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# Protein-Protein Interaction goes Multiplexed: Implications on Proteomes and Interactomes

**Chi Zhang***

Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts, USA

*Corresponding Author: Chi Zhang, Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts, USA; E-mail: chizhang@genetics.med.harvard.edu

The localization, function and activity of a protein are very often regulated by its interactions with other proteins in complexes, and as such, regulation of protein-protein interaction constitutes a central theme of numerous biological processes. For example, assembly of the DNA repair machinery at the loci of DNA damage involves specific recruitment of numerous proteins, such as Ataxia Telangiectasia Mutated (ATM) and Ataxia Telangiectasia and Rad3-related protein (ATR), via protein-protein interaction [1]. Therefore, studying protein-protein interactions is crucial for biological research.

Traditionally, a very commonly used method for analysis of protein interactions has relied on co-Immuno Precipitation (co-IP), which involves immobilizing the protein of interest on solid beads through affinity reagents such as antibodies, followed by detection of interacting protein via candidate approaches such as western blotting, or unbiased approaches such as mass spectrometry [2-5]. This approach of co-IP allows isolation of multi protein complexes in cell lysates that presumably preserve their physiological interactions. Additional methods involve using reporter systems such as yeast two-hybrid assay which uses transcriptional activity as a readout, or FRET (Fluorescence Resonance Energy Transfer) microscopy which relies on proximity information between putative interacting proteins. However, these methods suffer from various drawbacks. For example, yeast two-hybrid assay relies on expression of exogenous proteins tagged with various components in yeast, which may or may not be a physiological context for studying the interaction; FRET microscopy examines proximity of two proteins but not necessarily interaction between the two. As such, co-IP has become very popular for identification of protein-protein interactions.

One significant drawback of the current approaches is that they do not allow multiplexed detection of protein-protein interaction. For example, in co-IP, the researcher needs to immobilize the protein of interest, in order to pull down interacting proteins. In order to examine the interactome of a few proteins, parallel co-IP experiments need to be carried out using respective antibodies. This places a significant time and economic limitation on interactome studies on a large scale, and therefore new methods are needed that would allow multiplexed protein-protein interaction studies.

Single-Molecular-Interaction sequencing (SMI-seq) technology combines the power of protein engineering and polony-based DNA sequencing, and significantly improves the detection power of single-molecule protein-protein interaction [6] (Figure 1). This method takes advantage of the engineered enzyme tag Halo Tag, which allows conjugation of tagged protein with double-stranded DNA. Conjugation of individualized bar coding DNA onto each protein allows detection of individual proteins in a complex protein mixture

through in situ sequencing. To visualize protein-protein interaction, this method takes the advantage of the fact that bar coding DNAs of interacting proteins are physically colocalized, and can be amplified into colocalized polonies. Therefore the protein-protein interaction information can be read out by analyzing co localization of different barcodes in a random single-molecule protein array. Importantly, both colocalized and non-colocalized proteins are measured, and the co localization ratio is a quantitative measure of the relative strength of interaction. Additionally, this method can be easily adopted to assay multiple proteins in a single reaction, therefore dramatically increasing the power of proteomics research.

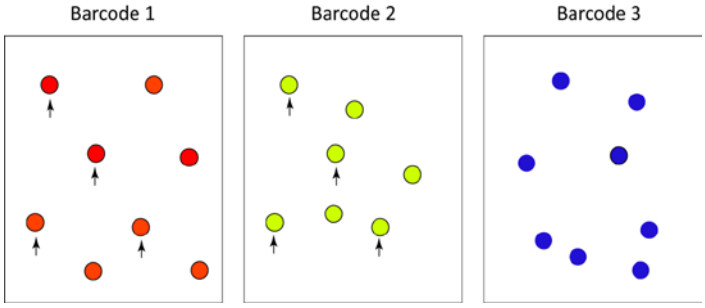


**Figure 1**: Illustration of co localization analysis in SMI-seq. 3 proteins are conjugated with 3 independent barcodes (shown as barcode 1, barcode 2 or barcode 3), and SMI-seq was used to detect co localization of barcodes. Barcodes 1 and 2 partially colocalize (shown by arrows), whereas barcode 3 failed to colocalize with the other two barcodes. Co localization of proteins labeled with different barcodes indicates interaction between respective proteins.

The SMI-seq technology enabled single molecule protein interactome studies via library-on-library screens, and will have broad potential applications in numerous biological questions. One direct application is to use this technology for systematic interactome mapping of genes implicated in human diseases, such as autism and psychiatric disorders [7]. This type of systematic interactome mapping has the potential of identifying common key players in diseases of relatively high genetic heterogeneity. SMI-seq might also enable in vitro identification of protein complex assemblies through examining co localization of multiple barcodes. On the other end, SMI-seq can also be rapidly adopted to screen for high affinity therapeutic agents such as antibody fragments or small molecules against many proteins in a single assay, and will therefore significantly increase the throughput of screening compared to available methods [8, 9].

In conclusion, SMI-seq can significantly advance the current methodology in studying protein-protein interactions, and enabled single-molecule, multiplex interaction profiling of the proteome. Application of this technology in areas such as human disease interactome and high affinity therapeutic reagent screening will very likely speed up discovery in both basic and clinical science.

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