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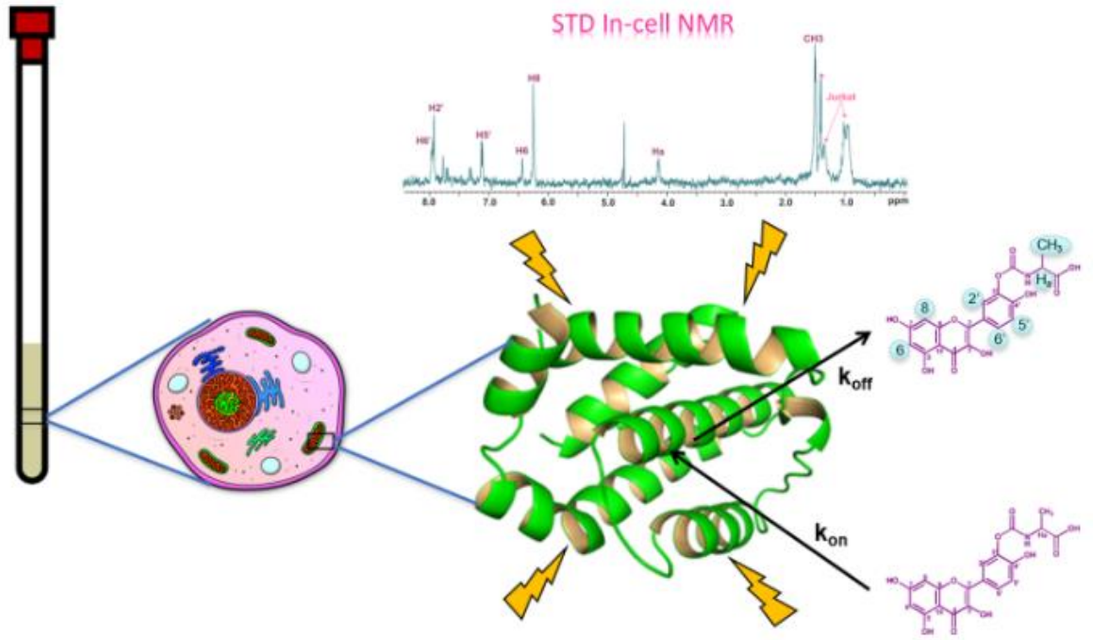
Description of the funded research project

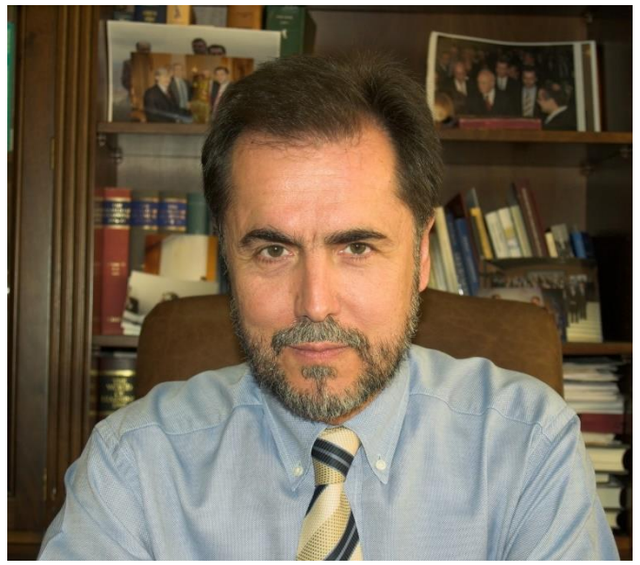
1st Call for H.F.R.I. Research Projects to Support Faculty Members & Researchers and Procure High-Value Research Equipment



Title of the research project: Investigation of interactions of unsaturated fatty acids with albumin and the anti-apoptotic protein Bcl-2 with emphasis in the use of NMR methodologies at a cellular level (in-cell NMR)

Principal Investigator: Ioannis P. Gerothanassis

Reader-friendly title: Lipid in-cell NMR



Scientific Area: Natural Sciences

Institution and Country: University of Ioannina, Greece

Host Institution: Unuverstiy of Ioannina

Collaborating Institution(s):

Budget: 180.000 €

Project webpage (if applicable):

Duration: 36 months

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Lipids are a broad and diverse group of biological molecules with numerous physicochemical and biological properties and important roles in nutrition and health. Lipids are the main structural components of cell membranes, of nervous tissue and act as important sources of energy in metabolic processes. A significant number of free fatty acids (FFAs), which are the building blocks of lipids, circulate in mammalian plasma and play important roles as ligands in protein-lipid interactions, in cell signaling processes, energy metabolism and inflammatory responses.

The antitumor activity of FFA has been attributed to their ability to promote apoptosis in various cancer cell lines. Interestingly, recent studies suggested that lipid anti-cancer properties should be attributed to their structural similarity with antagonists of the anti-apoptotic family of Bcl-2 proteins currently tested in clinical trials. Although there is strong indication of the pro-apoptotic activity of FFAs against cancer through affecting the expression levels of Bcl-2, the exact mechanism of this activity as well as of their interaction with Bcl-2 protein, at a molecular level, remain elusive. The main objectives and challenges of this research proposal are the following:

(A) Application of fast-screening NMR methodologies in the elucidation of the interactions and conformational changes of bioactive FFAs in solution with:

(i) serum albumin (SA), which is the main protein for the transportation of FFAs through systemic circulation and

(ii) the anti-apoptotic Bcl-2 protein target of their anticancer activity.

(B) Study of structural and conformational properties of the interactions of FFAs with the Bcl-2 protein into the intracellular environment of living human leukemia T cancer cells overexpressing the target protein using state-of-the-art in-cell NMR methodologies which were recently developed in our laboratory.

(C) Investigation of the in vitro cytotoxic activity of FFAs in cancer cell lines of leukemia.

The characterization of receptor–ligand interactions in their natural cellular environment would be a critical contribution to the efficacy of targeting approaches in bioorganic, medicinal and biological chemistry. The ligand-observed STD and TR-NOE NMR techniques rely on the rapid and efficient transfer of spectroscopic characteristics between the free and the bound state of a ligand (mM–μM affinity range). As these techniques require only small amounts of purified receptor (pM–nM concentrations), they are widely used to probe receptor–ligand interactions. Nevertheless, because of the complex network of macromolecules simultaneously exerting different biological activities at the cellular level, binding and structural studies using purified proteins in solution, often fail to reflect the true nature of the cellular environment.

The above significant drawbacks can be circumvented by using in-cell NMR spectroscopy, which has been particularly developed in recent years and focuses on structural studies of metabolites inside living cells. One of the recent applications of in-cell NMR spectroscopy, which has raised considerable interest of the scientific community as well as in the pharmaceutical research internationally, is its use in eukaryotic cell studies. This is particularly important as prokaryotic cells exhibit a limited range of biological activities and many cellular processes that determine significant aspects of modern biological research are absent in bacteria. This methodology will be applied in the present research proposal to study the interaction of selected unsaturated FFAs either isolated or in mixtures with the anti-apoptotic Bcl-2 protein at a cellular level. This protein has a particularly important role in the regulation of apoptosis and has been found to be overexpressed in various types of cancer, mainly in leukemia.

A. Fast screening of bioactive unsaturated lipids with the serum albumin protein (SA) and the anti-apoptotic Bcl-2 protein in vitro

The study of the interaction of bioactive unsaturated FFAs with the individual SA and Bcl-2 proteins in solution will be performed:

(i) by characterizing the epitope and the binding constant of FFA as well as their binding site to the protein and

(ii) by determining the preferred conformational orientation of these molecules upon binding.

B. Study of structural properties of FFAs interaction with the Bcl-2 target protein at the cellular level using in-cell NMR methodology

The fundamental research question which should be answered, is the investigation of the specific fatty acid components which interact with the target Bcl-2 receptor. A new methodology will be applied using selective inhibitors of the target protein that bind to specific sites through competition experiments. If this region coincides with the binding site of the bioactive molecule, the selective inhibitor will replace this molecule due to higher binding affinity. Consequently, the signals of the molecule binding epitope will be diminished upon addition of the selective inhibitor indicating that they have a common binding site. Furthermore, we have recently demonstrated that in-cell TR-NOESY NMR experiments can be utilized to investigate the interaction of a ligand (amino acid derivative of a flavonoid) with Bcl-2 in living cancer cells. The induced changes in the phase of the NOE cross-peaks can, thus, be utilized to investigate the biological conformation in living cells of free fatty acids.

C. Investigation of cytotoxic activity in vitro in cancer cell lines

In this part of the research proposal, the evaluation of the cytotoxic activity of free fatty acids will be performed for those that will have shown a significant interaction in the in-cell NMR experiments.

This research proposal will be expected:

(i) to strengthen my research group and to increase the reputation of our research in a field which is considered to be a hot topic internationally,

(ii) to give the opportunity of employment for a high-quality post-doctorate scientist with experience in-cell NMR methodologies, and of two PhD students,

(iii) To provide economic support for consumables, access to costs for equipment or other resources, dissemination and communication of our results.

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