Marina CASIRAGHI – CV

In the course of my training I have acquired multidisciplinary experience in biochemistry, molecular biology and structural biology, together with extensive expertise in a range of biophysical techniques including solution state nuclear magnetic resonance (NMR), Electron Paramagnetic Resonance (EPR) spectroscopy and single molecule fluorescence resonance energy transfer (smFRET). Being interested in protein biochemistry since my undergraduate degree, I conducted a four-months internship in the Laboratory of Paolo Tortora at the Department of Biotechnologies of University of Milano Bicocca, Italy. My project focused on ataxin-3, a soluble protein that in its aggregated form is responsible for the neurodegenerative disorder spinocerebellar ataxia type 3. During this internship, I learnt *E. coli* expression,protein purification, and general biochemistry methods for protein characterization. I got fascinated by membrane proteins during my MS research project, which took place during my student exchange year in Paris. The project, under the supervision of Bruno Miroux at the IBPC (Institute of Physico-Chemical Biology), consisted in the structural characterization of mitochondrial uncoupling proteins (UCPs). My goal was to find a strategy to target UCPs to the *E. coli* inner membrane for heterologous expression, facilitating further structural studies. With the tools I developed I was able to contribute to a review on membrane proteins expression protocols. I started to work on G-protein coupled receptors (GPCRs) during my Ph. D. at the IBPC under the supervision of Laurent Catoire. My goal was to optimize a strategy for GPCR expression and purification from *E. coli* for solution state NMR studies. The interest in *E. coli* as expression host is the possibility to fully deuterate the receptor, a key advantage for our NMR studies focusing on GPCR dynamics. I identified conditions to efficiently fold *in vitro* the GPCR purified from *E. coli* and I worked on a protocol to successfully insert the receptor into high-density lipoprotein (HDL) particles with varying lipid compositions. This strategydelivered fully active and functional GPCRs in HDLs and thank to a resolution never obtained in the field we were able to investigate GPCR conformational landscape and its modulation by ligands and lipids. This work has produced three first author publications and other peer-review scientific articles, and the protocol has been used to address complex biological questions with other biochemical techniques. For my postdoctoral studies, I was awarded a Marie Curie Individual Fellowship to join the laboratory of Brian Kobilka at Stanford University. At Stanford, I tackled two challenging open questions in the field: the molecular determinants of G protein specificity, as GPCRs can bind different G protein subtypes, and bias signaling, the ability of agonists to preferentially select a specific signaling pathway. To study these mechanisms, I focused on the beta2-adrenergic receptor (β_2AR), a well-characterized GPCR. My work has led to the identification of a bias agonist specific for Gi activation, that we have used to recently obtain the cryo-EM structure of a β_2AR-Gi complex. The structure elucidates the activation mechanism of β_2ARwhen bound to its inhibitory protein Gi. Further spectroscopic investigations show that bias agonist stabilizes a specific receptor conformation, that leads to the preferential binding of Gi. This work helps our understanding of bias agonism and the receptor conformations involved in G protein selectivity, which are important for the development of more specific drugs to target GPCRs. The experience at Kobilka lab has been an excellent training in biochemistry, pharmacology and structural biology, and contributed to develop my scientific thinking and vision. My career objective is to become and independent scientist. I am passionate about studying the relationship between the structure and function of membrane proteins. My goal is to apply my expertise in biochemistry,

structural biology and spectroscopy to continue studying the complex and perfectly integrated network of interactions between the extra-cellular stimuli, the membrane, and the intracellular partners, that altogether determine spatially and temporally the function of membrane proteins. During my PhD and my postdoc I have supervised students and fellow postdocs, and I am engaged in training and inspiring the young generations of scientists.

In 2023, I was the recipient of the Next Generation EU grant Young Researchers, and I moved to the University of Milano Statale in Milan to start my own line of research on GPCRs. My lab will focus on two main research directions i) GPCRs involved in obesity and metabolic diseases ii) addiction and nociception. The lab will use a combination of structural biology and biophysical techniques (fluorescence and single-molecule FRET, solution-state NMR) to understand GPCR signaling. This knowledge will be harnessed to design new small molecules to pharmaceutically target the receptors