UNIVERSITY OF COPENHAGEN

Biologics Seminar Series (BSS)

October-December 2016

Where: Universitetsparken 2, 2100 Copenhagen

Program

- Wednesday **12th October 2016** at **9am, Auditorioum 3**
  Prof. Martin Malmsten, Dept. of Pharmacy, Uppsala University, and Dept. of Pharmacy, KU
  "*Selective membrane activity through tryptophan end-tags: boosting of antimicrobial, anti-inflammatory, and anti-cancer effects"*

- Wednesday **9th November 2016** at **9am, , Benzon Auditorium,**
  Prof. Gregers Jungersen, Veterinary Institute, Technical University of Denmark
  "Tracking the elusive cytotoxic T cell response in pigs"

- Wednesday **7th December 2016** at **9am, Benzon Auditorium,**
  Prof. Tommy Nylander, Division of Physical Chemistry, Lund University
  "*The use of neutron and x-scattering techniques to study the non-lamellar lipid aqueous interface and interfacial films"*

The seminars will last for 45 min + 15 min questions and are free of charge. Please contact Vito Foderà (vito.fodera@sund.ku.dk) for further information.

Abstracts

Wednesday 12th October 2016 at 9am, Auditorioum 3

Prof. Martin Malmsten, Dept. of Pharmacy, Uppsala University, and Dept. of Pharmacy, KU

Selective membrane activity through tryptophan end-tags: boosting of antimicrobial, anti-inflammatory, and anti-cancer effects

End-tagging of antimicrobial peptides (AMPs) by tryptophan stretches provides a generally applicable approach for boosting membrane interactions of such peptides. In the context of infection, such end-tagging offers an approach to achieve high, but selective, AMP activity, e.g., allowing bacteria to be effectively killed in blood. The pronounced selectivity was demonstrated to be due to a combination of membrane charge density and the elastic penalty of insertion of bulky W/F residues in the presence of cholesterol. Through this, W/F-tagging offers opportunities for selective short AMPs, effective against a range of "superbugs", including methicillin-resistant S. aureus (MRSA), vancomycin-resistant enterococci, and multi-drug resistant P. aeruginosa, yet displaying limited toxicity. Analogously, W-tagging was demonstrated to promote anti-inflammatory properties of antimicrobial peptides, which was demonstrated to be due to increased peptide binding to bacterial lipopolysaccharide (LPS) and its lipid A component, membrane-localized LPS scavenging, and LPS disaggregation. Importantly, potent anti-inflammatory effects were observed at low cell toxicity, demonstrated for both monocytes and erythrocytes. W-tagging also represents a powerful way to increase selective peptide internalization in melanoma cells, resulting in toxicity against these, but not against non-malignant non-malignant keratinocytes, fibroblasts, and erythrocytes. These effects were shown to be due to increased peptide adsorption to the outer membrane in melanoma cells, caused by the presence of anionic lipids such as phosphatidylserine and ganglioside GM1, and to peptide effects on mitochondria membranes and resulting apoptosis. In addition, the possibility of using W-tagged peptides for targeted uptake of nanoparticles/drug carriers in melanoma was demonstrated, as was the possibility to open up the outer membrane of melanoma cells in order to facilitate uptake of low Mw anticancer drugs, here demonstrated for doxorubicin.

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Professor at Uppsala University, in October moving to the University of Copenhagen. Malmstens research has resulted in >250 papers and several books. Awarded, e.g., the Langmuir Lecture Award. Member of the Royal Swedish Academy of Engineering Science, Fellow of the Royal Chemical Society, guest professor at Charité University in Berlin, and Editor-in-Chief of the Journal of Colloid and Interface Science. Malmstens current research interests include microgels, nanomaterials for drug delivery, as well as antimicrobial, anti-inflammatory, and anticancer peptides.

Wednesday 9th November 2016 at 9am, , Benzon Auditorium,

Prof. Gregers Jungersen, Veterinary Institute, Technical University of Denmark

Tracking the elusive cytotoxic T cell response in pigs

Quantitative and qualitative assessment of antigen-specific cytotoxic T cell (CTL) responses in pigs is not a straightforward process. Through the years we have developed a series of reagents, tools and protocols to characterize peptide-specific CTL responses in pigs.

The most common recombinant SLA heavy chains were produced and peptide binding motifs were determined by assays measuring the affinity and stability of the peptide-SLA complex (pSLA) interaction. These results have been used to train neural networks to predict the binding of any pSLA (http://www.cbs.dtu.dk/services/). Recombinant SLA molecules complexed with verified binding peptides can be assembled to SLA multimers for staining of peptide-specific CTLs, and measured by flow cytometry, as we have shown with FMDV and influenza. This, however, requires SLA-matched pigs for which we have developed two methods: a sequence-based, high-resolution SLA genotyping method by standard PCR for specific detection of eight in-house SLA molecules; and a next-generation sequencing method for parallel detection of up to 50 samples of barcoded cDNA PCR products spanning exon 2 and 3. The latter for a wider characterization of expressed alleles in candidate pigs.

The in vivo generation of CTL responses to antigens following peptide immunizations is thought to require cross-presentation in appropriate dendritic cells (DC). In mice this was linked to targeting of CD103+DCs recruited after intraperitoneal immunizations. We have therefore developed a protocol for intraperitoneal delivery of peptides formulated in poly(I:C)/MMG-decorated liposomes (CAF09) to investigate the influence of peptide dose on the generation of CTL vs. antibody responses. Finally, the induced CTL killing was assessed by an in vivo cytotoxicity assay, where purified autologous PBMCs, fluorescently labeled and pulsed with target peptides, were reinjected into the donor. The in vivo killing of peptide-pulsed cells was measured by flow cytometry relative to non-pulsed PBMCs at different time points after cell transfer.

A native Dane, Professor Jungersen obtained his PhD in internal medicine from the Royal Veterinary and Agricultural University in Copenhagen in 1998 after 4 years as Veterinary Practitioner in the Southern part of Denmark. Since then he has worked at the National Veterinary Institute where he was appointed Professor in 2009. His expertise in immunology is founded on research in local and systemic immune responses to infectious diseases in cattle and pigs, and development of agent-specific tests to measure humoral and cell-mediated immune responses including direct identification of peptide-specific cytotoxic T cells by staining with recombinant produced MHC-peptide tetramer complexes. A goal of my research is to apply my immunological expertise to develop new and improved vaccines for livestock and to apply our large and advanced immunological tool box for porcine immunology to establish pigs as a relevant large animal model to study e.g. immunomodulatory vaccine constructs and accelerate vaccine development in human medicine.

Wednesday 7th December 2016 at 9am, Benzon Auditorium,

Prof. Tommy Nylander, Division of Physical Chemistry, Lund University

The use of neutron and x-scattering techniques to study the non-lamellar lipid aqueous interface and interfacial films

Biological membranes do not only occur as planar bilayer structures, but bilayers have also been shown to, depending on the lipid composition, curve into intriguing 3D structures. Understanding the biological implication as well as the application of such interfaces, for e.g. drug delivery and other biomedical application, requires the development of well-defined model system. We have previously demonstrated that the formation of fluid supported bilayers on vertical gallium phosphide nanowire (NW) forests using self-assembly from lipid vesicular dispersions.[1]

Here we will discuss the formation of non-lamellar lipid liquid crystalline surface layers prepared by spin-coating the constituting lipids followed by hydration of the lipid layer.[2] We will compare this approach by the one using adsorption of the corresponding reversed lipid liquid crystalline nanoparticles (LCNPs) of the cubic micellar (I_2) phase, which have high potential for drug delivery.[3] We will demonstrate that we can form non-lamellar liquid crystalline surfaces of different phases on the surface. The structure of films formed were studied both by specular and off-specular neutron reflectometry and the dynamics within the surface layer within nanosecond time scale was studied by grazing incidence neutron spin echo spectroscopy (GINSES).[4] The implications for biomedical delivery systems will be discussed.

[1]Dabkowska, A.P., Niman, C.S., Piret, G., Persson, H., Wacklin, H.P., Linke, H., Prinz, C.N. & Nylander, T. Fluid and highly curved model membranes on vertical nanowire arrays. Nano Letters, 2014, 14, 4286-4292.

[2]Wadsäter, M., Barauskas, J., Nylander, T., Tiberg, F. Nonlamellar lipid liquid crystalline model surfaces for biofunctional studies. Soft Matter, 2013, 9, 8815–8819.

[3]Chang, D. P., Dabkowska, A. P., Campbell, R. A., Wadsäter, M., Barauskas, J., Tiberg, F., Nylander, T. Phys. Interfacial properties of POPC/GDO liquid crystalline nanoparticles deposited on anionic and cationic silica surfaces. Chem. Chem. Phys., 2016, Advance Article, DOI: 10.1039/C6CP04506E

[4]Nylander, T., Soltwedel, O. Hirst, C., Holdaway, J. Yanez Arteta, M., Wadsäter, M., Barauskas, J., Frielinghaus, H., Holderer, O. Relationship between structure and fluctuations of lipid non-lamellar phases deposited at the solid/liquid interface. 2016, Manuscript in preparation

Prof. Nylander did his under graduate studies in Chemical Engineering with specialization in Food Technology at Lund University. He completed his PhD in Biophysical Technology at Lund University under the supervision of Prof. Kåre Larsson in 1987 with a thesis entitled "Proteins at the metal water interface- adsorption and solution behaviour". In 1990 he did a postdoc at Australian National University, Department of Applied Mathematics, Canberra, Australia with Prof. Barry Ninham. In 2000 he was appointed senior lecturer in Physical Chemistry, Lund University and in 2007 he was promoted to full professor at the same department.

The main theme of Prof. Nylander scientific activity has been to relate interfacial behaviour of surface-active molecule to their solution behaviour. The focus has been on molecules of biological origin, e.g. proteins and lipids, and the self-assembled structures they form in bulk and at interfaces. The relation between solution and interfacial behaviour is crucial in many system, in particular for lipids and proteins, but also on polymer-surfactant system. Focus is on interfacial techniques such as ellipsometry, surface film balance, QCM-D and neutron reflectometry as well as on other scattering techniques. Nylander has a large experience on application research and on international collaboration both with academia and industry. He has published 225 scientific articles and book chapters within his research area.