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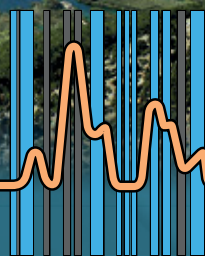
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Abstract Book

**A. Primikyri¹, N. Sayyad^{1,2}, G. Quilici³, E. I. Vrettos¹, K. Lim⁴, S.W. Chi⁴,
G. Musco³, I. P. Gerothanassis¹, A. G. Tzakos¹**

¹*Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Greece*

²*Department of Pharmaceutical Chemistry, College of Health Sciences, University of KwaZulu-Natal (Westville Campus), Durban, South Africa*

³*Biomolecular NMR Laboratory Genetics and Cell Biology, S. Raffaele Scientific Institute, Milan, Italy*

⁴*Disease Target Structure Research Center, KRIBB, Daejeon, Korea*

Email: aleprimik@gmail.com

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 ¹⁵N isotope-labeled proteins, overexpressed in *E. coli*, where ¹H-¹⁵N 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].

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References

- [1] Barbieri L, Luchinat E and Banci L (2016) *Nat Protoc* 11, 1101-1111.
- [2] Selenko P and Wagner G (2007) *J Struct Biol* 158, 244-253.
- [3] Li H and Sun H (2014) *Metallomics* 6, 69-76.
- [4] Primikyri A, Sayyad N, Quilici G, Vrettos EI, Lim K, Chi S-W, Musco G, Gerothanassis IP and Tzakos AG (2018) *FEBS Letters* 592, 3367-3379.

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