SCUOLA NORMALE SUPERIORE

RELAZIONE ATTIVITA' ANNUALE DEI PERFEZIONANDI/DOTTORANDI – PRIMO ANNO
REPORT ON THE PHD ACTIVITY – FIRST YEAR

NOME E COGNOME NAME AND SURNAME	FRANCESCO MARGHERITI
DISCIPLINA/PHD COURSE	Nanoscience

CORSI FREQUENTATI CON SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (WITH FINAL EXAM)	**VOTAZIONE RIPORTATA MARK**	**NUMERO DI ORE HOURS**
Introductory Quantum Physics	30	40
Fundamentals of Biophysics at the Nanoscale + MATLAB	29	60
Physics of the living cell	25	45

CORSI FREQUENTATI SENZA SOSTENIMENTO DI ESAME FINALE ATTENDED COURSES (ATTENDANCE ONLY)	**NUMERO DI ORE HOURS**
Ciclo di seminari – Biophysical Sciences	45

ALTRE ATTIVITÀ FORMATIVE (SEMINARI, WORKSHOP, SCUOLE ESTIVE, ECC.) – DESCRIZIONE OTHER PHD ORIENTED ACTIVITIES (SEMINARS, WORKSHOPS, SUMMER SCHOOLS, ETC) – DESCRIPTION	**NUMERO DI ORE HOURS**
Scientific Data Analysis School 2019.	27

ATTIVITÀ DI RICERCA EVENTUALMENTE SVOLTA (MAX. 3.000 CARATTERI)
RESEARCH ACTIVITY (MAX. 3000 CHARACTERS)

STRUCTURE OF MEMBRANE nanoBIOSTRUCTURES BY SINGLE PARTICLE CRYO-ELECTRON MICROSCOPY

My PhD program will be carried on in the group of Biostructures of Dr Gianpiero Garau and will be focused on the structural determination of selected membrane biostructures by advances approaches of single particle Cryo-electron Microscopy (SP cryo-EM).

Insight into biostructures at the molecular to atomic level is essential to understand how living organisms are built and work and to investigate the molecular bases of diseases for

novel therapeutic approaches. The most successful and powerful technique used to determine the structures of biomolecules remains X-ray crystallography, which requires the formation of crystals of the macromolecule of interest. This requirement has excluded the structure determination of many complexes that are either too flexible, too sensitive, or too large to form crystals. In the last few years, **cryo-Electron Microscopy of single-particle specimens** (SP Cryo-EM) has been used to determine the structure of proteins and macromolecular complexes when sample crystals useful for X-ray diffraction could not easily be formed [1]. For this purpose, the samples are plunge-frozen (in liquid ethane or propane) to ensure that the hydration of complex biological structures is maintained. The result is a collection of 2D pictures of macromolecules (or single particles) arranged in random orientations (and embedded into a layer of amorphous ice under near-native conditions), from which is possible to perform the 3D reconstruction of the cryoEM-density map, and thus of the biostructure. This technique was pioneered, developed, and refined by Jacques Dubochet (*Nobel Price in Chemistry 2017*). The recent development of a new generation of detectors capable of directly measuring incident electrons has finally revolutionized the field over the past five years and has enabled the determination of highly resolved structures of biological macromolecules [2].
In the coming years, SP cryo-EM and X-ray crystallography will be used as complementary technologies to investigate structures, interactions, and dynamics of biomacromolecules. Crystallography remains better suited to yield precise atomic coordinates of macromolecules under a few hundred kDa in size, while the ability to probe larger (> 200 kDa), potentially more disordered assemblies is a distinct advantage of cryo-EM [3].

Membrane proteins encoded by ~30% of the coding genes, play vital roles in numerous physiological processes. Membrane proteins are targets of more than half of the FDA-approved drugs and high-resolution structural studies under near-physiological conditions are required to provide an in-depth mechanistic understanding and to facilitate the discovery of innovative therapeutic approaches. In this field, different methodologies have been developed recently to support the structure determination of membrane proteins by sp cryo-EM. Among these, **lipid nanodiscs** are able to maintain a lipid-like environment for membrane proteins and facilitate their structural and functional studies [4], while **nanobodies** have been developed and used to stabilize protein complexes and lock specific conformational states [5].

The objectives of my PhD program are:

1. **Acquire knowledge for images acquisition, data processing and 3D reconstruction of membrane proteins by SP cryo-EM**. This objective will be achieved using a test model structure, known in the lab (human membrane protein NAPE-PLD).

2. **Determine the structure of the membrane receptor GPR119 by SP cryo-EM**, using nanodisc or nanobody technology, at the cutting edge in the field.

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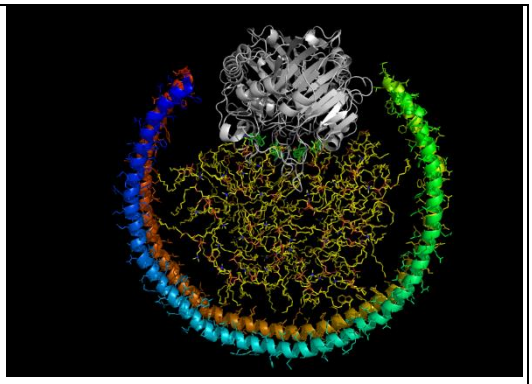

Starting from Summer 2020, I have initiated my PhD training in the Biostructures Lab of Dr Garau getting knowledge of basic procedures for the production at high yields and characterization of membrane protein targets, a requirement for following structural and functional studies by SP Cryo-EM.

(1) I will use samples of the human membrane protein NAPE-PLD in order to acquire the required **SP cryo-EM methodology** for (i) the nanodisk technology; (ii) data acquisition and images processing for the structural determination of membrane protein targets.
This work will also provide an in-depth advance in the field of lipid signalling mediated by NAPE-PLD, unveiling its association mode to neuronal lipidic membranes.

(2) The **human membrane receptor GPR119** belongs to the G protein-coupled receptor family and exhibits dual modes of action upon ligand-dependent activation: pancreatic secretion of insulin in a glucose-dependent manner and intestinal secretion of incretins. Hence, GPR119 has emerged as a promising target for treating obesity and type 2 diabetes mellitus, without causing dramatic hypoglycaemic events. However, despite continuous efforts by many major pharmaceutical companies, no structural characterization of GPR119 has been so far obtained. The final goal of my PhD project will be solving the molecular structure of GPR119 by SP Cryo-EM and provide structural and functional investigation of its role in diseases. Selective ligands against GPR119 have application for the treatment of obesity, type-2 diabetes and metabolic diseases.

[1] S. Raunser, “Cryo-EM Revolutionizes the Structure Determination of Biomolecules,” *Angew. Chemie Int. Ed.*, vol. 56, no. 52, pp. 16450–16452, Dec. 2017, doi: 10.1002/anie.201710679.

[2] Y. Cheng, N. Grigorieff, P. A. Penczek, and T. Walz, “A Primer to Single-Particle Cryo-Electron Microscopy,” *Cell*, vol. 161, no. 3, pp. 438–449, 2015, doi: https://doi.org/10.1016/j.cell.2015.03.050.

[3] S. C. Shoemaker and N. Ando, “X-rays in the Cryo-Electron Microscopy Era: Structural Biology’s Dynamic Future.,” *Biochemistry*, vol. 57, no. 3, pp. 277–285, Jan. 2018, doi: 10.1021/acs.biochem.7b01031.

[4] H. E. Autzen, D. Julius, and Y. Cheng, “Membrane mimetic systems in CryoEM: keeping membrane proteins in their native environment,” *Curr. Opin. Struct. Biol.*, vol. 58, pp. 259–268, 2019, doi: https://doi.org/10.1016/j.sbi.2019.05.022.

[5] T. Uchański, E. Pardon, and J. Steyaert, “Nanobodies to study protein conformational states,” *Curr. Opin. Struct. Biol.*, vol. 60, pp. 117–123, 2020, doi: https://doi.org/10.1016/j.sbi.2020.01.003.

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EVENTUALI PUBBLICAZIONI **PUBLICATIONS (IF AVAILABLE)**

DATA **DATE**	18/10/2020	**FIRMA** **SIGNATURE**