NUS/T1ρ-NMR techniques for studying receptor-ligand interactions on living cells surface

Clementina Acconcia[1], Biancamaria Farina[2], Maria Teresa Gentile[1], Sonia Di Gaetano[3], Domenica Capasso[4], Annarita Del Gatto[3], Antonella Paladino[5], Michele Saviano[6], Roberto Fattorusso[1], Laura Zaccaro[3] and Luigi Russo[1].

[1]Department of Environmental, Biological and Pharmaceutical Science and Technology, University of Campania - Luigi Vanvitelli, via Vivaldi 43, 81100 Caserta, Italy.
[2]Advanced Accelerator Applications, a Novartis Company, via Vivaldi 43, 81100 Caserta, Italy.
[3]Institute of Biostructures and Bioimaging-CNR, via Mezzocannone 16, 80134, Naples, Italy.
[4]Interdepartmental Center of Bioactive Peptide, University of Naples Federico II, Via Mezzocannone 16, 80134 Naples, Italy.
[5]Department of Science and Technology, University of Sannio, via Francesco de Sanctis, Benevento 82100, Italy.
[6]Institute of Crystallography-CNR, via Amendola 122/O, 70126 Bari, Italy.

*e-mail: clementina.acconcia@unicampania.it

Nuclear Magnetic Resonance Spectroscopy represents a powerful technique for studying, at atomic resolution, protein-protein and protein-ligand interactions, directly in the intracellular environment or on the membrane surface of living cells. In- and on- cell NMR methods for observations of ligands, including transfer NOESY (trNOESY) and saturation transfer difference (STD). However, the application of both NMR methodologie is limited by the short lifetime of cells in an NMR tube, which often prevents the acquisition of experiments longer than few hours [1,2]. To overcome these limitations, we developed an on-cell NMR-based strategy for describing the molecular determinants driving the receptor-ligand recognition process under native conditions. This approach relies on the combination of high-resolution NMR data with Molecular Dynamics simulations and Molecular Docking studies. The key point of our strategy is the application of Non-Uniform Sampling (NUS) and T1ρ-NMR techniques to collect atomic-resolution structural and dynamics information on the receptor-ligand interactions using living cells. We tested our approach to characterize the recognition mechanism of the $\alpha_v\beta_5$-integrin by RGDechi15D peptide [3,4]. Our data demonstrate that the developed strategy represents an alternative on-cell NMR tool for studying, at atomic resolution, receptor-ligand recognition mechanism on living cells surface.

References

[1] E. Luchinat, L. Banci IUCrJ. **2017**, 4, 108-18.
[2] E. Luchinat, L. Banci J Biol Chem. **2016**, 291, 3776-84.
[3] B. Farina, I.de Paola, L. Russo, D. Capasso, A. Liguoro, A. Del Gatto, et al. Chemistry **2016**, 22, 681-93.
[4] D. Capasso, A. Del Gatto, D. Comegna, L. Russo, R. Fattorusso, M. Saviano, et al. Molecules **2020**, 25.