Scientific report

4th International Singapore Lipid Symposium
March 13–16, 2012
Center for Life Sciences, National University of Singapore

Ajay K. Mahalka

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A recipient of 4th ISLS travel awards by the EUROCORES program of the European science foundation "EuroMEMBRANE" for oral presentation "Protein-Phospholipid Interactions: From Biophysics to Therapeutics"

Summary

The structurally most diverse class of molecular building block in all living creatures in their lipids, which in an average cell is estimated to consist of approximately 15000-18000 different species. Importantly, the specific distribution patterns of lipids in different cells and their organelle membranes in controlled by active mechanisms, and undergo rapid changes due to alterations in the functional state of the cells. On molecular level the catalytic activities of membranes associated protein can be controlled by lipid by different fundamental mechanisms. Lipid–protein interactions play a key role in a large number of cellular processes and are controlled by the membrane associated physicochemical properties and structure of membrane lipids.

4th INTERNATIONAL

March 13–16 2012

SINGAPORE LIPID SYMPOSIUM

ENABLING TECHNOLOGIES

Mass spectrometry • Imaging • Chemical Biology

APPLICATIONS

Model systems • Biomedicine • Plant fat

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4th International Singapore Lipid Symposium

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National University of Singapore
Center for Life Sciences
Organizer: Markus R Wenk

www.lipidprofiles.com

Once viewed simply as a reservoir for carbon storage, lipids are no longer cast as bystanders in the drama of biological systems. The emerging field of lipidomics is driven by technology, most notably mass spectrometry, but also by complementary approaches for the detection and characterization of lipids and their biosynthetic enzymes in living cells. The development of these integrated tools promises to greatly advance our understanding of the diverse biological roles of lipids.

Patricia Bassereau
Institut Curie, Paris

Mathieu Blanc
University of Edinburgh

Igor Butovich
UT Southwestern, Dallas

Chew Fook Tim
National University of Singapore

Chng Shu Sin
National University of Singapore

Chye Mee-Len
The University of Hong Kong

Neil Clarke
Genome Institute of Singapore

Giovanni D'Angelo
Institute of Protein Biochemistry, Naples

Toon de Kroon
Utrecht University

Ed Dennis
University of California, San Diego

Christian Eggeling
MPI Göttingen

Ivo Feussner
University of Göttingen

Anne-Claude Gavin
EMBL Heidelberg

Guan Xueli
Swiss TPH, Basel

John Harwood
Cardiff University

Gabriele Kastenmüller
Helmholtz Zentrum München

Harald Köfeler
Medical University of Graz

Benhur Lee
University of California, Los Angeles

Lok Hang Mak
Imperial College London

Ajay K. Mahalka
Aalto University, Espoo

Chakravarty BN Marella
NCBS Bangalore

Peter Meikle
Baker IDI Melbourne

Todd Mitchell
University of Wollongong

Yuki Nakamura
Academia Sinica, Taipei

Adam Orłowski
Tampere University of Technology

Herman Overkleeft
Leiden University

Rob Parton
The University of Queensland, Brisbane

Brendan Prideaux
Novartis, Basel

Thusitha Rupasinghe
The University of Melbourne

Dominik Schwudke
NCBS Bangalore

Andrej Shevchenko
MPI Dresden

Takao Shimizu
The University of Tokyo

Song Baoliang
Chinese Academy of Sciences, Shanghai

Shui Guanghou
National University of Singapore

Scott Summers
Duke-NUS, Singapore

Guillaume Thibault
Temasek Life Sciences Laboratory, Spingapore

Markus Wenk
National University of Singapore

Ioannis Xenarios
Swiss Institute of Bioinformatics, Lausanne

Xu Chenqi
Chinese Academy of Sciences, Shanghai

Xun Huang
Chinese Academy of Sciences, Beijing

Joanne Yew
Temasek Life Sciences Laboratory, Singapore

Andreas Zumbusch
University of Konstanz

Tue – Mar 13

10:00–12:00 WORKSHOP 1
Lipid metabolism and homeostasis – case studies cholesterol and ceramides
Song Baoliang
Scott Summers

12:00 *lunch*

13:00–15:00 WORKSHOP 2
Global standards for mass spectrometry based lipidomics
Harald Koefeler
Dominik Schwudke
Shui Guanghou
Todd Mitchell

15:00 *break*

15:30–17:30 WORKSHOP 3
Curating lipidomic information
Ed Dennis
Ioannis Xenarios
Andrej Shevchenko

19:00 *Reception at conference hotel (by invitation)*

Wed – Mar 14

09:00 *Opening Remarks*
09:20 Ed Dennis
10:00 Rob Parton
10:40 *break*
11:10 Xun Huang
11:50 Andreas Zumbusch

12:30 *lunch*

13:30 Peter Meikle
14:10 Andrej Shevchenko
14:50 Joanne Yew
15:30 *break*
16:00 Yuki Nakamura
16:20 Brendan Prideaux
16:40 Christian Eggeling
17:00 Guillaume Thibault

17:30 *posters and reception*

19:30 *bus to hotel*

Thu – Mar 15

09:00 Patricia Bassereau
09:40 Herman Overkleeft
10:20 *break*
10:50 Toon de Kroon
11:30 Anne-Claude Gavin
12:10 Markus Wenk

12:50 *lunch and posters*
12:50 AB Sciex lunch talk

14:00 Takao Shimizu
14:40 Gabriele Kastenmüller
15:20 Benhur Lee
16:00 *break*
16:30 Chakravarty BN Marella
16:50 Adam Orłowski
17:10 Ajay K. Mahalka
17:30 Lok Hang Mak

18:00 *bus to conference dinner*

18:30 *conference dinner*

21:00 *bus to hotel*

Fri – Mar 16

09:00 Xu Chenqi
09:40 Chng Shu Sin
10:20 *break*
10:50 Igor Butovich
11:30 John Harwood
12:10 Ivo Feussner

12:50 *lunch and posters*

14:00 Chye Mee-Len
14:40 Neil Clarke
15:20 Chew Fook Tim
16:00 *break*
16:30 Giovanni D'Angelo
16:50 Mathieu Blanc
17:10 Guan Xueli
17:30 Thusitha Rupasinghe

17:50 *closing remarks*

18:30 *bus to hotel*

A description of the scientific content of the event

The 4th International Singapore lipid symposium (ISLS) focuses on emerging field of lipidomics driven by technology, most notably mass spectrometry, but also by complementary approaches for the detection and characterization of lipids and their biosynthetic enzymes in living cells. Symposium was also dedicated on molecular level on physics of lipids, their properties, organization and interactions with proteins and other biopolymers, starting from fundamental principles.

The speaker and teacher were some of the leading pioneers in the physics of lipids and soft materials. Building on fundamental molecular and system level knowledge we will explore how lipid and membrane biophysics and cell biology manifests in the operation of biomembranes, their organization and physiological functions and pathological processes.

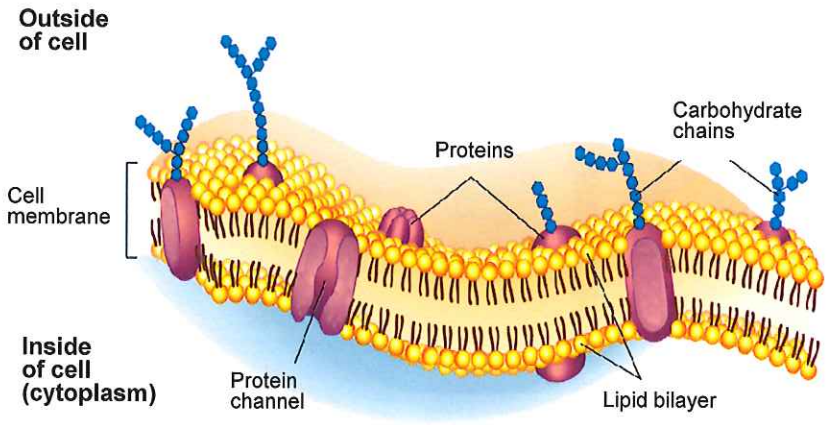
Protein-Phospholipid Interactions: From Biophysics to Therapeutics

Ajay K. Mahalka
Helsinki Biophysics and Biomembrane Group
School of Science, Aalto University
Finland

Mentor & supervisor
Prof. Paavo KJ Kinnunen

Aalto University
School of Science

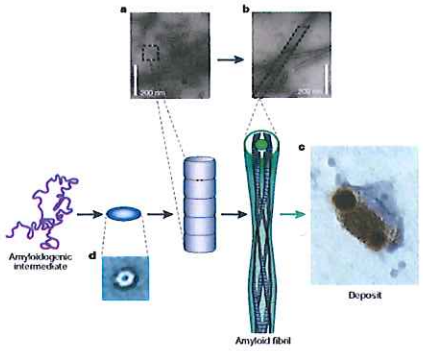
Cell Membrane



Content

- Oxidized phspholipid and gelsolin intercation: Amyloidosis
- Phspholipid-Hsp70 interaction in membrane stability in stress
- Hsp70 activates phospholipase A2 (PLA2)
- Lipid-protein interactions in therapeutics for amyloidosis, Niemann-pick disease, and cancer?

Amyloidosis

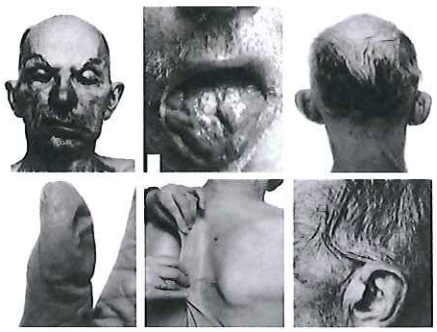


Lewy bodies associated with Parkinson's disease

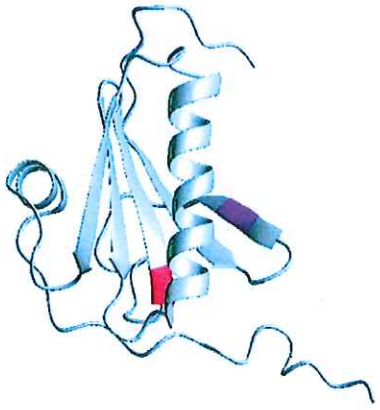
Pathological Conditions	Protein/Peptides
Alzheimer's disease (AD)	Aβ and tau
Age-related macular degeneration (AMD)	Aβ and others (drusen)
Parkinson's disease (PD)	α-synuclein
Type 2 diebetes mellitus (2DM)	IAPP
Prion disease	Prion
Finnish type familial amyloidosis (FAF)	gelsolin

Dobson, Nature 2003

Familial amyloidosis of Finnish type (FAF)



Familial amyloidosis of Finnish type (FAF)



S2 domain of D187N/Y gelsolin

Membrane/Lipid Accelerates amyloidosis

- Phopholipid membrane are primary targets of amyloid toxicity *in vivo*
- Amyloidogenesis always associated *in vivo* lipid rich environment
- Lipid are found in *ex vivo* amyloid plaque and pathological sample

Phospholipids membrane involves in

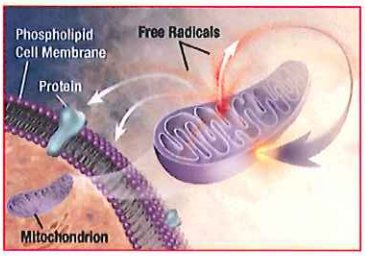
1. Structural transition of native structure (Misfolding)

2. Spatial enrichment of monomers (protein)

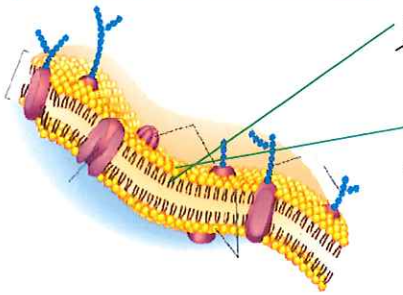
3. Alleviate electrostatic repulsion

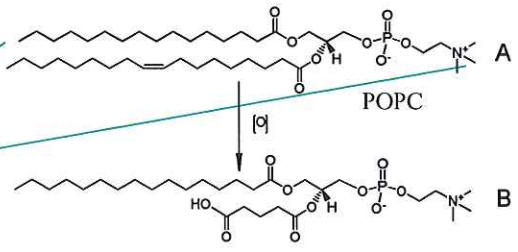
4. "Template" amyloid assembly

Elevated oxidative stress associated with amyloidosis



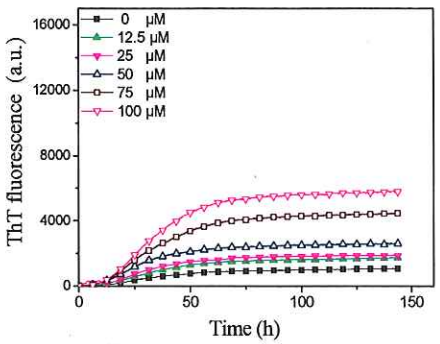
Studies at the Cellular and Molecular Level

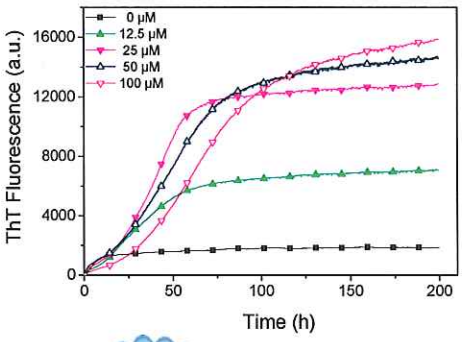


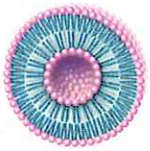


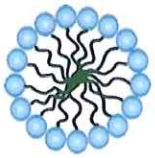
Oxidized phopholipid, PoxnoPC

Oxidized phospholipid accelerates the fibrillation of gelsolin



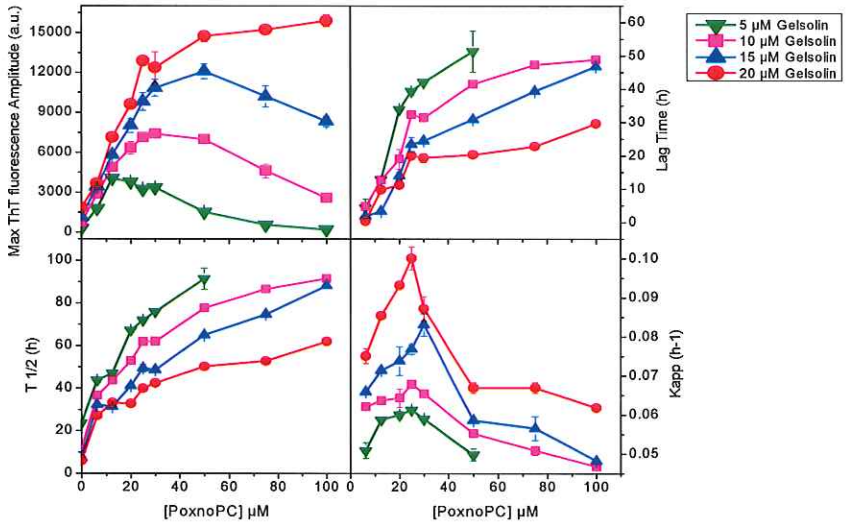






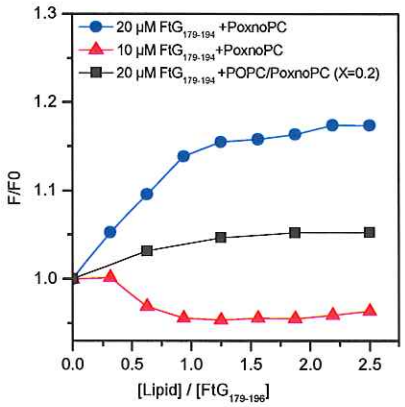
*Mahalka et al Biochemistry (2011) **50**, 4877–4889*

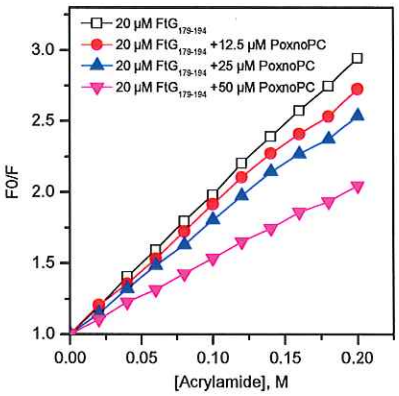
Oxidized phospholipid accelerates the fibrillation of gelsolin



*Mahalka et al Biochemistry (2011) **50**, 4877–4889*

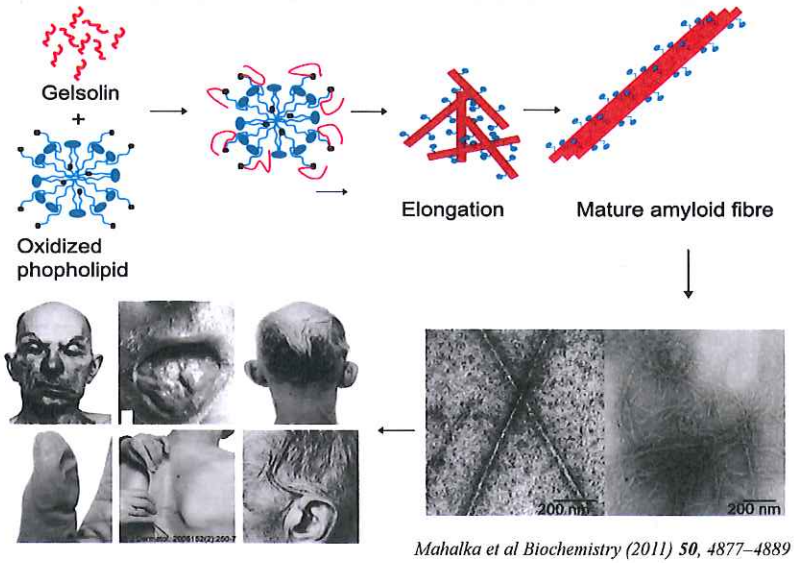
Oxidized phospholipid accelerates the fibrillation of gelsolin





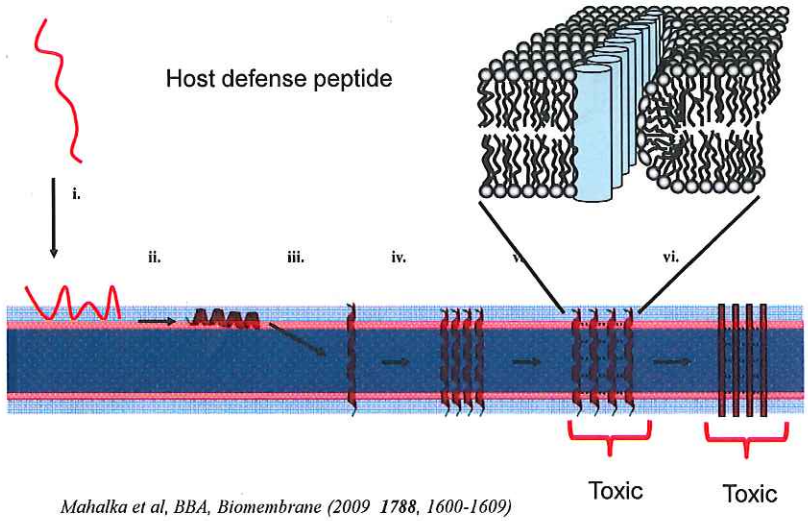
Mahalka et al Biochemistry (2011) ***50****, 4877–4889*

PoxnoPC, an Oxidized Phospholipid, Accelerates Finnish Type Familial Gelsolin Amyloidosis



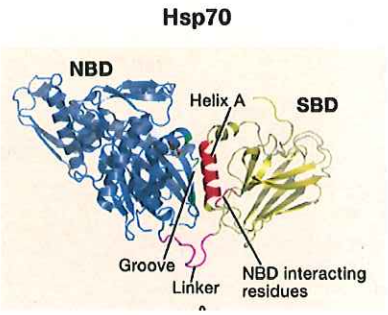
Mahalka et al Biochemistry (2011) ***50****, 4877–4889*

Membrane induced amyloid formation involved in the mechanism of action of HDP



*Mahalka et al, BBA, Biomembrane (2009 **1788**, 1600-1609)*

Heat shock protein 70 (Hsp70) is a molecular chaperone expressed in response to stress.



The house-keeping functions

Transport of proteins and vesicles

Degradation of unstable proteins

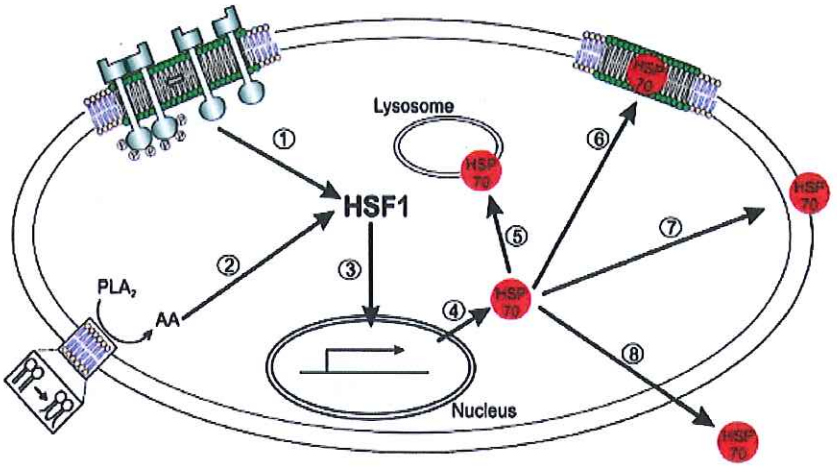
Folding and refolding of proteins

Control of regulatory proteins.

❖The most conserved protein in evolution (prokaryotic Hsp70 protein DnaK shares 50% aa identity with eukaryotic Hsp70)

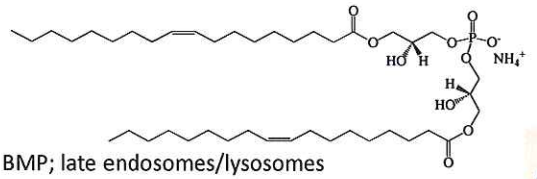
❖ There are eight human Hsp70 differ from each other by amino acid sequence, expression level and sub-cellular localization

Hsp70 promoter organelle stability by binding to membrane

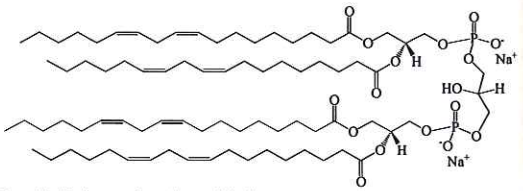


Laszlo Vigh, (2008), BBA Biomembrane

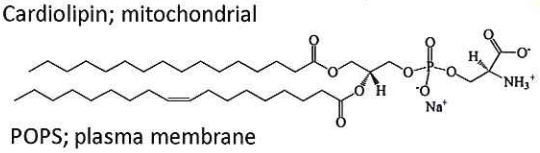
Hsp70 stabilizes membrane integrity by the association of phospholipid



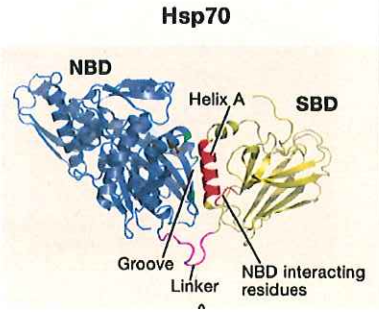
BMP; late endosomes/lysosomes



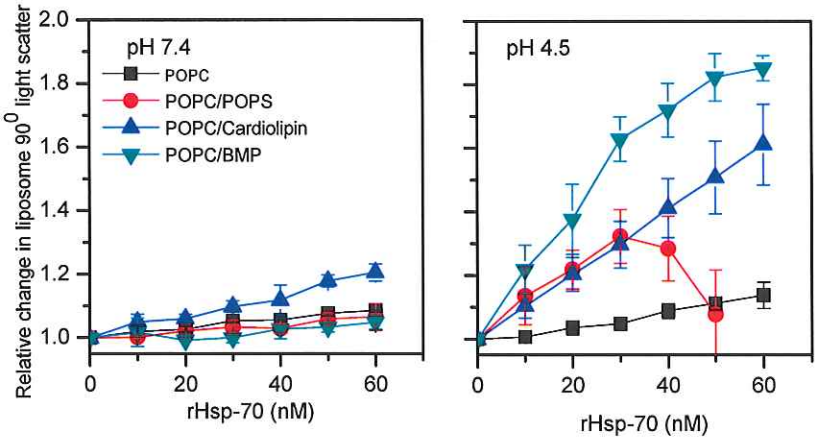
Cardiolipin; mitochondrial



POPS; plasma membrane

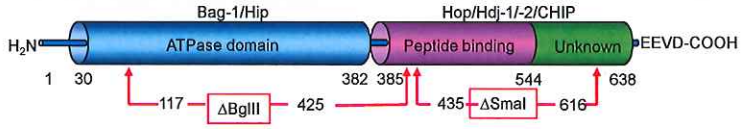


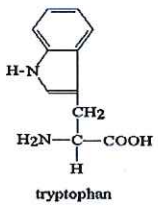
In acidic environment Hsp70 specifically binds to lysosomal phspholipid *i.e* BMP

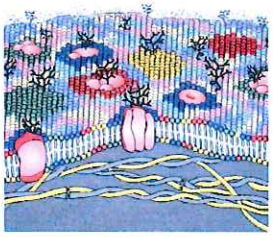


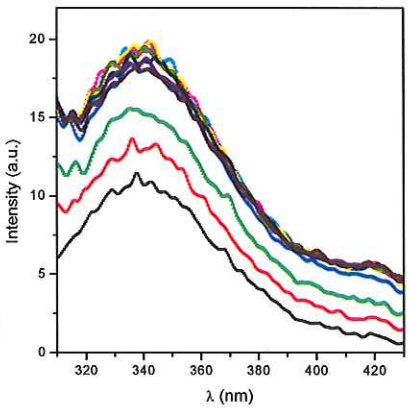
Nature **463**, 549-553 (2010)

Membrane protein interaction: tryptophan (W) as a fluoroscence probe



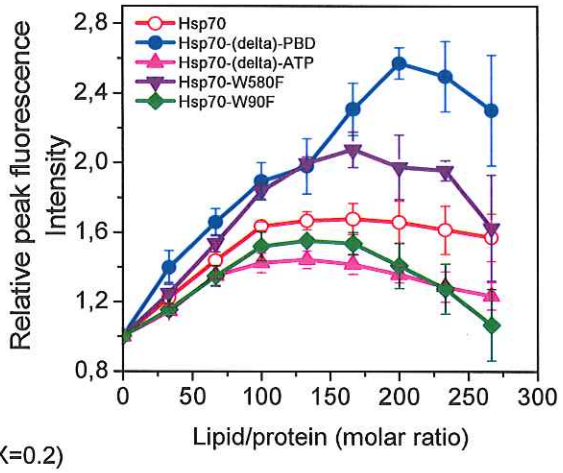






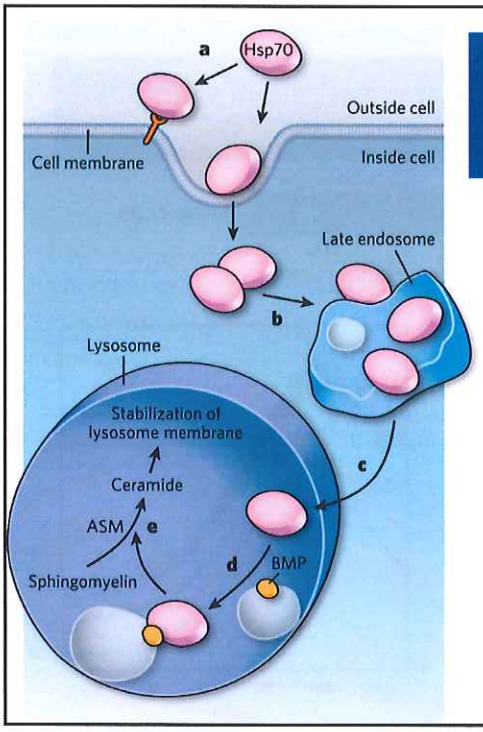
The quantum yield of Trp emission increases in a hydrophobic environment.

Hsp70 interacts with BMP containing membrane via ATPase domain



POPC/BMP (X=0.2)

Nature **463**, 549-553 (2010)

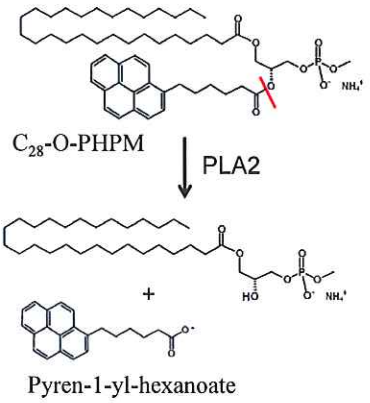


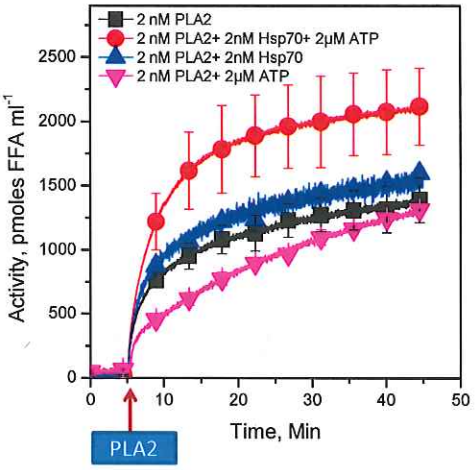
Hsp70 promotes cell survival, by stabilizing the membranes of lysosomes

- In the acidic environment Hsp70 interacts with BMP, an anionic phospholipid bound to the inner lysosomal membrane.
- Hsp70–BMP interaction activating the enzyme sphingomyelinase (ASM) that breaks down the lipid sphingomyelin to form ceramide.
- The ASM-mediated conversion of sphingomyelin to ceramide enhances membrane acyl chain order and increases lateral packing of lipids.

Nature **463**, 549-553 (2010)

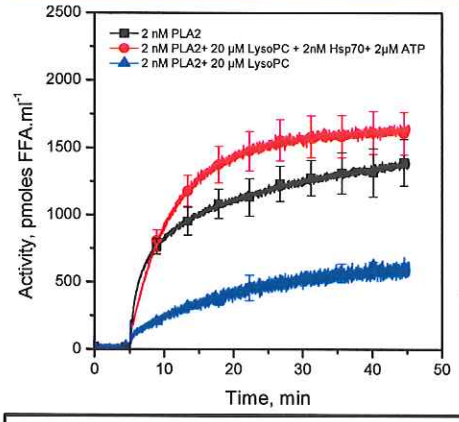
Hsp70 activates phospholipase A2 (PLA2) in presence of ATP

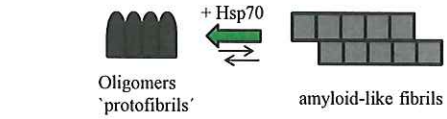




*Mahalka et al BBA **1808**, 2569-2572 (2011)*

Hsp70 can rescue the activity of PLA2 in an ATP dependent manner





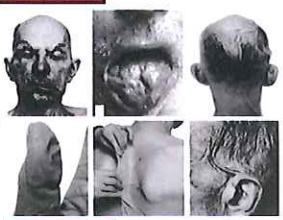
Activity ++++ (High) ---- (Low)

Hsp70 to sustain the lifetime of the active state of the enzyme oligomer by attenuating the conversion of the enzyme oligomers into inactive amyloid.

*Mahalka et al BBA **1808**, 2569-2572 (2011)*

Oxidized phospholipid accelerates amyloid formation

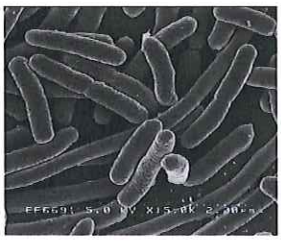
BAD



- Oxidized phospholipid membrane promote fibrillation of gelsolin and accelerates Familial amyloidosis of Finnish type (FAF).

Mahalka et al Biochemistry (2011) ***50****, 4877–4889*

GOOD

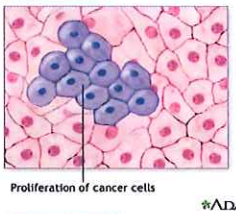


- The mechanism of microbial killing by host defence proteins (HDP) is similar to that of the amyloid forming cytotoxic peptidess

Mahalka et al BBA, Biomembrane(2009) 1788, 1600-1609
Kinnunen & Mahalka BBA , Biomembrane(2012) Revision

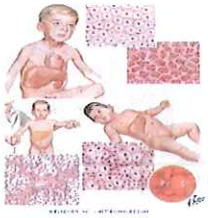
Phospholipid-Hsp70 interaction promotes stability by keeping enzyme oligomer in active state

BAD



- BMP-Hsp70 interaction attenuate lysosomal membrane permeabilization promote tumour-cell survival.

GOOD



- Hsp70-BMP interaction controls lysosome stability, providing a potential target to treat Niemann-Pick Disease-associated Lysosomal Pathology.

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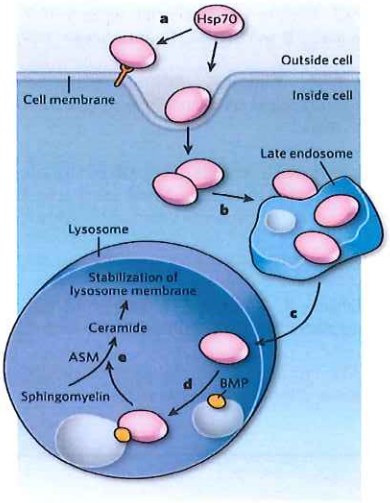
Protein-Phospholipid Interactions: From Biophysics to Therapeutics



Aalto University
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An assessment of the results and impact of the event on the EUROCORES Programme

Lipidomics may be defined as the large-scale study of pathways and networks of cellular lipids in biological systems. Thefield that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry and computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes.

Lipidomics research involves the identification and quantification of the thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. The growing attention on lipid research is also seen from the initiatives underway of the LIPID Metabolites and Pathways Strategy (LIPID MAPS Consortium) and The European Lipidomics Initiative (ELIfe).

Identification of functionality of different lipid and physiochemical property and impact on cellular process is necessary. The dynamic coupling between chemical composition, physical state, organization, and biological functions of lipid membrane assemblies, forming basis for the notion that membrane physical state of membrane is directly correlated with the physiological sate of cell.