Egypt. J. Biophys. Biomed. Eng. **Vol. 26**, pp: 89-96 (2025)

Egyptian Journal of Biophysics and Biomedical Engineering
https://ejbbe.journals.ekb.eg/

Computational Analysis of Doxorubicin-Encapsulated Zein Nanoparticles: A Synergistic Strategy for Targeted Cancer Drug Delivery

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Doxorubicin, a widely used chemotherapeutic drug of the anthracycline family, remains among the most efficient agents in cancer treatment. However, its clinical applications are often restricted by systemic toxicity, drug resistance, and poor targeting capacity, highlighting the necessity of advanced delivery systems. In this study, we introduce a novel approach based on doxorubicin-loaded zein nanoparticles (Doxorubicin-LNPs) as a carrier system for targeted drug delivery. Zein, a hydrophobic plant-derived protein, was selected as a carrier due to its biocompatibility, biodegradability, and capacity to interact with hydrophobic and amphiphilic molecules. Its integration with doxorubicin, an amphiphilic drug, demonstrates the adaptability of zein in encapsulating diverse therapeutic agents. To reinforce the stability of the encapsulation matrix, the cross-linker EDC was incorporated, ensuring enhanced structural integrity and sustained release. Extensive characterization studies confirmed favorable physicochemical properties, high encapsulation efficiency, and controlled release behavior of the formulated nanoparticles. Furthermore, molecular docking was conducted to explore the interactions among zein, doxorubicin, and EDC, providing molecular-level evidence of the stability and binding mechanisms within the system. The overall findings confirm that Doxorubicin-LNPs not only achieve efficient drug loading and prolonged release but also exhibit anticancer activity, making them a candidate for improving therapeutic outcomes. This innovative formulation paves the way for the development of advanced zein-based nanocarriers in targeted cancer therapy and broader biomedical applications.

Keywords: Zein, ECD, Doxorubicin, Amphiphilic, Molecular docking.

Introduction

Recently, protein-based systems have attracted growing interest as delivery vehicles for Bioactive compounds and functional food components, including minerals, vitamins, flavonoids, and carotenoids [1]. Zein, a hydrophobic prolamin protein obtained from corn, has shown strong potential as a multifunctional nanocarrier, efficiently facilitating the encapsulation of hydrophobic drugs within its nanoparticles.[2]. Zein is a naturally occurring, edible, and biocompatible protein with unique properties that make it highly suitable for drug delivery applications. Its pronounced hydrophobicity allows for effective encapsulation of nonpolar compounds, and its thermal and chemical stability confer protection against degradation in food matrices.[3]. However, achieving efficient encapsulation of hydrophilic drugs within zein nanoparticles still a major challenge. [4]. Zein, a member of the prolamin protein family, consists of several polypeptide fractions categorized into four types: α, β, γ, and δ. The α-zein fractions appear at 19 and 22 kDa, β-zein at 14 kDa, γ-zein at 16 and 27 kDa, and δ-zein at 10 kDa. Among these, α-zein represents the dominant component (70–85%), followed by γ-zein (10–20%), while β- and δ-zein each account for approximately 1–5%. All zein subtypes are amphiphilic, containing both hydrophilic and hydrophobic amino acid regions. The α-zein fraction is particularly distinguished by its repetitive sequence motifs and high α-helical content.[5] Doxorubicin (DOX) is a powerful chemotherapeutic agent used to treat several types of cancer, including breast, ovarian, and lung malignancies, as well as leukemia. However, its clinical use is limited by dose-dependent cardiotoxicity and the emergence of drug resistance, with the underlying mechanisms of its cardiac toxicity not yet completely elucidated.[6], [7]. Many FDA-approved drugs are used in cancer treatment, and among them, doxorubicin (DOX) — a water-soluble anticancer agent — has shown strong efficacy against several types of cancer. [8]. It was observed that the inclusion of zein accelerates the degradation of the coating throughout the investigated timeframe [9]. Nonetheless, DOX's clinical utility is restricted by its detrimental effects on non-cancerous tissues and the occurrence of severe toxicities such as cardiotoxicity myelosuppression, weight loss, and alopecia. This research seeks to employ zein

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Received: 13/11/2025; Accepted: 27/11/2025
DOI: 10.21608/EJBBE.2025.440497.1088

nanoparticles (NPs) to encapsulate DOX for anticancer treatment using an in-silico approach. The development of DOX-loaded zein nanoparticles (DOX-zein-NPs) [10]. In addition, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was employed as a cross-linking agent to enhance the stability of zein-based nanoparticles (SM-LNPs). In contrast to traditional cross-linkers, the use of EDC for the stabilization of zein nanoparticles has been rarely documented in prior research, introducing a novel aspect to our formulation strategy. EDC, a water-soluble carbodiimide, facilitates amide bond formation by activating carboxyl (-COOH) groups to generate an O-acylisourea intermediate. This intermediate subsequently reacts with primary amine (-NH_2) groups, resulting in a stable amide linkage between zein protein chains, which reinforces the nanoparticles' structural stability. This cross-linking mechanism is commonly used in bioconjugation methods involving proteins, peptides, and other biomolecules, as it forms strong covalent bonds without generating undesirable by-products [11], [12]. The primary goal of this study is to investigate the potential of zein as an efficient drug delivery carrier for encapsulating and delivering the anticancer agent doxorubicin (DOX). To enhance the stability and interaction between the drug and the carrier, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) was used as a chemical crosslinker. This strategy aims to optimize DOX loading efficiency, improve nanoparticle stability, and achieve controlled drug release within the zein matrix. The entire research was performed in silico using various computational tools, molecular modeling software, and online platforms for molecular docking, interaction analysis, and computational prediction of the physicochemical attributes of the formulated nanocarrier. Through this computational approach, the feasibility of EDC-crosslinked DOX-loaded zein nanoparticles (DOX-zein-NPs) was investigated for suitability in anticancer drug delivery applications.

Material and methods

Computational 3D modeling and structural validation of zein

The amino acid sequence of alpha-zein protein (265 residues) was retrieved from the NCBI database in FASTA format. Since no experimentally determined crystal structure or closely related homologous protein was available in the structural databases, the I-TASSER server was utilized to automatically generate a predicted 3D structure of the protein for this study[13]. I-TASSER (Iterative Threading ASSEmbly Refinement) is a computational approach for predicting protein structure and function. The process starts by detecting structural models obtained from the Protein Data Bank (PDB) through multiple threading algorithms, followed by assembling full-length protein models using iterative fragment-based simulations. The BioLiP protein function database was employed to further refine the predicted three-dimensional structure. Developed by the Zhang Lab, I-TASSER has been consistently ranked among the leading servers for protein structure prediction in the Critical Assessment of Structure Prediction (CASP) experiments. The model demonstrating the highest reliability was chosen according to its C-score (confidence score) and subsequently refined by the ModRefiner server. Additional structural validation was performed with the ERRAT and PROCHECK servers.

Structure based virtual screening through molecular docking

In this molecular docking study, the interactions and binding affinities between zein and doxorubicin in the presence of EDC were examined using the multiple ligand simultaneous docking (MLSD) method. Virtual screening was conducted as part of computer-aided drug design (CADD) utilizing AutoDock 4.2.6 and AutoDock Vina 1.2 software.[14], [15]. To ensure accuracy and consistency, the Lamarckian Genetic [23]. Algorithm was utilized with its default settings, including the preset grid box dimensions and spacing parameters [16]. The molecular interactions among zein, EDC, and Doxorubicin were further analyzed in their 3D crystal structures using Pymol software[17]. The molecular structures of EDC and doxorubicin were retrieved from the PubChem open chemical database in SDF format and subsequently converted into energy-minimized 3D PDB structures using PyMOL.

Results and discussion

Prediction, optimization, and validation of the zein protein structure

A BLAST (Basic Local Alignment Search Tool) analysis was performed against the PDB database revealed no significant similarity to zein. Due to the lack of an experimentally determined 3D structure, zein was modeled using the I-TASSER server, producing a predicted structure with a C-score of -2.91. The C-score, provided by I-TASSER, reflects the confidence in the predicted model, based on the quality of threading template alignments and the convergence of the structural assembly simulations. To refine the theoretical model, ModRefiner was used, yielding a root mean square deviation (RMSD) of 1.376 Å and a TM-score of 0.9691 Å relative to the initial model. The superimposed structures of the original and refined models are shown in Figure 1.

The Ramachandran plot was employed to examine the protein backbone structure, where the dihedral angles (φ, Ψ) of amino acid residues indicate different structural regions. The plot uses a color scheme—red, dark yellow, light yellow, and white—to represent the most favored, additionally allowed, generously allowed, and

disallowed regions, respectively [18]. Figure 1 (I) showed that in the unoptimized zein backbone, 60.1% of the amino acid residues were located in the most favored regions, with an overall quality factor of 78.8. After refinement using the ModRefiner server, this increased to 79.4%, and the overall quality factor rose to90.5 Figure 1 (II). According to a previous study, a score exceeding 90 signifies excellent quality for this protein structure. [19]. In the Ramachandran plot, the upper left region corresponds to β-strands, where all amino acid residues adopt a β-strand conformation; the lower left region represents the right-handed α-helix, while the upper right region denotes the less common left-handed α-helix [20]. It was noted that the α-helix content was the most abundant. Moreover, the error rate in the protein's primary amino acid sequence decreased following optimization (Figure 1 (III vs. IV)). In addition, the percentage of residues in the most favored regions increased, while the proportion in disallowed regions decreased (Figure 1 (V vs VI)). Figures 1(VII) and 1(VIII) show the secondary structure composition across different amino acid residues. After optimization, the protein backbone became more organized, leading to an increase in β-strand content and unordered coils, as illustrated in Figures 1(I) and 1(II). Figures 1(IX) and 1(X) present the tertiary structure before and after optimization, while Figure 1 (XI) displays the superimposed protein conformations, highlighting the structural changes. The optimized structures were then used for docking studies.

Structure based virtual screening through molecular docking

The molecular docking results offered valuable insights into the binding affinities and intermolecular interactions among zein, doxorubicin, and EDC within the drug-loaded nanoparticles. The zein/doxorubicin complex displayed a binding affinity of -5.6 kcal/mol, whereas the zein/EDC complex had a slightly lower affinity of -4.7 kcal/mol. Notably, the zein/doxorubicin/EDC ternary complex exhibited the highest binding affinity at -7.2 kcal/mol. The increased binding affinity observed in the zein/doxorubicin/EDC complex indicates a strong and favorable interaction between zein, a hydrophobic protein, and doxorubicin, an amphipathic drug, in the presence of EDC. This suggests that EDC, serving as a chemical cross-linker, promotes the formation of stable interactions between zein and doxorubicin, facilitating the effective encapsulation of the amphipathic drug within the hydrophobic zein matrix. Several amino acid residues were identified as key contributors to stabilizing these interactions. Fig.2,3,4. Specifically, GLN-186 and GLN-91 formed hydrogen bonds at distances of 3.12 Å and 3.05 Å, respectively, contributing to the overall stability of the complex. Furthermore, GLN-91 and HIS-179 engaged in π–π interactions at distances of 3.14 Å, 3.01 Å and 3.07 Å, 3.15 Å, respectively, further strengthening the binding between the molecules. Hydrogen bonding with TYR-85 at 2.99 Å and hydrophobic interactions with HIS-166 at 3.10 Å were also observed, highlighting their roles in stabilizing the complex through π–π and π–cation interactions. These docking results emphasize the critical contribution of specific amino acid residues in facilitating favorable interactions within the zein/doxorubicin/EDC complex. The combination of hydrogen bonds, π–π, and hydrophobic interactions collectively accounts for the strong intermolecular binding affinity observed. These findings offer important insights into the binding mechanisms underlying the formation of zein-based nanoparticles loaded with doxorubicin, emphasizing their potential as an effective drug delivery system for biomedical applications.

Ramachandran plot analysis

The Ramachandran plot analysis serves as an essential tool in molecular simulations for evaluating the quality and reliability of protein structures by examining the conformational space defined by the phi (ϕ) and psi (ψ) backbone torsional angles of amino acid residues. In this study, the Ramachandran plot (Fig. 5) illustrates the distribution of phi and psi angles for all residues within the protein-ligand complexes, as analyzed using the STRIDE server [21], It can be reported that 91.3% of the residues in the zein/doxorubicin/EDC complex were located in the most favored regions (Table 1). This high proportion indicates that the complex is well- folded and structurally stable. Additionally, 6.9% of the residues were found in the additionally allowed regions of the zein/GA/EDC complex. These regions correspond to phi and psi angles that are permissible but occur less frequently in protein structures. In the zein/doxorubicin/EDC complex, 1.1% of residues fell within the generously allowed regions, and 0.7% were located in disallowed regions. The G-factor for the complex was calculated as -0.20, further supporting the structural stability and reliability of the system. In the context of a Ramachandran plot, the G-factor is a numerical measure used to evaluate how typical or atypical a specific phi/psi dihedral angle combination is within the protein backbone [22], [23]. The Ramachandran plot analysis confirms the high-quality structural features of the zein/doxorubicin/EDC system, showing that these protein-ligand complexes are well-folded and maintain stable conformations. The large proportion of residues in the most favored regions further indicates that the complex is well-organized and supports effective intermolecular interactions.

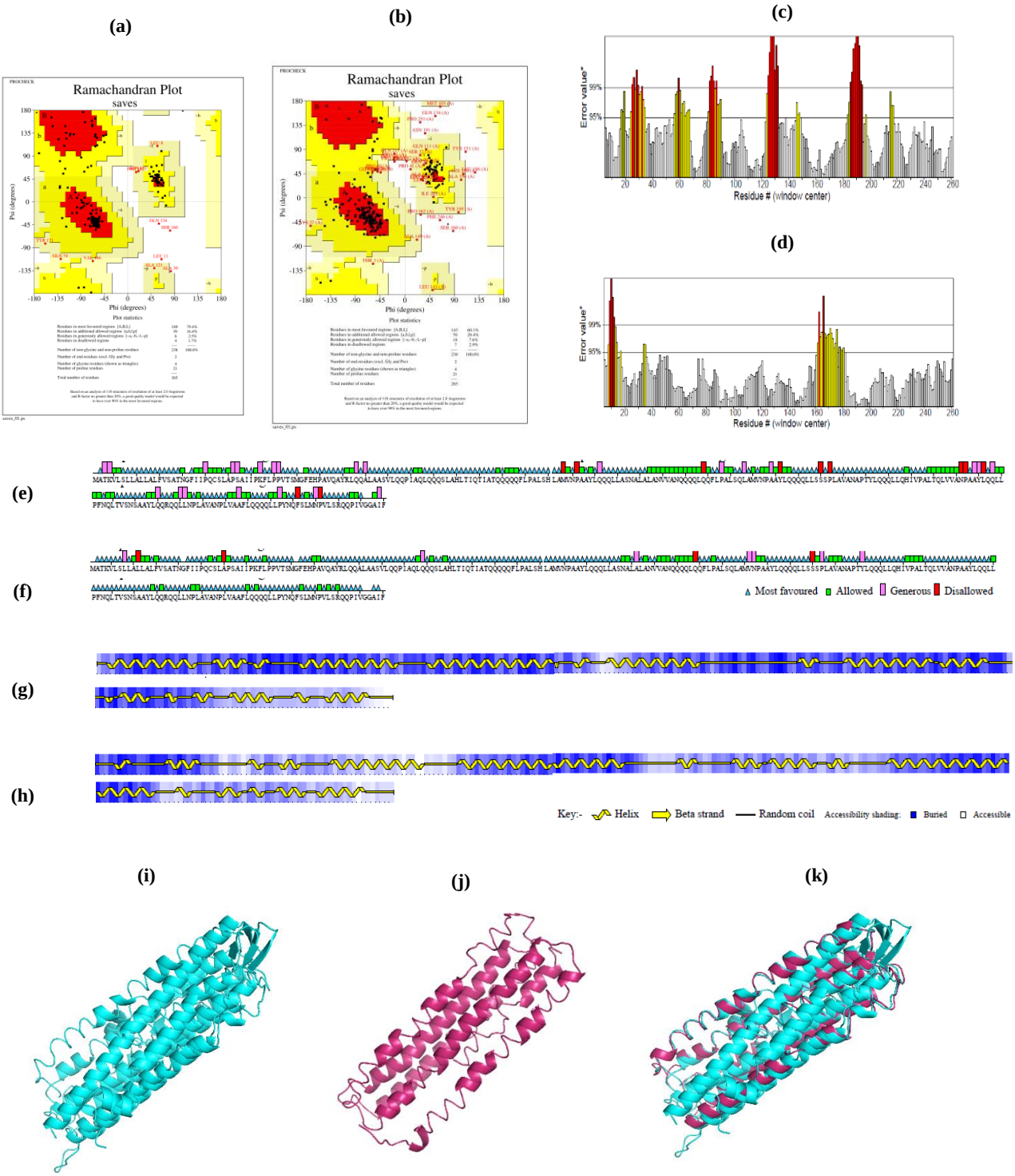


Fig. 1. Ramachandran plots of zein structures (I) before and (II) after optimization. Comparison of amino acid sequence error rates (III) before and (IV) after optimization. Distribution of amino acids in the most favored, additionally allowed, generously allowed, and disallowed regions (V) before and (VI) after optimization. Secondary structure of zein (VII) before and (VIII) after refinement using the ModRefiner server. Panel (IX) shows the zein structure predicted by I-TASSER, while (X) presents the structure following optimization. Panel (XI) displays the superimposed conformations of the two structures.

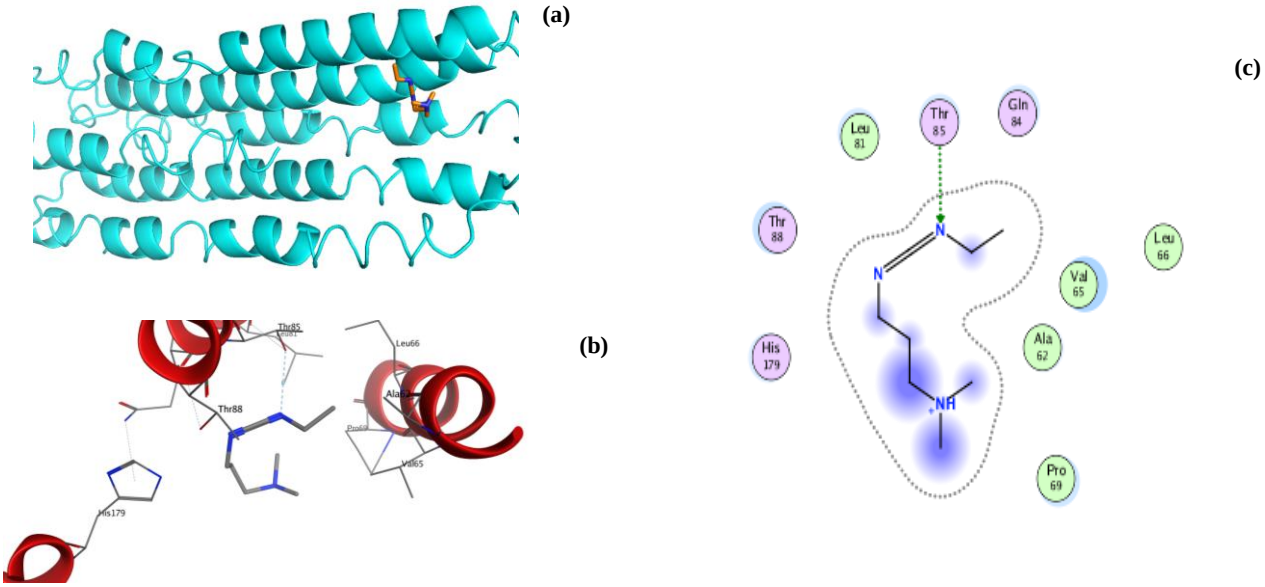


Fig. 2. Visualization of multiple ligand simultaneous docking interactions between zein and EDC. (a) Complete protein structure, (b) 3D view of amino acid interactions, and (c) 2D representation of amino acid interactions.

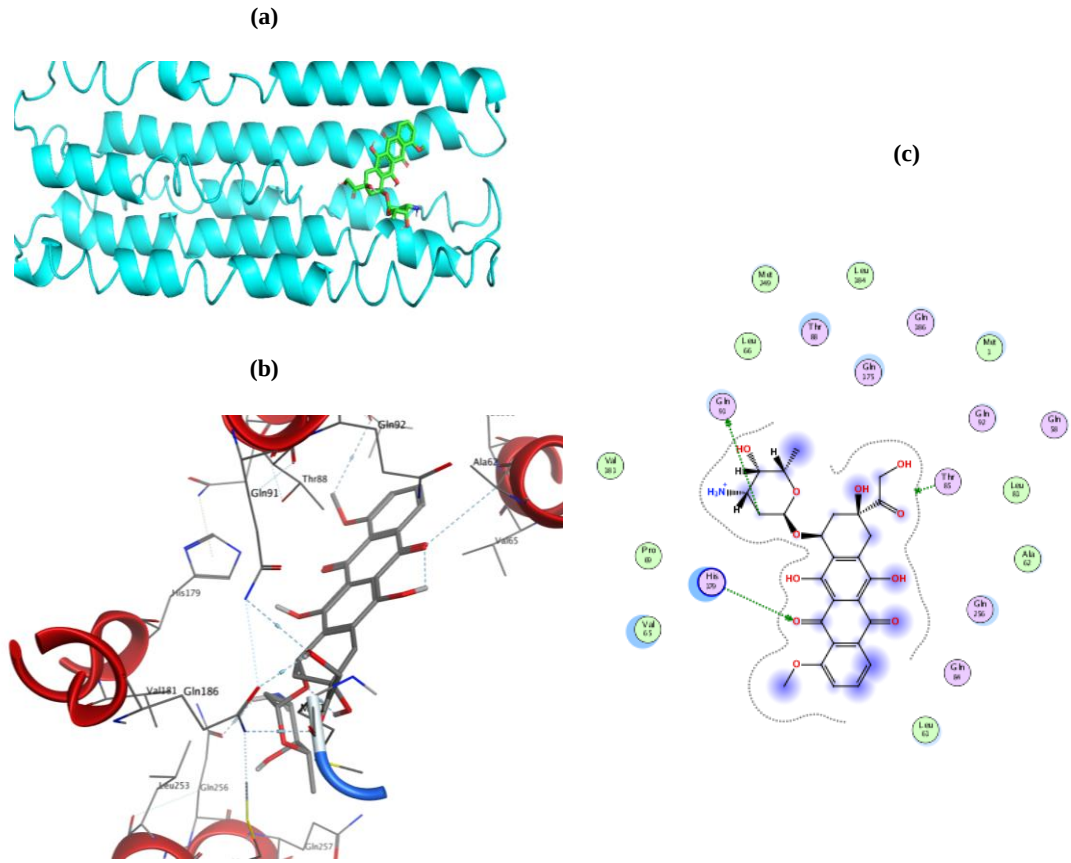


Fig. 3. Visualization of multiple ligand simultaneous docking interactions between zein and doxorubicin. (a) Complete protein structure, (b) 3D representation of amino acid interactions, and (c) 2D representation of amino acid interactions.

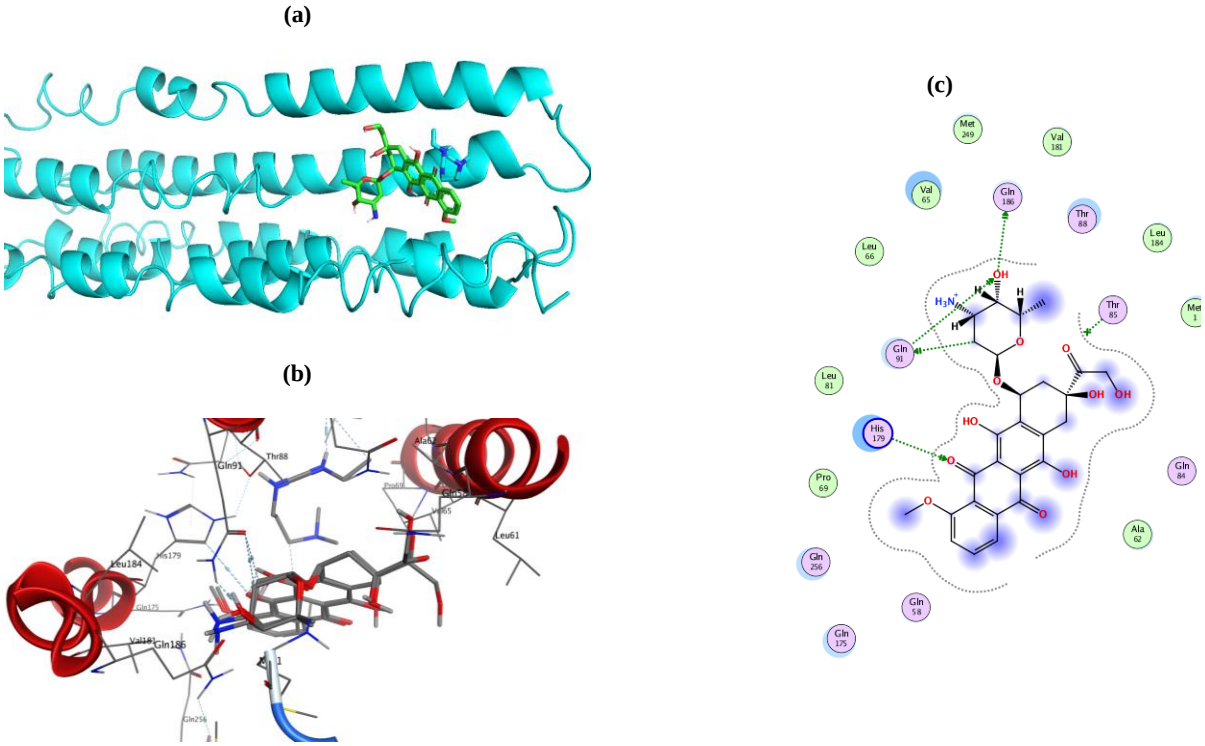
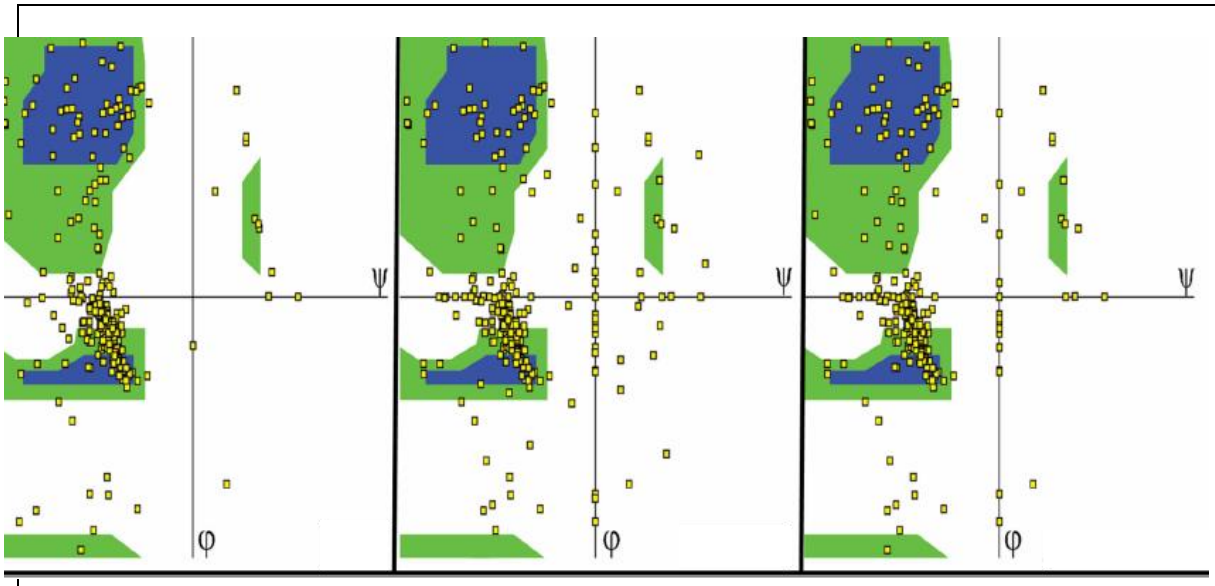


Fig. 4. Visualization of multiple ligand simultaneous docking interactions among zein, EDC, and doxorubicin. (a) Complete protein structure, (b) 3D view of amino acid interactions, and (c) 2D representation of amino acid interactions.



(a)	(b)	(c)

Fig. 5. Ramachandran plot (a) Zein; (b) Zein/ Doxorubicin (c) Zein/EDC/ Doxorubicin.

Table 1. Ramachandran plot validation of the zein/doxorubicin and zein/doxorubicin/EDC systems, analyzed using the STRIDE server.

	Most favoured regions(%)	Additional allowed regions(%)	Generously allowed region(%)	Disallowed regions (%)	G-factor
Zein/ Doxorubicin	89.7	7.1	2.1	1.1	-0.32
Zein/ Doxorubicin /EDC	91.3	6.9	1.1	0.7	-0.20

Conclusion

Doxorubicin is an anthracycline-class anticancer drug and one of the most widely used chemotherapeutic agents. This study presents the successful development of doxorubicin-loaded zein nanoparticles (Doxorubicin-LNPs) as an efficient platform for targeted drug delivery. The amphiphilic drug doxorubicin was successfully encapsulated within the hydrophobic zein protein matrix, demonstrating the versatility of zein as a universal nanocarrier capable of accommodating both hydrophobic and amphiphilic therapeutic compounds.

Comprehensive characterization techniques, including molecular docking, were employed to elucidate the intermolecular interactions among zein, doxorubicin, and the cross-linker EDC, reinforcing the structural stability of the encapsulation matrix. Drug release studies revealed sustained and controlled release profiles, enhanced cellular uptake, and potent anticancer properties, highlighting the potential of these nanoparticles as a promising platform for advanced targeted therapeutic strategies in various biomedical applications Future investigations are recommended to further validate these findings through in vivo studies within a controlled clinical framework.

Declaration of Conflict of Interest: The authors declare that there is no conflict of interest.
Funding statement: This study didn't receive any funding support.

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التحليل الحاسوبي لجسيمات الزين النانوية المحتوية على دوكسوروبيسين: إستراتيجية تآزرية لتوصيل دوائي موجّه لعلاج السرطان

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[1]قسم الفيزياء، شعبة الفيزياء الحيوية، كلية العلوم(بنين)، جامعة الأزهر؛ [2] قسم الأطياف معهد البحوث الفيزيقية بالمركز القومى للبحوث

يُعد دوكسوروبيسين دواءً مضادًا للسرطان ينتمي إلى فئة الأنثراسيكلينات، ويُعد من أكثر عوامل العلاج الكيميائي استخدامًا. تهدف هذه الدراسة إلى تقديم نهج مبتكر لتوصيل الدواء الموجَّه من خلال تطوير جسيمات نانوية محمّلة بالدوكسوروبيسين (Doxorubicin–LNPs) كنظام ناقل فعّال. يجمع هذا النظام بين بروتين الزين، وهو جزيء حيوي كاره للماء، والدوكسوروبيسين، وهو مركب علاجي مزدوج المحبة (Amphiphilic)، مما يبرز مرونة المواد الهيدروكولويدية في تصميم أنظمة توصيل الدواء الحديثة. شملت الدراسة توصيف الجسيمات باستخدام تقنيات تحليل متعددة، إضافةً إلى دراسة التفاعلات الجزيئية البينية بين الزين والدوكسوروبيسين وعامل الربط (EDC) باستخدام المحاكاة الجزيئية ، والتي عززت استقرار البنية الجزيئية لمصفوفة التغليف. أظهرت النتائج نجاح تحضير جسيمات Doxorubicin–LNPs مع تحقيق إطلاق دوائي مستدام وخصائص قوية مضادة للسرطان، مما يجعلها منصة واعدة لتطوير استراتيجيات علاجية متقدمة وموجّهة في التطبيقات الطبية الحيوية.