DOCKING STUDIES USING MEF 2A AND MYELOPEROXIDASE AS TARGET PROTEINS FOR MYOCARDIAL INFARCTION


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ABSTRACT

Cardiovascular disease is a class of diseases that involve the heart or blood vessels. They remain the biggest cause of deaths worldwide, though over the last two decades, cardiovascular mortality rates have declined in many high-income countries, it is still a major concern in today’s society. Cardiovascular diseases are the number 1 cause of death globally. About 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to Myocardial Infarction and 6.7 million were due to stroke. Myocardial infarction occurs due to the interruption of blood supply to a part of the heart, causing heart cells to die. This is most commonly due to occlusion of a coronary artery by a blood clot which results in ischemia and emanates oxygen shortage, finally leading to the death of cardiac muscle tissue (Myocardium). There are various drugs that can prevent Myocardial Infarction like Warfarin, Aspirin, Clopidogrel etc. By using bioinformatics as a basic tool we can understand how effective these drugs are in suppressing and counter act the protein targets like responsible for Myocardial Infarction. Docking studies are performed with respect to target and various drugs. These results help us in understanding the effectiveness of each drug on these protein targets.

Keywords: Cardiovascular Diseases, Myocardial Infarction, Protein targets, Docking

1. INTRODUCTION

Cardiovascular disease is a type of disease caused due to the irregularities caused in the heart muscles and blood vessels. They are one of the leading causes of death globally, it is a significant cause of worry for the general public. According to WHO, by 2030 it is expected that almost 23.6 million people will die from cardiovascular diseases manifesting from myocardial infarction, globally. Although there has been a significant decline in the mortality numbers in highly developed and high-income countries, the number of cardiovascular disease related deaths have increased at an
astonishingly fast rate in low- and middle-income countries\textsuperscript{[1]}. There are various types of cardiovascular diseases, some of these are Coronary heart disease → Myocardial Infarction, Cardiomyopathy, Hypertensive heart disease, Heart failure, Cor pulmonale, Cardiac dysrhythmias, Inflammatory heart disease, Valvular heart disease and Peripheral arterial disease. The first studies on cardiovascular health were performed in 1949 by Jerry Morris using occupational health data and were published in 1958. Various studies were done since then and it was clearly found that Myocardial Infarction causes close to 80% of the cardiovascular deaths in the developing world. Myocardial infarction occurs due to the interruption of blood supply to a part of the heart, causing heart cells to die. This is most commonly due to occlusion of a coronary artery by a blood clot which results in ischemia and ensuing oxygen shortage, followed by damage or death (infarction) of heart muscle tissue (myocardium).\textsuperscript{[2]}

The blood clot is usually caused by the development of a hard substance called plaque, which is made up of cholesterol and dead cells. A heart attack can occur as a result of plaque rupture due to (Figure 1):

\begin{enumerate}
  \item Development of cracks or tears in the plaque, causing the platelets to adhere and form clots, thus completely blocking supply of oxygen-enriched blood.
  \item Lack of oxygen-enriched blood due to slow formation of plaque.
\end{enumerate}

There are two basic types of MI based on the pathology viz.

\begin{enumerate}
  \item Transmural: It is associated with atherosclerosis involving a major coronary artery. Infarcts extend through the whole thickness of the heart muscle and our usually a result of complete occlusion of the area’s blood supply.
  \item Subendocardial: It involves a small area in the subendocardial wall of the left ventricle, ventricular septum or papillary muscles.
\end{enumerate}

Classical symptoms are sudden chest pain, Shortness of breath or dyspnea, Diaphoresis, Weakness, Light-headedness, Nausea, Vomiting and Palpitations.

\textbf{Figure 1 – Heart Attack Mechanism}

Myocardial infarction can be discovered using electrocardiograms, using blood enzymes tests or at autopsy. Although causes are unknown, heart attacks are recognized to occur in association with
Various procedures have been developed like Angioplasty, Thromolytic therapy has been done in order to identify and treat heart attack. Various types of drugs have also been used like Antiplatelet drugs (blood thinners), Beta-blockers, ACE inhibitors and Statins. Hence it is important to understand how computation biology helps us understand the interaction of these given set of drugs on the protein targets.

Computational methods have been widely developed and applied to pharmacological hypothesis development and testing. The term ‘in silico’ is a modern word used to mean experimentation performed by computer. The ‘in silico’ methods include use of databases, analyzing structure activity relationships, performing similarity searching, developing and understanding pharmacophores, obtaining homology models, data mining, network analysis tools and data analysis tools. These methods have been frequently used in the discovery and optimization of novel molecules and bioactive compounds with affinity to a target, the clarification of ADMET and toxicity properties as well as physicochemical characterization. In silico pharmacology for drug discovery can be applied with respect to targets or can be applied in terms of ligand screening. In silico methods can help in identifying drug targets via bioinformatics tools. They can be used to analyze the target structures for possible binding, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

The use of computers and computational methods permeates all aspects of drug discovery today and forms the core of structure based drug design. The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at lower cost.

2. TARGETS

A survey of literature was needed to understand which proteins play an important role in Myocardial Infarction. The evidence for heritability of myocardial infarction (MI) is very distinctive, with a positive family history being one of the most important risk factors for this complex trait. There have been seven such studies which showed a cumulatively result of more than 2,000 families. Each study had found different loci, but the results based on different populations differed substantially on the basis of sample size, ethnicity, age, and analysis program used to determine linkage. Furthermore, most used CAD as the phenotype rather than MI. Thus far, of the seven
studies, only one was able to determine the specific gene accounting for a significant linkage peak. A specific haplotype on the 15th chromosome near its telomere had more than 60 genes. Thus, MEF2A was considered as a potential candidate because of its known effect in the development of Myocardium, albeit without apprehension of coronary arterial impact. The functional genomics of this deletion mutation, leading to a seven aminoacid “in frame” truncation, was necessary to be carried out to show the inability for this transcription factor to localize in the nucleus, the presence and location of MEF2A in the endothelial layer of the artery, and the shutdown of transcription using a very standard assay technique. The drugs currently present in the market and this research domain will provide a more than sufficient relief to the sufferers of Myocardial Infarction.\[4\]

Coronary artery disease (CAD) that results from formation of lesions on the vascular walls is an essential cause of myocardial infarction (MI) and stroke. A human pedigree with a predisposition to CAD and MI was found to harbor a mutation with respect to the MEF2A transcription factor. These findings help us unveil a new function of this regulator for cardiovascular development and raise and peak curious and interesting questions about the underlying mechanisms of CAD.\[5\]

Positional cloning based on genome-wide linkage analysis with respect to large families helped us identify the first non-lipid-related disease-causing gene, MEF2A (encodes a transcriptional factor), for myocardial infarction and coronary artery disease. About 1.93% of diseases in the populations was due to the MEF2A mutations; thus the genetic testing based on the mutational analysis of MEF2A may soon be available for many coronary artery disease/Myocardial Infarction patients. Genetic studies provided several new insights into the pathogenic aspect of coronary artery disease and myocardial infarction.\[6\]

Myeloperoxidase (MPO) is one of the essential biomarkers of inflammation and oxidative stress which is produced by neutrophils, monocytes, and endothelial cells. Concentrations of MPO predicts the mortality in patients with heart related diseases. Myeloperoxidase (MPO) is released from activated monocytes and neutrophils during inflammation. The production of MPO has been recently demonstrated on endothelial cells with respect to its response to oxidative stress. MPO is one of the major contributors to vascular inflammation caused by depletion of vascular nitric oxide (NO) with resulting endothelial dysfunction, as well as by promoting the LDL oxidation.\[7\]

Oxidative stress plays an essential and critical role in the initiation and the further progression of atherosclerosis. Myeloperoxidase (MPO) is a major biomarker of oxidative stress. Further investigations were done to see if there is an increased serum concentration of MPO which is associated with an increased risk of incident coronary heart disease (CHD). Elevated concentrations of the oxidative stress marker MPO were independently associated with enhanced risk of incidents of CHD.\[8\]

It was found through rigorous survey that MEF2A and Myeloperoxidase were the key factors responsible for Myocardial Infarction and Coronary Heart Disease. By performing various computational studies we can figure out how these targets get affected when drugs are introduced into the biological system.
3. METHODS, DATABASES AND TOOLS

Methods

- Determine the virtual library to be used
- Determine the structure of drug target
- Filter the library if necessary
- Determine the interaction site on drug target

**Docking**: Is flexibility being considered?

**Scoring**: Which method to be used?

**Evaluation**: Do results make sense?

**Optimization**: Are there any focused libraries available?
Target Identification

Targets were identified from literature survey and by using sequence information of proteins in databanks that were further modelled and developed to get an optimum target which was used for further analysis.

Databanks like PDB and UniProtKB were used to screen and obtain structures of proteins, MEF2A and Myeloperoxidase. Since both of these structures were found to occur along with hetero-atoms/molecules, homology modelling was carried out in order to obtain a well-established and dependable model which would help us understand the structures at a greater depth.\textsuperscript{[9][10]}

Molecular modelling is a discipline that contributes to the understanding of these processes in a qualitative and sometimes in a quantitative way. It presents itself as a means for analyzing the details of the molecular machinery involved in a known biological system, also helps to understand the way the system functions and it further provides the necessary tools for predicting the potential possibilities of prototype suitor molecules.\textsuperscript{[11]}

Some applications of molecular modelling include,

- Investigating the structural, dynamics of the system, surface properties and thermodynamically applicable aspects of inorganic biological and polymeric systems.
- Investigation of biological activity like protein folding, enzyme catalysis, protein stability, conformational changes of the targets, molecular recognition of proteins, DNA and membrane complexes.

Three methods of molecular modelling are,

- Homology or Comparative modelling
- Fold recognition or threading methods
- Ab initio method with or without knowledge-based information\textsuperscript{[11]}

Comparative modelling of proteins refers to constructing and reconfiguration of the atomic resolution model of the target proteins that have been obtained from its amino acid sequence and an experimental 3-Dimensional structure of a related homologous protein. It relies on the identification of one or more known protein structures and likely resembles the structure of query sequence and on the production of an alignment that maps residues in the query sequence to residues in the template sequence. It has the following practicality,

- Protein-protein interaction prediction,
- Protein-protein docking,
- Molecular docking between the proteins and the ligand molecules,
- Functional annotation of genes that have been identified in an organism’s genome,
- To identify the subtle differences between all related proteins that have not been solved structurally.\textsuperscript{[12]}

Target Validation

The targets that were chosen were validated using literature review and by means of metabolomics approach. Metabolomics is the scientific study and approach of chemical processes that involve metabolites. This is a new, novel approach that can be used for the validation of proteins in drug designing and development.\textsuperscript{[13][14]}

Lead Identification

Lead molecules for Myocardial infarction was identified by filtering molecules which were enlisted in various databanks like Drugbank and Pubchem.\textsuperscript{[15][16]}
Lead Validation: Lipinski’s Rule of Five
The Rule of Five stated- “Poor absorption or permeation are more likely when there are.
• More than 5 H-bond donors
• The molecular weight is over 500
• The log P is over 5
• The sum of N’s and O’s is over 10
The partition coefficient, log P is the ratio of the concentration of a compound in the two phases of a mixture of two immiscible solvents at equilibrium. [17]

Docking
Docking is finding the binding geometry of two interacting molecules with known structures. It is a method which predicts the preferred orientation of one molecule to another when bound to form a stable complex. The process of docking tries to mimic the interaction between two molecules in its natural state as well in the biological system. The types of docking used in this project were
• Local docking - Local docking refers to finding the position of the ligand in the binding site which is already established.
• Global docking - Global docking refers to the method wherein the binding site is unknown,
• Rigid docking – Here, both the ligand and receptor are kept rigid
• Flexible docking – Here, flexibility is given for the receptor, ligand or both [11]

Active site identification: Grid docking
To speed up the process of energy evaluation during the docking process, a grid-based representation of the binding site is needed and used where every grid point corresponds to an affinity value calculated for the interaction of a probe atom with the protein. In Auto Dock, the incorporated empirical free energy function is always used to determine and calculate the grids. The primary grid values was scaled for proper combination with the intramolecular force field terms used by Auto Dock. The scaling up parameters were put to use and the grid was centered to a crystallographic binding mode. Sufficient grid spacing is done for the molecule to bind to the protein. [18][19]

Databases
The various databases used with respect to identifying the targets of Myocardial infarction.
Protein Data Bank- Structures of MEF2A and Myeloperoxidase were obtained.
UniProt- Protein sequences of MEF2A and Myeloperoxidase were obtained.
DrugBank- To obtain all the drugs that have been used to treat heart attack.
PubChem- To download the SDF files of all the compounds, which are then docked.
KEGG- To obtain the metabolic pathway of both MEF2A and Myeloperoxidase.
PubMed and PubMed Central- To download all the papers required for the aspect of the docking and target identification. [25][26]

Tools
Swiss Model- Online tool used for the modelling protein structures of MEF2A and Myeloperoxidase. [20][21][27]
Open Babel- Chemical tool box used for converting the chemical data files. [28]
RasMol- A program for molecular graphics visualization of the targets. [29]
Auto Dock- Used for Global and Local docking. [30]
Auto Dock PYRX- Used for performing Rigid and Flexible docking. [31][32]
4. RESULTS AND INTERPRETATION

The two macromolecules MEF2A and Myeloperoxidase were chosen as targets and their 3D structure were retrieved from PDB (Protein Data Bank). Lead molecules were screened from databanks like DrugBank and PubChem, to get a series of possible drug candidates shown in Table 1.

Table 1: Drugs for Myocardial Infarction

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Name of the compound</th>
<th>Mol. Wt.</th>
<th>XlogP</th>
<th>H₂bond donor</th>
<th>H₂ bond acceptor</th>
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<td>1</td>
<td>Ridogrel</td>
<td>366.33</td>
<td>4.3</td>
<td>1</td>
<td>5</td>
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<tr>
<td>2</td>
<td>Ticlopidine</td>
<td>263.786</td>
<td>2.9</td>
<td>1</td>
<td>0</td>
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<tr>
<td>3</td>
<td>Propranolol</td>
<td>259.3434</td>
<td>3.0</td>
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<td>3</td>
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<tr>
<td>4</td>
<td>Ramipril</td>
<td>416.50</td>
<td>2.9</td>
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<td>5</td>
<td>Benazepril</td>
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<td>2</td>
<td>5</td>
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<td>6</td>
<td>Metoprolol</td>
<td>267.36</td>
<td>1.6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Bisoprolol</td>
<td>325.44</td>
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<td>2</td>
<td>5</td>
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<tr>
<td>8</td>
<td>Losartan</td>
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<td>4.5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>Propafenone</td>
<td>341.4</td>
<td>3.2</td>
<td>2</td>
<td>4</td>
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<td>10</td>
<td>Arbutamine</td>
<td>317.37</td>
<td>2.9</td>
<td>5</td>
<td>5</td>
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<tr>
<td>11</td>
<td>Warfarin[22][23]</td>
<td>308.33</td>
<td>2.7</td>
<td>1</td>
<td>4</td>
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<tr>
<td>12</td>
<td>Oxyfedrin</td>
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<td>2.4</td>
<td>2</td>
<td>4</td>
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<td>Molsidomine</td>
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<td>14</td>
<td>DIB014</td>
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<td>15</td>
<td>Fluanisone</td>
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<tr>
<td>16</td>
<td>Stepholidine</td>
<td>327.37</td>
<td>2.6</td>
<td>2</td>
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<tr>
<td>17</td>
<td>Disopyramide</td>
<td>339.47</td>
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<tr>
<td>18</td>
<td>Phenoxybenzamine</td>
<td>303.826</td>
<td>4.4</td>
<td>0</td>
<td>2</td>
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</tbody>
</table>

Of these, 18 were further screened on the basis of Lipinski’s rule of five. On screening only four molecules qualified, Warfarin, Ticlopidine, Propranolol and Arbutamine[24]. Docking studies were carried out using Auto Dock by using Myeloperoxidase with warfarin, Myeloperoxidase with Arbutamine, Myeloperoxidase with Propranolol, MEF2A with Ticlopidine and MEF2A with Warfarin as the protein and the drug molecules respectively. Grid docking was used to identify active sites and the resultant docking models were found based on the lowest energy values. Following were the Dock results obtained.
Docking result of Myeloperoxidase with Warfarin

![Docking result of Myeloperoxidase with Warfarin](image)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tbody>
<tr>
<td>Binding energy</td>
<td>-0.61</td>
</tr>
<tr>
<td>Ligand efficiency</td>
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</tr>
<tr>
<td>Intermolecular energy</td>
<td>-11.65</td>
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<tr>
<td>Desolvation energy</td>
<td>-11.64</td>
</tr>
<tr>
<td>Torsional energy</td>
<td>11.04</td>
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<tr>
<td>RMS value</td>
<td>24.657</td>
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</tbody>
</table>

Docking results for Myeloperoxidase with Arbutamine

![Docking results for Myeloperoxidase with Arbutamine](image)

Figure 6 - Docking results for Myeloperoxidase with Arbutamine
Docking results for Myeloperoxidase with Propranolol

<p>| | |</p>
<table>
<thead>
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<th></th>
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<tbody>
<tr>
<td>Binding energy</td>
<td>-3.64</td>
</tr>
<tr>
<td>Ligand efficiency</td>
<td>-0.16</td>
</tr>
<tr>
<td>Intermolecular energy</td>
<td>-10.8</td>
</tr>
<tr>
<td>Desolvation energy</td>
<td>-10.9</td>
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<tr>
<td>Torsional energy</td>
<td>7.16</td>
</tr>
<tr>
<td>RMS value</td>
<td>5.52</td>
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</table>

Docking results for Myeloperoxidase with Propranolol

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Binding energy</td>
<td>-4.17</td>
</tr>
<tr>
<td>Ligand efficiency</td>
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<td>Intermolecular energy</td>
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<tr>
<td>Torsional energy</td>
<td>5.07</td>
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<tr>
<td>RMS</td>
<td>10.965</td>
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</table>

Figure 7 - Docking results for Myeloperoxidase with Propranolol
Docking results for MEF2A with Ticlopidine

![Docking results for MEF2A with Ticlopidine](image)

**Figure 8 - Docking results for MEF2A with Ticlopidine**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<td>Ligand efficiency</td>
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<tr>
<td>Intermolecular energy</td>
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<tr>
<td>Torsional energy</td>
<td>6.56</td>
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<tr>
<td>RMS</td>
<td>74.128</td>
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</table>

Docking results for MEF2A with Warfarin

![Docking results for MEF2A with Warfarin](image)

**Figure 9 - Docking results for MEF2A with Warfarin**
The Docking results for Myeloperoxidase with Arbutamine and Myeloperoxidase with Propranolol showed lowest energy values and therefore are found to have favourable binding efficiency. Hence the two molecules Arbutamine and Propranolol are identified as efficient drug molecules for Myocardial Infarction. Further ADME studies on these drug molecules can be performed for predicting their activity in the human body.

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