NANOSCIENCES

2nd YEAR PhD FINAL EXAM

Sara Chiarugi

Pisa, 17/10/2019

COURSES & OTHER ACTIVITIES

Attended courses	Hours
Seminar Cycle in Biophysical Sciences – Prof. F. Cardarelli	45
Topics in Structural Biology – Prof. A. Pastore	20

Seminars/Workshops	Hours
Seminars	31
EMBO Workshop (Hamburg): "Tools for Structural Biology of Membrane Proteins"	25

EMBO | EMBO Workshop

RESEARCH ACTIVITY

BioStructures Lab Dr. Gianpiero Garau

1

Design and engineering of a 3D protein-trapping nano-biostructure for X-ray crystallography

2

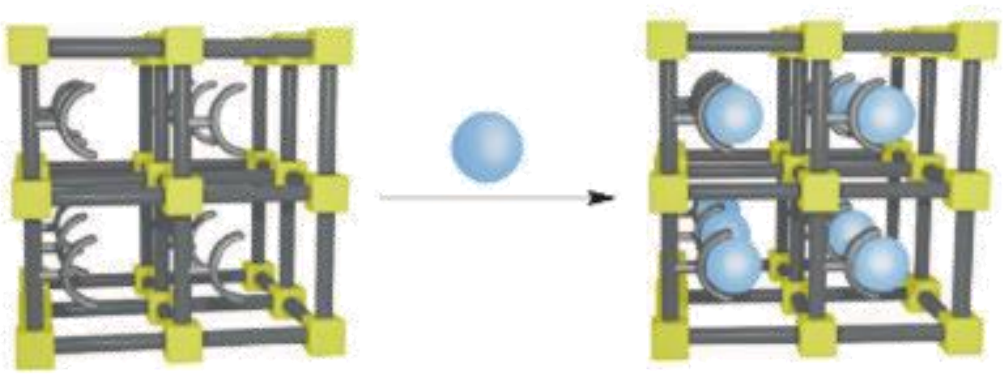
Discovery of ligands targeting the membrane protein NAPE-PLD

1 PROJECT GOAL & BACKGROUND

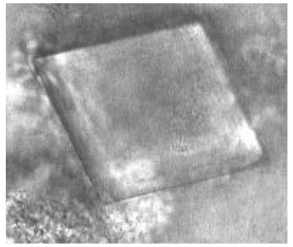
Efficient and innovative methodology for membrane protein crystallization and structure determination based on **trapping protein-nanocages**

Smart protein scaffolds (gCAD) able to:

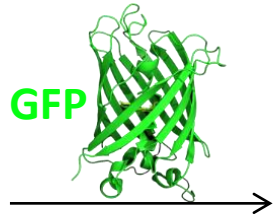
- ❑ Generate crystalline cages with internal cavities
- ❑ Crystallize easily and efficiently (known crystallization conditions)
- ❑ Trap guest target proteins

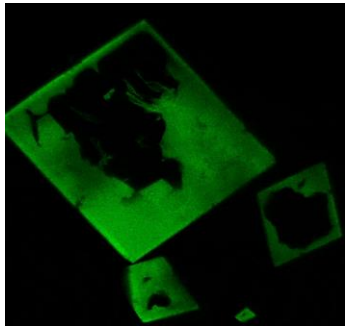


Scheme. Assembly model of the system



gCAD crystal



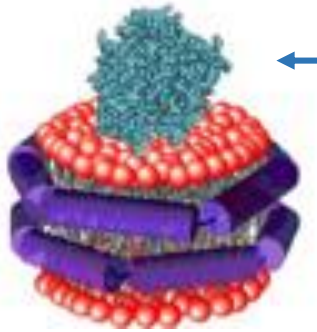


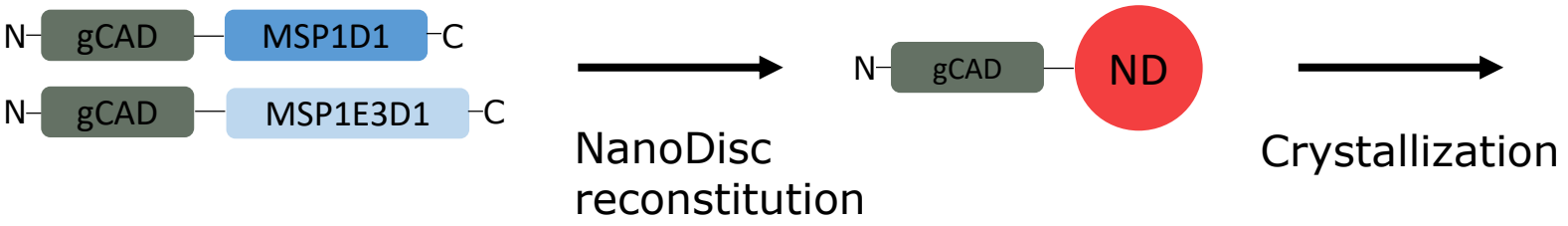
1 SYSTEM EVOLUTION

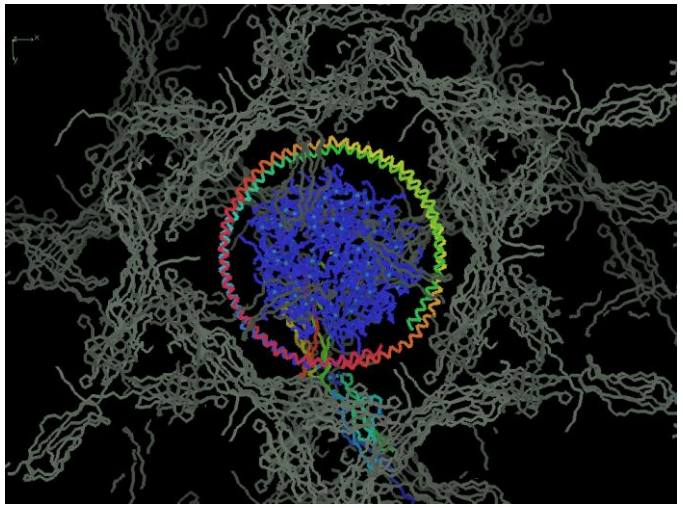
MEMBRANE PROTEIN TARGETS

- **Challenging** structure determination
- ~3% of crystal structures in the PDB

NANODISC TECHNOLOGY





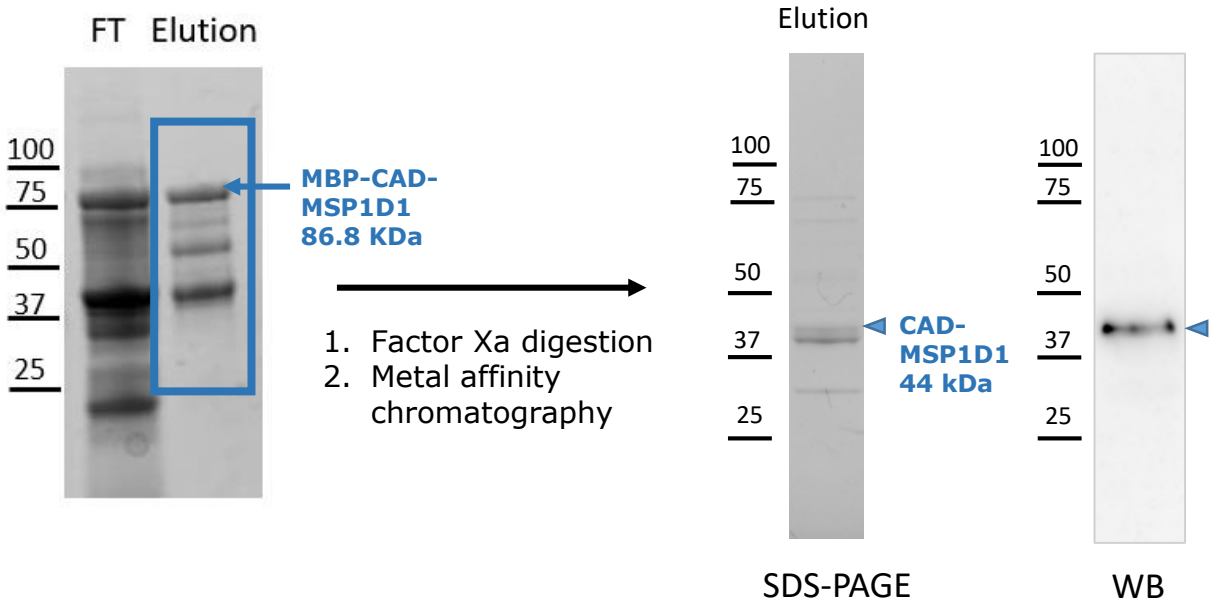


Model design

RESULTS

1

PROTEIN EXTRACTION AND PURIFICATION

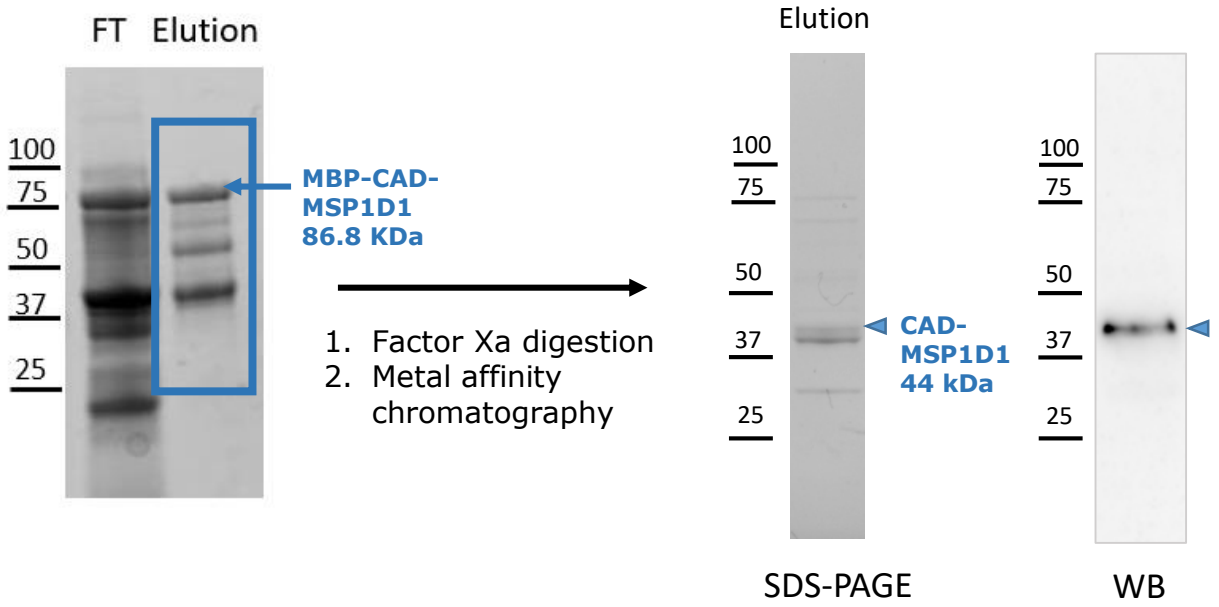


X Limited purity & protein yield

RESULTS

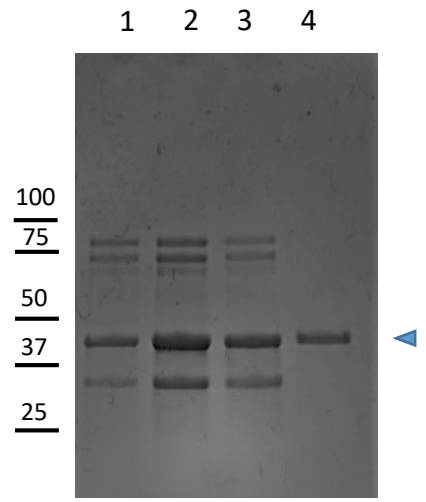
1

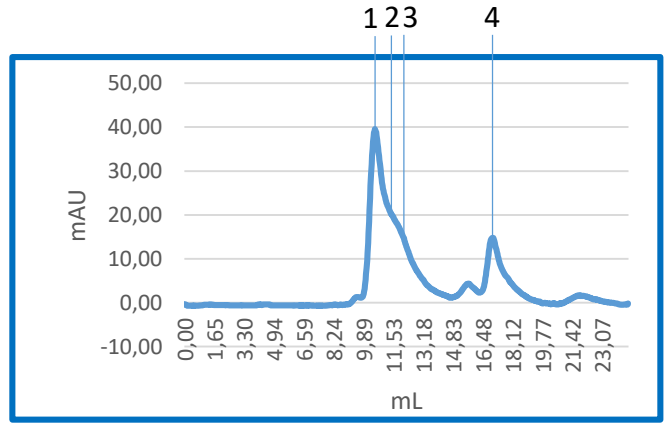
PROTEIN EXTRACTION AND PURIFICATION



X Limited purity & protein yield

NANODISC RECONSTITUTION

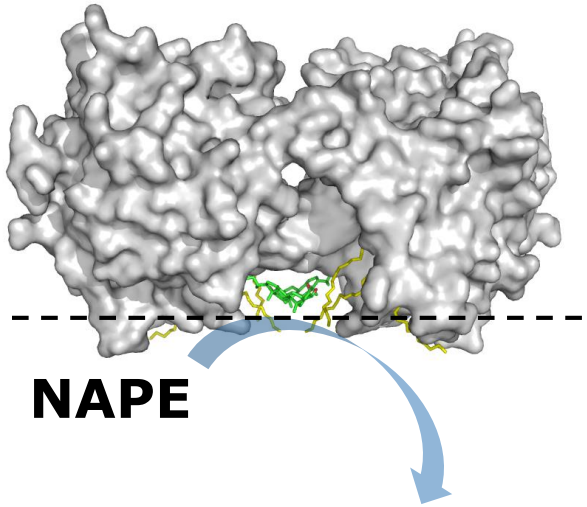


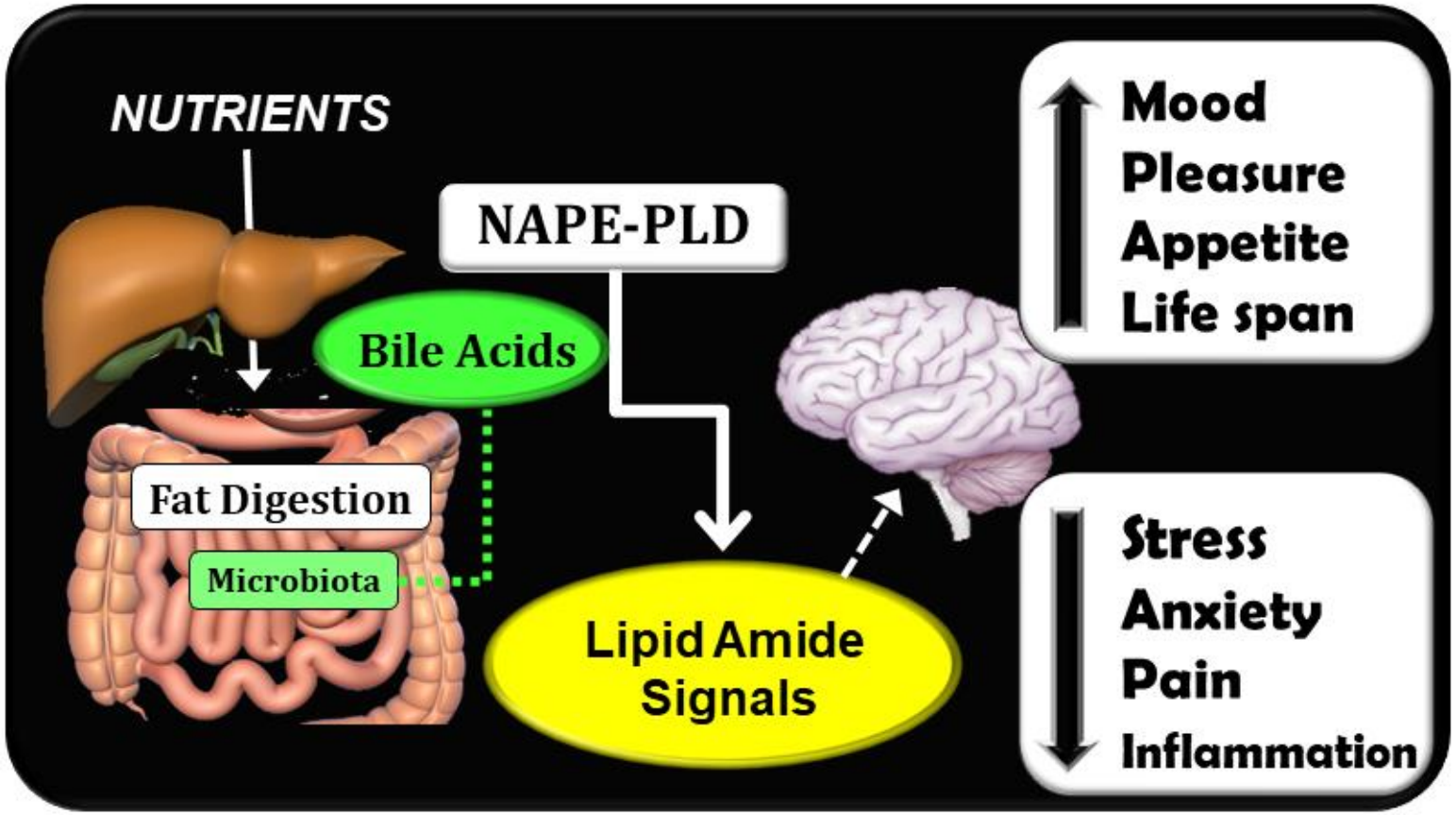


GFSEC NDs Reconstitution

NAPE-PLD: OVERVIEW

2

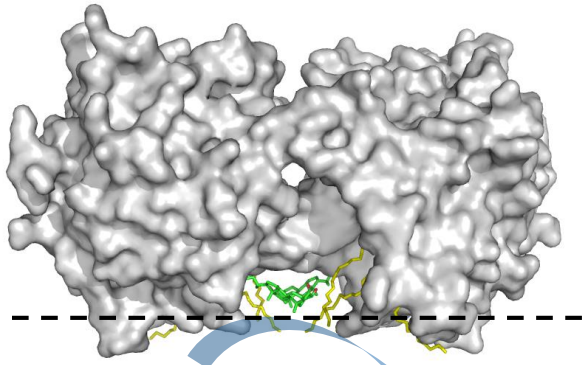




Ligand Discovery with applications in metabolic and eating disorders

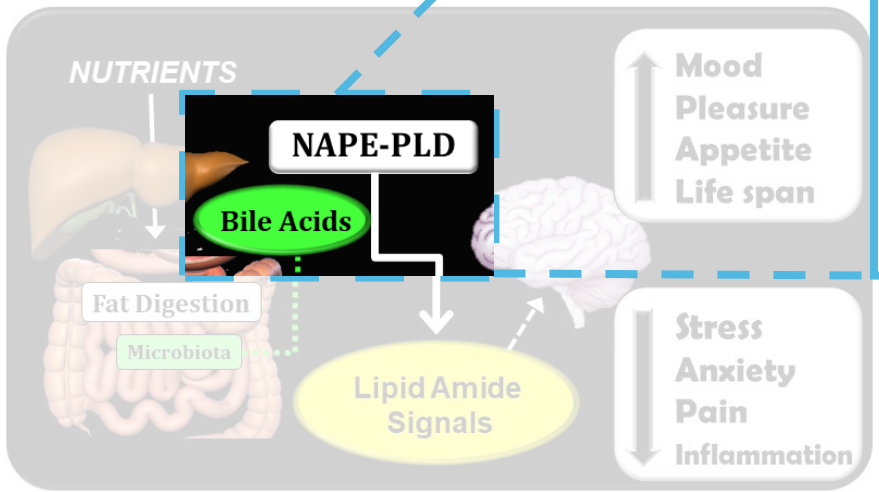
NAPE-PLD: OVERVIEW

2



NAPE

Lipid signaling amides



nature REVIEWS ENDOCRINOLOGY

Bile acids in glucose metabolism and insulin signalling — mechanisms and research needs

*Tiara R. Ahmad[1,2] and Rebecca A. Haeusler[1,2]**

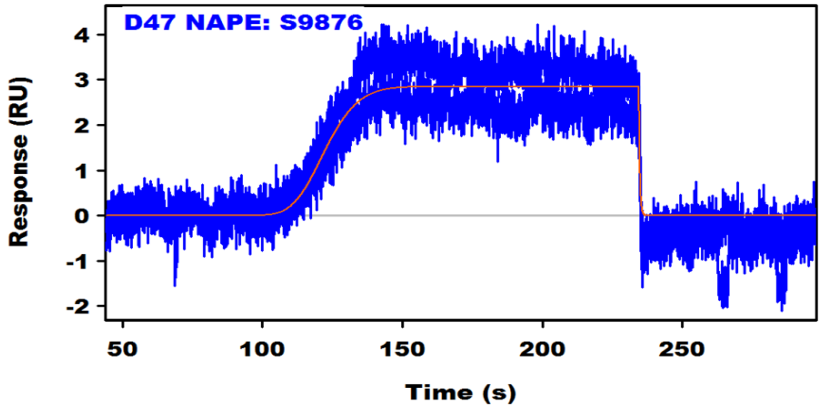
Abstract | Of all the novel glucoregulatory molecules discovered in the past 20 years, bile acids (BAs) are notable for the fact that they were hiding in plain sight. BAs were well known for their requirement in dietary lipid absorption and biliary cholesterol secretion, due to their micelle-forming properties. However, it was not until 1999 that BAs were discovered to be endogenous ligands for the nuclear receptor FXR. Since that time, BAs have been shown to act through multiple receptors (PXR, VDR, TGR5 and S1PR2), as well as to have receptor-independent mechanisms (membrane dynamics, allosteric modulation of *N*-acyl phosphatidylethanolamine phospholipase D). We now also have an appreciation of the range of physiological, pathophysiological and therapeutic conditions in which endogenous BAs are altered, raising the possibility that BAs contribute to the effects of these conditions on glycaemia. In this Review, we highlight the mechanisms by which BAs regulate glucose homeostasis and the settings in which endogenous BAs are altered, and provide suggestions for future research.

Ligand Discovery with applications in metabolic and eating disorders

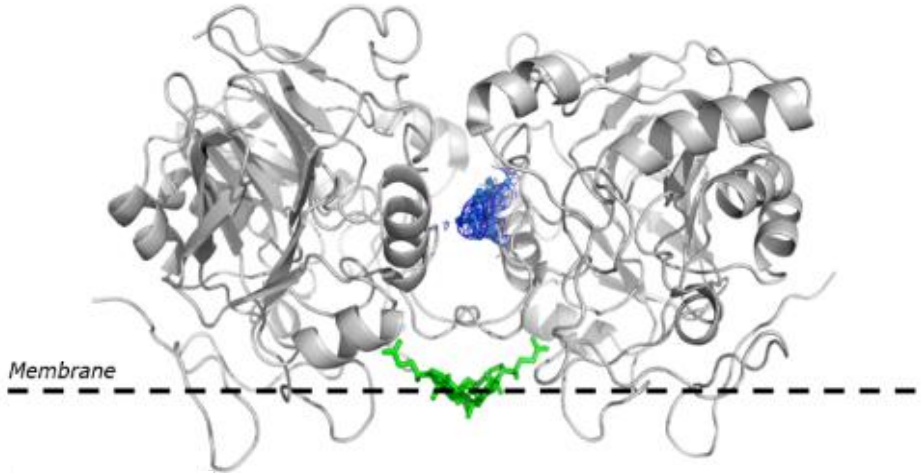
METHODS

2

- Ligands screening by SPR



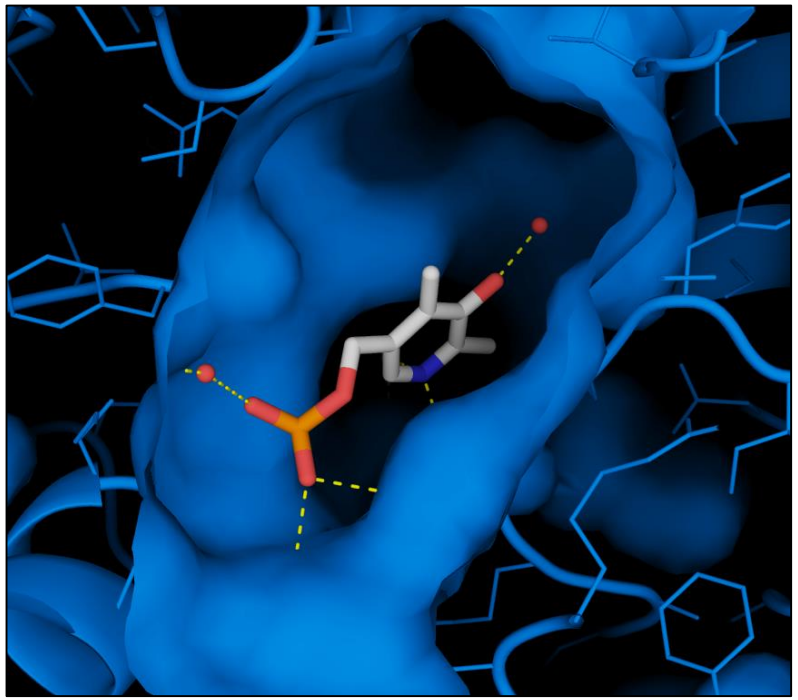
- Interactions at atomic resolution by X-ray diffraction

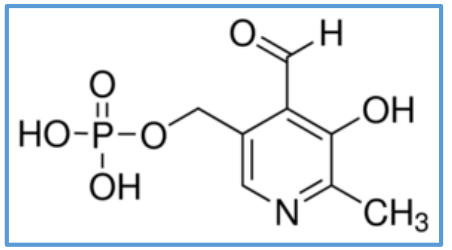


RESULTS

2

Structure of NAPE-PLD in complex with PLP





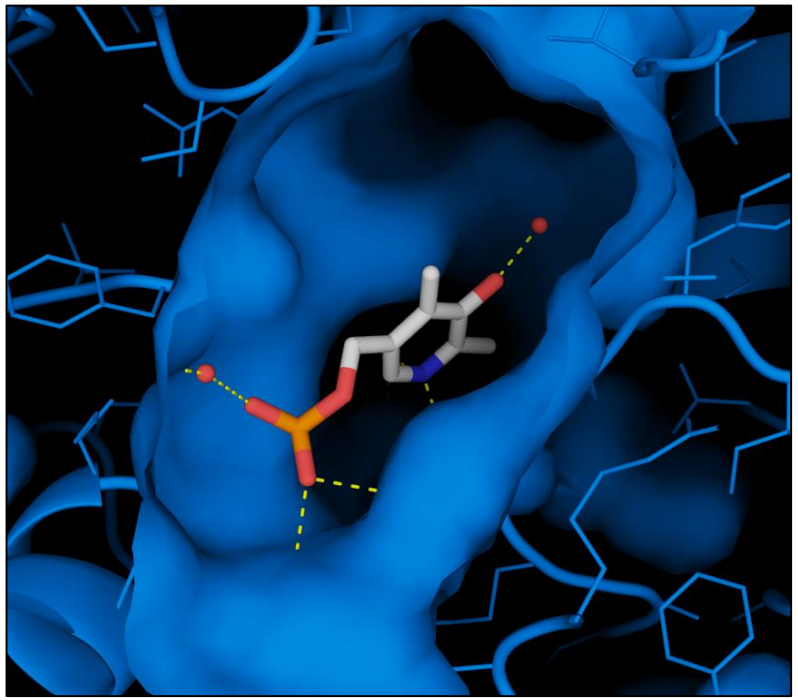
Pyridoxal-5’-phosphate

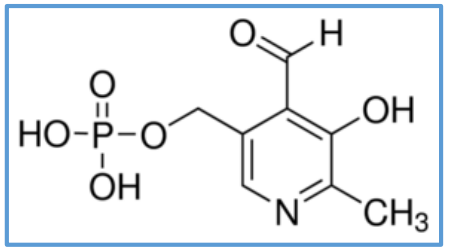
- Active form of Vitamin B6
- Coenzyme in different cellular pathways
- Involved in **neurotransmitters generation**

RESULTS

2

Structure of NAPE-PLD in complex with PLP





Pyridoxal-5'-phosphate

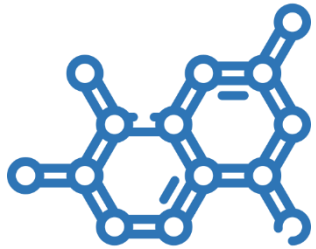
- Active form of Vitamin B6
- Coenzyme in different cellular pathways
- Involved in **neurotransmitters generation**

NAPE-PLD at the cross-talk between Lipid Amides and Bile Acid signaling

➡ **Hypothesis: modulation of neurotransmission**

2 WORKPLAN

VALIDATION



Design and synthesis of PLP analogues

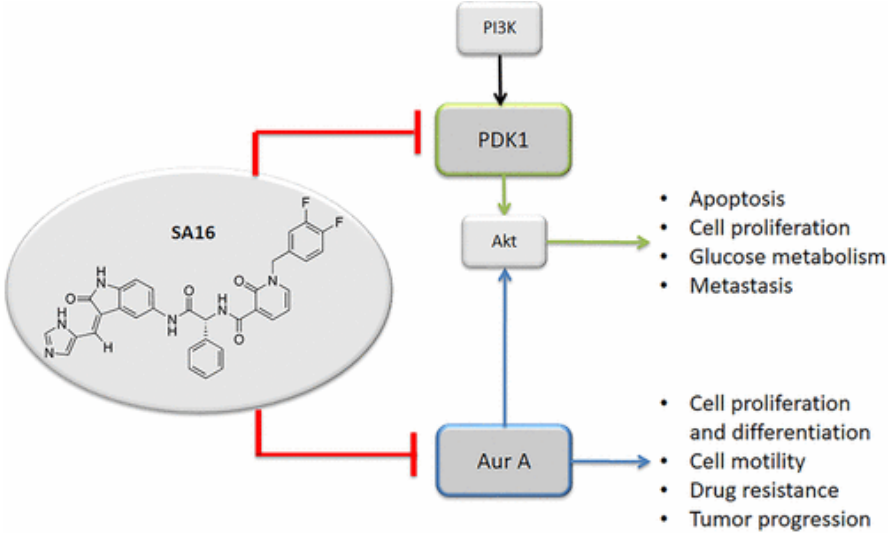
Prof. Simona Rapposelli
University of Pisa
Department of Pharmacy

***In vitro* studies** (binding & bioassays in living cells)

***In vivo* studies**

Structural, Functional & Physiological basis

AurA PROJECT



ACS Chemical Neuroscience

Article

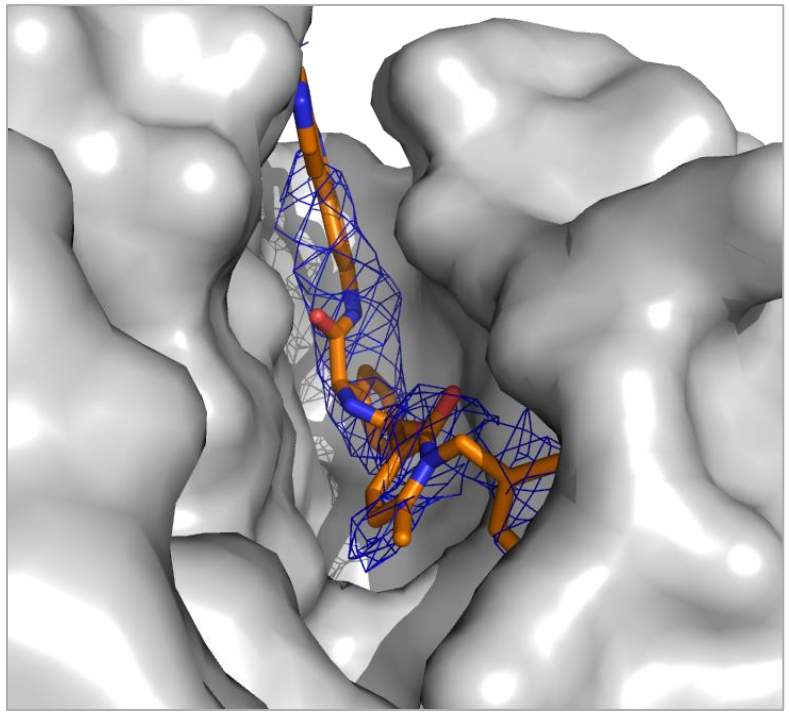
Dual inhibition of PDK1 and Aurora Kinase A: an effective strategy to induce differentiation and apoptosis of human glioblastoma multiforme stem cells

Simona Daniele, Simona Sestito, Deborah Pietrobono, Chiara Giacomelli, Grazia Chiellini, Danilo Di Maio, Luciana Marinelli, Ettore Novellino, Claudia Martini, and Simona Rapposelli

Glioblastoma Multiforme resistance & recurrence

Prof. Simona Rapposelli
University of Pisa
Department of Pharmacy

Crystal structure of AurA in complex with the lead compound VI8



(Manuscript in preparation)