A Computational Approach in Identifying Interaction of Chitosan Sodium Benzoate Hydrocolloid with Upper Gastrointestinal Protein Targets

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Abstract

The utility of functional hydrocolloids have envisaged new formulations and combination of biopolymers and their functionalization in nutraceutical industry. The study have examined chitosan sodium benzoate complex based hydrocolloid interacts with GI proteins. The interaction of polymers have been analysed with a Insilco approach where itaids in exploring the polymeric composites interact with gastric juice proteins and intestinal wall proteins. The interaction was found in the proteins like Taste receptor type 1 member 3, Adenylate cyclase type 1, Anion exchange transporter showed best docking scores against each ligand compound like chitosan, sodium benzoate and chitosan sodium benzoate composite. The overall interaction of these composites and gastric proteins could render new information for polymer protein interaction specifically to hydrocolloids that could be used for pharma and healthcare industry.

Keywords: chitosan, Sodium benzoate, Insilco docking , hydrcolloids

Introduction

Increasingly, researchers in the field of food science are concentrating on the development of novel colloidal systems for use in the food and pharmaceutical industries to improve rheology, quality, nutritional value, and delivery efficiency.Hydrocolloids are a type of colloidal substance that readily dissolves in liquid and chemically they are large, water-loving macromolecules.Hydrocolloids are used to stabilise and thicken formulations for technical and regulated uses that essentially operate to the viscosity, texture, and base of processed foods, and no other group of ingredients is as pervasive.Because of their inherent functional features and the

possibility of tailoring the nutritional properties (fat mimics produce low-fat foods and serve as dietary fibres), these biopolymers offer a remarkable set of health benefits.A chitosan-sodium benzoate composite was developed and was investigated for a functional hydrocolloid in the previous study.

Fourier transform infrared spectroscopy, X-ray diffraction, differential scanning calorimetry, and thermogravimetric analysis were used in a prior study to characterise the physiochemical properties of chitosan-sodium benzoate hydrocolloid (TGA). Compared to chitosan, sodium benzoate, and a physical combination, chitosan-sodium benzoate has a significantly different structure and crystallinity, as seen by FTIR and XRD. DSC and TGA were used to study the temperatures at which substances decomposed and the resulting mass losses. The effectiveness of the samples was also tested in antibacterial and antioxidant assays.

Chitosan has demonstrated controlled release properties with numerous tablet formulations, and it has been reported as an effective excipient in the pharmaceutical application for tablets' direct compression (Nigalaye et al., 1990, Nunthanid et al., 2004). The nanoscale structure of chitosan enhances the bioavailability and stability of bioactive ingredients. Also, there is proof that epithelial cells take in more of these positively charged polymeric nanoparticles (Akbari-Alavijeh et al., 2020). Its high capacity to bind fat in the gastrointestinal tract, combined with its antimicrobial and antioxidant activity in both its pristine and functionalized forms, makes chitosan a promising option for nutraceuticals (Abd El-Hack et al., 2020). Studies on the release of lidocaine using chitosan as a hydrocolloid have shown that a slow and sustained release can be achieved; this is believed to be due to the chitosan's influence on the extent to which lidocaine is reacetylated (Kristl et al., 1993).Because of these merits, chitosan is a model hydrocolloid with potential for wide application in pharmaceuticals and nutraceutics. An alternative choice material to the ubiquitous gelatin has been demonstrated in terms of accessibility, ease of extraction, inherent physicochemical properties, cost, and demonstrated efficacy as a drug delivery agent. They are superior to gelatin in many respects, including being cheaper, easier to work with, and more versatile as a polymer that can be functionalized through the manipulation of its constituent functional groups.

An effective method for visualising the molecular interactions between receptor and ligand molecules, molecular docking can also aid in understanding the potential molecular involvement of such interactions in achieving the desired functionality.To determine and get the binding affinity and interactive mode between ligand and receptor, molecular docking simulates the optimum conformation in accordance with complementarity and pre-organization.Molecules like chitosan serve a crucial role as a carrier medium in the nanoscale realm to improve the bio-availability and

hydrophilicity of these active chemicals. The fate of the product's design hinges on the compatibility of these two elements. It is nearly impossible to calculate the ideal balancing ratio for the efficient transport of the drug molecule to the target, as there are an infinite number of configurations between only two systems, the carrier and the drug.Standardizations in computational design aid in the construction of the nanocarrier in the desired formulation, which is determined by the interaction and conformational moieties in the target protein. Moreover, they would provide a foundation for learning about the molecule's potential metabolic destiny, which is necessary for designing effective in vivo experiments.Our working hypothesis is that the system's efficacy as a potent hydrocolloid in nanoscale machinery will improve upon incorporation of computational analysis from the synthesis step of nanoparticulated systems. For this reason, it could be said that the present study's fair consideration was to create biopolymeric chitosan nanoparticles with sodium benzoate for a functional hydrocolloid.

The study intends to study the interaction between a novel hydrocolloid and proteins in the gastrointestinal tract. The interaction would aid in exploring the possible interaction of these polymeric composites with proteins of gastric juices and the walls of intestinal walls. To the best of our knowledge, the probable binding sites of the hydrocolloid compounds with the proteins in the gastrointestinal tract were predicted through molecular docking for the first time.

MATERIALS AND METHODS

Materials

The computational toolsused wasPyMOL 2.4.1 from Schrodinger LLC (https://pymol.org/),AutodockVina 1.1.2 software from The Scripps Research Institute Inc(Trott & Olson, 2009),AutoDockTools 1.5.6 (Morris et al., 1998),Discovery Studio Visualizer v21.1.0.20298 from Dassault System BIOVIA (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/) and LigPlot(https://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/)

Protein Databank

The RCSB Protein Data Bank (http://rcsb.org) expands on PDB data to allow structural biology, computational biology, and beyond research and teaching. The Protein Data Bank (PDB) is the world's only repository for experimentally determined, atomic-level three dimensional structures of biological macromolecules (proteins, DNA, and RNA). The Worldwide Protein Data Bank organisation is in charge of the PDB repository (Altunkaya et al., 2016). When the PDB first started, there were few structures, they were tiny, and only X-ray crystallographic methods were

employed to discover the structures. The PDB already has structures with molecular weights in excess of two million, and new structure-determination techniques are on the way to join NMR and cryoEM in the PDB Exchange Dictionary (Berman et al., 2002).

PubChem database

PubChem (https://pubchem.ncbi.nlm.nih.gov) is a chemical information repository hosted by the National Centre for Biotechnology Information in the United States (Kim et al., 2019). PubChem is one of the most comprehensive collections of freely available chemical information. It has about 157 million depositor-provided chemical substance descriptions, 60 million distinct chemical structures, and 1 million biological test descriptions (as of September 2015), encompassing around 10,000 unique protein target sequences(Kim et al., 2016). With the increased amount and complexity of biological screening findings, the necessity for computational tools to obtain and evaluate such rich data becomes increasingly pressing (Wang et al., 2013).

PyMOL

PyMOL, an open source and cross-platform molecular graphics tool, has been widely used to visualise proteins, nucleic acids, tiny molecules, electron densities, surfaces, and trajectories in three dimensions (3D). PyMOL is developed in Python, one of the most popular programming languages, it is easily extensible to Python plugins. PyMOL can generate high-quality videos and photographs of macromolecules in a variety of formats like as ribbons, cartoons, dots, surfaces, spheres, sticks, and lines(Yuan et al., 2017).

Discovery Studio Visualizer

A variety of molecular docking software is now available on the market; however, a literature search revealed that Discovery Studio is a suite of tools for stimulating small molecule and macromolecule systems. Dassault system BIOVIA created and distributed it (formerly Accelerys). Because of its capacity to anticipate the binding-confirmation of small molecule ligands to the right target binding site, molecular docking is one of the most often utilised strategies in structure-based drug design. The discovery and development of a novel medicine is often regarded as a time-consuming and resource-intensive process. The Discovery Studio programme is ideal for stimulating tiny and macromolecule systems (Kemmish et al., 2017)

CASTp

Computed Atlas of Surface Topography of proteins (CASTp) (http://cast.engr.uic.edu) provides an online resource for locating, delineating and measuring concave surface regions on

threedimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins. The measurement includes the area and volume of pocket or void by solvent accessible surface model (Richards' surface) and by molecular surface model (Connolly's surface), all calculated analytically. CASTp can be used to study surface features and functional regions of proteins. CASTp includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures. CASTp is updated daily and can be accessed at (Binkowski et al., 2003)

Autodock MGL tools

The Autodock package is a popular free open-source programme for protein–ligand docking and virtual screening (VS). Overall, the results for pose prediction are promising, with binding free energy errors of 2–3 kcal/mol for small drug-like compounds in the absence of considerable receptor conformational change. Autodock Bias is built on the AutoDock4 and AutoDockTools foundations. It is based on a Python script that updates the appropriate energy grid maps and Docking Parameter File in the PDBQT file to include the bias and, if needed, the ligand-targeted atom(s) (Meldrum et al., 2019).

AutodockVina

One of the various protein-ligand docking programmes available is Autodock. It was initially published in 1990 and has since been improved. This is a significant benefit in research and education since it allows for a thorough knowledge of a tool via the study of its source code and facilitates replication(Gaillard, 2018). During the docking procedure, both the protein and ligands are considered as rigid. The results less than 1.0 Å in positional root-mean-square deviation (RMSD) was clustered together and represented by the result with the most favourable free energy of binding. The pose with lowest energy of binding or binding affinity was extracted and aligned with receptor structure for further analysis. The molecular docking was performed using the Vina programme with globally searching exhaustiveness of 8, 56, and 400, which correspond to short, medium, and long options, respectively. The maximum energy difference between the two docking modes was set at 7 kcal/mol(Nguyen et al., 2020)

METHODS

The Protein structure Preparation

The X-ray crystallographic structure of human Taste receptor type 1, Gastrin, Somatostatin, Anion exchange transporter, Inositol 1,4,5-triphosphate receptor type 1, Adenylate cyclase type 1were

retrieved from PDB (https://www.rcsb.org/). Removing water and bound molecules in the protein structure preparation using Discovery studio visualizer. Adding non-polar hydrogen atoms, Kollman and Gasteiger charges, for the conversion of macromolecule into pdbqt format using ADTTools 1.5.6 (https://ccsb.scripps.edu/mgltools/). The computational tools used are cited in the materials.

The Ligand structure Preparation

The three-dimensional structure of Chitosan (ID: 71853), and Sodium benzoate (ID: 517055)from a PubChem database (https://pubchem.ncbi.nlm.nih.gov/compound) were retrieved.Also, a synthesized compound chitosan sodium benzoate composite was prepared independently. The ligand structure was viewed independently in PyMOL 2.4.1 and converted to pdbformat. All non-polar hydrogens, Gasteiger charges were merged and rotatable bond was defined. Later, the ligand file was converted into pdbqt format using ADTTools 1.5.6.

Active site prediction:

The CASTp website (http://sts.bioe.uic.edu/castp/index.html?2pk9) was used to locate the active site of the target proteins and also identify all pockets and voids on a protein structure.

Grid generation and Virtual screening

Using the active site, grid generation was done using ADT Tools 1.5.6. AutodockVina 1.1.2 was used to perform Virtual screening (Trott & Olson, 2009). The grid box size was set to 126 × 126 × 126 Å dimensions with center_x =-22.583, center_y =-5.081, center_z =20.758 and the grid spacing of 0.547 Å for all the proteins. The other docking parameters were set to exhaustiveness = 8and energy_range = 4. At the end of virtual screening, 10 best models were attained for each ligand docked against the protein. To visualize their binding energy, amino acid residues, and protein-ligand interactions, Discovery studio Visualizer and LigPlotwas used.

RESULT

Many biological functions of protein depend upon the formation of protein-ligand complexes. The selected ligands and reference molecule were docked against protein. The results were analyzed at the end of molecular docking. Best docking models were achieved based on their binding affinities against protein.

From the PubChem database, Chitosan (ID: 71853),Sodium benzoate (ID: 517055) andchitosan sodium benzoate composite were docked. The compound chitosan showed highest binding affinity

withTaste receptor type 1 member 3 showed–13.2kcal/mol, whereas the compound sodium benzoate and chitosan sodium benzoate composite withAdenylate cyclase type 1 showed – 5.0 kcal/moland -7.7 kcal/mol, respectively. The conventional hydrogen bond interaction between proteinTaste receptor type 1 member 3 and ligand chitosan were found to be ARG A: 64, ARG A: 56, LEU A: 61, ARG A: 216, SER A: 104, ASP A: 216, SER A: 147, SER A: 146, SER A: 170, PHE A: 145, GLU A: 143, GLU A: 105, GLU A: 301, GLU A: 163. The conventional hydrogen bond interaction between proteinAdenylate cyclase type 1 and ligand sodium benzoate were found to be ASP A: 60. The conventional hydrogen bond interaction between protein Adenylate cyclase type 1 and ligand chitosan sodium benzoate composite were found to be THR A: 424, ASN A: 878, SER A: 924, THR A: 925, ILE A: 922, ARG A: 976, ASP A: 1010. The binding scores, amino acid interactions, number of hydrogen bonds, common amino acid interactions highlighted were listed in the below table.

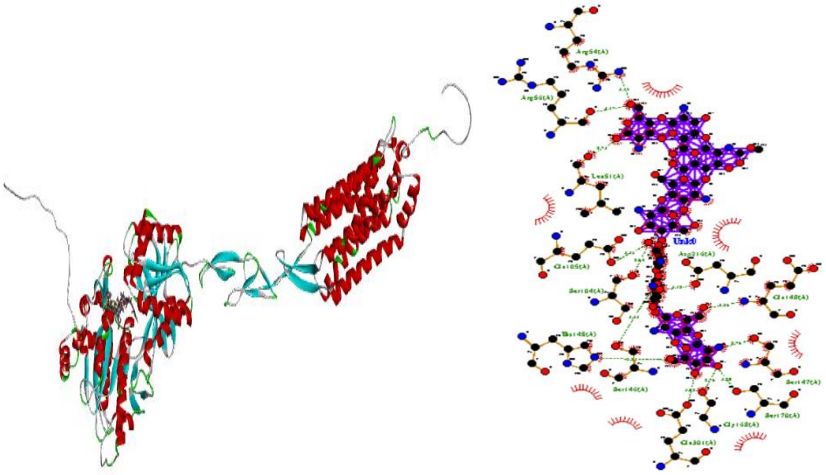
Table 1:

S.NO	PROTEIN NAME	DOCKING SCORE			HBOND INTERACTIONS
		CHITOSAN (ID: 71853)	SODIUM BENZOATE (ID: 517055)	CHITOSAN SODIUM BENZOATE COMPOSITE	
1	Taste receptor type 1 member 3	- 13.2	- 4.9	- 6.5	ALA A:383, LEU A:468, LYS A:457, ASP A:470, ASP A:455, ASP A:216, GLY A:328, HIS A:388, ASN A: 386, GLU A: 148, ARG A:64, ARG A:56, LEU A:61, ARG A:216, SER A:104, SER A:147
2	Gastrin	- 8.4	- 3.3	- 4.4	TYR A:87, TRP A:89, TRP A:79, GLU A: 84, GLU A:85, GLU A:82,
3	Somatostatin	- 9.9	- 4.2	- 4.9	ASP A:61, GLU A:64, THR A:63, CYS A: 28, ARG A: 32, SER A:38

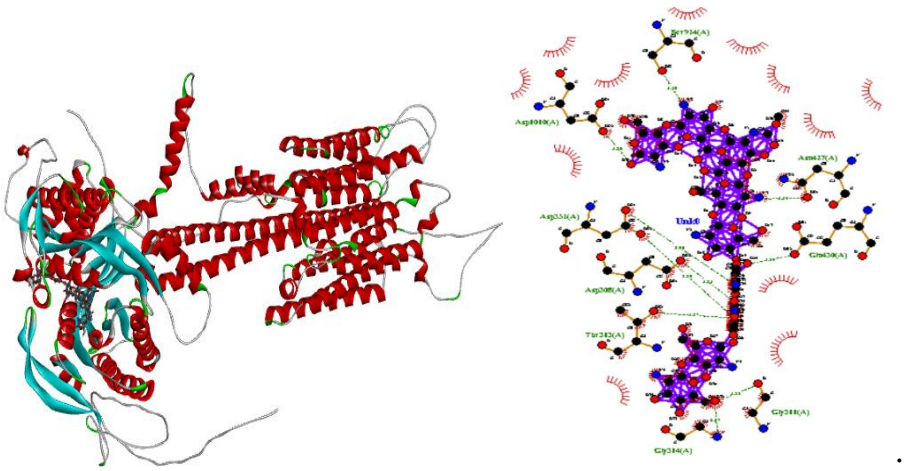
4	Anion exchange transporter	- 11.0	- 4.3	- 6.9	TYR A:197, GLN A:121, LYS A:196, GLU A:116, ARG A:144, GLU A:285, VAL A: 287, HIS A: 75, THR A: 369, HIS A:98, VAL A:99, LEU A: 372, VAL A: 381, ALA A:382, PHE A:85, CYS A: 387
5	Inositol 1,4,5-triphosphate receptor type -1	- 10.6	- 4.1	- 5.8	GLU A:243, ARG A: 19, ASP A:240, ARG A: 237, LYS A: 233, GLU A: 303, PHE A: 344, ALA A: 242
6	Adenylate cyclase type 1	- 12.8	- 5.0	- 7.7	ASP A:1010, ARG A:976, THR A:925, ILE A:922, SER A:924, THR A:424, ASN A: 878, ASP A:606, ASP A:437, ASP A:352, GLY A:314, GLY A:311, GLU A:430, THR A: 313

3D& 2D INTERACTION MAP OF TOP 3 COMPOUNDS - CHITOSAN

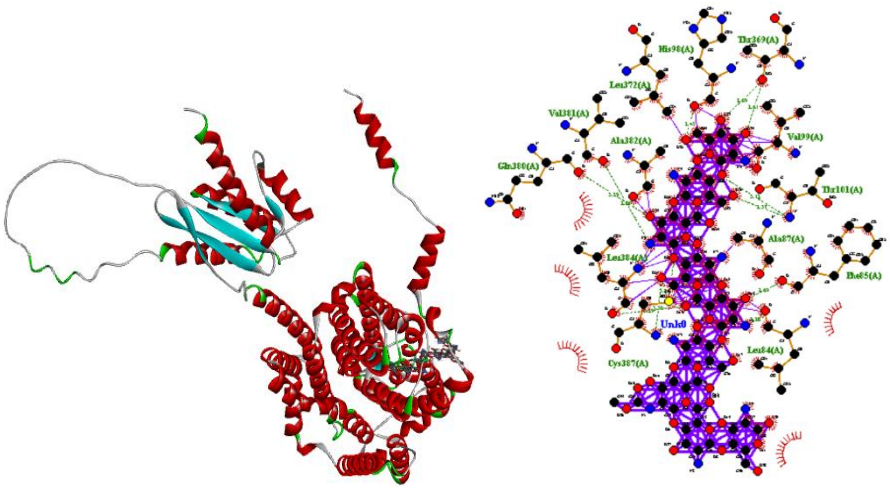
1) Taste receptor type 1 member 3 and Chitosan



2) Adenylate cyclase type 1 and Chitosan

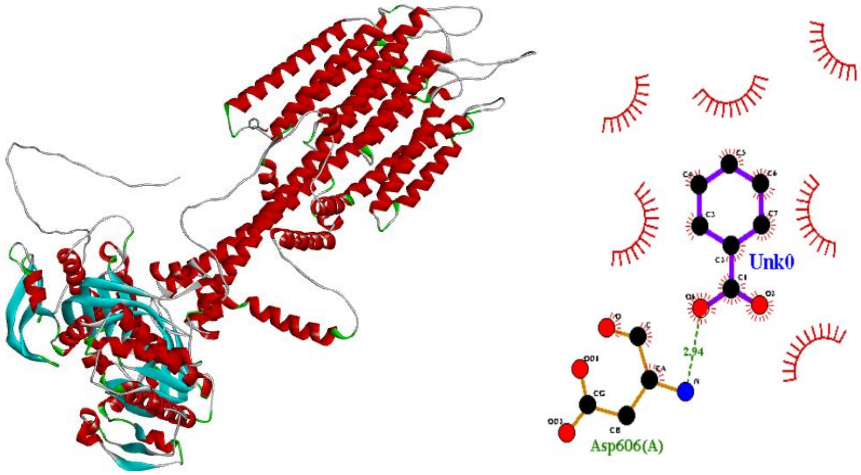


3) Anion exchange transporter and Chitosan

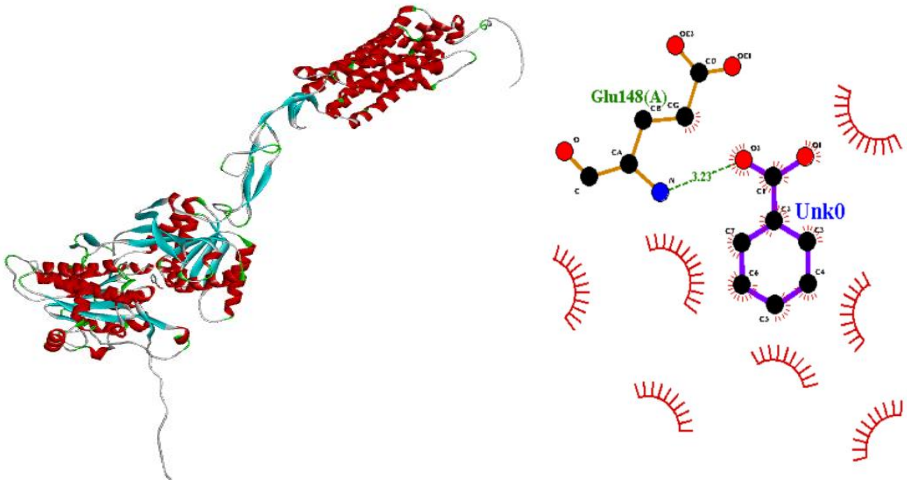


3D & 2D INTERACTION MAP OF TOP 3 COMPOUNDS - SODIUM BENZOATE

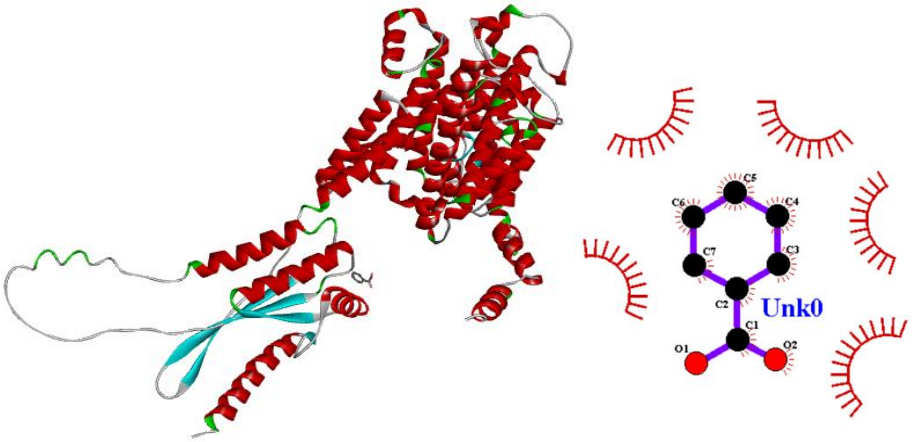
1) Adenylate cyclase type 1 and Sodium benzoate



2) Taste receptor type 1 member 3 and Sodium benzoate

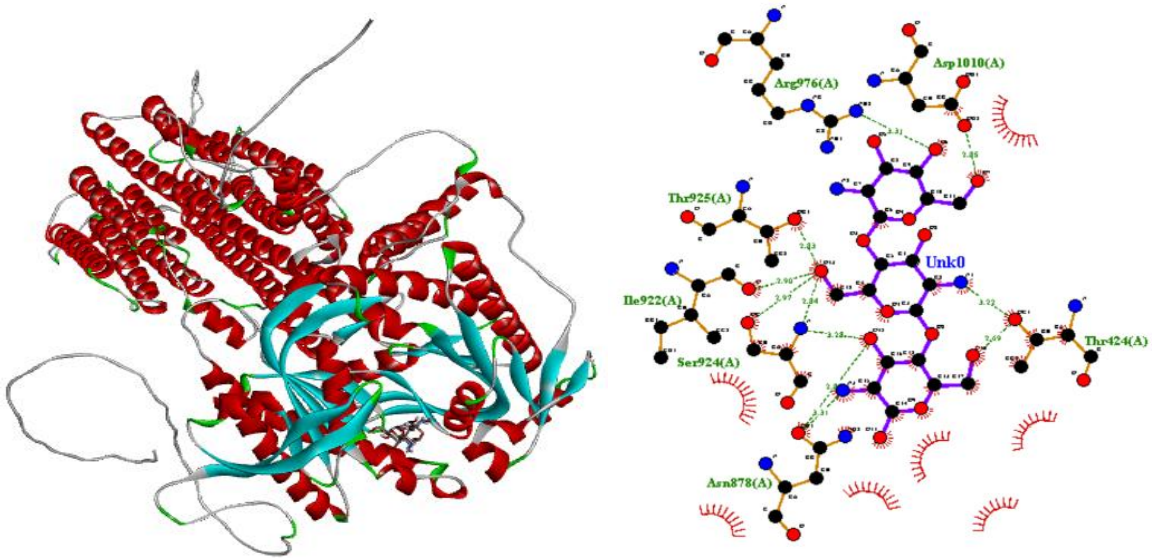


3) Anion exchange transporter and Chitosan

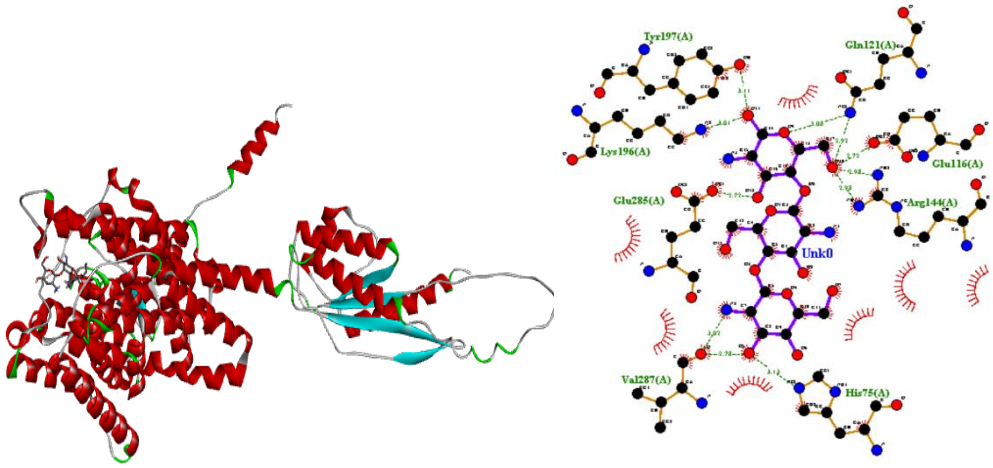


3D & 2D INTERACTION MAP OF TOP 3 COMPOUNDS – COMPOSITE

1) Adenylate cyclase type 1 and Chitosan Sodium benzoate composite



2) Anion exchange transporter and Chitosan



3) Taste receptor type 1 member 3 and Chitosan

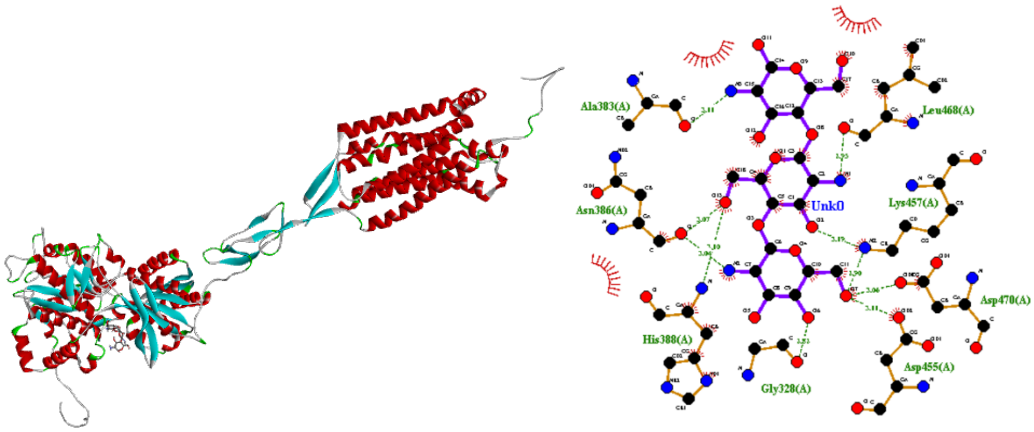


FIGURE : 2D and 3D interaction map of top 3 compounds like **Taste** receptor type 1 member 3, Adenylate cyclase type 1, Anion exchange transporter with chitosan, sodium benzoate and chitosan sodium benzoate composite.

From the above table and figures, the proteins like **Taste** receptor type 1 member 3, Adenylate cyclase type 1, Anion exchange transporter showed best docking scores against each ligand compound like chitosan, sodium benzoate and chitosan sodium benzoate composite. These compounds exhibit H-bond interactions and strong intermolecular force with the target protein as well.(extend this part)

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