MEMBAQ

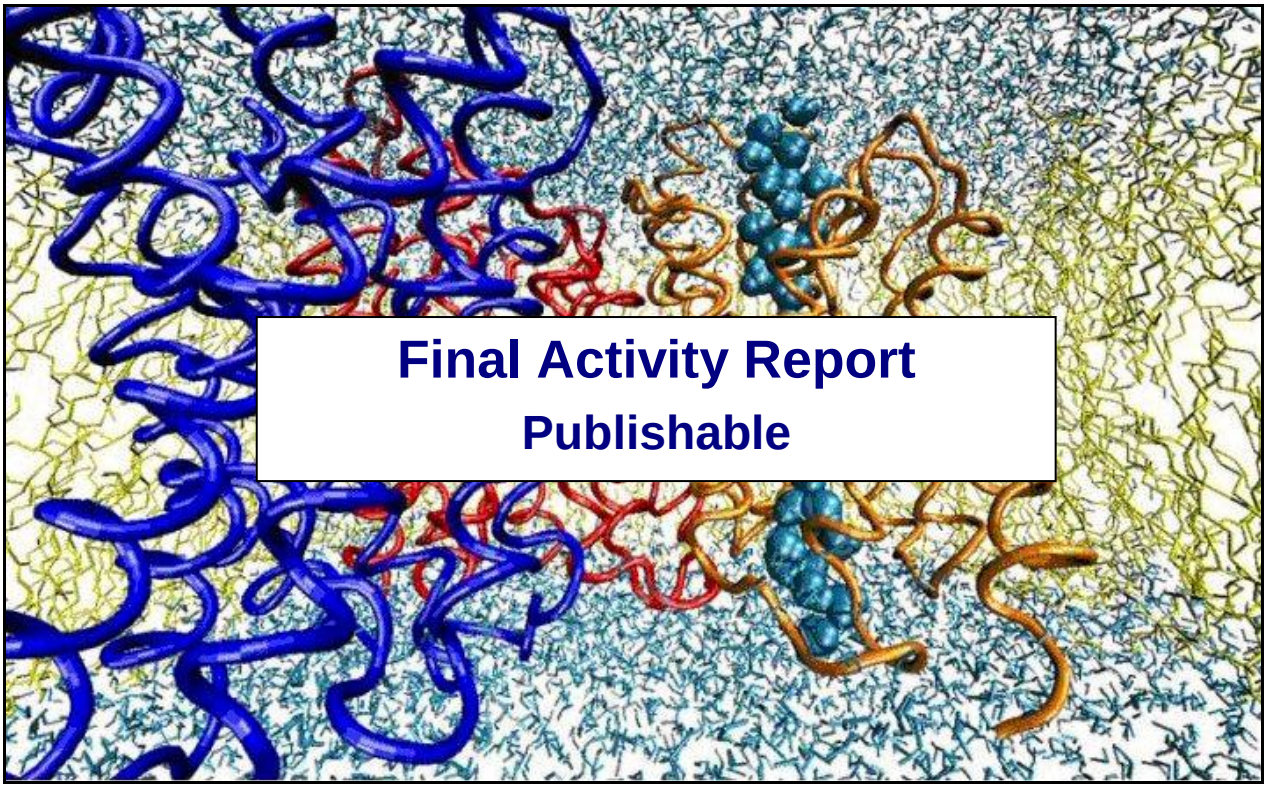
NMP4-CT-2006-033234

MEMBAQ

Incorporation of Aquaporins in Membranes for Industrial Applications

Specific Targeted Project

Priority 3: ***NMP Nanotechnology and nanosciences, knowledge-based multifunctional materials and new production processes and devices***



Final Activity Report

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Final Activity Report
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[1] This does not include subcontractors

PUBLISHABLE FINAL ACTIVITY REPORT

Background

In all living cells, channels transporting water – aquaporins – exist. Aquaporins are proteins, which only transport water i.e. pure H_2O molecules. They have a unique selectivity and are extremely efficient being nature's own water transporting systems.

Under a contract with the Directorate of General Research of the Commission of European Communities (DG-RCC), a specific targeted research project was carried out 2006-2010 within the NMP FP6 programme (Nanotechnologies and nanosciences, knowledge-based multifunctional materials and new production processes and devices). The name of the project was: MEMBAQ - Incorporation of Aquaporins in Membranes for Industrial Applications.

Objectives

The objective of the MEMBAQ project is to investigate a possibility to industrially exploit the unique features of aquaporin proteins. This is done by incorporating recombinant aquaporin molecules in different types of industrial membranes for water filtration. By this, the nature will be used as a model for the development of a novel nanobiotechnological water membrane technology. Tests of pilot membrane systems will be developed for three applications: A water purification application, an ocean energy application (salinity gradient energy) and an application for industrial wastewater reclamation and reuse. The applications will demonstrate the revolutionary industrial breakthrough in separation and filtration techniques using natural processes.

Participants

The nine partners of the MEMBAQ project include research institutes (DHI Water & Environment, project co-ordinator), SME technology company (AQUAporin ApS), universities (University of Southern Denmark, Ben-Gurion University of the Negev, Vilnius University, Lund University, Malaga University, Technical University of Denmark) and a global water company (Anjou Recherche, which is part of Veolia). The participant organisations represent molecular biology, biotechnology, computational chemistry, membrane science and engineering, water purification and water management.

Work activities

The research project is organised in five work packages (WPs) with the following tasks:

1. Production of recombinant aquaporin. Production of purified aquaporins is essential input for developing stable membrane films.
2. Design of new nanobiotechnological membrane materials by means of computer simulations (e.g. atomistic molecular dynamics). Simulation results are imperative for optimising stable membrane films and for initial characterisation.
3. Building of aquaporins into a stable membrane film, and incorporation of this layer into a composite (integral) membrane system. Samples of novel ultra thin membrane on a support with sufficient strength, permeability, durability, etc. are the output.
4. Characterisation and testing of the membranes towards critical parameters such as pressure, temperature, pH, fouling and fouling prevention etc. Documentation in terms of laboratory test results of the membrane samples is the output.

5. Application tests of small-scale membrane systems in water purification and salinity power production. Documentation from three different applications (water purification, energy production and wastewater reclamation and re-use) under realistic conditions is the output.

The project started in October 1st, 2006. The following is the description of the work done throughout the project and the achieved results after three and a half years.

Results

The overall objective of WP1 was to produce purified aquaporin to be used in experimental studies in WP3. This is divided into two sub-objectives: 1) production of recombinant expression and purification of plant aquaporins to be used as building blocks in water membrane and 2) purification f aquaporin, AQP-1, from a natural source. WP1 was succesfully completed, and batches of recombinant plant aquaporins SoPIP2;1 have been produced and shipped to WP3 participants. Research work (aided by computer simulations in WP2) has been carried out at identifying and constructing (expressing) new constitutively open plant aquaporins, which yield higher water flux. Stability of solubilized SoPIP2;1 have been investigated. All objectives were accomplished and deliverables were produced.

In WP2, the overall objective was to carry out computer simulation studies of aquaporin embedded in different nanotechnological membrane materials, and to use the data from computer simulations in the design of new nanotechnological membrane materials. Molecular Dynamics (MD) simulation of conduction across aquaporin membranes were carried out. Additional simulations to design solutions for improved permeability of SoPIP2;1 mutants of aquaporin were completed. Simulations of tritiated water to be used for effective water flux measurements were carried out using a.o. force field parameters for T_2O based on experimental expertise by WP4 participant (UMA). Artificial membrane constructs composed of bolalipids are known to be mechanically and thermally more resilient then conventional phospholipid membranes. Thus, calculation of mechanical properties of lipid bilayer composed of bolalipids and mixtures of bolalipids and phospholipids using atomistic and course-grained simulations were carried out. Finally, work in evaluating model reliability and accuracy by analyzing the influence of channel permeability on simulation parameters was done. Thus objectives in WP2 have been reached and milestones and deliverables have been accomplished.

The overall objective of WP3 was the design and fabrication of an artificial aquaporin water membrane, by 1) incorporation of aquaporins into a nanobiotechnological membrane material, 2) visualization of channel permeability and 3) measurements of water flux. Two designs for aquaporin membranes were initially outlined in the Description of Works of the contract (task 3.1 and task 3.2). For the first design, support membrane material based on perforated ETFE partition material has been developed. Investigations into how the perforation process may be up-scaled to industrial volumes have been completed. Protein incorporation and function studies have been extended. A fluorescence-based assay has been successfully developed to address protein folding (correct folding is a prerequisite for functionality). A microfluidic chamber has been constructed and the first membranes have been established in this device. Also large scale membrane arrays have been established with both lipids and polymers membranes. In the second design, the membrane structure consists of two layers: A bottom layer of a commercial nano-filtration (NF) membrane that serves as a robust mechanical support, and a second layer of a biomimiced membrane composed of phospholipids bilayer with embedded aquaporins. At low pH conditions, bilayer formation and surface coverage through vesicle fusion was successfully demonstrated. As an alternative support structure, carbon-nanotube CNT scaffold sub-layers on polymer membranes were tested as specified in the Description of Works but found to be too rough to use for aquaporin incorporation. The potential for the use of CNT and graphene membranes for energy extraction from the seawater based on reverse electrodialysis was discovered, and work was done to test a hydrovoltaic cell. Overall, the objective of having robust protein

functionality was successfully demonstrated in the project. Development of biomimetic membranes of sufficient quality (i.e. which are stable, reproducible, portable) was more comprehensive than envisaged in the Description of Works. The durability of aquaporin membranes is still limited although progress has been substantial towards the completion of the MEMBAQ project. Extended research and development in this field is therefore envisaged. Objectives and updated milestones and deliverables have been accomplished accordingly.

In WP4, the overall objective was membrane engineering design, i.e. to investigate and characterize the membranes produced in WP3 in relation to the possible future applications of such membranes. This included: Characterization of membranes produced in WP3 for selectivity and permeability; Investigation of such membranes in relation to life time and fouling studies; Feedback information on how such membranes can be engineered into larger scale and made applicable for practical use in relation to the objectives of WP5. Development and application of various characterization methods for supported biomimetic films with embedded aquaporins was carried out. Activities included characterization of composite support structures, establishing conditions for preparation a support layer for ETFE partition, investigation of water fluxes on hydrogels, and liquid membranes experiments. The porous support structures as well as initial aquaporin membranes developed in WP3 have been tested. Objectives, milestones and deliverables have been accomplished.

In WP5, application tests of the membranes under realistic conditions were planned. However, the accomplished stage of aquaporin membranes did not allow for such tests. The reason is that the developed aquaporin embedded membranes – despite comprehensive additional development work in WP3 and WP4 co-financed by partners themselves – remain to have a limited duration and stability outside the laboratory. Significant progress is made every month, and the partners are convinced that the future market potential for aquaporin membranes is intact with extended research and development. Financing of further research and development is partly secured through other research funding as well as private investment capital, see below.

Publishable results

The work in MEMBAQ has led to more than 15 submitted peer-reviewed manuscript, see below.

Journal paper title, books	Author	Journal, date
A support structure for biomimetic applications	Vogel et al	J. Micromech. Microeng. 19
Electrochemical characterisation of hydrogels for biomimetic applications	L. Peláez, V. Romero, S. Escalera, V. Scileira, S. Ibragimova, K. Stibius, J. Benavente, and C.H. Nielsen	Journal of Polymers for Advanced Technology, January 2010
Hydrogels for in-situ encapsulation of biomimetic membrane arrays	Sania Ibragimova, Karin Stibius, Piotr Szewczykowski, Mark Perry, Henrik Bohr, and Claus Hélix Nielsen	Journal of Polymers for Advanced Technology, January 2010
Supported lipid bilayer membranes for water purification by reverse osmosis	Yair Kaufman, Amir Berman, Viatcheslav Freger	Langmuir 2010, 26, (10), 7388-7395.)
Biomimetic Triblock Copolymer Membrane Arrays: A Stable Template for Functional Membrane Proteins,	Alfredo González-Pérez, Karin B. Stibius, Thomas Vissing, Claus H. Nielsen, Ole G. Mouritsen	Langmuir Letter 2009, 25(18), 10447-10450
Temperature dependence of micellar sphere-to-rod transition using adiabatic compressibility	Alfredo González-Pérez, Juan M. Ruso	Colloids and Surfaces A: Physicochem. Eng. Aspects 356 (2010) 84-88
Instrumentation for automated voltage clamp sampling and data processing	M. Perry, T. Vissing, T.P. Boesen, J.S. Hansen, J. Emnéus and C.H. Nielsen	Journal of Instrumentation, 2009
Biomimetic membranes for sensor and separation applications	Claus Helix Nielsen	Journal of Analytical and Bioanalytical Chemistry, Special Issue November 2009
Raman spectroscopy as a tool for investigating lipid-protein interactions	Frederic N. R. Petersen, Claus Helix Nielsen	Journal of Spectroscopy, www.spectroscopyonline.com
Aquaporin biomimetic membranes for sensor and separation applications	Claus Helix Nielsen	Chapter 2 in Jahn (ed.) MIPS and their role in the exchange of metalloids. Landes Bioscience Publishers, 2009
Large scale biomimetic membrane arrays	Jesper S. Hansen, Mark Perry, Jörg Vogel Jesper S. Groth, Thomas Vissing, Henrik Bohr, Oliver Geschke, Jenny Emneús and Claus H. Nielsen	Journal of Analytical and Bioanalytical Chemistry / Journal of Micromechanics and Microengineering
pH-dependend water peneration across CNT sub-layers arranged on the polycarbonate membrane filters	Jurgis Barkauskas	March 2009
CVD Synthesis and Purification of Carbon Nanotubes	J. Barkauskas, I. Stankevičienė, A. Selskis	Journal of Diamond and Related Materials, 2009

To gate or not to gate: Morphing gated plant aquaporins into constitutively open conformations	Himanshu Khandelia, Morten Ø. Jensen, Ole G. Mouritsen	J Phys Chem B. 2009. 113:5239-5244
Growth of nano-sized carbon particles on Ni: a parametric study.	J. Barkauskas, R. Kuodis, I. Grigoravičiūtė, R. Juškėnas, A. Selskis.	*Chemija*, 2008. vol. 19(3–4) p.p. 15–21.
Synthesis of vapour-grown micrometer-scale carbon fibers.	J. Barkauskas, I. Stankevičienė	*Mendeleev Communications*, 2009. vol. 19(3) p.p. 123-125.
A novel purification method of carbon nanotubes by high-temperature treatment with tetrachloromethane	J. Barkauskas, I. Stankevičienė, A. Selskis	*Separation and Purification Technology*, 2010, vol. 71(3) p.p. 331–336.
pH-dependent water penetration through CNT sub-layers arranged on the polycarbonate membrane filters	J. Barkauskas	*Carbon*, 2010, vol. 48(6), p.p 1858-1865

The results have also been presented at a number of conferences, see below.

Conf. paper, poster, talk, title, author	Conference, date
The Use of NF Membranes as Substrates for Supported Phospholipid Bilayers, Y. Kaufman, A. Berman, V. Freger	International Congress on Membranes and Membrane Processes (ICOM), Honolulu, Hawaii, July 12-18, 2008.
Electrochemical characterization of a regenerated cellulose/polypropylene membrane: Interfacial effects and bulk membrane transport parameters, L. Peláez, V. Romero, S. Escalera, A. Muñoz, M. I. Vázquez, J. Benavente	CITEM 2010 (Conferencia Iberoamericana en Ciencia y Tecnología de Membranas), Sintra, Portugal, April 2010
Modification of a regenerated cellulose membrane by lipid layer deposition and its effect on NaCl transport, M.I. Vázquez, V. Romero, S. Escalera, F.J. Casado, J. Hierrezuelo, M. R. López-Ramirez, J.M. López-Romero, J. Benavente	CITEM 2010 (Conferencia Iberoamericana en Ciencia y Tecnología de Membranas), Sintra, Portugal, April 2010
"New Insights Into the Gating Mechanism of Plant Aquaporins from Computer Simulations", Khandelia, H. & Mouritsen, O.G	The 2008 Biophysical Society, Long Beach, USA
"Simulations of water channels and ion pumps" , Khandelia, H. & Mouritsen, O.G	The 40th Sandbjerg Meeting on Membrane Transport, Sandbjerg Estate, May 2008, Denmark
Nature's own water membranes for industrial use (poster), Peter Holme Jensen, Hans Enggrob	EuroNanoForum 2007 in Düsseldorf
Development Of Novel Biomimetic Membrane Designs For Separation And Biosensor Applications (poster), Jesper S. Hansen, Mark Perry, Sania Ibragimova, Jörg Vogel, Thomas Vissing, Christian R. Hansen, Pierre-Yves Bollinger, Kamila J. Pszon, Jenny Emnéus1, Claus H. Nielsen	EuroNanoForum 2009 in Prague
	5th International Conf. on Aquaporins, Japan
Stability of the Spinach Aquaporin (SoPIP2;1)	The Biophysical Society's 53rd Annual Meet-

in Detergent Solution and Lipid Membranes, Plasencia, I., Johanson, U., Kjellbom, P, Mouritsen, O.G.	ing, February 28-March 4, 2009, Boston, Massachusetts
Free Standing Catanionic Membranes, Alfredo González-Pérez, Thomas Vissing, Claus H. Nielsen and Ole G. Mouritsen	Conference

Significant public interest has been devoted to the MEMBAQ project, and some of the news media events with MEMBAQ participation includes

Radio, TV, magazines, newspapers, title, author	Media name, date
Press conference: MEMBAQ nature's own water membranes for industrial use, Hans G. Enggrob, Sania Ibragimova	World press journalist conference in London June 2009
Press conference: Nature's own water membranes for industrial use, Peter Holme Jensen, Hans Enggrob	EuroNanoForum 2007 Nanotechnology in Industrial Applications, 19-21 June 2007 in Düsseldorf
Press conference: MEMBAQ nature's own water membranes for industrial use, Hans G. Enggrob, Sania Ibragimova	EuroNanoForum 2009 Nanotechnology for Sustainable Economy, 2 5 June 2009 in Prague
European Union publication: 99.999995% clean water - Development of revolutionary water filtration membranes, Hans G. Enggrob	Public Service Review, Issue 18, EU profile page 575
Nano magazine: Water filter – nature's pores in industrial purification, Hans G. Enggrob, Claus Helix Nielsen	NANO – the magazine for small science, issue 12, June 2009, ISSN 1757-2517
NANO-TV programme for DG Research, C.H. Nielsen	

Conclusions

The ambitions objectives to investigate the possibility to industrially exploit aquaporin proteins for water filtration were pursued by incorporating recombinant aquaporin molecules in different types of industrial membranes for water filtration. Through focused research and technology development, such aquaporin membranes have been successfully developed. Challenges in extending the durability (lifetime) of artificial biomimetic membranes outside laboratory environments remain, but the progress sofar confirms that this goal is indeed achievable. The remaining challenges caused a shift in the project activities towards membrane development and less pilot testing. More research and development is required to test the membranes in the field.

The use and dissemination of knowledge has been extremely efficient in the MEMBAQ project: At least three patents have paved the way for substantial private venture investments (more than EUR 10 mill. in the aquaporin membrane technology). More than 15 peer-reviewed papers were published together with a number of conference proceedings and posters. A number of large multinational companies within membrane manufacturing have met with different consortium members to investigate the possibilities for exploitation. Finally, a variety of public media such as radio, TV, news magazines, press conferences has shown considerable interests in the MEMBAQ project. All together, this has effectively profiled European research within membrane technologies.

At the end of the 3½-year project, a new revolutionary nanobiotechnological membrane is developed in the laboratory which could be an industrial break-through in water filtration and separation. This development of long-term visionary research activities produces step changes in the water systems towards sustainable solutions. This will in turn stimulate economic activities and the connected growth of the world water market. Further reference is made to the publish web page www.membaq.eu.

Information Sheet

EC-funded project: STRP MEMBAQ NMP4-CT-2006-033234
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EU funding: 2.1 million Euro
Duration: 40 months
Start date: 1st Oct. 2006
End date: 31st March 2010
More information: www.membaq.eu