

<b>SCHOOL OF ADVANCED STUDIES- 2011 Research topics</b>	
<b>Area of Studies</b>	<b>LIFE SCIENCES AND PUBLIC HEALTH</b>

<b>Curriculum:</b>	<b>AGEING AND NUTRITION</b>
<b>Supervisor:</b> <b>Prof. Carlo Polidori</b> <b>Fellowship in Co-Tutelle with the University “Paul Sabatier”, Toulouse, FRANCE</b> <b>Basic knowledge of French language is required</b>	<b>Research topic n.1 : FUNCTIONAL CHARACTERIZATION OF PROTEINS INVOLVED IN OBESITY</b>
<b>Supervisor:</b> <b>Dr. Rosita Gabbianelli</b>	<b>Research topic n. 2 : EFFECT OF MEMBRANE LIPID STATE ON ALHEIMER’S PEPTIDE</b> The development of Alzheimer's disease pathology has been proposed to be the result of A $\beta$ deposition in association with membrane structures. After cleavage, these peptides are involved in a self-assembly-triggered conformational change, which implicates the conversion of $\alpha$ -helical structures into $\beta$ - structures. This conformational change is linked in the nucleation-dependent polymerization process. The aggregates bind to the cell membrane and this event could influence in cellular degeneration. A $\beta$ is known to interact with the cell membrane and also with the membranes of subcellular organelles (lysosome, Golgi complex and endoplasmic reticulum). In consequence of its lipophilicity, A $\beta$ can interact strongly with the lipid bilayer leading to an increase in A $\beta$ fibrillogenesis and changes of bilayer properties . The size of the A $\beta$ aggregates and its hydrophobicity have been correlated with a decrease of membrane fluidity . Self-association of A $\beta$ into aggregates exposes hydrophobic sites and induces a change in model membrane fluidity. Both soluble and aggregated A $\beta$ 1 -40 significantly increased the synaptic plasma membrane bulk and protein annular fluidity . A $\beta$ fibrillogenesis was increased in the presence of plasmal lysosomal and endosomal membrane, and A $\beta$ 1 -40/42 decreased the fluidity of the

	<p>fatty acyl and head group of these membranes, which is consistent with A<math>\beta</math> insertion into bilayer. The aim of this study is to evaluate the effect of lipids on the aggregation processes of amyloid peptides. Liposomes prepared from brain cells (rats) will be oxidized and the influence of lipid hydroperoxides on aggregation process will be evaluated. The oxidation state of lipids will be measured by diphenyl-1-pyrenylphosphine (DPPP). DPPP, a fluorescent probe which localizes in the plasma membrane and shows increased fluorescence emission after its oxidation by lipid hydroperoxides, can be used for detection membrane lipid peroxidation. Moreover we will study the changes in physico-chemical state of hydrophilic-hydrophobic region of the bilayer employing laurdan which is a fluorescent probe that gives indication on changes in fluidity and polarity at the plasma membrane level.</p>
<p><b>Supervisor:</b> <b>Prof. Paolo Polidori</b></p>	<p><b>Research topic n. 3 : NUTRITIONAL CHARACTERIZATION OF DONKEY'S MILK</b></p> <p>Milk is fundamental in early infancy. Genetic, physiological, and nutritional factors as well as environmental conditions widely vary the milk composition (lipids, proteins and salts) of mammalian species, determining a different intake of nutrients according to the type of milk used.</p> <p>Newborns fed with donkey milk or with human milk show the same kidney load. Recent works suggest providing donkey milk to newborns allergic to cow milk, because donkey milk has a low <math>\beta</math>-lactoglobulin content. This protein has a high allergenic activity. No therapy exists for this allergy, and thus the only feasible response is to avoid assumption of milk and milk products. About 3% of milk is formed of proteins, divided into two main fractions, caseins and wheyproteins. The former are organized in micelles that give milk its opacity and constitute 80% of its proteins. The latter constitute the other 20% and contain two main components, <math>\beta</math>-lactoglobulines (8%) and <math>\alpha</math>-lactoalbumines (4%). Studies conducted on the serum of children with hypersensitivity to milk have shown that caseins are the proteins with the greater allergenic potential. However, in some cases, children have also shown hypersensitivity to the <math>\beta</math>-lactoglobulines and the <math>\alpha</math>-lactoalbumines.</p> <p>The aim of our study will be to characterize</p>

	<p>donkey's milk, nitrogen fraction and lipid composition .</p> <p>The casein (alpha, beta and k fractions) and wheyprotein (alpha lactoalbumin, beta lactoglobulin, lactoferrin, lactoperoxidase and lysozyme) contents will be quantitatively and qualitatively determined, considering the allergenic or nutraceutical (antibacterial) role of those components, whose amino acids composition will be also investigated.</p> <p>Lysozime content will be also determined in donkey's milk, considering the antimicrobial role of this nitrogen compound.</p> <p>Hydro- and lypo-soluble vitamins take part in various important biological activities, and are important for their antioxidant capacity .Vitamin content can vary because of the animal's diet, as well as the type of conservation and formulation of the milk, influencing the fatty acid composition. This study will evaluate the changes in unsaturated and monounsaturated fatty acid content and the antioxidant capacity of the milk following different treatment.</p>
<p><b>Supervisors:</b>  <b>Prof. Cantalamessa</b>  <b>Dr. Rosita Gabbianelli</b>  <b>Dr. Cinzia Nasuti</b></p>	<p><b>Research topic n. 4 : BEHAVIORAL AND BIOCHEMICAL CHANGES INDUCED BY NEONATAL PYRETHROID EXPOSURE IN RATS.</b></p> <p>The pyrethroid insecticides are members of a chemical class of heavily used compounds, and hazards from exposure to insecticides exist from their manufacture, storage, and spraying, as well as through contact with insecticide-contaminated food or areas. They can be divided into two classes. Type I has no cyano-group at the carboxyl <math>\alpha</math> position (<math>\alpha</math> -carboxyl), whereas Type II presents this cyano-group. Permethrin (PERM) is claimed to be one of the most potent Type I insecticides, whereas cypermethrin (CY) belongs to Type II. Studies have documented effects of the pyrethroids on dopaminergic nerve pathways, which may be a contributory factor in the etiology of environmentally induced Parkinson's disease. Although pyrethroid neurotoxicity to adults has been well characterized, and several comprehensive reviews of pyrethroid toxicity, metabolism, and actions are available, information regarding the potential developmental neurotoxicity of this class of compounds is limited. The relationships between biochemical alterations and pyrethroid-induced developmental neurotoxicity have yet to be established by better characterization of the</p>

	<p>neurochemical mode of action. Our previous studies on adult rats orally treated with different concentrations of CY and PERM, showed biochemical alterations, such as oxidative stress, in the plasma membrane and antioxidant enzymatic activity of erythrocytes (Nasuti et al., 2003) as well as DNA damage in lymphocytes. The aim of our work will be to investigate in rats the long-lasting effects after developmental exposure (from postnatal days 6 to postnatal days 15) to type I (PERM) or type II (CY) pyrethroids at a dose that does not induce acute toxicity in pups. Subsequently, open-field behaviours as well as striatal monoamine levels in adulthood will examine. In addition, in an attempt to assess whether pyrethroids can cause oxidative stress in striatum, and to shed some light on the mechanisms involved in the reported neurotoxicity of pyrethroids, we will perform an exhaustive investigation of oxidative stress caused by pyrethroid exposure, examining the effect of both pyrethroid types on plasma membrane fluidity, lipid peroxidation, protein oxidation in striatum, and erythrocytes of rats.</p>
<p><b>Supervisor:</b>  <b>Prof. Anna Maria Eleuteri, Prof. Mauro Angeletti</b></p>	<p><b>Research topic n. 5 : METABOLITES FROM VEGETABLE EXTRACTS AND THEIR EFFECTS ON ENZYMES.</b></p> <p>The aim of the project is the characterization of secondary metabolites (polyphenols) extracted from natural sources and the evaluation of their role in biological processes of biomedical interest. It will be developed as follows:</p> <p>a) <i>Analytical characterization of the polyphenolic content.</i> It will be obtained through different analytical techniques (HPLC and/or Capillary Electrophoresis) or spectrophotometric procedures.</p> <p>b) <i>Study of the polyphenols interaction with purified enzymatic systems</i></p> <p>Using different methodologies, it will be studied the interaction of polyphenols with enzymes retained heavily involved in the genesis of human pathologies such as:</p> <p>i) Proteasome 20 S  ii) 3-hydroxy-3-methylglutaryl CoA reductase  iii) Serine proteases</p> <p>Different experimental approaches will produce thermodynamic and/or kinetic parameters (Spectrophotometric assays, Surface Plasmonic Resonance, Docking analysis and QSAR).</p>

	<p><i>c) Effect of polyphenols on cellular systems.</i></p> <p><b>d)</b> Using the 20S Proteasome as biological target, it will be evaluated the polyphenols effect on normal as well as transformed cells. The aim of these studies is to demonstrate that polyphenols are able to selectively affect the apoptotic pathways of cancerous cells disclosing for them a potential use in cancer therapy and prevention.</p>
<p><b>Supervisors:</b>  <b>Prof. Anna Maria Eleuteri, Prof. Mauro Angeletti,</b></p>	<p><b>Research topic n. 6: CROSSTALK BETWEEN AMYLOID BETA AND AUTOPHAGIC AND PROTEASOMAL PROTEOLYTIC PATHWAYS IN CELLULAR AND ANIMAL MODELS</b></p> <p>Protein aggregates-associated pathologies are diseases with a high degree of impairment in the cellular protein degradation pathways. Amyloid beta peptides and their aggregates are responsible for damages and alterations to protein turnover thus resulting in an accumulation of further cytoplasmic aggregates and damaged proteins and in higher levels of reactive oxygen species (ROS). The ubiquitin-proteasome pathway mediates the removal of short lived, damaged and oxidative proteins. Lysosomes, instead, have a key role in degrading and recycling cellular components for energy production and biosynthesis and in degrading long-lived proteins, organelles and protein complexes enable to enter the proteasome inner channel. Treating the cells with different aggregation states of amyloid peptides induced a strong inhibition of the proteasomal complex, particularly evident in the presence of oligomers. Similarly, through the induction of high levels of ROS, amyloid aggregates are capable of injuring the lysosomal membrane leading to the release of enzymes with consequent damages to the cytoplasmic organelles.</p> <p>It has been demonstrated the presence of a crosstalk between the ubiquitin-proteasome system and autophagy, suggesting a coordinated and complementary relationship between these degradation systems, that becomes critical in times of cellular stress. The effects of amyloid treatment, soluble peptides and oligomeric structures, on the proteasome and lysosomal functionality, directly measuring the activity of their catalytic components, will be measured. A neuroblastoma cell line, the SH-SY5Y cells, and HEK 293 cells which overexpress a mutated and a wild type form of the amyloid precursor protein will be used as cellular model. It will be possible to better understand which proteolytic pathway is</p>

	<p>particularly influenced by the peptides in conditions which are similar to those observed in AD cells. The expression levels of HDAC6 and Hsp90 will be also evaluated, in order to check the status of these mediators in the crosstalk between proteasome and autophagy. The levels of intracellular ROS and the protein carbonyl content will be assayed in order to check the global oxidative status. Measurements of proteasome and lysosomal enzymes activities will be performed.</p> <p>Proteomic studies on the obtained cell lysates will be performed in order to check if the treatments are able to induce modifications in the protein expression profile. In vivo studies using transgenic mice TgCRND8, characterized by mutations in the amyloid precursor protein, will be performed.</p>
<p><b>Supervisors:</b>  <b>Prof. Anna Maria Eleuteri, Prof. Mauro Angeletti, Prof. Gilberto Mosconi</b></p>	<p><b>Research topic n. 7: USE OF POLYPHENOLS AS FEED INGREDIENTS TO IMPROVE QUALITY AND SAFETY OF FISHERY AND AQUACULTURE PRODUCTS</b></p> <p>On the basis of continuing demand for sustainable aquaculture, we are interested in investigating the biological effects of dietary plant extracts supplements on the quality and safety of fishery production. The aim of the study is to investigate the role of a large variety of plant phenolics presenting antioxidant properties on the stress response, disease resistance and growth rate of reared fish by using an integrated approach based on physiological, biochemical and molecular tools.</p> <p>It will be developed as follows:</p> <ul style="list-style-type: none"> <li>• the effects of polyphenols on the fish survival and growth will be evaluated by morphometric and molecular approach through the relative body weight, total lengths and gene expression of Myostatin and Thyroid receptors.</li> <li>• The effects on animal welfare will be estimated by cortisol level and both proopiomelanocortin (POMC) and 70KDa Heat Shock Protein (HSP70) gene expression analyses. Particular attention will be given to the evaluation of total antioxidant status in plasma and body fluids.</li> <li>• The effects of dietary supplements on fish immune system responses will be investigated by evaluating cytokine</li> </ul>

	<p>expression changes. The effects of plant extracts supplementation will be also evaluated on serum and tissue lipid profiles.</p>
<p><b>Supervisor:</b> <b>Prof. Giulio Lupidi</b> <b>Pro. Luca Agostino Vitali</b></p>	<p><b>Research topic n. 8 : STREPTOCOCCAL DIPEPTIDASES, CARIES, AND DIABETES</b></p> <p>The bacterial dipeptidases are important for the metabolism of bacteria and may play an important role in the pathogenesis contributing to the degradation of some tissues and the impairment of the immunological response mediated by regulatory peptides. The biochemical characterization of dipeptidil-prolyl peptidase IV (DPP-IV) enzymes and their activity in selected oral streptococci mainly involved in the cariogenic process, but also responsible of bacterial endocarditis, is the main object of the research. Then the definition of the in vitro and ex vivo activity of some specific DPP-IV inhibitors will permit to hypothesize on the effect of therapies against type 2 diabetes, which utilize DPP-IV inhibitors, on the composition of the gram-positive oral microbiota and the overall incidence of caries in diabetic patients.</p>
<p><b>Supervisor:</b> <b>Prof. Giulio Lupidi</b> <b>Prof. Massimo Bramucci</b> <b>Dr. Luana Quassinti</b></p>	<p><b>Research topic n. 9 : DEVELOPMENT OF NEW CATALYTIC MOLECULES (SOD-MIMICS) WITH SENSOR/EFFECTOR PROPERTIES.</b></p> <p>A range of human diseases, such as certain types of cancer, are associated with a disturbed intracellular redox-balance. The latter often results in exceptionally high intracellular concentrations of reactive oxygen species, such as hydrogen peroxide, and free metal ions. We have recently developed catalytic molecules which use intracellular stressors as their substrates. These compounds mimic the activity of the human enzyme, Superoxide dismutases, often referred to as SODs, that are a group of enzymes that contain a metal atom at the active site which catalyzes the dismutation of two superoxide anions into one oxygen and one hydrogen peroxide and is thus involved in protecting the cell from oxygen toxicity.</p> <p>The metal present in enzyme active site can be a copper/zinc combination, manganese, or iron. The focus of this study is to synthesize mimics of the active site of copper, copper/zinc and manganese SOD and see if they could be used for therapeutical treatments. By copying the active</p>

	<p>site only, it helps us to avoid having to make a copy of the rest of the bulk of the enzyme. In order to enhance efficiency, we have recently produced a second generation of catalysts combining new ligands derivatives of bis(4-methylpyrazolyl)/metals complexes (Cu,Cu-Zn, Mn) and evaluated their antioxidant activities. The resulting selectivity might provide a lead for the future development of selective, yet high efficient anti-cancer drugs.</p> <p>Future work will focus on combining two and more oxidative stressor response sites' in a single molecule. This approach should considerably enhance the efficiency of the compounds and also their selectivity for cells under oxidative stress.</p>
<p><b>Supervisor:</b> <b>Prof. Giancarlo Falcioni</b></p>	<p><b>Research topic n. 10</b></p> <p>Nanotechnology, defined as techniques aimed to design, characterize and produce materials on a nanometer scale, is a fast-growing field today. Nanomaterials are used in a wide range of industries, including food, clothing, electronics, cosmetics, medicine and agriculture.</p> <p>Increasing production and usage of nanomaterials in consumer products may lead to human exposures. Currently, little is known about the potential adverse health effects of these particles. Therefore, there is an urgent need to understand the potential impact of nanoparticle exposure on human health. Among the different exposure routes (e.g.: inhalation, skin contact, and ingestion) particle inhalation is the most important.</p> <p>Once translocated into the cells, nanoparticles may cause several biological responses including the generation of reactive oxygen species, DNA strand breaks and the enhanced expression of pro-inflammatory cytokines. There is increasing evidence that particulate pollutants act as adjuvant for allergic sensitization to common environmental. This raises the possibility that long term ambient particulate matter exposure may lead to increased prevalence of asthma and allergic diseases. There are accumulating evidences suggesting that nanoparticles may exert adverse effects on the lung and other organ systems (Bonner, 2010).</p> <p>Nanoparticles deposited in airways and alveoli are readily taken up by lung macrophage phagocytosis. Interstitial and surface (air-way and alveolar) macrophages are the pulmonary phagocytes that are recognized as first line of</p>

	<p>cellular host defence. Being exposed to the environment, surface macrophages are potential targets of inhaled nanoparticles; but they also are at the forefront of lung defence and their primary function is the clearance of foreign intruders and their disintegration.</p> <p>We intend to evaluate the damage induced by different nanoparticles (TiO<sub>2</sub>, ZnO, CuO etc.) exposure on DNA of alveolar macrophages and monocyte-derived macrophages. The effect of antioxidant supplementation will be also evaluated.</p> <p>In particular, we intend to examine on these cells:</p> <ol style="list-style-type: none"> <li>1.The DNA damage and the capacity of the DNA repair systems.</li> <li>2.The phagocytosis capacity.</li> </ol> <p>The objective is to gain deeper knowledge about the cellular insult in relation to stimulus represented by different nanoparticle exposures. Better understanding the nature of nanoparticle damage could help to hypothesize a preventive therapy to protect human health.</p>
<p><b>Supervisor: Prof. G. Lupidi</b></p>	<p><b>Research topic n. 11: OPTIMIZATION OF LIQUOR PRODUCTION FROM HERBS AND SEEDS: FROM THE CHARACTERIZATION OF THE PLANTS TO THE RECOVERY OF HIGH VALUABLE BIO-ACTIVE COMPOUNDS FROM LIQUORS INDUSTRIAL BY-PRODUCTS.</b></p> <p>.</p> <p>The project has two research lines. The first is the characterization of the different species and populations of the plants used in the liquors. The second is the characterization of bioactive molecules from extracts and wastes obtained by the processing of herbs during the production of bitters and liquors. The second line of the project is interdisciplinary as it requires the chemical and biochemical characterization of molecules and evaluation of their potential role in processes of biomedical interest. The project is co-funded by a private company.</p>