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Wissenschafts - und Erziehungskooperation
AKTION ČESKÁ REPUBLIKA - RAKOUSKO
spolupráce ve vědě a vzdělávání

PROJEKT ABSCHLUSSBERICHT / ZÁVĚREČNÁ ZPRÁVA PROJEKTU

Einreichstelle für Österreich und Tschechien: ***Místo pro podávání návrhů projektů pro ČR i Rakousko:*** DZS - AKTION Na Porici 1035/4 CZ - 110 00 Praha 1 Tel.: + 420-221 850 506 /513, 602 169 216 e-mail: aktion@dzs.cz , http://www.dzs.cz	***Projektnummer/číslo projektu:*** **64p1**

Projektbezeichnung / Název projektu :

Study of photosynthetic PsbI protein's interaction with the thylakoid membrane

Studium interakce fotosyntetického proteinu PsbI s thylakoidní membránou

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PROJEKT ABSCHLUSSBERICHT / ZÁVĚREČNÁ ZPRÁVA PROJEKTU

Oxygenic photosynthesis is one of the essential processes on Earth as it results in energy production with water and sunlight as inputs. It takes place on the thylakoid membranes of phototrophic organisms where the four major multicomponent protein-pigment engines are embedded. The first of these units is photosystem II (PSII), where water molecule is split into electrons and oxygen. The major attention is paid to examine the bigger and functionally most relevant subunits of PSII, but there is still little known about single-helical small subunits. One of these is PsbI, that is suspected [Dobáková, 2007] to be one of the first PSII subunit being inserted into the membrane and to alter its local composition. The photosynthetic membrane consists mainly of galactolipids, contrary to animal membranes, that are made of mainly from phopholipids.

The project was planned and written as a two years project, though financed by two separate projects, each for one year (project number 60p21 for the first years, this project 64p1 for the second). We fulfilled all steps planned within the first year, as reported in the final report of project 60p21. For this year the planned aim was to fine-tune the glycolipid parameters by confrontation with experimental NMR data, characterize the thylakoide membrane in *Synechocystis* and finalize the data analysis for PsbI.

For the final three-dimensional model of PsbI from cyanobacterium Synechocystis the sequence AAC43720.1 (GenBank code) was used as a target sequence and the coordinates for the PsbI chain were built on basis of the crystal structure 3ARC [Umena et al., 2011] (PSII from *Thermosynechococcus elongatus*), which is the crystal structure of PSII with the highest resolution (1.9 Å) available. For the coarse-grained (CG) simulations the best model was converted into the CG representation by "martinize", a script contained in the official distribution of the MARTINI force field.
For model refinement, sequence analysis based on all available PsbI protein sequences was performed. If necessary, transit peptides were removed and also incomplete sequences were left out. Only unique sequences were used for further work. All the sequences were aligned in ClustalX software interface [Larkin et al., 2007] and their sequence logo was made by a weblogo internet interface [Crooks et al., 2004].
The CG systems with one copy of PsbI were set up by the insane script, followed by the same minimization protocol as the membranes without protein (i.e. 1000 steps of steepest descent energy minimization, and series of 1000 steps of NVT followed by NpT MD simulations with the time steps of 1ns, 2ns, 5ns and 10ns). Simulations were performed in the Gromacs software package [Hess et al., 2008], with standard MARTINI force field for protein [Monticelli et al., 2008], solution and PG [Marrink et al., 2007] and with the parameters reported in this grant for MGDG, DGDG and SQDG. No elastic network was necessary for the secondary structure stabilization. Simulations ran with the time step of 20 fs (maximum possible time step for proteins) for 1.2 μs, while the temperature was kept at 310 K by separate connecting of two groups (water + ions and protein + membrane) to the rescale-velocities thermostat once per 0.5 ps. Pressure was coupled semi-isotropically by connecting the system each picosecond to a Berendsen barostat at 1 bar with the compressibility of 3×10^{-5} bar^{-1}. Center of mass motion was removed separately for the upper and lower leaflet of the membrane and for the rest of the system. Standard MARTINI maintainance (long range interactions were cut at 1.2 nm apart from the bead, shift algorithm was used to preserve

grouping of the charges at the cut-off distance) of long-range forces (Coulombic and van der Waals).
Mutual interactions of more than one PsbI unit were examined only in the thylakoid membrane of Wada's composition. Two different proportions of lipids to one PsbI unit were used. In the first – sparse – case the final frame of the simulation of one PsbI in the membrane was used as an input for building the bigger systems. The system was duplicated and 2 resp. 4 frames were put next to each other resp. into the grid of 2 × 2 frames. Than the system underwent the same minimization procedure as if it would have been built by the insane script. Both simulations ran for 5 μs.
To study the aggregation process by enabling simulations of more PsbI units, a building block composed of 1 PsbI protein, 29 LPMG, 10 LPDG, 7 DPSQ, 4 lPPG, 938 water beads (i.e. 18.8 water beads per lipid) and 10 sodium beads were generated by the insane script and minimized by the standard procedure. Then this block was multiplied and systems consisting of 2, 4, 9 and 16 of these blocks were made. Having been built these systems underwent the "after-insane" minimization again. The systems containing 2 and 4 proteins ran for 2.4 μs, the bigger ones with 9 resp. 16 PsbIs ran for 7.5 μs.

For atomistic control simulations, the atomistic model of Wada's thylakoid membrane after 150 ns relaxation was used as an input. The protein was inserted into the membrane by the g_membed [Wolf et al., 2010] routine of Gromacs, that removed 5 molecules of LPMG, 3 molecules of LPDG and 10 water molecules. After that the system was minimized by 5000 of the steepest descent minimization followed by a series of molecular dynamics simulations with the following settings: time step of 0.5 fs and τ_p = 5ps, for the next two simulations the time step was risen to 1 fs and the system was connected to the pressure bath one in 2 ps resp. 1.5 ps. All the three simulation ran for 0.5 ns and position restraints (F_r = 1000 N) were applied to the protein on the first two simulations. In the last minimizing simulation running for 0.15 ns, the system was connected to the pressure bath once a picosecond and the time step of the simulation was set to 1.5 fs. The production simulation ran with the time step of 2 fs for 250 ns and the pressure of the system was hold on the value 1 bar semi-isotropically by Berendsen barostat to which the system with compressibility $4.6e^{-5}$ bar^{-1} was connected each picosecond. All the simulations were performed in Gromacs suite with Gromos 45A4 force field for the sugar parts of the lipids and Gromos 53A6 force field for the rest of the system. Electrostatic interactions were cut at the 1.4 nm, beyond what the reaction field approximation with the relative permittivity of 62 bar^{-1} was used. Periodic boundary conditions with the distance of 0.9 nm for neighbor searching were used, neighbor list was updated every 5 steps. Temperature of the system was hold at 310 K by connecting the membrane grouped with protein and solution separately to the temperature bath (rescale-velocities thermostat) every 0.5 ps.

In summary, during this grant period, by intense collaboration between the experimental NMR lab in Linz, and the computational lab in Nove Hrady, glycolipid parameters were refined and the development of force field parameters for glycolipid-based thylakoide membranes was succesfully finished. The parameters were embedded into the MARTINI coarse-grained forcefield and published in César A. López, Zofie Sovova, Floris J. van Eerden, Alex H. de Vries, Siewert J. Marrink, Martini force field parameters for glycolipids, J. Chem. Th. Comp. The parametrization of all essential membrane lipids enabled us to study thylakoide membranes having a composition as seen in Synechocystis PCC6803, which we were able to characterize on coarse-grained and atomistic levels (using Gromos parameters for the atomistic simulations). Once characterized we used this membranes for the study of the PsbI-membrane system computationally (and experimentally to verify certain proprties of the protein observed in the simulations). To do so, we embedded various numbers of the PsbI

from cyanobacteria Synechocystis PCC6803 into the thylakoide membrane and were able to characterize not only the behaviour of a single protein in the membrane but the mutual interaction of the PsbI molecules in the membranes. Hereby, we get excellent agreement between the atomistic and coarse-grained simulations (paper in preparation), as well as with the expected behaviour predicted by experiments. Additionally, the collaboration allowed us to collect Phosphorous-31 NMR spectra to measure NAD binding to apoWrbA in the Linz facility and therefore this grant is acknowledged in the respective publication. Modeling studies, based on earlier experimental results performed in Linz of the lectin-like domain of Tumor Necrosis Factor during a long-term stay of the first author, lead this year to a publication in which Aktion is therefore acknowledged as well.
Further benefits of the collaboration were preparatory investigations of other proteins involved in the photosystem 2, in particular PsbP and PsbQ by NMR also involving some methodological deveelopments (accelerated NMR). These results will mostly be published in the coming years.

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Marrink S.J., Risselada H.J., Yefimov S., Tieleman D.P., de Vries A.H. (2007) The MARTINI force field: coarse grained model for biomolecular simulations. J Phys Chem B 111, 7812 – 7824

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Umena Y., Kawakami K., Shen J.R., Kamiya N. (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473; 55 – 60

Wolf M.G., Hoefling M., Aponte-Santamaria C., Grubmueller H., Groenhof G. (2010) g membed: Efficient insertion of a membrane protein into an equilibrated lipid bilayer with minimal perturbation. J Comput Chem 31; 2169 – 2174

This AKTION project built upon the past achievements (supported also by AKTION) of the applicant and the partner. Providing the financial means in a framework of the current project strengthened significantly the former collaboration among the research groups based in the Johannes Kepler University in Linz and the University of South Bohemia in Nové Hrady and helped to start the regional research infrastructure RERI-uasb. As one main focus of the project were the theoretical simulations and predictions, and their interplay with experimentally measureable predicted data, several research stays of the austrian experts in the Czech republic were necessary to evaluate the data and refine the membrane parameters (Norbert Muller, 11. July, 20. September, 21. September, 12.-14. Dezember, Maria Theresia Poeschko, 12.-14. Dezember, Maria Braeuer, 12.-14. Dezember) These stays were fully covered on the Czech side from the funded grant. One scientist from the Czech partner (Petr Rathner) was extensively using the newly availbale NMR instrumentation in Linz

(November/Dezember 2012, altogether 4 weeks). Thus this project opened new opportunities for joint research plans, based the individual knowledge on both sides on the same level, and will be continued. Results of this grant were discussed in a final meeting that was organized as a workshop including letcures of all participants of both partners in Nove Hrady in Dezember 2012 (workshop program attached to the report).

Doc.RNDr. Rudiger H. Ettrich, Ph.D.
řešitel

Nové Hrady, 30.1.2013

Univ.Prof. Mag. Dr. Norbert Müller

Linz, 31.1.2013

Regular scientific papers in impacted journals:

In 2011 (first year of the grant):

M Horničáková, J Kohoutová, J Schlagnitweit, C Wohlschlager, R Ettrich, R Fiala, W Schoefberger, N Müller (2011) Backbone assignment and secondary structure of the PsbQprotein from Photosystem II Biomolecular NMR Assignments 5: 2. 169-175.

In 2012 (this grant):

Cesar Augusto Lopez, Zofie Sovova, Floris J. van Eerden, Alex H. De Vries, and Siewert J. Marrink Martini force field parameters for glycolipids J. Chem. Theory Comput., 2013, DOI: 10.1021/ct3009655

Alexander Dulebo, Rüdiger Ettrich, Rudolf Lucas, David Kaftan (2012) A Computational Study of the Oligosaccharide Binding Sites in the Lectin-Like Domain of TumorNecrosis Factor and TIP Peptide Current Pharmaceutical Design 18 (27): 4236-4243.

Iryna Kishko, Balasubramanian Harish, Vasalina Zayats, David Reha, Brian Tenner, Dhananjay Beri, Tobias Gustavsson, Rüdiger Ettrich, Jannette Carey (2012) Biphasic kineticbehavior of FMN-dependent NAD(P)H:quinone oxidoreductase WrbA from E. coli PLOS One 7 (8): e43902

Schlagnitweit, J.; Horničáková, M; Zuckerstätter, G.; Müller, N. MQD - Multiplex-Quadrature Detection in Multi-Dimensional NMR. ChemPhysChem 2012, 13, 342-346.

Conference abstracts:

In 2011 (first year of the grant):

Schlagnitweit J, Hornicakova M, Mueller N.: Accerelerating Biomolecular NMR by Multiplex Techniques, Book of Abstracts: 1st Visegrad Symposium on Structural Systems Biology, Nove Hrady, Czech Republic, 2011 p. 17.

Sovova Z, de Vries AH, Ettrich, R, Marrink SJ.: DGDG bilayer as a first step in thylakoid membrane building, Book of Abstracts: 1st Visegrad Symposium on Structural Systems Biology, Nove Hrady, Czech Republic, 2011 p. 39.

In 2012 (this grant):

TALK: Z. Sovova, A.H. de Vries, R. Ettrich and S.J. Marrink; Thylakoid membrane studied by the means of molecular dynamics. Materials Structure, vol. 19, no.1 (2012), p. 47, 10th meeting of Czech and Slovak Structural Biologists named "Discussion in Structural Molecular Biology", March 22- 24, 2011, Nove Hrady, CZ

TALK: Z. Sovova, A.H. de Vries, R. Ettrich and S.J. Marrink; Characterization of the thylakoid membrane from Synechocystis PCC6803 by the means of molecular dynamics. Book of abstracts, p.5, 2nd Visegrad symposium on structural systems biology, June 13- 15, 2012, Gyongyostarjan, HU

TALK: Norbert Müller; The oxygen evolving complex and noise NMR - work in progress; Seminar Series on Modern Concepts in Structural Biology; Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, 31.5.2012

POSTER: Z. Sovova, A.H. de Vries, R. Ettrich and S.J. Marrink; Characterization of the thylakoid membrane from Synechocystis PCC6803 by the means of molecular dynamics.Vanderbilt/Columbia Molecular Modeling CyberCamp, sekce: Poster session with grad students and post docs

K. Chandra, A. Walnerova, P. Rathner and N. Müller; Towards assignment of PsbP and carbon detected reduced dimensionality 3D HNCACO; National Magnetic Resonance Symposium, Numbai, India, February 2013

Copies of the publications are attached to the final report.