Docking study of Paracetamol (N-acetyl-p-aminophenol) with Bovine Serum Albumin: A Case Study at the Undergraduate Level

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Abstract

This study mainly focuses on the molecular docking of Paracetamol with Bovine Serum Albumin. Both geometry optimisation of the Paracetamol molecule and docking were done using Argus Lab Software. Docking calculations showed that the binding free energy of -8.6759 Kcal/mol, which indicates a stable and favourable protein-ligand interaction. The study highlights the potential implications for paracetamol's pharmacokinetics and supports the educational value of molecular docking exercises in undergraduate research.

Keywords: Molecular Docking, Hydrogen Bonding, Binding Affinity, Computational Chemistry,Pharmacokinetics

INTRODUCTION

Under Molecular docking, studies of some popular & frequently used medicines with proteins in the biological systems are an area of interest for students at the UG level, as NEP 2020 [1] recommended for a four-year UG program, after which students, the young learners, can be encouraged to hypothesise the drug action and be provided with initial research training. Keeping the above in mind, a project titled "Docking Study of Paracetamol with Bovine Serum Albumin" was assigned to third-year students of B.Sc. (H) Chemistry. Molecular docking is a computational technique widely recognised as a fundamental tool in the fields of structural molecular biology and computer-assisted drug design [2]. This method predicts the preferred orientation of one molecule

relative to another when they bind together to form a stable complex.

The primary objective of molecular docking is to achieve an optimised conformation for both protein and ligand, as well as their relative orientation to minimise the system's free energy [3].

The main principle is molecular recognition, where we identify the complementarity between the surfaces of molecules, which governs the binding interaction. The docking process identifies the shape complementarity, electrostatic interactions, Van der Waals forces, hydrogen bonding, and hydrophobic interactions to measure the binding potential. The binding potential is represented by the docking score. This process is repeated to explore various ligand conformations till we achieve the minimum potential energy [4].

In the present study, Bovine Serum Albumin (BSA) was selected as the target protein, and Paracetamol as the ligand. BSA is a globular serum protein with 583 amino acids arranged in a single polypeptide chain and a molecular weight of approximately 66,400 Da [5]. It is frequently used as a model protein in molecular docking and pharmacokinetic studies due to its structural similarity with human serum albumin and its well-characterised ligand-binding properties [5,6].

Recently, the experimental investigations of Paracetamol with BSA using fluorescence and NMR studies have been done [7]. The docking studies of paracetamol with different proteins have also been studied [

Paracetamol is a widely used nonsteroidal anti-inflammatory drug (NSAID), analgesic and antipyretic agent with the molecular formula $C_8H_9NO_2$ and molecular weight of 151.17 Da [8-9].

It is an important molecule for computational drug design studies & molecular docking because of its well-established therapeutic profile and diverse molecular interactions [8-10].

OBJECTIVES OF THE PAPER

- To highlight the educational benefits and practical value of introducing molecular

docking experiments in undergraduate curricula, emphasising their role in enhancing interdisciplinary understanding, computational skills, and career readiness.

- To conduct a molecular docking experiment with the paracetamol molecule as ligand against BSA as target protein, considering enzymes and receptors involved in its pharmacological effects.
- To evaluate the binding affinity, key molecular interactions, and stability of the docked complexes with the help of computational tools
- To compare the docking results with standard ligands and inhibitors to assess the potential efficacy of paracetamol.

· **Software & Tools**: Argus Lab-free available software [11]

· **Protein Selection**: Obtained from the Protein Data Bank (PDB), having code 4F5S [12].

· **Ligand Preparation**: The 3D structure of the Paracetamol molecule used was optimised using Austin Model 1(AM1) on the same software Argus Lab.

METHODOLOGY

1. The BSA protein structure was downloaded from the Protein Data Bank in PDB format. The structure was then imported into Argus Lab software, where non-essential components, including water molecules and miscellaneous heteroatoms, were removed from the residue list to prepare the protein for docking simulations. (Figure 1)

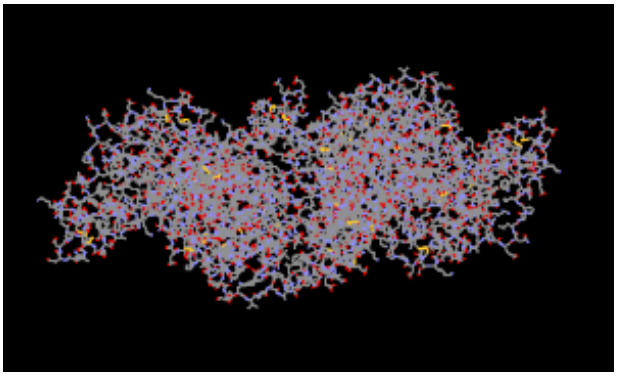

Figure 1: The BSA Protein from which water and misc. has removed.

2. All amino acid residues from chain A were selected in the residue panel, and hydrogen atoms were added to complete the structure

for docking preparation. (Figure 2)

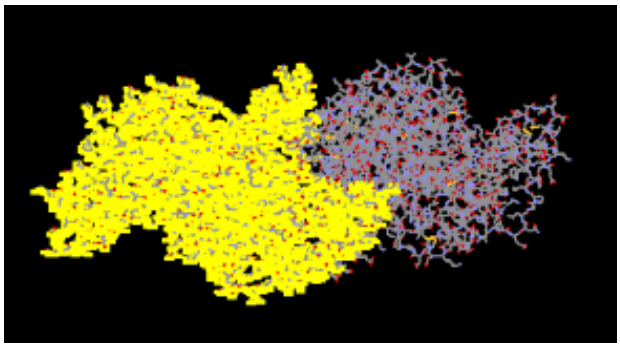

Figure 2: Chain A of BSA highlighted in yellow, representing amino acid residues with added hydrogen atoms for docking preparation.

3. The first amino acid of chain A was selected from the residue panel, and a binding site was defined by creating a group from the selected residue using the context menu. (Figure 3)

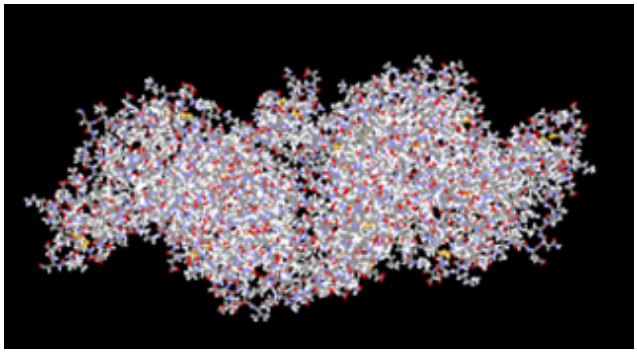

Figure 3: Amino acid residues of Chain A from the BSA protein. The first residue is highlighted to define the binding site for docking studies.

4. The optimised drug molecule (Final Geometrical Energy (-46024.7038 kcal/mol) was imported into the same Argus Lab workspace, where the 1DEF structure was selected and designated as a ligand by creating a ligand group via the context menu. (Figure 4)

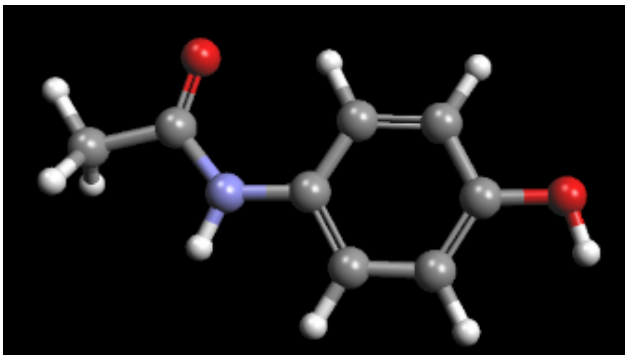

Figure 4: The optimised geometry of the paracetamol molecule.

5. **Orbital and ESP Visualisation:** Highest Occupied Molecular Orbitals (HOMO), Lowest Occupied Molecular Orbitals (LUMO), and Electrostatic Potential (ESP) maps were visualised using Argus software to identify electron-rich and

electron-deficient regions relevant to binding interactions. (Figure 4 (a,b,c))

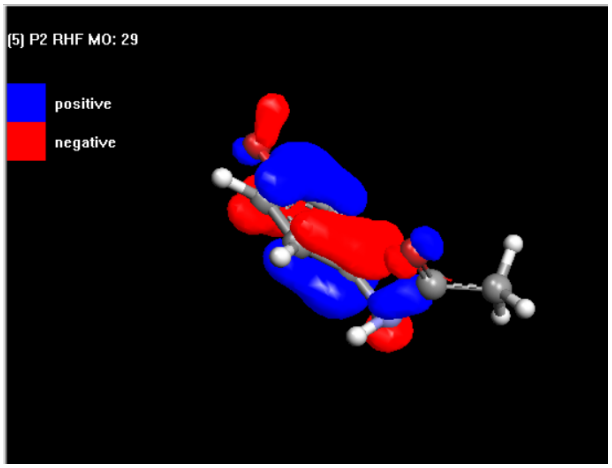

Figure 4a: HOMO (Highest Occupied Molecular Orbital) distribution of the paracetamol molecule obtained after AM1 geometry optimisation in ArgusLab.

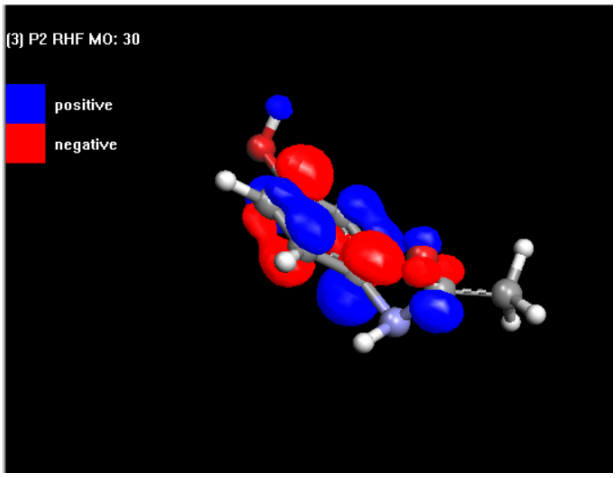

Figure 4b: LUMO (Lowest Unoccupied Molecular Orbital) distribution of the paracetamol molecule.

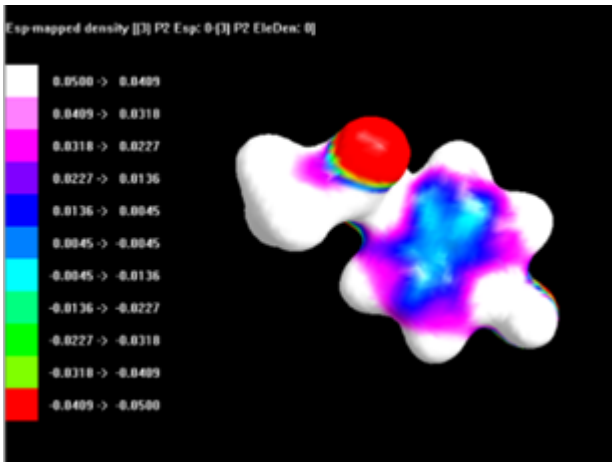

Figure 4c: Electrostatic potential (ESP) map of paracetamol visualising electron-rich (red) and electron-poor (blue) regions, indicating potential interaction sites with BSA.

Figure 4c:

6. The docking calculation was initiated by selecting the ligand and defining the calculation grid size. (Figure 5)

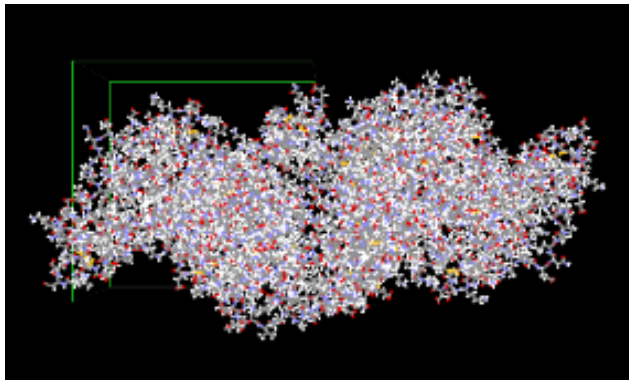

Figure 5: The 3D grid with the ligand for initiating the calculation.

7. After completing the docking calculation, the DEF ligand group within the 4F5S structure was selected from the group folder. The protein was then hidden to enhance visualisation, and the display settings were adjusted to 'ball and cylinder' mode with high resolution for clearer representation. (Figure 6)

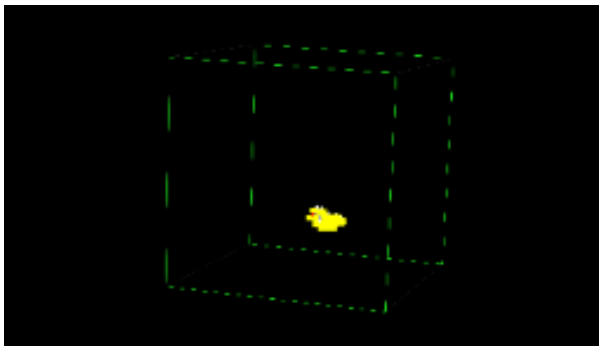

Figure 6: High-resolution view of the 12 DEF ligand docked in BSA (4F5S), shown in ball-and-cylinder mode with the protein hidden for clarity.

8. The 12 DEF ligand was right-clicked to create a binding site group and zoomed in for detailed inspection. Hydrogen bonds were visualised by selecting "Show Hydrogen Bonding" from the group folder, with interactions highlighted in red. (Figure 7)

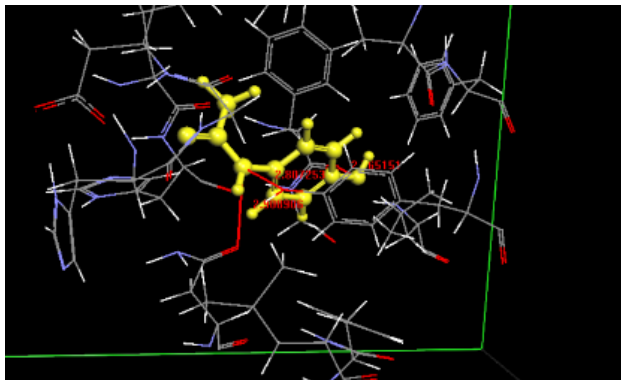

Figure 7: Zoomed view of the 12 DEF ligand in its binding site with hydrogen bonds highlighted in red.

9. **Visualisation of Hydrogen Bonding:** Images were generated to display the hydrogen bonding interactions between the 12 DEF ligand and the active site residues. (Figure 8(a, b, c))

Figure 8: Visualisation of hydrogen bonding interactions between the 12 DEF ligand and BSA residues.

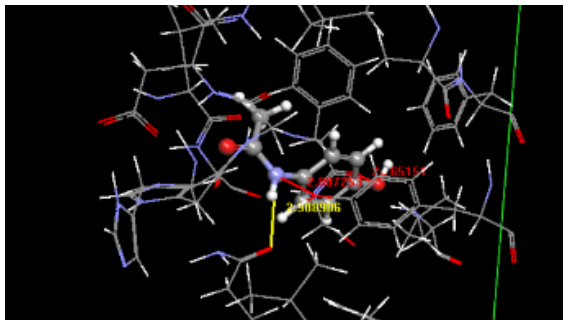

Figure 8(a)

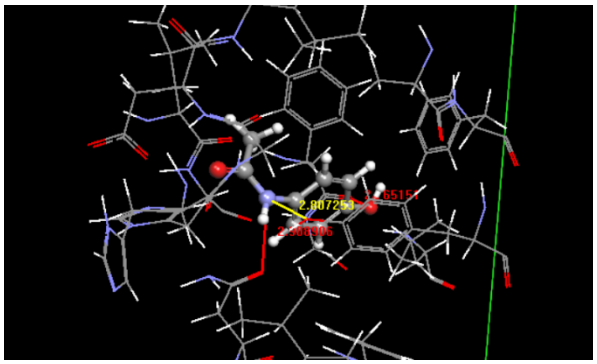

Figure 8(b)

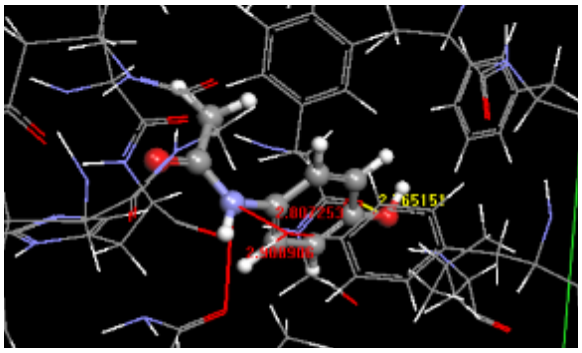

Figure 8(c)

10. Hardness and Softness Estimation:

The chemical hardness (η) and softness (S) were calculated from HOMO and LUMO energies using:

$$\eta = \frac{E_{HOMO} - E_{LUMO}}{2}, \quad S = \frac{1}{\eta}$$

RESULTS

1. The paracetamol molecular geometry was optimised using the semi-empirical quantum mechanical method, AM1 and reported in Table 1. These computed values are compared with corresponding literature-reported ranges based on experimental data such as X-ray crystallography and spectroscopic studies. The comparison helps assess the reliability and accuracy of the AM1 method for modelling the molecular structure of paracetamol.
2. The frontier molecular orbitals (HOMO and LUMO) of paracetamol were visualised post-AM1 optimisation to assess electron distribution and potential reactivity. These are shown in Figure 4 (a,b). The HOMO was primarily localised over the aromatic ring, while the LUMO was concentrated near the acetamide group, suggesting possible electron transfer or interaction with nucleophilic/polar residues in the BSA binding pocket. Additionally, the ESP (Fig. 4c) map revealed electron-rich zones around the hydroxyl and carbonyl groups, supporting their roles in hydrogen-bonding interactions

observed in the docking pose. (Figure 8a–8c) [13,14].

3. The electronic properties of paracetamol were evaluated using semi-empirical AM1 calculations in ArgusLab and reported in Table 2. The computed dipole moment was found to be 2.7378 Debye, indicating moderate polarity. This value suggests a balanced distribution of charge within the molecule, contributing to its ability to form hydrogen bonds and engage in polar interactions with amino acid residues in the active site of Bovine Serum Albumin (BSA). The observed dipole moment aligns well with the known pharmacokinetics of paracetamol, including its solubility and permeability characteristics [13,15].
4. The HOMO and LUMO energies (Table 2) of paracetamol were calculated as –9.514 eV and –0.122 eV, respectively. This yielded a ΔE of 9.39 eV, indicating a significant energy gap between the frontier orbitals. Based on this gap, the chemical hardness (η) was calculated to be 4.696 eV, and the corresponding chemical softness (S) was 0.213 $(eV)^{-1}$. The relatively large energy gap and high hardness value suggest that paracetamol is chemically stable and less reactive, reflecting its limited tendency for charge transfer interactions. This electronic stability may influence its binding dynamics within the BSA binding site. These results are in good agreement with earlier reported work. [13, 15-17].
5. The molecular docking calculations revealed binding interactions between paracetamol and BSA, with a binding free energy of -8.6759 kcal/mol. This binding affinity value exceeds typical experimental observations for paracetamol-albumin interactions (-5.6 to -6.7 kcal/mol) [13]. This binding free energy value lies in the range of -7 to -10 Kcal/mol, which indicates that paracetamol binds tightly and stably to BSA.Strong binding energy suggests that paracetamol is effectively transported in blood via BSA

protein. A significant fraction of the drug may remain bound, regulating its bioavailability and distribution. This also suggests binding is reversible, so the drug can still be released at the target tissues. This may be due to additional hydrogen bond formation by its phenolic OH and acetamide NH groups with polar residues present in the protein (Table 03). It may result in minor conformational changes in BSA.

6. This study provides the practical exposure to structure-based computational analysis of drug–protein interactions.

TABLES

Table 1: Calculated Equilibrium Geometry of Paracetamol using AM1 Method.

Structural Parameters	Calculated Values	Literature Range (a/b)
Bond Lengths (Å)		
C–H in CH_3	1.1131 Å	0.88–1.10[a]
O–H	1.0349 Å	0.96–0.97[a]
C=C in Ring	1.3982 Å	1.38–1.41[a]
C–O	1.4106 Å	1.36–1.39[a]
C–N (C of Ring)	1.4537 Å	1.35–1.41[a]
C=O	1.2654 Å	1.22–1.25[a]
N–H	1.0662 Å	0.90–1.01[a]
N–C (Amide C–N)	1.4523 Å	1.34–1.40[a]
Bond Angles (°)		
C–O–H	105.02°	107–109[a]
C–C–C (Aromatic)	119.90°	117–121[a]
O–C–C	120.05°	116–123.2[a]
C–N–C	105.42°	114–129[a] (planar N)

C–C–N	119.65°	116.7–117.1[a]
Dihedral Angles (°)		
C–C(=O)–N–C	177.42°	−179.99[b]
C(=O)–N–C–C	12.85°	0.0037, 179.98[b]
N–C–C–C	179.47°	−179[b]
O–C–C–C	−179.96°	−179.9[b]

Numbers in parentheses are taken from references.a -Reference [14,18], b-Reference [14]

Table 2: Electronic properties of paracetamol optimised using the AM1 method.

PROPERTY	VALUE
HOMO / eV	-9.5145
LUMO / eV	-0.1219
(ΔE) / eV (EHOMO–ELUMO)	9.3926
Dipole Moment / Debye	2.7378
Hardness (η) / eV	4.6963
Softness (S) / $(eV)^{-1}$	0.2129

Table 03: Hydrogen Bond Interactions Between Ligand and Receptor Residues.

Fig. No.	Donor Atom	Donor Residue (No.)	Acceptor Atom	Acceptor Residue (No.)	Distance (Å)
8(a)	N (Atom 18446)	Residue 3017	O (Atom 1526)	ASN 99	2.9089
8(b)	N (Atom 18446)	Residue 3017	O (Atom 482)	TYR 30	2.8075
8(c)	O (Atom 18445)	Residue 3017	O (Atom 1088)	PHE 70	2.7652

This table presents the hydrogen bond interactions formed between the optimised ligand (residue 3017) & amino acid residues in the receptor protein. Each row includes the donor and acceptor atom involved, their respective residues and numbering, and the bond distance in angstroms (Å).

CONCLUSION

This project serves as a valuable educational model at the undergraduate level, offering hands-on experience in structure-based drug design and molecular docking. It fosters interdisciplinary learning by integrating concepts from chemistry, biology, and computational science, and equips

students with essential skills for modern pharmaceutical and computational research.

This molecular docking study demonstrated strong binding affinity between paracetamol and Bovine Serum Albumin (BSA) with a binding free energy of -8.68 kcal/mol. The docked pose shows multiple hydrogen bonds via paracetamol's phenolic OH and acetamide NH groups with polar residues in BSA, emphasising the role of hydrogen bonding in stable protein-drug interactions. Minor conformational changes in BSA were also observed. Electronic property calculations indicate paracetamol's high chemical stability and moderate polarity, supporting its interaction potential. While rigid docking and solvent approximations may overestimate binding energy, key interaction patterns remain reliable.

FUTURE PERSPECTIVES

While this study establishes the binding affinity and key interactions between paracetamol and BSA using molecular docking, further refinement through molecular dynamics (MD) simulations is warranted to capture conformational flexibility and solvent effects. Incorporating explicit water models and free energy calculations (e.g., MM-PBSA) could improve accuracy in estimating binding energetics. Future work may also explore comparative docking with paracetamol metabolites or BSA site-directed mutants to better understand the specificity and transport dynamics of this widely used drug.

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