Dr. Avital Braiman,

Director of Editorial,

Journal of Visualized Experiments

Dear Dr. Braiman,

Please find enclosed a manuscript titled ‘***Distinguishing Allosteric effects from orthosteric binding in Protein-Ligand***’ to be considered for publication in *Journal of Visualized experiments*.

We thank the editors of Journal of Visualized experiments for inviting us to contribute this article on separating allosteric effects from orthosteric binding. Deciphering allosteric relays in protein complexes and distinguishing them from orthosteric effects is of great importance in drug discovery. HDXMS has proven to be a powerful tool to probe protein dynamics in response to various perturbations. Here, we have described the used of two different facets of HDXMS: to monitor orthosteric binding and effects, from differences in deuterium exchange, and to identify allosteric effects from kinetic analysis. Our results clearly demonstrate the use of this technique in identification of early orthosteric binding events and later allosteric events in the Heat shock protein, Hsp90, during ligand/fragment binding. This approach has a categorical implication in fragment based drug design and lead-compound optimization.

This manuscript is of great importance since it describes in detail an experimental HDXMS approach to distinguish between two distinct responses elicited by ligands/fragments: Orthosteric binding and Allosteric effects. These have broad significances in accelerating the understanding of allosteric effects and in fragment-based drug design. Importantly, this highlights the power of amide hydrogen/deuterium exchange mass spectrometry (HDXMS) in distinguishing between binding and allosteric events in protein-ligand interactions by deuterium uptake and kinetic analyses.

We hope you will find our manuscript of interest to readers of *Journal of Visualized experiments* and consider it for publication.

Thank you.

Sincerely

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