Vito Genna:

Research Experience

My interest in computational chemistry applied to the 'nucleic acids-driven' cancer research began in 2011 when I joined – thanks to the *"Erasmus Placement" fellowship* – the group of Prof. Gervasio at the Dept. of Structural and Computational Biology located at *Cnio Centro Nacional De Investigaciones Oncológicas*, Madrid (Spain). In Gervasio's group I was exposed to molecular dynamics (MD) simulations techniques, which allowed me to perform a structural investigation of HIV (type-I/II) reverse transcriptase for the discovery of new anti-viral drugs to treat HIV infections in patients with cancer. Results of research performed during this period are part of my Bachelor Thesis. During my Master in molecular biology, I have started a collaboration with the Molecular Modeling group, held by Prof. Giacomo Cao, at *Centro Ricerche Superiori Sardegna* (CRS4). In that period, I characterized the interactions between type-II human leukocyte antigen in complex with different peptides which have shown to modulate multiple sclerosis susceptibility. Results have been published in Molecular Biosystems Journal and the work has been also selected as Cover Article. Notably, despite my period abroad and the almost full-time collaboration with CRS4, I concluded my Master in 14 months with highest marks and, for this reason, the University of Cagliari awarded me as *Best Master Student of the Year* (2013).

Few days after my Master graduation, I joined the group of Prof. Dal Peraro at the Bioengineering Dept. of the *Swiss Federal Institute of Technology Lausanne (EPFL),* where I further consolidated my computational skills in statistical mechanics and enhanced sampling techniques such as well-tempered and steered MD. In the group of Prof. Dal Peraro, I began a project focused on the two-metal-aided catalysis performed by Human DNA Polymerase-η (Pol-η), an enzyme that bypasses DNA lesions ensuring correct genetic inheritance. Interestingly, in that period, Human DNA Pol-η was found aberrantly expressed in *melanoma* cells, hence the discovery of a potent Pol-η inhibitor was a challenge of great scientific interest. For this reason, in late 2013, I moved in the group of Dr. De Vivo at Dept. of Drug Discovery and Development of the Fondazione Istituto Italiano di Tecnologia (IIT) where I started my PhD in the field enzyme-mediated nucleic acids (NA) editing. Thanks to the collaboration between IIT and EPFL results of my research were published on *Nucl. Acid Res.*, *J. Am. Chem. Soc.*, *Nat. Rev*., *Structure* and *ACS Catalysis,* to name few. Once having described the physical properties of Pol-η, I decided to investigate the reaction mechanism operated by Pol-η to chemically extend damaged DNA. To do so, in 2015 – thanks to the "Marco Polo" fellowship for Excellence in Science – I moved in the group of Prof. Carloni, director of

the Institute of Advanced Simulation at *Forschungszentrum Jülich*, Germany. The group of Prof. Carloni is one of the most important players in the field Car-Parrinello quantum-mechanics/molecular-mechanics (CP QM/MM) simulations, an expensive computational approach which allows, differently to classical simulations, the detailed investigation of the breaking/forming chemical bonds involved in reaction mechanisms. To run CP QM/MM simulations I have designed and been awarded with two important grants (2014 and 2015) funded by the *John von Neumann Institute for Computing* (Germany). By means of CP QM/MM simulations we were able to dissect key features for the design of a potent and selective inhibitor of Human Pol-η. Also, our research led to the discovery of a unifying mechanism that drives the Pol-mediated polymerization of NA. Importantly, thanks to extensive bioinformatics analysis we have also demonstrated that such a mechanism is shared across each form of life; a breakthrough discovery in modern biochemistry. These results were recently published in two *J. Am. Chem. Soc.* with me as a first author. The works provoked a large echo in the scientific community and have been selected by *J. Am. Chem. Soc.* editors as Cover articles. Moreover, papers have also been highlighted in JACS spotlights. Thanks to these investigations, we gained precious insights on Pol-η catalytic activity and all this knowledge was used to begin a drug discovery project (Pol-η inhibitor) which, at the time of this proposal, is reached the *in-cellulo* stage at IIT. Concomitantly, at IIT, I started a project on the dissection of self-splicing group-II intron (a RNA machinery) in collaboration with Dr. Marcia at EMBL (Grenoble). We found that a common structural motif – critical for catalysis – features the active site of different biomolecule families spanning from RNAzymes, DNAzymes, Polymerases, Ribonucleases to Spliceosome. So, regardless the evolutionary path such different biomolecules have followed, all of them show a binuclear active site were strategically located positively and negatively charged residues perform both hydrolysis and formation of DNA/RNA. On the basis of this discoveries, a novel anticancer strategy might be initiated to efficiently arrest/enhance key NA-editing enzymes with prominent roles in cancer onset, growth and spread.

So, on the basis of this extensive expertise accumulated in nucleic acid properties and editing, in late 2017 I felt that the natural trajectory of my scientific career would have passed through the field of synthetic biology. There, bioengineering of native DNA and RNA would have allowed me to create new and programmable genome-made technologies (XNAzymes) to perform tasks such as the specific hydrolysis of foreign genomes belonging to viruses (e.g. SARS-CoV-2, Ebola, HCV) or endogenous and overexpressed human long-non-coding RNAs (lncRNA) with established roles in cancer

onset. To do so, I have conceived a research project and been awarded by the *European Molecular Biology Organization* (EMBO – Excellence in Life Sciences, Long-Term Fellowship) to degrade the carcinogenic MALAT1 lncRNA by means of XNAzyme. This challenge has been accepted by Prof. Orozco with a great enthusiasm leading us to an unceasing dissection of the physicochemical properties of XNAzyme and MALAT1 by both theoretical and experimental approaches. Our efforts resulted in two key discoveries: *i)* the A-rich tract that generates a triple helix in MALAT1, represents our target region to start the essential unfolding propedeutic to XNAzyme hybridization and subsequent MALAT1 cleavage and *ii)* two minimum structural requirements of XNAzyme (to be active against MALAT1) represented by an extra ribo-polyT portion and a strategically-poised adenine which, along with its surrounding environment, creates a peculiar motif that hosts the two catalytically-active Mg^{2+} ions mediating nuclease activity. Results were recently published in Chem (Cell Press) with me as a co-first author and another manuscript on the triplex hybrids properties has been submitted for publication. Moreover, by fruitfully collaborating with the group of Dr. Philip Holliger (MRC Laboratories, Cambridge UK) we have performed a set of investigations on some engineered Pols to biologically produce XNAs on DNA templates. We succeeded, and for the first time stereo-specific phosphonated-DNA has been synthetized on native DNA-template. Results where very recently published in Nature Chemistry. Collaboration is still ongoing and different projects have been started in the field of xeno-nucleic acids. My researches were also presented (posters or seminar) at several international conferences (CECAM, EMBO, Gordon Conferences) on the topic.

In the time required to accomplish the first stage of this project I have profoundly extended my knowledge of biophysics approaches for the *ad-hoc* and *de-novo* design of the XNAzyme. So, I have maturated a complete and independent professional maturity along with a more complete overview of all the modern and relevant issues in the field. This has generated the opportunity of applying for medium-term position in established institutes as junior group leader due to the high-level of expertise in one of the application field treated in this proposal. The knowledge and skills created by this project is suitable for setting up a new laboratory in institutes with an already established division of biochemistry or structural biology. The distinct topics of my first-author articles, published and highlighted in top-rate scientific journals, reflect my capacity of acquiring new knowledge and think independently and critically; essential skills for the good implementation of the proposed project. Therefore La-Caixa Fellowship represents a great opportunity for me to boost my career at the cutting-

edge of the nucleic-acids-based pharmaceutical field. Also, I am confident that the expertise of the group of Prof. Modesto Orozco in integrative modeling of NA coupled to the great opportunity to be constantly surrounded by experimentalists can be integrated with my competences in enzyme-mediated NA editing and enthusiasm for understanding XNAzymes catalysis and think transversally to find a new and accurate anticancer strategy to treat solid state tumors.

In Pills:

- Research Experience at EPFL Lausanne (Switzerland), Bioengineering Department **2013**
- Research Experience at FZJ Jülich (Germany), Advanced Simulations **2016**
- PhD in Computational Biochemistry in **2017**. University of Bologna, Italy jointly with the Italian Institute of Technology (IIT)
- EMBO (Excellence in Life-Sciences) Long-Term Fellowship to work on Synthetic and Catalytic Nucleic Acids, IRB Barcelona (Orozco's Lab) **2018**
- Gran from Spanish Minister of Health to face COVID19 pandemic. Topic: SARS-CoV-2 host selection mechanism and next move prediction. **2019**
- Established a collaboration with Top-player Wet Experimentalists on SARS-CoV-2 from San Raffaele Milano (Italy) **2020**
- Advanced *Juan De La Cierva* Fellowship for scientific and academic merits. **2019**