BIOINFORMATICS AND BIOMEDICAL RESEARCH JOURNAL
2021, Vol. 4, No. 2, 70 - 82
http://dx.doi.org/10.11594/bbrj.04.02.04

Review Article

Fundamentals of Protein-Protein Interactions and Their Methods of Characterization

Kholis A. Audah[1*]

[1]Department of Biomedical Engineering, Swiss German University, Tangerang, 15143, Indonesia

Article history:
Submission November 2021
Revised December 2021
Accepted December 2021

**Corresponding author:*
E-mail: kholis.audah@sgu.ac.id

ABSTRACT

Protein-protein interactions are ubiquitous biological phenomena in all types of organism and play vital roles in various metabolic processes. Therefore, it is very important to understand how protein-protein interactions take place and govern different mechanisms at cellular and molecular levels. Thus, determination of protein-protein interactions is the key in elucidating such mechanisms. In order to provide adequate knowledge and guidance in selecting appropriate methods to determine protein-protein interactions, this article will review basic principles, definitions, terminologies, parameters and classification of protein-protein interactions, obtained either from in silico or laboratory experimental works. Some examples of commonly used biochemical and biophysical methods for characterization of protein-protein interactions were also discussed.

Keywords: contacting residues, hot spots, interacting residues; protein-protein interfaces, residue interface propensities

Introduction

Protein-protein interactions are essentials in nearly all biological processes. They are essential for all intra and extracellular functions, and the technology to analyze it is widely applied in various field of biological science [1]. Any major research topics in biology such as the central dogma of DNA replication, transcription, and translation, vesicle transport, signal transduction, and drug or protein design are some examples in which protein-protein associations are essential components [2]. Moreover, the vast majority of proteins bind to other proteins at some time in their existence in order to perform various functions [3].

Protein-protein interactions can be generated from different association of proteins, such as polymerization of identical subunit of a protein that will result as homodimer, homotetramers, homohexamers proteins and even the proteins that contain high numbers (for example, 60, 180, and 240) of subunits such as found in the viral coat proteins. Trimers are relatively rare compared to dimers and tetramers based on certain number of proteins that have been studied [4] (Figure 1.19 if necessary). Protein complexes is a group of polypeptide chains linked by noncovalent protein-protein interactions (PPIs) [5]. Protein complexes can also be generated from multi subunits, enzyme-inhibitor complexes, antibody-protein complexes, or between two distinct types of proteins. Protein-protein associations that are generated from different proteins result two different types of complexes that can be divided as homocomplexes and heterocomplexes which include transient protein-protein

How to cite:
Audah, K. A. (2021). Fundamentals of Protein-Protein Interactions and Their Methods of Characterization. *Bioinformatics and Biomedical Research Journal 4*(2): 70 - 82. doi: 10.11594/bbrj.04.02.04

interactions [4]. Homocomplexes are usually permanent and optimized. Heterocomplexes can also have such properties, or they can be non-obligatory, being made and broken according to the environment or external factors and involve proteins that must also exist independently [6]. Transient protein-protein interactions which means protein interactions that are formed and broken easily, control a large number of cellular processes [7]. All modifications of proteins necessarily involve such transient protein-protein interactions. These include the interactions of protein kinases, protein phosphatases, glycosyl transferases, acyl transferases, and proteases with their substrate proteins [8].

Basic Principles of Protein-Protein Interactions

Numerous studies have addressed protein-protein interactions, yet the principles governing such interactions are not fully understood. From structural point of view, there is no general pattern observed in the motif of binding sites. One of the reasons is because similar proteins structures can associate in different ways, or the opposite situation could be the case in which proteins with globally different structures can associate in similar ways [9].

Some Definitions

Contacting residues are those responsible for the interactions across the interfaces. Two residues are defined to be contacting if the distance between any two atoms of the two residues from different chains is less than the sum of their corresponding van der Waals radii plus 0.5 Å [10,11]. A residue is defined to be nearby if the distance between its Cα atom and a Cα atom of any contacting residue is less than 6 Å [9]. Protein-protein interactions may be mediated by a small region of one protein fitting into a cleft in another protein or by two surfaces interacting over a large area. Most interfaces are composed of two protein surfaces with good shape and electrostatic complementary [12,13]. Almost of all these interfaces bury more than 600 Å2 of total surface area and it is often assumed that the energy protein-protein binding is directly related to buried hydrophobic surface area [14,15,16].

Hot Spots of Protein-Protein Interfaces

Alanine-scanning mutagenesis has shown insignificant correlation between buried surface area and free energy binding. Moreover, a highly uneven distribution of energetic contributions of individual residues is found across each interface and with certain residues responsible for the bulk of the binding energy [17]. These critical residues are called hot spots and defined as a residue that when mutated to alanine, gives rise to a distinct increase in the absolute binding energy (ΔΔG) of more than 2 kcal/mol [18, 19]. These hot spots are enriched in tryptophan, tyrosine, and arginine [19, 20, 21] with percentage of appearance more than 10%; 21% of tryptophan, 13.3% of arginine, and 12.3% of tyrosine residues from a set of proteins that have been studied [21]. It has been well established that most hydrophobic residues are found in the interior of proteins, while polar and charged residues are found on the surfaces. Interestingly, tryptophan and tyrosine residues are found in interiors and on surfaces with nearly identical frequencies, the only two hydrophobic residues for which this is true [19, 22]. A data set of as many as 2325 alanine mutants have been analyzed by using this hot spots experimental approach for which the change in free energy of binding upon mutation to alanine was measured [21]. These hot spots are surrounded by energetically less important residues that most likely serve to exclude bulk solvent from the hot spot by forming hydrogen bonds between residues. Exclusion of solvent is found to be a necessary condition for highly energetic interactions [21,9]. To a lesser extent, conservation of phenylalanine and methionine also imply a binding site. There is a positive correlation between energy hot spots and structurally conserved residues [23]. Further computational analysis on hot spots residues indicated that hot spots are not randomly spread along the protein-protein interfaces; rather, they tend to be clustered and are located within densely regions [9]. Within an assembly, the tightly packed hot spots form networks of interactions. These assembly regions are called hot regions. These regions contain residues that are moderately conserved that further suggesting the crucial role of the

conserved interactions in the local densely packed environment. An interface may contain a single, or a few hot regions [9, 18].

Parameters in measuring protein-protein interfaces

Protein-protein interfaces are defined based on the change in their solvent accessible surface area (ΔASA) when going from a monomeric to a dimeric state [24, 25]. The interface residues (atoms) were defined as those having ASAs that decreased by >1 Å2 on complexation [26]. Many studies have carried out analysis of the characteristics of protein-protein interfaces in an attempt to search for factors that contribute to the affinity and specificity of protein-protein interactions. There are several fundamental properties that characterize a protein-protein interface, which can be calculated from the coordinates of the complex [27]. First, size and shape. The size and shape of protein interfaces can be measured simply in absolute dimensions (Å) or, more accurately, in terms of the ΔASA on complexation. The ΔASA is often used, since there is a correlation between the hydrophobicity free energy of transfer from polar to a hydrophobic environment and the solvent ASA [28]. Calculating ΔASA may provide a measure of the binding strength. The shape of the interfaces can also be analyzed and is relevant to designing molecular mimics. Two protein subunits may interact and form a protein-protein interface with two relatively flat surfaces or form a twisted interface. A term "planarity", which is a measure of how far the interface residues deviated from a plane, is commonly used to assess how flat or how twisted the protein-protein interfaces are. A term "circularity" is often used to provide a rough guide to the shape of the interface. An interface with circularity ratio 1.0 indicates approximately circular interface. Second, electrostatic and shape complementary between surfaces. Different methods have been used to evaluate the electrostatic [29] and shape complementary of the interacting surfaces including the evaluation of gap index in the protein-protein interactions [30]. Based on gap index values from different types of protein complexes, the interacting surfaces in the homodimers, the enzyme-inhibitor complexes, and the permanent heterocomplexes are the most complementary, whereas the antibody-antigen complexes and the nonobligatory other heterocomplexes are the least complementary [31]. Third, residue interface propensities. The relative importance of different amino acids residues in the interfaces of complexes can give a general indication of the hydrophobicity, that can only be interpreted if the distribution of residues occurs in the interface are compared with the distribution of residues on the protein surface as a whole. Residue interface propensities for each amino acid is defined as the fraction of ASA that individual amino acid contributes to the interface compared with the fraction of ASA that individual amino acid contribute to the whole surface (exterior residues plus interface residues) [31]. A propensity of >1 denotes that a residue occurs more frequently in the interface than on the protein surface. The propensities for hydrophobic residues, with the exception of methionine, have shown a greater preference for the interfaces of homodimers than for those of heterocomplexes. The lower propensities for hydrophobic residues in the heterocomplex interfaces balancing by an increase propensity for the polar residues. Fourth, hydrophobicity and hydrogen bonding. The mean hydrophobicity value [28] of residues involved in protein-protein complexes will vary depend on the subunit compose the protein complexes. Studies have indicated that when the hydrophobicity values of the interface are compared between the homodimers and the heterocomplexes, the interfaces of the heterocomplexes are less hydrophobic than those of homodimers that is positively correlated with the residue propensities [31]. Major polar interactions between the components in the complexes can be predicted from the mean number of hydrogen bonds per 100 Å2 of ΔASA. Studies have shown that the heterocomplexes that exist as both monomers and complexes have relatively more intermolecular hydrogen bonds per ΔASA. This is positively correlated with the residue propensities in which the transient complexes (those with components that occur as both monomers and complexes) contained more hydrophilic residues in their interfaces than the permanent complexes [31]. Fifth, the binding constant. The strength of protein-

protein interactions can be measured through binding constant value that can be expressed in three different ways [31]. 1) Dissociation constant (Kd), the most commonly used binding constant, according to the following equations (Eq.9-10):

$$P_f + L_f \leftrightarrow PL$$

(Eq.9)

$$K_d = \frac{[P_f][L_f]}{[PL]}$$

(Eq.10)

with P refers to bound protein, L refers to bound ligand, and PL refers to protein-ligand complex, while [Pf] and [Lf] refer to the concentration of unbound P and L, respectively. The smaller the Kd value, the stronger the binding is. 2) Affinity constant (Ka), with Ka = 1/ Kd. 3) A ratio of two rate constants, that is between the rate of formation of PL and the rate of breakdown of PL. The rate of formation of PL is ka [Pf] [Lf], where ka is the association rate constant. The rate of breakdown of PL is kd [PL], where kd is the dissociation rate constant. At equilibrium, the rate of formation of PL equals the rate of breakdown of PL, and Kd = kd/ka.

The order of magnitude of Kd value of protein-protein interactions that have been studied that likely to be physiologically relevant, varies from the range of as week as 10-3 molar (M) to the strongest of 10-16 M range [32]. The Kd value of protein-protein interactions in the range of micromolar to nanomolar is considered a relatively tight interactions, and the protein-protein interactions are considered too tight when the Kd value less than 10-12 M [32]. Few examples of the very tight protein-protein interactions have been found between human placental RNase inhibitor (PRI) with both angiogenin (Kd = 7 □ 10-16 M) [33,34] and human placental RNase (Kd = 9 □ 10-16 M) [35].

Some Measurable Effects of Protein-Protein Interactions

Protein-protein interactions are essential for proper cellular function. Protein-protein interactions may lead to different physical or physiological changes of the interacting proteins. The effects of protein-protein interactions can be evaluated in different ways depend upon the nature of the proteins [31,36]. The most common effect of protein-protein interactions is alterations of kinetic properties of proteins, such as substrates binding including the change of protein specificity to its substrate, catalysis or altered allosteric properties of protein complexes, or even the alteration in mechanisms between single and coupled-enzyme reactions [37]. The alteration of kinetic properties of proteins is a strong indication of another effect of protein-protein interactions such as in the case of substrate channeling [38]. Substrate channeling is a common biological phenomenon that requires protein-protein interactions. Substrate channeling is a process of direct transfer of the product of an enzyme to another enzyme or to nearby cells as its substrate without equilibrium with the bulk phase [39]. This has been found in different enzyme systems from different organisms, such as tryptophan synthase from Salmonella typhimurium [38] and carbamoyl phosphate synthetase, which is found in eukaryotes and prokaryotes [40]. More examples of channeling mechanisms have been reported not only found in multifunctional or multisubunits enzymes, but also found in protein complexes between two distinct proteins [41,42]. Protein-protein associations can also cause conformational changes of proteins. Although, it is still not clear to what extent proteins change their conformation on forming a complex [43,31], it is possible to distinguish various levels of conformational change: no change, side chain movements, segment movement involving the mainchain, and domain movements (gross relative movements of the domains) [31]. Conformational changes of proteins on forming a complex may then lead to the formation of a new binding site, although this will not always be the case. The formation of a new binding site has been shown in α and β subunits of E. coli F1-ATPase [44], yeast hexokinase [45], and Bacillus stearothermophilus phosphofructokinase [46]. Inactivation of a protein is another effect of protein-protein interactions; such as in the case of the interaction of phage P22 repressor with its antirepressor [47], and with the interaction of trypsin with trypsin inhibitor [48].

Methods for Determination of Protein-Protein Interactions

There are numbers of methods that have been used to determine both in vivo and in vitro protein-protein interactions [49]. They can be largerly categorized as biophysical, library-based, genetic and in silico methods [50,51]. It is too vast to describe all the available methods in this section. Therefore, this review will be focusing only on some biophysical methods that widely used for detection of protein-protein interactions, from classical chromatography techniques to recent spectroscopy technologies. This literature study is not aimed to give an extensive review, rather serves as initial guidelines to select suitable methods for conducting protein-protein interactions experiments. In principle, there is no superiority nor preference in methods of choices in determining protein-protein interactions simply because it really depend upon the nature of the interactions as well as the availability of the instruments. In fact, they are frequently complementary one another.

The selection of methods for determination of protein-protein interactions will depend on at least the following three factors. First, different levels of informations which want to be obtained, : 1) the informations between two proteins interaction including the binding strength [52]; 2) determination of amino acids residues involved in complex formation; and 3) measuring the consequences of the interactions [53,54]. Second, the nature of the interacting proteins including physical properties of proteins (e.g. concentration, solubility, amino acids sequences, and structural information), cofactors, reactions catalyzed including characteristics of substrates, intermediate, and final products [55,56]. Third, the availability of instruments and cost of analysis. These are more general factors rather than specific ones. However, they are very important things for ones to take these into consideration before stepping forward in conducting protein-protein interactions researches. These experiments usually require a variety of instruments, expensive chemical reagents, and high cost of analysis. Although these factors should not become major problems in conducting researches, yet they are in certain conditions becoming the limiting factors. These three factors then will determine researchers to choose certain methods that fit best to their needs and conditions.

Affinity Chromatography

Protein-protein interactions are perhaps the most sophisticated of all the types of interactions that can be exploited for affinity chromatography. The basic of this experiment is simple. One protein has to have a large different binding affinity to a column matrix such as Sepharose compared to other proteins including the ligand in protein extract. Most proteins are washed off under low-salt conditions; the proteins that are retained then able to be eluted by high-salt solutions and can be judge as the interacting proteins. This method was first used more than 30 years ago to detect phage and host proteins that interacted with different forms of E. coli RNA polymerase [49,57,58,59]. Several excellent reviews have been published on this widely used technique including a number of strategic and important considerations [52,60]. There are at least five advantages of protein affinity chromatography as a technique for detecting protein-protein interactions. First, protein affinity chromatography is very sensitive. It can detect interactions with a binding constant as weak as 10-5 M [52]. Second, this technique tests all proteins in an extract equally; thus, extract proteins that are detected have successfully competed for the test protein with the rest of the population of proteins. Third, interactions that depend on a multisubunit tethered protein can be detected, unlike the case with protein blotting [32]. Fourth, it is easy to examine both the domains of a protein and the critical residues within it that are responsible for a specific interaction, by preparing mutant derivatives [61,62]. Fifth, this technique allows the modifications of proteins of interest due to the rapid developments on matrix column.

Chemical Cross-Linking

Chemical cross-linking is the process of chemically joining two or more molecules by a covalent bond. The molecules to be linked may be proteins, peptides, drugs, nucleic acids or even solid particles [63]. Chemical cross-

linking reagents and the intramolecular and intermolecular cross-

linking of proteins have been instrumental in the structural and functional characterization of proteins [63]. Cross-linking reagents contain at least two reactive groups that can be targeted for cross-linking to different functional groups such as primary amines and carboxyl groups on proteins or other molecules [64,65]. Cross-linking reagents have various spacer arms lengths that then can be used as molecular rulers for associations of biomolecules [66]. The selection of cross-linkers are based on the following characteristics: a) chemical specificity; b) spacer arm length; c) water solubility and cell membrane permeability; d) reactive groups such as same (homobifunctional) or different (heterobifunctional); e) spontaneously reactive or photoreactive groups; f) cleavability; and g) reagent contains moieties that can be radiolabeled or tagged with another label [67]. Chemical cross-linking method has been widely used to detect protein-protein interactions both in vivo and in vitro. It can be used to deduce the architecture of protein complexes that are readily isolated from the cell and to detect proteins that interact with a given test ligand by probing extracts, whole cells, or partially purified protein [68,69,70,71,72,73]. Cross-linked proteins can be analyzed by gel electrophoresis or other methods such as immunoprecipitation [68,74] and mass spectroscopy. Two-dimentional gels are used to deduce the architecture of proteins or assemblies that are readily isolated intact from the cell. The procedures involve three steps (Figure 1.19) [75]. 1) The protein complex is reacted with a cleavable bifunctional reagent of the form RSSR', and the R and R' groups react with susceptible amino acid side chains in the protein complex to form P-RSSR'-P' adduct, where SS is a form of a disulfide bond within the reagent. 2) The proteins are fractionated on an SDS-gel in the absence of reducing agents and the cross-linked proteins of the form P-RSSR'-P' migrates as species of greater molecular weight. 3) A second dimension of the SDS-gel is run after treatment of the gel with a reducing agent such as β-mercaptoethanol to cleave the S-S bond. Un-cross-linked species align along the diagonal, as their molecular weights do not change after reduction, while cross-linked proteins migrate off the diagonal as they migrate as molecules of the form P-RSH and P'-R'SH. The cross-links are then identified by their size, which corresponds to that of the un-cross-linked species P and P'. This technique has been used to study the architecture of multienzyme complexes such as CF1-ATPase [69], E. coli F1-ATPase[70], and ribosome [75,76].

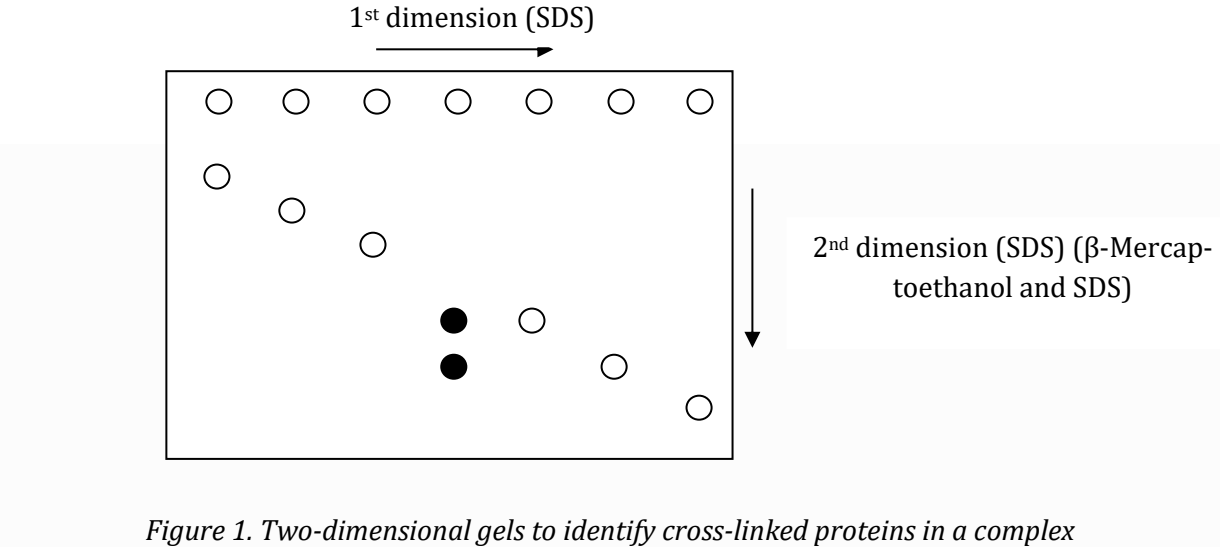

Figure 1. Two-dimensional gels to identify cross-linked proteins in a complex

There are three major advantages of using cross-linking methods to determine protein-protein interactions. First, cross-linking reagent can capture weak interactions that might not be visible by other methods. Second, it can capture transient contacts with different proteins at various stages by freezing the process at different stages. Third, it can be done in vivo with membrane-permeable cross-linking reagents. One major disadvantage of using any cross-linking technique to detect protein-protein interactions is that it can detect unspecific nearest neighbors within the spacer arm of the reagents. Therefore, the most reliable information is derived from zero-length reagents that induce a direct covalent link between cross-linked sites [77].

Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) relies upon the accurate measurement of heat changes caused by the interaction of molecules (e.g. protein-protein interactions and DNA-protein interactions) in solution and possess the advantage of not requiring labelling or immobilization of the components. The raw data consist of peaks of heat output generated by successive injections of ligand and, when integrated, these provide the total heat output per injection which provide the binding isotherm (Figure 1.20). Injection number is proportional to both ligand concentration and the ratio of ligand to receptor. As a result, curve fitting based upon a one or more site model provides the affinity as dissociation constant (Kd) and entalphy changes (ΔH) directly. The final calculable information consists of association constant (Ka), stoichiometry, Gibbs free energy (ΔG), entropy (ΔS) are obtained using the relationship $\Delta G = RT \ln Kd = \Delta H - T\Delta S$, and $Ka = 1/Kd$. Heat capacity (ΔCp) can also be obtained if the experiments are performed over a range of temperatures [78]. ITC method has been used to characterize the coupled folding and association of heterodimeric coiled coils (leucine zipper) [79], structural and mutation analysis of affinity-inert contact residues at the growth hormone-receptor interface [80]. and energetics of target peptide recognition by calmodulin [81]. One major disadvantage of this method is that it requires higher concentrations of protein than most other methods.

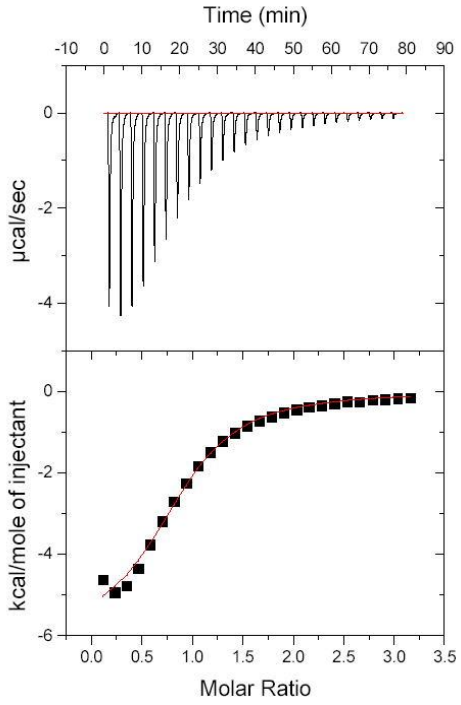

Figure 2. An example of typical raw data obtained from an ITC experiment

Fluorescence spectroscopy

Fluorescence spectroscopy and its various applications for the science of life has developed quite rapidly. This is because many technical advances in instrumentation and data analysis methods have been carried out, such as in the method of determining protein-protein interactions [82]. Fluorescence spectroscopy is the most important optical spectroscopic method for measuring protein-protein interactions. This because fluorophores such as tryptophan residues in proteins are highly sensitive probes of environmental changes [83] and can be used to measure changes in mobility associated with binding [84]. Several fluorescence methods have been used to detect and to measure protein-protein interactions. 1) Fluorescence spectrum. Complex formation between two proteins can be detected from a shift in the wavelength of maximum fluorescence emission or the changes in the fluorescence intensity caused by protein-protein associations. The changes in fluorescence intensity at a particular wavelength can be used to determine the dissociation constant of protein complexes. One example of this application that shows the changes in both parameters has been demonstrated in the interactions of the γ subunit of cGMP phosphodiesterase (PDEγ) subunit with the transducin α subunit (Tα) in the presence of GTPγS or GDP [85]. There are two limitations of using this technique. First, the fluorescence spectrum relies on the sum of the contribution of all the tryptophan residues. Therefore, the probability of detecting a change in the fluorescence spectrum decrease with the total number of tryptophan residues in the interacting proteins. Second, the sensitivity is limited by the intensity of fluorescence change that depends on the inherent sensitivity of fluorescence (in the order of nanomolar) and the change that is observed (which is not easily predictable). To overcome this limitation, a fluorescent tag needs to be added to increase the sensitivity of the interacting proteins as long as the fluorescent adducts do not adversely affect the function of the modified protein or its interaction with other proteins. 2) Fluorescence polarization or anisotropy with tagged molecules. The principle of this technique is based on rotational motion of molecules which occur in the lifetimes of excited fluorescent molecules (nanoseconds). This is accomplished experimentally by the use of plane-polarized light of excitation, followed by measurement of the emission at parallel and perpendicular planes. Rotational correlation times depend on the size of the molecule of approximately 1ns/2,400 Da for an idealized molecule. Therefore, this method can be used to measure the affinity of two proteins for one another because of the increased rotational correlation time of the complex. This method has been used to study separately the interaction of protein synthesis initiation factor 3 (IF3) and initiation factor 2 (IF2) with 30S ribosomal subunits by using fluorescein-labeled IF3 and fluorescein-labeled IF2, respectively [76,86].

Circular dichroism spectroscopy

Circular dichroism (CD) spectroscopy measures differences in the absorption of the left-handed polarized light versus right-handed polarized light when arise due to structural asymmetry. CD is used to determine the secondary structure of proteins. Therefore, this method can be used to detect protein-protein interactions only if the complex formation caused by secondary structural changes, in which the spectrum of the complex will differ from the sum of the individual components. This technique has been used to study the conformational stability of the dimerization domain of transcription factor LFB1 [87] and characterization of the B-box protein-protein interaction motif of the ETS-domain of transcription factor Elk1 [88].

Mass spectroscopy

Mass spectroscopy (MS) method is one of the most developed analytical technique and it has become a central for protein research [89]. This is due to its development with various ionization techniques such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) [90] and electrospray mass spectrometry (ES-MS) [91]. The development in ionization techniques have change dramatically the use of MS from restricted for small and thermostable compounds to macromolecules such as proteins. Recent technologies have allow to combine MS with

other analytical instruments to develop more efficient tools to study a large-scale analysis in protein research. Some to mention are liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [92]. The use of MS technique in protein-protein interactions study is generally coupled with affinity selection procedures used in molecular or cellular biology; for examples: cross-linking or affinity chromatography [93]. Affinity selection procedure followed by peptide digestion is performed prior to analysis by MS. The MS method has been used to detect protein-protein interactions in various biological systems [4].

Nuclear magnetic resonance spectroscopy

The advances in nuclear magnetic resonance (NMR) spectroscopy have alleviated the size limitations for the determination of biomolecular interactions in solution. There are two parameters commonly used in determination of biomolecular interactions by NMR; residual dipolar couplings (RDCs) [94,95] and chemical shift perturbations (CSPs) [96]. RDCs are providing structural information of molecular complexes. With recent technology, the structures of protein-protein and protein-nucleic acid complexes up to 50 kDa can be accurately determined. CSPs, which is much easier to obtain, provide informations on interaction surfaces. NMR is particularly powerful in mapping interfaces, allowing the study of weak and transient complexes that can be very difficult to study by other experimental techniques. The characterization of protein interactions has greatly benefited from the incorporation of interface mapping information in the computational modelling of complexes [97]. NMR studies of protein-protein interactions have varied from full structure determination to NMR-filtered docking and modeling using interface information. Several structures of protein-protein interactions have been determine completely de novo by NMR, for examples the structure of ubiquitin interactions of NZF zinc finger [98], HP1/PXVXL motif peptide interactions and HP1 localization to heterochromatin [99] and the tetrameric L27 domain complex [100]. The strength of NMR spectroscopy technique relies upon its sensitivity in detecting weak protein-protein interactions (with a Kd in the millimolar range) using a combination of intermolecular nuclear Overhauser effects (NOEs) and RDCs. However, it also has some disadvantages that it requires high concentrations of proteins and the limited size of macromolecus that can be detected up to 50 kDa only where the majority of protein complexes more likely have larger molecular weight than that. It is also clear that the data generated from NMR spectroscopy required strong computational analysis knowledge that interdisciplinary expertise is necessary.

Conclusions

Determination of protein-protein interactions plays vital roles in understanding different mechanism in a wide range of biological systems in which the success will rely on the methods fit best with the nature of the interacting proteins and other binding partners. The protein- protein interactions study becomes more important, more complex and more challenging with the emerging of various omics disciplines following the completion of human genome sequence.

Particularly, during this pandemic situation, through the revealation of the proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2), the cause of COVID-19 [101], protein-protein interactions study is very crucial in understanding host-pathogens interactions and their applications toward precision medicine.

Acknowledgments

Kholis A. Audah is a recipient of a research grant from the Ministry of Education and Culture, Research and Technology, Republic of Indonesia under contract number: 002/SP2H/RT-JAMAK/LL4/2021, C/ARCS/461/VII/2021 and the Swiss German University for financial support through the Faculty Research Fund. Author thanks to Ms. Amalda Siti Anisa for editing the manuscript.

Competing Interests

Author declares has no competing interest

References

1. Westermarck J, Ivaska J, Corthals GL 2013.Indentification of protein-interactions involved in cellular signaling. Molecular & Cellular Proteomics. 12(7):1752-1763. doi:10.1074/mcp.r113.027771
2. Vranken VD, Weiss GA 2012. Introduction to Bioorganic Chemistry and Chemical Biology. New York: Garland Science. doi:10.1201/9780203381090
3. Kastritus PL, Bonvin AMJJ. 2013. On the binding affinity of macromolecular interaction: daring to ask why proteins interact. Journal of The Royal Society Interface. 10(79):835. doi: 10.1098/rsif.2012.0835
4. Wetie N, Armand G, Izabela S, Alisa WG, Urmi R, Joseph AL, Costel DC. 2013. Investigation of stable and transient protein-protein interactions: past, present, and future. Proteomics. 13(3-4):538-557. doi:10.1002/pmic.201200328
5. Tolani P, Gupta S, Yadav K, Aggarwal S, Yadav AK. 2021. Big data, Integrative omics and network biology. Advance in Protein Chemistry and Structural Biology. 127:127-160. doi:10.1016/bs.apcsb.2021.03.006
6. Massoud TF, Paulmurugan R. 2021. Chapter 47- Molecular imaging of Protein-protein interaction and protein folding. Molecular Imaging (Second Edition): Academic Press. 897-928. doi: 10.1016/B978-0-12-8163863.00071-5
7. Perkins JR, Diboun I, Dessailly BH, Lees JG, Orengo C. 2010. Transient protein-protein interactions: structural, functional, and network properties. Structure. 18(10):1233-1243. doi:10.1016/j.str.2010.08.007
8. Ursula J, Richard K, Vladimir UN. 2014. Conditionally and transiently disordered proteins: awakening cryptic disorder to regulate protein function. Chemical Reviews. 114(13):6779-6805. doi:10.1021/cr400459c
9. Keskin O, Ma B, Nussinov R. 2005. Hot region in protein-protein interaction: the organization and contribution of sctructurally conserved hot spot residues. Journal of Molecular Biology. 345(5):1281-1294. doi:10.1016/j.jmb.2004.10.077
10. Keskin O, Tsai CJ, Wolfson H, Nussinov R. 2004. A new, structurally nonredundant, diverse data set of protein–protein interfaces and its implications. Protein Science. 13(4):1043-1055. doi:10.1110/ps.03484604
11. Tsai CJ, Lin SL, Wolfson HJ, Nussinov R. 1996. A dataset of protein-protein interface generated with a sequence-order independent comparison technique. Journal of Molecular Biology. 260(4):604-620. doi:10.1006/jmbi.1996.0424
12. Joël J. 1997. Specific versus non-specific contacts in protein crystals. Nature Structural & Molecular Biology. 4(12):973-974. doi:10.1038/nsb1297-
13. Janin J. 1995. Principles of protein-protein recognition from structure to thermodynamics. Biochimie. 77(7-8):497-505). doi:10.1016/0300-9084(96)88166-1
14. Horton N, Lewis M. 1992. Calculation of the free energy of association for protein complexes. Protein Science. 1(1):169-181. doi:10.1002/pro.5560010117
15. Cyrus C, Joël J. 1975. Principles of protein–protein recognition. Nature Structural & Molecular Biology. 256(5520):705-708. doi:10.1038/256705a0
16. Privalov PL. 1979. Stability Small Globular Proteins. Advance Protein Chemistry. 33:167-241. doi:10.1016/S0065-3233(08)60460-X
17. Zerbe BS, Hall DR, Vadja S, Whitty A, Kozakov D. 2012. Relationship between hot spot residues and ligand binding hot spot in protein-protein interface. Journal of Chemical Information and Modeling. 52(8):2236-2244. doi:10.1021/ci300175u
18. Golden MS, Cote SM, Sayeg M, Zerbe BS, Villar EA, Beglov D, Sazinsky SL, Georgiadis RM, Vaida S, Kozakov D, Whitty A. 2013. Comprehensive experimental and computational analysis of binding energy hot spot at the NF-kB essesntial modulator/IKKβ protein-protein surface. Journal of the American Chemical Society. 135(16):6242-6256. doi:10.1021./ja400914z
19. Cukuroglu E, Engin HB, Gursoy A, Keskin O. 2014. Hot spot in protein-protein interface: toward drug discovery. Progress in Biophysics and Molecular Biology. 116(2-3):165-173. doi:10.1016/j.pbiomolbio.2014.06.003
20. David A, Sternberg MJE. 2015. The contribution of missense mutations in core and rim residues of protein-protein interface to human disease. Journal of Molecular Biology. 427(17):2886-2898. doi:10.1016/j.jmb.2015.07.004
21. Bogan AA, Thom KS. 1998. Anatomy of hot spots in protein interfaces. Journal of Molecular Biology. 280(1):1-9. doi:10.1006/jmbi. 1998.1843
22. Tsai CJ, Lin SL, Wolfson HJ, Nussinov R. 1997. Studies of protein-protein interfaces: A statistical analysis of the hydrophobic effect. Protein Science. 6(1):53-64. doi:10.1002/pro.5560060106
23. Scott DE, Bayly AR, Abell C, Skidmore J. 2016. Small molecular, big targets, drug discovery faces the protein-protein interaction challenge. Nature Reviews Drug Discovery. 15:533-550. doi:10.1038/nrd.2016.29
24. Luo J, Guo Y, Zhong Y, Ma D, Li W, Li M. 2014. A functional feature analysis on diverse protein-protein interactions: application for the prediction of binding affinity. Journal of Computer-Aided Molecular Design. 28(6):619-629. doi:10.1007/s10822-014-9746-y
25. Thiruphati R, Sravanthi S, Kumar A, Prabakharan EN. 2011. Protein-protein complexes. Journal of the Indian Institue of Science. 91:497-520.
26. Chakravarty D, Janin J, Robert CH, Chakrabarti P. 2015. Changes in the protein structure at the interface accompanying complex formation. IUCrJ. 2(6):1-10. doi:10.1107/S2052252515015250
27. Yan C, Wu F, Jernigan RL, Dobbs D, Honavar V. 2008. Characterization of Protein–Protein Interfaces. Protein J. 27(1):59-70. doi:10.1007/s10930-007-9108-x
28. Cyrus C. 1974. Hydrophobic bonding and accessible surface area in proteins. Nature (London). 248(5446):338-339. doi:10.1038/248338a0
29. Ilya Dr, Vasker A, Alflalo C. 1994. Hydrophobic docking: A proposed enhancement to molecular recognition techniques. Genetics. 20(4):320-329. doi:10.1002/prot.340200405
30. Lawrence MC, Colman PM. 1993. Shape Complementarity at protein/protein interface. Journal of Molecular Biology. 234(4):946-950. doi:10.1006/jmbi.1993.1648

31. Jones S, Thornton JM. 1996. Principle of protein-protein interactions. Proceedings of the National Academy of Sciences. 93(1):13-20. doi:10.1073/pnas.93.1.13
32. Phizicky EM, Fields S. 1995. Protein-protein interactions: Methods for Detection and Anaysis. Microbiological Review. 59(1):94-123). doi:10.1128/mr.59.1.94-123.1995
33. Lee FS, Auld DS, Bert VL. 1989. Tryptophan fluorescence as a probe of placental ribonuclease inhibitor binding to angiogenin. Biochemistry. 28(1):219-224. doi:10.1021/bi00427a030
34. Lee FS, Robert S, Bert VL. 1989. Tight-binding inhibition of angiogenin and ribonuclease A by placental ribonuclease inhibitor. Biochemistry. 28(1):225-230. doi:10.1021/bi00427a031
35. Robert S, Bert VL. 1991. Interaction of human placental ribonuclease with placental ribonuclease inhibitor. Biochemistry. 30(8):2246-2255. doi:10.1021/bi00222a030
36. Speer SL, Zheng W, Jiang X, Chu I, Guseman AJ, Liu M, Pielak GJ, Li C. 2021. The intracellular environment affect protein-protein interactions. Proceedings of the National Academy of Sciences. 18(11):1-7. doi:10.1073/pnas.2019918118
37. Gao B, Ellis HR. 2005. Altered mechanism of the alkanesulfonate FMN reductase with the monooxygenase enzyme. Biochemical and Biophysical Research Communications. 331(4):1137-1145.
38. Abdurachim KA. 2007. Studies to Elucidate the Mechanism of Reduced Flavin Transfer in the Alkanesulfonate Monooxygenase System From Escherichia coli. [Thesis]. Alabama [US]: Department of Chemistry and Biochemistry. Auburn University
39. Zhang YHP. 2011. Substrate channeling and enzyme complexes for biotechnological applications. Biotechnology Advance. 29(6):715-725. doi:10.1016/j.biotechadv.2011.05.020
40. Raushel FM, Thonden JB, Holden HM. 2003. Enzymes with molecular tunnels. Accounts of Chemical Research. 36(7):539-548. doi:10.1021/ar020047k
41. Abdurachim K, Ellis HR. 2006. Detection of protein-protein interactions in the alkanesulfonate monooxygenase system from Escherichia colli. Journal of Bacteriology. 188(23):8153-8159. doi:10.1128/JB.00966-06
42. Jeffers CE, Nichols JC, Tu SC. 2003. Complex formation between vibrio harveyi luciferase and monomeric NADPH:FMN oxidoreductase. Biochemistry. 42(2):529-534. doi:10.1021/bi026877n
43. Wilson IA, Stanfield RL. 1994. Antibody-antigen interactions: new structures and new conformational changes. Current Opinion in Structural Biology. 4(6):857-867. doi:10.1016/0959-440x(94)90267-4
44. Weber J, Lee RS, Wilke-Mounts S, Grell E, Senior A. 1993. Combined application of site-directed mutagenesis, 2-azido-ATP labeling, and lin-benzo-ATP binding to study the noncatalytic sites of Escherichia coli F1-ATPase. Journal of Biologycal Chemistry. 268(9):6241-5247. doi:10.1016/S0021-9258(18)53245-2
45. Steitz TA, Anderson WF, Fletterick RJ, Anderson CM. 1977. High resolution crystal structure of yeast hexokinase complexes with substrates, activators, and inhibitors. Evindence for an allosteric control site. Journal of Biologycal Chemistry. 252(13):4494-4500. doi:10.1016/S0021-9258(17)40188-8
46. Evans PR, Farrants GW, Hudson PJ, Britton HG. 1981. Phosphofructokinase: structure and control [and discussion]. Philosophical Transactions of the Royal Society B: Biological Sciences. 293(1063):53-62. doi:10.1098/rstb.1981.0059
47. Sauer RT, Krovantin W, DeAnda J, Youderian P, Susskind MM. 1983. Primary structure of the immI immunity region of bacteriophage P22. Journal of Molecular Biology. 168(4):699-713. doi:10.1016/s0022-2836(83)80070-9
48. Vincent, Pierre J, Michel L. 1972. Trypsin-pancreatic trypsin inhibitor association. Dynamics of the interaction and role of disulfide bridges. Biochemistry. 11(16):2967-2977. doi:10.1021/bi00766a007
49. Rao VS, Srinivas K, Sujini GN, Kumar GNS. 2014. Protein-protein interaction detection: methods and analysis. International Journal of Proteomics. 12:2-10. doi:10.1155/2014/147648
50. Poluri KM, Gulati K, Sarkar S. 2021. Experimental Methods for Determination of Protein–Protein Interactions. In: Protein-Protein Interactions. Springer, Singapore. 197-264. doi:10.1007/978-981-16-1594-8_5
51. Berggard T, Linse S, James P. 2007. Methods for the detection and analysis of protein–protein interactions. Proteomics. 7(16):2833-2842. doi:10.1002/pmic.200700131
52. Formosa, Tim. 1991. Using protein affinity chromatography to probe structure of protein machines. Methods in Enzymol. 208:24-45. doi:10.1016/0076-6879(91)08005-3
53. Friedrich P. 1984. Supramolecular Enzym Organization: Quatemary Structure and Beyond. Pergamon Press, Oxford
54. Srere, Paul A. 1987. Complexes of sequential metabolic enzymes. Annual Review of Biochemistry. 56(1):89-124. doi:10.1146/annurev.bi.56.070187.0005
55. Goodsell DS, Olson AJ. 1993. Soluble proteins: Size, shape and function. Trends in Biochemical Sciences. 18(3):65-68. doi:10.1016/0968-0004(93)90153-E
56. Klotz IM, Darnall DW, Langerman NR. 1975. In: The proteins (Neurath, H. & Hill, R. L., eds). Academic Press. Inc, New York
57. Berggard T, Linse S, James P. 2007. Methods for the detection and analysis of protein-protein interactions. Proteomics. 7:2833-2842. doi:10.1002/pmic.200700131
58. Muronetz VL, Sholukh M, Korpela T. 2001. Use of protein–protein interactions in affinity chromatography. Journal of Biochemical and Biophysical Methods. 49(1-3):0-47. doi:10.1016/s0165-022x(01)00187-7
59. Ratner D. 1974. The interaction of bacterial and phage proteins with immobilized Escherichia coli RNA polymerase. Journal of Molecular Biology. 88(2):373-383. doi:10.1016/0022-2836(74)90488-4
60. Miller, Kathryn G. 1991. Use of actin filament and microtubule affinity chromatography to identify proteins that bind to the cytoskeleton. Methods in

Enzymol.196:303-319. doi:10.1016/0076-6879(91)96028-p
61. Cohen BD, Lowy DR, Schiller JT. 1993. The conserved C-terminal domain of the bovine papillomavirus E5 oncoprotein can associate with an alpha-adaptin-like molecule: a possible link between growth factor receptors and viral transformation. Molecular and Celullar Biology. 13:6462-6468. doi:10.1128/mcb.13.10.6462-6468.1993
62. Truan R, Xiao H, Ingles CJ, Blatt G. 1993. Direct interaction between the transcriptional activation domain of human p53 and the TATA box-binding protein. Journal of Biologycal Chemistry. 268:2284-2287. doi:10.1016/S0021-9258(18)53769-8
63. Fan C, Basri G, Simon W, Stephan FR, Alfredo iJ, Renato Z. 2016. Applying mass spectrometry to study non-covalent biomolecule complexes. Mass Spectrometry Reviews. 35(1):48–70. doi:10.1002/mas.21462
64. Baird BA, Hammes GG. 1976. Chemical cross-linking studies of chloroplast coupling factor 1. Journal of Biologycal Chemistry. 251(22): 6953-6962. doi:10.1016/S0021-9258(17)32927-7
65. Leitner , Joachimiak LA, Unverdorben P, Walzthoeni T, Frydman J, Forster F, Aebersold R. 2014. Chemical cross-linking/mass spectrometry targeting acidic residues in proteins and protein complexes. Proceedings of the National Academy of Sciences. 111(26):9455–9460. doi:10.1073/pnas.1320298111
66. Mayers G. 2020. XLMOD: Cross-linking and chromatography derivatization reagents ontology. Biomolecules. 1-10. doi:10.48550/arXiv.2003.00329
67. Rockford IL. 2006. Cross-linking reagents Technical Handbook. Pierce Biotechnology Inc.
68. Gunzburg Dj, Riehl R, Weinbarg RA. 1989. Identification of a protein associated with p21ras by chemical crosslinking. Proceedings of the National Academy of Sciences. 86(11):4007-4011. doi:10.1073/pnas.86.11.4007
69. Baird BA, Hammes GG. 1976. Chemical cross-linking studies of chloroplast coupling factor 1. Journal of Biologycal Chemistry. 251(22): 6953-6962. doi:10.1016/S0021-9258(17)32927-7
70. Bragg PD, Hou C. 1980. A cross-linking study of the Ca^{2+} activated adenosine trihosphatase of Escherichia coli. European Journal of Biochemistry. 106(2):495-503. doi:10.1111/j.1432-1033.1980.tb04596.x
71. Sarkar FH, Gupta SL. 1984. Receptors for human gamma interferon: binding and crosslinking of 125I-labeled recombinant human gamma interferon to receptors on WISH cells. Proceedings of the National Academy of Sciences. 81(16):5160-5164. doi:10.1073/pnas.81.16.5160
72. Scherer PE. Manning-Krieg UC, Jeno P, Schatz G, Horst M. 1992. Identification of a 45-kDa protein at the protein import site of the yeast mitochondrial inner membrane. Proceedings of the National Academy of Sciences. 89(24):11930-11934. doi:10.1073/pnas.89.24.11930
73. Sanders SL, Whitfield KM, Vogel JP, Rose MD, Schekman RW. 1992. Sec61p and BiP directly facilitate polypeptide translocation into the ER. Cell. 69(2):353-365. doi:10.1016/0092-8674(92)90415-9
74. Matthias M, Hendrickson RC, Akhilesh P. 2001. Analysis of Protein and Proteomes by mass spectrometry. Annual Review of Biochemistry. 70(1):437-473. doi:10.1146/annurev.biochem.70.1.437
75. Traut RR, Casiano C, Zecherle N. 1989. Crosslinking of Protein Subunit and Ligands by Introductions of disulfide bonds. In: Creighton (ed)
76. Cover JA, Lambert JM, Norman CM, Traut RR. 1981. Identification of proteins at the subunit interface of the Escherichia coli ribosome by cross-linking with dimethyl 3,3'-dithiobis(propionimidate). Biochemistry. 20(10):2843-2852.
77. Green NS, Reisier E, Houk KN. 2001. Quantitative evaluation of the lengths of homobifunctional protein cross-linking reagents used as molecular rulers. Protein Science. 10(7):1293-1304. doi:10.1110/ps.51201
78. O'Brien R, Ladbury JE, Chowdry BZ. 2000. Isothermal titration calorimetry of biomolecules. Chapter 10 in Protein-Ligand interactions: hydrodynamics and calorimetry Ed. Harding SE and Chowdry BZ. Oxford University Press.
79. Jelesarov L, Bosshard HR. 1996. Thermodynamic characterization of the coupled folding and association of heterodimeric coiled coils (Leucine zippers). Journal of Molecular Biology. 263(2):344-358. doi:10.1006/jmbi.1996.0579
80. Pearce KH, Ultsch MH, Kelley RF, De Vos AM, Wells JA. 1996. Structural and mutational analysis of affinity-inert contact residues at the growth hormone–receptor interface. Biochemistry. 35(32):10300-10307. doi:10.1021/bi960513b
81. Wintrode PL, Privalov PL. 1997. Energetics of target peptide recognition by calmodulin: A calorimetric study. Journal of Molecular Biology. 266(5):1050-1062. doi:10.1006/jmbi.1996.0785
82. Arroyo MIJ, Campos TJ, Hernández AA, McClements DJ. 2016. Characterization of flavonoid-protein interactions using fluorescence spectroscopy: Binding of pelargonidin to dairy proteins. Food Chemistry. 213:431–439. doi:10.1016/j.foodchem.2016.06.105
83. Reza AM, Ulrike H, Goody RS, Alferd W. 1997. Individual rate constants for the interaction of ras proteins with GTPase-activating proteins determined by fluorescence spectroscopy. Biochemistry. 36(15):4535-4541. doi:10.1021/bi962556y
84. Bai Y, Perez GM, Beechem JM, Weil PA. 1997. Structure-function analysis of TAF130: identification and characterization of a high-affinity TATA-binding protein interaction domain in the N terminus of yeast TAF(II)130. Molecular and Cellular Biology. 17(6):3081-3093. doi:10.1128/mcb.17.6.3081
85. Annie OB, Bruno A, Minh VT, Pierre C, Marc C. 1993. Interaction between the retinal cyclic GMP phosphodiesterase inhibitor and transducin. Kinetics and affinity studies. Biochemistry. 32(33):8636-8645. doi:10.1021/bi00084a035
86. Weiel J, Bershey JW. 1982. The binding of fluorescein-labeled protein synthesis initiation factor 2 to Escherichia coli 30 S ribosomal subunits determined by fluorescence polarization. Journal of Biological Chemistry. 257(3):1215-1220. doi:10.1016/s0021-9258(19)68177-9

87. Raffaele DF, Annalisa P, Giuseppe V, Riccardo C. 1991. Circular dichroism study on the conformational stability of the dimerization domain of transcription factor LFB1. Biochemistry. 30(1):143-147. doi:10.1021/bi00215a021
88. Ling Y. 1997. Molecular characterization of the B-box protein-protein interaction motif of the ETS-domain transcription factor Elk-1. The EMBO Journal. 16(9):2431-2440. doi:10.1093/emboj/16.9.2431
89. Gillette MA, Carr SA. 2012. Quantitative analysis of peptides and proteins in biomedicine by targeted mass spectrometry. Nature Methods. 10(1):28–34. doi:10.1038/NMETH.2309
90. Antony C, Guy P, Gilbert G. 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. 36(2):380–407. doi:10.1111/j.1574-6976.2011.00298.x
91. Van B, Gary J, Kertesz V. 2007. Using the Electrochemistry of the Electrospray Ion Source. Analytical Chemistry. 79(15): 5510-5520.
92. Keevil, Brian G. 2013. Novel liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for measuring steroids. Best Practice & Research Clinical Endocrinology & Metabolism. 27(5): 663–674. doi:10.1016/j.beem.2013.05.015
93. Mullari M, Lyon D, Jensen LJ, Nielsen ML. 2017. Specifying RNA-binding regions in proteins by peptide Cross-Linking and Affinity Purification. Journal of Proteome Research. 7b00042. doi:10.1021/acs.jproteome.7b00042
94. Prestegard JH, Kishore AI. 2001. Partial alignment of biomolecules: an aid to NMR characterization. Current Opinion in Chemical Biology. 5(5):584-590. doi:10.1016/s1367-5931(00)00247-7
95. [95] Blackledge M. 2005. Recent progress in the study of biomolecular structure and dynamics in solution from residual dipolar couplings. Progress in Nuclear Magnetic Resonance Spectroscopy. 46:23-61. doi:10.1016/j.pnmrs.2004.11.002
96. Zhuogin Y, Pengfei L, Kenneth M. 2017. Using ligand-induced protein chemical shift perturbations to determine protein–ligand structures. Biochemistry. 56(18):2349-2362. doi:10.1021/acs.biochem.7b00170
97. McCoy MA, Wyss DF. 2002. Spatial localization of ligand binding sites from electron current density surfaces calculated from NMR chemical shift perturbations. Journal of the American Chemical Society. 124(39):11758-11763. doi:10.1021/ja026166c
98. Welch BD, Blake BK, David DR, Meyer HH, Emr SD, Sundquist WI, Alam SL, Sun J, Payne M. 2004. Ubiquitin interactions of NZF zinc fingers. The EMBO Journal. 23(7):1411-1421. doi:10.1038/sj.emboj.7600114
99. Abarna T, Daniel N, Helen MR, Mitsuru O, Debbie L, Peter NR, Miriam H, Alain V, Natalia MV, Ernest DL. 2004. Structural basis of HP1/PXVXL motif peptide interactions and HP1 localisation to heterochromatin. The EMBO Journal. 23(3):489-499. doi:10.1038/sj.emboj.7600088
100. Wei F, Jia-Fu L, Jing-Song J, Tetsuya S, Mingjie Z. 2004. The tetrameric L27 domain complex as an organization platform for supramolecular assemblies. Nature Structural & Molecular Biology. 11(5):475-480. doi:10.1038/nsmb751
101. Yoshimoto FK. 2020. The Proteins of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2 or n-COVI9), the Cause of COVID-19. The Protein Journal. 39:198-216. doi:10.1007/s10930-020-09901-4