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Designing of Peptides for targeting CD14 receptor site which induce differentiation of monocytes to MΦ in U937 cancer cell line.

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ABSTRACT: Drugs used for cancer have some disadvantages prior among them is less specificity towards target leading to unwanted reactions. Cancer cells from U937 cell line are identified by the specific surface receptors, hence targeting those receptors can help in increasing the specificity of drug. Normally cancer cells are monocytes that do not differentiate to MΦ. The surface receptor CD14 helps in inducing the differentiation of monocytes to MΦ in cancer cells when its bind to LPS.LPS contain lipid and sugar complex molecule that attaches to the hydrophobic pocket of CD14. Lipid A region is the hydrophobic region in LPS, this region of LPS interacts with CD14. The Lipid A cannot fit for docking studies. So new peptide sequence resembles the LPS characteristics are designed by keeping hydrophobic interaction as a key. The designed peptide is docked with the surface receptor and the one with minimal binding energy which possess maximum binding score is been select and its interactions been observed by using dynamic stimulations (Gromacs).

KEYWORDS: U937 - (human histiocyticlymphoma cell line), CD14- Cluster of Differentiation 14,Gromacs.

I. INTRODUCTION

TheDrug designing, the process of exploring new and effective drugs for the treatment of diseases. Drugs are molecule that can be introduced into the organism to change the abnormal biological activity thereby bringing back to usual functions. The commercially available anti-cancerous drugs even though effective has some drawbacks, the prominent one is fewer targets specific. So increasing target specificity can increase the reactivity thereby giving good activity towards disease. The cancer cells from U937 cell line are identified by the specific surface receptors, hence targeting those receptors can help in increasing the specificity of drug. The surface receptor CD14 helps in inducing the differentiation of monocytes to MΦ in cancer cells when its bind to LPS. LPS contain lipid and sugar complex molecule that attaches to the hydrophobic pocket of CD14. Lipid A region is the hydrophobic region in LPS, this region of LPS interacts with CD14. The Lipid A cannot fit for docking studies. So new peptide sequence resembles the LPS characteristics are designed by keeping (hydrophobic interaction) as a key. The designed peptide is docked with the surface receptor and the one with minimal binding energy which possess maximum binding score is been select and its interactions been observed by using dynamic stimulations

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II. IDENTIFICATION OF SURFACE RECEPTOR:

It is identified that some surface receptors are present only in the cancer cells. Hence targeting these receptors in cancer cells can increase the specificity, thereby avoiding the interactions with other cells. Among those receptors, the receptor that helps in differentiation is CD14 – Cluster of Differentiation 14. CD14 is also known as the pattern recognition receptor. [1].CD14 helps in the differentiation of monocytes to macrophages. Cancer cells are usually monocytes that do not get converted to macrophages. This leads to continuous proliferation forming tumor. Inducing the receptor helpful for macrophage differentiation may initiate the macrophage formation [3]. From the literature it is identified that first 152 residues from amino terminal is responsible for differentiation in many organisms. And in human it is identified to be first 72 residues from N-terminal end. Mainly it is identified to be the first and second alpha helix with loops that are hydrophobic in nature. This region is responsible for inducing differentiation in human monocytes or cancer cells [5]. First 152 residues from amino terminal end viewed using Pymol software. Residues between first and second alpha helix with loops in between. From residues 49-72 the first hydrophobic pocket in the N-terminal helps in inducing differentiation of CD14. (YLLKRVTEADLGQFTDIKLS). Active site of CD14 shown in below figure 1.

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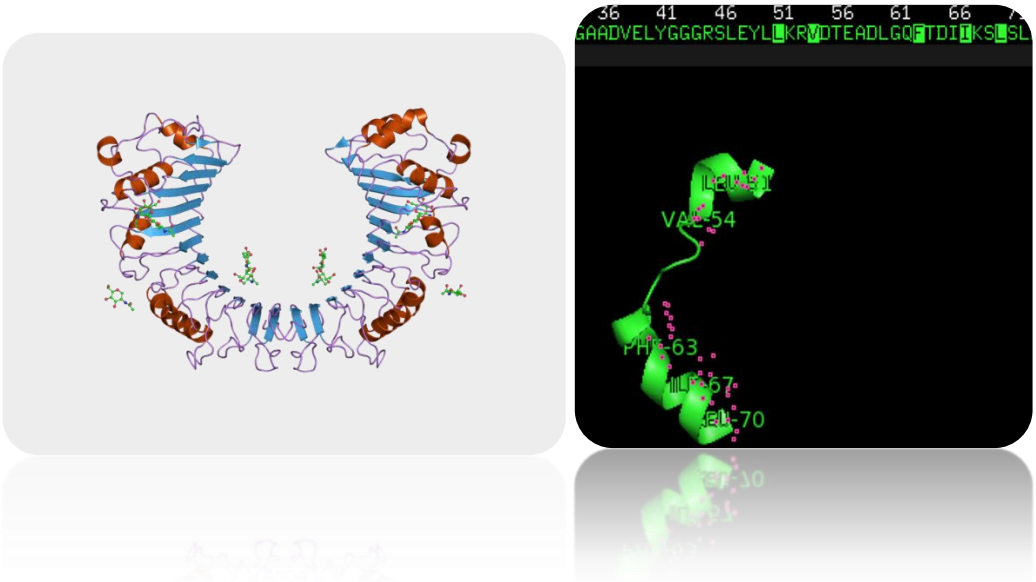

Figure 1: Active Site of CD14

III.MOLECULES INDUCING DIFFERENTIATION IN MONOCYTES:

Some molecules help in binding to the site which induces differentiation in monocytes. Such molecule is LPS-Lipopolysaccharide. LPS is a lipid and sugar complex molecule which is shown in the figure 2 that attaches to the hydrophobic pocket of CD14. Lipid A region is the hydrophobic region in LPS, this region of LPS interacts with the CD14 site. It has been proved in many wet laboratory experiments.[11]. Lipopolysaccharide binds with CD14 inducing differentiation. But LPS as a lipid cannot fit for docking studies. So a new peptide molecule that resembles the LPS characteristics is designed using various tools. [24].

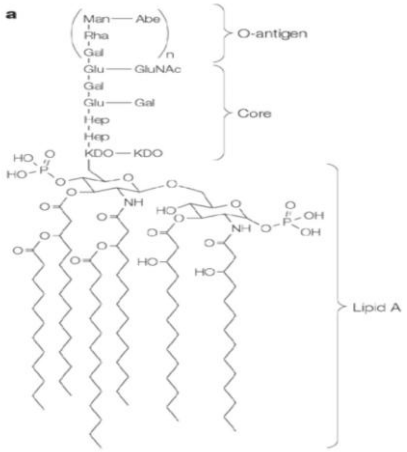

Figure 2: Structure of LPS

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IV. PREPARATION OF PEPTIDE SEQUENCE

The active sites of CD14 are highly hydrophobic in nature. These are the hydrophobicity amino acids (A, I, V, P, L, F, G).So the peptides are designed on the basis of permutation and combination concepts by keeping the hydrophobic nature as key. So 5040 combinations are retrieved from the database. - 50 Peptides structures which are shoen in the figure 3 are generated using predict-protein database and they are docked with 3D structure of surface receptor (CD14) which is downloaded from PDB databases.

Combination and permutation formula = $\frac{n!}{(n-r)!}$

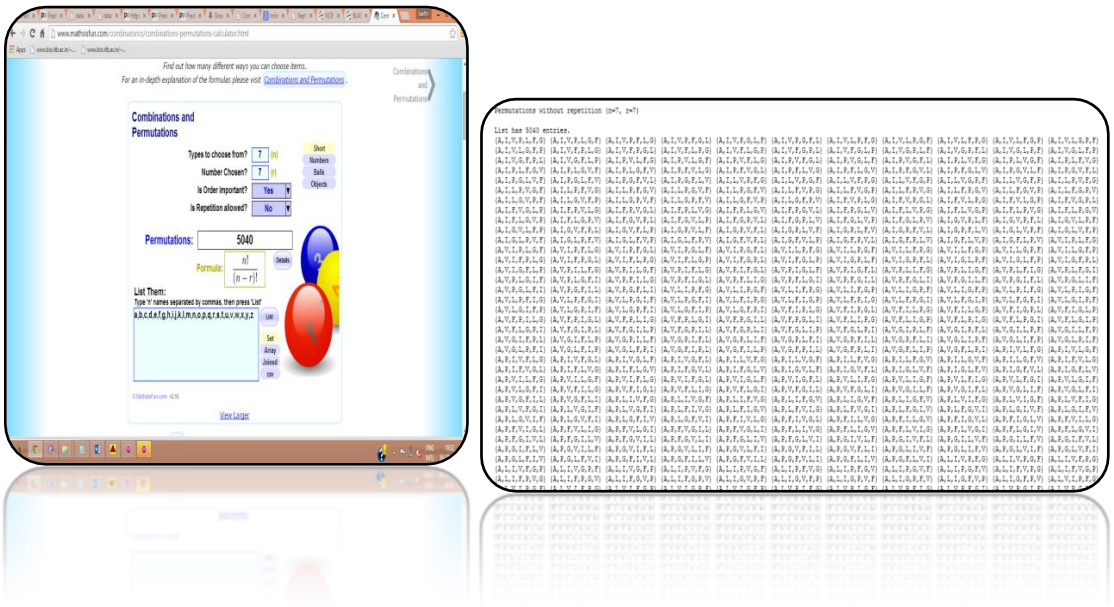

Figure 3:Retreived data from database

V. MOLECULAR DOCKING

Molecular docking is a computer simulation or computational tool that uses specific procedures to predicate the conformation of a receptor-ligand complex, where the receptor is usually a protein and the ligand is either a small molecule (drug) or another protein [8]. It is consider as the key step in identifying potential drug candidates [9]. In addition, it is known as the automated computer algorithm that determines how candidate ligands (peptide sequence) will be docked into the active site of the target protein. It involves several processes such as determination of orientation, conformational geometry and the scoring function. The real task of docking algorithm is to locate the peptide sequence in many different orientations and conformations in the active site of the selected protein and then calculate the score for each peptide sequence docked in the protein.

V. MOLECULAR DYNAMICS

One of the principal tools in the theoretical study of biological molecules is the method of molecular dynamics simulations (MD). This computational method calculates the time dependent behavior of a molecular system. MD simulations have provided detailed information on the fluctuations and conformational changes of proteins and nucleic acids. These methods are now routinely used to investigate the structure, dynamics and thermodynamics of biological

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molecules and their complexes. They are also used in the determination of structures from x-ray crystallography and from NMR experiments. GROMACS 4.5.4 is the software used for Molecular Dynamics.[28].

VII. METHODOLOGY

In this article we have conduct the experiment on various designed peptide models with CD14 receptor.

a. Protein Data Bank (PDB)

PDB is a worldwide repository for processing macromolecular structure data. It has more than 25000 structures determined by x-ray diffraction. It is a repository for the 3D structural data of large biological molecules – proteins and nucleic acids. The data, typically obtained by X- ray crystallography or NMR spectroscopy and submitted by biologists and biochemist from around the world. The pdb is a key resource in areas of structural biology, such as structural genomics.
URL: http://www.pdb.org/

b. PyMOL

PyMOL is python based visualization software.PyMOL is one of a few open-source visualization tools available for use in structural biology. PyMOL can produce high-quality 3D images of small molecules and biological macromolecules, such as proteins, nucleic acid. PyMOL supports most of the common representations for macromolecular structures: wire bonds, cylinders, spheres, ball-and-stick, dot surfaces, solid surfaces, wire mesh surfaces, backbone ribbons, and cartoon ribbons. Labels can be displayed for atoms, and dashed bonds can be used to indicate hydrogen bonding interactions and distances. Surfaces can be transparent, and molecules can be loaded from PDB files as well as several other common file formats.

c. AUTODOCK 4.2

In our case we involved in 'protein - protein docking'. This method is also called as rigid docking. AutoDock is a molecular modeling simulation software. AutoDock 4 comprises three major improvements: The docking results are more accurate and reliable. It can optionally model flexibility in the target macromolecule. It enables AutoDock's use in evaluating protein-protein interactions. AutoDock 4.0 not only is it faster than earlier versions, it allows side chains in the macromolecule to be flexible. As before, rigid docking is blindingly fast, and high-quality flexible docking can be done in around a minute. Up to 40,000 rigid dockings can be done in a day on one CPU. .In this case both the molecules are rigid and interaction produces no change in conformation [24]

d. GROMACS 4.5.4

GROMACS (GROningen Machine for Chemical Simulations) is a molecular dynamics package primarily designed for simulations of proteins, lipids and nucleic acids. It was originally developed in the Biophysical Chemistry department of University of Groningen, and is now maintained by contributors in universities and research center across the world. GROMACS is one of the fastest and most popular software packages available] and can run on CPUs as well as GPUs. It is free, open source released under the GNU General Public License. Starting from version 4.6, GROMACS is released under the GNU Lesser General Public License. Under a non-GPL license, GROMACS is widely used in the Folding home distributed computing project for simulations of protein folding, where it the base code for the project's largest and most regularly used series of calculation cores.[11][12]

VIII. RESULTS AND DISCUSSIONS

The active sites of CD14 are highly hydrophobic in nature. These are the hydrophobicity amino acids (A, I, V, P, L, F, G).So the peptides are designed on the basis of permutation and combination concepts by keeping the hydrophobic nature as key.So 5040 combinations are retrieved from the database. 50 Peptides structures are generated using predict protein database and they are docked with our receptor (CD14).Among that, Model 6 shows results with minimal binding energy and Hydrogen bonds -1 at valine 32 which is shown in the figure 4,5 and table 1

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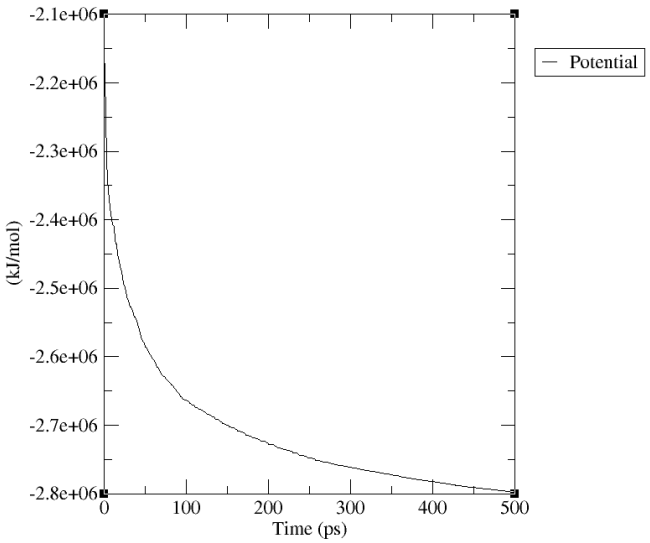
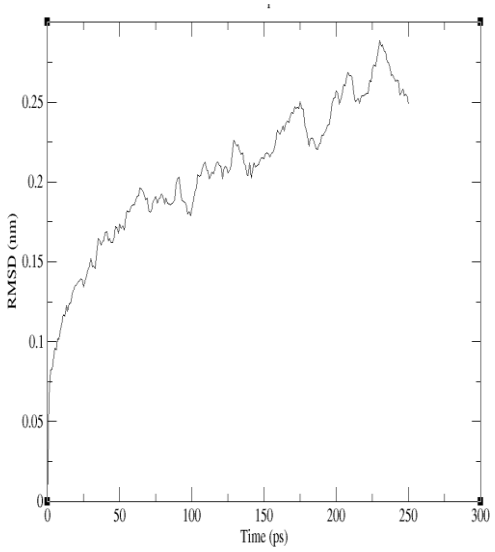
Models	Binding energy	Molecular Weight
06	-11.43	-1222.143

Table 1: Stimulation Results for model 06



Gromacs Energies



RMSD – Root Mean Square Deviation

Figure 4:Stimulation Results for model 06

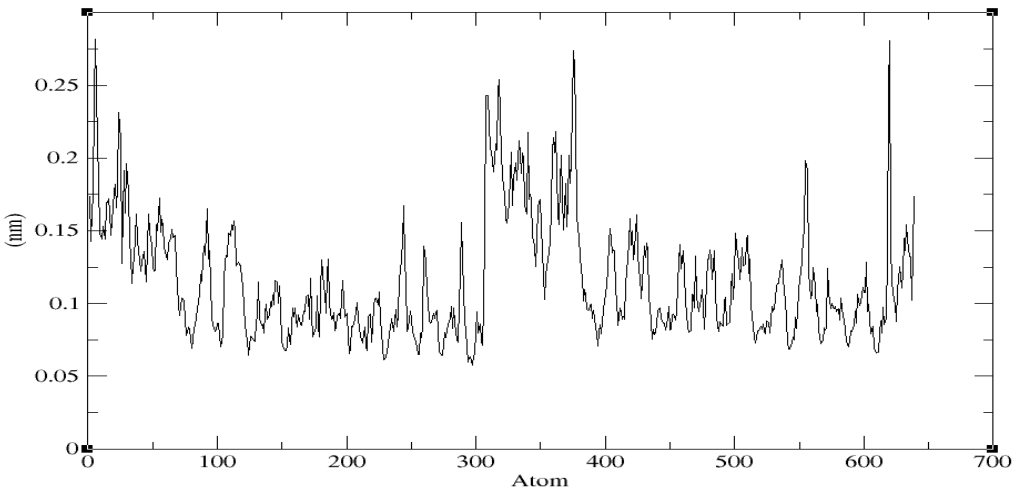


Figure 4:RMS Fluctuationfor model 06

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VIII. CONCLUSION

The surface receptor in cancer cell that helps in inducing differentiation is identified to be CD14-cluster of differentiation receptor14. Lipopolysaccharide which binds with the differentiation site of that surface receptor initiates monocytes to macrophage conversion. Among 5040 combination, 50 new peptides are generated and are docked with surface receptor to identify the effective peptide model. Among them, model six shows better targeting against the CD14 receptor.

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