



Structure and dynamics biomolecules studied by neutron scattering (examples from photosynthesis)

J. Pieper

University of Tartu, Estonia



European Union
European Social Fund



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Outline

Introduction/Photosynthesis

Solution Structures

Small Angle Neutron/X-Ray Scattering (SANS/SAXS)

- Oligomerization
- Complex Formation
- Detergent Shells / Solubilization of Membrane Proteins
- Light-Induced Structures

Molecular Dynamics

Neutron Spectroscopy (ENS, QENS)

- Temperature and hydration dependence
- Light-induced (functional) dynamics - time-resolved experiments

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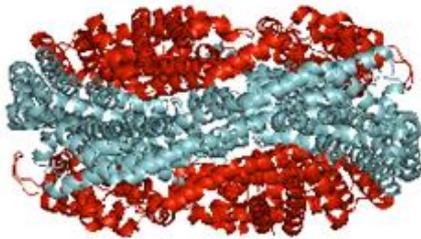
Neutron Spectroscopy (ENS, QENS)

- Temperature and hydration dependence
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Photosynthetic Protein Complexes

for studies of structure-dynamics-function relationships

Phycocyanin (PC)



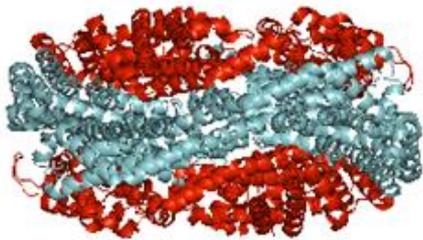
Fromme et al. IUCrJ (2015)

Light-harvesting protein
in cyanobacteria

Photosynthetic Protein Complexes

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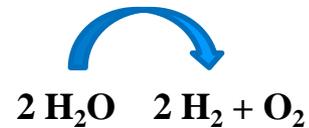
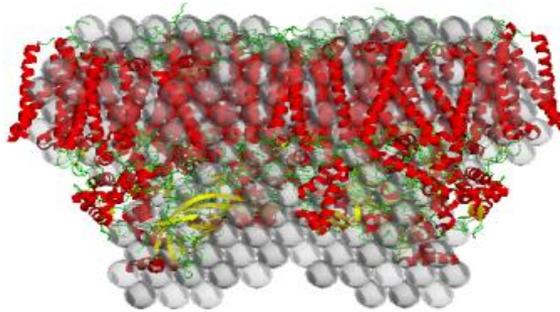
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Photosystem II (PS II)

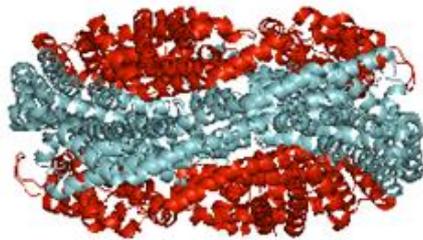


Water splitting into
oxygen and hydrogen
in plants/cyanobacteria

Photosynthetic Protein Complexes

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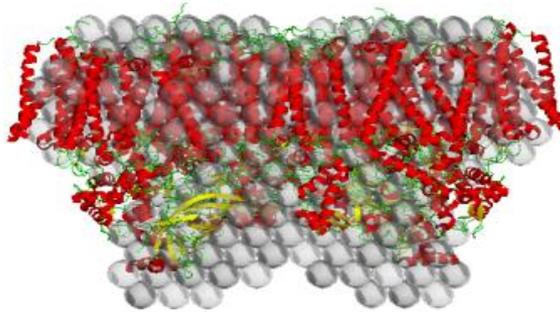
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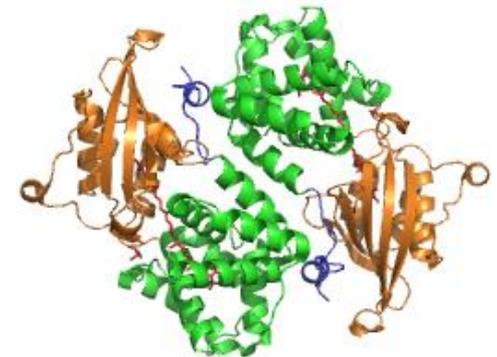
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Orange Carotenoid
Protein (OCP)



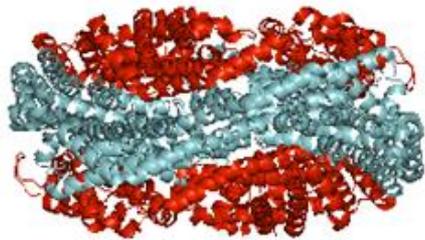
Wilson et al. J. Biol. Chem. (2010)

Protection of
cyanobacterial
photosystems against
photodamage

Photosynthetic Protein Complexes

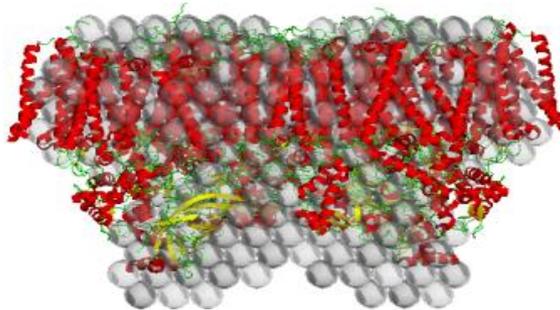
for studies of structure-dynamics-function relationships

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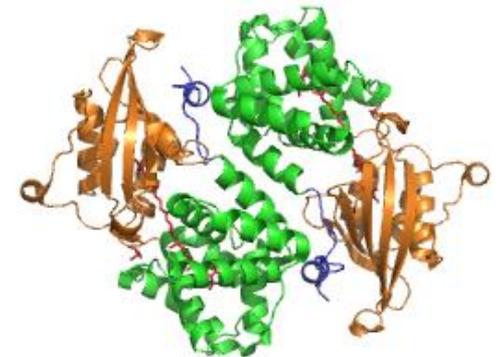
Fromme et al. IUCrJ (2015)

Photosystem II (PS II)



Zouni et al. Nature (2004)

Orange Carotenoid
Protein (OCP)



Wilson et al. J. Biol. Chem. (2010)

High-resolution crystal structures are often available, but...

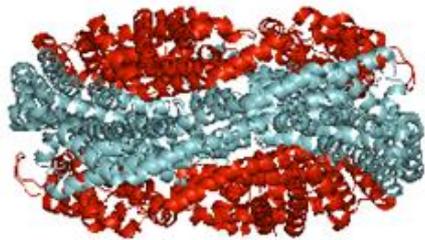
Solution structure ?

→ experiments on protein function are performed in solution

Photosynthetic Protein Complexes

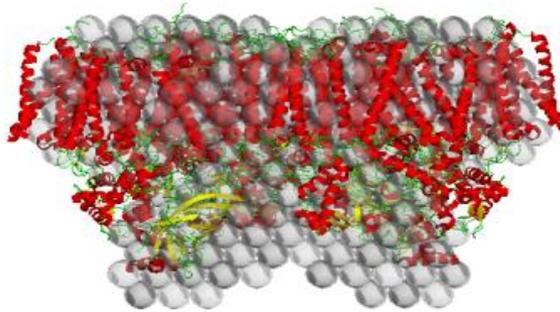
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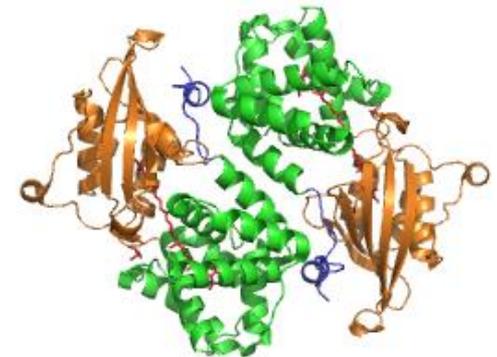
Fromme et al. IUCrJ (2015)

Photosystem II (PS II)



Zouni et al. Nature (2004)

Orange Carotenoid Protein (OCP)



Wilson et al. J. Biol. Chem. (2010)

specific questions/problems:

occurs in oligomers,
supercomplexes

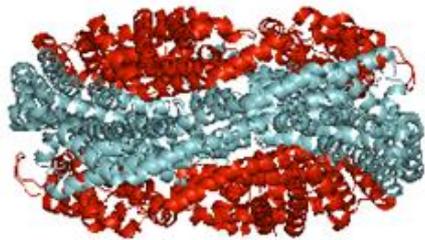
membrane protein
solubilized in detergent shell

light-induced
active state

Photosynthetic Protein Complexes

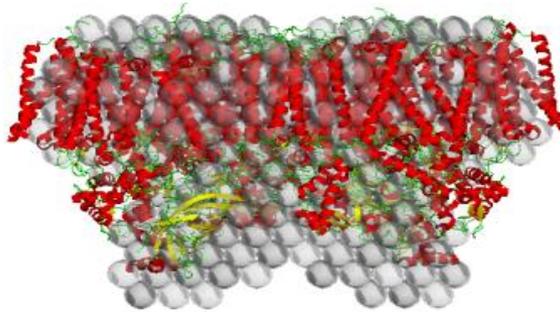
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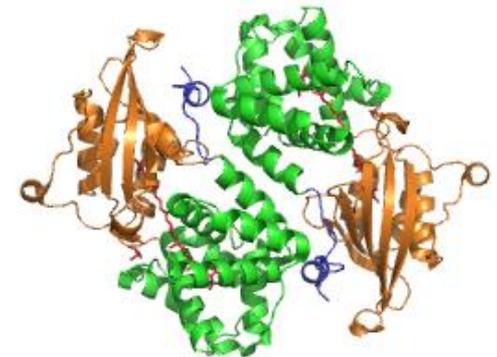
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Photosystem II (PS II)



Zouni et al. Nature (2004)

Orange Carotenoid
Protein (OCP)



Wilson et al. J. Biol. Chem. (2010)

**SANS/SAXS delivers structure in buffer solution
i.e. same conditions as for experiments on protein function**



Structure and function can be directly correlated



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Small Angle Neutron/X-Ray Scattering (SANS/SAXS)

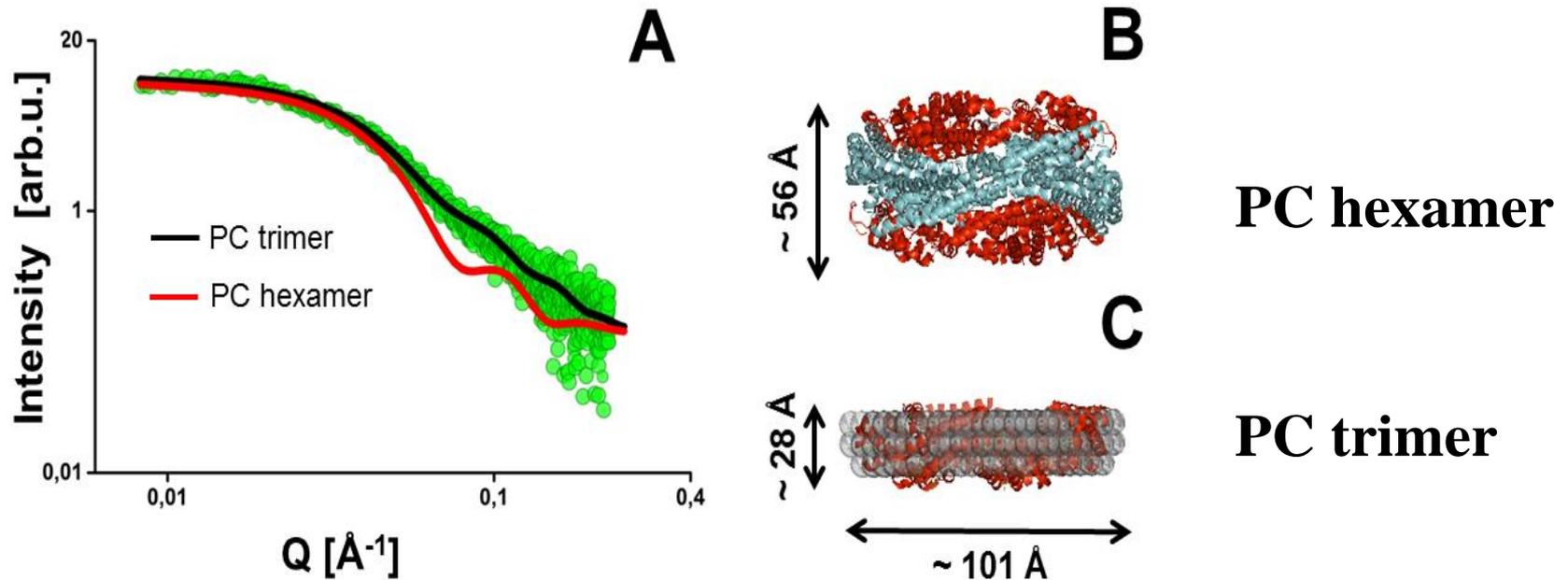
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- Detergent Shells
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Molecular Dynamics

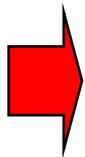
Neutron Spectroscopy (ENS, QENS)

- Temperature and hydration dependence
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Structure of Phycocyanin - SAXS/SANS



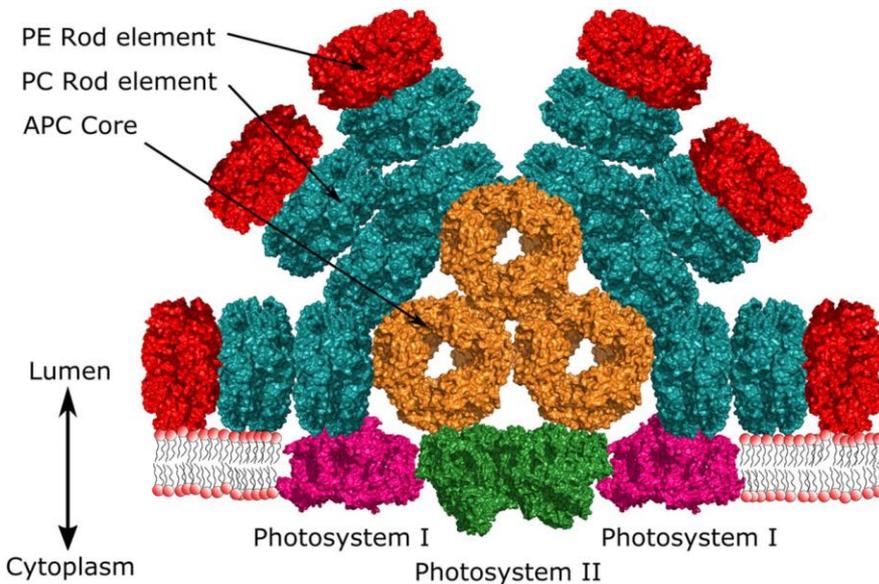
- SAXS data, SANS data from YuMo, FLNP Dubna, Russia
- CRY SOL simulation of PC trimer and hexamer
- structure reconstitution of PC in solution from SAXS/SANS



SANS/SAXS reveals oligomerization

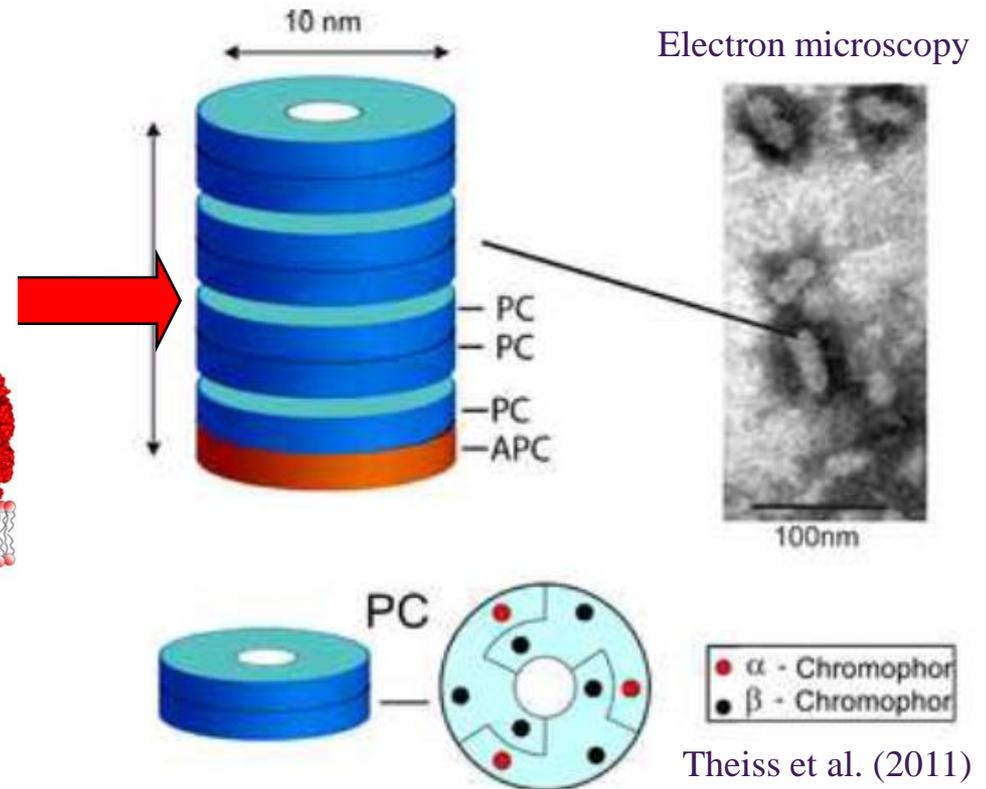
Structure of Phycobiliproteins

phycobilisomes
of usual cyanobacteria



according to Marquardt et al. (1997)

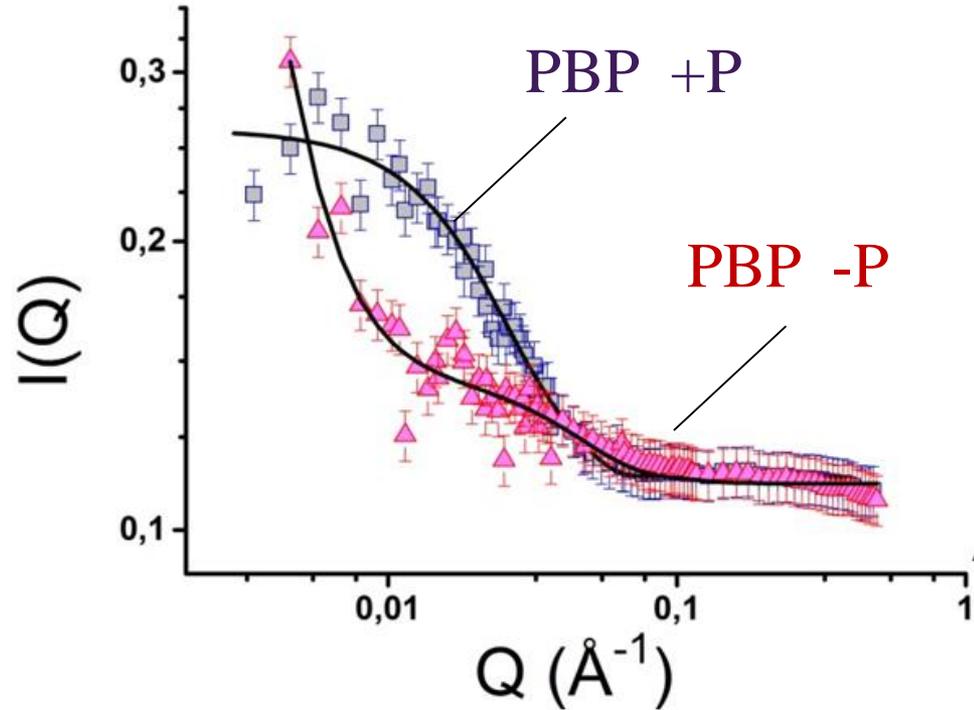
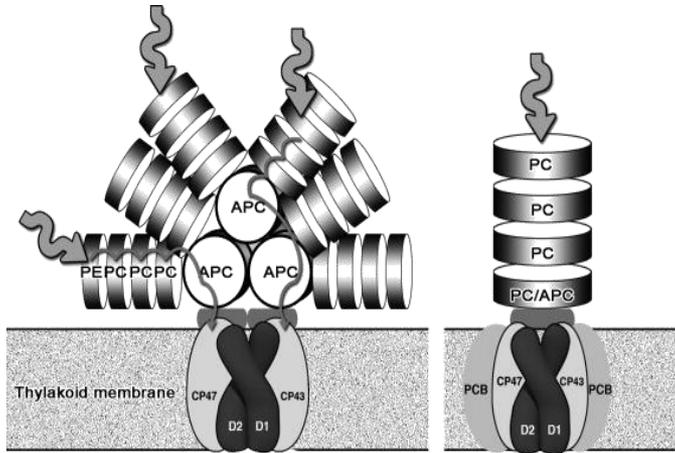
phycobiliproteins
of *A. marina*



Theiss et al. (2011)

Rod-shaped assembly ?

Structure of Phycobiliproteins by SANS



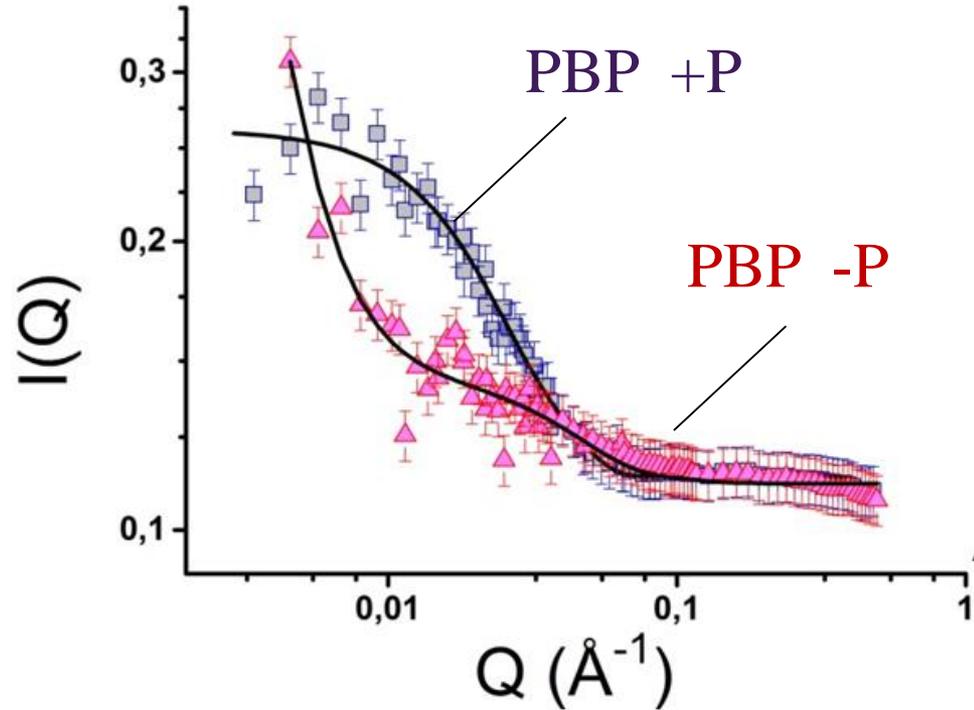
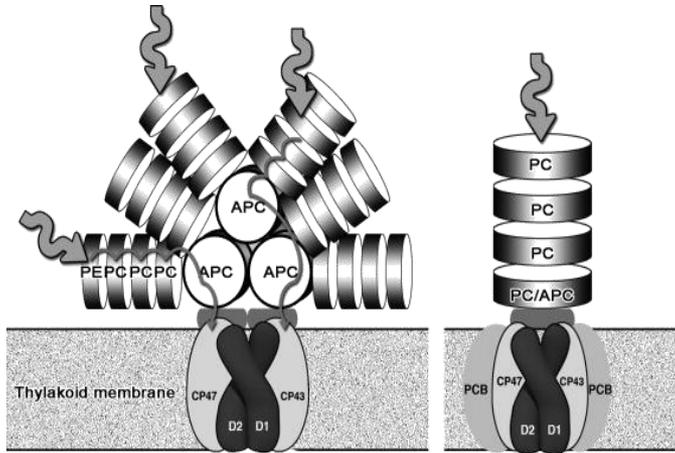
Cylinder (+ power law)

	Phosphate	MES
Length [Å]	225±10	28±2
Radius [Å]	50.5	50.5
SLDc [10⁻⁶ Å⁻²]	3.5	3.5



+P samples have rod-like (cylinder) structure

Structure of Phycobiliproteins by SANS



Cylinder (+ power law)

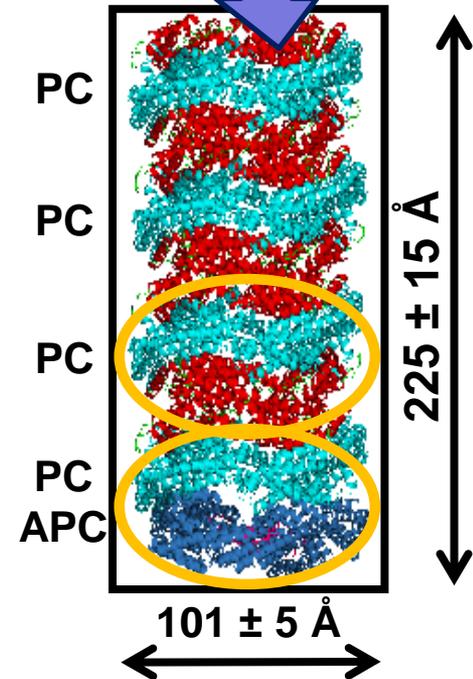
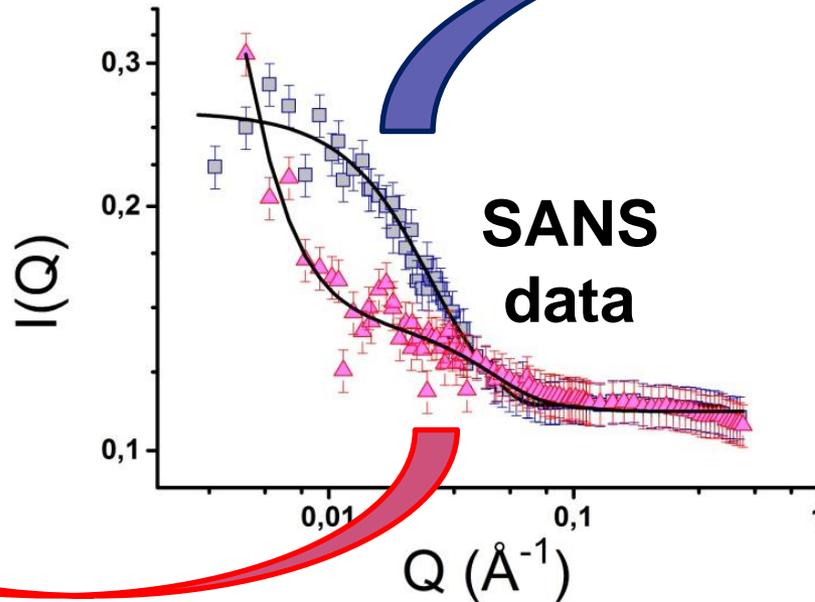
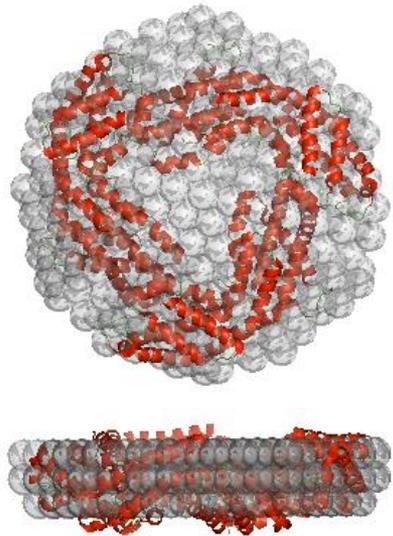
	Phosphate	MES
Length [Å]	225±10	28±2
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-P samples are decomposed
EET is impaired
partial aggregation

Summary PBP

Phycobiliproteins
in solution
retain a rod-shaped structure



upon phosphate removal
split into trimeric PC
Function is impaired !!

SANS/SAXS provides supercomplex



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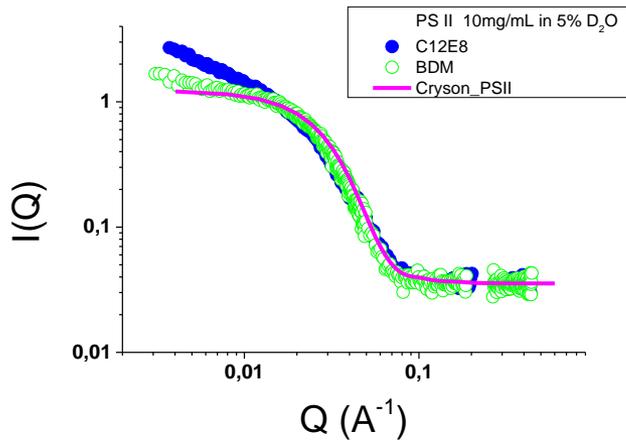
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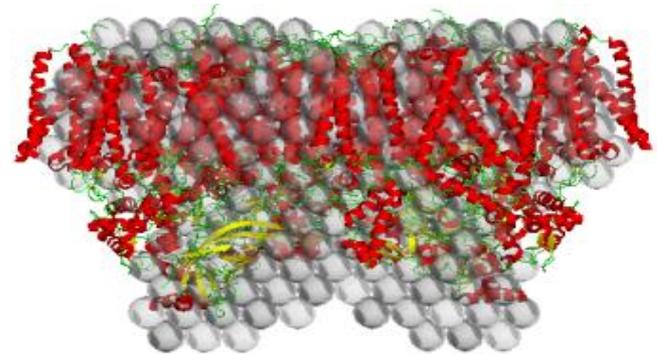
Solution Structures from SANS/SAXS



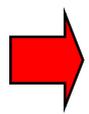
SANS in 5% D₂O



protein only

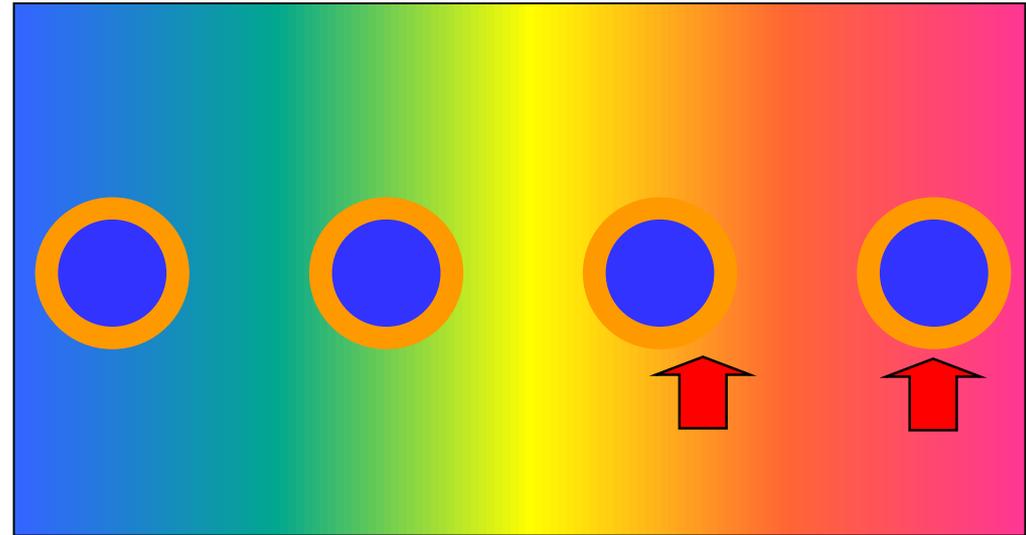
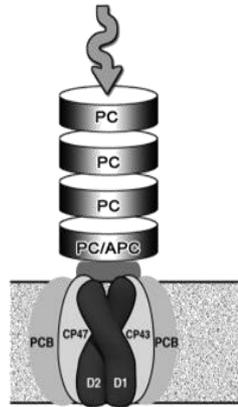
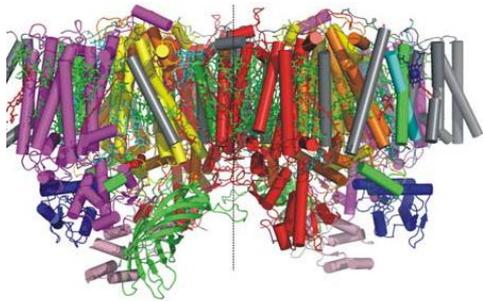


Golub et al. *JPC B* (2020)



Solution structure compares well to PSII X-ray structure
Why is detergent belt missing → contrast variation

Contrast Variation

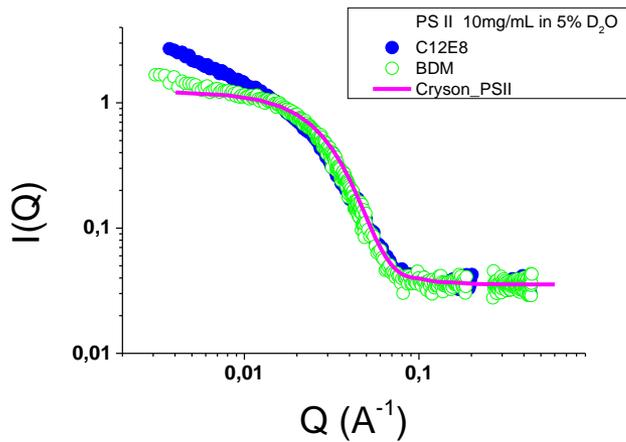


SANS provides : low-resolution structures (only), but:

- → **structure at close to in-vivo conditions**
- possible at room temperature
- **contrast variation** yields protein and detergent shell separately

Nagy, G., Garab, G., and Pieper, J. (2014) in:
Contemporary Problems of Photosynthesis (Editors: S.
Allakhverdiev, A. B. Rubin, V. A. Shuvalov)

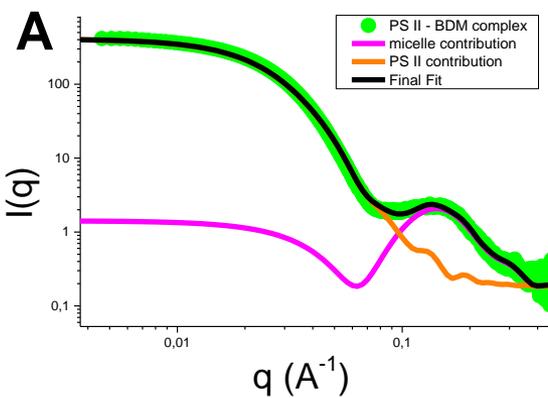
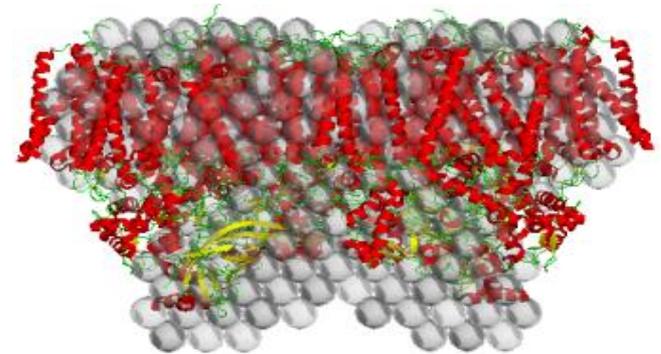
Solution Structures from SANS/SAXS



SANS in 5% D₂O



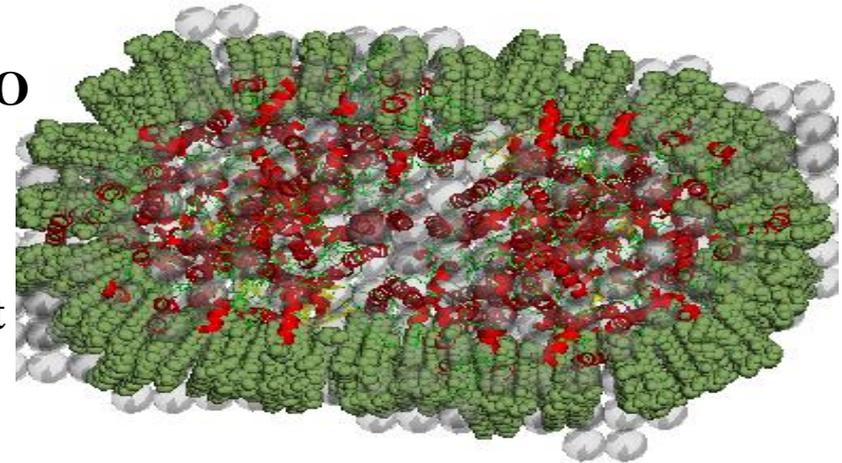
protein only



SANS in 100% D₂O
SAXS



protein-detergent
complex



SANS/SAXS provides detergent structure

Summary – SANS PS II

- ➔ **PS II in β -DM solution exhibits a structure close to the X-ray structure, however slightly expanded**
- ➔ **PS II in β -DM solution is surrounded by one detergent layer**
- ➔ **Combination of SAXS and SANS provides link between X-ray structure and experiments carried out in solution**

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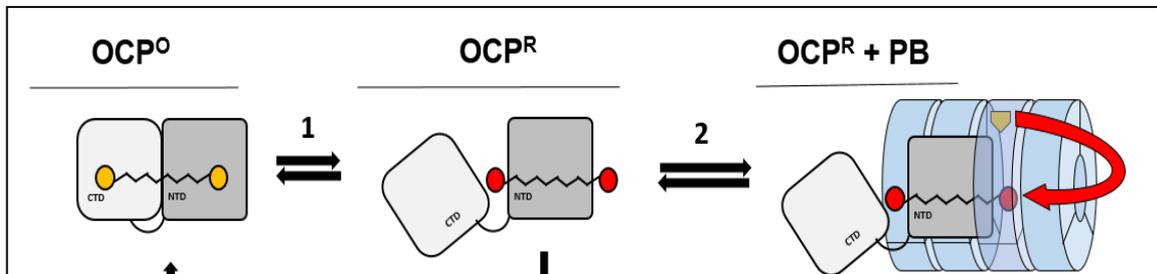
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Orange Carotenoid Protein

Structure and Dynamics from SANS and QENS with optical excitation



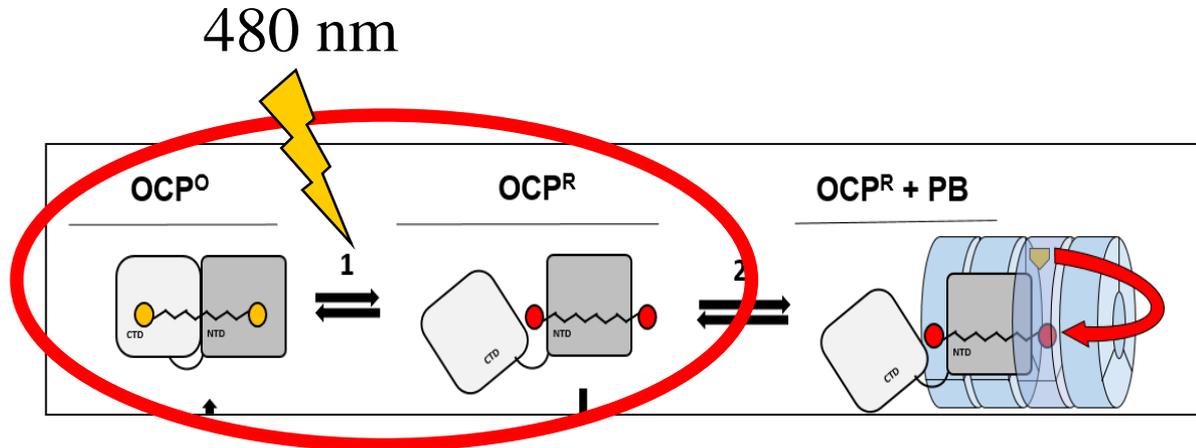
Functional role:

OCPwt is responsible for the non-photochemical quenching of phycobilisomes under intense light

Photodamage is prevented

Orange Carotenoid Protein

Structure and Dynamics from SANS and QENS with optical excitation



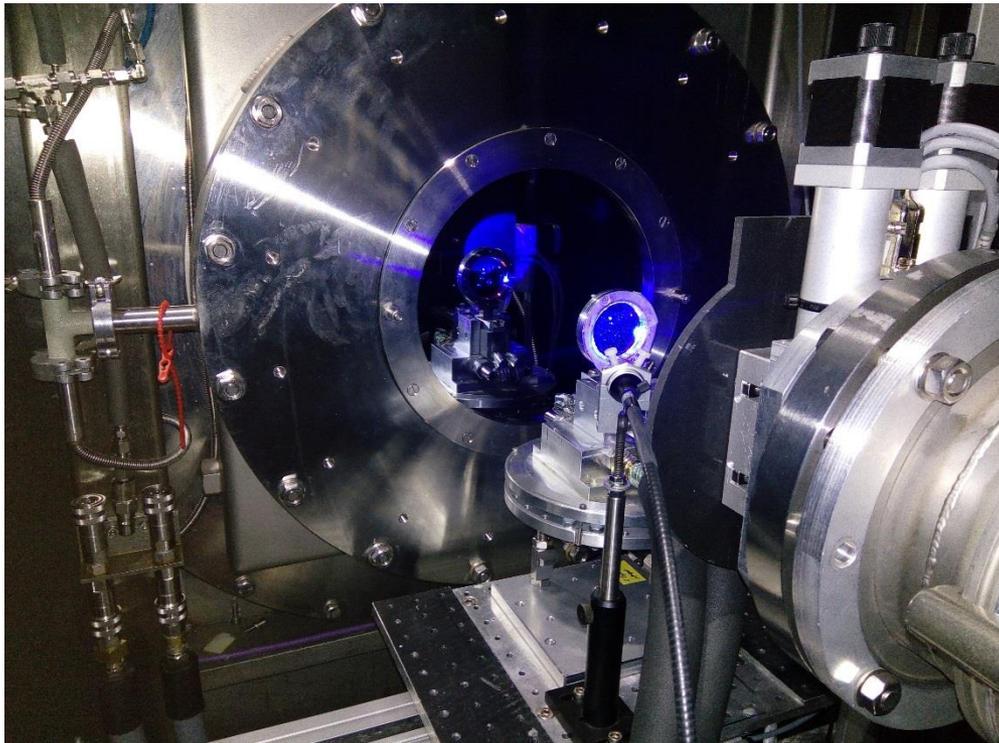
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Instrument
platform

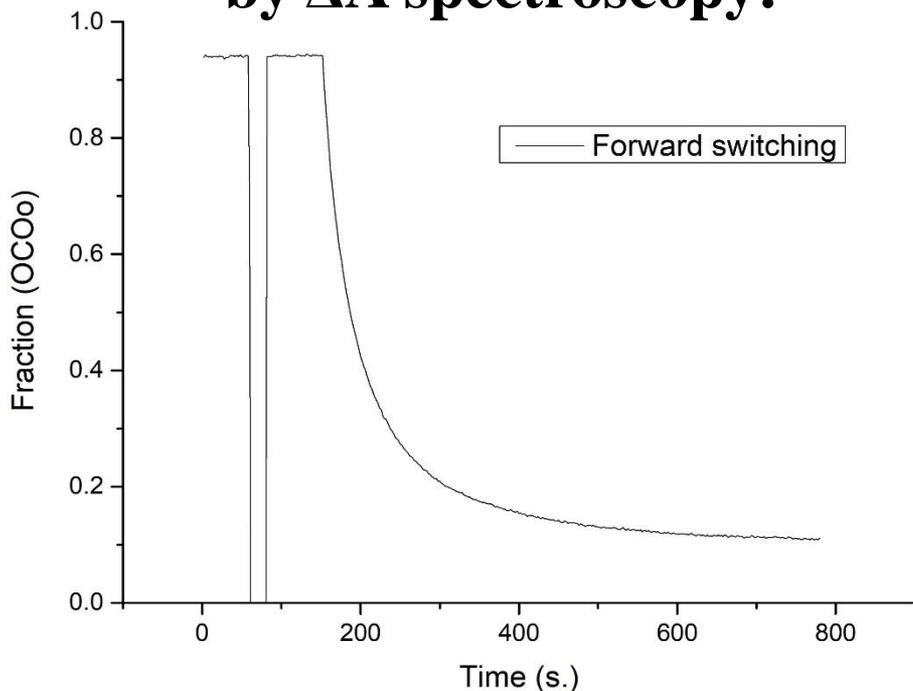
KWS 1 (FRMII)

Golub et al. **JPC B** (2019)

Orange Carotenoid Protein

Structure and Dynamics from SANS and QENS with optical excitation

Check of photoswitching
by ΔA spectroscopy:



Sample cell



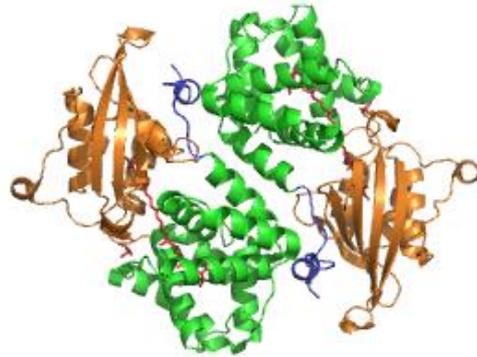
OCP_O

OCP_R

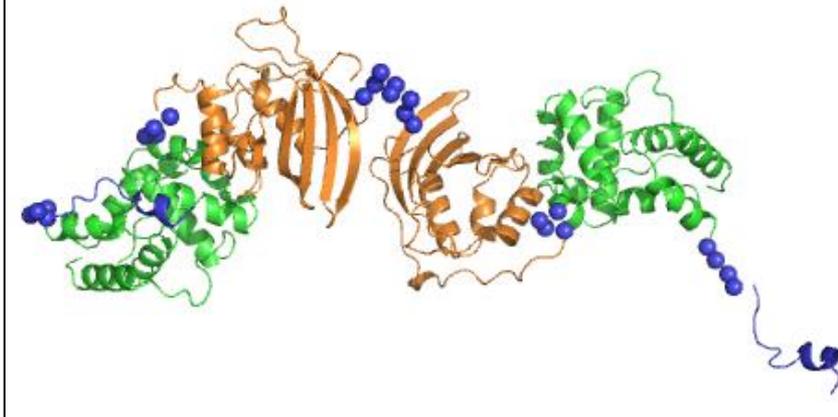
OCP is converted by >90%

OCP Structure: SANS Light Effect

OCP_o



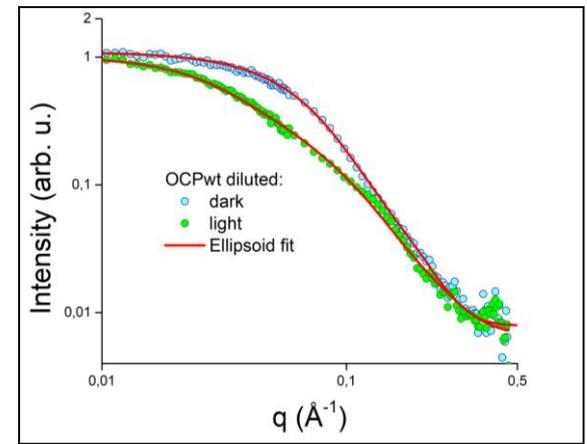
OCP_R



**OCP_R: domain separation,
unfolding of NTE**

SANS results:

OCPwt sample is monodisperse even at high concentrations
Clear structural change under constant light illumination



Golub et al. JPC B (2019)

Summary OCP

- ➔ **OCP is converted to active state under illumination**
- ➔ **OCP expands under illumination → domain separation**
- ➔ **OCP in its active state reveals higher flexibility**
- ➔ **using SANS**
Solution structure in active state can be well characterized

What is SANS/SAXS good for?

- ➔ **Check for oligomerization state / aggregation**
- ➔ **Solution structures at physiological temperatures**
- ➔ **Complex formation**
- ➔ **Large scale conformational changes**

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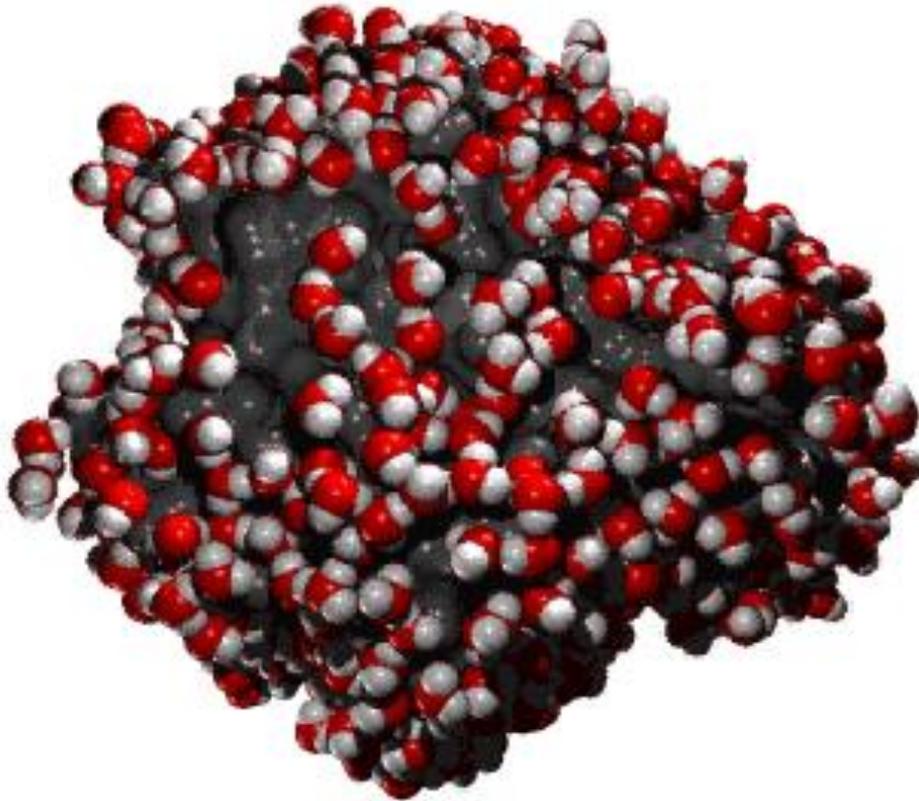
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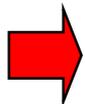
Molecular Dynamics



**MD simulation
of hydrated myoglobin**



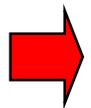
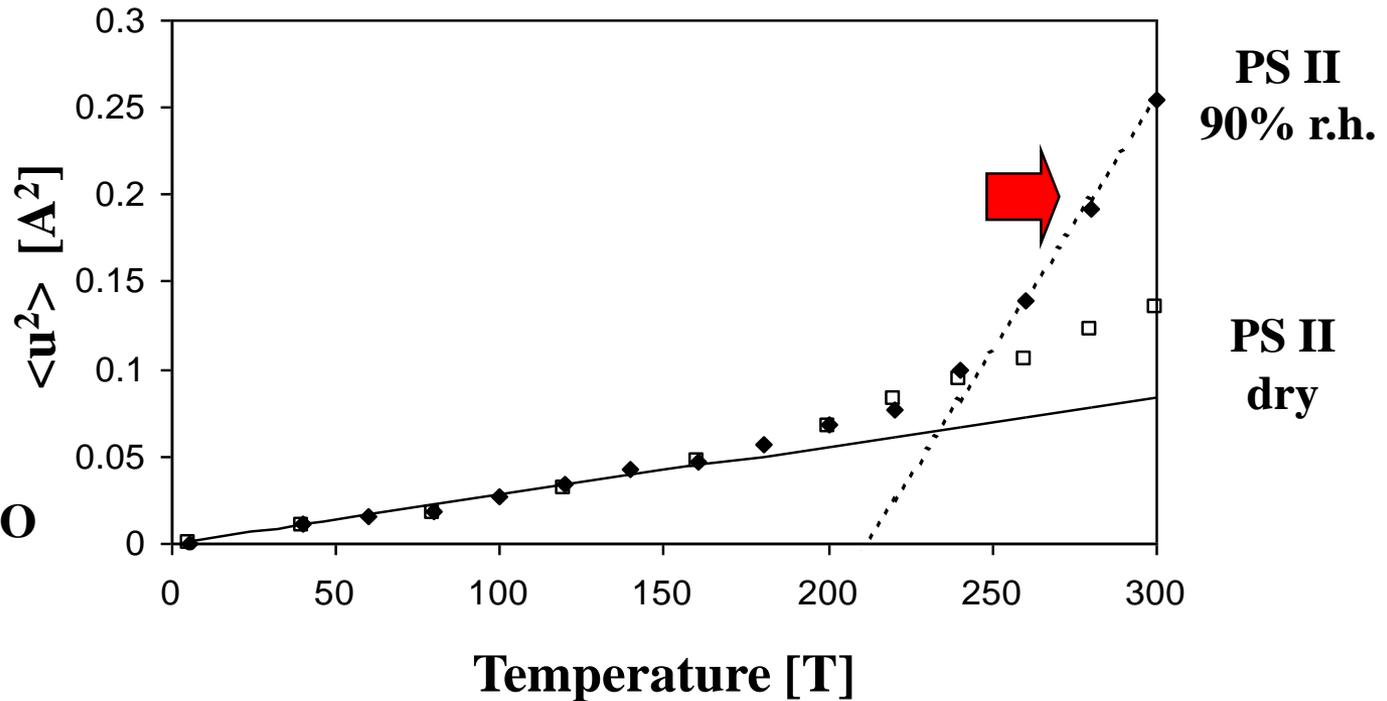
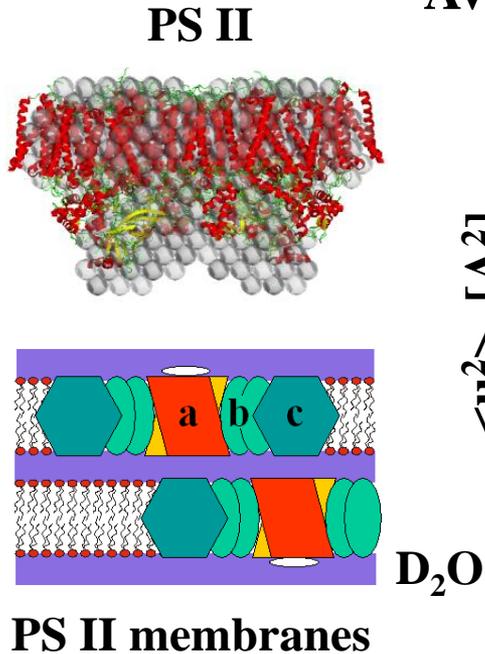
**Courtesy of
D. Tobias (UCI)**



**Proteins are flexible / motions on different time/length scales
Flexibility due to stochastic ps-motions of protein residues**

PS II Conformational Flexibility

Average atomic mean square displacement



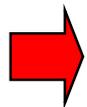
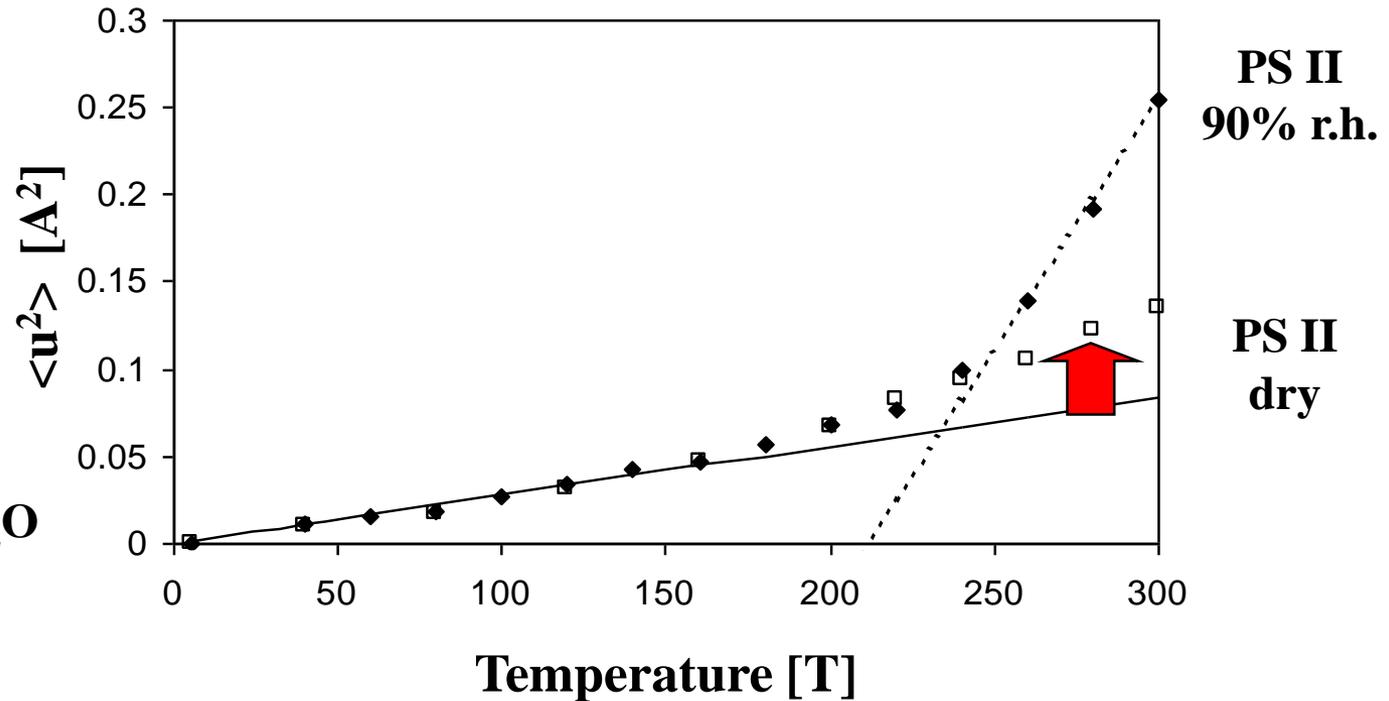
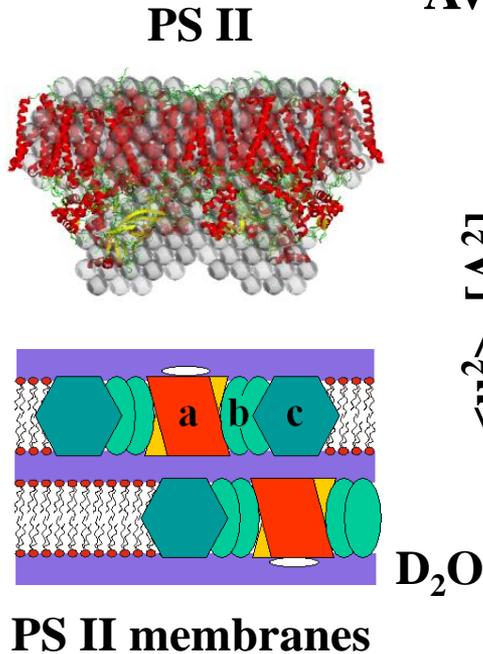
PS II membranes exhibit
„dynamical transition“ at ~ 240 K
 20 ps-resolution

Pieper et al.
 Biochemistry (2007), EPJ (2008),
 BBA (2012)

Vrandečić et al. JPC B (2015)
 Golub et al. JPC B (2018)

PS II Conformational Flexibility

Average atomic mean square displacement

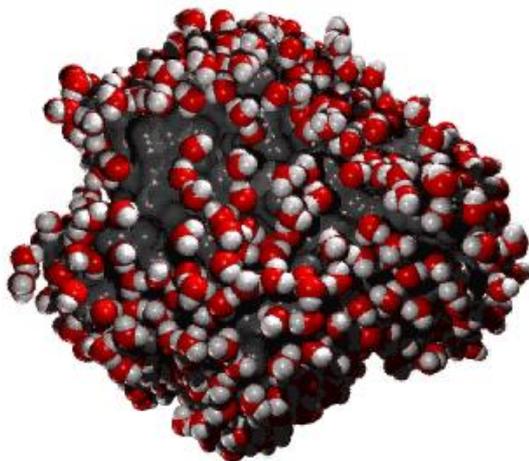


dry PS II membranes exhibit NO „dynamical transition“ at ~ 240 K
dehydration similar to freezing

Pieper et al.
Biochemistry (2007), EPJ (2008),
BBA (2012)

Vrandečić et al. JPC B (2015)
Golub et al. JPC B (2018), (2019)

Molecular Dynamics Simulations



MD simulation of hydrated myoglobin



D. Tobias (UCI)

VOLUME 88, NUMBER 13

PHYSICAL REVIEW LETTERS

1 APRIL 2002

Role of Protein-Water Hydrogen Bond Dynamics in the Protein Dynamical Transition

M. Tarek^{1,2} and D.J. Tobias³

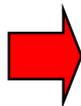
¹NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8562

²Chemistry Department, University of Pennsylvania, Philadelphia, Pennsylvania 19103-6323

³Department of Chemistry and Institute for Surface and Interface Science, University of California, Irvine, California 92697-2025

(Received 14 September 2001; published 14 March 2002)

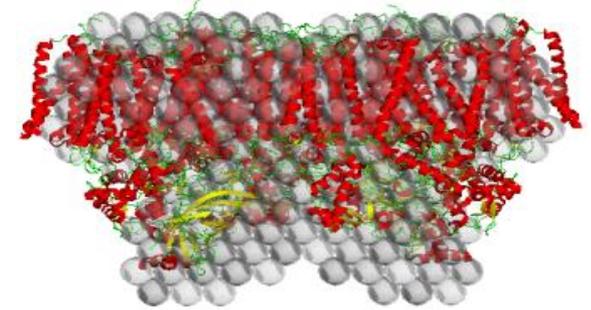
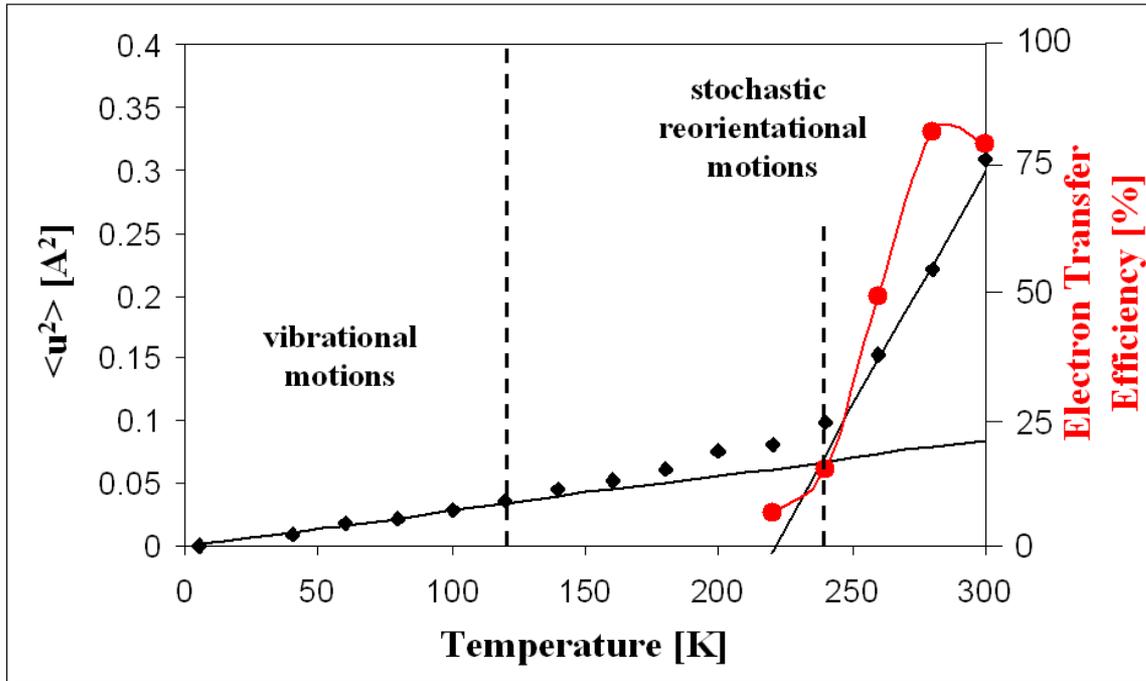
The role of water in protein dynamics has been investigated using molecular dynamics simulations of crystals and a dehydrated powder. On the 100 ps time scale, the anharmonic and diffusive motions involved in the protein structural relaxation are correlated with the protein-water hydrogen bond dynamics. The complete structural relaxation of the protein requires relaxation of the hydrogen bond network via solvent translational displacement. Inhibiting the solvent translational mobility, and therefore the protein-water hydrogen bond dynamics, has an effect on the protein relaxation similar to dehydration.



Translational motions of hydration water induce dynamics

Functional Relevance of Dynamics in PS II ?

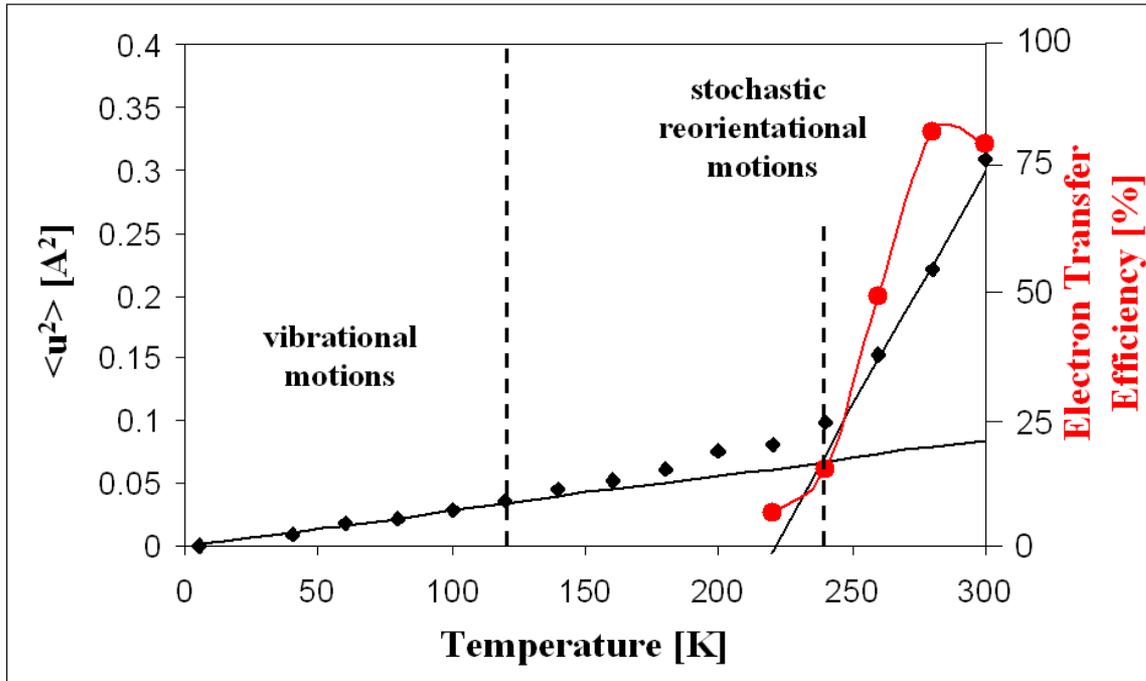
PSII Flash-Induced Fluorescence Yield



Pieper et al.
**Biochemistry (2007), EPJ (2008),
 BBA (2012)**

Functional Relevance of Dynamics in PS II

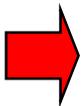
PSII Flash-Induced Fluorescence Yield



onset of protein conformational motions at ~240 K correlated with activation of $Q_A \rightarrow Q_B$ electron transfer
i.e. protein dynamics and hydration are prerequisite for PSII function

Pieper et al.
 Biochemistry (2007), EPJ (2008),
 BBA (2012)

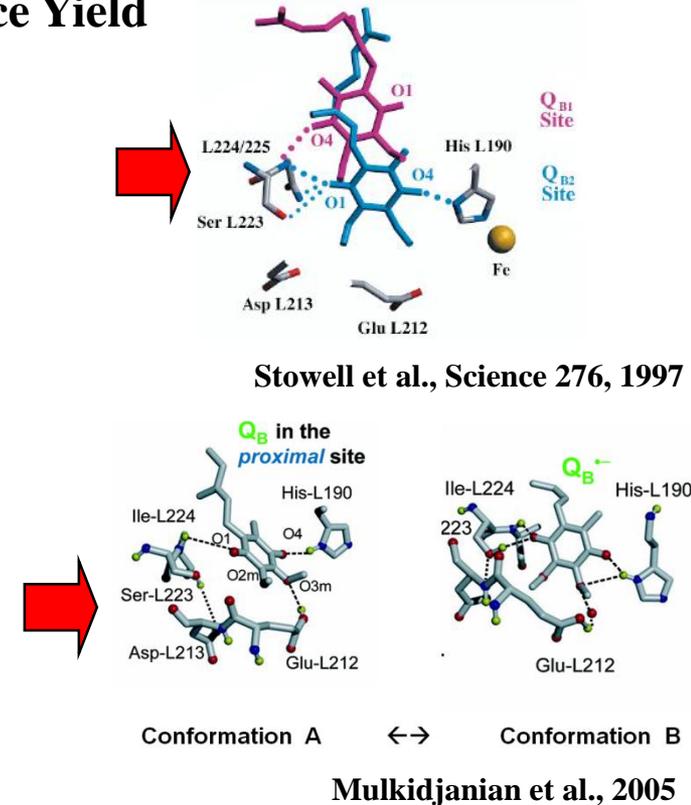
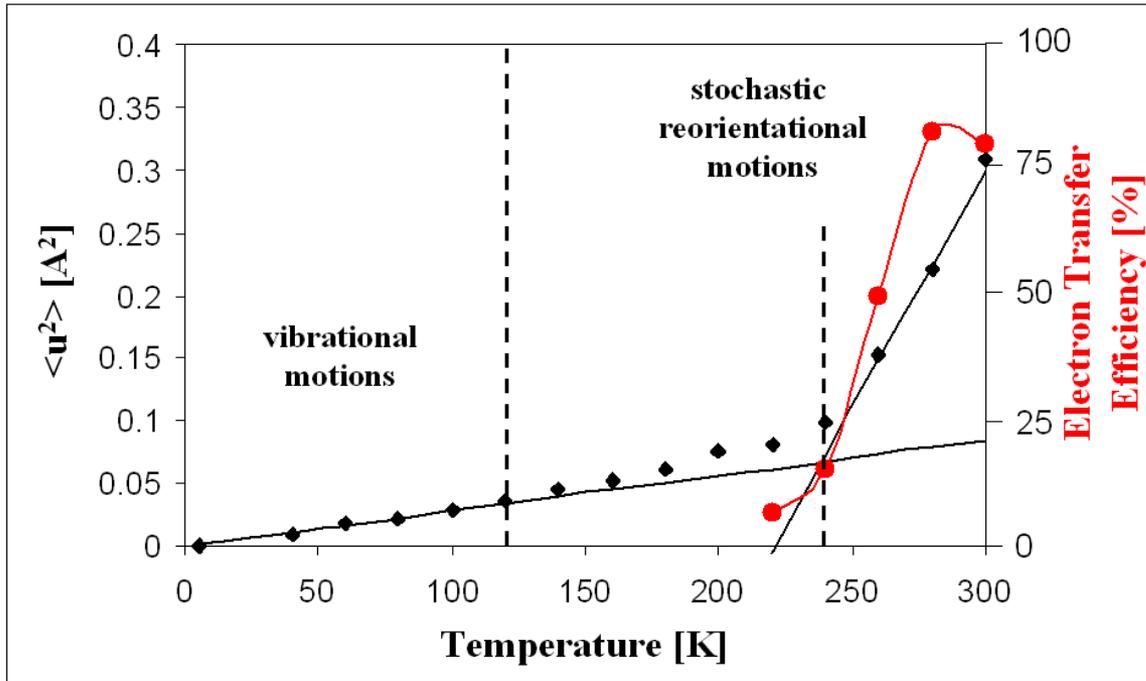
- Q_A - Q_B electron transfer requires protein flexibility
- possible reasons: Q_B isomerization and/or rearrangement of protein environment
 see e.g. Stowell et al., *Science* 276, 1997, 812-816
 Mulkidjanian et al., *Biochem. Soc. Trans.* 33, 2005, 845-850.



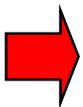
- **cf. bacteriorhodopsin:** retinal isomerization in photocycle (e.g. Fitter et al., JPC B 1999)

Functional Relevance of Dynamics in PS II

PSII Flash-Induced Fluorescence Yield



- Q_A - Q_B electron transfer requires protein flexibility
- possible reasons: Q_B isomerization and/or rearrangement of protein environment
see e.g. Stowell et al., *Science* 276, 1997, 812-816
Mulkiidjanian et al., *Biochem. Soc. Trans.* 33, 2005, 845-850.



- cf. bacteriorhodopsin: retinal isomerization in photocycle (e.g. Fitter et al., *JPC B* 1999)

Summary – PS II/water dynamics

- ➔ **PS II dynamics accomodates function**
- ➔ **PS II dynamics depends on temperature AND hydration**
- ➔ **First hydration water shell has to be completed to induce dynamics AND function**

2. Example

Time-resolved studies of BR under illumination

PRL 100, 228103 (2008)

PHYSICAL REVIEW LETTERS

week ending
6 JUNE 2008

Transient Protein Softening during the Working Cycle of a Molecular Machine

Jörg Pieper,^{1,*} Alexandra Buchsteiner,² Norbert A. Dencher,³ Ruep E. Lechner,^{2,3} and Thomas Haub^{2,3}

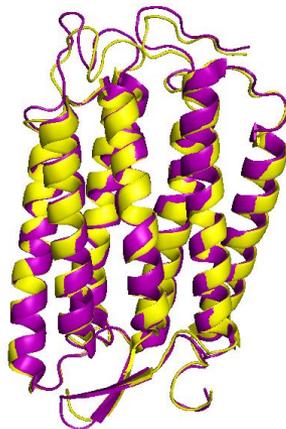
¹Max-Volmer-Laboratories for Biophysical Chemistry, Technische Universität Berlin, Strasse des 17. Juni 135, 10623 Berlin, Germany

²Hahn-Meitner-Institut Berlin, Glienicker Str. 100, 14109 Berlin, Germany

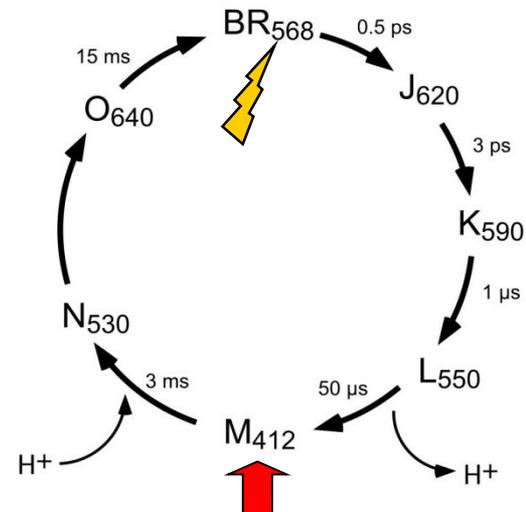
³Physical Biochemistry, Department of Chemistry, Technische Universität Darmstadt, Petersenstrasse 22, D-64287 Darmstadt, Germany

(Received 13 December 2007; published 3 June 2008)

Bacteriorhodopsin



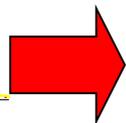
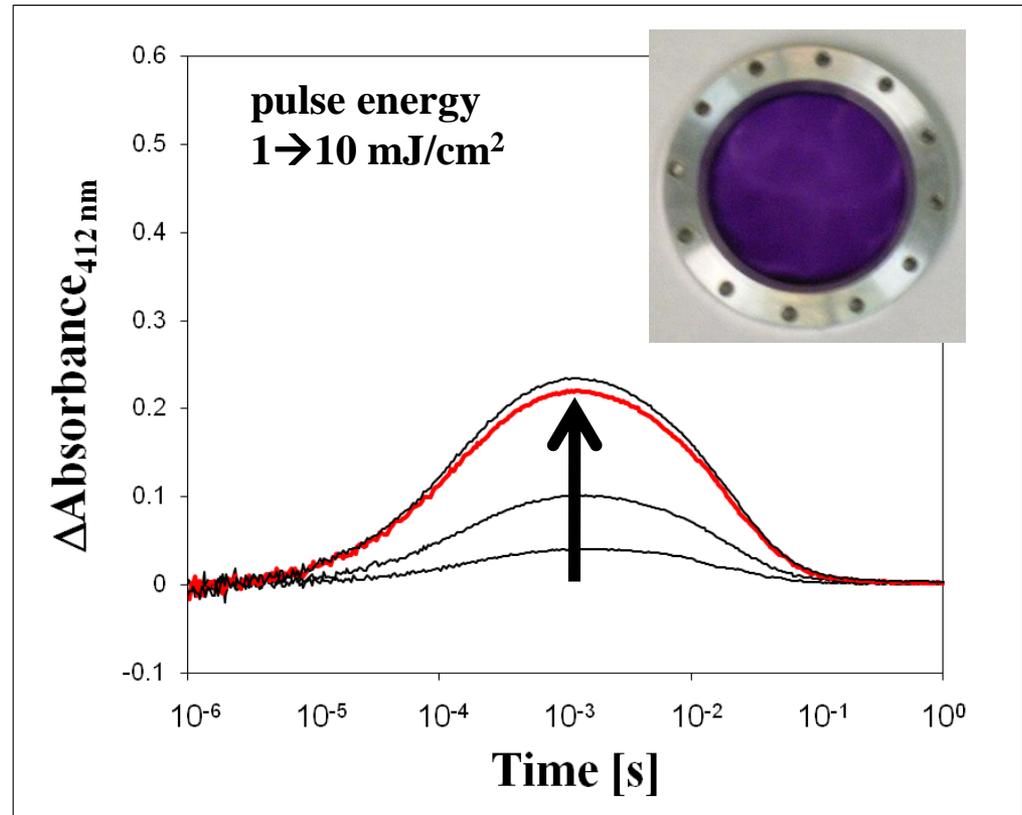
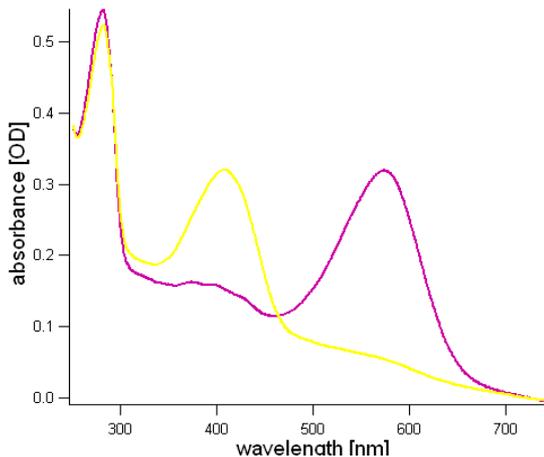
Sass et al. Nature (2000)



3D Conformational Change

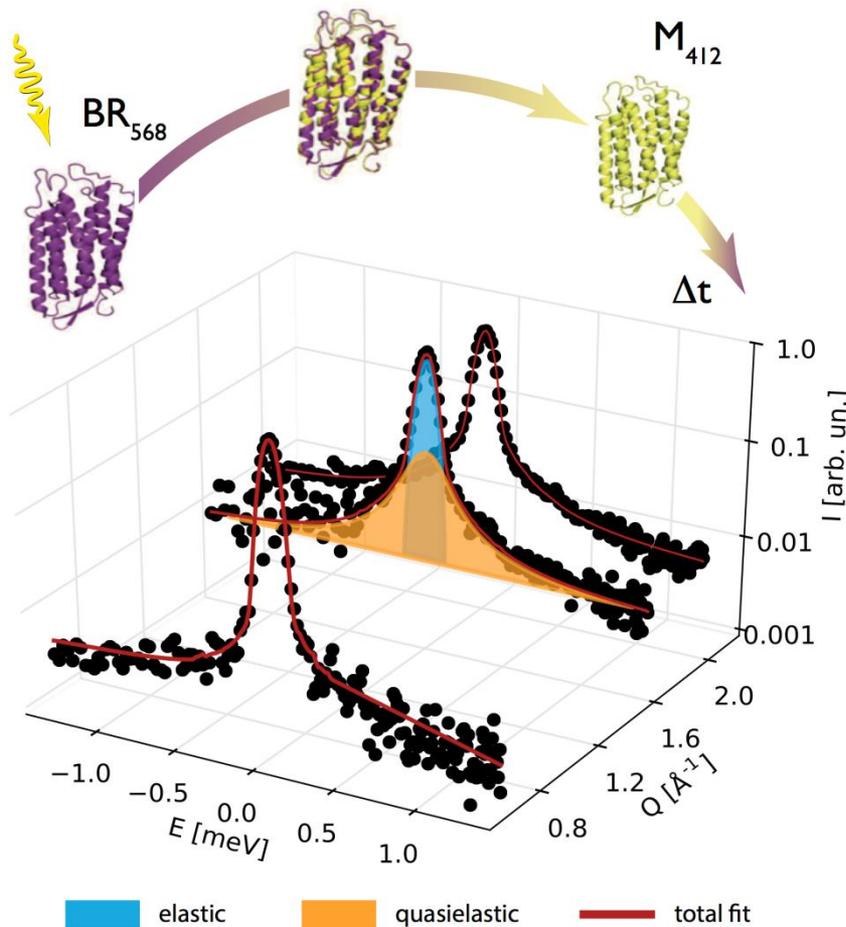
Excitation Conditions of QENS – Experiment

„Protein at Work“



**Linear increase of ΔOD with pulse energy up to threshold of $\sim 10 \text{ mJ/cm}^2$ & pulse
Protein remains functional**

Time resolved QENS data of BR



IN 5, ILL Grenoble

5Å, ca. 93μeV

2D simultaneous fit
Isotropic rotation
on a sphere

describes localized
protein motions

free parameter:

P_{mob}

IN5 experiments:

- analysis of Q-dependence to characterize protein motions
- scan photocycle separately at several delay times shorter than 7 ms

Conclusions BR

- ➔ **Pump-probe QENS experiments are feasible**
- ➔ **Permit preparation / selection of specific functional states after proper sample characterization**
- ➔ **However, time selection leads to large losses in neutron intensity → higher flux needed (pump-probe setup for ESS)**
- ➔ **Pump-probe experiments highlight active role of ps-protein dynamics in functional processes**



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Thank You for your attention !