Annual report 2014

FOM programme nr. 126
'The thylakoid membrane - a dynamic switch'

Foundation for Fundamental Research on Matter
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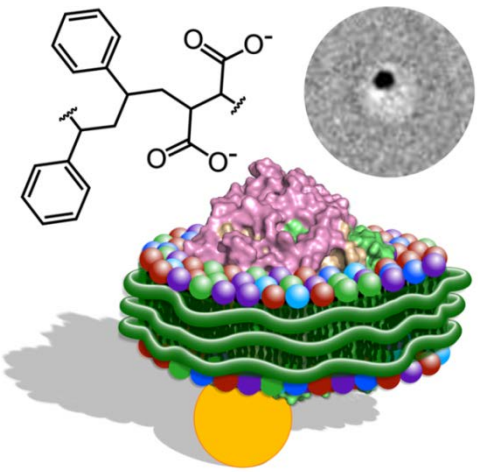


Schematic representation of the nanodisc with a reaction center from Rhodobacter sphaeroides. In the right-upper corner is an Electron Microscopy image of this nanodisc with RC, and in the upper-left corner is the chemical structure of the SMA co-polymer.

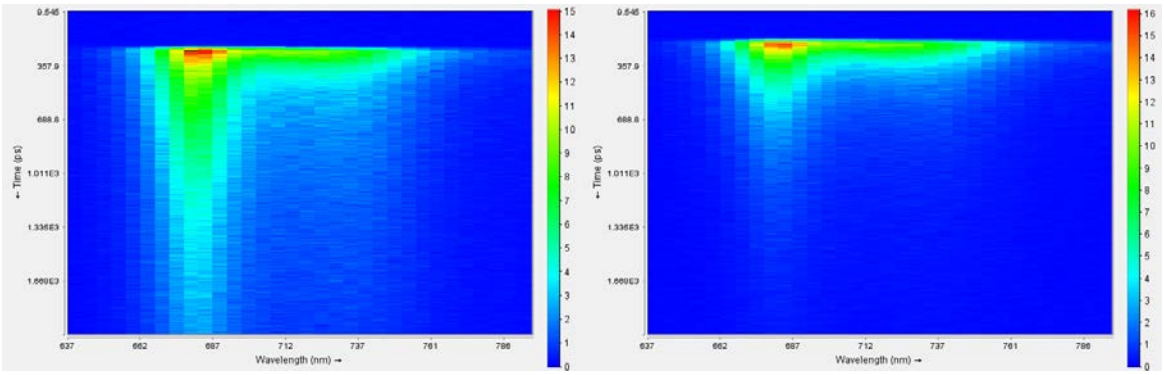
May 2015

Content

1. Scientific results 2014

The programme The Thylakoid Membrane- A Dynamic Switch has shown significant progress along a number of complementary research lines.
Team 10TM01 in collaboration with 10TM08 performed classical MD calculations on the major plant light-harvesting complex LHCII, an essential player in the dynamic thylakoid membrane and responsible for the switching between a photosynthetic light-harvesting state and a photoprotective quenched state. It was established that indeed solubilized LHCII is able to undergo significant rearrangements in its structure as obtained previously by X-ray crystallography. These results do suggest that LHCII is able to modulate its energetics and thereby its fluorescence quenching properties. These results have direct implications for the experimentally observed (by 10TM03) switching dynamics of LHCII, of Photosystem II supercomplexes and of FCP, the major light harvesting complex of diatoms and functionally and structurally related to LHCII.

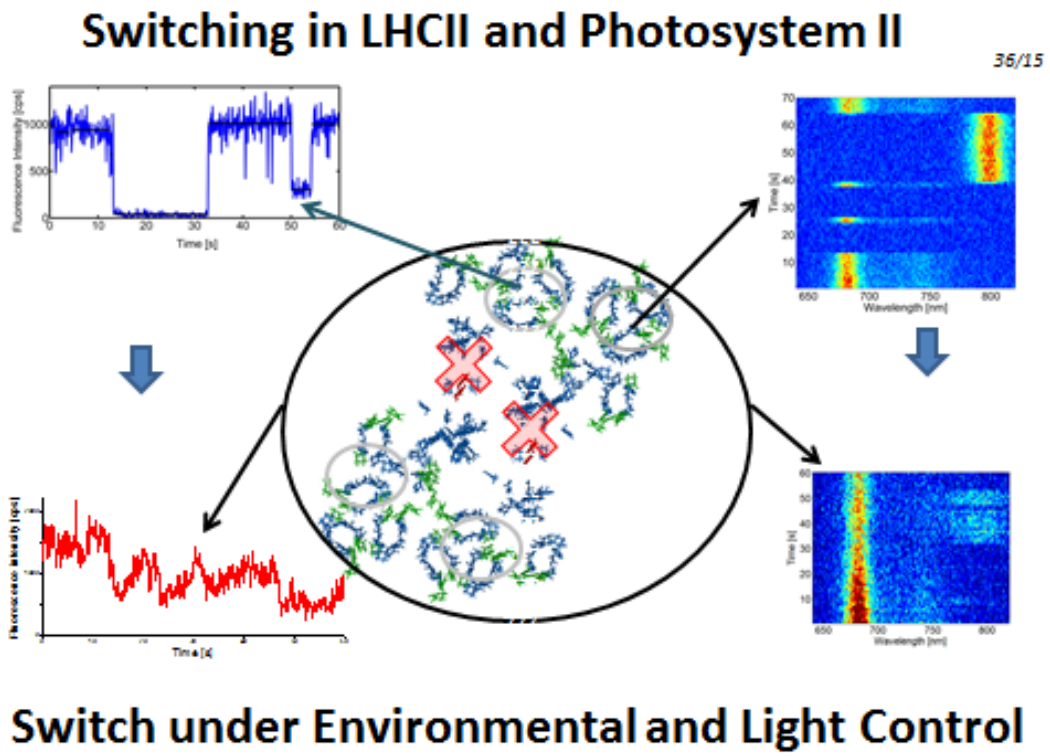
A second major development occurred in team 10TM06, in collaboration with 10TM03. Using the styrene maleic acid (SMA) co-polymer the bacterial photosynthetic reaction center, a photosynthetic pigment-protein was purified. This method yields nanodisc particles (~10 nm diameter) that are small patches of lipid bilayer having the membrane protein of interest in its native lipid composition. This, in contrast to detergents, offers a very stable host for purified membrane proteins and allows us to identify native protein-lipid interactions. This is the basis for present collaborations with the group of prof. Croce (Jan Dekker and Henny van Roon) on purification of proteins from spinach thylakoids and blebs, with the group of Prof. Marrink on simulation of the action of SMA copolymers in lipid membranes and with the group of Prof. Van Grondelle on (single molecule) spectroscopy on nanodiscs with proteins.



Streak-camera images of spinach leaves with closed reaction centers. Non-photochemical quenching is absent in the left figure and present in the right one. The time is picoseconds is plotted along the vertical axis and the fluorescence wavelengths are plotted along the horizontal axis. Fluorescence intensity is given in false colours. Fluorescence below 700 nm is mainly stemming from photosystem II and it is clear that upon quenching the excited-state lifetime decreases substantially. The fluorescence above 700 nm is mainly due to photosystem I and detailed analysis shows that it is not affected by NPQ.

The third major research line aims to measure dynamic changes in the intact thylakoid membrane related to rearrangement of the relevant proteins (10TM02), dynamics of lipids (10TM07), functional dynamics (10TM04, 10TM03). 10TM02 has initiated cryo-electronmicroscopy on Photosystem II supercomplexes with the aim to resolve a structure of such a complex and how this rearranges under stress. This combines very well with the MD simulations on Photosystem II complexes (10TM08), the *in vivo* fluorescence studies under different stress conditions (10TM04), the time-resolved single molecule fluorescence experiments on Photosystem II supercomplexes (10TM03) and the lipid and protein diffusion dynamics as measured by NMR (10TM07). Interes-

tingly by the latter technique the diffusion of lipids can be distinguished from that of proteins. Hopefully, in the near future, also the SMA copolymer method developed by 10TM06, that allows us to obtain small patches of membrane will start to work for plant thylakoids.
Finally, in 10TM05, we, in close collaboration with prof. Bruno Robert (VU and CEA), have made very significant progress in further developing super-resolution microscopy. Our approach is much simpler than other existing super-resolution techniques all based on complex laser excitation schemes. Basically we reconstruct the high resolution image from an ensemble of classical microscopy images. Our current spatial resolution is several 10s of nm, and can be improved. Since the concept is up for patenting not many details can be given here and will be presented later.



LHCII switches under Environmental and Light Control. The figure displays single particle fluorescence experiments on isolated LHCII top and on Photosystem II supercomplexes, of which LHCII is an essential component, bottom. Left: time traces that show the reversible variation in fluorescence intensity for purified LHCII (top) and Photosystem II supercomplexes (bottom). Right: the evolution of the emission spectrum of purified LHCII (top) and Photosystem II supercomplexes (bottom). Note the dramatic and reversible redshift of the fluorescence from around 680 nm to above 800 nm that occurs occasionally

2. Added value of the programme

The programme works very well. There are many collaborations between the participating groups, in fact often new collaborations originate from the frequent discussion meeting organized by the consortium. The programme builds on the biological material available within the consortium, the state-of-the-art biochemistry, the spectrum of advanced spectroscopic/structural techniques available (ultrafast spectroscopy, single molecule fluorescence, in vivo fluorescence, various NMR techniques, cryoEM, superresolution microscopy) , the excellent data-analysis and modelling facilities and last but not least the MD facility. Concerning the latter, it is remarkable to watch the pivotal role that the MD-group plays in connecting a variety of projects, a connection that is a major achievement of this programme.

A second excellent example of new and unexpected collaboration is the SMA-method for membrane-protein purification. The nanodisc method allows us to study protein-protein and lipid-protein interactions for proteins from photosynthetic membranes. This is the basis for present collaborations between the groups of Killian, Croce, Dekker (Henny van Roon), Marrink and van Grondelle on purification of proteins from spinach thylakoids and blebs using SMA copolymers, on the simulation of the action of SMA copolymers in lipid membranes and on (single molecule) spectroscopy on nanodiscs with proteins.

Within the consortium a variety of groups aim to observe and understand the structural rearrangements that occur under light (and other forms of) stress. The groups apply complementary methods (biochemical, fluorescence, single complex analysis, NMR, cryoEM) thereby reveal very different aspects of the dynamic thylakoid membrane. The aim is of course to integrate this information into a general and robust model amongst others by MD calculations in combination with spectroscopic modeling.

Finally, in 2016 the 17th International Photosynthesis Conference will be held in Maastricht (august 12-17). The Thylakoid Membrane-A Dynamic Switch consortium plays a leading role in its organization. Croce and Van Amerongen are joint chairs of the congress, several other members of the consortium participate in the programmecommittee and/or in the organization of satellites.

3. Personnel

All positions are occupied per today (no vacancies). The PhD projects proceed as planned and in all cases will lead to PhDs.

4. Publications

10TM01

Oral presentations

Gordon Seminar in Photosynthesis (Speaker), Vermont, 2014.
CHAINS conference (Speaker), Netherlands, 2014.
Jam Session in Photosynthesis workshop (Speaker), Netherlands, 2014.

Poster presentations

FOM Dutch Biophysics conferece (Poster), Netherlands, 2014.
ACMM/NSBM symposium (Poster), Netherlands, 2014.

Nicoletta Liguori, the PhD student appointed in 10TM01, was awarded the Poster prize at the Gordon Conference in Photosynthesis, Vermont, August 2014. For this prize, she received a mention in a special issue of the international journal Photosynthesis Research (DOI 10.1007/s11120-014-0058-9). Also, she was awarded the FOM Event Grant (2000 Euro) for the organization of the workshop Jam Session in Photosynthesis.

10TM02

Manuscripts in preparation.

10TM03

- T.P.J. Krüger, C. Ilioaia, P. Horton, M.T.A. Alexandre, R. van Grondelle (2014). How protein disorder controls non-photochemical fluorescence quenching. In: Non-Photochemical Quenching and Energy Dissipation in Plants, Algae and Cyanobacteria, Advances in Photosynthesis and Respiration Volume 40, 2014, pp 157-185, Springer.

- T.P.J. Krüger, C. Ilioaia, M.P. Johnson, A.V. Ruban, R. van Grondelle (2014).Disentangling the low-energy states of the major light-harvesting complex of plants and their role in photoprotection. Biochim. Biophys. Acta 1837(7), pp 1027-1038.
- M.T.A. Alexandre, K. Gundermann, A. A. Pascal, R. van Grondelle, C. Büchel, B. Robert (2014). Probing the carotenoid content of intact Cyclotella cells by resonance Raman spectroscopy. Photosynth. Res. 119, pp. 273-281.
- M.T.A. Alexandre, T.P.J. Krüger, C. Büchel, C. Ilioaia, R. van Grondelle (2014). Conformational switching of the fucoxanthin chlorophyll protein as observed by single molecule spectroscopy. Submitted to Proc. Natl. Acad Sci.
- E.A. Stojković, K.C. Toh, M.T.A. Alexandre, M. Baclayon, K. Moffat, J.T.M. Kennis (2014). FTIR Spectroscopy Revealing Light-Dependent Refolding of the Conserved Tongue Region of Bacteriophytochrome. J. Phys. Chem. Lett., 5 (15), pp 2512–2515.

10TM04
- Poster: Single molecule FRET imaging with improved time resolution using stroboscopic alternating laser excitation (sALEX). Veldhoven 29-30 Sept 2014.

10TM05
- L. Gerencser, B. Boros, V. Derrien, D. K. Hanson, C. A. Wraight, P. Sebban, and P. Maroti, Stigmatellin probes the electrostatic potential in the QB site of the photosynthetic reaction center, Biophys. J. 108:379-394.
- Laszlo Gerencser, Jan Dekker and Hans van Gorkom, The catalytic function of water-oxidizing complex of photosystem II monitored by time-resolved absorption measurement, FOM Physics Meeting Veldhoven.

10TM06
- Scheidelaar S., Koorengevel M.C., Dominguez-Pardo J., Meeldijk J.D., Breukink E., Killian J.A. (2015) Molecular model for the solubilization of membranes into nanodisks by styrene maleic acid copolymers, Biophys. J. 108, 279-290.
- Swainsbury D.J., Scheidelaar S., van Grondelle R., Killian J.A., Jones M.R. (2014) Bacterial Reaction Centers Purified with Styrene Maleic Acid Copolymer Retain Native Membrane Functional Properties and Display Enhanced Stability. Angew Chem Int Ed Engl. 53, 11803-11807.

10TM07
Two manuscripts in preparation.

10TM08
- F.J. van Eerden, D.H. de Jong, A.H. de Vries, T. A. Wassenaar, S.J. Marrink Characterization of thylakoid lipid membranes from cyanobacteria and higher plants by molecular dynamics simulations BBA-Biomembranes, 1848:1319-1330, 2015. Doi:10.1016/j.bbamem.2015.02.025
- http://www.c2w.nl/computer-kijkt-in-chloroplast.401133.lynkx
- H.I. Ingólfsson, M.N. Melo, F.J. van Eerden, C. Arnarez, C. A. López, T.A. Wassenaar, X. Periole, A.H. de Vries, D.P. Tieleman, S.J. Marrink *Lipid Organization of the Plasma Membrane* JACS, 136:14554–14559, 2014 DOI: 10.1021/ja507832e.

5. Valorisation and outreach

Nicoletta Liguori (10TM01)was the organizer of the Jam Session in Photosynthesis workshop, an international conference of experimental and theoretical biophysics of photosynthesis, held at the VU.

She was chair of the International PhD and Postdoc organization of the VU University Amsterdam (i-ProVU) from September-2013 to January-2015.(**http://provu.nl/2014/provund-the-grant**).
She was member of the advisory board BAC for the position of endowed professorship in experimental physics of behavior at the VU University Amsterdam.

Nicoletta Liguori (10TM01) was a speaker at the International Talent Event Amsterdam (ITEA) 2014-congress

The consortium is a very active participant in the BioSolarCells programme.

6. Vacancies

No vacancies.

FOM - 10.1719/4
datum: 01-01-2015

APPROVED FOM PROGRAMME

Number	126.
Title (code)	The thylakoid membrane - a dynamic switch (TM)
Executive organisational unit	BUW
Programme management	Prof.dr. R. van Grondelle
Duration	2011-2015
Cost estimate	M€ 2.6

Concise programme description

a. Objectives

The aim of the programme is to study the structure and dynamics of thylakoid membranes and its constituent pigment proteins under varying conditions. The main objectives are:

- Study the switching thylakoid membrane at nm-μm length scale and at fs-sec timescale.
- Develop quantitative models to understand the dynamic function, structure and organization of this biological switch at nm-μm length scale and at fs-sec timescale.
- Manipulate the switching thylakoid at atomic, molecular, supra-molecular and cellular level.

b. Background, relevance and implementation

Photosynthesis is the biological process performed by plants, algae and photosynthetic bacteria to convert the energy of sunlight into a form that can be used by the organism to grow, maintain, multiply. The ultimate efficiency of photosynthesis relies heavily on the ability of the photosynthetic apparatus to respond to extreme 'stress' conditions: intense light, drought, cold. The photosynthetic thylakoid membrane (TM) that contains all the essential components reacts strongly to stress conditions by switching rapidly between 'photosynthetic' and 'photo-protective' states by reorganizing the TM. Understanding the photosynthetic process thus requires exploring the dynamics of the system and especially the functional and structural flexibility of the TM and its constituting pigment-proteins. The elucidation of the molecular biophysical feed-back mechanism(s), which relate the sensing of photosynthetic activity to the re-organization of the membrane architecture, is a major contribution to understanding the efficiency of photosynthesis in oxygenic organisms in general. It is essential for understanding abiotic stress tolerance, which is relevant for developing strategies to optimize agriculture and biofuel production.

A set of 8 projects has been defined covering all aspects of this problem from the molecular level to the intact chloroplast. (1) biochemistry to produce photosynthetic complexes, membrane fragments and (reconstituted) membranes of increasing complexity and intactness combined with genetics to produce selectively and intelligently genetically engineered materials; (2) EM and EM-tomography to identify single complexes/membrane fragments and their organization in the membrane; (3) single molecule emission spectroscopy to identify the switching capacity of photosynthetic complexes of

increasing complexity; (4,5) fluorescence lifetime imaging (4) and non-linear (5) microscopy on photosynthetic membranes in vitro and in vivo to identify the functional organization at high resolution; (6) solid state NMR to study the dynamics of proteins and lipids in the TM; (7) in vivo NMR to understand the role of transport phenomena and membrane phase changes and (8) molecular dynamics simulations to make a quantitative model of the TM that relates the dynamic structure of supercomplexes/ membrane fragments/-membranes to the physical-chemical properties of the pigment proteins, lipid composition and functionality.

Funding

salarispeil per 01-07-2012

bedragen in k€	≤ 2014	2015	2016	2017	2018	2019	≥ 2020	Totaal
FOM-basisexploitatie	2.283	293	-	-	-	-	-	2.576
FOM-basisinvesteringen	-	-	-	-	-	-	-	-
Doelsubsidies NWO	-	-	-	-	-	-	-	-
Doelsubsidies derden	-	-	-	-	-	-	-	-
Totaal	2.283	293	-	-	-	-	-	2.576

Source documents and progress control

a) Original programme proposal: FOM-10.1237
b) Ex ante evaluation: FOM-10.1344
c) Decision Executive Board: FOM-10.1718

Remarks

The final evaluation of this programme will consist of a self-evaluation initiated by the programme leader and is foreseen for 2016.

SK par. HOZB

Subgebied: 100% FL

Historical overview of input en output

Input	personnel (in fte)				finances* (in k€)
	WP/V	WP/T	PhD	NWP	
2011	-	0.6	1.5	-	134
2012	-	1.9	5.6	0.9	578
2013	-	2.0	6.0	1.0	500
2014	-	1.4	6.0	1.0	553

Output	PhD theses	refereed publications	other publications & presentations	patents
2011	-	-	-	-
2012	-	2	14	-
2013	-	10	28	-
2014	-	24	36	-

* After closing the financial year.

PhD defences

2011
None.

2012
None.

2013
None.

2014
None.

Patents (new/changes)

2013
None.

2014
None.

Overview of projects and personnel

Workgroup FOM-G-29

Leader	Prof.dr. E.J. Boekema
Organisation	Groningen University
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Structural changes in the organization of Photosystem II: supercomplexes and membranes in response to stress 10TM02

FOM employees on this project

Name	Position	Start date	End date
L.S. van Bezouwen	PhD	01 May 2011	30 April 2015

Workgroup FOM-G-31

Leader	Prof.dr. S.J. Marrink
Organisation	Groningen University
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Computational modeling of the structure and dynamics of the PSII supercomplex 10TM08

FOM employees on this project

Name	Position	Start date	End date
F.J. van Eerden	PhD	01 September 2011	31 August 2015

Workgroup FOM-U-38

Leader	Prof.dr. J.A. Killian
Organisation	Utrecht University
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Role of lipids in structure and organization of photosynthetic membranes 10TM06

FOM employees on this project

Name	Position	Start date	End date
S. Scheidelaar	PhD	15 October 2011	14 October 2015

Workgroup FOM-V-05

Leader	Prof.dr. R. van Grondelle
Organisation	Vrije Universiteit Amsterdam
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Non-linear microscopy of the thylakoid membrane: Towards a high-resolution functional image 10TM05

FOM employees on this project

Name	Position	Start date	End date
M.T.A. Alexandre	postdoc	01 June 2011	31 May 2014

Leader	Prof.dr. R. van Grondelle
Organisation	Vrije Universiteit Amsterdam
Project leader	Dr. J.P. Dekker
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Single-molecule fluorescence microscopy of Photosystem II: complexes and membranes in response to stress (10TM03)

FOM employees on this project

Name	Position	Start date	End date
L. Gerencser	postdoc	1 February 2012	31 January 2015

Workgroup FOM-V-22

Leader	Prof.dr. R. Croce
Organisation	Vrije Universiteit Amsterdam
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Changes in the functional organization of Photosystem II: supercomplexes in response to stress 10TM01

FOM employees on this project

Name	Position	Start date	End date
L.M. Roy	TP/T	20 February 2012	19 February 2017
N. Liguori	PhD	15 January 2012	14 January 2016

Workgroup FOM-W-02

Leader	Prof.dr. H. van Amerongen
Organisation	Wageningen Universiteit en Researchcentrum
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	Picosecond microspectroscopy of photosynthetic complexes in vivo 10TM04

FOM employees on this project

Name	**Position**	**Start date**	**End date**
S. Farooq	PhD	01 May 2012	30 April 2016

Leader	Prof.dr. H. van Amerongen
Organisation	Wageningen Universiteit en Researchcentrum
Project leader	Dr. H. van As
Programme	The thylakoid membrane - a dynamic switch
Project (title + number)	1H and 31P in vivo NMR methods to study thylakoid membrane phase, time dependent lipid diffusion and exchange 10TM07

FOM employees on this project

Name	**Position**	**Start date**	**End date**
S. Pagadala	PhD	01 September 2011	31 August 2015