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Summary

The current thesis extends the applicability of *in-vitro* ligand-based solution-state ^{1}H NMR methods to analyze organophosphate pesticides (OP)-protein interaction and OP metabolite-protein interaction. The robustness of the NMR methods employed in the thesis emphasizes the utility and advantage of NMR approach in the field of medicine, agrochemicals, pharmaceuticals, and materials. The advantages of multi-spectroscopic methods coupled with computation techniques, are clearly demonstrated throughout the chapters by unraveling the kinetic parameters pertaining to the OP-protein interaction. The thesis reveals the impact of OP on the secondary targets and enables the extraction of quantitative parameters of such interactions by employing NMR spectroscopy complemented with ITC, fluorescence quenching and, molecular docking providing insight of the interaction. The thesis aims to demonstrate the usefulness of magnetization transfer-based ligand detected ^{1}H NMR method, namely the Saturation Transfer Difference (STD) NMR and the spin-lattice relaxation analysis for monitoring OP-protein interaction. It is established in the thesis that both these methods are equally capable of quantitatively characterizing the binding event. In one hand, relaxation analysis are suitable for ligands that degrade faster in solution while on the other hand STD NMR allows mapping of the spatial proximity of the ligands NMR active nuclei with the protein binding sites. The thesis successfully points out that the toxic effects of OP and their metabolites within humans and animals depend on the dose, frequency of exposure, and a chemical constituent of the OP.

The thesis evaluates the binding interaction of OP parent molecules with serum proteins employing STD NMR in a systematic approach. Determination of binding efficacy followed by Group Epitope Mapping (GEM) has been achieved through STD NMR. Besides GEM, dissociation constant (K_D), binding site determination by competition STD NMR, and also the competition STD NMR for a mixture of ligands are reported. To determine and support NMR results, molecular docking and ITC are performed for all the ligands. Further, to gain insight into OP metabolite-protein interaction, especially TCPy and PM interaction with BSA, the ^{1}H spin-lattice relaxation analysis approach is applied. This particular set of spin-lattice relaxation rate measurements are carried out to quantify the metabolite-protein interaction before the onset of degradation of the metabolite into further smaller fragments. Relaxation experiments have been proven to be useful specifically in case of unstable OP metabolites such as PM that have a very short half-life and thereby making STD NMR approaches invalid. The first two working chapters clearly point out the following: (i) the halogen-containing OP and its metabolite shows the highest binding affinity towards protein (ii) metabolites interaction is stronger than OP parents (iii) structure plays an important role in the binding of OP-serum albumin (iv) BSA imparts catalytic effect (degradation promoter) for OP oxons. The thesis further deals with the interaction of OP and their respective metabolites with a water-soluble globular digestive protein, namely trypsin. The interaction of OP with trypsin has been quantified by addressing the dissociate constant, GEM, and possible inhibitory effect of OP and metabolites on the enzymatic activity of trypsin. It is found that the OP metabolite interaction is stronger compared to OP parents-trypsin, and as expected, halogen-containing metabolite shows the highest affinity towards trypsin than others. Also, OP-trypsin interaction is less strong compared to that of OP-BSA. This is supported by the fact that in literature, it is already mentioned that OP exhibits less affinity towards enzymes than proteins.

The present thesis offers a valid and relatively fast protocol for OP-protein interaction analysis. Ligand-based solution-state ^{1}H NMR spectroscopy complemented with molecular

docking, ITC and, fluorescence quenching analysis provides a complete characterization of OP-protein binding event. The thesis silently compares the two NMR methods *viz.*, STD NMR, and relaxation measurements. It is clearly indicated that STD NMR is advantageous in terms of GEM, binding constant, and binding site determination, albeit, the limitation is in terms of long experimental time and less sensitivity. Also, STD NMR is non-functional for high-affinity complexes. In such cases, spin-lattice relaxation measurement becomes a viable alternative that provides information regarding kinetic parameters. The future perspective of the thesis would be to implement a similar set of experiments to study OP interaction with other biologically relevant proteins. Also, the design of biomarkers for OP and their metabolites will be undertaken based on the current findings. Toxicological details would be furnished by investigating these OP-protein systems in *in-vivo* fashion.

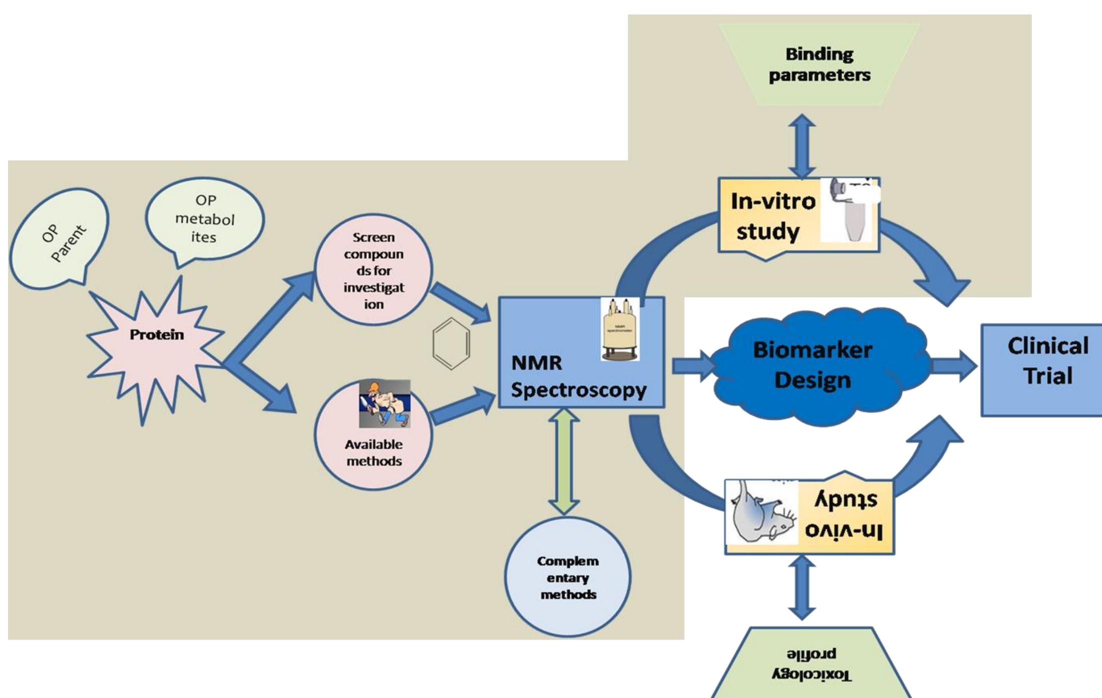

Figure 7.1 : The grey area represents the work completed, and the light area is to show the future work.