A novel integrated NMR-based approach for studying receptor-ligand interactions on living cells surface

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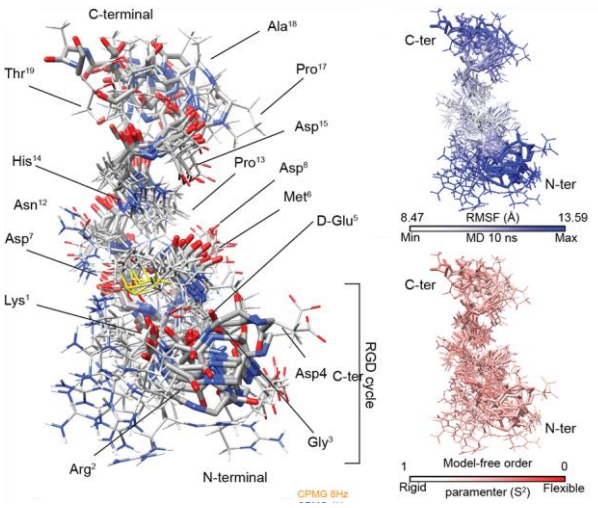
Introduction

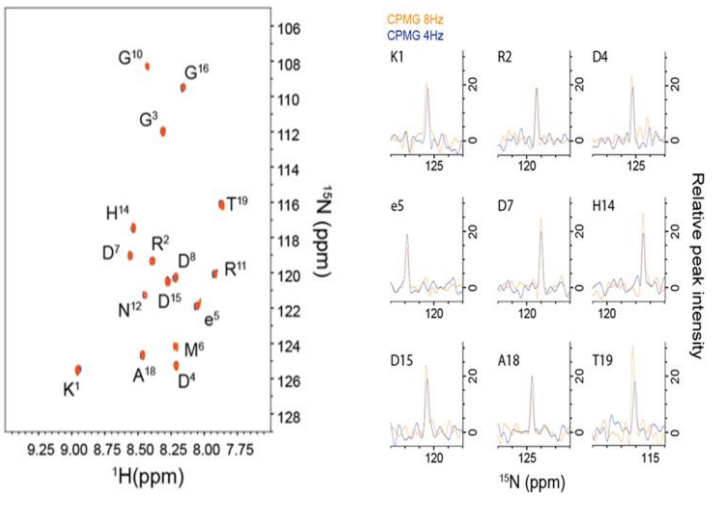
Nuclear Magnetic Resonance Spectroscopy represents a powerful technique for studying, at atomic resolution, protein-protein and protein-ligand interactions, directly in the intracellular environment or on the membrane surface of living cells. In- and on-cell NMR methods as transfer NOESY (trNOESY) and Saturation Transfer Difference (STD), are widely used to characterize binding of ligands to membrane receptors on the cell surface. However, the application of both NMR methodologies is limited by the short lifetime of cells in an NMR sample tube, which often prevents the acquisition of experiments longer than few hours [1,2]. Therefore, to overcome these drawbacks, we developed an alternative approach based on the application of Non-Uniform Sampling (NUS) and 1D T1ρ NMR techniques to collect structural and dynamics information on the receptor-ligand interactions with living cells, that in turn can be used as conformational constraints in computational studies. We tested our approach to explore the recognition mechanism of αvβ5-integrin by RGDechi15D [3,4]. This peptide is a selective cyclic molecule able to interfere with tumor proliferation and progression and to regulate angiogenesis in endothelial cells.

Results

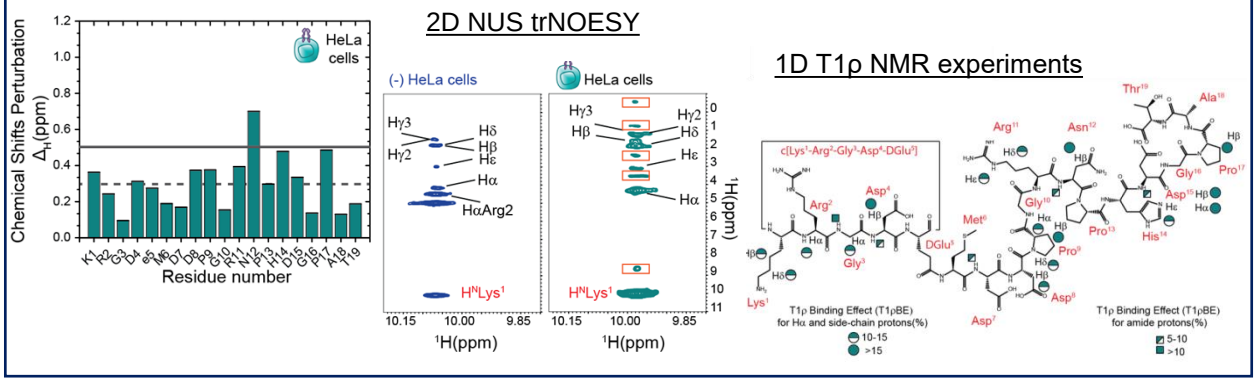
ps-ns dynamics of RGDechi15D



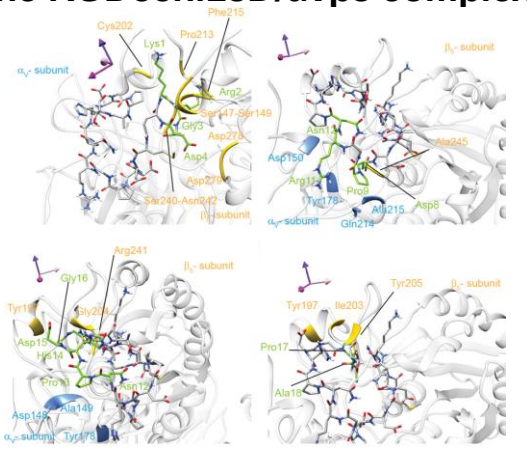
μs-ms dynamics of RGDechi15D by natural-abundance NMR spectroscopy



Mapping of the binding interface between RGDechi15D and αvβ5 integrin on living HeLa cancer cells surface



The RGDechi15D/αvβ5 complex



Conclusions

Herein, we developed and applied an alternative methodology for studying receptor-ligand interactions with living cells at atomic resolution. This application combines high-resolution structural and dynamics NMR data with Molecular Dynamics simulations and Molecular Docking studies. Our developed methodology was successfully applied in the investigation of the direct binding of the RGDechi15D peptide with the αvβ5 integrin with living human cancer cells. The structural model of RGDechi15D/αvβ5 complex indicates that the recognition mechanism is mainly mediated by the residues of the RGD motif and it is further stabilized by the residues located in the C-terminal region of the peptide. This approach represents an alternative 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.