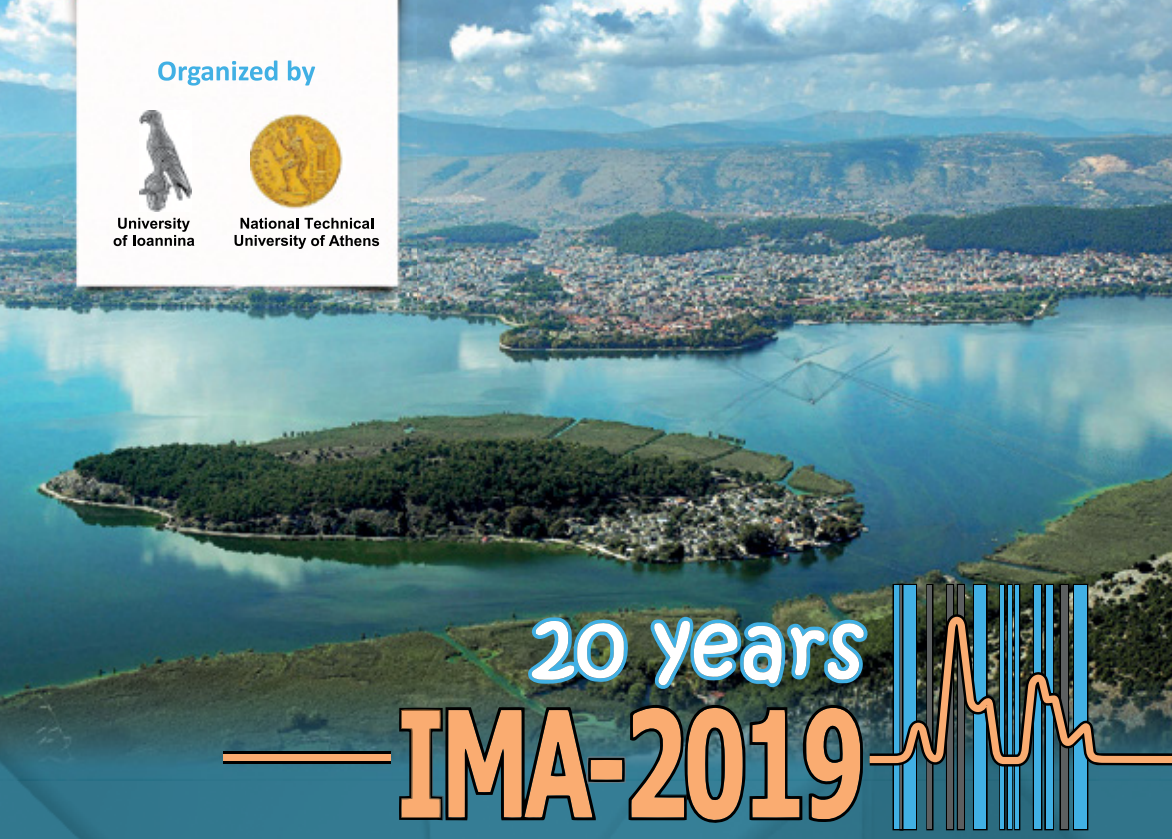

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Abstract Book

OP52 IN-CELL NMR SPECTROSCOPY APPLIED IN THE STUDY OF LIGAND-PROTEIN INTERACTIONS IN LIVING CANCER CELLS

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In-cell NMR spectroscopy is a noninvasive analytical technique, which reveals structural and conformational information for the study of protein-protein and ligand-protein interactions directly in the intracellular environment of living cells, at the atomic level, under physiological conditions [1,2]. This methodology has been successfully applied in the analysis of ^{15}N isotope-labeled proteins, overexpressed in *E. coli*, where ^{1}H-^{15}N HSQC in-cell NMR is performed directly in intact cells. However, current methodologies are inadequate at charting intracellular interactions of nonlabeled proteins [3].

Herein, we describe for the first time the application of in-cell NMR analytical methodology in the monitoring of the interaction of a bioconjugate of quercetin with the antiapoptotic protein Bcl-2 inside living human cancer cells without requiring prior isotopic labeling of the target protein. STD and Tr-NOESY NMR were employed to evaluate the direct binding of the ligand to the nonlabeled Bcl-2 protein intracellularly, which was further validated *in vitro* [4]. All the aromatic protons of the ligand were found to interact with receptors intracellularly, whereas competition experiments with a selective inhibitor of Bcl-2 clearly indicated the direct binding of the bioconjugate to the BH3 domain of the protein. Tr-NOESY in-cell NMR was recorded to investigate the preferred conformation of bound quercetin-alanine. Two new Tr-NOE crosspeaks of the ligand inside the intact cells were detected, suggesting the adaption of a new conformation of the bioconjugate upon binding. This approach has proved a very promising strategy for the real-time screening of the interaction profiling of drugs with their therapeutic targets in their native cellular environment in living eukaryotic cells, paving the way to the new field of intracellular rational drug design [4].

Acknowledgments

The research was implemented with an IKY fellowship from the State Scholarships Foundation of Greece, funded by the Act ‘Supporting Postdoctoral Researchers’ from the resources of the NF ‘Human Resources Development, Education and Lifelong Learning’ 2014-2020 and co-funded by European Social Fund-ESF and the Greek State.

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