UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.1

Proponent: Prof. Cristina Airoldi

Project Title: Development and application of advanced technologies for the structural characterization of biomolecules and their interactions.

Scientific background and 'open issues'

Biomolecular assembly plays a central role in nearly all life processes, from short-lived microscopic events, such as DNA replication, translation of genes into proteins and signalling within and between cells, to the formation of long-lived macroscopic architectures such as collagen networks and amyloid aggregates.[1]

The characterization of biomolecules' structures and the elucidation of how they interact is fundamental to understand key biological processes and for the identification/rational development of molecules capable of restoring their activity when altered under pathological conditions.

In recent years our group developed new advanced NMR (Nuclear Magnetic Resonance)-based methodologies making molecular recognition studies feasible in "unconventional" experimental conditions such as in the presence of living cells (cell-based NMR to study interactions involving membrane receptors),[2,3] calixarens,[4] dendrimers,[3,5] nanoparticles and liposomes.[6]

Furthermore, combining NMR and LC-MS (Liquid Chromatography Mass Spectrometry) we linked the metabolic profiling and chemical characterization of natural extracts to their screening aimed at identifying small molecules capable of binding and modulating the activity of a target protein involved in the etiology of a specific disease.[7–12]

These innovative technologies allowed us to identify/develop natural and synthetic ligands of different molecular targets, including amyloidogenic proteins,[8–10,12–14] oncoproteins[7] and constituents of the outer envelope of pathogenic bacteria,[4,5,15] which have shown potential applications for the prevention/diagnosis of neurodegenerative diseases, tumours, and bacterial infections.

Objectives

The main goal of the project will be the further development of the technologies mentioned above aimed at increasing their versatility and applicability to the widest possible range of biomolecular targets and ligands of different nature and chemical complexity.

For this purpose, different diseases and their related molecular biomarkers/etiological agents will be selected.

The list of potential biomolecular targets includes Aβ peptides (Alzheimer's disease), α-synuclein (Parkinson's disease) and the Ataxin-3 (Spinocerebellar Ataxia Type 3), the lectin FimH (uropathogenic strains of *E. coli*), the lipopolysaccharide (LPS) and other peculiar components of the bacterial envelop of the ESKAPE *Acinetobacter baumannii*.

Methodologies

The search for new inhibitors of amyloid proteins will focus on the screening of natural extracts, also from edible sources, rich in polyphenols, a class of compounds that in previous studies have proven to be the most promising for their ability to present multitarget activities (antiamyloidogenic, antioxidant, induction of autophagy).
The identification of new ligands of FimH and of the *A. baumanni* outer envelope will be based on the screening of both extracts from microorganisms and small libraries of multivalent compounds.
The project implementation will require the application/development of different techniques and methodologies and will offer to the PhD student the opportunity to acquire several and complementary skills concerning:

- *in vitro* protein expression and purification;
- transformation/transfection of bacterial/mammalian cell lines for on-cell NMR studies;
- application and development of new advanced NMR techniques for the elucidation of key molecular recognition processes (with the selected targets and ligands of interest);
- NMR spectroscopy and mass spectrometry coupled with chromatographic techniques for natural extract components' identification and isolation;
- application of biophysical, biochemical and biological assays to assess the biological activities of identified ligands;
- assays on molecules' ability to cross biological barriers (blood brain barrier and intestinal barrier).

Collaboration / Co-tutoring opportunities

Being a highly multidisciplinary project, the research group will take advantage of on-going collaborations (BTBS - Prof. M. E. Regonesi, Prof. A. Natalello, Prof. S. Brocca; School of Medicine and Surgery – Prof. Francesca Re, Prof. F. Mantegazza; UNIPR – Prof. A. Casnati; UNIMI – Prof. A. Polissi) to guarantee the PhD student support in terms of supply of materials and also technical supervision to carry out some specific experimental activities.
Additional advanced expertise will be acquired during the secondment(s) abroad (see next section).

Project's Sustainability & Mobility

The project topic fit perfectly with the main research lines and expertise of the proponent's laboratory (BioOrgNMR Lab) (see references 2-15).
The research and training activity will include a period abroad with a duration varying between six and nine months.
Possible joint supervision: Prof. Jimenez-Barbero (Center for Cooperative Research in Biosciences (CICbioGUNE) and University of the Basque Country (UPV/EHU)).
In addition, collaboration with research groups form other European Universities and Research Centres, as well as participation in international congresses, seminars and workshops is expected.

References

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