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IN SILICO RNA APTAMER DRUG DESIGN AND MODELLING

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ABSTRACT

In recent years it has been observed that aptamers are highly efficient target for drugs to inhibit the activity of a specific protein. Aptamers are present on various proteins in humans. To inhibit the activity of that specific protein, we can design the drug that binds to the aptamer which is present on that specific protein. We can conquer this task with in-silico drug designing (molecular docking). In this study we have taken the crystal structure of an RNA aptamer bound to human thrombin and check its binding affinity to dexamethasone. In this study we have checked the efficacy of dexamethasone as an anti-coagulant agent using autodock 4.0. The result shows promising but still further work is required in this to use the dexamethasone as an inhibitor for thrombin.Structural analysis in PyMOL platform for computational drug design representing macromolecule in different style including stick,sphere,ribbon.In nucleic acid modeling and in silico design, a full set of in silico methods can be applied, such as docking, molecular dynamics (MD), and statistical analysis ,docking score calculation as modeling workflow starts with structure prediction. Docking of target and aptamer is performed. which allows for an evaluation of the stability of aptamer/ligand complexes and determination of the binding energies with higher accuracy where aptamer/ligand interactions are analyzedinbetween mutations arestudied in aptamers.

***IndexTerms*- Aptamers, structural analysis,binding protein, in silico design ,molecular modelling,docking, molecular dynamic.**

INTRODUCTION

The scope of aptamer research is enlarging rapidly. Aptamers are short oligonucleotides which can bind to a specific molecule including biomolecules (proteins, peptides, carbohydrates) and chemical compounds. Aptamers are non-immunogenic and non-toxic single-stranded DNA (deoxyribose nucleic acid) or single- stranded RNA (ribose nucleic acid) molecules that can fold into the complex tertiary structure. Aptamer word is formed by joining two Latin words “aptus”, meaning to fit, and “meros”, meaning part. Aptamers bind to a target with very high specificity and selectivity becauseaptamers mimic antibodies that can bind to a specific target.Usually,aptamers are very small in size, due to this reason aptamers have ability to disrupt interaction between proteins. Aptamers are also capable of penetrating tissues then antibody because of their smaller size.The term aptamer is coined by The Andy Ellington. Aptamers with affinity for a desired target are selected from a large oligonucleotide library through a process called SELEX which stands for sequential evolution of ligands by exponential enrichment.[1][6][10][11][16]

In our study we have taken the aptamer of human thrombin. Thrombin is a naturally synthesize enzyme that is derived from prothrombin and plays a vital role in maintaining blood-coagulation. In blood-coagulation pathway thrombin converts fibrinogen to fibrin which forms the blood clot. Thrombin is a Na+ activated, allosteric serine protease.Thrombin isconsist of two polypeptide chain of 36 (A chain) and 259 (B chain) residues that are covalently linked through a disulphide bond.[4][5]The inhibitor of thrombin can be used as an anti-coagulant agent which plays an important role to prevent strokes and heart-attacks. Anti-coagulants are used to prevent the formation of blood clots and to maintain blood vessels open. In order to prevent the stroke, anticoagulation is a prime outlook of the codirection of atrial fibrillation.[2][8] Anti-coagulating agents has used to reduce various problematic condition of human body like cancer. In the treatment of cancer, the main problem is bleeding due to the chemotherapy. To eliminate this problem, we can use anti-coagulating agents.[3][8]

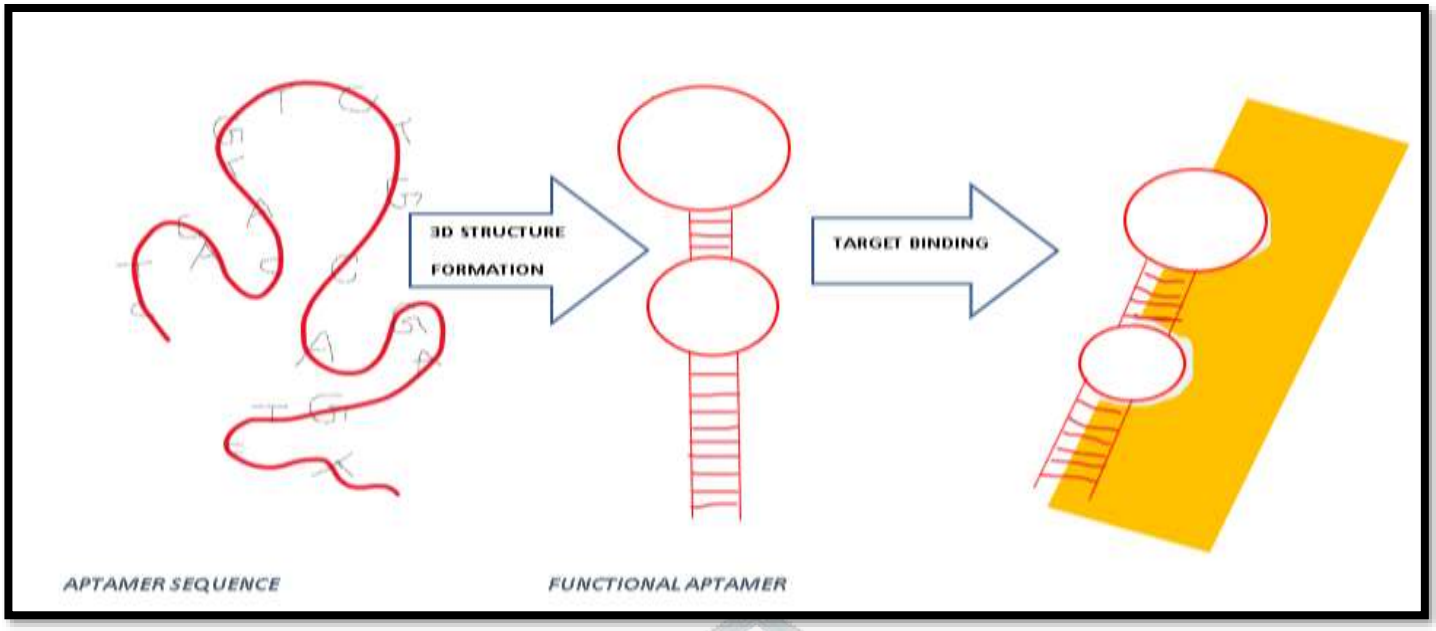

Figure 1 conversion of aptamer sequence to functional aptamer and its binding to a drug

For our study we have taken dexamethasone as an anti-coagulant agent. Dexamethasone is an odor-less, bitter taste crystallized powder which is used to treat some kinds of arthritis in human. It is an anti-inflammatory compound. It is an artificially synthesize member of the class of glucocorticoids. The glucocorticoids are a group of corticosteroids that help to inhibit pro-inflammatory signals, and supports inflammatory signals. Glucocorticoids are highly proficient in suppression of the inflammatory process through numerous pathways. They bind to specific intracellular receptor proteins in target tissues to revise the expression of corticosteroid-responsive genes.[7][9]

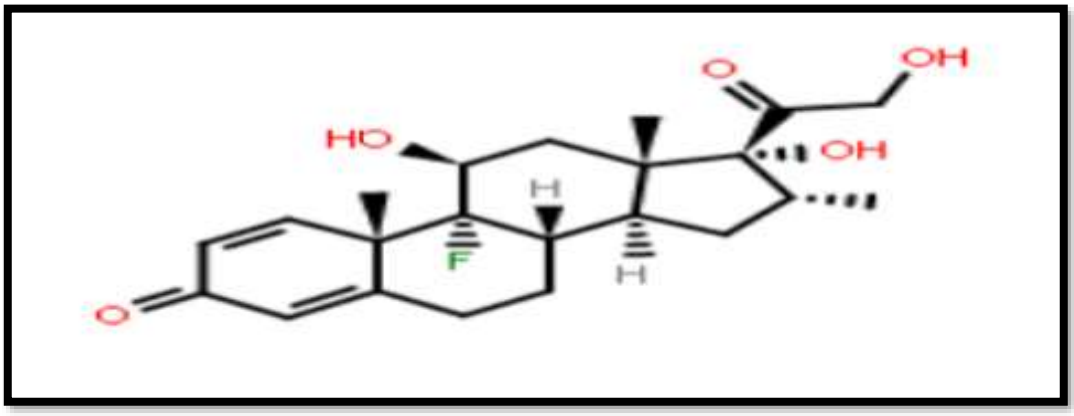

figure 2: chemical structure of dexamethasone

Now a days Dexamethasone is widely usedto cure state like arthritis, allergic reactions, skin diseases, eye problems, breathing problems, bowel disorders and cancer. [7]

SOME CHEMICAL PROPERTIES OF DEXAMETHASONE-

Table1:ligand properties

CHEMICAL PROPERTIES	**VALUE**
Molecular weight	392.45
Appearance	crystalline powder
Colour	White to yellowish-white
Odor	odorless
Chemical formula	C22 H29 F05
Hydrogen Bond Acceptor	6

Hydrogen Bond Donar	3
Rotatable Bond Count	2
Melting Point	504 to 507 °F
Vapor pressure	8.86X10-14 mm Hg at 25 °C

As we all can observe that there is an exponential increment in the number of heart and cancer patients in all over the world and in the treatment of both condition, anti-coagulation plays a key role. And this paper will help researchers and doctors to take a look on different drugs that can be a better option of conventional drugs used for anti-coagulation.[2][3][8]

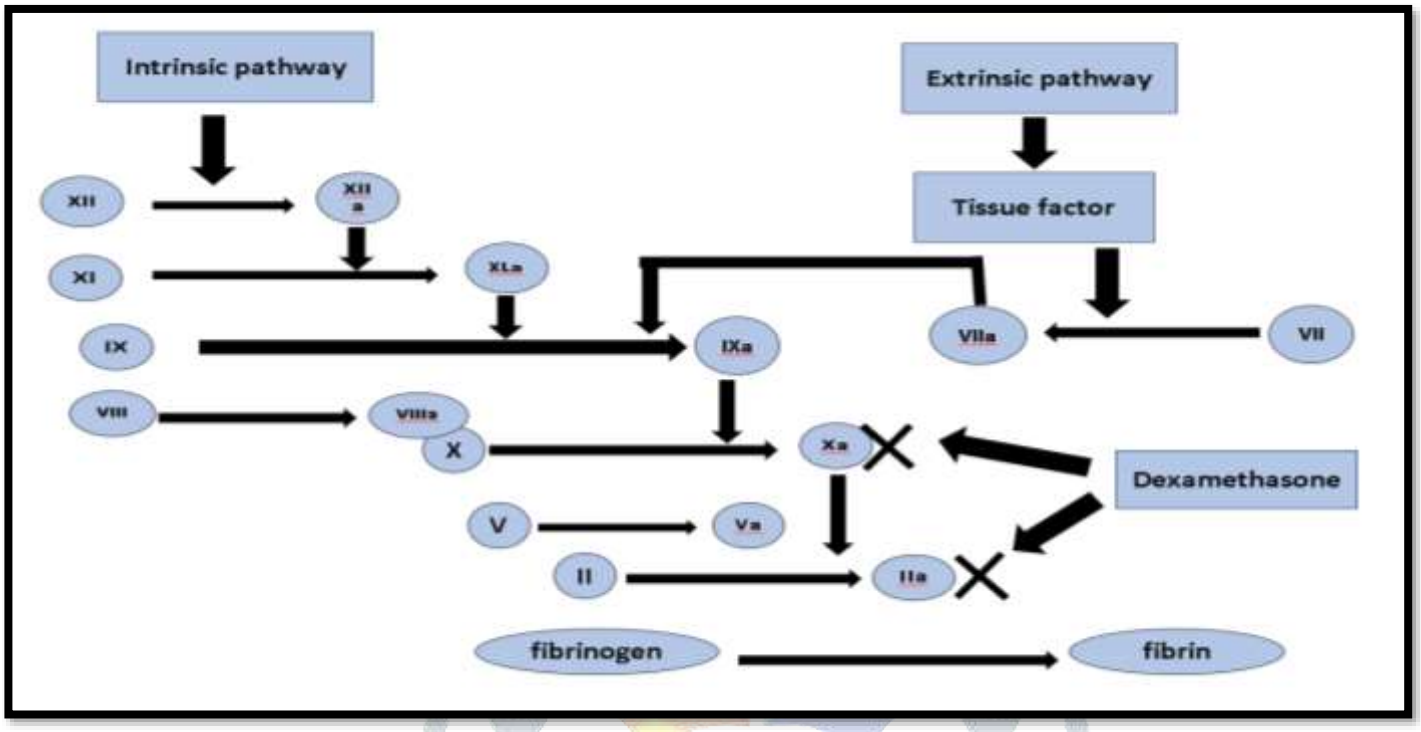

figure 3: The above given diagram shows the expected pathway of inhibition by dexamethasone

MATERIAL AND METHODS-

We have taken our RNA aptamer structure from PDB (protein data-bank) and dexamethasone structure from the pubchem database. RNA aptamer is downloaded in PDB format and dexamethasone is downloaded in SDF format. SDF stands for structure data file which contains the information about the atoms, bonds, connectivity and coordinates of Pubchem molecule which describes the structural relationships and properties of the atoms. We have prepared our aptamer and ligand structure using pymol. We have also performed the analysis of our protein structure using Rasmol software. Using Rasmol we have seen how many oxygen, nitrogen, hydrogen and carbon molecules are present in our structure. After analyzing the structure, we performed structure-baseddocking between aptamer and ligand using autodock 4.0. The aptamer that we have taken from the PDB is the RNA aptamer of human thrombin protein. The id of this aptamer is **3DD2** and the pubchem CID number of dexamethasone is **5743.**The PDB database was established in 1971 at Broakhaven national laboratory under leadership of Sir Walter Hamilton. PDB or protein data-bank is a database where we store all the structure of proteins of various organism that have been discovered till now. PDB has a large collection of proteins whose structures are obtained through various chemical methods.Pubchem is a database where we store the structure of various chemical compounds. It is a part of NCBI (national center for biotechnology information) which is freely available for everyone. Rasmol is a molecular graphics visualization tool which is used to visualize and analyze the various type of molecular structures like protein, nucleic acid, chemical compounds etc. Using Rasmol, we can visualize our structure in various format like wireframe, spacefill, ball and stick, backbone, ribbon, strands and cartoon.Pymol is also a visualization tool through which we can visualize the molecular structure. Pymol software is based on the python that is a computer programming language.[12]Autodock is a computer software program through which we can observe the binding between protein and ligand.Pymol, Rasmol and Autodock all these tools widely used in CADD (computer aided drug designing). Every virus, bacteria or fungus has protein or DNA or RNA that is infectious and fatigue to human, we can yield the structure of that infectious biomolecule through chemical method and store it in a biological databse, whenever any scientist or researcher need the structure information, they can obtain it through biological databases like PDB. After extracting the information, autodock is used to find a suitable drug for inhibition of that specific infectious biomolecule. During covid-19 many drugs has discovered using this approach. Now a days this in-silico drug designing technique has been used world-wide to minimize the cost and reduce the time of drug-discovery. Drug-discovery is a process consist of five steps target identification and Validation, Hit identification and Validation,Moving from a hit to a lead, Lead Optimization, Late Lead Optimization.[17][18][19]

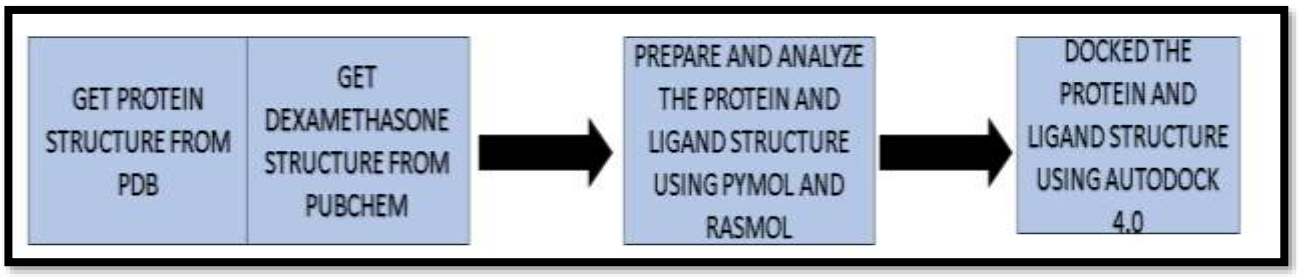

Figure 4: workflow of docking between ligand and target

RESULTS and DISCUSSION

In this paper we have docked the thrombin aptamer with dexamethasone. From this docking experiment, we can conclude that dexamethasone partially binds to the thrombin aptamer and shows very low efficacy of binding to the thrombin aptamer. We have analyzed the thrombin aptamer structure using Rasmol. Macromolecules like protein, nucleic acid and many more whose structures, we can get through X-ray crystallography, NMR spectroscopy, electron microscopy and circular diachroism, can store in biological databases and from biological databases we can yield these structures and visualize the structure using various software like pymol and rasmol. Rasmol is a command-line base as well as graphical user-interface based tool. Various commands are present in the rasmol like “color” command allows different objects (such as atoms, bonds and ribbon segments) to be given a specified colour. Rasmol also assigns colour to the molecule according to the atoms. Carbon is defined with grey colour, hydrogen is defined with white colour and likewise oxygen with red, nitrogen with blue and sulphur is defined with yellow. Colours assign to the molecules according to the type of their secondary protein structure. Alpha-helices contain magenta colour, beta sheets contain yellow colour, turns contain blue and all other residues show with white colour. There are various methods in rasmol through which we can assign colour to the molecules. The colours assign according to nucleotides, amino acids, C or N terminal and temperature. Adenine is express with light blue, cytosine with light orange, guanine with medium salmon and thymine with light green.Rasmolwas evoloved by Roger Sayle in the early 1990s. Molecular docking is a computational method in which ligand receptor bind can done. For extracting any information from the autodock we have to perform three steps: experiment, model and prediction. First we performed the experiment using autodock, then autodock will generate a model based on Lamarckian algorithm and then from that model, we analyze the binding between protein and ligand is possible or not, if not then we run the docking again with another drug. We can screen any number of drugs against any protein.Rasmol “amino” colour scheme colours amino acids according to traditional amino acid properties. The purpose of colouring is to identify amino acids in an unusual or surprising environment.[14][15]

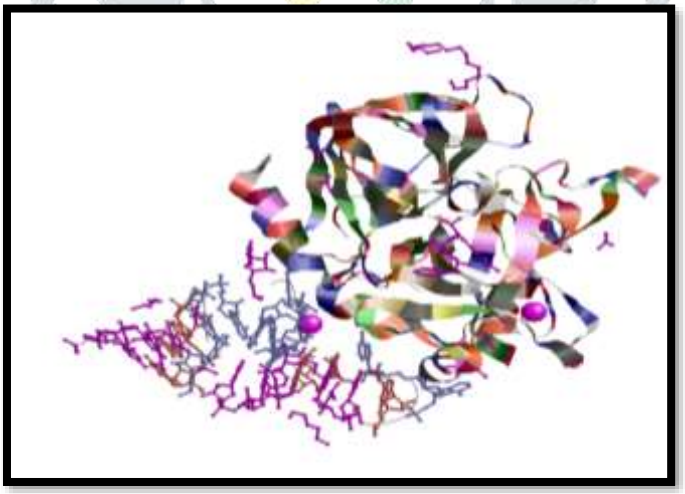

Figure 5:This is the structure of human thrombin protein visualize by Rasmol through shapely.

RasMol 'shapely' colour scheme colour codes residues by amino acid property. This scheme is based upon Bob Fletterick's "Shapely Models". Each amino acid and nucleic acid residue is given a unique colour. The 'shapely' colour scheme is used by David Bacon's Raster3D program.[14][15]

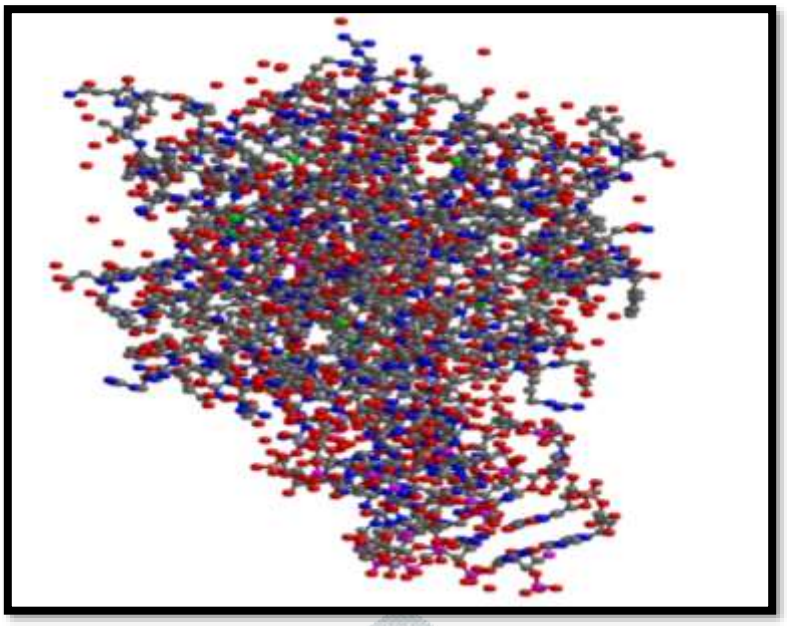

Figure 6: This structure is visualize by rasmol and using command line oxygen atoms show with red colour, carbon atom with grey, nitrogen with blue and sulphur with green colour.

Results obtained usingRasmol-

Table2: result from rasmol

ATOM NAME	OBSERVED NO. OF ATOMS
Oxygen	870
Carbon	1777
Nitrogen	515
Sulphur	15

Total atoms present in ligand molecule is 57.

PyMOL is a cross-platform molecular graphic tool and has been widely used for 3D visualization of macromolecules. The utilities of PyMOL have been extensively enhanced by various plugins, including macromolecular analysis, homology modeling, protein–ligand docking, pharmacophore modeling. PyMOL is freely available,open-source software developed by Warren Lyford DeLano. The homepage of pymol software consist main canvas, object list, GUI menus, Mouse commands, movie controls and command line. In the main canvas we visualize the structure and all the options that we need to prepare the protein for docking, present in the GUI menus. Some functions in pymolis present in scripting language. All options are not available in menus. Command-line interface is a form of interface between the operating system and the user in which the user types commands, using a special command language.[12][13]

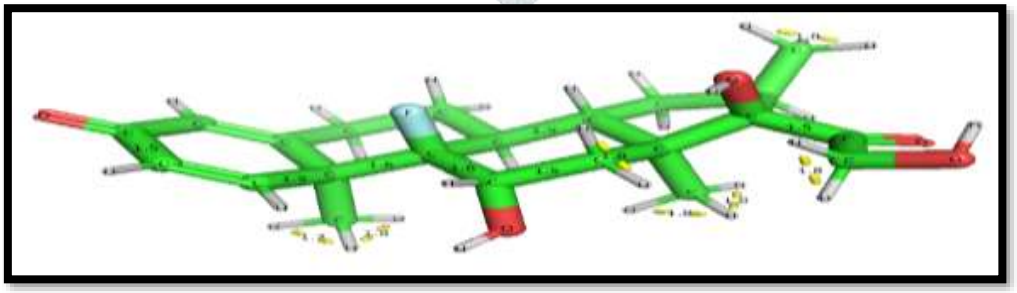

Figure 7: This figure has been taken through pymol. From this figure we can conclude that average distance between carbon-carbon atom is $1.5A^0$ and average distance between H-H bond is 1.8 $A^{0.}$

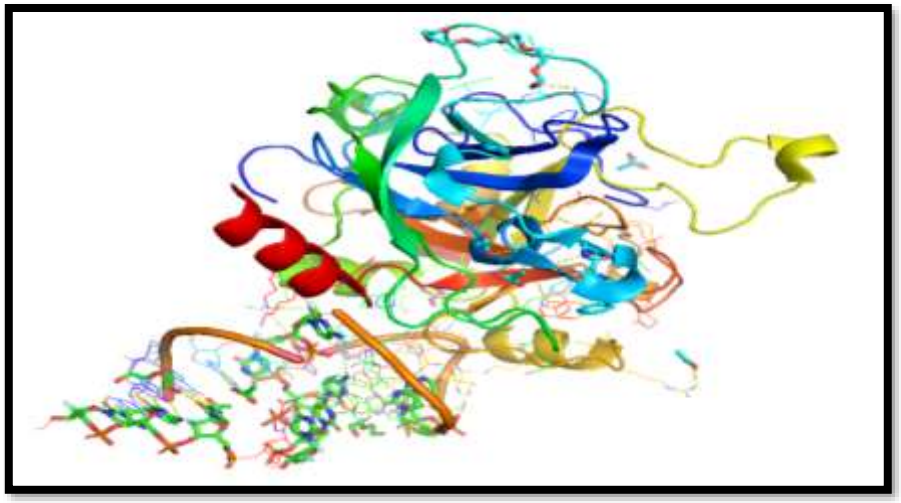

Figure 8:This is the structure of RNA aptamer of human thrombin visualize through pymol.Inpymol we can see the binding region of the aptamer has shown by the blue color and mutating region has shown by the red color

Pymol is written in python. Python is a computer programming language. Using pymol we can perform various tasks in our structure. In dexamethasone we have deleted water molecules and because water molecules create disturbance in binding of drug. We can also check where the ligand site is present in the protein using "ligand sites" option in the "present" menu. "Label" scheme is used to label the residue name, atom name or chain name. In the "Wizard" option we find "measurement" option which is used to measure the distance between any two atoms.[12][13]

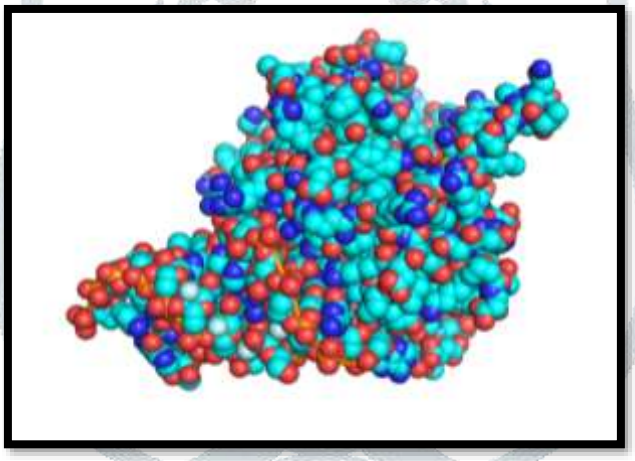

Figure 9: This is the structure of RNA aptamer of human thrombin visualize by pymol through "sphere" scheme.

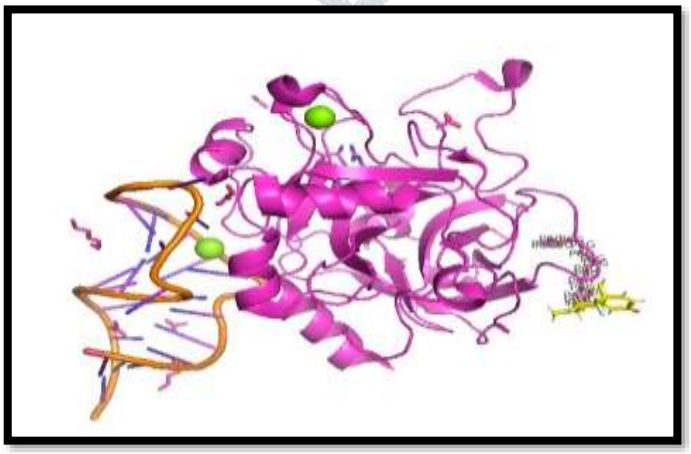

Figure 10: This image is captured by using pymol software in which we can observe the binding of the drug with protein.This image is taken through "cartoon" option

Docking is an attempt to find the best matching between two molecules. Docking predicts the preferred orientation of one ligand when bound in an active site to form a stable complex. There are various types of forces that can form interaction between target

and ligand. These forces can be electrostatic forces, electrodynamics forces or vanderwaals interaction, steric forces (caused by entropy) and solvent related forces (due to the structural changes of the solvent generated by ions, colloids, proteins etc.).[17][20]

Figure 11: Using pymol we can observe the amino acid or nucleotide present in the binding site

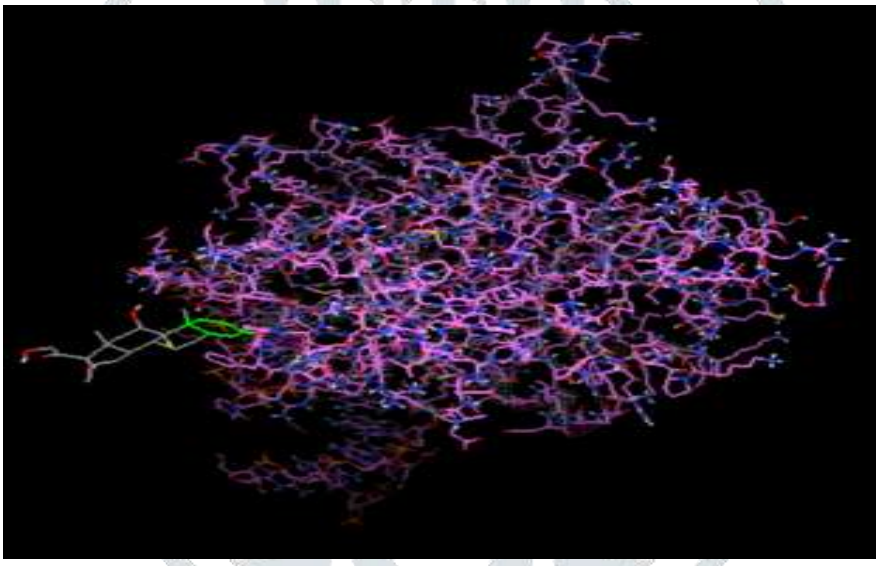

Figure 12: Structure based docking between dexamethasone and thrombin RNA aptamer by Autodock 4.0

Figure11 is the docking image of dexamethasone and thrombin aptamer.Autodock is a suite of automated docing tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. Autodock software read input files in PDB format. After opening the protein structure, we have added the polar hydrogen and kolman charges into structure. Polar hydrogens are hydrogens that are bonded to electronegative atoms like oxygen and nitrogen. In dexamethasone the value of total kolman charge added is '17.055'. Using 'input' option we upload our ligand or drug structure in autodock and check aromaticity and detect torsion root present in the drug candidate. After all these editing, we save our protein and ligand structure separately in PDBQT format. PDBQT file format is similar to PDB but it includes partial charges (Q) and autodock4 (AD4) atom types (T). There is one line for each atom in the ligand, plus special keywords indicating which atoms, if any, are to be flexible during the autodock experiment. After generating PDBQT file of both ligand as well as protein we generate grid file for both structure through "Grid Box" option. In the "Grid box" option set number of points in X, Y and Z coordinate to 60 and observe the binding between ligand and protein.[16]

CONCLUSION

From this research paper we can conclude that dexamethasone cannotuse as an anti-coagulating agent alone. It shows binding with thrombin but not stable binding. We can use this after some modification or with some another compound which helps the dexamethasone to bind to the thrombin aptamer. Thrombin is a serine protease that converts soluble fibrinogen into insoluble fibrin. Inregulation of thrombin enzymatic activity offers the possibility of controlling blood Coagulation.Thrombin-binding oligonucleotide aptamers have been used to inhibit thrombin activity.

REFERENCES-

1. Rabal, O., Pastor, F., Villanueva, H., Soldevilla, M. M., Hervas-Stubbs, S., & Oyarzabal, J. (2016). In Silico Aptamer Docking Studies: From a Retrospective Validation to a Prospective Case Study-TIM3 Aptamers Binding. *Molecular therapy. Nucleic acids*, *5*(10), e376. https://doi.org/10.1038/mtna.2016.84
2. Despotis, G. J., Gravlee, G., Filos, K., & Levy, J. (1999). Anticoagulation monitoring during cardiac surgery: a review of current and emerging techniques. *Anesthesiology*, *91*(4), 1122–1151. https://doi.org/10.1097/00000542-199910000-00031
3. De Lorenzo, F., Dotsenko, O., Scully, M. F., &Tymoshchuk, M. (2006). The role of anticoagulation in cancer patients: facts and figures. *Anti-cancer agents in medicinal chemistry*, *6*(6), 579–587. https://doi.org/10.2174/187152006778699112
4. Alquwaizani, M., Buckley, L., Adams, C., &Fanikos, J. (2013). Anticoagulants: A Review of the Pharmacology, Dosing, and Complications. *Current emergency and hospital medicine reports*, *1*(2), 83–97. https://doi.org/10.1007/s40138-013-0014-6
5. Di Cera E. (2008). Thrombin. *Molecular aspects of medicine*, *29*(4), 203–254. https://doi.org/10.1016/j.mam.2008.01.001
6. Bunka, D. H., Platonova, O., & Stockley, P. G. (2010). Development of aptamer therapeutics. *Current opinion in pharmacology*, *10*(5), 557–562.
7. Edward M. Cohen,Dexamethasone,Editor(s): Klaus Florey,Analytical Profiles of Drug Substances,Academic Press,Volume 2,1973,Pages 163-197,ISSN 0099-5428,ISBN 9780122608025,
8. DeWald, T. A., & Becker, R. C. (2014). The pharmacology of novel oral anticoagulants. *Journal of thrombosis and thrombolysis*, *37*(2), 217–233. https://doi.org/10.1007/s11239-013-0967-z
9. Lammers, T., Sofias, A. M., van der Meel, R., Schiffelers, R., Storm, G., Tacke, F., Koschmieder, S., Brümmendorf, T. H., Kiessling, F., &Metselaar, J. M. (2020). Dexamethasone nanomedicines for COVID-19. *Nature nanotechnology*, *15*(8), 622–624. https://doi.org/10.1038/s41565-020-0752-z
10. Dunn, M.R., Jimenez, R.M., &Chaput, J.C. (2017). Analysis of aptamer discovery and technology.
11. Ahirwar, R., Nahar, S., Aggarwal, S., Ramachandran, S., Maiti, S., & Nahar, P. (2016). In silico selection of an aptamer to estrogen receptor alpha using computational docking employing estrogen response elements as aptamer-alike molecules. *Scientific reports*, *6*, 21285. https://doi.org/10.1038/srep21285
12. Seeliger, D., & de Groot, B. L. (2010). Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *Journal of computer-aided molecular design*, *24*(5), 417–422. https://doi.org/10.1007/s10822-010-9352-6
13. Lill, M. A., & Danielson, M. L. (2011). Computer-aided drug design platform using PyMOL. *Journal of computer-aided molecular design*, *25*(1), 13–19. https://doi.org/10.1007/s10822-010-9395-8
14. Sayle, R. A., & Milner-White, E. J. (1995). RASMOL: biomolecular graphics for all. *Trends in biochemical sciences*, *20*(9), 374. https://doi.org/10.1016/s0968-0004(00)89080-5
15. Goodsell D. S. (2005). Representing structural information with RasMol. *Current protocols in bioinformatics*, *Chapter 5*, .https://doi.org/10.1002/0471250953.bi0504s11
16. Jing, M., & Bowser, M. T. (2011). Methods for measuring aptamer-protein equilibria: a review. *Analytica chimica acta*, *686*(1-2), 9–18. https://doi.org/10.1016/j.aca.2010.10.032
17. Chen Y. C. (2015). Beware of docking!. *Trends in pharmacological sciences*, *36*(2), 78–95. https://doi.org/10.1016/j.tips.2014.12.001
18. Eder, J., &Herrling, P. L. (2016). Trends in Modern Drug Discovery. *Handbook of experimental pharmacology*, *232*, 3–22. https://doi.org/10.1007/164_2015_20
19. Forli, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nature protocols*, *11*(5), 905–919. https://doi.org/10.1038/nprot.2016.051
20. Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. *Methods in molecular biology (Clifton, N.J.)*, *443*, 365–382. https://doi.org/10.1007/978-1-59745-177-2_19