SCREENING FOR POTENTIAL COMPOUNDS USING DRUG-REPURPOSING AND VIRTUAL SCREENING OF N-METHYL-S-ASPARTATE (NMDA) RECEPTOR FOR AUTISM SPECTRUM DISORDER (ASD)

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by

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LIST OF ABBREVIATIONS

ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ADHD	Hyperactivity disorder and attention deficit
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APA	American Psychiatric Association
AS	Asperger's Syndrome
ASD	Autism Spectrum Disorder
ATD	Amino-terminal domain
BBB	blood-brain barrier
BNDF	Brain-derived neurotrophic factor
Ca^{2+}	Calcium ion
Cbl	Cobalamin
CNS	Central nervous system
CTD	Carboxy-terminal domain
DSM-5	The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DPF	Docking parameter file
FDA	U.S Food and Drug Administration
GROMACS	GROningen MAchine for Chemical Simulations
GPCR	G-protein-coupled receptor
GPF	Grid parameter file
GSH	Glutathione
LBD	Ligand-binding domain
ID	Intellectual disability
MD simulation	Molecular Dynamic simulation
MeCbl	Methyl cobalamin
Mg^{2+}	Magnesium ion
NMDA	N-methyl-D-Aspartate
NPT	isothermal–isobaric ensemble, amount of substance (N), pressure (P) and temperature (T)
NVT	constant Number of atoms, Volume, and Temperature
PD	Parkinson's Disease
PDB	Protein Data Bank

RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
SDF	Spatial Data File
SSD	Schizophrenia Spectrum Disorders
SSRIs	Selective serotonin reuptake inhibitors
TMD	Transmembrane domain

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SARINGAN UNTUK SEBATIAN BERPOTENSI MENGGUNAKAN PENGGUNAAN SEMULA UBAT DAN SARINGAN MAYA RESEPTOR V-METIL-S-ASPARTET (NMDA) UNTUK SINDROM SPEKTRUM AUTISTIK (ASD)

ABSTRAK

Di Malaysia, kajian mengenai Sindrom Apektrum Autistik (ASD) amat terhad. Kebanyakan kajian yang dijalankan memfokuskan kepada gen seperti neuroligin 3 (NLGN3), NLGN4X, neurexin 1 (NRXN1), dan SH3. Kajian ini memberi fokus kepada reseptor N-Methyl-D-Aspartate (NMDA), yang dipercayai mempunyai kesan yang signifikan terhadap ASD. Dalam kajian ini, beberapa sebatian dan ubat yang berpotensi memulihkan fungsi reseptor dalam pesakit autistik telah dianalisis menggunakan kaedah *in silico* Menggunakan servis laman sesawang DrugReposER, sebatian atau ubat yang dipercayai mempunyai potensi yang boleh berinteraksi dengan NMDAR dikenal pasti, iaitu alitretinoin, 4-androstenedione, amprenavir, marimastat, dan nelfinavir. Penyaringan maya bagi sebatian ini telah dijalankan menggunakan Autodock Vina, Autodock, HDock, dan CB Dock. Simulasi dinamik molekul kemudian dilakukan dengan semua sebatian terpilih untuk menjelaskan mekanisme pengikatan. Analisis ADMET telah digunakan untuk menilai keberkesanan dan keselamatan sebatian tersebut, yang penting untuk kajian akan datang. Hasil daripada graf RMSD dan RMSF yang diperoleh melalui simulasi dinamik molekul menunjukkan bahawa protein mengalami perubahan bentuk apabila berinteraksi dengan ubat-ubatan terpilih. Kajian ini menunjukkan beberapa sebatian, termasuk alitretinoin, marimastat, asid salisilik, karbocistein, vitamin A, dan amprenavir, sebagai mempunyai potensi untuk memulihkan fungsi reseptor NMDA, AMPA, dan

DOCK4 di dalam otak individu dengan ASD. Walaupun kaedah *in silico* telah terbukti secara konsisten sebagai alat untuk penemuan ubat, melaksanakan kajian in vitro adalah penting untuk mengesahkan penemuan yang diperoleh.

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ABSTRACT

In Malaysia, studies on autism spectrum disorders (ASD) have been limited, primarily focusing on genes such as neuroligin 3 (NLGN3), NLGN4X, neurexin 1 (NRXN1), and SH3. This research aimed to investigate the role of the N-Methyl-D-Aspartate (NMDA) receptor, believed to have a significant effect on ASD. The potential compounds and drugs that could restore receptor function in individuals with autism were analyzed using an effective *in silico* method known as drug repurposing and virtual screening. The trusted server, Drug ReposER, was employed to identify potential compounds or drugs that bind to the NMDA receptor, including alitretinoin, 4-androstenedione, amprenavir, marimastat, and nelfinavir. Virtual screening of these compounds was conducted using Autodock Vina, Autodock, HDock, and CB Dock. Molecular dynamics simulations were then performed with all selected compounds to elucidate the binding mechanism. The ADMET analysis was utilized to evaluate the efficacy and safety of the compounds, which is critical for future studies. Results from the RMSD and RMSF graphs obtained through molecular dynamics simulations indicated that the protein underwent shape changes when interacting with the selected drugs. This study highlighted several compounds, including alitretinoin, marimastat, salicylic acid, carbocisteine, vitamin A, and amprenavir, as having the potential to restore NMDA, AMPA, and DOCK4 receptor function in the brains of individuals with ASD. Although the *in silico* method has reliably served as a tool for drug

discovery, conducting in vitro studies is crucial to further validate the obtained findings.

CHAPTER 1

INTRODUCTION

1.1 Research background

Autism Spectrum Disorder (ASD) is a complex neurological condition with significant long-lasting effects. It is relatively common and highly diverse, linked to genetic factors and environmental influences (Lord et al., 2020). Research has revealed numerous de novo mutations and advanced our understanding of epigenetics, polygenic risk, and gene-environment interactions (Lyall et al., 2017). ASD is classified as a spectrum disorder, with symptoms ranging from mild to severe. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), a diagnosis requires persistent deficits in social communication, restricted patterns of behaviour, early symptom onset, clinically significant impairment, and the presence of intellectual disability or global developmental delay (Lord et al., 2018). ASD frequently coexists with other mental health conditions, including schizophrenia and dementia (Starkstein et al., 2015; Ivashko-Pachima et al., 2021).

Individuals with autism may struggle with communication and social interactions, often becoming agitated by changes in routine and engaging in repetitive behaviours. The prevalence of ASD in developed countries is estimated to be at least 1.5%, with an increasing number of diagnoses, particularly among those without intellectual disabilities (Baxter et al., 2015; Christensen et al., 2016). Autism is more commonly diagnosed in men than women, with a reported ratio of approximately 3 to 4 males for every female diagnosed. However, this ratio may be skewed due to underdiagnosis in women, who often exhibit subtler symptoms. Researchers believe the true ratio could be closer to 2:1 (Loomes et al., 2017). Factors contributing to this gender difference include genetic and hormonal influences, such as the "female

protective effect," which posits that women may have greater resilience against mutations linked to autism. Furthermore, social and diagnostic biases often lead to women masking their symptoms, resulting in underdiagnosis (Jacquemont et al., 2014). Understanding these gender differences is crucial for improving diagnosis and tailoring interventions, ensuring that women receive adequate support.

While ASD is a biological disorder, treatment has primarily focused on social services, supplemented by medications to manage behaviour (Lord et al., 2018). This study emphasizes the importance of restoring N-methyl-D-aspartate (NMDA) receptor function, which plays a critical role in synaptic transmission and significantly impacts ASD phenotypes.

1.2 Problem statement

ASD is categorized as a spectrum disorder, indicating significant variability in symptoms and treatment responses among individuals. This heterogeneity complicates the identification of effective interventions, as treatments that may be beneficial for one individual might not be effective for another (Lord et al., 2018). Individuals with ASD frequently present with co-occurring mental health conditions, leading to the prescription of psychiatric medications, such as aripiprazole and risperidone, which are FDA-approved specifically for reducing irritability (Coleman et al., 2019; Fusar-Poli et al., 2019). However, these medications do not address the core symptoms of ASD, highlighting the limitations of current pharmacological options.

Concerns regarding the long-term effects of atypical antipsychotics include negative impacts on glucose and lipid metabolism, as well as an increased risk of type 2 diabetes and cardiovascular disease (Bobo et al., 2013). Additionally, the efficacy of selective serotonin reuptake inhibitors (SSRIs) in treating ASD has been questioned,

with some studies suggesting they may exacerbate symptoms (Williams et al., 2013). The absence of clear guidelines for treating adults with ASD further complicates the situation, as FDA-approved medications are primarily aimed at children. The DSM-5 outlines diagnostic criteria based on childhood presentations, making it challenging to identify ASD in adults, who may mask their symptoms. This highlights the urgent need for more research on medication and treatment strategies tailored for adults with ASD.

Moreover, emerging evidence suggests that individuals with ASD exhibit impaired synaptic transmission. Studies have indicated reductions in key synaptic components, such as NMDA and AMPA receptors, in mouse models exhibiting ASD phenotypes, correlating with social deficits (Takarae & Sweeney, 2017; Guo et al., 2019). Restoring synaptic transmission in these models has shown improvements in social behaviors, indicating that pharmacological interventions must focus not only on symptom management but also on addressing underlying biological factors, particularly the differences in synaptic transmission between individuals with and without ASD. Thus, a comprehensive approach is essential for understanding and treating this complex biological disorder, extending beyond medication and traditional social interventions.

1.3 Research aims and objectives

This research aims to restore NMDA receptor function through drug repurposing, as this approach is believed to help address the impairments observed in individuals with ASD.

1.3.1 Specific Objectives

1. To identify potential compounds or drugs using virtual screening and drug repurposing that have the potential to bind to the NMDA Receptor.

2. To evaluate the stability of the complex, analyse its trajectory, detect durable interatomic bonds, and determine the binding free energy using molecular dynamics simulation.

3. To analyse the selected drugs for their absorption ability, distribution throughout the body, metabolism, excretion, and toxicity effect using the ADMET predictor.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Autism Spectrum Disorder (ASD)

Autism spectrum disorder (ASD) seems to be a genetically pervasive developmental condition with a highly varied genetic architecture linked with human brain development (Baribeau & Anagnostou, 2021; Bhola et al., 2021). This syndrome caused childhood-onset delays in children aged 25 to 41 months, which more delayed than participants between the ages of 19 and 24 months and deferred than 12 to 18-month toddlers (Miller et al., 2021).

Not only that, ASD typically affects fine motor skills, communication, and social interaction deficit, restricted and peculiar interest, sensory hypersensitivity, and repetitive behaviours, which can affect one's day-to-day life (Lee et al., 2015; McCracken et al., 2021). However, this diverse population exhibits a broad ability. Many individuals on the autism spectrum, for instance, have above-average to average intellectual performance, with roughly 30% meeting intellectual disability nine conditions (ID) (Braconnier & Siper, 2021). In addition, multiple studies have estimated that about 69% to 79% of adults and 60% to 70% of children with ASD exhibited at minimum one prevalent mental disorder. For example, hyperactivity disorder and attention deficit (ADHD) or other mood disorders (Lever & Geurts, 2016).

2.2 Level in ASD

Based on the newest Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (2013) version, ASD can be split into levels 1, 2 and 3. These levels centre around two main criteria: restricted and repetitive behaviours and social

interaction. The levels assigned will help doctors and others to determine the proper support everyone needs.

Level 1 ASD is known as the high-functioning form. An individual with level 1 ASD usually experiences difficulties communicating and interacting with other people. For instance, they most likely say the wrong thing at the worst time possible, do not understand others' (inconsistent punctuation) body language, or even read the most prominent social cues. Because of this, making new friends would be almost impossible for them. A person with level 1 ASD can generally communicate and talk in complete phrases but struggles to converse with people continuously. On top of that, they may work with organisation and planning, hindering them from being as independent as other persons their age (Masi et al., 2017).

Individuals with ASD level 2 will have more noticeable social communication and interaction difficulties than those with ASD level 1. An individual in Level 2 will need more support compared to Level 1. Sometimes, even with assistance, speaking can be challenging, and they tend to respond in manners that normal neurotypical individuals find inappropriate (*Diagnostic and Statistical Manual of Mental Disorders*, 2013). They may, for example, turn away from the person they are conversing with. They usually speak in short and simple sentences and focus on particular topics. Furthermore, they may find daily routine challenging due to their difficulties adapting to surrounding changes. Because of this nature, they will suffer substantial distress when forced to face change (Masi et al., 2017).

Level 3 autism is the most extreme manifestation of the disorder. Individuals in this category will display the same characteristics as those in groups 1 and 2, except to a greater extent, and require the most support they can get (*Diagnostic and Statistical Manual of Mental Disorders*, 2013). It can be challenging for them to

operate, communicate socially, and adapt to a shift in focus or location due to the difficulties expressing themselves verbally and nonverbally. Another sign of level 3 ASD is a tendency to engage in repetitive behaviours. A person with autism spectrum disorder (ASD) level 3 will be unable to talk coherently and reluctant to engage in conversation with others awkwardly when it comes to it. Only the most direct social overtures will elicit a response from someone with level 3 (Masi et al., 2017).

2.3 Factors contribute to ASD

ASD is a complex neurodevelopmental condition influenced by a multitude of biological factors. One of the primary contributors is genetic factors, which play a significant role in the aetiology of ASD. Numerous studies indicate a higher prevalence of the disorder among individuals with a family history, suggesting that genetic factors account for approximately 40-80% of the risk for developing ASD (Tick et al., 2016). Specific genetic mutations, such as those found in the CHD8, MECP2, and FMR1 genes, have been associated with the disorder, further underscoring the biological pathways that may be disrupted in affected individuals (Abrahams & Geschwind, 2008).

In addition to genetic influences, environmental factors are critical in the development of ASD. Maternal exposure to environmental toxins such as pesticides and air pollution during pregnancy has been linked to an increased risk of ASD in offspring. Furthermore, complications during pregnancy, including gestational diabetes and preeclampsia, can also elevate this risk (Modabbernia et al., 2017). Nutritional deficiencies, particularly in folate, have been found to contribute to a higher likelihood of ASD, emphasizing the importance of prenatal care (Schmidt et al., 2012).

Brain structure and function are also pivotal in understanding ASD. Neuroimaging studies reveal atypical brain development characterized by increased overall brain volume and altered connectivity in specific regions associated with social communication, such as the frontal and temporal lobes (Ecker et al., 2015). Functional neuroimaging has shown altered activation patterns in areas related to social cognition, suggesting that disruptions in neural circuits may contribute to the symptoms of ASD (Schultz, 2005).

Neurochemical imbalances represent another significant factor. Dysregulation of neurotransmitter systems, particularly those involving serotonin, dopamine, and gamma-aminobutyric acid (GABA), has been implicated in ASD. Abnormal levels of serotonin, for example, may affect mood regulation and social behaviour, potentially exacerbating core symptoms of the disorder (Hollander et al., 2009). Furthermore, an imbalance between excitatory and inhibitory neurotransmission may lead to altered synaptic function, contributing to the behavioural manifestations observed in individuals with ASD (Rubenstein & Merzenich, 2003).

Epigenetic factors have also emerged as important contributors to ASD. These modifications influence gene expression without altering the underlying DNA sequence and can be influenced by environmental factors such as stress, diet, and exposure to toxins. Research suggests that epigenetic changes may play a significant role in the development of ASD, highlighting the interaction between genetic predispositions and environmental influences (Oztenekecioglu et al., 2021).

Other contributing factors include immune system dysregulation, which has been associated with neuroinflammation and altered brain development in some studies, and metabolic disorders such as mitochondrial dysfunction, which further underline the complex biological basis of ASD (Patterson, 2011).

In summary, the etiology of Autism Spectrum Disorder is multifactorial, involving a complex interplay of genetic, environmental, neurodevelopmental, and neurobiological factors. Understanding these contributions is essential for developing effective interventions and supports for individuals with ASD.

2.4 Molecular biology

2.4.1 Protein

Proteins are amino acid-based biomolecules in nearly every cellular activity (Bailey, 2020). Proteins are classified into seven types: transport proteins, antibodies, enzymes, hormonal proteins, storage proteins, contractile proteins, and structural proteins. They are highly complex substances in the cytoplasm when it comes to proteins. The translation is the process that allows proteins to be synthesised. Proteins are unique to each species. Species-specific proteins exist only within their genus. Organ-specific proteins, for example, differ from those found in the brain and liver of a single individual as compared to proteins in muscle. Approximately 20 different amino acids are found naturally in proteins (Whitford, 2013).

It can be globular or fibrous in protein structure, depending on its role. Proteins that are spherical and are dense yet soluble are called globular proteins. The stretched and insoluble nature of fibrous proteins is one of their defining 16 characteristics. A protein molecule is enormous when compared to sugar or salt molecules. However, most proteins share one characteristic they are composed of long chains of α-amino acids connected by peptide bonds to create lengthy chains, similar to how beads are strung on a string (Whitford, 2013). The R groups of the amino acids found in proteins each have their unique structure as the side chains. Polar or nonpolar groups repel or attract each other in the amino acid R groups, determining the protein's configuration

or conformation. Some 100-200 amino acid peptide chains form an inverted loop or helix. However, other segments may be straight or arranged in irregular coils (Kessel & Ben-Tal, 2018).

Proteins have four structural levels: primary, secondary, tertiary, and quaternary. The primary protein structure is determined by the sequence of amino acids, disregarding the peptide chain's spatial arrangement (Kessel & Ben-Tal, 2018). Unlike the primary structure, the secondary structure is determined entirely by the structural configuration of the core peptide chain, disregarding the side chains and other parts of the main chain. The tertiary structure is distinguished by its many neighbouring portions and both main chain's side chains, and it is unaffected by the existence of other peptide chains in the immediate vicinity. The arrangement of identical or distinct subunits of a significant protein, each of which is a different peptide chain, is referred to as its quaternary structure (Whitford, 2013). Proteins are essential because of their ability to fulfil specific activities. Examples include enzymes, which act as catalysts in all metabolic reactions and allow an organism to build up the chemical substances necessary for life, such as carbohydrates, proteins, lipids, and nucleic acids, and convert them into other substances while degrading them. It is not possible to live without enzymes. Next, vital regulatory functions are performed by protein hormones. For example, the respiratory protein haemoglobin functions as an oxygen carrier inside the blood through all vertebrates, delivering oxygen from the lung to tissues and organs (Kessel & Ben-Tal, 2018). In addition, proteins are sensitive to high temperatures, organic solvents, radiation exposure, bases, and acids. As a result, the chemical methods used to purify organic compounds are ineffective when applied to proteins. Most proteins are 17 insoluble in boiling water but are denatured. Because of that, they are irreversibly transformed into insoluble

substances. Heat denaturation cannot be used on connective tissue because collagen's primary structural protein is converted into water-soluble gelatine when water is heated to a high temperature (Kessel & Ben-Tal, 2018).

2.4.2 Receptors

Receptors are distinct protein classes that work by interacting with a ligand. Transmembrane proteins are receptors. Transmembrane receptor proteins are built into the phospholipid bilayer of the plasma membrane. These proteins' cytoplasmic and extracellular sides have hydrophobic and hydrophilic parts (Casem, 2016).

Receptors are chemical structures based on proteins that receive and transmit signals (Hall, 2016). These transmitters are chemical messengers that bind to a receptor and cause a biological change in tissue or cellular response, such as changes in a cell's chemical uptake. The receptor's action can be classified into three categories: signal relay, integration, and amplification. By relaying the signal forwards, integration incorporates the movement into another biochemical pathway, amplifying the single ligand effect (Alberts et al., 2014).

The location of receptor proteins can be used to identify them. G protein-coupled receptors, enzyme-linked hormone receptors, and ligand-gated ion channels are all examples of transmembrane receptors. Those found within the cell are called intracellular receptors, including nuclear receptors and cytoplasmic receptors. Each type is associated with distinct cellular biochemical pathways that correlate to the signal. While most cells contain numerous receptors, each receptor would bind to ligands with a specific structure. A cell's sensitivity to different molecules can be altered by upregulating or downregulating the number of receptors for a particular hormone or neurotransmitter it produces (Hall, 2016).

2.4.3 Ligands

The ligand is either an ion or a molecule that donates one or two electrons to the central metal ion or atom, causing the formation of the coordination complex. If the ligand 18 is linked to the metal by a single atom, it is known as a monodentate ligand; if two or more atoms attach it, it is known as a dentate or polydentate ligand (Britannica, 2010).

The bonding of the ligand to the metal is typically accomplished by donating one or more of the ligand's electron pairs, frequently via Lewis Bases (Burdge & Overby, 2020). wherein rare cases, Lewis acidic ligands were used (Miessler et al., 2013). Metal–ligand bonds can be covalent or ionic, depending on the nature of the bond. Metal–ligand bond order can also vary from one to three. The ligands determine the central atom's reactivity in a complex, including rates of ligand substitution, the ligands' reactivity, and redox.

When a ligand binds to a matched receptor, it either activates or inhibits the biochemical pathway associated with the receptor in question. In addition, ligands can change the receptor conformation and transmit a signal to the cell. Some receptors remain on the cell surface while the ligand diffuses away. In other instances, when a ligand binds to a receptor, it sets off a chain of processes leading to the receptor entering the cell. This is called receptor-mediated endocytosis, and the receptor captures a ligand complex (Casem, 2016).

2.5 N-methyl-D-Aspartate (NMDA) Receptor

N-methyl-D-Aspartate (NMDA) Receptors are ion channels, and neurons' glutamate receptors serve as an essential regulator of synaptic activity in the brain (Parsons & Raymond, 2014). NMDA receptor is one of the ionotropic glutamate

receptors which only allows ions to pass through the membrane. These receptors mediate excitatory neurotransmission of Ca2+ in the central nervous system (CNS). Additionally, NMDA receptors are involved in the pathophysiology of various CNS disorders and have been reported as a disease locus linked to genomic diversity (Hansen et al., 2018).

2.5.1 Structures of NMDA Receptor

The NMDA Receptors are heteromeric complexes made of the GluN1 (NR1), GluN2 (NR2), and GluN3 (NR3) subunits (Figure 1) that translate to form functional channels in the endoplasmic reticulum (Zhang et al., 2016). Two GluN1 and two GluN2 are the most common NMDA receptors form, creating a central ion channel that resembles an inverted potassium channel (Hansen et al., 2018). GluN1 (NR1) is expressed ubiquitously throughout the brain, with the highest concentration found in the hippocampus (Coutinho & Vincent, 2014).

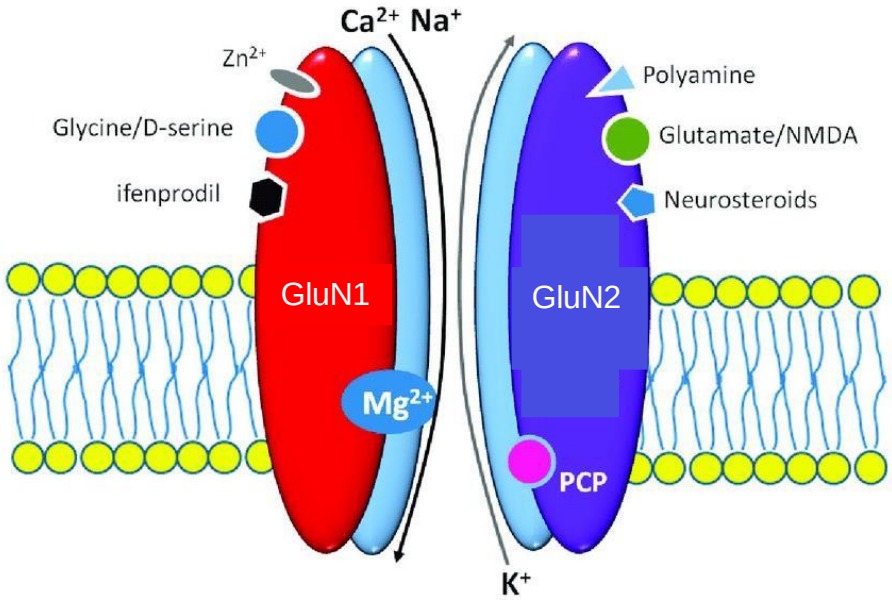

Figure 1.1 Schematic representation of NMDA Receptor structure with the binding sites (Krzystanek & Pałasz, 2019).

The extracellular amino-terminal domain (ATD) partakes in allosteric regulation and subunit arrangement and controls the deactivation speeds and opening of the ion channel. Next, an extracellular ligand-binding domain (LBD) is in charge of binding antagonists and agonists to regulate the ion channel opening. The glutamate binding site is posed in GluN2 LBDs, whereas the Glycine/D-serine co-agonist binding site is present in GluN1 LBDs (Hackos & Hanson, 2017). Three transmembrane segments and a re-entrant pore loop make up the transmembrane domain (TMD). TMD is a component of the channel pore contributes to the channel's calcium permeability, and mediates magnesium blockade (Paoletti et al., 2013; Karakas & Furukawa, 2014). Finally, carboxy-terminal domain (CTD) engages with several cytosolic proteins (Sanz-Clemente et al., 2013). GluN1 and GluN2 activate NMDA receptors when glutamate and glycine bind to the extracellular LBD and release the magnesium chunk by depolarizing the membrane at the TMD. (Regan et al., 2015). Positively charged residues on the surface of GluN2 LBD facilitate glutamate binding through a mechanism known as 'guided diffusion'. In comparison, glycine bound to the GluN1 LBD through a process called "unguided diffusion." In this process, glycine is mostly drawn to the binding site by spontaneous changes in temperature (Yu & Lau, 2018).

In conclusion, to open the channel, glutamate must bind to the receptor and depolarise the membrane in which NMDA receptors are located. This is due to the receptor being blocked by Mg^{2+} at the normal membrane resting state. Next, NMDA receptors allow for a significant influx of Ca^{2+}, and any rise in Ca^{2+} levels can activate a wide range of processes that modify the neuron's properties. However, an excessive amount of Ca^{2+} is also toxic, and it is believed that NMDA receptor hyperactivation causes the formation of several neurodegenerative diseases.

2.5.2 Functions of NMDA receptors

NMDA receptors are crucial in prolonged potentiation and regulating synaptic transportation plasticity, which are vital in the development of learning and memory (Coutinho & Vincent, 2014). NMDA receptors are commonly identified as an essential factor in focal and global neuronal death caused by ischemia. Hypoxia and focal ischemia result in increased NMDAR activation increased Ca^{2+} influx, and excitotoxic cell death (Zhang et al., 2016).

2.6 Roles of NMDA receptors

In the hippocampus, NMDA receptors are crucial for synaptic plasticity and transmission, which are thought to underpin learning and memory by transforming specific neurotransmission patterns into extended synapse function and structure changes. These receptors are necessary for nervous system development, function, and neurotoxicity. However, as one's age increases, the NMDA receptors function lower progressively hypofunctional, which contributes to the reduction in learning and memory performances (Liu et al., 2019).

Also, NMDA receptors can be found in non-neuronal cells such as bone, pancreas, kidney, central and peripheral glial cells, and endothelium (Hogan-Cann & Anderson, 2016). NMDA receptors in osteoblasts can support precursor differentiation and increase the mineralisation of bone, which leads to the deposition of bone matrix (Li et al., 2011). Astrocytes exhibit functional NMDA receptors that can respond to neuroinflammatory processes and glutamatergic neural inpu (Dzamba et al., 2013) t. The function of NMDA receptors in endothelial cells is located in the blood-brain barrier (BBB) area. A high level of glutamate in the brain can be toxic to neurons, damaging the functioning endothelial cell and disturbing BBB's integrity (Basuroy et

al., 2013). Next, in the lung, NMDA receptors are found in the airway smooth muscle cells involved with the hyper-reactivity of inflammatory bronchioles (Antošová & Strapková, 2013; Anaparti et al., 2015). NMDA receptors are expressed by insulin-producing islet β cells in the pancreas. The receptors affect the survival and function of β cells (Marquard et al., 2015).

2.7 Dysregulation of NMDA receptor

Memory deficit, learning impairment, psychosis, and, worst-case scenario excitotoxic brain injury is caused by an increasing level of NMDA receptor hypofunction in the brain (Newcomer et al., 2000). Not only that, but excessive NMDA receptor activation can also cause the pathophysiology of several acute CNS injury syndromes, including trauma, status epilepticus, and hypoxia-ischemia. The dysregulation in NMDA Receptor activity is also connected to ischemic stroke, Alzheimer's disease, Huntington's disease, and Parkinson's disease (Benarroch, 2011).

Chronic stress has also been proven capable of altering glutamatergic neurotransmission through NMDA Receptors, which then trigger the impaired brain-derived neurotrophic factor (BNDF) activity. When NMDA subtype GluN2 receptors are exposed to long-term stress, they become more phosphorylated and express themselves even more. These NMDA receptor subtypes have been linked to decreased hippocampal-dependent cognitive tasks as well as higher anxiety reactions (Costa-Nunes et al., 2014).

A study showed that perturbed synapses in handling Ca^{2+} led to synaptic dysfunction, which caused the NMDA Receptors' glutamate receptors to be overactivated (Mota et al., 2014). The primary excitatory neurotransmitter in the brain

is glutamate, which enables fast neural interaction at excitatory synapses. As a result, excessive glutamatergic signalling results in excitotoxicity (Traynelis et al., 2010). The toxic effects are caused mainly by too much Ca^{2+} entering the cell, mainly through NMDA Receptors, because they have a much higher calcium ion permeability than other iGluRs (Wang & Reddy, 2017).

2.8 Involvement of NMDA receptors in ASD

A study showed genes related to autism and cognitive disabilities located on the NMDA pathway (Marco et al., 2011). This situation can be observed when low NMDA receptors antagonists results in social and cognitive deficits that are comparable to those observed in ASD, including social incapacity and a lack of working memory (Saunders, 2012). In one study, current mediated by NMDA receptors was significantly reduced in ASD patients compared to control-induced neuronal cells due to the mutation that takes place in the receptors (Lim et al., 2021)

Multiple studies have identified that the GRIN2B gene that encoded the GluN2 subunit is linked to ASD (Kenny et al., 2014). Furthermore, since assembled NMDA Receptors are made up of four subunits, each with its own set of properties, ASD-associated GRIN2A/GRIN2B variants are likely to alter the biological functions of the GluN2 subunit (Paoletti et al., 2013).

2.9 Virtual screening

Virtual screening is a computational approach used in drug discovery to identify potential drug candidates by simulating their interactions with a biological target, such as a protein or enzyme. This technique involves docking a library of compounds into the target's binding site using algorithms that predict how well each

molecule binds and how effective it might be. The results are scored based on predicted binding affinity and interaction quality, enabling researchers to prioritise the most promising candidates for further experimental testing. This method enhances efficiency in the drug discovery process by narrowing down the number of compounds that need to be tested in the lab, thereby saving time and resources (da Silva Rocha et al., 2019).

2.9.1 Types of virtual screening

Two main approaches to virtual screening exist: ligand-based and structure-based methods. Ligand-based virtual screening relies on the knowledge of existing ligands that bind to the target, utilizing this information to find similar compounds that may exhibit comparable binding properties. Techniques such as quantitative structure-activity relationship (QSAR) modeling and pharmacophore mapping are commonly employed in this approach (Jang et al., 2018).

In contrast, structure-based virtual screening utilizes the three-dimensional structure of the target protein to identify compounds that can bind effectively within its active site. Molecular docking, which predicts the preferred orientation of a ligand when bound to a target, is a key technique in this category. The results from docking studies provide insights into binding affinity and can guide further optimization of lead compounds (Liu et al., 2020).

2.9.2 Advantages and limitation of virtual screening

The advantages of virtual screening are manifold. Firstly, it enables the rapid evaluation of large chemical libraries, drastically reducing the number of compounds that need to be tested experimentally. This not only saves time but also minimizes the use of resources and materials in the lab. Secondly, virtual screening can enhance the success rates of hit identification, as it allows researchers to prioritize compounds with

a higher likelihood of biological activity (Oliveira et al., 2023). Additionally, the insights gained from virtual screening can guide medicinal chemistry efforts to design and optimize new drug candidates (Lokwani et al., 2023).

Despite its advantages, virtual screening has limitations. The accuracy of the predictions largely depends on the quality of the target structure and the algorithms used. Additionally, false positives and negatives can occur, necessitating subsequent validation through experimental methods. However, ongoing advancements in computational power and algorithms continue to improve the reliability and efficacy of virtual screening in drug discovery (Reddy et al., 2007).

In conclusion, virtual screening is a powerful tool in the drug discovery process, allowing for the efficient identification of promising compounds by simulating their interactions with biological targets. By integrating computational techniques with experimental validation, researchers can accelerate the development of new therapeutics for various diseases

2.10 Drug repurposing

Drug repurposing, also known as drug repositioning, is a strategy used to identify new therapeutic uses for existing medications. This approach leverages the known safety profiles and pharmacological properties of approved drugs to explore their efficacy in treating different diseases or conditions beyond their original indications. The process typically involves screening existing drugs against new targets or diseases, often using computational methods, laboratory experiments, or clinical trials to evaluate their potential for repurposing. Drug repurposing can significantly accelerate the drug development timeline and reduce costs, as it avoids

the lengthy and expensive process of developing a new drug from scratch (Hua et al., 2022).

2.10.1 Importance of Drug repurposing

Drug repurposing, the strategy of identifying new therapeutic uses for existing drugs, has gained significant attention in recent years due to several compelling advantages it offers in the field of drug development. One of the most notable benefits is the potential for shortened development timelines. Since repurposed drugs have already undergone extensive safety and pharmacokinetic assessments, they can move more rapidly through the regulatory process compared to novel compounds, significantly reducing the time required to bring new treatments to market (Krishnamurthy et al., 2022).

Additionally, drug repurposing often involves a lower financial burden. The cost of developing a new drug can exceed billions of dollars, largely due to the extensive research and clinical trials required. In contrast, repurposed drugs can leverage existing data and clinical experiences, making the process more cost-effective (Rodrigues et al., 2022).

Moreover, repurposing can be particularly beneficial in addressing unmet medical needs. Many diseases, including rare conditions and complex disorders such as neurodegenerative diseases and cancer, lack effective treatments. By identifying new applications for existing drugs, researchers can provide therapeutic options more quickly for these challenging conditions (Kulkarni et al., 2023).

Another important aspect of drug repurposing is its ability to exploit the inherent knowledge of the drug's pharmacology and mechanism of action. This understanding allows researchers to predict the potential efficacy of a drug in treating different diseases, facilitating the identification of suitable candidates for repurposing.

Drug repurposing has been pivotal in responding to urgent public health challenges, such as the COVID-19 pandemic, where existing medications were quickly evaluated for effectiveness against the novel virus. This rapid response exemplifies how repurposing can be a critical strategy in addressing emerging health crises (Singh et al., 2020).

In summary, drug repurposing plays a vital role in modern medicine by accelerating the availability of treatments, reducing costs, and addressing unmet medical needs while leveraging existing knowledge of drug safety and mechanisms. This approach not only enhances the efficiency of drug development but also holds significant promise for improving patient outcomes across a wide range of conditions

2.11 Introduction to Bioinformatics

The amount of biological data being collected has never been higher. As a result, many biological challenges have been transformed into computing challenges due to this explosion in data. Bioinformatics is the large-scale application of computational techniques derived from statistics, applied math, and computer science to analyse information linked to biomolecules. Since its inception, bioinformatics has grown to encompass a broad topic in molecular biology ranging from structural biology to genomics to gene expression studies. The combination of biology and technology is because biology is an information technology with the physiology and behaviour of organisms determined by their genes, which can be thought of as digital information repositories (Luscombe et al., 2001).

There are three main goals in bioinformatics. The first goal is to coordinate the information so researchers can retrieve current information and contribute new entries as they are developed, like the Protein DataBank for 3D macromolecular structures.

While collecting is an important job, the data kept in these platforms is worthless till it is analysed. Hence, the bioinformatics objectives go far beyond simple volume control (Berman, 2000). The second goal is to create tools and resources to help data analysis. For instance, examining a newly sequenced protein to know sequences is fascinating. This part requires anything other than a straightforward database search. PSI-BLAST and FASTA programs must consider the biological significance their similarity. Creating such resources needs a solid foundation in computational theory and biology. The third goal is to utilize this technology biologically relevantly to analyse and interpret the information. In the past, biological research focused on a single system and compared it to similar ones. When conducting global analyses of all available data in bioinformatics, we can look for patterns shared by many methods and identify distinct features (Luscombe et al., 2001).

Table 2. 1 List of databases and URLs that are important in bioinformatics studies

Database type and name	**Uniform Resource Locator (URL)**
Functional genomic data	
ArrayExpress	https://www.ebi.ac.uk/arrayexpress/
Gene Expression Omnibus (GEO)	https://www.ncbi.nlm.nih.gov/geo/
Genome sequences	
Database of Clusters of Orthologous Genes (COGs)	https://www.ncbi.nlm.nih.gov/research/cog
Genome - NCBI	https://www.ncbi.nlm.nih.gov/genome
GeneCensus	http://bioinfo.mbb.yale.edu/genome/

Integrated database	
InterPro	http://www.ebi.ac.uk/interpro/
Sequence Retrieval System (SRS)	https://www.expasy.org/
Macromolecular structures	
CATH / Gene3D v4.3	https://www.cathdb.info/
Chem-BLAST	https://randr.nist.gov/chemblast/default.aspx
Structural Classification of Proteins (SCOP)	https://scop.mrc-lmb.cam.ac.uk/
PubChem	https://pubchem.ncbi.nlm.nih.gov/
Protein Data Bank (PDB)	https://www.rcsb.org/
Nucleotide sequences	
EMBL-EBI	https://www.ebi.ac.uk/pdbe/node/1
GenBank	https://www.ncbi.nlm.nih.gov/genbank/
DDBJ	https://www.ddbj.nig.ac.jp/index-e.html
Primary protein sequence	
UniProt/Swiss-Prot	https://www.expasy.org/resources/uniprotkb-swiss-prot
NCBI	https://www.ncbi.nlm.nih.gov/

Secondary protein sequence	
Pfam	http://pfam.xfam.org/ncbiseq/398365647
PROSITE	https://prosite.expasy.org/
PRINTS	http://130.88.97.239/PRINTS/index.php
Composite protein sequence	
OWL	http://130.88.97.239/OWL/

2.11.1 Molecular docking

Structure-based drug design in docking studies has become an essential bioinformatics method for drug discovery since the early 1980s. Molecular docking studies can be done with several different programs based on different algorithms. These algorithms make docking far more approachable and easier to do. The molecular docking method can interact with a small molecule and a protein at the atomic level. These interactions allow researchers to study how small molecules behave in the target protein binding pocket and show how essential biochemical reactions work. Its primary application is in structure-based virtual screening to discover novel active compounds directed against a specific target protein, resulting in several success stories. However, molecular docking is not typically the only technique used when designing the drug, but rather as part of a larger workflow that includes other *in silico* and experimental methods (Romano T. and Kroemer, 2007). The main goal of molecular docking is to use computers to figure out the structure of the ligand-receptor complex. Docking is done in two steps that depend on each other: first, ligand conformations in the active site of the protein are sampled, and then, these conformations are ranked using a scoring function. In a perfect world, sampling