International Journal Of Multidisciplinary Research In Science, Engineering and Technology (IJMRSET)

| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 4.988

| Volume 4, Issue 5, May 2021 |

New Biochemistry Method for Pharmaceutical Analysis in Proteins

Shuchita Bajpai

Assistant Professor, School of Pharmacy, Master in Pharmaceutical Analysis and Quality Assurance, Raffles University, Neemrana, Rajasthan, India

ABSTRACT: Proteins are essential pillars of primary structural organization of an organism and perform a wide variety of functions like- DNA replication, cellular networking, metabolic attributes, growth, transportation and signaling, apoptosis [1, 2]. Most of the cellular functions are accomplished by protein-protein interactions (PPI) with other proteinaceous and non-proteinaceous moieties [3]. The function of a protein is represented by the functional group present like- amide, thiol, carboxyl-amine, hydroxyl, *etc*., which also modulates the activity of a protein [4].
The study of protein-protein interactions (PPI's) is summarized under the title of 'Interactome Analysis' [6]. The interactome studies have drawn the attention of the majority of system biologists due to its crucial role in cellular networking and signal transduction. It is reported that more than 80% of proteins do not function alone, and they are inevitably found in close associations with other proteins or non-protein molecules *in-vivo* conditions [7, 8]. Based on their surface properties, the interactions can be transient (occurring for short duration) or permanent (stable for a longer time) having either homo or hetero-oligomeric residues. Transient PPI's will serve the purpose in signaling pathways, for instance, where protein-receptor complex assembles, they pass the signal/information and then dissociate back to individual components once the signal is being relayed. Permanent PPI's are either fixed or at least stand for a considerable time duration, contributing to their higher significance and non-substitutable nature. Based on stability factors, the size of PPI's complex may be larger or smaller to facilitate important cellular functions. Those proteins that serve as the nucleus for multiple associations have been reported to function as either enzyme complexes, central molecules in the signaling cascade or as transcriptional mediators. [5]

I. INTRODUCTION

In *in-vivo* based approach, the whole organism is used to carry out the experimental procedure to study PPI's in the cellular premise in their native interacting state. It includes techniques like Yeast Two-Hybrid systems (Y2H and Y3H) and Fluorescence Resonance Energy Transfer (FRET). Yeast Two-Hybrid system lies on the principle of binding between the interacting proteins, which in turn can activate the transcription of the reporter gene. The reporter gene product can be a fluorescent protein or chemiluminescent moiety. Different variants of the Yeast Two-Hybrid system have been worked out, giving a better answer to the complex puzzle of the functioning of the cellular arena. Fluorescence Resonance Energy Transfer (FRET) is another variant of Y2H system which uses Fluorescent surface epitopes that absorb light of one wavelength and emits it at another wavelength[1]. The different wavelength emitted falls in the distinct spectra of light (mostly in the visible range) which can be observed either directly or using computer-based software.

Several efficient protein interaction studies have been carried out using the pull-down assay [2].

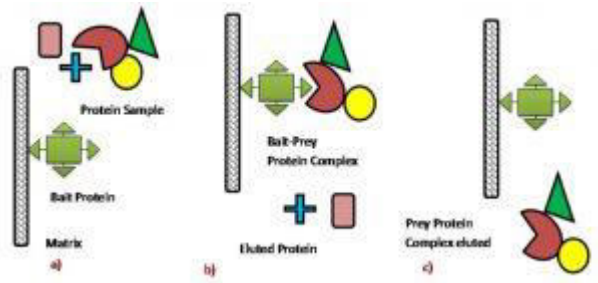

Fig.1: STEPS IN PULL DOWN ASSAY

They identified strategies to characterize individual macromolecular complexes by Affinity purification - Mass Spectrometry (AP-MS) by either high-density data acquisition (indirect approach) or by biochemical coupling of AP-

| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 4.988

| Volume 4, Issue 5, May 2021 |

MS (direct approach). Moreover, studies in *S. cerevisiae* showed that AP-MS could be used efficiently for high throughput interactome studies [1]. The cellular fraction contains all the protein complexes, which can be purified using the concept of affinity purification [16]. Currently, the major challenge faced is not of protein characterization but protein purification.

II. DISCUSSION

The protein interactome studies deal with the identification of the target protein from the total cell fraction and its purification by a variety of approaches, one being pull-down assay The proteins interacting with the prey protein can also be co-precipitated along with it, thus giving an insight into the structural organization of the prey protein complex additional to the prey protein interactome assembly. The bait protein can be attached to the column in two different ways; - (i) using a ligand or (ii) using an Antibody specific for the bait protein.

Ligand must be firmly bound covalently to the bait protein so that it does not get dissociated with the bait protein at the time of elution. Also, it should not alter the specificity of the active site in the bait protein. Various polymers can effectively serve the purpose of a linker. Antibodies can be used to immobilize the bait protein in the column. The specificity provided by antibodies is more to ligands used. A group of researchers experimentally expressed MBP-tagged CagX protein from *H. pylori* in *E. coil* strain DH10β. Specific antibodies namely, Anti-His and Anti-MBP were used to find out the His-CagX (prey protein) and another MBP/MBP-tag (used as a bait protein) [3]. Points to keep in consideration while dealing with antibodies is that chances of cross-reactivity are high.

III. GENERAL STEPS INVOLVED IN PULLDOWN ASSAY

a) Binding of bait protein to the matrix. This binding is the generally strong covalent type where the Bait protein is attached to the matrix before the experiment. b) Formation of Bait-Prey Protein complex. The bait protein has a selective affinity towards the target protein, which causes it to stop and stand by the matrix. c) Elution of the Prey Protein Complex.

Thus the Pull -down assay can help to resolve many challenges faced till date which includes;

- Functional activity of the protein can be studied
- Used for those protein complexes that cannot be studied using recombinant methods
- Promises to improve sensitivity, time consumption and accuracy in term of western blot analysis
- Cost-effective.

Despite having such a high efficiency of purification, this technique is not entirely free from flaws. Since it relies on fluorescent signals emitted, any contamination or similar protein can give false results. Moreover, the separation results are based on affinity interaction and not size and density. Also, the data obtained from software analysis requires correct interpretations. Overcoming these hurdles can make pull-down assay as an ideal technique for the study and analysis of PPI's.

B) Co-Immuno precipitation:

Co-Immuno precipitation (Co-P) is an analogs technique to immune precipitation. A specific antibody is used to bind appropriately to the domains of the target protein. Upon binding of prey protein to the antibody, other interacting proteins also elute out together. Washing steps result in the isolation of the desired binding complex . The binding complex can then be resolved using SDS-PAGE and Western Blotting technique [4].

Advantages:

- Used to confirm novel protein-protein interactions.
- Analyze the effect of mutant protein on a normal binding.
- Being cost-effective stands as a gold standard to study protein interactions.

Major limitation faced include; to confront the transient type of protein interactions, sometimes protein interacting site may be overlapping with the antibody binding site, which gives a false negative result for no binding. Variations in antibody binding fashion to the same protein due to different affinities and domains present. Epitope tags can hinder in complex formation or the appropriate antibody binding. Additionally, the technique cannot function in the presence of strong ionic detergents like SDS. When interacting proteins Expression levels are low, a false signal can be obtained

| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 4.988

| Volume 4, Issue 5, May 2021 |

due to low binding or weak binding [5]. Efforts are desired to enhance the Co-P technique so that rapid and number of protein or peptide bindings can be studied.

C) Co-expression Studies: Cellular dynamics have active networks where different biomolecules interact to carry out a specific function. Proteins have different motifs and domains contributing to its solubility in the aqueous medium. When a protein has hydrophilic residues in the motif, they become readily soluble in the aqueous medium, but it is not the same case with hydrophobic proteins.
Protein A and B initially forms inclusion bodies, thus hampering in their studies. When the genes for particular proteins are co-expressed in a common vector, the product formed is in a conjugated form masking the hydrophobic residues or making it soluble in the solution which can then be isolated using Pull down or Western Blot analysis.

D) Circular Dichroism: In the cell, proteins not only interact with other proteins but also to some non-proteinaceous molecules making their analysis and isolation even more difficult. Rapid detection and characterization of novel proteins are of great importance in the field of Genomics and Proteomics. High throughput techniques like X-ray crystallography and NMR sometimes becomes impractical or difficult in application. In such a situation, Circular Dichroism (CD) can demonstrate its potential to resolve the complex 3-D and 2-D structure of a protein [6].

Advantages:

- To compare macromolecules similar in structure.
- To check the correct folding of a new or purified protein.
- Folding pattern in a mutant protein concerning the wild-type.
- To check the activity of a biopharma product.
- Can resolve the conformation of soluble proteins [7].

E) Density Gradient Centrifugation: In the world of genomics and proteomics, large macromolecular complexes can be sorted into their monomeric units based on their molecular masses and the order of varying densities [1]. Density gradient centrifugation (DGC) involves centrifugation of biomolecules in a medium having a spectrum of densities, ranging from lighter to denser. When the interacting proteins are subjected to graded densities, under the influence of the centrifugal field, they migrate through the medium and localize themselves according to their densities

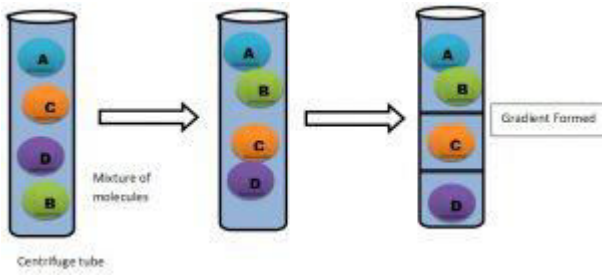

FIG. 2: DENSITY GRADIENT CENTRIFUGATION

IV. CONCLUSION

The proteins are mischievous moieties that switch their functions almost immediately even with minor changes in the surroundings, and so their native state isolation pose a big issue, and that is what needs to be addressed.[9] Techniques to isolate these interacting complexes serve as the tool which can be used to dig out the answers to various unsolved puzzles of cell biology. Some techniques may give excellent yield for one set and adverse outcomes for another type of proteins. It does not mean that one technique is better than the other. The choice must depend on the physio-chemical and topological properties of the protein of interest. Based on the knowledge gained, different groups can extend their findings and can help to place a piece of the puzzle, eventually completing the picture of cellular, molecular dynamics which is governed predominantly by the proteins. Also, a better understanding of the principles can help to design new outlets which can promise even better results in the future.[10]

| ISSN: 2582-7219 | www.ijmrset.com | Impact Factor: 4.988

| Volume 4, Issue 5, May 2021 |

REFERENCES

1. Aze A and Maiorano D: Recent advances in understanding DNA replication: cell type-specific adaptation of the DNA replication program. F1000 Research 2018; 7.
2. Phizicky EM and Fields S: Protein-protein interactions: methods for detection and analysis. Microbiol Mol Biol Rev 1995; 59(1): 94-23.
3. Ardejani MS, Powers ET and Kelly JW: Using cooperatively folded peptides to measure interaction energies and conformational propensities. Accounts of Chemical Research 2017; 50(8): 1875-82.
4. Kluger R and Alagic A: Chemical cross-linking and protein-protein interactions-a review with illustrative protocols. Bioorganic Chemistry 2004; 32(6): 451-72.
5. Watson BS, Hazlett TL, Eccleston JF, Davis C, Jameson DM and Johnson AE: Macromolecular arrangement in the aminoacyl-tRNA. cntdot. elongation factor Tu. cntdot. GTP ternary complex. A fluorescence energy transfer study. Biochemistry 1995; 34(24): 7904-12.
6. Ng SK and Tan SH: Discovering protein-protein interactions. Journal of Bioinformatics and Computational Biology 2004; 1(04): 711-41.
7. Berggård T, Linse S and James P: Methods for the detection and analysis of protein-protein interactions. Proteomics 2007; 7(16): 2833-42.
8. Yanagida M: Functional proteomics; current achievement. Journal of Chromatography B 2002; 771(1-2): 89-06.
9. Pedamallu CS and Posfai J: Open source tool for prediction of genome-wide protein-protein interaction network based on ortholog information. Source Code for Biology and Medicine 2010; 5(1): 8.
10. Sarmady M, Dampier W and Tozeren A: HIV protein sequence hotspots for crosstalk with host hub proteins. PLoS One 2011; 6(8): e23293.