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A Comprehensive Study on Interactions Between Protein Molecules and Their Importance in Drug Discovery

Taneesha Gupta[1], Pankaj Malhotra[2,*]

Abstract

Protein-protein interactions (PPIs) are extremely specialized chemical and physical interactions that occur when two or more protein molecules come into contact with one another with the help of biochemical processes impacted by interactions between them, such as hydrogen bonding, electrostatic forces, and the hydrogen effect. Many interactions that occur in a specific bimolecular environment within a cell or living creature entail physical contact as well as molecular connections between chains. PPIs have a major impact on both the target protein's function and a molecule's capacity to bind into the targeted sites that show beneficial effects. The majority of proteins and genes understand that the final phenotype is a result of several interactions. PPIs include signal transmission, muscle contraction, membrane transport including active and passive transport, cell metabolism, and electron transport proteins. The understanding of protein interaction networks is extremely beneficial to the study of the signal transduction pathway. The majority of proteins and genes regard the actions that result in the phenotype as a group of interplays. There are some methods to carry out the procedure, which will include affinity purification, yeast 2 hybrid, tandem affinity purification, and other in vitro and in vivo methods, depending on factors such as cost, time, and other variables. When it comes to annotating the function of pharmacological molecules, the resulting data sets are also noisy and have a larger proportion of false positives. As a result, in silico methods, including chromosome proximity, gene fusion, sequence-based, and structure-based approaches, were created. This study will help to know more about PPIs and the database that is available to carry out the further interactions that happened in the protein molecules and also helpful in minimizing them.

Keywords: In vitro and in vivo methodologies, protein-protein interaction, databases on protein-protein interaction, experimental and computational methods, therapeutic targets

***Author for Correspondence**
Pankaj Malhotra
E-mail: malhotrap29@gmail.com

[1]Research Scholar, Department of Pharmacy, School of Health Sciences, Sushant University, Gurugram, Haryana India
[2]Assistant Professor, Department of Pharmacy, School of Health Sciences, Sushant University, Gurugram, Haryana, India

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INTRODUCTION

Protein-protein complex structure serves a variety of natural purposes. Cell-to-cell interactions, metabolic regulation, and experimental control are only a few of the many natural processes that protein-protein connections (PPIs) manage [1]. Trade between proteins is gradually becoming one of the central objectives of system biology. The non-covalent linkages between side chains of residues serve as the basis for PPI, protein folding, and protein assembly [2]. These linkages cause the proteins to form a variety of partnerships and associations. PPIs' distinct

structural and functional characteristics allow for a variety of classifications [3]. Based on their classification, they can be classified as homo- or heterooligomeric; obligate or non-obligate based on stability; flash, or unending based on continuity [4]. One instance of a homi complex is cytochrome c' [5]. The enzyme-asset complex between trypsin and the chymotrypsin is an illustration of a hetero complex [6].

A given PPI could be any combination of these three different dyads. Endless relations form a stable protein complex, whereas flash relations form signaling pathways. More than 80% of proteins, according to some reports, do not work alone [7]. Instead, they typically interact with other proteins or molecules like DNA or RNA to carry out specific biological tasks. It has been stated that proteins are the primary representation of natural function [8].

PPIs were characterized by De Las Rivas and Fontanillo as "physical connections with molecular docking between the proteins that do in a cell or in a living organism in vivo." The distinct physical links that certain proteins have evolved to fulfill are what give them their particular functions. Any structure can interact with other proteins, including double molecules, multiprotein complexes, and long chains [9]. Protein interactions can decode the result of nearly all biological operations. By examining the pathogen-host exchange network, scientists can uncover implicit medication targets with the use of PPI information [10, 11]. It is crucial to study PPIs to understand the functions that proteins perform in living things, such as cells.

AN ILLUSTRATION OF A PROTEIN-PROTEIN INTERACTION

PPIs are the new era of studying the interactions between molecules. When we are studying the methods that are helpful for studying or carrying out the experimental studies among them will include the electron transfer protein, contraction of muscles, and transduction of signals, which are described below.

Electron Transfer Protein

In several metabolic processes, an enzyme that acts as an electron carrier's reductase binds to a protein that serves as an electron carrier. It dissociates and binds to the incoming enzyme, which functions as its oxidase (i.e., an electron acceptor), after receiving an electron. To ensure efficient electron transfer, these relationships between proteins rely on a highly specialized list. Cytochrome c-reductase, cytochrome c, and cytochrome c oxidase are examples of system factors for the mitochondrial oxidative phosphorylation chain, microsomal, and mitochondrial P450 system [12].

In the instance of the mitochondrial P450 systems, the specific remainders implicated in the connection of the electron transfer protein adrenodoxin to its reductase were connected to two initial Arg remainders on the reductase's face and two acidic Asp remainders on the adrenodoxin [13]. Recent studies on the phylogeny of reductase have shown that the residues implicated in PPIs have not changed throughout the enzymes' existence [14].

Contraction of Muscles

The mechanics of muscle compression involve multiple relationships. By binding to actin, myosin fibers, which serve as molecular motors, enable hair to slide [15]. In cadaverous muscle, the family of liquid drop-associated proteins binds to regulate lipolysis within the muscle. Adipose triglyceride lipase and its coactivator gene identification-58 are among those that are activated in this way.

Transduction of Signals

The activity of the cell is managed by extracellular signals. Signals within and/or along the interior of cells are carried via interactions between the various signaling molecules. In addition to being crucial for many biological processes, signal transduction—the term for the recruitment of signaling pathways through PPIs is also important for diseases including Parkinson's disease and cancer.

GROUPING TECHNIQUES FOR IDENTIFYING INTERACTIONS BETWEEN PROTEINS

The in vitro, in vivo, and in silico methods are the three categories of PPI finding methods. An in vitro procedure is performed in a controlled setting away from a living organism. The in vitro techniques utilized in PPI identification include tandem affinity sanctification, affinity chromatography, coimmunoprecipitation, protein arrays, protein scrap complementation, phage display, X-ray crystallography, and nuclear magnetic resonance (NMR) spectroscopy [16]. The entire living organism is utilized throughout an in vivo process. In PPI discovery, yeast two-hybrid (Y2H, Y3H) and synthetic lethality are the in vivo styles [17]. On a computer (or) by computer simulation, in silico methods are carried out. The in silico techniques employed in PPI discovery, chromosome propinquity, gene emulsion, in silico two hybrid, glass tree, phylogenetic tree, and gene expression-grounded approaches include sequence-grounded approaches, structure-grounded approaches, and other approaches [18].

Method for Predicting Protein-Protein Interaction In Vitro

The development of valve trailing allowed for the study of PPIs in the natural cell environment. To test the yeast interactome, Gavin et al. [19] initially attempted the valve-trailing system in a high-return method [19]. The system's cornerstone is the target protein's double-trailing on its chromosomal locus, which is followed by a two-step sanctification procedure. Proteins that stay attached to the target protein can also be analyzed and linked using mass spectrometry analysis and SDS (sodium dodecyl sulphate)-runner [20], which identifies the PPI collaborator of the original protein interest. One major benefit of valve trailing is its capacity to measure the activity of monomeric or multimeric protein complexes that exit in vivo and to identify a wide variety of protein complexes [21].

The valve can be used to discover protein complexes and relations when used in conjunction with mass spectrometry (MS). Coimmunoprecipitation uses a whole cell extract to detect native proteins within a complex combination of biological components that may be required for a successful connection, hence verifying the relationship. Furthermore, post-translational modification is made possible by employing eukaryotic cells, which is essential for exchange but not possible in prokaryotic expression methods. Protein microarrays are quickly becoming recognized as a crucial tool for the analysis of proteins, the study of their expression conditions, and the investigation of protein relationships and activities. A protein microarray is a glass plate coated in colorful protein molecules placed at different locations in an ordered pattern [22].

X-ray crystallography [23] is a kind of true high-resolution microscopy that allows us to observe protein structures at the microscopic level, which advances our understanding of how proteins work. It illustrates, in particular, the conformational changes that occur in enzymes and the interactions that proteins have with other molecules. With this knowledge, we can also create novel medications that specifically target a target protein. Recently, researchers have become interested in using NMR spectroscopy to analyze PPI. One crucial component of the protein interaction analysis is the list interface's location. The foundation of NMR spectroscopy is the idea that materials that are magnetically active and subjected to intense magnetic fields absorb electromagnetic radiation at a certain frequency that is based on their chemical makeup [24, 25].

In Vivo Methods for Predicting the Interaction of Proteins

An in vivo approach used to find PPIs is called the Y2H system [17]. Two different protein disciplines are needed for the Y2H assay: (i) an activation sphere (announcement), which speeds up DNA replication, and (ii) a DNA binding sphere (DBD), which helps bind to DNA. A journalist's gene summary requires both disciplines [18]. Y2H analysis allows for the direct recognition of PPI between protein dyads. There is a potential, though, that the algorithm will steer clear of many false positive connections. Moreover, the proteins could not fold correctly to interact if they were not in their native physiological environment [26]. In the past 10 years, Y2H has undergone modifications

through the creation of novel expression vectors to facilitate the transformation of yeast cells into hybrid proteins and novel yeast strains with numerous reporter genes [27]. Additional widely used methods with significant apparatus include bioluminescence resonance energy transfer (BRET), luminescence resonance energy transfers (STEM), and bimolecular luminescent complementation (BiFC). STEW (Scanning Transmission Electron Microscope) predicts protein relationships using time-identified single-photon counting [28]. Synthetic lethality, which seeks to understand the mechanism underlying phenotypic stability in the face of inheritable variation, environmental fluctuations, and random occurrences analogous to mutations, is a significant type of in vivo inheritable web research. This process results in mutations or elisions in two or more genes that, while viable individually, under certain circumstances combine to generate mortality [29–33].

Protein-Protein Interaction Prediction Using the In Silico Method

Various in vitro and in vivo techniques, such as the yeast two-hybrid (Y2H) system, have broadened the scope of developing useful instruments for identifying PPIs among specific proteins that may have different functions. Still, because of the lack of potential PPIs, the data produced using these methods could not be accurate.

TYPES OF PROTEIN-PROTEIN INTERACTIONS

Proteins with different PPIs can interact with other proteins in two ways: either in “stable” ways that result in complexes that become molecular machinery in living systems, or in “flash” ways that cause specific effects quickly, such as signal transduction. The assembly of a human complex can influence the configuration of homo- or hetero-oligomeric complexes. It is possible to build relationships between sphere-sphere and sphere peptide, in addition to the conventional complexes like enzyme-asset and antibody-antigen.

Homo-oligomers vs Hetero-oligomers

Homo-oligomers are macromolecular complexes composed of a single type of protein component. The assembly of protein subunits is guided by the creation of non-covalent bonds in the quaternary structure of the protein. Denaturation of the complex is often necessary for the dislocation of homo-oligomers to return to the original individual monomers [34]. Numerous enzymes, scaffolding proteins, carrier proteins, and transcriptional nonsupervisory factors are all filled by homo-oligomers. Hetero-oligomers are assemblies of different protein subunits that interact and are required for the regulation of several biological processes. It is obvious during cell signaling processes, and the structural differences between the proteins are the only reason why comparable relationships are conceivable.

Permanent Versus Ephemeral Interactions

Proteins that interact over long periods to carry out functional activities and that take part as subunits in infinite complexes are involved in stable interactions. This is usually the case for homo-oligomers like ATPase subunits and some hetero-oligomers like cytochromes. A protein, however, can interact compactly and reversibly with other proteins in only certain cellular surrounds, such as cell type, cell cycle stage, external factors, presence of other binding proteins, etc. These interactions are known as flash relations. This is the case with most of the proteins involved in biochemical falls. For instance, some Gq-coupled receptors, such as muscarinic [35], bind to Gi/o proteins only momentarily when they are triggered by external ligands [36], in contrast to certain G protein-coupled receptors. Flash relations are those that exist between intrinsically disordered protein areas and spherical protein disciplines, such as MoRFs (molecular recognition features) [37].

Covalent Versus Non-Covalent

Covalent bonds, which are formed by disulfide or electron sharing, are the strongest kind of bonds. These relationships, however uncommon, are crucial for some posttranslational modifications, such as SUMOylation and ubiquitination. Weaker ties, such as hydrophobic bonds, ionic relations, van der Waals forces, or hydrogen bonds, are formed during flash relations [38].

DATABASES OF PROTEIN-PROTEIN INTERACTIONS

The amount of PPI data generated by high-throughput technologies is growing, hence it is necessary to create biological repositories where this data may be efficiently and methodically kept. Analyzing various interaction kinds in light of our concerns is made considerably simpler by the data in the PPI databases that are accessible to the general public [39]. Regarding PPI data, there are about 100 online sources available [40].

The General Repository of Biology for Interaction Datasets

Experimentally determined PPI data for almost all relevant model organisms are available in the Biological General Repository for Interaction Datasets (BioGRID), a large and readily accessible resource [41]. According to the February 2021 version, it includes inheritable relations and 1740000 non-redundant proteins that were compiled from 70000 publications [42]. It has consistently been made more efficient. As of right now (v4.3.194), the BioGRID interpretation's themed curation systems are focused on curated relationships of various illnesses, such as autophagy, glioblastoma, Fanconi anemia, ubiquitin-proteosome system, and coronavirus disease 2019 (COVID-19).

Search Tool for Retrieval of Interacting Genes

The search tool for retrieving interacting genes (STRING) provides access to the complete database of functional interactions between proteins. The highest number of organisms represented by any PPI database, 5090, are included in the commerce data of the current interpretation of STRING v11.0. The main features of the STRING database are its comprehensive content, the relations' confidence scores, and its user-friendly interface [43, 44]. The database now includes 3123056667 PPIs, which are the total of relations with high and low confidence. Thanks to their ability to perform gene ontology and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses of their input, druggies may easily execute gene-set enrichment analysis, which is a significant development in the present interpretation of STRING [45].

Host-Pathogen Interaction Database (HPIDB)

Data about host-pathogen commerce is contained in the carefully managed database HPIDB. Designed in 2010, it undergoes annual streamlining and features fresh performances. As of right now, it includes information on protein trade between 668 infectious disease species and 66 hosts. As of the most recent update (July 29, 2019), there are 69787 distinct relations. Pathogenic organisms such as herpes simplex, papillomaviruses, *Saccharomyces cerevisiae*, influenza, and several others can establish superabundantly in HPIDB [46].

IntAct Database

Established in 2002, IntAct is a freely available repository for molecular interaction data, containing material curated from published publications or directly contributed by researchers. To increase the coverage and curation output, IntAct and MINT merged their efforts in 2013 and launched the MINTACT initiative [47].

International Molecular Exchange Consortium Databases

An international organization called the International Molecular Exchange Consortium (IMEx) was founded by the combined efforts of several major public commerce databases, such as DIP, IntAct, HPIDB, MINT, BioGRID, MatrixDB, and I2D. Members of IMEx, BIND and MPIDB, were once sizable PPI databases; however, they are not currently operational. Every experimenter has the option to delete and re-evaluate the data in IMEx, a vast network of databases that captures PPI meta data in a standard PSL-MS format. IMEx is the sole source offering up-to-the-nanosecond information on protein connections and reflections among all the funds. Over the past 20 years, protein trade data has risen substantially [48, 49]. Certain protein trade databases, such as HCVPro [50], are dedicated to a particular viral disease and contain information on PPIs between HCV and mortal. VirHostNet [51] includes close to 22,000 virus-human PPIs and covers a wide spectrum of human-specific contagions.

Practises for Experiments

It is also important to carry out the experiments for identifying the techniques for the interaction of the molecules and there are some methods available for them and they are described below.

Hybrid Screening with Yeast Two

Different types of methods are available to locate them in a specific site and each strategy has pros and cons of its own, especially in terms of the sensitivity and specificity of the procedure [52]. The most common and conventional high-throughput methods are affinity purification combined with mass spectrometry and yeast two-hybrid screening.

Saccharomyces cerevisiae was used as a natural model in Fields and Song's 1989 description of this system [53, 54]. With yeast two-hybrid, paired PPIs (double system) can be identified in vivo and evaluated for biophysically direct trade between the two proteins. The Y2H is based on the posterior activation of a finicky journalist akin to His3 and the functional reconstitution of the incentive recap factor Gal4. To test two proteins for commercial usage, two protein expression constructs are made. Two proteins have been fused together: Y has been fused to the Gal4 activation sphere (announcement), and X has been fused to the Gal4 DNA-binding sphere (DB). These constructs are utilized in the experiment to convert incentive cells. The purpose of reporter genes cannot be fulfilled unless there is interaction between the bait (DNA-Binding DB-X) and prey (Activation Domain AD-Y) to create a functional Gal4 recap factor. Consequently, the existence of the products attendant to the reporter gene expression suggests that there is interaction between proteins [55].

A relationship between the two proteins under inquiry is suggested by incentive growth under selective media circumstances when the reporter gene creates enzymes that allow the incentive to synthesize sufficient amino acids or nucleotides. Recently, programs to categorize and rank protein relationships have been released [56, 57]. The yeast two-hybrid method has drawbacks despite its usefulness. Its primary host system is an incentive, which presents an issue for analyzing proteins with post-translational modifications unique to mammals. Due to a high false negative rate [58] and understatement of membrane proteins, for example [59, 60], the number of PPIs connected is typically minimal. When DB-X activates the reporter gene without AD-Y present, for example, appropriate controls for false positives (false negatives) were consistently neglected in the initial Y2H investigations, resulting in a higher-than-average false positive rate. To account for these false negatives, an empirical framework needs to be in place [61].

The emergence of incentive two-hybrid variants, such as the split-ubiquitin system and the membrane yeast two-hybrid (MYTH), which are not restricted to relationships that occur in the nexus, and the bacterial two-hybrid system, which is carried out in bacteria, has resulted in limitations in the lower content of membrane proteins [62].

Nucleic Acid Programmable Protein Array (NAPPA)

LaBaer and colleagues initially created this technique in 2004 utilizing an in vitro recap and restatement technology. Using DNA template garbling, the target gene coupled with GST (Glutathione-S-transferase) protein was rendered paralyzed in the solid face. An aminopropyltriethoxysilane (APTES)-carpeted slide was used to contain the biotinylated plasmid DNA and anti-GST antibody boundaries. BSA (Bovine serum albumin) has the ability to improve DNA binding efficiency. Avitin bound the biotinylated plasmid DNA. Rabbit reticulocyte lysate (RRL), a cell-free expression system, was used to synthesize the novel protein. It was additionally bound on the slide by an anti-GST antibody. The targeted protein cDNA and the query protein cDNA were paralyzed in the same carpeting slide in order to investigate PPI. Targeted and query proteins were synthesized from the same extract utilizing an in vitro recap and restatement method. The slide's antibody carpet attached the targeted protein to the array, and the array was probed using the query protein. The hemagglutinin (HA) epitope was attached to the query protein. Consequently, the antibody against HA was used to picture the trade between the two proteins [63, 64].

TARGETS FOR THERAPEUTICS

The scientific community is beginning to pay more attention to the difficult task of PPI modulation [65]. Medicine-design efforts have included a number of PPI chunks that are comparable to allosteric regions and hotspots [66, 67]. However, Food and Drug Administration (FDA)-approved small-patches PPI barriers directly target a very large number of PPIs, highlighting a significant unrealized opportunity for drug discovery. Recently, Jaiswal and colleagues were able to use protein-protein interaction investigations to create 30 peptides that block telomerase reclamation towards telomeres [68–71].

CONCLUSION

The aforementioned study will aid in understanding the significance of protein-protein interaction, the databases that are available to finish the identification of its molecules' binding with others, and how to find these molecules using various databases that are also accessible and that researchers are using to finish the identification test. In addition, study is required to determine whether other kinds of molecules interact with proteins; this will facilitate the synthesis or review of other studies.

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