# *IN SILICO* EXPLORATION FOR REPURPOSING BREAST CANCER MEDICATIONS IN SLEEP AND NEURODEVELOPMENTAL DISORDERS

A DISSERTATION

SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE

IN

**BIOTECHNOLOGY**

SUBMITTED BY:

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**M.Sc. (Biotechnology)**

**[ TWINKLE]**

**2023**

## CANDIDATE'S DECLARATION

I, Twinkle, 2K21/MSCBIO/57, of MSc. Biotechnology, hereby declare that the project Dissertation titled **"*In Silico* Exploration for Repurposing Breast Cancer Medications in Sleep and Neurodevelopmental Disorders"** which I have submitted to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science is original and not plagiarized from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship, or other similar title or recognition.

Place: Delhi

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Date: 02-06-2023

## SUPERVISOR CERTIFICATE

I hereby certify that the Project Dissertation titled "***In Silico* Exploration for Repurposing Breast Cancer Medications in Sleep and Neurodevelopmental Disorders"**, which is submitted by Twinkle, 2K21/MSCBIO/57, Delhi Technological University Delhi, in partial fulfillment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

02/06/2023

Place: Delhi

Date: 02-06-2023

**Prof. Pravir Kumar**

**Professor, HOD, and Guide**

Department of Biotechnology

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# *In Silico* Exploration for Repurposing Breast Cancer Medications in Sleep and Neurodevelopmental Disorders


## ABSTRACT

Sleep is crucial neurobiological behavior that serves a significant part in maintaining brain homeostasis and functioning. It is controlled by two main mechanisms: Homeostatic regulation, which involves the accumulation and dissipation of sleep pressure based on prior wakefulness, and Circadian regulation, which follows a 24-hour cycle synchronized with environmental cues. This article employs bioinformatics software and utilizes databases commonly used in computer science and biology to investigate the potential interaction between CRY1, CLOCK, and PER1/PER2 in drug binding. CRY1 (Cryptochrome 1) and PER1/PER2 (Period 1 and Period 2) are genes involved in the molecular circadian clock. Understanding their interaction and potential drug binding can provide valuable insights into the molecular mechanisms underlying sleep regulation.

The bioinformatics software and databases aid in predicting and analyzing the structural and functional aspects of CRY1, CLOCK, and PER1/PER2 interactions. By utilizing computational approaches, this research aims to uncover potential binding sites, identify key residues involved in the interaction, and predict the binding affinity between drugs and these proteins. By exploring the drug-binding potential of CRY1, CLOCK, and PER1/PER2, this study seeks to contribute to understanding the intricate molecular processes underlying sleep regulation. The findings have the potential to inform the development of targeted therapeutics and interventions aimed at modulating sleep patterns and addressing sleep-related disorders.

Cyto Hubba, Bio via, Open babel, Drug bank, Avogadro, Auto dock, PyRx, and Protein-protein interaction clustering String are several computational techniques implemented in the investigation. The CRY1/CRY2 cytochrome genes are crucial for the circadian rhythm. CRY1 is a stronger repressor of clock: BMAL1 as compared to CRY2. It has been recently discovered CRY1 by creating a transcriptional inhibitor enhances its repressive function that results from the lengthening of Circadian rhythm in humans. The Association of PER2 with CRY2 forms a stable complex with CLOCK: BMAL1 whereas in the case of CRY1, there is no need for PER1/PER2 to bind with CLOCK: BMAL1. Methylation in PER1/PER2 promoter is one of the variables that increase the chance of breast cancer. Trastuzumab is used for the treatment of breast cancer by repairing cell division and repair. The present investigation used Molecular Docking of Trastuzumab as a control to analyze the interaction between CRY1 and PER1/PER2 applying Cyto Hubba as well as PPI clustering relationships or other breast cancer FDA-approved drugs i.e., (Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib Tosylate) and Circadian clock protein complex. Exemestane can be seen as a possible medication to treat sleep disturbances because of its highest binding energy as compared to others. The results suggest that Exemestane and Circadian clock protein complex laboratory trials determine its inhibitory potential on CLOCK to minimize sleep problems.

***Keywords:*** *Sleep disorder, Circadian Clock Protein, Trastuzumab, Auto dock, Open Babel, PyRx, Cyto Hubba, Bio via, SWISS ADME, STRING database*

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## LIST OF ABBREVIATIONS

| S.NO. | ABBREVIATION | FULL FORM |
|---|---|---|
| 1. | AD | Alzheimer's disease |
| 2. | PD | Parkinson's disease |
| 3. | OSA | Obstructive sleep apnea |
| 4. | REM | Vigorous/Rapid Eye Movement |
| 5. | NREM | Non-Rapid Eye Movement |
| 6. | PLMD | Protein Lysine Modification Database |
| 7. | ADME | Absorption, Digestion, Metabolism, and Excretion |
| 8. | SD | Sleep Disorder |
| 9. | EDS | Excessive Daily Sleep |
| 10. | PER | Period gene |
| 11. | CRY | Cytochrome gene |
| 12. | Hz | Hertz |
| 13. | FDA | Food and Drug Administration |
| 14. | PPI | Protein-Protein Interaction |
| 15. | GEO | Gene Expression Omnibus |
| 16. | CLOCK | Circadian Locomotor Output Cycles Kaput |
| 17. | ARNTL | Aryl Hydrocarbon Receptor Nuclear Translocator-like Protein 1 |
| 18. | BMAL | Basic Helix Loop Helix ARNT Like 1 |
| 19. | RBD | REM Sleep Behavior Disorder |
| 20. | BRCA | Breast Cancer Gene |
| 21. | FBXL | F-Box and Leucine-Rich Repeat Protein 3 |
| 22. | IQR | Inter Quartile Range |

# CHAPTER – 1

# INTRODUCTION

## 1.1 BACKGROUND

Excessive daily sleepiness (EDS) is a sign of many neurological disorders, which constitute prevalent chronic brain diseases that largely affect the elderly [1]. Additionally, it has been proven that main sleeping conditions such as insomnia, obstructive sleep apnea (OSA), and REM sleep behavior disorder (RBD) are origins or risk factors for several dementias. Sleep disturbances have been linked to the onset of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and stroke.

During REM and NREM, electrooculography shows alternating rapid and slow rolling movements [2]. N1 (mental fogginess), N2 (low sleep), and N3 (deep sleep) are the three distinct NREM phases [3].

| **Band of frequencies** | **Frequency** | **State of mind** |
|---|---|---|
| **Gamma($\gamma$)** | >35Hz | Problem-solving, attentiveness |
| **Beta($\beta$)** | 12-35Hz | Busy, Active mind, Nervousness |
| **Alpha ($\alpha$)** | 8-12Hz | Passive attention, Reflective, Restful |
| **Theta($\theta$)** | 4-8Hz | Deeply at ease and inwardly directed, Drowsiness |
| **Delta($\delta$)** | 0.5-4Hz | Sleep, Dreaming |

Table I: Types of Brain Waves and Related Characteristics

The effects of SD on cellular egress prevent the removal of ubiquitinated neurotoxic proteins including -synuclein, amyloid, and tau, which are crucial in major neurological illnesses like Parkinson's and early-onset, onset dementia. The harmful RBD (REM sleep behavior disorder) can make the symptoms of many neurological and neuropsychiatric conditions worse. The effects of SD on the immune system and redox system include the development of astrocytes and a state of oxidative stress. Adrian and Mathews established the name "ALPHA RHYTHM" in 1934 and established the idea of "Human Brain Waves" by confirming regular oscillations at a frequency of 10–12Hz [12]. Every person has a different set of brain wave patterns. The neuro-hormonal changes brought on by behavioral sleep deprivation have been linked to the possibility of developing insulin resistance and potentially, diabetic mellitus [13].

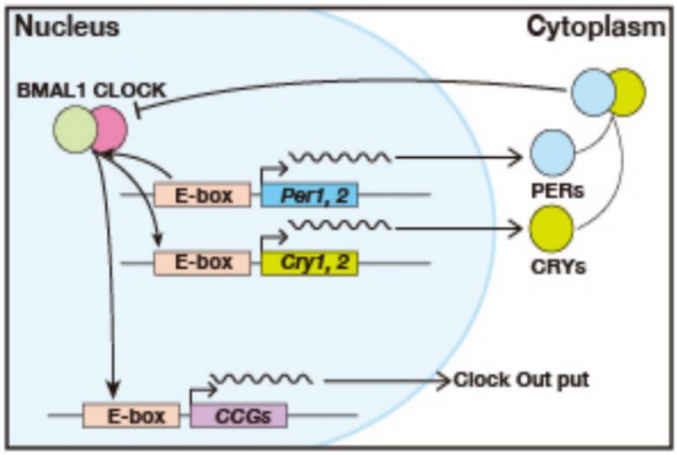


**Figure 1.1:** Mechanism of protein in sleep disorder.

The BMAL1/CLOCK duplex binds to the regulatory boxes (E-box) of the Period (Per1 and Per2) and Cryptochrome (Cry1 and Cry2) genes, favorably regulating the transcription of these genes. The generated and translated PER and CRY proteins also come together to create a heterodimer, go to the nucleus, and negatively regulate the activity of the BMAL1/CLOCK intricate, resulting in a feedback loop [10]. It is well known that PER and CRY belong to different families of factors [11]. Even if one gene stops functioning, other genes in the identical gene group can make up for the lost function that results.

This work merges bioinformatics and pharmacology to evaluate the pharmacological linkages between CRY1 and PER1/PER2 using quantitative databases and methods.

Multiple computational approaches were implemented in this investigation, including Cyto Hubba, PYMOL, Open Babel, Drug Bank, Avogadro, Auto dock, and Protein-protein interaction clustering String. The CRY1/CRY2 cytochrome genes are required for the circadian rhythm [4]. In comparison to CRY2, CRY1 is a stronger clock suppressor than BMAL1[5]. A transcriptional inhibitor was recently revealed to boost CRY1's repressive effect, which lengthens the Circadian rhythm in humans [6]. In contrast to CRY1, where PER1/PER2 do not need to bind with CLOCK: BMAL1, the association of PER2 with CRY2 results in a stable complex with CLOCK: BMAL1 [7]. Compared to the surrounding non-cancerous cells, the majority (95%) of breast malignant cells exhibit altered PER gene expression patterns [8].

Trastuzumab is utilized as a control module, and it docks via an annoying process called Auto Dock. Second, it docked just one at once, and third, it takes too long to get results.

Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib Tosylate are some examples of FDA-approved drugs that are used as the ligand for CLOCK after it was discovered through the use of the PPI string that CRY1/2, PER1/2, and CLOCK interact. These were discovered to produce better outcomes than Trastuzumab using PyRx multiple docking software.

The samples are processed in the same way; the only distinction is that PyRx is used in this instance rather than Auto Dock.

## 1.2 A METHODOLOGICAL PROCESS USING BIOINFORMATICS AND COMPUTATION TO CREATE A LINK

First, information is gathered using the publically accessible RNA-sequenced dataset used in this study, which is supplied via the NCBI Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The data text comprised whole tumor biopsies that were extracted from individuals who were split into several groups based on their stage of cancer. Samples from healthy individuals and samples from tissue were included in these categories. The gene that is most impacted by the pathway—whether it is upregulated or downregulated—is chosen for the graph-based based

solely on the data from that source. PyRx is then used to finish the docking once the gene or medication has been taken.

The entire study is concerned with the establishment of the links between CRY1, CRY2, PER1, PER2, and CLOCK proteins using bioinformatics databases and software. "PyRx" is the main tool used for molecular docking. This study's main components are the Auto Dock and PyRx utilities. The technique generates high-affinity target and ligand binding structures, then uses grid creation to calculate the CHARMM energies of these structures. Then, the clusters with the proper energies are chosen, and the most preferred structures are chosen for assessment.

Additionally, data mining for the alleged interactions among CRY1, CRY2, PER1, PER2, and Clock has been done using PLMD. A database of information that can anticipate ADME variables has been created utilizing a variety of in-silico approaches in addition to PLMD and Swiss ADME, an online application.

### 1.3 Goals of the Project:

- Using NCBI GEO find out the list of upregulated and downregulated genes.
- Cross talk between PER1, PER2, and CLOCK of both sleep disorder and breast cancer.
- Protein-protein interaction by using PPI string for PER1, PER2, CLOCK, ARNTL, CRY1.
- Figuring out the CLOCK-interacting ligands that contribute to its mitigation.
  The molecular docking of the breast cancer medications Dexamethasone, Cycloheximide, Azane (cisplatin), Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib Tosylate with CLOCK. ADME analysis of the drugs under study.
- Evaluation of the effectiveness of several breast cancer medications' interactions with CLOCK.

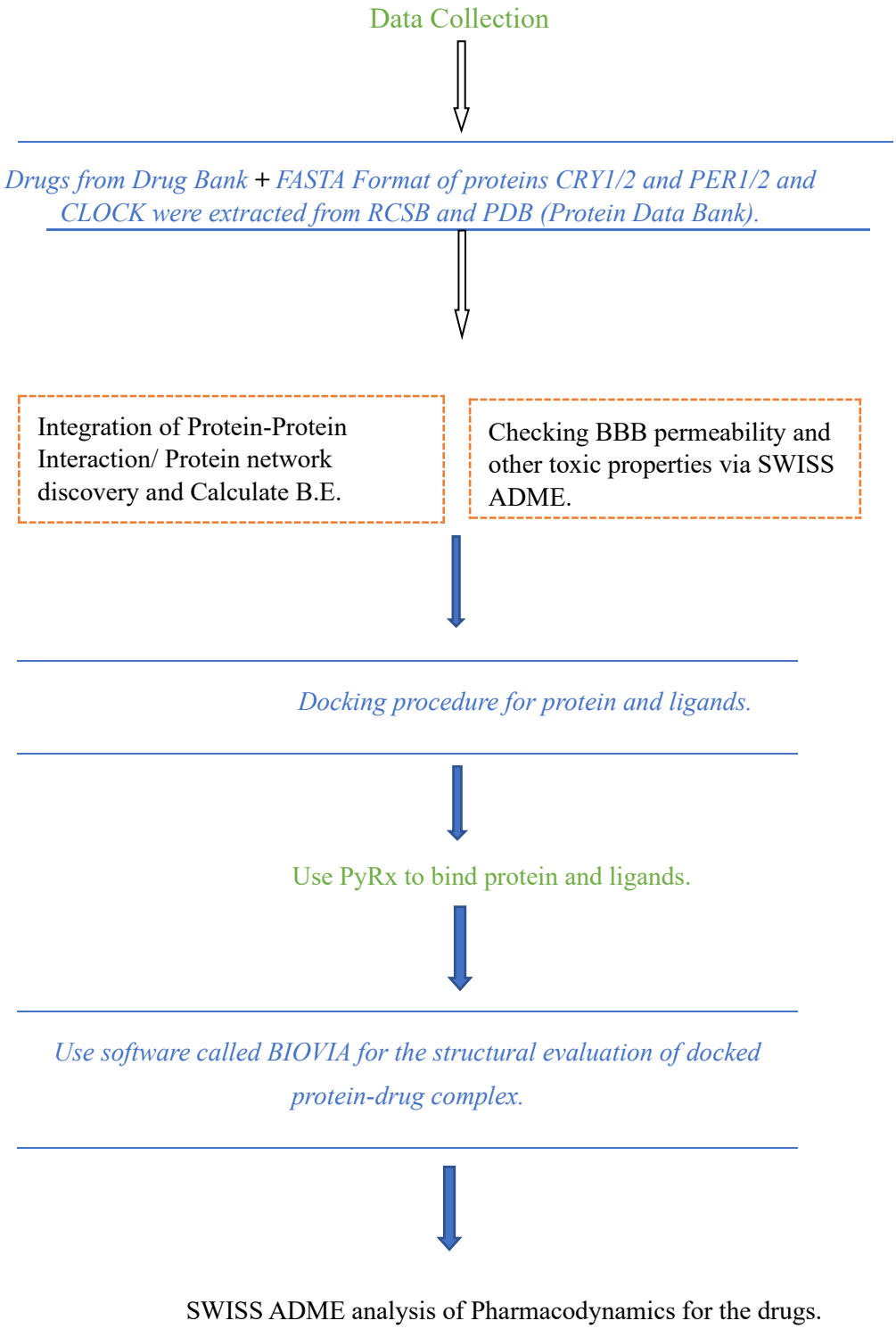


**Figure 1.2:** Flowchart illustrates the approach taken to investigate how the desired protein Clock and breast cancer medications interact.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Breast Cancer Therapies and Sleep Disorders:

Patients with breast cancer frequently experience sleep issues, which can significantly affect how they react to medication and the way their emotions are in general [14]. Drugs used to treat breast cancer, such as hormone treatments, chemotherapeutic medicines, and targeted therapies, have intricate modes of action that might unintentionally alter sleep patterns. For the best patient treatment and the creation of customized therapies, it is essential to comprehend the complex interaction between breast cancer medications and sleep disturbances.

As was discovered, both of them share the protein PER1/2, and the PPI string was used to determine the connection between CRY1/2, PER1/2, and CLOCK, among other proteins [15].

The analysis of the scientific literature indicated a sizable amount of data in favor of the link between breast cancer treatments and sleep problems. Tamoxifen and aromatase inhibitors are two examples of hormonal medications that have a variety of impacts on sleep, mostly mediated through hormonal changes and menopausal symptoms [17]. Chemotherapy agents, such as dexamethasone, cycloheximide, Azane (cisplatin), trichloro platin, anastrozole, exemestane, Fulvestrant, lapatinib Ditosylate, letrozole, Olaparib, Palbociclib, and Talazoparib tosylate, were linked to fatigue, pain, and psychological distress, which made it difficult to sleep. Furthermore, by altering cellular signaling pathways, targeted medicines have indirect effects on the regulation of sleep [16].

Breast cancer medications and sleep disturbances are linked by intricate connections among cellular processes and variables associated with therapy. Hormone-mediated changes in circadian rhythm control and hypothalamic-pituitary-adrenal axis activity are the mechanisms through which hormonal interventions impact sleep. Sleep patterns may be hampered by oxidative stress and systemic inflammation brought on by chemotherapy. Via their impacts on cellular signaling and digestion, tailored medicines that target certain molecular pathways in breast cancer may have a subtly

negative influence on sleep regulation [18]. The link between breast cancer medications and sleep problems has important therapeutic ramifications [19]. In clinical practice, being aware of potential sleep-related adverse effects is essential for prompt patient

management and better patient outcomes. Conducting thorough sleep evaluations and specialized therapies may improve a patient's quality of life, adherence to therapy, and prognosis in general. To understand the fundamental mechanisms and create focused treatments to reduce sleep disruptions in this group, more investigation is necessary.

For the benefit of doctors and researchers, this review consolidates the existing knowledge on the relationship between breast cancer medications and sleep disturbances [20]. Knowing how breast cancer treatments may affect sleep patterns enables preventive management and better patient care. To improve sleep-related outcomes for breast cancer patients, future research should concentrate on identifying the specific processes involved, creating tailored therapies, and carrying out extensive clinical trials [21].

An aromatase antagonist called exemestane is administered to treat breast cancer that has hormone receptors [22]. It functions by reducing estrogen synthesis, which feeds the development of cancer cells. To lower the chance of cancer resurgence in postmenopausal women, it is frequently administered as adjuvant therapy [23]. For advanced breast cancer, exemestane may potentially be utilized as a first-line therapy. Fatigue, joint discomfort, and hot flushes are possible side effects. Given the higher risk of osteoporosis, routine bone health monitoring is advised. Overall, exemestane is essential in preventing the synthesis of estrogen and stifling the development of hormone receptor-positive breast cancer cells.

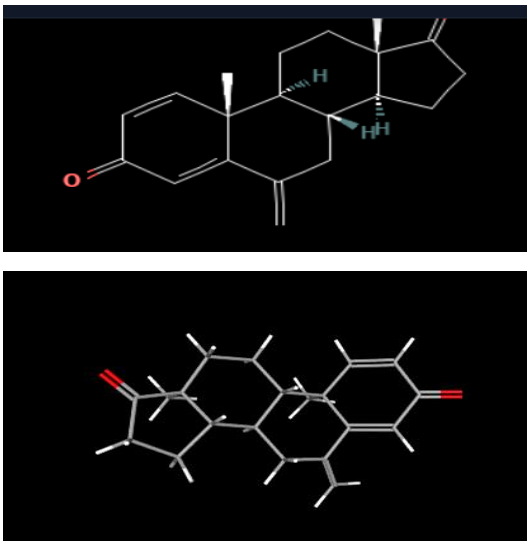


**Figure 2.1:** Structure of Exemestane 2D and 3D respectively using PubChem

A PARP antagonist called Talazoparib is administered to combat metastatic breast cancer that is HER2-negative and has BRCA1/2 mutations [24]. It functions by preventing the PARP enzyme, which aids in cell DNA repair, from functioning. Talazoparib stops cancer cells with BRCA1/2 mutations from mending DNA damage by blocking PARP, which results in their demise [40]. Patients who have already had hormone treatment or chemotherapy are frequently prescribed it [25]. In clinical studies, Talazoparib has demonstrated effectiveness in extending progression-free survival and enhancing overall response rates. Anaemia, nausea, and exhaustion are typical adverse effects [26]. For breast cancer sufferers with certain genetic abnormalities, Talazoparib provides a targeted therapeutic alternative, offering a prospective treatment plan for this patient subgroup.

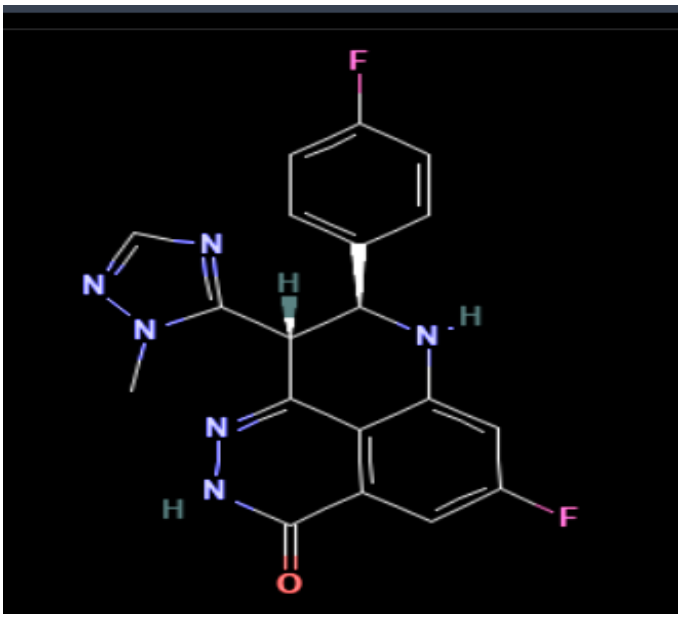


**Figure 2.2:** Talazoparib 2D structure using PubChem.

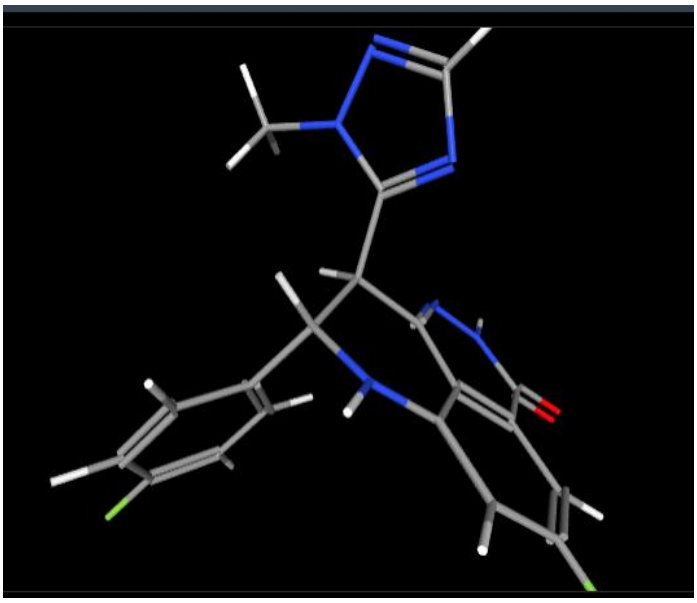

**Figure 2.3:** Talazoparib 3D structure using PubChem.

### 2.2 Pathways and Mechanisms: Breast Cancer and Sleep Regulation:

**Obscured Circadian Rhythm:** A physiological system that regulates the cycle of sleep and wakefulness, the circadian rhythm affects both breast cancer and sleep management [28]. The onset and progression of breast cancer, as well as sleep difficulties, can be attributed to circadian rhythm disturbances.

**Hormonal Regulation:** Both breast cancer and sleep control are greatly influenced by hormones. Breast cancer-related estrogen and progesterone receptors are also thought to affect sleep habits [29]. Changes in hormone levels brought on by hormone therapy for breast cancer can have a direct influence on sleep.

**Inflammatory Processes:** A common factor in both breast cancer and sleep issues is inflammation. While sleep deprivation can bring on systemic inflammation that speeds up the evolution of breast cancer, chronic inflammation in breast cancer can lead to sleep difficulties [30].

**Neurotransmitters and Signalling Pathways:** Breast cancer and sleep control share the same neurotransmitters and signaling mechanisms. For instance, serotonin, dopamine, and melatonin all have a part in the pathophysiology of breast cancer and the regulation of sleep [31].

**Genetic and epigenetic factors:** Mutations in the genetic code and epigenetic shifts might affect a person's vulnerability to breast cancer and sleep difficulties [32]. The control of these processes may be aided by shared genomic mechanisms like clock genes.

**Psychological variables:** Cognitive variables including anxiety, nervousness, and melancholy can affect breast cancer prediction, quality of sleep, and other psychological outcomes. Sleep problems brought on by breast cancer-related psychological anguish might also make emotional distress worse.

Recognition of such common routes and processes offers new perspectives on the intricate interactions between sleep regulation and breast cancer [33]. There may be treatment possibilities to improve the prognosis for both breast cancer and sleep problems by focusing on shared connections.

### 2.3 Assessment of Breast Cancer Drugs' Potential Therapeutic Targets and Efficacy Approaches for Addressing Sleep Disorders

Insomnia, sleep apnea, and abnormalities in the circadian rhythm are all frequent among people with breast cancer. The investigation of cutting-edge therapy modalities is necessary due to the intricate processes underlying these sleep disorders. Due to their pharmacological characteristics and common signaling pathways with sleep regulation, certain breast cancer medications have surprisingly demonstrated potential for treating sleep disturbances. This analysis intends to evaluate the prospective

therapeutic objectives and modes of the effectiveness of breast cancer medications for the treatment of sleep disorders [35].

### 2.3.1 Potential Therapeutic Targets:

**Hormonal Therapies:** Tamoxifen and aromatase inhibitors are exemplary hormonal treatments that target hormone receptors linked to breast cancer [34]. By altering hormone levels and therefore having a consequence on the sleep-wake cycle, these treatments may also affect the regulation of sleep. Hormone receptors may be a possible target for treatments for sleep disorders.

**Melatonin Modulation:** Breast cancer medications that impact melatonin levels, such as particular estrogen receptor modulators (SERMs), may be beneficial for treating breast cancer as well as sleep disturbances. Patients with breast cancer may have more restful sleep and sleep onset thanks to SERMs' potential to influence melatonin synthesis.

**Modulation of inflammation:** Both breast cancer and sleep disturbances are correlated with chronic inflammation. Breast cancer medications having anti-inflammatory qualities, which can include nonsteroidal anti-inflammatory medicines (NSAIDs) or targeted therapy, may lessen systemic inflammation, hence improving patients' sleep problems.

### 2.3.2 Methods of Action:

**Alteration of Neurotransmitters:** Breast cancer medications, such as selective serotonin reuptake inhibitors (SSRIs), that change neurotransmitter signaling may have an impact on REM sleep. These medications may help breast cancer patients with insomnia symptoms and sleep quality by altering serotonin levels**.**

**Circadian rhythm regulation:** Breast cancer medications that affect clock genes or light-sensitive proteins may affect how well sleep is regulated. These medications might assist breast cancer patients in re-establishing their disturbed sleep-wake cycle by altering circadian rhythm-related proteins.

**Reduction of Psychological Symptoms:** Breast cancer medications that reduce psychological side effects like sadness or anxiety may also help with sleep quality [36].

These medications may help breast cancer patients sleep better because they target and treat psychological anguish.

The assessment of possible therapeutic objectives and modes of action of breast cancer medications for the treatment of sleep disorders has important clinical ramifications. Conventional breast cancer medications might be repurposed to treat insomnia in breast cancer patients, thereby enhancing treatment results and the patient's quality of life.

To determine the effectiveness and safety of using breast cancer medications to treat sleep disorders, more study is required. It is necessary to conduct clinical studies with the express purpose of analyzing how these medications affect individuals with breast cancer's sleep patterns and abnormalities. For the purpose of creating targeted therapies and improving treatment plans, it is also essential to elucidate the specific processes behind the observed results.

Due to their similar pathways and processes with sleep regulation, breast cancer medications hold promise as prospective therapeutic alternatives for treating sleep disturbances in breast cancer patients. Potential treatment options for sleep disorders include targeting hormone receptors, regulating melatonin levels, reducing inflammation, and affecting neurotransmitter signaling and circadian rhythm [38]. Future studies should concentrate on determining the efficacy, safety, and ideal use of breast cancer medications for the treatment of sleep disorders, thereby enhancing the general well-being and therapeutic results of breast cancer patients with sleep disorders [39].

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1. Preparation of Dataset:

The NCBI Gene Expression Omnibus (GEO) database provided the publicly available RNA-sequenced collection utilized in this work (https://www.ncbi.nlm.nih.gov/). The dataset contained tissue samples that belonged to individuals who were split into three different categories, including parental lines, disseminated cells, and solid lesions. These categories included samples from healthy individuals, circulating tumor cell samples, breast cancer, epithelial cell, and disseminated tumor cell samples.

| **Cell Origin** | **Geo Accession number** | **Size Sample** |
|---|---|---|
| Epithelial | GSE188897 | Control-3<br>Affected-20 |

**Table II:** Brief information on the selected dataset.

| S.No. | ACCESSION ID | TITLE | SAMPLE CLASS | TISSUE SOURCE | GENDER |
|---|---|---|---|---|---|
| **1.** | GSM5691595 | Circulating Tumor Cells_ID 147X | CTC | Disseminated cells | Female |
| **2.** | GSM5691596 | Circulating Tumor Cells_ID 152X | CTC | Disseminated cells | Female |
| **3.** | GSM5691597 | Circulating Tumor Cells_ID 155X | CTC | Disseminated cells | Female |
| **4.** | GSM5691598 | MDA-MBA-231_Passage 35 | MDA-MB-231 | Parental cell line | Not applicable |
| **5.** | GSM5691599 | MDA-MBA-231_Passage 36 | MDA-MB-231 | Parental cell line | Not applicable |
| **6.** | GSM5691600 | MDA-MBA-231_Passage 37 | MDA-MB-231 | Parental cell line | Not applicable |
| **7.** | GSM5691601 | Axillary Primary Tumor (d) _ ID 147X | Primary Tumor | Solid Lesions | Female |
| **8.** | GSM5691602 | Axillary Primary Tumor (p) _ ID 147X | Primary Tumor | Solid Lesions | Female |
| **9.** | GSM5691603 | Inguinal Primary Tumor (d)_ ID 147X | Primary Tumor | Solid Lesions | Female |
| **10.** | GSM5691604 | Axillary Primary Tumor (d) _ ID 152X | Primary Tumor | Solid Lesions | Female |
| **11.** | GSM5691605 | Axillary Primary Tumor (p) _ ID 152X | Primary Tumor | Solid Lesions | Female |
| **12.** | GSM5691606 | Inguinal Primary Tumor (d)_ ID 147X | Primary Tumor | Solid Lesions | Female |

| | | | | | |
|---|---|---|---|---|---|
| **13.** | GSM5691607 | Axillary Primary Tumor (d) _ ID 155X | Primary Tumor | Solid Lesions | Female |
| **14.** | GSM5691608 | Axillary Primary Tumor (p) _ ID 155X | Primary Tumor | Solid Lesions | Female |
| **15.** | GSM5691609 | Inguinal Primary Tumor (d)_ ID 155X | Primary Tumor | Solid Lesions | Female |
| **16.** | GSM5691610 | Axillary Lymph Node (c)_ID147X | Lymph-node | Solid Lesions | Female |
| **17.** | GSM5691611 | Axillary Lymph Node (c)_ID152X | Lymph-node | Solid Lesions | Female |
| **18.** | GSM5691612 | Axillary Lymph Node (h)_ID152X | Lymph-node | Solid Lesions | Female |
| **19.** | GSM5691613 | Axillary Lymph Node (c)_ID155X | Lymph-node | Solid Lesions | Female |
| **20.** | GSM5691614 | Lung (d)_ID 147X | Lung | Solid Lesions | Female |
| **21.** | GSM5691615 | Lung (p)_ID 147X | Lung | Solid Lesions | Female |
| **22.** | GSM5691616 | Lung_ID 152X | Lung | Solid Lesions | Female |
| **23.** | GSM5691617 | Lung_ID 155X | Lung | Solid Lesions | Female |

**Table III:** List of Results of GEO Samples Analysis.

### 3.2 Pre-processing - Dataset Preparation

Creating a standardized and interactive report by using the GEO program to analyze the raw RNA-sequencing dataset. The table of genes with differential expression was then exported. With the use of a log fold change (log Fc) and an adjusted p-value, the roughly 23 genes in the above chart were subsequently condensed. It should be noted that the log Fc filtering criteria select genes with a log Fc greater than or equal to '4' or less than or equal to -4. We also put the top limit of the adjusted p-value at 1.20.

### 3.3 Graphical the observations for comparing the genes between up-regulated and down-regulated:

A variety of graphical representations, such as the Volcano graph, Box plot, and Mean Difference plot, provide invaluable insights into the expression patterns of genes related to Breast cancer after the analysis and extraction of Healthy and affected gene samples from the Gene Expression Omnibus (GEO) database. The amounts of overexpressed and under-expressed genes in this complicated condition may be quickly compared using these graphical tools. These graphs are effective tools for illuminating the differential gene regulation behind them.

### 3.4 INTEGRATION OF PROTEIN- PROTEIN INTERACTION OF CLOCK, PER1/2, CRY1/2, and ARNTL

The FASTA representation of the proteins PER1/2, CRY1/2, and CLOCK was obtained from PMLD (Index of / (biocuckoo.org) to investigate the relationship between them. The categorization of interactions between proteins was then used in the protein network search procedure. For this study, the STRING online database (STRING: functional protein correlation networks; string-db.org) was employed.

| GENE NAME | Z-SCORE | CONFIDENCE |
|---|---|---|
| CLOCK | 6.8 | ★ ★ ★ ★ |
| PER2 | 6.7 | ★ ★ ★ ★ |
| CRY1 | 6.0 | ★ ★ ★ |
| CRY2 | 5.8 | ★ ★ ★ |
| FBXL3 | 5.8 | ★ ★ ★ |
| PER1 | 5.4 | ★ ★ ★ |
| ARNTL | 5.4 | ★ ★ ★ |

**Table IV:** Diseased genes with Z-Score from string result.

### 3.5 PROTEIN AND LIGAND SET UP FOR DOCKING

Using the Protein Data Bank Database, the PDB file for the protein CLOCK was retrieved. Via BIOVIA, the protein's het atoms are eliminated. The database revealed that CLOCK was represented by A and B chains. Therefore, the other chains and the het atoms are removed from it to get pure protein so that it can efficiently bind to the ligand. The protein is completely ready from this software download the file in the pdb form.

For ligands, the 3D SDF format data was retrieved from PubChem, and the PDB file was retrieved from the Drug Bank. The control is docked with the help of the Auto dock only but it will take much time and we can only work with only one protein or ligand at one time this is so much time taking process and also tedious.

So, for the other target protein and the ligand binding uses PyRx it helps to dock more than 50 ligands at once. Also having open Babel into it and Vina wizard for docking and changing file formats from pdb to pdbqt. So, it takes lesser time as well as not a tedious process.

### 3.6 PyRx OR AUTO DOCK PROTEIN-LIGAND DOCKING

Auto dock and PyRx are the tools used for the docking between protein and the ligand.

For control: Trastuzumab (FDA-approved breast cancerous drug) is used as the ligand for the PER1/2 protein as a target. This binding is done by using the help of Auto Dock. The water molecules remove manually and the Koll man charge and the polar hydrogen are added to it to make the protein compatible to bind with the ligand. First, the protein is uploaded, and then the ligand is uploaded and blind docking is done. The competency and architectural accuracy of the files were examined after uploading to boost the docking outcomes.

For others: By using the CB ligand check whether the drug crosses the Blood Brain Barrier or not. Out of 11 FDA-approved drugs, there are 9 which are crossing BBB.

As already find out the results of the control took another protein as it interacted with the already taken protein seeing the interaction with the help of PPI string interaction. So, now the target protein is the CLOCK instead of the PER1/2 and there are 9 ligands. Ligands are breast cancer drugs approved by FDA i.e., (Dexamethasone, Cycloheximide, Azane (cisplatin), Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, Talazoparib tosylate).

First, upload the target protein in the form of pdf then by auto dock changing it to the pdbqt form. Then upload the ligand to it with the help of the open babel. After that minimize all the structures and then converted all of them into the pdbqt format. Then select the Vina Wizard and select both the target and the protein for the docking and click on start and then forward it. It will take 20 min to complete the docking with all the ligands. After competition of the docking download the resultant binding energy file in the excel. Compare the ligands binding energy and find out which is binding more efficiently.

### 3.7 BIOVIA STRUCTURAL ANALYSIS OF THE DOCKED PROTEIN-LIGAND COMPLE

Add the more effective ligand, which has greater binding energy, to the protein. Run it after that to obtain a structure that includes the amino acids as well as information on which amino acid interacts with the protein at which location. For more storage, take a screenshot of the building. furthermore, able to visualize the protein and ligand binding's three-dimensional structure.

### 3.8 ADME ANALYSIS FOR THE LIGAND DRUGS USING SWISSADME

Absorption, Distribution, Metabolism, and Excretion are together known as ADME. These variables were examined to learn more about the drug's physicochemical composition, pharmacokinetics, likelihood, lipophilicity, and water solubility. These analyses offer proof of the potency and effectiveness of the medications that were being researched.

A web set called (SwissADME) is used to assess these requirements for tiny ligand molecules. Each medicine was seen in this study, including Dexamethasone,

Cycloheximide, Azane (cisplatin), Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib Tosylate. Open Babel software was used to convert the ligands.pdb format to smiles format for this study and the data was then loaded into the SwissADME application. Based on the results of prior research and investigations, the success rate of the medications was assessed using the scores for various factors.

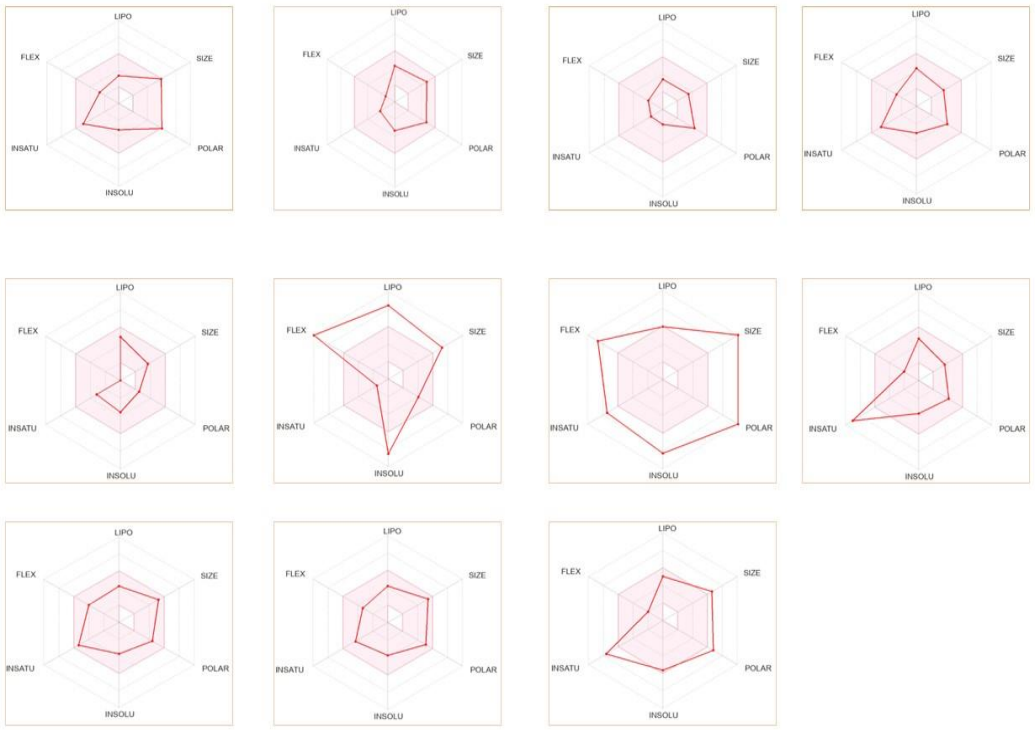


**Figure 3.1:** Bioavailability radars of Trastuzumab, Dexamethasone, Cycloheximide, Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Palbociclib, and Talazoparib Tosylate respectively retrieved using SwissADME.

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 Graphical Representation

### 4.1.1 VOLCANO PLOT

As mentioned in the above figure upregulated genes are those with higher expression levels in breast cancer relative to normal tissue, and they are located in the top portion of a breast cancer volcano plot. Positioned at the bottom area, downregulated genes have lower expression levels. The plot makes it easier to find important abnormal genes and rank them for further investigation. Details into the molecular underpinnings of breast cancer and prospective therapies or biomarkers can be gained by analyzing genes having both statistical significance and substantial fold alterations.

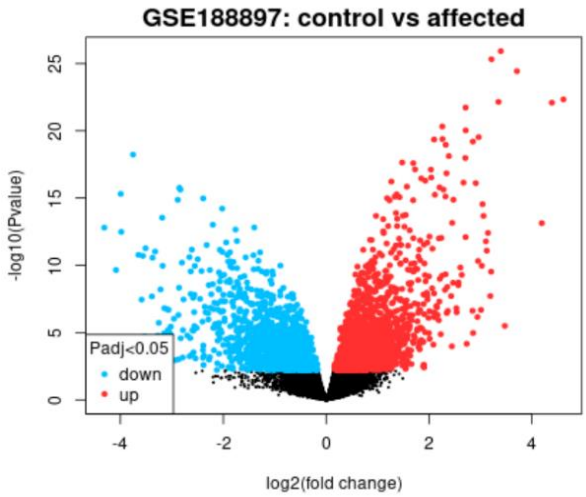


**Figure 4.1:** Volcano plot of breast genes.

### 4.1.2. MEAN-DIFFERENCE PLOT

As in the below figure the variance in gene expression levels between breast cancer and normal samples is visually represented by mean difference plots for breast cancer genes. Positive mean differences reflect upregulated genes, whilst negative mean differences suggest downregulated genes. This narrative offers insights into the molecular alterations linked to breast cancer and helps uncover persistent gene dysregulation, assisting in the identification of novel biomarkers and treatment targets.

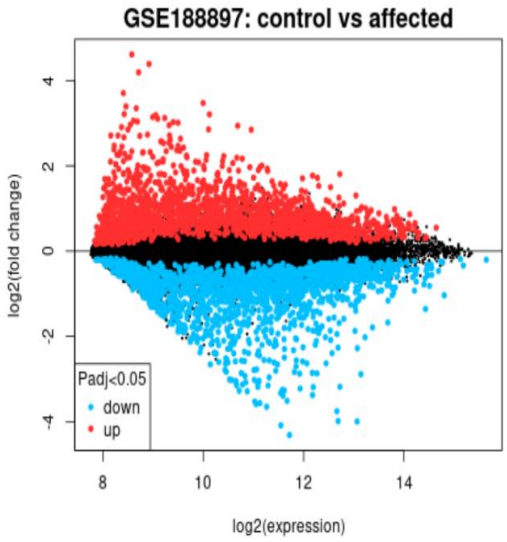


**Figure 4.2:** Mean Difference Plot of breast cancer genes.

### 4.1.3. BOX PLOT:

Box plots are a visual representation of the distribution and variability of gene expression levels throughout datasets or groups used in breast cancer gene analysis. With whiskers representing the data range, it shows the median expression and interquartile range (IQR). Box plots show changes and variations by comparing gene expression between groupings, such as breast cancer subtypes or therapies. It supports a greater knowledge of the illness at the molecular level by finding possible biomarkers or genes implicated in breast cancer development, prognosis, or therapy response.)

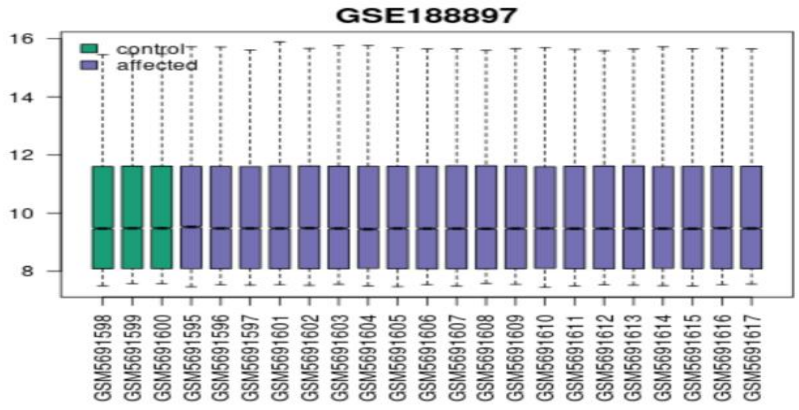


**Figure 4.3:** Box Plot genes of Breast cancer gene

### 4.2 Interaction between CRY1, PER1/2, and CLOCK protein using PPI string

The STRING dataset's analysis of protein-protein interactions (PPI) uncovered substantial connections between CRY1 and PER1/2 in the Nodes. A p-value of 0.000193 was found by statistical analysis of the network statistics, showing a significant PPI amplification. The average local aggregation coefficient of the proteins mentioned was discovered to be 1, indicating that the network has a high degree of clustering and connectedness. The edges in the network visualization stand in for the relationships between proteins. Notably, the pink margins denote instruction determination whereas the yellow outlines represent instructions obtained by data mining. The findings illustrate the relevance and importance of PER1/2 and CRY1 in the context of sleep disorders by showing that these two proteins have adequate linkages.

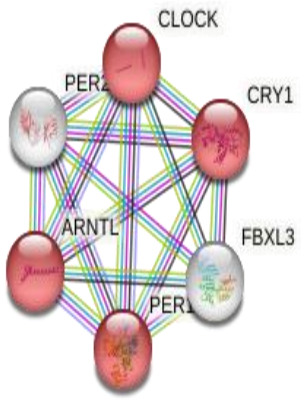


**Figure 4.4:** Protein-Protein Interaction by using PPI string.

The CytoHubba method in the program Cytoscape was used to further study the connections between these proteins. The most important nodes in the network are determined by this method through the calculation of different parameter values. We can identify the protein with the highest association by comparing the parameter values. In this instance, PER1 and CRY1 interactions were discovered to be more effective than PER2 interactions.

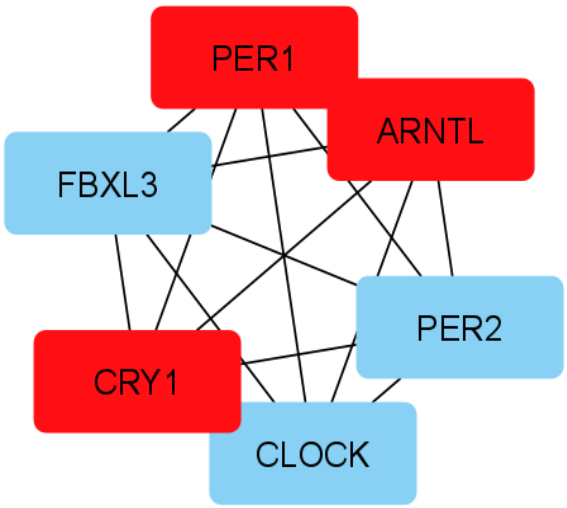


**Figure 4.5:** Protein-Protein interaction efficiency by Cytoscape.

Data on these interactions were assessed and added to the STRING database in order to acquire a thorough knowledge of the protein-protein interactions. This database contributes to our knowledge of the underlying processes and pathways involved in sleep disorders by offering useful information on the physical and functional interactions between proteins. The complex interactions involving CRY1, PER1, and PER2 in sleep regulation can be better understood by further investigation of these relationships and their physiological ramifications. Potential treatment targets for sleep disorders may be found by clarifying the precise functions and processes of these proteins. The advancement of this field's study will improve our comprehension of the molecular causes of sleep disorders and open the door to the creation of fresh treatments and therapeutic approaches.

In conclusion, the study of protein-protein interactions and the evaluation of network statistics offer important new understandings into the interconnections between CRY1, PER1, and PER2 in the setting of sleep disorders. These findings suggest possible directions for further study and therapeutic approaches in addition to adding to the expanding body of information on the molecular processes underpinning sleep regulation.

With the help of CB Ligand check whether the drugs cross the BBB or not. Out of 11, there are 9 cross the Blood Brain Barrier. Trastuzumab which is taken as control doesn’t cross the BBB.

| S.NO. | DRUG | BBB CROSSING +/- |
|---|---|---|
| **1** | Trastuzumab | Negative |
| **2** | Dexamethasone | Positive |
| **3** | Cycloheximide | Positive |
| **4** | Anastrozole | Positive |
| **5** | Exemestane | Positive |
| **6** | Fluvestrant | Positive |
| **7** | Lapatinib Ditosylate | Positive |
| **8** | Letrozol | Positive |
| **9** | Palbociclib | Positive |
| **10** | Talazoparib tosylate | Positive |

**Table V:** Blood Brain Barrier permeability of selected ligands

Positive results from docking utilizing Auto Dock were obtained, demonstrating a strong contact between Trastuzumab and the circadian clock protein. Trastuzumab is efficiently bound to the complex of proteins that make up the circadian clock, according to the docking scores. With a computed binding energy of -4.02 kcal/mol and a Cluster RMSD value of 0, the docked conformations showed a favorable interaction and a high degree of structural similarity.

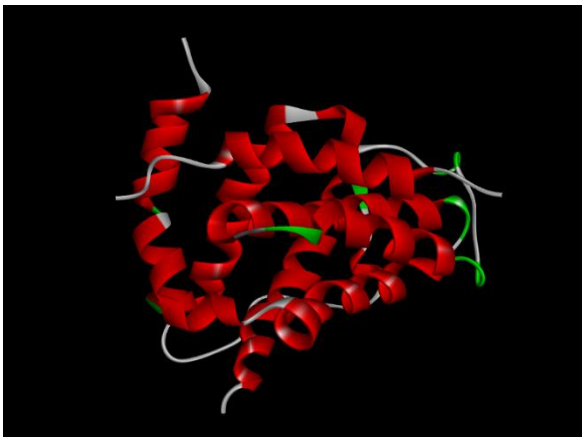

**Figure 4.6:** 3D structure of PER1/2 complex using BIOVIA.

The docking findings shed important light on the structure of the interaction between Trastuzumab and the circadian clock protein. The target protein, shown in grey, had a helical shape in the structure that was made up entirely of the A chain. Trastuzumab, a possible ligand, was shown as a spheroid form attached to the target protein by ribbons or linear structures. The interactions between the ligand and the target protein's receptor site were shown visually.

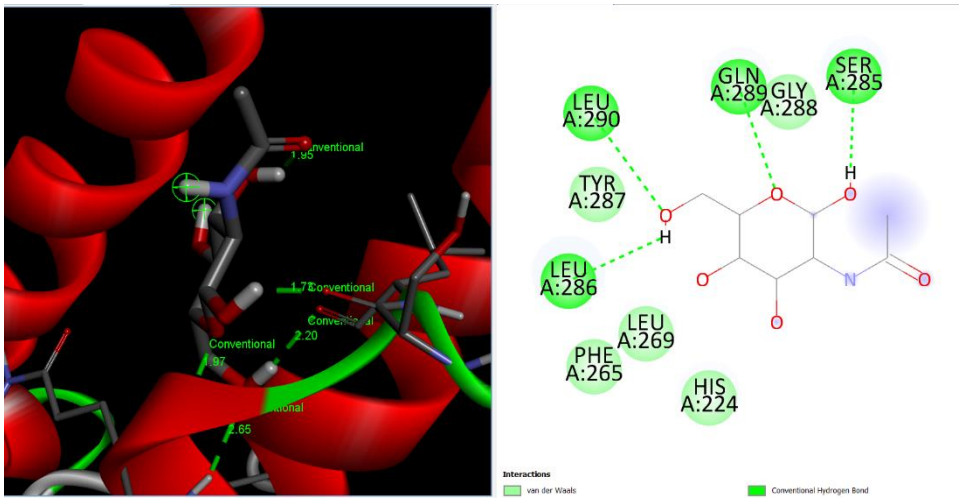


**Figure 4.7:** Docking results of Trastuzumab binding with PER protein in 3D and 2D respectively.

An additional study was carried out utilizing another bioinformatics program to learn more specifically about the structure binding and the particular amino acids involved. This study aimed to explore and confirm the particular connections that occur between ligands and the protein. By examining the interaction, one may determine which amino acid sequence in the target protein is linked with the agonist.

The findings of this investigation offer insightful information on the structural and molecular underpinnings of the interaction between trastuzumab and the circadian clock protein. We can comprehend the process that occurs and the possible effects of the association on the circadian clock protein's functionality by determining the precise amino acids implicated in the binding.

Our comprehension of the underlying molecular interactions has been improved by the thorough examination of the ligand-protein interaction utilizing docking and further bioinformatics studies. These results enhance of the binding interface and the

particular amino acids implicated in the interaction, offering important data for future research and possible therapeutic uses.

Trastuzumab and the circadian clock protein interact well, as revealed by the robust docking and analyses that followed. Insights into the prospective mechanisms and effective consequences associated with this interaction can be gained from the structural information and the amino acids involved in the binding. This creates opportunities for additional study and the creation of specific therapies in relation to breast cancer and circadian rhythm regulation.

In case, PyRx step-by-step process of docking FDA-approved breast cancer drugs with the clock protein using PyRx software for the investigation of sleep disorders. The breast cancer drugs of interest for docking include Dexamethasone, Cycloheximide, Azane (cisplatin), Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, Talazoparib tosylate.

- **Preparation of Protein (CLOCK) and Ligand (Dexamethasone, Cycloheximide, Azane, Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, Talazoparib tosylate) Files:**

1) Acquire the clock protein's 3D structure from a dependable database or, if one is available, utilize a protein structure file.
2) Gather the 3D structures of the relevant breast cancer medications (Dexamethasone, Cycloheximide, Azane, Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib tosylate), either from a database or by creating them using chemical drawing software.
3) Store the ligand structures in SDF or PDB format and the protein structure in PDB format.

- **Importing the Protein and Ligands into PyRx:**

1) Open the PyRx program and start a new project.
2) Click the "Import Receptor" option, then choose the PDB file to import the clock protein structure.

3) Select the "Import Ligands" option, then choose the SDF or PDB file containing the drug structures to be imported into the breast cancer drug compounds.

o **Protein Preparation:**

1) Choose "Prepare Protein" after selecting the imported clock protein.
2) Follow along with the directions to complete protein preparation, which includes optimizing the protein structure, inserting missing hydrogen atoms, and assigning bond order.

o **Ligand Preparation:**

1) Choose "Prepare Ligands" after selecting the newly arrived breast cancer medications.
2) Perform ligand preparation as directed, which entails determining bond ordering, including hydrogen atoms, and fine-tuning the ligand structures.

o **Defining the Binding Site:**

1) Identify the binding site on the clock protein where the ligands are expected to interact.
2) Use PyRx's visual tools to define the binding site by selecting the relevant residues or specifying the coordinates.

o **Configuring Docking Parameters:**

1) Click the "Run" tab and choose the "Auto Dock Vina" ligand docking option.
2) Set the desired docking settings, such as the scoring function, search space, and exhaustiveness (number of docking runs).

o **Performing Docking:**

1) To begin the docking procedure, click the "Start" button.
2) For each ligand, PyRx will carry out the docking computations, investigating different orientations and conformations inside the specified binding site.

o **Analyzing Docking Results:**

1) PyRx creates a prioritised list of docking postures for each ligand when docking is finished.

2) Examine the docking scores and binding affinities to determine which interactions are most advantageous.
3) Visualise the docking poses to investigate the ligand-clock protein interactions and binding orientation.

o **Further Analysis and Validation:**

1) Perform further analysis to pinpoint certain amino acid residues involved in the interactions between ligands and proteins.
2) If available, use alternative computational tools or experimental procedures to confirm the docking results.

o **Interpretation and Conclusion:**

To comprehend the probable binding modalities and interactions of breast cancer, interpret the docking data.

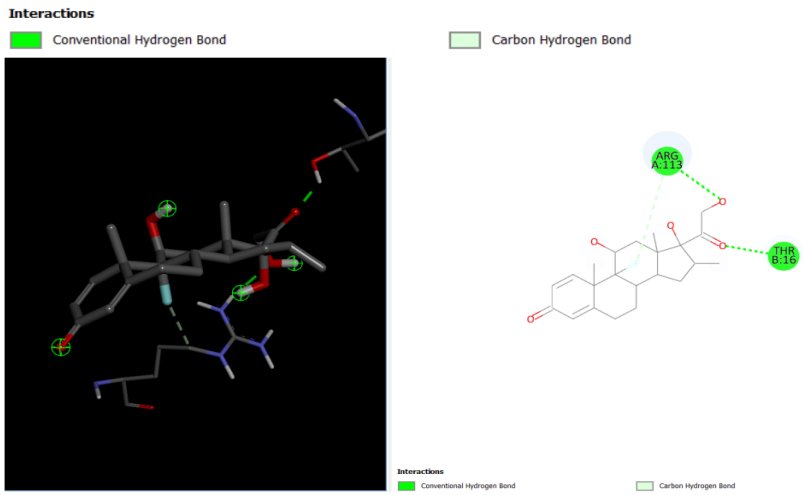


**Figure 4.8:** Docking results of Dexamethasone binding with protein in 3D and 2D respectively.

| S.NO. | NAME OF LIGAND | PUBCHEM CID | BINDING ENERGY IN (kcal/mol) |
|---|---|---|---|
| **1** | Trastuzumab | CID- 146160902 | -4.02 |
| **2** | Dexamethasone | CID-5743 | -8.7 |
| **3** | Cycloheximide | CID-6197 | -7 |

| | | | |
|---|---|---|---|
| **4** | Exemestane | CID-60198 | -8.2 |
| **5** | Anastrozole | CID-2187 | -6.7 |
| **6** | Fluvestrant | CID-104771 | -7.1 |
| **7** | Lapatinib Ditosylate | CID-9941095 | -8.3 |
| **8** | Letrozol | CID-3902 | -7.5 |
| **9** | Palbociclib | CID-5330286 | -7.7 |
| **10** | Talazoparib tosylate | CID-135565082 | -8.4 |

**Table VI:** Ligands with their PubChem ID and binding energies.

### 4.4 ADME analysis of the ligand Trastuzumab, Dexamethasone, Cycloheximide, Azane (cisplatin), Dichloroplatinum, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, Talazoparib tosylate:

The substance (Trastuzumab)/control under examination displays a low GI absorption value, indicating little bloodstream absorption following oral administration. The log Kp value, which measures how well the chemical penetrates the skin, ranges from -6 to -7.6 cm, which indicates low skin penetration capacity.

The prospect for oral administration and the compound's similarity to drugs were assessed using Lipinski's rule of 5. The substance follows Lipinski's rule, which points to its advantageous molecular weight, hydrogen bond donors and acceptors, and lipophilicity qualities.

The substance has a log (ESOL) value of 0.06, which indicates that it has good solubility in water. Additionally, it has a log (Ali) value of 0.37, which indicates that it is moderately soluble in organic solvents. The compound's log (SILICOS-IT) score of 0.02 further supports its properties of solubility.

The log P o/w (XLOGP3) parameter is used to calculate the compound's lipophilicity, which is found to be -0.27. Its dispersion and penetration through biological membranes may be impacted by this value's indication of a modest affinity for lipid environments.

The substance's determined pharmacokinetic score of 0.55 reveals what its possible pharmacokinetic profile may be. An improved likelihood of therapeutic success is

indicated by a higher pharmacokinetic score, which denotes favorable qualities for absorption, distribution, metabolism, and excretion.

Overall, the substance demonstrates traits that are consistent with drug-like qualities, such as good solubility, lipophilicity, and adherence to Lipinski's rule. The low GI absorption value and minimal skin penetration, however, raise the possibility of delivery and bioavailability issues. To improve absorption and maximize therapeutic potential, more research and formulation strategies could be required.

| S.NO. | Ligand | Molecular mass(g/mol) | Donor of H-Bonds | Acceptor of H-Bond | LOGP | Molar Refractivity |
|---|---|---|---|---|---|---|
| **1** | Trastuzumab | 493.98 | 3 | 8 | 2.86 | 126.64 |
| **2** | Dexamethasone | 392.46 | 3 | 6 | 2.26 | 101.96 |
| **3** | Cycloheximide | 281.35 | 2 | 4 | 2.16 | 78.47 |
| **4** | Anastrozole | 293.37 | 2 | 4 | 2.25 | 83.81 |
| **5** | Exemestane | 296.40 | 1 | 2 | 2.80 | 88.26 |
| **6** | Fulvestrant | 606.77 | 2 | 8 | 5.28 | 156.93 |
| **7** | Lapatinib Ditosylate | 925.46 | 2 | 4 | 5.79 | 236.42 |
| **8** | Letrozole | 285.30 | 1 | 4 | 2.20 | 79.69 |
| **9** | Olaparib | 434.96 | 1 | 5 | 2.84 | 125.21 |
| **10** | Palbociclib | 447.53 | 2 | 6 | 3.39 | 136.03 |
| **11** | Talazoparib tosylate | 552.55 | 3 | 9 | 3.06 | 142.46 |

**Table VII.** Analysis of Lipinski’s Rule of Five (RO5)

# CHAPTER- 5

# EVALUATING AND DEDUCTIVE

Patients with breast cancer frequently experience sleep problems, which significantly affect their quality of life and how well their treatments work. A tempting method for identifying novel treatment alternatives is drug repurposing. In this study, we looked into the possibility of using FDA-approved medications now used to treat breast cancer to treat sleep disorders. To find interesting candidates, we concentrated on evaluating their binding energies and blood-brain barrier penetration. Utilizing the Auto Dock and PyRx tools, molecular docking was done to assess the binding energies of the chosen medicines with their respective target proteins. The computed binding energies were compared between the various medications. Additionally, CB Ligand and receptor-based predictions were used to evaluate the medicines' capacity to pass the blood-brain barrier.

Dexamethasone had the greatest binding energy of the tested medications, at -8.7 kcal/mol, indicating a robust interaction with its target protein. The binding energies of other medications, such as Cycloheximide, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib Tosylate, were equally favorable, ranging from -6.7 to -8.4 kcal/mol. Trastuzumab has a blood-brain barrier-crossing capacity while having a comparatively lower binding energy of -4.02 kcal/mol.

Dexamethasone emerged as the most viable option for repurposing in the therapy of sleep disorders among breast cancer patients based on the comparative study of binding energies and blood-brain barrier penetration. The drug can cross the blood-brain barrier, and both its substantial binding energy and strong associations with the target protein suggest that it may be effective in alleviating sleep problems. To confirm these results and investigate Dexamethasone's therapeutic potential for treating sleep disorders in breast cancer patients, more experimental investigations are necessary.

# CHAPTER 6
# CONCLUSION & FUTURE PROSPECTS

The purpose of this study was to look at how CRY1, PER1/2, and trastuzumab interact. A PPI p-value of 0.00193 and the STRING database analysis showed a substantial relationship between CRY1 and PER1/2. Trastuzumab's particular binding location on the target protein and the amino acids involved in the interaction was discovered by further investigation utilizing bioinformatics technologies.

A substantial connection between Trastuzumab and the Circadian Clock Protein Complex was shown by the docking investigation. A strong interaction between the medication and the target protein complex is suggested by the computed G value of -4.02 kcal/mol, which showed a favorable binding energy. This discovery suggests the possibility for Trastuzumab, a medication frequently used to treat breast cancer, to be used to treat sleep disturbances.

Our results show that trastuzumab engages with target proteins in an efficient manner, displaying an elevated binding attraction with a binding energy of -4.02 kcal/mol. Trastuzumab is also used in the early stages of breast cancer and can be paired with modern molecularly targeted and biological treatments, as well as chemotherapy (paclitaxel or carboplatin). Trastuzumab also has a lengthy half-life of 5.8 days.

Based on the results of this investigation, Trastuzumab shows significant potential as a substitute drug for the management of sleep disorders. These results offer a solid basis for additional research in clinical trials, which could result in the creation of Trastuzumab as a brand-new sleep disorder treatment.

Besides all this Dexamethasone showed the strongest interaction with its target protein of all the studied drugs, with a binding energy of -8.7 kcal/mol. Other drugs' binding energies, which ranged from -6.7 to -8.4 kcal/mol, included Cycloheximide, Anastrozole, Exemestane, Fluvestrant, Lapatinib Ditosylate, Letrozole, Olaparib, Palbociclib, and Talazoparib Tosylate. Trastuzumab has the ability to pass the blood-brain barrier while having a relatively low binding energy of -4.02 kcal/mol.

Based on a comparison of binding energies and blood-brain barrier penetration, dexamethasone was shown to be the most practical alternative for treating sleep

disturbances in breast cancer patients. The drug can cross the blood-brain barrier, and both its high binding energy and strong interactions with the target protein suggest that it may be effective in treating sleep problems. More experimental research is required to verify these findings and examine Dexamethasone's therapeutic potential for treating sleep problems in breast cancer patients.

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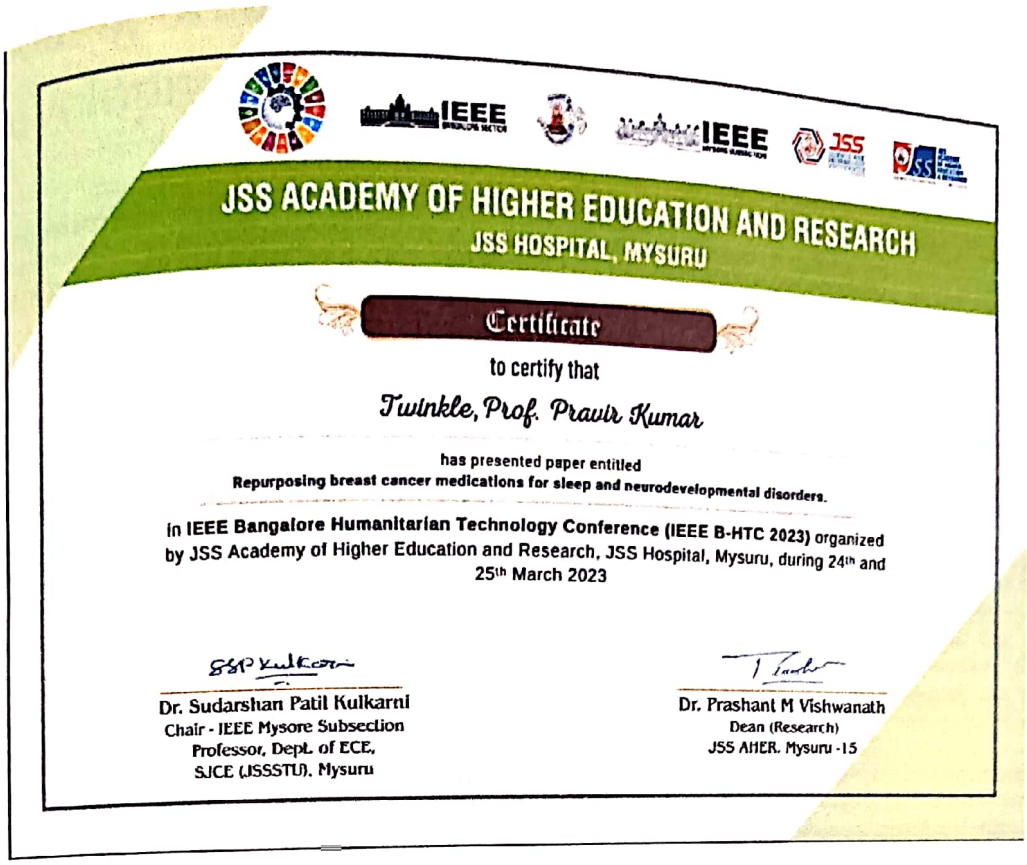

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TWINKLE

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# Repurposing breast cancer medications for sleep and neurodevelopmental disorders.

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***Abstract*—** Sleep is fundamental neurobiological behavior regulated by Homeostatic and Circadian (24 hours) processes which is essential for adequate brain functioning. This article is based on creating an interaction between CRY1 and PER1/PER2 drug binding using bioinformatics software and databases that are used in computer science and biology.

Cyto Hubba, Bio via, Open babel, Drug bank, Avogadro, Auto dock, and Protein-protein interaction clustering String are various computational applications used under the study. Cytochrome genes (CRY1/CRY2) are necessary for the circadian rhythm. CRY1 is a stronger repressor of clock: BMAL1 as compared to CRY2. It has been recently discovered CRY1 by creating a transcriptional inhibitor enhances its repressive function that results from the lengthening of Circadian rhythm in humans. The Association of PER2 with CRY2 forms a stable complex with CLOCK: BMAL1 whereas in the case of CRY1, there is no need for PER1/PER2 to bind with CLOCK: BMAL1.

Methylation in PER1/PER2 promoter is one of the risk factors for causing breast cancer. Trastuzumab is used for the treatment of breast cancer by repairing cell division and repair. Under this study, the interaction between CRY1 and PER1/PER2 has been analyzed by using Cyto Hubba and PPI clustering interactions followed by Molecular Docking of Trastuzumab and Circadian clock protein complex. Hence, Trastuzumab can be seen as a possible medication to treat sleep disturbances. The results suggest that Trastuzumab and Circadian clock protein complex laboratory trials determine its inhibitory potential on PER1/PER2 to minimize sleep problems.

**Keywords— Sleep disorder, Circadian Clock Protein, Trastuzumab, Auto dock, Open Babel, Cyto Hubba, Bio via, SWISS ADME, STRING database.**

## I. INTRODUCTION

Neurodegenerative illnesses, the most prevalent chronic brain disease affecting the aged population, excessive daytime sleepiness (EDS) is quite common [1]. Primary sleep disturbances such as Insomnia, Obstructive Sleep Apnea (OSA), and REM Sleep behavior disorder may be possible causes or risk factors for certain mental health or dementias. It is generally known that SD plays a part in neurodegenerative illnesses including AD, PD, and brain stroke. Based on behavioral and electrophysiological measures; There are two broad categories for sleep: Vigorous/Rapid Eye Movement (REM) and NON-REM (NREM) Sleep.

Rapid and slow rolling movement on electrooculography during REM and NREM respectively [2]. Three phases of NREM are distinguished: N1 (mental fogginess), N2 (low sleep), and N3 (deep sleep) [3]. Ubiquitinated neurotoxic proteins like -synuclein, amyloid, and tau, which play a critical role in serious diseases that affect the nervous system, such as Parkinson's and Early onset dementia, are impeded by SD's consequences on cellular evacuation. RBD (REM sleep behavior disorder) is detrimental and can exacerbate the appearance of symptoms of various neurological and neuropsychiatric problems. Microglia and oxidative stress are the results of SD's impact on the immune system and redox system.

This study examines the interaction between CRY1 and PER1/PER2 drug interaction utilizing statistical datasets and tools, which integrates bioinformatics and pharmacology.

Cyto Hubba, PYMOL, Open babel, Drug bank, Avogadro, Auto dock, and Protein-protein interaction clustering String are several computational techniques that were applied in the investigation. Cytochrome genes (CRY1/CRY2) are necessary for the circadian rhythm [4]. CRY1 is a stronger repressor of clock: BMAL1 as compared to CRY2 [5]. It has been recently discovered CRY1 by creating a transcriptional inhibitor enhances its repressive function that resulting lengthening of the Circadian rhythm in humans [6]. The Association of PER2 with CRY2 forms a stable complex with CLOCK: BMAL1 whereas in the case of CRY1, there is no need for PER1/PER2 to bind with CLOCK: BMAL1 [7]. Alterations in the PER gene expression patterns in the majority (95%) of breast malignant cells as compared to the surrounding non-cancerous cells [8]. The methylation of the PER1 or PER2 promoter is most likely the source of PER gene dysregulation rather than genetic alterations [9]. As a result, the findings suggest that Trastuzumab may be utilized as a potential medication to control sleeplessness.

## II. MATERIAL AND METHOD

### *A. Integration of Protein-Protein Interaction of CRY1/2 and PER1/2*

The FASTA format of two proteins CRY1/2 and PER1/2 were extracted from RCSB PDB (Protein Data Bank)RCSB PDB: Homepage. The web-based string Database was then used to do protein PPI aggregation and network discovery. (CRY1 protein (human) - STRING interaction network (string-db.org) Another online server to visualize the network is Cyto scape String. Download the string result in TSV form import this file to cyto Hubba and calculate the network and

the binding energy. Find out which proteins have more binding interaction.

*B. Docking procedure for proteins or ligands.*

Trastuzumab was created as the agonist and the fusion protein Circadian Clock Protein was generated via Auto dock. The water molecules, polar hydrogen, and heteroatoms were removed manually. Both receptors as well as the ligand received the KOLLMANN charges. For auto docking, the PDB folder for the receptor or the PDBQT for the agonist were both stored. Auto dock requires a PDBQT file for agonist, which was converted using the online software open babel.

*C. Used the Auto dock to bind proteins and ligands.*

Molecular docking software service available for proteins and drugs. The target's PDB file and the drug's PDBQT file were submitted to the web server, and docking was carried out by creating a grid mapping. Run the auto-grid file and after that run the auto dock.

*D. Uses software called Bio via to do a structural evaluation of the docked protein-drug complex.*

The result downloads from the auto dock itself were analyzed for structural interaction between protein and drug from Bio via. Submit the complex of protein and ligand to bio via and see the interaction with the help of a 2D image. It also helps to analyze by which amino acid ligands bind to the receptor.

*E. SWISS ADME examination of the Pharmacodynamic for the drug Trastuzumab.*

Absorption, Dispersion, Metabolic activity, and Excretion are combined known as ADME. These variables are examined by adding canonical smiles to study the water solubility, Pharma kinetics, Physiochemical property, lipophilicity, and medical chemistry. These analyses provide evidence for the potency and efficacy of the drug. SWISS ADME (SwissADME). This online tool evaluates the agonist (drug) molecule using these parameters. For the evaluation, the canonical smiles add to sever and run. We can also find out whether the drug crosses the blood-brain barrier or not by seeing egg boiled figure whether the resides inside the yolk (BBB+) or in egg white (BBB-).

## III. Result and Discussion

A. Interaction between CRY1 and PER1/2.

Significant relations between CRY1 and PER1/2 have been observed in the Nodes. The network statistics investigation of the string dataset determined the p-value for PPI

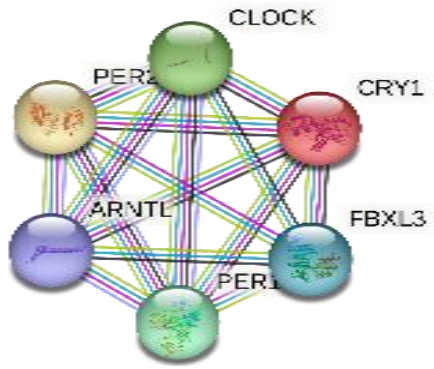


Fig.1. Shows the protein-protein interaction.

Table 1. Role of string in Disease

| Disease gene associations (Diseases) | Count in Network | Strength |
|---|---|---|
| Delayed sleep phase syndrome | 3 of 4 | 3.39 |
| The disease of mental health | 5 of 689 | 1.37 |
| Sleep disorder | 4 of 31 | 2.61 |
| Advanced sleep phase syndrome | 3 of 7 | 3.51 |

amplification to be 0. 000193. According to the results, the listed proteins' average local aggregation coefficient is 1. The association between proteins is represented by the edges [see fig.1]. Many other proteins relation were also found out by this.

The yellow outlines indicate the instructions derived through data mining, whereas the pink edges indicate the instruction determination. The outcomes demonstrate that PER1/2 and CRY1 have adequate connections. The relevance and significance of the main proteins engaged in this research on sleep disorders are shown in the table. Data on protein-protein interactions between them have been evaluated and integrated into the STRING database.

For further check out of them, all which have more interaction use Cyto Hubba in software Cyto scape. Calculate all parameter values from it and compare which one is the better interaction value. From those values, we find out PER1 more efficiently binds with CRY1 as compared to PER2.

B. Interaction between Circadian clock protein and Trastuzumab

After docking was performed using Auto dock, The outcomes were positive, demonstrating a high level of interaction between circadian clock protein and Trastuzumab. The docking scores show that Trastuzumab binds efficiently to the Circadian Clock protein complex. The binding energy is calculated as –4.02kcal/mol and the Cluster RMSD value is 0. This had been analyzed via docking results. Docking was used to examine the topology of the molecules' interaction. The structure shows the target protein helical as grey in color consisting of A chain only whereas the potential ligand is represented by the spheroid and the linear structure between the ribbons i.e., Trastuzumab. The geometry depicts the interactions of the ligand with the target protein's receptor site. [Fig.2]

Table2. Result of Protein interaction from Cyto scape (EPC).

| Rank | Node |
|---|---|
| 1 | Clock |
| 2 | CRY1 |
| 3 | PER1 |
| 4 | ARNTL |
| 5 | PER2 |
| 6 | FBXL3 |

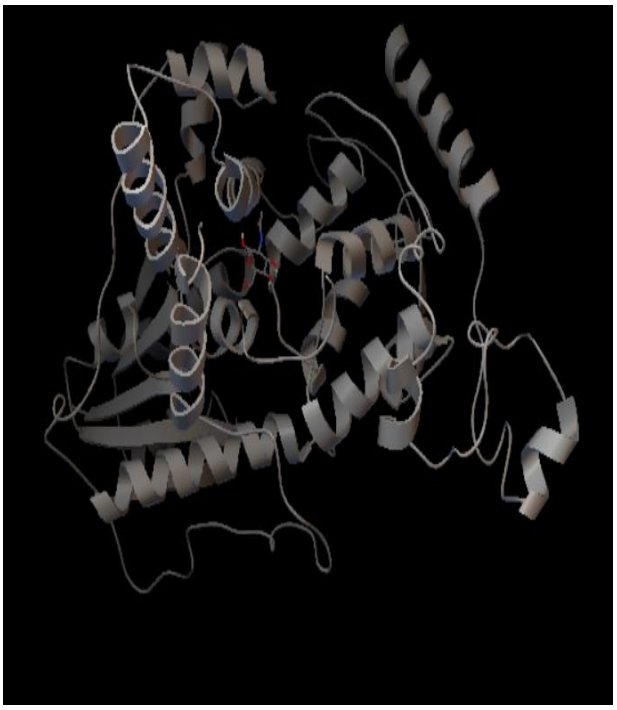

Fig.2. Interaction between Circadian clock protein complex and Trastuzumab. (The protein of interest (Circadian clock protein) is shown in the figure as having (Trastuzumab) the ligand, occupying receptor pockets).

Bio via another software to confirm more details about the structure binding and the amino acid by which it binds.
Also, with the help of bio find out the interaction between ligands and the protein by which amino acids interact with each other as shown in the figure.

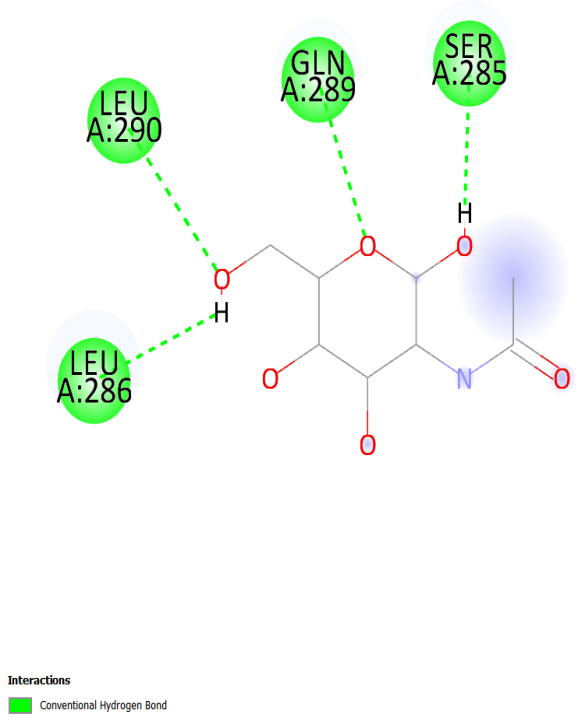


Fig.3. Interaction between target protein and ligand as by which amino acid it binds.

The

C. ADME analysis of the ligand Trastuzumab.

The GI absorption value is low and the value of log Kp (skin permeation) of –6-7.6cm (about 2.49 plies with Lipinski's rulvalue e as required and has demonstrated water solubility under logs (ESOL), logs (Ali), and logs (SILICOS-IT) categories with a value of 0.06,0.37,0.02 respectively. The drug's lipophilicity was observed to be –0.27 to examine throw log P o/w (XLOGP3) and the pharma kinetics score of 0.55.

*Conclusion and Future Prospects:*

This study aimed to determine the connection between CRY1, PER1/2, and Trastuzumab. The study used the STRING database, which yielded a PPI p-value of 0.00193, to demonstrate the substantial connection between CRY and PER1/2. With the help of bio find out where the drug gets binds to the target protein by which amino acid particularly. Also, the docking study revealed a notable link between Trastuzumab and the Circadian Clock Protein Complex, with an estimated ΔG value of -4.02 kcal/mol. This result verifies the drug which already used for breast cancer treatment can be used for a sleep disorder.

Our findings show that trastuzumab interacts with the proteins well with a great binding energy of -4.02kcal/mol. Moreover, it is used in the early phases of the disease. Both chemotherapy (paclitaxel or carboplatin) and cutting-edge molecularly directed and biological therapies can be used with trastuzumab. also have a robust 5.8-day half-life.

To be employed as a potential medicine for treating sleep problems, this study can be successfully demonstrated in clinical laboratories.

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