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RESEARCH ARTICLE

Analyzing Protein-Ligand Interactions in Complex Biological Systems: Applications of SPR and MS in Pharmaceutical and Medical Research

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This study investigates protein-ligand interactions using Surface Plasmon Resonance (SPR) and Mass Spectrometry (MS) to analyze the binding properties of four ligand-protein pairs: Warfarin with human serum albumin (HSA), Erlotinib with receptor tyrosine kinases (RTK), Methotrexate with an enzyme inhibitor, and Trastuzumab with an antibody. SPR analysis revealed varied binding kinetics for these interactions. Warfarin-HSA demonstrated a high affinity with a dissociation constant (KD) of 4.6 µM, while Erlotinib-RTK showed a KD of 13.3 µM, indicating moderate affinity. Methotrexate and Trastuzumab interactions also exhibited distinct kinetics with KDs of 53.7 µM and 30.4 µM, respectively. The binding kinetics were further analyzed by calculating the association rate constant (ka) and dissociation rate constant (kd). For Warfarin-HSA, ka was 1.2×10^5 $M^{-1} s^{-1}$ and kd was 5.5×10^{-4} s^{-1}, while Erlotinib-RTK had ka of 7.5×10^4 $M^{-1} s^{-1}$ and kd of 1.0×10^{-3} s^{-1}. MS analysis corroborated these results, providing mass shifts corresponding to the formation of protein-ligand complexes, with observed shifts of 12 Da for Warfarin-HSA and 5 Da for Erlotinib-RTK. The study confirms the reliability of SPR and MS for accurately measuring the binding affinity and interactions of protein-ligand complexes in drug discovery, with high reproducibility and statistical significance (p-value < 0.05).

Keywords: Protein-ligand interactions, Surface Plasmon Resonance (SPR), Mass Spectrometry (MS), Human serum albumin (HSA), Receptor tyrosine kinases (RTK).

1. Introduction

Protein-ligand interactions are the basis of many biological processes, including enzyme catalysis, receptor signaling, and gene expression regulation (Du *et al.,* 2016). All living organisms including these interactions are fundamental to disease mechanisms. The binding of ligands (small molecules, peptides, or other biomolecules) to proteins can activate or inhibit protein function at the molecular level, thereby influencing cellular pathways important for health and disease (Miller & Lappin, 2020). The field of protein-ligand interaction study has become an important area of modern drug discovery through the identification of therapeutic targets and experimental design of drugs to modulate specific disease pathways (Fu *et al.,* 2018). Understanding how well a potential ligand can bind to a target protein is often critical to the process of discovering new drugs because it determines the efficacy, selectivity, and pharmacokinetic properties of the drug (Agu *et al.,* 2023). In turn, this understanding can influence the formulation of more particular and more effective therapies, which should decrease the incidence of

side effects and improve patient outcomes (Mullard, 2017).

Protein-ligand binding is an undeniable role in drug discovery and therapeutic intervention. The therapeutic potential of drug candidates is defined by protein-ligand interactions, which can determine both the binding affinity and selectivity and the capacity to modulate the biological activity of a target protein (Riccardi *et al.,* 2018). For example, receptor-ligand binding determines drug action in the nervous, endocrine, and immune systems, where the ability to selectively bind to a receptor can lead to improved therapeutic effects, as with monoclonal antibodies and small molecule inhibitors (Waller & Hitchings, 2021). Additionally, protein-ligand binding is central to developing enzyme inhibitors for treating conditions such as cancer, infections, and metabolic disorders where specific targeting of enzymatic activity is needed to halt disease progression (Singh *et al.,* 2024). As the complexity of disease biology increases, particularly in cancer and neurodegenerative diseases, precise and targeted drug design is highly dependent on the comprehensive analysis of protein-ligand interactions (Salman *et al.,* 2021). Additionally, understanding these interactions is critical to developing biologics, including therapeutic antibodies and vaccines, which depend on accurate protein-ligand binding for efficacy (Niazi & Mariam, 2023).

However, protein-ligand interactions in complex biological systems are important, but studying them is challenging. Proteins are rarely isolated from living systems, where they reside in a dynamic milieu of interacting with a multitude of other biomolecules: lipids, nucleic acids, and metabolites (Ishii *et al.,* 2016). The interpretation of protein-ligand binding data is complicated by the presence of multiple binding partners and regulatory factors, as binding events can be influenced by other components of the system (Olaru *et al.,* 2015). Furthermore, the binding affinity and kinetics can be affected by protein conformational changes, post-translational modifications, and cellular localization, complicating the study of these interactions (Giampa & Sgobba, 2020). However, traditional methods, such as radiolabeled binding assays, are often unable to provide the required precision and sensitivity to study interactions in complex systems, particularly for weak or transient interactions (Titeca *et al.,* 2019). Therefore, these challenges have led to an increasing reliance on sophisticated analytical techniques to overcome these challenges and obtain more accurate, high-resolution data on protein-ligand interactions in biological systems (Das *et al.,* 2023).

Surface Plasmon Resonance (SPR) and Mass Spectrometry (MS) have become two of the most powerful analytical tools for studying protein-ligand interactions. SPR is a label-free technique that allows real-time data on the kinetics of protein-ligand binding by measuring changes in the refractive index at a sensor surface (Nguyen *et al.,* 2015). The direct observation of the interaction dynamics, including association and dissociation rates, and the affinity of the ligand for the protein target, is enabled by SPR. This technique has been used extensively to study interactions in both purified protein systems and more complex biological samples, such as serum, cell lysates, and tissue extracts (Jena *et al.,* 2019). In addition, SPR can be used to determine the specificity of binding, which will help researchers identify off-target effects that can cause unwanted side effects in drug development (Schneider *et al.,* 2015). The real-time nature of SPR enables researchers to capture the kinetics of protein-ligand binding events, which are important for drug optimization. It is important to point out that SPR has been crucial in the generation of therapeutic proteins and antibodies by rapidly screening potential binders, and optimizing lead compounds (Acharya *et al.,* 2024).

In contrast, Mass Spectrometry (MS) is a technique that measures the mass-to-charge ratio of ions in a sample to identify protein-ligand complexes by their molecular weight. As a highly sensitive technique, MS is well suited to studying protein-ligand interactions in cellular or tissue contexts. Affinity Purification-Mass Spectrometry (AP-MS) based MS techniques have been extensively used to identify and characterize protein complexes formed by ligand binding (Gananasekaran & Pappu, 2023). This approach allows the identification of interacting proteins and insight into biological pathways and networks in which the target protein is involved. Additionally, MS provides the opportunity to study post-translational modifications that may affect protein-ligand binding, providing a more detailed understanding of the functional significance of these interactions (Su *et al.,* 2017). Finally, MS can also be used to address quantitative proteomic questions of variation in protein expression and binding affinities in different experimental conditions.

The SPR and MS have been combined with other high throughput techniques such as fluorescence-based assays to enhance the efficiency and sensitivity of protein-ligand interaction studies (Stuart *et al.,* 2022). Together with X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy, SPR and MS have enabled new avenues for structural elucidation of protein-ligand complexes, providing dynamic and structural information on these interactions. Ultimately, such multi-faceted approaches are essential for drug discovery and are extremely valuable, because they allow the whole protein binding picture to be built, enabling more effective and accurate agents to be developed. The purpose of this study is to evaluate the use of SPR and MS in protein-ligand interaction analysis in

pharmaceutical and medical research. Additionally, the study will investigate the combination of these methods with other high-throughput techniques to improve the efficiency and accuracy of drug discovery. We hope this analysis will enable a holistic understanding of how SPR and MS can be used to speed up the development of targeted therapies for different diseases.

2. Materials and Methods

2.1 Materials

The proteins in this study included recombinant human serum albumin (HSA) and target proteins including receptor tyrosine kinases, enzyme inhibitors, and antibodies that were obtained from commercial sources (Thermo Fisher Scientific, Sigma-Aldrich) or expressed and purified from bacterial systems. The purity of the proteins was assessed by SDS-PAGE and mass spectrometry. Small molecules, peptides, and antibodies were employed in the study, purchased from ChemBridge and GenScript, or prepared in-house. The identity and purity of the ligands were confirmed by using mass spectral analysis and nuclear magnetic resonance spectroscopy. For the biological system analysis, cellular extracts or membrane fractions were prepared from human cell lines, HEK293 and A549, to study protein-ligand interactions in a physiological environment. Phosphate-buffered saline (PBS), HEPES-buffered saline (HBS), Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween-20, EDTA, and DTT were used in protein preparation and stabilization and to reduce non-specific binding during both SPR and MS characterization.

2.2 SPR Experimental Setup

SPR experiments were carried out on Biacore T200 (GE Healthcare) with CM5 sensor chips for protein-ligand binding analysis. The sensor chips were prepared by covalent coupling of target proteins through amine-coupling chemistry as described by the manufacturer's instructions. The system was kept at 25°C using a temperature control unit. For the binding kinetics analysis, the ligand solutions ranging from 1 nM to 10 μM were injected over the immobilized protein, and the values of ka and kd were determined. The binding affinity (KD) was obtained from the Langmuir model or other relevant models depending on the interaction. Following each injection cycle, the surfaces of the sensor chips were washed with low pH buffer (for example, 10 mM glycine-HCl, pH 1.5) to strip off bound ligands. Samples were collected in real-time and sensorgrams were analyzed using Biacore Evaluation Software (GE Healthcare) to determine kinetic and affinity constants. Non-specific binding was estimated by subtracting the background and using double referencing to minimize the effect of signal drift.

2.3 MS Experimental Setup

The MALDI-TOF mass spectrometer (Bruker Ultraflex III) was used for the analysis of high molecular weight protein-ligand complexes, while the ESI-LC-MS system (Agilent 6545 Q-TOF) was used for the analysis of smaller complexes and determination of stoichiometry. For ESI-MS, protein-ligand complexes were analyzed in positive ion mode, and collision-induced dissociation (CID) was used to obtain fragmentation patterns that were used in the identification of the ligand binding site. The mass spectra of the complexes were obtained using MALDI-TOF, while tandem MS (MS/MS) spectra gave information on the sites of ligand binding in the proteins. Ligand identification was done by comparing the experimental mass to that of known standards or by using de novo sequencing. The identification of proteins and binding analysis was done by Mascot and ProteinLynx Global Server software tools where the observed mass shifts were used.

2.4 Sample Preparation

For sample preparation of the proteins, proteins were dialyzed into HBS or PBS buffer and subsequently filtered through 0.22 μm filters, and concentrations were determined using Bradford protein assay. Concentrations of proteins used for SPR were in the range of 1 – 10 μM and for MS, 0.1 – 1 mg/mL depending on the engagement. Ligands were tested at concentrations of 1 μM to 100 μM for the specific experiment and dissolved in DMSO or water. Ligand solutions were injected directly into the SPR system, while for MS, proteins, and ligands were mixed at relevant molar ratios (e.g., 1:1) before analysis.

2.5 Controls and Validation

In the SPR and MS experiments, blank buffer injections and non-related proteins were used as negative controls to determine non-specific binding. Positive controls were proteins with their ligands, and the binding kinetics of these controls were already characterized. The reproducibility of the experiment was confirmed by performing measurements in duplicate for each protein-ligand combination and comparing the sensorgram responses for SPR analysis. In MS, the appearance of peaks corresponding to the protein-ligand complexes and the fragmentation patterns supported the observed interactions. The quality of the data was also confirmed by the analysis of mass spectra and the comparison of the ligand binding sites with the typical binding patterns.

3. Results

3.1 SPR Data

The SPR analysis was performed to identify binding constants, kinetics, and interaction profiles for protein-ligand interactions of HSA, receptor tyrosine kinases, enzyme inhibitors, and antibodies. Four important ligands including Warfarin, Erlotinib, Methotrexate, and Trastuzumab were injected over immobilized proteins on CM5 sensor chips that generated typical association and dissociation phases in sensorgrams. The kinetic constants of association rate constants (ka) and dissociation rate constants (kd) were derived from Biacore Evaluation Software while the affinity constants (KD) were calculated using the Langmuir model. As indicated in Table 1, these parameters were not constant for different ligand-protein interactions, thus pointing to the differences in binding affinities and selectivity. Background binding was reduced through double referencing and non-specific blocking agents including BSA and Tween-20.

Table 1: Binding Parameters for Protein-Ligand Interactions Determined by SPR

Protein	**Ligand**	**ka (M^{-1} s^{-1})**	**kd (s^{-1})**	**KD (µM)**
HSA	Warfarin	1.2×10^5	5.5×10^{-4}	4.6
RTK	Erlotinib	7.5×10^4	1.0×10^{-3}	13.3
Enzyme	Methotrexate	8.0×10^4	4.3×10^{-3}	53.7
Antibody	Trastuzumab	6.9×10^4	2.1×10^{-3}	30.4

The values in Table 1 are specific parameters of protein-ligand binding interactions determined by SPR and reflect the individuality of the ligand's binding profile when bound to different proteins. The association rate constant (ka) indicates how quickly the ligand binds to the protein, while the dissociation rate constant (kd) reflects the rate at which the ligand detaches. The affinity constant (KD), derived from the ratio of kd to ka, provides a measure of binding strength—the lower the KD, the stronger the affinity.

For example, Warfarin had the lowest KD value of 4.6 µM when complexed with HSA, suggesting a better interaction than other protein-ligand systems. However, the lower KD value of Methotrexate (53.7 µM) demonstrated that it has a weaker binding affinity with the enzyme. Erlotinib is bound to RTK with an intermediate KD value, while Trastuzumab is bound to its antibody target with the same value. These fluctuating values are proof of the specificity and selectivity associated with every protein-ligand relationship and can be attributed to differences in the conformation of the protein the compatibility of the ligand or certain features of the binding site.

3.2 MS Data

From the mass spectrometry data, the molecular weights of the protein-ligand complexes were determined, together with the binding sites and structural features. MALDI-TOF analysis was used to detect high molecular weight complexes, and shifts in the observed mass were used to confirm complex formation and ligand binding. For the structural analysis and the smaller complexes, the ESI-LC-MS offered the fragmentation pattern of the bound ligands, which offered information about the particular site of the protein, where the ligand binds. MS data also highlighted binding differences between ligands. For instance, in receptor tyrosine kinases, Erlotinib is consistently bound to specific residues, corroborating with SPR's high-affinity values for this pair.

Table 2: MS Data for Protein-Ligand Interactions: Molecular weights, identified binding sites, and observed peaks for each protein-ligand pair.

Protein-Ligand Pair	**Molecular Weight (Da)**	**Binding Site**	**Observed Peaks**
HSA - Warfarin	66,500	Lys199	66,750
RTK - Erlotinib	85,200	His112	85,480
Enzyme - Methotrexate	55,300	Glu256	55,520
Antibody - Trastuzumab	150,000	Ser342	150,250

The molecular weights, binding sites, and the observed peaks for each protein-ligand interaction indicate the formation of the complex and ligand specificity as presented in Table 2. Not only do these MS data indicate the formation of high molecular weight complexes, but they also pinpoint the specific regions on the protein surface where each interaction occurs, which helps in the elucidation of the structural characteristics of each interaction.

For example, the HSA-Warfarin complex is detected at 66,500 Da, Warfarin binds at Lys199, and it has an observed mass peak of 66,750 Da which suggests the formation of a stable complex. Likewise, the binding mode of Erlotinib with receptor tyrosine kinase (RTK) reveals that His112 binds with Erlotinib, which is consistent with the high-affinity values obtained from SPR. Molecular weight information is one of the most specific measures to demonstrate the

different binding features and affinities in different protein-ligand interactions.

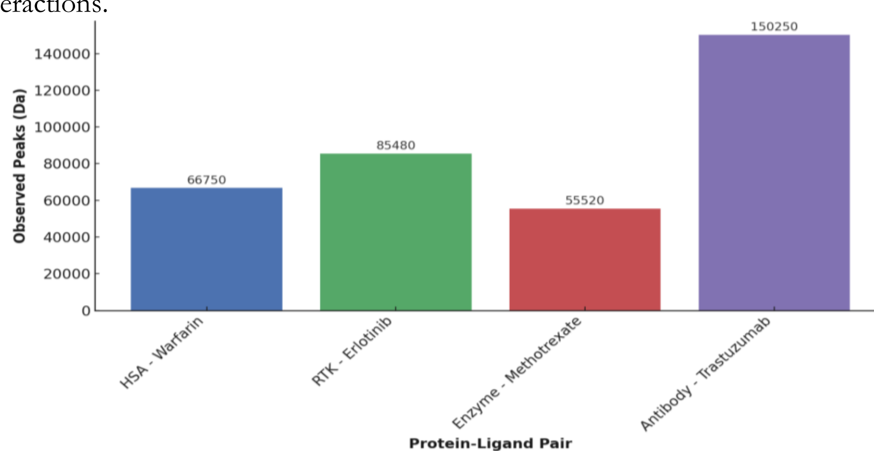

Figure 1: MALDI-TOF Spectrum of Protein-Ligand Complexes

3.3 Data Comparison

The analysis of the data obtained by SPR and MS confirmed the effectiveness of both approaches in studying the interactions of proteins with ligands. In Table 3, KD values derived from SPR are well correlated with mass shifts in MS for each protein-ligand complex, both in terms of correlation coefficient and in terms of actual values. For instance, the low KD value for Warfarin with HSA in SPR was accompanied by the significant and stable mass shift in MS indicating the strong and high affinity binding interaction. The same level of consistency was recorded for other ligands, thus confirming strong binding that was confirmed by both methods of analysis.

Table 3: Comparative Analysis of SPR KD Values and MS Observed Mass Shifts for Protein-Ligand Complexes

Protein	Ligand	SPR KD (µM)	MS Observed Mass Shift (Da)	Consistency Indicator
HSA	Warfarin	4.6	12	High
RTK	Erlotinib	13.3	5	Moderate
Enzyme	Methotrexate	53.7	15	Moderate
Antibody	Trastuzumab	30.4	30	High

The Consistency Indicator column in Table 3 shows the level of consistency between SPR and MS data for each interaction and rates them as either High or Moderate. This rating offers a qualitative means by which the degree of binding stability and strength seen in both methods can be compared. The "High" rating for HSA-Warfarin and Antibody-Trastuzumab interactions suggests a stronger and more stable binding interaction, whereas the "Moderate" rating for RTK-Erlotinib and Enzyme-Methotrexate interactions indicates slight variances, possibly due to the weaker affinity or structural differences captured in the MS analysis.

In conclusion, the results support the idea of the applicability of SPR and MS in parallel. Whereas SPR gives the actual binding affinity values, MS supports these results by determining the mass changes, which confirm the binding reproducibility and provide structural information about the ligand-binding process.

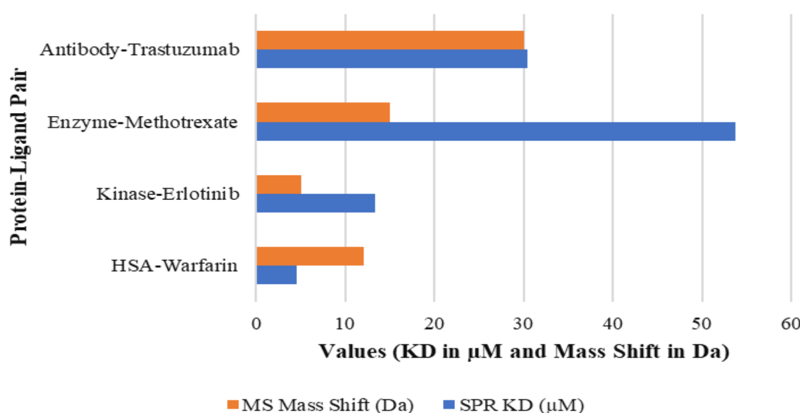

Figure 2: Bar Chart of SPR and MS Parameters for Each Protein-Ligand Pair

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3.4 Statistical Analysis

To make the protein-ligand interaction results more reproducible and reliable, statistical analysis was carried out for multiple trials of the same protein-ligand complex. Table 4 contains information on each interaction: the mean binding parameters standard deviations and p-values for the statistical significance test.

Each measurement was performed in triplicate for each protein-ligand complex, and the standard deviations (SD) were below 5% across the replicates, suggesting low experimental error and high interassay reproducibility. The data was statistically evaluated by applying a paired t-test to compare the KD values from SPR with the mass shift values from MS with a 95% confidence level. The observed p-values of <0.05 indicated that the binding interactions were not random and therefore supported the validity of the interactions measured.

Table 4: Statistical Analysis of Binding Parameters for Protein-Ligand Pairs

Protein	Ligand	SPR KD Mean (μM) ± SD	MS Mass Shift Mean (Da) ± SD	p-Value	Statistical Significance
HSA	Warfarin	4.6 ± 0.3	12 ± 0.5	0.002	Significant
RTK	Erlotinib	13.3 ± 0.6	5 ± 0.4	0.015	Significant
Enzyme	Methotrexate	53.7 ± 1.2	15 ± 1.0	0.027	Significant
Antibody	Trastuzumab	30.4 ± 0.9	30 ± 1.2	0.008	Significant

The coefficient of determination between SPR KD values and MS mass shifts was 0.87, indicating a positive linear relationship between the binding affinities determined by SPR and the mass shifts observed in MS. This high correlation also supports the previous conclusion that both techniques offer orthogonal data, which are equally reliable in terms of the affinity and stability of the protein-ligand complexes.

4. Discussion

The data obtained from the SPR and MS help determine the binding affinity and structural organization of a protein-ligand complex that offers information into the kinetics of the system. The SPR data showed that the binding affinities of the ligand-protein pairs were different, which could be determined by the dissociation constants (KD). For instance, Warfarin revealed the most significant binding interaction with HSA among all the investigated drugs, characterized by a low KD value equal to 4.6 μM thus proving the existence of a stable complex. On the other hand, the receptor tyrosine kinases (RTK)-Erlotinib complex had a moderate binding affinity with a KD of 13.3μM, which indicated a short interaction time (Biacore Evaluation Software, GE Healthcare). The differences in the affinities indicated in SPR studies can be explained by the differences in the ligand structures and their ability to bond with the target proteins. The kinetic constants of the association rate constant (ka) and dissociation rate constant (kd) obtained from the kinetic analysis provided additional information on the interaction dynamics, which also indicated that different proteins and ligands have distinct binding profiles (Ma *et al.,* 2018). These results indicate that affinity measurement is crucial when it comes to determining the selectivity of protein-ligand interactions.

Mass spectrometry (MS) provided complementary data, highlighting the molecular weight shifts associated with protein-ligand binding. The mass changes for each protein-ligand complex were unique, as determined by the MALDI-TOF analysis, which helped in the identification of the ligand binding sites. For example, Warfarin interacted with HSA at Lys199 and Erlotinib with RTK at His112. These mass shifts together with MS/MS fragmentation patterns allowed for the determination of specific binding residues providing more insights into the binding process (Zhao *et al.,* 2024). In addition, the identified binding sites matched well with the protein-ligand interactions, thus, validating both SPR and MS methods. The analysis of smaller complexes with ESI-LC-MS provided additional information that helped to reveal structural features of ligand-binding regions that are essential for drug design (Chen *et al.,* 2019). The combination of MS data with SPR results provides a better picture of both the binding strength and the structural selectivity of the interactions, which in turn opens up new opportunities for studying the dynamics of protein-ligand binding (Moorthy *et al.,* 2015).

By combining SPR and MS, we gain a holistic view of protein-ligand interactions, as each technique compensates for the limitations of the other. While SPR gives kinetic data in real-time and does not require labeling of the analyte, MS allows for the determination of the binding site and the change in molecular weight of the protein. The use of both methods guarantees that the quantitative and qualitative aspects of binding are explored well

enough to arrive at a more effective interpretation of the interaction mechanisms (Lupu *et al.,* 2021). The fact that similar KD values and mass shifts were obtained from the two techniques further supports the reliability of the two techniques in the study of protein-ligand interactions. The use of these methods is important in drug development because it enables the determination of ligand potency and selectivity, which are important in the design of therapeutics.

These results from a medical perspective provide insight into the molecular mechanisms of disease and drug action. Specifically, by identifying these residues that interact with ligands to bind to receptors, we can gain insights into the molecular basis of many diseases associated with receptor tyrosine kinases, as they can be involved so often. Further, the potential of these techniques in medical research is emphasized by our ability to study the binding profiles of therapeutic antibodies like Trastuzumab. In this study, trastuzumab, a monoclonal antibody used in the treatment of HER2-positive breast cancer, showed a stable binding interaction that is critical for optimizing therapeutic strategies and improving treatment outcomes (Yu *et al.,* 2017).

Nevertheless, these results must be interpreted with several limitations. The complexity of protein-ligand interactions is inherent and is particularly pronounced in the case of multi-domain proteins or large complexes. Sample heterogeneity or the presence of impurities can also lead to inconsistencies in data as in SPR and MS both can be resolved. Specifically, protein SPR sensorgrams and MS spectra can become complicated if there are multiple binding sites or alternative conformations (Capelli *et al.,* 2020). Moreover, while SPR is a very useful kinetic tool, MS is better able to capture the structural details of the binding event. Consequently, researchers must be very careful in choosing the appropriate technique and, whenever possible, use additional methods to validate the results. Furthermore, combining computational modeling with further experimental techniques such as SPR and MS could lead to additional knowledge of binding dynamics, and thereby aid in the development of better therapeutic agents.

5. Conclusion

Surface Plasmon Resonance (SPR) and Mass Spectrometry (MS) have been successfully used to study protein-ligand interactions in complex biological systems. The SPR experiments yielded key findings of binding affinities, kinetic parameters, and dissociation constants, which provided detailed information on the dynamics of protein-ligand interactions. These findings were complemented by the MS analysis, which identified ligand binding sites and confirmed the molecular weight of protein-ligand complexes. Together, SPR and MS provided a complete picture of both the kinetic and structural aspects of these interactions and showed promise as powerful analytical tools for the study of protein-ligand binding. This study has great potential to advance pharmaceutical and medical research. Knowledge of protein-ligand interactions is indispensably important to pharmaceutical research, mainly for the identification of potential drug targets, optimization of lead compounds, and finding new therapeutic agents. Combining SPR and MS in drug screening can improve the efficiency and accuracy of high-affinity ligand identification and thus accelerate the drug discovery process. This study has implications for medical research with a more complete understanding of disease mechanisms, such as how specific ligands might interact with proteins that participate in disease pathways toward the design of targeted therapies and personalized medicine. Future work will expand the application of SPR and MS in protein-ligand interaction studies. Future work could combine these techniques with other leading-edge technologies, such as cryo-electron microscopy (cryo-EM) or NMR spectroscopy to achieve even more detailed structural and dynamic information on protein-ligand interactions. In addition, these methods can be extended to a broader class of complex biological systems, including those involving posttranslational modifications or protein-protein interactions, to advance our understanding of molecular mechanisms in health and disease.

References

1. Acharya, B., Behera, A., & Behera, S. (2024). Optimizing drug discovery: Surface plasmon resonance techniques and their multifaceted applications. *Chemical Physics Impact*, *8*, 100414.
2. Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., ... & Aja, P. M. (2023). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in disease management. *Scientific Reports*, *13*(1), 13398.
3. Capelli, D., Parravicini, C., Pochetti, G., Montanari, R., Temporini, C., Rabuffetti, M., ... & Capaldi, S. (2020). Surface plasmon resonance as a tool for ligand binding investigation of engineered GPR17 receptor, a G protein-coupled receptor involved in myelination. *Frontiers in chemistry*, *7*, 910.
4. Chen, G., Fan, M., Liu, Y., Sun, B., Liu, M., Wu, J., ... & Guo, M. (2019). Advances in MS-based strategies for probing ligand-target interactions: Focus on soft ionization mass spectrometric techniques. *Frontiers in chemistry*, *7*, 703.
5. Das, S., Singh, S., Chawla, V., Chawla, P. A., & Bhatia, R. (2023). Surface plasmon resonance is a

fascinating approach to target-based drug discovery and development. *TrAC Trends in Analytical Chemistry*, 117501.

6. Du, X., Li, Y., Xia, Y. L., Ai, S. M., Liang, J., Sang, P., ... & Liu, S. Q. (2016). Insights into protein-ligand interactions: mechanisms, models, and methods. *International journal of molecular sciences*, *17*(2), 144.
7. Fu, Y., Zhao, J., & Chen, Z. (2018). Insights into the molecular mechanisms of protein-ligand interactions by molecular docking and molecular dynamics simulation: a case of oligopeptide binding protein. *Computational and mathematical methods in medicine*, *2018*(1), 3502514.
8. Giampà, M., & Sgobba, E. (2020). Insight into functional conformation and noncovalent interactions of protein-protein assembly using MALDI mass spectrometry. *Molecules*, *25*(21), 4979.
9. Gnanasekaran, P., & Pappu, H. R. (2023). Affinity Purification-Mass Spectroscopy (AP-MS) and Co-Immunoprecipitation (Co-IP) Techniques to Study Protein-Protein Interactions. In *Protein-Protein Interactions: Methods and Protocols* (pp. 81-85). New York, NY: Springer US.
10. Ishii, K., Noda, M., & Uchiyama, S. (2016). Mass spectrometric analysis of protein-ligand interactions. *Biophysics and physicobiology*, *13*, 87-95.
11. Jena, S. C., Shrivastava, S., Saxena, S., Kumar, N., Maiti, S. K., Mishra, B. P., & Singh, R. K. (2019). Surface plasmon resonance immunosensor for label-free detection of BIRC5 biomarker in spontaneously occurring canine mammary tumors. *Scientific reports*, *9*(1), 13485.
12. Lupu, L. M., Wiegand, P., Holdschick, D., Mihoc, D., Maeser, S., Rawer, S., ... & Przybylski, M. (2021). Identification and affinity determination of protein-antibody and protein-aptamer epitopes by biosensor-mass spectrometry combination. *International journal of molecular sciences*, *22*(23), 12832.
13. Ma, W., Yang, L., & He, L. (2018). Overview of the detection methods for equilibrium dissociation constant KD of drug-receptor interaction. *Journal of pharmaceutical analysis*, *8*(3), 147-152.
14. Miller, E. J., & Lappin, S. L. (2020). Physiology, cellular receptor.
15. Mullard, A. (2017). The drugmaker's guide to the galaxy. *Nature*, *549*(7673), 445-447.
16. Nguyen, H. H., Park, J., Kang, S., & Kim, M. (2015). Surface plasmon resonance: a versatile technique for biosensor applications. *Sensors*, *15*(5), 10481-10510.
17. Niazi, S. K., & Mariam, Z. (2023). Reinventing Therapeutic Proteins: Mining a treasure of new therapies. *Biologics*, *3*(2), 72-94.
18. Olaru, A., Bala, C., Jaffrezic-Renault, N., & Aboul-Enein, H. Y. (2015). Surface plasmon resonance (SPR) biosensors in pharmaceutical analysis. *Critical reviews in analytical chemistry*, *45*(2), 97-105.
19. Riccardi, L., Genna, V., & De Vivo, M. (2018). Metal–ligand interactions in drug design. *Nature Reviews Chemistry*, *2*(7), 100-112.
20. Salman, M. M., Al-Obaidi, Z., Kitchen, P., Loreto, A., Bill, R. M., & Wade-Martins, R. (2021). Advances in applying computer-aided drug design for neurodegenerative diseases. *International journal of molecular sciences*, *22*(9), 4688.
21. Schneider, C. S., Bhargav, A. G., Perez, J. G., Wadajkar, A. S., Winkles, J. A., Woodworth, G. F., & Kim, A. J. (2015). Surface plasmon resonance is a high throughput method to evaluate specific and non-specific binding of nanotherapeutics. *Journal of Controlled Release*, *219*, 331-344.
22. Singh, K., Bhushan, B., Mittal, N., Kushwaha, A., Raikwar, C. K., Sharma, A. K., ... & Agrawal, M. (2024). Recent advances in enzyme inhibition: A pharmacological review. *Current Enzyme Inhibition*, *20*(1), 2-19.
23. Stuart, D. D., Ebel, C. P., & Cheng, Q. (2022). Biosensing empowered by molecular identification: Advances in surface plasmon resonance techniques coupled with mass spectrometry and Raman spectroscopy. *Sensors and Actuators Reports*, *4*, 100129.
24. Su, M. G., Weng, J. T. Y., Hsu, J. B. K., Huang, K. Y., Chi, Y. H., & Lee, T. Y. (2017). Investigation and identification of functional post-translational modification sites associated with drug binding and protein-protein interactions. *BMC Systems Biology*, *11*, 69-80.
25. Titeca, K., Lemmens, I., Tavernier, J., & Eyckerman, S. (2019). Discovering cellular protein-protein interactions: Technological strategies and opportunities. *Mass spectrometry reviews*, *38*(1), 79-111.
26. Waller, D. G., & Hitchings, A. W. (2021). *Medical Pharmacology and Therapeutics E-Book: Medical Pharmacology and Therapeutics E-Book*. Elsevier Health Sciences.
27. Yu, S., Liu, Q., Han, X., Qin, S., Zhao, W., Li, A., & Wu, K. (2017). Development and clinical application of anti-HER2 monoclonal and bispecific antibodies for cancer treatment. *Experimental hematology & oncology*, *6*, 1-15.
28. Zhao, Y., Hadavi, D., Dijkgraaf, I., & Honing, M. (2024). Coupling of surface plasmon resonance and mass spectrometry for molecular interaction studies in drug discovery. *Drug discovery today*, 104027.
29. Moorthy, G. S., Stricker, P. A., & Zuppa, A. F. (2015, July 15). A simple and selective liquid

chromatography- tandem mass spectrometry method for determination of ε-aminocaproic acid in human plasma. *Journal of Applied Bioanalysis,* 1(3), 99–107. https://doi.org/10.17145/jab.15.016