Project 5

Name/title of the PhD course	**Chemical Sciences**
Name of the PhD coordinator	Prof. Angelina Lombardi
Name/Title of the PhD project	***Peptides for microbiota modulation***
Department of reference	Department of Chemical Sciences, University of Naples Federico II, Naples, Italy. http://www.scienzechimiche.unina.it/home)
Working conditions, research team, infrastructures, equipment	The project will be carried out at the Department of Chemical Sciences which hosts about 100 researchers and 20 units of technicians and administrative personnel. The main location of the Department is in the campus of Monte S. Angelo. The research activities cover several areas of chemistry, spanning from the design and synthesis of new molecules, from small molecules to macromolecules, the purification and characterization of natural and synthetic molecules, the structural characterization through X-ray diffraction, nuclear magnetic resonance, optical and spin electron spectroscopy techniques, mass spectroscopy. The research team where the recruited PhD will work with is currently composed of 3 full professors, 4 associate professors, 7 research associates, 11 PhD students. The team has been organized to encompass research units with outstanding and worldwide experience in different and not overlapping research areas, making them highly qualified in their specific role. The team provides a top-notch scientific environment, which combine interdisciplinary competencies in peptide and protein chemistry, biochemistry, physical chemistry, cell biology, synthetic biology, bioengineering, microbiology, computation and protein engineering. The main projects the team works with focus on: design of peptides and peptidomimetics as drugs; development of innovative antimicrobials for biotechnological applications; structural and thermodynamic characterization of biological model membranes and their interactions with peptides and proteins; characterization of nanosystems, nanoparticles and nanostructured materials; formulation and characterization of colloidal systems; development of artificial metalloenzymes.
Scientific context	The human microbiota inhabits in homeostatic balance with the host. The microbial communities growing on or in human body produce small bioactive molecules or peptides that interact with other bacteria to inhibit/modify their growth and colonization, or with the host to modulate the host immune response Imbalances in the relative abundance of certain microbes in the microbiota may cause changes in the host health status. Prebiotics are frequently used to encourage the repopulation of a healthy microbiota, thus restoring the host health. An alternative to this is the use of peptides, which play key roles in the microbiome landscape. The host body biochemistry uses antimicrobial peptides (AMPs) to modulate the microbiota, by favoring microbes that would help in re-establishing homeostasis. These peptides are expressed either by the host metabolism or the interacting microbiota. The host body naturally produces specific peptides (lactoferrin bactericidal/permeability- increasing protein, cathelicidins and defensins), that suppress the growth of pathogenic microbes. There is increasing evidence that gut neuropeptides are one the axes of communication between the gut microbiome and host and they might also be playing a role as antimicrobial agents. Gut neuropeptides are structurally like regular antimicrobial peptides: they are small (<10kDa), cationic and amphipathic molecules and have similarities with other AMPs in their mode of action. Several studies on gut neuropeptides, such as NPY, SP, melanocyte stimulating hormone (MSH), vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and adrenomedullin (AM), suggested that they have an important role in the regulation of the gut microbiota composition Indeed, even though they usually act as neurotransmitters, they have recently been shown to serve as antimicrobial agents. For these behaviors, NPs look like promising therapeutic tools to modulate microbiota. However, detailed knowledge of the antimicrobial actions by natural NPs, their effects on the immune system and their activity in the presence of other host defense molecules need to be acquired. The antimicrobial activity of gut neuropeptides has mainly been tested against pathogenic bacteria. Although of high relevance, it is necessary to study antimicrobial activity of gut neuropeptides on commensal bacteria. Not only inflammation can be caused by colonization of pathogens but also certain strains of commensal bacteria, such as *E.coli*, can trigger the production of cytokines and induce an inflammatory state in the gut that can translate to the brain. Therefore, deep investigation on the role of these peptides as antimicrobial agents should be investigated to understand their potential impact on the host health. The common structural features of the host defense peptides and antimicrobial gut neuropeptides suggest that they exert the function through the same mechanisms of action. These include membrane disruption, interference with cell division, and or disruption of ATP synthesis. Furthermore, gut neuropeptides have an additional capacity of interacting with the neuro- and immune system which ultimately causes the release of other molecules with antimicrobial activity. This combined action raises a great potential for gut neuropeptides to be used with therapeutic purposes to treat diseases associated with microbiota alterations, especially considering the increasing resistance to conventional antibiotics.
Project Research plan	The final goal of the project is the development of NPs active in microbiota modulation. It is planned to first clarify the mechanism of action of natural NPs (as Substance P; Neurokinins; neuropeptide Y, NPY) on the microbiota, and then to design modified peptides, with improved activity, in serum stability and reduced toxicity. In details, biophysical studies of the interaction of NPs with model membranes (liposomes and supported bilayer) mimicking both pathogens and commensal bacteria, will be performed. The binding of the peptides to the model membrane systems, changes in membrane structure and dynamics will be analyzed, in combination with *in vitro* and *in vivo* analysis of microbe cell growth. Experimental data, derived from the initial selected peptides, will be entered into rounds of molecular modeling, which in turn will generate new targets. Computational tools will be essential to ascertain, at molecular level, the factors responsible

	for the observed activity and to find solution to reshape and guide redesign of potential therapeutic agents. To reach the final goal, the project is organized into four specific Work Packages: *WP1. Structural and functional characterization of natural NPs.* *WP2. Design and synthesis of modified NPs.* *WP3. Structural and functional characterization of designed NPs* *WP4. Analysis of NPs and AMPs activity on gut and skin microbiome*
Research and Training Innovative aspects	The development of synthetic peptide for microbiota modulation is a challenging and timely field of research. Although NPs/AMPs are promising alternatives to prebiotics, several questions prevent their use, including low stability, toxicity, and bacterial resistance. The lack of detailed information on their mechanism of action further limits their potential applications. The rational design of AMPs/NPs can help solving these issues and open the way to their use as new therapeutic tools. In particular, the identification of peptides able to modulate gut or skin microbiota might have great therapeutic potentialities. Indeed, it is increasingly emerging that advancing our understanding of the highly balanced and sometimes imbalanced interactions between gut microbiota and host AMPs should have great therapeutic implications for different intestinal disorders and neurodegenerative diseases. In this scenario, designed peptides able to regulate gut microbiota have received considerable attention for the treatment of neurodegenerative disorders. Recent findings also suggest the importance of the interactions between cutaneous microbiota and skin-derived AMPs pointing out that a fine-tuned and well-balanced AMP-microbiota interplay on the skin surface may be crucial for skin health. Hence, the proposed identification and production of peptides able to modulate gut and skin microbiota could have a great impact in the treatment of disorders caused by an imbalance of the equilibrium between microbiota and endogenous AMPs, and may afford new molecules for the treatment of infection and inflammation. Thus, this research by exploring the role of NPs/AMPs as new therapeutic agents directly contributes to the United Nation SDG "GOAL 3" (good health and well-being).
Inter-Multidisciplinary aspects	The proposed research project relies on a multidisciplinary approach to face the different aspects of microbiome modulation. The selected PhD will gain expertise and skills in interdisciplinary fields, ranging from molecular design, chemical synthesis, biophysical methods, microbiology, thanks to the possibility of carry out his/her research project within the above-described research units. He/she will benefit from the strong background of the research team in the investigation of the structure, mechanism of action and physico-chemical properties of proteins and peptides, thanks to the most advanced techniques for structure determination (X-ray and NMR), spectroscopic and calorimetric means for investigation of peptide activity, peptide-membrane interactions, molecular biology techniques for the cloning and expression of recombinant proteins and peptides and computational methods for molecular dynamics simulations
Secondment opportunities	**University of Pennsylvani**a: US first University, with a history that dates back to 1740. De la Fuente research group research activities are focused on artificial antibiotics, discovering new antibiotics properties, generating technologies for microbiome engineering, developing tools for synthetic neuromicrobiology. **https://delafuentelab.seas.upenn.edu/research/** ✓ *Main PI/co-supervisor*: Prof. Cesar de la Fuente ✓ *Length of the foreseen secondment*: 6 months ✓ *Activities that the PhD will perform during the secondment*: Analysis of AMPs activity on gut and skin microbiota by in vitro assays on commensal and pathogenic bacterial strains and in vivo analyses on suitable mouse models. **The Institut Laue-Langevin (ILL)** is an international research center, founded by France, Germany and UK in partnership with other Countries, at the leading edge of neutron science and technology. **https://www.ill.eu/users/scientific-groups/large-scale-structures/people/giovanna-fragneto** ✓ *Main PI/co-supervisor:* Dr. Giovanna Fragneto ✓ *Length of the foreseen secondment*: 3 months ✓ *Activities that the PhD will perform during secondment:* Physico-chemical characterization of peptide interaction on lipid bilayer structure and dynamics by SANS, neutron reflectivity, ellipsometry, Langmuir trough, QCM-D.
Main Supervisor: Prof. Angelina Lombardi (https://www.docenti.unina.it/angelina.lombardi); **Co-supervisor**: Prof. Angela Arciello (https://www.docenti.unina.it/angela.arciello)	
Brief CV	**Prof Angelina Lombardi** (Orcid ID: 0000-0002-2013-3009; current bibliometric (Google Scholar): h-index 38, total citations: 5466) received her graduation (Laurea) in Industrial Chemistry, summa con laude, in 1986, and the Ph.D. in Chemistry in 1990. In the same year she became Research Assistant Professor, at the University of Naples Federico II, where she is now Full Professor. Angelina Lombardi spent sabbatical periods abroad: she was visiting fellow at the National Institute of Health in Bethesda (MD), visiting scientific consultant at the DuPont Merck Pharmaceutical Company, Wilmington (DE), at the "Department of Biochemistry and Biophysics, University of Pennsylvania (PA)", and at the "Department of Pharmaceutical Chemistry, University of California, San Francisco (CA)". Angelina Lombardi has a solid background of research activity in interdisciplinary areas, ranging from transition metal chemistry to peptide and protein chemistry. During her career, she has demonstrated the ability to generate highly novel and exciting research topics, and to go significantly beyond the state of the art. During her earliest research activities, she acquired broad competencies in the area of inorganic and peptide chemistry, which were valuable for the future studies. In particular, she was interested in developing appropriate "molecular tools" able to freeze linear and cyclic peptides in a well-defined three-dimensional structure. These studies have been basic for the design of new molecules capable of reproducing the chemical, structural and catalytic properties of several natural systems. Recently, she described for the first time the de novo design of an allosterically regulated phenol oxidase that responds to the binding of a synthetic porphyrin (PNAS 2020). *Experience in supervising PhD students*

	Ten PhD thesis and eight postdocs supervised; currently supervising four PhD students. Vice Chair of the Action and Member of the Management Committee as Italy Representative: COST Action CM1003: Biological oxidation reactions - mechanisms and design of new catalysts (https://www.cost.eu/actions/CM1003/). Co-supervisor is Prof **Angela Arciello.**(Orcid ID: 0000-0001-8269-6459; current bibliometric (Google scholar): h-index 21, total citations 1640).
Publications	Angelina Lombardi is co-author of almost 130 publications in leading scientific journals and several patents. She is also author of invited comments, review papers and book chapters (e.g. Acc. Chem. Res. 2019; TIBS 2019; Chem. Soc. Rev. 2016; Ann. Rev. Biochem. 1999). **Most significant /recent 5 publications in the microbiome field** -R. Oliva, M. Chino, **A. Lombardi**, F. Nastri, E. Notomista, L. Petraccone, P. Del Vecchio "Similarities and differences for membranotropic action of three unnatural antimicrobial peptides" *J Pep Sci.*26:e3270 (2020). -R. Oliva, M. Chino, K. Pane, V. Pistorio, A. De Santis, E. Pizzo, G. D'Errico, V. Pavone, **A. Lombardi, P**. Del Vecchio, E. Notomista, F. Nastri and L. Petraccone "Exploring the role of unnatural amino acids in antimicrobial peptides" *Sci Rep*, 8, 8888 (2018). -R. Gaglione, E. Dell'Olmo, A. Bosso, M. Chino, K. Pane, F. Ascione, F. Itri, S. Caserta, A. Amoresano, **A. Lombardi**, H. P Haagsman, R. Piccoli, E. Pizzo, E. JA Veldhuizen, E. Notomista, A. Arciello "Novel human bioactive peptides identified in Apolipoprotein B: Evaluation of their therapeutic potential" *Biochemical Pharmacology* 130, 34-50 (2017). -V. Pavone, S.-Q. Zhang, A. Merlino, **A. Lombardi**, Y. Wu, W.F. DeGrado "Crystal structure of an amphiphilic foldamer reveals a 48-mer assembly comprising a hollow truncated octahedron" *Nature Comm*. 5, (2014). -A. Zanfardino, A. Migliardi, D. D'Alonzo, **A. Lombardi**, M. Varcamonti, A. Cordone "Inactivation of MSMEG_0412 gene drastically affects surface related properties of Mycobacterium smegmatis" *BMC Microbiology* 16, 267 (2016).
Projects participation	European Union, POR Campania - FESR 2014-2020: High-tech districts, public-private aggregations and laboratories to strengthen the scientific and technological potential of the Campania Region. Project title: New strategies for medical and molecular diagnostics and for the traceability and monitoring of food products. Unit research PI. CUP B63D18000350007. Unit research budget: € 709.000 European Union (FSE, PON Research and Innovation 2014-2020, Azione I.1 "Dottorati Innovativi con caratterizzazione Industriale"), (XXXVII cycle); role: supervisor; industrial partner: Giotto Biotech (Florence, Italy); international partner: Department of Chemistry, University of Cambridge, UK.