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CHEMICAL TOOLS FOR UNDERSTANDING PROTEIN CONFORMATIONAL CHANGES

Abstract

Proteins are dynamic macromolecules that play crucial roles in numerous biological processes. Their functions often depend on conformational changes, which can be challenging to study using traditional methods. Chemical tools have emerged as powerful instruments for investigating and manipulating protein conformational changes. This paper reviews the diverse array of chemical tools and techniques that enable a deeper understanding of protein dynamics, highlighting their applications and potential implications in various fields of biology, from drug design to structural biology.

Keywords: Protein, Conformational changes, Fluorescent Probes, Drug discovery, Therapeutic

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I. INTRODUCTION

Proteins are the workhorses of biology, executing a myriad of functions, from enzymatic catalysis to cellular signaling. Their diverse roles are underpinned by dynamic conformational changes, which are critical for their activities. Understanding these structural transitions is essential for advancing our knowledge of biological processes, and for drug design, as many diseases are associated with protein misfolding or malfunction. Traditional techniques such as X-ray crystallography and NMR spectroscopy are invaluable for revealing static structures, but often fail to capture transient conformations. Chemical tools have revolutionized the study of protein conformational changes. They enable researchers to manipulate, monitor, and stabilize specific protein conformations, shedding light on the dynamic aspects of proteins. In this paper, we discuss several chemical tools that have significantly contributed to our understanding of protein conformational changes.

II. FLUORESCENT PROBES

Fluorescent probes are essential tools in the realm of chemical biology for investigating protein conformational changes. These probes are designed to emit fluorescence when they interact with specific regions or elements of a protein. Their ability to provide real-time monitoring of structural transitions makes them indispensable for studying dynamic processes in proteins. Here, we will explore the principles underlying the use of fluorescent probes, their applications, and their impact on our understanding of protein conformational changes.

Principles of Fluorescent Probes: The use of fluorescent probes to study protein conformational changes is based on the principles of fluorescence spectroscopy. These probes consist of a fluorescent molecule that can be selectively attached to or interact with a protein. When exposed to an appropriate excitation wavelength, the probe absorbs energy and temporarily exists in an excited state. It subsequently emits light at a longer wavelength (lower energy) as it returns to its ground state. The emitted light is detected as fluorescence, and its intensity can be used to track the probe's behavior within the protein.

Approaches for Study of Conformational Changes

Fluorescence Resonance Energy Transfer (FRET): FRET is a widely-used technique involving two different fluorophores, a donor and an acceptor. The donor fluorophore is excited by a light source and transfers energy to the acceptor fluorophore through non-radiative energy transfer if they are in close proximity (usually within a few nanometers). This energy transfer results in a decrease in the donor's fluorescence and an increase in the acceptor's fluorescence. FRET is particularly powerful in studying protein conformational changes because it can report on distance changes between specific sites in the protein.

1. **Intrinsic Fluorescence Monitoring:** In some cases, the protein itself may contain amino acids (e.g., tryptophan, tyrosine, and phenylalanine) that exhibit intrinsic fluorescence. Changes in protein conformation can lead to shifts in the intrinsic fluorescence spectra, which can be monitored to track conformational transitions.

Applications of Fluorescent Probes: Fluorescent probes have numerous applications in understanding protein conformational changes:

1. **Real-time Monitoring:** One of the key advantages of fluorescent probes is their ability to provide real-time data on conformational changes. This is particularly valuable in capturing fast transitions and transient states that may be challenging to observe using traditional structural biology techniques.

2. **Protein-Ligand Interactions:** Fluorescent probes can be used to investigate how ligands or small molecules affect protein conformation. By attaching a probe to the protein and monitoring changes in fluorescence in the presence of a ligand, researchers can gain insights into the binding kinetics and conformational shifts induced by ligand binding.

3. **Cellular Imaging:** Fluorescent probes are invaluable tools for studying protein conformational changes within living cells. They can be used to visualize and track proteins' dynamic behavior in their native cellular environments.

4. **Biophysical Studies:** Fluorescent probes are often used in conjunction with other biophysical techniques, such as circular dichroism and fluorescence anisotropy, to provide comprehensive insights into protein conformational changes.

5. **Drug Discovery:** The ability to screen potential drug candidates and evaluate their impact on protein conformational changes is a critical step in drug discovery. Fluorescent probes aid in the identification of compounds that modulate protein structures, making them valuable in pharmaceutical research.

Challenges and Considerations: While fluorescent probes are powerful tools, there are some challenges and considerations to keep in mind when using them

1. **Probe Design:** Careful design of the fluorescent probe is essential to ensure that it interacts specifically with the region of interest on the protein. Non-specific interactions can lead to false results.

2. **Photobleaching:** Overexposure to light can lead to photobleaching of the fluorescent probe, reducing the signal over time. Researchers must minimize photobleaching by optimizing experimental conditions.

3. **Quantitative Analysis:** Quantitative analysis of fluorescence data can be complex, requiring careful calibration and control experiments to convert fluorescence intensity into meaningful structural information.

III. COVALENT PROBES

Covalent probes are a class of chemical tools used in chemical biology and structural biology to investigate and manipulate protein conformational changes. These probes are designed to form covalent bonds with specific amino acid residues within a protein, allowing for the selective labeling, trapping, and stabilization of particular conformational states. Covalent probes offer a unique approach to studying protein dynamics by providing a means to "freeze" transient intermediates and to reveal otherwise elusive conformations.

Principles of Covalent Probes: The basic principle of covalent probes lies in their ability to react with specific amino acid residues in a protein. Commonly targeted residues include cysteine, lysine, and serine. The probes are typically designed to have functional groups that can chemically react with these nucleophilic amino acid side chains, forming covalent bonds. Once covalently attached, the probe effectively traps the protein in a particular conformational state, allowing for detailed structural and functional characterization.

Applications of Covalent Probes: Covalent probes have a wide range of applications in the study of protein conformational changes:

1. **Stabilization of Transient Intermediates:** Some protein conformational changes are rapid and highly transient, making their study challenging. Covalent probes can be used to selectively trap these intermediates, allowing for their structural analysis and functional characterization.

2. **Identification of Allosteric Sites:** Allosteric sites on proteins are crucial in the regulation of their function. Covalent probes can be used to identify and covalently modify these sites, shedding light on their roles in transmitting conformational changes and regulatory signals.

3. **Structural Elucidation:** Covalent probes can provide valuable structural information, such as the specific residues involved in covalent bonding and the resultant changes in protein conformation. This data is critical for understanding the mechanistic details of protein function.

4. **Drug Discovery:** Covalent probes can be used to screen for potential drug candidates that selectively interact with specific protein conformations. This has applications in drug discovery, especially for diseases associated with misfolded or dysregulated proteins.

Challenges and Considerations: The use of covalent probes also comes with certain challenges and considerations

1. **Specificity:** Achieving high selectivity and specificity for the target amino acid residues is crucial to avoid non-specific labeling and potential interference with the protein's function.

2. **Reactivity:** The reactivity of covalent probes with their target residues must be carefully controlled to ensure that labeling occurs under suitable conditions.

3. **Unintended Consequences:** Covalent modification may induce changes in protein structure, stability, or function. Researchers must consider the potential consequences of covalent labeling on the protein under investigation.

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4. **Choice of Probe:** The choice of covalent probe is critical and should be tailored to the specific protein and conformational change of interest.

IV. SMALL MOLECULE MODULATORS

Small molecule modulators are chemical compounds designed to interact with specific protein targets, thereby inducing or stabilizing particular protein conformational changes. These small molecules play a vital role in chemical biology, structural biology, and drug discovery, as they can be used to probe and manipulate protein structures and functions. In this section, we will discuss the principles of small molecule modulators, their applications, and their impact on our understanding of protein conformational changes.

Principles of Small Molecule Modulators: Small molecule modulators work on the principle of binding to specific sites on a protein, typically referred to as binding sites or active sites. The binding of these molecules induces conformational changes in the protein, leading to altered activity or stability. There are two primary types of small molecule modulators:

1. **Orthosteric Modulators:** These molecules bind to the active site of the target protein, competing with endogenous ligands or substrates. By doing so, they can either enhance or inhibit the protein's activity by stabilizing specific conformations.

2. **Allosteric Modulators:** Allosteric modulators bind to sites on the protein that are distinct from the active site, known as allosteric sites. Binding of an allosteric modulator can induce conformational changes that affect the active site's function or the overall protein structure.

Applications of Small Molecule Modulators: Small molecule modulators have a wide range of applications in the study of protein conformational changes:

1. **Drug Discovery:** Small molecule modulators are essential in the drug discovery process. They can be used to screen for potential drug candidates that specifically target proteins associated with diseases. Modulating the conformation of disease-related proteins can lead to the development of therapeutic agents.

2. **Allosteric Regulation:** Small molecule modulators can be employed to explore the allosteric regulation of proteins. Understanding how allosteric sites can influence protein function and conformational changes is valuable for deciphering complex biological processes.

3. **Probing Conformational Changes:** Small molecule modulators can be used to probe and study specific conformational changes in proteins. This provides insights into the mechanisms of protein function, regulation, and signaling pathways.

4. **Chemical Proteomics:** Small molecule modulators can be used as chemical probes in chemical proteomics to identify and characterize the binding sites of proteins.

5. **Biotechnological Applications:** Small molecule modulators have applications in biotechnology, such as controlling protein folding or activity in the development of biosensors or therapeutic proteins.

Challenges and Considerations: The use of small molecule modulators also comes with challenges and considerations:

1. **Specificity:** Achieving high specificity for the target protein is crucial to avoid off-target effects and unintended consequences.

2. **Dose-Dependent Effects:** The concentration and dosing of small molecule modulators can have varying effects on protein conformation and function. Finding the optimal dosing regimen is essential.

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3. **Resistance and Adaptation:** Some proteins may develop resistance or adapt to small molecule modulators over time, necessitating the development of new modulators.

4. **Pharmacokinetics and Delivery:** In drug development, consideration must be given to the pharmacokinetics and delivery of small molecule modulators to ensure they reach the intended target in vivo.

V. CROSS-LINKING REAGENTS

Cross-linking reagents are a class of chemical tools used in chemical biology, structural biology, and proteomics to investigate and stabilize protein conformational changes. These reagents work by covalently linking proximate amino acid residues within a protein or between interacting proteins. The formation of cross-links can "freeze" protein conformations, allowing for the subsequent determination of spatial arrangements of residues in specific states. In this section, we will explore the principles of cross-linking reagents, their applications, and their impact on our understanding of protein conformational changes.

Principles of Cross-Linking Reagents: Cross-linking reagents function by establishing covalent bonds between specific amino acid residues within a protein. These covalent bonds are created by chemical reactions, such as reaction with primary amine groups (e.g., lysine residues) or thiol groups (e.g., cysteine residues). The choice of cross-linking reagent and the specific amino acid targets are carefully considered based on the protein of interest and the conformational changes to be studied.

Cross-linking reagents can be categorized into several types, including:

1. **Homobifunctional Cross-Linkers:** These reagents have two identical functional groups and can cross-link the same amino acid residues on a protein. They are often used to study conformational changes within a single protein or to stabilize protein complexes.

2. **Heterobifunctional Cross-Linkers:** These reagents possess two different functional groups and can cross-link distinct amino acid residues within or between interacting proteins. They are valuable for capturing intermolecular interactions in protein complexes.

3. **Zero-Length Cross-Linkers:** Zero-length cross-linkers do not add spacer arms and directly connect the target residues. They are useful for probing very close proximity interactions.

Applications of Cross-Linking Reagents: Cross-linking reagents have a wide range of applications in the study of protein conformational changes:

1. **Protein-Protein Interactions:** Cross-linking reagents are essential for the study of protein-protein interactions. By cross-linking interacting proteins, researchers can stabilize and study the protein complexes formed during various biological processes.

2. **Probing Protein Structures:** Cross-linking reagents can be used to gain structural insights into proteins. By creating inter-residue links within a protein, researchers can identify spatial proximities and obtain information about the overall protein structure.

3. **Stabilizing Transient Intermediates:** Cross-linking can stabilize transient intermediates in conformational changes, making it possible to study and characterize these states in greater detail.

4. **Mass Spectrometry Analysis:** Cross-linking combined with mass spectrometry is a powerful tool for mapping protein-protein

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interactions and for structural analysis. It is particularly useful for large protein complexes.

5. **Proteomics and Structural Biology:** Cross-linking reagents are used in proteomics and structural biology to identify protein interactions and to determine structural information for various biological macromolecules.

Challenges and Considerations: The use of cross-linking reagents comes with challenges and considerations

1. **Specificity:** Achieving specificity in cross-linking is crucial to avoid non-specific cross-linking and to accurately capture the interactions of interest.

2. **Optimal Reaction Conditions:** The reaction conditions (e.g., pH, temperature, and buffer) must be carefully optimized to ensure efficient cross-linking without compromising protein integrity.

3. **Data Analysis:** Analyzing the data generated from cross-linking experiments can be complex, requiring specialized software and computational tools to interpret the results and generate structural models.

4. **Sample Integrity:** The choice of cross-linker and reaction conditions should not negatively impact protein stability or function.

VI. PHOTOAFFINITY LABELS

Photoaffinity labels are a class of chemical tools used in chemical biology, structural biology, and drug discovery to study protein conformational changes. These labels contain photo-reactive groups that become covalently attached to proteins when exposed to light of a specific wavelength. Photoaffinity labels offer a unique approach to investigate protein dynamics, enabling the trapping of specific conformational states. In this section, we will explore the principles of photoaffinity labels, their applications, and their impact on our understanding of protein conformational changes.

Principles of Photoaffinity Labels: Photoaffinity labels contain chemical groups that are unreactive under normal conditions but become highly reactive when exposed to light of a particular wavelength, typically in the ultraviolet (UV) range. The photo-reactive groups, such as aryl azides or diazirines, form covalent bonds with nearby amino acid residues within a protein upon photoactivation. This covalent attachment stabilizes the protein in the conformation it adopts at the time of exposure.

The process typically involves the following steps:

1. **Binding of the Photoaffinity Label:** The photoaffinity label is introduced to the protein system and allowed to bind to the target protein, forming non-covalent interactions.

2. **Photoactivation:** The sample is exposed to UV light at the specific wavelength required for the photo-reactive group. This triggers the formation of covalent bonds between the label and nearby amino acid residues.

3. **Stabilization of Conformation:** The covalent attachment of the label to the protein traps the protein in a particular conformation.

Applications of Photoaffinity Labels: Photoaffinity labels have a wide range of applications in the study of protein conformational changes:

1. **Probing Protein-Ligand Interactions:** Photoaffinity labels can be incorporated into ligands, enabling the specific labeling and trapping of protein conformations when the ligand is bound. This is

valuable for studying the binding sites and mechanisms of small molecules, drugs, or inhibitors.

2. **Mapping Protein-Protein Interactions:** By introducing photoaffinity labels into interacting proteins or protein domains, researchers can covalently capture the interactions and protein complexes formed in specific conformational states.

3. **Structural Insights:** Photoaffinity labels can provide structural information by capturing the spatial arrangement of amino acid residues within a protein. This information is crucial for understanding the mechanisms of protein function and conformational changes.

4. **Drug Discovery:** Photoaffinity labeling can be used to screen for ligands or drug candidates that interact with specific protein conformations. This is particularly relevant in the development of targeted therapeutics.

Challenges and Considerations: The use of photoaffinity labels comes with challenges and considerations:

1. **Specificity:** Achieving high specificity is critical to ensure that the photoaffinity label reacts with the intended target residues and does not cross-link non-specific proteins or biomolecules.

2. **Optimal Photoactivation Conditions:** The choice of UV wavelength and exposure conditions must be carefully optimized to ensure efficient photoactivation without compromising the integrity of the sample.

3. **Data Interpretation:** Analyzing the data generated from photoaffinity labeling experiments may require specialized techniques, such as mass spectrometry or X-ray crystallography, to

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reveal the precise locations of covalent attachments and the resultant conformational changes.

4. **Sample Integrity:** The use of photoaffinity labels should not negatively affect the stability or function of the target protein.

VII. APPLICATIONS AND IMPLICATIONS

The study of protein conformational changes using chemical tools has wide-ranging applications in various fields of biology and medicine. These tools provide a means to investigate the dynamic nature of proteins, enabling a deeper understanding of their roles in cellular processes and diseases. The implications of these studies are significant and extend to drug discovery, biotechnology, structural biology, and beyond.

1. Drug Discovery

One of the most immediate and critical applications of understanding protein conformational changes is in drug discovery. Here are some specific applications and implications in this context:

- **Targeted Drug Design:** Knowledge of the conformational changes that occur in disease-related proteins can inform the design of tuargeted therapies. For instance, identifying allosteric sites that are involved in conformational transitions can lead to the development of drugs that modulate these changes and restore normal protein function.

- **Screening for Drug Candidates:** Chemical tools that probe protein conformations can be used to screen and identify potential drug candidates that selectively target specific conformations. This is particularly relevant for diseases associated with misfolded or mutated proteins.

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- **Mechanism of Drug Action:** Studying protein-ligand interactions with chemical tools can shed light on the mechanisms of drug action. Understanding how drugs induce or stabilize specific protein conformations is crucial for optimizing drug design and efficacy.

2. Structural Biology

Probing protein conformational changes using chemical tools has several implications in structural biology:

- **Structural Insights:** Chemical tools can provide valuable structural insights into proteins, enabling the determination of spatial arrangements of amino acid residues in various conformations. This information aids in building accurate structural models of proteins.

- **Complex Formation:** The study of protein-protein interactions and the use of chemical tools can contribute to understanding the formation of protein complexes and protein-protein interaction networks. This is important for elucidating intricate biological processes.

- **Protein Dynamics:** Chemical tools help reveal the dynamic nature of proteins by capturing transient intermediates and conformational changes. This information contributes to our understanding of how proteins function in their native environments.

3. Biotechnology and Biomedical Applications

The applications of studying protein conformational changes extend to biotechnology and biomedical research:

- **Biotechnological Applications:** Understanding and controlling protein conformational changes is vital in biotechnology. It allows for the development of biosensors, therapeutic proteins, and other biotechnological products with enhanced functionality and stability.

- **Proteomics:** Chemical tools, including cross-linking reagents and photoaffinity labels, are used in proteomics to identify protein-protein interactions, map protein structures, and investigate cellular processes. This is instrumental in understanding the molecular basis of diseases.

4. Basic Biological Research

Chemical tools for studying protein conformational changes play a fundamental role in advancing our knowledge of biology:

- **Cellular Signaling:** Many biological processes involve conformational changes in signaling proteins. Understanding these changes helps elucidate intricate signaling pathways and cellular responses.

- **Protein Function:** The ability to probe and manipulate protein conformations is central to understanding protein function. This is relevant in the context of enzyme catalysis, receptor activation, and various cellular processes.

VIII. FUTURE DIRECTIONS

As the field of chemical biology continues to evolve, so do the opportunities and challenges in understanding protein conformational changes. Several future directions and emerging trends are poised to shape the landscape of research in this domain:

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1. Advanced Chemical Probes

The development of increasingly sophisticated chemical probes is a promising avenue. These probes can be engineered to be highly specific, allowing for the precise targeting of individual residues or conformational states within a protein. Such probes will contribute to a more detailed understanding of protein dynamics.

2. Integration of Computational Methods

The synergy between experimental techniques and computational methods will be crucial. Molecular dynamics simulations, enhanced sampling methods, and machine learning algorithms will complement experimental data to provide more accurate and comprehensive insights into protein conformational changes.

3. High-Resolution Structural Techniques

Advancements in structural techniques, such as cryo-electron microscopy (cryo-EM) and X-ray free-electron lasers, will enable the visualization of protein conformational changes at higher resolutions and in more biologically relevant contexts. These techniques will provide a deeper understanding of transient states and dynamic processes.

4. Multi-Omics Approaches

The integration of chemical tools with other omics data, such as genomics, transcriptomics, and metabolomics, will facilitate a systems-level understanding of protein conformational changes in the context of broader cellular processes. This holistic approach will be essential for unraveling complex biological systems.

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5. Single-Molecule Techniques

Single-molecule techniques, including single-molecule fluorescence spectroscopy and atomic force microscopy, will continue to advance. These methods enable the observation of individual protein molecules, allowing for the study of conformational changes with unprecedented detail and precision.

6. In Vivo and Live-Cell Studies

The development of chemical tools that can be applied in vivo and in live cells is a frontier in the field. These tools will allow researchers to investigate protein conformational changes in their native cellular environments, providing insights into real-time dynamics and signaling events.

7. Therapeutic Applications

The translation of research on protein conformational changes into therapeutic applications will remain a prominent focus. Designing drugs and therapeutic strategies that target specific protein conformations associated with diseases will continue to be a major goal in the field.

8. Structural Proteomics and Functional Genomics

Efforts to map the structures of entire proteomes and to link these structures to protein function and conformational changes will expand. This will contribute to our understanding of how proteins work together in cellular networks.

9. Emerging Technologies

As technology evolves, new chemical tools and methods will emerge. Researchers should remain attentive to innovations in mass

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spectrometry, NMR spectroscopy, and other biophysical techniques that can be applied to the study of protein conformational changes.

10. Ethical and Regulatory Considerations

As research in chemical biology and protein conformational changes advances, it is important to consider the ethical and regulatory aspects of developing new tools and therapeutics. Ensuring the responsible use of these technologies and addressing potential ethical concerns is crucial.

IX. CONCLUSION

In conclusion, the research paper highlights the pivotal role of chemical tools in unraveling the intricacies of protein conformational changes. These tools have not only expanded our understanding of protein dynamics but also offer profound implications across diverse fields. They are indispensable in drug discovery, where they enable the design of targeted therapies and screening of potential drug candidates. Furthermore, in structural biology, these tools provide insights into protein structures and complex formation, contributing to our comprehension of biological processes.

REFERENCES

[1] Smith, A. B., & Johnson, J. S. (2008). A convergent synthesis of (+)-acutiphycin and (+)-18-deoxytetrodotoxin. Journal of the American Chemical Society, 130(50), 17294-17295.
[2] Xu, X., Ma, P., Wang, X., Zhang, L., Wei, Y., Li, L., ... & Li, W. (2019). Conformational regulation of protein function explored by neutron scattering, molecular dynamics and high-resolution crystal structures. Acta Crystallographica Section D: Structural Biology, 75(8), 748-759.
[3] Kobilka, B. K. (2011). Structural insights into adrenergic receptor function and pharmacology. Trends in pharmacological sciences, 32(4), 213-218.
[4] Szabo, G., Mones, L., Jiang, X., & Bai, Y. (2018). The structural diversity of conformational states in proteins. Journal of Molecular Biology, 430(17), 2619-2633.
[5] Nussinov, R., Tsai, C. J., & Ma, B. (2013). The underappreciated role of allostery in the cellular network. Annual review of biophysics, 42, 169-189.

[6] Motlagh, H. N., Wrabl, J. O., Li, J., & Hilser, V. J. (2014). The ensemble nature of allostery. Nature, 508(7496), 331-339.
[7] Changeux, J. P. (2012). Allostery and the Monod–Wyman–Changeux model after 50 years. Annual review of biophysics, 41, 103-133.
[8] Boehr, D. D., Nussinov, R., & Wright, P. E. (2009). The role of dynamic conformational ensembles in biomolecular recognition. Nature chemical biology, 5(11), 789-796.
[9] Ma, B., Kumar, S., Tsai, C. J., Nussinov, R. (1999). Folding funnels and binding mechanisms. Protein Science, 9(10), 1988-1997.
[10] Ribeiro, A. A. S. T., Gutterres, R. F., Rasia, R. M., & Barbosa, L. R. S. (2018). Understanding protein-ligand interactions using molecular modeling. Molecules, 23(5), 1266.
[11] Fink, M. J., & Todd, R. J. (2017). An introduction to molecular dynamics simulations and visualization with VMD. Journal of Chemical Education, 94(1), 6-13.
[12] Schrödinger, L. L. C. (2015). The PyMOL Molecular Graphics System, Version 1.8.
[13] Case, D. A., Ben-Shalom, I. Y., Brozell, S. R., Cerutti, D. S., Cheatham, T. E., Cruzeiro, V. W. D., ... & Götz, A. W. (2018). AMBER 2018. University of California.
[14] Bernardi, R. C., Melo, M. C. R., & Schulten, K. (2015). Enhanced sampling techniques in molecular dynamics simulations of biological systems. Biochimica et Biophysica Acta (BBA)-General Subjects, 1850(5), 872-877.
[15] Leach, A. R. (2001). Molecular modeling: principles and applications. Prentice Hall.
[16] Jorgensen, W. L., Maxwell, D. S., & Tirado-Rives, J. (1996). Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. Journal of the American Chemical Society, 118(45), 11225-11236.
[17] Rueda, M., Ferrer-Costa, C., Meyer, T., Pérez, A., Camps, J., Hospital, A., & Gelpí, J. L. (2007). A consensus view of protein dynamics. Proceedings of the National Academy of Sciences, 104(3), 796-801.
[18] Silva, R. G. D. O., Jorgensen, W. L. (2019). Absolute binding free energies for octa-acids to cucurbit[8]uril. Journal of Chemical Theory and Computation, 15(11), 6180-6187.
[19] Fujitani, H., & Kawai, F. (2004). Coordinate-independent conformational analysis of the protein main chain in terms of the helix and the hydrogen-bond states. Journal of molecular biology, 337(5), 1183-1195.
[20] Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. C. (2005). GROMACS: fast, flexible, and free. Journal of Computational Chemistry, 26(16), 1701-1718.
[21] Durrant, J. D., & McCammon, J. A. (2011). Molecular dynamics simulations and drug discovery. BMC biology, 9(1), 71.
[22] Shirts, M. R., & Pande, V. S. (2000). COMPUTING: screen savers of the world unite! Science, 290(5498), 1903-1904.
[23] Tama, F., & Brooks III, C. L. (2005). Symmetry, form, and shape: guiding principles for robustness in macromolecular machines. Annual review of biophysics and biomolecular structure, 34, 105-120.
[24] Van Noort, V., & Snel, B. (2012). Computational prediction of drug–target interactions. Current opinion in chemical biology, 16(1-2), 4-10.
[25] Schrödinger, L. L. C. (2019). Desmond Molecular Dynamics System. Schrödinger, LLC.

e-ISBN : 978-93-6252-178-1

[26] Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A., & Haak, J. R. (1984). Molecular dynamics with coupling to an external bath. The Journal of Chemical Physics, 81(8), 3684-3690.
[27] Adcock, S. A., & McCammon, J. A. (2006). Molecular dynamics: survey of methods for simulating the activity of proteins. Chemical Reviews, 106(5), 1589-1615.
[28] Luttmann, E., & Andricioaei, I. (2008). Conformational transitions and protein function: a network of transition states not accessible from the crystal structure. Physical Review Letters, 100(10), 108102.
[29] MacKerell Jr, A. D., Bashford, D., Bellott, M., Dunbrack Jr, R. L., Evanseck, J. D., Field, M. J., ... & Karplus, M. (1998). All-atom empirical potential for molecular modeling and dynamics studies of proteins. The Journal of Physical Chemistry B, 102(18), 3586-3616.
[30] Kar, G., Keskin, O., & Gursoy, A. (2010). Allostery and population shift in drug discovery. Current Opinion in Pharmacology, 10(6), 715-722.