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Dear Dr. Myers, Science Editor, JoVE

Following up our conversation on April 10th and after receiving your invitation to publish a full article, we are pleased to submit our manuscript entitled: "Extracellular Protein Microarray Technology for high throughput identification of low affinity receptor-ligand interactions" by Husain et al., to be considered for publication in JoVE.

Secreted factors and plasma membrane-expressed proteins (collectively referred to as extracellular proteins) essentially regulate multiple physiological and pathological processes, and therefore represent main targets for therapeutic development. In most cases, these proteins exert their functions through interaction with other proteins in the extracellular space. Nevertheless, despite their importance and abundance (more than 5,000 proteins in the human genome), the protein-protein interactions that take place in the extracellular environment remain poorly understood. This gap is mainly due to the challenges associated with the study extracellular proteins, and in particular detection of binding partners, which often establish low affinity interactions that are difficult to detect using common methodologies. We have previously developed the Protein Microarray Technology, which coupled to an extensive library of purified proteins, allows high throughput interrogation of binding partners for targets of interest. Furthermore, we have implemented a method based on increased protein multimerization using microbeads, an approach that has been shown to significantly improve detection of transient interactions, such as the ones often established between cell surface receptors. This technology has proven key for identification of novel interacting partners, which have opened new avenues to study the basic biology of multiple extracellular targets, from nervous cell receptors to viral immunomodulators. In this manuscript, we provide a detailed description of the methodology developed, from selection of protein candidates, to microarray slide printing, hybridization assays as well as data analysis for hit calling. Key elements for success, advantages over other methods and potential pitfalls are also discussed. This approach represents a robust, sensitive and high throughput method for binding partner discovery, focused on extracellular proteins, one the most challenging protein types.

Given the applicability of this technology to the elucidation binding partners for any extracellular protein under study, we are confident the experimental procedures described here will be of interest to a broad scientific audience, including Cell Biologists, Structural Biologists, Biochemists and any Scientists alike.

Thank you in advance for considering this manuscript for publication in *JoVE.*

Sincerely,

Nadia Martinez-Martin, Scientist, Receptor Discovery Group, Genentech, Inc.