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Original article

Investigating mechanistic insights of curcumin in blocking the Interleukin-8 signaling pathway associated with Breast Cancer: An in-silico approach

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ABSTRACT

Interleukin-8 (IL-8) is a chemokine, a type of signaling molecule that has a role in immunological responses and inflammation. In recent years, IL-8 is additionally related to cancer growth and recurrence. Breast cancer growth, progression, and metastatic development are all linked to IL-8. Breast cancer cells are known to develop faster when IL-8 stimulates their proliferation and survival. It can also cause angiogenesis, or the creation of new blood vessels, which is necessary for tumor nutrition and growth. IL-8 and curcumin have been subjects of interest in drug design, particularly in the context of inflammation-related disorders and cancer. This study aims to give an overview of the role of IL-8. Inhibitor-based treatment approaches were being used to target IL-8 with curcumin. Molecular docking method was employed to find a potential interaction to supress competitive inhibition of IL-8 with curcumin. PASS analysis and ADMET characteristics were also being carried out. In the end, IL-8 complexed with curcumin is chosen for MD simulations. Overall, our results showed that during the simulation, the complex stayed comparatively stable. It is also possible to investigate curcumin further as a possible treatment option. The combined results imply that IL-8 and their genetic alterations can be studied in precision cancer therapeutic treatments, utilizing target-driven therapy and early diagnosis.

1. Introduction

Breast tissue is predominantly affected by breast cancer is a potentially fatal disease. In the world, it is very common type of cancer in women. Modification in the expression and operation of genes play role in the cell cycle regulation have been linked to BC, among other molecular modifications. One of the protein which is associated to breast cancer is IL-8. (Singh et al., 2013). Interleukin-8 (IL-8) is a minor protein in the chemokine family. In response to inflammation, it is produced through different cells, such as epithelial cells, immune cells, and cancer cells, in response to inflammation or other stimuli. IL-8 functions as a chemotactic factor (Mukaida et al., 1998), meaning it attracts and activates certain immune cells, particularly neutrophils, to the site of inflammation or injury.

IL-8 promotes breast cancer cell growth by stimulating their proliferation and survival (Freund et al., 2003). Additionally, in drawing endothelial cells to the tumor site, it plays a crucial part in angiogenesis, new blood vessels formation. This encourages the tumour's blood supply to be established, which helps the tumor grow and spread. Breast cancers have an inflammatory microenvironment that is influenced by IL-8. It brings immune cells to the tumor site, including macrophages and neutrophils, which results in long-term inflammation (Ren et al., 2023). In addition to facilitating the growth of tumors, inflammation can depress the immune system and impair the body's potential to fight cancer cells. IL-8 help to promote of breast cancer metastasis. It makes breast cancer cells more invasive, enabling them to separate from the main tumor and infiltrate nearby organs. IL-8 is also involved in the migration of cancer cells to distant sites through the bloodstream (Itoh

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et al., 2005) or lymphatic system, contributing to the formation of secondary tumors. IL-8 has been linked with resistance to certain treatments in breast cancer (Shi et al., 2012). It can promote the survival of cancer cells and protect them from the cytotoxic effects of therapy, thereby contributing to treatment resistance (Blakely et al., 2023). Elevated IL-8 levels have been associated to a worse outcome in breast cancer patients (Mohammed Bakheet et al., 2024). Higher levels of IL-8 expression have been linked to later stages of the disease, bigger tumor size, metastasis of lymph node, and lower overall survival rates.

Given its involvement in breast cancer, targeting IL-8 or its signaling pathways has emerged as a potential therapeutic strategy (Stanilov et al., 2024). Preclinical and clinical studies are exploring approaches to inhibit IL-8 or block its receptors as a means to suppress tumor growth (Barry et al., 2023), angiogenesis, and metastasis, and enhance the effectiveness of existing treatments. IL-8 (Interleukin-8) and curcumin have been subjects of interest in drug design, particularly in the context of inflammation-related disorders and cancer (Waugh and Wilson, 2008).

Curcumin, a natural small molecule found in turmeric, has gained considerable attention for its therapeutic properties. Curcumin has anti-inflammatory properties due to its ability to block several inflammatory pathways and reduce the generation of pro-inflammatory molecules (Jurenka, 2009; Menon and Sudheer, 2007). It has the ability to inhibit the activity of transcription factors such as NF-B, which play an crucial role in the inflamed response (Lin and Lin, 2008; Lin et al., 2007; Yu et al., 2011). By modulating these pathways, curcumin has shown potential in managing inflammatory conditions such as rheumatoid arthritis (Mohammadian Haftcheshmeh et al., 2021), inflammatory bowel disease (Vecchi Brumatti et al., 2014), and chronic inflammatory disorders (Razavi et al., 2021). Curcumin has been extensively reported for its potential anti-cancer properties (Shi et al., 2006). It exhibits various mechanisms that can interfere with cancer progression (Jin et al., 2015), including inhibition of tumor growth, induction of apoptosis (Pillai et al., 2004) and suppression of angiogenesis. Additionally, curcumin has been investigated for its potential to sensitize cancer cells to radiation and Chemotherapy, enhancing their effectiveness. Recent researches have shown curcumin as natural phenol and therapeutic role in cancer (Kah et al., 2023).Since many decades curcumin is being used as therapeutic agents in various cancer including lung cancer (Lin et al., 2009), ovarian cancer (Shi et al., 2006) etc.

Targeting a specific protein offers significant advantages, and advancements in bioinformatics have made this achievable (Beg et al., 2023; Beg and Parveen, 2021). inhibition of IL-8 has gained attention as a potential therapeutic approach for the treatment of breast cancer. The complicated role of IL-8 in breast cancer varies depending on the molecular subtype and features of the disease. To gain a deeper insight of the specific processes and possible therapeutic implications of IL-8 in the development and treatment of breast cancer, more study needs to be being conducted.

2. Methodology

2.1. Receptor and compound preparation

The structure of IL-8 protein was retrieved by accessing protein database bank using PDB ID: 5D14 and structure was visualized in PyMol (DeLano, 2002) visualizer tool, to make sure that the structure was complete. The protein structure was then pre-processed waters and additional co crystalized ligand entity were removed to vacant IL-8 binding sites or allosteric sites. The small molecule curcumin was downloaded from drug bank (ID- DB11672). It adheres to the five rules of Lipinski (Lipinski, 2004).

2.2. Molecular docking

Molecular docking reduces the time and cost involved in developing and delivering innovative medicine candidates, has been acknowledged as one of the most significant methodologies. After preparing the protein and compound in pdbqt format, we carried out molecular docking. It is possible to use docking programs like Gold and Gem Dock, Auto $pKi = -\log(Ki_{pred})$ Dock Vina, InstaDock, and others. Here InstaDock (Mohammad et al., 2021) was utilized resulting the output files produced after the compound was docked and analysis showed favourable interactions and binding affinities.

2.3. Inhibition constant (Ki), and pKi calculation

The calculated inhibition constant (Ki; nM) is a parameter of potency to inhibit (Mohammad et al., 2021; Tiwari et al., 2021); a less Ki value denotes effective inhibitor. By this formula (Eq1), the Ki was calculated from the ΔG parameter:

$$Ki = EXP((\Delta G \cdot *1000)/(R*T)) \quad (1)$$

$$\Delta G = dockingenergy, \cdot R = 1.98719 \cdot cal \cdot K^{-1} \cdot mol^{-1;T \cdot 298.15^{\circ}k}$$

The pKi, that is the negative decimal logarithm of inhibition constants (Shityakov and Förster, 2014), was estimated from the G parameter. (Eq2):

$$\Delta G = RT(Ln \cdot Ki_{pred}) \quad (2)$$

$$Ki_{pred} = e^{(\Delta G/RT)}$$

$$pKi = -\log(Ki_{pred})$$

ΔG is the binding affinity (kcal/mol), T (room temperature) is 298.15 Kelvin, R (gas constant) is 1.98 cal*(mol*K)$^{-1}$, Ki_{pred} is the predicted inhibitory constant.

2.4. ADMET analysis

The primary consideration in the creation of novel drugs is safety, which was established for a few pharmaceuticals and their efficacies utilizing ADMET. The platforms ADMETlab 2.0 (Xiong et al., 2021) and SwissADME (Daina et al., 2017) were used to evaluate the ADMET properties of the curcumin molecule. Because both web sites support it, SMILE IDs were collected from the databases linked with the molecules in order to access the databases.

2.5. PASS analysis

Pass is a web based server (Lagunin et al., 2000) that analyses use, side effect, and mechanism of action for any given drug. The biological function of compound is also evaluated on this website using structure–activity relationships. It uses a range of biological activities to compare the expected structure with an internal training set. It assesses the recommended framework against a pre-programmed instructional set of biological processes. The biological activities were calculated by dividing the "probability to stay inactive (pi)" by the "probability to be active (pa)," with a chemical under examination having a higher probability of being a biological active.

2.6. Molecular dynamic simulations

Based upon a basic physics model underpinning interatomic interactions, MD simulations provide detail how all the atoms in protein molecules or associated molecular systems will move with function of time (Beg et al., 2019). Before launching the simulation, the following steps should be executed in order to generate the protein data, translating the PDB to gmx and constructing the topology, defining the box, protein solvation, addition of ions, and minimizing of energy. Gromacs 2020.6 version was used and charmm36-jul2022 force field was utilized.

To generate topology of the compound, we utilized the CGenFF program and python script. Each system was placed in a cubic box with a 10 Å edges distance, and the SPCE water model was used. The equilibration process was executed in two stages: isothermal/isochoric (NVT) and isobaric (NPT). During the first stage, we maintained a constant NVT ensemble for 100 ps at 300 K. In the second stage, after temperature stabilization, we used a constant NPT ensemble for 100 ps to stabilize the system's pressure. Finally, we performed a 200 ns MD simulation, resulting analyzing the resulting trajectory files with GROMACS built-in tools.

2.7. PCA and FEL analysis

PCA has been demonstrated to be an excellent method for measuring underlying protein movements and evaluating folding mechanism (DeLano, 2002). PCA is a mathematical method that uses covariance matrices to reduce a set with several dimensions of variables to a lower dimension. It employs diagonalization of eigenvector for the covariance matrix, enabling us to explore IL-8 conformation selection and its relationships with curcumin. To explore the folding behavior of IL-8 in apo and complex form, FELs were produced utilizing IL-8 and curcumin. We learned more about the stability of IL-8 when it was complexed with curcumin.

3. Results

3.1. Molecules preparation and molecular docking

Using the PyMol visualizer tool, we were able to explore the structure of the IL-8 protein, which we ensured to be complete with no missing residues. The structure was retrieved by accessing protein data bank. After that, the structure was visualized using PyMol to clear out water and other ligand entity in order to unoccupied allosteric or IL-8 binding sites. The ability of molecular docking techniques to provide the binding pattern of compound that function as ligands to the adequate binding of the target site is a crucial part of in silico drug design. Given this, our study's objective was to molecularly dock the essential protein curcumin for possible IL-8 inhibition. Moreover, molecular docking executed using the InstaDock program following the preparation of receptor and small compound. This technique provides data on the binding energy and interaction pattern and distance between residues and atom. The automatic standalone program InstaDock delivers unique energy files for the greatest hits. We determined the inhibition constant (Ki) and pKi of the hit using the formulas Eqs. (1) and (2). Table 1 shows the energy values of curcumin's interaction with the IL-8 protein, which was −5.4 kcal/mol. Strong affinity for binding is exhibited by curcumin, as seen in Fig. 1. Critical bonds were formed by the compound with residues CYS5, CYS7, THR10, CYS32, ASN34, GLU36, and LYS52, including hydrogen bonds (Fig. 1B). Other interactions like alkyl, pi-alkyl, etc. was also formed.

3.2. ADMET analysis

ADMET properties play significant role in the decision-making and progression of drug-like compounds. Compounds which satisfy the physicochemical and ADMET test are more likely to be approved for clinical use. (Table 2). It followed the Lipinski, Ghose, Veber and BMS criteria, and no PAINS warning was generated. Other aspects of the compound exhibit drug-like characteristics. The MW (g/mol) is 368.38, the consensus log P is 3.03, HB acceptors and donors are 6 and 2 respectively, and topological polar surface area (2) is 93.06.

Table 1
Curcumin bind with IL-8 protein, with energy values −5.4 kcal/mol.

S. No.	Target Protein	Compound	Binding Affinity (kcal/Mol)	ki	pKi
	IL-8	Curcumin	−5.4	0.000110089	3.96

3.3. PASS analysis

The biological properties of chemical compound should be explored to assure the possibility of compound with the needed qualities. In this research article, it was employed to assess the biological properties of curcumin. Table 3 summarizes the biological functions of curcumin and whether it is active.

3.4. MD simulations

The GROMACS 2020.6 version simulation suit was utilized for atomic level MD stimulations at 300 K. To simulate both the systems apo and complex form, the charmm36-jul2022 force field was applied. We accessed the CGenFF program and python script to generate the compound's topology files. In each system edges distance were centralized in a 10 Å cubic box and the SPCE water model was utilized.

3.5. Calculation of potential energy of system

The mean potential energy of IL-8 and the IL-8-curcumin complex was calculated to assess system equilibration. Average potential energy at temperature fluctuations around 300 K for both systems suggested that the MD simulations exhibited stability and accuracy. IL-8, and IL-8-curcumin complex have average potential energies of −338631 kJ/mol and −232628 kJ/mol, respectively (Table 4).

3.6. Structural changes

A protein's RMSD is among the most widely used fundamental properties to figure out if the protein is structurally stable and close to its native structure. The protein IL-8 alone had an average RMSD value of 0.293 nm. Ligand binding may result in some conformational changes in the structure of protein. When IL-8 was complexed with curcumin, the average RMSD value was determined to be 0.275 (Table 4). The inhibitor IL-8-curcumin deviation was initially smaller than 0.2 nm, but as time passed, the deviation decreased while simultaneously increasing, with the maximum peak seen at 0.4 nm. Overall, we observed that there is relatively little variation in ligand binding. (Fig. 2**A**).

The fluctuations around the point of stable structure are not arbitrary, instead they based on the flexibility of the structure. By graphing the RMSF of the IL-8 after drug interaction as a function of residue number, the average variation of all residues throughout the entire simulation was determined (Table 4). The RMSF plot indicated that residual aberrations exist throughout the structure of IL-8. The fluctuations in residues were shown to be the less when IL-8 and curcumin were interacted, and the smallest when IL-8 and curcumin were bound to distinct regions of the protein. The largest RMSF fluctuation was seen at 0.1 nm following curcumin binding (Fig. 2B). Overall, there was relatively little variation observed.

The Rg is a parameter that has been utilized to obtain understanding the stability of protein in a biological system. It is connected to the volume of a protein's secondary structure (Beg et al., 2019). A protein's radius of gyration must be bigger due to the less tight packing. The average Rg of IL-8 and when complexed with curcumin was 1.29 (Table 4). The Rg plot demonstrates that there is no variation in IL-8 ligand binding. (Fig. 3**A and** Fig. 3**C**).

3.7. Solvent accessible surface area

The segment of a protein's interface that connect with its surrounding solvent molecules is known to as its SASA. Average SASA for IL-8 and with curcumin were measured during 200 ns MD simulations and were 57.5 and 57.15, respectively (Table 4). According to the SASA

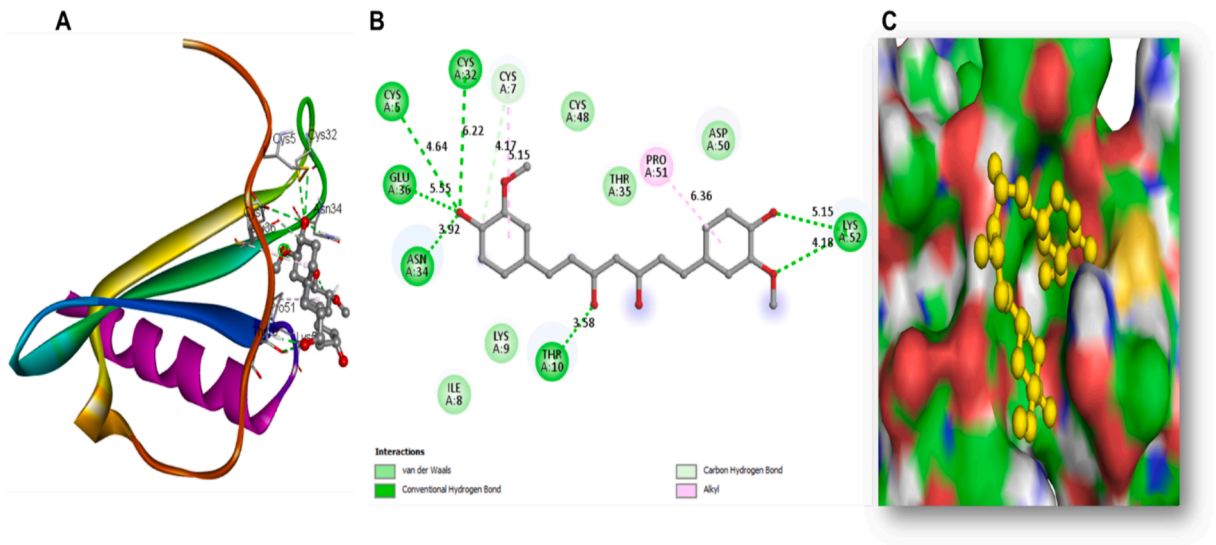

Fig. 1. The 3-D structure of IL-8 with curcumin. (A) A cartoon representation of IL-8-curcumin binding complex. (B) A 2D view of the IL-8 docking groove, displaying the binding amino acid residues with the curcumin. (C) Magnified surface view of IL-8 conserved substrate-binding pocket.

Table 2
Compound's ADMET properties.

S. No.	Compound ID	Compound	Absorption *GI Absorption*	Distribution *P-gp substrate*	Metabolism *CYP2C19 inhibitor/subst.*	Excretion *OCT2 substrate*	Toxicity *hERG I inhibitor*
1.	DB11672	Curcumin	High	NO	NO	NO	NO

Table 3
PASS analysis of curcumin.

S. No.	Phytochemical	Pa	Pi	Activity
	Curcumin	0,6770	0,019	Anti inflammatory
		0,610	0,004	Antioxidant
		0,611	0,012	Anticarcinogenic
		0,554	0,006	Prostate cancer treatment
		0,440	0,013	Interleukin antagonist
		0,435	0,043	Antiallergic
		0,411	0,020	Antiseptic

plot, there is no variation in total SASA values of IL-8 and complex with curcumin (Fig. 3B).

3.8. H-bond analysis

H-Bonds within proteins and compounds allows directionality and specificity of interaction, both of which are required for molecular recognition. We found that number of average intramolecular hydrogen bonds was 39, which increased to 41 after interaction with the curcumin compound (Fig. 4A&C). Average intermolecular Hydrogen bonds between IL and 8-curcumin were 3 with higher distribution as PDF shows (Fig. 4B&D).

3.9. Secondary structure changes

The changes in secondary structure determine the protein's secondary structure over time. 49–52 % of the residues participated in secondary structure development throughout the simulations (Table 5, Fig. 5). The ratio of coils involvement almost remains constant in complex and apo protein, but β-sheet, β-bridge, and bend changes. This may imply that when IL-8 binds to curcumin, it somewhat unfolds.

3.10. PCA and FEL

PCA was utilized to examine a protein's collective movements and structural space exploration using virtual trajectories. We utilized PCA to investigate the structural sampling of IL-8 and IL-8-curcumin complex employing the essential dynamics method (Maisuradze et al., 2009). Fig. 6 illustrates the conformational samples projected onto the Ca atoms of both systems (IL-8, IL-8-curcumin) in the critical subspace.

The IL-8-curcumin complex occupied a subspace similar to that of IL-8 alone. The reduced flexibility observed in the IL-8-curcumin complex on EV1, along with the smaller flexibility of IL-8 on EV2, contributes to improved stability of the complex across simulations, as illustrated in Fig. 6**A and 6B**. Principal component analysis and examination of the free energy landscape were utilized to investigate overall motions in both the free protein and complex. These analyses validated the optimal binding of curcumin with the IL-8 protein.

Free energy landscapes (FELs) are being used to show the manner in

Table 4
Overview of different parameters of MD simulations.

Complex	RMSD (nm)	RMSF (nm)	*Rg* (nm)	SASA (nm^2)	Kinetic Energy (kJ/mol)	Potential energy (kJ/mol)	Enthalpy kJ/mol	Volume (nm^3)	Density (kg/m3)
IL-8	0.293	0.149	1.29	57.55	56160.9	−338631	−282457	220.341	1015.77
IL-8-Curcumin	0.275	0.130	1.29	57.15	39383.1	−232628	−193236	152.922	1023.94

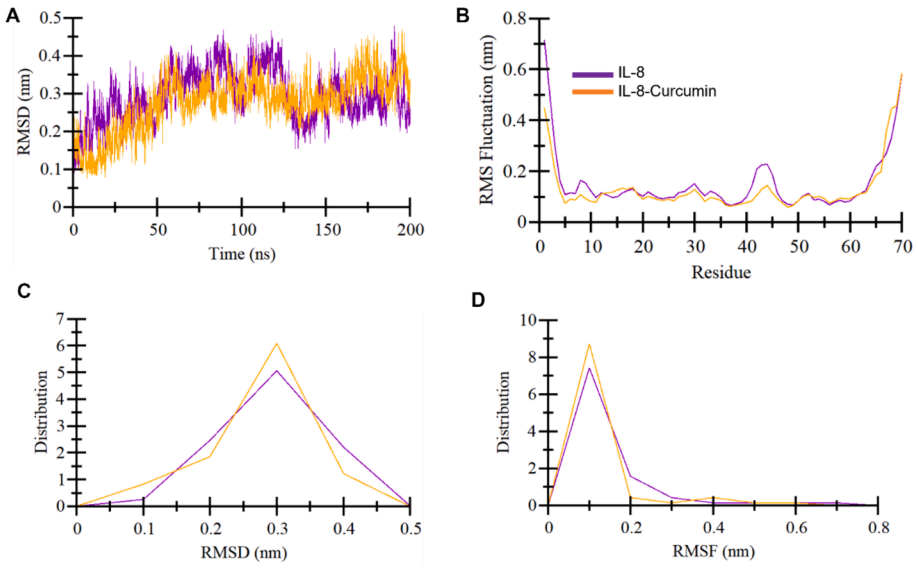

Fig. 2. Structural stability analysis. **(A)** RMSD graph for IL-8 in apo form and with curcumin. **(B)** RMSF graph for IL-8 in apo form and with curcumin. **(C)** and **(D)** in the lower panels indicates the probability as PDF.

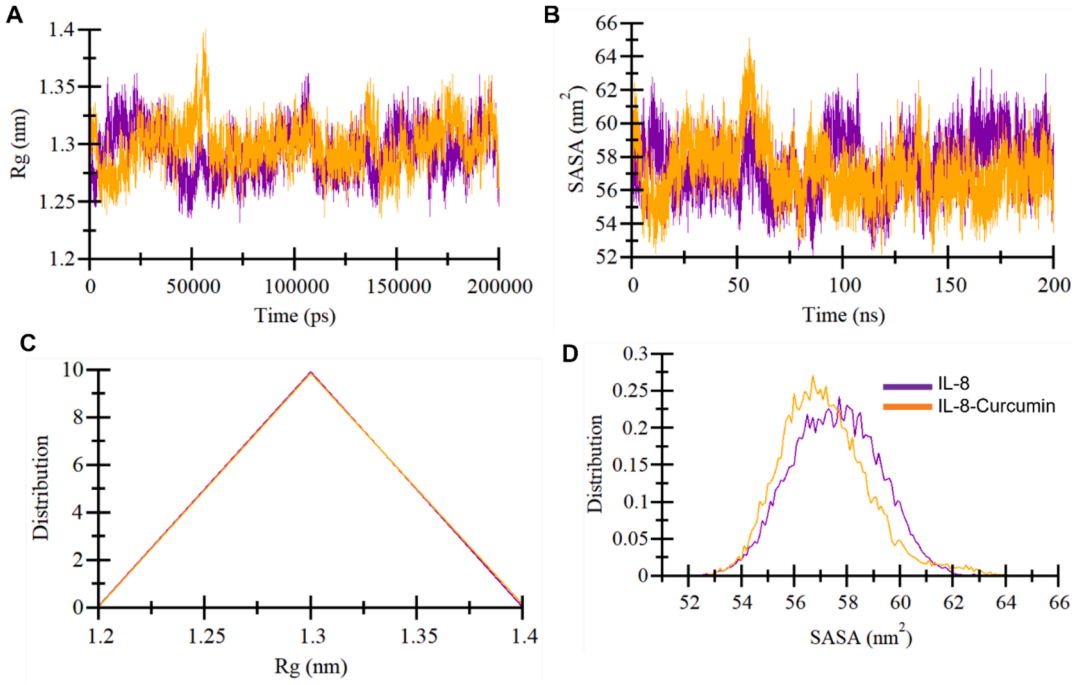

Fig. 3. Structural compactness assessment. **(A)** Radius of gyration (Rg) plot for IL-8 with curcumin. **(B)** SASA plot for IL-8 with curcumin. **(C)** and **(D)** in the lower panels display the PDF values.

which proteins wrap into their native form and eventually denature. Using MD simulation trajectories, it has been applied to study protein stabilization and receptor-ligand complex stability in the presence of solvent. We recreated the structure landscape and energy minima of the IL-8 and IL-8-curcumin complexes using three PCs Fig. 7A-B.

The binding of curcumin with IL-8 has little impact on the dimension and orientation of the phases encompassed persistent global minima, according to energy plots. In FELs, a dark blue color implies a low energy configuration close to the native state (Fig. 8). The graphic shows that IL-8 shown to be constrained to a single global minima that spans multiple basins. Similarly, IL-8-Curcumin produces nearly identical states to IL-8, but with smaller global minima and discrete basins with differing populations (Fig. 8A and B). In conclusion, the simulation and critical dynamics of IL-8 in conjunction with IL-8-curcumin complexes reveal that they gain stability for 200 ns simulations with minor structural flipping.

4. Discussion

IL-8 plays important role in metastasis of BC. It boost the invasive capabilities of BC cells, allowing them to break off from the original tumor and invade neighboring tissues (Singh et al., 2013). IL-8 also encourages cancer cell migration to distant sites through the bloodstream or lymphatic system, facilitating the establishment of secondary tumors. IL-8 can recruit immune cells to the tumor microenvironment, leading to chronic inflammation. This inflammatory response can

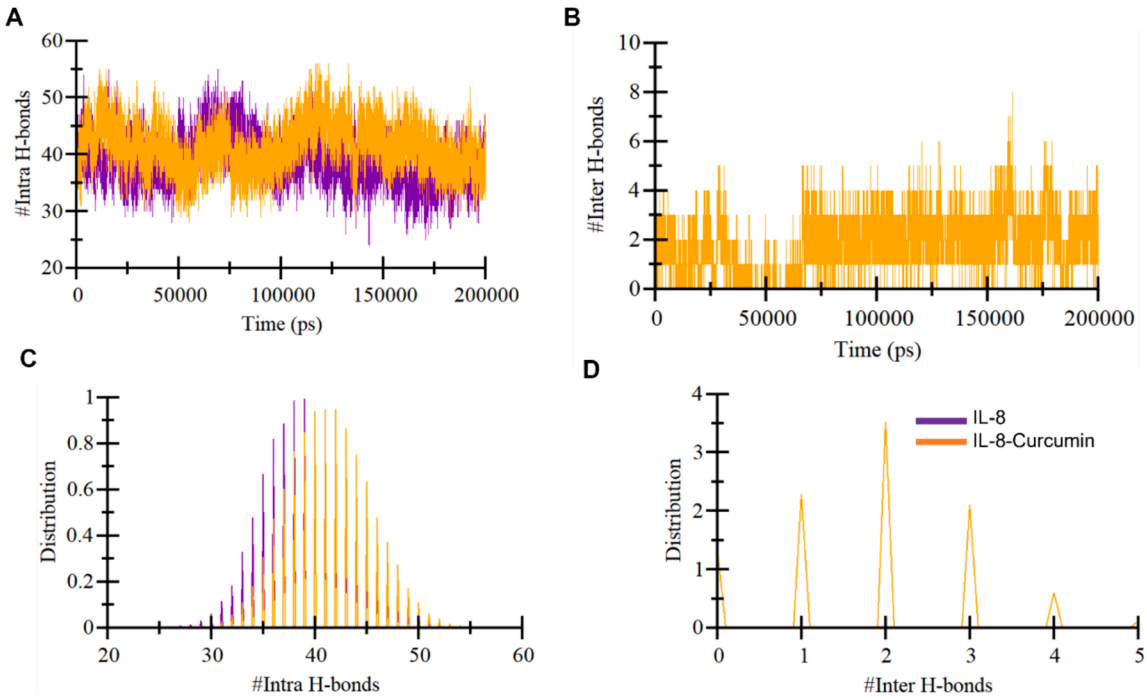

Fig. 4. Dynamics of H-bonds. **(A)** Time dependent intramolecular hydrogen bonds. **(B)** Time evolution of intermolecular hydrogen bonds. **(C)** Distribution of intramolecular hydrogen bonds in IL-8 and curcumin. **(D)** Distribution of hydrogen bonds between IL and 8 and curcumin.

Table 5
The percentage of residues that usually involved in the formation of the protein's secondary structure.

System	Structure	Coil	β-sheet	β-bridge	Bend	Turn	A-helix	5-helix	3-helix
IL-8	0.49	0.32	0.21	0.02	0.16	0.11	0.15	0.02	0.01
IL-8-Curcumin	0.52	0.31	0.24	0.01	0.14	0.11	0.17	0.01	0.02

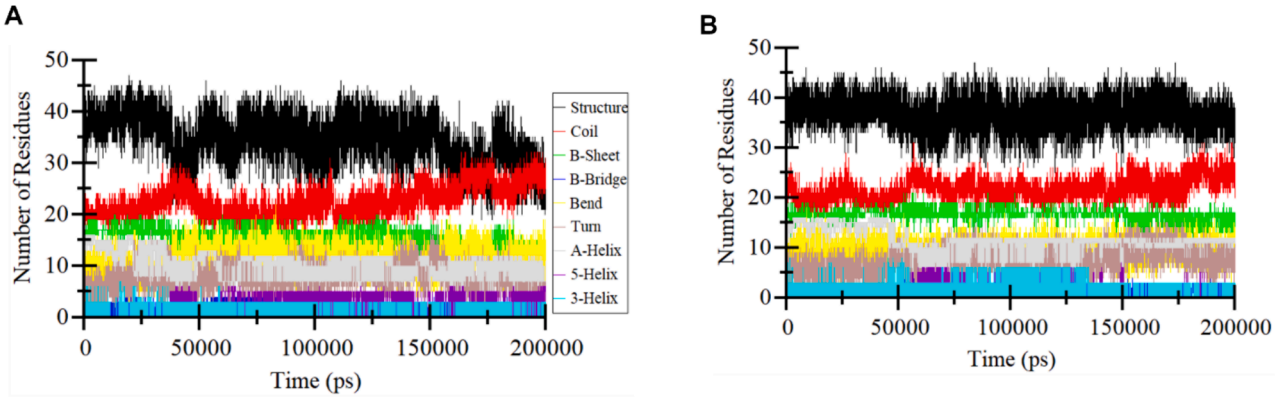

Fig. 5. Time-based secondary structure creation of IL-8 before and after curcumin binding. **(A)** IL-8 protein alone. **(B)** IL-8 protein following curcumin binding.

promote tumor progression and contribute to immune evasion by creating an immunosuppressive environment that hampers the immune system's ability to identify and eradicate cancer cells. Researchers are finding strategies to inhibit IL-8 signaling to suppress metastasis and enhance the efficacy of cancer treatments. It can enhance the growth and survival of ER-positive BC cells, potentially influencing their response to hormone-based therapies. Elevated IL-8 levels have been linked to a worse outcome in patients of breast cancer. Elevated expression of IL-8 is linked to advanced disease stages, bigger tumor size, lymph node metastases, and lower overall survival rates. Blocking IL-8 signaling has been investigated as a potential therapeutic method after studying IL-8 role in BC. Preclinical studies have shown promising results when inhibiting IL-8 or its receptors, either alone or in combination with other treatments, in reducing growth of tumor and metastasis in models of breast cancer.

Curcumin is a naturally occurring spice found in turmeric, commonly used in traditional medication. It has demonstrated anti-inflammatory, antioxidant, and anticancer properties (Zoi et al., 2021). It is used as a possible medicinal agent in various diseases due to its ability to modulate multiple molecular pathways (Wang et al., 2018). It can interfere with the production and activity of pro-inflammatory molecules, including IL-8, thereby suppressing inflammation (Mansouri et al., 2020; Wang et al., 2021). Curcumin has been demonstrated to inhibit IL-8 expression and release in a wide range of types of cells, particularly

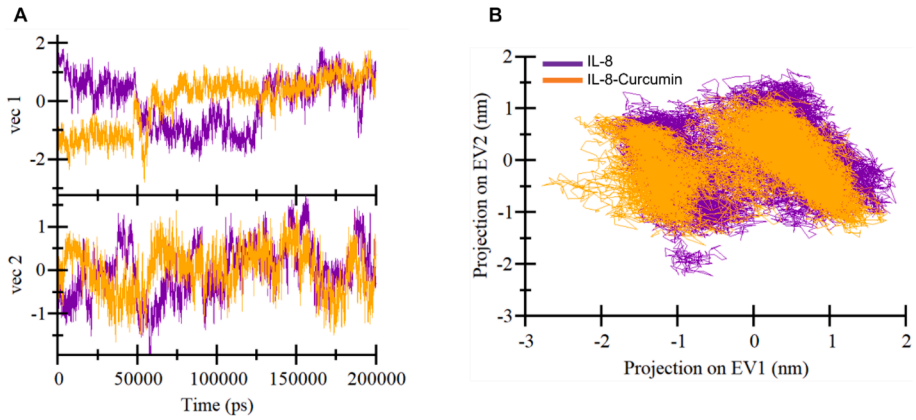

Fig. 6. PCA evaluation. (A) 2D presentations of trajectory on eigenvectors revealed distinct projections of IL-8 over curcumin binding. (B) Time projections of trajectory on eigenvectors. The violet color represents free IL-8 values, while the orange color represents IL-8 and curcumin projection. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

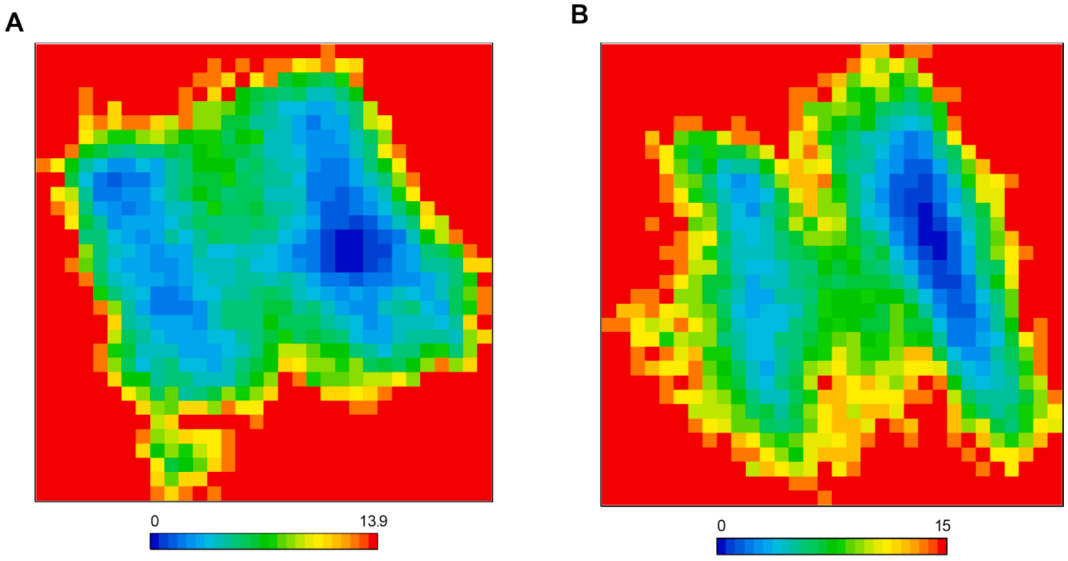

Fig. 7. The picture depicts energy maps of the systems (A) IL-8, (B) IL-8-Curcumin Complex.

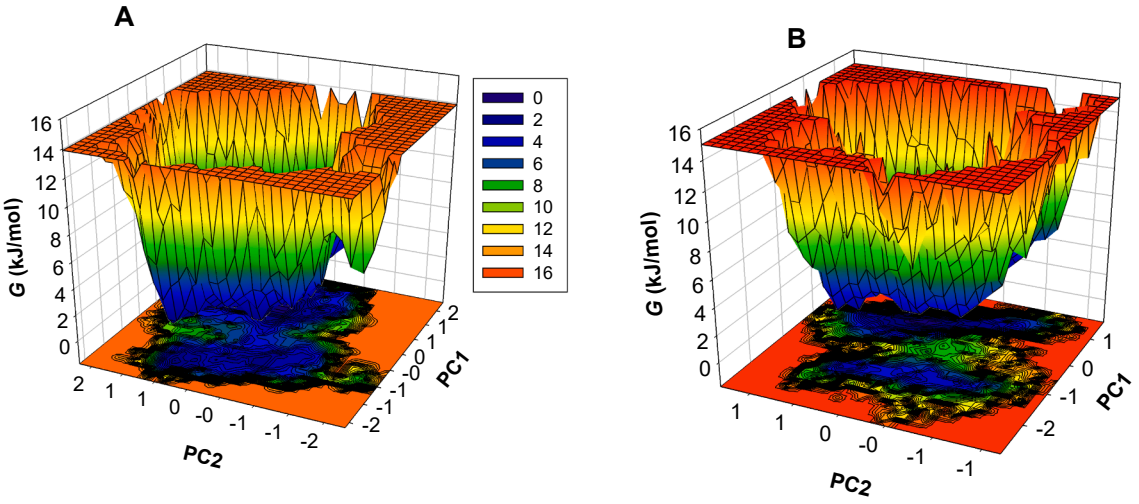

Fig. 8. 3D FEL maps of (A) free IL-8 (B) IL-8-Curcumin complex.

cancer cells. (Jin-Ying Wong et al., 2020; Shehzad et al., 2013). It can inhibit the activation of gene transcription factors like NF-B, which are responsible for the generation of IL-8 (Hidaka et al., 2002). By reducing IL-8 levels, curcumin may help alleviate inflammation and contribute to its therapeutic effects. It is therefore we applied molecular docking and MD simulation studied to study the effect of curcumin on IL-8.

The IL-8 protein was used in the present research to perform in silico medication repurposing against curcumin. The docking procedure is a widely recognized structure-based in silico method used in development of drugs. Docking allows for the discovery of new drugs as well as the molecular prediction of ligand-target relationships. We ran a simulated screen of curcumin compound against the IL-8 protein. The biological activities were determined using SWISS-Admet and the ADMETlab web server.

The protein IL-8 (5D14) was downloaded from RCSB protein database bank and visualised in PyMol (DeLano, 2002) visualizer tool, where it was found that the 3D-structure was complete. The additional ligand entries and waters were removed to vacant IL-8 binding sites.

The small molecule curcumin was downloaded from drug bank (ID-DB11672). It adheres to the Lipinski rule of five. Here InstaDock was used to get the most favourable binding affinities. Curcumin interacted with the IL-8 protein, with energy values −5.4 kcal/mol (Table 1). Curcumin binds with strong affinity showing its binding pockets (Fig. 1). The compound formed important bonds such as H-Bonds with the residues CYS5, CYS7, THR10, CYS32, ASN34, GLU36, and LYS52 (Fig. 1B).

Curcumin's physicochemical and ADMET properties were calculated using online tools like SwissADME and ADMETlab (Table 3). It followed the Lipinski, Ghose, Veber and BMS criteria, and no PAINS warning was generated. Other aspects of the compound exhibit drug-like characteristics. The MW (g/mol) is 368.38, the consensus log P is 3.03, the number of H-Bond acceptors and donors are 6 and 2 respectively, and the TPSA (2) is 93.06. Furthermore, we carried out PASS analysis to ensure the biological features of an elucidated chemical. After having assured with the ADMET and PASS analysis, MD simulation is being performed.

The GROMACS simulation software is utilized for MD stimulations at 300 K. A generalised table of all the parameters of MD-Simulations is provided in Table 4. The observed average potential energy of IL-8 and IL-8-Curcumin complex was calculated to determine system equilibration.

The protein IL-8 alone had an average RMSD value of 0. 293 nm. Ligand binding may result in some changes in the binding pocket of proteins. When IL-8 was complexed with curcumin, the average RMSD value was calculated to be 0.275. The inhibitor IL-8-curcumin deviation was initially smaller than 0.2 nm, but as time passed, the deviation decreased while simultaneously increasing, with the maximum peak seen at 0.4 nm. Overall, we can claim that there is relatively less variation in ligand binding. (Fig. 2**A**). Similarly, the average variance of all residues throughout simulation was observed using RMSF of the IL-8 and post ligand binding. The RMSF plot revealed that residual aberrations occur throughout the IL-8 structure. The RMSF graph shows that there are residual differences in several locations of the IL-8 structure of the protein. These fluctuations were observed to be maximal following curcumin binding. In the end, there was relatively little variation found. The A protein's radius of gyration must be bigger due to the less tight packing (Beg et al., 2019). The average Rg of IL-8 and complexed with curcumin were 1.29. The Rg plot demonstrates that there is no variation in IL-8 packing following ligand binding. (Fig. 3**A and** Fig. 3**C**). According to the SASA plot, there minor variation in total SASA values of IL-8 and complex with curcumin (Fig. 3B). Hydrogen bond analysis was performed for the molecular recognition of protein and its complex, and we discovered that curcumin was strongly attached in the active site of IL-8 with 6H- bonds with little variations. Furthermore, we investigated secondary structure modifications in response to ligand binding and showed that the average amount of residues participation in secondary structure development after binding was slightly enhanced due to an increase in the percentage of β-sheet.

The MD trajectory was utilized to determine the total number of intramolecular H-bonds formed in IL-8 with time. The result enables us to compare the consistency in number of intramolecular hydrogen bond formed with IL-8 and when complexed with curcumin. The graph suggests that H-bonds formed by IL-8 in complexes with curcumin were durable and contributed to the protein's structure. During the simulation, the PDF for intramolecular H-bonds demonstrated a significant amount of consistency in all systems. Similarly Intermolecular Hydrogen bonds between IL-8 and curcumin were 3 with higher distribution as PDF shows.

The PCA analysis or essential dynamics (ED) of a protein indicates its overall progression during different simulations (Maisuradze et al., 2009). PCA determines massively average movements of a protein, unveiling the architecture beneath the atomic fluctuations (David and Jacobs, 2014). We employed PCA to analyze the conformational sample of the IL-8, IL-8-curcumin complex using the essential dynamics technique. The conformational sample projected of Ca atoms of the systems in the critical subspace.

In the apo state, the IL-8-curcumin complex occupied the same essential subspace as IL-8. The reduced flexibility of theIL-8-curcumin complex on both EVs, as well as the key subspace on EV1 covered by the IL-8-curcumin complex, support complex stability throughout simulations. The process of protein folding and how it denatures, is described using free energy landscapes. Using MD simulation trajectories, it has been utilized to assess the stability of the systems in the availability of solvent. Using two PCs, we rebuilt the energy minima and conformational basins of the IL-8, IL-8-curcumin complex. Figs. 7 and 8 show that the MD simulation and critical dynamics of IL-8 in combination with curcumin are stable through 200 ns simulations with low structural flipping.

Conclusively, molecular docking was executed to predict inhibition of IL-8 with curcumin for possible drug combination. Further they undergone ADMET and PASS analysis. Finally, curcumin was chosen to run MD simulations. Overall, our results show that during the simulation, the IL-8-curcumin complex stayed stable. The results imply that target-driven therapy and early diagnosis can be used in customized oncology clinical trials to investigate IL-8 and its genetic variants.

CRediT authorship contribution statement

Bader S. Alotaibi: Conceptualization, Methodology, Writing – original draft. **Mohammed Ageeli Hakami:** Data curation, Software, Writing – review & editing. **Ali Hazazi:** Formal analysis, Software, Writing – review & editing. **Ahad Amer Alsaiari:** Formal analysis, Methodology, Writing – review & editing. **Mohammad Khalid:** Software, Visualization. **Anam Beg:** Conceptualization, Data curation, Supervision, Writing – review & editing.

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