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The two-state switch of chaperone BIP: A druggable target for endoplasmic reticulum stress in cancer and neurodegeneration

Şaperon BIP'nin iki durumlu anahtarı: Kanser ve nörodejenerasyonda endoplazmik retikulum stresi için ilaçlanabilir bir hedef

Zekeriya Düzgün[1,2] Zuhal Eroğlu[2]

[1]Department of Medical Biology, School of Medicine, Giresun University, Giresun/Türkiye

[2]Department of Medical Biology, School of Medicine, Ege University, Izmir/Türkiye

ABSTRACT

Aim: The endoplasmic reticulum (ER) chaperone BiP is a central node in the unfolded protein response and a key therapeutic target in diseases driven by ER stress, such as cancer and neurodegeneration. However, therapeutic development is hampered by an incomplete understanding of the conformational dynamics that govern its substrate binding and release cycle.

Materials and Methods: We performed a 100-nanosecond all-atomic molecular dynamics simulation of human BiP (PDB: 5e84) to characterize the flexibility of its substrate binding domain alpha (SBDα). We analyzed RMSD, inter-domain distances, and the free energy landscape to map its conformational states.

Results: Our simulations revealed that BiP exists in two distinct, low-energy conformational states: a predominant "extended" state (90% occupancy) and a transient "bent" state. The SBDα undergoes a rapid bending-restoring motion, with distances between key domains collapsing from ~25 Å to 3 Å within a 10 ns timescale. This motion represents a molecular switch with a moderate energy barrier of 25-30 kJ/mol, making it accessible at physiological temperatures.

Conclusions: The identified two-state conformational switch is a functionally critical feature that explains BiP's dynamic substrate handling. This molecular motion represents a "druggable" mechanism. Stabilizing either the extended or bent state with small molecules offers a tangible therapeutic strategy for modulating BiP activity, such as inhibiting its pro-survival function in cancer or enhancing its protective role in neurodegeneration.

Keywords: HSPA5 protein, endoplasmic reticulum stress, molecular dynamics simulation, protein conformation, neoplasms

ÖZ

Amaç: *Endoplazmik retikulum (ER) şaperonu BiP, katlanmamış protein yanıtının merkezi bir düğümüdür ve ER stresinin tetiklediği kanser ve nörodejenerasyon gibi hastalıklarda kilit bir terapötik hedeftir. Ancak, substrat bağlama ve salıverme döngüsünü yöneten konformasyonel dinamiklerin tam olarak anlaşılamaması, terapötik geliştirmeyi güçleştirmektedir.*

Gereç ve Yöntem: *İnsan BiP'sinin (PDB: 5e84) substrat bağlama domaini alfa (SBDα) esnekliğini karakterize etmek için 100 nanosaniyelik tüm-atom moleküler dinamik simülasyonu gerçekleştirdik. Konformasyonel durumları haritalamak için RMSD, domainler arası mesafeler ve serbest enerji yüzeyini analiz ettik.*

Bulgular: *Simülasyonlarımız, BiP'nin iki ayrı, düşük enerjili konformasyonel durumda var olduğunu ortaya koydu: baskın "uzamış" durum (%90 doluluk oranı) ve geçici "bükülmüş" durum. SBDα, ~10 ns zaman ölçeğinde, kilit domainler arasındaki mesafelerin ~25 Å'dan ~3 Å'a çöktüğü hızlı bir bükülme-*

Corresponding author: Zekeriya Düzgün
Department of Medical Biology, Faculty of Medicine, Giresun University, Giresun/Türkiye
E-mail: *zduzgun@gmail.com*
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geri-dönüş hareketi sergiler. Bu hareket, 25–30 kJ/mol düzeyinde orta şiddette bir enerji bariyerine sahip moleküler bir anahtarı temsil eder ve fizyolojik sıcaklıklarda erişilebilir kılar.

Sonuç: *Tanımlanan iki-durumlu konformasyonel anahtar, BiP'in dinamik substrat yönetimini açıklayan işlevsel açıdan kritik bir özelliktir ve "ilaçlanabilir" bir mekanizma sunar. Uzamış veya bükülmüş durumun küçük moleküllerle stabilize edilmesi, BiP aktivitesinin modülasyonu için somut bir terapötik strateji sağlar; örneğin kanserdeki hayatta kalma yanlısı işlevin inhibisyonu ya da nörodejenerasyondaki koruyucu rolün güçlendirilmesi gibi.*

Anahtar Sözcükler: *HSPA5 proteini, endoplazmik retikulum stresi, moleküler dinamik simülasyonu, protein konformasyonu, neoplazmlar*

INTRODUCTION

Recent investigations have demonstrated that BiP conformational dynamics are crucial for immune responses, viral infections, and cellular transformation processes (1,2), positioning it as a key node in diseases driven by endoplasmic reticulum (ER) stress. Central to the ER stress response is BiP (Binding immunoglobulin protein, HSPA5), a 78-kDa Hsp70 family chaperone essential for restoring ER homeostasis by managing protein folding, assembly, and translocation (2,3). Consequently, novel therapeutic approaches have begun to target the flexibility in the SBD region, which is essential for client protein recognition and chaperone regulation (4).

Despite its importance as a drug target, rational drug design is hampered because the SBDα dynamic behavior remains poorly understood. BiP functions through an allosteric mechanism where ATP-dependent conformational changes in its Nucleotide Binding Domain (NBD) and Substrate Binding Domain (SBD) modulate substrate affinity (4,5). However, static structural methods like X-ray crystallography consistently encounter difficulties resolving the flexible SBDα "lid" region, suggesting that functionally relevant conformational states are difficult to capture experimentally, even though complete structures under specific conditions serve as valuable references (6)**.**

This knowledge gap highlights the need for dynamic approaches. Molecular dynamics simulations provide a powerful tool for investigating protein flexibility at atomic resolution, capturing conformational evolution inaccessible to static methods (7,8). In this study, we therefore employed all-atomic molecular dynamics to investigate BiP conformational dynamics, focusing on SBDα behavior. Through analysis of a 100-nanosecond trajectory, we aim to characterize SBDα flexibility and identify its distinct conformational states, thereby providing a structural blueprint that explains its experimentally observed variability and informs future therapeutic strategies.

MATERIALS and METHODS

Protein Structure Preparation and System Setup

Human BiP protein structure was obtained from the Protein Data Bank (PDB ID: 5e84) (6). Missing atoms in residues THR-428, VAL-429, VAL-433, LYS-576, LEU-579, and LYS-582 were reconstructed using the Dunbrack Rotamer Library method in UCSF Chimera software (version 1.11, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, USA) (9,10). The completed structure was inspected for proper geometry and absence of steric clashes.

All-atomic MD simulations were performed using GROMACS software (version 5.1.4, developed by the University of Groningen, Netherlands, and Uppsala University, Sweden) (11) with the AMBER99SB-ILDN force field (12) and TIP3P water model. The protein was placed in a rectangular simulation box with 12 Å minimum distance to edges, solvated with approximately 45,000 water molecules, and neutralized with 0.15 M NaCl, resulting in approximately 150,000 total atoms.

Molecular Dynamics Simulation Protocol

Energy minimization was performed using the steepest descent algorithm for 5,000 steps. Two-stage equilibration included 100 ps NVT equilibration at 300 K using V-rescale thermostat (13), followed by 100 ps NPT equilibration at 300 K and 1 bar using Berendsen barostat (14). Position restraints (1000 kJ mol^{-1} nm^{-2}) were applied to protein heavy atoms during equilibration phases.

Production simulation was conducted for 100 ns under NPT conditions using Nosé-Hoover

thermostat (15) and Parrinello-Rahman barostat (16). Electrostatic interactions were calculated using the Particle Mesh Ewald (PME) method (17,18) with 10 Å cutoff, van der Waals interactions with 10 Å cutoff, and LINCS constraints (19) allowed 2 fs timestep. Coordinates were saved every 2 ps, providing 50,000 frames for analysis.

Trajectory Analysis Methods

Root mean square deviation (RMSD) was calculated for backbone atoms (Cα, C, N) after optimal superposition to monitor overall conformational changes. Distance analysis monitored critical residue pairs 113-114 and 578-582 (Cα atoms) to track SBDα domain flexibility. Hydrogen bonds were identified using geometric criteria: donor-acceptor distance ≤ 3.5 Å and donor-hydrogen-acceptor angle ≥ 150°.

Normal mode analysis was performed using the ProDy Python package (version 1.10, developed by the University of Pittsburgh, USA) (20) with Anisotropic Network Model (15 Å cutoff). The first 20 normal modes were calculated and visualized using Normal Mode Wizard (NMWiz) plugin (version 1.2) for VMD software (version 1.9.3, developed by the Theoretical and Computational Biophysics Group at the University of Illinois at Urbana-Champaign, USA) (21) to identify intrinsic flexibility patterns.

Free Energy Calculations

MM/PBSA analysis was performed using the g_mmpbsa tool (22) on trajectory snapshots extracted at 10 ns intervals. Free energy was decomposed into molecular mechanics, polar solvation (Poisson-Boltzmann equation, 0.5 Å grid spacing), and non-polar solvation components. A three-dimensional free energy surface was constructed using RMSD, inter-residue distance, and MM/PBSA energy as coordinates, with visualization performed in OriginPro 2018 software.

RESULTS

Overall Conformational Behavior During 100 ns Simulation

The all-atomic MD simulation revealed significant conformational flexibility in BiP throughout the trajectory. RMSD analysis showed a major transition at approximately 40 ns, characterized by rapid increase from 4 Å to 10 Å, followed by equally rapid return (Figure 1A). This pattern indicates BiP samples multiple conformational states rather than adopting a single rigid structure. The RMSD profile demonstrated two phases: initial stability (0-40 ns) around 4 Å, and transition period (40-50 ns) with dramatic rearrangement, followed by stabilization at 5-6 Å.

SBDα Domain Exhibits Rapid Bending-Restoring Movement

Major structural changes were localized to the SBDα subdomain. Distance measurements between residue pairs 113-114 and 578-582 provided quantitative evidence for domain-specific flexibility (Figure 1B). During the 40 ns transition, distances decreased dramatically from ~25 Å to 3 Å, indicating rapid SBDα bending accompanied by hydrogen bond formation. Movement kinetics were fast, occurring within 10 ns with asymmetric characteristics: gradual bending over 5-6 ns, followed by rapid restoration within 3-4 ns (Figure-1C).

Normal Mode Analysis Confirms SBDα Mobility

Normal mode analysis using the Anisotropic Network Model identified SBDα as the most mobile protein region (Figure 2). Principal component analysis revealed dominant motions involving large-amplitude SBDα movements, consistent with MD observations. The mobility pattern extended from residue 549, corresponding precisely to regions unresolved by **Preissler et al. (2017)** due to high flexibility, validating computational results.

Free Energy Landscape Reveals Two Stable States

MM/PBSA calculations identified two distinct low-energy conformational basins (Figure 3). State 1 (extended SBDα) characterized by large inter-residue distances (>20 Å) and low NBD-SBD interactions, and State 2 (bent SBDα) showing short distances (~3 Å) and increased inter-domain interactions. Free energy difference between states was 15-20 kJ/mol, with transition barriers of 25-30 kJ/mol, indicating both conformations are physiologically relevant.

Temporal and Interaction Characteristics

BiP spent ~90% of simulation time in extended conformation and ~10% in bent/transition states, with stochastic switching patterns. The extended state maintained minimal NBD-SBD interactions with open SBDα allowing substrate access, while the bent state established new contacts creating compact structure. Rapid transition completion within 10 ns suggests intermediate conformations

are energetically unfavorable, with the system preferentially occupying stable states compatible with cellular timescales.

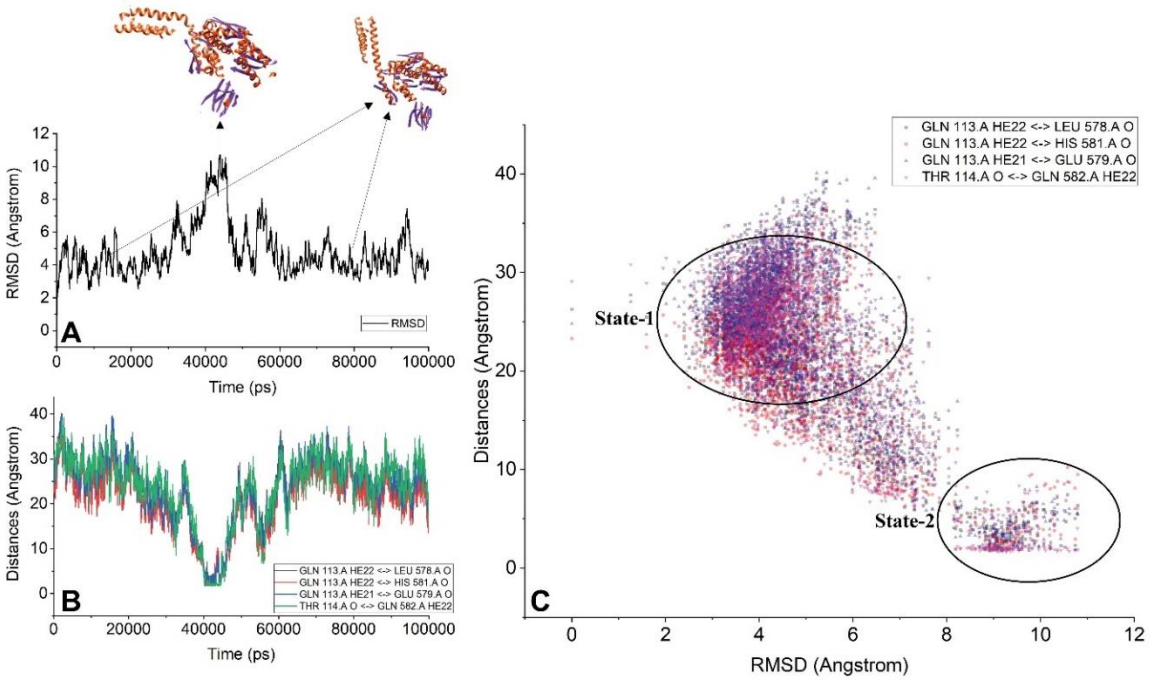

Figure-1. RMSD and distance graph of all atomic MD simulation of the BIP protein (A&B) RMSD-distance graph of the BIP protein at the end of the 100 ns all atomic MD simulation (C). Each point in the graph C represents the 40 ps time zone in the MD simulation.

DISCUSSION

The Two-State Switch: A Key Mechanism Regulating BiP's Chaperone Cycle

Our identification of two distinct, interconverting conformational states provides crucial insights into the mechanisms of BiP's chaperone function. The simulation reveals a rapid bending-restoring movement within the SBDα domain, which acts as a "molecular switch" that is central to the substrate binding and release cycle. The "extended" state, which predominates with ~90% occupancy, likely facilitates substrate access to the binding pocket, while the transient "bent" state may represent a conformation for substrate retention or release assistance. This dynamic switching mechanism complements the classical Hsp70 allosteric model by providing an additional layer of regulation (4). With a low energy barrier of 25-30 kJ/mol, this conformational transition is spontaneous at physiological temperatures, and its rapid 10 ns timescale is well-suited for the kinetics of cellular protein folding and chaperone turnover (23).

Clinical and Therapeutic Implications of the Conformational Switch

The discovery of this two-state conformational switch has significant clinical and therapeutic implications, as it provides a concrete mechanism for modulating BiP's activity in ER stress-related diseases. In many cancers, where BiP is overexpressed to help tumor cells manage proteotoxic stress, the "bent" (substrate-retaining) state may represent a pro-survival conformation. Therefore, developing small molecules that prevent this transition or lock BiP in the "extended" (substrate-receptive) state could be a viable strategy to sensitize cancer cells to therapy and inhibit cellular transformation processes (1,2,4). Conversely, in several neurodegenerative diseases characterized by the accumulation of misfolded proteins, the goal might be to enhance BiP's chaperone capacity. In this context,

molecules that stabilize the "bent" state could potentially improve the clearance of toxic protein aggregates (24). Furthermore, given BiP's role in facilitating the entry and replication of viruses, including coronaviruses, designing inhibitors that disrupt this conformational flexibility could serve as a broad-spectrum antiviral strategy (2). Our two-state model thus provides a clear structural framework for the rational design of state-specific modulators tailored to different pathological conditions.

A Dynamic Explanation for Static Crystallographic Data

Our findings also provide a clear molecular-level explanation for the longstanding difficulties in resolving the SBDα domain in X-ray crystallography studies. The inherent, rapid flexibility between the "extended" and "bent" states revealed by our simulation accounts for the poor electron density frequently reported for this region (24). Notably, our analysis identified residue 549 as a key boundary point for this flexibility, which correlates precisely with the regions that often remain unresolved in experimentally determined structures (6). This resolves a significant ambiguity in the field, validating that complete structures captured under specific conditions represent one of the physiologically relevant states rather than an artifact. More broadly, our results suggest that such "unresolved" regions in static structures should not be dismissed, but rather investigated as functionally important, dynamic elements whose characterization requires computational approaches like ours (25).

Limitations and Future Directions

While our all-atomic MD simulation successfully captured the intrinsic dynamics of BiP and provided a robust model, we must acknowledge its limitations. The primary limitation is that our simulation was performed on the apo-protein, in the absence of client substrates, co-chaperones, or post-translational modifications, all of which could influence the equilibrium and kinetics of the conformational switch in a cellular context. Despite this, the strong correlation between our flexibility predictions and experimental data provides confidence in the fundamental nature of the two-state mechanism.

While our 100 ns simulation provides initial insights into BiP’s conformational dynamics, we acknowledge methodological limitations inherent to the approach. The use of a single trajectory may not fully capture state occupancies and transition kinetics, which could be better characterized through replicated, longer simulations or enhanced sampling methods such as metadynamics or replica exchange. Additionally, the MM/PBSA method offers an approximate free energy landscape but lacks the rigor of more advanced thermodynamic techniques like umbrella sampling. Finally, our state definitions, based on RMSD and distance metrics, could be refined using systematic clustering algorithms (e.g., k-means or hierarchical clustering) in future work. These avenues will build upon the current findings to provide a more comprehensive understanding of BiP’s allosteric mechanisms.

These limitations illuminate clear and exciting future directions. The next step is to perform simulations that incorporate client peptides or disease-associated mutations to investigate how these factors modulate the balance between the "extended" and "bent" states. Furthermore, our two-state model provides an ideal framework for computational drug discovery. *In silico* screening campaigns can now be designed to specifically search for small molecules that either stabilize or disrupt one of these conformations. Such studies will be crucial for translating our fundamental insights into state-specific therapeutics, paving the way for personalized treatments for a range of ER stress-related diseases.

CONCLUSION

In this study, we employed all-atomic molecular dynamics simulations to resolve the conformational dynamics of BiP's SBDα domain. Our 100-nanosecond simulation revealed that this domain is not simply disordered, but functions as a sophisticated "molecular switch" between two distinct conformational states: a predominant "extended" state and a transient "bent" state. This rapid, spontaneous switching provides a molecular-level explanation for both BiP's dynamic substrate handling and the longstanding difficulties in its crystallographic resolution.

From a functional and therapeutic perspective, the identification of this two-state switch is highly significant. It shifts the paradigm from viewing BiP as a single entity to a dynamic machine with distinct, targetable states. This framework provides a concrete basis for the rational design of novel therapeutics for ER stress-related diseases. The ability to selectively stabilize the "extended" or

"bent" state with small molecules presents a powerful strategy to either inhibit BiP's pro-survival role in cancer or enhance its protective chaperone function in neurodegeneration. In conclusion, this work not only elucidates a fundamental mechanism of Hsp70 chaperone function but also provides a clear, structural blueprint for developing the next generation of state-specific BiP modulators.

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Authorship Contributions

Zekeriya Düzgün conceived and designed the study, performed the molecular dynamics simulations, analyzed the data, and wrote the manuscript. All authors contributed to the interpretation of results and revision of the manuscript.

Data availability statement

The data supporting the conclusions of this article are available from the corresponding author upon reasonable request.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics statement

This study did not involve human subjects, animal experiments, or other ethical considerations requiring institutional review board approval.

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References

1. Fernández L, Kong CS, Alkhoury M, Tryfonos M, Brighton PJ, et al. The endoplasmic reticulum protein HSPA5/BiP is essential for decidual transformation of human endometrial stromal cells. Sci Rep. 2024;14(1).
2. Li T, Fu J, Cheng J, Elfiky AA, Wei C, et al. New progresses on cell surface protein HSPA5/BiP/GRP78 in cancers and COVID-19. Front Immunol. 2023;14:1166680.
3. Chen X, Shi C, He M, Xiong S, Xia X. Endoplasmic reticulum stress: molecular mechanism and therapeutic targets. Signal Transduct Target Ther. 2023;8(1):352.
4. Obaseki I, Ndolo CC, Adedeji AA, Popoola HO, Kravats AN. The structural and functional dynamics of BiP and Grp94: opportunities for therapeutic discovery. Trends Pharmacol Sci. 2025;46(5):453–67.
5. Hendershot LM, Buck TM, Brodsky JL. The Essential Functions of Molecular Chaperones and Folding Enzymes in Maintaining Endoplasmic Reticulum Homeostasis. J Mol Biol. 2024;436(14).
6. Yang J, Nune M, Zong Y, Zhou L, Liu Q. Close and Allosteric Opening of the Polypeptide-Binding Site in a Human Hsp70 Chaperone BiP. Structure. 2015;23(12):2191–203.
7. Brown BP, Stein RA, Meiler J, Mchaourab HS. Approximating Projections of Conformational Boltzmann Distributions with AlphaFold2 Predictions: Opportunities and Limitations. J Chem Theory Comput. 2024;20(3):1434–47.
8. Meersche Y Vander, Cretin G, Gheeraert A, Gelly JC, Galochkina T. ATLAS: protein flexibility description from atomistic molecular dynamics simulations. Nucleic Acids Res. 2024;52(D1):D384–92.
9. Dunbrack RL, Karplus M. Backbone-dependent Rotamer Library for Proteins Application to Side-chain Prediction. J Mol Biol. 1993;230(2):543–74.
10. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, et al. UCSF Chimera - A visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605–12.

11. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 2015;1–2:19–25.
12. Lindorff-Larsen K, Piana S, Palmo K, Maragakis P, Klepeis JL, et al. Improved side-chain torsion potentials for the Amber ff99SB protein force field. Proteins Struct Funct Bioinforma. 2010;78(8):1950–8.
13. Bussi G, Donadio D, Parrinello M. Canonical sampling through velocity rescaling. J Chem Phys. 2007;126(1):014101.
14. Berendsen HJCC, Postma JPMM van, Van Gunsteren WF, Dinola A, Haak JR. Molecular dynamics with coupling to an external bath. J Chem Phys. 1984;81(8):3684–90.
15. Nosé S. A molecular dynamics method for simulations in the canonical ensemble. Mol Phys. 1984;52(2):255–68.
16. Parrinello M, Rahman A. Polymorphic transitions in single crystals: A new molecular dynamics method. J Appl Phys. 1981;52(12):7182–90.
17. Darden T, York D, Pedersen L. Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. J Chem Phys. 1993;98(12):10089–92.
18. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, et al. A smooth particle mesh Ewald method. J Chem Phys. 1995;103(19):8577–93.
19. Hess B, Bekker H, Berendsen HJCC, Fraaije JGEMEM. LINCS: A linear constraint solver for molecular simulations. J Comput Chem. 1997;18(12):1463–72.
20. Bakan A, Meireles LM, Bahar I. ProDy: Protein Dynamics Inferred from Theory and Experiments. Bioinformatics. 2011;27(11):1575–7.
21. Humphrey W, Dalke A, Schulten K. VMD: Visual molecular dynamics. J Mol Graph. 1996;14(1):33–8.
22. Kumari R, Kumar R, Lynn A. G-mmpbsa -A GROMACS tool for high-throughput MM-PBSA calculations. J Chem Inf Model. 2014;54(7):1951–62.
23. Bard JAM, Drummond DA. Chaperone regulation of biomolecular condensates. Front Biophys. 2024;2:1342506.
24. Preissler S, Rohland L, Yan Y, Chen R, Read RJ, et al. AMPylation targets the rate-limiting step of BiP's ATPase cycle for its functional inactivation. Elife. 2017;6.
25. Dawes S, Hurst N, Grey G, Wieteska L, Wright N V., et al. Chaperone BiP controls ER stress sensor Ire1 through interactions with its oligomers. Life Sci Alliance. 2024;7(10):e202402702.