Biomolecular NMR Spectroscopy - An in Sight to Protein Structure and Protein-Ligand Interactions

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ABSTRACT

Nuclear magnetic resonance (NMR) spectroscopy is a very powerful technique for the investigation of structure, chemical kinetics and dynamics of biomolecules. It is very useful techniques to find out molecular interactions, such as protein-protein and protein-ligand interactions. The process of determining a solution structure of macromolecules by NMR is by measuring thousands of short and long proton-proton distances and angles, and restraining the protein structure using this information computationally. We have carried out NMR based structural and functional studies of various proteins from methicillin resistant Staphylococcus aureus. Chemical shift mapping was also performed to investigate new compounds that can interaction with these proteins and can serve as potential lead to treat bacterial infections.

Saturation Transfer Difference (STD)-NMR is a biophysical technique for the identification of moderate to weak affinity ligands. It requires a large excess of ligand to protein ratio, and ligand must be in a fast exchange regime on chemical shift on the NMR timescale (i.e. ~ ms time scale). In this experiment, the resonances of protein are irradiated (saturated) selectively, this saturation is spread over the entire protein through spin diffusion, and from there to protons of ligands (if bound), because it behaves as part of the large protein complex and thus has a reduced mobility in solution. Ligands that are in exchange between the bound and the free form, will transfer this saturation into solution where it is detected. Information embedded in STD-NMR can be used to map the proximity of ligand protons on the protein surface (group epitope mapping, GEM). Protons which are close in proximity to protein will be saturated to the highest degree, and show give strong STD intensities, while protons which are located at longer distances to protein will appear with weaker STD intensities.

We have used STD-NMR spectroscopy as the primary technique for the identification of new anti-viral agents against SARS-CoV-2 Main protease (Mpro) and Papain Like Protease (PLpro), Non-Structural protein of dengue virus. These viral proteins were expressed in *E. coli* (BL21(DE3)), and Rosetta competent cells and purified using affinity and size exclusion chromatography. Following the STD-NMR studies, compounds that showed interaction with enzyme urease were evaluated for their thermal stability through DSF, which further validated the results of STD-NMR screening.

In the presentation the application of NMR spectroscopy for biomolecular structure determination and molecular interactions will be discussed.