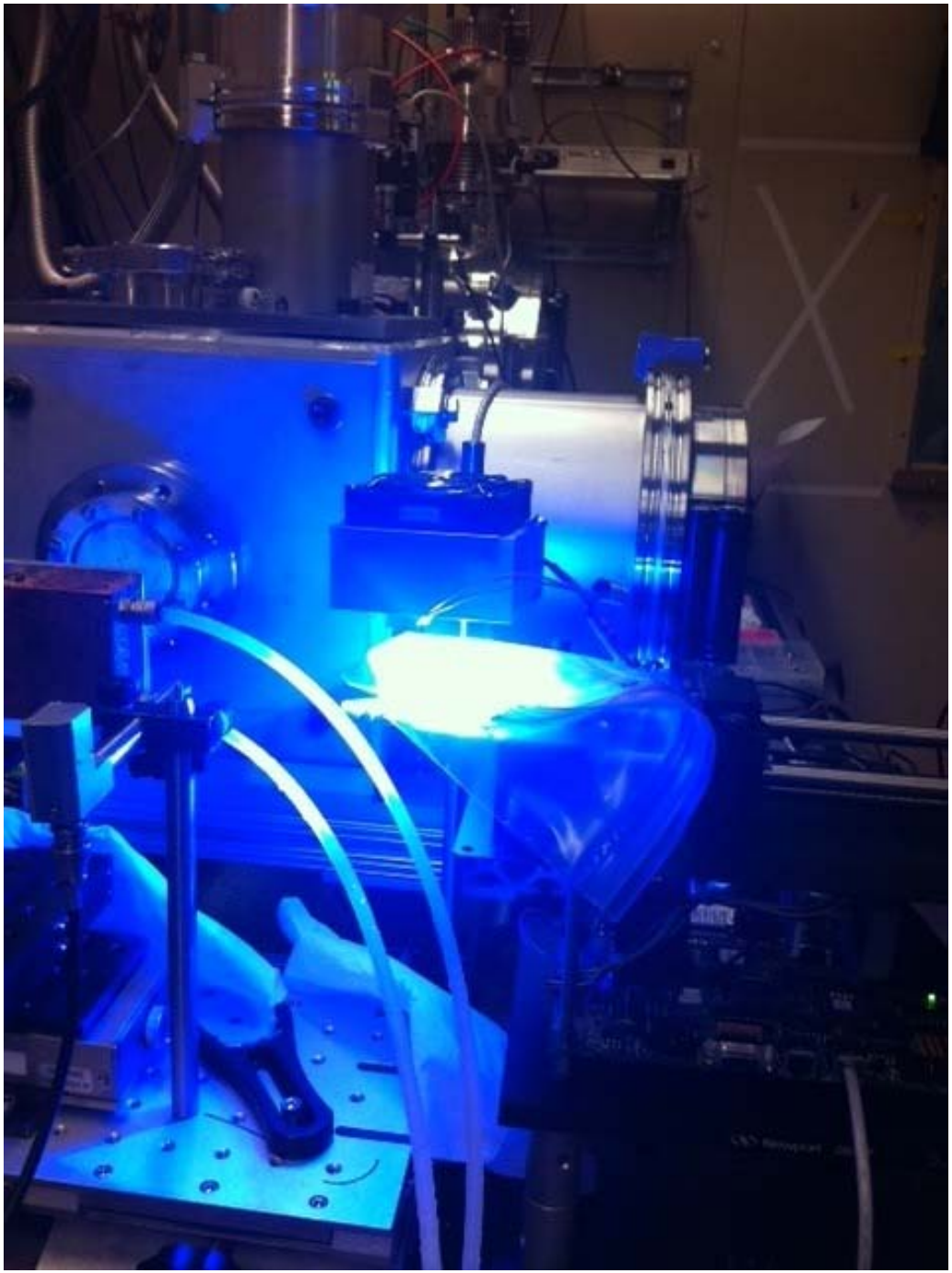
Slide 10

7 Ryan Leverenz, 1/16/2014

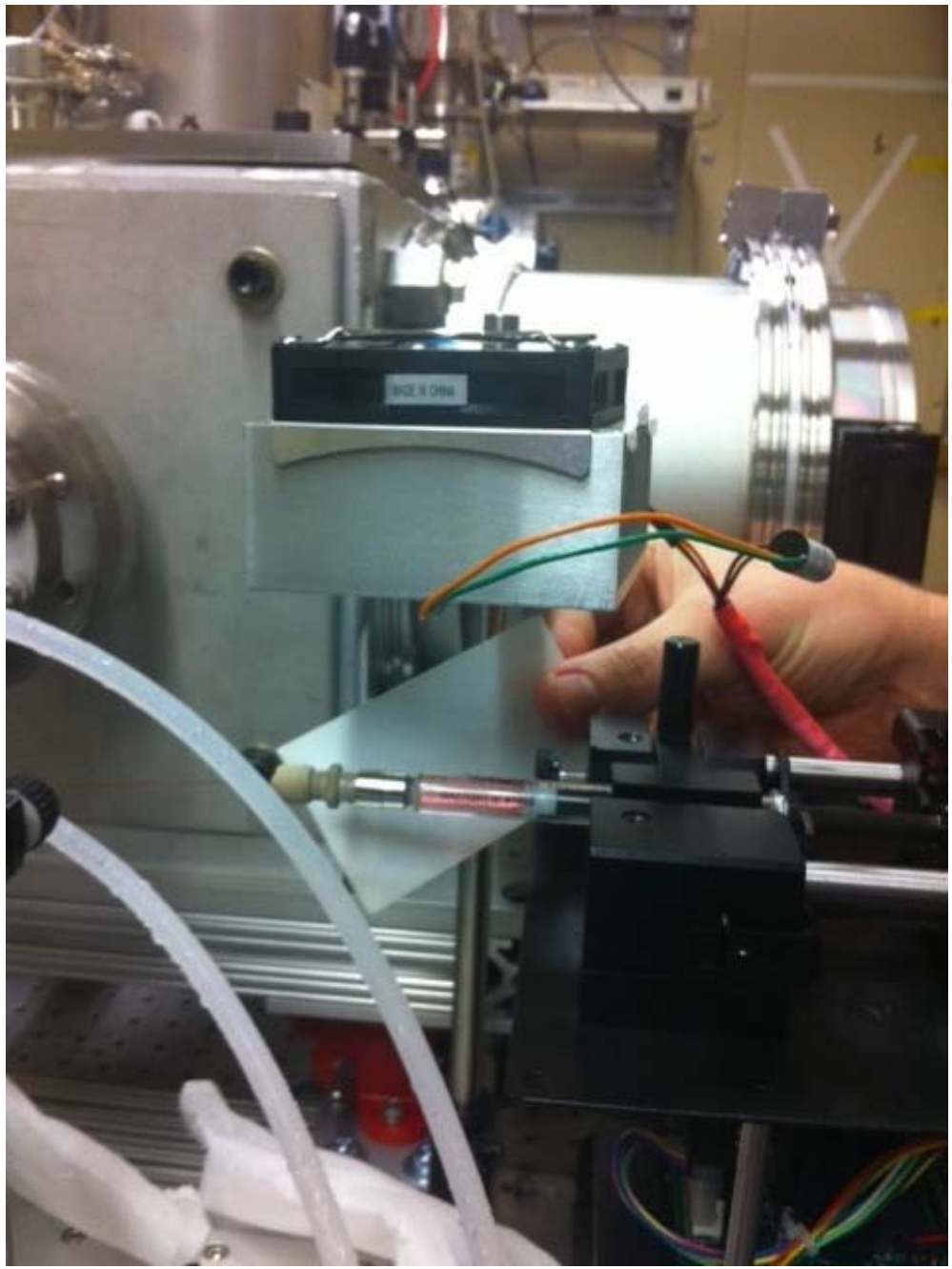


Expose OCP^O samples (in darkness) at different exposure times by varying flow rate.

Collect samples for MS (0.5 mL @ ~ 0.1 mg/mL for full data set).



Illuminate 10 min w/ blue LED (chill with ice bag)



Expose OCP^R samples. Collect for MS.

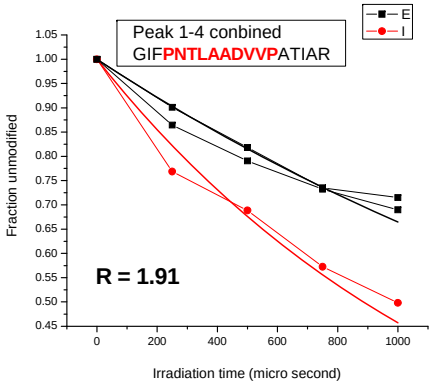
Collect samples for MS (0.5 mL @ ~ 0.1 mg/mL for full data set).

Data Processing

- Trypsin/Glu-C digestion and LC-MS/MS

- For each peptide, plot fraction unmodified as function of irradiation time. Calculate rate constant (k = 1/t) for modification using fit to single exponential function, y = A*exp(-x/t). The **Solvent Accessibility Change, R** = $k(OCP^O)/k(OCP^R)$

Sample Data Pro 13-22



Data: GIFPNTLAADVVP_E,GIFPNTLAADVVP_I
Model: ExpDec1
Equation: y = A1*exp(-x/t1) + y0
Weighting:
y(1) No weighting
y(2) No weighting

Chi^2/DoF = 0.00055
R^2 = 0.97923

y0	0	±0	
A1	1	±0	
t1	2448.96948		±194.80737
y0_2	0	±0	
A1_2	1	±0	
t1_2	1277.53117		±66.63494

Excellent OCP Sequence Coverage w/ MS using dual protease digests

Missing peptide regions in early XRF mass-spec data (w/ trypsin digest alone) covered well w/ V8-E digest

Example trypsin digest coverage (MS matched peptides in **BOLD**):

1 M**PFTIDSARG IFPNTLAADV VPATIARFSQ LNAEDQLALI WFAYLEMGKT**
51 LTIAAPGAAS MQLAENALKE IQAMGPLQQT QAMCDLANRA DTPLCRTYAS
101 WSPNIKLGFW YRLGELME**QG FVAPIPAGYQ LSANANAVLA TIQGLE**SGQQ
151 ITVLRNAVVD MGFTAGKDGK RIAEPVVPPQ DTASRTKVSI E**GVTNATVLN**
201 YMDNLNANDF DTLIELFTSD GALQPPFQRP IVGKENVLRF FREECQNLKL
251 IPERGVTEPA EDGFTQIKVT GKVQTPWFGG NVGMNIAWRF **LLNPEGKIFF**
301 VAIDLLASPK ELLNFAVHHH HHH

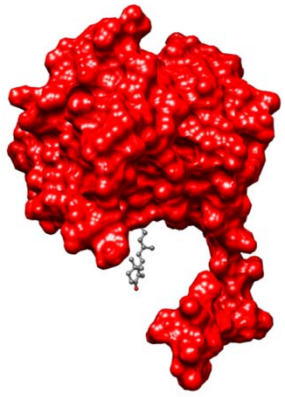
Example V8-E protease digest coverage:

1 M**PFTIDSARG IFPNTLAADV VPATIARFSQ LNAEDQLALI WFAYLEMGKT**
51 LTIAAPGAAS MQLAENALKE IQAMGPLQQT QAMCDLANRA DTPLCRTYAS
101 WSPNIKLGFW YRLGELMEQG FVAPIPAGYQ LSANANAVLA TIQGLESGQQ
151 ITVLR**NAVVD MGFTAGKDGK** R**IAEPVVPPQ DTASR**TKVSI EGVTNATVLN
201 YMDNLNANDF DTLIELFTSD GALQPPFQRP IVGK**ENVLR**F FR**EECQNLKL**
251 IPERGVTEPA EDGFTQIKVT GKVQTPWFGG NVGMNIAWRF LLNPEGKIFF
301 VAIDLLASPK ELLNFAVHHH HHH

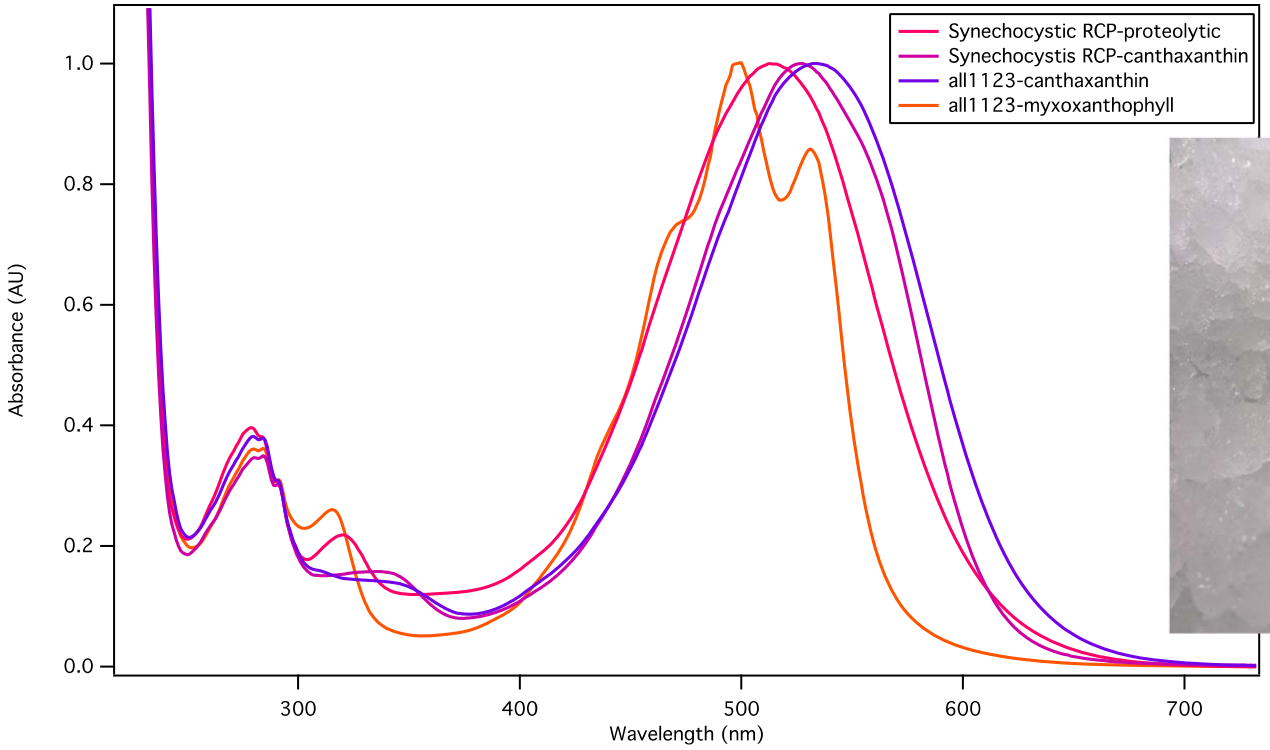
Use XRF to Probe Carotenoid Protein Interactions in OCP-R

- In OCPO- to OCPR conversion we propose that the carotenoid protein interactions change

- XRF can test this hypothesis: are solvent accessibilities the same in RCP and OCPR?



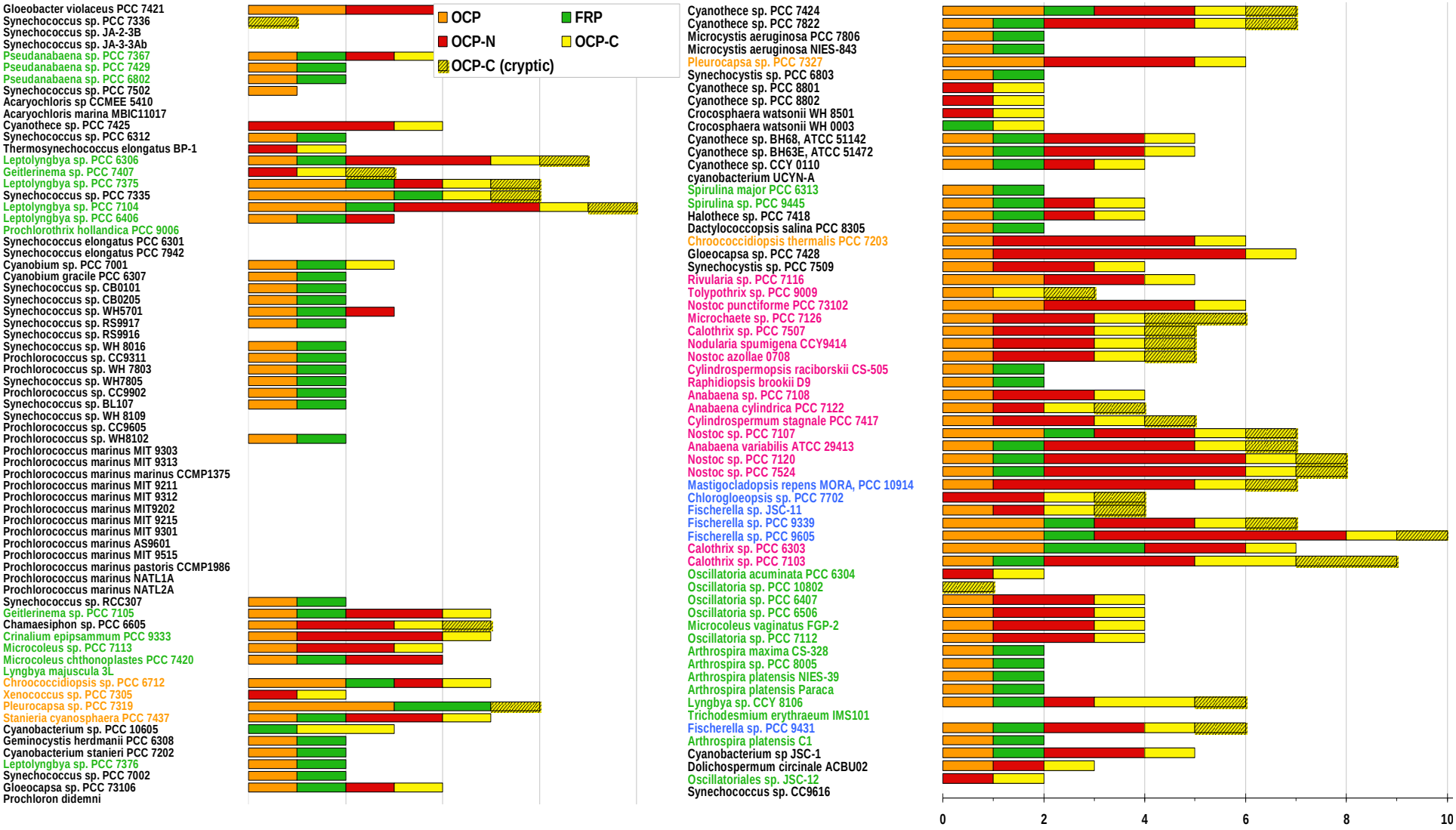
The N-terminal Domain: RCP
A Constitutively Active Quencher



From left to right: Proteolytic Synechocystis RCP - echinenone, Synechocystis RCP-canthaxanthin, all1123-canthaxanthin, all1123-myxoxanthophyll

Leverenz et al., *Plant Cell* 2014

Distribution of OCP, RCP (N-term domain) and Carotenoid Binding NTF2 (C-term domain) ORFs in Cyanobacteria



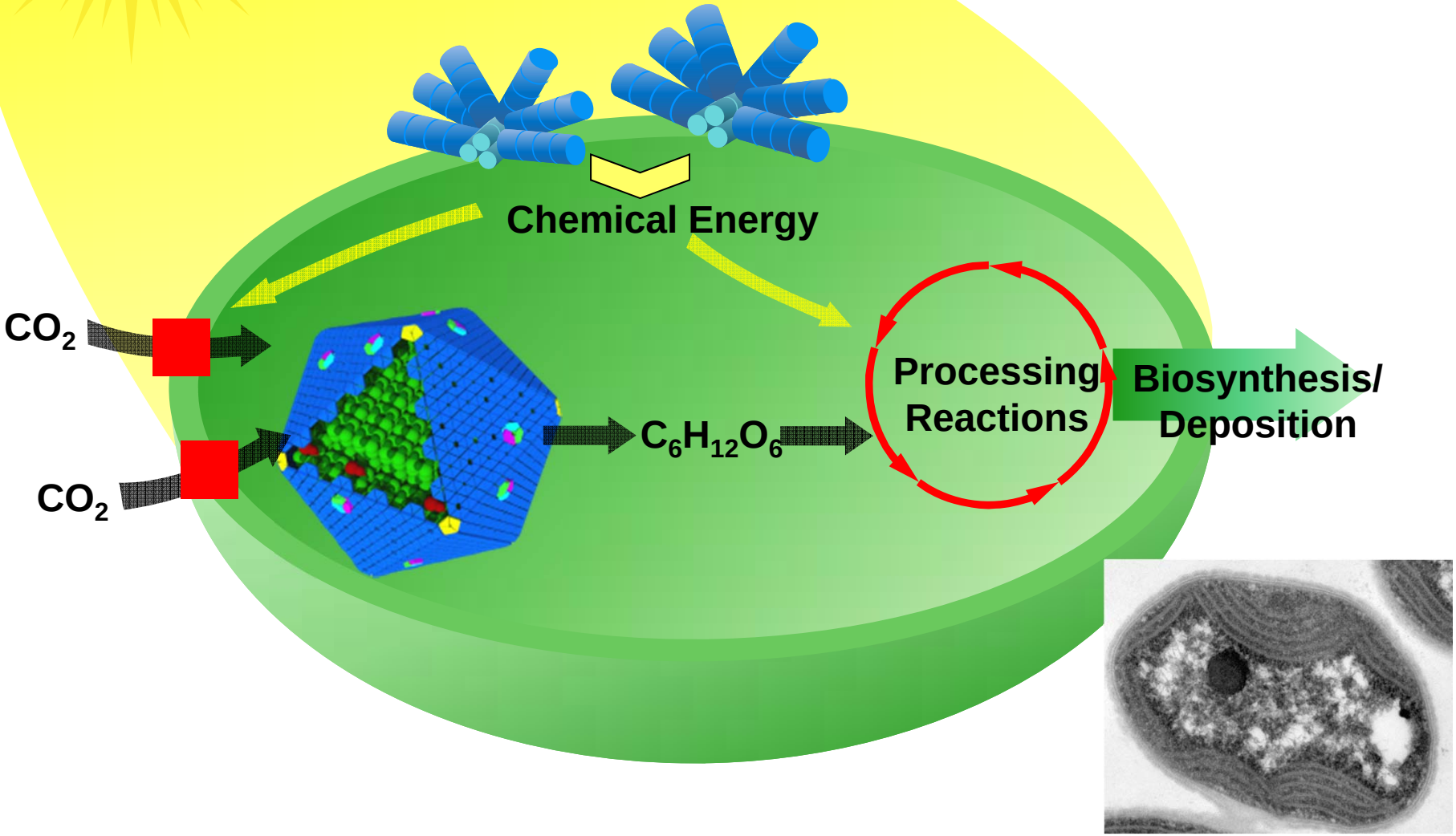
Kerfeld and Kirilovsky, *Photochemistry and Photobiology*, 2013

Using XRF to Compare Pigment Protein Interactions

- Comparison of OCP^R to isolated N-terminal domains (aka RCP)

- In both, the active quenching form shows similar, specific changes in carotenoid protein interactions

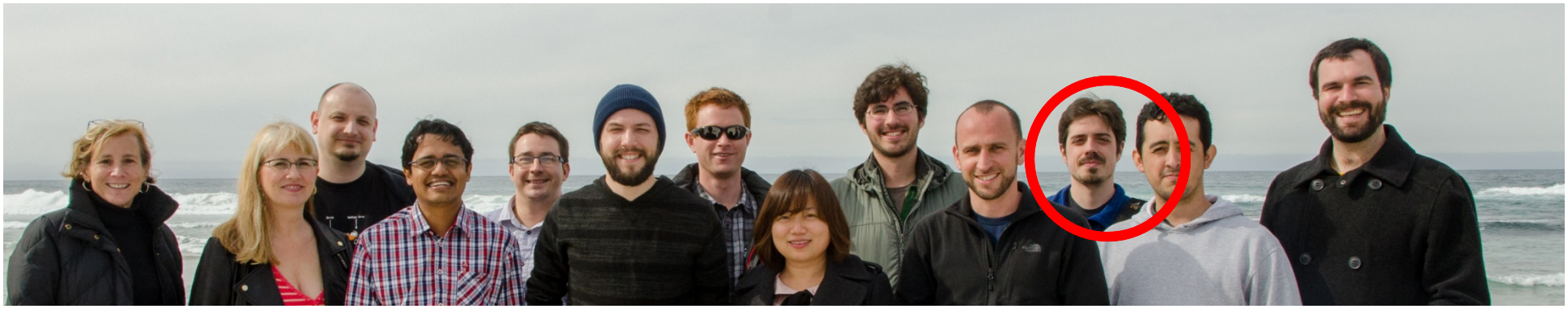
Modules in Cyanobacterial Photosynthesis



Summary

- XRF allows interrogation of short-lived structural state in a photoactive protein
- Next steps are to probe protein-protein interactions involved in energy dissipation
- Implications for engineering photoprotection in production strains of cyanobacteria

Acknowledgements



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