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Study of protein interactions by using in-cell NMR spectroscopy

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In the past few decades, most of the biological processes, such as protein-protein/ligand interactions and atomic resolution structures, have been studied under physiological or "near-physiological" conditions using *in vitro* NMR spectroscopy. With the advent of in-cell NMR spectroscopy, most of the biological interactions can now be studied within a cellular environment and provide information at atomic level resolution under physiological conditions. The critical components and considerations required to study protein-protein structural interactions inside a living cell by using NMR spectroscopy (STINT-NMR) will be described. STINT-NMR entails sequentially expressing two (or more) proteins within a single bacterial cell in a time-controlled manner and monitoring their interactions by using in-cell NMR spectroscopy. The resulting spectra provide a complete titration of the interaction and define structural details of the interacting surfaces at the level of single amino acid residues. We discuss the advantages and limitations of STINT-NMR, the differences between studying macromolecular interactions *in vitro* and *in vivo* (in-cell), the design of STINT-NMR experiments, focusing on selecting appropriate overexpression plasmid vectors, sample requirements and instrumentation, and the analysis of STINT-NMR data, with specific examples drawn from published works. Applications of STINT-NMR, including an in-cell methodology to post-translationally modify interactor proteins and an in-cell NMR assay for screening small molecule interactor libraries (SMILI-NMR) are presented.

Biography

Alexander Shekhtman has completed his Ph.D. at the age of 30 from the State University of New York at Albany and postdoctoral studies from the Rockefeller University in New York. He is an Associate Professor in the Department of Chemistry at the State University of New York at Albany and CEO of Palm Biologicals, LLC. He has published more than 50 papers in peer reviewed journals and is a series editor of Methods in Molecular Biology.

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