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RELAZIONE ATTIVITA' ANNUALE DEI PERFEZIONANDI/DOTTORANDI – TERZO/QUARTO ANNO
REPORT ON THE PHD ACTIVITY – THIRD/FORTH YEAR

NOME E COGNOME NAME AND SURNAME	Sara Chiarugi
DISCIPLINA PHD COURSE	Nanosciences (CNI)

CORSI FREQUENTATI CON SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (WITH FINAL EXAM)	**VOTAZIONE RIPORTATA MARK**	**NUMERO DI ORE HOURS**
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CORSI FREQUENTATI SENZA SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (ATTENDANCE ONLY)	**NUMERO DI ORE HOURS**
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ALTRE ATTIVITÀ FORMATIVE (SEMINARI, WORKSHOP, SCUOLE ESTIVE, ECC.) – DESCRIZIONE OTHER PHD ORIENTED ACTIVITIES (SEMINARS, WORKSHOPS, SUMMER SCHOOLS, ETC) – DESCRIPTION	**NUMERO DI ORE HOURS**
23/10/2019 IIT Seminar – Dr. Sacha De Carlo – "The current state of Micro-Electron Diffraction"	2
12/12/2019 Conference - Accelerating the Drug Discovery Process	9
24/02/2020 IIT Seminar – Dr. Eleonora Margheritis – "AlleyCat: an example of protein design"	2
05/05/2020 Nature Research on line seminar – "Structure-guided design of SARS-CoV-2 antivirals"	2
22-25/09/2020 Online School - GeCry School: "From Gene to Protein Crystal Structure"	34
29/09/2020 Giovani Cristallografi Italiani online event - GCI@HOME BioCrystallography section. Oral presentation: "Insights into human membrane NAPE-PLD interactions"	4

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ATTIVITÀ DI RICERCA SVOLTA (MAX. 8.000 CARATTERI)*
RESEARCH ACTIVITY (MAX. 8000 CHARACTERS)

SMALL-MOLECULE MODULATION OF HUMAN NAPE-PLD SIGNALING

My PhD research project focuses on the structural and functional investigation of the human protein target NAPE-PLD (N-acylphosphatidylethanolamine phospholipase D) by small molecule modulators for target validation. NAPE-PLD is a membrane-associated zinc enzyme that generates from membrane precursors a series of key lipid signaling molecules (fatty acid ethanolamides, FAE), which promote essential neurological, cytoprotective, and metabolic actions, including motivation and reward, mood regulation, appetite, cognitive function, lipid and glucose metabolism[1-4]. Among these, the endogenous cannabinoid anandamide has potent anxiolytic and analgesic actions, enhances mood, stimulates appetite and brain synaptogenesis. Modulation of NAPE-PLD can have potential application in several metabolic and neurological disorders[1].

To understand how NAPE-PLD works and the molecular bases of FAE biosynthesis, recently the group of Dr. Gianpiero Garau (Biostructures Lab@NEST) has solved the crystal structure of human membrane NAPE-PLD at resolution of 2.6 Å[1]. X-ray diffraction analysis and molecular biophysics studies have revealed not only the mechanism of lipid amide biogenesis, but unexpectedly specific binding sites for bile acids, the natural detergents secreted by the liver to aid the digestion of dietary fats in the intestine. The membrane dimeric enzyme is turned on by the interaction with bile acids at physiological concentrations (µM range), and the bound bile acid molecules act as structural cofactors at membrane interface[2]. This unexpected important discovery brings together bile acid physiology and lipid amide signaling, linking major players in lipid homeostasis with major players in lipid signaling[5]. The unique molecular architecture of NAPE-PLD offers two obvious targets for pharmacological modulation, the catalytic active site (containing a dimeric zinc center) and the allosteric bile acid-binding site (Figure 1).

During the previous year, my collaborators and I have discovered different classes of ligands that can bind and inhibit NAPE-PLD in the low micromolar range by surface plasmon resonance. Last year I successfully obtained the crystal structures of the membrane enzyme in complex with the ligand naphthalene trisulfonic acid (N3SA), with the inhibitor compound ARN4707[6], and with the compound pyridoxal-5'-phosphate (PLP), the metabolically active form of Vitamin B6 (Figure 1). These are the first structures of NAPE-PLD in the presence of chemical modulators so far obtained. We are currently drafting a first manuscript on the first two structural complexes.

Surprisingly, the crystal structure of the complex with ARN4707 unveils that the class of inhibitors bearing a quinazoline sulfonamide scaffold binds at the dimer internal channel of the enzyme, and not at the enzyme active site as previously shown by computational investigations[6]. Overall, the three crystal structures demonstrate that the dimer internal channel of the membrane enzyme is indeed a key interaction site for NAPE-PLD modulation.

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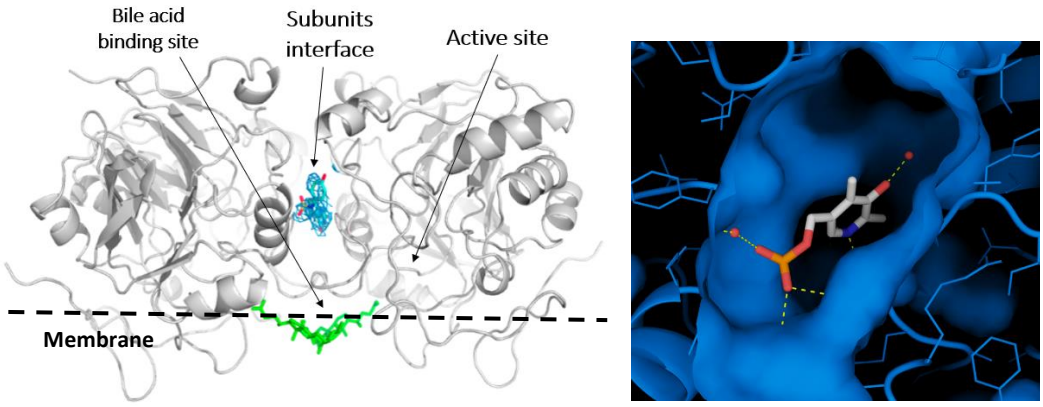

Figure 1. Crystal structure of human membrane NAPE-PLD in complex with ARN 4707 (left). The bile acid molecules (atom in green) bind to the enzyme, creating a lipid substructure at membrane interface, while the ligand binds to the enzyme at the subunits interface. The inset on the right shows the PLP molecule and its interactions with the enzyme.
(right).

Pyridoxal- 5'-phosphate (PLP), the active form of Vitamin B6, functions as a coenzyme in many enzymatic processes[7]. PLP-dependent enzymes are involved in many key cellular processes and metabolism and PLP is required for the synthesis of the neurotransmitters serotonin, norepinephrine, epinephrine, and γ-aminobutyric acid (GABA). **The discovery of the interaction of PLP with NAPE-PLD using different structural biophysics approaches** suggests a captivating physiological scenario where the process of lipid amide biogenesis in cells of different tissues (e.g. brain, heart, intestine, liver and immune system) might be linked directly to (i) the biosynthesis of neurotransmitters, and (ii) macronutrient metabolism (i.e. lipid metabolism and gene expression), where Vitamin B6 is well known to have a key role.
We need now to investigate:

1. The action of NAPE-PLD modulation in the biosynthesis of neurotransmitters.
2. The mechanism by which the PLP (or its analogues) might affects the membrane enzyme modulation in lipid metabolism and gene expression.

In order to carry on these studies, in collaboration with Prof. Simona Rapposelli (University of Pisa) we designed a series of PLP analogues. As a part of this collaboration, I have started with the chemical synthesis of the structure-based PLP-derivatives in the laboratory of Prof. Simona Rapposelli. Surface Plasmon Resonance analysis with these new ligands will be performed to assess their affinity for the NAPE-PLD. The best compounds will be used for i) LC-MS analysis to evaluate their ability to modulate the enzyme activity, and ii) further structural investigations by X-ray diffraction.
Finally, during 2020 we started a collaboration with Prof. Mario van der Stelt of the University of Leiden on a novel class of pyrimidine-derivatives that strongly inhibits NAPE-PLD in the nanomolar range (e.g. compound LEI-401), modulating animal emotional behavior[8]. Structure investigation of complexes by X-ray diffraction would shed light on the interaction the class with NAPE-PLD for their med-chem optimization and evolution.

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References:

1. Magotti P et al. 2015. Structure of human N-acylphosphatidylethanolamine-hydrolyzing phospholipase D: regulation of fatty acid ethanolamide biosynthesis by bile acids. Structure 23:598.
2. Margheritis E et al. 2016. Bile Acid Recognition by NAPE-PLD. ACS Chem Biol 11:2908.
3. Geurts L et al. 2015. Adipose tissue NAPE-PLD controls fat mass development by altering the browning process and gut microbiota. Nature Comm 6:6495.
4. Minor KM et al. 2018. Canine NAPEPLD-associated models of human myelin disorders. Scientific Rep 8:5818.
5. Ahmad TR & Haeusler RA 2019. Bile acids in glucose metabolism and insulin signalling. Nature Rev Endocrinol 15, 701-712.
6. Castellani B et al. 2017. Synthesis and characterization of the first inhibitor of N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD). Chem Comm 53(95):12814-12817.
7. Eliot A C et al. 2004. Pyridoxal phosphate enzymes: mechanistic, structural, and evolutionary considerations. Annu Rev Biochem 73:383-415
8. Mock ED et al. 2020. Discovery of a NAPE-PLD inhibitor that modulates emotional behavior in mice. Nature Chem Biol 16(6):667-675.

*se si intende sottoporre una relazione di ricerca più estesa, utilizzare il campo per una descrizione sintetica e allegare il documento in formato .pdf

If you are going to submit a longer report, please fill the box with a synthetic abstract and attach a document in pdf format

EVENTUALI PUBBLICAZIONI
PUBLICATIONS (IF AVAILABLE)

NOME DEL RELATORE
THESIS ADVISOR

Dr. Gianpiero Garau (IIT)

Prof. Gian Michele Ratto (SNS)

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DATA **DATE**	17/10/2020	**FIRMA** **SIGNATURE**