Dear MBIers,

Let me first congratulate Nathalie Reuter and her colleagues Anne-Sophie Schillinger, Cedric Grauffel, Hanif Muhammad Khan and Øyvind Halskau with this year's Paper Award; a very nice example of collaborative and multidisciplinary work. Congratulations also to Øyvind Strømland for his prize-winning contribution to the "Molecule of the Month". More on this inside this issue. We also celebrate that Øyvind Halskau has been granted a new FRIPRO project; a great achievement in the fierce competition for these precious grants, where 14% (37 of 257) of the projects passed through the eye of the needle. In this competition it is not sufficient to be rated excellent, projects must be outstanding!

By now, all exams are done for this semester, although a few piles of exams are still to be read and assessed. I thank everyone who have contributed to the teaching in all our courses and I wish all students the very best for their results. I also wish to thank all those who have been involved in the preparations for the new experimental courses, MOL221 and MOL222 (replacing MOL202). This strengthening of the practical component in our bachelor program underscores the importance of experiments in molecular biology. We also congratulate one new master this semester and a total of seven new PhDs this year.

Next year MBI will participate for the first time with a two-day module in a new course in natural sciences (naturfag) for school teachers organised by our faculty's Skolelaboratorium. This is part of the Government's efforts to invest in the school teacher's competences (Lærerløftet). The fact that we have been invited to participate reflects the increasing importance of molecular biology and biotechnology in the society. Last week we also visited our new neighbour, Amalie Skram Videregående Skole (just across the lake), which joined UiB in a new partnership for education earlier this year. The biology teachers are very eager to come in contact with us and start doing things together.

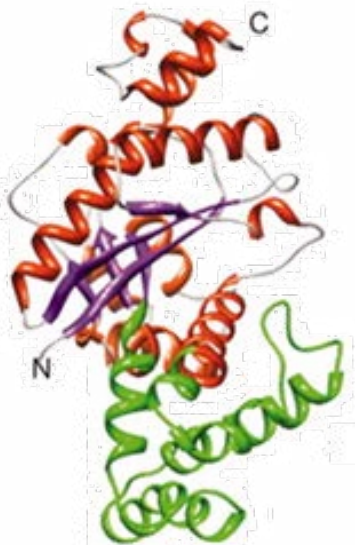
With this, I wish you all a nice juleferie and hope that you will have a good time with family and friends.

Nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2)

By Sergiy Kostenko

Nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT) proteins are the central enzymes for nicotinamide adenine dinucleotide (NAD) synthesis. In mammals, three different NMNAT's have been recognized: nuclear NMNAT1, mitochondrial NMNAT3 and NMNAT2 which is localized to the Golgi complex and undergoes fast, bidirectional axonal transport by population of Golgi-derived vesicles. NMNAT2 is critical for axon survival in primary culture and its depletion causes spontaneous neurite degeneration without injury, which endogenous NMNAT1 and NMNAT3 cannot prevent. After injury, endogenous NMNAT2 is depleted rapidly in the distal stump of neurites, initiating the process of Wallerian degeneration. Exogenous NMNAT2 can protect injured neurites when expressed at high enough levels to overcome its short half-life. Several gene-array studies found that NMNAT2 levels were reduced in brain specimens from patients with Alzheimer's disease (AD). Recently, it was shown that NMNAT2 attenuates phosphorylation of Tau, key protein for AD progression. Moreover, NMNAT2 was reduced by overexpressed inducible human mutant tau (TauP301L) in the mice forebrain (rTg4510mice). Overexpression of NMNAT2 in rTg4510 hippocampi significantly reduced neurodegeneration caused by TauP301L. So NMNAT2 may serve as a novel therapeutic target for Alzheimer's disease.

The reduction in nmnat2 transcription in rTg4510 mice likely results from reduced cAMP-response element binding protein (CREB) activity, suggesting that NMNAT2 is a direct target of CREB. CREB can be phosphorylated by another therapeutic agent for Alzheimer's disease, nerve growth factor (NGF), which is commonly used to differentiate PC12 cells. Therefore AD and cell differentiation can be regulated by similar signalling pathways. Role of NMNAT2 in cell differentiation is illusive and is a subject for the current research.



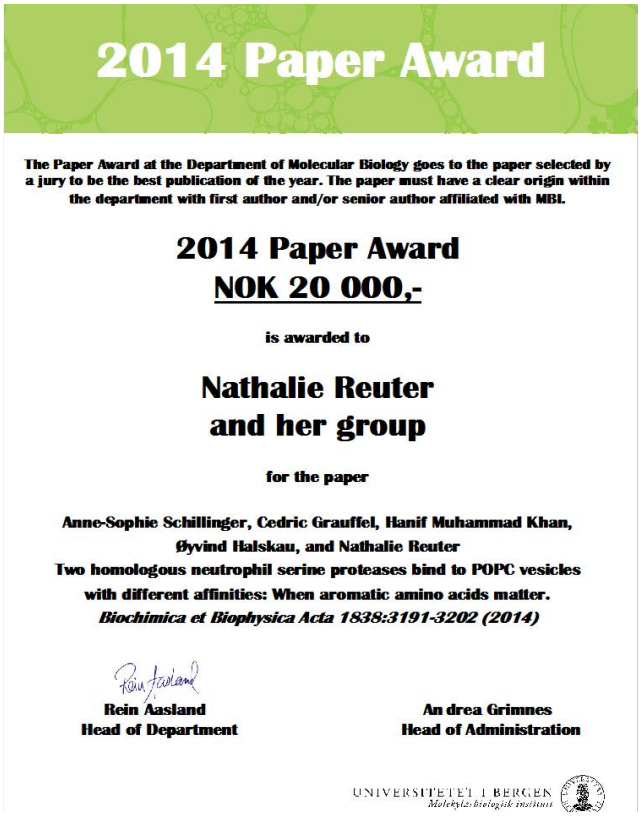
NMNAT2 model. Adapted from Brunetti et al. (2010)

MBI annual paper award 2014

This year's paper award was awarded to Nathalie Reuter and her group for the paper:

"Two homologous neutrophil serine proteases bind to POPC vesicles with different affinities: When aromatic amino acids matter." by Anne-Sophie Schillinger, Cedric Grauffel, Hanif Muhammad Khan, Øyvind Halskau, and Nathalie Reuter, published in Biochimica et Biophysica Acta 1838:3191-3202 (2014).

The jury said that the article was chosen "because this work represents a solid piece of work highlighting a great example of 1) collaborative work not only within MBI but also between 2 research programs and 2) multidisciplinary accomplishment."



2014 Paper Award

The Paper Award at the Department of Molecular Biology goes to the paper selected by a jury to be the best publication of the year. The paper must have a clear origin within the department with first author and/or senior author affiliated with MBI.

2014 Paper Award

NOK 20 000,-

is awarded to

Nathalie Reuter and her group

for the paper

Anne-Sophie Schillinger, Cedric Grauffel, Hanif Muhammad Khan, Øyvind Halskau, and Nathalie Reuter

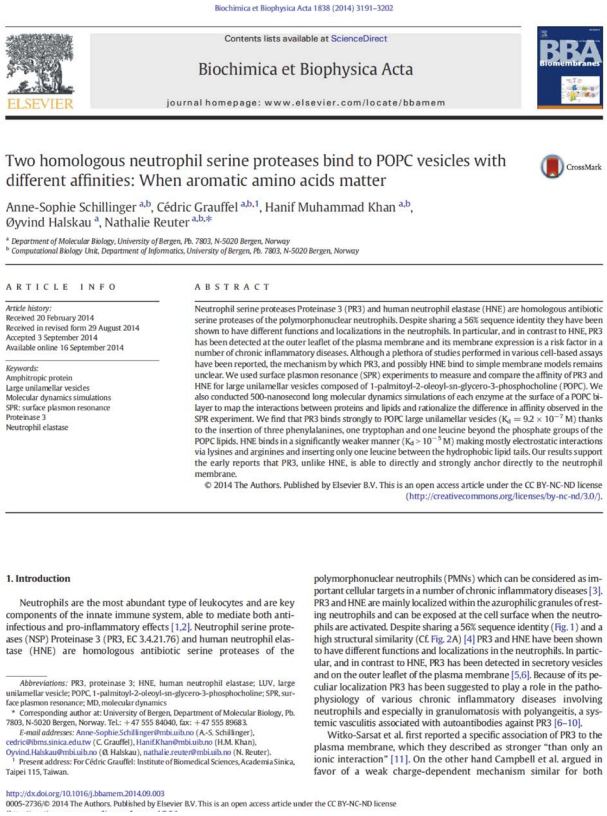
Two homologous neutrophil serine proteases bind to POPC vesicles with different affinities: When aromatic amino acids matter.

Biochimica et Biophysica Acta 1838:3191-3202 (2014)

Rein Aasland
Head of Department

An drea Grimnes
Head of Administration

UNIVERSITETET I BERGEN
Molekylær biologisk institutt



Biochimica et Biophysica Acta 1838 (2014) 3191–3202

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Two homologous neutrophil serine proteases bind to POPC vesicles with different affinities: When aromatic amino acids matter

Anne-Sophie Schillinger [a,b], Cédric Grauffel [a,b,1], Hanif Muhammad Khan [a,b], Øyvind Halskau [a], Nathalie Reuter [a,b,*]

[a] Department of Molecular Biology, University of Bergen, Pb. 7803, N-5020 Bergen, Norway
[b] Computational Biology Unit, Department of Informatics, University of Bergen, Pb. 7803, N-5020 Bergen, Norway

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 29 August 2014
Accepted 3 September 2014
Available online 16 September 2014

Keywords:
Amphitropic protein
Large unilamellar vesicles
Molecular dynamics simulations
SPR: surface plasmon resonance
Proteinase 3
Neutrophil elastase

ABSTRACT

Neutrophil serine proteases Proteinase 3 (PR3) and human neutrophil elastase (HNE) are homologous antibiotic serine proteases of the polymorphonuclear neutrophils. Despite sharing a 56% sequence identity they have been shown to have different functions and localizations in the neutrophils. In particular, and in contrast to HNE, PR3 has been detected at the outer leaflet of the plasma membrane and its membrane expression is a risk factor in a number of chronic inflammatory diseases. Although a plethora of studies performed in various cell-based assays have been reported, the mechanism by which PR3, and possibly HNE bind to simple membrane models remains unclear. We used surface plasmon resonance (SPR) experiments to measure and compare the affinity of PR3 and HNE for large unilamellar vesicles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). We also conducted 500-nanosecond long molecular dynamics simulations of each enzyme at the surface of a POPC bilayer to map the interactions between proteins and lipids and rationalize the difference in affinity observed in the SPR experiment. We find that PR3 binds strongly to POPC large unilamellar vesicles ($K_d = 9.2 \times 10^{-7}$ M) thanks to the insertion of three phenylalanines, one tryptophan and one leucine beyond the phosphate groups of the POPC lipids. HNE binds in a significantly weaker manner ($K_d > 10^{-5}$ M) making mostly electrostatic interactions via lysines and arginines and inserting only one leucine between the hydrophobic lipid tails. Our results support the early reports that PR3, unlike HNE, is able to directly and strongly anchor directly to the neutrophil membrane.

1. Introduction

Neutrophils are the most abundant type of leukocytes and are key components of the innate immune system, able to mediate both anti-infectious and pro-inflammatory effects [1,2]. Neutrophil serine proteases (NSP) Proteinase 3 (PR3, EC 3.4.21.76) and human neutrophil elastase (HNE) are homologous antibiotic serine proteases of the polymorphonuclear neutrophils (PMNs) which can be considered as important cellular targets in a number of chronic inflammatory diseases [3]. PR3 and HNE are mainly localized within the azurophilic granules of resting neutrophils and can be exposed at the cell surface when the neutrophils are activated. Despite sharing a 56% sequence identity (Fig. 1) and a high structural similarity (Cf. Fig. 2A) [4] PR3 and HNE have been shown to have different functions and localizations in the neutrophils. In particular, and in contrast to HNE, PR3 has been detected in secretory vesicles and on the outer leaflet of the plasma membrane [5,6]. Because of its peculiar localization PR3 has been suggested to play a role in the pathophysiology of various chronic inflammatory diseases involving neutrophils and especially in granulomatosis with polyangeitis, a systemic vasculitis associated with autoantibodies against PR3 [6–10].

Witko-Sarsat et al. first reported a specific association of PR3 to the plasma membrane, which they described as stronger "than only an ionic interaction" [11]. On the other hand Campbell et al. argued in favor of a weak charge-dependent mechanism similar for both

Abbreviations: PR3, proteinase 3; HNE, human neutrophil elastase; LUV, large unilamellar vesicle; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; SPR, surface plasmon resonance; MD, molecular dynamics

* Corresponding author at: University of Bergen, Department of Molecular Biology, Pb. 7803, N-5020 Bergen, Norway. Tel.: +47 555 84040, fax: +47 555 89683.
E-mail addresses: Anne-Sophie.Schillinger@mbi.uib.no (A.-S. Schillinger), cedric@ibms.sinica.edu.tw (C. Grauffel), HanifKhan@mbi.uib.no (H.M. Khan), Oyvind.Halskau@mbi.uib.no (Ø. Halskau), nathalie.reuter@mbi.uib.no (N. Reuter).
[1] Present address: For Cédric Grauffel: Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan.

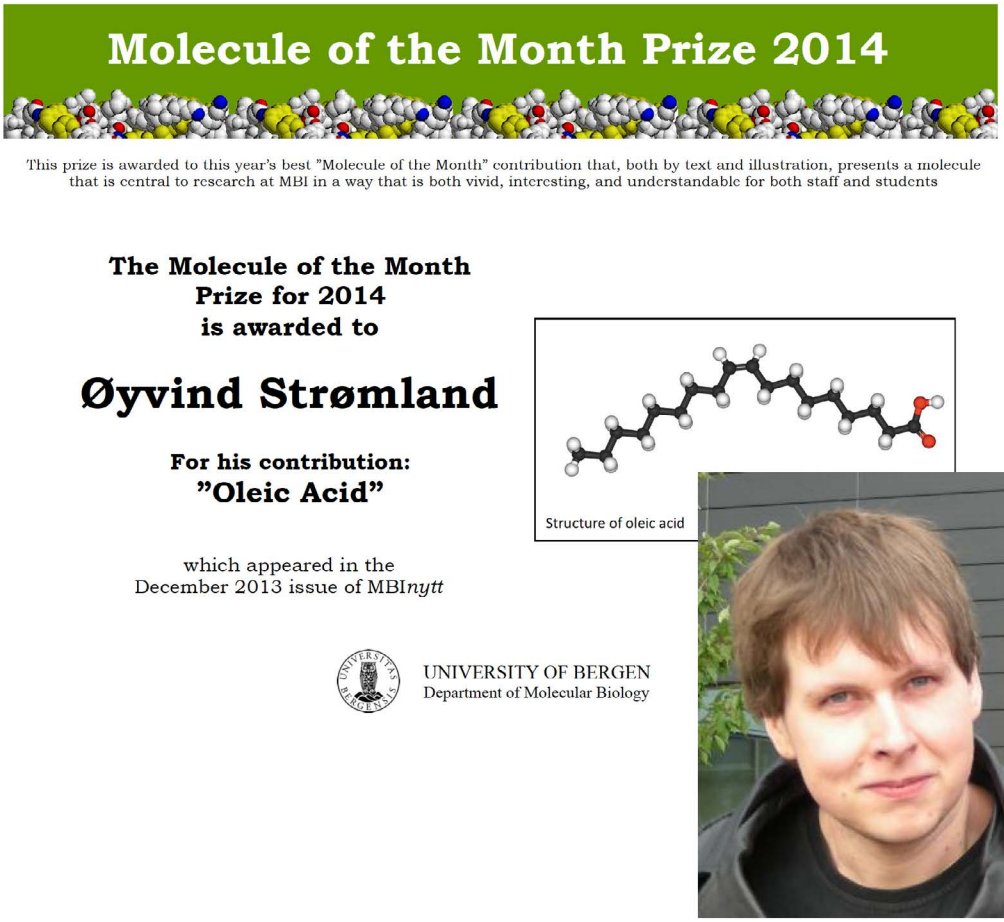
http://dx.doi.org/10.1016/j.bbamem.2014.09.003
0005-2736/

New research project

Øyvind Halskau has received a grant from the Norwegian reserach council (FRIPRO) for the project *The membrane as a catalyst of damaging protein misfolding events.* The project is for three years, and includes support for one post doc and one PhD-fellow, in addition to running costs.

MBI prize for best "Molecule of the Month" contribution

Øyvind Strømland has been awarded this year's prize for best "Molecule of the Month" contribution to MBI Nytt for his contribution "Oleic Acid". Besides fulfilling the criteria of being being engaging, interesting, and understandable for both staff and students, the jury said it was an exciting story with a hint of (dark) humour.



New papers

Line M. Myklebust, Petra Van Damme, Svein I. Støve, Max J. Dörfel, Angèle Abboud, Thomas V. Kalvik, Cedric Grauffel, Veronique Jonckheere, Yiyang Wu, Jeffrey Swensen, Hanna Kaasa, Glen Liszczak, Ronen Marmorstein, Nathalie Reuter, Gholson J. Lyon, Kris Gevaert, and Thomas Arnesen. Biochemical and cellular analysis of Ogden syndrome reveals downstream Nt-acetylation defects Human Molecular Genetics [Epub ahead of print]

Gossmann TI, Ziegler M. Sequence divergence and diversity suggests ongoing functional diversification of vertebrate NAD metabolism. DNA Repair (Amst). 2014 Nov;23:39-48. doi: 10.1016/j.dnarep.2014.07.005. Epub 2014 Jul 29.

Budnjo, S.Narawane, C.Grauffel, A.S. Schillinger, T. Fossen, N.Reuter, B.E.Haug. Reversible Ketomethylene-Based Inhibitors of Human Neutrophil Proteinase 3 A.. Journal of Medicinal Chemistry (2014) J. Med. Chem., 2014, 57 (22), pp 9396–9408. DOI: 10.1021/jm500782s

E. Fuglebakk, S.P. Tiwari, N. Reuter. Comparing the Intrinsic Dynamics of

New faces

Fedor Kryuchkov, MetaSig/NAD-gruppen

Multiple Protein Structures Using Elastic Network Models. Biochim Biophys Acta - General Subjects (2014) in press. doi:10.1016/j.bbagen.2014.09.021

A.S. Schillinger, C. Grauffel, H.M. Khan, O. Halskau, N. Reuter. Two homologous neutrophil serine proteases bind to POPC vesicles with different affinities: When aromatic amino acids matter. Biochim Biophys Acta - Biomembranes (2014) 1838(12): 3191-3202 DOI: 10.1016/j.bbamem.2014.09.003

"Hairclip" protein mechanism explained! -- Sandhya Tiwari and Nathalie Reuter and their colleagues at EMBL-EBI and MRC-LMB publish a cool and very interesting story in Science on how mutations can act from a distance to influence the function of a protein. MBI's SAB member, Sarah Teichmann, now at EBI, is senior coauthor on the paper.

Tina Perica, Yasushi Kondo, Sandhya P. Tiwari, Stephen H. McLaughlin, Katherine R. Kemplen, Xiuwei Zhang, Annette Steward, Nathalie Reuter, Jane Clarke, Sarah A. Teichmann. Evolution of oligomeric state through allosteric pathways that mimic ligand binding, Science 19 December 2014: Vol. 346 no. 6216 DOI: 10.1126/science.1254346

New PhD's

We congratulate **Anne-Sophie Schillinger**, **Bente Kjeilen** and **Hanzhen Wen** who have all finished their PhD's in November and December!

New Master degree

We congratulate **Øyvind Ødegård** who finished his master degree in Molecular biology 12 December!

Qaiser Waheed, MetaSig/CBU

Siri Strømsøy, NAT-gruppen

Nina Glomnes, NAT-gruppen

Sandhya P. Tiwari, MetaSig/CBU

Staff news

Fedor Kryuchkov is employed in a 4-year university post doc position affiliated with MetaSig/the NAD group, starting 1. December 2014. In addition to his own research, he will be assigned to the set-up of the new LC-MS machine.

Qaiser Waheed is employed in a 4-year university post doc position in Meta Sig/CBU, with Nathalie Reuters group, starting 4. December 2014.

Olena Dobrovolska is employed in a 4-year university post doc position in NucReg. She will start 1. February 2015. She will be presented in the next issue of MBINytt.

Sandhya P. Tiwari is engaged in a temporary position as a scientific assistant at MetaSig/CBU for three months starting mid-November. She is affiliated with Nathalie Reuter's group.

Nina Glomnes and **Siri Strømsøy** will both join the NAT-group, and have their main workplace at MBI. Nina is a technician and Siri is a PhD-fellow formally affiliated with the Faculty of Medicine and Dentistry and the Department of Clinical Chemistry (Klinisk institutt 2). Both work on gentic and molecular biological investigations of endocrine tumours based on an endocrine biobank and a collaboration with the Department of Surgery at Haukeland university hospital.

Vacancies

-4-year PhD-fellow with NucReg, affiliated with Øyvind Halskau in cooperation with Rein Aasland. Deadline for applications 5 January:
-4 year PhD-fellow with MetaSig, affiliated with Mathias Ziegler in cooperation with Nathalie Reuter. Deadline for applications 31 December:
-Another 4-year PhD-fellowship is soon to be published with MetaSig, affiliated with Nathalie Reuter in cooperation with Thomas Arnesen.

Molecular Biology Day 2015

Molecular Biology Day is a yearly event where MBI welcomes pupils from second and third year of high school into our labs. In 2015, this will be a part of "Open Day" at the whole university on 5 March. The whole department is expected to participate in one way or another, and it is a good idea not to plan to do any experiments that day. Think about how you want to participate, and do not be surprised if you are contacted regarding Molecular Biology Day.

Greetings from the administration

We wish to thank everyone for your cooperation in 2014, and wish you all a merry Christmas!

There will be people in the administration all working days during Christmas except for Christmas eve.

22, 23, 29, 30 December and 2 January the doors and elevators will be open as usual. All other days including Christmas eve, the elevators and main doors will be closed. Remember your key-card and pin-code!

God jul!