IN-SILICO STUDIES OF RBP7, CALML3, C35, LGALSL, S100A3 PROTEINS INVOLVED IN BREAST CANCER

Ms. Mini Fernandez
Assistant Professor
Department of Biotechnology,
St. Francis College for Women, Hyderabad

Ms. D. Keerthana Goud
Department of Biotechnology, St. Francis College for Women, Hyderabad

https://doi.org/10.34293/ctbtls.2025.ch013

Abstract

The most important reason for death caused by cancer in females around the globe is breast cancer. Early diagnosis, proper treatment are fundamental in order to enhance the outcomes of patients. This study aims to investigate various proteins that contribute to the development and progression of breast cancer. Proteins that were chosen for this work are: Retinoid-binding protein 7, Calmodulin-like protein 3, C35 protein, Galectin-related protein and S100-A3 protein. In addition, three drugs: Raloxifene, Tamoxifen, and Fulvestrant were chosen to check if they have therapeutic effects on the above-mentioned proteins. By studying how these proteins interact with drugs, we can gain important information that could help create effective treatment. The protein sequences were retrieved from biological databases. Homology modelling was done to produce models for the selected proteins using SWISS-MODEL, then validated with help of 'SAVES v6.0' and 'ProSA'. To identify places where drug would bind to the protein, surface pockets were characterized with the employment of 'CASTp'. Each protein was then docked to three drugs using Vina in 'PyRx' and the most suitable conformation of the ligand was selected via splitting the ligand using 'AutoDock Vina'. The protein-drug interactions were then visualised in 'Biovia Discovery Studio'. The models were generated for proteins and were docked to the ligands. As predicted, the ligands successfully attached to amino acid residues in active site of the proteins by Hydrophobic bond, Hydrogen bond, Electrostatic Bond. By studying interactions between the proteins and the drugs, it is analysed about how the drug help to slow the progression of breast cancer. This highlights their potential as targeted treatments that could help stop tumor growth. This study also interprets which drug has more or less therapeutic effect for each protein that help in better treatment.

Keywords: *Breast cancer, Effective treatment, Homology modelling, Drug docking, Protein-drug interactions.*

Introduction

Breast cancer is a common cancer that affects many people but can be severe or fatal. It is a heterogeneous disease caused by genetic and environmental factors. (Khadijeh Barzaman 1, 2020) The breasts are two glands of different sizes and densities, sitting atop the chest muscles. (Ashley Alex 1, 2020) Breast cancer typically begins in the milk ducts, known as ductal carcinoma, but it can also start in the milk-producing lobules, called lobular carcinoma. Usually detected by a physical examination, imaging tests, and by biopsy; treatment depends on each case, and may include surgery, chemotherapy, radiation, hormonal therapy, and now, immunotherapy. (Dharambir Kashyap 1, 2022)

There are many different types of breast cancer, which can be identified through genetic and molecular testing. Tumors are often made up of different types of cells, so a tissue biopsy might not capture the full genetic diversity of the cancer. (Arielle J Medford 1 2, 2018)

The pathogenesis of breast cancer stem cells has helped us better understand how the disease develops and why it can become resistant to treatment. (Yi-Sheng Sun 1, 2017) A number of risk factors have been well established, including race, ethnicity, family history of cancer, genetic traits and lifestyle factors.

We have also found that mutations of some genes such as BRCA1, BRCA2, and others, might be involved in some cases of breast cancer. (Coughlin, 2019)

Among various diagnosis techniques, imaging techniques are the best methods for the identification of the breast cancer. Techniques like Mammography, Magnetic resonance imaging (MRI), Computes tomography (CT), positron-emission tomography (PET), and single-photon emission computed tomography (SPECT) are used for diagnosis in various stages. (Seyed Hamed Jafari, 2018)

Different types of breast cancer and different stages consists of different treatment. The treatment of ductal carcinoma in situ is normally breast conserving surgery followed by radiotherapy. In stage I and II breast cancer, surgery with conservation of the breast with radiation therapy is the routine treatment. The original tumor may be eliminated by either breast-conserving surgery known as mastectomy. (Karen L Maughan, 2010)

Retinoid-binding protein 7 (RBP7), which belongs to the cellular retinol-binding protein (CRBP) family, is involved in breast cancer development. The current study seeks to investigate the prognostic value of RBP7 expression and its possible regulatory pathways in breast cancer. (Hong Lin, 2022)

Human calmodulin-like protein (CLP) is one of the calcium-binding proteins that usually get downregulated in various laboratory models of mammary tumorigenesis as well as in most breast cancers in man. (M S Rogers, 2001)

It is known that many invasive breast cancers generally overexpress C35, and in cell lines, it acts as an oncogene. It is expressed in normal breast epithelium, but it gets downregulated in breast cancer. (Wen Che, 2017) C35 is expressed in all four subtypes of breast cancer. (Kun Yin, 2015)

Galectin like protein (LGALSL) is a family of carbohydrate-binding proteins. (Mandika Chetry, 2022) Galectin-like protein is also implicated in tumorigenesis, metastasis, and immune evasion, all the more confirming its role in breast cancer pathogenesis. (Doudou Georges Massar Niang, 2022)

S-100 protein has been identified in a number of normal and neoplastic cells, including malignant ones among them. It has been found in normal and malignant breast tissues. In benign breast tissue, S-100 protein was located in myo-epithelial cells and epithelial cells of terminal duct and lobule. (S Dwarakanath, 2015)

Materials and Methods

1) Homology Modelling

The target and template proteins were selected using biological databases. They are NCBI, BLAST, PDB, and ExPASy. Selection criteria included proteins with fewer than 250 amino acids, those that had been reviewed in UniProtKB and those with relevant publications.

2) Model Generation and Validation

3D models were generated using tools like SWISS-MODEL, a server which is fully automated tool for protein structure homology modeling, available through the Expasy web platform.

Biovia discovery studio, a software used for simulations small molecules and macromolecules. PROCHECK, VERIFY 3D of the SAVES v6.1, a server tool used to evaluate the stereochemical quality of the protein. ProSa, a web tool used to identify any potential structural errors.

3) Active Site Prediction

The active sites of the generated models were predicted using CASTpFold, a platform which primarily facilitated the analytical identification of the pockets and cavities of the proteins.

4) Selection of Ligands

Ligands were selected from PubChem, a online database managed by National Library of Medicine (NLM) at the National Institute of Health (NIH). It is a resource that contains detailed information on chemicals, including their structural properties and biological effects. The selected drugs are Raloxifene, Tamoxifen and Fulvestrant.

5) Docking of Ligands and Macromolecules

Docking was performed using PyRx, a virtual screening tool used for docking target drugs with macromolecules for Drug Discovery. Splitting ligands was employed by AutoDock Vina through Command prompt. Finally, Biovia discovery studio used to study the interactions between the ligands and the the proteins.

Results and Discussion

1) Homology Modelling

Selection of target proteins: The accession numbers: NP_443192, NP_005176, AAR92035, NP_054900 and NP_002951 were obtained from NCBI of the RBP7(Retinoid-binding protein 7), CALML3 (Calmodulin like protein 3), C35 protein, LGALSL(Galectin like protein), S100-A3 protein and their respective FASTA sequences were obtained from UniProtKB.

Selection of template proteins: The accession codes obtained from BLAST (6AT8_A, 1GGZ_A, 1LJK_A, 2JJ6_A, 3NSL_A) with query coverage over 70% and identity above 27%. These codes were used to search PDB, where the template proteins were examined for the

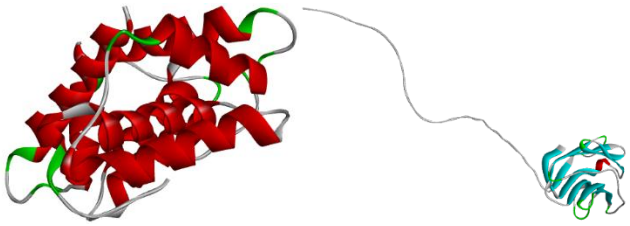
percentage of the unmodeled region in the 'sequence' section. The PDB files for the respective proteins were then downloaded.

Multiple sequence alignment: Multiple sequence alignments were performed using Clustal Omega, with the output format set to the 'Pearson/Fasta' format. Additionally, with the output format set to 'ClustalW with character counts' comparing the target proteins to the template proteins.

2) Model Generation

The models were generated for each protein accordingly using swiss model using the target sequences that were retrieved from blast searches.

(a) (b) (c)



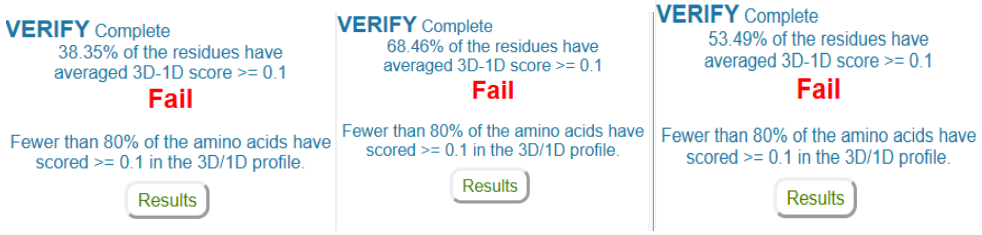
d) e)

Figure 1: The Generated Models of the Proteins a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3 using SWISS-MODEL

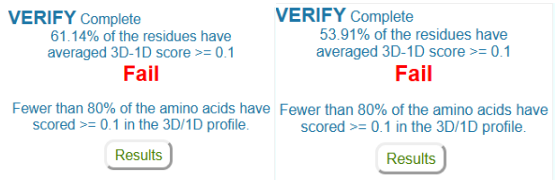
3) Model Validation

The modelled proteins were uploaded to SAVES v6.0, where the 'VERIFY 3D' and 'PROCHECK' programs were applied to each model. The resulting scores from 'VERIFY 3D', the PROCHECK summary, and the Ramachandran plots for the generated models are presented below:

Verify 3D



a) b) c)



d) e)

Figure 2: Verify 3D Results Obtained for the Protein Models a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

Procheck Summaries

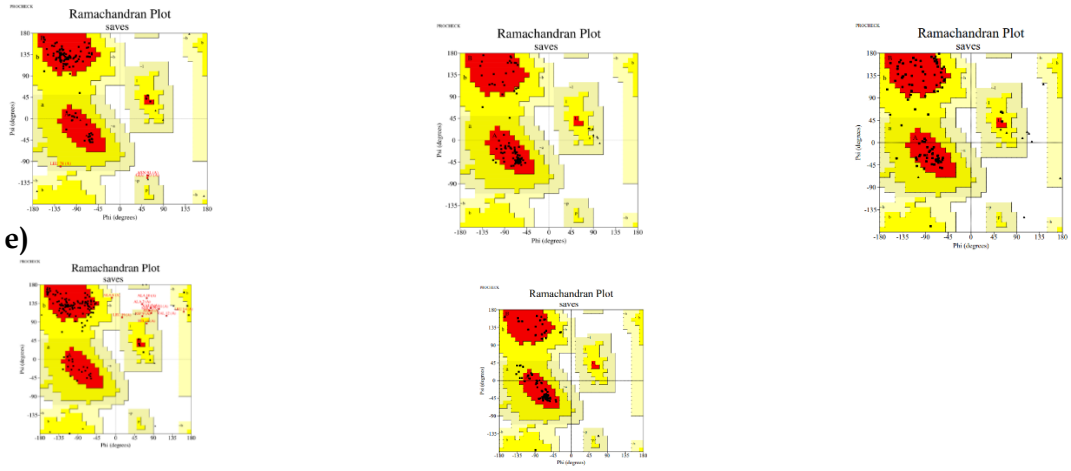
a) b) c)

d) e)

Figure 3: PROCHECK Summaries for the Models a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

Ramachandran Plots

a) b) c)



d) e)

Figure 4: RAMACHANDRAN PLOTS for the models a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

The three-dimensional (3D) profile of a protein is a table derived from its atomic structure, which is utilized to determine how accurately a specific amino acid sequence accommodates in that structure and also to check the quality of the model. The 3D profile can be applied to verify the correctness of a protein model. For additional verification, Procheck is utilized to check for more parameters. (D Eisenberg, 1997) As each model has more than 90% of its residues in the strongly favored area of the Ramachandran plot, they are considered valid for further studies.

The generated models were further validated using ProSA, and the results obtained are as follows:

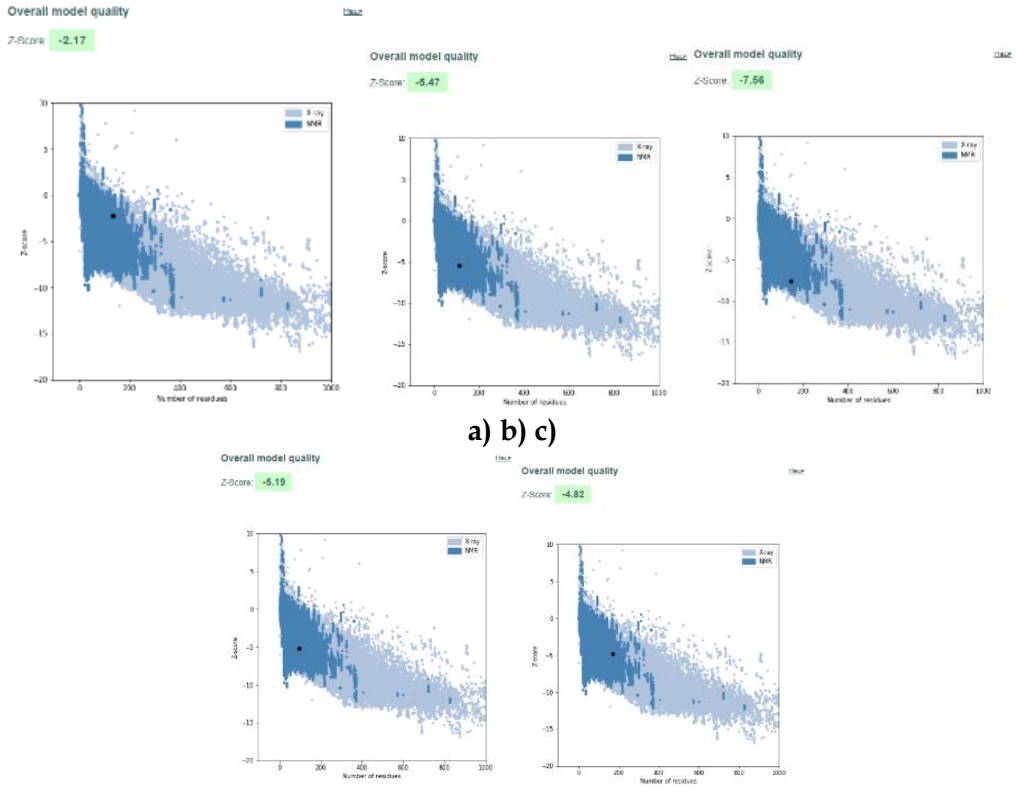


Figure 5: Z-score Graphs Obtained for the Protein Models a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

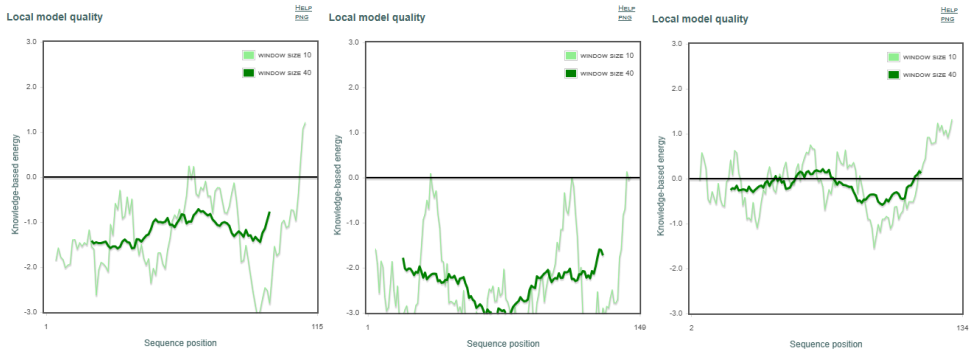


Figure 6: Local Model Quality Graphs Obtained for the Models a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

The ProSA (Protein Structure Analysis) software is also a popular resource used for quality improvement and refinement of experimental protein structures, validation, structure modeling, and structure prediction. ProSA's interactive web-based servers yield visual depictions of the scores and the energy plots with which to visualize problems in proteins. (Markus Wiederstein, 2007)

4) Active Site Prediction

The active sites in the generated models are predicted using CASTpFold are as follows:

Table 1: Summary of Volume and Amino Acid Residues

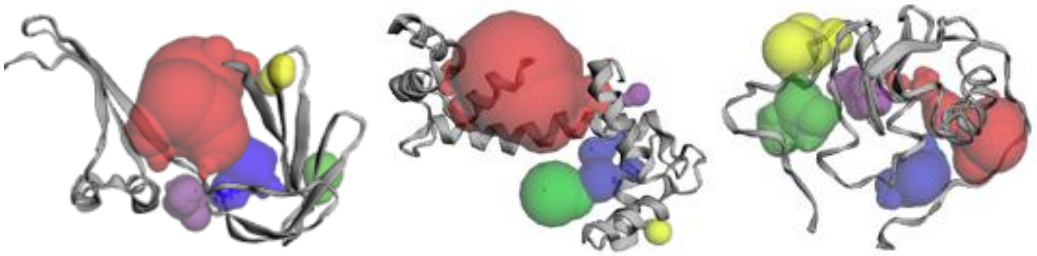
Pocket ID	Area (SA) (Å²)	Volume (SA) (Å³)
1	310.384	184.297
2	80.866	97.106
3	12.461	23.991
4	22.629	9.195
5	12.357	5.974

Pocket ID	Area (SA) (Å²)	Volume (SA) (Å³)
1	814.878	1855.467
2	185.967	78.691
3	13.854	32.912
4	11.495	4.568
5	16.678	3.33

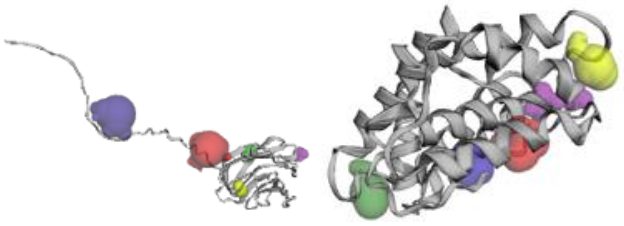
Pocket ID	Area (SA) (Å²)	Volume (SA) (Å³)
1	235.872	186.166
2	114.236	86.246
3	149.626	80.811
4	101.686	73.787
5	96.533	31.944

Pocket ID	Area (SA) (Å²)	Volume (SA) (Å³)
1	219.465	289.659
2	90.907	204.178
3	30.596	11.772
4	25.151	8.356
5	23.663	7.877

Pocket ID	Area (SA) (Å²)	Volume (SA) (Å³)
1	61.767	21.47
2	57.066	19.407
3	40.535	18.081
4	39.946	17.59
5	41.595	7.081



(a) (b) (c)

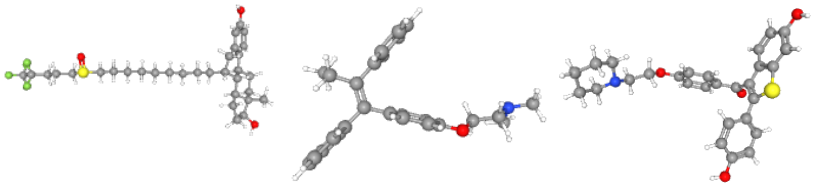


(d) (e)

Figure 7: Pockets of the models a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

The Computed Atlas of Surface Topography of Proteins (CASTp) is a popular web server intended to find, locate, and quantify the geometric and topological properties of protein structures.

These properties, such as surface pockets, interior cavities, and cross channels, are vital for protein activity. CASTp offers accurate and thorough analysis of protein topography to assist in the investigation of their structural and functional characteristics. (Bowei Ye, 2024)



a) b) c)

Figure 8: 3D Structures of the Drugs: a) Raloxifene b) Tamoxifen c) Fulvestrant

5) Selection of Ligands

Through a literature review on PubMed, three drugs: Raloxifene, Tamoxifen and Fulvestrant were identified for the treatment of Breast cancer.

PubChem is an open repository containing data on small molecules and their biological activities. It offers 3D conformer models for 92.3% of the compounds in its repository. The drugs were saved in SDF format and later converted to PDB format for docking studies, enabling researchers to analyze interactions between every drug (as a ligand) and target proteins.

6) Docking of Ligand and Macromolecule

The three selected ligands were docked to each protein in PyRx using Vina. Once the program was run, the results which showed the binding affinity and RMSD values were then analyzed.

Table 2: A Summary of the Binding Affinity and RMSD Values for the Different Conformations of the Ligands Docked to the Proteins: a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
calmudin.pdb_raloxifene	-7.4	0	0
calmudin.pdb_raloxifene	-7.4	5.126	1.134
calmudin.pdb_raloxifene	-7	16.913	14.806
calmudin.pdb_raloxifene	-6.8	17.381	14.576
calmudin.pdb_raloxifene	-6.8	16.451	14.572
calmudin.pdb_raloxifene	-6.7	17.179	14.316
calmudin.pdb_raloxifene	-6.6	16.005	13.996
calmudin.pdb_raloxifene	-6.6	17.749	14.973
calmudin.pdb_raloxifene	-6.4	15.139	12.978
calmudin.pdb_tamoxifen	-6.2	0	0
calmudin.pdb_tamoxifen	-6.1	4.642	1.195
calmudin.pdb_tamoxifen	-5.9	24.574	20.812
calmudin.pdb_tamoxifen	-5.7	17.411	14.828
calmudin.pdb_tamoxifen	-5.6	23.567	20.413
calmudin.pdb_tamoxifen	-5.5	2.84	1.825
calmudin.pdb_tamoxifen	-5.5	4.959	1.983
calmudin.pdb_tamoxifen	-5.4	17.213	14.622
calmudin.pdb_tamoxifen	-5.4	6.702	3.541
calmudin.pdb_fulvestrant	-7.4	0	0
calmudin.pdb_fulvestrant	-7.3	6.331	2.999
calmudin.pdb_fulvestrant	-7.1	7.897	5.082
calmudin.pdb_fulvestrant	-7	9.831	5.763
calmudin.pdb_fulvestrant	-6.9	19.344	15.556
calmudin.pdb_fulvestrant	-6.8	18.495	15.916
calmudin.pdb_fulvestrant	-6.8	18.219	15.078
calmudin.pdb_fulvestrant	-6.8	17.941	15.592
calmudin.pdb_fulvestrant	-6.7	18.059	15.634

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
retinoid.pdb_raloxifene	-8.1	0	0
retinoid.pdb_raloxifene	-7.2	9.503	3.946
retinoid.pdb_raloxifene	-6.8	30.105	27.772
retinoid.pdb_raloxifene	-6.8	7.482	3.117
retinoid.pdb_raloxifene	-6.6	31.096	28.81
retinoid.pdb_raloxifene	-6.6	15.211	11.413
retinoid.pdb_raloxifene	-6.6	7.447	5.991
retinoid.pdb_raloxifene	-6.3	16.619	14.315
retinoid.pdb_raloxifene	-6.2	7.996	3.821
retinoid.pdb_tamoxifen	-6	0	0
retinoid.pdb_tamoxifen	-5.8	4.807	3.265
retinoid.pdb_tamoxifen	-5.5	5.469	3.459
retinoid.pdb_tamoxifen	-5.5	30.992	27.996
retinoid.pdb_tamoxifen	-5.5	16.848	13.427
retinoid.pdb_tamoxifen	-5.5	31.76	28.764
retinoid.pdb_tamoxifen	-5.5	6.928	3.938
retinoid.pdb_tamoxifen	-5.5	31.857	28.729
retinoid.pdb_tamoxifen	-5.4	31.638	28.512
retinoid.pdb_fulvestrant	-5.4	0	0
retinoid.pdb_fulvestrant	-5.1	7.522	3.177
retinoid.pdb_fulvestrant	-5.1	3.836	3.199
retinoid.pdb_fulvestrant	-4.9	6.268	2.834
retinoid.pdb_fulvestrant	-4.9	6.309	3.293
retinoid.pdb_fulvestrant	-4.8	5.249	2.471
retinoid.pdb_fulvestrant	-4.8	6.131	2.326
retinoid.pdb_fulvestrant	-4.8	6.668	3.91
retinoid.pdb_fulvestrant	-4.7	6.799	3.427

(a) (b)

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
c35_raloxifene	-7.3	0	0
c35_raloxifene	-7.2	13.484	10.17
c35_raloxifene	-7.1	5.105	1.77
c35_raloxifene	-7	13.834	10.089
c35_raloxifene	-6.9	14.319	11.945
c35_raloxifene	-6.9	12.387	6.446
c35_raloxifene	-6.9	7.475	3.324
c35_raloxifene	-6.8	15.113	12.38
c35_raloxifene	-6.8	13.919	11.002
c35_tamoxifen	-6.3	0	0
c35_tamoxifen	-6	6.346	3.032
c35_tamoxifen	-5.9	17	15.13
c35_tamoxifen	-5.8	14.121	11.822
c35_tamoxifen	-5.8	6.988	3.403
c35_tamoxifen	-5.7	6.558	2.911
c35_tamoxifen	-5.7	6.627	3.26
c35_tamoxifen	-5.7	14.018	11.607
c35_tamoxifen	-5.6	4.973	3.197
c35_fulvestrant	-6.9	0	0
c35_fulvestrant	-6.7	9.872	4.443
c35_fulvestrant	-6.3	9.715	4.259
c35_fulvestrant	-6.3	3.678	2.366
c35_fulvestrant	-6.3	10.106	4.11
c35_fulvestrant	-6.3	5.655	2.737
c35_fulvestrant	-6.2	3.146	2.217
c35_fulvestrant	-6.1	9.895	4.588
c35_fulvestrant	-6.1	19.935	17.871

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
galection.pdb_raloxifene	-6	0	0
galection.pdb_raloxifene	-5.8	20.385	16.497
galection.pdb_raloxifene	-5.8	20.842	16.819
galection.pdb_raloxifene	-5.7	8.138	4.429
galection.pdb_raloxifene	-5.6	24.479	19.849
galection.pdb_raloxifene	-5.4	18.83	14.98
galection.pdb_raloxifene	-5.4	8.619	4.296
galection.pdb_raloxifene	-5.3	11.801	6.729
galection.pdb_raloxifene	-5	16.941	13.689
galection.pdb_tamoxifen	-5.5	0	0
galection.pdb_tamoxifen	-5.2	5.866	4.038
galection.pdb_tamoxifen	-5	15.105	13.148
galection.pdb_tamoxifen	-5	23.113	20.418
galection.pdb_tamoxifen	-4.9	4.396	2.918
galection.pdb_tamoxifen	-4.9	18.824	15.005
galection.pdb_tamoxifen	-4.9	5.334	2.236
galection.pdb_tamoxifen	-4.8	30.542	26.859
galection.pdb_tamoxifen	-4.7	31.298	27.917
galection.pdb_fulvestrant	-5.6	0	0
galection.pdb_fulvestrant	-5.4	3.469	2.713
galection.pdb_fulvestrant	-4.6	22.448	19.805
galection.pdb_fulvestrant	-4.6	31.369	28.107
galection.pdb_fulvestrant	-4.5	31.926	28.402
galection.pdb_fulvestrant	-4.4	22.537	19.113
galection.pdb_fulvestrant	-4.3	5.013	3.724
galection.pdb_fulvestrant	-4.3	33.489	30.77
galection.pdb_fulvestrant	-4.3	15.432	11.999

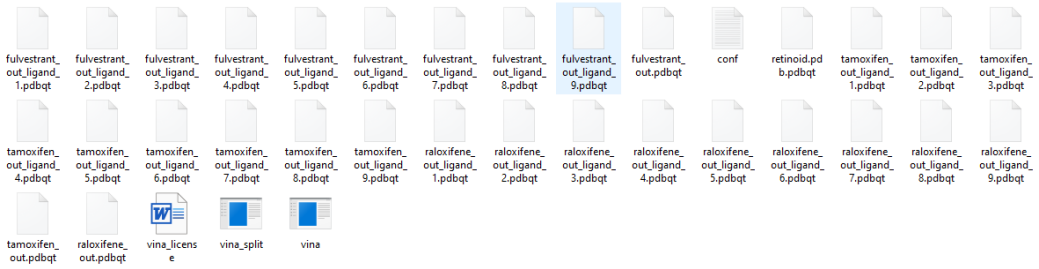
c) d)

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
S100A3_raloxifene	-7.3	0	0
S100A3_raloxifene	-7.2	29.304	24.585
S100A3_raloxifene	-7	24.237	22.288
S100A3_raloxifene	-6.9	6.072	3.128
S100A3_raloxifene	-6.8	34.919	33.01
S100A3_raloxifene	-6.6	25.175	22.299
S100A3_raloxifene	-6.6	34.402	32.466
S100A3_raloxifene	-6.6	29.618	25.706
S100A3_raloxifene	-6.5	25.058	22.217
S100A3_tamoxifen	-6.1	0	0
S100A3_tamoxifen	-6	16.232	13.582
S100A3_tamoxifen	-5.9	15.702	10.863
S100A3_tamoxifen	-5.9	6.715	2.918
S100A3_tamoxifen	-5.8	4.613	1.068
S100A3_tamoxifen	-5.7	27.114	22.844
S100A3_tamoxifen	-5.6	29.315	24.376
S100A3_tamoxifen	-5.5	14.028	11.464
S100A3_tamoxifen	-5.5	33.157	29.31
S100-A3_fulvestrant	-5.3	0	0
S100-A3_fulvestrant	-5.2	34.769	32.261
S100-A3_fulvestrant	-5.2	24.712	21.561
S100-A3_fulvestrant	-5.1	24.547	21.339
S100-A3_fulvestrant	-5.1	26.273	22.974
S100-A3_fulvestrant	-5	25.903	22.237
S100-A3_fulvestrant	-5	24.339	21.189
S100-A3_fulvestrant	-5	25.275	21.522
S100-A3_fulvestrant	-4.9	2.127	1.532

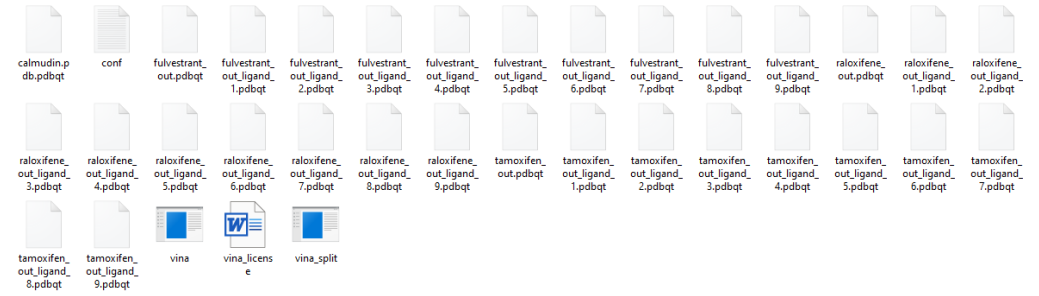
e)

7) Splitting of Ligands

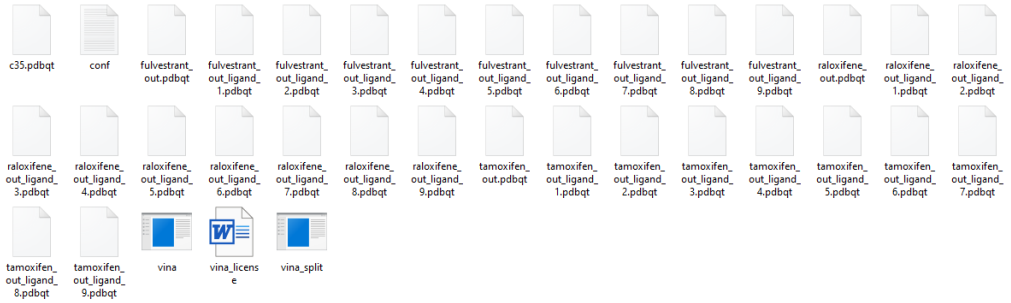
The docking data for each protein-ligand interaction was saved in the folder in the following path: 'C:\Users\ADMIN\.mgltools\PyRx\Macromolecules'



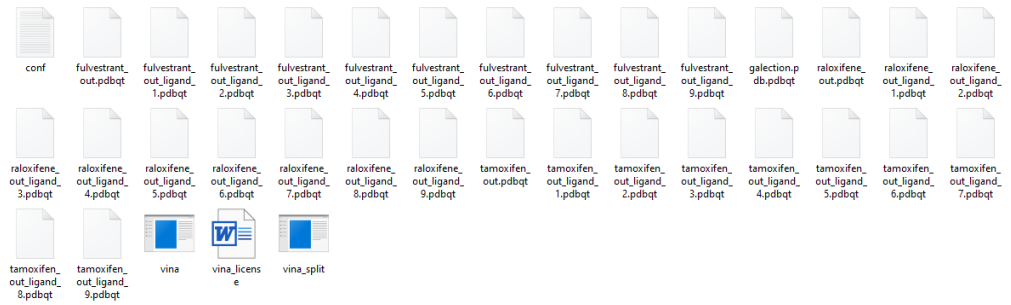
a)



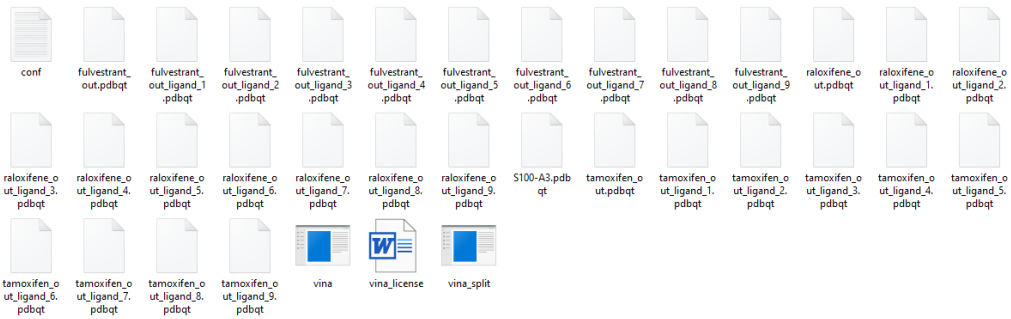
b)



c)



d)



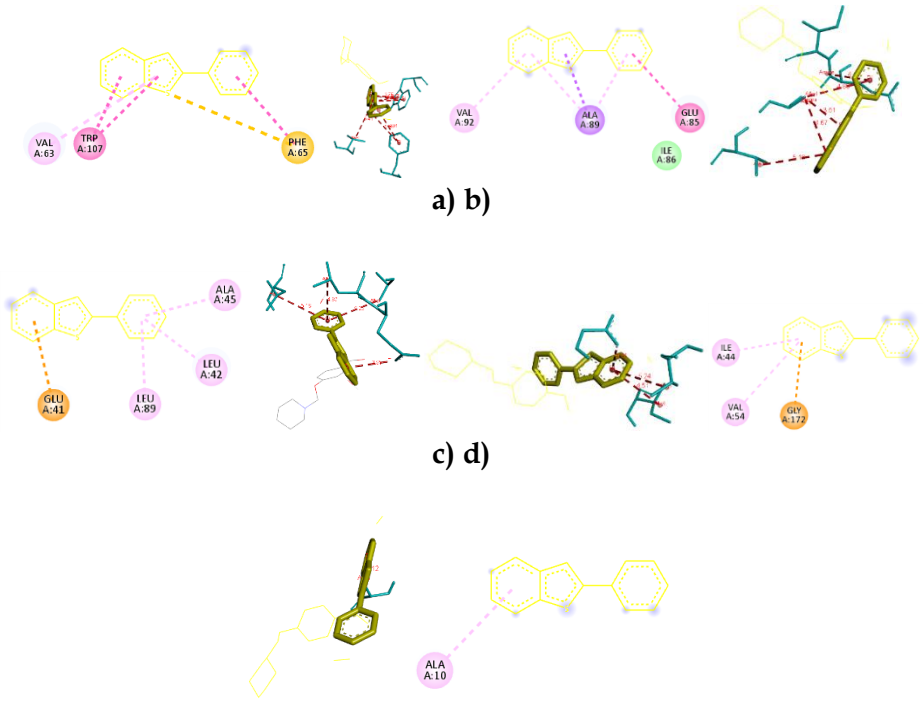
e)

Figure 9: Split Ligand Files in Different Conformations for Protein Accordingly

AutoDock Vina is used to split the ligands into different conformations in the 'pdbqt' format, considering the RMSD values and binding energies. The conformation with the lowest binding energy and 0 RMSD is chosen for the final step, where the interactions between the drug and protein are visualized.

8) Visualization of Interactions between Ligands and Proteins

The interactions between the drugs and each protein were visualized using Discover Studio.



a) b)

c) d)

e)

Figure 10: 2D and 3D representations of the Interactions between Raloxifene and a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

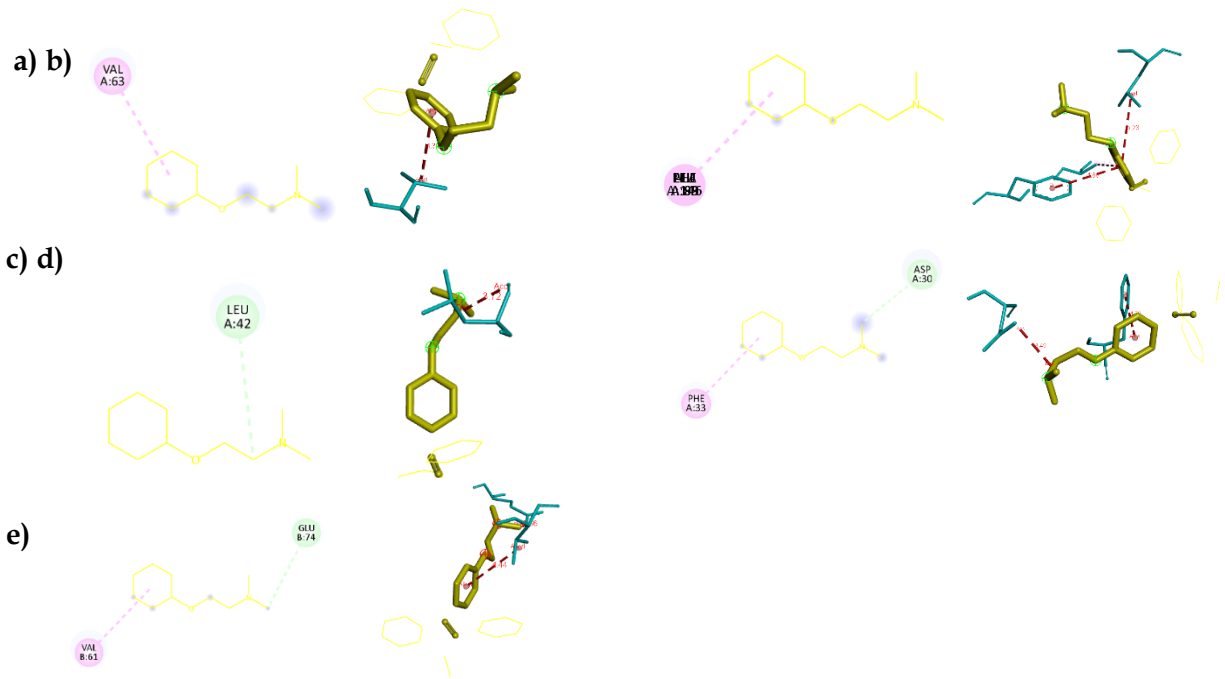


Figure 11: 2D and 3D Representations of the Interactions between Tamoxifen and a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

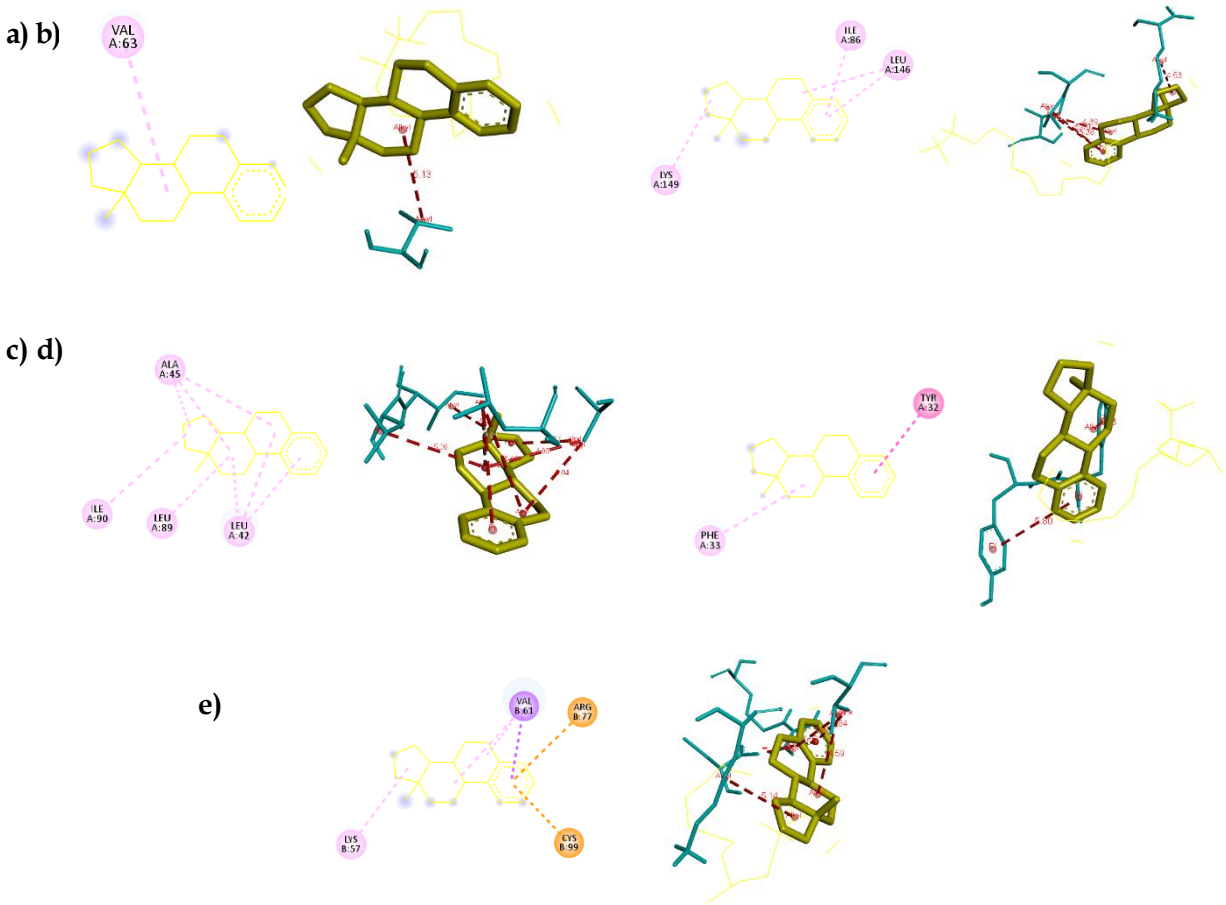


Figure 12: 2D and 3D Representations of the Interactions between Fulvestrant and a) RBP7 b) CALML3 c) C35 d) LGALSL e) S100-A3

The interactions between the ligand molecules (drugs) and all the five proteins were visualized in 2D and 3D using Biovia Discovery Studio. Bonds such as hydrogen bonds, hydrophobic and electrostatic interactions were seen between the ligands and certain residues of the proteins that indicate the possible binding affinity of the ligand-protein complex. Also, the existence of the salt bridges between the drug and amino acid residues indicates possible stabilization of the ligand-protein complex. Existence of traditional hydrogen bonds, is responsible for the stability of the drug- protein complex. All of these interactions are defined by pi-sigma and pi-alkyl bonds, proposes favorable binding between the ligand and the protein that modulates the function of the protein and interfere with cancer cell growth and survival pathways.

Conclusion

This study aims to understand the proteins and their role in breast cancer as well as protein-ligand interactions in order to find possible therapeutic medications. Molecular docking was successfully accomplished to assess the binding affinities between the proteins and ligands. The findings unveiled that the ligand showed favorable binding interactions

with target protein's active site with a binding affinity score. This implies that the ligand might be a viable option for more medication research or therapeutic uses.

Furthermore, the docking research revealed important interactions that affect the complex's stability and specificity, including hydrophobic, electrostatic, and hydrogen bonds. To confirm these results and evaluate the ligand's pharmacological potential, more experimental research will be required.

References

1. Alex, A., Bhandary, E., & McGuire, K. P. (2020). Anatomy and Physiology of the Breast during Pregnancy and Lactation. *Advances in experimental medicine and biology*, *1252*, 3–7. https://doi.org/10.1007/978-3-030-41596-9_1
2. Che, W., Bao, Y., & Tang, F. (2017). Down-regulation of C35 decreased the cell viability and migration of breast ductal carcinoma cells. *PloS one*, *12*(8), e0183941. https://doi.org/10.1371/journal.pone.0183941
3. Debald, M., Schildberg, F. A., Linke, A., Walgenbach, K., Kuhn, W., Hartmann, G., & Walgenbach-Brünagel, G. (2013). Specific expression of k63-linked ubiquitination of calmodulin-like protein 5 in breast cancer of premenopausal patients. *Journal of cancer research and clinical oncology*, *139*(12), 2125–2132. https://doi.org/10.1007/s00432-013-1541-y
4. Dwarakanath, S., Lee, A. K., Delellis, R. A., Silverman, M. L., Frasca, L., & Wolfe, H. J. (1987). S-100 protein positivity in breast carcinomas: a potential pitfall in diagnostic immunohistochemistry. *Human pathology*, *18*(11), 1144–1148. https://doi.org/10.1016/s0046-8177(87)80382-9
5. Evans, E. E., Henn, A. D., Jonason, A., Paris, M. J., Schiffhauer, L. M., Borrello, M. A., Smith, E. S., Sahasrabudhe, D. M., & Zauderer, M. (2006). C35 (C17orf37) is a novel tumor biomarker abundantly expressed in breast cancer. *Molecular cancer therapeutics*, *5*(11), 2919–2930. https://doi.org/10.1158/1535-7163.MCT-06-0389
6. Jafari, S. H., Saadatpour, Z., Salmaninejad, A., Momeni, F., Mokhtari, M., Nahand, J. S., Rahmati, M., Mirzaei, H., & Kianmehr, M. (2018). Breast cancer diagnosis: Imaging techniques and biochemical markers. *Journal of cellular physiology*, *233*(7), 5200–5213. https://doi.org/10.1002/jcp.26379
7. Kashyap, D., Pal, D., Sharma, R., Garg, V. K., Goel, N., Koundal, D., Zaguia, A., Koundal, S., & Belay, A. (2022). Global Increase in Breast Cancer Incidence: Risk Factors and Preventive Measures. *BioMed research international*, *2022*, 9605439. https://doi.org/10.1155/2022/9605439 (Retraction published Biomed Res Int. 2023 Dec 29; 2023:9872034. doi: 10.1155/2023/9872034.)

8. Lin, H., Han, Q., Wang, J., Zhong, Z., Luo, H., Hao, Y., & Jiang, Y. (2022). Methylation-Mediated Silencing of *RBP7* Promotes Breast Cancer Progression through PPAR and PI3K/AKT Pathway. *Journal of oncology*, *2022*, 9039110. https://doi.org/10.1155/2022/9039110
9. Maughan, K. L., Lutterbie, M. A., & Ham, P. S. (2010). Treatment of breast cancer. *American family physician*, *81*(11), 1339–1346.
10. Medford, A. J., Gillani, R. N., & Park, B. H. (2018). Detection of Cancer DNA in Early Stage and Metastatic Breast Cancer Patients. *Methods in molecular biology (Clifton, N.J.)*, *1768*, 209–227. https://doi.org/10.1007/978-1-4939-7778-9_13
11. Niang, D. G. M., Gaba, F. M., Diouf, A., Hendricks, J., Diallo, R. N., Niang, M. D. S., Mbengue, B., & Dieye, A. (2022). Galectin-3 as a biomarker in breast neoplasms: Mechanisms and applications in patient care. *Journal of leukocyte biology*, *112*(5), 1041–1052. https://doi.org/10.1002/JLB.5MR0822-673R
12. Veronesi, U., Boyle, P., Goldhirsch, A., Orecchia, R., & Viale, G. (2005). Breast cancer. *Lancet (London, England)*, *365*(9472), 1727–1741. https://doi.org/10.1016/S0140-6736(05)66546-4
13. Veronesi, U., Boyle, P., Goldhirsch, A., Orecchia, R., & Viale, G. (2005). Breast cancer. *Lancet (London, England)*, *365*(9472), 1727–1741. https://doi.org/10.1016/S0140-6736(05)66546-4
14. Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic acids research*, *35*(Web Server issue), W407–W410. https://doi.org/10.1093/nar/gkm290