SFB Molekulare Bioenergetik -Teilprojekt P27

Primary Photoreactions of Membrane Proteins in Energy and Signal Transduction : Report

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The primary reaction of proteorhodopsin

The goal was the real time observation of the ultrafast photoisomerisation of the retinal in proteorhodopsin and of the subsequent dynamic processes, like vibrational relaxation and conformational dynamics.

Excitation energy and electron transfer in photosynthetic units

Investigation of the light reaction of native and modified photosynthetic units (psu) of *R. rubrum*. The SPUHK1 mutant lacks the reaction center H subunit. Differences in the temporal behaviour of the energy transfer steps after photoexcitation were investigated.

fs vis-pump/mid-IR-probe spectrometer

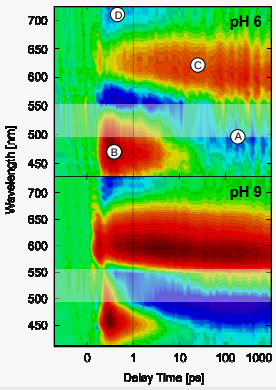
A VIS pump mid-IR probe setup with a time resolution of of 150 fs was assembled to obtain insights in ultrafast structural changes during photoreactions.

pH dependent primary reaction of proteorhodopsin

Proteorhodopsin is a light driven proton pump found in marine bacterioplankton. The retinal class I protein generates a chemiosmotic membrane potential and may be part of an unexpected bacterial energy conversion pathway in the oceanic surface waters worldwide.

primary photoreaction: *all trans* → 13 *cis* isomerisation of the chromophore retinal.
inversion of pumping direction at low pH (see P1, Bamberg): protonation state of the primary proton acceptor Asp 97 (pK_a 7,6) seems to be important.

Transient absorption spectra of PR



A: ground state bleach
B: excited state (S1) absorption
C: product state (J/K) absorption
D: stimulated emission [1]

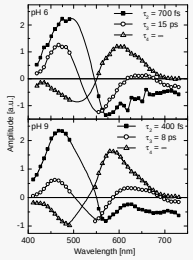
pH-dependent initial reaction effects:

reduced overall reaction speed at pH 6

electrostatic control of isomeristation reaction? [2]

reduced quantum yield at pH 6

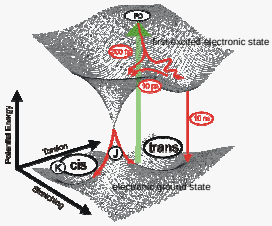
Multiexponential global fit analysis



ultrafast initial process with a timeconstant τ_1 < 200 fs

biphasic decay of excited state and stimulated emission
biphasic rise of product state

General potential surface reaction scheme for PR



isomerisation: first C=C streching motion, then torsion [3]

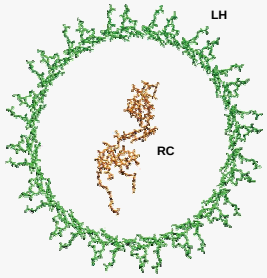
splitting of the population on the excited state potential surface

relaxation either through a conical intersection or by fluorescence

Energy transfer and dynamic differences in photosynthetic subunits of *R. rubrum*

Chromophore Arrangement

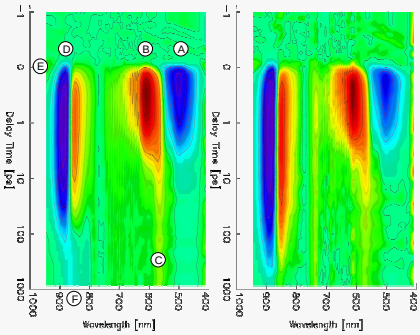
The LH apparatus of *Rhodospirillum* (*Rs.*) *rubrum* is composed of only one type of antenna, the LH1 complex, which surrounds the RC in a ring-like array of 16 αβ-polypeptide subunits. Each subunit binds two bacteriochlorophylls and one spirilloxanthin [4].



Deletion of the H subunit of *Rhodospirillum rubrum*:

The SPUHK1 mutant was created by deleting the reaction center H subunit during site-directed interposon mutagenesis [5].

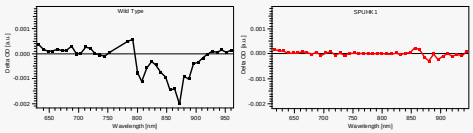
Spectral Regions of the Wild Type and SPUHK1



A: ground state bleach
B: excited state absorption (carotenoid)
C: triplet state T_1 (spirilloxanthin)
D: BChl bleaching / Electrochromic shift
E: carotenoid cation formation from S_2
F: RC bleaching

RC investigation within photosynthetic unit

Using time resolved difference absorption spectroscopy, LH-RC interaction can be measured directly in the photosystem unit, without being necessary to remove the antennae.



At this delay time, almost all of the energy transfer and charge separation processes are expected to be completed. Therefore, the spectra reflect the differences in absorption between the charge-separated and ground states.

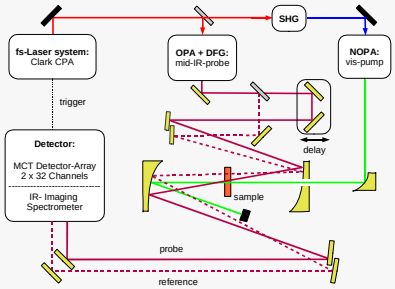
Results and Conclusinos

Transient Absorption Spectra and Global Fit analysis in the NIR region prove the existence of the RC for WT.

Energy transfer from the Carotenoid to the Bacterio-chlorophyll of the Light Harvesting Antenna takes place for both systems, WT and SPUHK1.

Energy transfer in LH1 does not critically depend on proper RC formation and/or insertion.

fs vis-pump/mid-IR-probe spectrometer



Fs-Laser system:
Wavelength: 775 nm
Pulse duration: 160 fs
Pulse energy: 800 µJ
Repetition rate: 1 kHz

Vis (pump):
Wavelength range: 480 – 700 nm
Pulse duration: < 50 fs
Pulse energy: 10 µJ

Mid-IR (probe):
Wavelength Range: 3 – 10 µm
Pulse Duration: < 150 fs
Pulse Energy: 0,5 – 1 µJ

Detection system:
• 250 mm Czerny Turner Spectrograph
• 2 x 32 element MCT Detector Array

Data Aquisition:
Multichannel pulse integrator supports simultaneous aquisition of probe and reference signal on single shot basis.

References

[1] Lenz M. O., *et al.*, to be submitted

[2] Song L., *et al.*, (1993) Science261, 891-894

[3] Garavelli M., *et al.*, (1998) JACS 120, 1285-1288

[4] Karrasch R., *et al.*, (1995) EMBO J. 14, 631–638

[5] Lupo D.*et al.*, (2004) J. Bacteriol 186, 5585