CHAPTER I

INTRODUCTION

1.1 Background

Over the last century, the world has seen a great increase in people's life expectancy, which has doubled since 1990 (Roser *et al.*, 2013). However, shifting generations and lifestyles has also brought about new challenges, and more modern medical issues. In 2010, it was reported that one in four people globally suffer from cardiovascular issues, or more specifically thromboembolic conditions (Lozano *et al*., 2012). Among the top three causes of death globally (which makes up 33% of the mortality cases), are: ischemic heart disease, stroke and chronic pulmonary obstructive disease (World Health Organization, 2020). One of the reasons why those conditions may occur is due to a blood clot which obstructs blood flow and occurs in the blood vessels, or also known as thromboembolism. (Fedan, 2016).

The risk factors and causes of medical conditions which lead to thrombosis (such as ischemic heart disease or stroke) are partly genetically predisposed, and other factors brought upon by the choices in one's lifestyle or certain incidents (such as pregnancy, surgery, etc.) (Center for Disease Control and Prevention, 2020). Certain medicine has been developed in order to mitigate the formation of a thrombus or an embolism, and to degrade the thrombus/embolism. Common drugs manufactured to tackle the problem include fibrinolytic drugs, in order to rapidly break down the thrombus in the blood vessel. Such medicine includes: streptokinase, which forms a 1:1 complex with plasminogen, and induces a

conformational change in plasminogen that exposes its active site and converts additional plasminogen to plasmin; urokinase, a two-chain serine protease that converts plasminogen to plasmin directly by cleaving the bond; alteplase, a recombinant form of single-chain tPA that preferentially activates plasminogen in the presence of fibrin (Jameson, et al., 2018). Most of these fibrinolytic agents are not specific in nature, and have certain side effects on certain conditions. It is in great urgency then, that newer and safer research and discovery of other drugs should be undergone. Meanwhile, microbial sourced fibrinolytic enzymes show great potential due to its low cost and safer use (Bode, 1996). Multiple research has shown to support this, such as nattokinase produced by *Bacillus natto,* and subtisilin DFE and DJ-4 produced by *Bacillus amyloliquefaciens,* amongst the better well-known ones. Fibrinolytic enzymes have also been shown to be produced by fungi, actinomyces, and algae (Peng *et al.*, 2005).

In a previous study done by Pinontoan, *et al.*, 2021, it was shown that *B. subtilis* G8 produced fibrinolytic enzymes which were then further identified through its genome. One particular enzyme was identified with high similarity to minor extracellular serine protease Vpr. The enzyme Vpr was computationally 3D-modelled in order to theoretically predict the docking of its structure with fibrin chains, the catalytic activity of the enzyme's amino acids was analyzed, as well as the enzyme-fibrin interactions. The docking and modelling of Vpr is important in order to understand the enzyme's mechanism and predict how the enzyme cleaves fibrin, such is the process other drugs undergo *in silico* in the design and development stage. Previous better known computational modelling and docking

has been done by Syahbanu, *et al.*, 2020 using subtilisin as its enzyme from bacteria *B. subtilis* K2, isolated from Indonesian food Moromi. No research has been done on the molecular docking of Vpr with fibrin itself.

1.2 Problem Statement

There remains a lack of information regarding the molecular structural analysis of Vpr, as well as its interactions with fibrin in its enzymatic activity in order to cleave the fibrin chains. Hence, a computational docking simulation would prove beneficial in order to predict the folding of the enzyme itself, as well as to understand molecularly how the enzyme interacts with the fibrin chains.

1.3 Purpose

1.3.1 General Purpose

The purpose of this research is to analyze and model the structure of the Vpr enzyme *in silico*, as well as its docking analysis in regards to fibrin in order to analyze its mechanism.

1.3.2. Specified Purpose

The specified purpose of this study, is to:

1. Analyze the gene sequence of Vpr through bioinformatics tools: Basic Local Alignment Search Tool (BLAST), ProtParam and Prosite.
2. Simulate the 3D model of the Vpr enzyme using SWISS-MODEL & Robetta.
3. Analyze the accuracy of the structure using model verification tools: PyMOL, Self-Optimized Prediction Method with Alignment (SOPMA), PROCHECK, Protein Structure Analysis (ProSA) and Verify-3D.

4. Dock the enzyme with fibrin fragments of known cleavage sites, and analyze the interactions between the enzyme residues with the fibrin fragments with docking tools and residue interaction analyzers: Consensus Prediction of interface Residues in Transient complexes (CPORT), High Ambiguity Driven bio molecular DOCKing (HADDOCK), PROtein binDIng enerGY Prediction (PRODIGY), LigPlot+.