In silico docking analysis of Curcumin as a ferroptosis inducer in Triple Negative Breast Cancer (TNBC)

A Dissertation Report submitted

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By

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ANNEXURE 1

CERTIFICATE

This is to certify that this dissertation work entitled **"In silico docking analysis of Curcumin as a ferroptosis inducer in Triple Negative Breast Cancer (TNBC)"** was successfully carried out by **Mr. Chintan Bagariya** towards the partial fulfilment of requirements for the degree of **Master of Science** in **Biotechnology** of Atmiya University, Rajkot. It is an authentic record of his own work, carried out by him under the guidance of **Dr. Anmol Kumar** during the academic year of **2022-2023**. The content of this report, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other University.

Dr. Nutan Prakash V.

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Head of the Department

Dr. Anmol Kumar

Name & Signature:
Supervisor

ANNEXURE 2

DECLARATION

I hereby declare that the work incorporated in the present dissertation report entitled **"In silico docking analysis of Curcumin as a ferroptosis inducer in Triple Negative Breast Cancer (TNBC)"** is my own work and is original. This work (in part or in full) has not been submitted to any University for the award of any Degree or a Diploma.

Date: 07/04/2023

Name and signature of Student

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In silico docking analysis of Curcumin as a ferroptosis inducer in Triple Negative Breast Cancer (TNBC)

Abstract:

Cancer is the leading cause of death worldwide after cardiac disease, with breast cancer being a major contributor to the mortality rate among women. The subtype of breast cancer known as Triple Negative Breast Cancer (TNBC) is particularly aggressive and shows poor prognosis. Due to the heterogeneity of TNBC and poor response to current treatments, there is an urgent need for new targeted therapies that are less toxic and cause minimal side-effects. Regulated cell death (RCDs), especially ferroptosis, have shown promising outcomes in combating cancer. Ferroptosis is a newly discovered iron-dependent non-apoptotic form of programmed cell death characterized by the accumulation of redox-active iron, loss of antioxidant defense, and lipid peroxidation. Phytochemicals, such as curcumin, have also attracted attention from scientists due to their potential in treating TNBC by reducing the risk of recurrence and drug resistance. Curcumin has been found to possess anti-cancer properties by interfering with various oncogenic signaling pathways and stimulating ferroptosis through solute carrier family 1 member 5 (SLC1A5) mediation. In this study, an in-silico analysis was conducted on curcumin and its potential interaction with various target proteins involved in ferroptosis. Molecular docking revealed its potential binding energy with specific targets, indicating its usefulness in the treatment of TNBC. However, further validation through in vitro studies is required.

Keyword: TNBC, RCDs, Ferroptosis, Curcumin**,** Molecular Docking

1. Introduction:

Every year 19 million peoples are diagnosed with Cancer and approximately 9.9 million peoples die due to cancer as per GLOBOCAN 2020 Statistics. Thus, Cancer is leading cause of death worldwide after cardiac disease. In 2020, around 2.26 million people diagnosed with breast cancer and 6.84 lakhs people die due to this. Breast cancer is the principal cause of mortality in women according to statistics[1]. Even in India, total number of new breast cancer cases were around 1.78 lakhs amongst them 90,000 people die due to this in 2020[2]. According to the National Cancer Registry programme 2021, Breast cancer accounts 25.4 % relative to all sites of cancer in women[3].

The major subtypes of breast cancer are Luminal A, Luminal B, HER2 Positive and TNBC (Triple Negative Breast Cancer). TNBC comprise around 20% of all breast cancer. TNBC is distinguished from the other breast cancer types by the absence of Estrogen Receptor(ER), Progesterone Receptor(PR) and Human epidermal growth factor receptor 2 Receptor (HER2)[4]. TNBC is highly metastatic, more aggressive, and shows a poor prognosis[5]. The mortality rate of TNBC is higher than any other subtype of breast cancer. The approximate survival rate of TNBC is studied to be 5 years after the diagnosis[6].

TNBC is further subdivided into six molecular subtypes: mesenchymal (M), basal-like (BL1 and BL2), mesenchymal stem-like (MSL), luminal androgen receptor (LAR) and immunomodulatory (IM), as well as an unspecified group (UNS) this diversity in markers makes TNBC a more heterogeneous type of cancer[7].

Certain endocrine therapy can suppress tumor growth in normal breast cancer types but due to absence of hormonal receptors conventional hormone therapies are not efficient. Combined chemotherapy treatment with taxanes or anthracyclines exhibits good tumor suppression rates but also carried out tumor recurrence 5 years after therapy [8]. These traditional treatment options work to some extent but drug toxicity, resistance to drugs, tumor recurrence and multiple side effects remain major drawbacks[8].

Considering the heterogeneity of disease there is no single standard therapy available for treatment. Thus, due to heterogeneity and poor response to current chemotherapies, there is an urgent need to develop new targeted combinational therapy which is less toxic, causes minimum side effects and reduces tumor recurrence.

Regulated cell death (RCDs) are being studied to treat various types of cancer like apoptosis, necroptosis, pyroptosis and ferroptosis[9]. Among these, ferroptosis is a special variety of RCD that inspired many researchers to study it to combat cancer and produced promising outcomes.

Ferroptosis is a newly discovered unique type of iron-dependent non–apoptotic form of programmed cell death[10]. This ferroptosis differs from other forms of RCD at their genetic, biochemical and morphological levels. It ruptures the outer membrane of mitochondria and reduced the number of cristae by altering the cytosolic redox equilibrium. The biochemical hallmarks of ferroptosis are Accumulation of redox-active iron, loss of antioxidant defence and lipid peroxidation[11].

The ferroptosis mechanism involved the activation of ROS-forming components and inhibition of antioxidant defence (ferroptosis defence mechanism). Accumulation of iron and ROS induce a Fenton reaction that further leads to lipid peroxidation. Another mechanism that regulates ferroptosis is System Xc- antiporter (cystine/glutamate antiporter). System Xc- involved in the uptake of cystine and further formation of Glutathione (GSH: a cofactor of GPX4, is synthesized from glutamate, glycine, and cysteine). GPX4 (Glutathione Peroxidase 4) inhibit ferroptosis by preventing lipid peroxidation by reducing the lipid peroxides to lipid alcohols.

Thus, Inhibitors of System Xc- antiporter and GPX4 can lead to ferroptosis in tumor cells. So, to develop therapies against cancer we can target the pathways that are involved in ferroptosis regulation. According to several studies, we can promote ferroptosis in breast cancer by targeting the system Xc- antiport[12][13].

Phytochemicals, which are plant-based secondary metabolites, have garnered considerable attention from scientists due to their potential in treating Triple-Negative Breast Cancer (TNBC) and reducing the risk of recurrence as well as drug resistance[14].

Curcumin is a plant based secondary metabolite can be isolated from rhizome of *Curcuma longa L.* (Plant of curcuminoids family). Curcumin is also known as diferuloylmethane[15].

According to recent research, curcumin has been found to possess anti-cancer properties due to its impact on numerous biological pathways that are essential in mutagenesis, oncogene expression, tumorigenesis, cell cycle regulation, apoptosis, and metastasis[16][17]. Several anticancer activities have been observed in cancer cell lines, including prostate, breast, and colon cancer cells, when exposed to curcumin derivatives[18][19].

Curcumin has been shown to have potential in interfering with various oncogenic signalling pathways (i.e., NF-κB, Akt, and mTOR Pathways), which can lead to the regulation of several important cellular processes such as cell survival and proliferation, angiogenesis, metastasis, cancer stem cells (i.e., Wnt/β-catenin signalling pathway), & regulated cell death (Apoptosis). This makes curcumin a promising candidate for various types of breast cancers, including triple-negative breast cancer (TNBC)[20].

Recent study also shown that, Curcumin has the ability to impede breast cancer cell survival by stimulating ferroptosis through solute carrier family 1 member 5 (SLC1A5) mediation and intensifying reactive oxygen species levels, MDA accumulation, and intracellular Fe2+ levels[21].

The exact mechanism by which curcumin promotes ferroptosis and its binding locations are currently in the study.

In our study, we conducted an *in-silico* analysis of Curcumin and its potential interaction with various target proteins and macromolecules involved in the signalling pathway of ferroptosis. By performing molecular docking, we were able to calculate the binding energy of the ligand with its target protein in different conformations. Based on this data, we hypothesized that Curcumin may bind to a specific target and induce or inhibit certain cascades, potentially useful in the treatment of TNBC.

However, further validation of these in silico findings is required through in vitro studies.

2. Tools & Resources:

2.1 PubChem

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information. Curcumin structure downloaded from this site for molecular docking. **(URL: https://pubchem.ncbi.nlm.nih.gov)**

2.2 RCSB PDB

The RCSB Protein Data Bank is a database for the three-dimensional structural data of macromolecules, such as proteins and nucleic acids. Structure of proteins/macromolecules were retrieved from RCSB PDB for docking analysis. **(URL: https://www.rcsb.org)**

2.3 AutoDock Vina

AutoDock Vina is an open-source program for doing molecular docking. It was designed and implemented by Dr. Oleg Trott in the Molecular Graphics Lab at The Scripps Research Institute. (**URL: http://vina.scripps.edu)**

2.4 PyRx

PyRx is essentially a Graphical User Interface that uses a large body of established open-source software like AutoDock 4 and AutoDock Vina. It is a virtual Screening tool for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. For this study PyRx 0.8 version is used. **(URL: https://pyrx.sourceforge.io)**

2.5 PyMOL

PyMOL is an open-source molecular visualization system created by Warren Lyford Delano. It is maintained and distributed by Schrödinger, Inc. PyMOL can produce 3D images of small molecules and biological macromolecules, such as proteins. For this study PyMOL 2.4.2 version is used. **(URL: https://pymol.org)**

3. Methodology:

3.1 Selection of Target molecules:

Molecules for the docking were identify from the ferroptosis pathway regulation involved in TNBC. In this study, we have selected Curcumin as a ligand [Figure 1]. PDB id for each target used in the study is mentioned with protein name in **Table 1.** Following is the selection of molecules, classified based on their role in respective pathway…

(A) System Xc: In Triple Negative Breast Cancer (TNBC), System Xc has been found to play an important role in maintaining redox homeostasis and supporting tumor growth[22]. System Xc structure was determined by Electron Microscopy with the Resolution: 6.20 Å by respective authors[23].

(B) FSP1: Ferroptosis is controlled by FSP1 in TNBC cells. According to the research, FSP1 was discovered to be significantly expressed in TNBC tumors, and its suppression led to an increase in lipid peroxidation and a reduction in tumour growth[24]. Structure was downloaded from Alpha fold Database[25].

(C) NCOA4: According to one study, NCOA4 knockdown increased cellular sensitivity to ferroptosis while NCOA4 expression was found to be inversely linked with the activation of ferroptosis in TNBC cells[26]. Structure was downloaded from Alpha fold Database.

(D) NOX4: Ferroptosis can be encouraged in cancer cells, particularly TNBC, via NOX4-mediated ROS generation. Recent study showed that NOX4 inhibition increases lipid peroxidation and sensitises TNBC cells to ferroptosis by inhibiting GPX4 activity[27]. Structure was downloaded from Alpha fold Database.

3.2 Molecular Docking:

AutoDock Vina is the program used as a docking tool for this study. Vina was plugged into the PyRx. Steps followed for Docking are...

a. At first, Curcumin was energy minimized in Open Babel using PyRx.
b. Then each molecule is loaded to the AutoDock macromolecules tab.
c. Then particular macromolecule is selected along with a ligand.
d. Then Exhaustiveness of AutoDock Vina is set to 8 and Dimensions for Vina Search Space were maximized and the Vina program was executed.

Results were saved for each molecule in .csv format along with the snapshots of conformations with the least binding affinity (in kcal/mol).

3.3 Observation of Molecular Interactions:

PyMOL is used to observe the molecular interactions between ligand and target molecules. In this study, we have mostly focused on polar interactions of molecules. Docked structures were loaded into PyMOL and then polar bonds were identified. Ray-Traced transparent images were exported representing these interactions.

4. Results:

In this section results obtained in our study are represented including the images representing binding affinities of ligand and target molecules, their binding poses at minimum binding affinities.

(A) FSP1: FSP1 represented a minimum binding affinity of -9 kcal/mol with Curcumin. Various conformations with their binding affinities are mentioned in Table 2. The binding pose with the minimum binding affinity is also mentioned in Figure (2).

(B) System Xc: System Xc represented a minimum binding affinity of -7.6 kcal/mol with Curcumin. Various conformations with their binding affinities are mentioned in Table 3. The binding pose with the minimum binding affinity is also mentioned in Figure (3).

(C) NCOA4: NCOA4 represented a minimum binding affinity of -6.7 kcal/mol with Curcumin. Various conformations with their binding affinities are mentioned in Table 4. The binding pose with the minimum binding affinity is also mentioned in Figure (4).

(D) NOX4: NOX4 represented a minimum binding affinity of -5.5 kcal/mol with Curcumin. Various conformations with their binding affinities are mentioned in Table 5. The binding pose with the minimum binding affinity is also mentioned in Figure (5).

5. Discussion:

In this study, FSP1 has shown minimum binding affinity -9 kcal/mol and NOX4 showed maximum binding affinity. FSP1 prevent the ferroptosis in the cell and inhibiting it by Curcumin can be induced the ferroptosis[28]. System Xc also prevent the ferroptosis by GPX4 pathways by inhibiting the System Xc we can induce the ferroptosis[29]. Similarly, NCAO4 and NOX4 is negative regulator of ferroptosis by targeting with curcumin we can induce the ferroptosis in TNBC.

6. Conclusion:

The study identifies FSP1 and System Xc as two pathways that prevent ferroptosis in cells. By inhibiting these pathways with curcumin, the researchers were able to induce ferroptosis, which could potentially be a novel approach for treating TNBC. The study also highlights the role of NCAO4 and NOX4 as negative regulators of ferroptosis. These proteins showed a lower binding affinity compared to FSP1, indicating their potential as targets for ferroptosis induction. Targeting these proteins with curcumin could provide a mechanism for inducing ferroptosis in TNBC. As this is an in-silico study, we need to test Curcumin's actual effect by performing experiment on various cell lines for the validation. This study shows us a good impression to move ahead and check the effects of Curcumin as ferroptosis inducer in-vitro.

7. Supplementary Material:

7.1 Figures:

Figure 1. Curcumin structure: PubChem

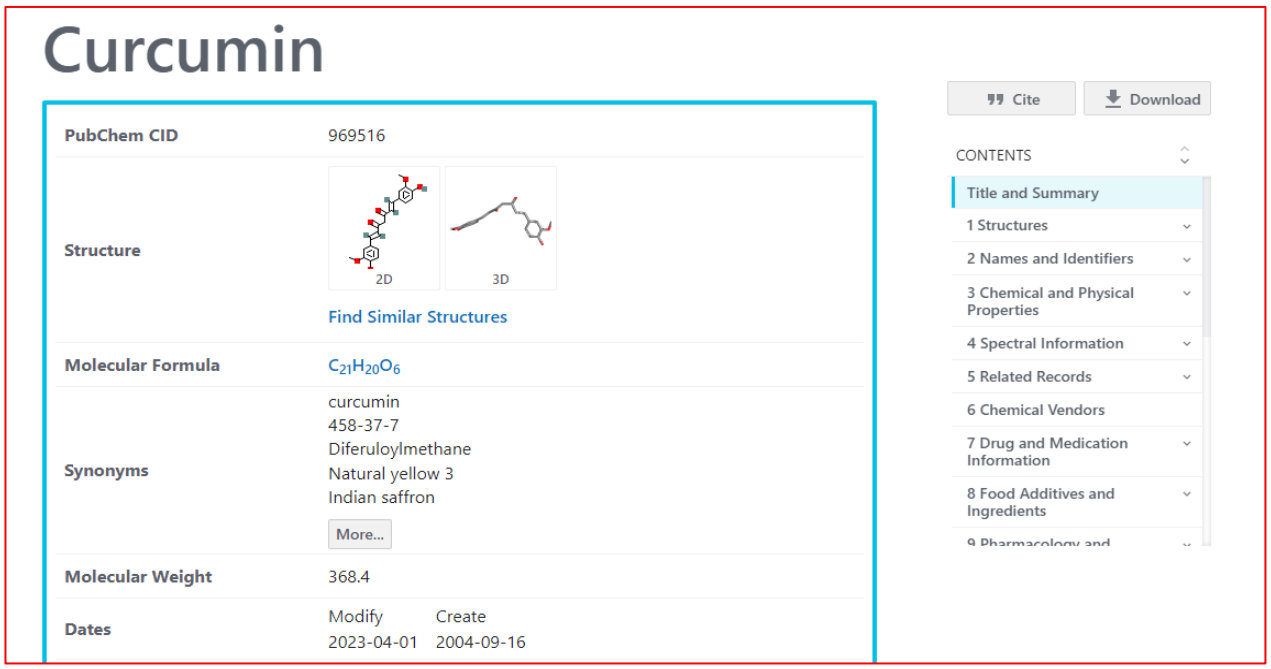
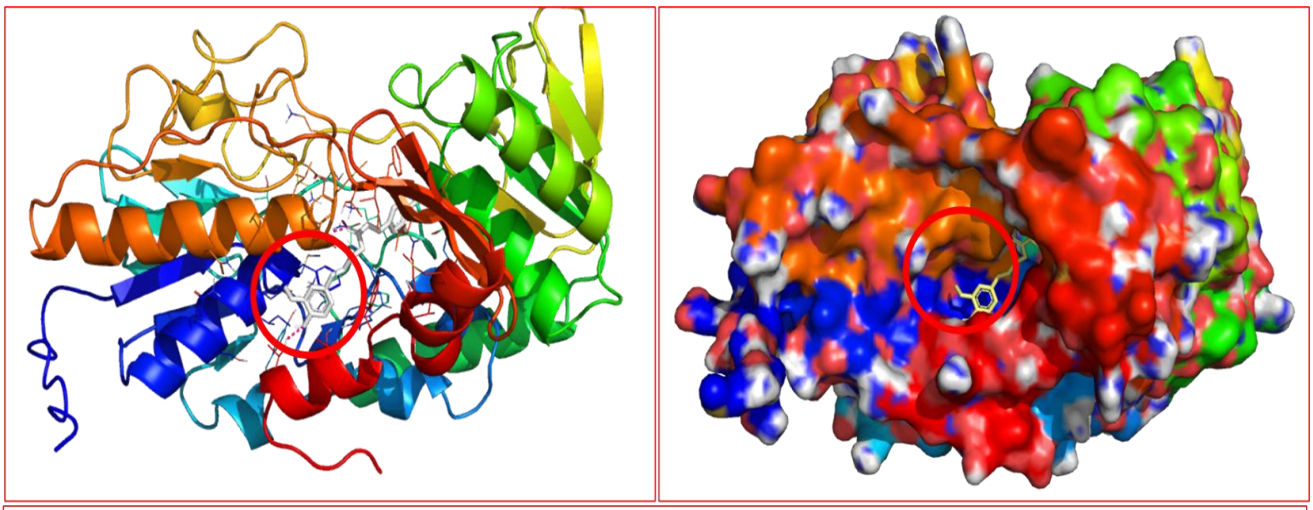
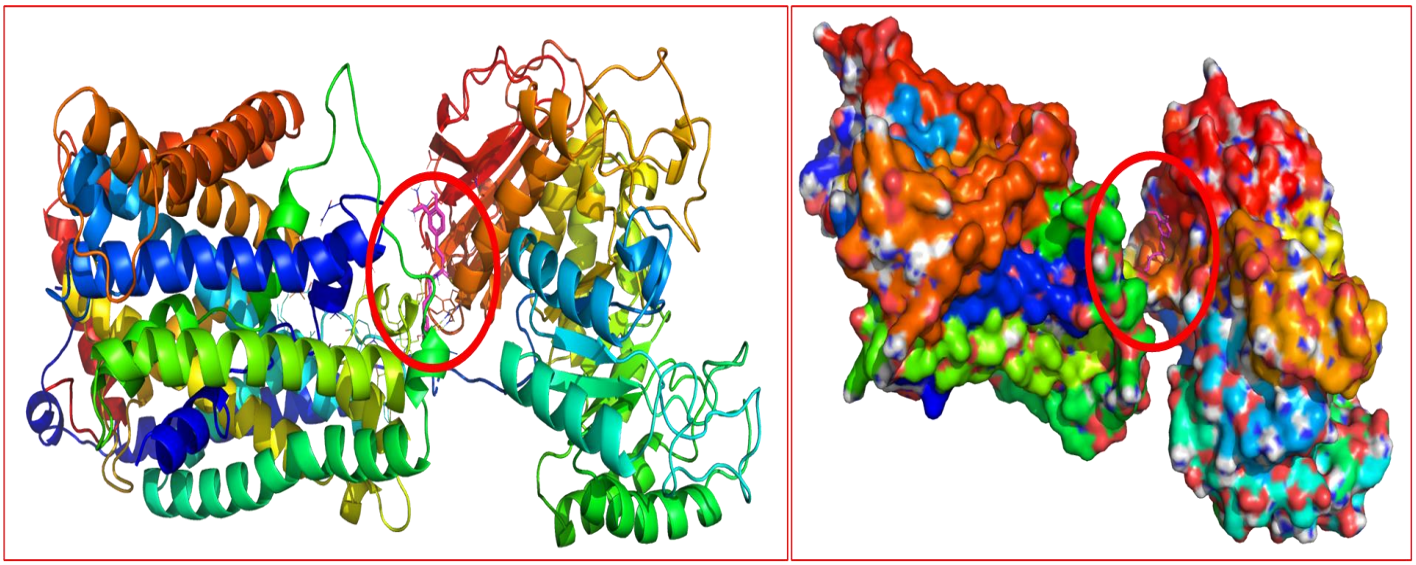


Figure 2. FSP1 binding pose:



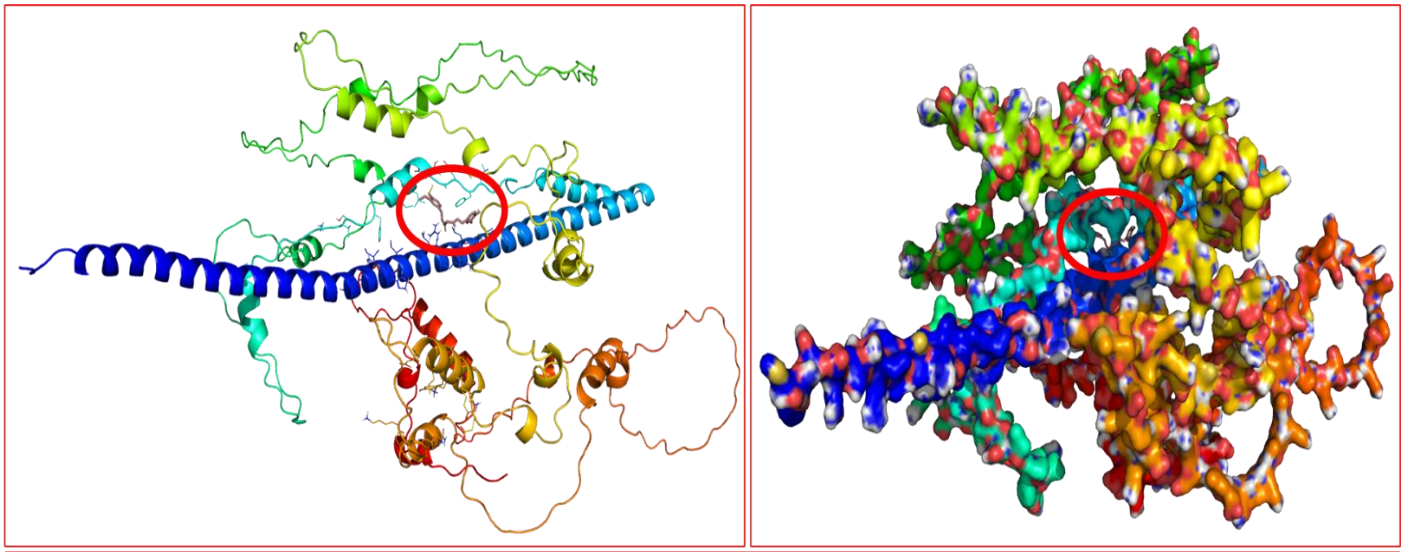
(A) Snapshot of binding pose of Curcumin with FSP1 taken in PyRx. Red ring represents the location of binding.
(B) Ray-traced image of Binding pose of Curcumin with FSP1, rendered using PyMOL. Red ring represents the location of binding.

Figure 3. System Xc binding pose:



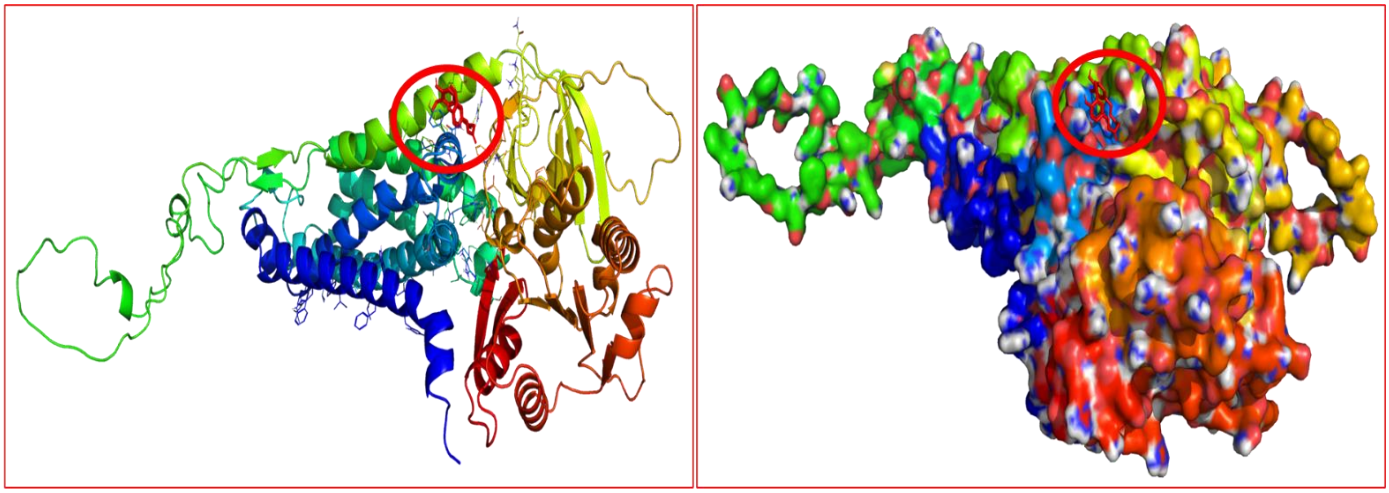
(A) Snapshot of binding pose of Curcumin with System Xc taken in PyRx. Red ring represents the location of binding.
(B) Ray-traced image of Binding pose of Curcumin with System Xc, rendered using PyMOL. Red ring represents the location of binding.

Figure 4. NCAO4 binding pose:



(A) Snapshot of binding pose of Curcumin with NCAO4 taken in PyRx. Red ring represents the location of binding.
(B) Ray-traced image of Binding pose of Curcumin with NCAO4, rendered using PyMOL. Red ring represents the location of binding.

Figure 5. NOX4 binding pose:



(A) Snapshot of binding pose of Curcumin with NOX4 taken in PyRx. Red ring represents the location of binding.
(B) Ray-traced image of Binding pose of Curcumin with NOX4, rendered using PyMOL. Red ring represents the location of binding.

7.2 Tables:

Table 1: Proteins focused in this study with their PDB/Alpha Fold IDs

Target Protein	PDB/Alpha Fold IDs
FSP1	**Q9BRQ8**
System Xc	**7CCS**
NCAO4	**Q13772**
NOX4	**Q9NPH5**

Table 2: Binding Affinities of FSP1 with Curcumin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
FSP1_model1_969516_uff_E=272.07	-9	0	0
FSP1_model1_969516_uff_E=272.07	-8.7	8.718	7.389
FSP1_model1_969516_uff_E=272.07	-8.7	10.616	7.422
FSP1_model1_969516_uff_E=272.07	-8.5	9.913	1.489
FSP1_model1_969516_uff_E=272.07	-8.5	10.025	4.12
FSP1_model1_969516_uff_E=272.07	-8.5	11.771	3.03
FSP1_model1_969516_uff_E=272.07	-8.4	5.764	3.009
FSP1_model1_969516_uff_E=272.07	-8.4	8.598	3.917
FSP1_model1_969516_uff_E=272.07	-8.4	8.076	3.871

Table 3: Binding Affinities of System Xc with Curcumin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
7ccs_969516_uff_E=272.07	-7.6	0	0
7ccs_969516_uff_E=272.07	-7.6	2.277	1.739
7ccs_969516_uff_E=272.07	-7.6	4.764	3.399
7ccs_969516_uff_E=272.07	-7.5	18.432	15.598
7ccs_969516_uff_E=272.07	-7.5	2.531	1.782
7ccs_969516_uff_E=272.07	-7.2	9.209	3.371
7ccs_969516_uff_E=272.07	-7.2	11.266	4.5
7ccs_969516_uff_E=272.07	-7.2	5.406	3.676
7ccs_969516_uff_E=272.07	-7.1	8.863	3.354

Table 4: Binding Affinities of NCAO4 with Curcumin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-6.7	0	0
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-6.4	2.913	1.533
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-6	49.537	45.635
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-6	2.959	2.436
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-5.9	6.822	4.147
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-5.8	8.799	1.715
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-5.7	49.427	45.453
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-5.5	22.974	18.776
AF-Q13772-F1-model_v4_model1_969516_uff_E=272.07	-5.4	49.614	45.823

Table 5: Binding Affinities of NOX4 with Curcumin

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.5	0	0
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.5	15.378	12.111
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.5	30.326	27.371
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.5	5.231	3.049
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.4	5.776	3.851
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.3	33.805	31.188
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.2	38.076	34.365
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.2	41.385	37.714
AF-Q9NPH5-F1-model_v4_model1_969516_uff_E=272.07	-5.2	16.888	13.774

7.3 Abbreviations:

AKT: Protein Kinase B, Serine/Threonine-specific protein kinase

ER: Estrogen Receptor

FSP1: Ferroptosis Suppressor protein 1

GPX4: Glutathione Peroxidase 4

GSH: Glutathione

HER2: Human Epidermal Growth Factor Receptor

MDA: Malondialdehyde

mTOR: Mammalian target of Rapamycin /Mechanistic target of Rapamycin

NCOA4: Nuclear receptor Coactivator 4

NF-κB: Nuclear factor kappa B

NOX4: NADPH oxidase 4

ROS: Reactive Oxygen Spices

SLC1A5: Solute Carrier family 1 member 5

System Xc-: Cystine/Glutamate antiporter

TNBC: Triple Negative Breast Cancer

Wnt: Wingless-related integration site, “Int/Wingless” Family

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