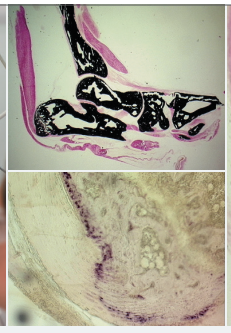
| GROUP

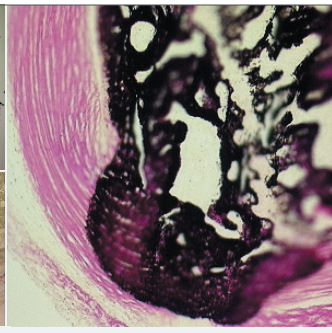
BIOCHEMISTRY OF PHYSIOPATHOLOGICAL MINERALIZATION AND ENZYMATIC LIPOLYSIS (BIOMPLE)

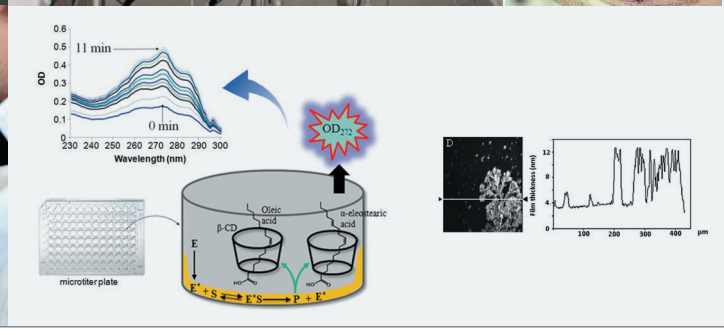
ICBMS - UMR 5246, UNIVERSITÉ CLAUDE BERNARD LYON 1 - CNRS - INSA LYON - CPE LYON

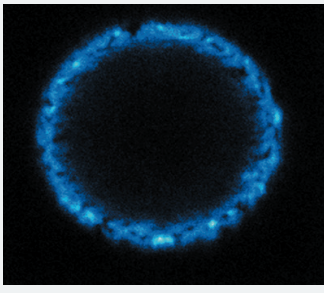
DAVID MAGNE

Professor









MOLECULAR AND INTERFACIAL MECHANISMS OF (PHOSPHO)LIPASES

STATE OF ART

The heterogeneous lipolysis reaction occurs in different cellular compartments, for example in membranes, involving phospholipases A1, A2 and D, and in lipid droplets of adipose tissue with adipose- and hormone-sensitive lipase.

OBJECTIVES

Understanding the functional specificities of (phospho) lipases is a major challenge, since these lipolytic enzymes have many biotechnological and medical applications. They are therapeutic targets for the treatment of obesity and type-2 diabetes. We are developing high throughput screening assays applicable to screen inhibitors of these (phospho) lipases.

BIOLOGICAL MEMBRANES AND RAFT-LIKE STRUCTURES

STATE OF ART

Cell membranes show considerable structural diversity. Membrane microdomains, lipid rafts or raft-like structures play a role in numerous cellular processes including signaling, lipid trafficking, vesicular transport, membrane protein activity interaction with pathogens, viral infection, etc.

OBJECTIVES

Understand the role of extrinsic proteins such as guanidino kinases in the dynamics and topology of phospholipidic membranes. Identify the nature of lipid-lipopeptide interactions to know how fungicides and antibiotics function. Design of fluorophore probes for high resolution cell imaging to visualize raft-like structures as well as structural details of plasma membranes and vesicles.

BIOMINERALIZATION AND VASCULAR CALCIFICATION

STATE OF ART

Pathological calcification in soft tissues such as tumor and vascular calcifications are characterized by the deposition of calcium salts. Due to the population ageing, this problem will be of increasing importance in the coming years.

OBJECTIVES

Determine the molecular and cellular mechanisms involved in the bone mineralization and in pathological calcifications (arteries, articular cartilage, tendons and ligaments) using primary cells and cell lines. Identify signaling pathways involved during calcification. Develop inhibitors of alkaline phosphatase – a mineralization marker- as possible drug therapy to treat vascular calcifications.

| Figure

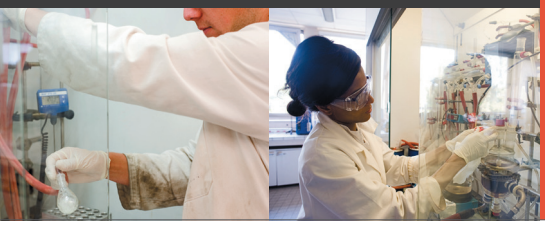
Centre bottom: High throughput screening assays schematic representation of the assay reaction showing the hydrolysis of ß-eleostearic acid-containing phosphatidylcholine coated in a microwell.

Right top: Models of calcifications in joints of mice and fibrochondrocytes.

Right bottom: Brewster angle microscopy of a model of internal mitochondrial membrane and confocal microscopy of proteins domains on the surface of giant vesicles.

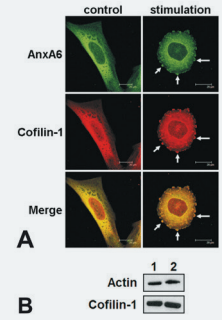
| EQUIPMENT

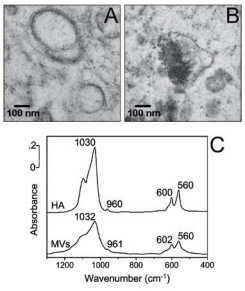
› Black room, cold room,
› Cell culture laboratory, Inverted microscopy
› Chromatography, gel electrophoresis
› Langmuir balance, on line data acquisition
› RT-PCR, molecular biology laboratory
› Ultracentrifugation, microfuge
› UV-visible and infrared spectrometers, Fluorescence and plate reader



| EXPERTISES

› Interfacial enzymology, reaction mechanisms
› Expression and purification of recombinant proteins or enzyme such as (phospho) lipases and guanidine kinases
› Development of enzyme assays applicable to high throughput screening of activities and of inhibitors
› Membrane biology and biophysics, purification of lipid rafts and matrix vesicles
› Membrane models (monolayers, vesicles)
› Molecular mechanisms of calcifications, bone biology
› Smooth muscle cells, chondrocytes and osteoblasts, primary cells and cell lines





COLLABORATION

Our research group belongs to the Institute of Molecular and Supramolecular Chemistry and Biochemistry (ICBMS), to the Institute of Multidisciplinary Biochemistry of Lipid (IMBL) and to the Institut Carnot LISA (Lipids for the industries and for the health) We perform biological analysis for the Biochemistry Platform of Sanofi Pasteur. We are collaborating at local level with Organist chemists at ICBMS; at national level with Institut Pasteur at Lille; and at international level with the Department of Biochemistry at Hong Kong University, with Jilin University at Chongchun (China), with the Nencki Institute of Experimental Biology at Warsaw (Poland) and with Lebanese University at Beirut (Lebanon).

KEYWORDS

Alkaline phosphatase – Brewster angle microscopy - Calcification – Chondrocyte – Fluorescence – Fluorescent probe - Guanidino kinase – High throughput screening – Infrared - Inhibitor - Lipase – Lipid – Lipid monolayer - Lipid raft – Liposome – Lipopetide - Mineralization – Matrix vesicle - Smooth muscle cell - Osteoblast – Phospholipase – Vascular calcification

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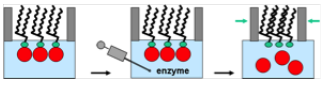
0-2 Post-Docs
6-9 Phd Student
0-2 Graduate Students
3-6 Undergraduate Students



Measure of phospholipase D activity using phospholipid monolayer at the air/water interface

| Figures

Right top : Localization of two proteins in osteoblasts

Right bottom: Matrix vesicles initiate hydroxyapatite formation

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