Projects available for Union College Students on the 2013 Sicily Science Term Abroad

Project # 1 (IBF - Fabio Librizzi)

In the framework of protein aggregation, two lines of experiments can be followed:

1) A class of small peptides, known as β-sheet breakers has been shown to be able to reduce the aggregation propensity of the amyloid β-peptide, whose aggregation process, as is very well known, is strongly involved in Alzheimer's disease [1,2].

2) Due to its high tendency to form amyloid aggregates, the bean agglutinin Concanavalin A is a very good model system to study the mechanisms underlying the aggregation process in general and the effects on it of external parameters such us temperature, pH, ionic strength of the solvent, etc. [3-5].

[1] Soto C., et al. Inhibition of Alzheimer's amyloidosis by peptides that prevent β-sheet conformation. Biochem. Biophys. Res. Comm. 226: 672-680 (1996).

[2] Giordano C. et al. Synthesis and activity of fibrillogenesis peptide inhibitors related to the 17-21 β-amyloid sequence. Eur. J. Med. Chem. 44: 179-189 (2009).

[3] Vetri V. et al, Amyloid fibrils formation and amorphous aggregation in Concanavalin A. Biophys. Chem. 125: 184-190 (2007).

[4] Carrotta R. et al. Amyloid fibrils formation of Concanavalin A at basic pH. J. Phys. Chem. B 115: 2691-2698 (2011).

[5] Carrotta R. et al. a-casein inhibition mechanism in Concanavalin A aggregation process. J. Phys. Chem B 116: 14700-14707 (2012).

Project # 2 (IBF - Silvia Vilasi)

Hsp60 is a molecular chaperone, highly conserved during evolution, known to assist protein folding in prokaryotes and in eukaryotic cells [1]. Similarly to its bacterial homolog Groel, Hsp60 is a 60 kDa protein, and under normal physiological conditions it assembles in a complex arranged as two stacked heptameric rings [2]. A recent work from Chandra et al. [3] brings into focus some important points concerning Hsp60's role. They suggest that Hsp60 has a pro-survival function if released from mitochondria to cytosol, and a pro-death function if it accumulates in the cytoplasm without mitochondrial secretion. Cytosolic and mitochondrial Hsp60 differ by a 26 amino acid signal sequence at the N terminus of the protein [4]. Their different functions involve a different protein binding to procaspase-3, whose direct interaction mechanism was proved by pulldown assays [3]. We propose to perform measurements by different biophysical techniques (circular dichroism, fluorescence, light scattering, calorimetry) to compare Hsp60 to Groel stability and to analyze the complex Hsp60/procaspase-3. This will shed light on the interaction of Hsp60 with procaspase-3, as affected by the signal sequence at the N-terminus region, and on the crucial features that determine the pro-death or pro-survival Hsp60 functional role.

[1] Ranson NA et al. (1998), *Review: Chaperonins*. Biochem. J. 333: 233–242.

[2] Cappello F et al. (2008) *Hsp60 expression, new locations, functions and perspectives for cancer diagnosis and therapy.* Cancer Biology and Therapy 7(6): 801-809.

[3] Chandra D et al. (2007) *Cytosolic accumulation of Hsp60 during apoptosis with or without apparent mitochondrial release.* Jbiol.Chem. 43:31289-31301.

[4] Itoh H et al. (2002) *Mammalian Hsp60 is quickly sorted into the mitochondria under conditions of dehydration.* European Journal of Biochemistry, 269(23): 5931–5938.

Project # 3 (IBF – Rita Carrotta)

Liposome as a membrane model. Production, characterization and protein interactions.

Membranes are fundamental constituents of living cells. Their function is related to compartment creation, with the important property of a selective permeability to ions/molecules that regulate the living processes in cells. Membranes are built of lipid molecules and they host as main protagonist proteins, working as receptors, pumps, transporters, etc …

Liposomes are simple lipid aggregates that are often used as membrane environment model systems. Lipids are anphypatic molecules that in water aggregate indeed by forming different structures, depending on preparation methods and/or physico-chemical conditions.
Large unilamellar vesicles (LUV) can be created in our lab. These aggregates are characterized by a lipid double layer, separating an inner water solvent filled part from the external bulk water. In order to study the interactions of such liposomes with proteins, we would like to entrap into the inner hydrophilic space of LUV, fluorescent molecules (such as calcein), which change their properties when are inside the liposome or outside in the solvent space. Therefore the measure of such change can give information about any eventual leakage due to liposome permeability. Such permeability can be induced by breaking the liposome structure with "soap", but also due to an interaction of the liposome surface with active protein components.
In particular our interest is to study the interaction between liposome systems and amyloid beta-protein in different aggregation states.

Ref:
Carrotta et al. 2012, Biochim.Biophys.Acta 1820: 124-132.
Kajed et al. 2004, JBiol.Chem. 279 (45): 46363-46366.
Wong et al. 2009, JMol.Biol. 386 (1): 81-96.
Dante et al. 2011, Biochim.Biophys.Acta 1808:2646-2655.
Sponne et al. 2004, FASEB J 18(7):836-8.
Alarcon et al. 2006, PEPTIDES 27: 95-104.

Project # 4 (IBF – Mariuccia Mangione)

The aggregation processes of the proteins involved in neurodegenerative diseases is our field of study. The goal of the research is the understanding of the pathological pathway, the characterization and isolation of the aggregate intermediate and the role of potential inhibitors in these processes. In particular, the protein studied, at the moment, is the amyloid beta protein (involved in Alzheimer's disease) and the techniques most used are: High Performance Liquid Chromatography (HPLC) and fluorescence spectroscopy. The determination of cytotoxicity, observed by other colleagues, can be an important parameter to distinguish effective drugs/inhibitors.

Project # 5 (IBF – Valeria Guarrasi)

The aim of our research is an identification and characterization of polyphenolic compounds drawn from fruits produced in Sicily, e.g., apple, mango and pomegranate. The goal is the collection of such chemical compounds, extracted from fruits, for medical applications.
Polyphenolic compounds are known for their potential benefits on human health, such as antioxidant (*Leontowicz et al. 2002*), antitumor (*Femia et al 2005*) and anti-inflammatory effects (*Terra et al. 2011*).
The polyphenols extracted from apples, mainly contain procyanidins (PCs), have anti-Aβ aggregative effects in vitro (*Toshihiko et al 2011*). Mice treated with pomegranate juice had significantly less (~50%) accumulation of soluble $A\beta_{42}$ and amyloid deposition in the hippocampus as compared to control mice (*Hartman et al. 2006*). Mango's polyphenolics have high antioxidative activity which is capable of attenuating amyloid toxicity (*Masibo et al. 2008*).
The study could provide a new powerful therapy for neurodegenerative disease as Alzheimer's.

Femia A.P., Caderni G., Vignali F., Salvadori M., Giannini A., Biggeri A., Gee J., Przybylska K., Cheynier V., Dolara P. Effect of polyphenolic extracts from red wine and 4-OH-coumaric acid on 1,2-

dimethylhydrazine-induced colon carcinogenesis in rats. *European Journal of Nutrition, vol. 44, pp. 79-84, 2005.*
Hartman R.E., Shah A., Fagan A.M., Schwetye K.E., Parsadanian M., Schulman R.N., Finn M.B. and Holtzman D.M. Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. *Neurobiology of Disease, vol. 24, pp. 506-515, 2006.*
Leontowicz H., Gorinstein S., Lojek A., Leontowicz M., Ciz M., Soliva-Fortuny R.C., Park Y-S., Jung S-T., Trakhtenberg S. and Martin-Belloso O. Comparative content of some bioactive compounds in apples, peaches and pears and their influence on lipids and antioxidant capacity in rats. *Journal of Nutritional Biochemistry, vol. 13, pp. 603-610, 2002.*
Masibo M. and He Q. Major Mango Polyphenols and Their Potential Significance to Human Health. *Comprensive Reviews in Food Science and Food Safety, vol. 7, pp. 309-319, 2008.*
Terra X., Palozza P., Fernandez-Larrea J., Ardevol A., Blade C., Pujadas G., Salvado J., Arola L. and Blay M.T. Procyanidin dimer B1 and trimer C1 impair inflammatory response signalling in human monocytes. *Free Radical Research, vol. 45, pp. 611-619, 2011.*
Toda T., Sunagawa T., Kanda T., Tagashira M., Shirasawa T. and Shimizu T.. Apple Procyanidins Suppress Amyloid β-protein Aggregation. *Biochemistry Research International, vol. 2011, pp. 784698, 2011.*

Project # 6 (IBIM – Di Carlo group)

Our research group is interested in the neuroscience field, in particular in the study of Alzheimer's disease and linked pathologies such as Diabetes and Obesity. We also study the effect of pro- and antioxidant molecules to use as potential therapy. In collaboration with IBF we study the aggregation process of beta amyloid protein alone or in presence of different molecules.
We analyze the molecular mechanisms underlying these pathologies, such as oxidative stress, apoptosis, and cell signaling using different experimental approaches.
As model systems we utilize neuroblastoma cell cultures, mice, and human lymphocytes.
The principal used techniques are:
Cell cultures
Cytotoxicity assays
Extraction of proteins and nucleic acids
Western blot
Immunohistochemistry
Real Time PCR
Oxidative stress assays
Apoptosis assays
Microscopy

References:

Aging Cell (2011) 10, pp832–843
www.pnas.org_cgi_doi_10.1073_pnas.0807991106
***Current Alzheimer Research,* 2012*, 9,* 35-66**
Journal of Diabetes Science and Technology Volume 2, Issue 6, November 2008
http://dx.doi.org/10.1016/j.mce.2011.08.019
PLoS ONE 7(1): e30378. doi:10.1371/journal.pone.0030378
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 29, pp. 24573–24584, July 13, 2012
Oxid Antioxid Med Sci 2012; 1(1):1-4